Agilent MassHunter Workstation Software

Qualitative Analysis

Familiarization Guide

Agilent Technologies

Notices

Manual Part Number

G3336-90018

Edition

Revision A, September 2011

Printed in USA

Agilent Technologies, Inc.
5301 Stevens Creek Blvd.
Santa Clara, CA 95051 USA

Software Revision

This guide is valid for B.05.00 and later revisions of the Agilent MassHunter Workstation Software - Qualitative Analysis program, until superseded.

Warranty

The material contained in this document is provided "as is," and is subject to being changed, without notice, in future editions. Further, to the maximum extent permitted by applicable law, Agilent disclaims all warranties, either express or implied, with regard to this manual and any information contained herein, including but not limited to the implied warranties of merchantability and fitness for a particular purpose. Agilent shall not be liable for errors or for incidental or consequential damages in connection with the furnishing, use, or performance of this document or of any information contained herein. Should Agilent and the user have a separate written agreement with warranty terms covering the material in this document that conflict with these terms, the warranty terms in the separate agreement shall control.

Technology Licenses

The hardware and/or software described in this document are furnished under a license and may be used or copied only in accordance with the terms of such license.

Restricted Rights Legend

U.S. Government Restricted Rights. Software and technical data rights granted to the federal government include only those rights customarily provided to end user customers. Agilent provides this customary commercial license in Software and technical data pursuant to FAR 12.211 (Technical Data) and 12.212 (Computer Software) and, for the Department of Defense, DFARS 252.227-7015 (Technical Data - Commercial Items) and DFARS 227.7202-3 (Rights in Commercial Computer Software or Computer Software Documentation).

Safety Notices

CAUTION

A **CAUTION** notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in damage to the product or loss of important data. Do not proceed beyond a **CAUTION** notice until the indicated conditions are fully understood and met.

WARNING

A WARNING notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in personal injury or death. Do not proceed beyond a WARNING notice until the indicated conditions are fully understood and met.

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

Exercise 4 Qualitative Analysis Wizards

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

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

before running the Chromatogram Peak Survey without Analysis Report action.

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

Reference

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

What's New

in B.05.00 (Qualitative Analysis)

- Qualitative Analysis is supported on Windows 7 Professional (64 bit) only.
- Reviewing of compound results is faster.
- Extracting MS profile spectra and EIC is faster.
- Extracting multiple MS EIC (centroid and profile) is faster.
- Finding compounds using the Find by Formula algorithm is faster.
- You can manually extract a Find by Formula compound.
- You can specify in the method how many compounds to report for each compound.
- You can specify in the method to automatically increase the number of matches reported for isomeric compounds.
- You can specify in the method to only generate compounds for matched formulas.
- You can specify to give a warning or error for a Find by Formula compound if the Score is less than the entered value.
- You can specify to give a warning or error for a Find by Formula compound if a second ion is expected and not found, and the abundance of the second ion is supposed to be greater than or equal to the value entered.
- You can specify the contribution to the overall Score that the Retention time score makes.
- You can specify to use both the MS spectrum with a background spectrum subtracted and the MS spectrum with no background spectrum subtracted. The best result is returned.
- You can either export only the highlighted results or all results when exporting a CEF file.
- You can extract an MS/MS spectrum either for each collision energy or an average MS/MS spectrum for all collision energies.

in B.04.00 (Qualitative Analysis)

- The Qualitative Analysis program is supported on Windows 7.
- Excel 2010 is supported on Windows 7.
- You can annotate chromatograms and spectra with text and graphics.
- You can add calipers to spectra in the MS Spectrum Results and the UV Spectrum Results window.
- You can add Amino Acid calipers and Modifications calipers to deconvoluted spectra.
- You can find compounds using the information from an MRM data file. The Qualitative Analysis program picks up compound names for MRM transitions from the Data Acquisition method.
- The Noise region in the signal-to-noise calculation can be determined automatically for LC/MS and GC/MS data.
- The Auto-RMS algorithm is available for calculating signal-to-noise.
- The information about the compounds is now displayed in an updated Compound List window.
- The identification results for a compound are shown together in the Compound Identification Results window.
- You can filter the identification results based on the identification techniques to show only compounds identified by a specific technique.
- The identification results for a spectrum are shown together in the Spectrum Identification Results window.
- You can manually add identification information to a spectrum or compound. You can also clear this information by clearing the identification results.
- You can extract an MS/MS spectrum automatically when you run the Find by Molecular Feature algorithm. You can select to extract a separate MS/MS spectrum for each collision energy or to extract one spectrum for all collision energies. You can also opt to deisotope the spectrum for protein applications.

- You can subtract a chromatogram from a chromatogram.
- You can subtract a deconvoluted spectrum.
- You can display glycan structures.
- You can see a summary of the modifications and links in the Chemical Data Dictionary.
- Several wizards have been added to show you the different parameters you need to modify for different workflows.
- The Agile integrator is supported for all chromatograms.
- System suitability calculations can be done for MS, MS/MS, UV and GC chromatograms when they are integrated.
- You can specify Peak Filters for the Find by Molecular Feature algorithm.
- You can specify Mass Filters for the Find by Chromatogram Deconvolution algorithm.
- You can specify a minimum Forward Search score and a minimum Reverse Search score for the Search Accurate Mass Library algorithm.
- You can export to an MS/MS inclusion list which can be included in the MassHunter Acquisition program.
- Exporting data in different formats is easier to do.
- You can filter the columns in the Compound List window and the Compound Identification Results table.
- You have more options available when you are switching to a new workflow, including whether or not to save the current method, whether or not to reload the current method and whether or not to use the current layout.
- The Glycan isotope model is supported when you are exporting an MGF or mzData file.
- The Isotope Distribution Calculator program is included.
- If BioConfirm is installed, the MassHunter Comparative Analysis program is available to compare the Match Sequence between a single reference data file and one or more sample data files. You can only compare the reference data file to one sample data file at a time.

- If BioConfirm is installed, then you can specify Scoring parameters for the Define and Match Sequences algorithm.
- If BioConfirm is installed, then you can find compounds by maximum entropy.
- If BioConfirm is installed, you can do MS/MS confirmation.

Before you begin these exercises...

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

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

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

Contents

Exercise 1
Learn basics of qualitative analysis

In this exercise, you explore some of the many powerful capabilities of the Qualitative Analysis program for working with 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.
- In Task 6, you add a text annotation to a chromatogram.

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 7. Extract chromatograms (MS only), you extract chromatograms at various levels and merge the EICs.
- In Task 8. Interactively integrate a chromatogram (MS only), you integrate chromatograms, change the integration parameters and calculate the S/N ratio for integrated peaks.
- In Task 9. 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 Task 10. Add a caliper, you add a delta mass caliper to the extracted peak spectrum. You can use these instructions to add a spectrum to any MS or MS/MS spectrum.

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

- Task 11. Extract chromatograms (LC/MS and LC/MS/MS)
- Task 12. Interactively integrate a chromatogram (LC/MS and LC/MS/MS)
- Task 13. Extract spectra from a chromatogram (LC/MS and LC/MS/MS)

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

- Task 14. Extract chromatograms (MS and UV)

- Task 15. Interactively integrate a chromatogram (UV) and calculate System Suitability values (MS and UV)
- Task 16. Extract spectra from a chromatogram (UV)

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

- Task 17. Configure User Interface for GC
- Task 18. Extract chromatograms from a GC/MS data file
- Task 19. Interactively integrate a GC/MS chromatogram
- Task 20. 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

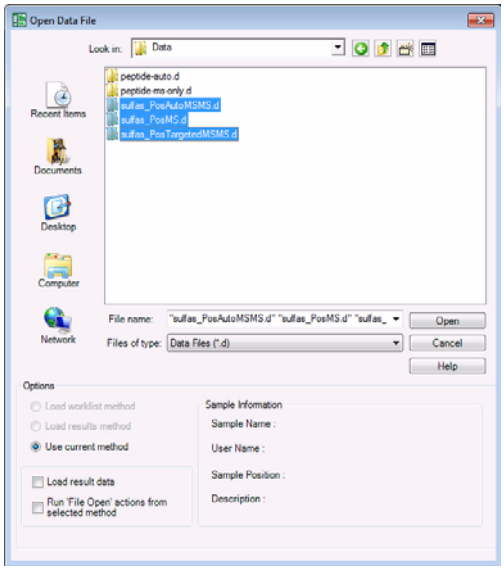
Steps	Detailed Instructions	Comments
1 Open the Qualitative Analysis program. • Open the data files, **sulfas-PosAutoMSMS**, **sulfas-PosMS.d** and **sulfas-PosTargetedMSMS.d** in the folder **\\MassHunter\Data**, or in the folder where you copied them.	**a** Double-click the **Agilent MassHunter Qualitative Analysis B.05.00** 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 the **Use current method** button is clicked. • Make sure that the **Load result data** check box is clear. • Make sure that the **Run 'File Open' actions from selected method** check box is clear.		

Figure 1 Open data files when opening software

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

Steps	Detailed Instructions	Comments
	c Press and hold the **Shift** key while you click **sulfas_PosAutoMSMS**, **sulfas_PosMS.d** and **sulfas-PosTargetedMSMS.d**. **d** Click **Open**. All three data files are displayed in the Data Navigator window, and 1 to 3 chromatograms are displayed in the Chromatogram Results window. **e** Click the **List Mode** icon  in the Chromatogram Results toolbar.	• If you press the **Ctrl** key, you can pick files which are not directly next to each other in the list. • What you see in the main window at this point depends on the method, layout, display and plot settings used before you opened these files. • When you click the List Mode icon, the background of the icon changes to orange.

Figure 2 Qualitative Analysis main window

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

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

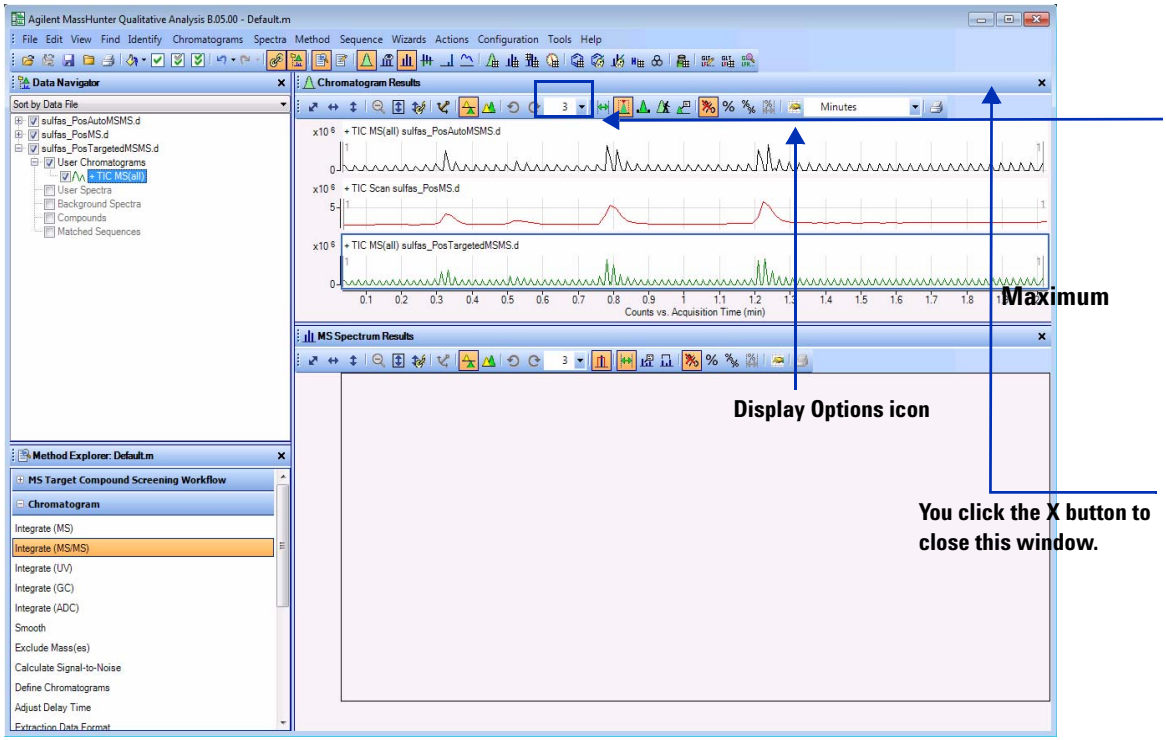


Figure 3 Qualitative Analysis main window with the General Workflow selected.

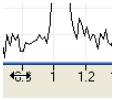
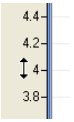
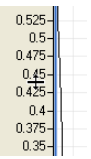
Task 2. Zoom in and out of the chromatogram

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

Task 2. Zoom in and out of the chromatogram

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

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

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

Task 3. Anchor a chromatogram

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

Task 3. Anchor a chromatogram

Steps	Detailed Instructions	Comments

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

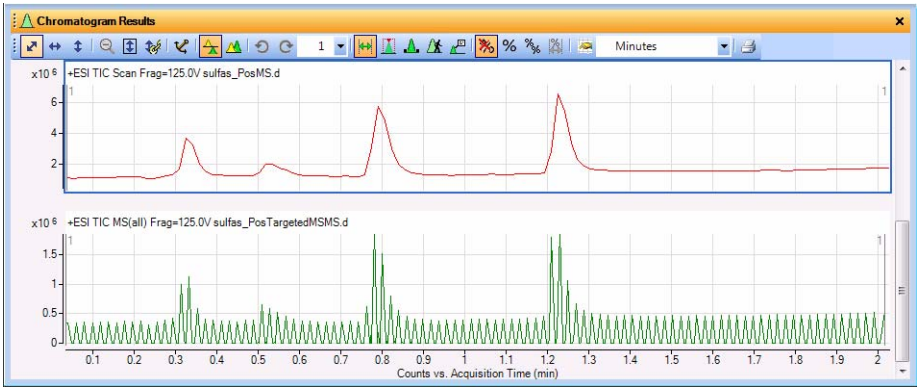


Figure 4 Anchored TIC in the Chromatogram Results window

Task 4. Change window layouts

In this task, you move windows within the Main View and create various window layouts.

Task 4. Change window layout

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

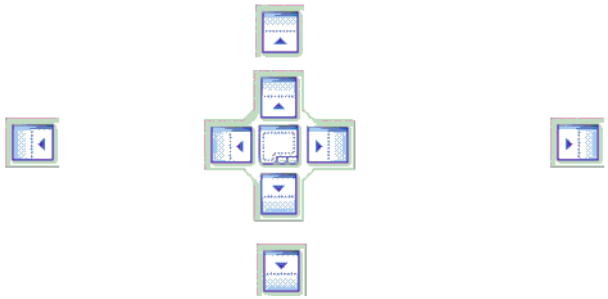


Figure 5 Window repositioning tools

Task 4. Change window layout (continued)

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

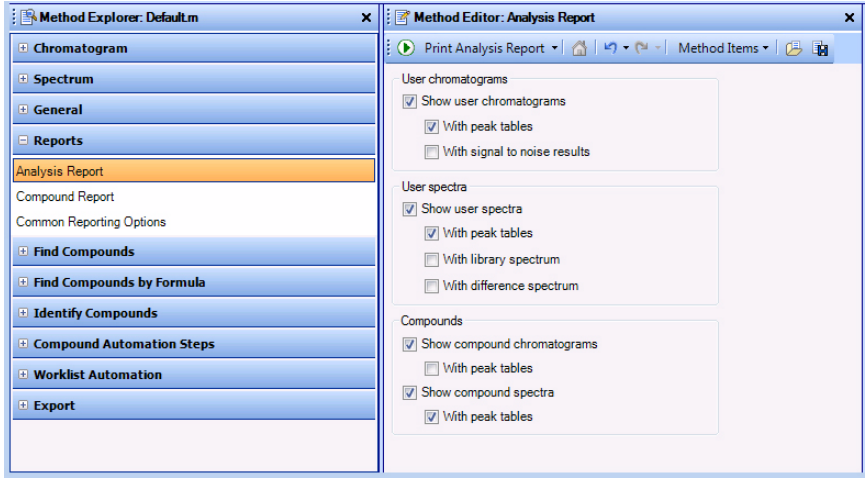
Task 5. Print an analysis report

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

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

Task 5. Print an analysis report

Steps	Detailed Instructions	Comments
1 Change the analysis report selections: • Mark the check boxes for the chromatograms, spectra or tables you want to print. • Clear the check boxes for the chromatograms, spectra or tables you do not want to print.	**a** In the Method Explorer window, click **Reports > Analysis Report**. **b** Mark the check boxes for any additional selections you want to print. **c** Clear any chromatogram and spectra choices you do not want to print.	• The Analysis report only contains the information that you mark in this section. • If some results are not available, then those results are not included, even if those results are marked in this section. For example, if you have not integrated the chromatogram, then the peak table is not included.



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

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

Task 5. Print an analysis report (continued)

Steps	Detailed Instructions	Comments
2 Print the report.	**a** You can interactively print the report in multiple ways: • From the main menu, click **File > Print > Analysis Report.** • From the main toolbar, click the Printer icon. • Click the **Print Analysis Report** icon,  in the Method Editor toolbar when the Analysis Report section is selected. • Right-click the Analysis Report section in the Method Editor, and click **Print Analysis Report**. • From the data file shortcut menu in the Data Navigator, click **Analysis Report**. **b** Click the **Report contents**. **c** Mark the **Print report** check box and select a printer. **d** Mark the **Print preview** check box. **e** Click the **OK** button. **f** Review the report. **g** Click the **Close Print Preview** icon in the toolbar.	• The Run icon  in the Method Editor toolbar sometimes allows you to choose an action from a set of possible actions. For example, if you switch to the Reports > Common Reporting Options section of the Method Editor window, four different actions are possible when you click the Run icon. If you click the arrow, a list of possible actions is shown, and you can choose which action to do. Choosing a different action from the list changes the default action. If you simply click the Run button, the current default action is performed.

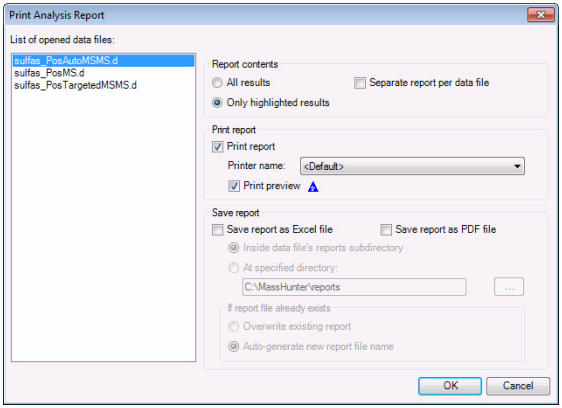


Figure 7 Print Analysis Report dialog box

Task 6. Add an annotation

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

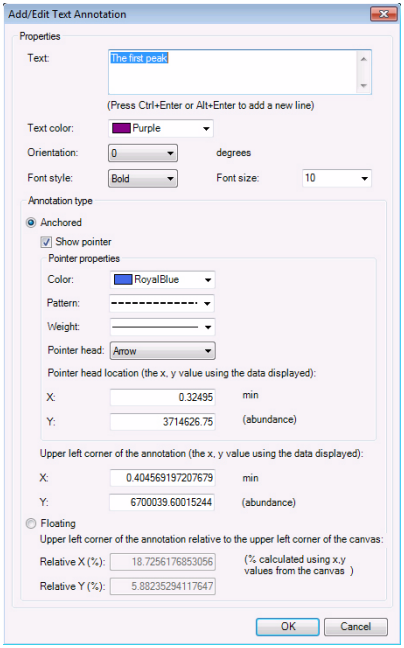
- Chromatogram Results window
- MS Spectrum Results window
- Difference Results window
- Deconvolution Results window
- UV Spectrum Results window

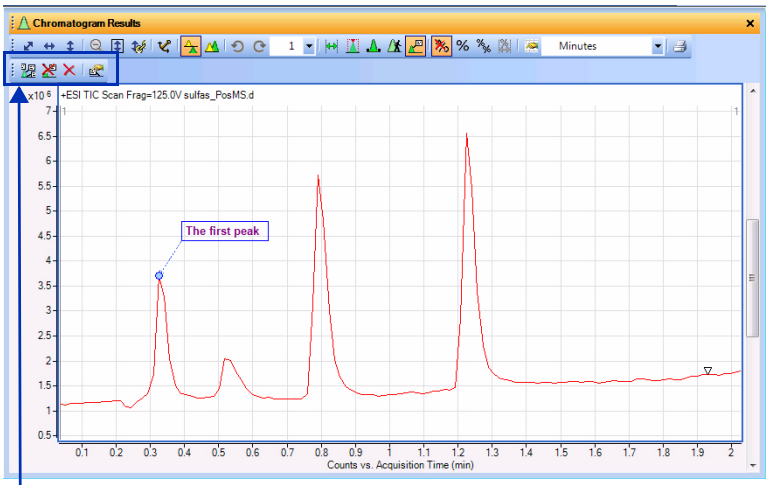
Task 6. Add an annotation

Steps	Detailed Instructions	Comments
1 Select the location in the chromatogram.	**a** In the Chromatogram Results window, click the **Annotation** tool ()in the toolbar. **b** Move the cursor to the location in the chromatogram pane where you want to add the annotation. **c** Right-click and then click **Add Text Annotation**.	• The cursor changes to a cross-hair. You use this cursor to select the exact location to add the annotation. •
2 Add the information about the text annotation in the Add/Edit Text Annotation dialog box.	**a** Type the **Text** for the annotation. **b** Select the **Text color**. **c** Select the **Orientation**. **d** Select the **Font style** and **Font size**. **e** Click either **Anchored** or **Floating**. If you click **Anchored**, select the options for the pointer to the text annotation. If you click **Floating**, you can change the relative position. It is easier to change the position interactively in the graphics window. **f** Click **OK**.	• You can add multiple annotations to a chromatogram or spectrum. • You can use the icons in the Annotate toolbar to select all of the annotations, delete annotations and edit annotations.

Task 6. Add an annotation (continued)

Steps	Detailed Instructions	Comments





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

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

3 Switch back to the Range Select tool in the Chromatogram Results window. Delete the annotation first.

a Click the icon to remove all annotations.

b Click the (Range Select) icon in the Chromatogram Results toolbar.

- You can switch between five different tools in the Chromatogram Results toolbar. Refer to the online Help for more information. The five tools are:
  - Range Select
  - Peak Select
  - Manual Integration
  - Walk Chromatogram
  - Annotation

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

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

Task 7. Extract chromatograms (MS only)

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

Task 7. Extract chromatograms (MS only)

Steps	Detailed Instructions	Comments
1 Extract and merge extracted ion chromatograms (EICs) from two masses in the **sulfas-PosMS.d** data file. • The m/z values are 279.09102 and 311.08085. • Merge the peaks from the individual masses into one chromatogram.	**a** In the Data Navigator window, clear the check boxes for the data files except for **sulfas-PosMS.d**. **b** Open the Extract Chromatograms dialog box, using the option below or one of the options to the right: • Click **Chromatograms > Extract Chromatograms**. **c** In the **List of opened data files**, click **sulfas-PosMS.d.** **d** In the **Type** list box, select **EIC**. **e** In the **m/z value(s)** 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 7. Extract chromatograms (MS only) (continued)

Steps	Detailed Instructions	Comments

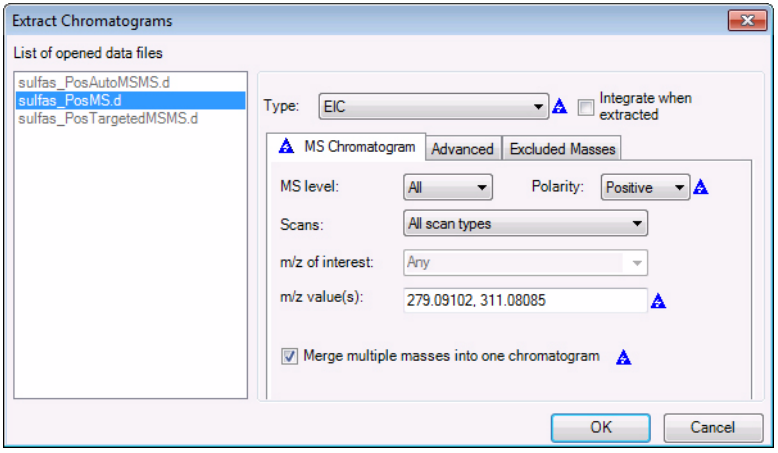


Figure 9 The Extract Chromatograms dialog box

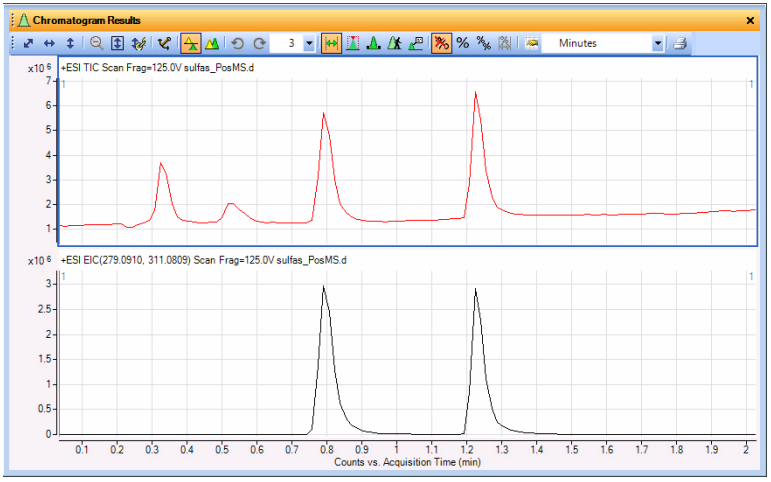


Figure 10 Merged extracted ion chromatograms (EICs) compared to the original TIC

Task 8. Interactively integrate a chromatogram (MS only)

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

Task 8. Interactively integrate a chromatogram (MS only)

Steps	Detailed Instructions	Comments
1 Integrate the **sulfas_PosMS.d** TIC chromatogram.	• Integrate the **sulfas_PosMS.d** chromatogram, using any of the following options. ◦ From the main menu, click **Chromatograms > Integrate Chromatogram**. ◦ Highlight the chromatogram. Then, right-click the chromatogram, and click **Integrate Chromatogram**. ◦ In Data Navigator, highlight **TIC Scan** in the **sulfas_PosMS.d > User Chromatograms** section**.** Then, right-click 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) > Integrator tab in the Method Editor window. • Note that the integration with default parameters is detecting very small peaks.

