Agilent MassHunter Workstation Software

Qualitative Analysis

Familiarization Guide

Agilent Technologies

Notices

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Software Revision

This guide is valid for B.03.00 and later revisions of the Agilent MassHunter Workstation Software - Qualitative Analysis program, until superseded.

If you have comments about this guide, please send an e-mail to feedback_lcms@agilent.com.

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In This Guide...

This guide contains information to learn to use your Agilent MassHunter Workstation Software - Qualitative Analysis .

Before you begin the exercises, please read the instructions in “Before you begin these exercises..." on page 8.

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 this exercise, 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.

Reference

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

What's New

in B.03.01 (Qualitative Analysis)

- The Find Compounds by Formula algorithm now supports finding compounds using a compound exchange file (CEF).
- You can export compounds in compound exchange format (CEF) format.
- You can import compounds that are in compound exchange format (CEF) format.
- You can convert a profile spectrum to a centroid spectrum.
- You can search an accurate mass library for MS/MS spectra.
- A structure is displayed in a spectrum pane after doing a library search or a database search, if the structure is available.
- When you copy a spectrum to the Clipboard, it is copied as a bitmap and as a MOL file. You can copy the MOL file directly into the Library Editor program.
- For the Find Compounds by Targeted MS/MS results, you can either extract a separate MS/MS spectrum for each collision energy or an average MS/MS spectrum for all collision energies.
- For the Find Compounds by Auto MS/SM algorithm, you can filter results by fragment masses or neutral losses.
- System suitability values are also calculated for MS signals.
- The Chemical Data Dictionary is available as part of the Qualitative Analysis program. This tool is used with the MassHunter Optimizer program.
- You can now define and match sequences in the Qualitative Analysis program. This tool is used with the MassHunter Optimizer program.
- You can click on a CAS number, a KEGG number, an HMP number or an LMP number and automatically search a site on the internet for that number. You can specify which web site to use.

in B.03.00 (Qualitative Analysis)

- GC/QQQ data is supported.
- MS library search and MS/MS library search are supported.
- If a structure is available in the library, then you can view it in the Structure Viewer window and include it in reports.
- Difference spectra are automatically displayed in the difference window after a library search is done.
- Chromatogram Deconvolution, a new Find Compounds algorithm, is supported.
- The Universal integrator is supported.

in B.02.00 (Qualitative Analysis)

- The Agilent MassHunter BioConfirm Software is supported. This software allows you to create, edit and import sequences and then match sequences. Sequences can be provided in the protein column in the worklist. The Chemical Data Dictionary Editor is now included.
- Four different workflows are supported, including BioConfirm. Each workflow has its own method, layout and special section in the Method Explorer to help you do your tasks more easily.
- System suitability calculations can be created when integrating UV chromatograms. You can specify different pharmacopoeia.
- You can automatically subtract an averaged spectrum from a designated time range when extracting a peak spectrum.
- When determining Charge State, three different isotope models are supported: common organic molecules, peptides, and unbiased.
- When determining Charge State, you can specify to treat ion with an unassigned charge state as singly-charged.
- The Maximum Entropy Deconvolution is improved to create cleaner spectra. You can increase the number of iterations and specify the singlet width.
- For compounds with resolved isotopes, you can deconvolute using the new Deconvolute: Resolved Isotope algorithm.

- Three different target data type are available for the Find Compounds by Molecular Feature algorithm.
- You can filter the compounds found by the Find Compounds by Molecular Feature algorithm before these compounds are written to the MHD file. This MHD file is used by Mass Profiler and GeneSpring.
- You can extract EIC and Raw Spectrum automatically when using the Find Compounds by Molecular Feature algorithm.
- You can specify a list of masses to exclude or include in the Find Compounds by Molecular Feature algorithm. You can either type this list in directly or specify a database to use.
- When determining Charge State in the Find Compounds by Molecular Feature algorithm, you can specify to treat ion with an unassigned charge state as singly-charged.
- You can specify whether or not to include ions with only one ion in the results for the Find Compounds by Molecular Feature algorithm.
- When using the Find Compounds by Formula algorithm, you can include sample purity calculations. You can calculate based on area or height, and you can use any of all of the following algorithms: TIC %, EIC/TIC %, ADC %, UV A %, UV B %, UV C %, or TWC %.
- In the Search Database algorithm, the scoring now is based upon accurate mass of monoisotopic ion, isotope spacing and isotope ratios. You can control how these three scores are weighted when calculating the overall score.
- The Find Compounds by Molecular Feature algorithm has been improved for cases with exotic elements. For example, the algorithm has been improved when the compound has many isotopes with strange patterns or a first isotope with a low abundance.
- In the Find Compounds by Molecular Formula algorithm, you can control how the different scores are weighted when calculating the overall score. The three parts of the score are mass position, isotope abundance and isotope spacing.
- The Compound Automation algorithm includes the option to Match Sequences. You can also specify whether to include only identified compounds.

- You can save a report as a PDF file using the Microsoft PDF Generator Add-in. You can download this Excel 2007 add-in free from Microsoft.
- You can export in multiple formats: ASR, Compound Summary CSV, MGF and mzData. The ASR format includes Find by Formula results with Sample Purity (either one file per data file or merged). The Compound Summary CSV format includes mass lists (one file per data file or merged). The MGF format includes peptides with MS/MS spectrum and precursor (one file per data file or merged). The mzData format now has more options available.
- When printing graphics, you can select to print all graphics, only highlighted graphics, or only graphics that are visible. You can also print directly from the toolbar of any of the graphics windows.
- The new Excel 2007 Report Designer Add-in is supported. This add-in supports printing graphics side-by-side.
- Easy Access version B.02.00 is supported.
- System performance is improved.
- You can configure the compound label. You can choose from 11 different peak labels. You can select to only include the first attribute that is defined or to include all of the defined attributes.
- You can now configure the user interface. You can select what types of features to show in the user interface based upon separation types, mass accuracy, non-ms detectors, BioConfirm features, and Advanced features.

in B.01.03 (Qualitative Analysis)

- Dual mode data is supported.
- UV data files can be opened.
- You can search a database for molecular formula, mass or mass and retention time.
- You can find compounds in a data file based on formula that are specified.
- You can specify what parts of an analysis report and compound report to include.

- Excel 2007 is supported for Qualitative Analysis.
- You can integrate UV and other types of chromatograms.
- You can view UV spectra.
- You can automate the creation of a compound report including which find compounds algorithm to use and which compound identification algorithm to use.
- You can automate the creation of an analysis report including which chromatograms to extract, which spectra to extract and which spectra identification algorithms to use.

Before you begin these exercises...

- 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

Contents

Exercise 1
Learn basics of qualitative analysis

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

First, you perform tasks whose instructions are independent of the data type.

- In Task 1, you open the program with multiple data files.
- In Task 2, you zoom in and out on specific points of data.
- In Task 3, you anchor a chromatogram so it never disappears from view when scrolling.
- In Task 4, you change the layout of the windows.
- In Task 5, you print an analysis report.

Then you choose whether to work with MS-only data, combined MS and MS/MS data, combined MS and UV data or GC/MS data.

In these tasks, you work with MS-only data:

- In Task 6. Extract chromatograms (MS only), you extract chromatograms at various levels and merge the EICs.
- In Task 7. Interactively integrate a chromatogram (MS only), you integrate chromatograms, change the integration parameters and calculate the S/N ratio for integrated peaks.
- In Task 8. Extract spectra from a chromatogram (MS only), you extract spectra from specific points and ranges in a chromatogram, learning to average them and subtract background data.

In these tasks, you work with combined MS and MS/MS data:

- Task 9. Extract chromatograms (LC/MS and LC/MS/MS)
- Task 10. Interactively integrate a chromatogram (LC/MS and LC/MS/MS)
- Task 11. Extract spectra from a chromatogram (LC/MS and LC/MS/MS)

In these tasks, you work with combined MS and UV data:

- Task 12. Extract chromatograms (MS and UV)
- Task 13. Interactively integrate a chromatogram (UV) and calculate System Suitability values (MS and UV)
- Task 14. Extract spectra from a chromatogram (UV)

In these tasks, you work with GC/MS data:

- Task 15. Configure User Interface for GC
- Task 16. Extract chromatograms from a GC/MS data file
- Task 17. Interactively integrate a GC/MS chromatogram
- Task 18. Basic tasks for a GC/MS data file

Each exercise is presented in a table with three columns:

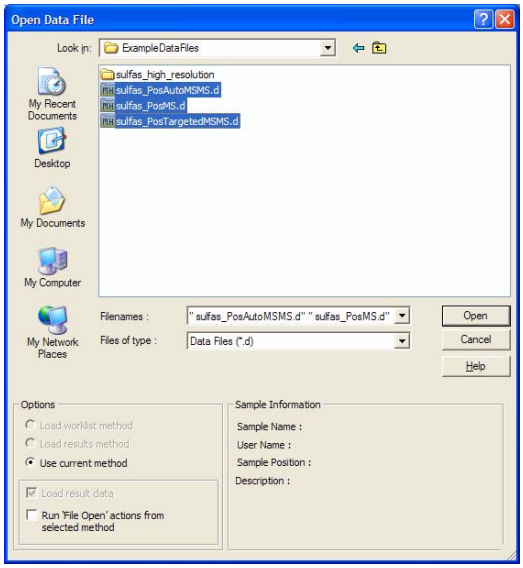
- Steps – Use these general instructions to proceed on your own to explore the program.
- Detailed Instructions – Use these if you need help or prefer to use a step-by-step learning process.
- Comments – Read these to learn tips and additional information about each step in the exercise.

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**, **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** icon . The system displays the Open Data Files dialog box. **b** Go to the folder **\\MassHunter\Data\LC** 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 **Use current method** is clicked. • Make sure that the check box for **Run 'File Open' actions from selected method** 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**, **sulfas_PosMS.d** and **sulfas-PosTargetedMSMS.d**. **d** Click **Open**. All three data files are displayed in Data Navigator, and 1-3 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 instead, 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.

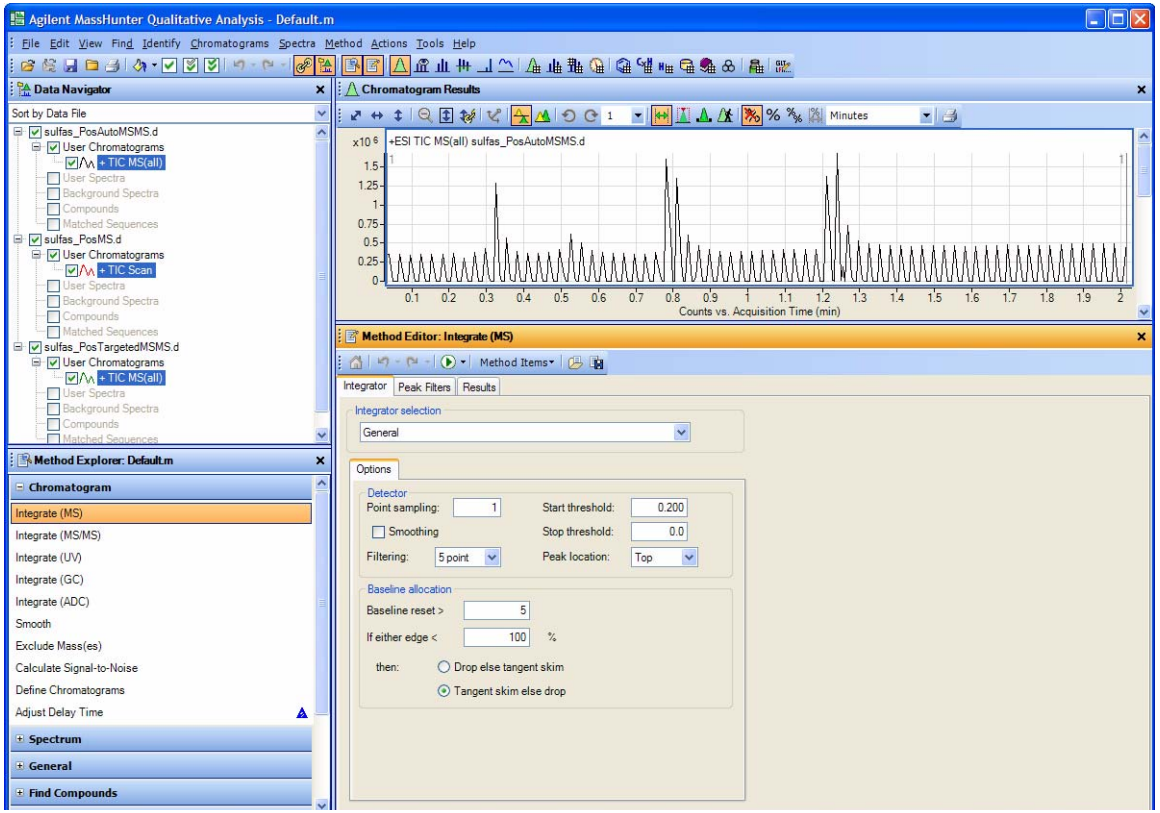


Figure 2 Qualitative Analysis main window

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

Steps	Detailed Instructions	Comments
2 Return the main window to the default worfklow, General. The default method and layout are loaded. • Make sure you can see all three chromatograms.	**a** If necessary, click **Tools > Configure for Workflow > General**. **b** 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 the Plot Display Options dialog box. • You can change the layout by clicking **View > Window Layouts > Load Layout**.

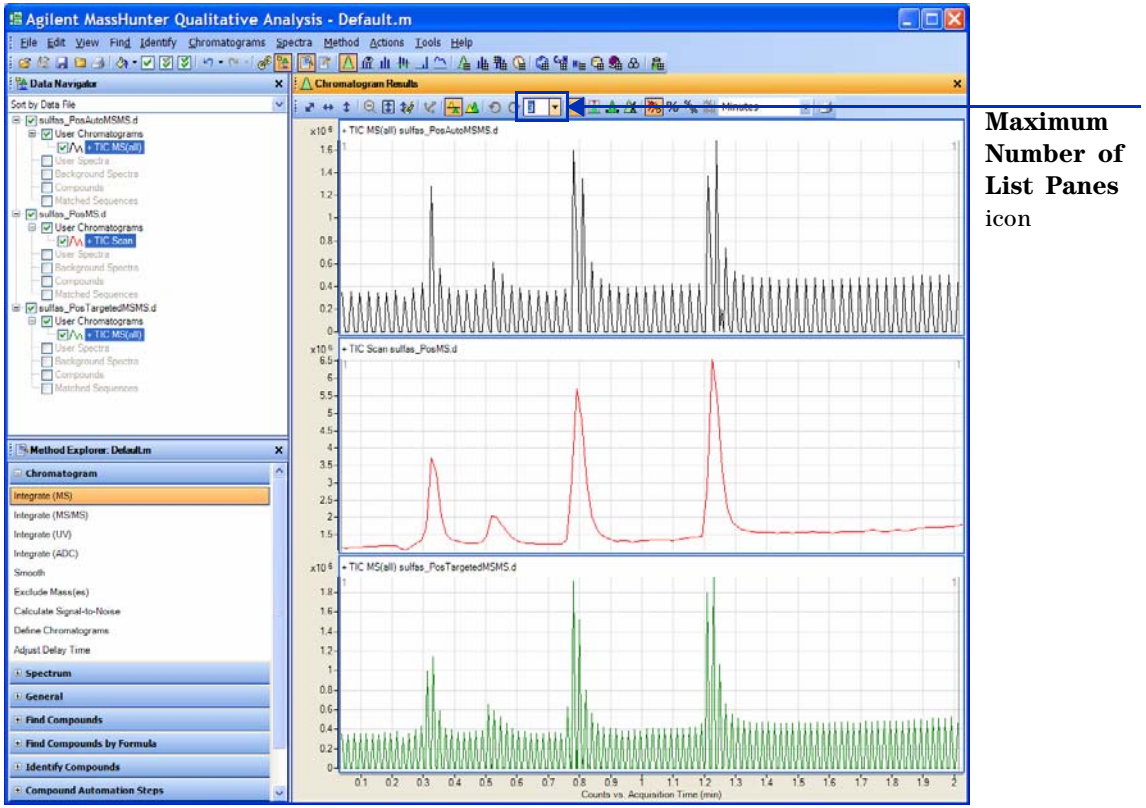


Figure 3 Qualitative Analysis main window with the General Workflow selected.

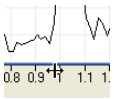
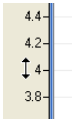
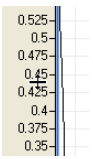
Task 2. Zoom in and out of the chromatogram

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 out 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 that line 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 and the UV 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 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 Data Navigator 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 then click **Clear Anchor** in the shortcut menu.

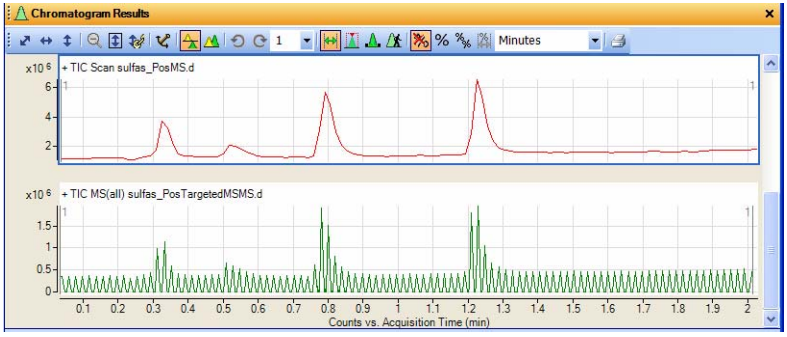


Figure 4 Anchored TIC

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 **View > Window Layouts > Save Layout**. • To unlock a layout, click **View > Window Layouts > Lock Layout**. • To make a window float, right-click the title bar of the Chromatogram Results window, and click **Floating** from the shortcut menu. • To move a window, click on the title bar of the Chromatogram Results 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 an icon in 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 four different workflows. Each workflow loads a different layout. Switching to a different workflow also changes the layout. • If the BioConfirm program is installed, it also has a different workflow and layout.

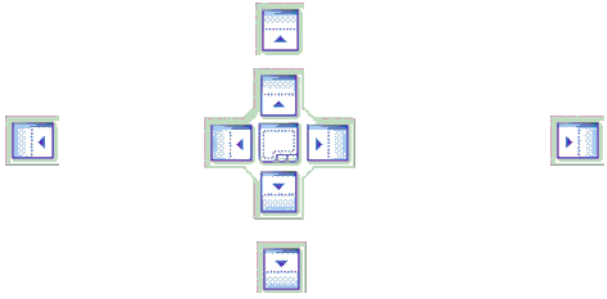


Figure 5 Window repositioning tools

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 **View > 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 Method Explorer, click **General > 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. • Also, 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.

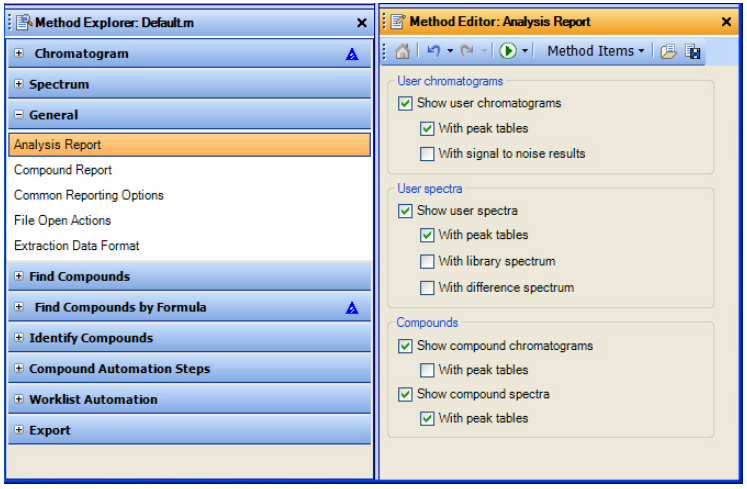


Figure 6 Analysis Report window in Method Editor

Task 5. Print an analysis report (continued)

Steps	Detailed Instructions	Comments
2 Print the report.	• 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. ◦ 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 **Print Analysis Report**.	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 General > Common Reporting Options section, 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 default action is performed.

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 instrument.

Task 6. Extract chromatograms (MS only)

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

Task 6. 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, click **EIC**. **e** In the **m/z value(s)** field, 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**, 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 6. Extract chromatograms (MS only) (continued)

Steps	Detailed Instructions	Comments

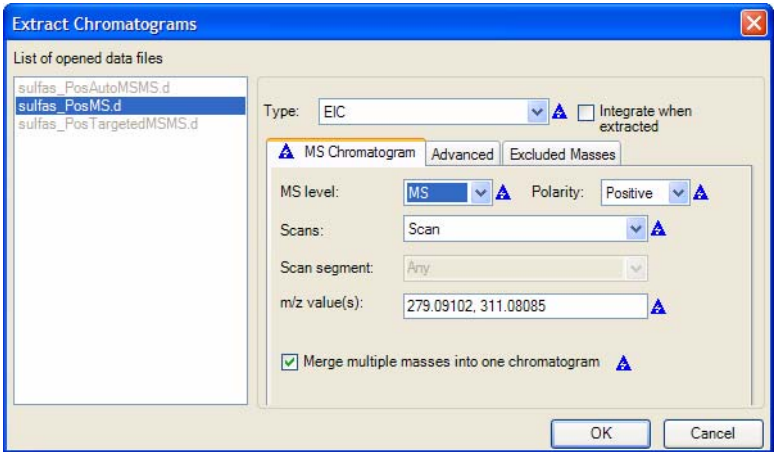


Figure 7 The Extract Chromatograms dialog box.

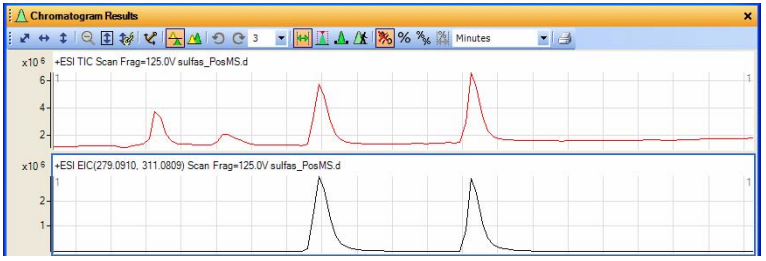


Figure 8 Merged extracted ion chromatograms (EICs) compared to the original TIC

Task 7. 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 7. 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 TIC Scan and click **Integrate Chromatogram**.	• The integration uses the General Integrator, because that is the integrator selected in the method default.m. You can change this value in the Chromatogram > Integrate (MS) > Integration tab. • Note that the integration with default parameters is detecting very small peaks.

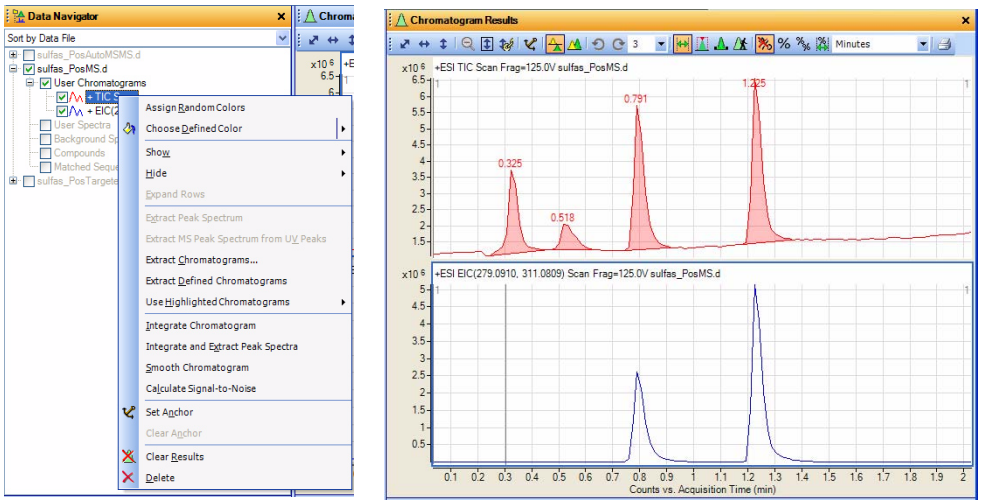


Figure 9 Shortcut menu from Data Navigator and integrated sulfas_PosMS.d TIC chromatogram

Task 7. 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**.	• Normally you would 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 **Limit (by height) to the largest,** if necessary, and type in 2. **d** Click the **TIC Scan** in the Data Navigator window.	• 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.

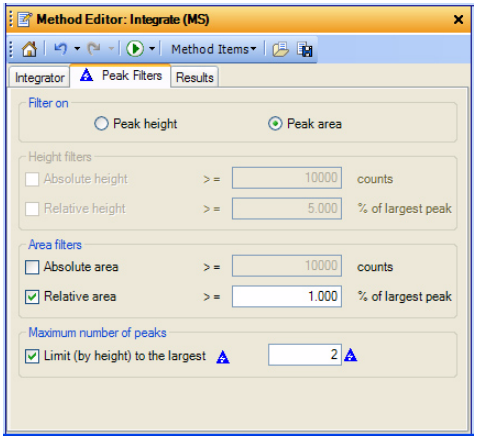


Figure 10 Peak Filters tab with **Limit (by height) to the largest** marked

4 Reintegrate the chromatogram.	• Click the **Integrate Chromatogram** icon on the Method Editor toolbar to integrate using the new setting.	• Note that only the two largest peaks are now integrated.

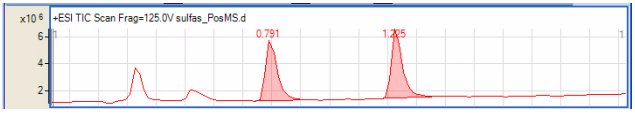
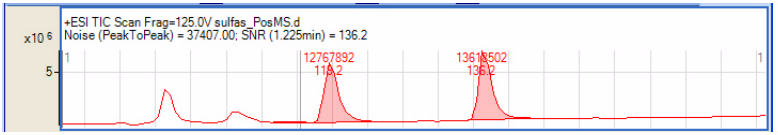


Figure 11 Integration results with limited number of peaks

Task 7. 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 **Tools > Plot 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** Type `0.63 – 0.73` for the **Noise regions**, and click the **Calculate Signal to Noise** icon.	• 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 12 Integrated TIC with Area and Signal-to-Noise labels	
6 Restore the settings for the default method, and close Method Editor.	**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 **Method Editor**.	
7 Return the peak labels to Retention Time.	**a** Click **Tools > Plot Display Options**. **b** Click the Chromatogram tab. **c** Set the first Peak Label to **Retention Time** and the second Peak Label to **None**. **d** Click **OK**.	

