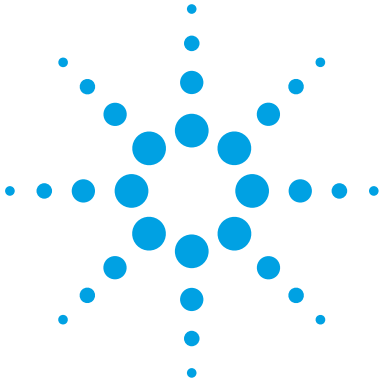


Agilent MassHunter Workstation – Data Acquisition for 6400 Series Triple Quadrupole LC/MS

Familiarization Guide

Agilent Technologies

Use the exercises in this guide to learn how to use the Agilent 6400 Series Triple Quad LC/MS. You can do these exercises with the demo data files, SulfaDrugs, shipped with the system (in the **Data** folder of your Qualitative Analysis installation disk), or with data you acquire.

In Exercise 1, you learn how to determine the best acquisition settings for analyzing your compounds of interest. These instructions help you understand not only how to set up a worklist to optimize instrument parameters for best sensitivity in acquisition, but also how to use the Agilent MassHunter Qualitative Analysis program to identify parameter values producing optimum signal response. You can also learn about the Qualitative Analysis program by using the *Qualitative Analysis Familiarization Guide* or the Qualitative Analysis *online Help*.

In Exercise 2, you learn how to use either an acquired data file or the Quantitative Analysis report results to update a Dynamic MRM method. This method allows you to easily set up a Dynamic MRM method.

In Exercise 3, you learn how to create a triggered Dynamic MRM method.

In Exercise 4, you learn how to use two programs to optimize parameters. Agilent MassHunter Optimizer helps you optimize acquisition parameters. Specifically, it automates the selection of the best precursor ion and the fragmentor voltage for the most abundant precursor ion, selection of the best product ions, and optimization of collision energy values for each transition for a list of compounds you specify. Agilent Source and iFunnel Optimizer helps you to find the optimal source and iFunnel parameters.

NOTE See the *Concepts Guide* to learn more about how the triple quadrupole mass spectrometer works and why the fragmentor and collision energy voltages are important. For background information, see Chapter 3, "Triple Quadrupole MS and Sensitivity", in the *Concepts Guide*. See the *online Help* for detailed information on how the program works.

Each task 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.

Before you begin

Before you begin, you need to check that your system is ready. If you plan to acquire data, you also need to set up the instrument.

Prepare your system

1 Check that:

- The Data Acquisition program has been installed.
- The LC modules and the 6400 Series Triple Quad LC/MS have been configured.
- The performance has been verified.
- The system has been turned on.

If these actions have not yet been done, see the *Installation Guide* for your instrument.

2 Copy the data files to your PC.

Copy the folder named **SulfaDrugs** in the **Data** folder on your Qualitative Analysis installation disk to any location on your hard disk. This folder contains all the data files needed for this exercise.

NOTE Do not re-use the sulfa drug 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. Data files that are already on the system may contain processed results, leading to different behavior during the exercises in this guide.

Prepare to acquire data

If you do not intend to acquire data but want to learn how to use the Qualitative Analysis program for method development, you can skip this step, which tells you how to prepare the demo sample. You then do those tasks that show you how to use the Qualitative Analysis program with the sulfa drug data files shipped with the system.

Parts List

The exercise in this guide uses this equipment and materials:

- Agilent 1200, 1260 Infinity or 1290 Infinity LC modules: well-plate sampler, binary pump, thermostatted column compartment, DAD
- Zorbax column (see Table 1 on page 4)
- A 1 ng/µL concentration of the sulfa mix sample (prepared in this step)

Table 1 Zorbax column

Triple Quadrupole	Column Description	Particle Size	Pore Size	Part Number
6410B, 6420, 6430, 6460, 6490, and 6495	RRHD Eclipse Plus C18.2.1 mm x 50 mm	1.8 µm	95Å	959757-902

1 Prepare the LC solvents.

For the A channel, add 1 mL of 5M ammonium formate to a 1-liter reservoir filled with HPLC-grade water.

For the B channel, add 1 mL of 5M ammonium formate to a 1-liter reservoir filled with 90:10 acetonitrile and HPLC-grade water.

2 Prepare the sample.

a Add 10 µL of the sulfa mix from one of the ampoules (500 µL) to 990 µL of solvent A in a 2 mL glass sample vial so that the final concentration is 1 ng/µL.

b Cap the vial and place in a sample location in the autosampler.

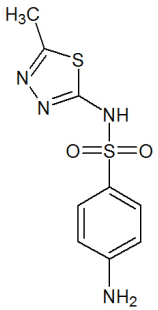
3 Set up the LC column.

Use the column from Table 1. Other columns and instrument parameters may be used in these exercises, but some parameters may need adjustment, and the results will differ.

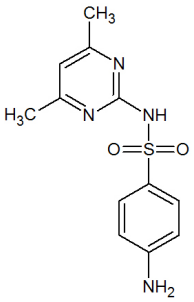
4 Set the column temperature to 60°C. Lower temperatures may be used; however, the retention times will be longer, and the pump pressure may exceed the limit of some LC systems.

The Electrospray LC Demo Sample (P/N 59987-20033) contains five ampoules with 100 ng/µL each of sulfamethizole $(M+H)^+$ = 271, sulfamethazine $(M+H)^+$ = 279, sulfachloropyridazine $(M+H)^+$ = 285, and sulfadimethoxine $(M+H)^+$ = 311.

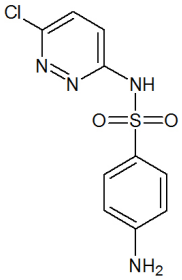
Sulfamethizole



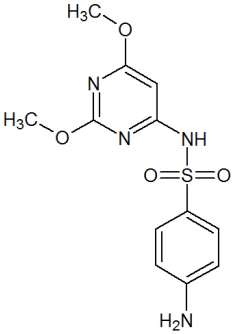
Sulfamethazine



Sulfachloropyridazine



Sulfadimethoxine



NOTE

Determining optimal parameter values for acquiring sample compound data requires that the Triple Quadrupole instrument already be tuned on the Tuning Mix calibrant ions. Before proceeding with this exercise, make sure you have used Checktune or Autotune to verify that calibrant ions each have the proper mass assignment, peak width, and signal intensity.

See the *Quick Start Guide, Installation Guide* or *online Help* for instructions on tuning the instrument.

Exercise 1 – Develop an acquisition method

For this exercise you analyze a mixture of four sulfonamide compounds. These tasks show you how to manually select the acquisition parameters including using the Qualitative Analysis program to analyze the data files. You can instead use the automated process to select some of the acquisition parameters. See "Exercise 4 – Optimize Acquisition parameters" on page 58 to learn how to automate this process.

Task 1. Enter acquisition parameters and acquire data

In this exercise, you enter the conditions for the analysis of the sulfa drug mix.

Steps	Detailed Instructions	Comments
1 Enter LC parameters appropriate for sulfa drug mix. See Table 2.	**a** Double-click the **Data Acquisition** icon. **b** Make sure that **Acquisition** appears as the selection in the **Context** text box. If Tune is the selection, click **Acquisition** from the **Context** dropdown menu in the Combo bar. **c** Enter the LC parameters listed in the Table 2.	• The Data Acquisition window appears. See Figure 1 on page 8.

Table 2 LC parameters for sulfa drug mix

Parameter	LC Parameter
PUMP	
• Flowrate	800 µL/min
• Solvent A	5 mM ammonium formate in water
• Solvent B	5 mM ammonium formate in 90:10 acetonitrile:water
• Gradient (min - %B)	0 min - 13% 1.80 min - 60% 2 min - 60%

Table 2 LC parameters for sulfa drug mix (continued)

Parameter	LC Parameter
• Stop Time	2.5 min
• Post Time	3.0 min
INJECTOR	
• Inj. Vol.	2.0 µL
• Injection	Standard
• Draw Position	0.0 mm
UV DETECTOR	
• Ch A	254 nm (4 nm BW on DAD)
• REF A (DAD only)	400 nm (80 nm BW)
COL THERM	
• Temp	60 °C for the 6460, 6490 and 6495 with Agilent Jet Stream Technology 40 °C for other instruments

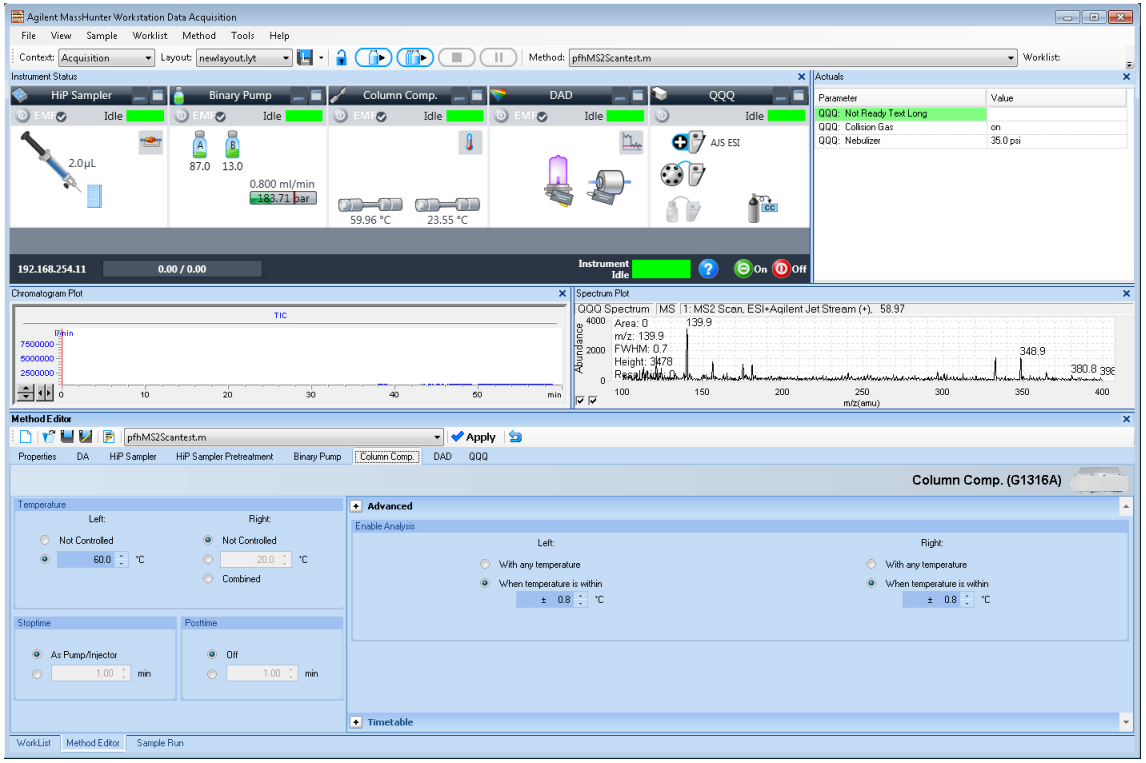


Figure 1 MassHunter Workstation – Data Acquisition window

Steps	Detailed Instructions	Comments
2 Enter MS parameters appropriate for sulfa drug mix and save the method as ***iii*MS2Scantest.m**, where ***iii*** are your initials. See Table 3 on page 9.	**a** Click the **QQQ tab** in the **Method Editor** window. **b** Select **MS2Scan** from the **Scan Type** list in the Time Segments table. **c** Enter the other MS parameters as listed in Table 3. These parameters are in either the Acquisition or the Source tabs. **d** Save the method as ***iii*MS2Scantest.m**, where ***iii*** are your initials.	

Table 3 MS parameters for sulfa drug mix

Parameter	Value (ESI)	Value (AJS ESI)
• Inlet	ESI (positive polarity)	AJS ESI (positive polarity)
• Scan Type	MS2Scan	MS2Scan
• Delta EMV pos	400 V	200 V
• Mass Range	100 to 400	100 to 400
• Cell Acceleration Voltage	7 V	7 V
• Gas Temp	350 °C 250 °C for 6490 and 6495 QQQ	350 °C 250 °C for 6490 and 6495 QQQ
• Gas Flow	12 L/min 14 L/min for 6490 and 6495 QQQ	10 L/min 14 L/min for 6490 and 6495 QQQ
• Nebulizer	50 psi	35 psi
• Sheath Gas Temperature	not applicable	400 °C
• Sheath Gas Flow	not applicable	12 L/min
• Nozzle Voltage	not applicable	0 V
• Capillary Voltage positive	4000 V	4000 V
• Fragmentor	100 V (not adjustable on 6490 nor 6495, comes from the Tune file)	100 V (not adjustable on 6490 nor 6495, comes from the Tune file)

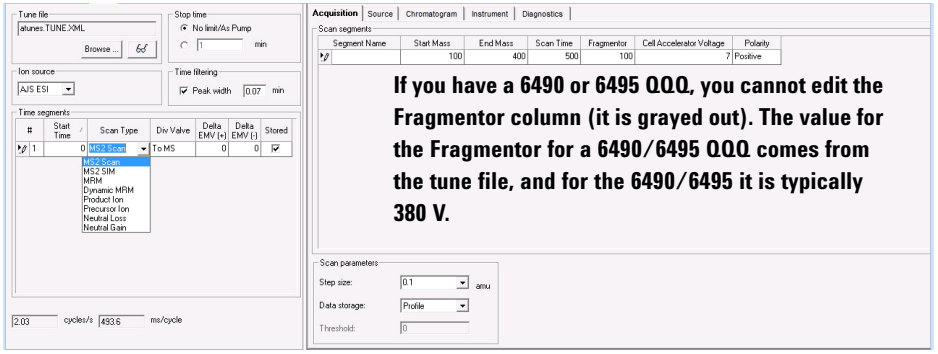
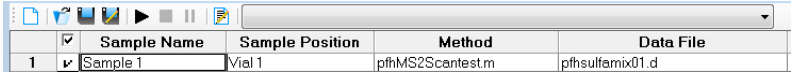


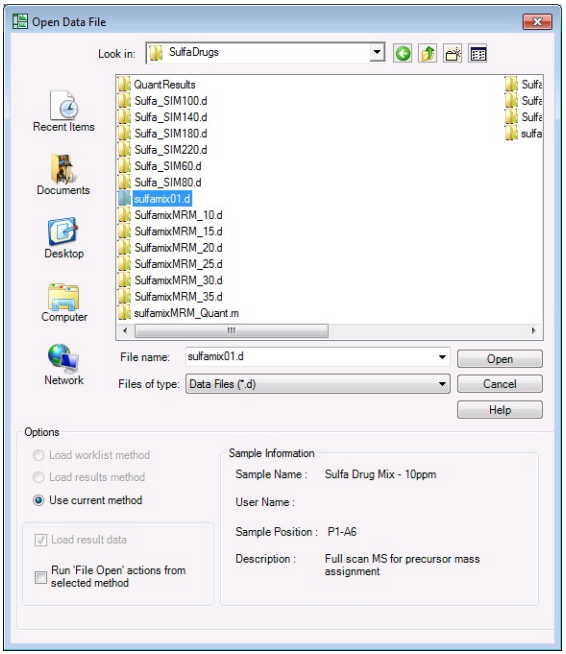
Figure 2 Select **Scan Type** of MS2 Scan in the QQQ tab

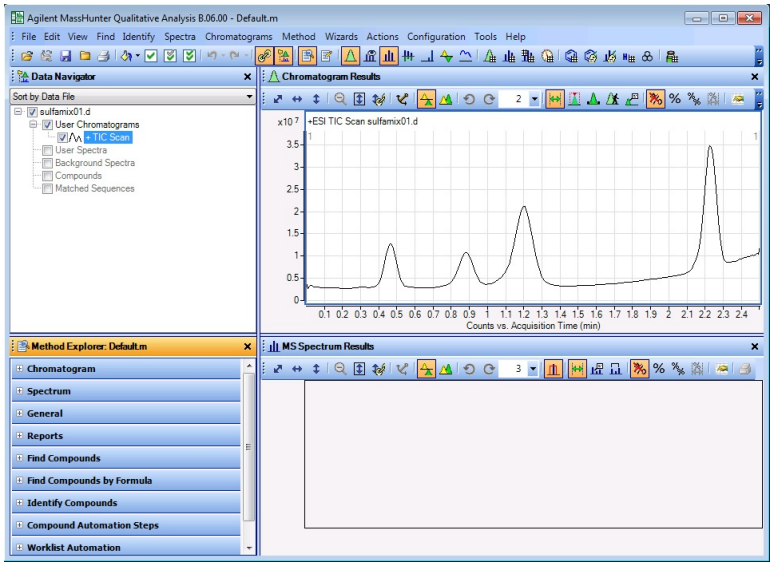
Steps	Detailed Instructions	Comments
3 Acquire data (optional). • Set up a one-line worklist with the method you just created. • Name the data file ***iii*sulfamix01.d**, where ***iii*** are your initials. • Designate a directory path to hold your data files and method.	**a** If necessary, click **View > Worklist** to display the Worklist window. **b** Click **Worklist > Worklist Run Parameters.** Verify that the parameters are set properly. Click **OK**. **c** Click **Worklist > Add Multiple Samples**. **d** Type `iiisulfamix01.d` as the data file name **e** Select **iiiMS2Scantest.m** as the method name. **f** Click the **Sample Position** tab. **g** Select the Autosampler, Well-plate or Vial Tray. **h** In the graphic, select a single position. Click **OK.** **i** In the Worklist window, mark the check box to the left of the sample.  **j** Click the **Start Worklist Run** icon in the main toolbar, the **Run Worklist** icon in the Worklist toolbar or click the **Worklist > Run** command.	• The Worklist window is tabbed with the Method Editor window by default. Click the **Worklist** tab to show the Worklist window. • The **Number of samples** is set to 1. • You have just acquired a full scan MS data file to see what ions are being formed from the sample. • This step is optional because you can perform the next step with an example data file that comes with the program. If you prefer, you can create your own data file as described in this step.

Task 2. Determine precursor ion masses

In this exercise, you determine the precursor ions for each of the sulfa drugs in the acquired data file.

Steps	Detailed Instructions	Comments
1 Open the acquired data file. • In the Qualitative Analysis program, open either the example file, **sulfamix01.d,** or the data file you created in "Task 1. Enter acquisition parameters and acquire data" on page 6.	**a** Double-click the **Qualitative Analysis** icon.  The program displays the "Open Data File" dialog box.	• When you open the sulfa drug directory after installation, the **Load result data** (lower left corner) check box is grayed out. • If you see the check box marked, this means that the data file(s) already contains results. ***Clear this check box before opening the file***.

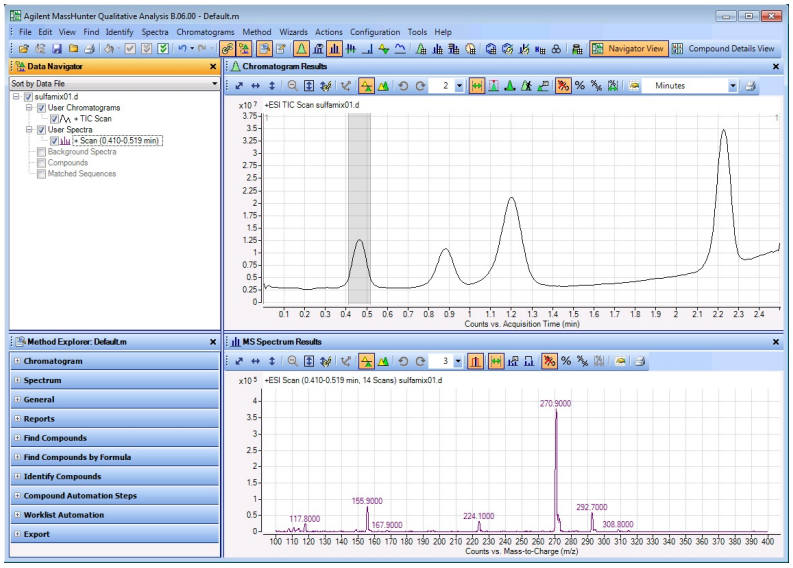


Steps	Detailed Instructions	Comments
	b Do one of the following: • Select the example data file **sulfamix01.d**, and click **Open**. • Select the data file you created in "Task 1. Enter acquisition parameters and acquire data" on page 6, and click **Open**. By default, the system displays the Total Ion Chromatogram (TIC).	• The figure below shows the default layout. • The Qualitative Analysis program displays a newly opened data file with the same layout and display settings used for the previous data file. Therefore, you MUST make sure to return to the default settings for this exercise.
		Before you begin, make sure that all previous settings are returned to their default values: • Restore default layouts ◦ Click **Configuration > Window Layouts > Restore Default Layout**. • Make sure the method is **default.m**. (see title bar) ◦ Click **Method > Open**. ◦ Select **default.m**, and click **Open**. • Return display options to default settings. ◦ In the **Configuration** menu, click each of the **Display Options** commands. ◦ Click **Default**, and then click **OK**. Or... • Restore the General layout. ◦ Click **Configuration > Configure for Workflow > General**. ◦ Click **OK**. ◦ (optional) Save method changes if needed. • Return display options to default settings. ◦ In the **Configuration** menu, click each of the **Display Options** commands. ◦ Click **Default**, and then **OK**.