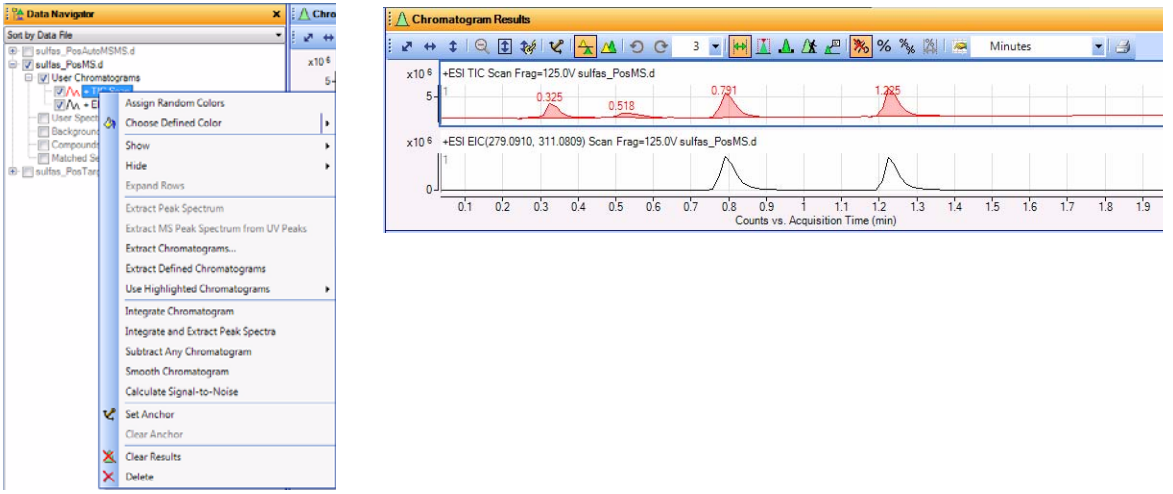


Figure 11 Shortcut menu in the Data Navigator and the integrated sulfas_PosMS.d TIC chromatogram

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

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

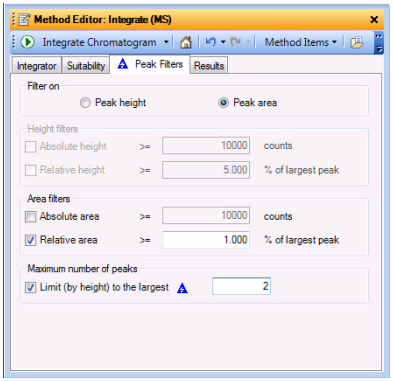


Figure 12 Peak Filters tab with **Limit (by height) to the largest** marked

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

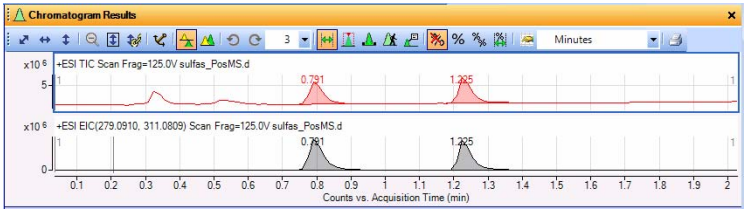
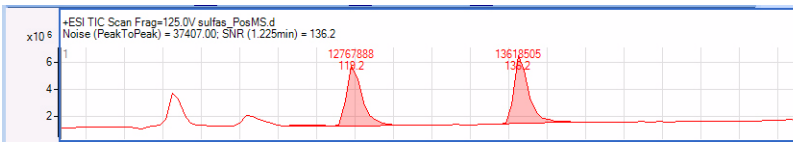


Figure 13 Integration results with limited number of peaks

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

Steps	Detailed Instructions	Comments
5 Calculate the signal-to-noise ratio. • Select the **sulfas_PosMS.d** TIC. • Set the first Peak Label to **Area** and the second Peak Label for the chromatographic peaks to **Signal-to-noise.** • Open the Method Editor. • Use `0.63 – 0.73` for the noise region, and calculate the signal-to-noise ratio for the integrated peaks.	**a** Click **Configuration > Chromatogram Display Options**. **b** Click the **Chromatogram** tab. **c** Set the first **Peak labels** to **Area** and the second **Peak labels** to **Signal-to-Noise**. **d** Click **OK**. **e** In the Method Explorer, click **Chromatogram > Calculate Signal-to-Noise**. **f** Click the **Specific noise regions** button. **g** Type `0.63 – 0.73` for the **Noise regions**, and click the **Calculate Signal to Noise** icon.	• You can also click the icon in the Chromatogram Results window to display the Chromatogram Display Options dialog box. • Make sure the TIC is highlighted before you calculate the signal-to-noise. • The area that you specified to be the noise region is drawn in bold in the Chromatogram Results window.
		
	Figure 14 Integrated TIC with Area and Signal-to-Noise labels	
6 Restore the settings for the default method, and close Method Editor.	**a** To cancel your changes and restore the values from the default method, click the **Restore to last saved values from file** button on the Method Editor toolbar. **b** Close the **Method Editor** window.	• The online Help describes each of the Signal-to-Noise algorithms.
7 Return the peak labels to Retention Time.	**a** Click **Configuration > Chromatogram Display Options**. **b** Click the **Chromatogram** tab. **c** Set the first Peak label to **Retention Time** and the second Peak label to **Compound Label**. **d** Click **OK**.	• You can also click the **Default** button to restore the original values in this dialog box.

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

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

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

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

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

Steps	Detailed Instructions	Comments

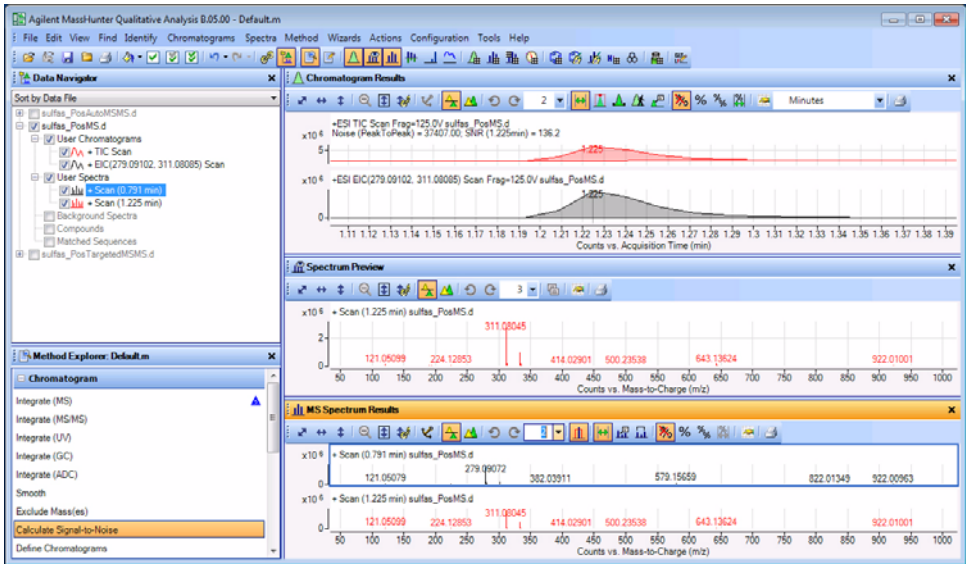


Figure 15 Main window with extracted spectra from both integrated peaks in the sulfas_PosMS.d file

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

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

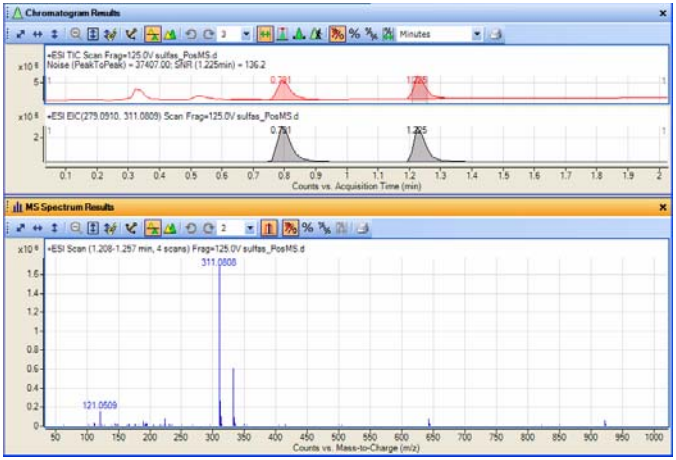


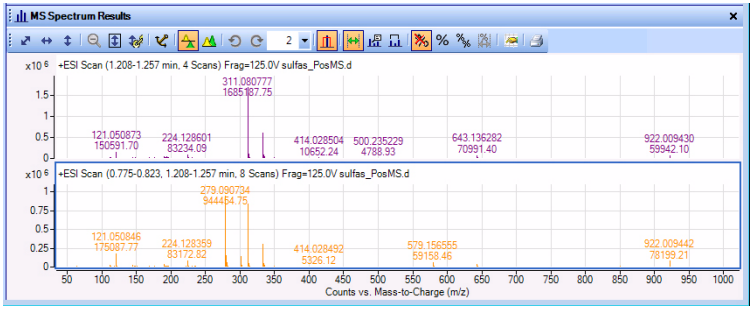
Figure 16 Average spectrum extracted from selected range for last peak

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

Steps	Detailed Instructions	Comments
3 Extract a spectrum that averages the ranges of integrated peaks 1 and 2 together for the **sulfas_PosMS.d** data file. • Hint: Use the **Range Select** icon and the **Ctrl** key to select the Peak 1 range taken from the halfway point. • Extract the spectrum, using any of the options on the right.	**a** Click the **Chromatogram Results** window title bar. The Chromatogram Results window becomes the active window, and the selected area is not lost. **b** Press and hold the **Ctrl** key. **c** Click at the halfway point on the left side of the first integrated peak, and drag over to the halfway point on the right. **d** Release the mouse. **e** Release the **Ctrl** key. **f** Extract the average spectrum using this option or the one on the right: • Double-click inside the selected range in either peak.	• Remember that the second peak already has a range selected from step 2. • You can also extract a spectrum by right-clicking anywhere in the chromatogram, and then click **Extract MS Spectrum**. The Extract Spectrum dialog box is shown. Click **Extract**.

Figure 17 An averaged spectrum created from multiple ranges.

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

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

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

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

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

Steps	Detailed Instructions	Comments

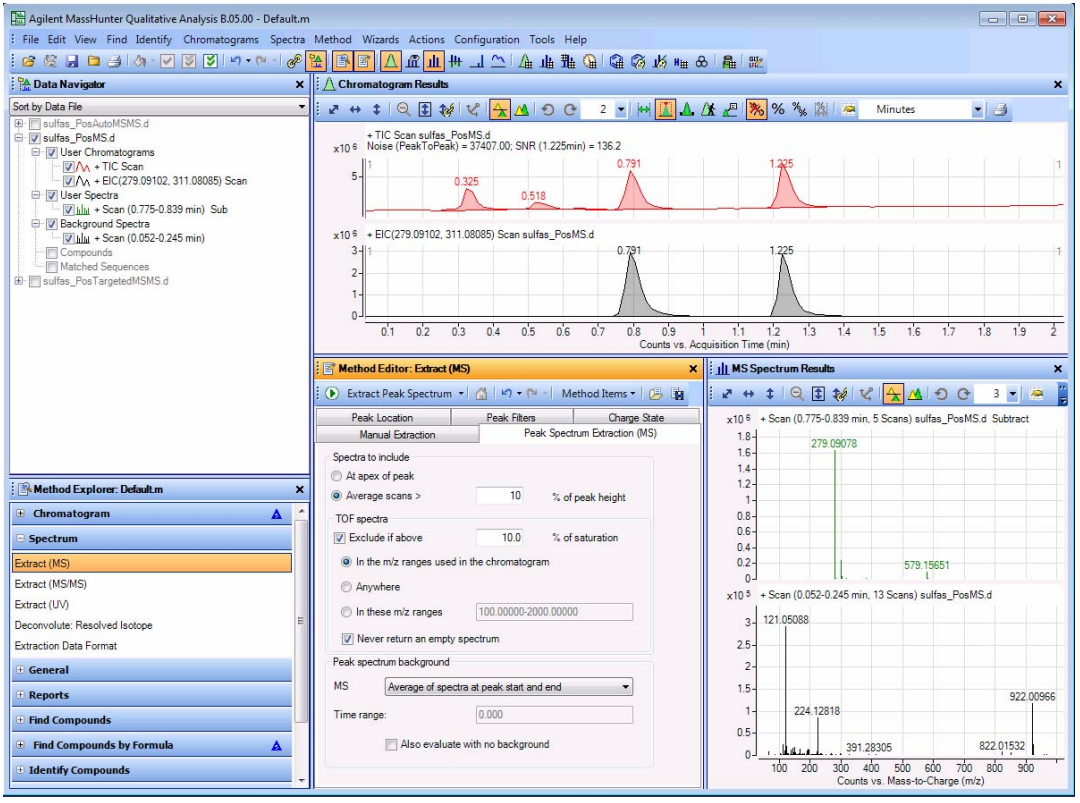


Figure 18 Spectrum with background subtracted

Task 10. Add a caliper

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

- MS Spectrum Results window
- Deconvolution Results window

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

Task 10. Add a caliper

Steps	Detailed Instructions	Comments
1 Add the caliper to the peak spectrum created in the previous task.	**a** In the MS Spectrum Results window, click the **Caliper** tool ()in the toolbar. **b** Select **Profile Point to Point** for the type of caliper in the Caliper toolbar. **c** Move the cursor to the location in the spectrum pane where you want to add the caliper. **d** Drag the cursor to the end point of caliper in the spectrum. As you drag the cursor, the value of the delta mass changes. When you release the mouse button, the caliper is added.	• See "Task 9. Extract spectra from a chromatogram (MS only)" on page 33 to extract an MS spectrum. • The cursor changes to an arrow. You use this cursor to select the start and end point of the caliper.
2 Modify the caliper to use a different color.	**a** Click the caliper created in the previous step. **b** Click the Caliper Properties button () in the MS Spectrum Results Caliper toolbar. **c** (optional) Type the **Start X** and **Start Y** values. **d** Select the **Text color**. **e** Select the **Font style** and **Font size**. **f** Click **OK**.	• You can add multiple calipers to a spectrum. • You can use the icons in the Caliper toolbar to select all of the calipers, delete calipers and edit calipers.

Task 10. Add a caliper (continued)

Steps	Detailed Instructions	Comments

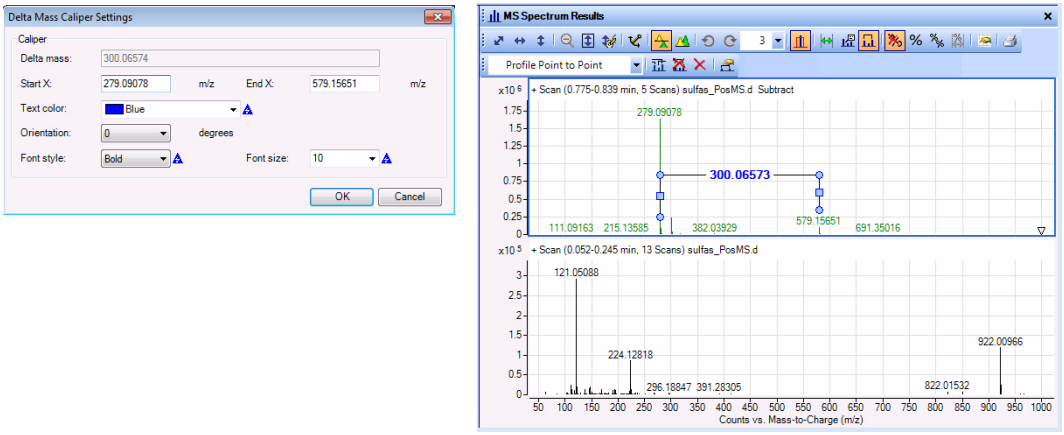


Figure 19 Delta Mass Caliper Settings dialog box and the MS Spectrum Results window

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

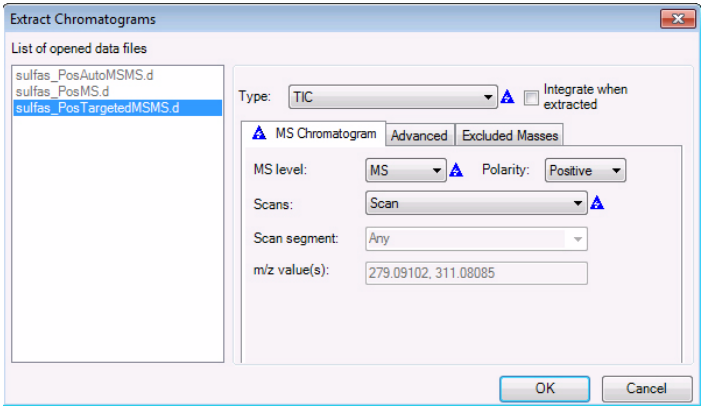
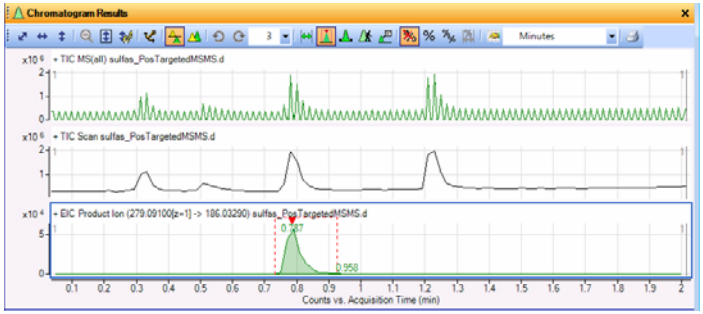
Task 11. Extract chromatograms (LC/MS and LC/MS/MS)

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

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

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

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

Steps	Detailed Instructions	Comments
		
	Figure 20 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, select 279.09100. **f** In the m/z value(s) text box, type `186.03299`. **g** Mark the **Integrate when extracted** check box. **h** Click **OK**.	• In the m/z value(s) text box, you can also type a range (for example, `100 - 300`)
		
	Figure 21 TIC for MS and EIC for MS/MS data compared to the original TIC	

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

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

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

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

Steps	Detailed Instructions	Comments
1 Integrate the TIC Scan chromatogram for the **sulfas_PosTargetedMSMS.d** data file, using any of the options listed at right.	**a** Highlight the TIC Scan chromatogram, and choose from any one of the following commands to integrate the chromatogram. • From the menu bar click **Chromatograms > Integrate Chromatogram**. • Right-click anywhere in the chromatogram window, and click **Integrate Chromatogram**. • In the Data Navigator window, select **sulfas_PosTargetedMSMS.d > User Chromatograms > TIC Scan,** then right-click the TIC Scan and click **Integrate Chromatogram**.	• Note that the program integrated 4 peaks in the chromatogram. • You select the integrator to use for MS data, MS/MS data, UV data and ADC data in the Method Editor window.
2 Change the threshold to integrate fewer peaks. • Change the threshold to retain only the two largest peaks.	**a** From the Method Explorer window, click **Chromatogram > Integrate (MS)** to display the Integrator tab. **b** 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 12. Interactively integrate a chromatogram (LC/MS and LC/MS/MS) (continued)

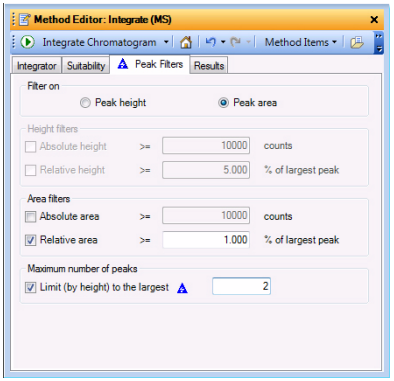
Steps	Detailed Instructions	Comments
		

Figure 22 Peak Filters tab with the **Limit (by height) to the largest** check box marked

3 Reintegrate the chromatogram.	**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.

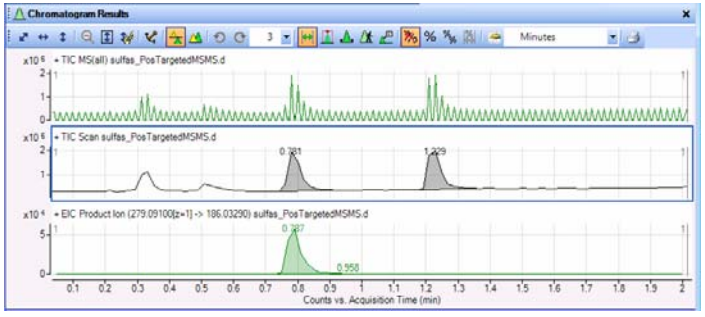


Figure 23 Integrated TIC MS and MS/MS chromatograms with limited peaks integrated

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

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

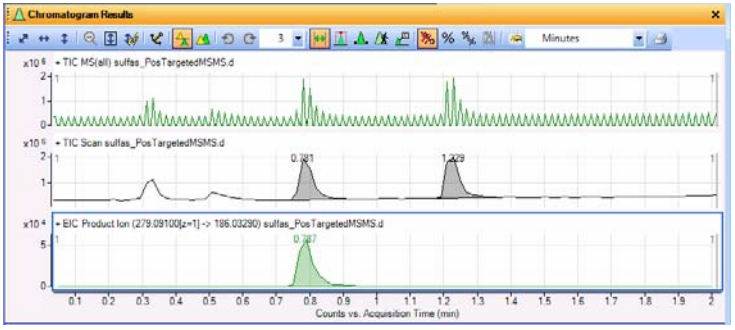


Figure 24 Integrated TIC MS and MS/MS chromatograms with higher threshold setting

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

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

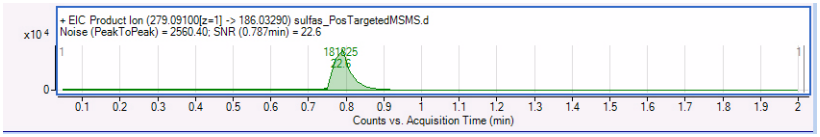


Figure 25 Signal-to-Noise results for MS/MS EIC Product Ion

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

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

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

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

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

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

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

Steps	Detailed Instructions	Comments
1 Walk a chromatogram to view the precursor ion and product ion for the last peak of **sulfas_PosTargetedMSMS.d**. • Zoom in on the region between 1.15 and 1.35 minutes. • Use the Walk Chromatogram icon. • Review the spectra starting at about 1.15 minutes, and move the arrow to the right.	**a** Click the **TIC MS(all)** chromatogram 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, press the right and left arrow keys on your keyboard.	• The Walk Chromatogram tool is particularly useful on MS/MS data for identifying precursor and product ions. • The spectrum for each point you click in the Chromatogram Results window is automatically displayed in the Spectrum Preview window, which is opened automatically.

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

Steps	Detailed Instructions	Comments

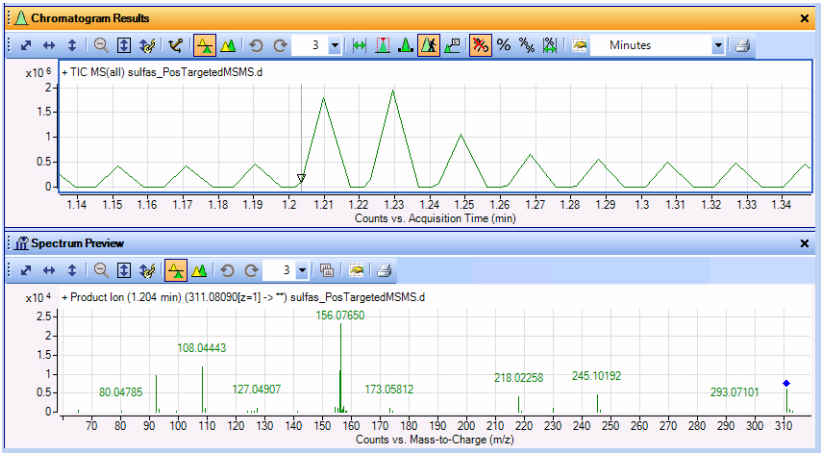
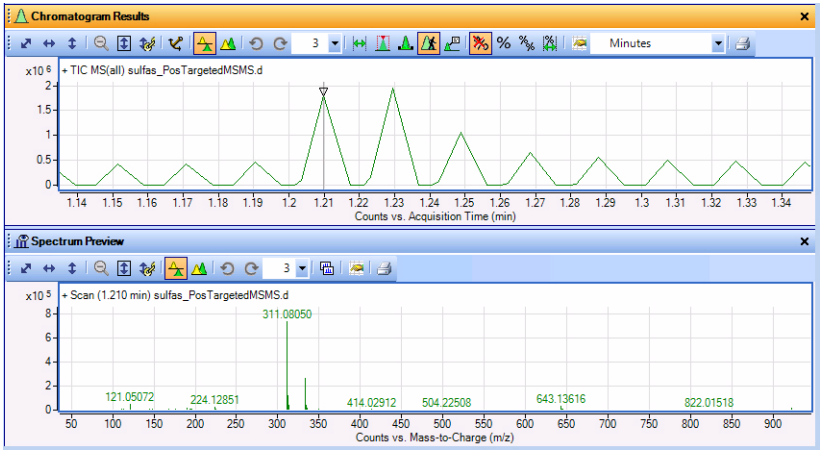


Figure 26 Walk chromatogram to view the MS/MS product ion at 1.204 minutes



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

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

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

Steps	Detailed Instructions	Comments
2 Extract spectra on specific data points for the peak at 0.33 minutes and the last peak of the **sulfas_PosTargetedMSMS.d** data file. • After zooming in on the region between 0.3 and 0.4 min., extract a spectrum from one of the peaks (MS) at or near 0.33 min. and then one of the valleys (MS/MS), using any one of the options described under Comments. • After zooming in on the region between 1.15 and 1.25 min., extract a spectrum from one of the peaks at or near 1.23 min. (not the valley yet) • Change the display to show at least three spectra.	**a** Click the **Range Select** icon from the Chromatogram Results toolbar. **b** Close the Spectrum Preview window. **c** Click the **Zoom Out** icon, , in the Chromatogram Results toolbar. **d** To zoom in to the first peak, right-click the mouse above the peak at 0.3 min. and drag it to 0.4 min., then release. **e** On a peak near 0.33 min. extract a spectrum in any of the ways listed in the Comments column. **f** On a valley near 0.34 minutes, extract a spectrum. **g** Click the **Zoom Out** icon, , in the Chromatogram Results toolbar. **h** Zoom into the region between 1.15 and 1.25 min. **i** On a peak near 1.23 minutes, extract a spectrum in any of the ways listed in the Comments column. (Do not extract the valley spectrum yet.) **j** If necessary, click the arrow next to the **Maximum number of list panes** icon in the MS Spectrum Results toolbar, and select **3**.	• 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 13. Extract spectra from a chromatogram (LC/MS and LC/MS/MS) (continued)

Steps	Detailed Instructions	Comments

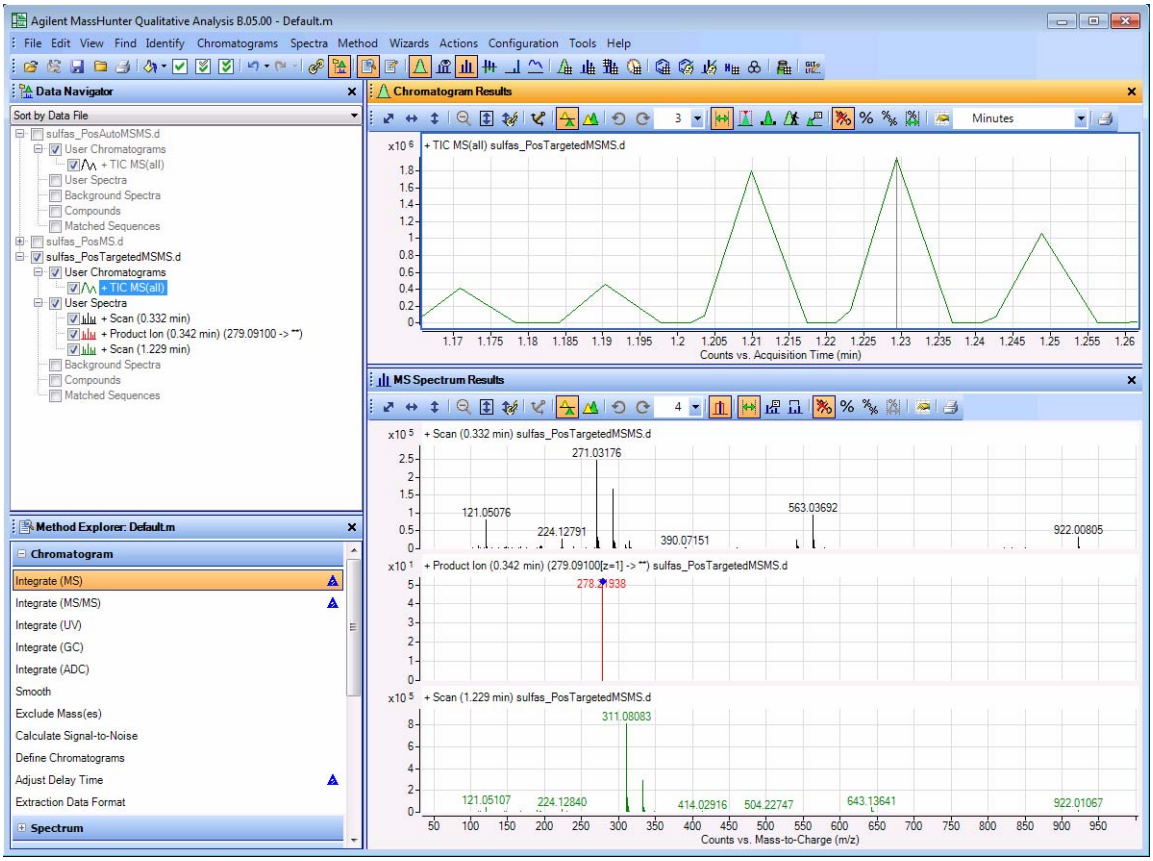


Figure 28 The Qualitative Analysis program with MS Scan and Product Ion spectra from the first peak and MS Scan spectrum from the last peak

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

Steps	Detailed Instructions	Comments
3 Extract a product ion spectrum for the last peak of the **sulfas_PosTargetedMSMS.d** data file. • View the Spectrum Preview window. • Extract a spectrum from the valley at RT 1.237 min. • Copy this spectrum to the User Spectra folder. • Change the display to show 4 spectra. • Turn off Spectrum Preview.	**a** Click the **Spectrum Preview** icon, in the main toolbar. **b** On a valley near 1.23 minutes extract a spectrum. **c** Right-click the spectrum in the Spectrum Preview window, and click **Copy to User Spectra**. **d** Select 4 for the **Maximum number of list panes** in the MS Spectrum Results window. **e** Close the Spectrum Preview window.	• When Spectrum Preview is enabled, the system displays any manually-selected spectrum in the Spectrum Preview window but not in the User Spectra section of Data Navigator. • With the Spectrum Preview window open, Qualitative Analysis overwrites the previous spectrum when you extract a new spectrum. • Spectrum Preview mode is useful when you quickly want to review the spectra in your chromatogram and save only a few of the spectra.