Task 8. Extract spectra from a chromatogram (MS only)

In this task, you extract a spectrum from exactly where you specify in the chromatogram. You can tell the Qualitative Analysis program to 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 8. 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 first peak, 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 min. 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** To open Spectrum Preview, click the **Spectrum Preview** icon,  **e** Zoom into the region between 1.1 and 1.4 min. **f** On the peak near 1.22 min. extract a spectrum in any of the ways listed in the Comments column. The spectrum is shown in the Spectrum Preview window. **g** Right-click the spectrum in the Spectrum Preview window, and click **Copy to User Spectra**. The Spectrum Preview window is not closed. **h** If necessary, click the arrow next to the **Maximum number of list panes** icon in the MS Spectrum Results toolbar, and click 2. **i** 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 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 MS 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 on, Qualitative Analysis overwrites the previous spectrum when you extract a new spectrum.

Task 8. Extract spectra from a chromatogram (MS only) (continued)

Steps	Detailed Instructions	Comments

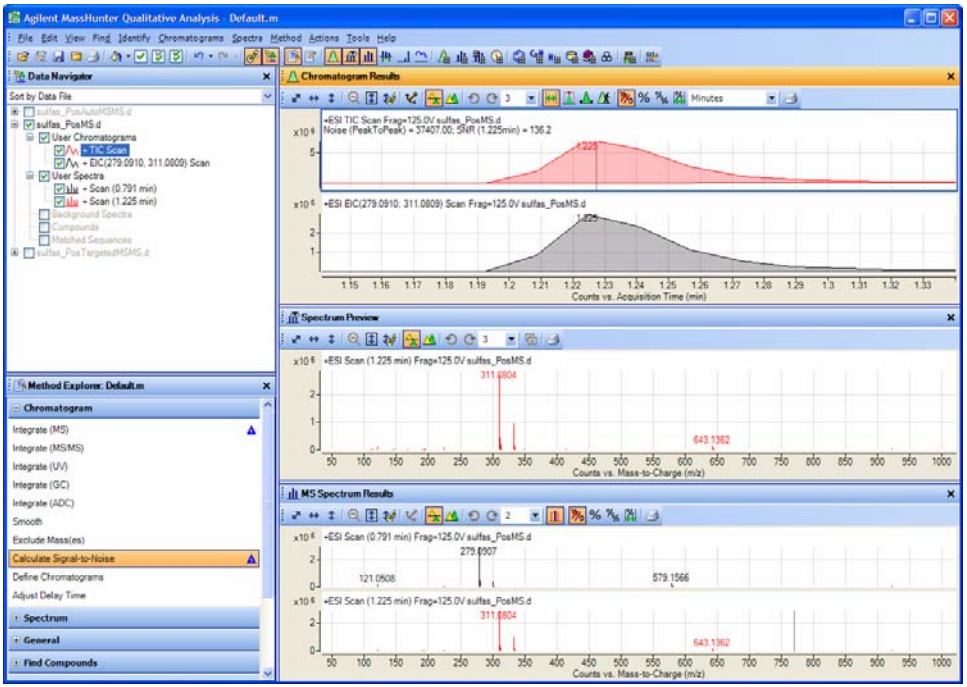


Figure 13 Main window with extracted spectra from both integrated peaks in the sulfas_PosMS.d file

Task 8. 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 (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** 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 from the **Tools > Message Box Options** command. • The Extract Spectrum dialog box is only shown if more than one data file is loaded.

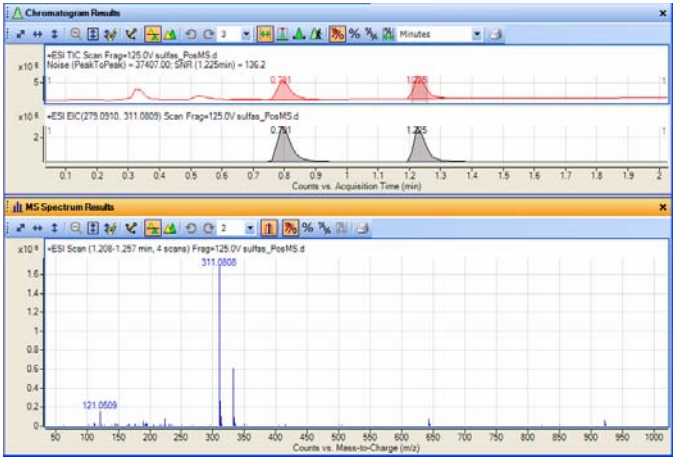


Figure 14 Average spectrum extracted from selected range for last peak

Task 8. 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**.

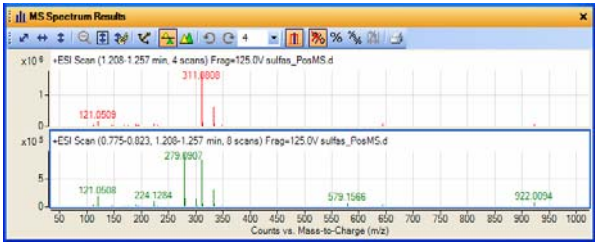
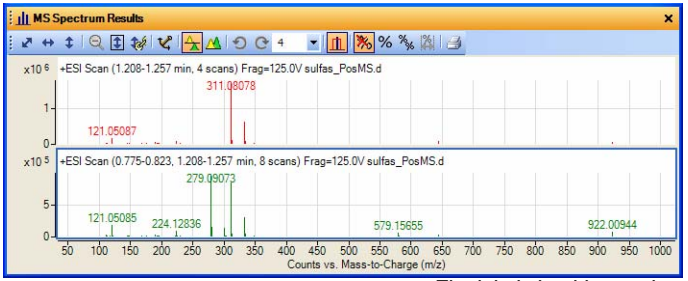


Figure 15 An averaged spectrum created from multiple ranges.

Task 8. 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 **Tools > Plot 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 **OK**.	• Note that the label now shows *m/z* with one more digit.
		• The label should now show the original number of digits.
	e Repeat steps a and b, then set **Digits after the decimal** to one less than the current setting. **f** Click **OK**.	

Task 8. Extract spectra from a chromatogram (MS only) (continued)

Steps	Detailed Instructions	Comments
5 Subtract a background spectrum every time you extract a peak spectrum in **sulfas_PosMS.d**. • 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** Make sure the **Range Select** icon is selected in the Chromatogram Results toolbar, and 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 **OK**. **f** In Method Explorer click **Spectrum > Extract MS**. **g** Click the **Manual Extraction** tab. **h** Under **Manual 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**.	• 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 8. Extract spectra from a chromatogram (MS only) (continued)

Steps	Detailed Instructions	Comments

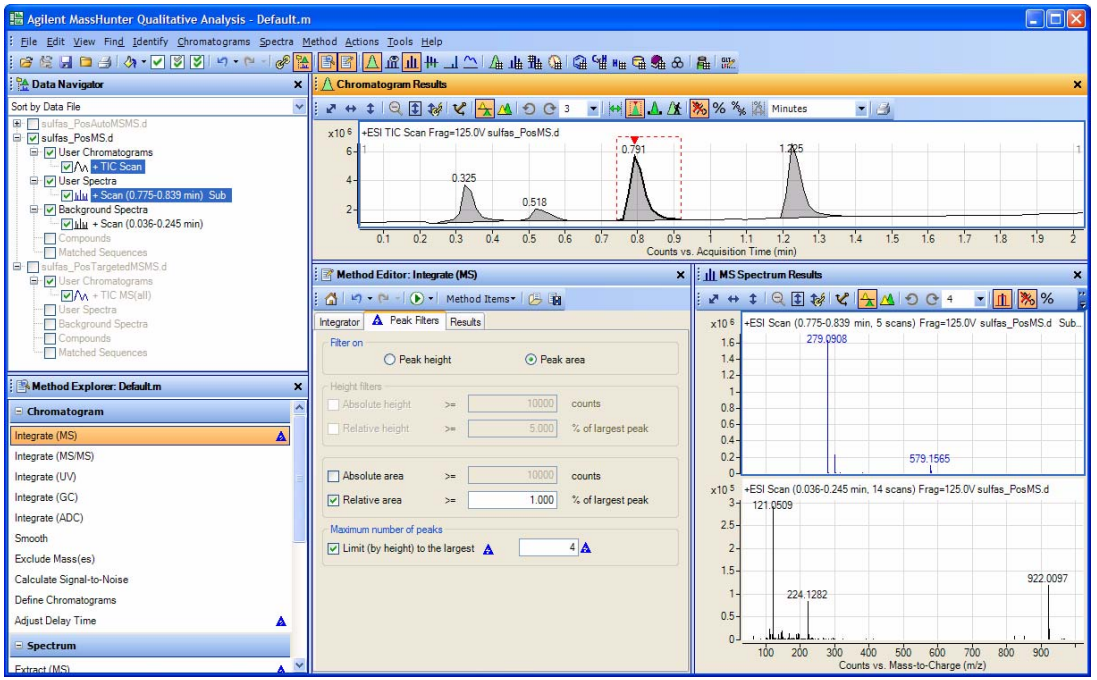


Figure 16 Spectrum with background subtracted

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

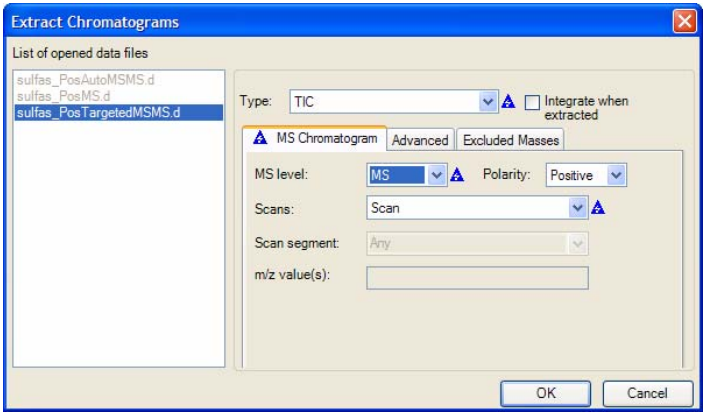
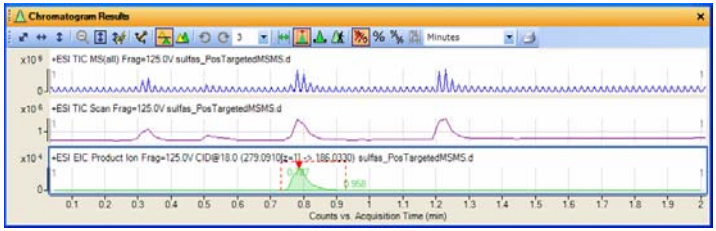
Task 9. Extract chromatograms (LC/MS and LC/MS/MS)

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 9. 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, 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 inside 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 from mass spectra.

Task 9. Extract chromatograms (MS and MS/MS) (continued)

Steps	Detailed Instructions	Comments
		
	Figure 17 The Extract Chromatograms dialog box.	
2 Extract another chromatogram but based on a product ion for the MS/MS data. • This time choose to integrate the extracted chromatogram.	**a** Repeat steps b-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, click 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**.	• In the m/z value(s) text box, you can also type a range (for example, `100 - 300`)
		
	Figure 18 TIC for MS and EIC for MS/MS data compared to the original TIC	

Task 10. Interactively integrate a chromatogram (LC/MS and LC/MS/MS)

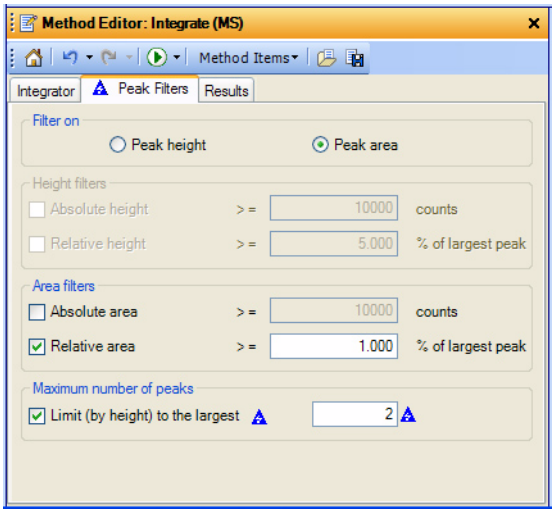
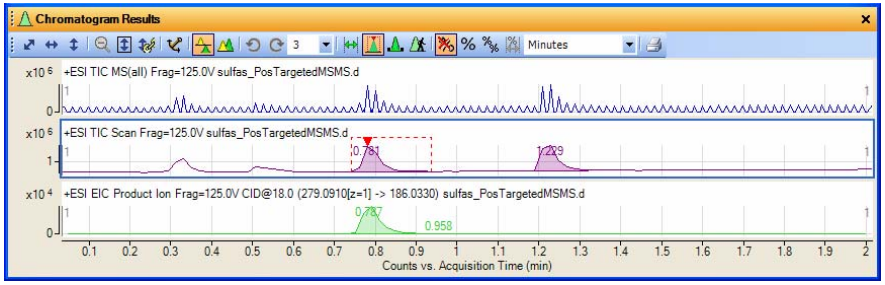
In this task, you learn different ways to integrate a chromatogram, change integration parameters to modify the results and calculate the S/N for the integrated peaks for MS/MS data.

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

Task 10. 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,** then right-click the TIC Scan 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 and ADC data in the Method Editor window.
2 Change the threshold to integrate fewer peaks. • 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** In the Maximum number of peaks box, mark **Limit (by height) to the largest,** if necessary, and type in 2.	• 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 10. Interactively integrate a chromatogram (LC/MS and LC/MS/MS) (continued)

Steps	Detailed Instructions	Comments
		
	Figure 19 Peak Filters tab with **Limit (by height) to the largest** marked	
3 Reintegrate the chromatogram.	**d** 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 20 Integrated TIC MS and MS/MS chromatograms with higher threshold setting	

Task 10. 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 and ADC data in the Method Editor window.
5 Change the filter to filter on height. • Display the Integration Method Editor window from Method Explorer for MS/MS data. • Change the Filter on value to peak height • Mark the **Absolute height** check box.	**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.	• 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 button on the Method Editor toolbar to integrate using the new setting.	• Note that only the largest peak is now integrated.

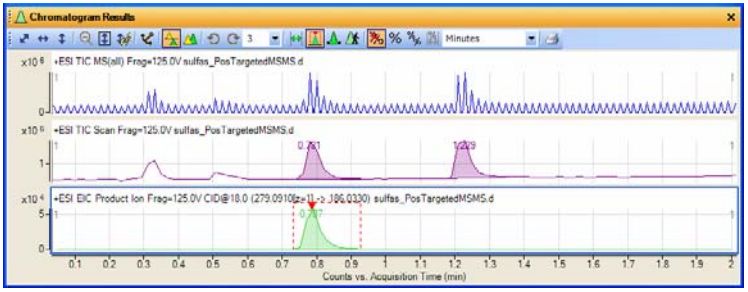


Figure 21 Integrated TIC MS and MS/MS chromatograms with higher threshold setting

Task 10. 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 **Tools > Plot Display Options**, and set the first Peak label to **Area** and the second Peak label to **Signal-to-Noise**. **b** In Method Explorer in the Chromatogram section, select **Calculate Signal to Noise**. **c** Type `0.0 – 0.76` for the **Noise regions**, and click the **Calculate Signal to Noise** icon.	• 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.

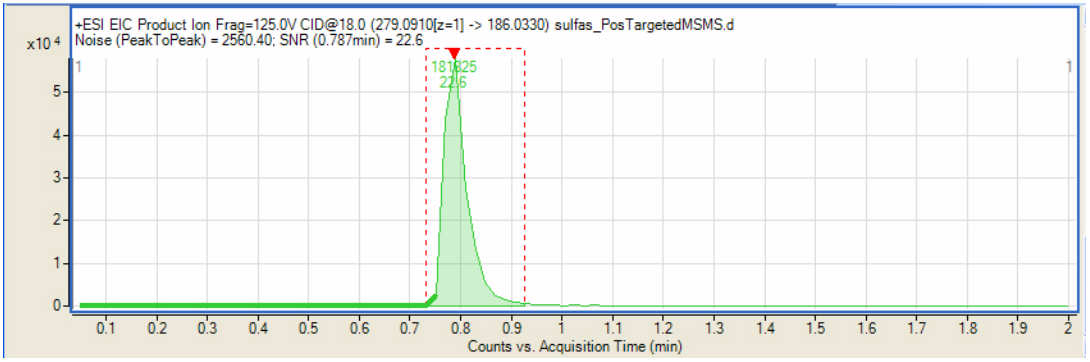


Figure 22 Signal-to-Noise results for MS/MS EIC Product Ion

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 on 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 on the Method Editor toolbar.

Task 10. Interactively integrate a chromatogram (LC/MS and LC/MS/MS) (continued)

Steps	Detailed Instructions	Comments
9 Return the peak labels to Retention Time.	**a** Click **Tools > Plot Display Options**. **b** Select **Retention Time** for the first Peak label and **None** for the second Peak label. **c** Click **OK**.	
10 Delete all chromatograms except the original.	**a** Under User Chromatograms in the Data Navigator window, highlight all the chromatograms except the original. **b** Right-click the highlighted chromatograms, and click **Delete**.	

Task 11. 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. You can tell the Qualitative Analysis program to 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 11. 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 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. **f** To navigate from spectrum to spectrum, use the right and left arrow keys on your keyboard.	• The Walk Chromatogram tool is particularly useful on MS/MS data for identifying precursor and product ions. • The spectrum for each point you click in the Chromatogram Results window is automatically displayed in the Spectrum Preview window, which is opened automatically.

Task 11. Extract spectra from a chromatogram (LC/MS and LC/MS/MS) (continued)

Steps	Detailed Instructions	Comments

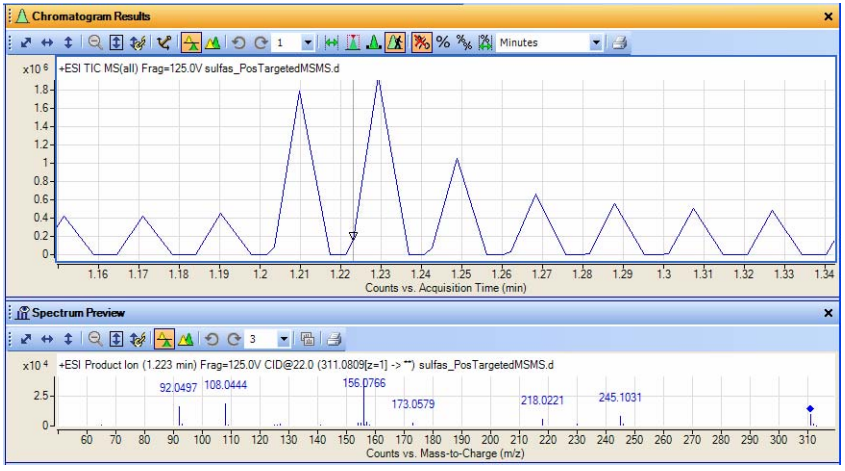


Figure 23 Walk chromatogram to view the MS/MS and product ion for the background at 1.223 minutes

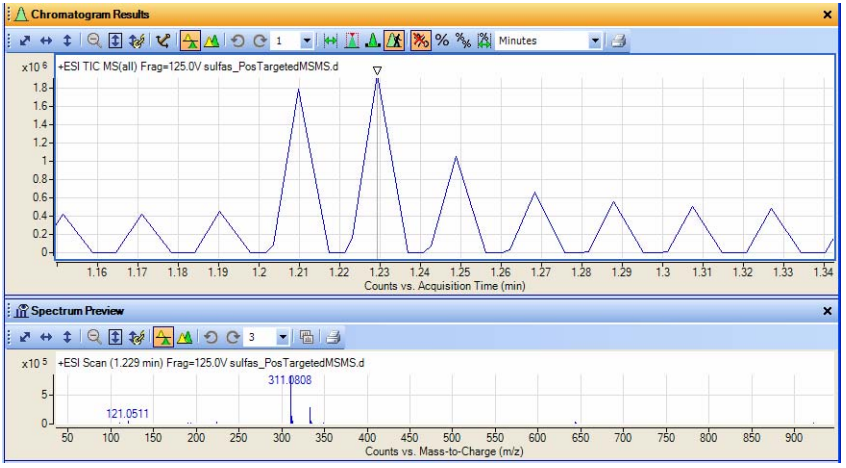


Figure 24 Walk chromatogram to view the MS scan for the peak at 1.229 minutes

If you want the Fragmentor voltage included in the chromatogram title and the spectrum title, you mark the Expanded check box in the Plot Display Options dialog box. You mark this check box on the Chromatogram tab and the MS and MS/MS Spectrum tab.

Task 11. 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 .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 min., extract the 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 click 3.	• 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 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. All subsequent extracted spectra appear in both places as well.

Task 11. Extract spectra from a chromatogram (LC/MS and LC/MS/MS) (continued)

Steps	Detailed Instructions	Comments

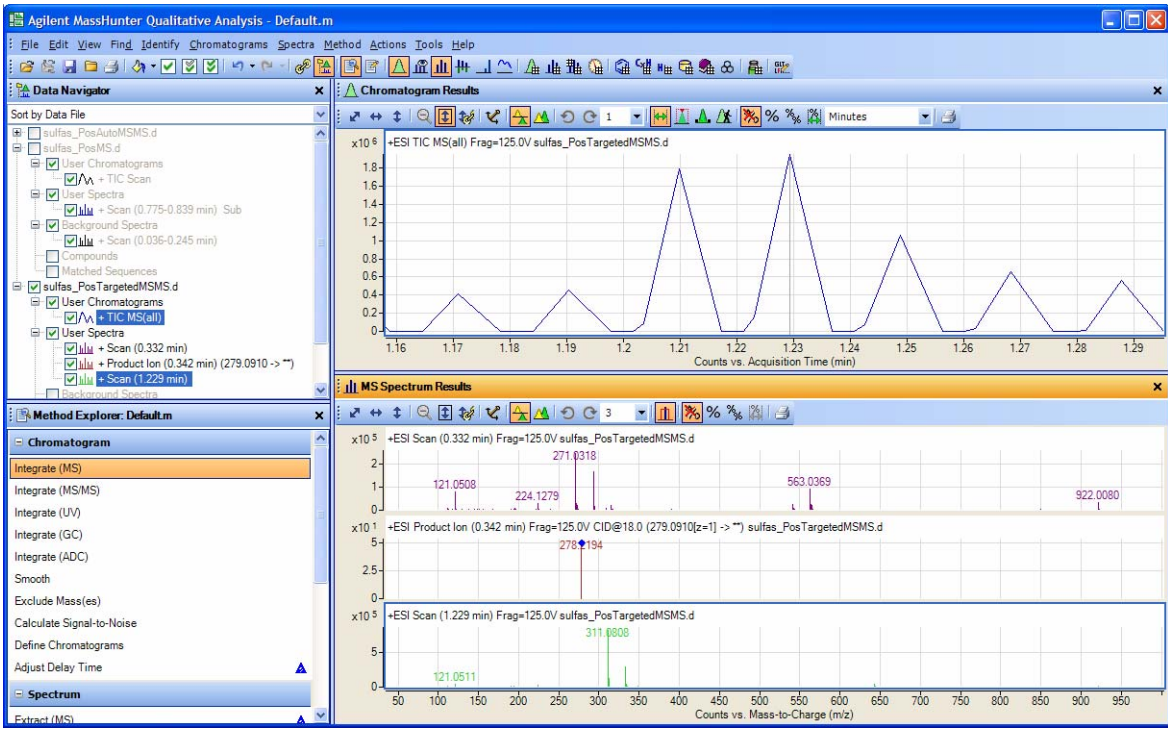


Figure 25 The Qualitative Analysis program with MS Scan and Product Ion spectra from the first peak and MS Scan spectrum from the last peak

Task 11. 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**. The Spectrum Preview window is between the Chromatogram Results window and the MS Spectrum Results window. **d** Click the down arrow next to the spectrum pane list, and select 4. **e** Close the Spectrum Preview window.	• When Spectrum Preview is enabled, the system displays any manually-selected spectrum in the Spectrum Preview window but not in the User Spectra section of Data Navigator. • With Spectrum Preview on, Qualitative Analysis overwrites the previous spectrum when you extract a new spectrum. • Spectrum Preview mode is useful when you quickly want to review the spectra in your chromatogram and save only a few of the spectra.

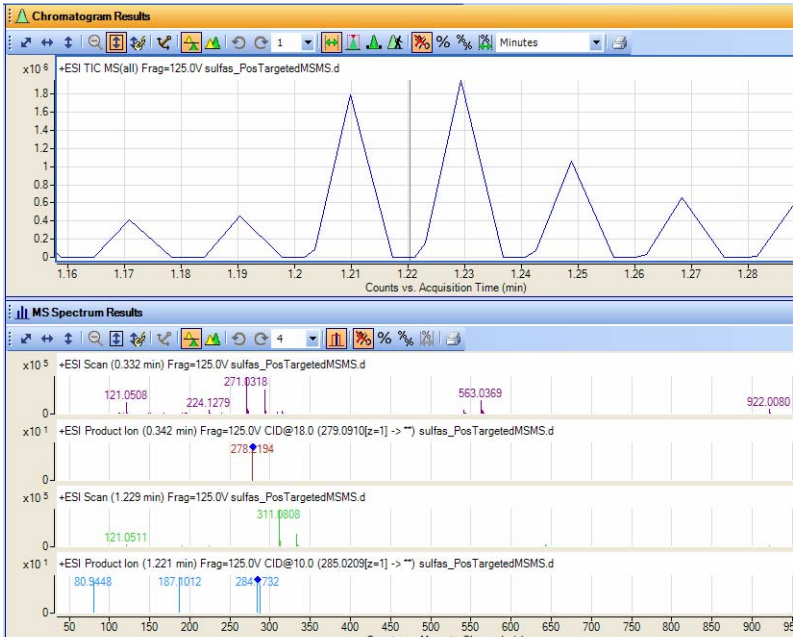


Figure 26 Main window with product ion spectrum from the last peak in the chromatogram

Task 11. 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, 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.

