

MassHunter Workstation Data Acquisition for Triple Quad LC/MS

Familiarization Guide

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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 or Resources media), 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 Navigator program to identify parameter values producing optimum signal response. You can also learn about the Qualitative Analysis programs by using the *MassHunter Qualitative Analysis Familiarization Guide*, the *MassHunter Qualitative Analysis eFamiliarization 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.

These steps are done by your Agilent Field Service Engineer at installation.

2 Copy the data files to your PC.

Copy the folder named **SulfaDrugs** in the **Data** folder on your Qualitative Analysis installation or Resources media 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 product media, 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 Navigator 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
- RRHD Eclipse Plus C18.2.1 mm x 50 mm Zorbax column (p/n 959757-902)
- A 1 ng/μL concentration of the sulfa mix sample (prepared in this step)

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 ampules (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.

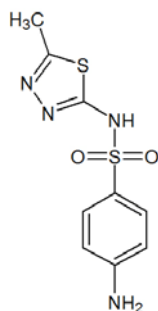
Use the column listed in the **Parts List**. Other columns and instrument parameters may be used in these exercises, but some parameters may need adjustment, and the results will differ.

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.

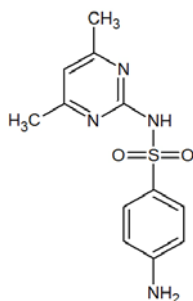
The Electrospray LC Demo Sample (P/N 59987-20033) contains five ampules with 100 ng/μL each of sulfamethizole (M+H)⁺ = 271, sulfamethazine (M+H)⁺ = 279, sulfachloropyridazine (M+H)⁺ = 285, and sulfadimethoxine (M+H)⁺ = 311.

Before you begin
Prepare to acquire data

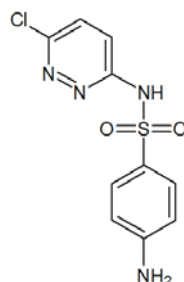
Sulfamethizole



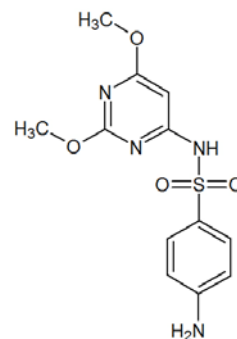
Sulfamethazine



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* or *online Help* for instructions on tuning the instrument.

Exercise 1 – Develop an acquisition method

Task 1. Enter acquisition parameters and acquire data

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 Navigator 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 1 .	a Double-click the Data Acquisition icon. b On the toolbar, make sure Context is set to Acquisition . c Enter the LC parameters listed in the Table 1 .	• The Data Acquisition window appears. See Figure 1 on page 8.

Exercise 1 – Develop an acquisition method

Task 1. Enter acquisition parameters and acquire data

Table 1 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%
• 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, 6470, 6490, and 6495 if you have an AJS ESI source 40 °C for other instruments

Exercise 1 – Develop an acquisition method

Task 1. Enter acquisition parameters and acquire data

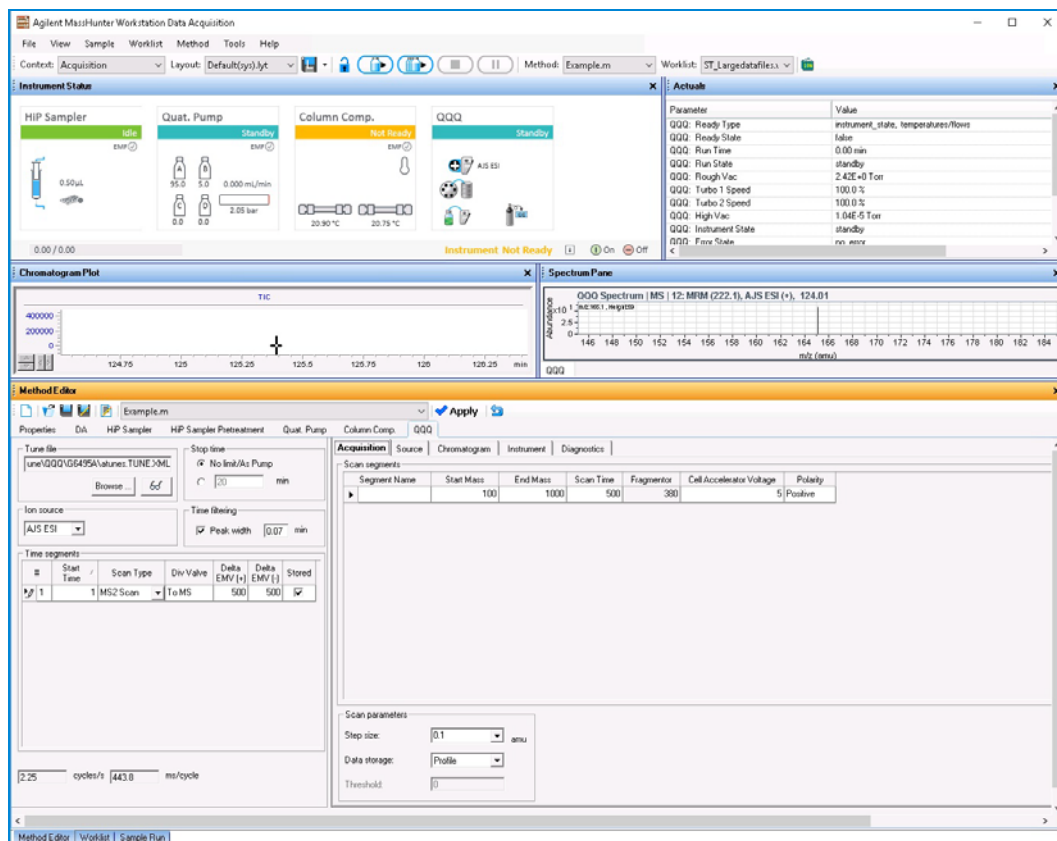


Figure 1. MassHunter Workstation – Data Acquisition window

Steps	Detailed Instructions	Comments
2	Enter MS parameters appropriate for sulfa drug mix and save the method as iiiMS2Scantest.m, where iii are your initials. See Table 2 on page 9.	a Click the QQQ tab in the Method Editor window. b Select MS2 Scan from the Scan Type list in the Time Segments table. c Enter the other MS parameters as listed in Table 2. These parameters are in either the Acquisition or the Source tabs. d Save the method as iiiMS2Scantest.m, where iii are your initials.

Exercise 1 – Develop an acquisition method

Task 1. Enter acquisition parameters and acquire data

Table 2 MS parameters for sulfa drug mix

Parameter	Value (ESI)	Value (AJS ESI)
• Inlet	ESI (positive polarity)	AJS ESI (positive polarity)
• Scan Type	MS2 Scan	MS2 Scan
• Delta EMV (+)	400 V	200 V
• Mass Range	100 to 400	100 to 400
• Cell Accelerator 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)

Acquisition | Source | Chromatogram | Instrument | Diagnostics

Scan segments

Segment Name	Start Mass	End Mass	Scan Time	Fragmentor	Cell Accelerator Voltage	Polarity
1	100	400	500	100	7	Positive

If you have a 6490 or 6495 QQQ, you cannot edit the Fragmentor column (it is unavailable). The value for the Fragmentor for a 6490/6495 QQQ comes from the tune file, and for the 6490/6495 it is typically 380 V.

Scan parameters

Step size: 0.1 amu

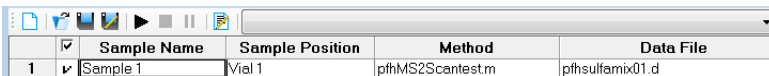
Data storage: Profile

Threshold: 0

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

Exercise 1 – Develop an acquisition method

Task 1. Enter acquisition parameters and acquire data

Steps	Detailed Instructions	Comments
3 Acquire data (optional). - Set up a one-line worklist with the method you just created. - Name the data file iiisulfamix01.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 On the Sample Position tab, select the Autosampler, Well-plate or Vial Tray. g In the graphic, select a single position. Click OK. h In the Worklist window, mark the check box to the left of the sample.	- 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.
	 The screenshot shows the Worklist window with a toolbar at the top containing icons for file operations and execution. Below the toolbar is a table with four columns: Sample Name, Sample Position, Method, and Data File. The first row contains the following data: Sample Name: Sample 1, Sample Position: Vial 1, Method: pfmMS2Scantest.m, Data File: pfmSulfamix01.d. A checkmark is visible in the first column of the first row.	
	i Click the Start Worklist Run icon in the main toolbar, the Run Worklist icon in the Worklist toolbar or click the Worklist > Run command.	

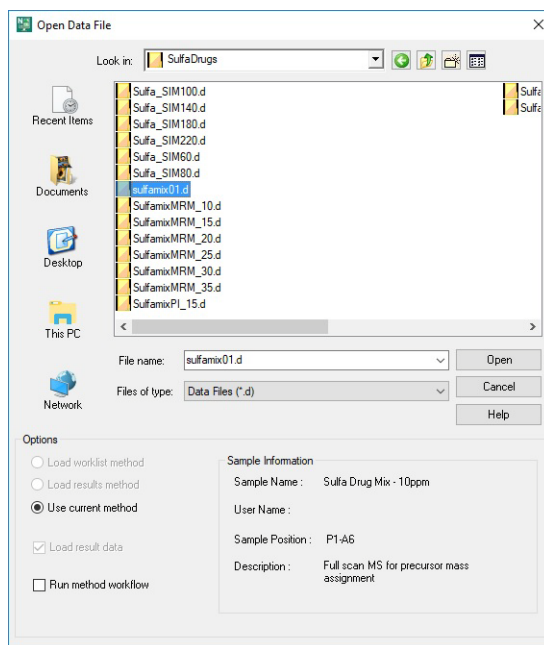
Exercise 1 – Develop an acquisition method

Task 2. Determine precursor ion masses

Task 2. Determine precursor ion masses

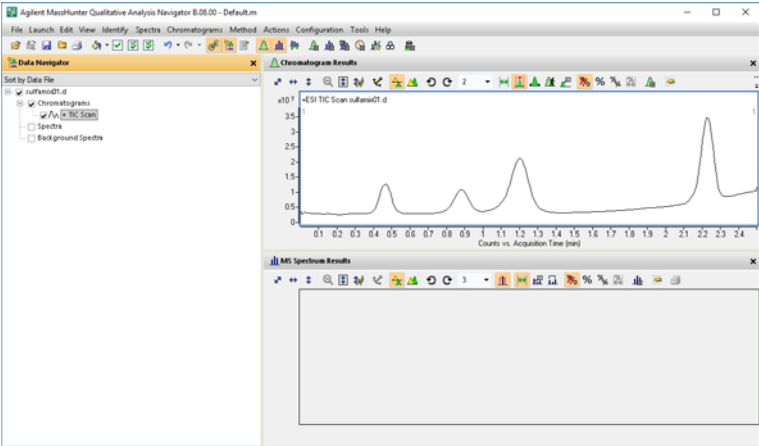
In this exercise, you determine the precursor ions for each of the sulfa drugs in the acquired data file.