Steps	Detailed Instructions	Comments
2 Determine precursor ion masses for all four peaks. • You have determined them correctly if you find the values are similar to those shown in this table:	**a** In the Chromatogram Results window, make sure that the Range Select icon in the toolbar  is on. **b** Click the left mouse button and drag the cursor across the first peak to produce a shaded region, as in the figure below. **c** Right-click the shaded area, and click **Extract MS Spectrum** from the shortcut menu.	• The system displays an averaged spectrum across the peak in the MS Spectrum Results window. • The precursor mass of the first compound, sulfamethizole, is determined to be m/z 270.9. • To obtain a single scan, double-click the apex of the peak.

Compound	RT	*m/z*
Sulfamethizole	0.47	270.9
Sulfachloropyridazine	0.88	284.9
Sulfamethazine	1.20	279.0
Sulfadimethoxine	2.23	311.0

• If you acquired the data file using the Agilent Jet Stream Technology, the retention times may be different.
• The sulfamix01.d data file was acquired with a different column so your retention times are different.
• Close the data file after finding the precursor ion masses.



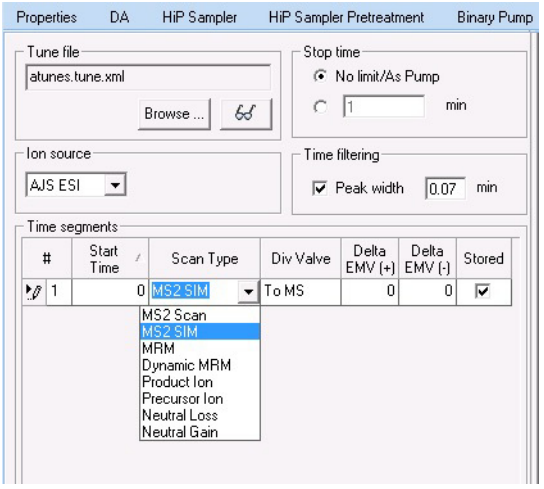
d Repeat step a through step c for the other compounds.
The precursor ion masses should match those in the table in step 2.
e Click **File > Close Data File**.
f When asked if you want to save the results, click **No**.

• Some compounds form sodium (Na) and/or potassium (K) adducts as well, corresponding to M + 23 and M + 39 masses respectively. Seeing these masses along with the M + H can make for an easy confirmation of which ion is the pseudo-molecular ion (M + H)+.

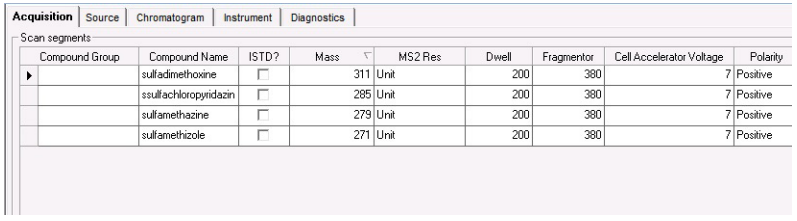
Task 3. Find optimum fragmentor voltage for maximum response

Task 3 shows you how to carry out the optimization for fragmentor voltage by creating selected ion-monitoring experiments for each compound within a method and setting up multiple methods with varying fragmentor voltages.

The Fragmentor Voltage for the 6490 and 6495 is set automatically during Autotune, and it cannot be set in the Data Acquisition program. If your instrument is a 6490 or a 6495, skip to "Task 4. Determine product ion masses". You can do the Qualitative Analysis part of this task by using the data files that were shipped with the software.

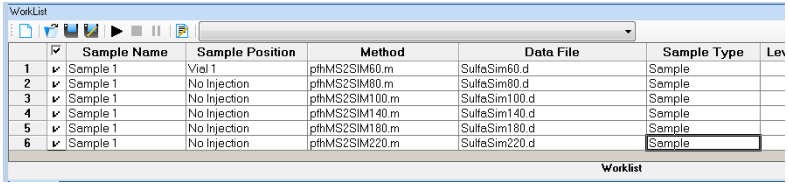
Steps	Detailed Instructions	Comments
1 Set up six methods for six different fragmentor voltages. • Change to a SIM experiment. • Use 60, 80, 100, 140, 180 and 220 volts as the fragmentor voltages for the six methods. • Save the methods as ***iii*MS2SIM*xxx*.m**, where ***iii*** are your initials and ***xxx*** is the voltage.	**a** In the **Scan Type** dropdown list, click **MS2 SIM**. 	

Steps	Detailed Instructions	Comments
	b In the **Acquisition** tab, enter the **Compound Name** and **Mass** (precursor ion mass) for sulfadimethoxine (311 *m/z*). **c** Right-click anywhere in the Scan segments section, and click **Add Row**. **d** Type the **Compound Name** and the **Mass** for sulfachloropyridazine (285 *m/z*). **e** Repeat steps c and d for sulfamethazine (279 *m/z*) and sulfamethizole (271 *m/z*). **f** Save the method as ***iii*MS2SIM140.m**, where ***iii*** are your initials**.** **g** Change the fragmentor voltage to 60, and save the method as ***iii*MS2SIM060**, where ***iii*** are your initials. **h** Repeat step g for voltages 80, 100, 180 and 220, saving the methods as ***iii*MS2SIM080, *iii*MS2SIM100, *iii*MS2SIM180 and *iii*MS2SIM220,** where ***iii*** are your initials.	• With the MS2SIM Scan Type set, a different set of columns appears in the Acquisition window. • The Instrument Control and Data Acquisition program creates a SIM experiment for each compound mass, starting with a default fragmentor voltage of 140. See the example below. • The Fragmentor column is grayed out if the instrument type is a 6490 or 6495.



Compound Group	Compound Name	ISTD?	Mass	MS2 Res	Dwell	Fragmentor	Cell Accelerator Voltage	Polarity
	sulfadimethoxine	☐	311	Unit	200	380	7	Positive
	ssulfachloropyridazin	☐	285	Unit	200	380	7	Positive
	sulfamethazine	☐	279	Unit	200	380	7	Positive
	sulfamethizole	☐	271	Unit	200	380	7	Positive

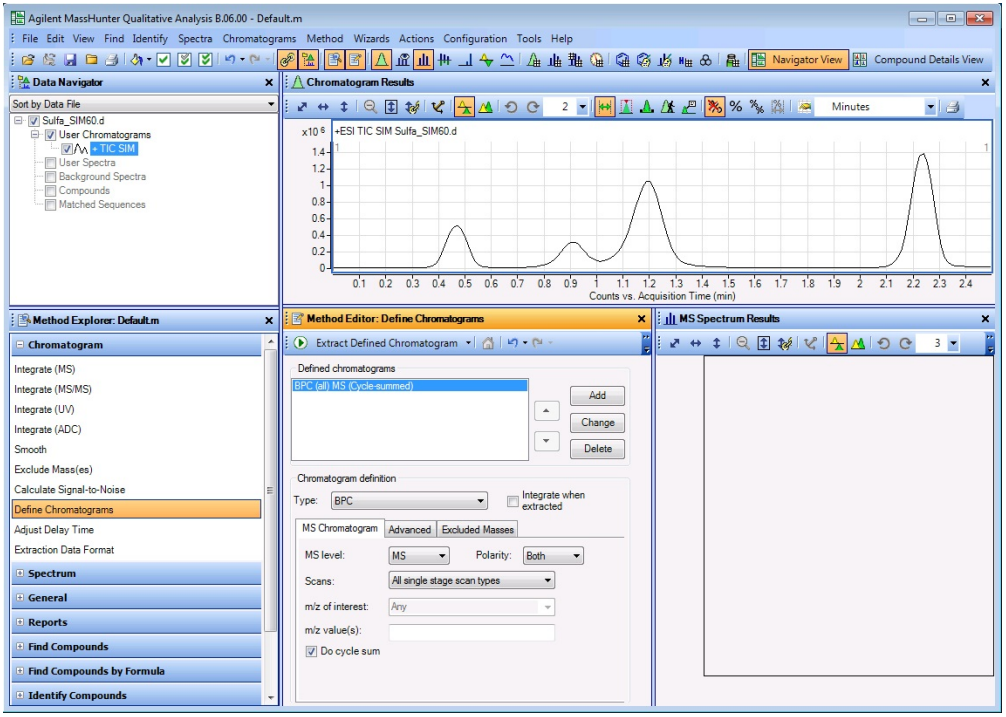
Steps	Detailed Instructions	Comments
2 Set up and run the worklist (optional). • Set up six samples with Sample Name SulfaDrugMix to inject 1ul from vials 1-6 or the ones you choose. • Specify the data files as ***iii*SulfaSIM*xxx*.d**, where ***iii*** are your initials and ***xxx*** is the voltage.	**a** Click the **Worklist** icon if necessary to make sure the worklist is visible. **b** Click **Worklist > New** to start a new worklist. You do not need to save the last worklist. **c** To set up the run, right-click the upper left corner of the worklist, and click **Worklist Run Parameters**. **d** Type the paths for the method and data files. **e** Type the information for the 60 voltage run. **f** Click **Worklist > Add Sample**. Another sample is added to the Worklist. Add five samples to the worklist for voltages 80-220. **g** Mark the checkbox to the left of the Sample Name for each of the six samples.	• This step is optional because you can use data files shipped with the system to perform many of the tasks in this exercise.



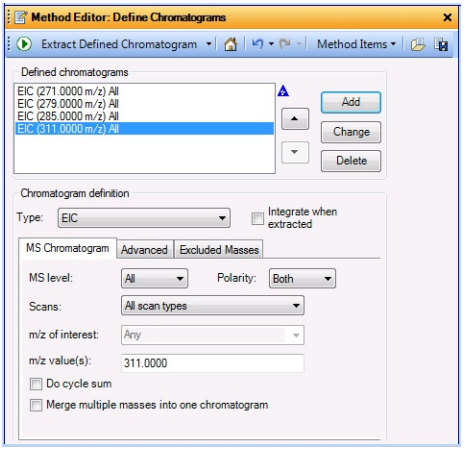
		Sample Name	Sample Position	Method	Data File	Sample Type	Lev
1	✔	Sample 1	Vial 1	pthMS2SIM60.m	SulfaSim60.d	Sample	
2	✔	Sample 1	No Injection	pthMS2SIM80.m	SulfaSim80.d	Sample	
3	✔	Sample 1	No Injection	pthMS2SIM100.m	SulfaSim100.d	Sample	
4	✔	Sample 1	No Injection	pthMS2SIM140.m	SulfaSim140.d	Sample	
5	✔	Sample 1	No Injection	pthMS2SIM180.m	SulfaSim180.d	Sample	
6	✔	Sample 1	No Injection	pthMS2SIM220.m	SulfaSim220.d	Sample	

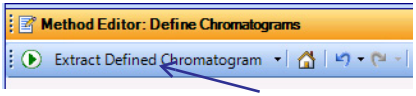
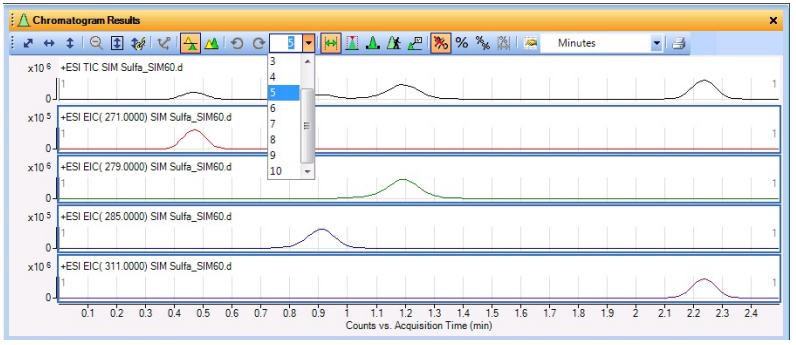
Steps	Detailed Instructions	Comments
	h Start the worklist. • Click **Worklist > Run**. • Click the  icon in the main toolbar. • Click the  icon in the worklist toolbar.	• Note that the program only runs those samples that are marked with a checkmark. • You can also run the worklist in locked mode by clicking the lock button in the main toolbar (the icon looks like this icon when it is locked). 

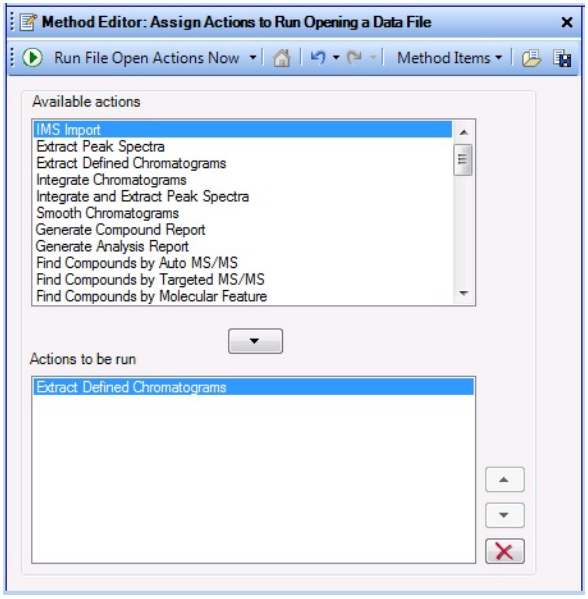
Steps	Detailed Instructions	Comments
3 Set up a qualitative method to view the EIC data automatically. • Open the data file **Sulfa_SIM60.d** or your own ***iii*Sulfa_SIM60.d**, where ***iii*** are your initials. • In the Method Editor, add in the EICs corresponding to the precursor ion masses of 271, 279, 285, and 311. • Save the method as *iii*Exercise1, where "*iii*" are your initials.	**a** Click **File > Open Data File**. The system displays the Open Data File dialog box **b** Select either **Sulfa_SIM60.d** or ***iii*Sulfa_SIM60.d**, and click **Open**. **c** Click **Method > Method Editor** or **View > Method Editor**. The system displays the Method Editor window.	• The Qualitative Analysis program should be open. If not, see "Double-click the Qualitative Analysis icon." on page 11. • By default, the Method Editor is a floating window. The window is docked for these images. See the *online Help* for more information on floating and docking windows.



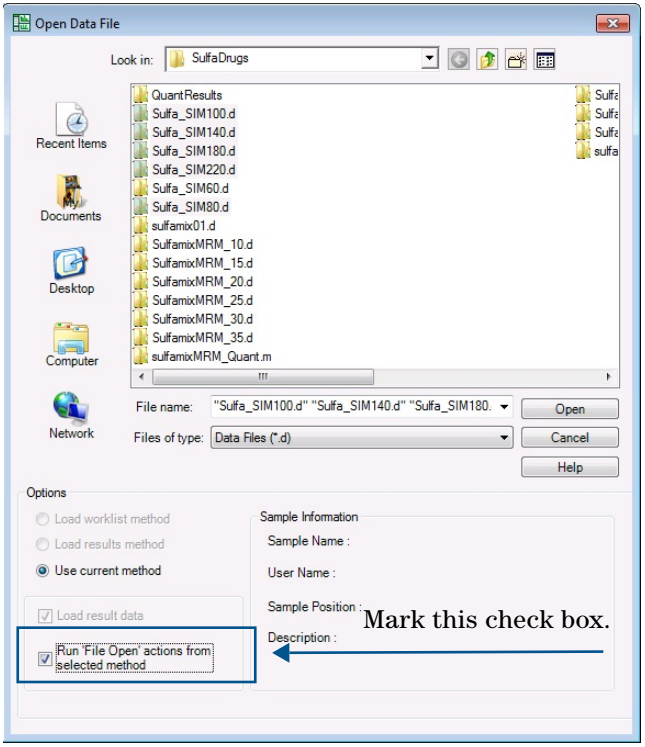
Steps	Detailed Instructions	Comments
	d If necessary, click **Define Chromatograms** in the Chromatogram section of the Method Explorer. **e** To delete the BPC chromatogram, click **Delete** in the Method Editor window. **f** Select **EIC** for the **Chromatogram Definition Type**, **g** In the MS Chromatogram tab, make sure **MS Level** is set to **All** and **Scans** is set to **All scan types**. **h** Clear the **Do cycle sum** check box. **i** Type `271` as the **m/z value**. **j** Click **Add**. **k** Repeat steps i and j for the other precursor ions, `279`, `285` and `311`. **l** Click **Method > Save As**. The system opens the Save As dialog box **m** Save the method as ***iii*Exercise 1.m**. **n** Click **Save**.	• The default Method Editor list selection after installation is **Integrate (MS)**. • You can also select Define Chromatograms from the Method Items list in the Method Editor window. • When you are defining chromatograms, instead of deleting the Defined chromatogram, you can select EIC. Then, you enter the m/z value and click the **Change** button.



Steps	Detailed Instructions	Comments
4 Extract the chromatogram for the data file and view the results. • Make sure you can see all five chromatograms, the TIC and four EICs.	**a** Click the **Run** button on the Method Editor toolbar to run the **Extract Defined Chromatogram** command.  **b** To see the TIC and four EICs, click the arrow next to the Maximum Number of List Panes icon in the Chromatogram Results toolbar, as shown in the example below. **c** Select **5** to view five chromatograms simultaneously. The system displays chromatogram results as shown below.  	• You can also click the **Chromatograms > Extract Defined Chromatograms** command to extract the defined chromatograms.

Steps	Detailed Instructions	Comments
5 Extract the remaining ion chromatograms automatically. • Extract Defined Chromatograms should be the default action for Assign File Open Actions. • Open the remaining data files, Sulfa_SIM80.d through Sulfa_SIM220.d. • Close the Method Explorer.	**a** Select **File Open Actions** from the General section in the Method Explorer. **b** Make sure that **Actions to be run** list only contains **Extract Defined Chromatograms.**  **c** Click **File > Open Data File.** The system displays the Open Data File dialog box. **d** Select the data files to be opened, *Sulfa_SIM80.d* through *Sulfa_SIM220.d*. **e** Mark the **Run 'File Open' actions from selected method** check box. (lower left corner)	• The Qualitative Analysis Method Editor lets you define actions to be performed automatically upon opening a data file(s).

Steps	Detailed Instructions	Comments

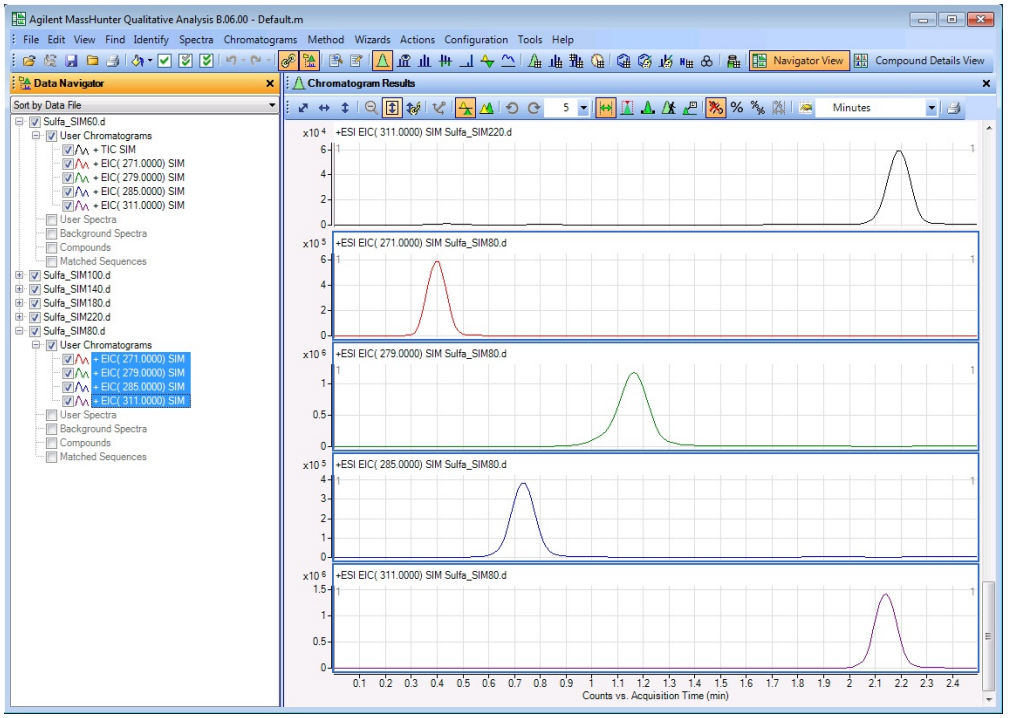


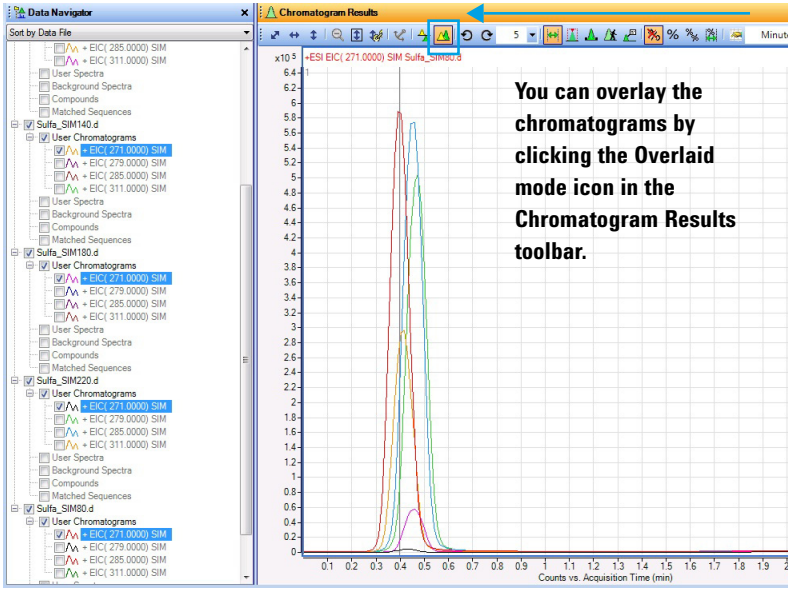
f Click **Open.**
The Qualitative Analysis program displays all the EICs for all the data files selected.

g To close the Method Explorer, Method Editor and MS Spectrum Results windows, click the **X** in the upper right corner of each window.