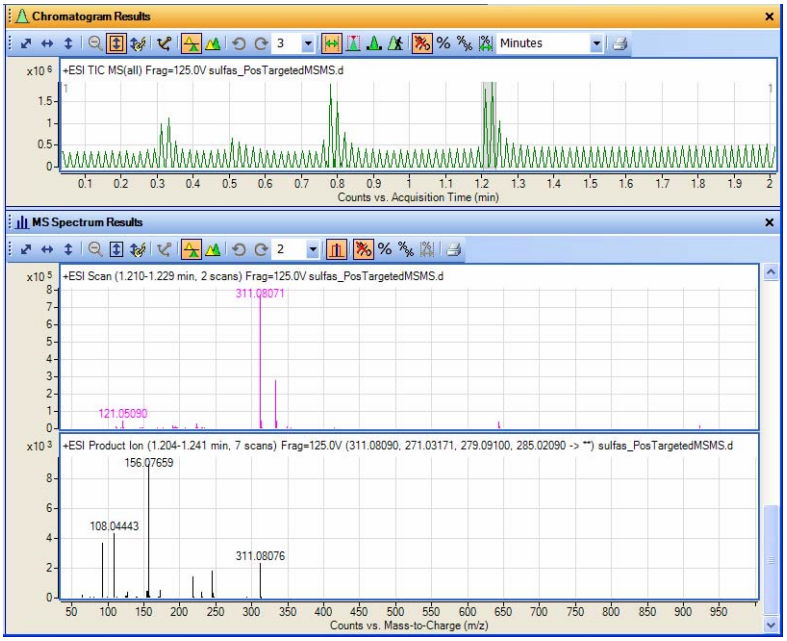


Figure 27 Averaged spectra extracted from selected range for last peak

Task 11. 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_PosTargeted.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.

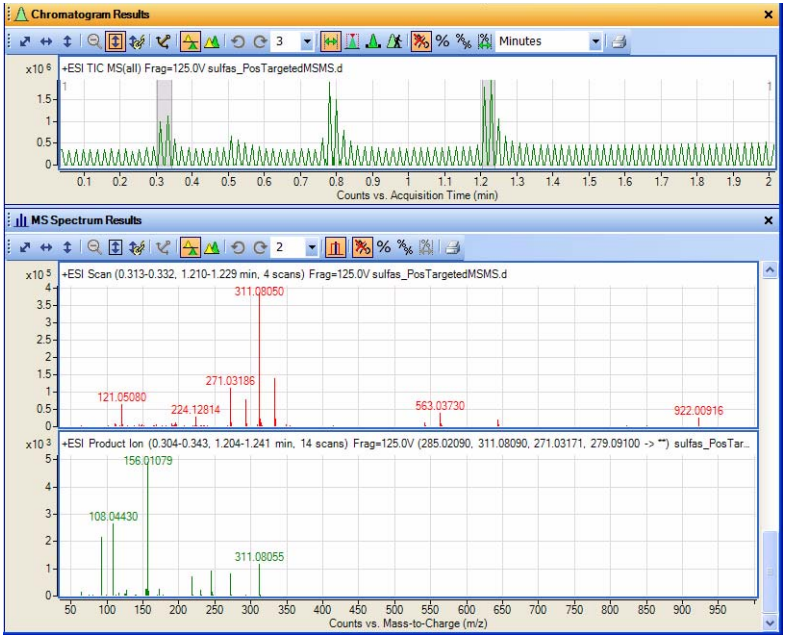


Figure 28 Averaged MS and MS/MS spectra created from multiple ranges.

Task 11. 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**. **c** Extract an integrated MS/MS EIC of ions 279.09100 with an m/z range of 100-300 (see "Task 9. Extract chromatograms (LC/MS and LC/MS/MS)" on page 38) **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 at 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.

Task 11. Extract spectra from a chromatogram (LC/MS and LC/MS/MS) (continued)

Steps	Detailed Instructions	Comments

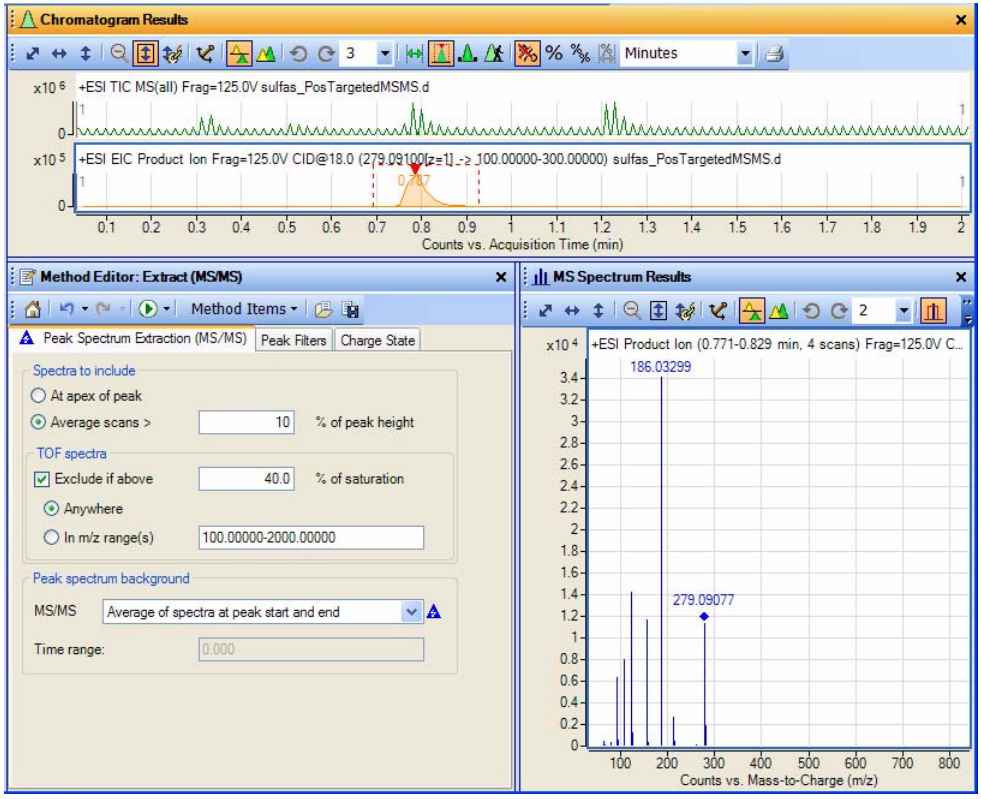


Figure 29 Product ion (MS/MS) spectra with background subtracted

7 Extract an MS/MS EIC based on the product ion spectra, 186.03299 from Step 6.

- Choose to integrate after extraction.

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 Type `186.03396,156.07760` into the m/z values box.
g Mark the **Merge multiple masses into one chromatogram** check box.
h 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.

Task 11. Extract spectra from a chromatogram (LC/MS and LC/MS/MS) (continued)

Steps	Detailed Instructions	Comments

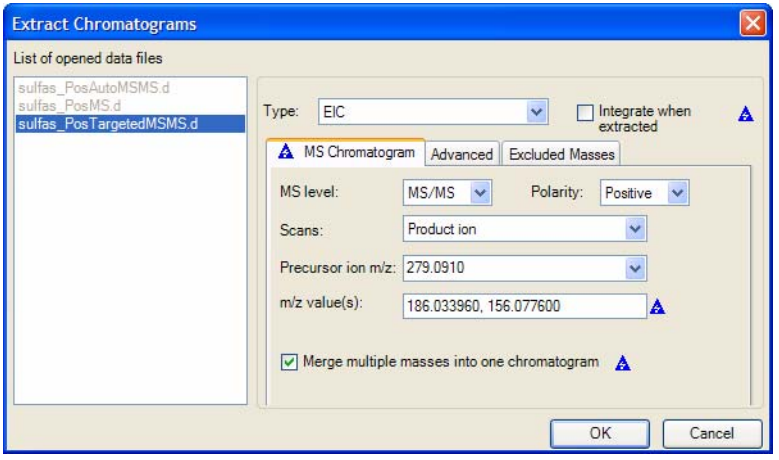


Figure 30 Extract Chromatograms dialog box for EIC based on product ions

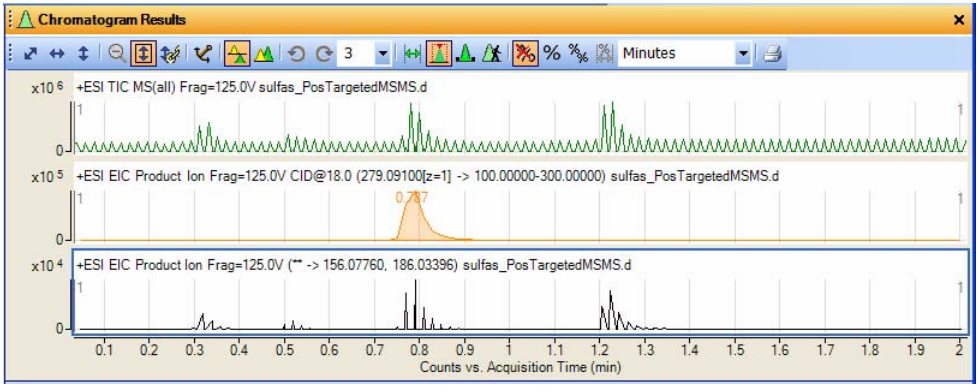


Figure 31 EIC based on background-subtracted product ion spectra

Tasks for MS and UV Data

Task 12. Extract chromatograms (MS and UV)

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

Task 12. 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 inside the chromatogram, and click **Extract Chromatograms**. • From Data Navigator, highlight the **TIC Scan** for **sulfas_PosMS.d**, then right-click **TIC Scan** and click **Extract Chromatograms**. • Note that you can also choose to have the extracted chromatogram automatically integrated after extraction.

Task 12. Extract chromatograms (MS and UV) (continued)

Steps	Detailed Instructions	Comments

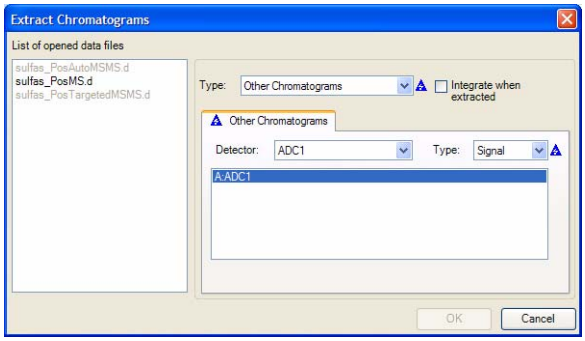


Figure 32 The Extract Chromatograms dialog box with **Type** Other Chromatograms.

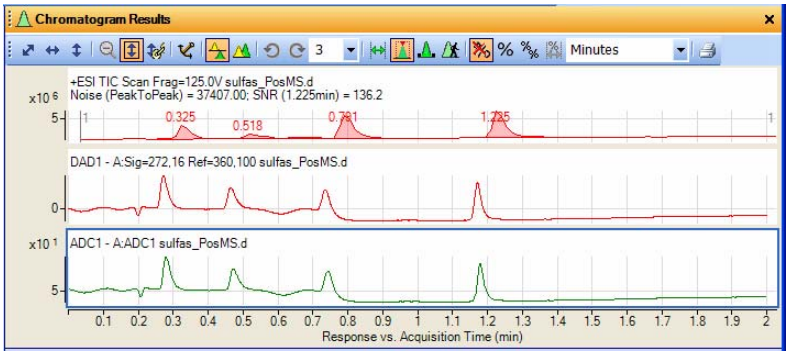


Figure 33 DAD1 and ADC1 compared to the original TIC

Task 13. 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.

Task 13. 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** Integrate the **sulfas_PosMS.d** UV 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 **DAD1** and **ADC1** in the **sulfas_PosMS.d > User Chromatograms** section. Then, right-click either chromatogram and click **Integrate Chromatogram**.	• The integration uses the General Integrator, because that is the integrator selected in the method default.m. You can change this value in the Chromatogram > Integrate (UV) > Integrator tab. • Note that the integration with default parameters is detecting very small peaks.

Task 13. Interactively integrate a chromatogram (MS and UV) (continued)

Steps	Detailed Instructions	Comments

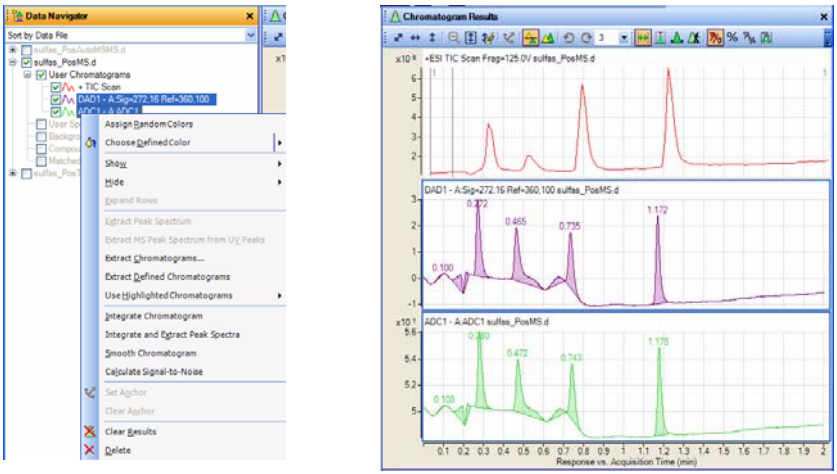


Figure 34 One of the shortcut menus in the Data Navigator and integrated sulfas_PosMS.d chromatograms

2 Enable system suitability calculations.

- Display the Chromatogram > Integrate (UV) > Suitability tab.
- Enable Suitability calculations.

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 `500`.

- 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.

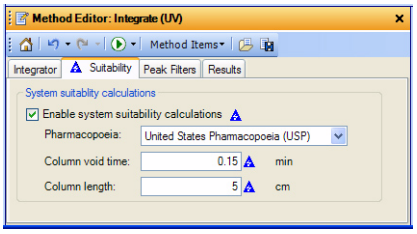


Figure 35 Chromatogram > Integrate (UV) Suitability tab

Task 13. Interactively integrate a chromatogram (MS and UV) (continued)

Steps	Detailed Instructions	Comments
3 Reintegrate the chromatogram.	• Click the **Integrate Chromatogram** icon on the Method Editor toolbar to integrate using the new setting.	
4 View the system suitability calculations. • 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.
	Peaks: DAD1 - A:Sig=272,16 Ref=360,100	

Peak	RT	Area	Area%	Height	Max Y	Width	k'	Plates	Plates/M	Resolution	Symmetry	Tailing factor
1	0.1	0.1	2.07	0.01	0.18	0.043	-0.3	1602	320.4	-20	23.5	0.5
2	0.185	1.03	21.16	0.61	0.16	0.057	0.2	266	53.2	4.8	3.14	0.7
3	0.214	0.45	9.19	0.46	0.25	0.028	0.4	1325	265	0.8	1	1
4	0.272	4.39	90.03	2.91	3	0.089	0.8	815	163	1.9	0.45	1.7
5	0.465	4.88	100	2.02	1.94	0.169	2.1	1692	338.4	4.6	0.18	3.4
6	0.676	0.81	16.53	0.25	0.05	0.095	3.5	334	66.8	2.2	1.77	0.8
7	0.735	3.5	71.74	2.11	1.76	0.08	3.9	4688	937.6	0.6	0.62	1.3
8	1.172	4.72	96.71	3.37	2.4	0.085	6.8	19207	3841.4	11.4	0.58	1.5

Figure 36 Integrated TIC with Area and Signal-to-Noise labels

5 Restore the settings for the default method, and close the Method Editor window and the Integration Peak List window.	**a** To cancel your changes and restore the values from the default method, click the **Restore to last saved values from file** icon on the Method Editor toolbar. **b** Close **Method Editor**. **c** Right-click the header 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.

Task 14. Extract spectra from a chromatogram (UV)

In this task, you extract a spectrum from exactly where you specify in the chromatogram. You can tell the Qualitative Analysis program to 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 14. Extract spectra from a chromatogram (MS and UV)

Steps	Detailed Instructions	Comments

1 Extract spectra on specific data points for the peak at 1.2 min. and the last peak 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 first peak, right-click the mouse above the peak at 0.17 min. and drag it to below the curve at 0.31 minutes, then 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 1.22 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 when you extract a new spectrum.

Task 14. Extract spectra from a chromatogram (MS and UV) (continued)

Steps	Detailed Instructions	Comments

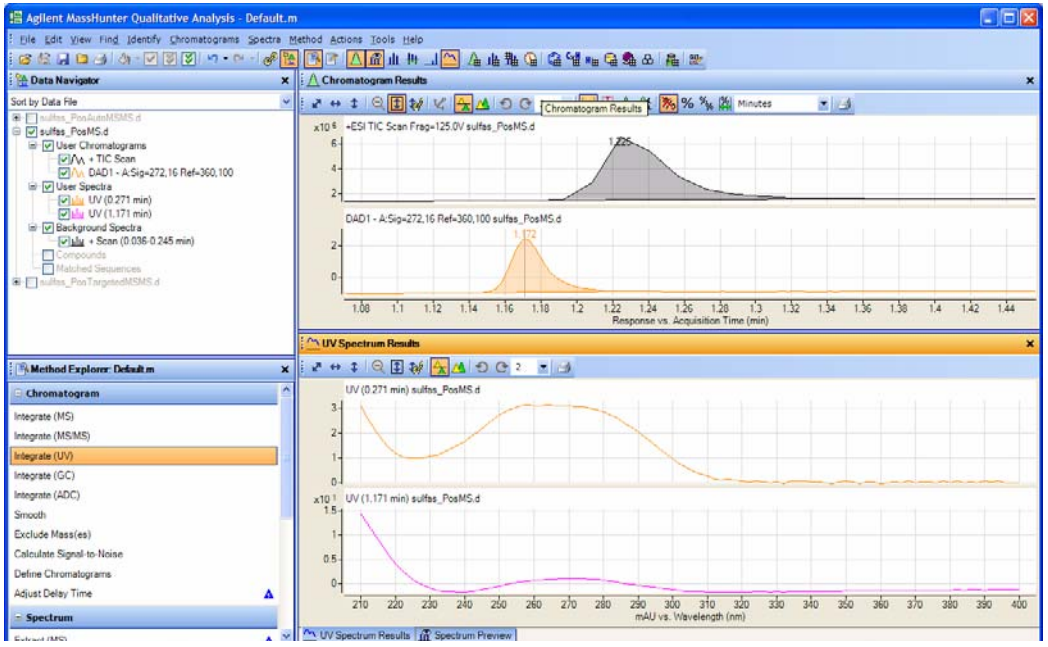


Figure 37 Main window with extracted UV spectra from two integrated peaks in the sulfas_PosMS.d file

Task 14. 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.

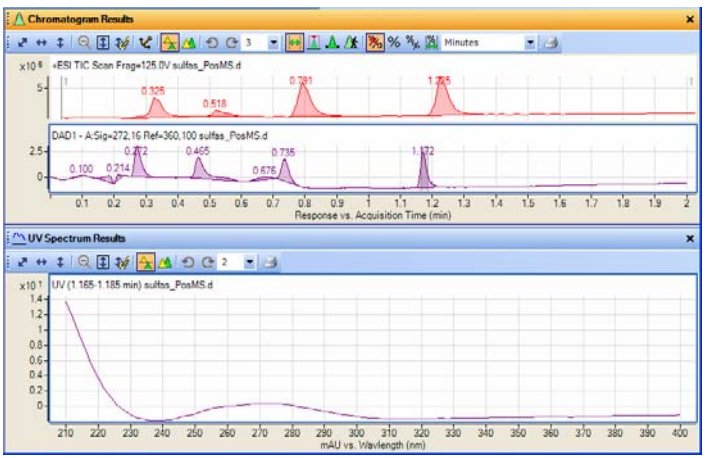


Figure 38 Average spectrum extracted from selected range for last peak

Task 14. 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 (Use **Ctrl**). **b** Right-click the spectra, and click **Delete**. **c** Click **Yes**. **d** Highlight the DAD1 Chromatogram. **e** Click **Chromatogram > Integrate Chromatogram.** **f** Click the **Peak Select** icon in the Chromatogram Results toolbar. **g** Click the third integrated peak (at 0.225 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.

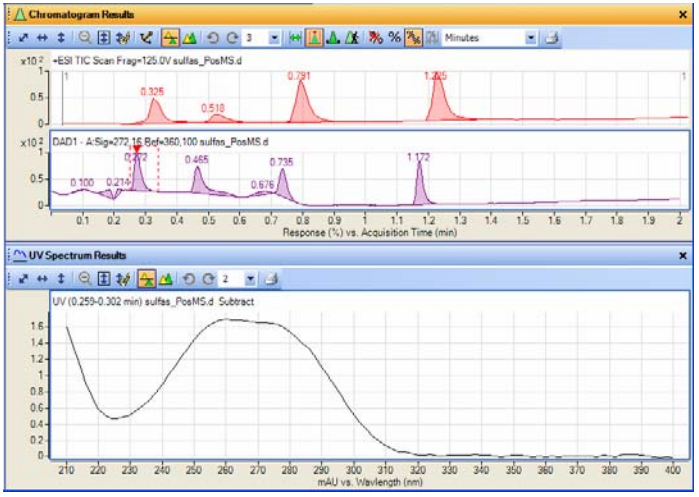


Figure 39 Integrated DAD1 chromatogram and UV Peak Spectrum

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

Tasks for GC-MS data

Task 15. Configure User Interface for GC

In this task, you switch to the General workflow. This is the only workflow that supports analyzing GC/MS data. Then, you open the User Interface Configuration dialog box and mark the appropriate check boxes for a GC QQQ system.

Task 15. Configure User Interface for GC

Steps	Detailed Instructions	Comments
1 Open the Qualitative Analysis program.	**a** Double-click the **Agilent MassHunter Qualitative Analysis** icon . The system displays the Open Data Files dialog box. **b** Click **Cancel** in the Open Data Files dialog box.	• You can get help for any window, dialog box, or tab by pressing the `F1` key when that window is active.
2 Switch to the General Workflow.	**a** Click the **View > Configure for Workflow > General** command. **b** Click the **List Mode** icon in the Chromatogram Results toolbar.	• If the Data Acquisition program for GC-QQQ is installed on the same computer, the software configures the User Interface automatically. • By default, chromatograms are overlaid. For these examples, the chromatograms are shown in **List Mode**.

Task 15. Configure User Interface for GC

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

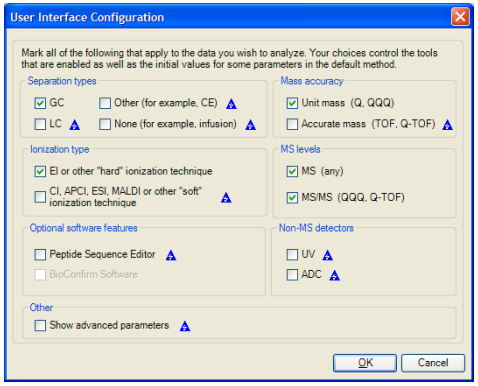


Figure 40 Configuring the user interface for a GC Triple Quadrupole

Task 16. Extract chromatograms from a GC/MS data file

In this task, you extract one BPC chromatogram from a GC/MS data file. You also extract an EIC chromatogram from two GC/MS/MS data file.

Task 16. Extract chromatograms from a GC/MS data file

Steps	Detailed Instructions	Comments
1 Open the three example GC data files. • Open the data files, **Pest - 200 - Scan.D, Pest - STD 200 MRM.D,** and **Pest Strawb-01 SPIKED 1 ppb - 1 ul inj.D** in the folder **\MassHunter\Data\GC**, or in the folder where you copied them.	**a** Click **File > Close All**. **b** Click **No** in the Save dialog box. **c** Click **File > Open Data File**. **d** Go to the folder **\MassHunter\Data\GC** or the folder where the example files are located. **e** Select the three data files. **f** Clear the **Load result data** check box. **g** Click **Open**.	• First, close any other data file that was loaded. • The Pest - 200 -Scan.D file contains MS (GC/MS) data, and the Pest - STD 200 MRM.D and Pest Strawb-01 SPIKED 1 ppb - 1 ul inj.D files contain both MS and MS/MS (GC/MS) data. • You can get help for any window, dialog box, or tab by pressing the `F1` key when that window is active.
2 Configure the user interface to work with GC data.	• Follow the instructions in Task 15. Configure User Interface for GC 64.	
3 Extract a BPC for the GC/MS data in the **Pest - 200 - Scan.d** data file.	**a** In the Data Navigator, mark the check box for **Pest - 200 - Scan.d** and clear the check boxes for the other data files. **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 **Pest - 200 - Scan.d**, if necessary. **d** Make sure the **Type** is **BPC**. **e** Click **OK**.	• You can also extract chromatograms in one of the following ways: • Right-click inside the chromatogram, and click **Extract Chromatograms**. • From Data Navigator, click one of the chromatograms in the **User Chromatograms** section, then right-click and click **Extract Chromatograms**. • You can also extract chromatograms based upon a mass spectrum.

Task 16. Extract chromatograms from a GC/MS data file (continued)

Steps	Detailed Instructions	Comments

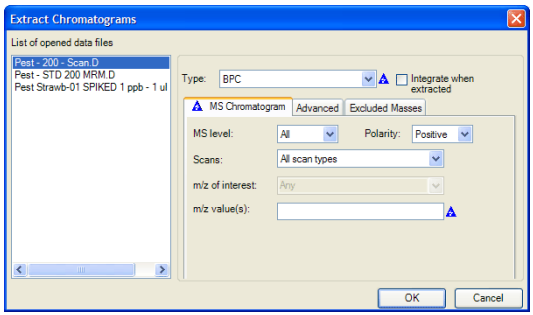


Figure 41 The Extract Chromatograms dialog box.