Steps	Detailed Instructions	Comments
1 Open the acquired data file. In the Qualitative Navigator 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 Navigator B.08.00 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 unavailable. - If you see the check box marked, this means that the data file(s) already contains results. <i>Clear this check box before opening the file.</i>



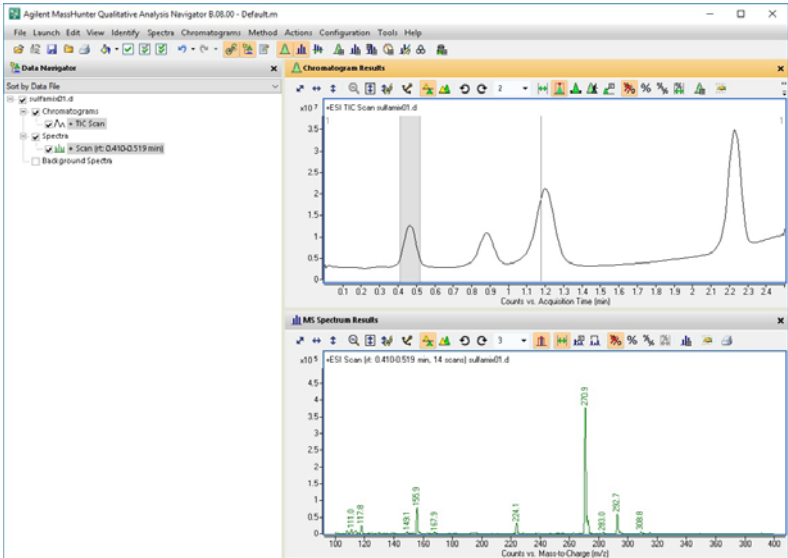
Exercise 1 – Develop an acquisition method

Task 2. Determine precursor ion masses

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 Navigator 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. - Click  in the Chromatogram Results toolbar. Click Default and click OK. - Click the  in the MS Spectrum Results toolbar. Click Default and click OK.

Exercise 1 – Develop an acquisition method

Task 2. Determine precursor ion masses

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: <table border="1"> <thead> <tr> <th>Compound</th><th>RT</th><th>m/z</th></tr> </thead> <tbody> <tr> <td>Sulfamethizole</td><td>0.47</td><td>270.9</td></tr> <tr> <td>Sulfachloropyridazine</td><td>0.88</td><td>284.9</td></tr> <tr> <td>Sulfamethazine</td><td>1.2</td><td>279.0</td></tr> <tr> <td>Sulfadimethoxine</td><td>2.23</td><td>311.0</td></tr> </tbody> </table> - 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.	Compound	RT	m/z	Sulfamethizole	0.47	270.9	Sulfachloropyridazine	0.88	284.9	Sulfamethazine	1.2	279.0	Sulfadimethoxine	2.23	311.0	a In the Chromatogram Results window, make sure that the Range Select icon on the toolbar  is on. b Click the 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	1.2	279.0															
Sulfadimethoxine	2.23	311.0															
	 The screenshot shows the Agilent MassHunter Qualitative Analysis Navigator interface. The top window displays a Total Ion Chromatogram (TIC) with four distinct peaks. The first peak is shaded, and a region is selected around it. The bottom window shows the extracted MS Spectrum Results for the selected peak, displaying a mass spectrum with a base peak at m/z 270.9 and several other labeled peaks at m/z 114.6, 117.6, 148.1, 155.3, 197.9, 224.1, 281.0, 292.7, and 308.8.	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)+.															

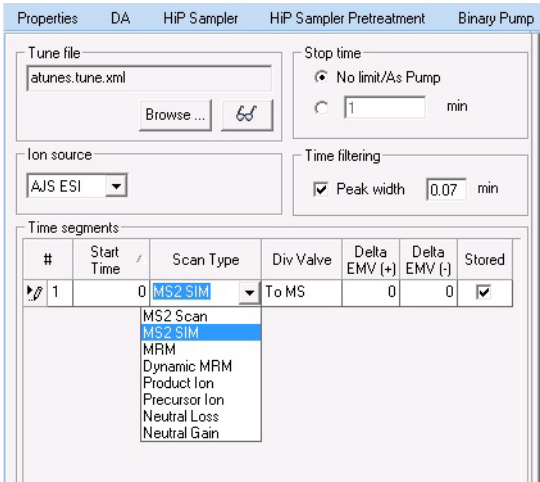
Exercise 1 – Develop an acquisition method

Task 3. Find optimum fragmentor voltage for maximum response

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 Navigator 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 iiiMS2SIMxxx.m, where iii are your initials and xxx is the voltage.	a In the Scan Type dropdown list, select MS2 SIM .  The screenshot shows the 'Properties' tab of the Data Acquisition software. The 'Tune file' is set to 'atunes.tune.xml'. The 'Ion source' is set to 'AJS ESI'. The 'Stop time' is set to 'No limit/As Pump'. The 'Time filtering' is set to 'Peak width 0.07 min'. The 'Time segments' table shows a single segment with 'Scan Type' set to 'MS2 SIM'.	

Exercise 1 – Develop an acquisition method

Task 3. Find optimum fragmentor voltage for maximum response

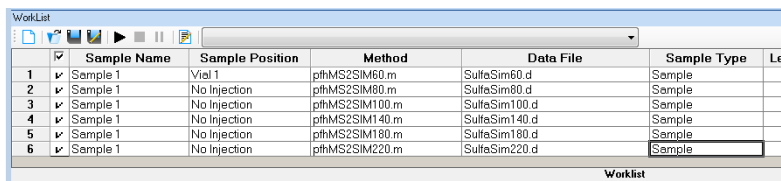
Steps	Detailed Instructions	Comments
b	On the Acquisition tab, enter the Compound Name and Mass (precursor ion mass) for sulfadimethoxine (311 <i>m/z</i>).	• With the MS2SIM Scan Type set, a different set of columns appears in the Acquisition window.
c	Right-click anywhere in the Scan segments section, and click Add Row .	• 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.
d	Type the Compound Name and the Mass for sulfachloropyridazine (285 <i>m/z</i>).	• The Fragmentor column is unavailable if the instrument type is a 6490 or 6495.
e	Repeat steps c and d for sulfamethazine (279 <i>m/z</i>) and sulfamethizole (271 <i>m/z</i>).	
f	Save the method as iiiMS2SIM140.m , where <i>iii</i> are your initials.	
g	Change the fragmentor voltage to 60, and save the method as iiiMS2SIM060 , where <i>iii</i> are your initials.	
h	Repeat step g for voltages 80, 100, 180 and 220, saving the methods as iiiMS2SIM080 , iiiMS2SIM100 , iiiMS2SIM180 and iiiMS2SIM220 , where <i>iii</i> are your initials.	

Acquisition Source Chromatogram Instrument Diagnostics									
Scan segments									
Compound Group	Compound Name	ISTD?	Mass	MS2 Res	Dwell	Fragmentor	Cell Accelerator Voltage	Polarity	
▶	sulfadimethoxine	☐	311	Unit	200	380	7	Positive	
	sulfachloropyridazin	☐	285	Unit	200	380	7	Positive	
	sulfamethazine	☐	279	Unit	200	380	7	Positive	
	sulfamethizole	☐	271	Unit	200	380	7	Positive	

Exercise 1 – Develop an acquisition method

Task 3. Find optimum fragmentor voltage for maximum response

Steps	Detailed Instructions	Comments
2 Set up and run the worklist (optional). - Set up six samples with Sample Name SulfaDrugMix to inject 1 µl from vials 1-6 or the ones you choose. - Specify the data files as iiiSulfaSIMxxx.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	pHMS2SIM60.m	SulfaSim60.d	Sample	
2	☒	Sample 1	No Injection	pHMS2SIM80.m	SulfaSim80.d	Sample	
3	☒	Sample 1	No Injection	pHMS2SIM100.m	SulfaSim100.d	Sample	
4	☒	Sample 1	No Injection	pHMS2SIM140.m	SulfaSim140.d	Sample	
5	☒	Sample 1	No Injection	pHMS2SIM180.m	SulfaSim180.d	Sample	
6	☒	Sample 1	No Injection	pHMS2SIM220.m	SulfaSim220.d	Sample	

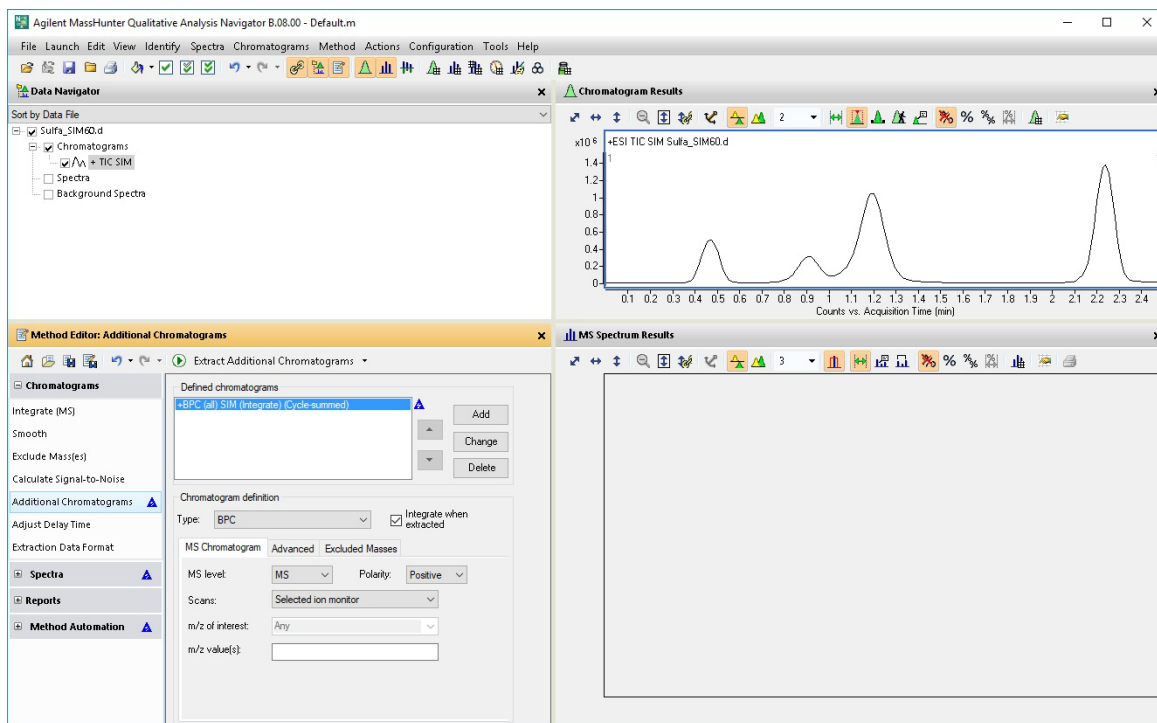
- h** Start the worklist.
- Click **Worklist > Run**.
 - Click  in the main toolbar.
 - Click  in the worklist toolbar.
- Note that the program only runs those samples that are marked with a checkmark.
 - You can also run the worklist in locked mode by clicking the lock button in the main toolbar (the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