- You can instead click **View > Method Explorer**, **View > Method Editor,** and **View > MS Spectrum Results**.

Steps	Detailed Instructions	Comments

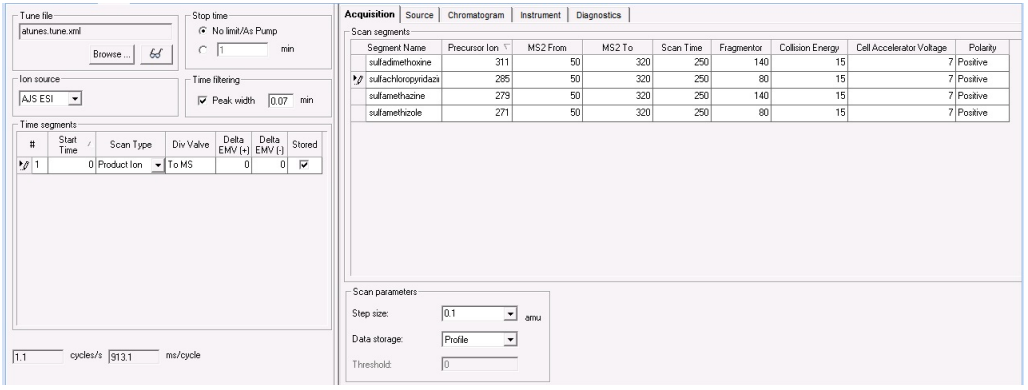


Steps	Detailed Instructions	Comments
6 Select the fragmentor voltage that produces the maximum response for each of the precursor ions. • Close the data files after you determine the optimum voltage.	**a** In the Data Navigator window, highlight the EICs for 271.0 *m/z*. **b** Click the **Show only the highlighted items** icon, . Only the 271 m/z check boxes are now marked. **c** Look at the relative intensities of each peak to determine which fragmentor voltage setting will be best to use for the 271 precursor.	• You press the **Ctrl** key to be able to select multiple objects from the Data Navigator window. • You press the **Shift** key to be able to select a group of objects. • A fragmentor voltage of 100 should be sufficient for each precursor ion. • You can now determine the product ions that are available for the multiple-reaction monitoring experiments to maximize sensitivity
		
d Repeat step a through step c for the other three base peaks or precursor ions. **e** Click **File > Close All**. **f** Click **No** in the Save dialog box.		• You click **Method > Save** or **Method > Save As** to save the method changes. • Click an EIC in the Data Navigator window to change which chromatogram is labeled in the Chromatogram Results window. When the chromatogram label color matches the color of the chromatogram that has the highest intensity, you use the Fragmentor voltage that was used for that file.

Task 4. Determine product ion masses

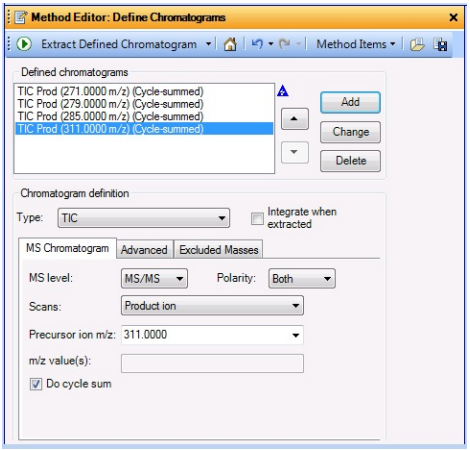
In this part of the method development, we will use three collision energies to determine the best fragment ions to use for the eventual Multiple Reaction Monitoring (MRMs) acquisition.

Steps	Detailed Instructions	Comments
1 Set up three product ion acquisition methods and acquire data. • Use the MS parameters in the example below, but change the Fragmentor voltage to the optimum voltage you determined in the previous task. • Save methods as ***iii*Sulfamix PI_*xx*.m**, where ***iii*** are your initials and ***xx*** is the collision energy.	**a** Click the **QQQ** tab in the Method Editor pane. **b** Select **Product Ion** in the **Scan Type** combo box to scan each precursor ion for all its product ions. **c** Enter all MS parameters as listed in the example below, making sure the **Collision Energy** is set to `15` and the Fragmentor voltage is set to the optimum voltage determined in Task 3. **d** Save the method as ***iii*Sulfamix PI_15.m**. **e** Repeat step c and step d for collision energies of 30 and 45.	• When you change the **Scan Type** in the **Time Segments** table, the **Scan segments** table is reset. If you want to copy the **Scan segments** to the new **Scan segments** table, highlight all of the lines in the **Scan segments** table and then right-click the Scan segments table and click **Copy**. After you select a new Scan Type, right-click the Scan segments table and click Paste from Clipboard. • You cannot copy and paste the **Scan segments** table between all **Scan Types**.



2 Set up and run the worklist (optional). • Specify the data files as ***iii*Sulfamix PI_*xx*.d**, where ***iii*** are your initials and ***xx*** is the collision energy.	**a** Click the **Worklist** tab. **b** Add three samples to the worklist for collision energies 15, 30 and 45. **c** Mark the check box to the left of the Sample Name for each sample you are adding. **d** Click **Worklist > Run**.	• This step is optional because you can determine the product ion masses from the data files shipped with the system. • Use the instructions in Step 2 of Task 3 to set up the worklist.

Steps	Detailed Instructions	Comments
3 Set up a qualitative method to integrate and extract product ion spectra. • Use the data files **SulfamixPI_*xx*.d,** where *xx* is the collision energy, or your own data files, ***iii*SulfamixPI_*xx*.d**. • Open Method Explorer and Method Editor. • Use TICs set up for MS/MS, product ion and each of the precursor ions 271, 279, 285, 311. • Make sure the MS/MS integrator has been selected and the maximum number of peaks has been limited to the largest 100 peaks. • Add the ability to integrate and extract peak spectra to the file actions run upon data opening. • Save the changes to the current method.	**a** Click the **Open Data File** icon in the toolbar. **b** Select **SulfamixPI_15.d**. **c** Clear the **Run File Open Actions from Specified Method** check box, and click **Open**. **d** Make sure the Method Explorer and the Method Editor windows are displayed; otherwise, click the **Method Explorer** and then **Method Editor** icons.  **e** In the Chromatogram section in the Method Explorer window, select **Define Chromatograms.** **f** Delete any existing chromatograms in the **Defined Chromatograms** list. **g** Select **TIC** from the **Type** list in the **Define chromatograms** section. **h** For **MS level**, select **MS/MS**. **i** Mark the **Do cycle sum** check box. **j** For **Scans**, select **Product ion**. **k** For **Precursor ion m/z**, type `271`. **l** Click the **Add** button. **m** Repeat steps j and k for each ion.	• The Qualitative Analysis program should already be open and contain *iii*exercise 1.m as the method.



Steps	Detailed Instructions	Comments
	n From the Method Explorer in the Chromatogram section, click **Integrate (MS/MS)**. **o** Select **MS/MS** as the **Integrator selection**, if necessary.	• These data files contain MS/MS data, so you need to modify the parameters in the Integrate (MS/MS) section. If the data file contained only MS data, you would need to modify the parameters in the Integrate (MS) section.

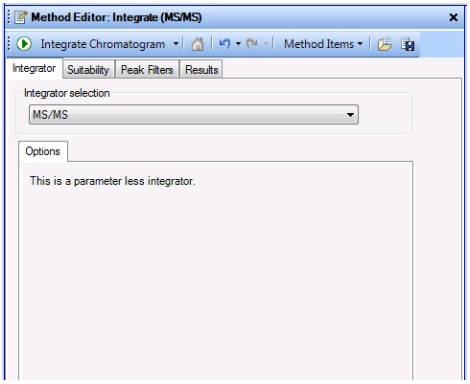


Figure 3 Integrate (MS/MS) > Integrator Tab

p Click the **Peak Filters** tab. Make sure that the **Limit (by height) to the largest** check box is marked and set to the value `100` as shown below.

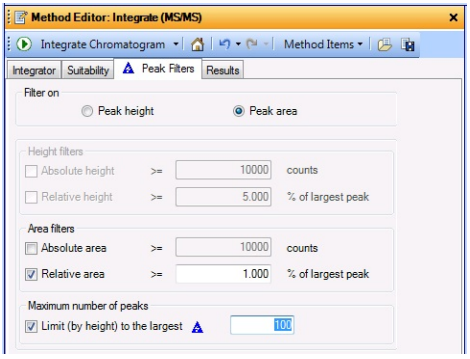


Figure 4 Integrate (MS/MS) > Peak Filters tab

Steps	Detailed Instructions	Comments
	q Click **General** in Method Explorer, and then click **File Open Actions**. **r** Select **Integrate and extract peak spectra** from the Available actions list and click  to add this to **Actions to be run**.	

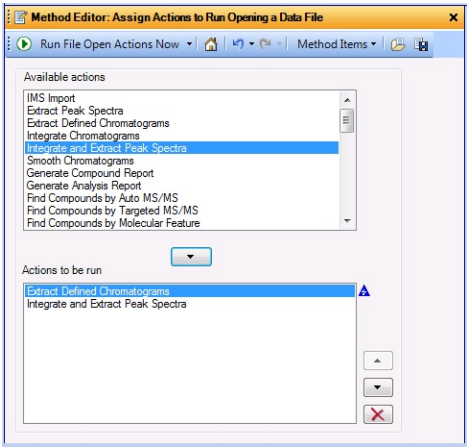


Figure 5 General > File Open Actions tab

	s To apply the changes to the current method, *iii*exercise1.m, click the **Save Method** icon.  You can also click **Method > Save**.	
4 Run the qualitative method on the current data file.	• In the Method Editor toolbar, click the **Run** button,  . When the Assign Actions to Run Opening A Data File section is displayed, the **Actions to be run** list is run.	• The program first extracts the product ion chromatograms for each precursor ion in the data file. • Next, it finds the largest peak in the total ion chromatograms, and integrates and extracts peak spectra from each integrated peak. • See Figure 6 on page 28.

Steps	Detailed Instructions	Comments

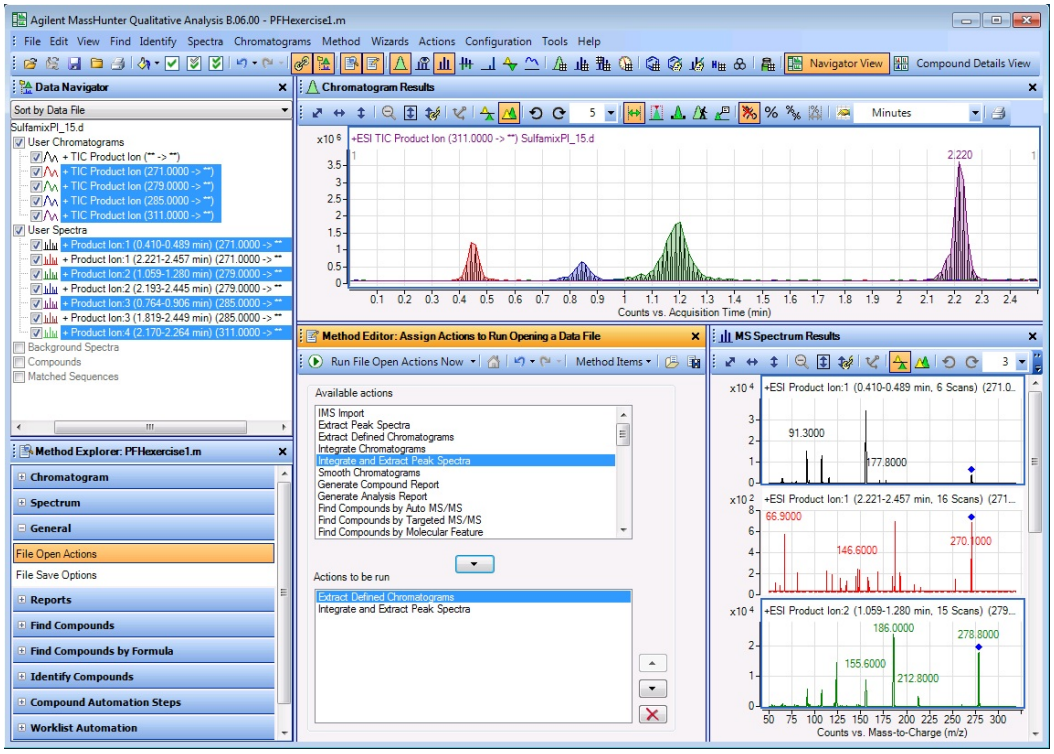


Figure 6 Results for integration and extraction of peak spectra.

5 Run the 'File Open' actions on the remaining product ion data files.

- Use either the example files, **Sulfamix PI_*xx*.d**, or the data files you acquired in step 2.

a Click **File > Open Data File.**
The system displays the Open Data File dialog box.

b Hold the **Ctrl** key and do one of these:

- Select the two data files **Sulfamix PI_30.d,** and **Sulfamix PI_45.d**.
- Select the data files you acquired in step 2.

c Mark the **Run 'File Open' actions from selected method** check box in the Open Data File dialog box, and click **Open**.

- After the data files open, the Qualitative Analysis method first extracts the product ion chromatograms for each precursor ion.
- Next, it integrates each total ion chromatogram and extracts peak spectra from each integrated peak.

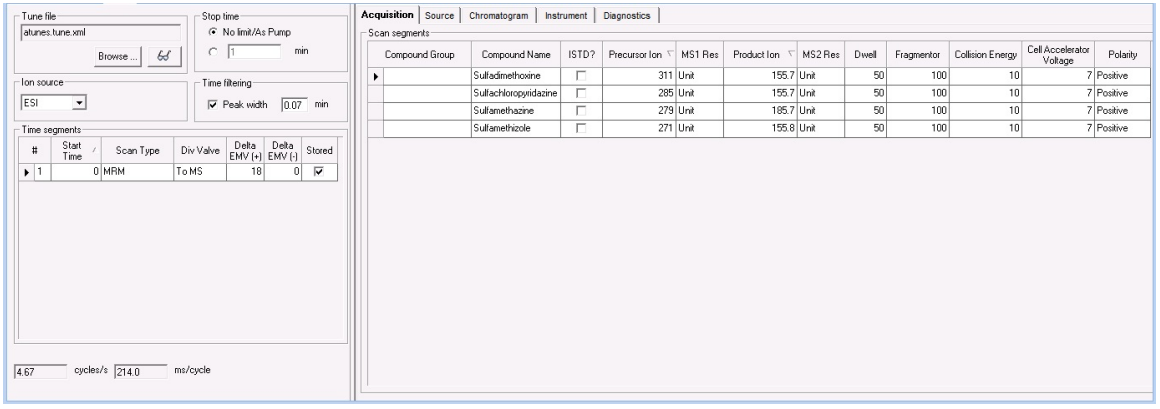
Steps	Detailed Instructions	Comments
6 Identify product ions. • View each set of TICs and spectra individually (e.g., 271 m/z first). • Close the data files.	**a** In the Data Navigator, select the TICs and spectra for the 271 m/z precursor ion. **b** Click the **Show only the highlighted items** icon,  . **c** Click **View > MS Spectrum Peak List 1**. **d** Examine the spectra to see which fragment ions are produced at which collision energies. **e** Repeat steps a to d until all the product ions are identified.	• The m/z 155.7 product ion is the most abundant of any product ion and the highest signal is recorded at 15 V. This means that a good choice for the MRM for sulfamethizole would be 271.0 > 155.7 when the collision energy is around 15 V. • The peak may not be labeled if the peak is too wide.

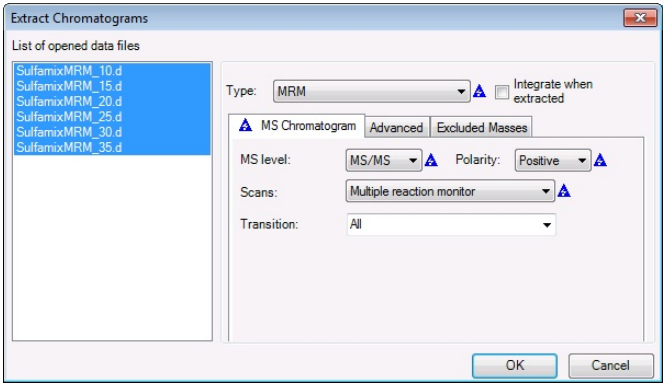
	f Click the **Close Data File** icon in the main toolbar, and click **Close** when the dialog box containing the list of data files pops up.	• The product ions appear to be: Sulfamethizole-271.0 > 155.7 Sulfamethazine-279.0 > 185.7 Sulfachloropyridazine-285.0 > 155.7 Sulfadimethoxine-311.0 > 155.7

Task 5. Find optimum collision energy for MRM acquisition

In this task, you set up MRM acquisition methods for the sulfa drugs for different collision energies. By examining the spectra and comparing peak intensities, you determine the optimal collision energy settings for the compounds.

Steps	Detailed Instructions	Comments
1 Set up three MRM acquisition methods. • Use all the MS parameters in the example below except for the collision energy value. • Use collision energies of 10, 15 and 20. • Save methods as ***iii*Sulfamix MRM_*xx*.m**, where ***iii*** are your initials and ***xx*** is the collision energy.	**a** Click the **QQQ** tab. **b** Set **Scan Type** to **MRM**. **c** Enter all MS parameters shown in the example below except for the collision energy value. **d** In the collision energy column, type `10` for each compound. **e** Save the method as ***iii*Sulfamix MRM_10.m**. **f** Repeat step d and step e for collision energies of 15, 20, 25, 30 and 35 saving the methods as ***iii*Sulfamix MRM_*xx*.m**, where ***iii*** are your initials and ***xx*** is the collision energy.	• Because the largest peaks were produced with a collision energy of 15 in the previous exercise, you will look at only those collision energies to either side of 15.



Steps	Detailed Instructions	Comments
2 Set up and run the worklist (optional). • Specify the data files as ***iii*Sulfamix MRM_*xx*.d**, where ***iii*** are your initials and ***xx*** is the collision energy.	**a** Click the **Worklist** tab to make the worklist visible. **b** Add six samples to the worklist for collision energies 10, 15, 20, 25, 30, 35. **c** Mark the check box to the left of the Sample Name for each of the three samples. **d** Click **Worklist > Run**.	• This step is optional because you can use the six example data files in the next step.
3 Compare the compound transition intensities at different collision energies. • Open the MRM data files: SulfamixMRM_10.d SulfamixMRM_15.d SulfamixMRM_20.d SulfamixMRM_25.d SulfamixMRM_30.d SulfamixMRM_35.d • Set the MRM chromatogram extraction parameters as shown at right for all transitions. • Disable the TICs for clarity and examine the peak intensities. • Compare the intensities of each compound transition obtained at one collision energy with the same compound transition obtained at another collision energy. (Do this in Overlaid Mode with all the MRM chromatograms.) • Close the data files but don't save results. • Refer to Table 4 on page 32 for optimal method settings for each compound.	**a** Open the **Qualitative Analysis** program. **b** Clear the **Run 'File Open' actions...** check box. **c** Open the MRM data files in the Qualitative Analysis program. **d** Right-click the Chromatogram Results window, and click **Extract Chromatograms** from the shortcut menu. **e** To select all data files, click the last file while holding down the **Shift** key. **f** Enter the parameters as listed in the example below, and click **OK**. **g** Clear the TIC check boxes to make the MRM chromatograms easier to view. 	• Why a spectrum for MRM? It's a feature of the program to show spectra even for MRM experiments and can be quite handy for comparing relative intensities of product ions generated from the same precursor. • You can also click **Chromatograms > Extract Chromatograms** to start this dialog box.