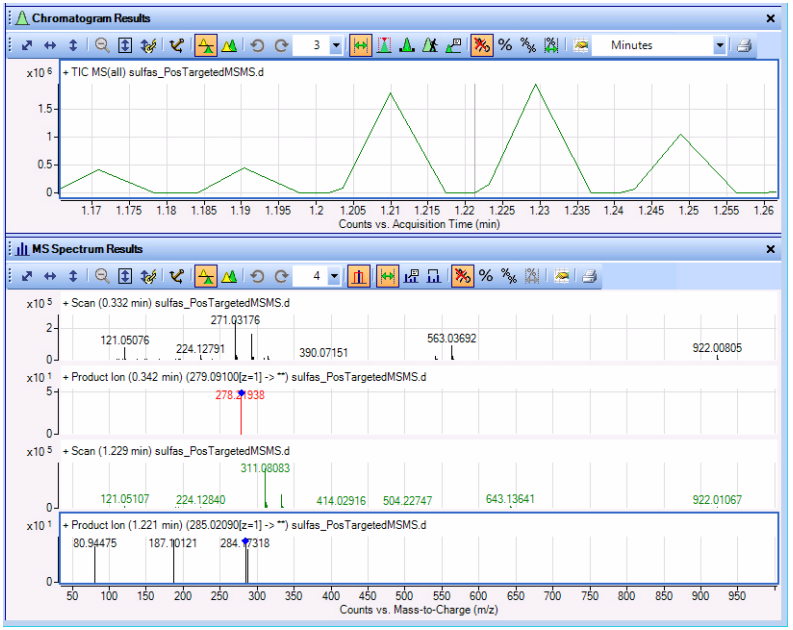


Figure 29 Chromatogram Results and MS Spectrum Results windows with product ion spectrum from the last peak in the chromatogram

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

Steps	Detailed Instructions	Comments
4 Extract a spectrum that averages all points within a specified range for the last peak for the **sulfas_PosTargeted.d** data file: • Zoom out. • Use the Range Select icon on the Chromatogram toolbar. • Set the range across the entire peak. • Extract the spectrum, using any of the options listed.	**a** Click the **Autoscale X-axis and Y-axis** icon in the Chromatogram Results toolbar to zoom out completely. **b** Click the **Range Select** icon on the Chromatogram toolbar. **c** Click at about 1.21 minutes of the last peak and drag over to about 1.229 minutes on the right. **d** Extract the average spectrum using one of the options on the right. **e** Click the down arrow next to the Maximum number of list panes icon, and select **2**.	• You can extract an average spectrum by double-clicking the selected range in the chromatogram. • Or, right-click anywhere in the chromatogram, and click **Extract MS Spectrum** from the shortcut menu. Then, click **Extract**. • Note that both the averaged MS spectrum and averaged MS/MS spectrum appear.

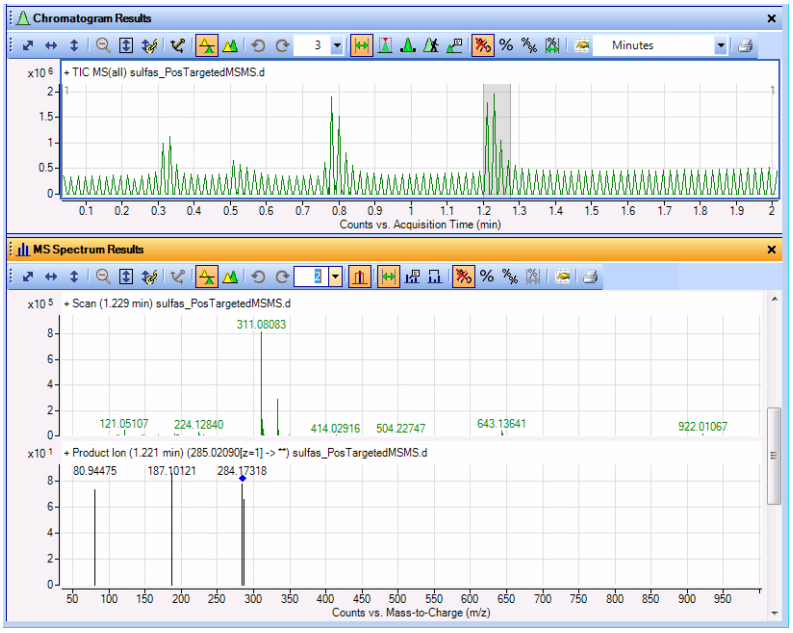


Figure 30 Averaged spectra extracted from selected range for last peak

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

Steps	Detailed Instructions	Comments
5 Extract spectra that average the ranges of peaks 1 and 4 together for the **sulfas_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 31 Averaged MS and MS/MS spectra created from multiple ranges.

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

Steps	Detailed Instructions	Comments
6 Subtract a background spectrum every time you extract a peak spectrum for an MS/MS EIC extracted from **sulfas_PosTargetedMSMS.d**. • Delete any scans under User Spectra in Data Navigator. • Extract a background spectrum that is the average of a spectrum at the start of the peak and a spectrum at the end of the peak. • Extract a peak spectrum from the integrated peaks.	**a** Under **User Spectra** in Data Navigator, right-click the spectra, and click **Delete**. **b** Click **Yes** in the Delete message box. **c** Extract an integrated MS/MS EIC of ions 279.09100 with an m/z range of 100-300 (see "Task 11. Extract chromatograms (LC/MS and LC/MS/MS)" on page 42) **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 13. Extract spectra from a chromatogram (LC/MS and LC/MS/MS) (continued)

Steps	Detailed Instructions	Comments

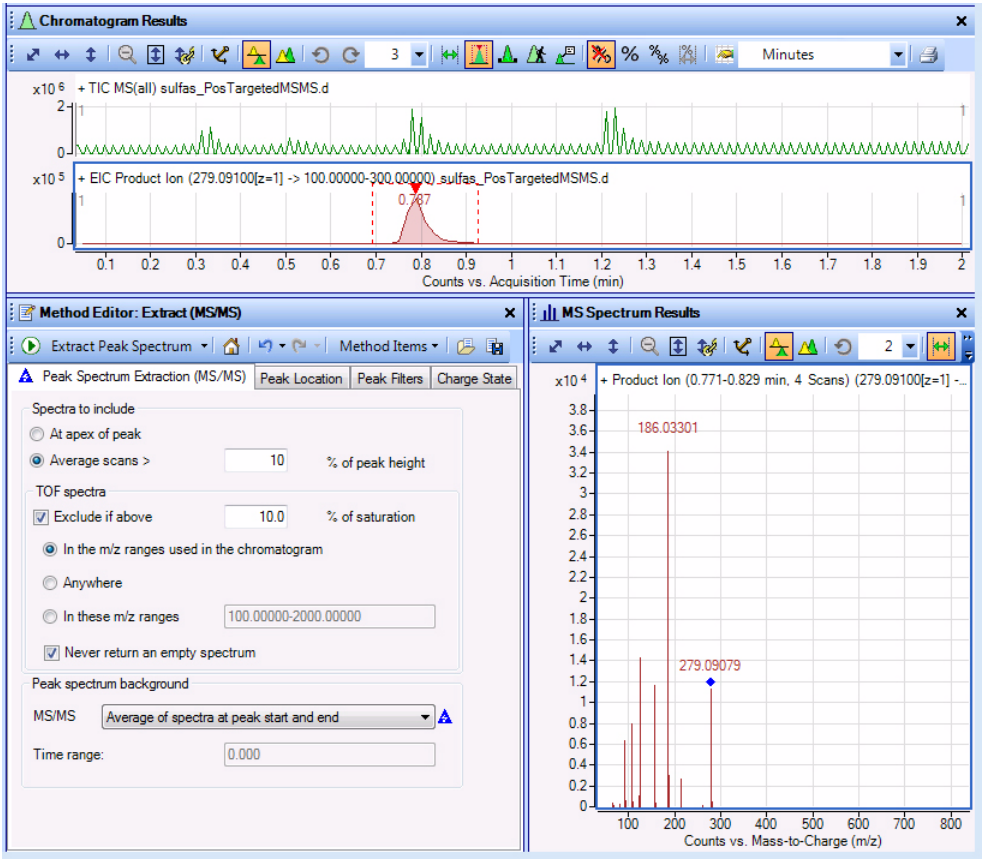


Figure 32 Product ion (MS/MS) spectra with background subtracted

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

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

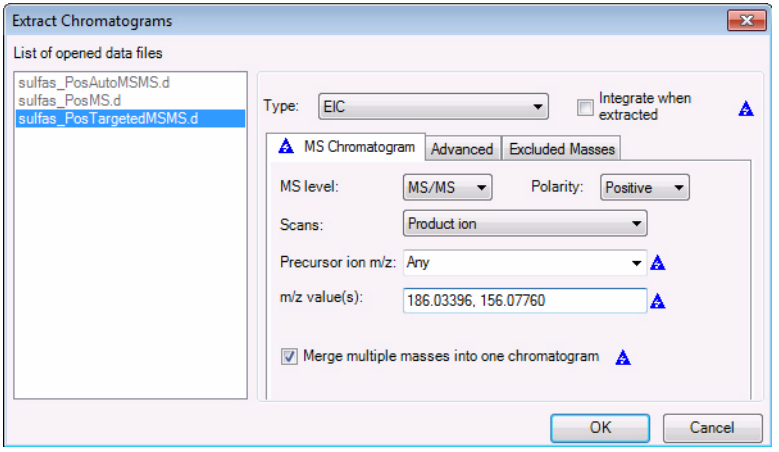


Figure 33 Extract Chromatograms dialog box for EIC based on product ions

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

Steps	Detailed Instructions	Comments

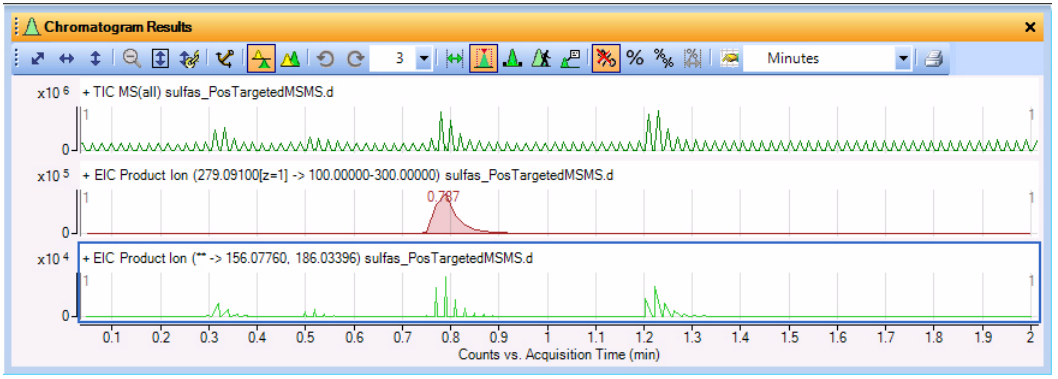
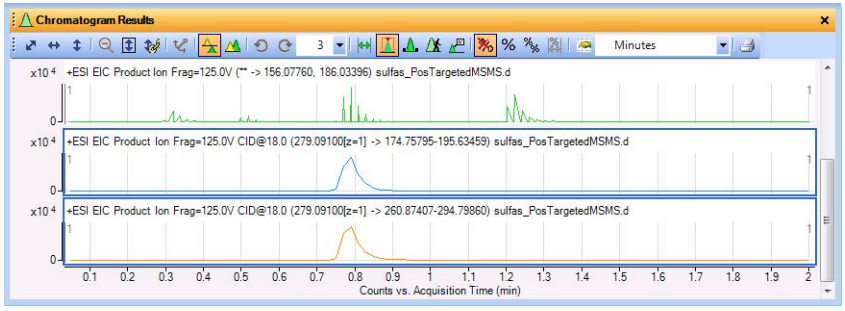


Figure 34 Product Ion EIC

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

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

- A separate chromatogram is extracted for each range in the spectrum.
- The product ion range is set to the range selected in the MS Spectrum Results window.



Expanded titles are enabled in the Chromatogram Display Options dialog box. The expanded title includes ionization, fragmentor voltage and collision energy.

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

Tasks for MS and UV Data

Task 14. Extract chromatograms (MS and UV)

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

Task 14. Extract chromatograms (MS and UV)

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

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

Steps	Detailed Instructions	Comments

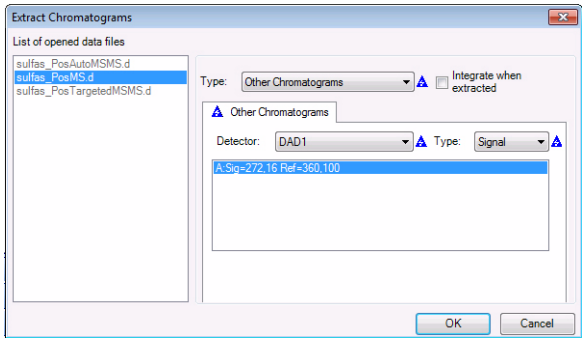


Figure 36 The Extract Chromatograms dialog box with **Type** Other Chromatograms.

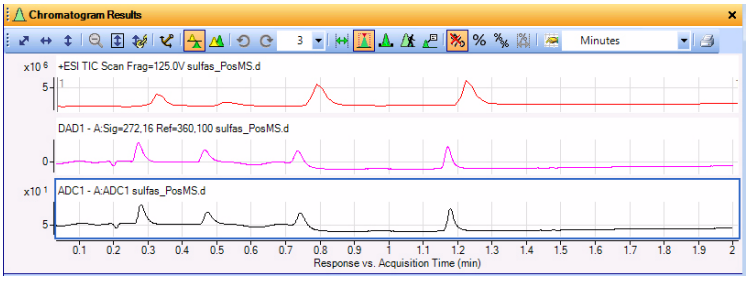


Figure 37 Chromatogram Results window with the DAD1, the ADC1, and the original TIC

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

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

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

Steps	Detailed Instructions	Comments
1 Integrate the **sulfas_PosMS.d** UV chromatograms, using any of the options listed at right. • Highlight the DAD1 and ADC1 chromatogram. • Integrate the chromatograms.	**a** Highlight the DAD1 and ADC1 chromatograms. **b** Integrate the **sulfas_PosMS.d** UV chromatograms, using any of the following options. • From the main menu, click **Chromatograms > Integrate Chromatogram**. • Highlight the chromatogram. Then, right-click the chromatogram, and click **Integrate Chromatogram**. • In Data Navigator, highlight **DAD1** and **ADC1** in the **sulfas_PosMS.d > User Chromatograms** section. Then, right-click either chromatogram and click **Integrate Chromatogram**. **c** If needed, highlight the MS chromatogram and integrate.	• 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. • If the Chromatogram > Integrate (UV) section is not available, then you need to mark the UV check box in the User Interface Configuration dialog box. • Note that the integration with default parameters is detecting very small peaks.

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

Steps	Detailed Instructions	Comments

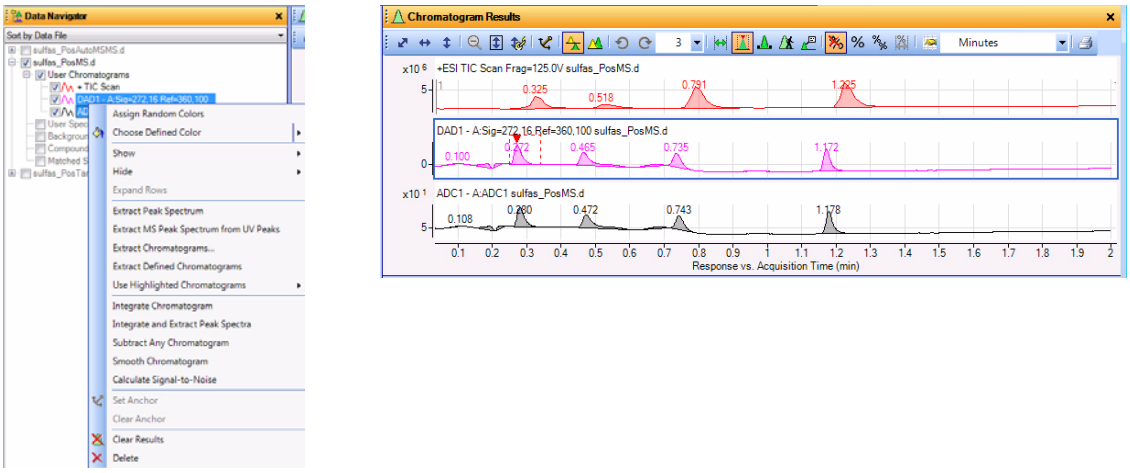


Figure 38 One of the shortcut menus in the Data Navigator and integrated sulfas_PosMS.d chromatograms

2 Enable system suitability calculations for UV chromatograms.

a From Method Explorer, select **Chromatogram > Integrate (UV)** to display the Integrator tab.
b Click the **Suitability** tab.
c Mark **Enable system suitability calculations**.
d Select the **United States Pharmacopoeia (USP)**.
e In the Column void time box, type `0.15`.
f In the Column length box, type `5`.

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

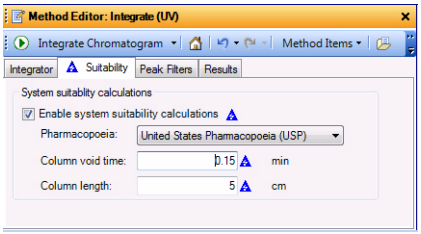
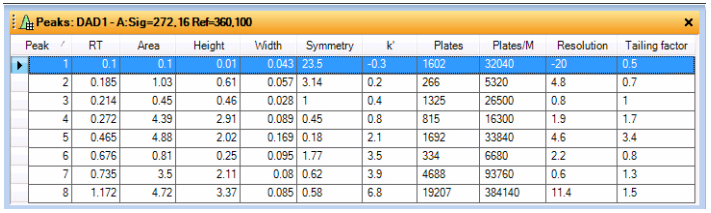


Figure 39 Chromatogram > Integrate (UV) Suitability tab

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

Steps	Detailed Instructions	Comments
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. • You can also enable system suitability calculations for an MS, an MS/MS and a GC chromatogram.
		
5 Restore the settings for the default method, and close the Method Editor window and the Integration Peak List window.	**a** To cancel your changes and restore the values from the default method, click the **Restore to last saved values from file** icon on the Method Editor toolbar. **b** Close the **Method Editor** window. **c** Right-click the title of the Integration Peak List window and click **Floating**. **d** Click **View > Integration Peak List**.	• When you click the Floating command in the shortcut menu the second time, the Integration Peak List window is docked where it was originally.

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

Peak	RT	Area	Height	Width	Symmetry	k'	Plates	Plates/M	Resolution	Tailing factor
1	0.1	0.1	0.01	0.043	23.5	-0.3	1602	32040	-20	0.5
2	0.185	1.03	0.61	0.057	3.14	0.2	266	5320	4.8	0.7
3	0.214	0.45	0.46	0.028	1	0.4	1325	26500	0.8	1
4	0.272	4.39	2.91	0.089	0.45	0.8	815	16300	1.9	1.7
5	0.465	4.88	2.02	0.169	0.18	2.1	1692	33840	4.6	3.4
6	0.676	0.81	0.25	0.095	1.77	3.5	334	6680	2.2	0.8
7	0.735	3.5	2.11	0.08	0.62	3.9	4688	93760	0.6	1.3
8	1.172	4.72	3.37	0.085	0.58	6.8	19207	384140	11.4	1.5

Figure 40 Integrated Peaks table with system suitability values

Task 16. Extract spectra from a chromatogram (UV)

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

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

Steps	Detailed Instructions	Comments

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

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

Steps	Detailed Instructions	Comments

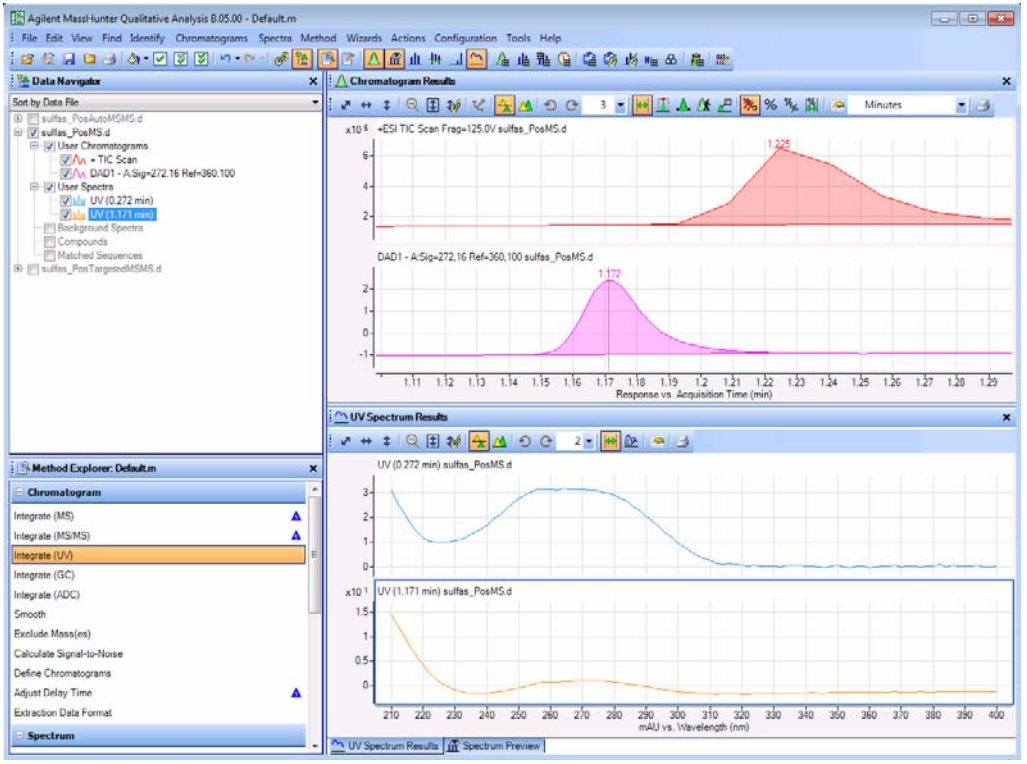


Figure 41 Main window with extracted UV spectra from two integrated peaks in the sulfas_PosMS.d file

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

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

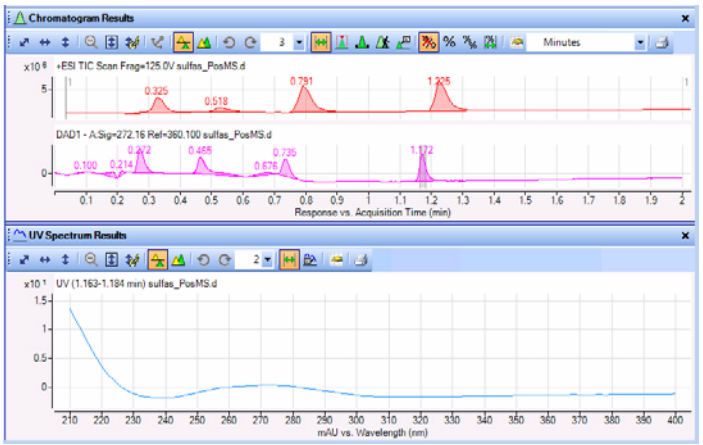


Figure 42 Average spectrum extracted from selected range for last peak

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

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

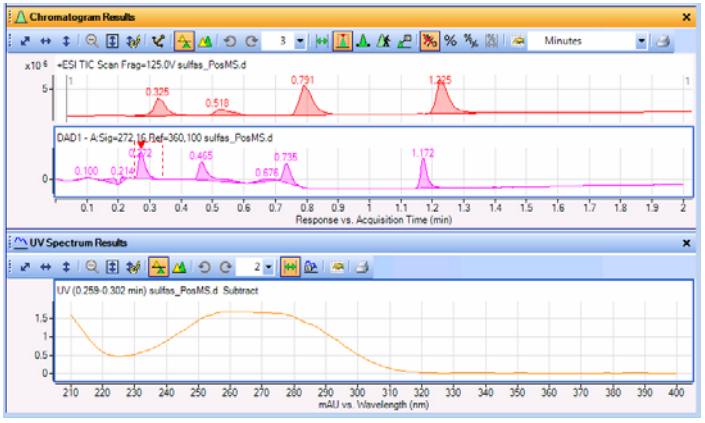


Figure 43 Integrated DAD1 chromatogram and UV Peak Spectrum

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

Tasks for GC-MS data

Task 17. 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 17. 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 **Configuration > Configure for Workflow > General** command. **b** Click the **Load workflow's default method** button and the **Load workflow's default layout** button. **c** Click **OK**. **d** Click 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 17. Configure User Interface for GC

Steps	Detailed Instructions	Comments
3 Configure the user interface to show GC features only.	**a** Click **Configuration > 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 (Q, QQQ)** check box. **e** Under Optional software features, clear the **Peptide Sequence Editor** check box and the **BioConfirm Software** check box. **f** Under Non-MS detectors, clear the **UV** and **ADC** check boxes. **g** Mark 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.

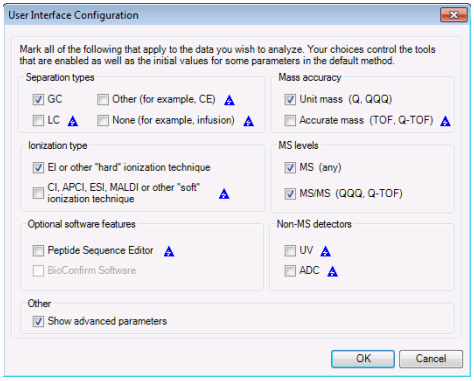


Figure 44 Configuring the user interface for a GC Triple Quadrupole

Task 18. 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 18. 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 17. Configure User Interface for GC" on page 69.	
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** Select **BPC** for the **Type**. **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 18. Extract chromatograms from a GC/MS data file (continued)

Steps	Detailed Instructions	Comments

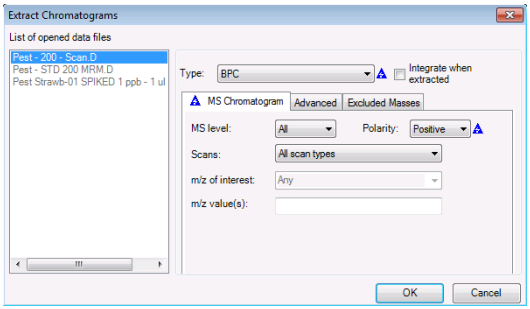


Figure 45 The Extract Chromatograms dialog box

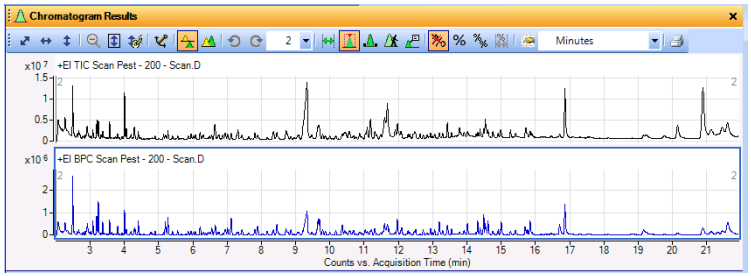


Figure 46 TIC and BPC for GC/MS data file

Task 18. 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: • 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**.	• To select multiple files, you press the **Ctrl** key or the **Shift** key while selecting additional files. • When you first select EIC, a red error warning icon is shown next to the m/z value(s) text box. This error icon is removed when you enter an *m/z* value. • 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.