Exercise 1 – Develop an acquisition method

Task 3. Find optimum fragmentor voltage for maximum response

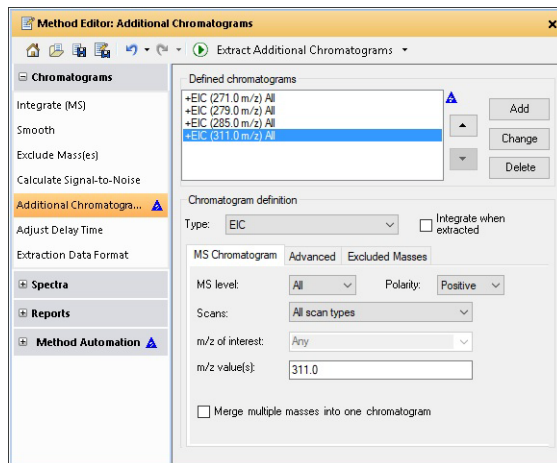
Steps	Detailed Instructions	Comments
3 Set up a qualitative method to view the EIC data automatically. - Open the data file Sulfa_SIM60.d or your own iiiSulfa_SIM60.d, where iii are your initials. - In the Method Editor, add in the EICs corresponding to the precursor ion masses of 271, 279, 285, and 311. - Save the method as iiiExercise1, 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 iiiSulfa_SIM60.d , and click Open . c Click Method > Method Editor or View > Method Editor . The system displays the Method Editor window.	- The Qualitative Navigator program should be open. If not, see "Double-click the Qualitative Navigator B.08.00 icon." on page 11. - The window is docked for these images. See the <i>online Help</i> for more information on floating and docking windows.



Exercise 1 – Develop an acquisition method

Task 3. Find optimum fragmentor voltage for maximum response

Steps	Detailed Instructions	Comments
d	If necessary, click Additional Chromatograms in the Chromatograms section of the Method Editor.	- The default Method Editor list selection after installation is Integrate (MS). - When you are defining chromatograms, instead of deleting the Defined chromatogram, you can select EIC. Then, you enter the m/z value and click Change.
e	To delete the BPC chromatogram, click Delete in the Method Editor window.	
f	Select EIC for the Chromatogram definition Type .	
g	On the MS Chromatogram tab, make sure MS Level is set to All and Scans is set to All scan types .	
h	Clear the Integrate when extracted 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 Exercise1.m .	
n	Click Save .	



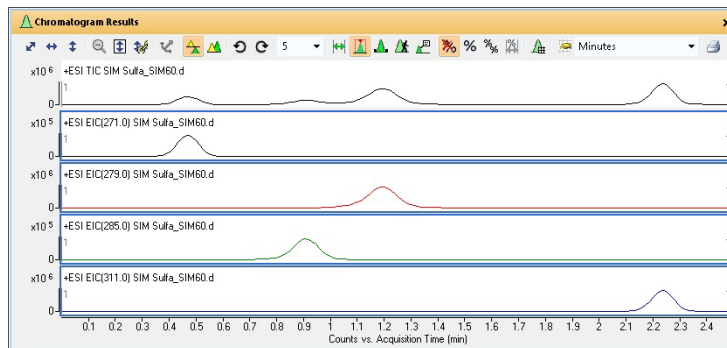
Exercise 1 – Develop an acquisition method

Task 3. Find optimum fragmentor voltage for maximum response

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 Run on the Method Editor toolbar to run the Extract Additional Chromatograms command.	You can also click the Chromatograms > Extract Additional Chromatograms command to extract the additional chromatograms.

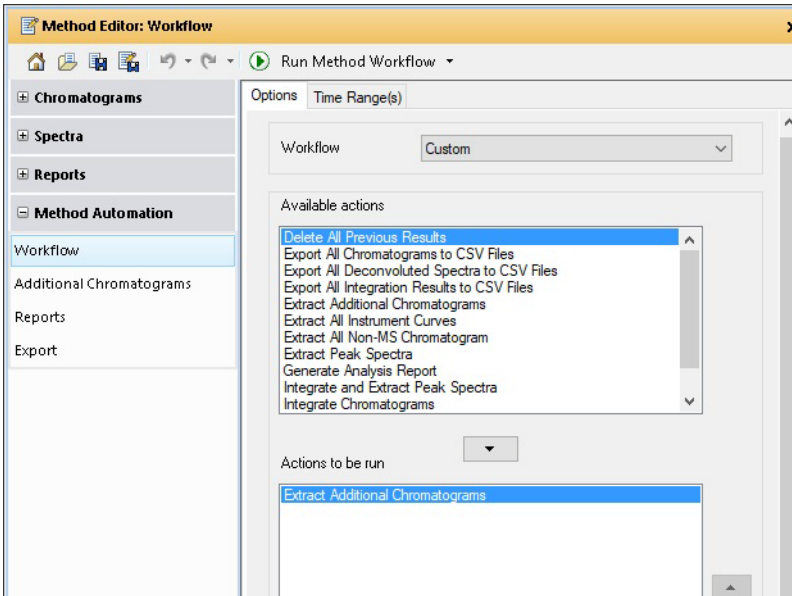


- b** To see the TIC and four EICs, click the arrow next to the **Maximum Number of List Panes** button in the Chromatogram Results toolbar, as shown in the example below.
- c** Select **5** to view five chromatograms simultaneously.
The system displays chromatogram results as shown below.



Exercise 1 – Develop an acquisition method

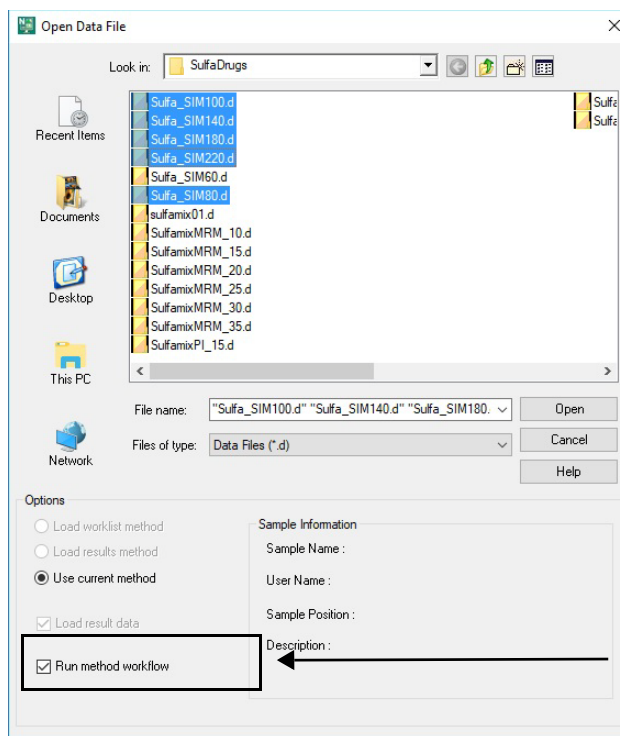
Task 3. Find optimum fragmentor voltage for maximum response

Steps	Detailed Instructions	Comments
5 Extract the remaining ion chromatograms automatically. - Extract Additional Chromatograms should be one of the default action for the Custom Workflow. - Open the remaining data files, Sulfa_SIM80.d through Sulfa_SIM220.d. - Close the Method Editor.	a Select Workflows from the Method Automation section in the Method Editor window. b Make sure that Actions to be run list only contains Extract Additional Chromatograms.	The Qualitative Navigator Method Editor lets you define a workflow to be performed automatically upon opening a data file(s).
		
	c Click File > Open Data File. The system displays the Open Data File dialog box. d Select the data files to be opened, <i>Sulfa_SIM80.d</i> through <i>Sulfa_SIM220.d</i>. e Mark the Run method workflow check box. (lower left corner)	

Exercise 1 – Develop an acquisition method

Task 3. Find optimum fragmentor voltage for maximum response

Steps	Detailed Instructions	Comments
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f Click **Open**.

The Qualitative Navigator program displays all the EICs for all the data files selected.

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

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

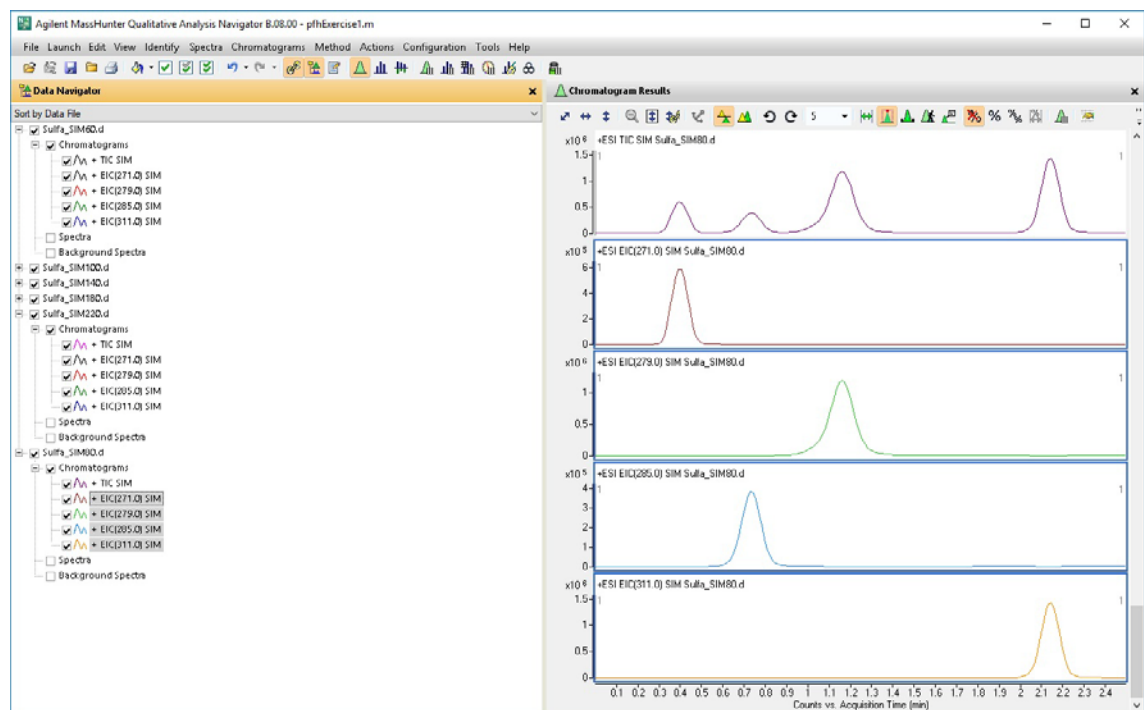
Exercise 1 – Develop an acquisition method