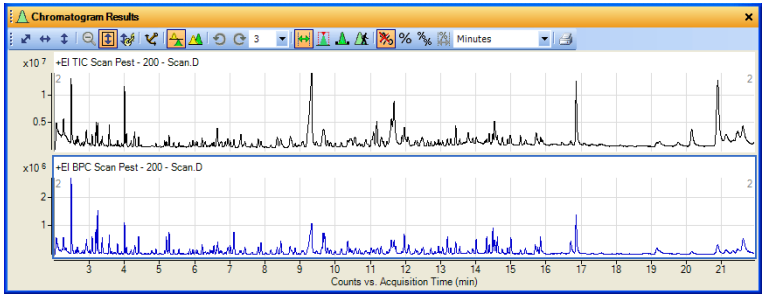


Figure 42 TIC for GC/MS and BPC for GC/MS data

Task 16. Extract chromatograms from a GC/MS data file (continued)

Steps	Detailed Instructions	Comments
4 Extract the 160 -> 133 EIC from the MS/MS data files. • This time choose to integrate the extracted chromatogram.	**a** In the Data Navigator, mark the check box for **Pest - STD 200 MRM.d** and **Pest Strawb-01 SPIKED 1 ppb - 1 ul inj.D** and clear the check box for **Pest - 200 - Scan.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 **Pest - STD 200 MRM.d** and **Pest Strawb-01 SPIKED 1 ppb - 1 ul inj.D**, if necessary. **d** Select **EIC** as the **Type**. **e** From the **MS Level** list, select **MS/MS**. **f** From the Scans list, select **Multiple reaction monitor**. **g** From the **Precursor ion m/z**, select 160. **h** In the **m/z value(s)** box, type `133`. **i** Mark the **Integrate when extracted** check box. **j** Clear the **Do cycle sum** and **Merge multiple masses into one chromatogram** check boxes. **k** Click **OK**.	• In the m/z value(s) text box, you can also type a range (for example, `100 - 300`) or multiple values (for example, `133, 139`). • If you type a single m/z value, it is automatically converted to a range using the parameters on the Advanced tab.

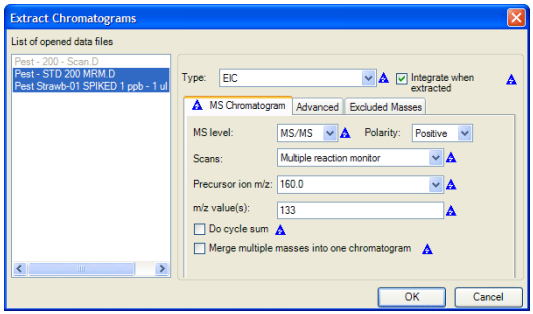
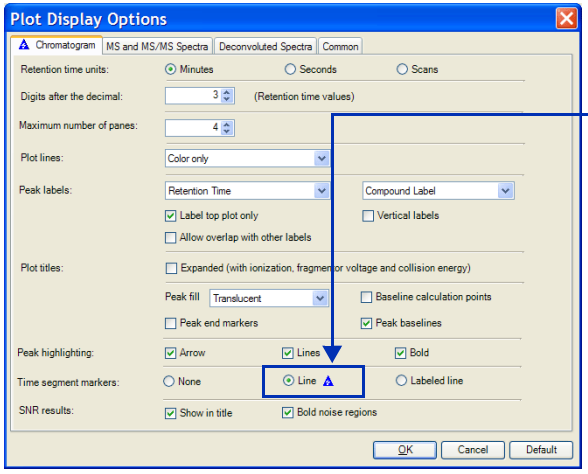


Figure 43 The Extract Chromatograms dialog box.

Task 16. Extract chromatograms from a GC/MS data file (continued)

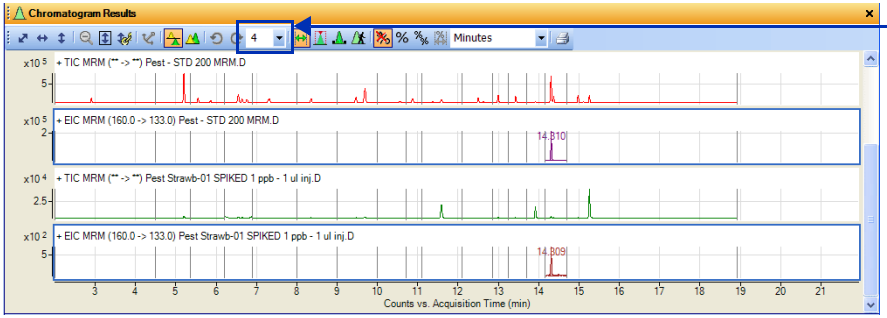
Steps	Detailed Instructions	Comments
5 Change the plot display options to not label the Time segment markers.	**a** Click **Tools > Plot Display Options**. **b** In the Plot Display Options dialog box, click **Line** for the **Time segment markers**.	• You can customize how graphics are displayed in many different ways by modifying values in this dialog box.



The example data files have many time segments. You remove the labels from the Time segment markers by clicking the Line button.

Figure 44 Plot Display Options dialog box

6 Display all four chromatograms from the MS/MS data files at the same time.	• Select **4** in the **Maximum number of list panes** box in the Chromatogram Results Toolbar.	



Maximum number of list panes box

The two EIC MRM chromatograms are integrated when they are extracted.

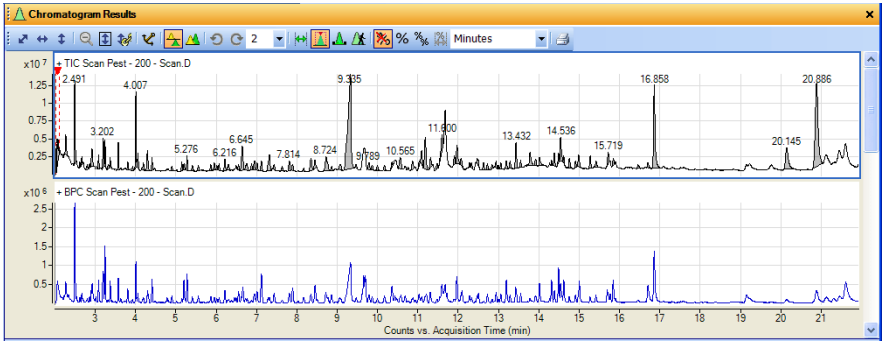
Figure 45 TIC MRM Chromatograms and EIC MRM Chromatograms for MS/MS data files

Task 17. Interactively integrate a GC/MS chromatogram

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

Task 17. Interactively integrate a chromatogram (GC/MS)

Steps	Detailed Instructions	Comments
1 Integrate the TIC Scan chromatogram for the **Pest - 200 - Scan.d** data file, using any of the options listed at right.	**a** Mark the Pest - 200 - Scan.D line in the Data Navigator window. **b** Highlight the TIC Scan chromatogram, and use one of the following commands: • 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,** then right-click the TIC Scan 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 and ADC data in the Method Editor window. • This chromatogram is an MS chromatogram, so the values that are set in the Integrate (MS) section of the Method Editor are used when integrating this chromatogram.
2 Display only two chromatograms at the same time.	• Select **2** in the **Maximum number of list panes** box in the Chromatogram Results Toolbar.	



Many small peaks are integrated.

Figure 46 Integrated TIC Scan Chromatogram with many small peaks

Task 17. Interactively integrate a chromatogram (GC/MS) (continued)

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

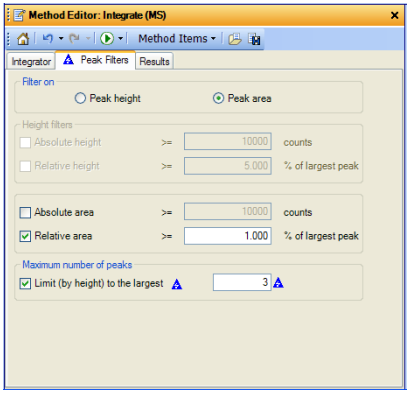


Figure 47 Peak Filters tab with **Limit (by height) to the largest** marked

4 Reintegrate the chromatogram	**d** Click the button on the Method Editor toolbar to integrate using the new setting.	• Note that only the three largest peaks are now integrated.

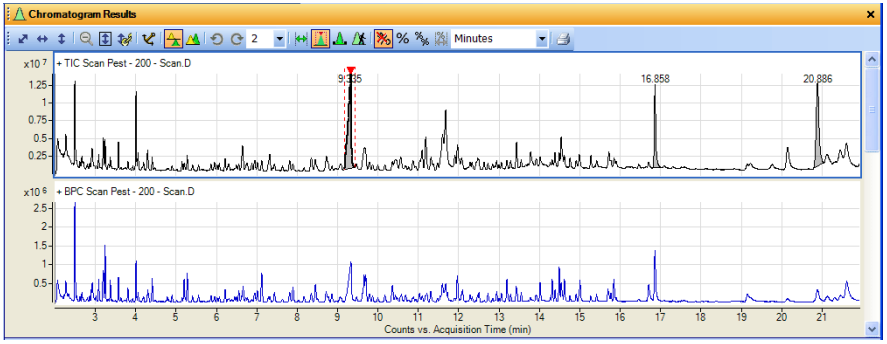


Figure 48 Integrated TIC Scan chromatogram when limiting the number of peaks

Task 17. Interactively integrate a chromatogram (GC/MS) (continued)

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

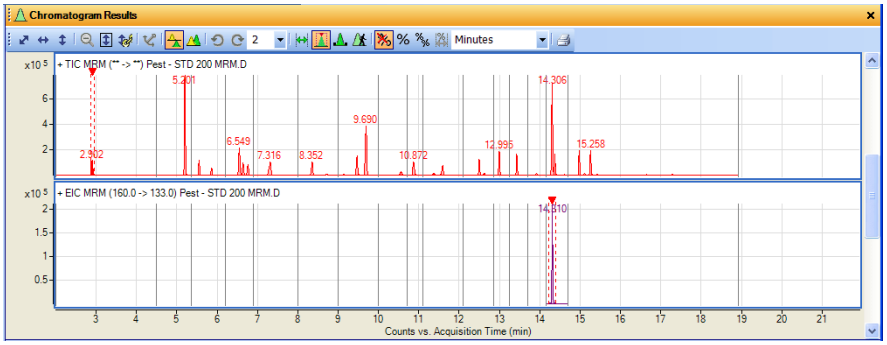


Figure 49 Integrated MRM chromatograms

6 Select the **MS/MS (GC)** integrator. Change the filter to only accept peaks with an absolute height greater or equal to 10,000.	**a** From Method Explorer, select **Chromatogram > Integrate (MS/MS)**. **b** Select **MS/MS (GC)**. **c** Click the **Peak Filters** tab. **d** Under **Filter on**, click **Peak height**. **e** Under Height filters, mark the **Absolute height** check box. **f** Type `10000` as the **Absolute height**.	• Note the blue triangle that appears when you change a setting from the value saved in the current method. When you save the method, the triangles disappear.

Task 17. Interactively integrate a chromatogram (GC/MS) (continued)

Steps	Detailed Instructions	Comments

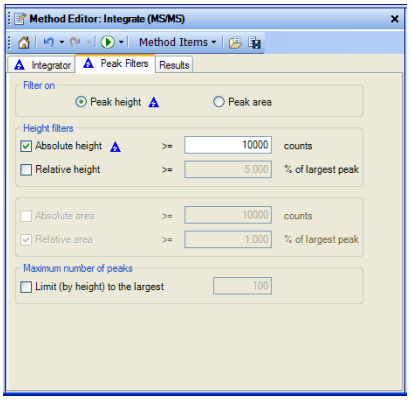


Figure 50 Peak Filters tab with **Absolute height** marked

7 Reintegrate the chromatogram	**g** Click the button on the Method Editor toolbar.	• Note that only the largest peaks are now integrated.

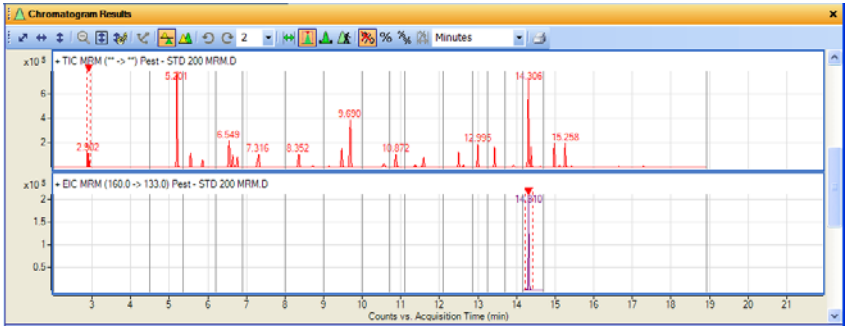


Figure 51 Integrated TIC MS and MS/MS chromatograms with higher threshold setting

8 Restore the settings that are saved for the current method and close Method Editor.	**a** Select the Chromatogram > Integrate (MS/MS) section in the Method Explorer. **b** Click the icon . **c** Select the Chromatogram > Integrate (MS) section in the Method Explorer. **d** Click the icon . **e** 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 on the Method Editor toolbar.

Task 17. Interactively integrate a chromatogram (GC/MS) (continued)

Steps	Detailed Instructions	Comments
9 Delete all chromatograms except the original. Delete the integration results on the original chromatogram.	**a** Under User Chromatograms in the Data Navigator window, highlight all the chromatograms except the original. **b** Right-click the highlighted chromatograms, and click **Delete**. **c** Click **Chromatograms >Clear Results**.	

Task 18. Basic tasks for a GC/MS data file

In this task, you extract a spectrum from exactly where you specify in the chromatogram. You can tell the Qualitative Analysis program to 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, subtract the background spectrum and integrate and extract peak spectra.

Task 18. Basic tasks for a GC/MS data file

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

Task 18. Basic tasks for a GC/MS data file

Steps	Detailed Instructions	Comments

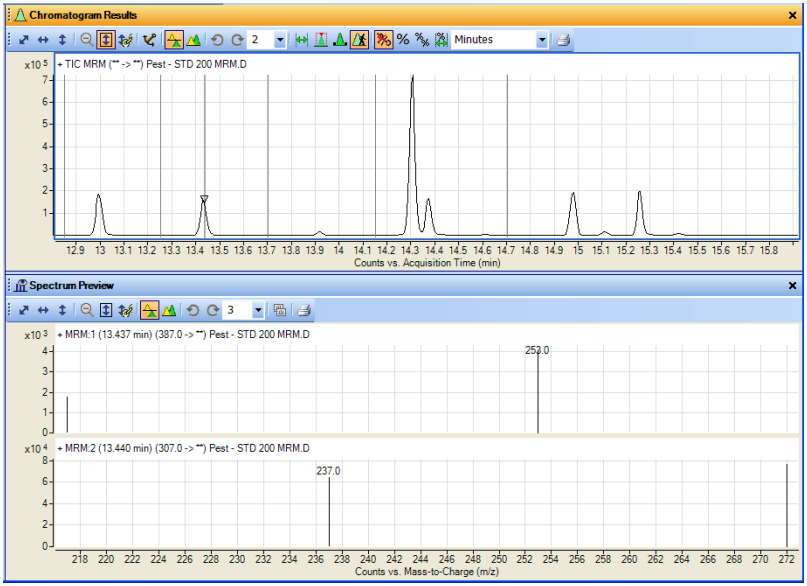


Figure 52 Walk chromatogram to view the two MRM spectra for the peak at 13. 431 minutes

Task 18. Basic tasks for a GC/MS data file

Steps	Detailed Instructions	Comments
2 Extract spectra on specific data points for the peak at 5.2 minutes and the peak at 14.3 minutes of the **Pest - STD 200 MRM.d** data file. • Extract a spectrum from the peak at or near 5.2 min. and then one of the valleys, using any one of the options described under Comments. • Extract a spectrum from the peak at or near 14.3 minutes. (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 peak at 5.2 minutes, right-click the mouse above the peak at 4.0 min. and drag it to 6.0 min., then release. **e** On a peak near 5.2 min. extract a spectrum in any of the ways listed in the Comments column. **f** On a valley near 5.1 min., extract the spectrum. **g** Click the **Zoom Out** icon, , in the Chromatogram Results toolbar. **h** Zoom into the region between 14 and 15 min. **i** On a peak near 14.3 minutes, extract a spectrum in any of the ways listed in the Comments column. (Do not extract the valley spectrum yet.) **j** If necessary, select 4 in the **Maximum number of list panes** icon in the MS Spectrum Results toolbar.	• When you zoom, make sure the AutoScale Y-axis during Zoom icon, is "on". The background of the icon is orange when it is on. • 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. All subsequent extracted spectra appear in both places as well.

Task 18. Basic tasks for a GC/MS data file

Steps	Detailed Instructions	Comments

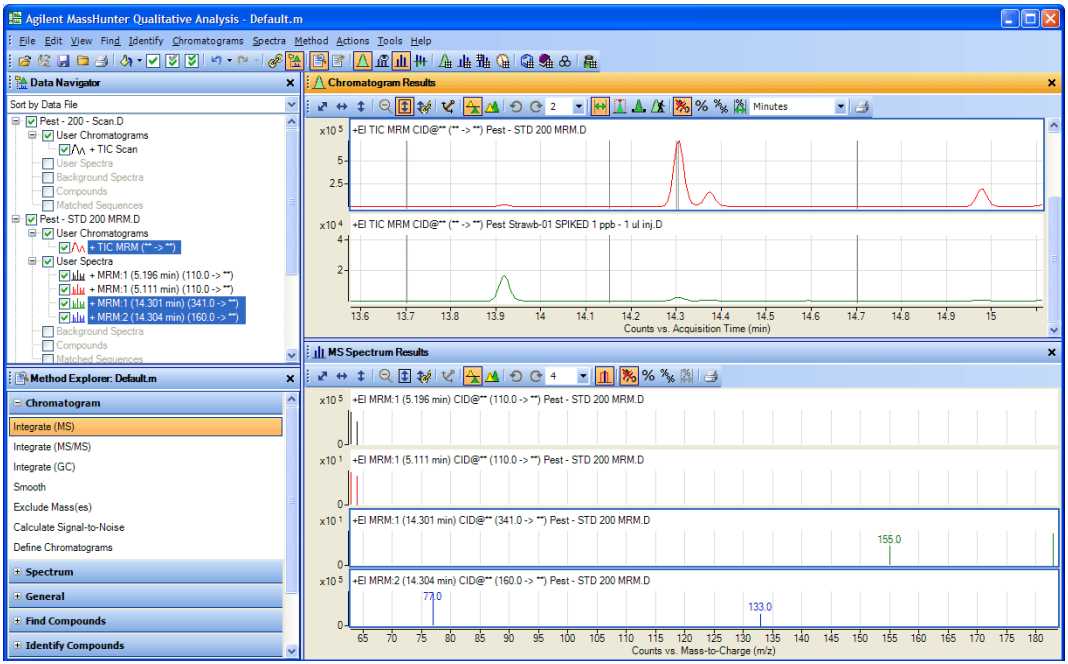


Figure 53 Main window with two MRM spectra from the peak at 5.2 minutes and two MRM spectra from the peak at 14.3 minutes

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

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

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

b Un a valley near 14.3 minutes extract a spectrum.

c Right-click the spectrum in the Spectrum Preview window, and click **Copy to User Spectra**.
Spectrum Preview is above the MS Spectrum Results window.

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

e Close the Spectrum Preview window.

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

Task 18. Basic tasks for a GC/MS data file

Steps	Detailed Instructions	Comments

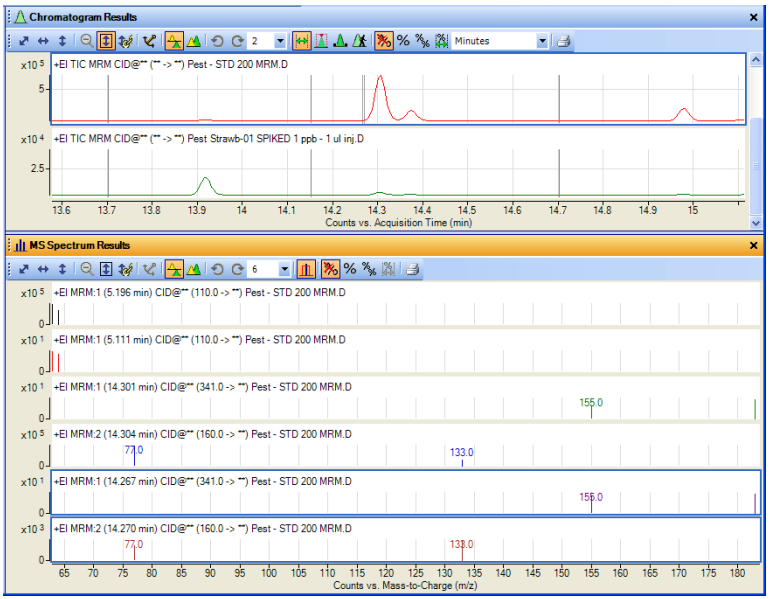


Figure 54 Chromatogram Results and MS Spectrum Results windows

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

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

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

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

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

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

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

Task 18. Basic tasks for a GC/MS data file

Steps	Detailed Instructions	Comments

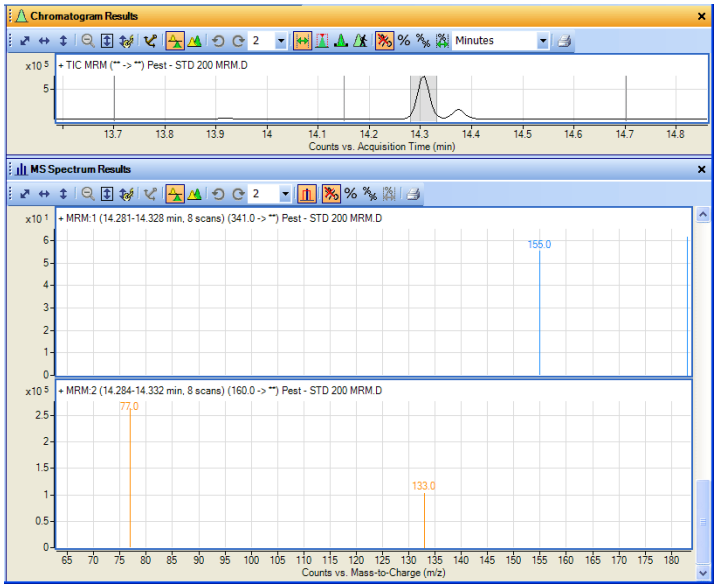


Figure 55 Chromatogram Results and MS Spectrum Results showing two averaged spectra

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

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

a Click the **Zoom Out** icon, , in the Chromatogram Results toolbar.

b Press the **Ctrl** key.

c Click at about 5.0 min. on the left side of the first peak and drag over to about 5.3 min. on the right, and release the mouse.

d Release the **Ctrl** key.

e Extract the averaged spectra using this option or the one on the right:

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

Task 18. Basic tasks for a GC/MS data file

Steps	Detailed Instructions	Comments

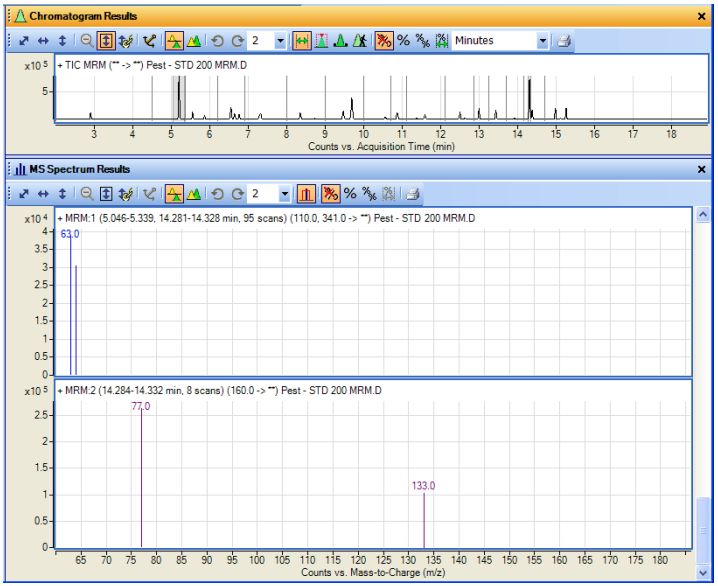


Figure 56 Two averaged spectra from two different ranges in the chromatogram

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

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

a Click the User Spectra line in the Data Navigator. Right-click the User Spectra line, and click **Delete**.

b Click **Yes**.

c In Method Explorer, select **Spectrum > Extract (MS/MS)**.

d Click the **Peak Spectrum Extraction (MS/MS)** tab, if not visible.

e In the **Peak spectrum background** box, select **Average of spectra at peak start and end**.

f In the Chromatogram Results toolbar, click the **Peak Select** icon, .

g Select the peak at 14.306 minutes.

h Right-click and click **Extract Peak Spectrum** from the shortcut menu.

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

Task 18. Basic tasks for a GC/MS data file

Steps	Detailed Instructions	Comments

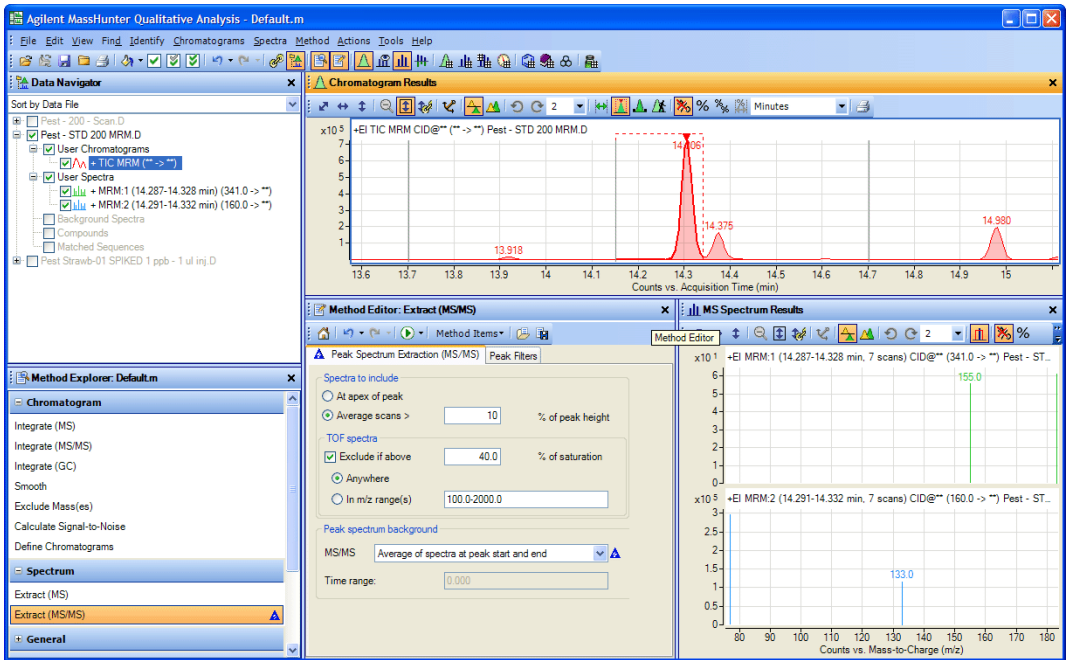


Figure 57 Peak spectra with background subtracted

7	Integrate and extract peak spectra from the Pest - STD 200 MRM.d data file.	**a** Click the TIC MRM chromatogram in the Data Navigator window. **b** Click **Chromatograms > Integrate and Extract Peak Spectra**.