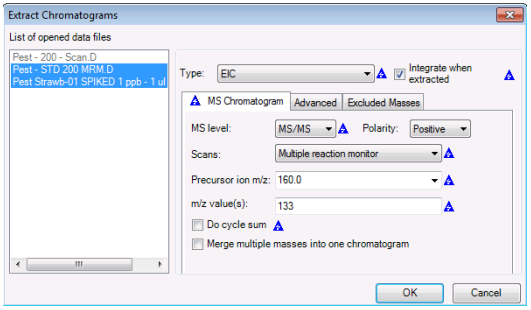
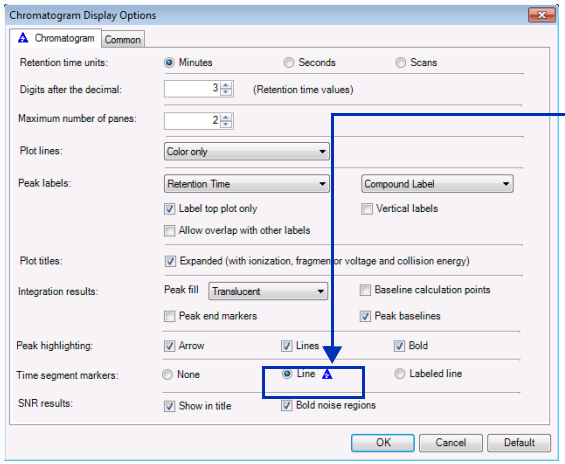


Figure 47 The Extract Chromatograms dialog box

Task 18. Extract chromatograms from a GC/MS data file (continued)

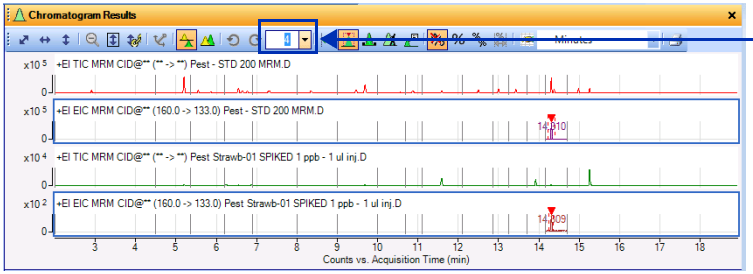
Steps	Detailed Instructions	Comments
5 Change the plot display options to not label the Time segment markers.	**a** Click **Configuration > Chromatogram Display Options**. **b** Click **Line** for the **Time segment markers**. **c** Clear the **Expanded** check box. **d** Click the **OK** button.	• You can customize how chromatograms 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 48 Chromatogram 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.

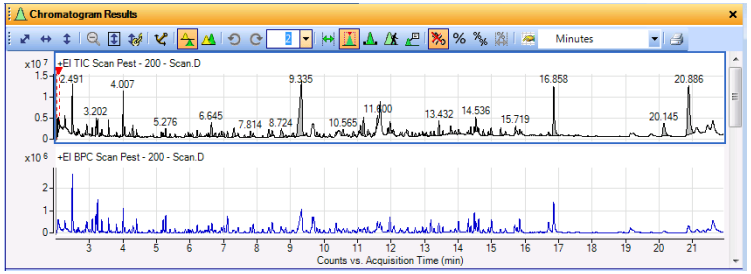
Figure 49 TIC MRM Chromatograms and EIC MRM Chromatograms for MS/MS data files

Task 19. Interactively integrate a GC/MS chromatogram

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

Task 19. 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 50 Integrated TIC Scan Chromatogram with many small peaks

Task 19. 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 the Method Explorer window, 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.

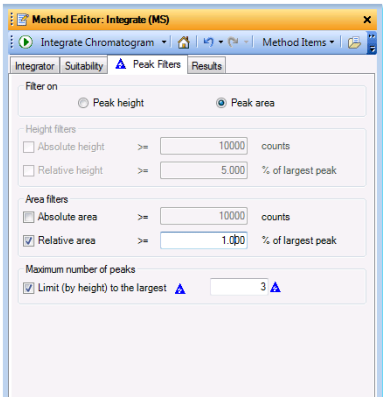


Figure 51 Peak Filters tab with **Limit (by height) to the largest** marked

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.

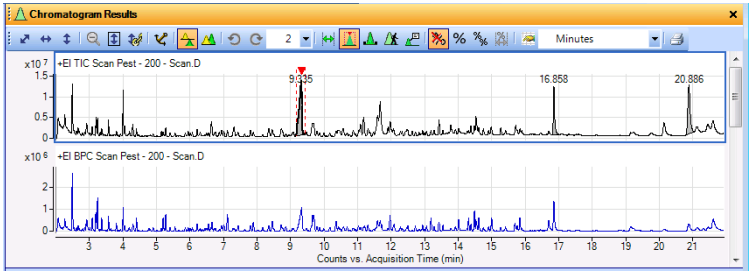


Figure 52 Integrated TIC Scan chromatogram when limiting the number of peaks

Task 19. 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 53 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 the Method Explorer window, select **Chromatogram > Integrate (MS/MS)**. **b** Select **MS/MS (GC)** as the **Integrator**. **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 `200000` 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 19. Interactively integrate a chromatogram (GC/MS) (continued)

Steps	Detailed Instructions	Comments

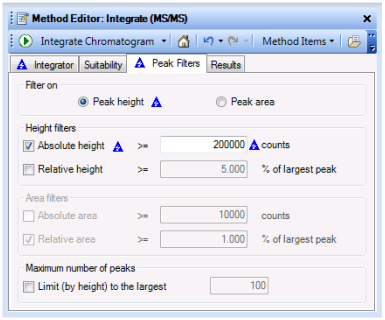


Figure 54 Peak Filters tab with **Absolute height** marked

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

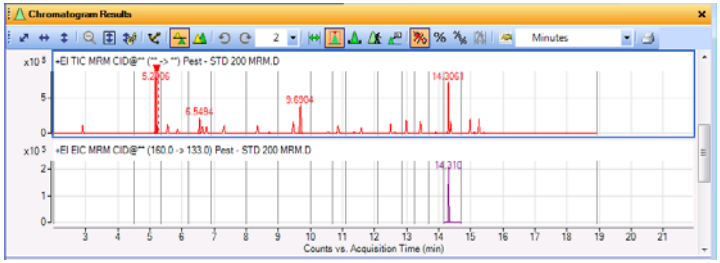


Figure 55 Integrated TIC and EIC MS/MS chromatograms with higher threshold setting

8 Restore the settings that are saved for the current method and close Method Editor.	**a** Select the Chromatogram > Integrate (MS/MS) section in the Method Explorer. **b** Click the icon in the Method Editor. **c** Select the Chromatogram > Integrate (MS) section in the Method Explorer. **d** Click the . icon in the Method Editor. **e** Close the **Method Editor** window.	• To cancel your changes and restore the values from the method that is loaded, click the **Restore to last saved values from file** icon on the Method Editor toolbar.

Task 19. 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** Select all of the TIC chromatograms. **d** Click **Chromatograms >Clear Results**.	• When you use the Clear Results command, the chromatograms are not deleted; the results that are connected to the chromatograms are removed. In this case, the integration values are cleared.

Task 20. Basic tasks for a GC/MS data file

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

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 20. 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** Mark the **Pest - 200 - MRM.D** line in the Data Navigator window. **b** Click on the **TIC MRM** chromatogram in the Data Navigator window. **c** Close the Method Editor window. **d** Close the MS Spectrum Results window. **e** Click the **Autoscale Y-axis during Zoom** icon  in the Chromatogram Results toolbar. **f** 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. **g** Click the **Walk Chromatogram** icon  in the Chromatogram Results toolbar. **h** Move the Walk Chromatogram cursor to above the X axis at about 13 minutes, and click. **i** 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 20. Basic tasks for a GC/MS data file

Steps	Detailed Instructions	Comments

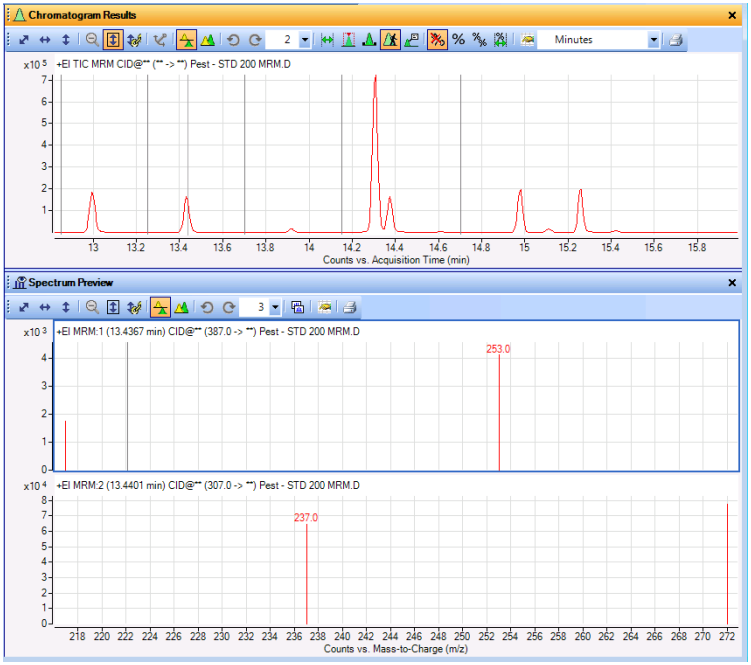


Figure 56 Walk chromatogram to view the two MRM spectra for the peak at 13. 43 minutes

Task 20. 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 20. Basic tasks for a GC/MS data file

Steps	Detailed Instructions	Comments

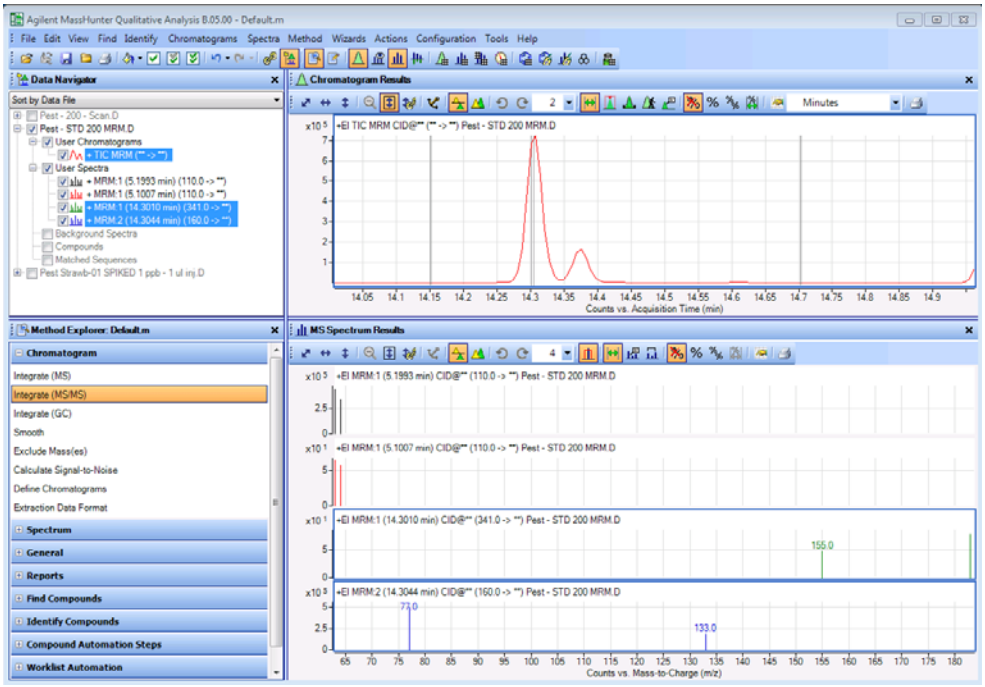


Figure 57 Main window with two MRM spectra from the peak at 5.2 minutes and two MRM spectra from the peak at 14.3 minutes

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**. The spectra are copied to the User Spectra section in the Data Navigator and are shown in the MS Spectrum Results window.

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

e Close the Spectrum Preview window.

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

Task 20. Basic tasks for a GC/MS data file

Steps	Detailed Instructions	Comments

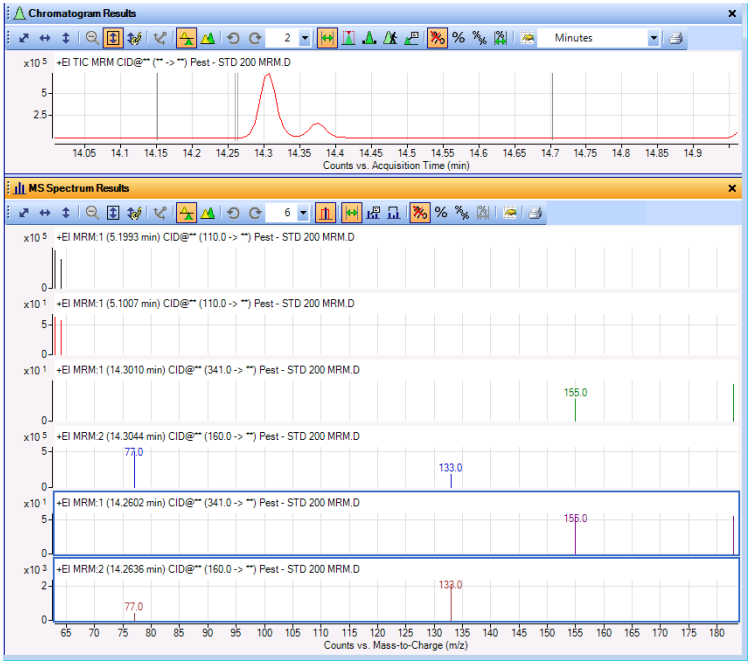


Figure 58 Chromatogram Results and MS Spectrum Results windows

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 20. Basic tasks for a GC/MS data file

Steps	Detailed Instructions	Comments

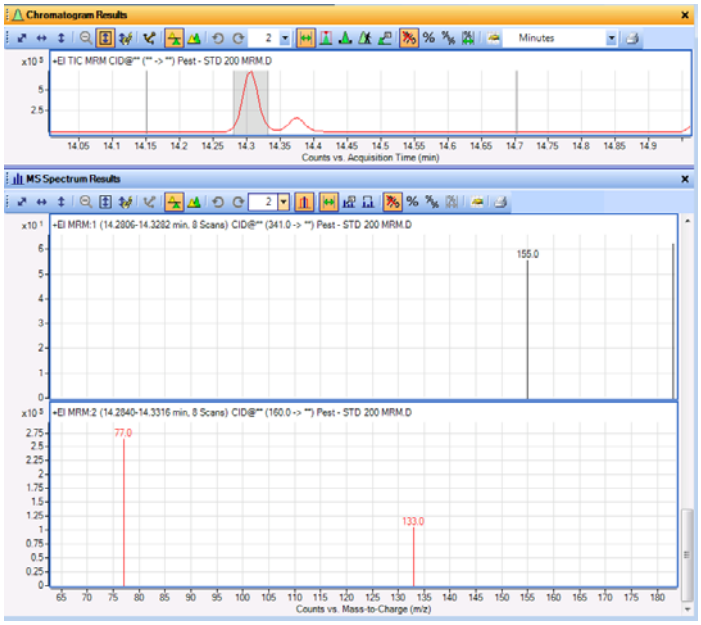


Figure 59 Chromatogram Results and MS Spectrum Results showing two averaged spectra

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 20. Basic tasks for a GC/MS data file

Steps	Detailed Instructions	Comments

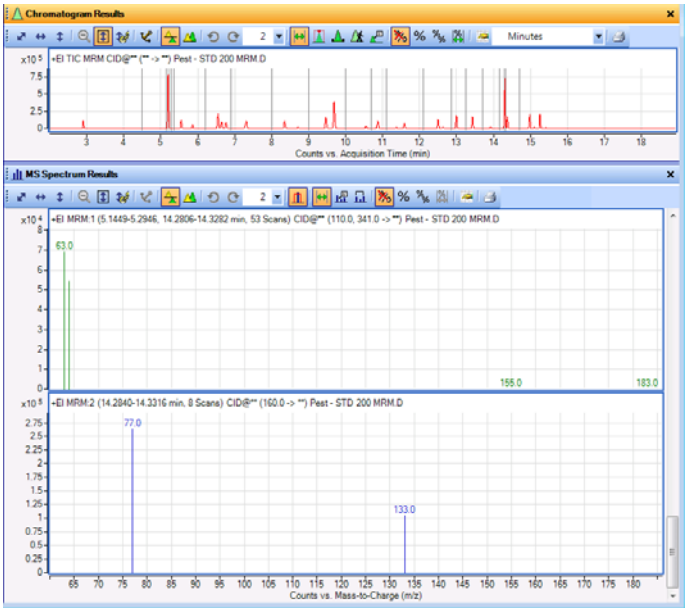


Figure 60 Two averaged spectra from two different ranges in the chromatogram

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 Click the **Chromatograms > Integrate** command.

h Select the peak at 5.206 minutes.

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

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

Task 20. Basic tasks for a GC/MS data file

Steps	Detailed Instructions	Comments

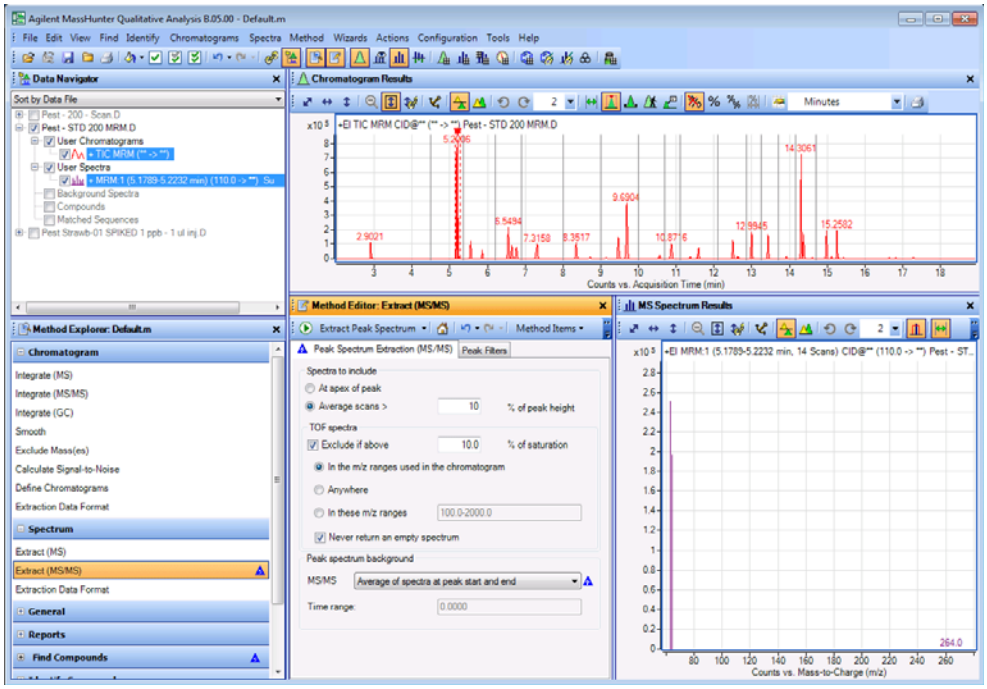


Figure 61 Peak spectra with a background peak spectrum subtracted

Task 20. Basic tasks for a GC/MS data file

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

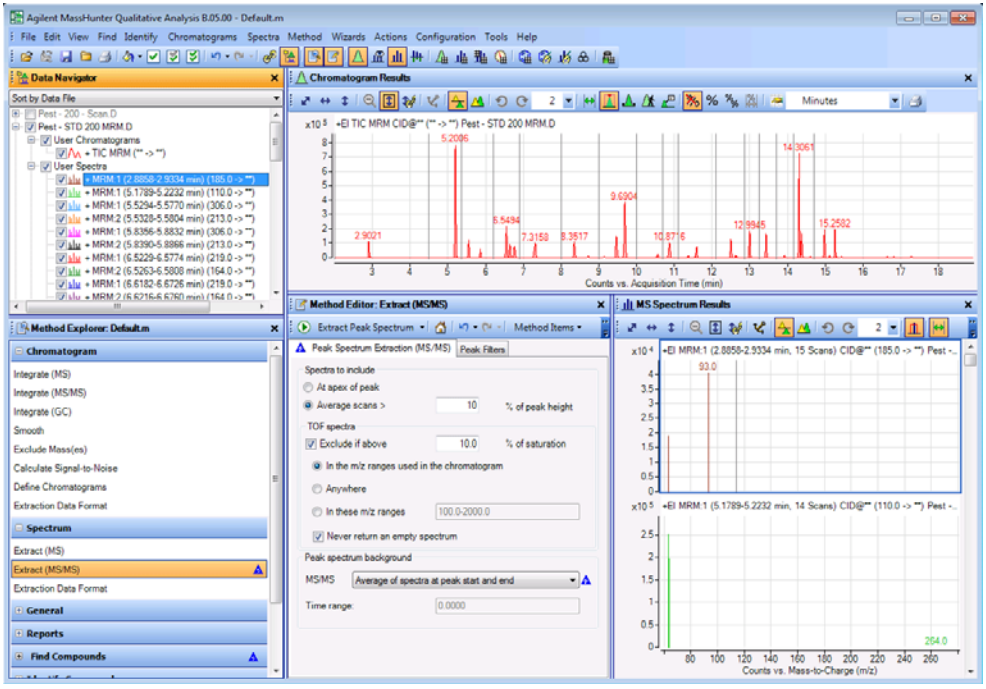


Figure 62 Integrate and Extract Peak Spectra

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 **Configuration > User Interface Configuration**. **e** Mark all check boxes. **f** Click **OK**. **g** Click **No** when asked whether to save the method changes.	• 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)

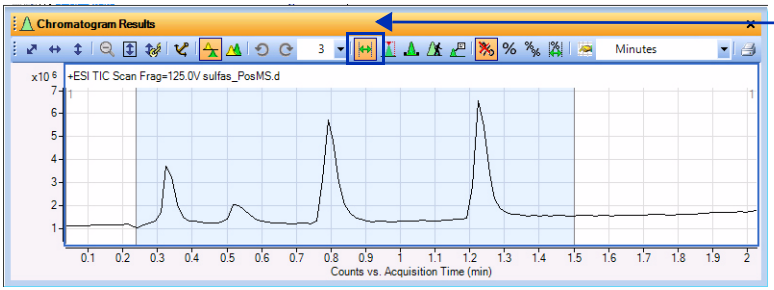
The FindCompounds algorithms find compounds in data and create averaged MS spectra for each compound. This functionality is an easy way to "mine" information from complex data. This algorithm only works with data that contains MS scan data. It does not work on data with unit mass resolution (for example, 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** Select the **sulfa_PosMS.d** data file in the example data file directory. Clear the **Load result data** check box and click **Open**. **c** Click **Configuration > Configure for Workflow > General**. The Workflow Configuration dialog box is opened. **d** Clear the **Save current method** check box if you don't want to save the changes to the method. **e** Click the **Load workflow's default method** button. **f** Click the **Load workflow's default layout** button. **g** Click **OK**. **h** Click the **Range Select** tool, and select the region from 0.24 to 1.5 minutes.	• The method Default.m is loaded automatically. To load this method interactively, click **Method > Open**. Select Default.m and click **Open**. • You can get help for any window, dialog box, or tab by using the `F1` key when that window is active. • When you switch between workflows, the Workflow Configuration dialog box is opened. • If you mark the **Save current method** check box, the method is automatically saved to the current method name. If the method is default.m, then the Save Method dialog box is opened. (you cannot overwrite this method).

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

Step	Detailed Instructions	Comments



Range Select tool

Figure 63 Selecting a time range in the TIC chromatogram

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

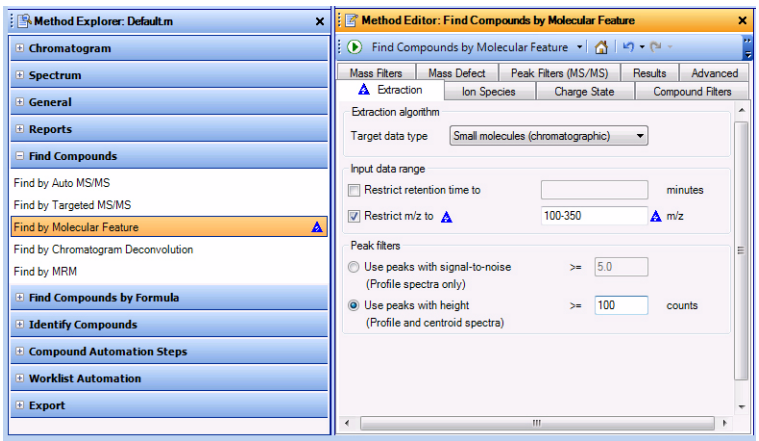
a In the Method Explorer window, click **Find Compounds > Find Compounds by Molecular Feature.**

b Select **Small molecules (chromatographic)** as the Target data type.

c Mark the **Restrict m/z to** check box.

d Type `100-350`.

- Learn more about **Target data type** in the online Help.
- You choose the region of the chromatogram from which to find compounds. See Figure 63.
- 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 LMFE and the Advanced tabs are only available if the Advanced check box is marked in the User Interface Configuration dialog box.

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

Figure 64 Restricting mass range for finding compounds by molecular feature

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

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

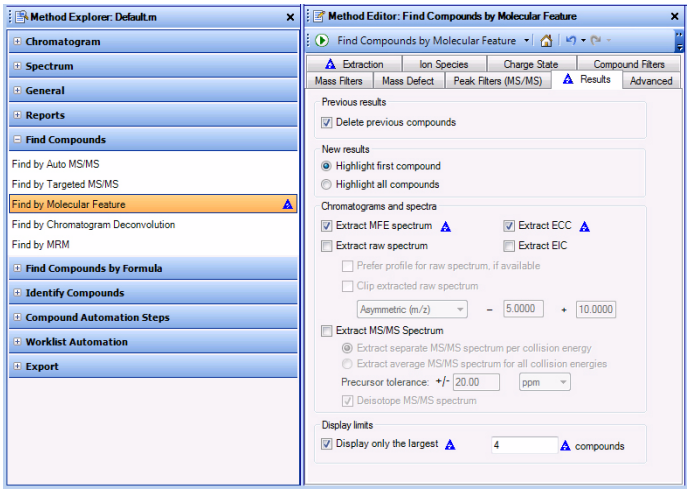


Figure 65 Changing the values in the Find by Molecular Feature > Results tab

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

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

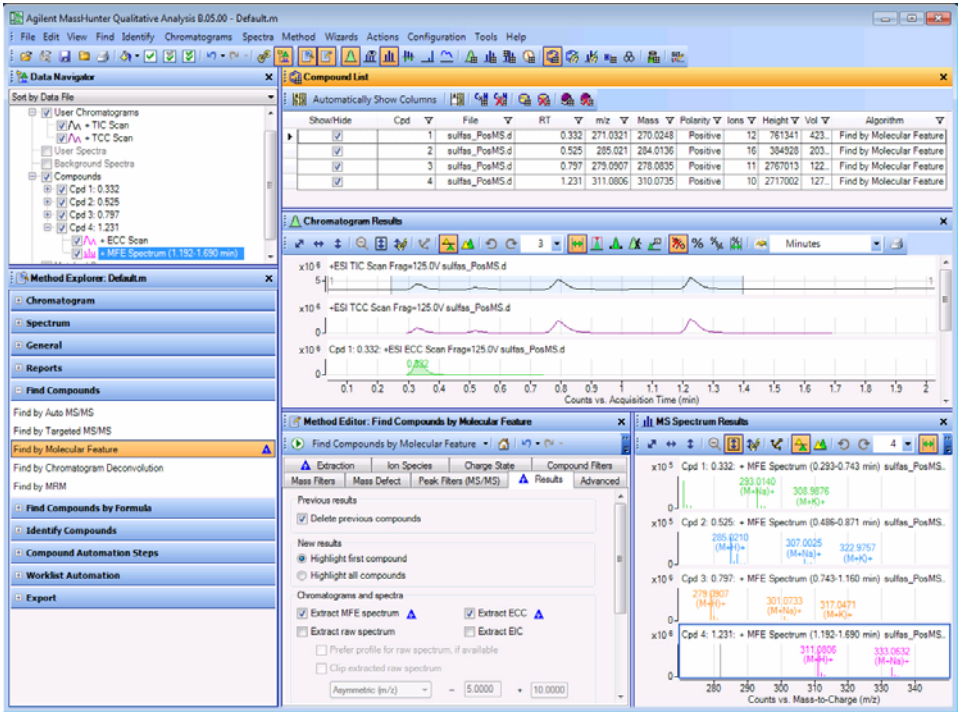


Figure 66 Finding all four compounds in the sulfa drug mix

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 68, make sure you have selected **Common organic molecules** as the Isotope model.	**a** In the Method Explorer window, click **Identify Compounds > Generate Formulas**. **b** In the Method Editor window, click the **Charge State** tab, and select **Common organic molecules** as the Isotope model. **c** In the Data Navigator window, click **Compounds** to highlight all of the compounds. **d** Click the **Identify > Generate Formulas** command or the **Generate Formulas from Compound** icon to run the algorithm. **e** If necessary, click the Compound Identification Results icon, , or click the **View > Compound Identification Results** command. **f** If necessary, click **View > Compound List**. **g** In the Compound List window, click the **Automatically Show Columns button** in the toolbar. **h** In the Compound Identification Results window, click the **Automatically Show Columns** button in the toolbar. **i** Click the Hide Empty Columns icon, ,in the Compound List and the Compound Identification Results window. **j** Close the Method Editor window and the MS Spectrum Results window.	• By default, the MS Formula Results window is tabbed with the Chromatogram Results window. Click on the tab at the bottom of the window to switch between windows. • You can see the predicted isotope abundance ratios on the spectrum plot when you zoom in at the appropriate m/z. See the online Help for more information. • The Run icon in the Method Editor toolbar sometimes allows you to choose an action from a set of possible actions. For example, two different actions are possible when you click the Run icon in this section. If you click the arrow, a list of possible actions is shown, and you can choose which action to do. Choosing a different action from the list changes the default action. If you simply click the Run button, the default action is performed. • You can change the width of a column by dragging the line that separates adjacent columns. • You can move a column by dragging the column header. • You can delete a column by clicking **Remove column** in the shortcut menu.