Task 3. Find optimum fragmentor voltage for maximum response

Steps

Detailed Instructions

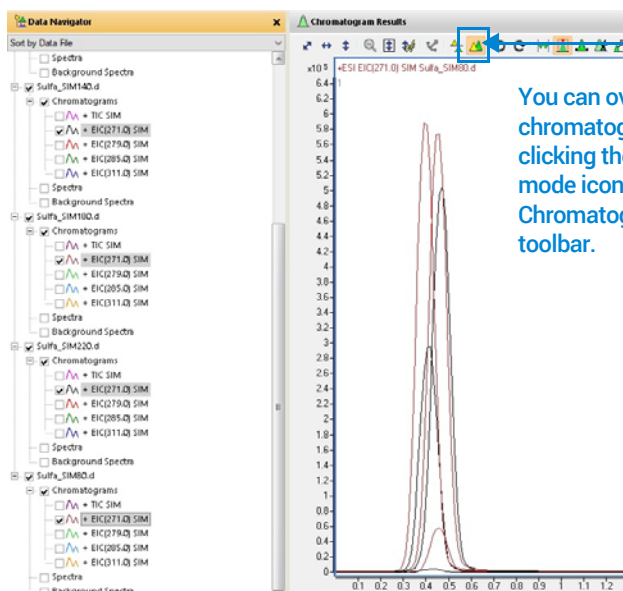
Comments



Exercise 1 – Develop an acquisition method

Task 3. Find optimum fragmentor voltage for maximum response

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 <i>m/z</i> . b Click the Show only the highlighted items button,  . Only the 271 <i>m/z</i> 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 for the analysis.



- | | |
|--|--|
| <p>d Repeat step a through step c for the other three base peaks or precursor ions.</p> <p>e Click File > Close All.</p> <p>f Click No in the Save dialog box.</p> | <ul style="list-style-type: none"> 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. |
|--|--|

Exercise 1 – Develop an acquisition method

Task 4. Determine product ion masses

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 iiiSulfamix PL_{xx}.m, where iii are your initials and xx is the collision energy.	a Click the QQQ tab in the Method Editor window. 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. Find optimum fragmentor voltage for maximum response ” on page 14. d Save the method as iiiSulfamix PL₁₅.m . e Repeat step b and step c 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.

The screenshot shows the Agilent Method Editor interface. The **Acquisition** tab is selected. The **Scan segments** table is populated with the following data:

Segment Name	Precursor Ion	MS2 From	MS2 To	Scan Time	Fragmentor	Collision Energy	Cell Accelerator Voltage	Polarity
sulfadimethoxine	311	50	300	250	140	15		7 Positive
sulfachloropyridazine	265	50	300	250	80	15		7 Positive
sulfamethoxazole	279	50	300	250	140	15		7 Positive
sulfamethizole	271	50	300	250	80	15		7 Positive

The **Scan parameters** section shows:

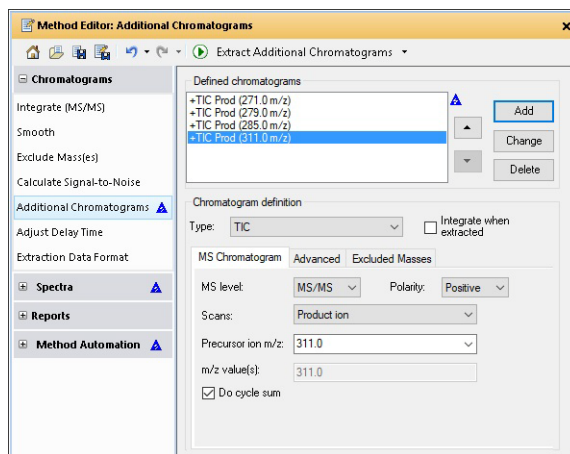
- Step size: 0.1 amu
- Data storage: Profile
- Threshold: 0

2 Set up and run the worklist (optional). Specify the data files as iiiSulfamix PL_{xx}.d, where iii are your initials and xx is the collision energy.	a On the Worklist tab, add three samples to the worklist for collision energies 15, 30 and 45. b Mark the check box to the left of the Sample Name for each sample you are adding. c 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.
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Exercise 1 – Develop an acquisition method

Task 4. Determine product ion masses

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, iiiSulfamixPI_xx.d. - Open 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.	- Click the Open Data File button in the main toolbar. - Select SulfamixPI_15.d. - Clear the Run Method Workflow check box, and click Open. - Make sure the Method Editor window is displayed; otherwise, click the Method Editor button.  - In the Chromatograms section select Additional Chromatograms. - Delete any existing chromatograms in the Defined Chromatograms list. - Select TIC from the Type list in the Define chromatograms section. - For MS level, select MS/MS. - Mark the Do cycle sum check box. - Clear the Integrate when extracted check box. - For Scans, select Product ion. - For Precursor ion m/z, type 271. - Click Add. - Repeat steps j and k for each ion.	The Qualitative Navigator program should already be open and contain iiiexercise 1.m as the method.



Exercise 1 – Develop an acquisition method

Task 4. Determine product ion masses

Steps	Detailed Instructions	Comments
	- o In the Method Editor window in the Chromatograms section, click Integrate (MS/MS). - p Select Agile 2 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.

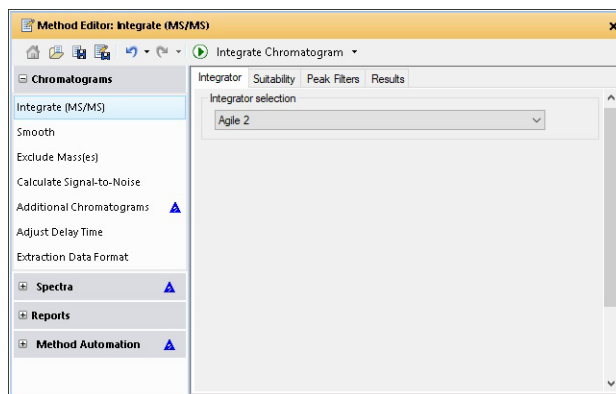


Figure 3. Integrate (MS/MS) > Integrator Tab

- q On 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.

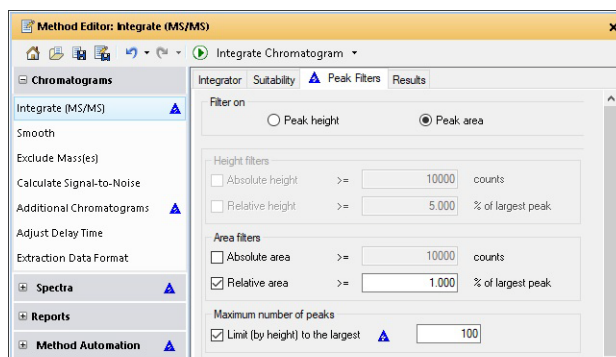


Figure 4. Integrate (MS/MS) > Peak Filters tab

Exercise 1 – Develop an acquisition method

Task 4. Determine product ion masses

Steps	Detailed Instructions	Comments
r	Click Method Automation , and then click Workflow .	
s	Select Integrate and extract peak spectra from the Available actions list and click  to add this to Actions to be run .	

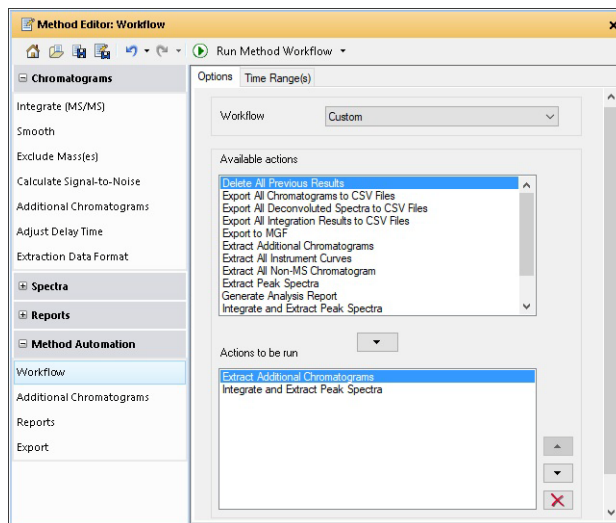


Figure 5. Method Automation > Workflow section

- t To apply the changes to the current method, *exercise1.m*, click the **Save Method** button.  You can also click **Method > Save**.

Exercise 1 – Develop an acquisition method

Task 4. Determine product ion masses

Steps	Detailed Instructions	Comments
4 Run the qualitative method on the current data file.	In the Method Editor toolbar, click Run (🟢). When the Workflow section is displayed, the Method Workflow runs.	- 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.