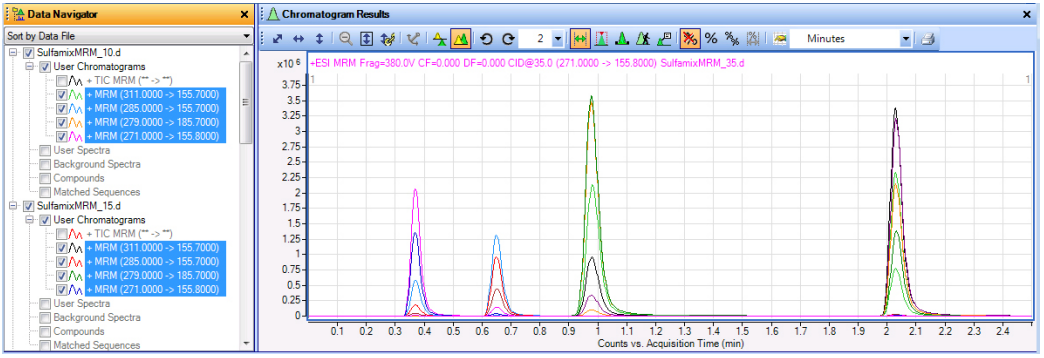
Steps	Detailed Instructions	Comments
	h Click the **Overlaid Mode** icon,  . **i** Compare peak intensities for each compound transition in each data file in the Chromatogram Results window.	• Compare the colors shown in Chromatogram Results with the color next to the MRM transition name in the Data Navigator. • You can also right-click the Chromatogram Results window header and compare the colors of the chromatograms to the colors of the titles in the shortcut menu.
		
	Unless you decide to acquire MRMs at lower collision energies, you should find that the optimal method settings are as shown in Table 4. **j** Click the **Close Data File** icon in the main toolbar, and click **Close** when the Close Data File dialog box appears.	• You now have all the information you need to do an MRM acquisition experiment of the sulfa drug mixture. Consider doing at least one more run with those settings.

Table 4 Compounds and Collision Energy

Compounds	MRM Transition	Collision Energy (V)
Sulfamethizole	271.0 > 155.8	10
Sulfamethazine	279.0 > 185.7	15
Sulfachloropyridazine	285.0 > 155.7	10
Sulfadimethoxine	311.0 > 155.7	15

Exercise 2 – Develop a Dynamic MRM acquisition method from an MRM acquisition data file or an MRM method

The purpose of this exercise is to create a Dynamic MRM method from an acquired MRM data file for sulfamix_MRM data files with the correct retention times for Dynamic MRM using the Quantitative Analysis program.

For this exercise, you have three main tasks:

- "Task 1. Create a batch file from an existing MRM data file" on page 33
- "Task 2. Print a report in the Quantitative Analysis program" on page 36
- "Task 3. Create a Dynamic MRM method using Update dMRM" on page 37

You can easily create a Dynamic MRM method from an existing MRM method.

- "Task 4. Create a Dynamic MRM method from an MRM method" on page 39

Task 1. Create a batch file from an existing MRM data file

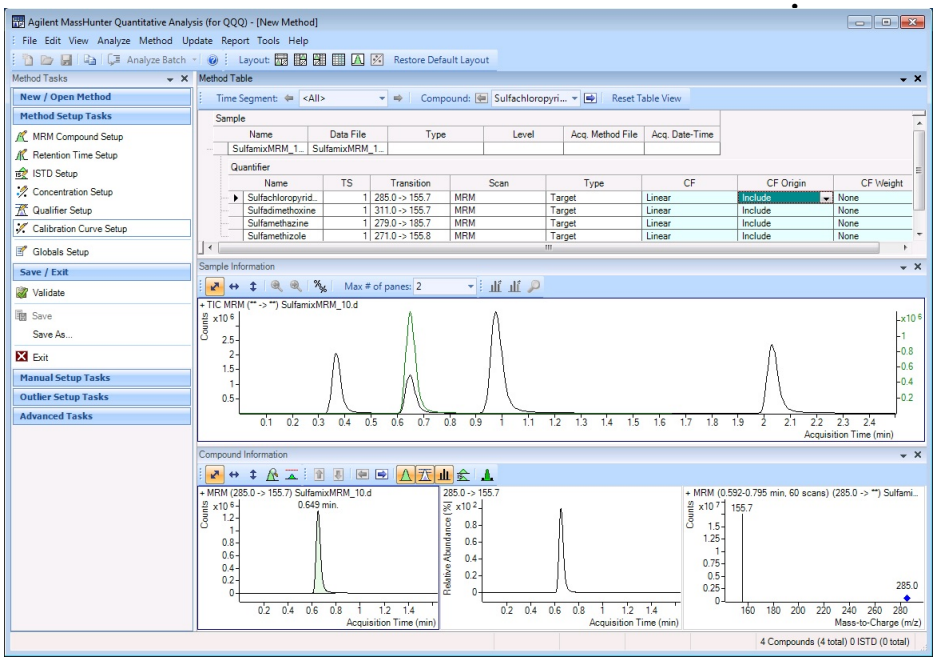
In this exercise, you create a batch and a method from an existing MRM data file.

Steps	Detailed Instructions	Comments
1 Open the Quantitative Analysis program and create a batch file with one sample file, SulfamixMRM_10.d. • Copy the data file SulfamixMRM_10.d from the installation disk to the **\MassHunter\Data\MRM_to_DMRM** folder.	**a** Double-click the **QQQ Quantitative Analysis** icon or the **Drug Quant (QQQ)** icon. **b** Click **File > New Batch**. **c** Navigate to the **\MassHunter\Data\MRM_to_DMRM** folder. **d** Type `MRM_to_DMRM` in the **File name** text box. **e** Click **Open**. **f** If the **Add Samples** dialog box does not open, click **File > Add Samples**. **g** Select the file **SulfamixMRM_10.d**. **h** Click **OK**.	• The file **SulfamixMRM_10.d** is on the installation disk in the **\Support\Data** folder. Copy this entire folder to the **\MassHunter\Data\MRM_to_DMRM** folder.

Exercise 2 – Develop a Dynamic MRM acquisition method from an MRM acquisition data file or an MRM method

Task 1. Create a batch file from an existing MRM data file

Steps	Detailed Instructions	Comments
2 Create a method for that batch using MRM data.	**a** Click **Method > New > New Method from Acquired MRM Data**. **b** Select the **SulfamixMRM_10.d** data file. **c** Click **Open**.	
3 Set the Concentration Setup, Qualifier Setup, and Calibration Curve Setup. • Add calibration level 1 with a concentration of 10000. • Set the **Uncertainty** to Relative for all qualifiers. • Set the **Curve Fit** to Linear. • Set the **Curve Fit Origin** to Include. • Set the **Curve Fit Weight** to None.	**a** Select **Concentration Setup** in the Manual Setup Tasks section in the Method Tasks pane. **b** Select the first compound in the table. **c** Right-click the compound row and click **New Calibration Level** from the shortcut menu. **d** Enter `A1` in the **Level** column and `10` in the **Conc.** column. **e** Right-click in the Level box and click **Copy Calibration Levels To**. **f** Click **Select All**. Click **OK**. **g** Select **Qualifier Setup** in the Manual Setup Tasks section in the Method Tasks pane. **h** Verify that the **Uncertainty** is Relative. **i** Select **Calibration Curve Setup** in the Manual Setup Tasks section in the Method Tasks pane. **j** Set **Curve Fit** to **Linear** for all compounds. **k** Set **CF Origin** to **Include** for all compounds. **l** Set **CF Weight** to **None** for all compounds.	• Refer to the *online Help* in the Quantitative Analysis program for additional help on these tasks. • You can also click **Method > Copy Calibration Levels To** to display the **Copy Calibration Levels To** dialog box. • To enter the same value in all cells in a column, you can change the value in the first row, and then right-click that value in the first row and click **Fill Down**. • The compound names in Quantitative Analysis need to exactly match the Compound Name in the QQQ Acquisition program. If you capitalized the **Compound Name** in the Data Acquisition program, then you need to make sure that the **Name** in the Quantitative Analysis program is also capitalized.

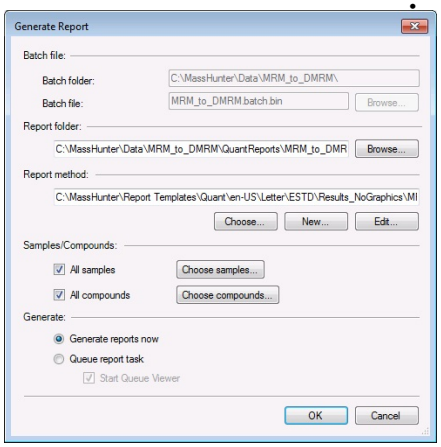
Steps	Detailed Instructions	Comments
		
4 Verify method and then save the method and apply the method to the batch.	**a** Click **Method > Validate.** **b** Click **OK** on the message box. Fix any errors, if necessary. **c** Click **Method > Save As**. **d** Enter `MRM_to_DMRM`. **e** Click the **Save** button. **f** Click **Method > Exit**. **g** (optional) Click **Analyze** in the **Apply Method** dialog box. **h** Click **Yes** to apply the method to the batch.	• In the **Apply Method** dialog box, you can click **Analyze** to automatically start additional batch processing.
5 Analyze and save the batch.	**a** Click **Analyze > Analyze Batch.** **b** Click **File > Save Batch**.	

Task 2. Print a report in the Quantitative Analysis program

In this task, you print a report using any template.

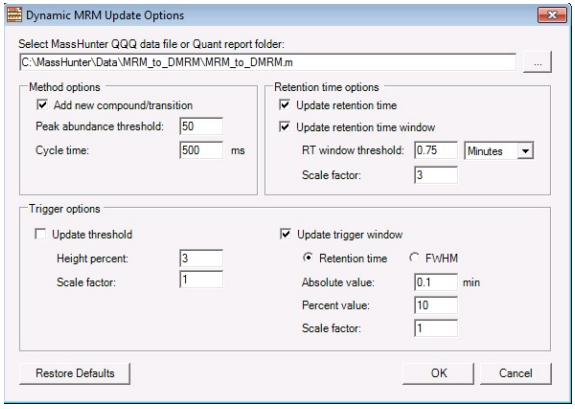
You can update a Dynamic MRM method using either a data file or a quantitation report folder, so this task creates the quantitation report folder.

Steps	Detailed Instructions	Comments
1 Print a report using the template MRM_to_DMRM.xltx.	**a** Click **File > Save Batch**. **b** Click **Report > Generate**. The system displays the **Generate Report** dialog box. **c** Select the **Report folder**. This folder name will be used in the next task. **d** Select the **Report method**. **e** Mark **All samples**. **f** Mark **All compounds**. **g** Click **OK**.	• Copy the **MRM_to_DMRM.xltx** template from the **\Support\Data** folder on the installation disk. • For this report, you do not need to print the report. • If you have not created a Report Method, see the *online Help* for Quantitative Analysis for instructions on how to create a report method.
2 Check the status of the report using the Queue Viewer program.	**a** Click **Report > Queue Viewer**. **b** Wait for the report to finish printing. **c** Close the **Task Queue Viewer** program.	



Task 3. Create a Dynamic MRM method using Update dMRM

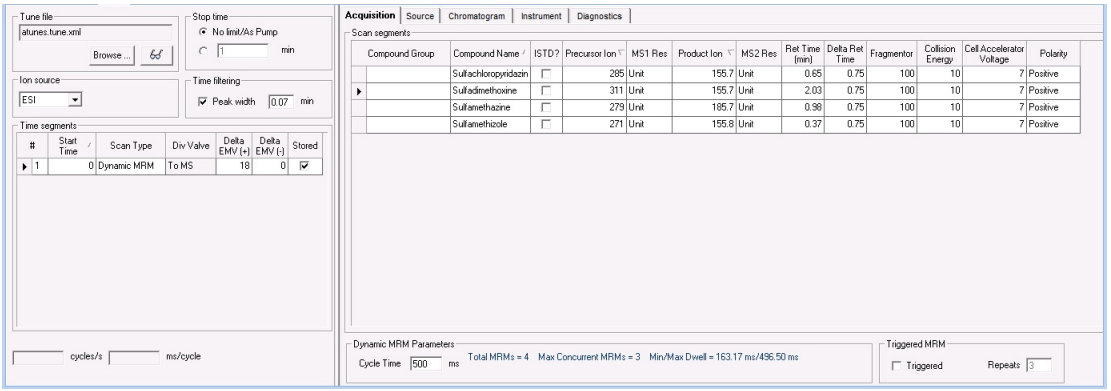
You can create a Dynamic MRM method from an MRM data file or a Quantitative Analysis method. You first set the **Scan Type** to Dynamic MRM, and then you use the Update MRM Method dialog box.

Steps	Detailed Instructions	Comments
1 Open the method *iii*Sulfamix MRM_10.m and save it to a new name with the format ***iii*Sulfamix dMRM.m**, where ***iii*** are your initials.	**a** Click **File > Open > Method**. **b** Select the *iii*Sulfamix MRM_10.m method. Click **OK**. **c** Click **Method > Save As**. **d** Type the new method name with the format ***iii*Sulfamix_dMRM.m.**	• In this example, the batch is in the **\MassHunter\Data\ MRM_to_DMRM** folder.
2 Change the method to a dynamic MRM method with the same compounds. You can either use a data file or the report that was generated in the last task.	**a** Click the **Acquisition** tab in the QQQ tab in the Method Editor window. **b** Right-click the **Scan segments** table and click **Update DMRM Method**. The **Dynamic MRM Update Options** dialog box opens. **c** Select the folder containing the *report.results.xml* file or the data file ***iiiSulfamix MRM_10.d***. **d** Mark **Add new compound/transition**. **e** Mark **Update retention time**. **f** Mark **Update trigger window**. **g** Click **OK**.	• The Dynamic MRM Update tool automatically sets the **Scan type** to **Dynamic MRM**. • You can select either a data file that was acquired with a **Scan Type** of **MRM** or a Quant Report folder as the input to this dialog box. The Scan segments are created from one of these two input sources.
		• **You can update the compounds in the Scan segments table by using a QQQ data file or a Quantitative analysis report folder.**

Exercise 2 – Develop a Dynamic MRM acquisition method from an MRM acquisition data file or an MRM method

Task 3. Create a Dynamic MRM method using Update dMRM

Steps	Detailed Instructions	Comments



The compounds from the data file or quantitation report are automatically added to the Scan segments table.

h Verify that each row has a **Compound Name**. A blank Compound Name is not allowed.

i Click **Method > Save**.

Task 4. Create a Dynamic MRM method from an MRM method

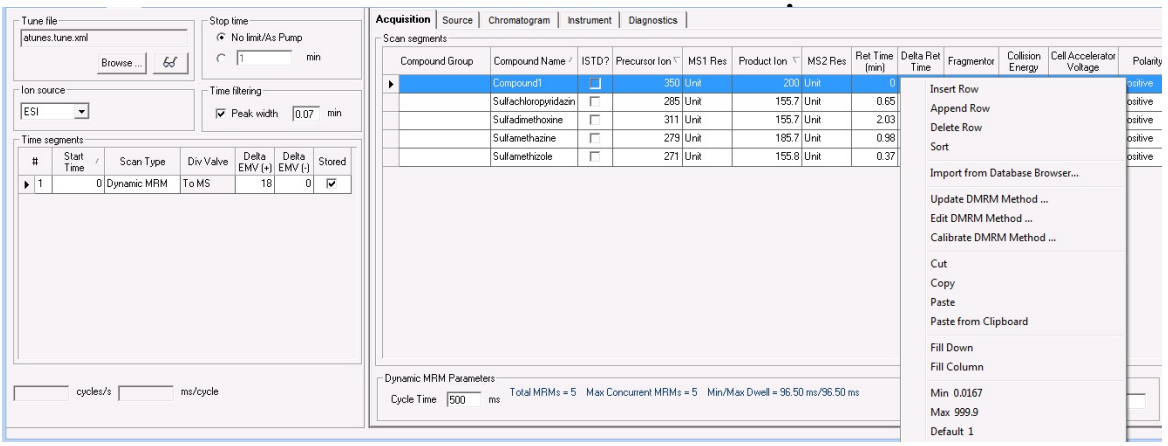
You can create a Dynamic MRM method directly from an MRM method by using the **Paste from Clipboard** command from the shortcut menu. You need to manually enter the retention time.

Steps	Detailed Instructions	Comments
1 Open the method *iii*Sulfamix MRM_10.m and save it to a new name with the format ***iii*Sulfamix dMRM2.m**, where ***iii*** are your initials.	**a** Click **File > Open > Method**. **b** Select the *iii*Sulfamix MRM_10.m method. **c** Click **OK**. **d** Click **Method > Save As**. **e** Type the new method name with the format ***iii*Sulfamix_dMRM2.m**. **f** Click the **Save** button.	
2 Copy all compounds from the Scan segments table in the MRM method.	**a** Click the **Acquisition** tab in the QQQ tab in the Method Editor. **b** Select all of the rows in the Scan segments table. **c** Right-click the Scan segments table and click **Copy**.	• To select all of the rows in the Scan segments table, you select the first row in the table, Then, you scroll to the last row in the Scan segments table. Press the **Shift** key and select the last row in the table.
3 Change the Scan Type to Dynamic MRM and paste the rows into the new Scan segments table.	**a** Select **Dynamic MRM** for the **Scan Type**. **b** Right-click the Scan segments table and click **Paste from Clipboard**. **c** Click **Method > Save**.	• To combine multiple Time Segments into one Dynamic MRM Time Segment, you paste the Scan segments into Excel and create one long list. Then, you copy all of the Scan segments in Excel.
4 Enter the retention times and delete the original compound.	**a** Type the value for the **Ret. Time (min)** for Sulfachloropyridazine. **b** Type the value for the **Ret. Time (min)** for Sulfadimethoxine. **c** Type the value for the **Ret. Time (min)** for Sulfamethazine. **d** Type the value for the **Ret. Time (min)** for Sulfamethizole. **e** Select the original compound in the Scan segments table. **f** Right-click and click **Delete Row**. **g** Click **Method > Save**.	• Retention time is different for different systems and columns. For the example data files, enter the following values: ◦ Sulfachloropyridazine: 0.65 minutes ◦ Sulfadimethoxine: 2.03 minutes ◦ Sulfamethazine: 0.98 minutes ◦ Sulfamethizole: 0.37 minutes

Exercise 2 – Develop a Dynamic MRM acquisition method from an MRM acquisition data file or an MRM method

Task 4. Create a Dynamic MRM method from an MRM method

Steps	Detailed Instructions	Comments



Exercise 3 – Create a Triggered Dynamic MRM acquisition method

For this exercise you analyze a mixture of four sulfonamide compounds.

Task 1. Create a Triggered Dynamic MRM method from a Dynamic MRM method manually

You can create a Triggered Dynamic MRM method directly from a Dynamic MRM method. In a Triggered Dynamic MRM method, you specify some of the transitions to be primary transitions. These transitions are acquired for the entire retention time window. Some of these primary transitions are also marked as triggers. As the data is acquired, the program checks whether or not the abundances of the trigger transitions are higher than the threshold. If the abundances are higher than the thresholds and other additional conditions are met, then the secondary transitions are acquired. These other conditions are described in the *Concepts Guide*.

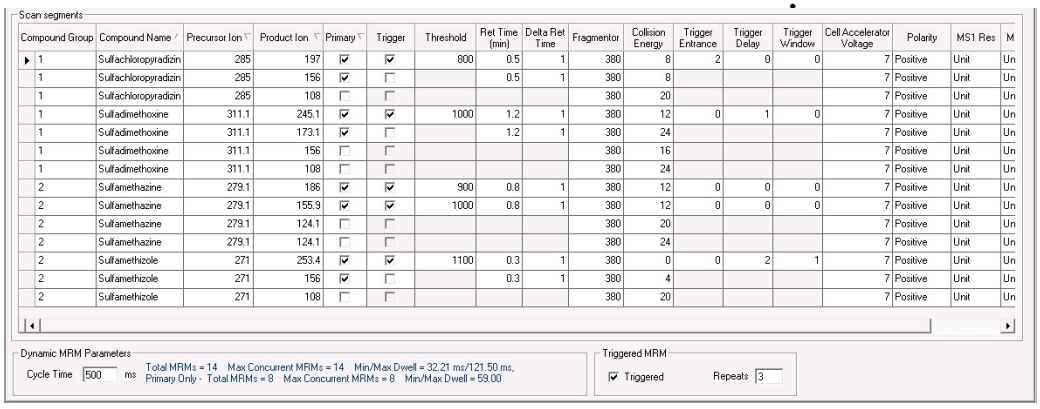
Steps	Detailed Instructions	Comments
1 Open the method ***iii*Sulfamix dMRM2.m**, where ***iii*** are your initials.	**a** Click **File > Open > Method**. **b** Select the *iii*Sulfamix_dMRM2.m method. **c** Click **OK**. **d** Click **Method > Save As**. **e** Type the new method name with the format ***iii*Sulfamix_TriggeredDMRM.m.** **f** Click the **Save** button.	• A Triggered Dynamic MRM method is a type of Dynamic MRM method. The **Scan Type** for both methods is **Dynamic MRM**. • The Dynamic MRM method is the template method for the optimization.