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

Step	Detailed Instructions	Comments

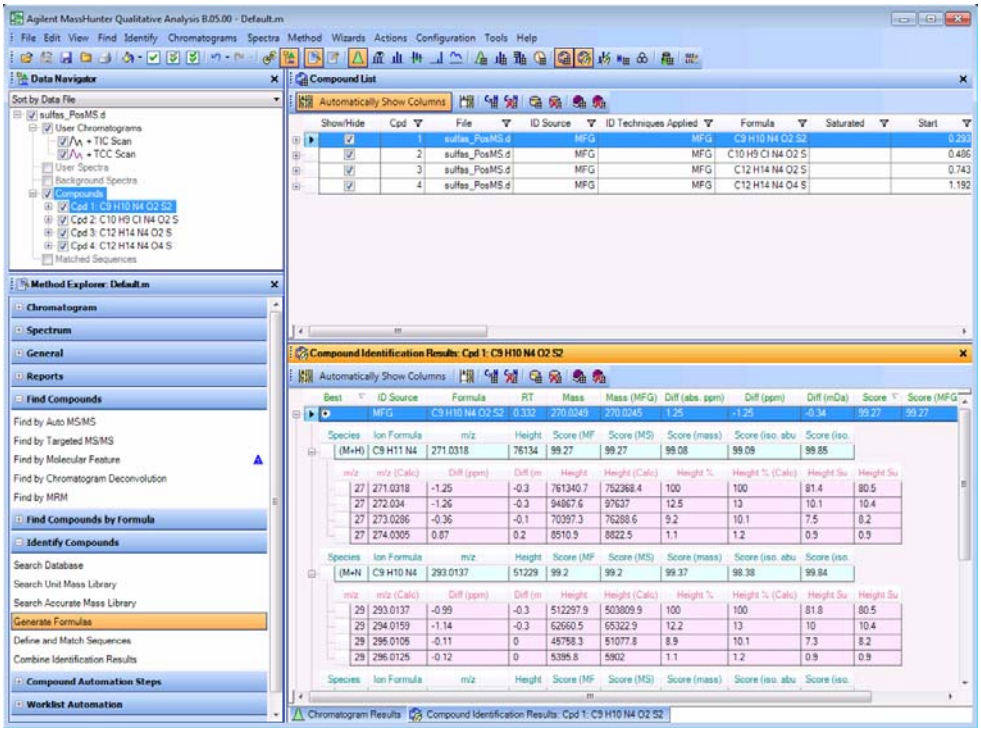


Figure 67 Generate Formula results for Compounds 1 to 4 in sulfas_PosMS.d

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

- Base search on formula.

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

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

c Under Search Criteria click **Molecular formula**.

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

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

- The Method Editor is opened automatically when you click a section in the Method Explorer.
- Note in the Compound List that all four sulfa drugs have been identified (See Figure 68).
- All of the identification results for compounds are shown in the Compound Identification Results window.
- Some identification results are also shown in the Compound List window.

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

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

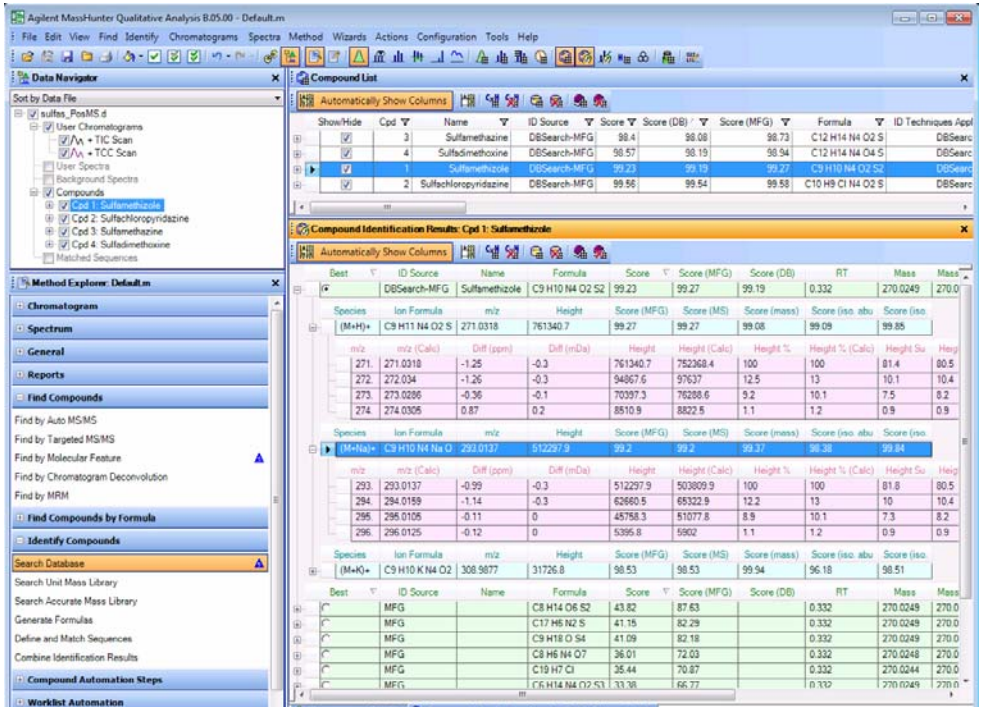


Figure 68 Results for Database Search and Generate Formulas for Compounds 1 to 4 in sulfas_PosMS.d

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)** 91 and identified in **Task 2. Generate formulas and identify compounds (LC/MS - MS only)** 95.

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

Step	Detailed Instructions	Comments
1 Change some of the selections in the method for compound reports: • Turn off viewing the MS spectra zoomed in on special peaks. • Turn off the MS/MS options in the report.	**a** In Method Explorer, click **Reports > Compound Report**. **b** Clear the **Show MS spectrum** check box. **c** Clear the **Show MS/MS spectrum** check box. **d** Clear the **Show MS/MS peak table** check box.	• These check boxes allow you to specify what information to include in a report if it is available. If the information is not available, that section is automatically skipped. For example, MS/MS results are never included when the data file only has MS data.

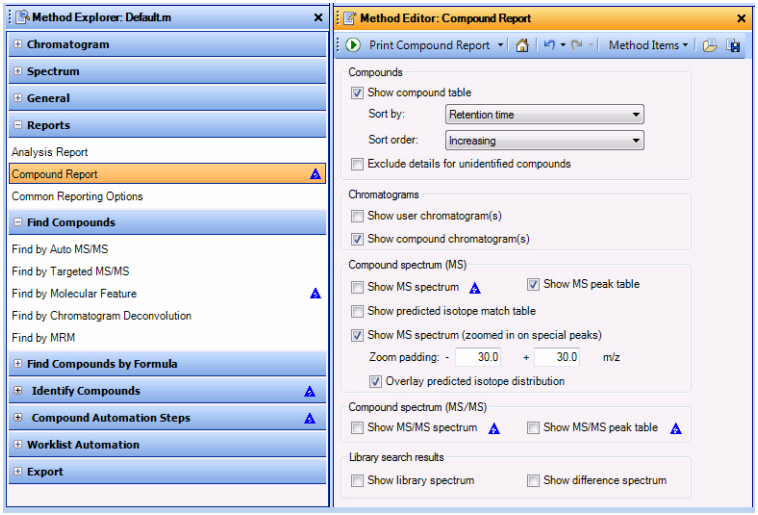
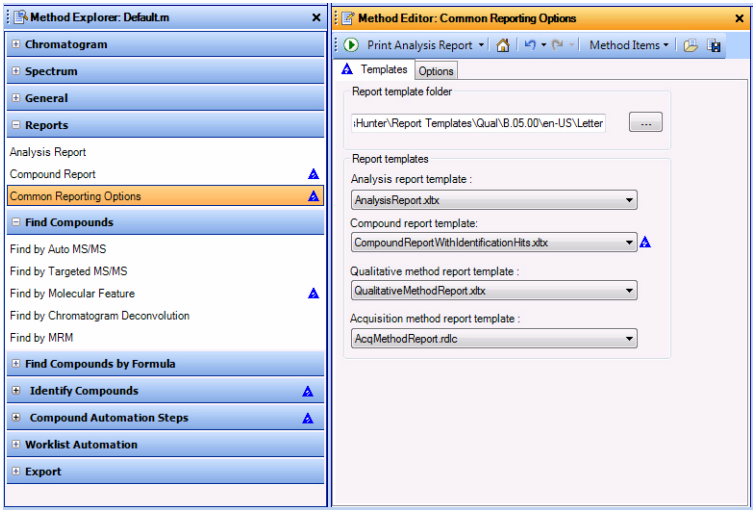


Figure 69 Compound Report section in the Method Editor

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

Step	Detailed Instructions	Comments
2 (optional) Choose a different compound report template.	**a** In the Method Explorer window, click **Reports > Common Reporting Options.** **b** Select **CompoundReport WithIdentificationHits.xlsx** as the Compound report template.	• Several different report templates are included with the software. • You can customize a report template using Excel and the Report Designer add-in.



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

Figure 70 Common Reporting Options section in the Method Editor

3 Print the report.	**a** Click **File > Print > Compound Report** or click the arrow in the **Print Analysis Report** icon and click **Print Compound Report** to print the compound report. **b** Mark the **Print preview** check box. **c** Click **OK**. Examine the report. **d** Click the **Close Print Preview** icon.	• In the Print Compound Report dialog box, you can select a different printer, select to save the report to a PDF or Excel file, select whether to print all results or only the highlighted results, and whether or not to combine different data files into one report. • See the online Help or the Report Designer Training DVD for additional information.
4 Close the data file without saving results.	**a** Click **File > Close Data File**. **b** Click **No** when asked if you want to save the results.	

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

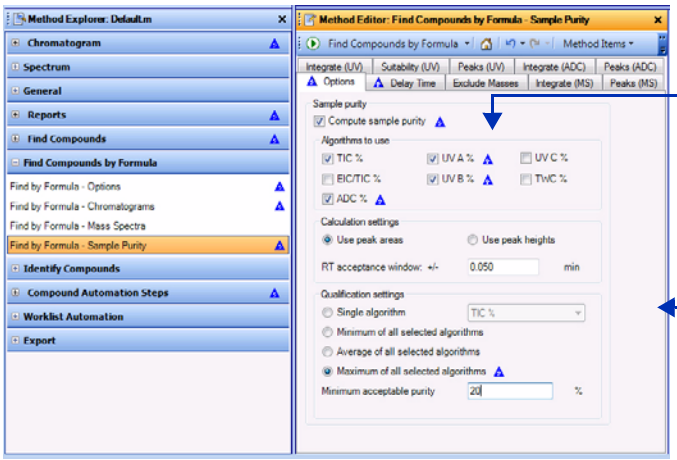
The Find Compounds algorithms find compounds in data and create averaged MS spectra for each compound. This functionality is an easy way to "mine" information from complex data. You can also compute sample purity.

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**. See "Open the sulfas_PosMS.d chromatogram." on page 91 for more information. **d** Click the **Autoscale Y-axis during Zoom** icon,  , in the Chromatogram Results toolbar. **e** Click the **Range Select** tool, and select the region from 0.2 to 1.5 minutes.	• If you switch to the Formula Confirmation and Sample Purity workflow, the Compound List table automatically shows the sample purity columns. • The Find by Formula sections are included in the **Formula Confirmation and Sample Purity Workflow** section.
2 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 the Method Explorer window, click the **Find Compounds by Formula > Find by Formula - Options** section. **b** Click **Database** as the **Source of formulas to confirm** and select default.csv. **c** In the Method Explorer window, click **Find Compounds by Formula > Find by Formula - Sample Purity** section. **d** Mark the **Compute sample purity** check box. **e** Mark the **TIC %**, **ADC %**, **UV A%** and **UV B%** check boxes. **f** Click **Maximum of all selected algorithms**. **g** In the **Minimum acceptable purity** box, type `20`.	• You double-click the title bar to anchor a window that is floating. By default in the General workflow, the Method Editor window is floating. You can also right-click the title of the window and then click **Floating**. • The blue triangle appears when you change a setting from the value that is saved in the current method. When you save the method, the triangles disappear. • This data file contains multiple sulfa drugs which is why the expected purity is 20%.

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

Step	Detailed Instructions	Comments



All of the algorithms that are marked are calculated, even if they are not used to determine the sample purity.

You specify how to determine the sample purity in this section.

Figure 71 Setting sample purity options for the Find Compounds by Formula algorithm

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

Step	Detailed Instructions	Comments
	h Click  to run the **Find Compounds by Formula** algorithm on the data file. **i** Change the Maximum number of list panes to 3 in the MS Spectrum Results windows. **j** Click **View > Compound List** to open the Compound List window. **k** In the Compound List window, if the **Automatically Show Columns** icon in the toolbar is not on, click the icon. **l** Click the Hide Empty Columns icon,  ,in the Compound List window. **m** In the Compound List window, clear the Automatically Show Columns check box. **n** Remove columns from the table that you don't want to include.	• The Qualitative Analysis program finds 6 major compounds in the selected range. • When you click the Category column, the columns are shown with columns from the same algorithm together. They are shown alphabetically within each section. • The Compound List was docked at the top of the Qualitative Analysis window so that more columns are visible. See "Task 4. Change window layouts" on page 22 for more information on moving windows. • If the Automatically Show Columns icon is not on, then you can manually display the Purity columns: **a** Right-click the Algorithm column, and click **Add/Remove Columns** to open the Compound Columns dialog box. **b** Click the **Category** column header to sort the possible columns. **c** Mark the **Purity Value** column, the **Purity Result** column, the **ADC% Area** column, the **TIC% Area** column, the **UVA% Area** column, and the **UVB% Area** column. **d** Click the **OK** button.

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

Step	Detailed Instructions	Comments

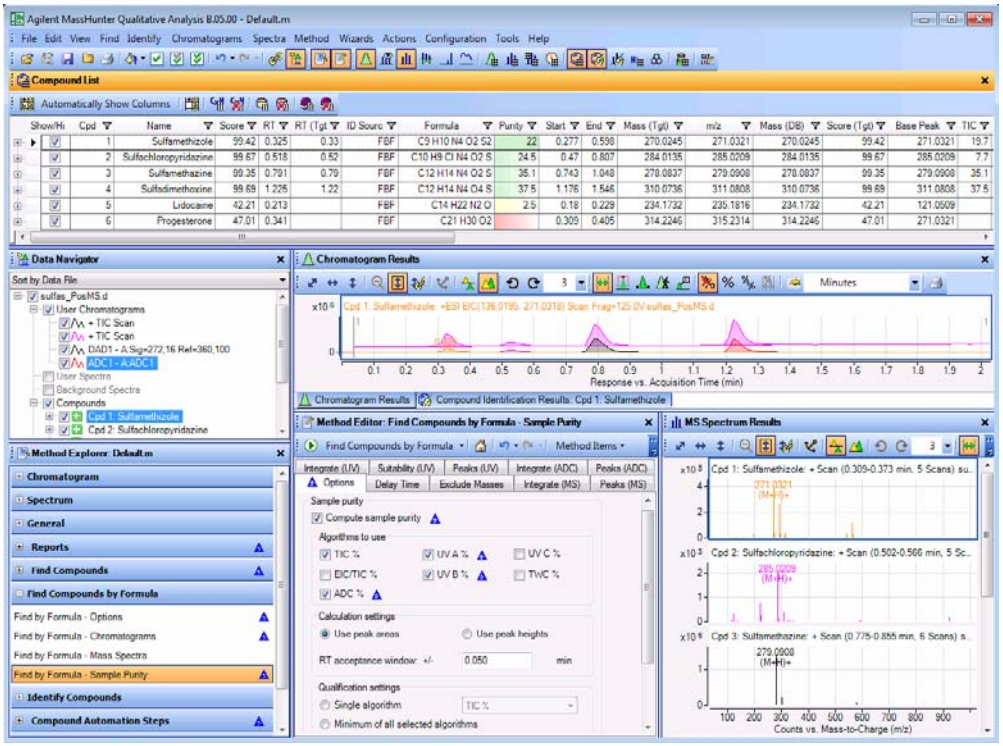


Figure 72 Finding all four compounds in the sulfa drug mix

- 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 Enable Peptide Sequence Editor features.	**a** Click **Configuration > Configure for Workflows > General**. **b** Click the **Load workflow's default method** button and the **Load workflow's default layout** button. **c** Click the **OK** button. **d** Click **Configuration > User Interface Configuration**. **e** Mark the **Peptide Sequence Editor** check box. **f** Click the **OK** button.	• The Peptides option in the Charge State tab is not available unless the **Peptide Sequence Editor** or the **BioConfirm** check box is marked. • You switch to the General workflow to change the layout and the visible Compound columns back to the defaults.
2 Do a molecular feature extraction for the data file **peptide-ms-only.d** with these parameters: • Time range is 2.5 to 4 minutes. • Specify that the Isotope model is peptides. • Filter to show only the largest 20 compounds in abundance. • Change the window layout to match that of Figure 73 (next page).	**a** Open the **peptide-ms-only.d** data file. **b** In the Method Explorer window, click **Find Compounds > Find by Molecular Feature** to display the parameters in the Method Explorer window. **c** In the **Extraction** tab, mark the **Restrict retention time to** check box. **d** Type `2.5 - 4`. **e** Clear the **Restrict m/z to** check box, if necessary. **f** On the **Charge State** tab, select **Peptides** in the Isotope model 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 MFE spectrum** and **Extract ECC** check boxes. **i** Click  to run the **Find Compounds by Molecular Feature** algorithm on the data file.	• The **Limit to the largest** filter does not limit the number of features extracted. It just limits the number of compounds displayed in Qualitative Analysis. • You extract features using the Qualitative Analysis Molecular Feature algorithm. Then, you can compare sets of different compounds using Agilent Mass Profiler Professional software. • You export the compounds to a CEF file using the **File > Export > Export CEF Options** command. • If you are going to use the Match Sequences algorithm, you also mark the **Extract MS/MS** check box. If you do not, the columns are not displayed in the Compound List window and the Compound Identification Results window.

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

Step	Detailed Instructions	Comments
3 Find the compound spectrum for the *m/z* 570.7362 ion and determine the charge state, mass and ion species.	**a** In the MS Spectrum Results window, scroll to find the spectrum containing the *m/z* 570.7362 ion. **b** Find the charge state. **c** Find the ion species. **d** Find this compound in the Compound List window. **e** Find the mass.	• Compound 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 Species**.

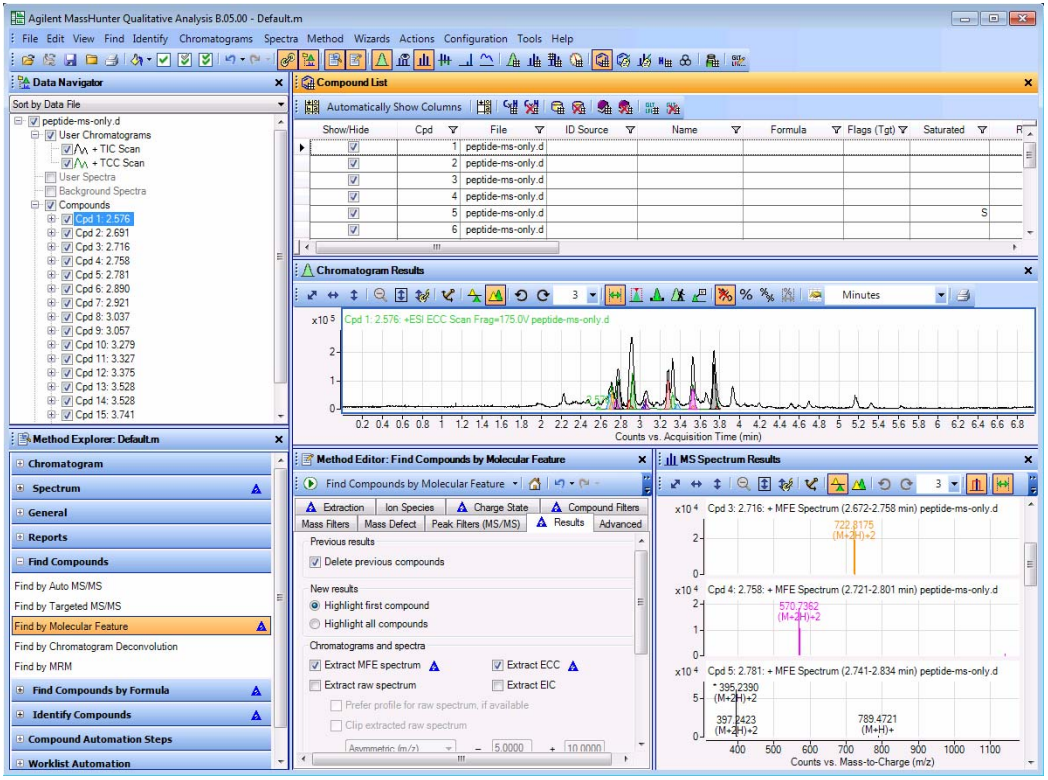
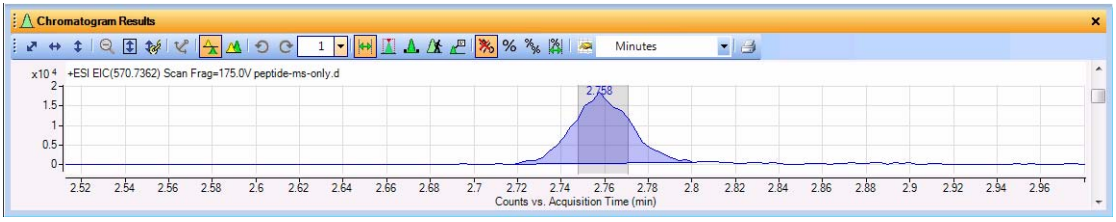
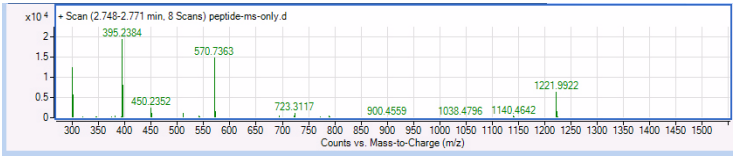


Figure 73 Find Compounds by Molecular Feature with Qualitative Analysis

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

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

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

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

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

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

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

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

Step	Detailed Instructions	Comments

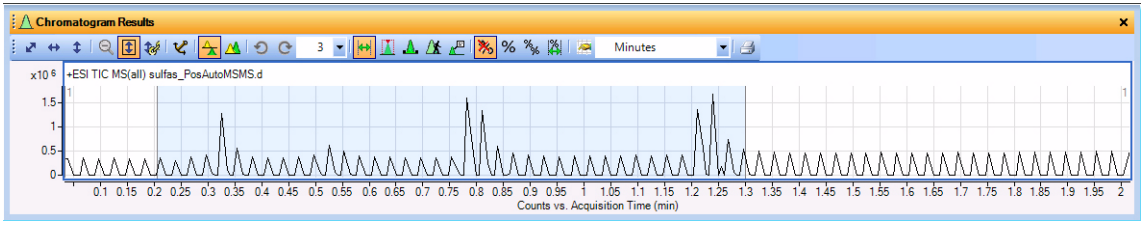


Figure 74 Zoomed range for TIC chromatogram of sulfas-PosAutoMSMS.d data file

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

a In Method Explorer click **Find Compounds > Find by Auto MS/MS.**
b 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 can choose the region of the chromatogram from which you intend to find compounds. See Figure 74.
- You can extract the complete result set for a compound after it is found by using the **Compounds > Extract Complete Result Set** menu item when a compound is highlighted.

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

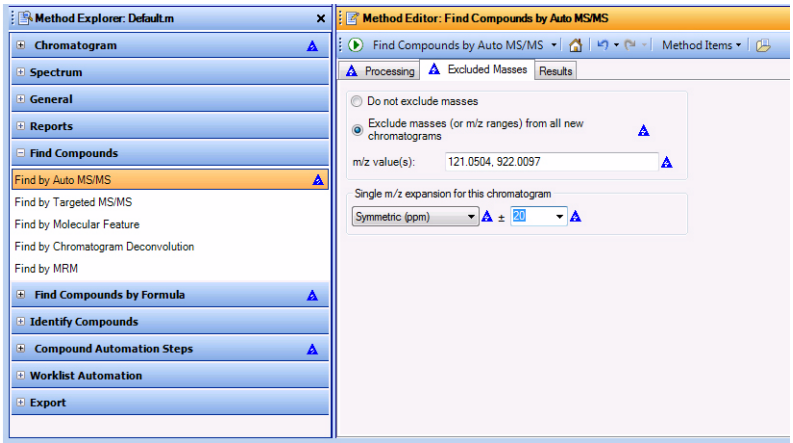


Figure 75 Excluded Masses tab of Find Compounds by AutoMS/MS

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

Step	Detailed Instructions	Comments
• Select to extract EIC, MS spectra and MS/MS spectra.	**h** Click the **Results** tab. **i** Mark the **Extract EIC**, **Extract MS**, and **Extract MS/MS** check boxes. **j** Clear the **Extract ECC** check box. **k** Click to run the **Find Compounds by Auto MS/MS** algorithm on the data file.	• You can also click **Find > Find Compounds by Auto MS/MS > Over Selected Ranges**. • The Qualitative Analysis program will find 4 compounds in the selected range under these conditions. • In the next task you identify which compounds are the sulfa drugs.
3 Display both spectra for Compound 4 only. See Figure 76.	**a** Highlight Compound 4 only. **b** Click the **Show only the highlighted items** tool in the main toolbar. **c** Expand Compound 4 to see the chromatogram and two spectra. You click the Plus sign next to the compound in the Data Navigator window to see the labels for the chromatogram and spectra.	• Showing both spectra is a convenient way to display all the information for a single compound. • Note that both the precursor and product spectra are extracted for each compound. The diamond represents the precursor ion. You can change the color to use for the MS/MS precursor ion symbol in the MS and MS/MS Spectra Display Options dialog box.

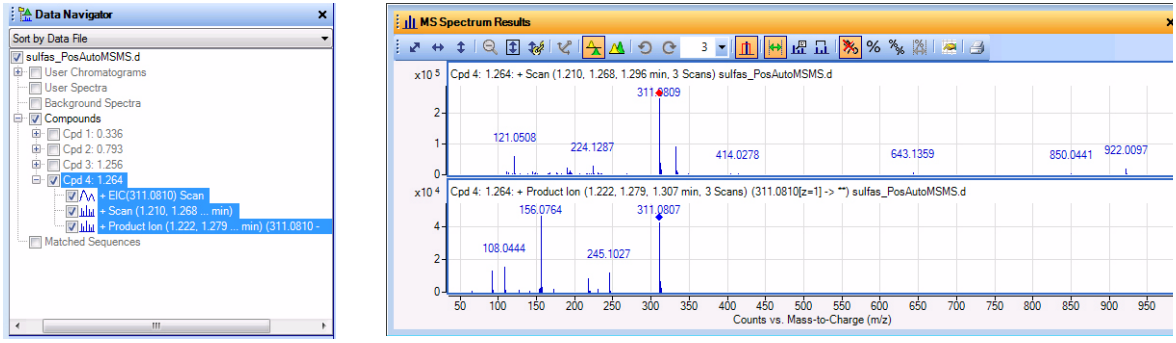


Figure 76 Data Navigator window and MS Spectrum Results window showing MS and MS/MS spectra for Compound 4

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

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

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

Step	Detailed Instructions	Comments
1 Do a database search of Compounds 1 to 4 based on masses. See Figure 77 on page 111.	**a** Highlight all compounds in the Data Navigator window. **b** In Method Explorer, click **Identify Compounds > Search Database**. **c** In the Method Editor window in the Search Criteria tab, click **Mass**. **d** Click **Identify > Search Database for Compounds** from the main menu. You can instead click the **Search Database for Compounds** icon to run the algorithm. **e** If the Compound List is not showing, click **View > Compound List**. **f** If the Compound Identification Results window is not showing, click **View > Compound Identification Results**. **g** Mark the **Show/Hide** check boxes for compounds 1 to 3 in the Compound List. Compounds 1 to 3 were hidden in the last task. Or, click the **Show all highlighted items** tool in the main toolbar.	• Note that three of the sulfa drugs have been identified in the Compound List (See Figure 78 on page 112). • Note that no compound name was found for Compound 3 after running the database search algorithm.