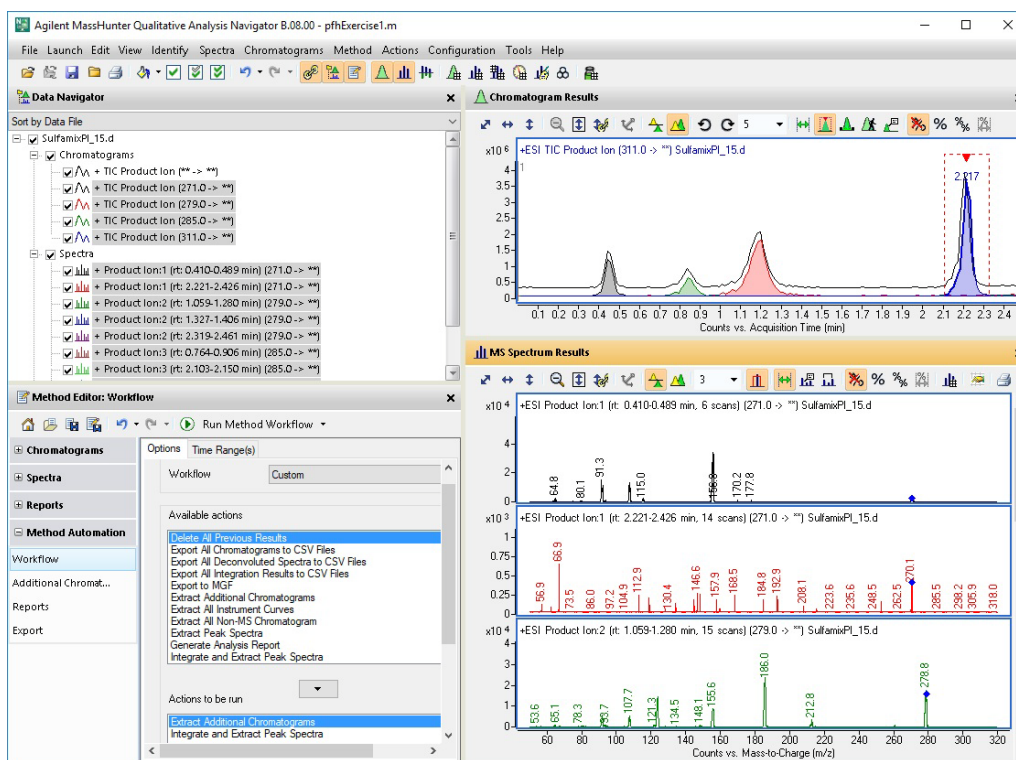


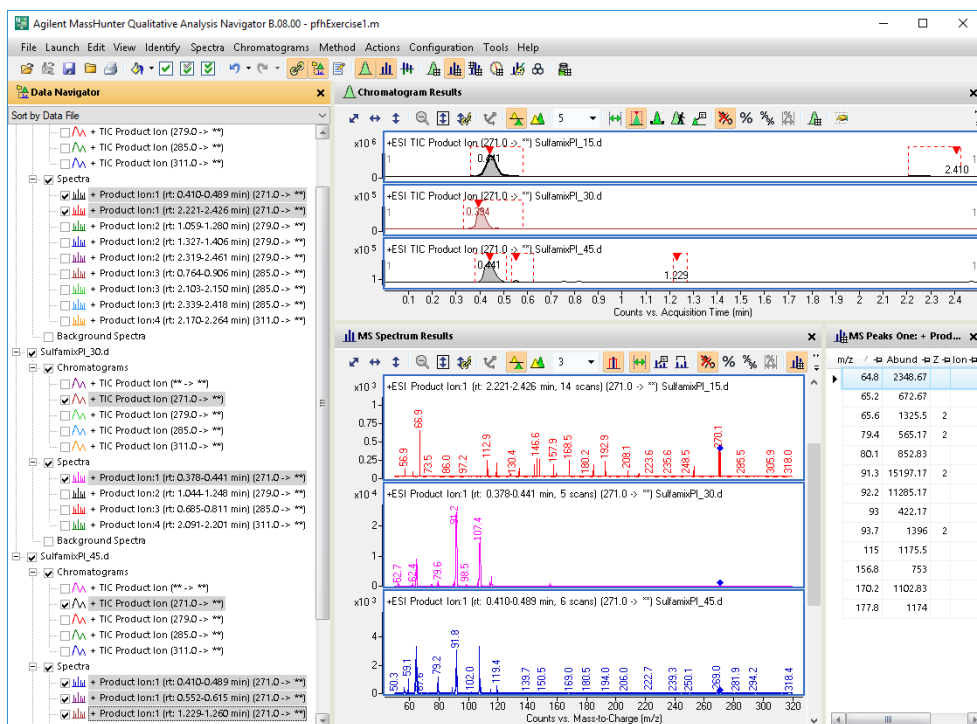
Figure 6. Results for integration and extraction of peak spectra.

- 5 Run the workflow 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 method workflow** check box in the **Open Data File** dialog box, and click **Open**.
- After the data files open, the Qualitative Navigator 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.

Exercise 1 – Develop an acquisition method

Task 4. Determine product ion masses

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 button, . 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** button 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

Exercise 1 – Develop an acquisition method

Task 5. Find optimum collision energy for MRM acquisition

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 iiiSulfamix MRM_xx.m, where iii are your initials and xx is the collision energy.	a On the QQQ tab, set Scan Type to MRM . b Enter all MS parameters shown in the example below except for the collision energy value. c In the collision energy column, type 10 for each compound. d Save the method as iiiSulfamix MRM_10.m . e Repeat step c and step d for collision energies of 15, 20, 25, 30 and 35 saving the methods as iiiSulfamix 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.

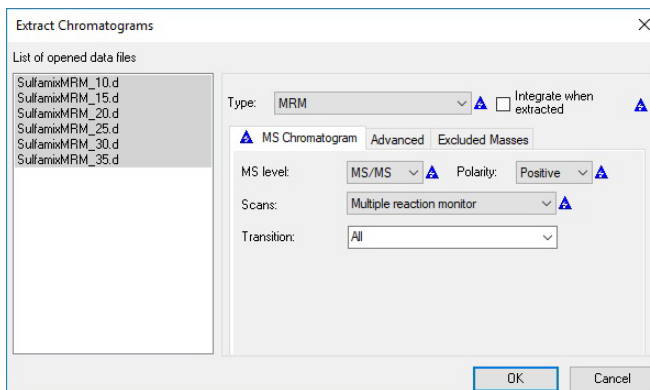
Compound Group	Compound Name	ISTD?	Precursor Ion	MS1 Ret	Product Ion	MS2 Ret	Dwell	Fragmentor	Collision Energy	Cell Accelerator Voltage	Polarity
	Sulfamethoxazole	☐	211	Unit	155.7	Unit	50	100	10		7 Positive
	Sulfachloropyridazine	☐	285	Unit	155.7	Unit	50	100	10		7 Positive
	Sulfamethazine	☐	279	Unit	185.7	Unit	50	100	10		7 Positive
	Sulfamethizole	☐	271	Unit	155.0	Unit	50	100	10		7 Positive

2 Set up and run the worklist (optional). Specify the data files as iiiSulfamix MRM_xx.d, where iii are your initials and xx is the collision energy.	a Click the Worklist tab to show worklist. 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.
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Exercise 1 – Develop an acquisition method

Task 5. Find optimum collision energy for MRM acquisition

Steps	Detailed Instructions	Comments
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 3 on page 32 for optimal method settings for each compound.	a Open the Qualitative Navigator program. b Clear the Run method workflow check box. c Open the MRM data files in the Qualitative Navigator 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.



Exercise 1 – Develop an acquisition method

Task 5. Find optimum collision energy for MRM acquisition

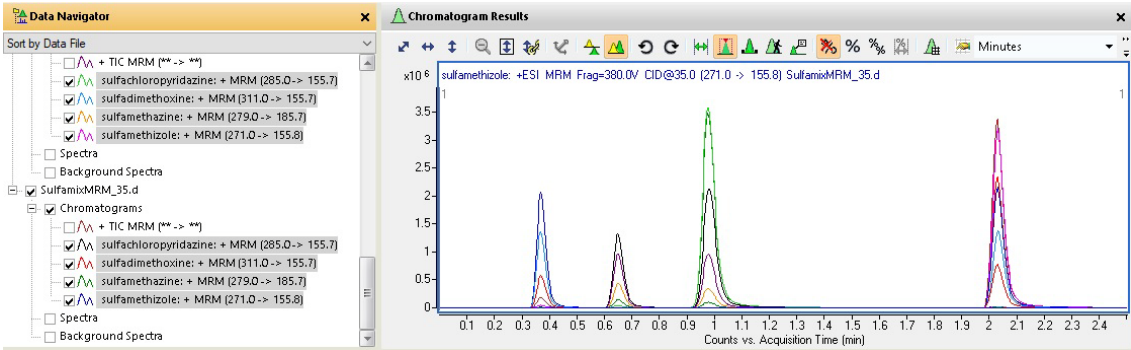
Steps	Detailed Instructions	Comments
	h Click the Overlaid Mode button, . i Compare peak intensities for each compound transition in each data file in the Chromatogram Results window.	- Compare the colors shown in Chromatogram Results with the color next to the MRM transition name in the Data Navigator. - 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 3. j Click the Close Data File button 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 3 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 method

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

Exercise 2 – Develop a Dynamic 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 38

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 40

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 media to the \MassHunter\Data\MRM_to_DMRM folder.	• You can instead use the classic view for the QQQ Quantitative Analysis program. • The file SulfamixMRM_10.d is on the installation media in the \Support\Data folder. Copy this entire folder to the \MassHunter\Data\MRM_to_DMRM folder.
	a Double-click the QQQ Quantitative Analysis (Quant-My-Way) icon or the Drug Quant (Quant-My-Way) icon. b On the Home tab click New Batch and then click Browse. c Navigate to the \MassHunter\Data\MRM_to_DMRM folder. d Type MRM_to_DMRM in the File name text box. e Click Create. f If the Add Samples dialog box does not open, click File > Add Samples. g Select the file SulfamixMRM_10.d. h Click OK.	

Exercise 2 – Develop a Dynamic 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 On the Method tab, click New > New Method from Acquired MRM Data. b Select the SulfamixMRM_10.d data file. c Click Open.	- If the Method Tasks is not shown as the left pane, do the following: - Click the View tab on the ribbon. - Click Panes > Method Development Tasks.
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 Method Setup Tasks section in the Method Tasks pane. b Select the first compound in the table. c On the Method tab, click Manual Setup Tasks > New Calibration Level. You can instead right-click the compound row and click New Calibration Level from the shortcut menu. d Enter A1 in the Level column and 1.0 in the Conc. column. e On the Method tab, click Calibration Curve > Copy Calibration Levels To. You can instead 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 Method Setup Tasks section in the Method Tasks pane. j Set CF to Linear for all compounds. CF means curve fit. k Set CF Origin to Include for all compounds. l Set CF Weight to None for all compounds.	- Refer to the <i>online Help</i> 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.

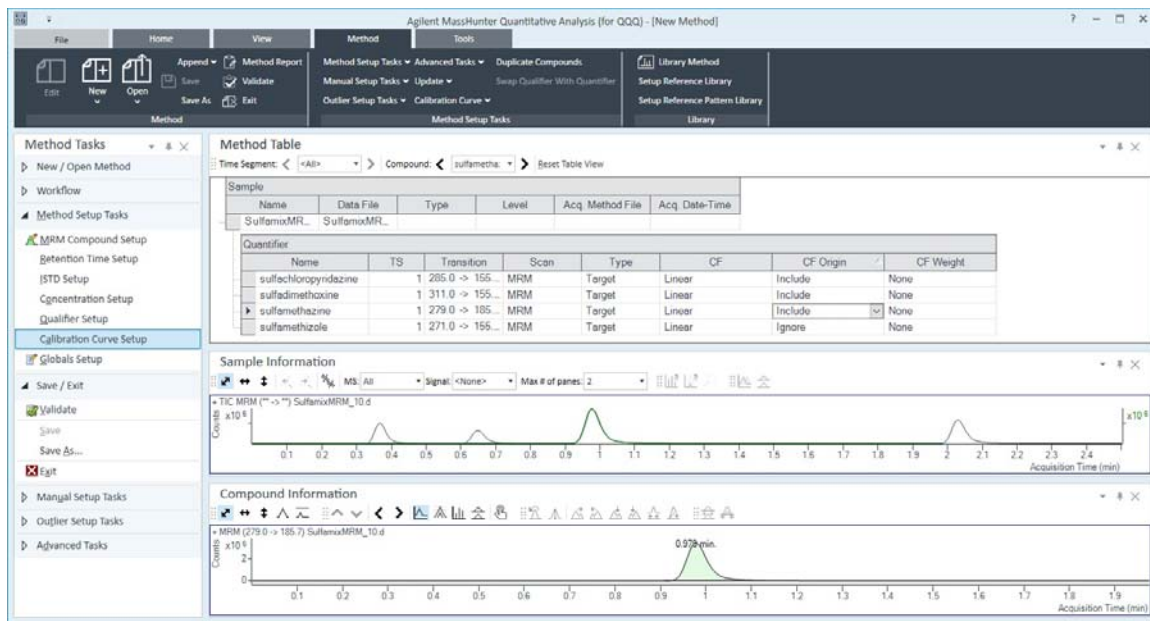
Exercise 2 – Develop a Dynamic MRM method

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

Steps

Detailed Instructions

Comments



- 4 Verify method and then save the method and apply the method to the batch.
 - a From the Save / Exit task section, click **Validate**.
 - b Click **OK** on the message box. Fix any errors, if necessary.
 - c On the **Method** tab on the ribbon, click **Save As**.
 - d Enter **MRM_t_o_DMRM**.
 - e Click **Save**.
 - f On the **Method** tab, click **Exit**.
 - g (optional) Click **Analyze** in the **Apply Method** dialog box.
 - h Click **Yes** to apply the method to the batch.
 - 5 Analyze and save the batch.
 - a On the **Home** tab on the ribbon, click **Analyze Batch**.
 - b Click **File > Save Batch**.
- If any errors are discovered, the Method Error List window is displayed. Double-click an error to see where in the Method Table the parameter needs to be changed.
 - In the **Apply Method** dialog box, you can click **Analyze** to automatically start additional batch processing.