Exercise 3 – Create a Triggered Dynamic MRM acquisition method

Task 1. Create a Triggered Dynamic MRM method from a Dynamic MRM method manually

Steps	Detailed Instructions	Comments
2 Change the method to a triggered dynamic MRM method.	**a** Click the **Acquisition** tab in the QQQ tab in the Method Editor. **b** Mark the **Triggered** check box in the Triggered MRM section. This section is only available if the **Scan Type** is **Dynamic MRM**. **c** Select whether to automatically mark the highest product ion as the Primary. **d** Enter the value for **Repeats**.	• Several columns are added to the **Scan segments** table. These columns only apply to a triggered dynamic MRM method. • The value **Repeats** is the number of times to acquire each of the secondary transitions when the triggering conditions are met.
3 Add additional transitions. See the next image.	**a** Select the first compound. Right-click and click **Insert Row**. Repeat to insert another row **b** Modify the information in these rows to match the information in the next image. **c** Repeat for each compound.	• For each compound, we are going to add additional transitions.
4 Select the transitions that are the **Primary** transitions. See the next image.	**a** For each transition, mark the **Primary** check box if it is a **Primary** transition. **b** Verify that you have marked at least one transition as the **Primary** transition for each **Compound Name**.	• You can select multiple transitions from each compound to be **Primary** transitions. If a transition has the same **Compound Name**, then it is part of the same compound. You must mark at least one transition as a **Primary** transition for each compound. • Typically, the most abundant ion is the **Trigger** transition.

Steps	Detailed Instructions	Comments
5 Select the transitions that are the **Trigger** transitions and set the trigger conditions.	**a** For each compound, mark the **Trigger** check box if it is a Trigger transition. **b** (optional) Mark a second **Trigger** transition. **c** Enter the **Threshold** value for each **Trigger** transition. **d** Enter the **Trigger Entrance** for each **Trigger** transition. **e** Enter the **Trigger Delay** for each **Trigger** transition. **f** Enter the **Trigger Window** for each **Trigger** transition.	• For each compound, you can have two Trigger transitions. • If the **Trigger** transition has an abundance over the **Threshold**, then that triggering condition is met. • By default, the **Trigger Entrance**, the **Trigger Delay** and the **Trigger Window** are set to 0. If these values are 0, then these triggering conditions are not enabled. • The threshold is established when the method is updated using a data file. The other values are also selected based on results. You must collect data at different settings to establish what works best. These values are very important to the success of the method. •



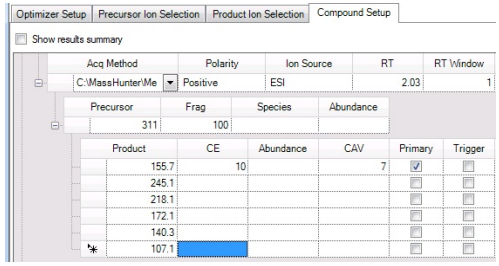
Task 2. Add/Modify compounds in an existing database

You can also manually add compounds to a database and modify the compounds in the database. In the next task, you create a Triggered Dynamic MRM method from the compounds in the database.

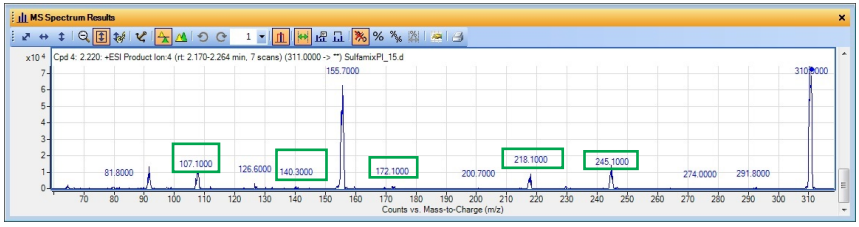
Steps	Detailed Instructions	Comments
1 Review the ***iii*Sulfamix_dMRM2.m**, where ***iii*** are your initials.	**a** Click **File > Open > Method**. **b** Select the **iiiSulfamix_dMRM2.m** method. **c** Click **OK**. **d** Review the parameters.	• A Triggered Dynamic MRM method is a type of Dynamic MRM method. The **Scan Type** for both methods is **Dynamic MRM**.
2 Start the MassHunter Optimizer program.	• Double-click the **Optimizer** icon.  .	• If you are optimizing peptides, use the **Optimizer for Peptides** program.
3 Set parameters on the Optimizer Setup tab.	**a** Click the **Optimizer Setup** tab. **b** Click the **Injection (with or without column)** button. **c** Set the **CE** range from 4 to 48. **d** Set the **Cell Accelerator Voltage** to 7. **e** Right-click the table and click **Add Method**. **f** Select the **iiiSulfamix_dMRM2.m** method.	• To create low mass product ions from a precursor ion near 300 *m/z*, you need fairly high collision energies.
4 Set parameters on the Precursor Ion Selection tab.	**a** Click the **Precursor Ion Selection** tab. **b** Verify that +H is marked for the **Positive ions (with priorities)** list.	
5 Set parameters on the Product Ion Selection tab.	**a** Click the **Product Ion Selection** tab. **b** Click the **Mass (m/z)** button under Low mass cut-off. **c** Enter 60 for the low mass cut-off.	• On the Product Ion Selection, you can automatically add up to 4 product ions per compound (for instance, 2 primaries and 2 secondaries). You want 8 to 10 peaks in the composite spectrum to prove that this is indicative of the compound, so you need to add at least some of the product ions manually.

Steps	Detailed Instructions	Comments
6 Set parameters on the Compound Setup tab and add additional transitions. • For Precursor ion 311, add the following product ions: 245.1, 218.1, 172.1, 140.3, 107.1 • For Precursor 285, add the following product ions: 108.1, 92.1, 80.1, 65.1, 39.2 • For Precursor 279, add the following product ions: 185.7, 155.6, 107.7, 92.1 • For Precursor 271, add the following product ions: 177.8, 115, 91.3, 80.1, 64.8	**a** Click the **Compound Setup** tab. **b** Click the **Import/Export > Import from Acquisition Methods** command. **c** Select the **iiiSulfamix_dMRM2.m** method and click **Open**. **d** (optional) Right-click the tab and click **Expand/Collapse All Rows**. **e** Select one of the **Product** rows for one of the compounds. In this example, select the **Product** row 155.7 for **Precursor** 311. **f** Right-click the Product row and click **Add Product Ion**. In this example, you add 5 product ion rows. **g** Enter the **Product** in each of the product ion rows that were added. See "To determine product ions in the Qualitative Analysis program:" on page 46. **h** Add product ions for the other three compounds.	• For each compound, we are going to add additional transitions. • In the Qualitative Analysis program, you examine Product Ion data files which you acquired previously to determine additional transitions to add. See "Task 4. Determine product ion masses" on page 24. • You can use the arrow keys to move between rows in the Product table. • Some of the product ions were determined from the Veterinarian Drugs tMRM database. • See Table 5 on page 53 for values to use in the method.

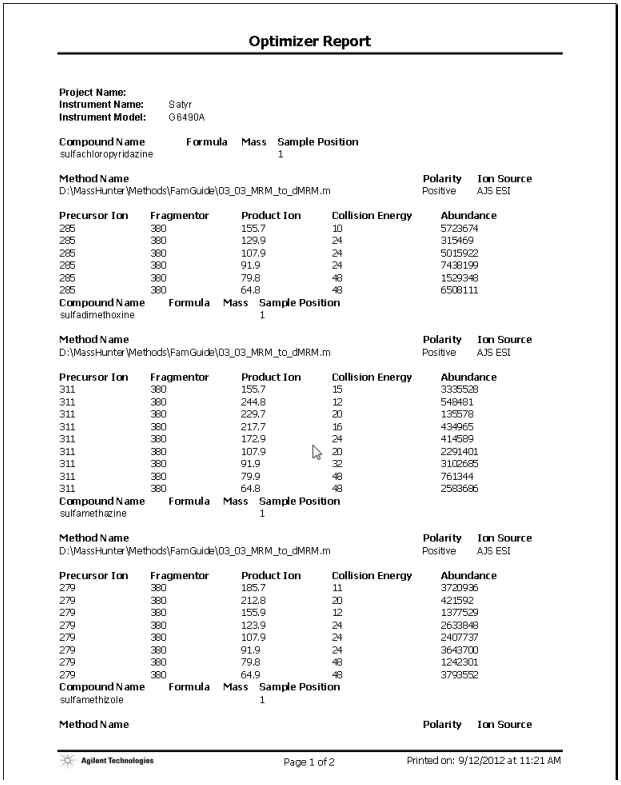
This product ion scan has a precursor mass of 311. You examine the MS spectrum to determine the product ions to add to the Product ion section of the Compound Setup table.



The product ions that are manually added as additional Product ions in Optimizer are shown in the MS Spectrum Results window. The green boxes were added in this guide to show which product ions were used.

Steps	Detailed Instructions	Comments
• To determine product ions in the Qualitative Analysis program:	**a** Open the **SulfamixPI_15.d** from "Task 4. Determine product ion masses" on page 24. **b** Click **Find > Find Compounds by Targeted MS/MS**. **c** Close the Compound List window. **d** Select a compound in the Data Navigator window. For this example, click **Cpd 4**. **e** Click the **Autoscale Y-axis** icon in the MS Spectrum Results toolbar. **f** Right-click and drag to zoom in on the MS spectrum.	• If possible, rearrange the windows on the screen so you can see the Optimizer program and the Qualitative Analysis program at the same time. • See Table 5 on page 53 for values to use in the method.
	**This product ion scan has a precursor mass of 311. You examine the MS spectrum to determine the product ions to add to the Product ion section of the Compound**	
7 Set other parameters in the Compound Setup tab and start the optimization. • You cannot perform a multi-compound run. • You have to mark each row in the table to use.	**a** Mark the check box in the left column at the top of the table. The check box for every row in the table is marked. **b** Clear the **Perform multi-compound run** check box in the right column. **c** Click the **Start Optimization** button in the Optimizer toolbar.	• You cannot perform a multi-compound run with the number of transitions that were added. If you mark this check box, then the Expected peak width (base) is automatically set to almost 80 seconds wide. If you clear this check box, then the Expected peak width is calculated to be around 9 seconds which is more appropriate. • See Table 5 on page 53 for values to use in the method.

Steps	Detailed Instructions	Comments
8 Examine the Optimizer Report.	**a** Examine the Collision Energy for each Product Ion. **b** Print or save the report.	



Optimizer Report

Project Name:
Instrument Name: Satyr
Instrument Model: G6490A

Compound Name	Formula	Mass	Sample Position
sulfachloropyridazine			1

Method Name	Polarity	Ion Source
D:\MassHunter\Methods\FamGuide\03_03_MRM_to_dMRM.m	Positive	AJS ESI

Precursor Ion	Fragmentor	Product Ion	Collision Energy	Abundance
285	380	155.7	10	5723674
285	380	129.9	24	315469
285	380	107.9	24	5015922
285	380	91.9	24	7438199
285	380	79.8	48	1529348
285	380	64.8	48	6508111

Compound Name	Formula	Mass	Sample Position
sulfadimethoxine			1

Method Name	Polarity	Ion Source
D:\MassHunter\Methods\FamGuide\03_03_MRM_to_dMRM.m	Positive	AJS ESI

Precursor Ion	Fragmentor	Product Ion	Collision Energy	Abundance
311	380	155.7	15	3335528
311	380	244.8	12	549481
311	380	229.7	20	135578
311	380	217.7	16	434965
311	380	172.9	24	414589
311	380	107.9	20	2291401
311	380	91.9	32	3102685
311	380	79.9	48	761344
311	380	64.8	48	2583686

Compound Name	Formula	Mass	Sample Position
sulfamethazine			1

Method Name	Polarity	Ion Source
D:\MassHunter\Methods\FamGuide\03_03_MRM_to_dMRM.m	Positive	AJS ESI

Precursor Ion	Fragmentor	Product Ion	Collision Energy	Abundance
279	380	185.7	11	3720936
279	380	212.8	20	421592
279	380	155.9	12	1377529
279	380	123.9	24	2633848
279	380	107.9	24	2407737
279	380	91.9	24	3643700
279	380	79.8	48	1242301
279	380	64.9	48	3793552

Compound Name	Formula	Mass	Sample Position
sulfamethizole			1

Method Name	Polarity	Ion Source

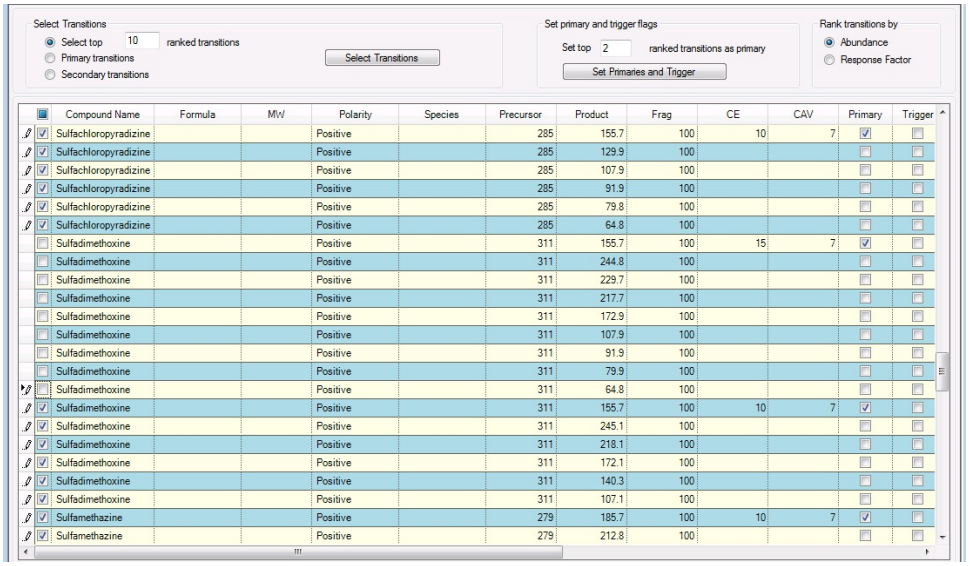
Agilent Technologies Page 1 of 2 Printed on: 9/12/2012 at 11:21 AM

As a general rule, as the Product Ions get smaller, the optimal Collision Energy gets larger. However, when you also examine the abundance, you can see that if the Collision Energy is set to 48 for the smallest product ion, the smallest product ion can become the dominant peak. The collision energies are further adjusted later in this task.

This report was generated with a different set of transitions.

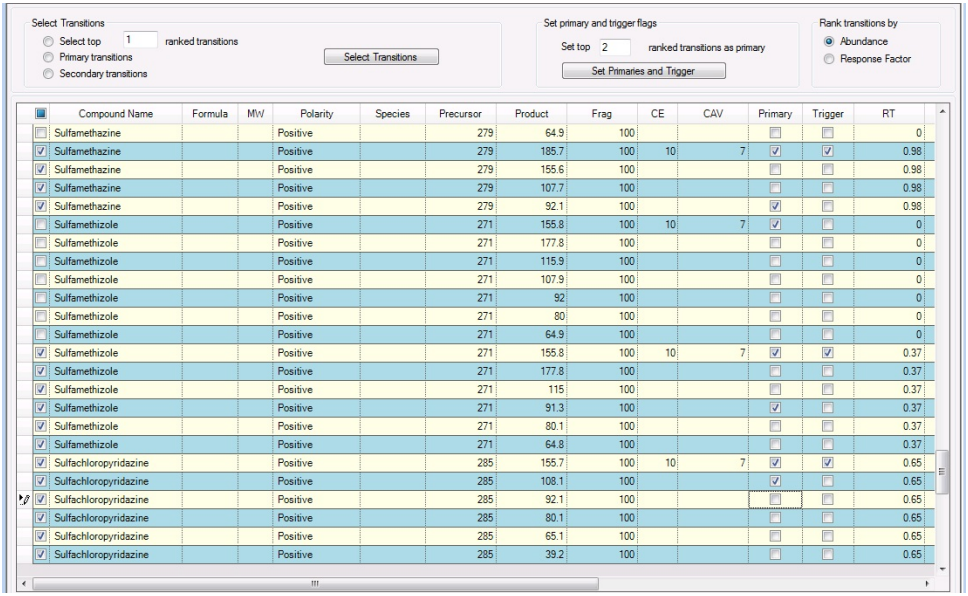
9 Save the compounds.	• Click the **File > Save Compounds** command.	
10 Import compounds from a database.	• Click the **Import/Export > Import from Database** command. The Database Browser program opens.	• You can also import compounds that were distributed as part of a database.

Steps	Detailed Instructions	Comments
11 In the Database Browser program, select the transitions.	**a** Mark the **Show All Records** check box. **b** Click the **Select top** button under Select Transitions. **c** Type `10` for the ranked transitions. **d** Click the **Select Transitions** button.	• All the transitions that you typed in are visible. • The tools to allow you to set up **Primary transitions** and **Secondary transitions** are available in this program. • If any of the compounds have two collision energies, clear the check boxes for one of these values. • See Table 5 on page 53 for values to use in the method.



Compound Name	Formula	MW	Polarity	Species	Precursor	Product	Frag	CE	CAV	Primary	Trigger
Sulfachloropyradizine			Positive		285	155.7	100	10	7	☑	☐
Sulfachloropyradizine			Positive		285	129.9	100			☐	☐
Sulfachloropyradizine			Positive		285	107.9	100			☐	☐
Sulfachloropyradizine			Positive		285	91.9	100			☐	☐
Sulfachloropyradizine			Positive		285	79.8	100			☐	☐
Sulfachloropyradizine			Positive		285	64.8	100			☐	☐
Sulfadimethoxine			Positive		311	155.7	100	15	7	☑	☐
Sulfadimethoxine			Positive		311	244.8	100			☐	☐
Sulfadimethoxine			Positive		311	229.7	100			☐	☐
Sulfadimethoxine			Positive		311	217.7	100			☐	☐
Sulfadimethoxine			Positive		311	172.9	100			☐	☐
Sulfadimethoxine			Positive		311	107.9	100			☐	☐
Sulfadimethoxine			Positive		311	91.9	100			☐	☐
Sulfadimethoxine			Positive		311	79.9	100			☐	☐
Sulfadimethoxine			Positive		311	64.8	100			☐	☐
Sulfadimethoxine			Positive		311	155.7	100	10	7	☑	☐
Sulfadimethoxine			Positive		311	245.1	100			☐	☐
Sulfadimethoxine			Positive		311	218.1	100			☐	☐
Sulfadimethoxine			Positive		311	172.1	100			☐	☐
Sulfadimethoxine			Positive		311	140.3	100			☐	☐
Sulfadimethoxine			Positive		311	107.1	100			☐	☐
Sulfamethazine			Positive		279	185.7	100	10	7	☑	☐
Sulfamethazine			Positive		279	212.8	100			☐	☐

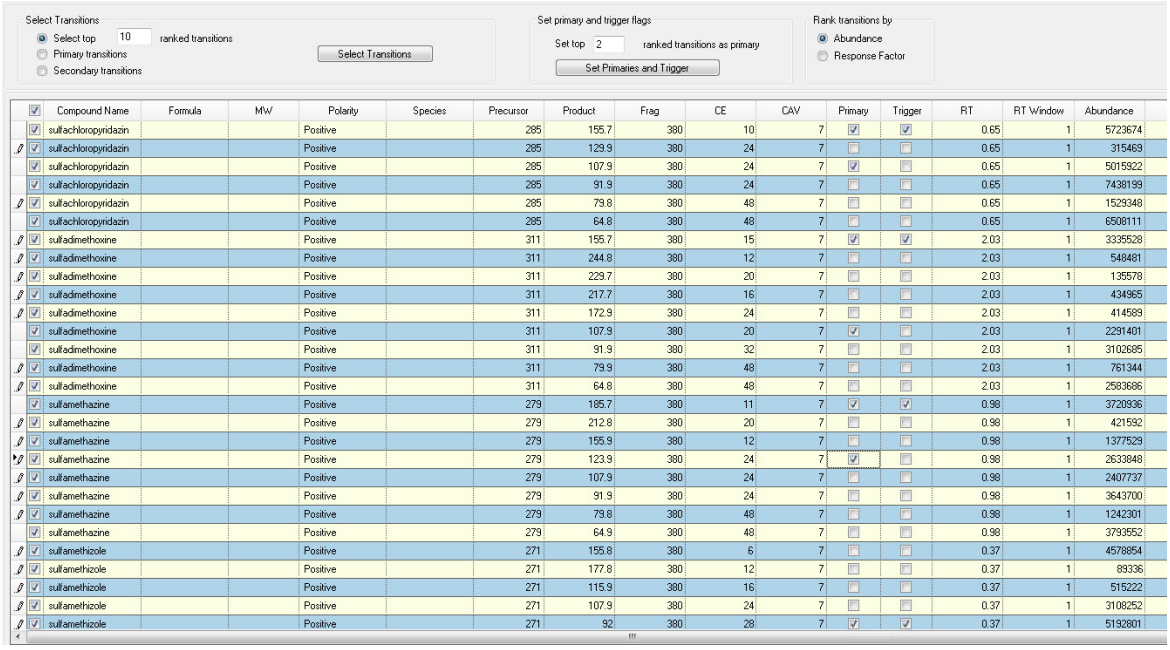
Steps	Detailed Instructions	Comments
12 In the Database Browser program, automatically select the **Primary** transitions and **Trigger** transition.	**a** In the **Set top ranked transitions as primary** box, enter 2. **b** Click the **Set Primaries and Trigger** button.	• The program automatically selects the two most abundant transitions as the Primary transitions. • The program also selects the most abundant transition as the **Trigger**. • You can manually select a second **Trigger** transition. • See Table 5 on page 53 for values to use in the method.