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

Step	Detailed Instructions	Comments

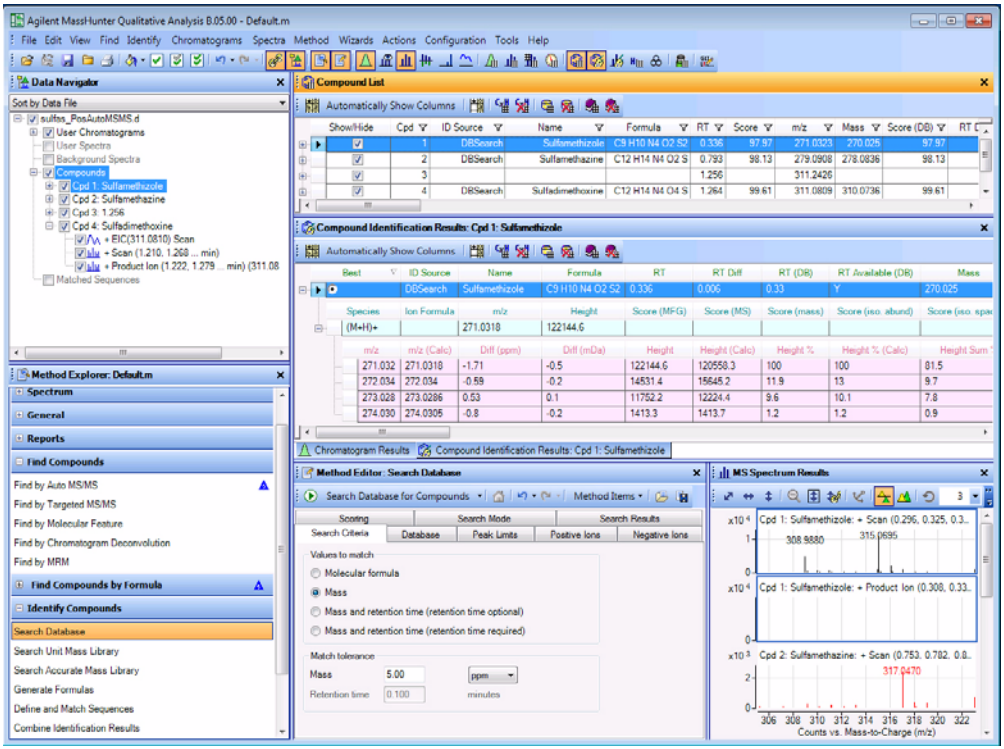


Figure 77 Compounds in sulfas-PosAutoMSMS.d data file identified by searching a database

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

Step	Detailed Instructions	Comments
2 Generate formulas for Compounds 1 to 4. • View the Compound List. • View the Compound Identification Results list. • Close the MS Spectrum Results window. Hint: To obtain the same results as in Figure 78, make sure you have selected **Common organic molecules** for the Isotope model.	**a** In the Method Explorer window, click **Identify Compounds > Generate Formulas**. **b** Click the **Charge State** tab, and select **Common organic molecules**. **c** Highlight all four compounds. **d** Click the **Generate Formulas from Compound** tool ( ) to run the algorithm or click the **Identify > Generate Formulas from Compounds** command. **e** In the Data Navigator window, highlight the compound that you want to see. **f** Use the scroll bar in the Compound Identification Results window to see the Generate Formulas results (MFG). The second level of the table shows several Score columns. The ID Source column shows that the result was found by both the Database Search (DB) algorithm and the Generate Formulas algorithm (MFG).	• By default, the Compound Identification Results window is tabbed with the Chromatogram Results window. Click on the tab at the bottom of the window to switch between windows. • You see the predicted isotope abundance ratios on the spectrum plot when you zoom in at the appropriate m/z. • Note that one or more formula were found for all compounds. • Click the Hide Empty Columns icon to automatically hide empty columns. You can also use the Remove column shortcut command. • Note that the formula from the database search is the same as the formula determined by the Generate Formulas algorithm. • Click **Configuration > Compound Label Configuration** to change the compound label.

Figure 78 Compound Identification Results window and MS/MS Details window for Compound 4

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

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

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

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

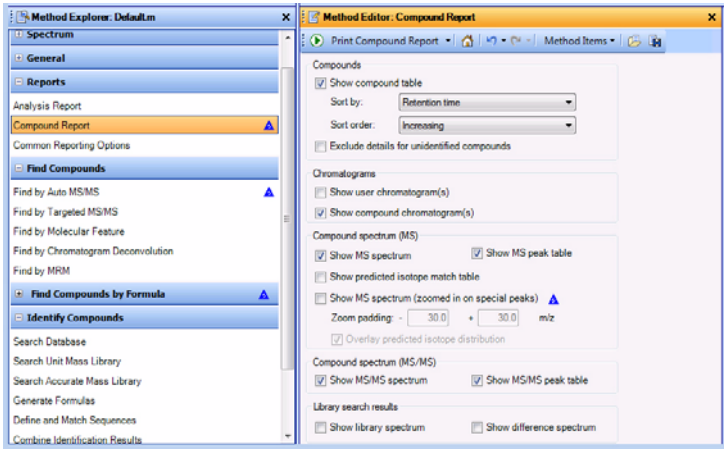
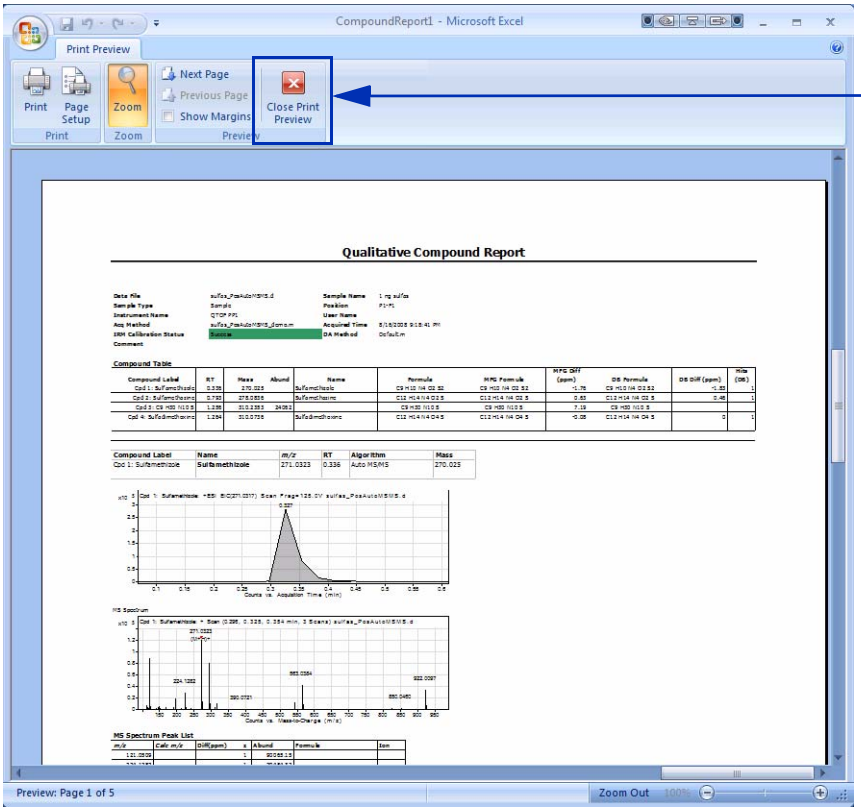


Figure 79 Compound Report window in the Method Editor

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

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

Step	Detailed Instructions	Comments



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

Figure 80 Print Preview of a Compound Report

3 Close the Print Preview window.

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

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

4 Close the data file without saving results.

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

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

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

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

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

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

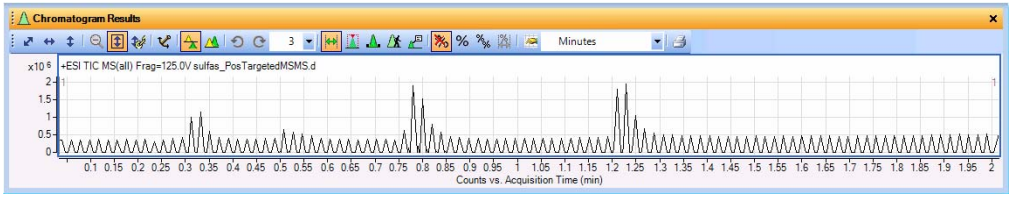


Figure 81 The TIC chromatogram for sulfas-PosTargetedMSMS.d data file

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

Step	Detailed Instructions	Comments
2 Find compounds using the Targeted MS/MS algorithm. • Select to extract an MS/MS chromatogram and MS/MS spectra.	**a** In the Method Explorer window, click **Find Compounds > Find by Targeted MS/MS.** **b** Click the **Results** tab. **c** Mark the **Extract MS/MS chromatogram** and **Extract MS/MS spectrum** check boxes. **d** Click **Find > Find Compounds by Targeted MS/MS**.	• You can extract the complete result set for a compound after it is found by using the **Compounds > Extract Complete Result Set** menu item when a compound is highlighted. • The Qualitative Analysis program will find 4 compounds under these conditions.
3 Search each compound using the Search Accurate Mass Library algorithm. • Select the SulfasLib.CDB library. • If this library is not available, then install the Personal Compound Database and Library (PCDL) program. • Lower the minimum match score to 50.	**a** In the Method Explorer window, click **Identify Compounds > Search Accurate Mass Library.** **b** Click the Browse button. **c** Select **Sulfas_AM_PCDL.cdb**. **d** Click the **Open** button. **e** Click the **Search Results** tab. **f** Type 50 in the **Minimum reverse score** box. **g** Mark the Minimum forward score check box. Type 20 as the minimum forward score. **h** Highlight all compounds in the Data Navigator window. **i** Click **Identify > Search Library for Compounds**. **j** If not showing, click **View > Compound List**. **k** If not showing, click **View > Compound Identification Results**.	• If the selected library has the *CDB* extension, then the Search Accurate Mass Library algorithm is run when you search a library. If the selected library has the *L* extension, then the Search Unit Mass Library algorithm is run when you search a library. • You can also right-click the Compounds line in the Data Navigator window and then click **Search Library for Compounds**. • 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

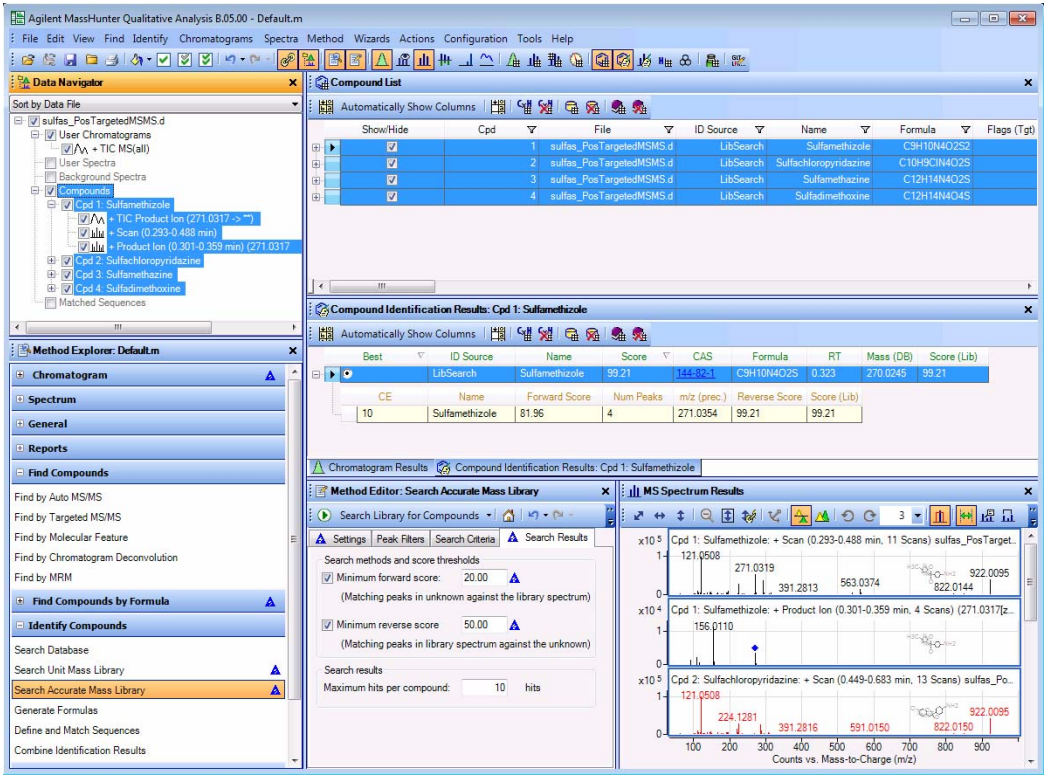


Figure 82 Results after running the Search Accurate Mass Library algorithm.

4 Close the data file without saving results.

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

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

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

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

Step	Detailed Instructions	Comments
1 Do a molecular feature extraction in the data file **peptide-auto.d** with these parameters: • Make sure the layout is returned to the Default Layout. • Time range is 2.5 to 4 minutes. • Set the isotope model to peptides. • Filter to show only the largest 20 compounds in abundance. • Change the window layout to match that of Figure 83 (next page).	**a** Open the **peptide-auto.d** data file. **b** Click the **Configuration > Configure for Workflow > General** command. **c** Click **OK**. **d** Click **Find Compounds > Find by Molecular Feature** in the Method Explorer to display the parameters in the Method Editor window. **e** In the **Extraction** tab, select **Small molecules (chromatographic)** as the Target data type. **f** Mark the **Restrict retention time to** check box. Then, type `2.5 - 4`. **g** On the **Charge State** tab, select **Peptides** as the **Isotope model**. **h** On the **Compound Filters** tab, mark the **Limit to the largest** check box and type `20` for the number of compounds. **i** On the Results tab, mark the **Extract MFE spectrum** and **Extract ECC** check boxes. **j** Click  to run the **Find Compounds by Molecular Feature** algorithm on the data file. **k** Click the List Mode tool in the Chromatogram Results toolbar. **l** If necessary, select **3** in the **Maximum number of list panes** box in the Chromatogram Results toolbar.	• To return the layout to the default layout, click **Configuration > Window Layouts > Restore Default Layout**. • The **Limit to the largest** filter does not limit the number of features extracted. It just limits the number of compounds displayed in Qualitative Analysis. • If Peptides is not one of the options for the **Isotope model**, you enable this feature by marking the **Peptide Sequence Editor** check box in the User Interface Configuration dialog box. Click **Configuration > User Interface Configuration** to display this dialog box. To display the LMFE and Advanced tabs for the Find by Molecular Feature section, you mark the **Advanced** check box. • You extract features using the Molecular Feature algorithm. Then, you can compare sets of data from different extractions using Agilent MassHunter Profiling software or GeneSpring MS software. • By default, chromatograms are displayed overlaid.
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

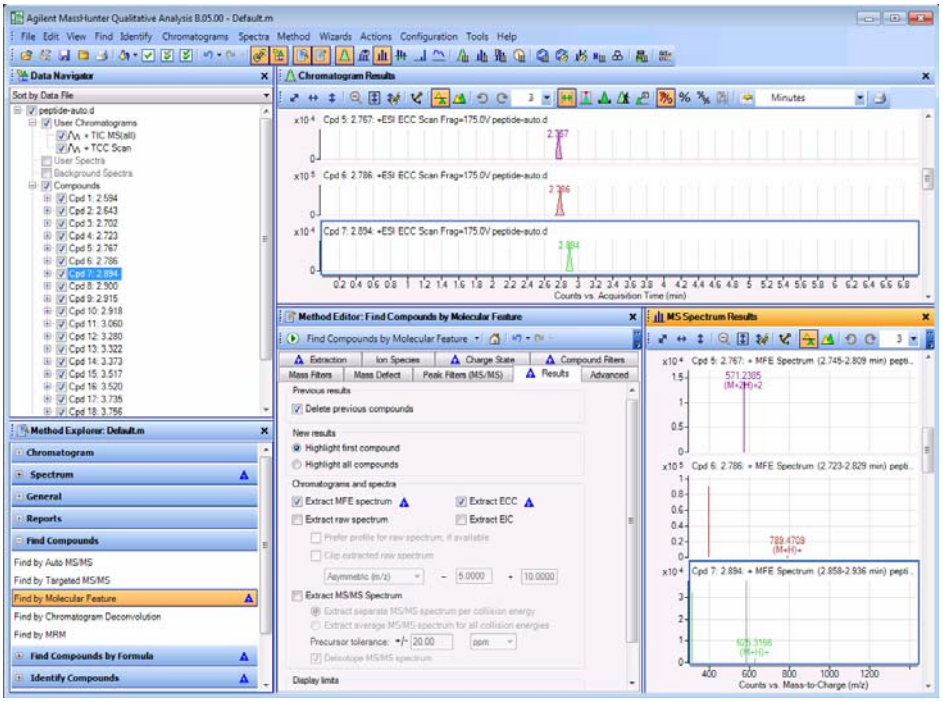


Figure 83 Find Compounds by Molecular Feature for a protein digest with auto MS/MS data

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)

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)

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.

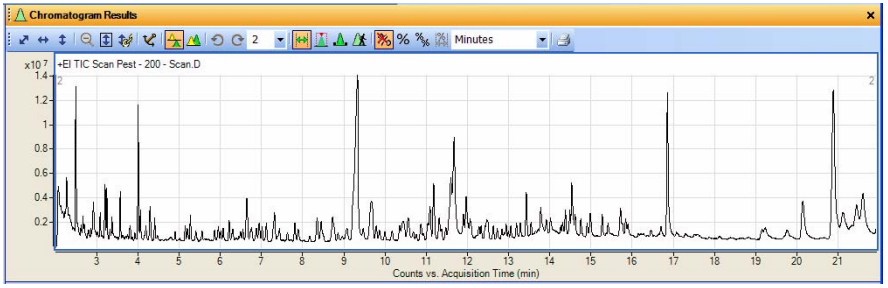


Figure 84 TIC chromatogram from Pest - 200 - Scan.d

2 Configure the user interface to work with GC data.	• Follow the instructions in Task 17. Configure User Interface for GC 69.	

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

Step	Detailed Instructions	Comments
3 Find compounds using the chromatogram deconvolution algorithm. • Select the Agile integrator. • Enter an SNR threshold of 20.	**a** In the Method Explorer window, select **Chromatogram > Integrate (MS)**. **b** Select **Agile** as the Integrator selection. **c** Select **Find Compounds > Find by Chromatogram Deconvolution.** **d** On the Settings tab under Peak filter, type 20 for the **SNR threshold**.	• 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.

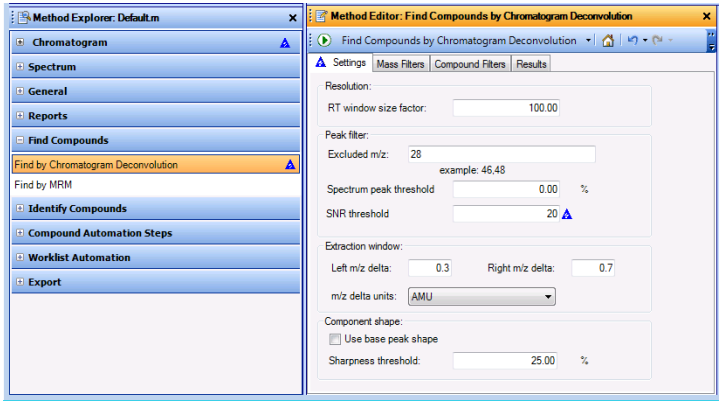


Figure 85 Settings tab in the Find by Chromatogram Deconvolution section

• Select to extract EIC, MS spectra and MS/MS spectra.	**e** Click the **Results** tab. **f** Mark the **Extract EIC, Extract ECC, Extract cleaned spectrum** and **Extract raw spectrum** check boxes. **g** Click to run the **Find Compounds by Chromatogram Deconvolution** algorithm on the data file. **h** Click the **View > Compound List** command.	• The Qualitative Analysis program finds 132 compounds under these conditions. • In the next task you identify these compounds by searching the *NIST08.L* library.

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

Step	Detailed Instructions	Comments
4 Examine the compounds. See Figure 76.	**a** Select **2** in the **Maximum number of list panes** box in the MS Spectrum Results toolbar. **b** Click the Hide Empty Columns icon in the Compound List window. **c** Click the first compound in the Data Navigator window. **d** When the Data Navigator window is selected, use the arrow keys to switch compounds.	• Showing both spectra is a convenient way to display all the information for a single compound. • Note that both the cleaned spectrum and the raw spectrum are shown.

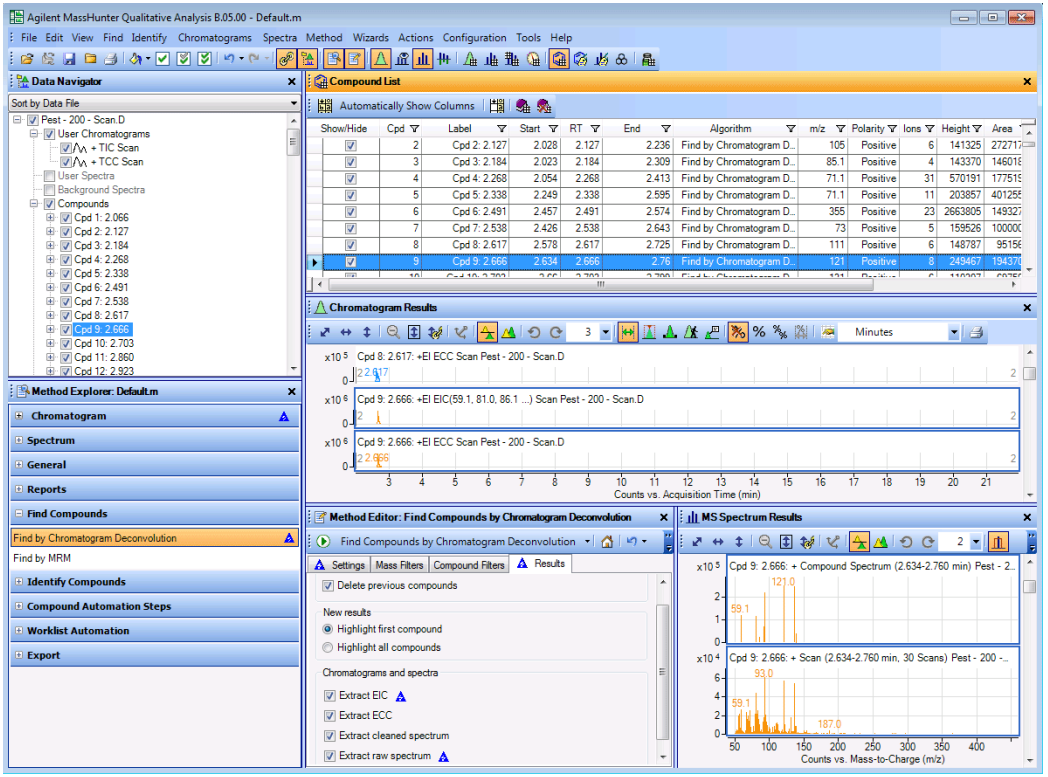


Figure 86 Chromatogram Deconvolution results

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

In this task, you identify and generate formulas for the compounds found in Task 1. Find compounds by chromatogram deconvolution (GC/MS) 120. You can do this task if you have purchased the *NIST08.l* library or if you use the *demo.l* library.

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

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 the Method Explorer window, click **Identify Compounds > Search Unit Mass Library**. **c** In the Settings tab, click the ... button. Select the **NIST08.l** library and click the **OK** button. **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** Click **View > Difference Results**. **f** Click **View > Structure Viewer**. **g** Click **View > Compound Identification Results**, if necessary to display this window. **h** If necessary, click the **Compound Identification Results** window. This window is tabbed with the Chromatogram Results window. **i** Click the Hide Empty Columns icon in the Compound Identification Results toolbar.	• Note that many of the compounds are identified after searching the *NIST08.l* library. • If you do not have the *NIST08.l* library, select the *demo.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 PCDL program which is included when you purchase the Qualitative Analysis program. See the online Help for the PCDL program for more information.

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

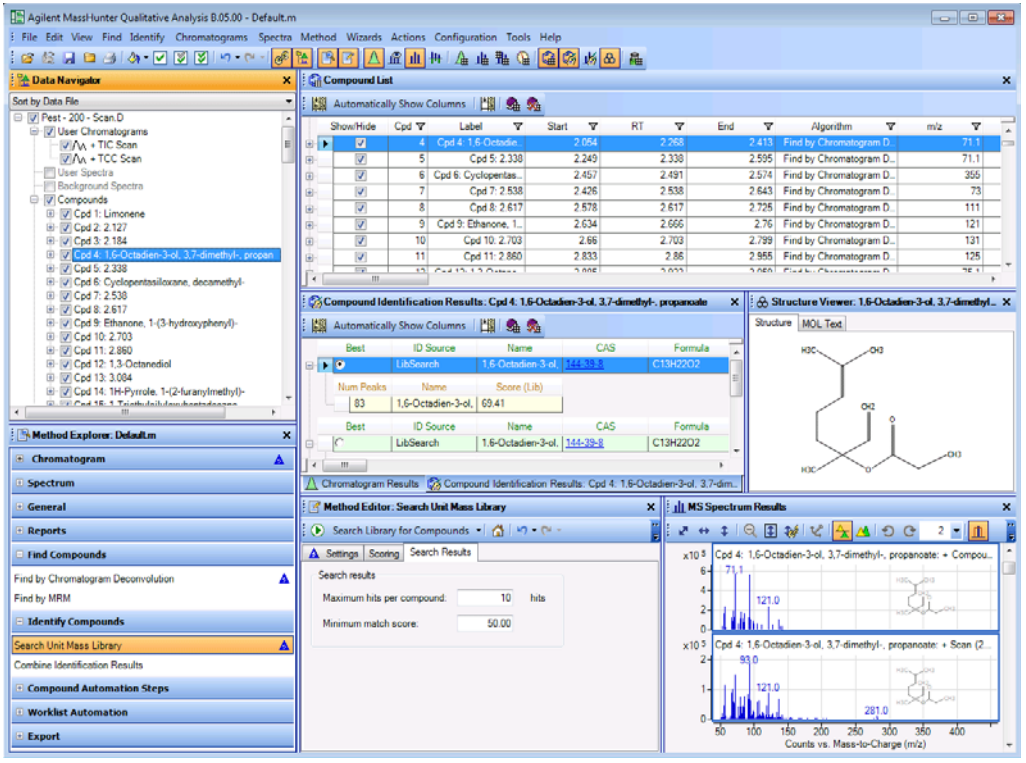
Step	Detailed Instructions	Comments
		
2 Close data files and return to LC/MS/MS user interface configuration.	**a** Click **File > Close Data File**. **b** Select all files. **c** Click **Close**. **d** Click **Configuration > User Interface Configuration**. **e** Mark all check boxes. **f** Click **OK**.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

Figure 87 Compounds in Pest - 200 - Scan.D data file and the library search results

Task 3. Find compounds by MRM (GC/MS - MRM only)

The Find Compounds by MRM algorithm identifies compounds in GC/MRM data. The algorithm searches for compounds using the MRM transitions. All of the compounds in the acquisition method are extracted and shown in the Compound List. Compounds are not eliminated based on chromatogram integration results. You can only use the Find Compounds by MRM algorithm on data that was acquired using MRM transitions.

Task 3. Find compounds using MRM (GC/MS - MRM only)

Step	Detailed Instructions	Comments
1 Open the TIC for the **Pest - STD 200 MRM.d** data file.	**a** If the program is not open, double-click the **Mass Hunter Qualitative Analysis icon**. Otherwise, click **File > Open Data File**. **b** Click the **Pest - STD 200 MRM.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.
2 Configure the user interface to work with GC data.	• Follow the instructions in "Task 17. Configure User Interface for GC" on page 69.	
3 Find compounds using the MRM algorithm.	**a** In the Method Explorer window, select **Find Compounds > Find by MRM.** **b** Click the **Group transitions by compound name** button. **c** Click the **Integrator** tab. **d** Select the **Agile** integrator.	• 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.