Exercise 2 – Develop a Dynamic MRM method

Task 2. Print a report in the Quantitative Analysis program

Task 2. Print a report in the Quantitative Analysis program

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

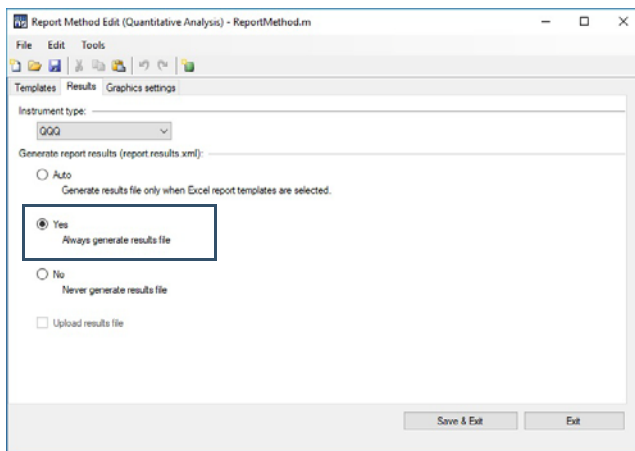
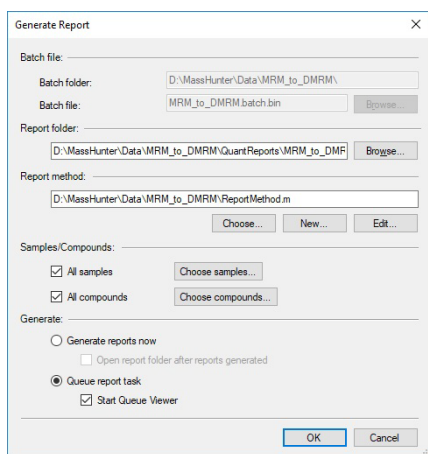
You can update a Dynamic MRM method using either a data file or a quantitation report folder, so this task creates the quantitation report folder.

Steps	Detailed Instructions	Comments
1 Print a report using the template MRM_to_DMRM.xltx.	a Click File > Save Batch. b On the Home tab on the ribbon, click Generate Report. The system displays the Generate Report dialog box. c Select the Report folder. This folder name will be used in the next task. d Click New for the Report method. The Report Method Edit dialog box opens. e Click Add Template. f Navigate to the template you wish to begin with, and click Open. g On the Results tab, click Yes Always generate results file. h Click Save & Exit. i Enter a File name for the method report. j Click Save. k Mark All samples. l Mark All compounds. m Click OK.	• Copy the MRM_to_DMRM.xltx template from the \Support\Data folder on the installation media. • For this report, you do not need to print the report. • If you have not created a Report Method, see the <i>online Help</i> for Quantitative Analysis for instructions on how to create a report method. • The Data Acquisition program uses the results file in the Quant reports folder to update the method, so you must generate this file if you want to update your method with it.

Exercise 2 – Develop a Dynamic MRM method

Task 2. Print a report in the Quantitative Analysis program

Steps	Detailed Instructions	Comments
2 (optional) Check the status of the report using the Queue Viewer program.	- Click Report > Queue Viewer. - Wait for the report to finish printing. - Close the Task Queue Viewer program.	
You only use this option if you clicked the Queue report task option in the Generate Report dialog box.		



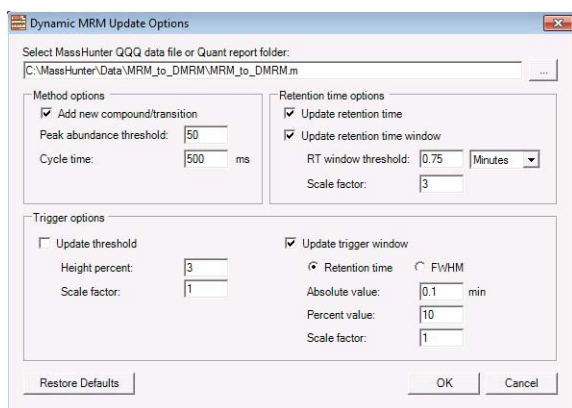
Exercise 2 – Develop a Dynamic MRM method

Task 3. Create a Dynamic MRM method using Update dMRM

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 **Dynamic MRM Update Options** dialog box.

Steps	Detailed Instructions	Comments
1 Open the method <i>iii</i> Sulfamix MRM_10.m and save it to a new name with the format <i>iii</i> Sulfamix dMRM.m, where <i>iii</i> are your initials.	- Click File > Open > Method. - Select the <i>iii</i>Sulfamix MRM_10.m method. Click OK. - Click Method > Save As. - Type the new method name with the format <i>iii</i>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.	- Click the Acquisition tab in the QQQ tab in the Method Editor window. - Right-click the Scan segments table and click Update DMRM Method. The Dynamic MRM Update Options dialog box opens. - Select the folder containing the <i>report.results.xml</i> file or the data file <i>iii</i>Sulfamix MRM_10.d. - Mark Add new compound/transition. - Mark Update retention time. - Mark Update trigger window. - Click OK.	- The Dynamic MRM Update Options 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.

If you select a Quantitative analysis report folder, you need to make sure to generate the results file. See “Task 2. Print a report in the Quantitative Analysis program” on page 36 to learn how to always generate the results file.

Exercise 2 – Develop a Dynamic MRM method

Task 3. Create a Dynamic MRM method using Update dMRM

Steps	Detailed Instructions	Comments
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Tune file
alanes.tune.xml
Browse ... 6f

Ion source
ESI

Time segments

#	Start Time	Scan Type	Div Valve	Delta EMV (+)	Delta EMV (-)	Stored
1	0	Dynamic MRM	To MS	18	0	☒

cycles/s ms/cycle

Stop time
☒ No limit/As Pump
☐ min

Time filtering
☒ Peak width 0.07 min

Acquisition Source Chromatogram Instrument Diagnostics

Scan segments

Compound Group	Compound Name	ISTD?	Precursor Ion	MS1 Res	Product Ion	MS2 Res	Ret Time (min)	Delta Ret Time	Fragmentor	Collision Energy	Cell Accelerator Voltage	Polarity
	Sulfachloropyridazin	☐	285	Unit	155.7	Unit	0.85	0.75	100	10	7	Positive
	Sulfamethoxazole	☐	211	Unit	155.7	Unit	2.03	0.75	100	10	7	Positive
	Sulfamethazine	☐	279	Unit	185.7	Unit	0.98	0.75	100	10	7	Positive
	Sulfamethizole	☐	271	Unit	155.8	Unit	0.37	0.75	100	10	7	Positive

Dynamic MRM Parameters
Cycle Time 500 ms Total MRMz = 4 Max Concurrent MRMz = 3 Min/Max Dwell = 163.17 ms/496.50 ms

Triggered MRM
☐ Triggered Repeats 3

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**.

Exercise 2 – Develop a Dynamic MRM method

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

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 <i>iiiSulfamix</i> MRM_10.m and save it to a new name with the format <i>iiiSulfamix</i> dMRM2.m, where <i>iii</i> are your initials.	- a Click File > Open > Method. - b Select the <i>iiiSulfamix</i> MRM_10.m method. - c Click OK. - d Click Method > Save As. - e Type the new method name with the format <i>iiiSulfamix</i>_dMRM2.m. - f Click Save.	
2 Copy all compounds from the Scan segments table in the MRM method.	- a Click the Acquisition tab on 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.

Exercise 2 – Develop a Dynamic MRM method

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

Steps	Detailed Instructions	Comments
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

The screenshot displays the Agilent Triple Quad LC/MS software interface. The 'Acquisition' tab is selected, showing a table of scan segments. The table has columns for Compound Group, Compound Name, ISTD?, Precursor Ion, MS1 Res, Product Ion, MS2 Res, Ret Time (min), Delta Ret Time, Fragmentor, Collision Energy, Cell Accelerator Voltage, and Polarity. The table contains four rows for Sulfachloropyridazine, Sulfadimethoxine, Sulfamethazine, and Sulfamethizole. A right-click context menu is open over the table, showing options like 'Insert Row', 'Append Row', 'Delete Row', 'Sort', 'Import from Database Browser...', 'Update DMRM Method...', 'Edit DMRM Method...', 'Calibrate DMRM Method...', 'Cut', 'Copy', 'Paste', 'Paste from Clipboard', 'Fill Down', and 'Fill Column'. The 'Dynamic MRM Parameters' section at the bottom shows Cycle Time: 500 ms, Total MRM: 5, Max Concurrent MRM: 5, Min/Max Dwell: 96.50 ms/96.50 ms, Min: 0.0167, Max: 999.9, and Default: 1.

Exercise 3 – Create a Triggered Dynamic MRM acquisition method

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

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 <i>iiiSulfamix dMRM2.m</i> , where <i>iii</i> are your initials.	a Click File > Open > Method. b Select the <i>iiiSulfamix_dMRM2.m</i> method. c Click OK. d Click Method > Save As. e Type the new method name with the format <i>iiiSulfamix_TriggeredDMRM.m</i>. f Click Save.	• 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 on the QQQ tab in the Method Editor. b Under Triggered MRM, mark the Triggered check box. 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.

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
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.