Compound Name	Formula	MW	Polarity	Species	Precursor	Product	Frag	CE	CAV	Primary	Trigger	RT
Sulfamethazine			Positive		279	64.9	100			☐	☐	0
Sulfamethazine			Positive		279	185.7	100	10	7	☑	☑	0.98
Sulfamethazine			Positive		279	155.6	100			☐	☐	0.98
Sulfamethazine			Positive		279	107.7	100			☐	☐	0.98
Sulfamethazine			Positive		279	92.1	100			☑	☐	0.98
Sulfamethizole			Positive		271	155.8	100	10	7	☑	☐	0
Sulfamethizole			Positive		271	177.8	100			☐	☐	0
Sulfamethizole			Positive		271	115.9	100			☐	☐	0
Sulfamethizole			Positive		271	107.9	100			☐	☐	0
Sulfamethizole			Positive		271	92	100			☐	☐	0
Sulfamethizole			Positive		271	80	100			☐	☐	0
Sulfamethizole			Positive		271	64.9	100			☐	☐	0
Sulfamethizole			Positive		271	155.8	100	10	7	☑	☑	0.37
Sulfamethizole			Positive		271	177.8	100			☐	☐	0.37
Sulfamethizole			Positive		271	115	100			☐	☐	0.37
Sulfamethizole			Positive		271	91.3	100			☑	☐	0.37
Sulfamethizole			Positive		271	80.1	100			☐	☐	0.37
Sulfamethizole			Positive		271	64.8	100			☐	☐	0.37
Sulfachloropyridazine			Positive		285	155.7	100	10	7	☑	☑	0.65
Sulfachloropyridazine			Positive		285	108.1	100			☑	☐	0.65
Sulfachloropyridazine			Positive		285	92.1	100			☐	☐	0.65
Sulfachloropyridazine			Positive		285	80.1	100			☐	☐	0.65
Sulfachloropyridazine			Positive		285	65.1	100			☐	☐	0.65
Sulfachloropyridazine			Positive		285	39.2	100			☐	☐	0.65

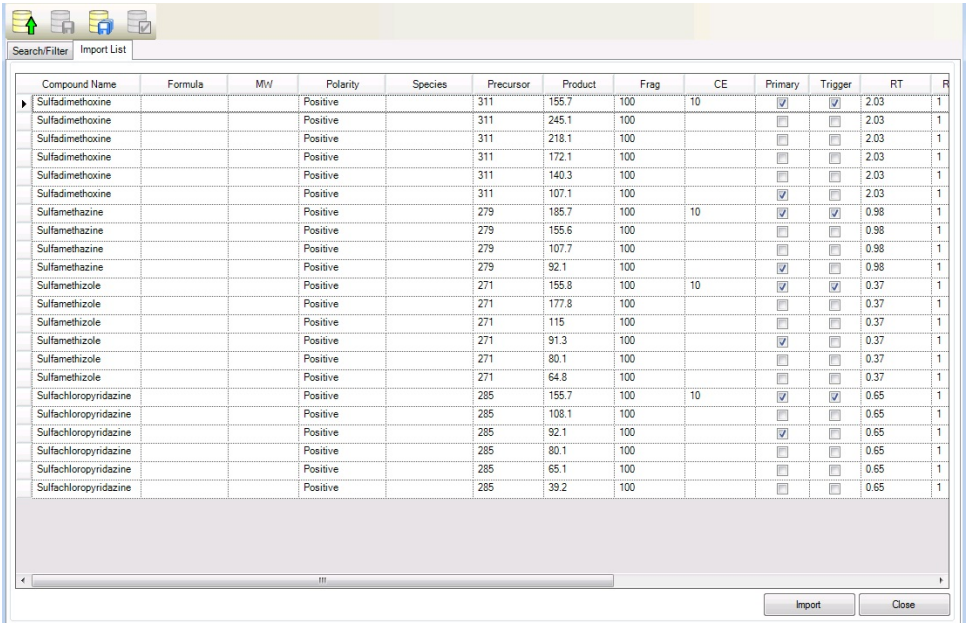
You examine the Primary column and the Trigger column to determine which transitions are selected. You can select one or two Trigger transitions. You can select multiple Primary transitions.

Steps	Detailed Instructions	Comments
13 Review the Primary transitions and Trigger transitions. • For sulfachloropyridazine, select 285 *m/z* -> 155.7 *m/z* transition as the Primary and Trigger transition. • For sulfadimethoxine, select 311 *m/z* -> 155.7 *m/z* transition as the Primary and Trigger transition. • For sulfamethazine, select 279 *m/z* -> 185.7 *m/z* transition as the Primary and Trigger transition. • For sulfamethizole, select 271 *m/z* -> 155.8 *m/z* transition as the Primary and Trigger transition.	• Review each compound. Change the Primary and Trigger transitions to the transitions listed in the left column. • Change the other Primary transitions as shown below.	• The program selected the most abundant transitions which in this example often had a low *m/z* for the Product Ion. A very abundant low *m/z* ion may be unsuitable as a Primary transition. • You can select two Primary transitions as triggers for a compound. • In the example below, all of the columns have values. The Fragmentor voltages and collision energy values were set in Optimizer. • See Table 5 on page 53 for values to use in the method.



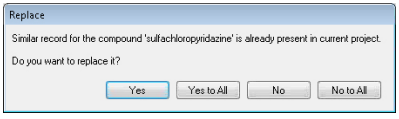
Compound Name	Formula	MW	Polarity	Species	Precursor	Product	Frag	CE	CAV	Primary	Trigger	RT	RT Window	Abundance
sulfachloropyridazin			Positive		285	155.7	380	10	7	☑	☑	0.65	1	5723674
sulfachloropyridazin			Positive		285	129.9	380	24	7	☐	☐	0.65	1	315469
sulfachloropyridazin			Positive		285	107.9	380	24	7	☑	☐	0.65	1	5015922
sulfachloropyridazin			Positive		285	91.9	380	24	7	☐	☐	0.65	1	7438199
sulfachloropyridazin			Positive		285	79.8	380	48	7	☐	☐	0.65	1	1529348
sulfachloropyridazin			Positive		285	64.8	380	48	7	☐	☐	0.65	1	6508111
sulfadimethoxine			Positive		311	155.7	380	15	7	☑	☑	2.03	1	3335528
sulfadimethoxine			Positive		311	244.8	380	12	7	☐	☐	2.03	1	548481
sulfadimethoxine			Positive		311	229.7	380	20	7	☐	☐	2.03	1	135578
sulfadimethoxine			Positive		311	217.7	380	16	7	☐	☐	2.03	1	434965
sulfadimethoxine			Positive		311	172.9	380	24	7	☐	☐	2.03	1	414589
sulfadimethoxine			Positive		311	107.9	380	20	7	☑	☐	2.03	1	2291401
sulfadimethoxine			Positive		311	91.9	380	32	7	☐	☐	2.03	1	3102685
sulfadimethoxine			Positive		311	79.9	380	48	7	☐	☐	2.03	1	761344
sulfadimethoxine			Positive		311	64.8	380	48	7	☐	☐	2.03	1	2583686
sulfamethazine			Positive		279	185.7	380	11	7	☑	☑	0.98	1	3720936
sulfamethazine			Positive		279	212.8	380	20	7	☐	☐	0.98	1	421592
sulfamethazine			Positive		279	155.9	380	12	7	☐	☐	0.98	1	1377529
sulfamethazine			Positive		279	123.9	380	24	7	☑	☐	0.98	1	2633848
sulfamethazine			Positive		279	107.9	380	24	7	☐	☐	0.98	1	2407737
sulfamethazine			Positive		279	91.9	380	24	7	☐	☐	0.98	1	3643700
sulfamethazine			Positive		279	79.8	380	48	7	☐	☐	0.98	1	1242301
sulfamethazine			Positive		279	64.9	380	48	7	☐	☐	0.98	1	3793552
sulfamethizole			Positive		271	155.8	380	6	7	☐	☐	0.37	1	4578854
sulfamethizole			Positive		271	177.8	380	12	7	☐	☐	0.37	1	89336
sulfamethizole			Positive		271	115.9	380	16	7	☐	☐	0.37	1	515222
sulfamethizole			Positive		271	107.9	380	24	7	☐	☐	0.37	1	3108252
sulfamethizole			Positive		271	92	380	28	7	☑	☑	0.37	1	5192801

Steps	Detailed Instructions	Comments
14 Review the **Import List** table on the Import List tab.	**a** Click the **Add to Import List** button. **b** Click the **Import List** tab. **c** Review the **Import List** table.	• In this example, you are importing from the database to the Import List. Then, you are importing from Database Browser to Optimizer.



Compound Name	Formula	MW	Polarity	Species	Precursor	Product	Frag	CE	Primary	Trigger	RT	R
Sulfadimethoxine			Positive		311	155.7	100	10	☑	☑	2.03	1
Sulfadimethoxine			Positive		311	245.1	100		☐	☐	2.03	1
Sulfadimethoxine			Positive		311	218.1	100		☐	☐	2.03	1
Sulfadimethoxine			Positive		311	172.1	100		☐	☐	2.03	1
Sulfadimethoxine			Positive		311	140.3	100		☐	☐	2.03	1
Sulfadimethoxine			Positive		311	107.1	100		☑	☐	2.03	1
Sulfamethazine			Positive		279	185.7	100	10	☑	☑	0.98	1
Sulfamethazine			Positive		279	155.6	100		☐	☐	0.98	1
Sulfamethazine			Positive		279	107.7	100		☐	☐	0.98	1
Sulfamethazine			Positive		279	92.1	100		☑	☐	0.98	1
Sulfamethizole			Positive		271	155.8	100	10	☑	☑	0.37	1
Sulfamethizole			Positive		271	177.8	100		☐	☐	0.37	1
Sulfamethizole			Positive		271	115	100		☐	☐	0.37	1
Sulfamethizole			Positive		271	91.3	100		☑	☐	0.37	1
Sulfamethizole			Positive		271	80.1	100		☐	☐	0.37	1
Sulfamethizole			Positive		271	64.8	100		☐	☐	0.37	1
Sulfachloropyridazine			Positive		285	155.7	100	10	☑	☑	0.65	1
Sulfachloropyridazine			Positive		285	108.1	100		☐	☐	0.65	1
Sulfachloropyridazine			Positive		285	92.1	100		☑	☐	0.65	1
Sulfachloropyridazine			Positive		285	80.1	100		☐	☐	0.65	1
Sulfachloropyridazine			Positive		285	65.1	100		☐	☐	0.65	1
Sulfachloropyridazine			Positive		285	39.2	100		☐	☐	0.65	1

Steps	Detailed Instructions	Comments
15 Review the Compound Setup table in Optimizer. You replace all compounds with the compounds from the Database Browser program.	**a** Click the **Import** button. **b** Click the **Yes to All** button. **c** In the **Compound Setup** tab in Optimizer, review the compounds.	• The compounds in Optimizer are overwritten by the compounds that you updated in the Database Browser program.



Steps	Detailed Instructions	Comments
16 Save the new compound parameters to the database.	• Click the **File > Save Compounds** command to save all of the changes to the database.	• You cannot see these results by default, but the Primary and Trigger transitions are updated in the project. • The **Primary** column, **Trigger** column, **Trigger Entrance Delay** column, **Trigger Delay** column, **Trigger Window** column and **Trigger MRM Threshold** column are available in the Compound Setup tab. They may be hidden.

Table 5 Information for TMRM method for sulfa drugs

Compound	Precursor	Product Ion	Primary	Trigger	Threshold	Collision Energy
Sulfachloropyridazine	285	155.7	Yes	Yes	500	12
Sulfachloropyridazine	285	92.1	Yes	No		24
Sulfachloropyridazine	285	108.1	No	No		24
Sulfachloropyridazine	285	80.1	No	No		60
Sulfachloropyridazine	285	65.1	No	No		60
Sulfadimethoxine	311	155.7	Yes	Yes	500	16
Sulfadimethoxine	311	107.1	Yes	No		28
Sulfadimethoxine	311	245.1	No	No		16
Sulfadimethoxine	311	218.1	No	No		16
Sulfadimethoxine	311	172.1	No	No		32
Sulfadimethoxine	311	140.3	No	No		40
Sulfamethazine	279	185.7	Yes	Yes	500	12
Sulfamethazine	279	92.1	Yes	No		32
Sulfamethazine	279	155.6	No	No		16
Sulfamethazine	279	107.7	No	No		28
Sulfamethizole	271	155.8	Yes	Yes	500	10
Sulfamethizole	271	91.3	Yes	No		40
Sulfamethizole	271	177.8	No	No		10
Sulfamethizole	271	115	No	No		20
Sulfamethizole	271	80.1	No	No		40
Sulfamethizole	271	64.8	No	No		40

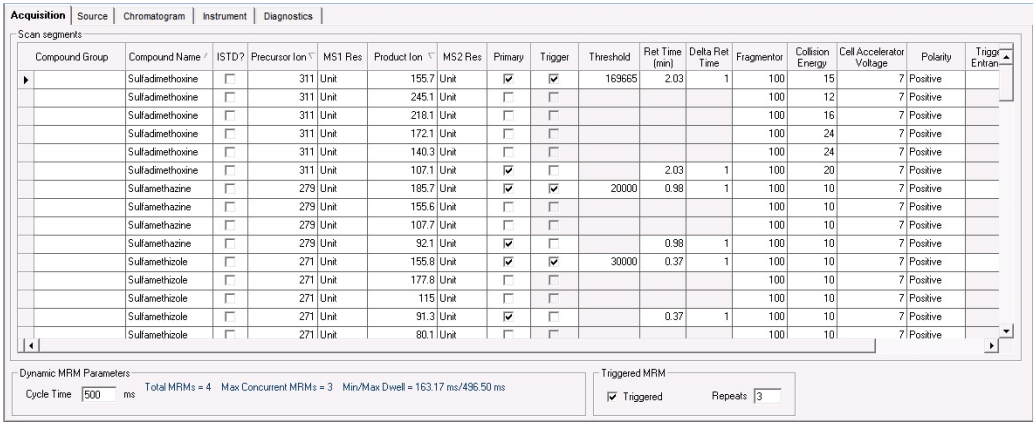
You select the most abundant transition as the Trigger. The threshold is set automatically when any acquisition method is updated. You can set the threshold manually, also.

Task 3. Create a Triggered Dynamic MRM method from an existing database

You can create a Triggered Dynamic MRM method from a database such as the **Pesticide Triggered MRM Database and Library**, the **Forensics and Toxicology Triggered MRM Database and Library**, or the **Veterinary Drug Triggered MRM Database and Library**. These databases can be purchased from Agilent. You can also copy the information from an Excel spreadsheet, but that method is not described in this guide.

Steps	Detailed Instructions	Comments
1 In the Data Acquisition program, you now import the updated compounds from the database. These compounds have optimized collision energies and also Primary and Trigger transitions marked.	**a** Switch to the Data Acquisition program. **b** Open the **iiiSulfamix_dMRM2.m** method. **c** In the **QQQ** tab, click the **Acquisition** tab. The Scan segments table contains four rows which are deleted later. **d** Right-click the Scan Segments table and click **Import from Database Browser**. The Database Browser program opens. **e** Mark the **Show All Records** check box. **f** Mark all of the transitions for the four sulfa drug compounds. Clear the check boxes next to any unwanted compounds. **g** Click the **Add to Import List** button. **h** Click the **Import List** tab. **i** Review the Import List table. **j** Click the **Import** button. **k** Delete the original compounds from the Scan segments table. **l** Mark the **Triggered** check box under Triggered MRM.	• Before you import compounds from Database Browser, the Scan segments table contains at least one row. After importing compounds from the Database Browser, you need to remove any original rows. • The Scan segments table always has to have at least one row. • The triggering information is loaded from the Database Browser program even if the Triggered check box is clear. • See the *online Help* for the Data Acquisition program and the QQQ *Concepts Guide* for an explanation of the other triggering conditions: **Trigger Entrance**, **Trigger Delay**, and **Trigger Window**.

Steps	Detailed Instructions	Comments

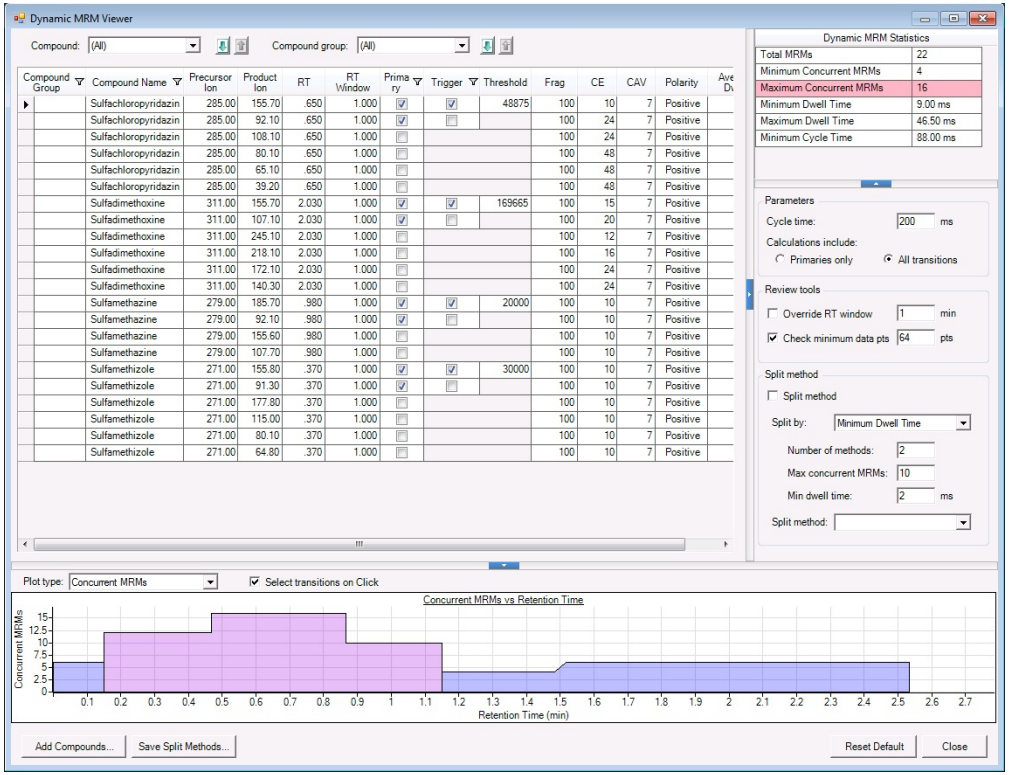


Compound Group	Compound Name	ISTD?	Precursor Ion	MS1 Res	Product Ion	MS2 Res	Primary	Trigger	Threshold	Ret Time (min)	Delta Ret Time	Fragmentor	Collision Energy	Cell Accelerator Voltage	Polarity	Trigg Entran
	Sulfadimethoxine		311	Unit	155.7	Unit	☑	☑	169665	2.03	1	100	15	7	Positive	
	Sulfadimethoxine		311	Unit	245.1	Unit						100	12	7	Positive	
	Sulfadimethoxine		311	Unit	218.1	Unit						100	16	7	Positive	
	Sulfadimethoxine		311	Unit	172.1	Unit						100	24	7	Positive	
	Sulfadimethoxine		311	Unit	140.3	Unit						100	24	7	Positive	
	Sulfadimethoxine		311	Unit	107.1	Unit	☑			2.03	1	100	20	7	Positive	
	Sulfamethazine		279	Unit	185.7	Unit	☑	☑	20000	0.98	1	100	10	7	Positive	
	Sulfamethazine		279	Unit	155.6	Unit						100	10	7	Positive	
	Sulfamethazine		279	Unit	107.7	Unit						100	10	7	Positive	
	Sulfamethazine		279	Unit	92.1	Unit	☑			0.98	1	100	10	7	Positive	
	Sulfamethizole		271	Unit	155.8	Unit	☑	☑	30000	0.37	1	100	10	7	Positive	
	Sulfamethizole		271	Unit	177.8	Unit						100	10	7	Positive	
	Sulfamethizole		271	Unit	115	Unit						100	10	7	Positive	
	Sulfamethizole		271	Unit	91.3	Unit	☑			0.37	1	100	10	7	Positive	
	Sulfamethizole		271	Unit	80.1	Unit						100	10	7	Positive	

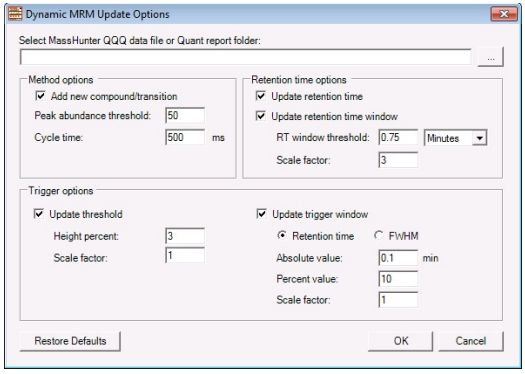
2 Save the method to a new method name, ***iii*Sulfas_TriggerOpt.m**, where ***iii*** are your initials.

a Click the **Method > Save Method** command.
b Type **iiiSulfas_TriggerOpt.m**.
c Click the **Save** button.