Task 18. Basic tasks for a GC/MS data file

Steps	Detailed Instructions	Comments

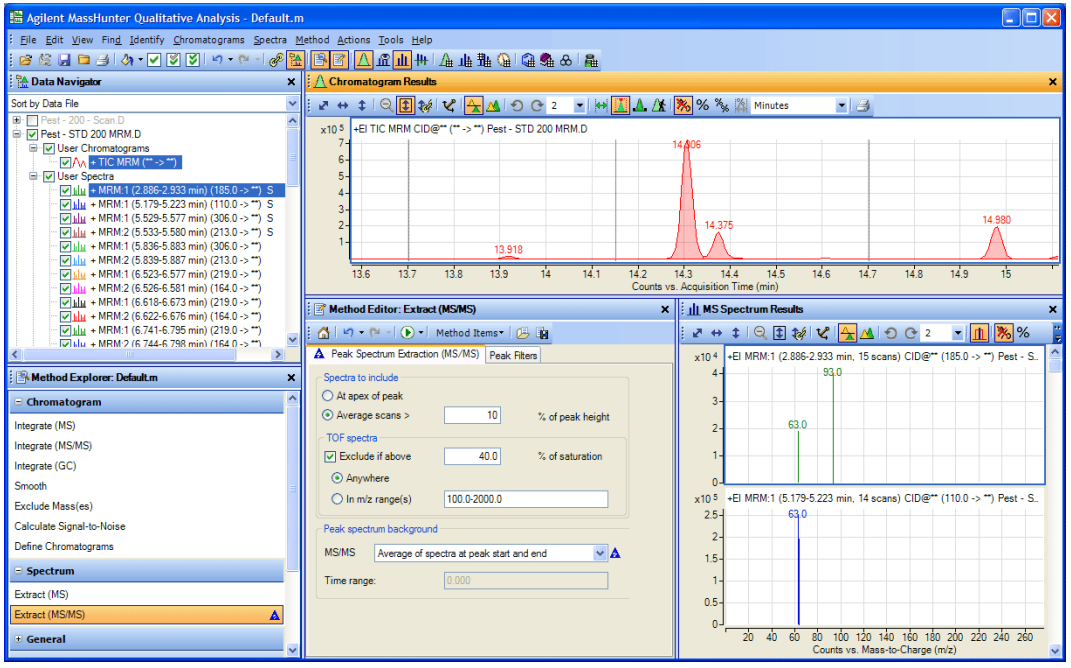


Figure 58 Integrate and Extract Peak Spectra

8 Close data files and return to LC/MS/MS user interface configuration.	**a** Click File > Close Data File. **b** Select all files. **c** Click **Close**. **d** Click **Tools > User Interface Configuration**. **e** Mark all check boxes. **f** Click **OK**.	• If these check boxes are not marked, then some of the algorithms are not available.

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.

In the third set of tasks, you find and identify compounds in a GC/MS pesticide data file. You find compounds using the Find Compounds by Chromatogram Deconvolution algorithm. You identify these compounds using the Search Unit Mass Library algorithm.

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** Click the **sulfa_PosMS.d** data file in the example data file directory. Clear the **Load result data** check box and click **Open**. **c** Click **View > Configure for Workflow > General**. **d** 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.

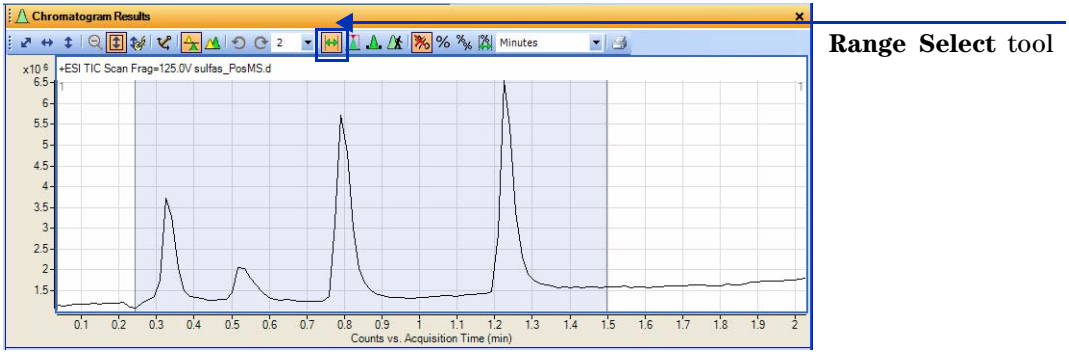
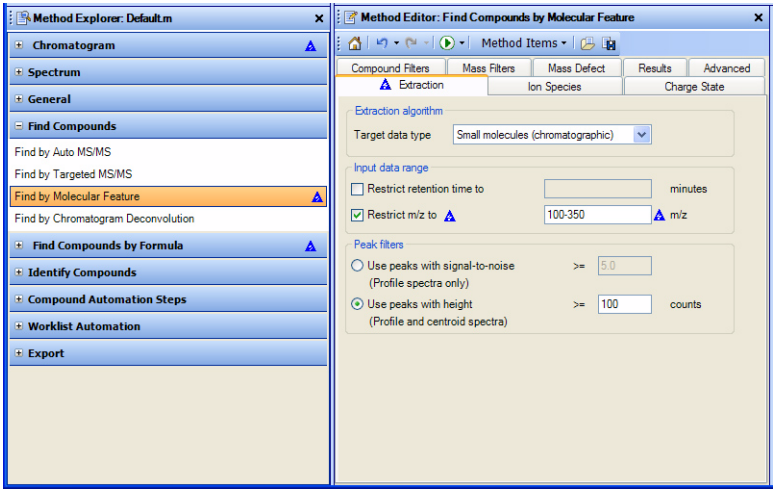


Figure 59 Selecting a time range in the TIC chromatogram

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

Step	Detailed Instructions	Comments
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 Method Explorer 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 for which you intend to find compounds. See Figure 59 on page 87. • 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.



The Advanced tab is only available if the Advanced check box is marked in the User Interface Configuration dialog box.

Figure 60 Restricting mass range for finding compounds by molecular feature

	e Click the **Results** tab. **f** Mark **Extract ECC** and **Extract MFE spectrum**. **g** Mark **Display only the largest** 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 a compound is highlighted.

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

Step	Detailed Instructions	Comments

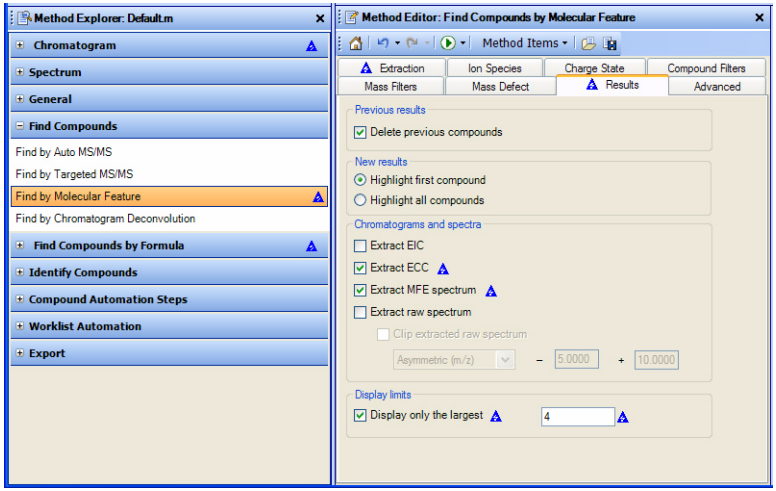


Figure 61 Changing the values in the Find Compounds by Molecular Feature > Results tab

h Click ▶ to run the **Find Compounds by Molecular Feature** algorithm on the data file.

i Change the number of panes to view in both the Chromatogram Results and MS Spectrum Results windows to **4**.

j Click the **Autoscale Y-axis during Zoom** icon, ↕, in the MS Spectrum Results toolbar.

k Zoom in on the m/z range from 200 to 350.

- The Qualitative Analysis program should find 4 major compounds in the selected range.
- The selected range is automatically 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**.

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

Step	Detailed Instructions	Comments

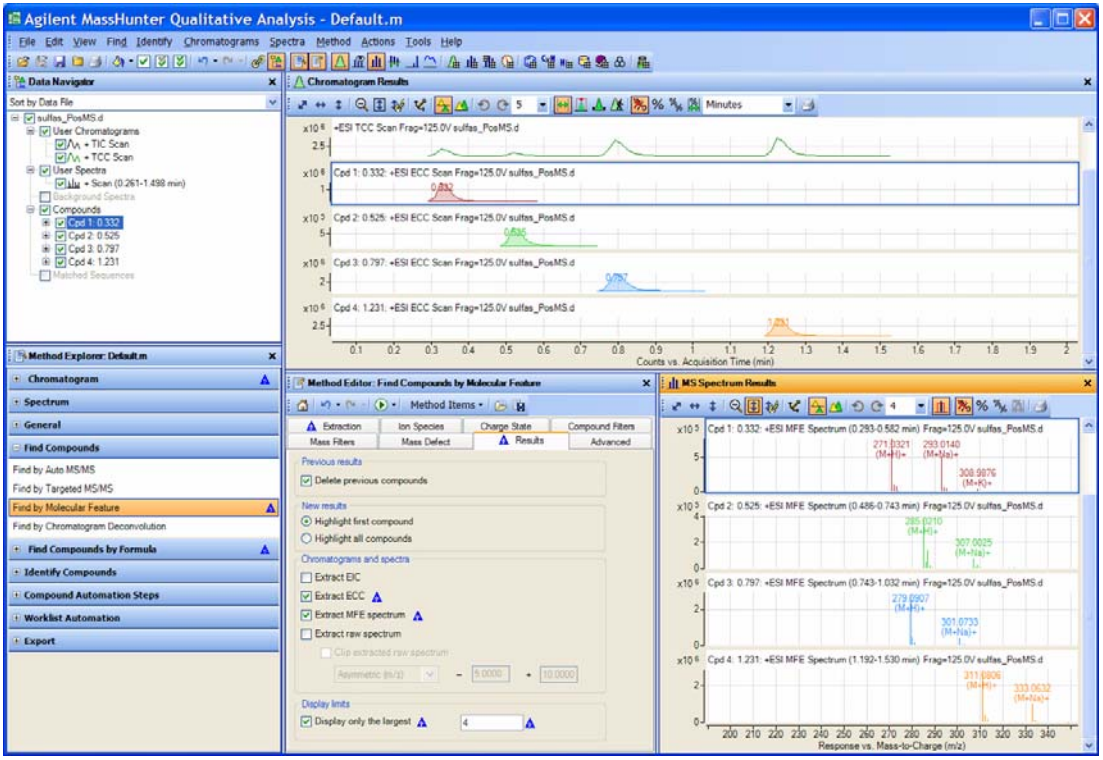


Figure 62 Finding all four compounds in the sulfa drug mix

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 63, make sure you have selected **Common organic molecules** as the Isotope model.	**a** In Method Explorer, click **Identify Compounds > Generate Formulas**. **b** 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 **Generate Formulas from Compound** icon to run the algorithm. **e** Click a compound in the Data Navigator to see the **MS Formula Results** for that compound. **f** If necessary, click **View > Compound List**.	• 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.
2 Do a database search based on formulas on Compounds 1-4. • Base search on formula.	**a** In the Data Navigator window, click **Compounds**. **b** In Method Explorer, click **Identify Compounds > Search Database**. **c** Under Search Criteria click **Molecular formula**. **d** Click **Identify > Search Database for Compounds** in the main menu.	• Note in the Compound List that all four sulfa drugs have been identified (See Figure 63). • If the DB Search Results window is not displayed, click **View > DB Search Results**.

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

Step	Detailed Instructions	Comments

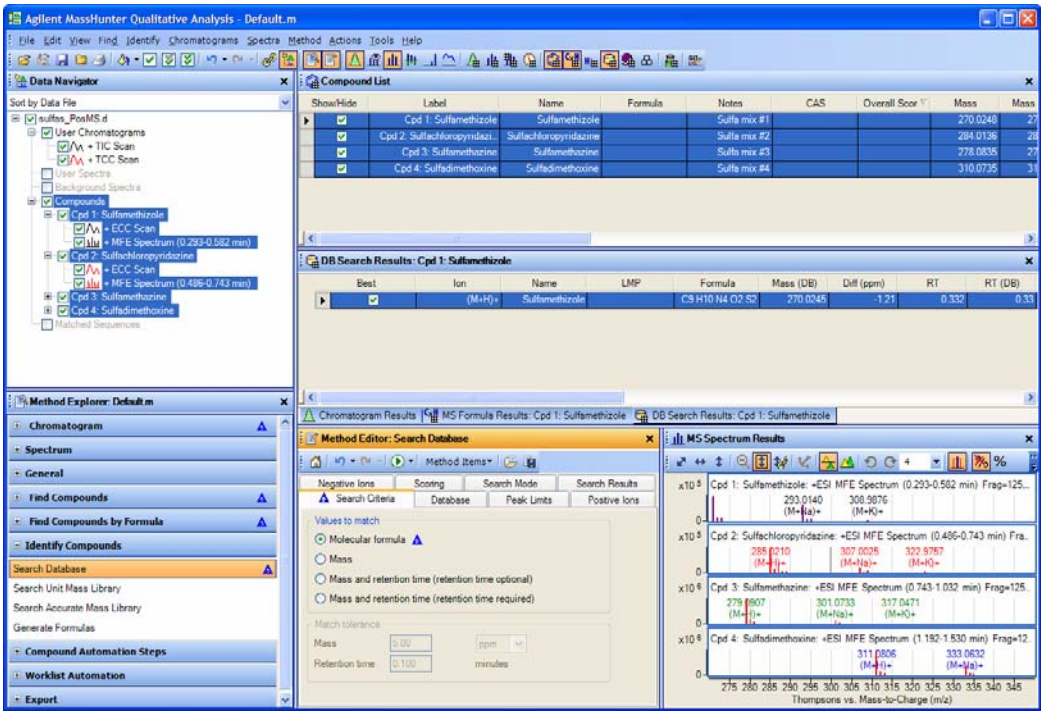


Figure 63 DB Search Results for Compounds 1-4 in sulfas_PosMS.d

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)** 87 and identified in **Task 2. Generate formulas and identify compounds (LC/MS - MS only)** 91.

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 **General > 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. **e** Click the **Print Compound Report** icon to run the algorithm.	• 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.

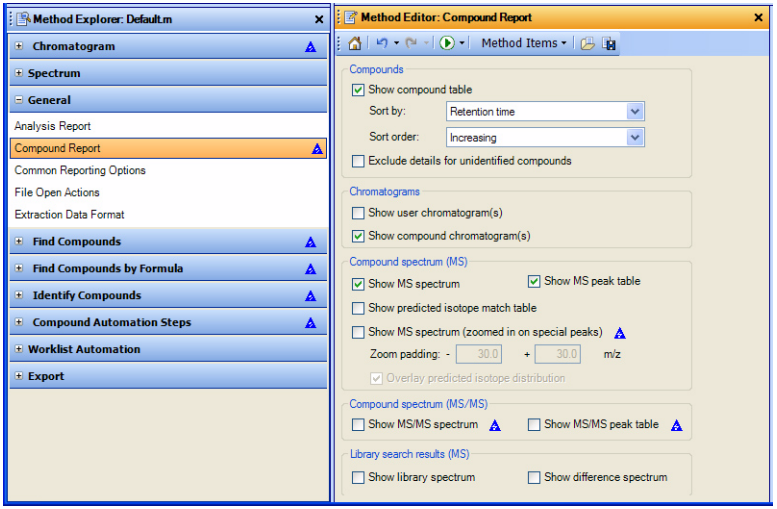


Figure 64 Compound Report window in the Method Editor

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

Step	Detailed Instructions	Comments
2 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.

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 **View > Configure for Workflow > General**. **d** Click **Yes** to switch workflows. **e** Click **No** to save method parameters. **f** Click the **Autoscale Y-Axis during zoom button** in the Chromatogram Results toolbar. **g** 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 Find compounds within the specified range on the chromatogram. • Enable sample purity calculations. • Calculate the TIC %, ADC %, UV A%, and UV B% purity values. • Use the maximum value as the purity value. • Add columns to the Compound List window. • Review results.	**a** In Method Explorer click **Find Compounds by Formula > Find by Formula - Options** tab. **b** Click **Database** as the Source of formulas to confirm. **c** In Method Explorer click **Find Compounds by Formula > Find by Formula - Sample Purity** tab. **d** Mark the **Compute sample purity** check box. **e** Mark the **TIC %**, **ADC %**, **UV A%** and **UV B%** check boxes. **f** Click **Maximum of all selected algorithms**. **g** In the **Minimum acceptable purity** box, type 20.	• 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

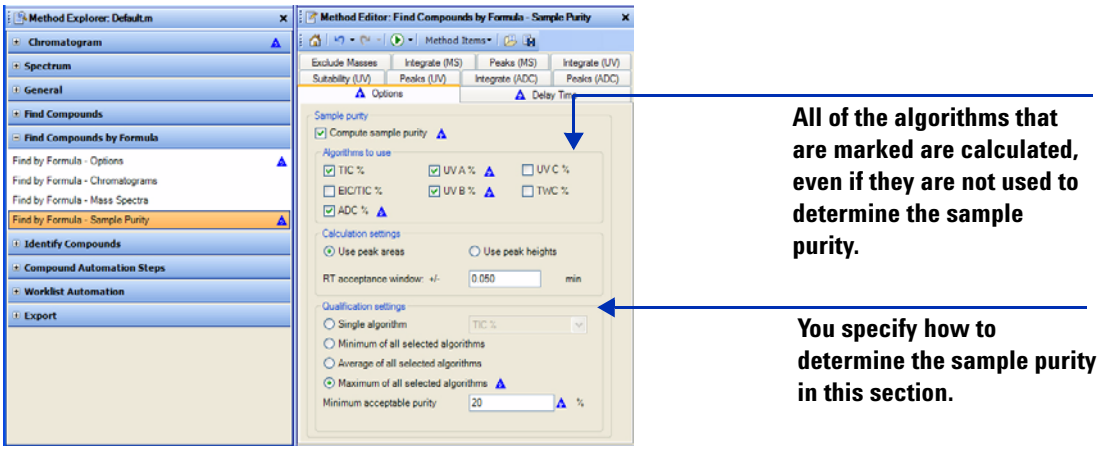


Figure 65 Setting sample purity options for the Find Compounds by Formula algorithm

h Click  to run the **Find Compounds by Formula** algorithm on the data file.

i Change the number of panes to view in both the Chromatogram Results and MS Spectrum Results windows to 3.

j Click **View > Compound List** to open the Compound List window.

k Right-click the Algorithm column, and click **Add/Remove Columns** to open the Compound Columns dialog box.

l Click the Category column header to sort the possible columns.

m 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.

n Click **OK**.

- The Qualitative Analysis program finds 6 major compounds in the selected range.
- Other columns were removed from the Compound List table to show you the Sample Purity results.
- 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 22 for more information on moving windows.

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

Step	Detailed Instructions	Comments

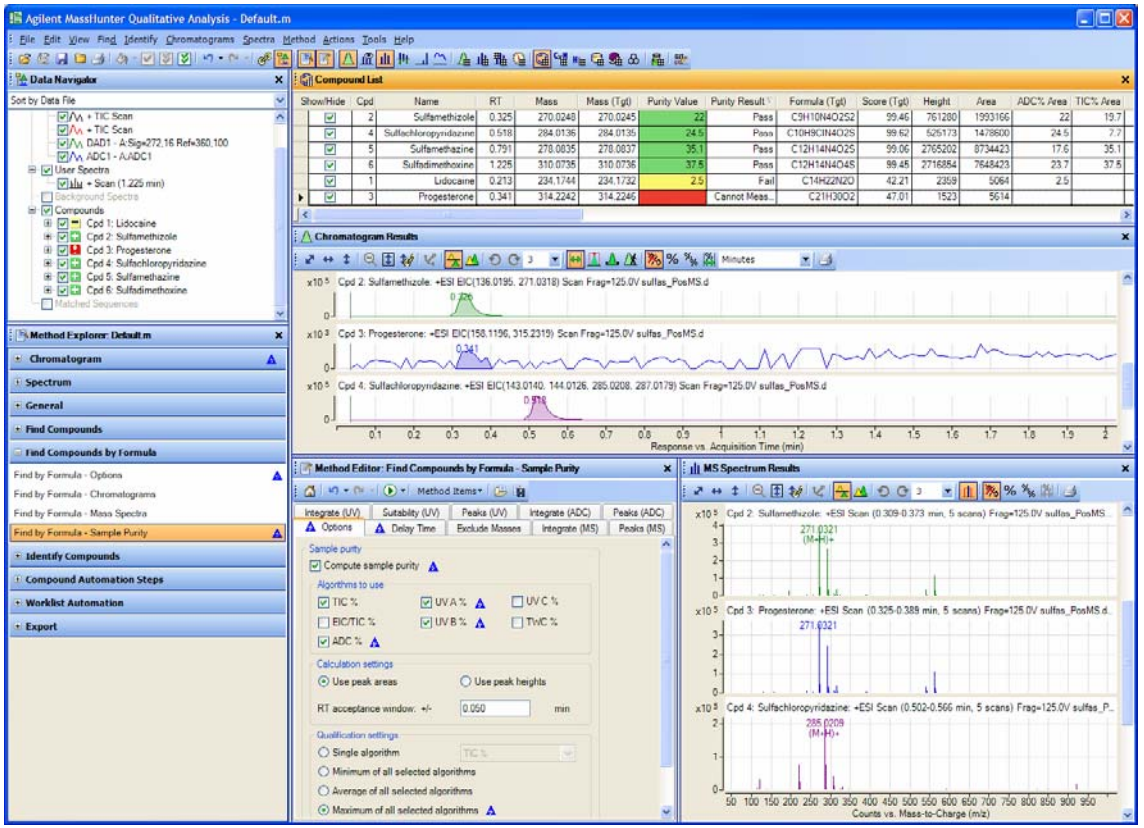


Figure 66 Finding all four compounds in the sulfa drug mix

- 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

3 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 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 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 67 (next page).	**a** Open the **peptide-ms-only.d** data file. **b** Click **Find Compounds > Find by Molecular Feature** in the Method Explorer to display the parameters in the Method Editor 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 box. **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 ECC** and **Extract MFE spectrum** check boxes. **i** Click  to run the **Find Compounds by Molecular Feature** algorithm on the data file. **j** If necessary, click **2** in the **Maximum number of list panes** in the Chromatogram Results toolbar.	• 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. • The resulting .mhd files are stored in the Results directory under the data file directory. • You extract features using the Qualitative Analysis Molecular Feature algorithm. Then, you can compare sets of data from different extractions using Agilent MassHunter Profiling software or GeneSpring MS software. • If you click Apply all filters to MHD file, then only compounds that pass the filters are written to the MHD file. Otherwise, compounds are written to the MHD file before the filters are applied. The **Limit to the largest** filter does not ever apply to the MHD file.
2 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. **e** Find the mass.	• Compound 4 has a spectrum containing this ion with a charge state of +2. • The mass is 1139.4577. 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**.

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

Step	Detailed Instructions	Comments

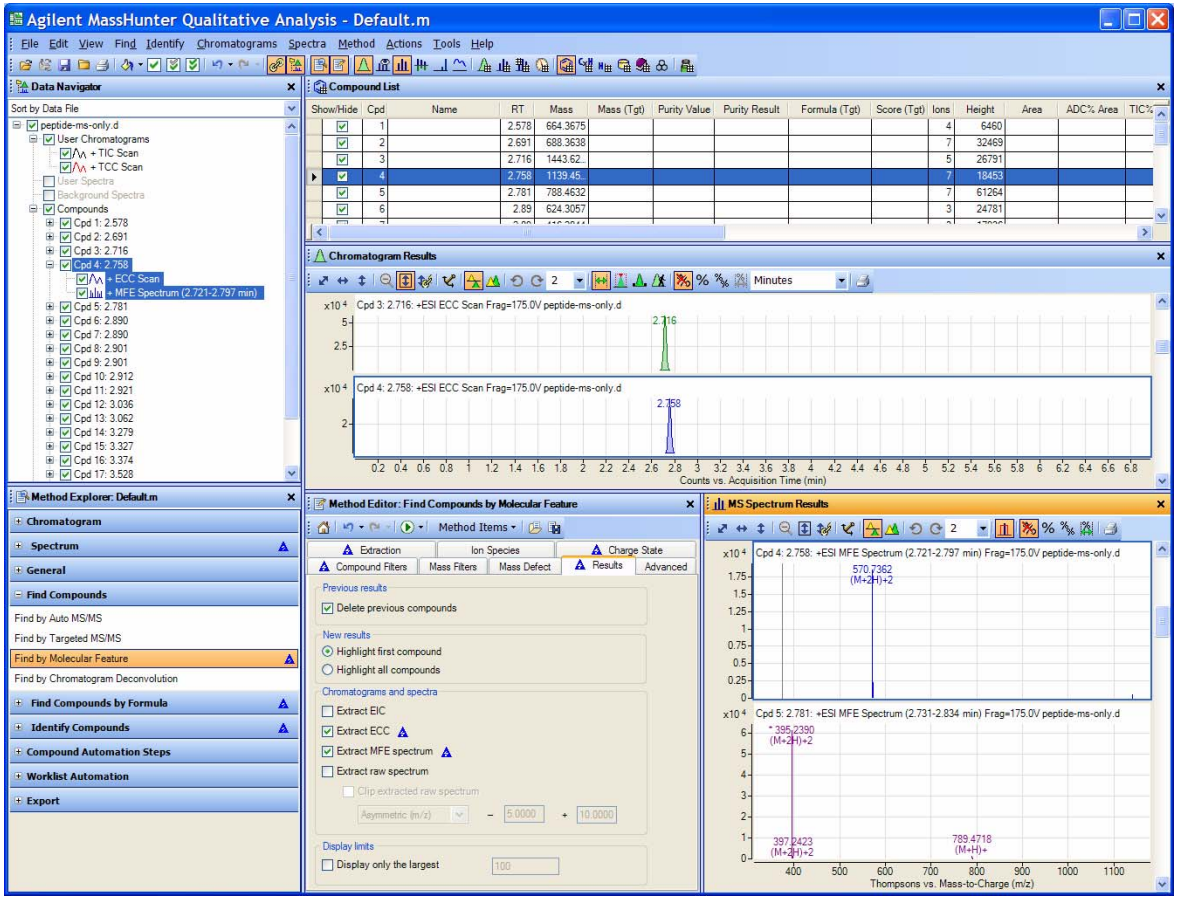


Figure 67 Find Compounds by Molecular Feature with Qualitative Analysis

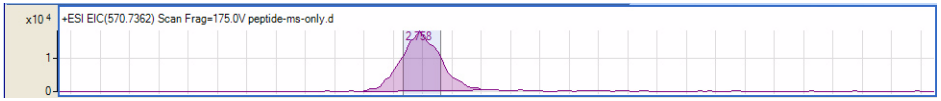
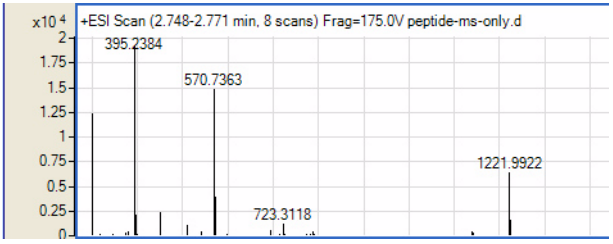
3 Extract an integrated EIC for this peptide.