Scan segments

Compound Group	Compound Name	Precursor Ion	Product Ion	Primary	Trigger	Threshold	Ret Time (min)	Delta Ret Time	Fragmentor	Collision Energy	Trigger Entrance	Trigger Delay	Trigger Window	Cell Accelerator Voltage	Polarity	MS1 Res	M
1	Sulfachloropyridazin	285	197	☒	☒	800	0.5	1	380	8	2	0	0	7	Positive	Unit	Un
1	Sulfachloropyridazin	285	156	☒	☐		0.5	1	380	8				7	Positive	Unit	Un
1	Sulfachloropyridazin	285	108	☐	☐				380	20				7	Positive	Unit	Un
1	Sulfadimethoxine	311.1	245.1	☒	☒	1000	1.2	1	380	12	0	1	0	7	Positive	Unit	Un
1	Sulfadimethoxine	311.1	173.1	☒	☐		1.2	1	380	24				7	Positive	Unit	Un
1	Sulfadimethoxine	311.1	156	☐	☐				380	16				7	Positive	Unit	Un
1	Sulfadimethoxine	311.1	108	☐	☐				380	24				7	Positive	Unit	Un
2	Sulfamethazine	279.1	186	☒	☒	900	0.8	1	380	12	0	0	0	7	Positive	Unit	Un
2	Sulfamethazine	279.1	155.9	☒	☒	1000	0.8	1	380	12	0	0	0	7	Positive	Unit	Un
2	Sulfamethazine	279.1	124.1	☐	☐				380	20				7	Positive	Unit	Un
2	Sulfamethazine	279.1	124.1	☐	☐				380	24				7	Positive	Unit	Un
2	Sulfamethizole	271	253.4	☒	☒	1100	0.3	1	380	0	0	2	1	7	Positive	Unit	Un
2	Sulfamethizole	271	156	☒	☐		0.3	1	380	4				7	Positive	Unit	Un
2	Sulfamethizole	271	108	☐	☐				380	20				7	Positive	Unit	Un

Dynamic MRM Parameters

Cycle Time 500 ms

Total MRMs = 14 Max Concurrent MRMs = 14 Min/Max Dwell = 32.21 ms/121.50 ms.
Primary Only - Total MRMs = 8 Max Concurrent MRMs = 8 Min/Max Dwell = 53.00

Triggered MRM

☒ Triggered

Repeats 3

Task 2. Add/Modify compounds in an existing database

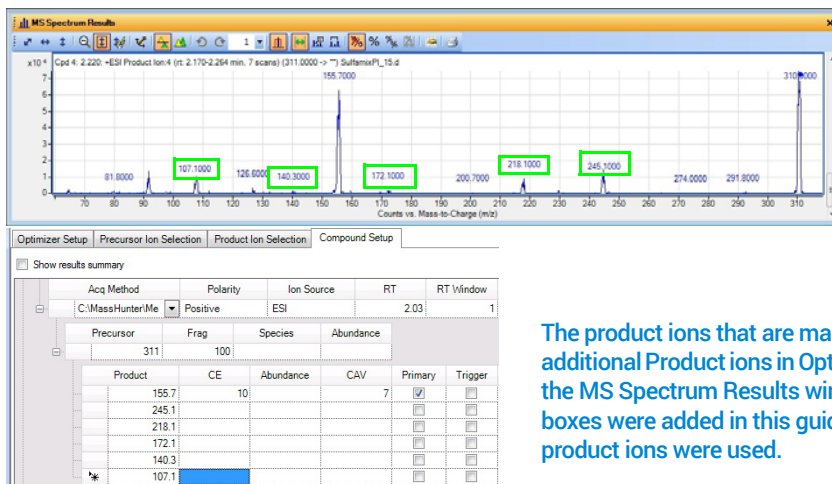
You can also manually add compounds to a database and modify the compounds in the database. In the next task, you create a Triggered Dynamic MRM method from the compounds in the database.

Steps	Detailed Instructions	Comments
1 Review the <i>iiiSulfamix_dMRM2.m</i> , where <i>iii</i> are your initials.	- Click File > Open > Method. - Select the <i>iiiSulfamix_dMRM2.m</i> method. - Click OK. - 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.	- On the Optimizer Setup tab, click the Injection (with or without column). - Set the CE range from 4 to 48. - Set the Cell Accelerator Voltage to 7. - Right-click the table and click Add Method. - Select the <i>iiiSulfamix_dMRM2.m</i> method.	To create low mass product ions from a precursor ion near 300 <i>m/z</i>, you need fairly high collision energies.
4 Set parameters on the Precursor Ion Selection tab.	On the Precursor Ion Selection tab, verify that +H is marked for the Positive ions (with priorities) list.	
5 Set parameters on the Product Ion Selection tab.	- On the Product Ion Selection tab, under Low mass cut-off, click Mass (m/z). - 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.

Exercise 3 – Create a Triggered Dynamic MRM acquisition method

Task 2. Add/Modify compounds in an existing database

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 On the Compound Setup tab, click the Import/Export > Import from Acquisition Methods. b Select the ///Sulfamix_dMRM2.m method and click Open. c (optional) Right-click the tab and click Expand/Collapse All Rows. d Select one of the Product rows for one of the compounds. In this example, select the Product row 155.7 for Precursor 311. e Right-click the Product row and click Add Product Ion. In this example, you add 5 product ion rows. f Enter the Product in each of the product ion rows that were added. See “To determine product ions in the Qualitative Navigator program:” on page 47. g Add product ions for the other three compounds.	- For each compound, we are going to add additional transitions. - In the Qualitative Navigator 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 4 on page 53 for values to use in the method.

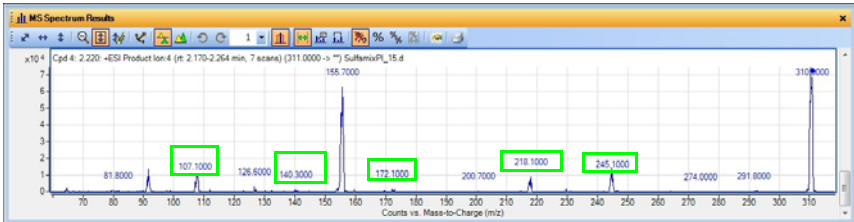


This product ion scan has a precursor mass of 311. You examine the MS spectrum to determine the product ions to add to the Product ion section of the Compound Setup table.

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.

Exercise 3 – Create a Triggered Dynamic MRM acquisition method

Task 2. Add/Modify compounds in an existing database

Steps	Detailed Instructions	Comments
To determine product ions in the Qualitative Navigator program:	- Open the SulfamixPI_15.d from “Task 4. Determine product ion masses” on page 24. - Click Chromatograms > Integrate and Extract Peak Spectra. - Select the spectrum Product Ion:4 (rt: 0.422-0.500 min, 6 scans). - Click the Autoscale Y-axis icon in the MS Spectrum Results toolbar. - Select 1 for the Maximum Number of List Panes. - 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 Navigator program at the same time. - See Table 4 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.
- 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.	- 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. - Clear the Perform multi-compound run check box in the right column. - Click Start Optimization 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 4 on page 53 for values to use in the method.

Exercise 3 – Create a Triggered Dynamic MRM acquisition method

Task 2. Add/Modify compounds in an existing database

Steps	Detailed Instructions	Comments
8	Examine the Optimizer Report. - Examine the Collision Energy for each Product Ion. - 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	300	129.9	24	315469
205	300	107.9	24	5015922
285	380	91.9	24	7438199
285	380	79.8	48	1520948
285	380	64.8	48	6508111

Compound Name Formula Mass Sample Position
sulfadimethazine 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	548481
311	380	229.7	20	135578
311	380	217.7	16	434965
311	300	172.9	24	414599
311	380	107.9	20	2291401
311	380	91.9	32	3102685
311	380	79.9	48	761344
311	380	64.8	48	2503606

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	3737936
279	380	212.8	20	421592
279	380	155.9	12	1377529
279	380	123.9	24	2633040
279	380	107.9	24	2407737
279	380	91.9	24	3645700
279	380	79.8	48	1242301
279	380	64.9	48	3792552

Compound Name Formula Mass Sample Position
sulfamethizole 1

Method Name Polarity Ion Source

Agilent Technologies

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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.	

Exercise 3 – Create a Triggered Dynamic MRM acquisition method

Task 2. Add/Modify compounds in an existing database

Steps	Detailed Instructions	Comments
11 In the Database Browser program, select the transitions.	a Mark the Show All Records check box. b Under Select Transitions, click Select top. c Type 10 for the ranked transitions. d Click Select Transitions.	- 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 4 on page 53 for values to use in the method.

Select Transitions
☒ Select top 10 ranked transitions
☐ Primary transitions
☐ Secondary transitions
Select Transitions

Set primary and trigger flags
Set top 2 ranked transitions as primary
Set Primaries and Trigger

Rank transitions by
☒ Abundance
☐ Response Factor

	Compound Name	Formula	MW	Polarity	Species	Precursor	Product	Frag	CE	CAV	Primary	Trigger
☒	Sulfachloropyridazine			Positive		285	155.7	100	10	7	☒	☐
☒	Sulfachloropyridazine			Positive		285	129.9	100			☐	☐
☒	Sulfachloropyridazine			Positive		285	107.9	100			☐	☐
☒	Sulfachloropyridazine			Positive		285	91.9	100			☐	☐
☒	Sulfachloropyridazine			Positive		285	79.8	100			☐	☐
☒	Sulfachloropyridazine			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			☐	☐

Exercise 3 – Create a Triggered Dynamic MRM acquisition method

Task 2. Add/Modify compounds in an existing database

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 Set Primaries and Trigger.	- 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 4 on page 53 for values to use in the method.

Select Transitions
☐ Select top 1 ranked transitions
☒ Primary transitions
☐ Secondary transitions
Select Transitions

Set primary and trigger flags
Set top 2 ranked transitions as primary
Set Primaries and Trigger

Rank transitions by
☒ Abundance
☐ Response Factor

	Compound Name	Formula	M/W	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.

Exercise 3 – Create a Triggered Dynamic MRM acquisition method

Task 2. Add/Modify compounds in an existing database

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 4 on page 53 for values to use in the method.