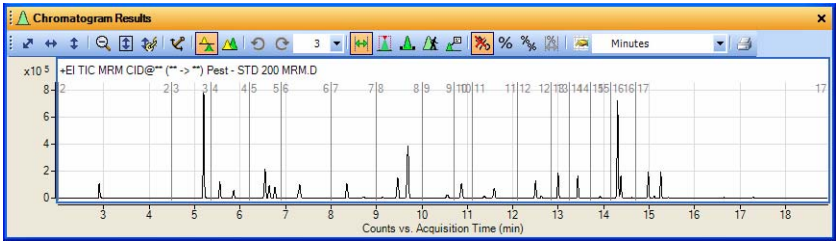


Figure 88 TIC chromatogram from Pest - STD 200 MRM.d

Task 3. Find compounds using MRM (GC/MS - MRM only)

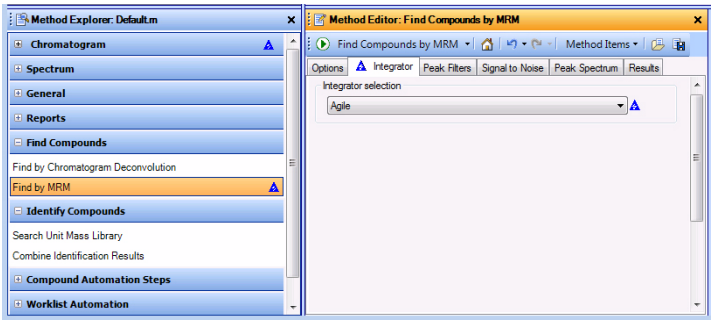
Step	Detailed Instructions	Comments
		
	e Click to run the **Find Compounds by MRM** algorithm on the data file. **f** If necessary, click the **View > Compound List** command. **g** Click **View > Compound Identification Results** to open this window.	• The Qualitative Analysis program finds 28 compounds under these conditions. • If you have a library in the XML format, you can use the Search Unit Mass Library algorithm.
4 Examine the compounds. See Figure 76.	**a** Select **2** in the **Maximum number of list panes** box in the MS Spectrum Results toolbar. **b** Click the Hide Empty Columns icon in the Compound List window and in the Compound Identification Results window. **c** Click the first compound in the Data Navigator window. **d** When the Data Navigator window is selected, use the arrow keys to switch compounds.	• The precursor ion is displayed in the Precursor (Acq Method) column, and the product ion is displayed in the Product (Acq Method) column in the Compound Identification Results window.

Figure 89 Integrator tab in the Find by MRM section of the Method Editor

Task 3. Find compounds using MRM (GC/MS - MRM only)

Step	Detailed Instructions	Comments

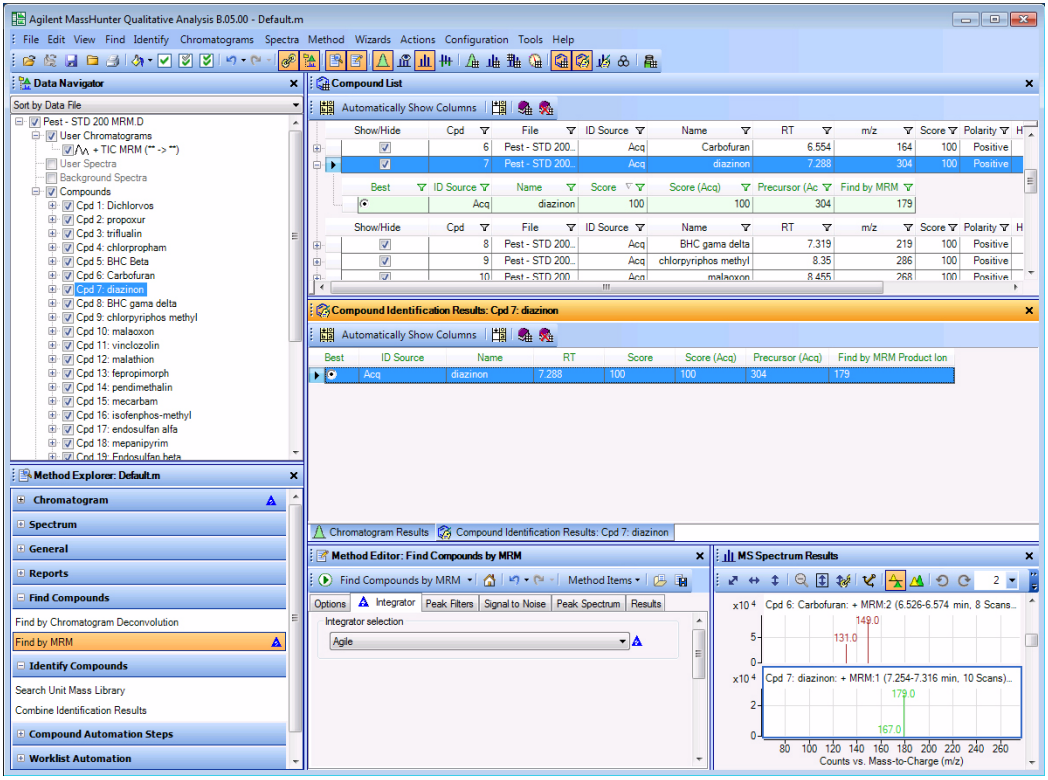


Figure 90 Find by MRM results

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

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

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

Different workflows are used for these examples. You can explore these different workflows before deciding which one best matches your tasks. See "Workflows" on page 171 for more information.

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

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

Each exercise is presented in a table with three columns:

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

Task 1. Set up and run a qualitative analysis method using the general workflow

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

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

Task 1. Set up and run a qualitative analysis method

Steps	Detailed Instructions	Comments
1 Open the **sulfas_PosMS.d** data file. • Make sure that the program will not run any file actions when the data file is open. • Make sure the method is Default.m. • Make sure the window layout is the default layout.	**a** Double-click the **Qualitative Analysis** icon on your desktop. **b** In the Open Data File dialog box, select **sulfas_PosMS.d**, **c** If necessary, clear the **Run 'File Open' actions from selected method** check box. **d** If necessary, clear the **Load result data** check box. **e** Click **Open**. **f** Click the **Configuration > Configure for Workflow > General** command. **g** Click the **Load workflow's default method** button and the **Load workflow's default layout** button. **h** Click **OK**. **i** Click **Configuration > User Interface Configuration**. **j** Mark all of the check boxes so all options are available. **k** Click the **OK** button.	• The default layout for the General workflow is automatically loaded. If you want to return to this default layout, click **View > Window Layouts > Restore Default Layout**. This command always restores the layout that is used with the General workflow. • To load a method, do this: ◦ Click **Method > Open**. ◦ Select the method ◦ Click **Open**. • As you noticed in the last exercise, every time a change is made to a method, a blue triangle appears next to the change and in the Method Explorer next to the section which has changed.
2 Set up the method to extract a TIC chromatogram. • Define a TIC chromatogram for MS data. • Turn off cycle sum since this is an MS-only data file.	**a** In the Method Explorer window, select **Chromatogram > Define Chromatograms**. **b** Delete the **BPC** chromatogram. **c** Select **TIC** as the **Type**. **d** Make sure the MS Level is **MS**. **e** Clear the **Do cycle sum** check box. **f** Click **Add**.	
3 Edit the method to integrate the data. • Limit the integration to the four highest peaks.	**a** In the Method Explorer window, click **Chromatogram > Integrate (MS)**. **b** Click the **Peak Filters** tab. **c** In the Maximum number of peaks section, mark the **Limit (by height) to the largest** check box. **d** Type 4.	• Updating a value in the Peak Filters tab in the Chromatogram > Integrate (MS) section also updates values in other sections of the Method Explorer. Blue triangles appear to show these other sections.

Task 1. Set up and run a qualitative analysis method

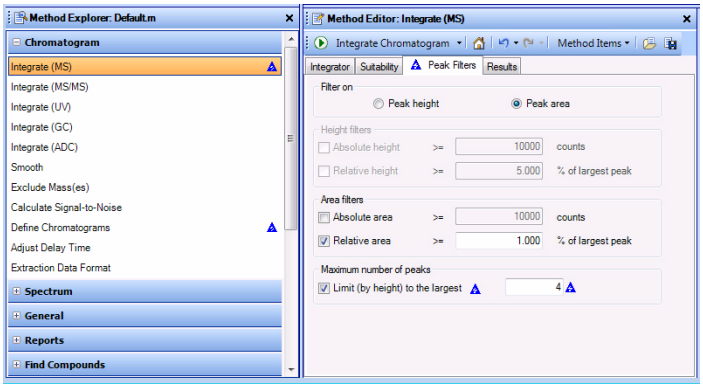
Steps	Detailed Instructions	Comments
		**You can click the Save Method icon to save the current method.**

Figure 91 The Spectrum > Extract (MS) > Peak Spectrum Extraction (MS) tab

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

Task 1. Set up and run a qualitative analysis method

Steps	Detailed Instructions	Comments

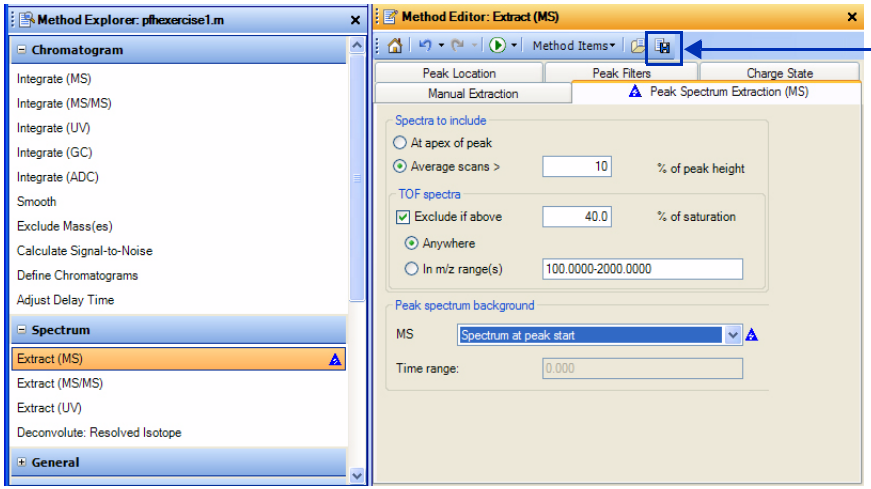
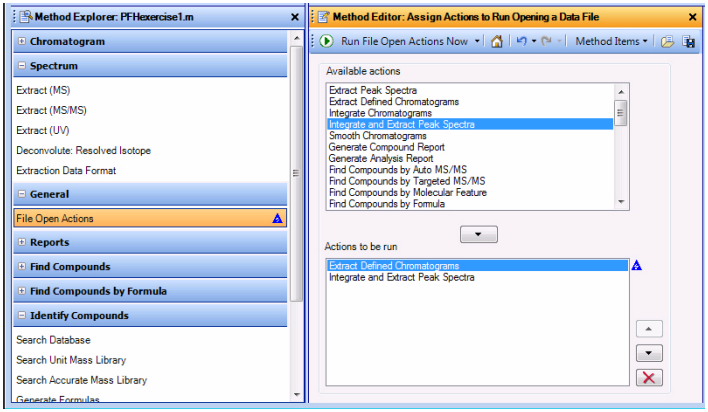


Figure 92 The Spectrum > Extract (MS) > Peak Spectrum Extraction (MS) tab

7 Test the MS spectrum extraction to make sure a background spectrum is subtracted.	• Click the **Extract Peak Spectrum** icon to run the action on the selected peak in the data file.	
8 Save the method.	• Save the method in one of three ways: ◦Click the **Save Method** icon in the Method Editor. ◦Right-click the Method Editor, and click **Save Method**. • From the top menu click **Method > Save**.	• The Save Method icon is shown in Figure 92 on page 133

Task 1. Set up and run a qualitative analysis method

Steps	Detailed Instructions	Comments
9 Set up the method to automate the actions whose parameters you just changed when you open a data file. • List the actions to be performed when this or another data file is opened. Hint: Look under General in Method Explorer.	**a** In the Method Explorer window, select **General > File Open Actions**. **b** Select **Integrate and Extract Peak Spectra** from the **Available actions** list. **c** Click the **Add** button, , to move the selected action to the **Actions to be run** list. You can also double-click on the selected action to move it to the other list.	
10 Test the File Open Actions.	• Click the **Run File Open Actions Now** icon to run the actions on the data file.	• The chromatograms and spectra are not overwritten. New chromatograms and spectra are added.



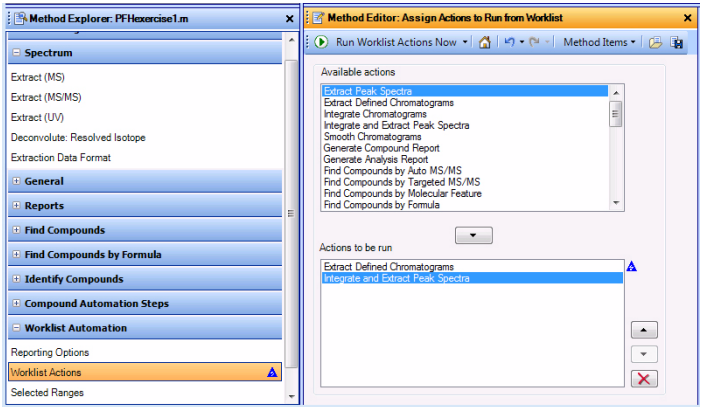
Two different actions are part of the Actions to be run list. The first action is to extract the defined chromatograms. Then, that chromatogram is integrated and peaks are extracted.

Figure 93 The General > File Open Actions section in the Method Editor

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

Task 1. Set up and run a qualitative analysis method

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



Two different lists of actions are included in a method. The first list of actions (File Open Actions) can be run when a data file is opened. The second list of actions (Worklist Actions) is run when the method is run as part of a worklist.

Figure 94 The Worklist Automation > Worklist Actions section in the Method Editor

14 Save the method and close the data file without saving results.	**a** Click the **Save Method** icon in Method Editor, **b** Click **File > Close Data File,** and click **No** when asked to save results.	

Task 2. Set up and run a method to automate an analysis using the Chromatogram Peak Survey workflow

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

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

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

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

Steps	Detailed Instructions	Comments
1 Open the **sulfas_PosMS**.d again. • Make sure that the method will not perform any actions on the data file when opening the file. • Make sure the method is *iii*exercise1.m.	**a** Click the **Configuration > Configure for Workflow > Chromatogram Peak Survey** command. **b** Click the **Load workflow's default method** button and the **Load workflow's default layout** button. **c** Click **OK**. **d** Click **Configuration > User Interface Configuration**. **e** Mark all of the check boxes so all options are available. **f** Click the **OK** button. **g** Click **File > Open Data File**. **h** In the Open Data File dialog box, select **sulfas_PosMS.d**. **i** Clear the **Run 'File Open' actions from selected method** check box. **j** Click **Open**. **k** Click **Method > Open**, select the iiiexercise1.m method, then click **Open**.	• Make sure the **Load result data** check box is either clear or grayed out. • When you switch to a different workflow, a new method can be loaded, a new window layout can be loaded, and a new section is added to the Method Explorer. • If you are prompted to save changes to the method, click **No**. • The method may load with red exclamation marks. These errors may be caused if the MassHunter folder is not on the D: drive. You can fix these errors by changing the folder that is specified for the database and library.

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

Steps	Detailed Instructions	Comments
2 Look at the sections for the Chromatogram Peak Survey algorithm.	• In the Method Explorer window, click **Chromatogram Peak Survey Workflow**.	• Note the eleven sections in this workflow. Most of these sections are duplicates of sections in the General workflow.
3 Make sure that new results will overwrite previous results.	**a** In the Method Explorer window, select **Previous Results**. **b** Mark the **Delete all previous results** check box.	• Note that blue triangles appear in other sections of Method Explorer. These indicate that the same parameter values have been changed elsewhere as well.
4 Make sure that a TIC will be extracted, and the four largest peaks integrated.	**a** Select **Chromatogram Extraction**. **b** Click the **Chromatograms** tab. **c** Make sure that **TIC** has been selected as the Chromatogram used to find mass spectra. **d** Mark **Signal A** under Additional chromatograms to extract. **e** Select 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**.	
6 Choose to search a database and generate formulas for all spectrum peaks. • Don't change the Molecular Formula Generation nor the Database Search parameter values.	**a** Select **Spectrum Peak Identification** in the Method Explorer. **b** Mark the **Search a database for each peak** check box. **c** Mark the **Generate formula for each peak** check box. **d** Click the **All peaks** button.	• Note that the "Spectrum Peak Identification" section is unique. You cannot enter this information anywhere else in the Method Editor.

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

Steps	Detailed Instructions	Comments
7 Test the automated analysis process up to this point.	• Click the **Run Chromatogram Peak Survey** icon from the Spectrum Peak Identification section.	• If you click the icon from the Molecular Formula Generation section, you click the arrow first and select **Run Chromatogram Peak Survey** from the list of possible action. By default, the action that is run in this section is **Generate Formulas from Spectrum Peaks**. Several other sections also have different default actions.
8 Open the Spectrum Identification Results window for viewing: • This list is tabbed along with the Chromatogram Results window as in Figure 95 • Save the method if the automation worked.	**a** If necessary, click **View > Spectrum Identification Results**. **b** Review the results for each MS scan to make sure that all actions in the Chromatogram Peak Survey algorithm were performed.	• SeeTask 4. Change window layouts 22 to learn how to move windows on the main screen. • The Spectrum Identification Results window is tabbed with the Chromatogram Results window. You can click on the tab if the Spectrum Identification Results window is not visible. • You can also use the icons in the main toolbar to display these windows.

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

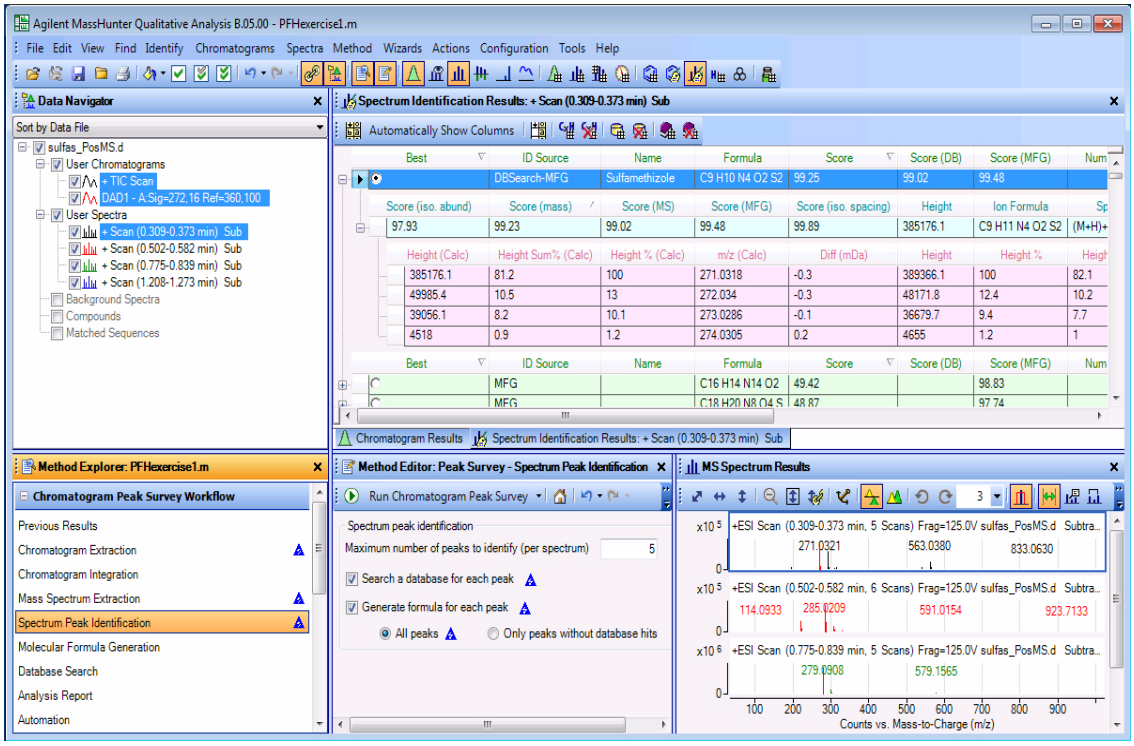
Steps	Detailed Instructions	Comments
		

Figure 95 Tabbed results from running automated analysis steps

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

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

Steps	Detailed Instructions	Comments
11 Set up the method to run the automated analysis when the data file is opened • Save the method.	**a** Select **Automation** in the Method Explorer. **b** Click **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 the Method Editor, Method Explorer and Data Navigator windows. • Move the windows so they look like the layout in Figure 96. • Close the data file, and do not save results.	**a** Click the **Close** button for the Method Editor, Method Explorer and Data Navigator window. **b** Move the windows so they look like Figure 96. **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 96.	**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

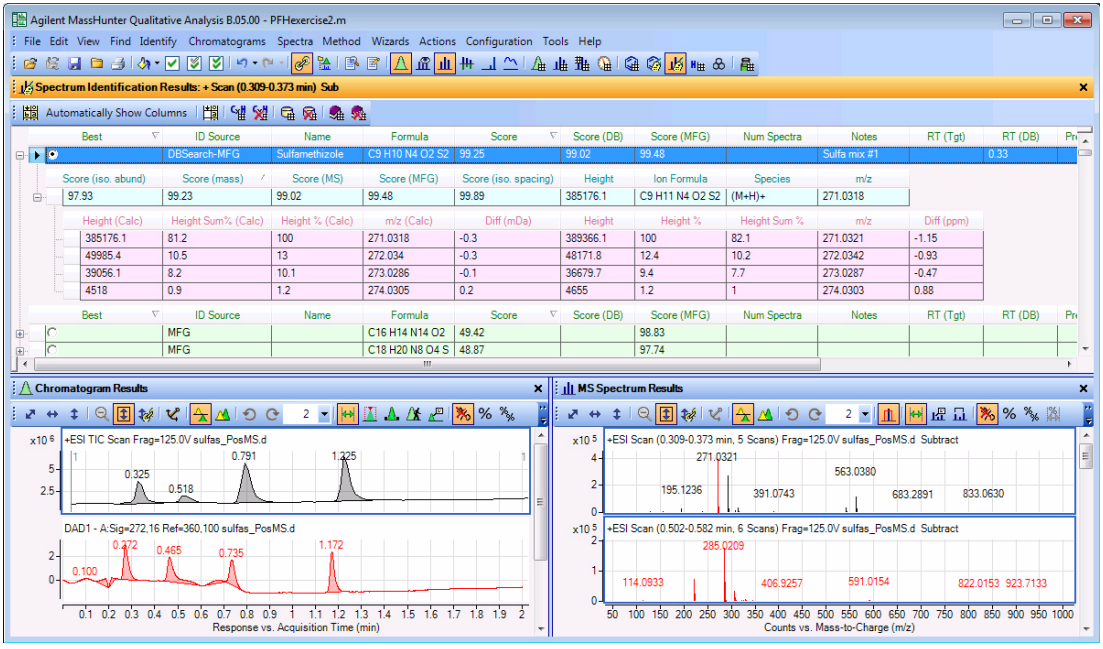


Figure 96 Results of Chromatogram Peak Survey action when opening the sulfas_PosMS.d data file

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

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

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

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

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

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

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

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

Steps	Detailed Instructions	Comments
2 Look at the automation steps for finding and identifying compounds. • Tab the Method Editor window in a convenient location.	**a** In the Method Explorer window, click **MS Target Compound Screening Workflow > Automation**. **b** (optional) Tab the Method Editor window with the Data Navigator window. **c** Close the **Compound List** window.	• In this workflow, the Method Editor is a floating window. You can either leave it as a floating window or tab it with another window, such as the Data Navigator window.
3 Choose to search a database and generate formulas for all compounds. • Make sure you are finding compounds by molecular feature.	**a** Click the **Analysis Options** tab. **b** Select **Find by Molecular Feature**. **c** Mark the **Search a database for each compound** check box. **d** Mark the **Generate formulas for each compound** check box. **e** Click **All compounds**. **f** Mark the **Show only identified compounds** check box.	• A compound can be identified by the Search Database algorithm, the Generate Formulas algorithm, the Search library algorithm or if the compound was found using the Find by Formula algorithm. If MassHunter BioConfirm software is installed, then a compound can also be identified by the Match Sequences algorithm.
4 Make sure that new results will overwrite previous results.	**a** Click the **Results** tab. **b** Mark the **Delete all previous results** check box.	
5 Test the automation process up to this point.	• Click the **Run Compound Automation Steps** icon  from any of the MS Target Compound Screening Workflow > Automation sections.	
6 Open these windows for viewing: • Compound List • Compound Identification Results • Make sure the windows are displayed as in Figure 97 • Review each list for each compound (except for Compounds 1 and 2).	**a** (if necessary) Click **View > Compound List**. **b** (if necessary) Click **View > Compound Identification Results**. **c** Clear the Compound 1 and Compound 2 check boxes in the Data Navigator. Or, you can clear the check boxes for Compound 1 and Compound 4 in the **Show/Hide** column in the Compound List window **d** 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 Compound Identification Results window is tabbed with the Chromatogram Results window in Figure 97.

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

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

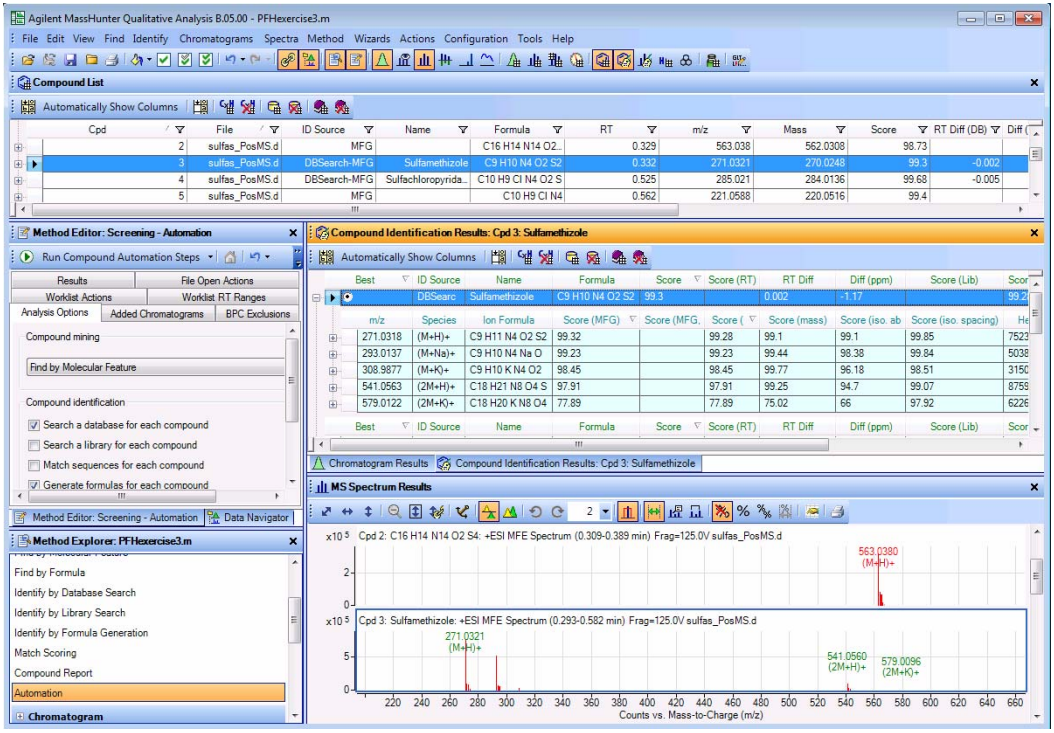


Figure 97 Tabbed results from running compound automation identification steps

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

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

Steps	Detailed Instructions	Comments
9 Set up the method to run the automated compound identification when the data file is opened • Save the method.	**a** Select **MS Target Compound Screening Workflow > Automation > File Open Actions**. **b** Select 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 98. • 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 98. **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 98. • Hide Compounds 1 and 2 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 2 in the Compound List.	• Compounds 1, 2, 5, 6, and 8 are not found by the database search algorithm, but they do have formulas generated by the formula generation algorithm.