- 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, click **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.

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

Step	Detailed Instructions	Comments
4 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** Right-click the EIC, and drag the cursor to zoom in around the first integrated peak. **b** Make sure that the **Range Select** icon has been selected, and select a range across the peak at the midpoint.	
		
	c Double-click within the shaded region of the peak to extract an averaged spectrum.	
		
5 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 **sulfa-PosAutoMSMS.d** data file in the example data file directory, and click **Open**. **c** Click the **View > Configure for Workflow > General** command. **d** Click the **Range Select** icon in the Chromatogram Results toolbar, if necessary. **e** Click the **Auto-scale Y-axis during Zoom** icon in the Chromatogram Results toolbar, if necessary. **f** 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. This data file also contains DAD and ADC data. You may ignore these blue triangles unless you want to enter a delay time.

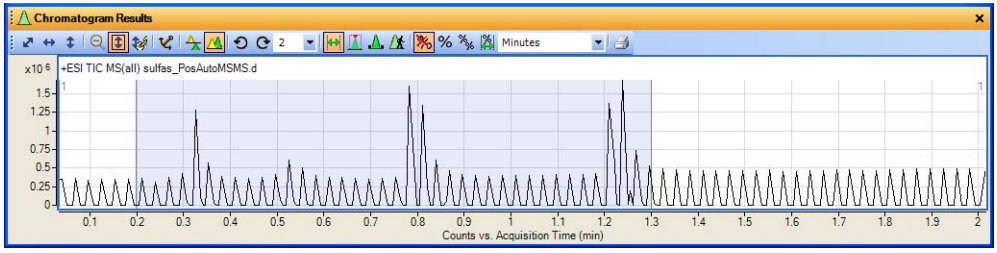


Figure 68 Zoomed range for TIC chromatogram of sulfas-PosAutoMSMS.d data file

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

Step	Detailed Instructions	Comments
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** Under Processing, 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 choose the region of the chromatogram from which you intend to find compounds. See Figure 68. • 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.
• Select to extract EIC, MS spectra and MS/MS spectra.	**h** Click the **Results** tab. **i** Mark the **Extract EIC, MS and MS/MS** check boxes. **j** 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.

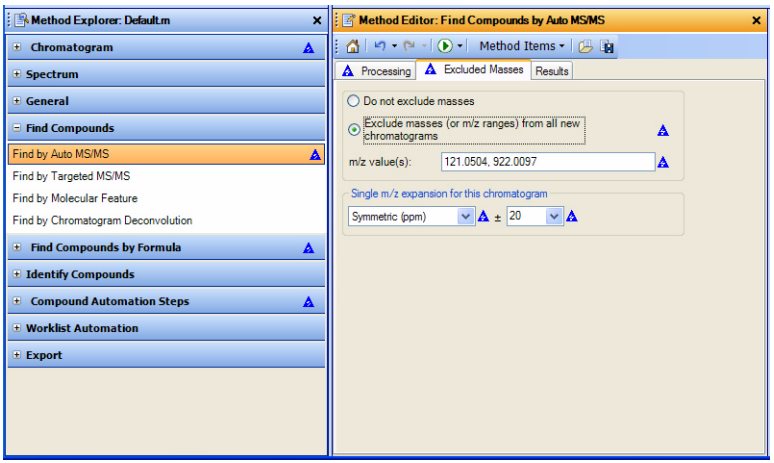


Figure 69 Excluded Masses tab of Find Compounds by AutoMS/MS

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

Step	Detailed Instructions	Comments
3 Display both spectra for Compound 4 only. See Figure 70.	**a** Highlight Compound 4 only. **b** Click the **Show only the highlighted items** icon in the main toolbar. **c** Expand Compound 4 to see the chromatogram and two 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 red diamond represents the precursor ion.

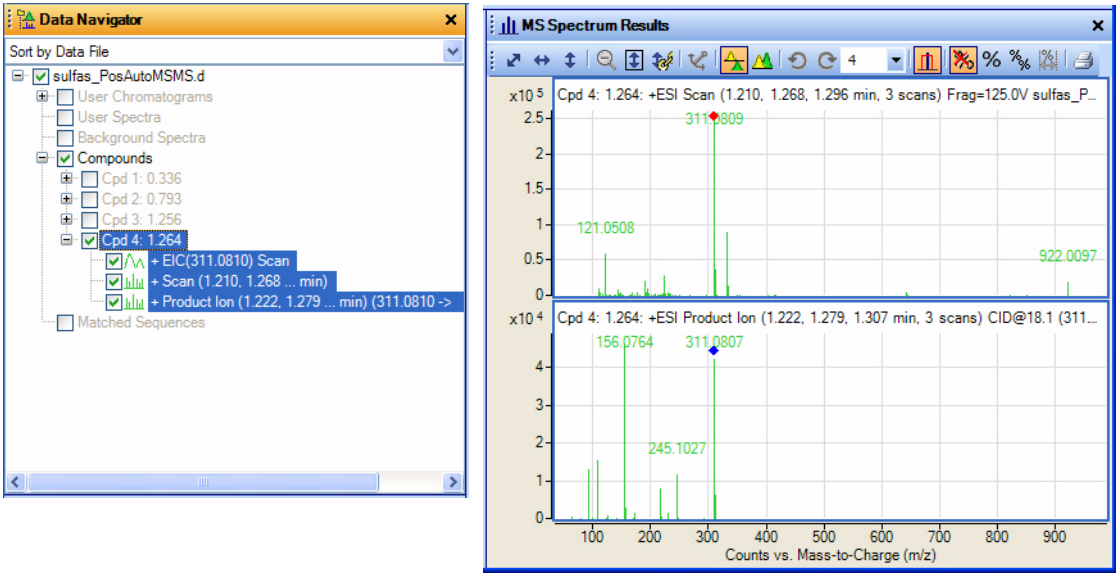


Figure 70 Data Navigator window and MS Spectrum Results window showing MS and MS/MS spectra for Compound 4

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 1-4 based on masses.	**a** Highlight all compounds in the Data Navigator window. **b** In Method Explorer, click **Identify Compounds > Search Database**. **c** In the Search Criteria tab, click **Mass**. **d** Click **Identify > Search Database for Compounds** from the main menu. You can instead click the **Search Database for Compounds** icon to run the algorithm. **e** Click **View > Compound List**. **f** Mark the **Show/Hide** check boxes for each compound in the Compound List. Compounds 1 - 4 were hidden in the last task. Or, use the **Show all highlighted items** icon in the main toolbar. **g** Display the Chromatogram Results window by clicking on the Chromatogram Results tab. This window is tabbed with the MS Formula Results window and the DB Search Results window.	• Note that three sulfa drugs have been identified in the Compound List (See Figure 72 on page 106). • Note that no compound name was found for Compound 3 in the database search.

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

Step	Detailed Instructions	Comments

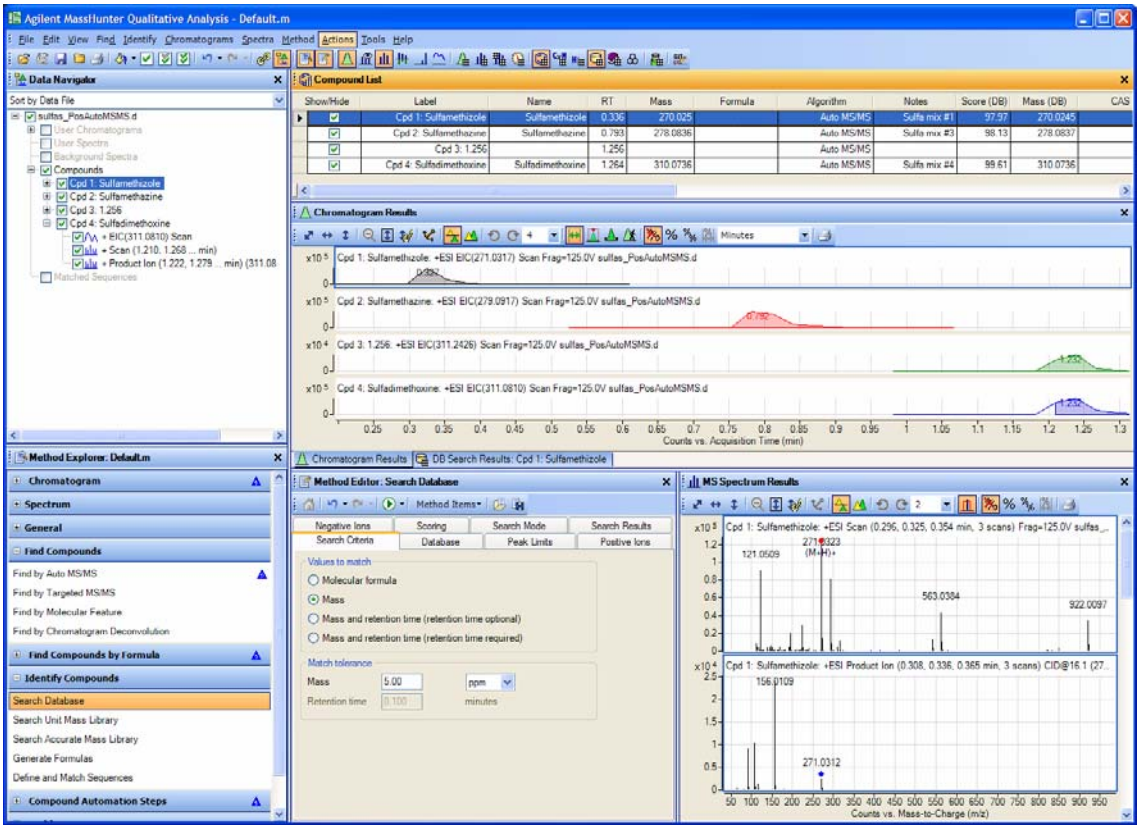


Figure 71 Compounds in sulfas-PosAutoMSMS.d data file identified by searching a database

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

Step	Detailed Instructions	Comments
2 Generate formulas for Compounds 1- 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 72, make sure you have selected **Common organic molecules** for the Isotope model.	**a** In Method Explorer, 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** icon to run the algorithm. **e** Click on the Plus sign icon in the MS Formula Results window to expand the table. **f** Click **View > MS/MS Formula Details**. **g** Move this window to beside that of the MS Formula Results window. **h** In Data Navigator highlight the compound whose **MS Formula Results** and **MS/MS Formula Details** you want to see. **i** Use the scroll bar in the Compound List window to see the MFG results.	• 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. • Note that one or more formula were found for all compounds. • Use the Remove column shortcut command to remove empty columns from the Compound Table. • Note that the formula from the database search is the same as the formula determined by the Generate Formulas algorithm. • Click **Tools > Compound Label Configuration** to change the compound label.

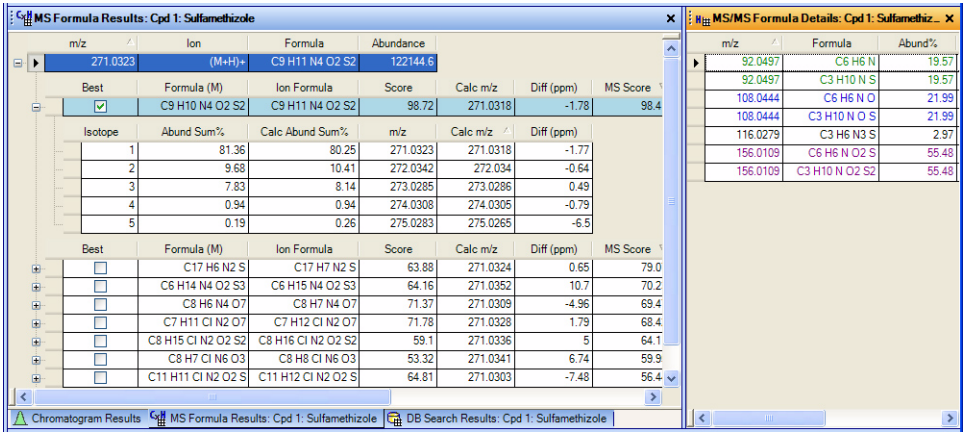


Figure 72 MS Formula Results and MS/MS Formula Details for Compound 1 in sulfas_PosAutoMSMS.d

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 Method Explorer, click **General > 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.

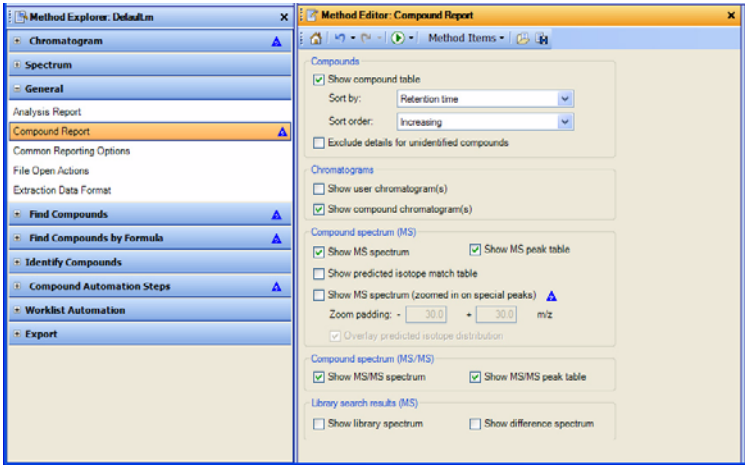
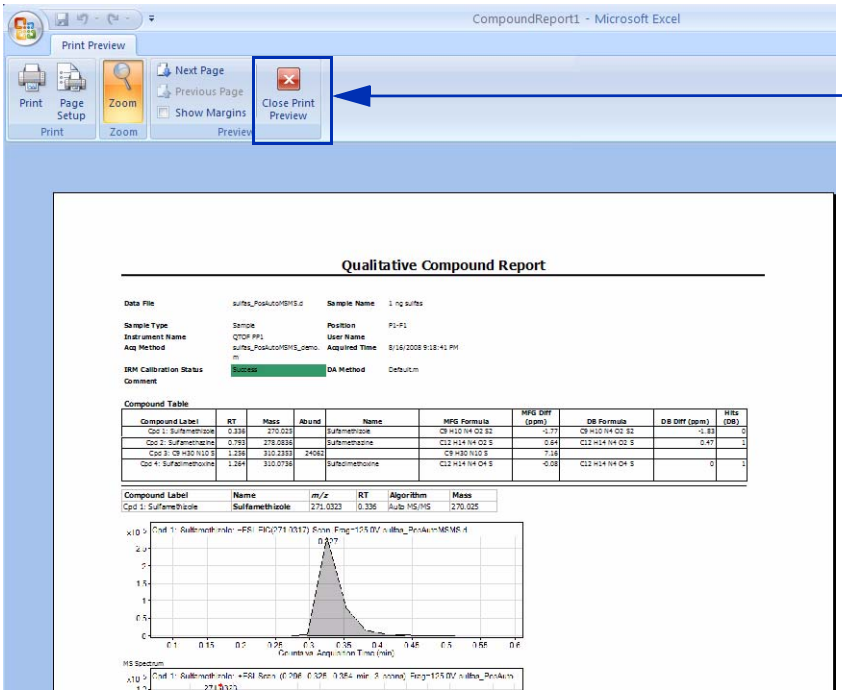


Figure 73 Compound Report window in the Method Editor

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 **All results**. **c** Mark **Print report**. **d** Mark **Print preview**. **e** Click **OK**.	• You can also create a PDF file by marking 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 74 Compound Report window in the Method Editor

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 earlier 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 Accurate Mass Library (LC/MS - MS/MS)

The FindCompounds 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-PosAutoMSMS.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 **View > Configure for Workflow > General** command. **c** Click **Yes** to switch workflows. **d** Click **File > Open Data File**. **e** Click **sulfa-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.	• 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. This data file also contains DAD and ADC data. You may ignore these blue triangles unless you want to enter a delay time.

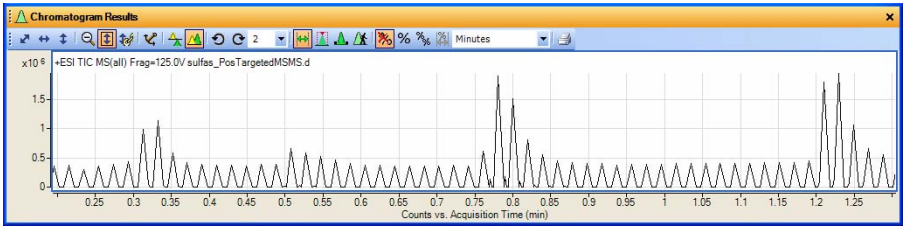


Figure 75 Zoomed range for TIC chromatogram of sulfas-PosAutoMSMS.d data file

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 Method Explorer 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 SulfasLib.CDB library • Lower the minimum match score to 50.	**a** In Method Explorer click **Identify Compounds > Search Accurate Mass Library.** **b** Click the Browse button. Every other field on this tab is grayed out. **c** Select SulfasLib.cdb. **d** Click Open. **e** Click the **Search Results** tab. **f** Type `50` in the **Minimum match score** box. **g** Highlight all compounds in the Data Navigator window. **h** Click **Identify > Search Library for Compounds**.	• 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**. • To see all of the parameters that affect the Search Accurate Mass Library algorithm, you mark the Advanced check box in the User Interface Configuration dialog box. Then, the Search Criteria tab is shown. You use this tab to filter the library entries that are searched on Ionization mode, Instrument type, and Collision energy.

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

Step	Detailed Instructions	Comments

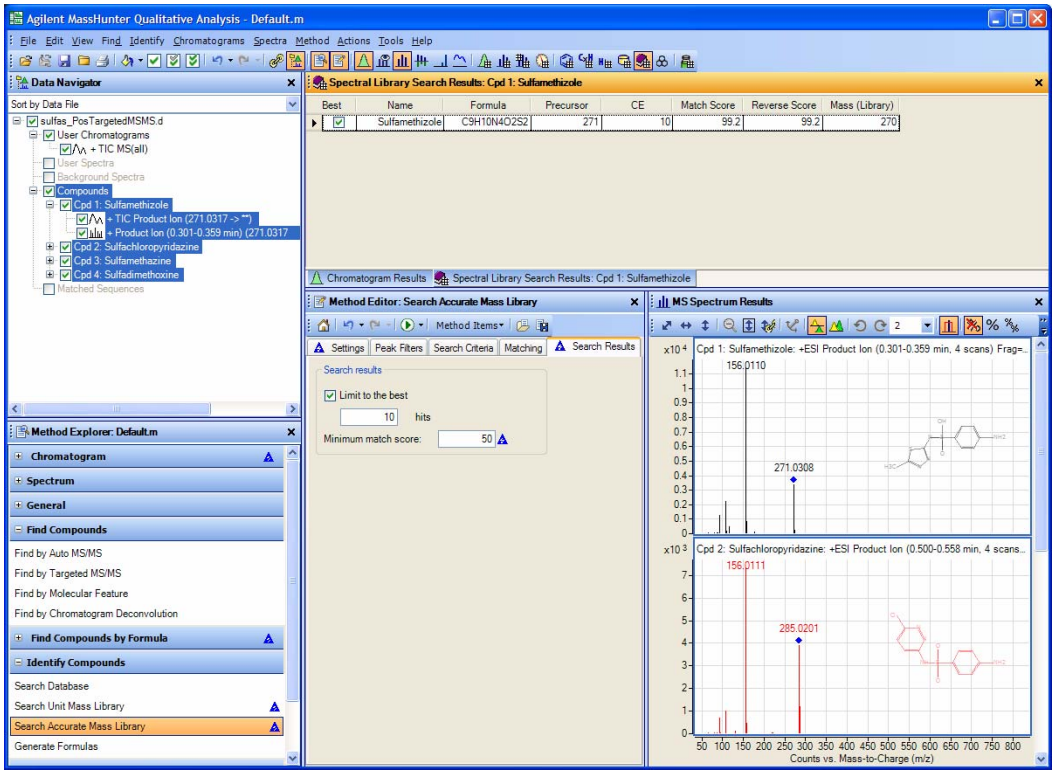


Figure 76 Results after running the Search Accurate Mass Library algorithm.

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 77 (next page).	**a** Open the **peptide-auto.d** data file. **b** Click the **View > Configure for Workflow > General** command. **c** Click **OK** to continue. **d** Click **No** to save method changes. **e** Click **Find Compounds > Find by Molecular Feature** in the Method Explorer to display the parameters in the Method Editor window. **f** In the **Extraction** tab, select **Small molecules (chromatographic)** as the Target data type. **g** Mark the **Restrict retention time to** check box. **h** Type `2.5 - 4`. **i** On the **Charge State** tab, select **Peptides** as the **Isotope model**. **j** On the **Compound Filters** tab, mark the **Limit to the largest** check box and type `20` for the number of compounds. **k** On the Results tab, mark the **Extract ECC** and **Extract MFE spectrum** check boxes. **l** Click  to run the **Find Compounds by Molecular Feature** algorithm on the data file. **m** If necessary, select **3** in the **Maximum number of list panes** box in the Chromatogram Results toolbar.	• To return the layout to the default layout, click **View > 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. • You extract features using the Qualitative Analysis Molecular Feature algorithm. Then, you can compare sets of data from different extractions using Agilent MassHunter Profiling software or GeneSpring MS software. • The resulting .mhd files are stored in the Results directory under the data file directory.
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)

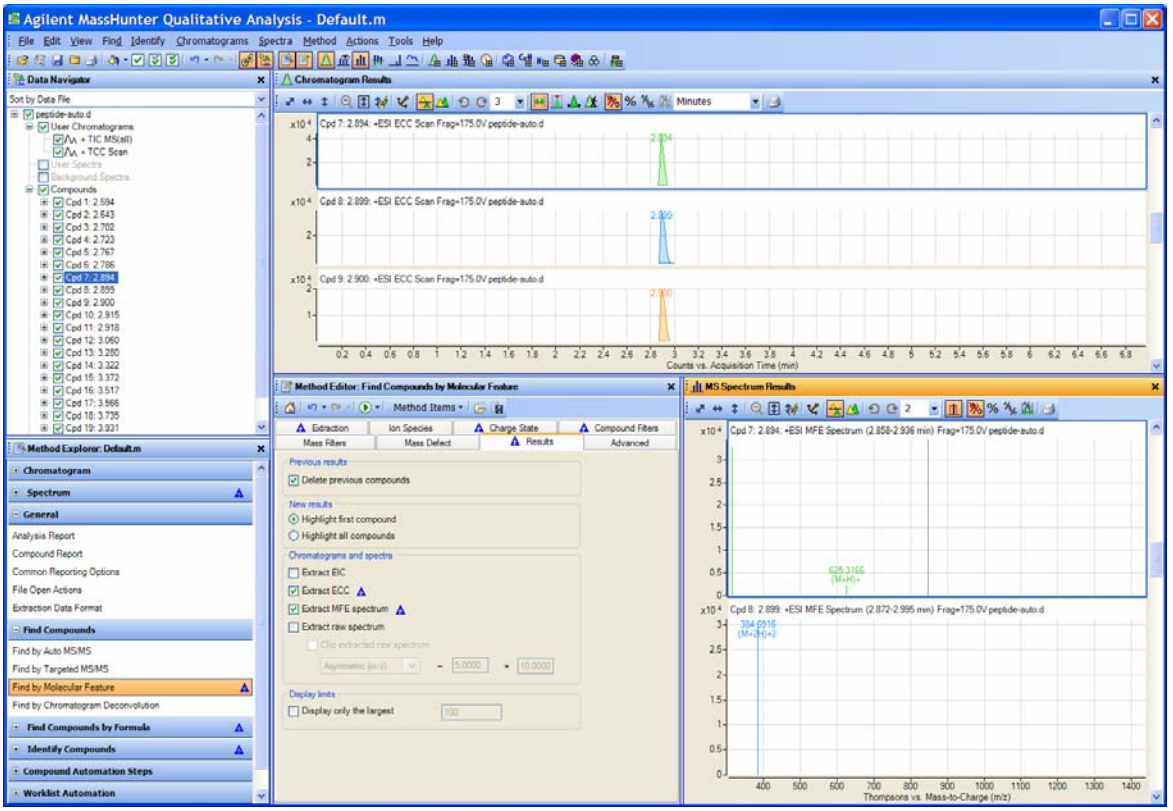
Step	Detailed Instructions	Comments
		

Figure 77 Find Compounds by Molecular Feature for a protein digest with auto MS/MS data

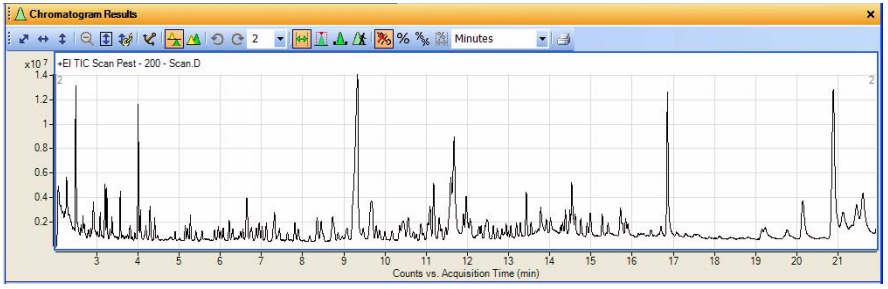
3 Close the data file without saving results.	**a** Click **File > Close Data File**. **b** Click **No** when asked to save the results.	