Steps	Detailed Instructions	Comments
3 Review the method in the Dynamic MRM Viewer dialog box.	**a** Right-click the Scan segments table and click **Edit DMRM Method**. The **Dynamic MRM Viewer** dialog box is opened. **b** Type 200 for the **Cycle time**. This value is shown in the Acquisition tab. **c** Click between the **Primaries only** button and the **All transitions** button if the **Dynamic MRM Statistics** information is not updating. Then, click the **All transitions** button. **d** Click **Close**.	• The compounds in Optimizer were overwritten by the compounds that you updated in the Database Browser program. • You can modify the **Cycle time** and see how the **Minimum Dwell Time** is changed. If the **Minimum Dwell Time** is less than 5 ms, and especially if it is less than 2 ms, then signal-to-noise is poor. • A **Dwell Time** of 8 ms per transition is fine.



Steps	Detailed Instructions	Comments
4 Review the Trigger Thresholds to verify that they are appropriate.	**a** Do an injection to make sure that the Trigger Thresholds are set properly. **b** Right-click the Scan segments table and click **Update DMRM Method**. **c** In the MRM Update Options dialog box, select **True** for **Update threshold**. **d** Enter the value for the **Percent of Height** for the Trigger Threshold. **e** Select the data file that you just acquired. **f** Click **OK**.	



Exercise 4 – Optimize Acquisition parameters

For this exercise you optimize a mixture of four sulfonamide compounds.

Task 1. Use Optimizer to optimize acquisition parameters

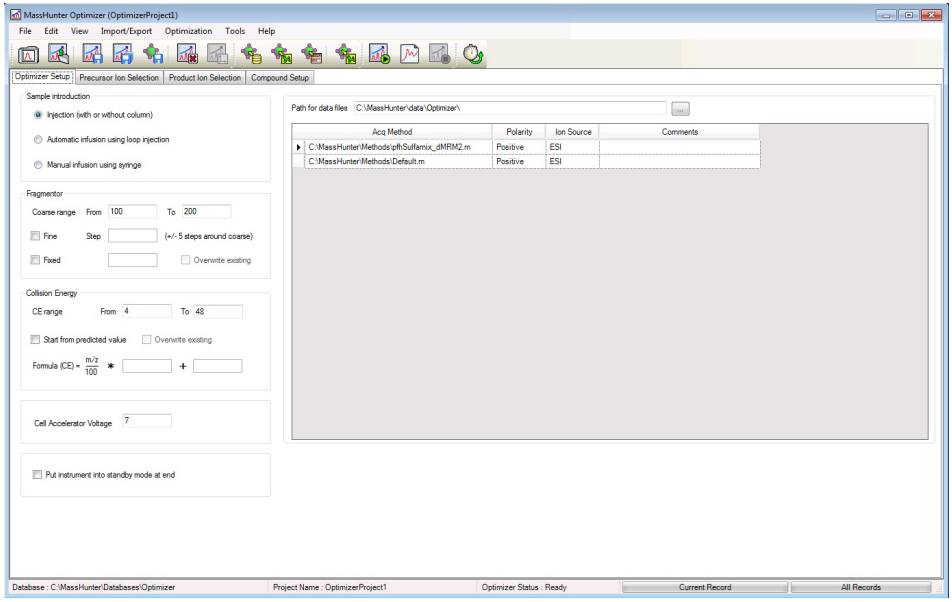
Optimizer helps you optimize acquisition parameters. Specifically, it automates the selection of the best precursor ions, the optimization of the fragmentor voltage for each precursor ion, selection of the best product ions, and optimization of collision energy values for each transition for a list of compounds you specify.

To do this task, you first need to create the method ***iii*Sulfamix MRM_10.m** in **"Task 5. Find optimum collision energy for MRM acquisition"** on page 30. You do not need to acquire the data file.

The Fragmentor Voltage for the 6490 and the 6495 is set automatically during Autotune. The Fragmentor voltage for a 6490 and for a 6495 is not optimized. The Fragmentor parameters and results will not be displayed for a 6490 instrument or a 6495 instrument.

Steps	Detailed Instructions	Comments
1 Start the MassHunter Optimizer program.	• Double-click the **Optimizer** icon.  .	• If you are optimizing peptides, use the **Optimizer for Peptides** program.

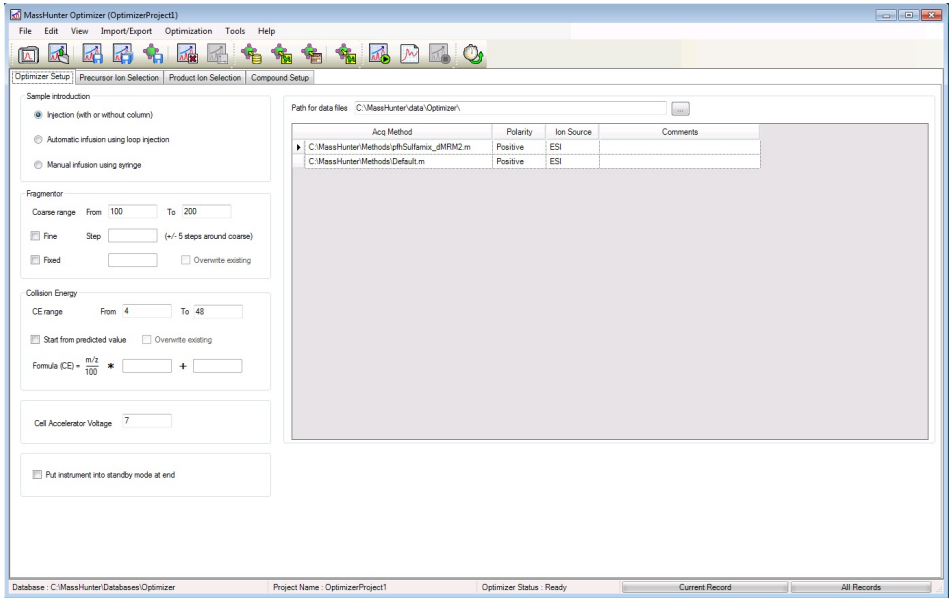
Steps	Detailed Instructions	Comments



Exercise 4 – Optimize Acquisition parameters

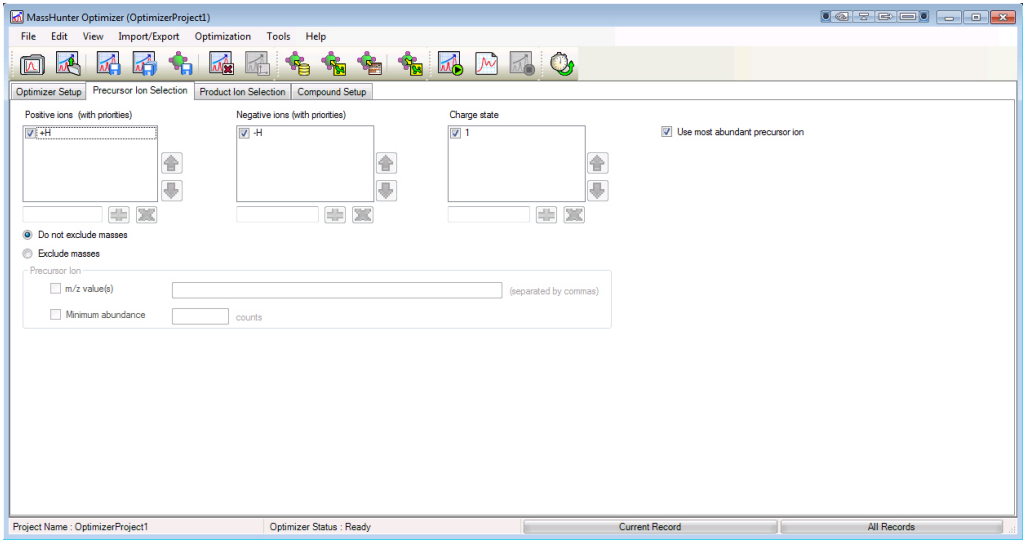
Task 1. Use Optimizer to optimize acquisition parameters

Steps	Detailed Instructions	Comments

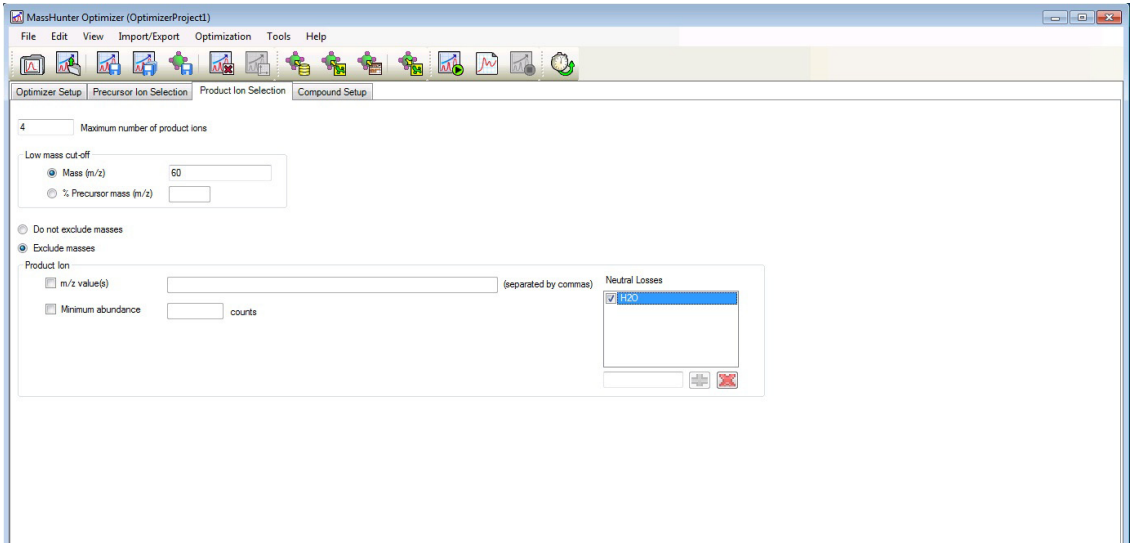


Steps	Detailed Instructions	Comments
2 Set the optimization parameters.	**a** Click the **Optimizer Setup** tab. **b** Set the **Sample introduction** method to **Injection (with or without column)**. **c** If you do not have a 6490 or a 6495, then set the Fragmentor ramp parameters as follows: • Set the range for ramping the **Fragmentor** values from 90 to 135. • Clear the **Fragmentor Fine** check box. **d** Set the range for ramping the Collision Energy from 0 to 40 V. **e** Select a Path for data files to store the optimization run data. **f** Right-click the table on the right and select **Add Method** from the shortcut menu. **g** Click the button on the right side of the Acq Method cell to open the Open Method dialog box. **h** Select the method created in the previous exercise ***iii*Sulfamix MRM_10.m** and click **OK**. The Polarity and Ion Source will be filled in from the values set in the selected method. **i** Check to make sure that the Ion Source from the method matches the physical configuration of your instrument. **j** Repeat step f to step i to select additional methods.	• Fine optimization refines the coarse ramping values and provides better optimization but takes longer to run. • The data can be displayed later with MassHunter Qualitative Analysis program. • The Fragmentor Voltage is not optimized for a 6490 or 6495 QQQ. It is set automatically when you Autotune. The Fragmentor parameters and results for a 6490 or 6495 are not shown in Optimizer.

Steps	Detailed Instructions	Comments
3 Select the precursor ions	**a** Click the **Precursor Ion Selection** tab. **b** Select the **Positive ions** +H adduct. **c** Select the **Charge state** of **1**. **d** Set the search priority of the precursor ions. **e** (optional) To exclude certain masses from consideration, click **Exclude masses** at the bottom of the screen. Enter the **m/z Values** to exclude separated by commas and/or enter a **Minimum abundance value** in counts.	• Mark the **Use most abundant precursor ion** check box to use the most abundant precursor ion. • Clear the **Use most abundant precursor ion** check box and use the **Up** and **Down** arrow buttons to set the search order (ions at the top of the list are given more priority). • You can also enter Neutral Losses to exclude (for example H_2O).

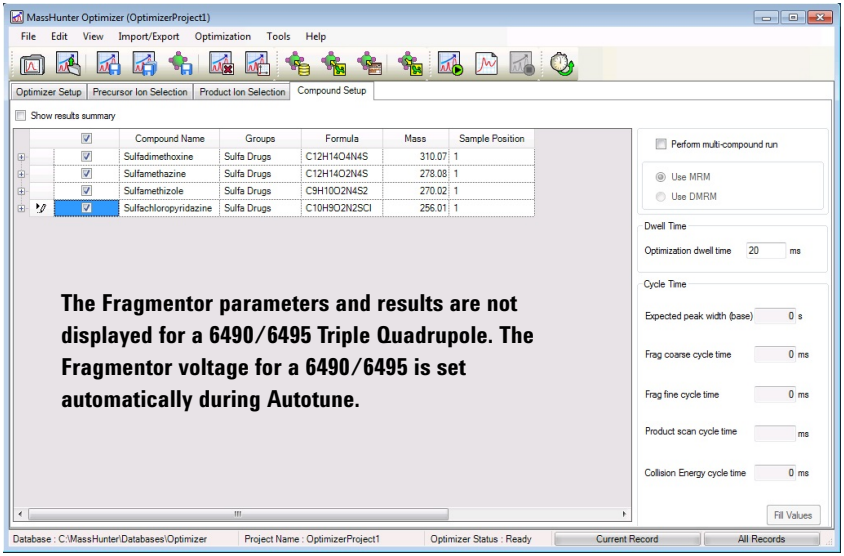


Steps	Detailed Instructions	Comments
4 Select the product ions	**a** Click the **Product Ion Selection** tab. **b** Enter a **Low mass cut-off** value. Select **Mass (m/z)** of 60 *m/z*. **c** To exclude certain masses from consideration, click **Exclude masses** option at the bottom of the screen. Enter the **m/z Values** to exclude separated by commas and/or enter a **Minimum abundance value** in counts. **d** If desired, you can also enter **Neutral Losses** to exclude, for example H_20. Enter a formula in the box and click the button to add it to the list.	• You want to set the **Low mass cut-off** value because you do not want to optimize low mass non-specific ions. For the sulfa drugs, significant ions are above 60 *m/z*.



Steps	Detailed Instructions	Comments
5 Set up a compound list. The formula for the four Sulfa Drugs are: • Sulfamethizole $C_9H_{10}O_2N_4S_2$ • Sulfamethazine $C_{12}H_{14}O_2N_4S$ • Sulfachloropyridazine $C_{10}H_9O_2N_4SCl$ • Sulfadimethoxine $C_{12}H_{14}O_4N_4S$	**a** Click the **Compound Setup** tab. **b** Clear the **Show results summary** check box above the table while you set up the compound list. **c** Right-click the table and select **Add Compound** from the shortcut menu to add a row to the end of the table. **d** Enter `Sulfamethizole` as the **Compound Name**. **e** Enter `Sulfa drugs` as the group name in the **Groups** column. **f** Enter `C9H10O2N4S2` as the **Formula** of the compound. The mass is calculated. **g** Enter the **Sample Position** for the new compound. **h** (optional) Enter an **Optimization dwell time** value to set longer or shorter cycle times. **i** Repeat the steps above to add the other three sulfa drugs to the table. **j** Mark the **Select** columns for the compounds (rows) to use for optimization. **k** Save the compound list to the database or to the current project.	• Compounds are global to all projects. Compound information such as name, group, formula, and mass in one project will be reflected in the entire database. • If no methods or ions are specified here, then optimization for the compound uses the methods from the Optimizer Setup tab and information from the Precursor Ion Selection and Product Ion Selection tabs to generate the ions. • You can also enter the monoisotopic mass in the **Mass** column instead of the **Formula**.

Steps	Detailed Instructions	Comments



6 Start the optimization process.

- Click the Start Optimization button ( ) on the toolbar

or

- Click the **Ion Breakdown Profile** button ( ) on the toolbar.

7 Review results.

a Click the **Compound Setup** tab.
b Mark the **Show results summary** check box above the table.
c Review the following values for each transition ion in the Compound Table:
- Fragmentor
- Collision Energy

d Review the printed optimization report.

- (optional) Use the MassHunter Workstation Qualitative Analysis program to look at the data.
- See the *online Help* for Optimizer or the Optimizer *Quick Start Guide* to learn how to import optimization results to acquisition for MRM time segments.

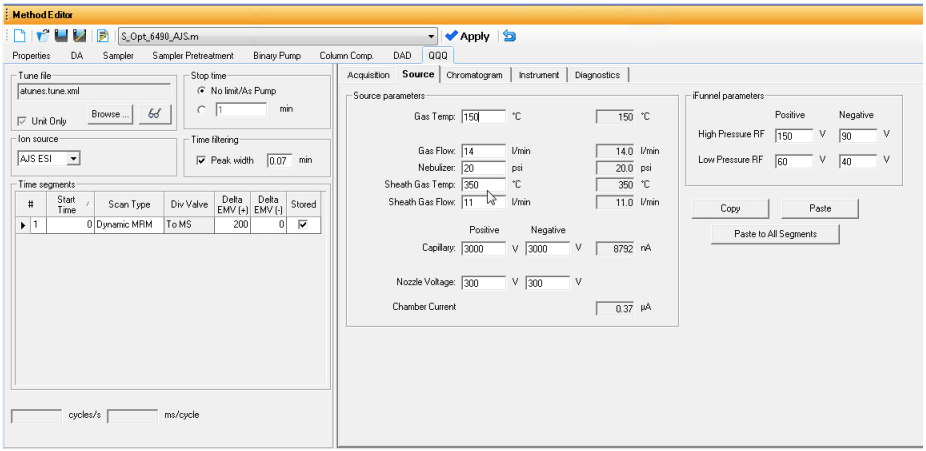
Task 2. Use Source and iFunnel Optimizer to optimize acquisition parameters

Source and iFunnel Optimizer helps you optimize acquisition parameters for the source and iFunnel.

To do this task, you first need to create the method ***iii*Sulfamix_dMRM2.m** in "Task 4. Create a Dynamic MRM method from an MRM method" on page 39. You do not need to acquire the data file. When you use this program, a worklist for each of the parameters being optimized is added to the Study Manager program.

Steps	Detailed Instructions	Comments
1 Start the MassHunter Data Acquisition program and load the iiiSulfamix_dMRM2.m method. Save this method to a new name.	**a** Start the Data Acquisition program. **b** Make sure that Acquisition appears as the selection in the **Context** text box. If Tune is the selection, click **Acquisition** from the **Context** dropdown menu in the Combo bar. **c** Load the ***iii*Sulfamix_dMRM2.m** method. **d** Save this method to the name ***iii*Sulfamix_SourceOpt.m**. **e** View the Method Editor window.	• The first step is to create a template method. This method is used when you are optimizing the source and iFunnel parameters.
2 Edit the Source parameters.	**a** Click the **QQQ** tab. **b** Click the **Source** tab on the QQQ tab. **c** Modify the parameters to the recommended starting parameters for source optimization. These parameters are shown in the following image.	