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

Steps	Detailed Instructions	Comments

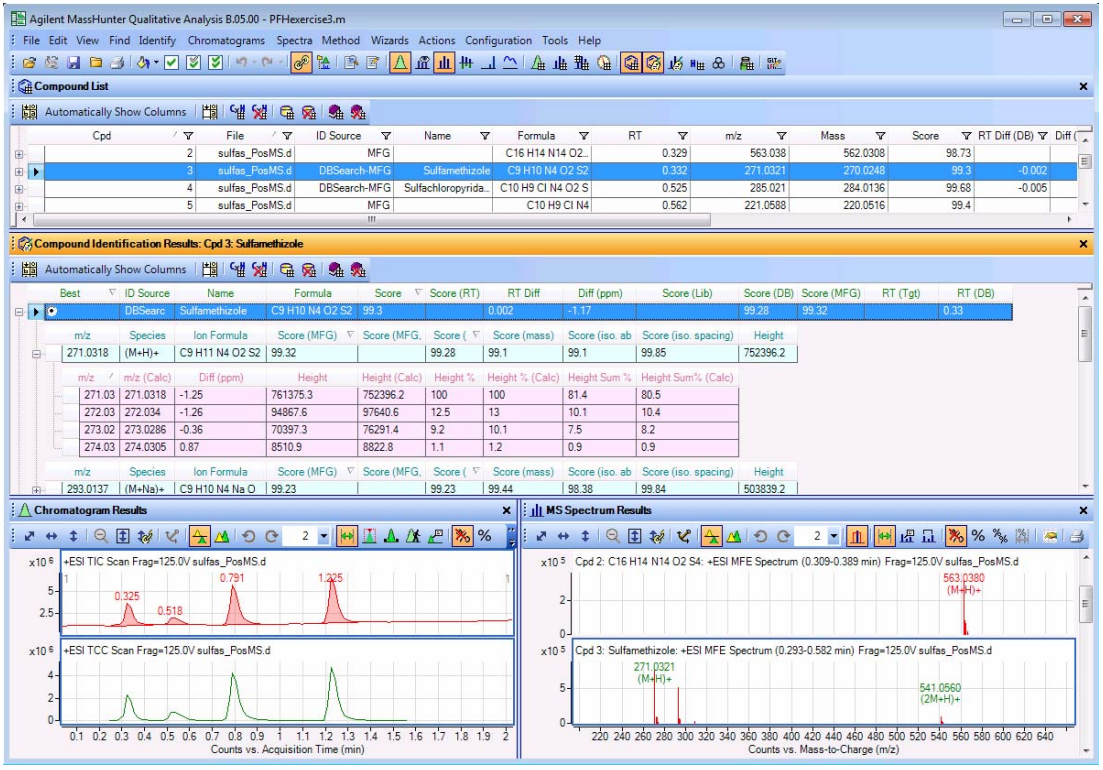


Figure 98 Results of automated compound identification when opening the sulfas_PosMS.d data file

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

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

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

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

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

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

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

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

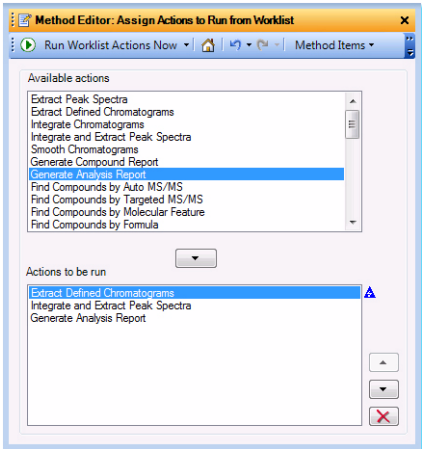


Figure 99 Method Editor with Worklist Actions section displayed

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

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

Exercise 4
Qualitative Analysis Wizards

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

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

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

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

Each exercise is presented in a table with three columns:

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

Task 1. Run the Identify Chromatogram Peaks Wizard

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

Task 1. Run the Identify Chromatogram Peaks Wizard

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

Task 1. Run the Identify Chromatogram Peaks Wizard

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

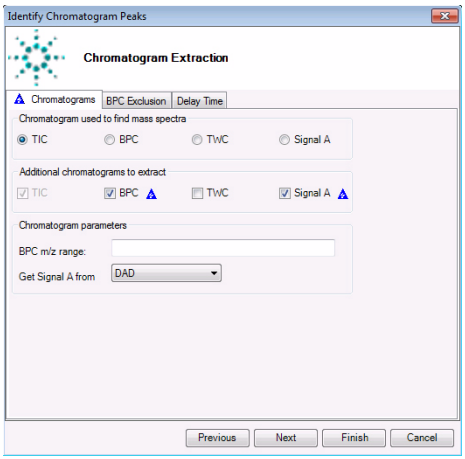


Figure 100 The Chromatogram Extraction page in the Identify Chromatogram Peaks wizard

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

Task 1. Run the Identify Chromatogram Peaks Wizard

Steps	Detailed Instructions	Comments

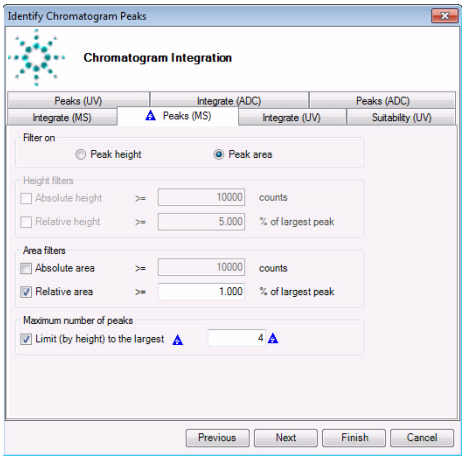


Figure 101 The Chromatogram Integration page in the Identify Chromatogram Peaks wizard

5 Review the parameters on the Extraction Data Format page.

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

b Click the **Next** button.

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

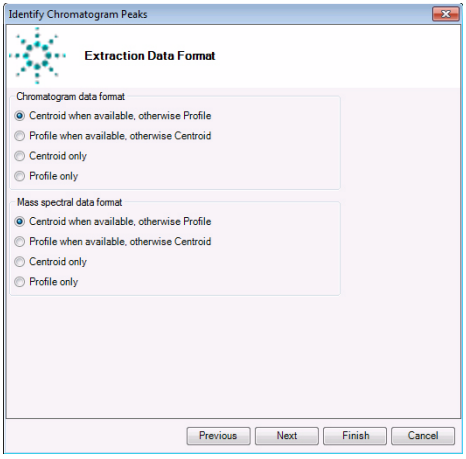


Figure 102 The Extraction Data Format page in the Identify Chromatogram Peaks wizard

Task 1. Run the Identify Chromatogram Peaks Wizard

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

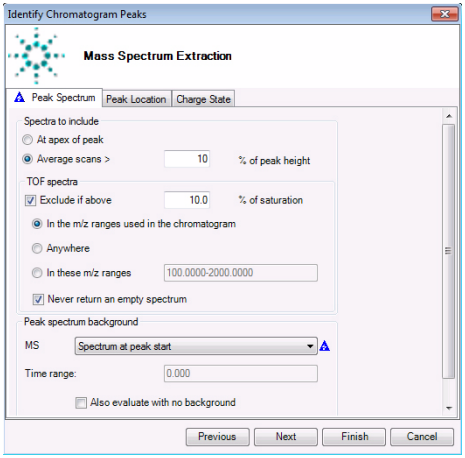


Figure 103 The Mass Spectrum Extraction page in the Identify Chromatogram Peaks wizard

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

Task 1. Run the Identify Chromatogram Peaks Wizard

Steps	Detailed Instructions	Comments

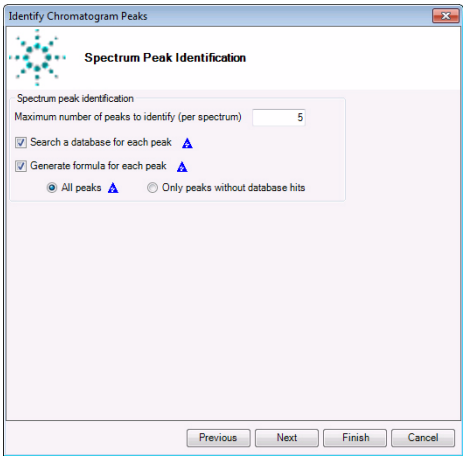


Figure 104 The Spectrum Peak Identification page in the Identify Chromatogram Peaks wizard

8 Review the parameters on the Database Search page.

a In the Database Search page, review the parameters.

b Click the **Next** button.

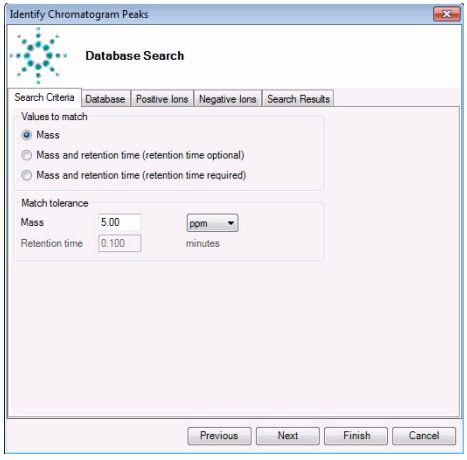


Figure 105 The Database Search page in the Identify Chromatogram Peaks wizard

Task 1. Run the Identify Chromatogram Peaks Wizard

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

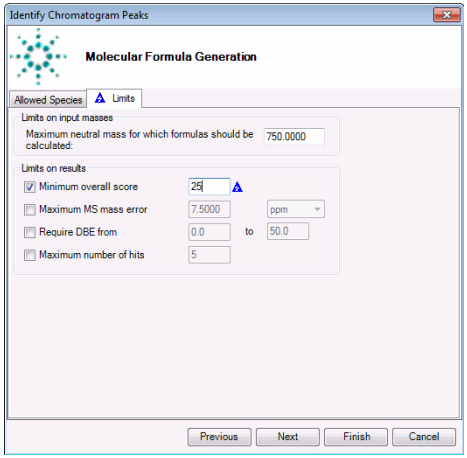


Figure 106 The Molecular Formula Generation page in the Identify Chromatogram Peaks wizard

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

Task 1. Run the Identify Chromatogram Peaks Wizard

Steps	Detailed Instructions	Comments

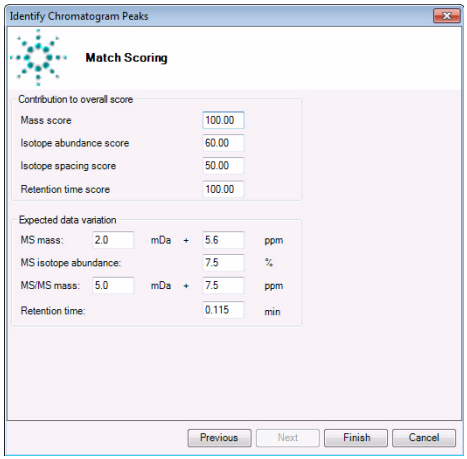


Figure 107 The Match Scoring page in the Identify Chromatogram Peaks wizard

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

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

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

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

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

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

Steps	Detailed Instructions	Comments

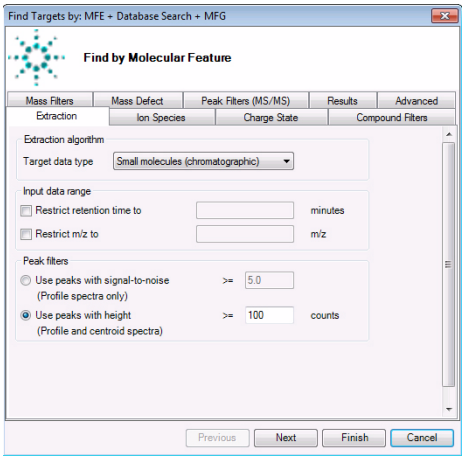


Figure 108 The Find by Molecular Feature page in the Find Targets by: MFE + Database Search wizard

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

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

Steps	Detailed Instructions	Comments

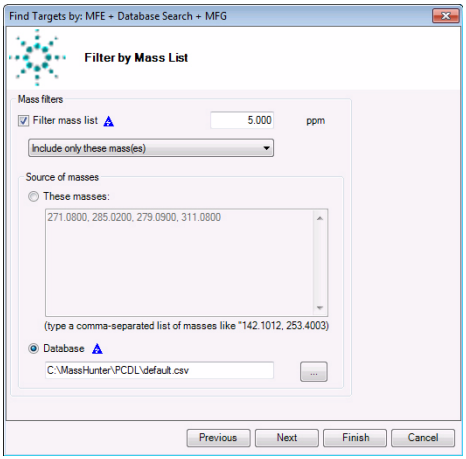


Figure 109 The Filter by Mass List page in the Find Targets by: MFE + Database Search wizard

4 Review the parameters on the Search Database page.

a Review the parameters.
b Click the **Next** button.

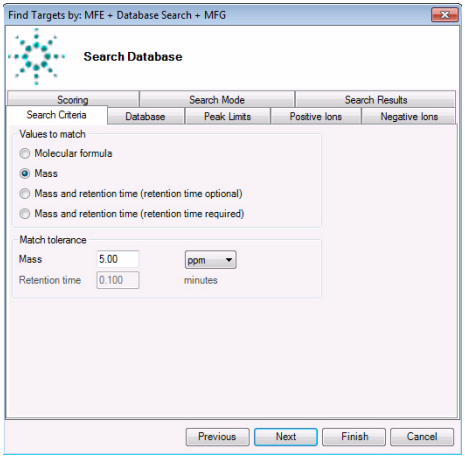


Figure 110 The Search Database page in the Find Targets by: MFE + Database Search wizard

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

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

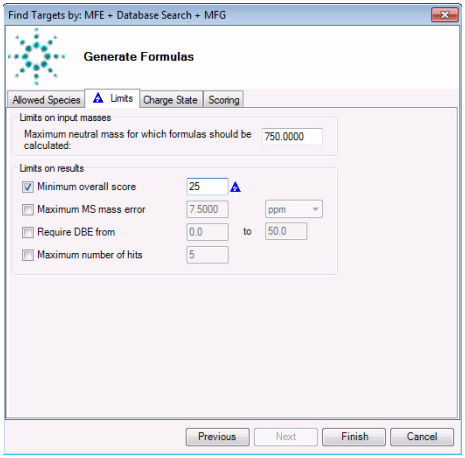


Figure 111 The Generate Formulas page in the Find Targets by: MFE + Database Search wizard

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

Reference

Work with windows

When you first open the Qualitative Analysis program, you see four windows in the default layout: Data Navigator, Method Explorer, Chromatogram Results and MS Spectrum Results. You can bring up sixteen other windows using the View menu:

- Method Editor - allows you to edit method parameters separated into different tabs
- Spectrum Preview - allows you to quickly scan the spectra in a data file
- MS Spectrum Results - shows the MS and MS/MS spectra
- Difference Results - shows the difference results after a library search
- Deconvolution Results - shows the deconvoluted spectra
- UV Spectrum Results - shows the UV spectra
- Integration Peak List - shows the integration results in a table
- MS Spectrum Peak List 1 - shows the peak table for the first spectrum selected
- MS Spectrum Peak List 2 - shows the peak table for the second spectrum selected
- MS Actuals - shows acquisition information for the highlighted spectrum
- Compound List - shows the compounds that are found using one of the Find Compounds algorithms
- Compound Identification Results - shows the identification information for the selected compound
- Spectrum Identification Results - shows the identification information for the selected spectra
- MS/MS Formula Details - shows a table containing possible formulas calculated for fragments seen in an MS/MS spectrum
- Structure Viewer - shows the structure associated with the current compound or spectra
- Sample Information - shows information about the highlighted data file
- Sequence Editor - allows you to edit a method sequence

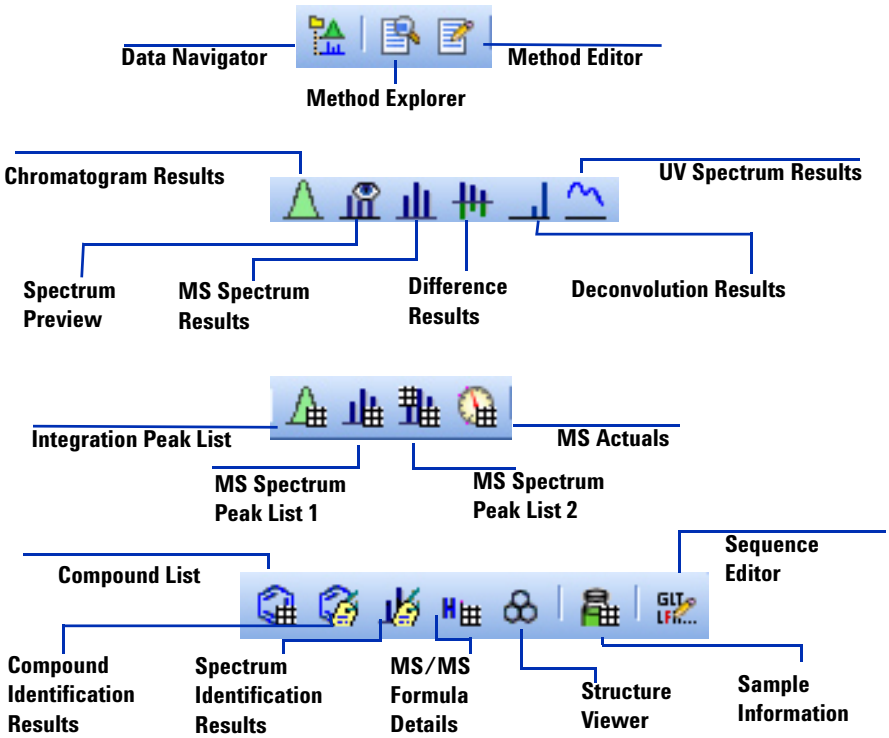
You can also display three tool windows which are displayed when you start using the associated tool:

- Formula Calculator
- Mass Calculator

- Recalibrate

Window Icons in the Main Toolbar

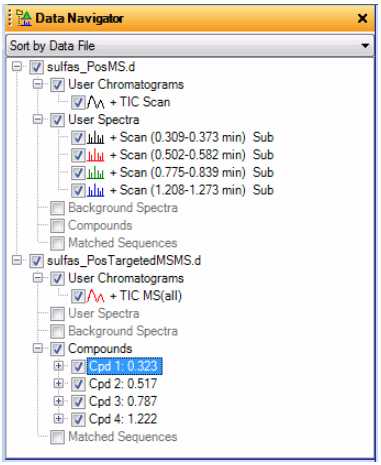
You open and close the windows with these icons on the main toolbar. Additional icons are available when the MassHunter BioConfirm software is installed. Commands in the View menu can also be used to open these windows.



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 graphic results are also highlighted. Linked Navigation only works if you have used the Integrate and Extract Peak Spectra menu item from the Chromatograms Menu or have run any of the Compounds algorithms.

Check Mark Tools

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

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

Dual check marks – Marks all check boxes.

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

Perform operations on the chromatogram

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

Action	Menu Item
Change peak labels in chromatogram	Configuration > Chromatogram Display Options
Extract a chromatogram	Chromatograms > Extract Chromatograms
Extract defined chromatograms	Chromatograms > Extract Defined Chromatograms
Integrate the chromatogram	Chromatograms > Integrate Chromatogram
Integrate and extract peak spectra	Chromatograms > Integrate and Extract Peak Spectra
Smooth the chromatogram	Chromatograms > Smooth Chromatogram
Calculate Signal-to-Noise	Chromatograms > Calculate Signal-to-Noise
Subtract any chromatogram	Chromatograms > Subtract Any Chromatogram
Find compounds from auto MS/MS data	Find > Find Compounds by Auto MS/MS
Find compounds from targeted MS/MS data	Find > Find Compounds by Targeted MS/MS
Find compounds for MS(1) data	Find > Find Compounds by Molecular Feature
Find compounds for MRM data	Find > Find Compounds by MRM
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 tool () in the Chromatogram Results toolbar.

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

3 Right-click anywhere in the chromatogram, and click the operation from the shortcut menu.

Save results to the data file(s)

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

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

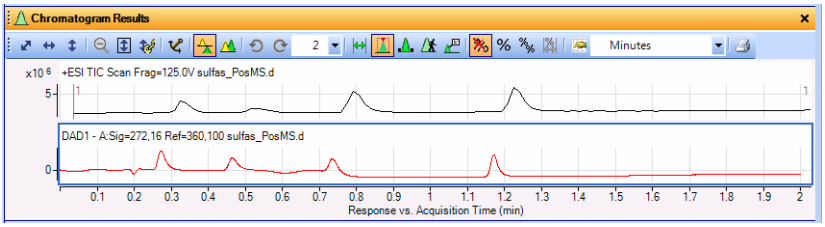
Perform operations on an MS or MS/MS spectrum

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

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

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

Range Select – When **On**, you can draw a range for chromatogram, for which you can perform actions.

Peak Select – When **On**, you can select spectrum of an integrated peak at apex.

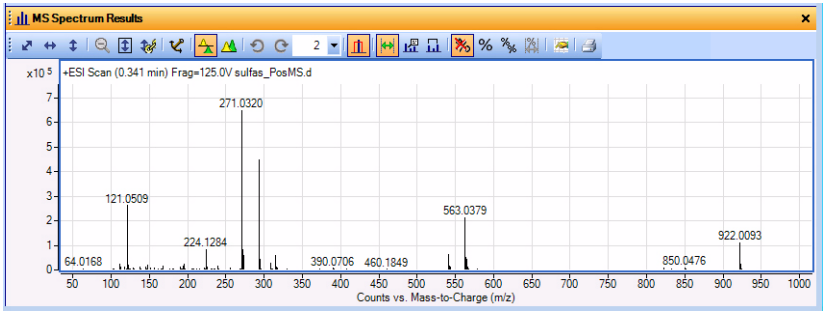
Manual Integration – When **On**, you can integrate interactively.

Walk Chromatogram – When **On**, you can see individual spectra as you click each point or use the left and right arrows on the keyboard.

Annotation – When **On**, you can add image and text annotations to the chromatograms.

Work with spectral visual data

MS Spectrum Results Window



MS Spectrum 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

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

Range Select – When **On**, you can draw a range for chromatogram, for which you can perform actions

Annotation – When **On**, you can add image and text annotations to the chromatograms

Calipers – When **On**, you can add a Delta Mass caliper to the selected spectrum. In the Deconvolution Results window, you can also add an Amino Acid caliper or a Modifications caliper. See the online Help for more information.

Workflows

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

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

- General
- BioConfirm - These workflows are only available if the BioConfirm software is installed and marked in the User Interface Configuration dialog box. BioConfirm has several possible workflows, depending on the type of analysis that you want to do.
- 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 one of the BioConfirm workflows, the **Target data type** for the Find Compounds by Molecular Feature algorithm is set to **Large molecules (proteins, oligos)**. This setting is appropriate for the BioConfirm 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
- Any filter that has been added to the table (only available for the Compound List table, the Compound Identification Results table, and the Spectrum Identification Results window).

Specific section in the Method Explorer and Method Editor

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

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

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

Workflow methods and layouts

Additional default methods and layouts are provided for each workflow.

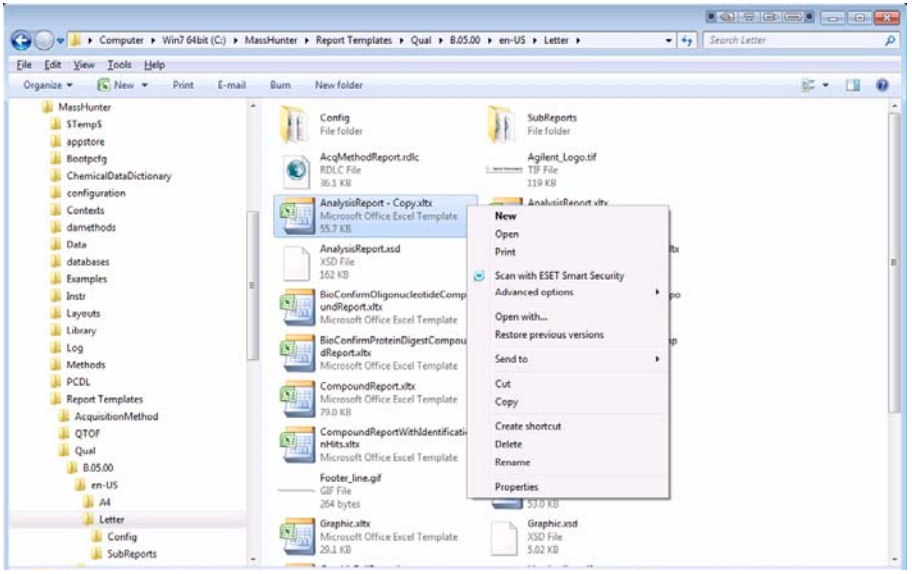
Workflow	Method	Layout	Method Editor Section
General	default.m	Default.xml	None
BioConfirm Intact Protein	BioConfirm IntactProtein-Default.m	BioConfirm-IntactProtein-MaximumEntropy-Default.xml	BioConfirm Workflow
BioConfirm High Mass Intact Protein	BioConfirm IntactProtein HighMass Default.m	BioConfirm IntactProtein LMFE.xml	BioConfirm Workflow
BioConfirm Small Oligonucleotides	BioConfirmOligo nucleotideSmall.m	BioConfirmOligo-nucleotide.xml	BioConfirm Workflow
BioConfirm Large Oligonucleotides	BioConfirmOligo nucleotideLarge-Default.m	BioConfirmOligo-nucleotide.xml	BioConfirm Workflow
BioConfirm Protein Digest	BioConfirmProtein Digest-Default.m	BioConfirm ProteinDigest.xml	BioConfirm Workflow
BioConfirm Synthetic Peptide	BioConfirmSynthetic Peptide-Default.m	BioConfirm SyntheticPeptide.xml	BioConfirm Workflow
Chromatogram Peak Survey	ChromPeakSurvey-Default.m	Default.xml	Chromatogram Peak Survey Workflow
Formula Confirmation and Sample Purity	SamplePurity-Default.m	SamplePurity-Default.xml	Formula Confirmation and Sample Purity Workflow
MS Target Compound Screening	Screening-Default.m	Screening-Default.xml	MS Target Compound Screening Workflow

Customize a report template

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

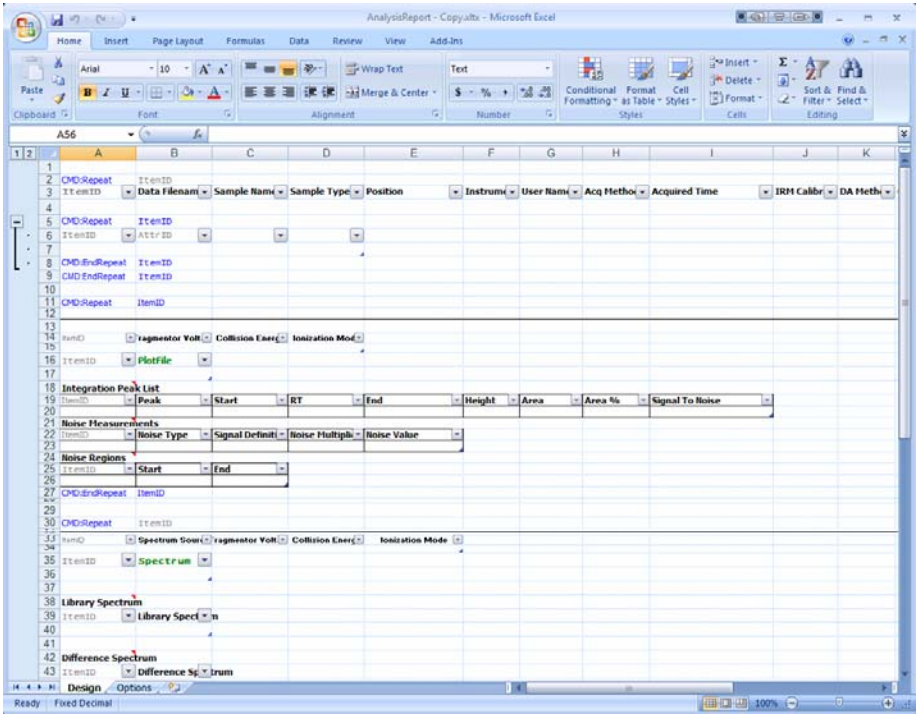
1 Go to the folder that contains the report templates. By default, this folder is **\MassHunter\Report Templates\Qual\B.05.00\en-US\Letter**. You can select a different folder in the Method Explorer in the General > Common Reporting Options > Templates tab.

2 Make a copy of the template which you intend to modify. Right-click the copy and click **Properties**. If necessary, clear the **Read-only** check box. Then, right-click the copy and click **Open** from the shortcut menu.



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**.

www.agilent.com

In This Book

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

Revision A, September 2011

G3336-90018

Agilent Technologies