Tasks for GC/MS Data (Triple Quad)

Task 1. Find compounds by chromatogram deconvolution (GC/MS - MS only)

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

Task 1. Find compounds using Chromatogram Deconvolution (GC/MS - MS only)

Step	Detailed Instructions	Comments
1 Open the TIC for the **Pest - 200 - Scan.d** data file.	**a** If the program is not open, double-click the **Mass Hunter Qualitative Analysis icon**. Otherwise, click **File > Open Data File**. **b** Click the **Pest - 200 - Scan.d** data file in the **GC** example data file folder. **c** Clear the **Load result data** check box and click **Open**.	• You only use the General Workflow when working with GC/MS data.
		
Figure 78 TIC chromatogram from Pest - 200 - Scan.d		
2 Configure the user interface to work with GC data.	• Follow the instructions in Task 15. Configure User Interface for GC 64.	

Task 1. Find compounds using Chromatogram Deconvolution (GC/MS - MS only)

Step	Detailed Instructions	Comments
3 Find compounds using the chromatogram deconvolution algorithm. • Enter an SNR threshold of 20.	**a** In Method Explorer select **Find Compounds > Find by Chromatogram Deconvolution.** **b** Under Peak filter, in the **SNR threshold**, type 20.	• You can choose the region of the chromatogram from which you intend to find compounds. • You can extract the complete result set for a compound after it is found by using the **Compounds > Extract Complete Result Set** menu item when a compound is highlighted.

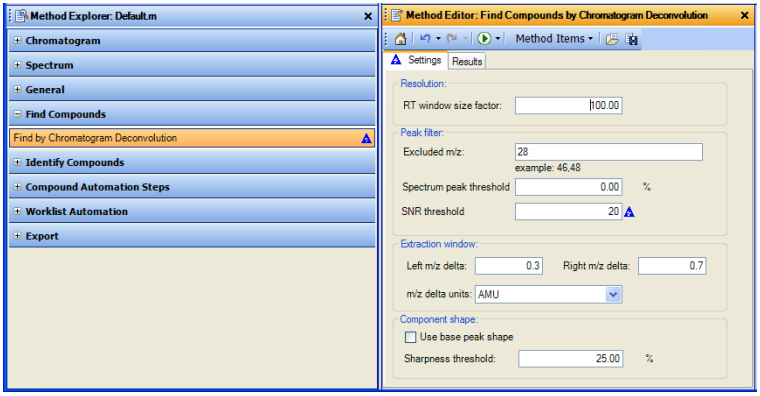


Figure 79 Settings tab of Find by Chromatogram Deconvolution

• Select to extract EIC, MS spectra and MS/MS spectra.	**c** Click the **Results** tab. **d** Mark the **Extract EIC, Extract ECC, Extract cleaned spectrum** and **Extract raw spectrum** check boxes. **e** Click ▶ to run the **Find Compounds by Chromatogram Deconvolution** algorithm on the data file.	• The Qualitative Analysis program finds 69 compounds under these conditions. • In the next task you identify these compounds by searching the NIST08.L library.
4 Examine the compounds. See Figure 70.	**a** Select **2** in the Maximum spectra panes box in the MS Spectrum Results toolbar. **b** Click the first compound in the Data Navigator window. **c** When the Data Navigator window is selected, use the arrow keys to switch compounds.	• Showing both spectra is a convenient way to display all the information for a single compound. • Note that both the cleaned spectrum and the raw spectrum are shown.

Task 1. Find compounds using Chromatogram Deconvolution (GC/MS - MS only)

Step	Detailed Instructions	Comments

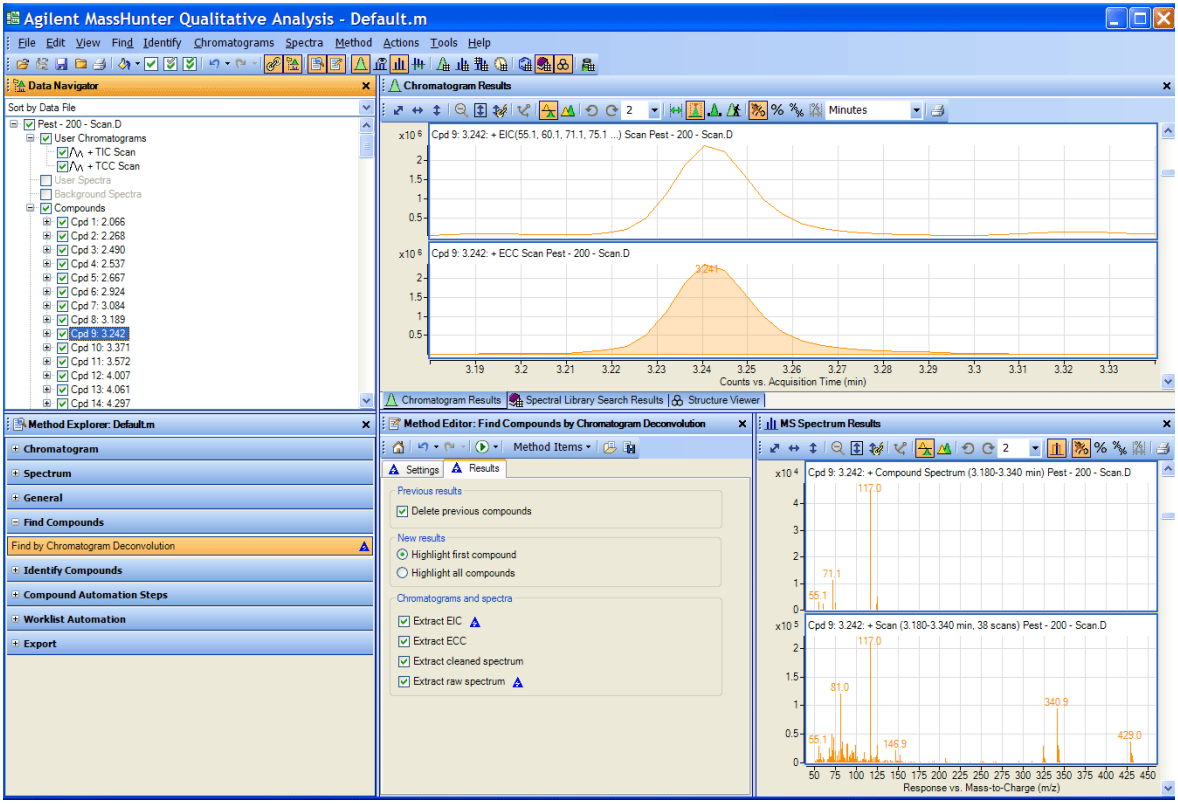


Figure 80 Using the arrow keys in the Data Navigator window to switch compounds

Task 2. Identify compounds using the Search Library algorithm (GC/MS - MS only)

In this task, you identify and generate formulas for the compounds found in Task 1. Find compounds by chromatogram deconvolution (GC/MS - MS only) 114. You can only do this task if you have purchased the NIST08.l library.

Task 2. Identify compounds using the Search Library algorithm (GC/MS - MS only)

Step	Detailed Instructions	Comments
1 Do a library search of all of the compounds.	**a** Highlight all compounds in the Data Navigator window. **b** In Method Explorer, click **Identify Compounds > Search Unit Mass Library**. **c** In the Settings tab, click the ... button. Select the **NIST08.l** library and click **OK**. **d** Click **Identify > Search Library for Compounds** from the main menu. You can instead click the **Search Library for Compounds** icon to run the algorithm. **e** Close the **Compound List** window. **f** Close the MS Spectrum results window by clicking **View > MS Spectrum Results**. **g** Display the Chromatogram Results window by clicking on the Chromatogram Results tab. This window is tabbed with the MS Spectral Library Search Results window. **h** Click **View > Difference Results**. **i** Click **View > Structure Viewer**.	• Note that many of the compounds are identified after searching the NIST08.l library. • You can use the search library algorithm on an MS/MS spectrum if you have an XML library. You can create and edit an XML library using the Library Editor program which is installed with the Quantitative Analysis program. See the online Help for more information.

Task 2. Identify compounds using the Search Library algorithm (GC/MS - MS only)

Step	Detailed Instructions	Comments

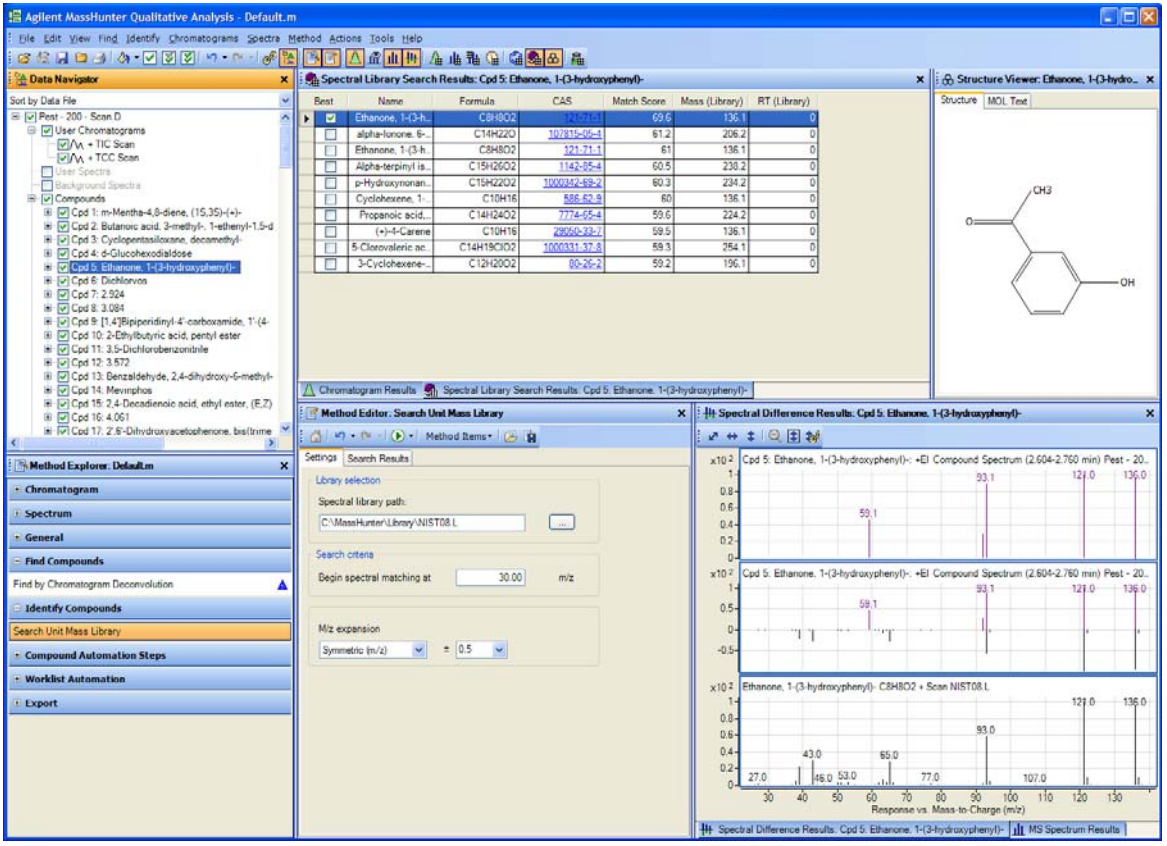


Figure 81 Compounds in Pest - 200 - Scan.D data file and the library search results

2 Close data files and return to LC/MS/MS user interface configuration.	**a** Click **File > Close Data File**. **b** Select all files. **c** Click **Close**. **d** Click **Tools > User Interface Configuration**. **e** Mark all check boxes. **f** Click **OK**.	• If these check boxes are not marked, then some of the algorithms are not available.

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

In this exercise, 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 148 for more information.

The General workflow supports both GC/MS and LC/MS data. The other workflows only support LC/MS data.

Each exercise is presented in a table with three columns:

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

Task 1. 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 Set up the method to extract a TIC chromatogram.
• 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.
• Define a TIC chromatogram for MS data.
• Turn off cycle sum since this is an MS-only data file.
	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 **View > Configure for Workflow > General** command.
	g Click **OK** to continue loading the workflow.
	h Click **No** to save the method changes.
	i In Method Explorer, select **Chromatogram > Define Chromatograms**.
	j Delete the BPC selection.
	k Select **TIC** as the **Type**.
	l Make sure the MS Level is **MS**.
	m Clear the **Do cycle sum** check box.
	n Click **Add**.
		• 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.
		• You can also change the workflow by clicking a command in the Tools > Configure for Workflow menu.

2 Edit the method to integrate the data. • Limit the integration to the four highest peaks.	**a** In Method Explorer, 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.
3 Test the integration to make sure that only 4 integrated peaks appear.	• Click the **Integrate Chromatogram** icon  to integrate the data file.	
4 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 **Save**.	• Note that saving the method causes all the blue triangles indicating value changes in the opened method to disappear.

Task 1. Set up and run a qualitative analysis method

Steps	Detailed Instructions	Comments
5 Change the peak spectrum background to use the spectrum at the start of a peak.	**a** In Method Explorer, click **Spectrum > Extract (MS)**. **b** Click **Peak Spectrum Extraction (MS)**. **c** For the Peak spectrum background, select **Spectrum at peak start**.	• Any changes after saving will produce more blue triangles.

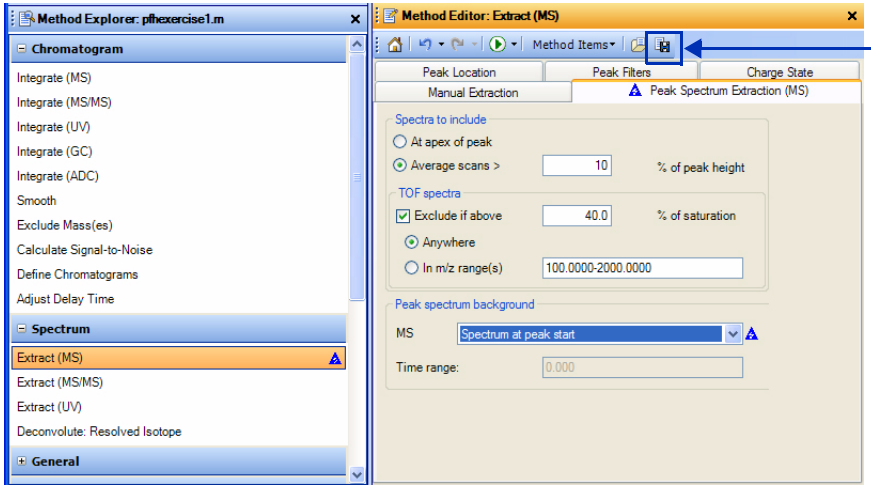
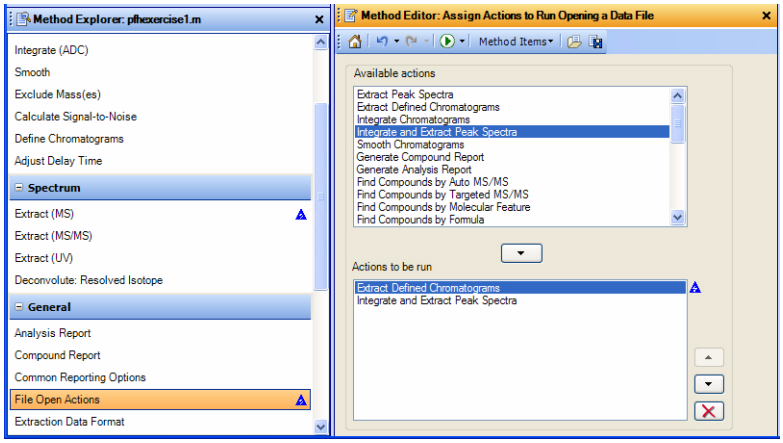


Figure 82 The Spectrum > Extract (MS) > Peak Spectrum Extraction (MS) tab

6 Test the MS spectrum extraction to make sure a background spectrum is subtracted.	• Click the **Extract MS spectrum** icon to run the action on the data file.	
7 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 82 on page 124

Task 1. Set up and run a qualitative analysis method

Steps	Detailed Instructions	Comments
8 Set up the method to automate the actions whose parameters you just changed. • 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, 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.	
9 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.

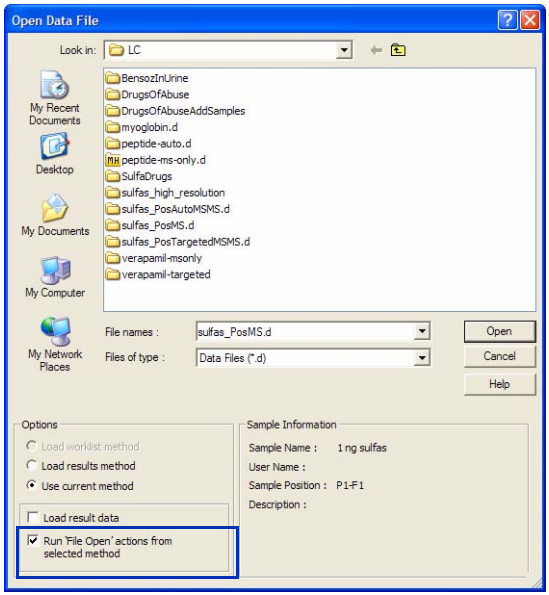


Two different actions are part of the Actions to be run list. The first action is to extract the defined chromatograms. Then, that chromatogram is integrated and peaks are extracted.

Figure 83 The General > File Open Actions section in the Method Editor

10 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 1. Set up and run a qualitative analysis method

Steps	Detailed Instructions	Comments
11 Open the data file again and run the actions specified.	**a** Click **File > Open Data File**, and select **sulfas_PosMS.d**. **b** Mark the **Run 'File Open' actions from selected method** check box.	• If you have saved the previous results by mistake, clear the **Load Result data** check box.
		
	Figure 84 The Open Data File dialog box with "Run 'File Open' actions..." marked	
	c Click **Open**.	• The screen should look like the one in Figure 85. (You may have to move window boundaries.)

Task 1. Set up and run a qualitative analysis method

Steps	Detailed Instructions	Comments

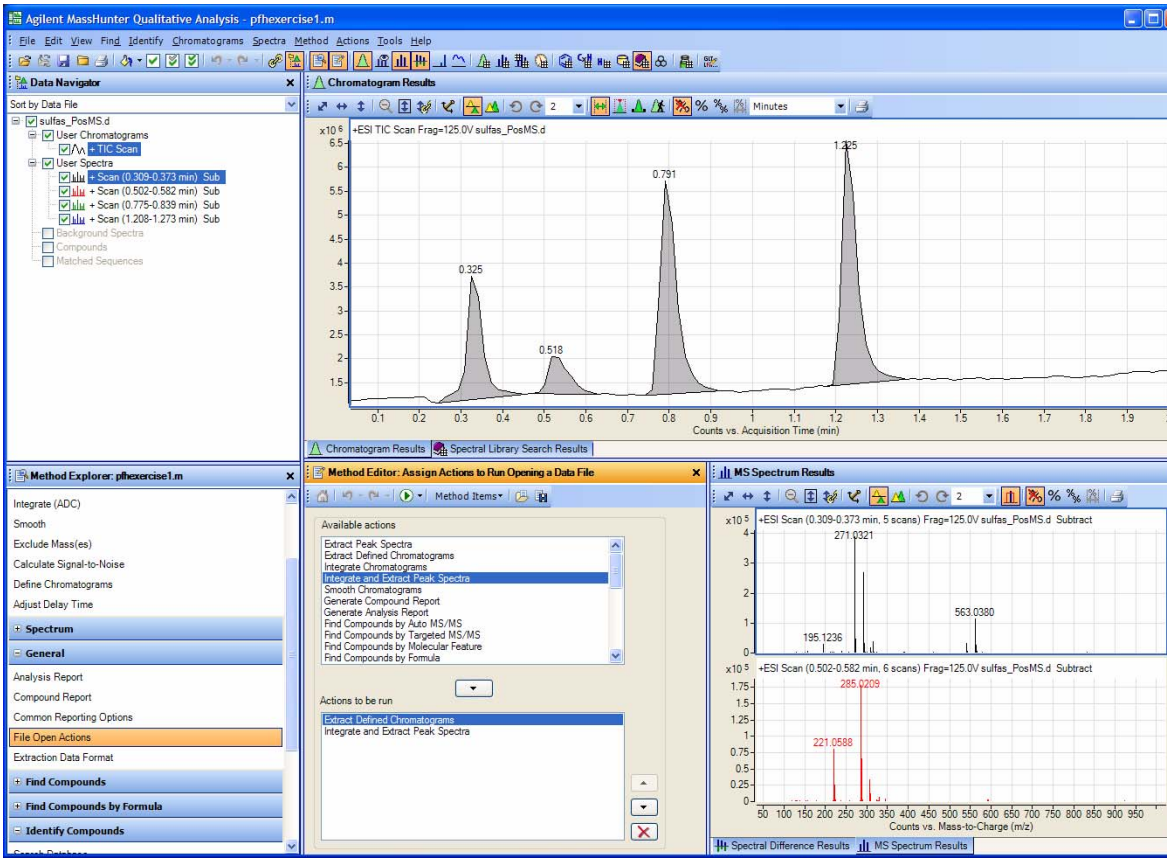


Figure 85 Integrated TIC and background-subtracted spectra – result when sulfas_PosMS.d data file opened

12 Close the data file again, not saving the results.	**a** Click **File > Close Data File**. **b** 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 **View > Configure for Workflow > Chromatogram Peak Survey Workflow** command. **b** Click **OK** to switch the workflow. **c** Click **No** to save the method changes. **d** Click **File > Open Data File**. **e** In the Open Data File dialog box, select **sulfas_PosMS.d**. **f** Clear the **Run 'File Open' actions from selected method** check box. **g** Click **Open**. **h** 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**.
2 Look at the sections for the Chromatogram Peak Survey algorithm.	• In Method Explorer, click **Chromatogram Peak Survey Workflow**.	• Note the eleven sections in this workflow. Most of these sections are duplicates of sections in the General workflow.
3 Make sure that new results will overwrite previous results.	**a** Select **Previous Results** in the Method Explorer. **b** Mark **Delete all previous results**.	

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

Steps	Detailed Instructions	Comments
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 the **Chromatogram Integration** section in the Method Explorer. **f** 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.
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**.	• Note that blue triangles appear in other sections of Method Explorer. These indicate that the same parameter values have been changed elsewhere as well.
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 **All peaks**.	• Note that the "Spectrum Peak Identification" section is unique. You cannot enter this information anywhere else in the Method Editor.
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.

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

Steps	Detailed Instructions	Comments
8 Open these windows for viewing: • DB Search Results list • MS Formula Results list • These lists are tabbed along with Chromatogram Results as in Figure 86 • Review each list for each MS scan. • Save the method if the automation worked.	**a** Click **View > DB Search Results**. **b** Click **View > MS Formula Results**. **c** Move these windows so they are tabbed with the Chromatogram Results window as in Figure 86. **d** Review the results for each MS scan to make sure that all actions in the Chromatogram Peak Survey algorithm were performed.	• SeeTask 4. Change window layouts 22 to learn how to move windows on the main screen. • You can also use the icons in the main toolbar to display these windows.

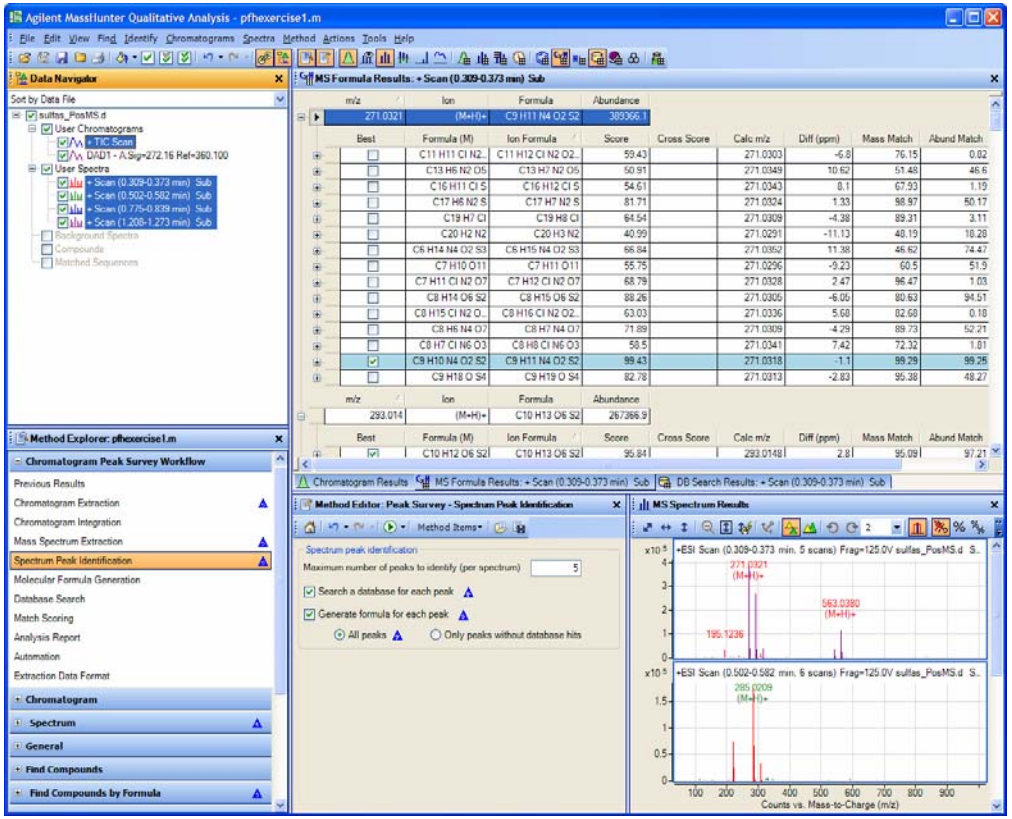


Figure 86 Tabbed results from running automated analysis steps

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

Steps	Detailed Instructions	Comments
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** Make any changes you want. **c** Click the **Print Analysis Report** icon. **d** 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.
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 **File Open Actions**. **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.
12 Close Method Editor, Method Explorer and Data Navigator. • Move the windows so they look like the layout in Figure 87. • 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 87. **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 87.	**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**.	