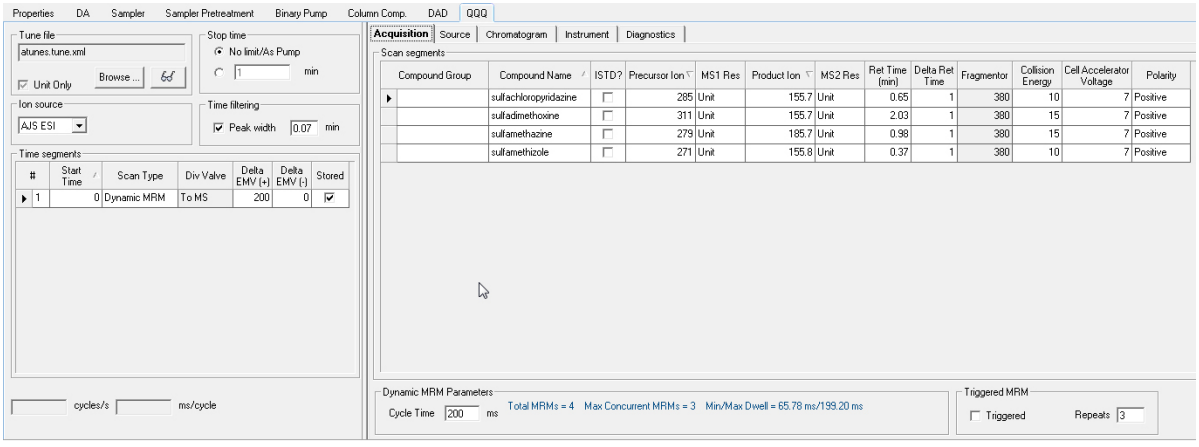
Steps	Detailed Instructions	Comments



3 Create template method.

a Click the **Acquisition** tab.
b Select a single ion for each compound for the optimization.
c Save the method.

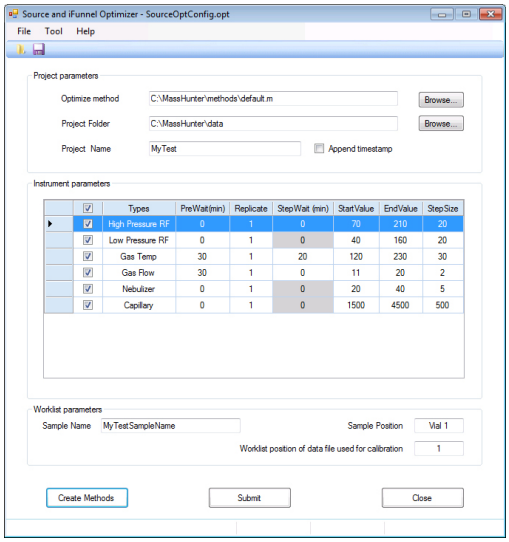
- A transition for each compound is already included in the Dynamic MRM method.



4 Start the Source and iFunnel Optimizer program.

- Double-click the **Source Optimizer** icon ( ).

Steps	Detailed Instructions	Comments

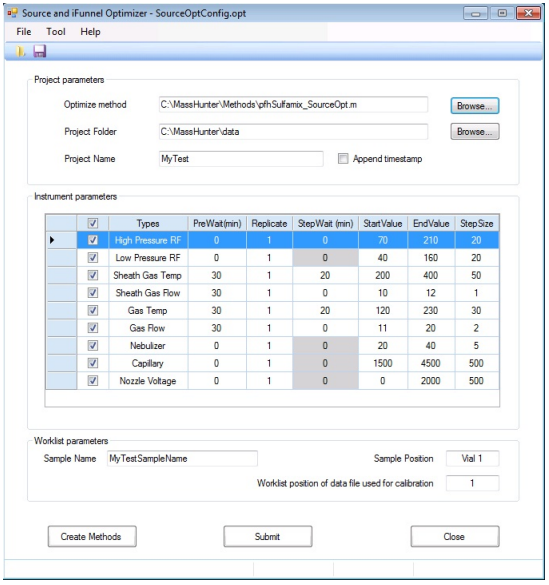


When this program starts, it automatically selects the default.m method. This method is not set up for an Agilent Jet Stream source, so no Agilent Jet Stream parameters are shown in the Instrument parameters table. If the method was set up for an Agilent Jet Stream source, then the Sheath Gas Flow, the Sheath Gas Temp and the Nozzle Voltage are included in this table.

5 Select the template method, ***iii*Sulfamix_SourceOpt.m.**

a Click the **Browse** button. The Browse For Folder dialog box is opened.
b Select the ***iii*Sulfamix_SourceOpt.m** method. Click the **OK** button.
c If the ion source in the method is different than the ion source in the Instrument parameters list, a warning message is opened. Click **OK**.

Steps	Detailed Instructions	Comments



The Sheath Gas Temp, Sheath Gas Flow, and Nozzle Voltage are all specific to the Agilent Jet Stream. If you do not have an Agilent Jet Stream source, these rows are not included in the table.

The High Pressure RF and Low Pressure RF are only included if the QQQ model is a 6490 or a 6495.

6 Change the order of the rows in the Instrument parameters table to the following:

- Ion Funnel parameters
- Sheath Gas temperature and flow
- Gas temperature and flow
- Nebulizer
- Capillary
- Nozzle Voltage

a If necessary, select the row that shows the **High Pressure RF** parameter.

b Drag this row to the top of the table. Both of the Ion Funnel parameters are moved together.

c Verify that the order of the rows in your table is as indicated.

- The order of the parameters in the Instrument parameters table is the order that the parameters are optimized. You want to optimize the parameters that have the greatest effect on the source optimization first. The Ion Funnel parameters have the greatest effect, so you move those parameters to the top of the list.
- By default, the parameters are in the optimized list.

Steps	Detailed Instructions	Comments

Instrument parameters

	Types	PreWait (min)	Replicate	StepWait (min)	StartValue	EndValue	StepSize
▸	High Pressure RF	0	1	0	70	210	20
	Low Pressure RF	0	1	0	40	160	20
	Sheath Gas Temp	30	1	20	200	400	50
	Sheath Gas Flow	30	1	0	10	12	1
	Gas Temp	30	1	20	120	230	30
	Gas Flow	30	1	0	11	20	2
	Nebulizer	0	1	0	20	40	5
	Capillary	0	1	0	1500	4500	500
	Nozzle Voltage	0	1	0	0	2000	500

The High Pressure RF and the Low Pressure RF are always optimized together. If you move one of these rows in the table, the other row is also moved. All possible combinations of the High Pressure RF and Low Pressure RF are tried to find the optimal values. For this example, the High Pressure RF parameter has 8 different parameter settings (70, 90, 110, etc.). The Low Pressure RF parameter has 7 different parameter settings. So, the program automatically creates 56 (8 * 7) different methods (1 for each parameter combination) just for optimizing the ion

7 Review the values for each parameter in the **Instrument parameters** table.

For each row in the table, verify:

- **PreWait** (in minutes).
- **Replicate**.
- **StepWait** (in minutes).
- **StartValue**.
- **EndValue**.
- **StepSize**.

- When the study for each parameter is loaded for the first time but before you run the first run, you wait the **PreWait** number of minutes before starting the run. Some parameters (that are electronic) stabilize almost instantly (in milliseconds), so you do not need to wait. For flows and temperatures, you want to have a **PreWait** before you run the study.
- You also want to wait for temperature parameters in between changing the parameter to a different value, so you also set the **StepWait** (in minutes).
- If you optimize the Gas Temp or the Sheath Gas Temp, then the test takes a long time because the temperature needs to stabilize for the time specified in StepWait before it moves to the next temperature.

8 Save the Instrument parameters.

a Click **File > Save As (*.opt)**.
b Enter the name for this set of instrument parameters.
c Click **OK**.

Steps	Detailed Instructions	Comments
9 Modify the Instrument parameters table to only modify one parameter for this task. This task only optimizes the **Capillary** voltage.	**a** Mark the check box next to the **Capillary**. **b** Clear the check boxes next to all of the other parameters.	• For this example, you optimize the **Capillary** voltage. Usually, you optimize the parameters in the order specified in the Instrument parameters table.

Instrument parameters

		Types	PreWait(min)	Replicate	StepWait (min)	StartValue	EndValue	StepSize
	☐	High Pressure RF	0	1	0	70	210	20
	☐	Low Pressure RF	0	1	0	40	160	20
	☐	Sheath Gas Temp	30	1	20	200	400	50
	☐	Sheath Gas Flow	30	1	0	10	12	1
	☐	Gas Temp	30	1	20	120	230	30
	☐	Gas Flow	30	1	0	11	20	2
	☐	Nebulizer	0	1	0	20	40	5
▶	☑	Capillary	0	1	0	1500	4500	500
	☐	Nozzle Voltage	0	1	0	0	2000	500

The capillary voltage is optimized from 1500 V to 4500 V with a step size of 500 V.

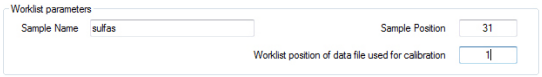
10 Set the **Project Folder** and the **Project Name**.	**a** Select the **Project Folder**. In this example, select *\MassHunter\Data*. **b** Enter a **Project Name**. **c** (optional) Mark the **Append timestamp** check box.	• If you mark the **Append timestamp** check box, then a time stamp is automatically added to the **Project Name** when you click the **Submit** button.

Project parameters

Optimize method	C:\MassHunter\Methods\pfhSulfamix_SourceOpt.m	Browse...
Project Folder	C:\MassHunter\data	Browse...
Project Name	MyTest	☑ Append timestamp

11 Set the **Worklist parameters**.	**a** Type the **Sample Name**. **b** Type the **Sample Position.** **c** Type the **Worklist position of data file used for calibration**.	• If you mark the **Append timestamp** check box, then a time stamp is automatically added to the **Project Name** when you click **Submit**. • For each parameter that is optimized, a batch file is created for Quantitative Analysis. One of the injections is considered 100% of the starting value. The value of **Worklist position of data file used for calibration** states which data file to use. If you enter `1`, then the data file from the first row is used.

Steps	Detailed Instructions	Comments



12 Create the methods and submit the study to the Study Manager.

a Click **Create Methods**.
b Click **Submit**.

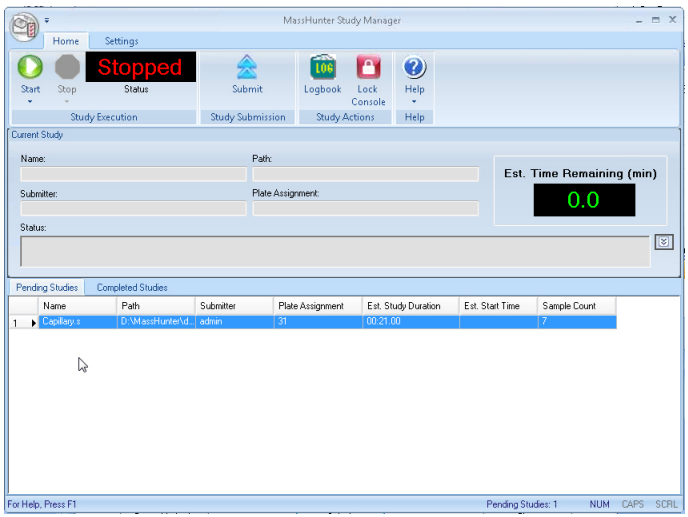
- When you click **Create Methods**, a message at the bottom of the main window states how many **Methods** were created, how many **Injections** are involved, and the **Estimated time**. The **Estimated time** is only an approximation.

The estimated time includes the Stoptime for the method plus one minute per injection. It does not consider the Posttime specified in the method. Also, it does not include the PreWait nor the StepWait that you entered in the Instrument parameters table.

13 Review the study (or studies) submitted to Study Manager.

a Open the Study Manager program.
b Select a row in the Pending Studies table.
c Right-click the row and click **Edit Worklist From Study**.
d Review the worklist in the Edit Worklist dialog box. Click **Save**.

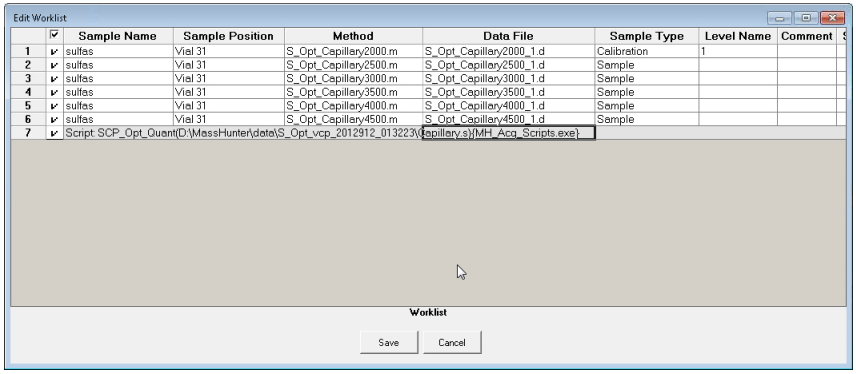
- A study is submitted for each parameter that you marked in the Instrument parameters table.
- Only one study is created for the **High Pressure RF** and **Low Pressure RF** parameters.



The name of the study is the Instrument parameter that is being optimized. A separate study is added for each parameter that is being optimized.

You can examine or edit the worklist for the study. You right-click the line in the Pending Studies table and click Edit Worklist from Study.

Steps	Detailed Instructions	Comments

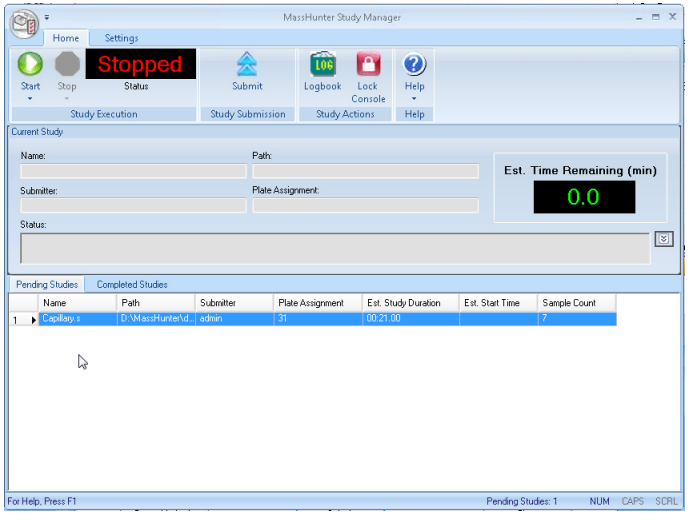


The script that is run at the end of the worklist creates the Quantitative Analysis batch file.

14 Modify the Study Manager parameters to run a standby script when the study completes and then start the Study Manager.

a Click the **Settings** tab in the Ribbon.
b Mark the **Enable standby script execution on idle or error** check box.
c Click the "..." button to select the script to run.
d Select **SCP_InstrumentStandby** and click the **OK** button.
e Enter 1 for the **Wait for** time.
f Click the **Start** button if necessary.

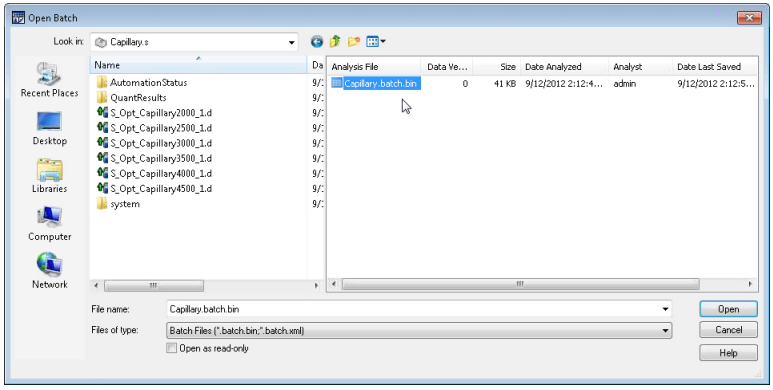
- When the Study Manager is not running a study for the time specified, the script you select is run.



The name of the study is the Instrument parameter that is being optimized. A separate study is added for each parameter that is being optimized.

You can examine or edit the worklist for the study. You right-click the line in the Pending Studies table and click Edit Worklist from Study.

Steps	Detailed Instructions	Comments
15 When the study completes, stop the Study Manager queue and exit from the Study Manager program.	**a** Click the **Stop > Immediately** command. **b** Close the Study Manager program.	
16 Open the data in the Quantitative Analysis program.	**a** Start the Quantitative Analysis program. **b** Click **File > Open Batch**. **c** Navigate to the location of the study. **d** Select the **Batch file** named *Capillary.batch.bin* and click **Open**.	

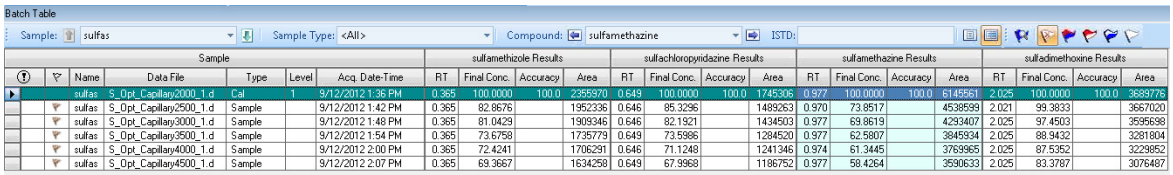


You specified the Project Folder and the Project Name in the Source and iFunnel Optimizer program before you submitted the study.

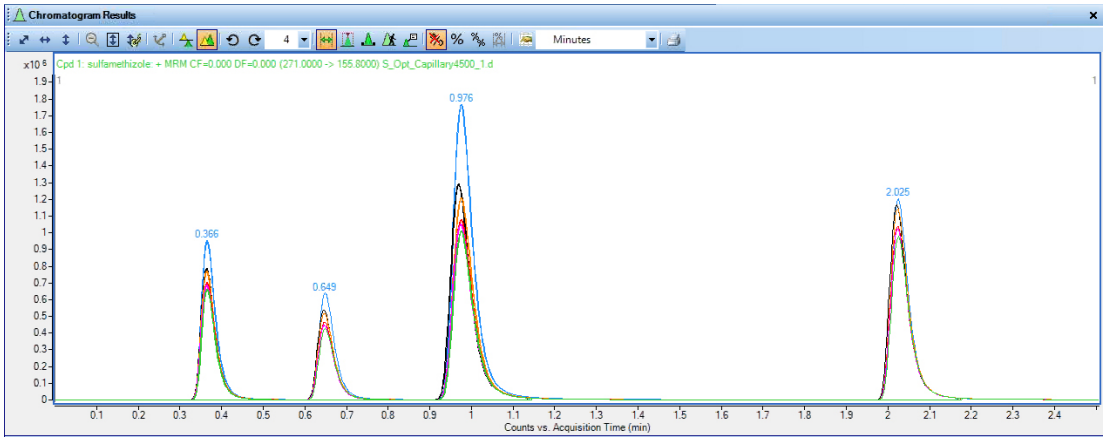
The batch file is created automatically at the end of the study.

The "system" folder contains all of the methods that were used in this study.

17 Review the Batch Table.	**a** Switch to **Multiple Compound View**. **b** Add the **Area** column to the table. **c** For each compound, right-click the **Final Conc.** column and click **Plot this column**. **d** Examine the Area column and the Final Conc. graph to determine the best capillary voltage. **e** Close the Quantitative Analysis program.	• Refer to the *online Help* for the Quantitative Analysis program to learn how to do these tasks. • In this case, all four compounds optimize at the same setting. Often, different compounds have different optimal settings, and you have to compromise.



Steps	Detailed Instructions	Comments
18 (optional) Review the data files in the Qualitative Analysis program.	**a** Start the Qualitative Analysis program. **b** Open all of the data files in the study. **c** Click **Find > Find Compounds by MRM.** **d** Select all of the data files and click the **Find** button. **e** Click the **Edit > Auto-Color Mode > Single Color per Data File** menu item. **f** Clear the check boxes next to the **TIC** for each data file. **g** Examine the results in the Chromatogram Results window. **h** Close the Qualitative Analysis program.	• By default, the program selects different colors for different transitions. • It is clear that the conditions used for the blue chromatograms are the best, and the blue chromatograms are for the data file with the capillary voltage set to 2000.



www.agilent.com

In This Book

The exercises in this guide help you learn to use the Agilent 6400 Series Triple Quadrupole LC/MS system. In this guide, you acquire data and then analyze the results using the MassHunter Qualitative Analysis program to learn how to develop an acquisition method.

You also learn about Agilent MassHunter Optimizer and Agilent Source and iFunnel Optimizer.

Printed in USA
Revision A, May 2014

G3335-90167

Agilent Technologies