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

Steps	Detailed Instructions	Comments

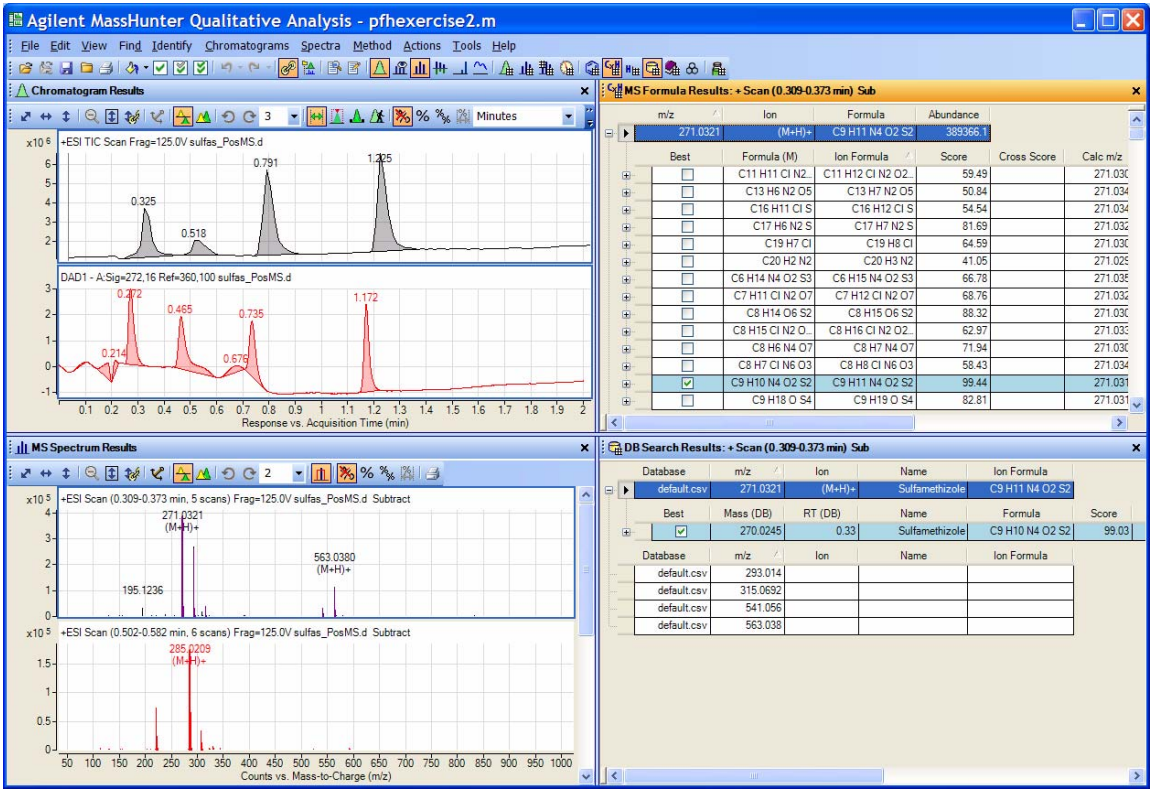


Figure 87 Results of Chromatogram Peak Survey action when opening the sulfas_PosMS.d data file

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 **View > Configure for Workflow > MS Target Compound Screening**. **b** Click **OK** to switch the workflow. **c** Click **No** to save the method changes. **d** Click **File > Open Data File**. **e** In the Open Data File dialog box, select **sulfas_PosMS.d**. **f** Clear the **Run 'File Open' actions from selected method** check box. **g** Click **Open**. **h** Click **Method > Open.** Select the *iii*exercise2.m method. **i** Click **Open.** **j** 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.
2 Look at the automation steps for finding and identifying compounds. • Tab the Method Editor window in a convenient location.	**a** In Method Explorer, 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.

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

Steps	Detailed Instructions	Comments
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** Click **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 • DB Search List • MS Formula Results List • Make sure the results lists are tabbed along with Chromatogram Results as in Figure 88 • Review each list for each compound (except for Compounds 1 and 4).	**a** Click **View > Compound List**. **b** (if necessary) Click **View > DB Search Results**. **c** (if necessary) Click **View > MS Formula Results**. **d** Clear the Compound 1 and Compound 4 check boxes in the Data Navigator. Or, you can clear the check boxes for Compound 1 and Compound 4 in the **Show/Hide** column in the Compound List window **e** Review each list for each identified compound to make sure that all actions in the Compound Automation Steps were performed.	• See Exercise 1 Task 4 to learn how to move windows on the main screen. • The MS Formula Results window and the DB Search Results window are tabbed with the Chromatogram Results window in Figure 88.
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.

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

Steps	Detailed Instructions	Comments

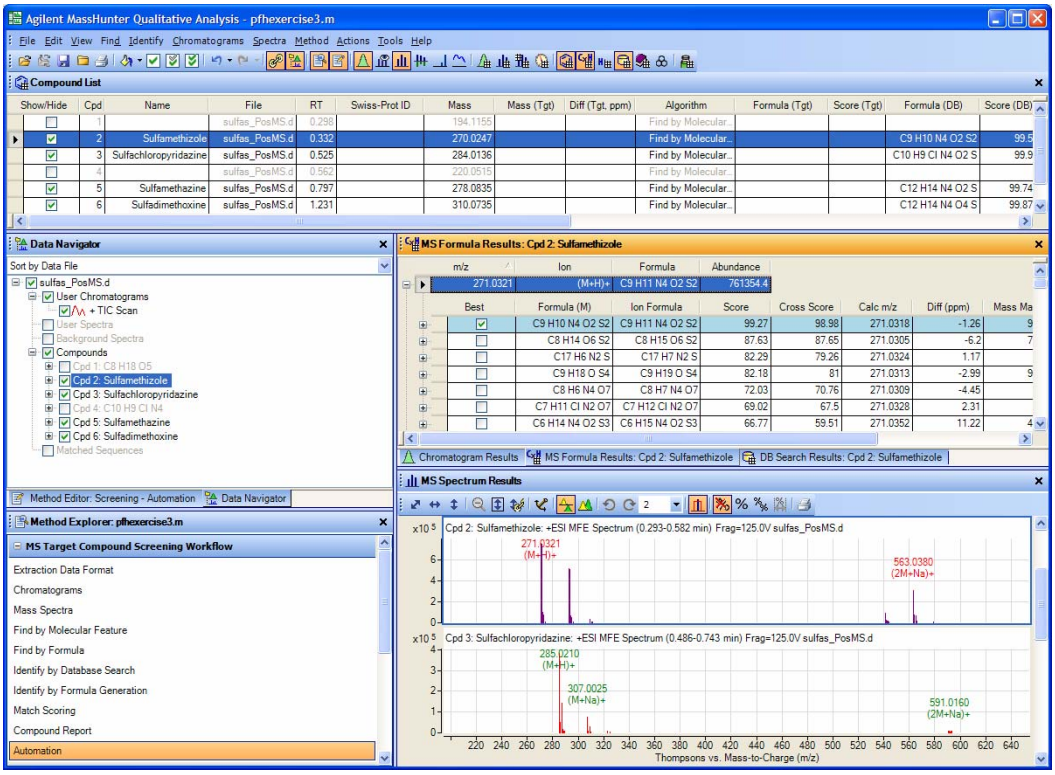


Figure 88 Tabbed results from running compound automation identification steps

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** If necessary, click the **Save Method** icon in Method Editor.	

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 any 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 89. • 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 89. **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 89. • Hide Compounds 1 and 4 in the Compound List.	**a** Click **File > Open Data File** **b** Mark the **Run 'File Open' actions from selected method** check box. **c** Click **Open**. **d** Clear the **Show/Hide** check boxes for Compounds 1 and 4 in the Compound List.	• Compounds 1 and 4 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

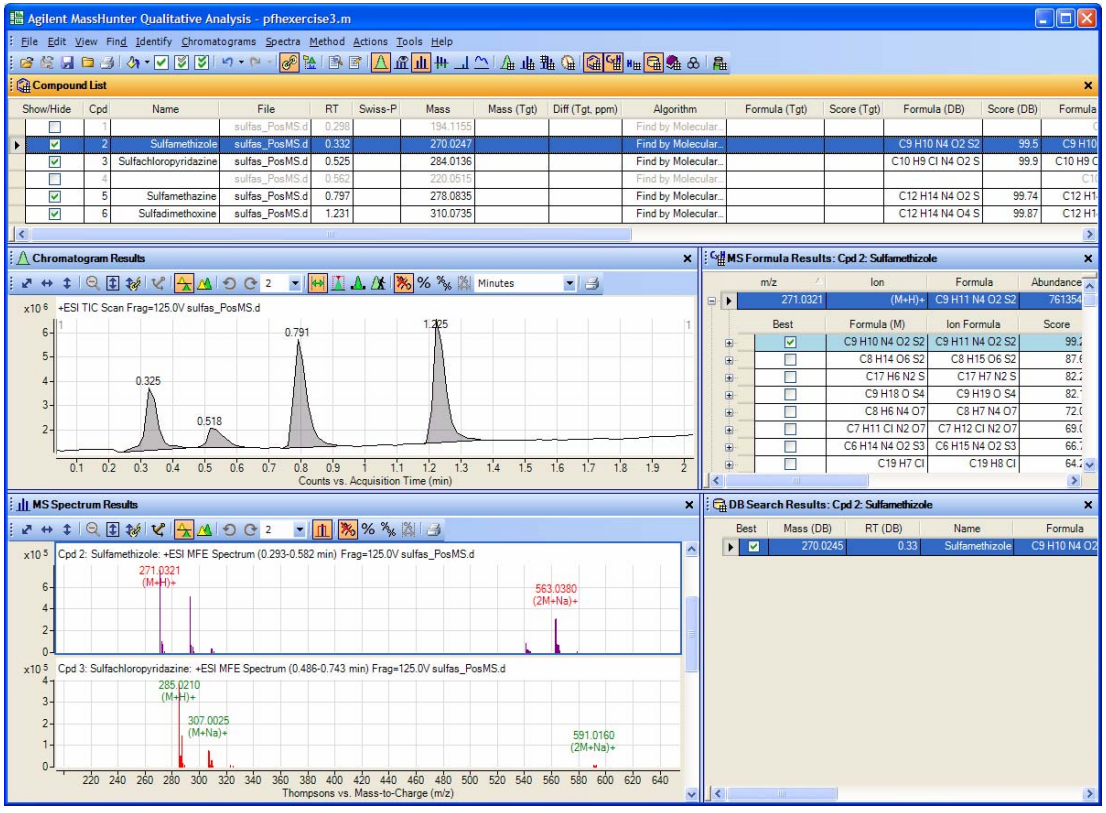


Figure 89 Results of automated compound identification when opening the sulfas_PosMS.d data file

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

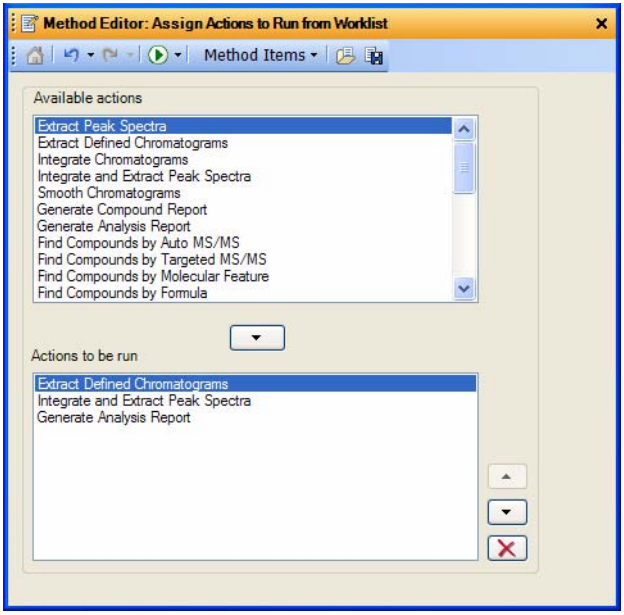
Task 4. Set up a qualitative method to run with a worklist

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.

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

Steps	Detailed Instructions	Comments
1 Set up a method to automatically execute upon completion of every run in the worklist. • Open the **sulfas_PosMS.d** data file with the method you saved in Task 2. • Make sure actions are not run when you open the file. • Restore the default window layout. 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** To restore the default workflow, click **View > 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** Click **Open**. **g** Load the method ***iii*Exercise2.m**. **h** In the Method Explorer, select **Worklist Automation > Worklist Actions** to display the **Assign Actions to Run from Worklist** section. **i** 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 **j** 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. **k** If necessary, select any actions in the **Actions to be run** list that are not in the above list, and click the **Remove** icon  .	• 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
		
	Figure 90 Method Editor with Worklist Actions section displayed	
2 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.

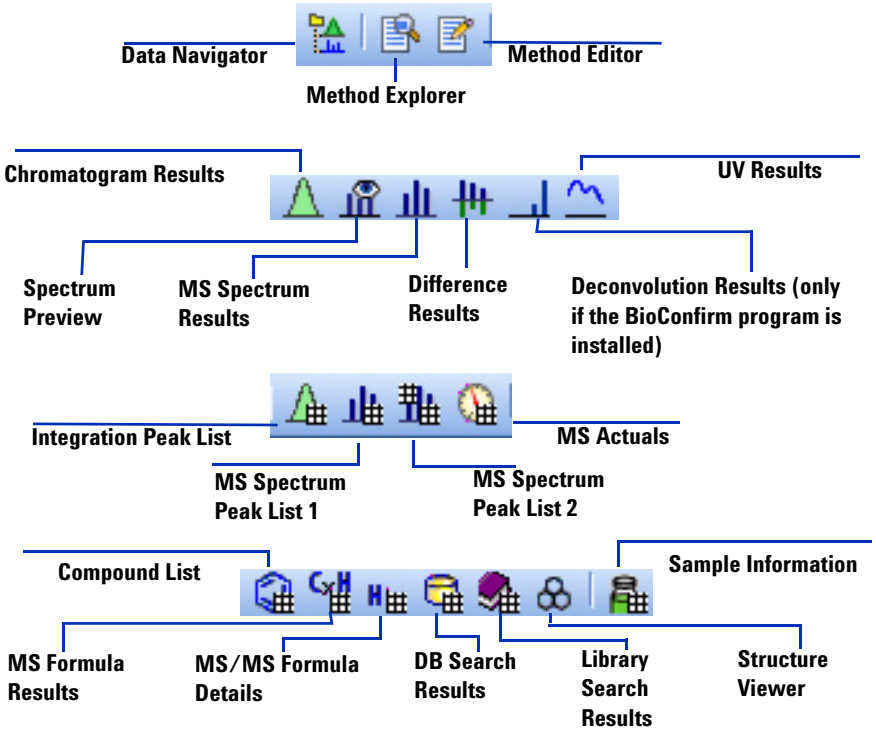
Reference

Work with windows

When you first open the Qualitative Analysis program, you see three windows as the default layout: Data Navigator, Method Explorer and Chromatogram Results. You can bring up seventeen other windows using the View menu: Method Editor, Spectrum Preview, MS Spectrum Results, Difference Results, UV Results, Integration Peak List, MS Spectrum Peak List 1, MS Spectrum Peak List 2, MS Actuals, Compound List, MS Formula Results, MS/MS Formula Details, DB Search Results, Library Search, Structure Viewer and Sample Information. You can also display three tool windows which are displayed when you start using the associated tool: Formula Calculator, Mass Calculator, and Recalibrate.

Window Icons in the Main Toolbar

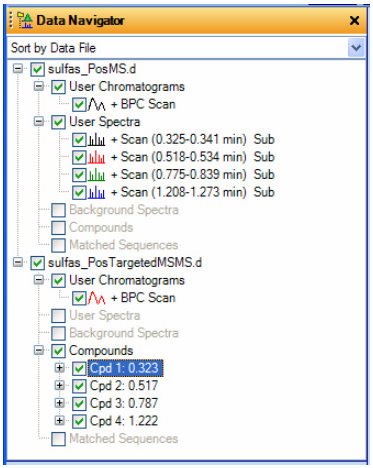
You open and close the Qualitative Analysis windows with these icons on the main toolbar. Additional icons are available when the MassHunter BioConfirm software is installed. Commands in the View menu can also be used to open these windows.


Data Navigator
Method Editor
Method Explorer
Chromatogram Results
UV Results
Spectrum Preview
MS Spectrum Results
Difference Results
Deconvolution Results (only if the BioConfirm program is installed)
Integration Peak List
MS Actuals
MS Spectrum Peak List 1
MS Spectrum Peak List 2
Compound List
Sample Information
MS Formula Results
MS/MS Formula Details
DB Search Results
Library Search Results
Structure Viewer

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.



Linked Navigation Icon

When activated (default), highlighting a chromatogram in Data Navigator also highlights the corresponding spectra. The corresponding chromatogram and spectrum visual 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
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
Smooth the chromatogram	Chromatograms > Smooth 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 by Chromatogram deconvolution	Find > Find Compounds by Chromatogram Deconvolution
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 icon in the Chromatogram Results toolbar.

2 Click the left mouse button at the point where you want to start the range, drag the cursor over a range, and release the 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.

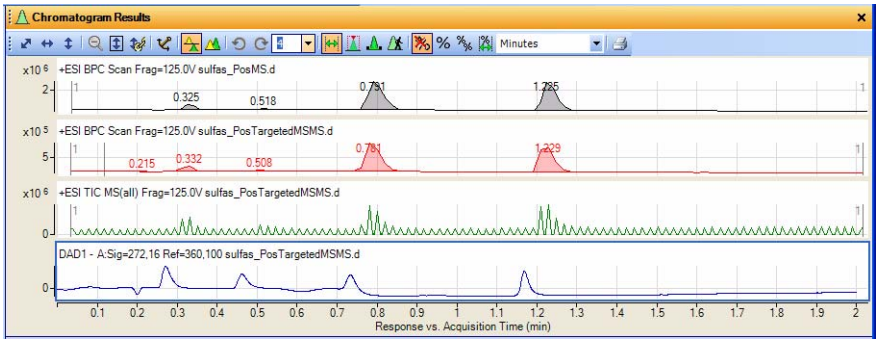
Perform operations on an MS or MS/MS spectrum

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 a spectrum	View >MS Spectrum Peak List 1
Subtract the background spectrum	Spectra > Subtract Background Spectrum
Add two spectra together	Spectra > Add Any Spectrum (and then click another spectrum)
Search a database for compounds 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)
Deconvolute using the Resolved Isotope algorithm	Spectra > Deconvolute (Resolved Isotope)
Search Library	Identify > Search Library for Spectra

Work with chromatographic visual data

Chromatogram Results Window



Chromatogram Results Tools

Zoom Tools in order

Autoscale X-axis and Y-axis

Autoscale X-axis

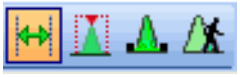
Autoscale Y-axis

Unzoom

Autoscale Y-axis during Zoom

Linked Y-axis mode

Select Tools in order



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.

To clear a tool selection, click another tool or icon.

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.

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

- General
- BioConfirm - This workflow is only available if the BioConfirm software is installed and marked in the User Interface Configuration dialog box.
- Chromatogram Peak Survey
- Formula Confirmation and Sample Purity
- MS Target Compound Screening

If you are working with GC/MS data, you can only select the General workflow. If you are working with LC/MS data, you can select any of the workflows.

Specific Method

Each workflow loads a specific default method with more appropriate settings for that workflow. For example, if you switch to the BioConfirm workflow, 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 workflow 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 and the UV 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

Specific section in the Method Explorer and Method Editor

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

Each of the four workflows changes the sections available in the Method Explorer. Each 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 **Spectrum Peak Identification** section and the **Database Search > Search Criteria** 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**.

BioConfirm methods and layouts

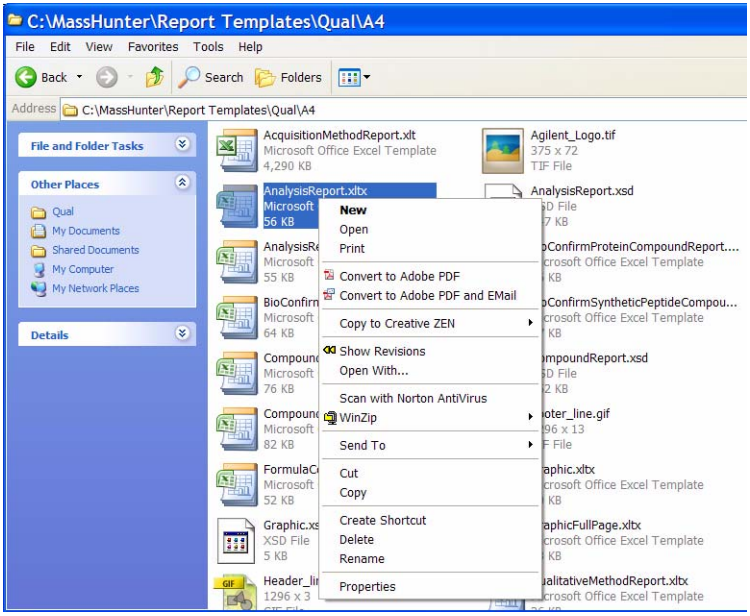
Additional default methods and layouts are provided with BioConfirm software to use as starting points for creating your own customized methods for Sequence Matching.

Workflow	Method	Layout	Method Editor Section
General	default.m	Default.xml	None
BioConfirm	BioConfirm IntactProtein-Default.m	BioConfirm-IntactProtein-MaximumEntropy-Default.xml	BioConfirm Workflow
Chromatogram Peak Survey	ChromPeakSurvey-Default.m	Default.xml	Chromatogram Peak Survey Workflow
MS Target Compound Screening	Screening-Default.m	Screening-Default.xml	MS Target Compound Screening Workflow
Formula Confirmation and Sample Purity	SamplePurity-Default.m	SamplePurity-Default.xml	Formula Confirmation and Sample Purity Workflow

Customize a report template

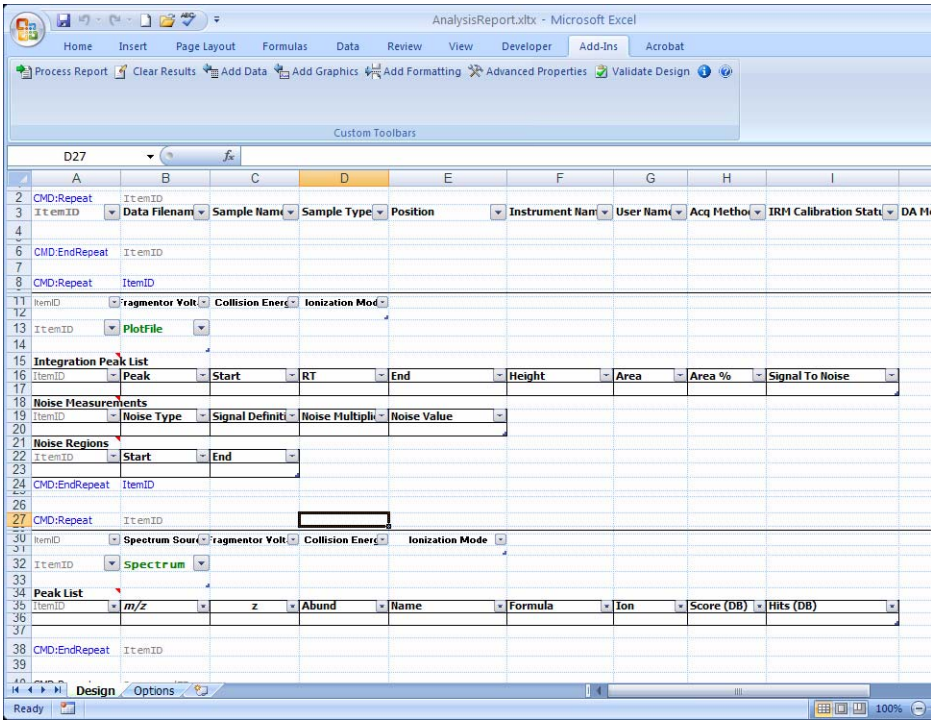
Please refer to either the online Help for the MassHunter Report Designer Add-in or the Reporting Training DVD for detailed information on how to modify a report template. The following steps give you a quick look at what it means to customize a template.

1. Go to the folder that contains the report templates. By default, this folder is **\MassHunter\Report Templates\Qual\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. Right-click the copy and click **Properties**. Clear the **Read-only** check box. Then, right-click the copy and click **Open** from the shortcut menu.



Opening the template this way lets Excel know that this file is a template file. When the template is open, you can modify headers and footers and add, remove or move parameter columns. Refer to the online Help for more information. All Qualitative Analysis templates are marked Read-only. You change this property before you edit a template.

Many templates are installed with the Qualitative Analysis program. Refer to the Qualitative Analysis online Help for more information about the content of each report template.

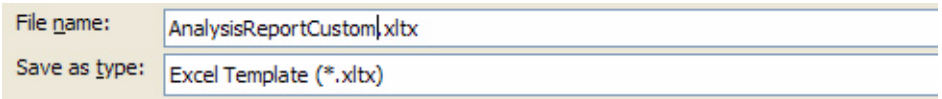


3 Make the changes you want to make.

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*.

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

5 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 .

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