Select Transitions

Select top

10

ranked transitions

Primary transitions

Secondary transitions

Select Transitions

Set primary and trigger flags

Set top

2

ranked transitions as primary

Set Primaries and Trigger

Rank transitions by

Abundance

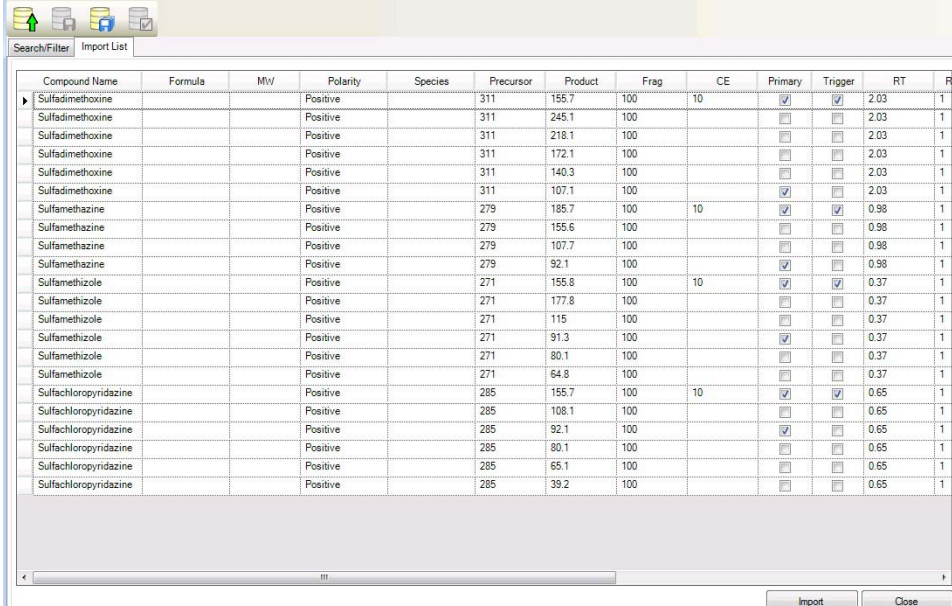
Response Factor

☒	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	7436199
☒	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	263848
☒	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

Exercise 3 – Create a Triggered Dynamic MRM acquisition method

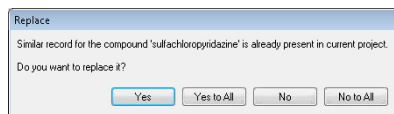
Task 2. Add/Modify compounds in an existing database

Steps	Detailed Instructions	Comments
14 Review the Import List table on the Import List tab.	a Click Add to Import List . b Click Import List . 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
Sulfamethazole			Positive		271	155.8	100	10	☒	☒	0.37	1
Sulfamethazole			Positive		271	177.8	100		☐	☐	0.37	1
Sulfamethazole			Positive		271	115	100		☐	☐	0.37	1
Sulfamethazole			Positive		271	91.3	100		☒	☐	0.37	1
Sulfamethazole			Positive		271	80.1	100		☐	☐	0.37	1
Sulfamethazole			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

15 Review the Compound Setup table in Optimizer. You replace all compounds with the compounds from the Database Browser program.	a Click Import . b Click Yes to All . c On 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.
--	---	---



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.
--	---	--

Exercise 3 – Create a Triggered Dynamic MRM acquisition method

Task 2. Add/Modify compounds in an existing database

Table 4 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 On the QQQ tab, click Acquisition. 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 Add to Import List. h Click Import List. i Review the Import List table. j Click Import. 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 <i>online Help</i> for the Data Acquisition program and the <i>QQQ Concepts Guide</i> for an explanation of the other triggering conditions: Trigger Entrance, Trigger Delay, and Trigger Window.

Exercise 3 – Create a Triggered Dynamic MRM acquisition method

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

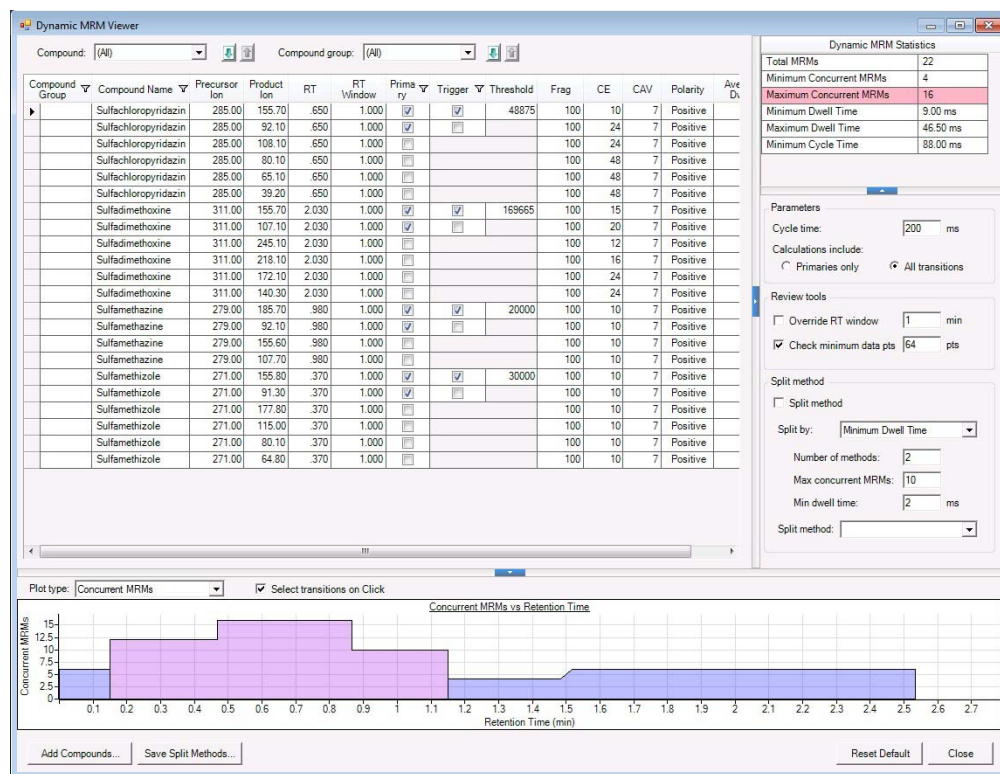
Steps	Detailed Instructions	Comments																																																																																																																																																																																																																																																																																
Acquisition Source Chromatogram Instrument Diagnostics Scan segments <table border="1"> <thead> <tr> <th>Compound Group</th> <th>Compound Name</th> <th>ISTD?</th> <th>Precursor Ion</th> <th>M51 Res</th> <th>Product Ion</th> <th>M52 Res</th> <th>Primary</th> <th>Trigger</th> <th>Threshold</th> <th>Ret Time (min)</th> <th>Delta Ret Time</th> <th>Fragmentor</th> <th>Collision Energy</th> <th>Cell Accelerator Voltage</th> <th>Polarity</th> <th>Trigger Enable</th> </tr> </thead> <tbody> <tr><td></td><td>Sulfadimethoxine</td><td>☐</td><td>311</td><td>Unit</td><td>155.7</td><td>Unit</td><td>☒</td><td>☒</td><td>16365</td><td>2.03</td><td></td><td>1</td><td>100</td><td>15</td><td>7</td><td>Positive</td></tr> <tr><td></td><td>Sulfadimethoxine</td><td>☐</td><td>311</td><td>Unit</td><td>245.1</td><td>Unit</td><td>☐</td><td>☐</td><td></td><td></td><td></td><td></td><td>100</td><td>12</td><td>7</td><td>Positive</td></tr> <tr><td></td><td>Sulfadimethoxine</td><td>☐</td><td>311</td><td>Unit</td><td>218.1</td><td>Unit</td><td>☐</td><td>☐</td><td></td><td></td><td></td><td></td><td>100</td><td>16</td><td>7</td><td>Positive</td></tr> <tr><td></td><td>Sulfadimethoxine</td><td>☐</td><td>311</td><td>Unit</td><td>172.1</td><td>Unit</td><td>☐</td><td>☐</td><td></td><td></td><td></td><td></td><td>100</td><td>24</td><td>7</td><td>Positive</td></tr> <tr><td></td><td>Sulfadimethoxine</td><td>☐</td><td>311</td><td>Unit</td><td>140.3</td><td>Unit</td><td>☐</td><td>☐</td><td></td><td></td><td></td><td></td><td>100</td><td>24</td><td>7</td><td>Positive</td></tr> <tr><td></td><td>Sulfadimethoxine</td><td>☐</td><td>311</td><td>Unit</td><td>107.1</td><td>Unit</td><td>☒</td><td>☐</td><td></td><td>2.03</td><td></td><td>1</td><td>100</td><td>20</td><td>7</td><td>Positive</td></tr> <tr><td></td><td>Sulfamethazine</td><td>☐</td><td>279</td><td>Unit</td><td>165.7</td><td>Unit</td><td>☒</td><td>☒</td><td>20000</td><td>0.98</td><td></td><td>1</td><td>100</td><td>10</td><td>7</td><td>Positive</td></tr> <tr><td></td><td>Sulfamethazine</td><td>☐</td><td>279</td><td>Unit</td><td>155.6</td><td>Unit</td><td>☐</td><td>☐</td><td></td><td></td><td></td><td></td><td>100</td><td>10</td><td>7</td><td>Positive</td></tr> <tr><td></td><td>Sulfamethazine</td><td>☐</td><td>279</td><td>Unit</td><td>107.7</td><td>Unit</td><td>☐</td><td>☐</td><td></td><td></td><td></td><td></td><td>100</td><td>10</td><td>7</td><td>Positive</td></tr> <tr><td></td><td>Sulfamethazine</td><td>☐</td><td>279</td><td>Unit</td><td>92.1</td><td>Unit</td><td>☒</td><td>☐</td><td></td><td>0.98</td><td></td><td>1</td><td>100</td><td>10</td><td>7</td><td>Positive</td></tr> <tr><td></td><td>Sulfamethazole</td><td>☐</td><td>271</td><td>Unit</td><td>155.8</td><td>Unit</td><td>☒</td><td>☒</td><td>30000</td><td>0.37</td><td></td><td>1</td><td>100</td><td>10</td><td>7</td><td>Positive</td></tr> <tr><td></td><td>Sulfamethazole</td><td>☐</td><td>271</td><td>Unit</td><td>177.8</td><td>Unit</td><td>☐</td><td>☐</td><td></td><td></td><td></td><td></td><td>100</td><td>10</td><td>7</td><td>Positive</td></tr> <tr><td></td><td>Sulfamethazole</td><td>☐</td><td>271</td><td>Unit</td><td>115</td><td>Unit</td><td>☐</td><td>☐</td><td></td><td></td><td></td><td></td><td>100</td><td>10</td><td>7</td><td>Positive</td></tr> <tr><td></td><td>Sulfamethazole</td><td>☐</td><td>271</td><td>Unit</td><td>91.3</td><td>Unit</td><td>☒</td><td>☐</td><td></td><td>0.37</td><td></td><td>1</td><td>100</td><td>10</td><td>7</td><td>Positive</td></tr> <tr><td></td><td>Sulfamethazole</td><td>☐</td><td>271</td><td>Unit</td><td>80.1</td><td>Unit</td><td>☐</td><td>☐</td><td></td><td></td><td></td><td></td><td>100</td><td>10</td><td>7</td><td>Positive</td></tr> </tbody> </table> Dynamic MRM Parameters Cycle Time 500 ms Total MRMs = 4 Max Concurrent MRMs = 3 Min/Max Dwell = 163.17 ms/496.50 ms Triggered MRM ☒ Triggered Repeats 3			Compound Group	Compound Name	ISTD?	Precursor Ion	M51 Res	Product Ion	M52 Res	Primary	Trigger	Threshold	Ret Time (min)	Delta Ret Time	Fragmentor	Collision Energy	Cell Accelerator Voltage	Polarity	Trigger Enable		Sulfadimethoxine	☐	311	Unit	155.7	Unit	☒	☒	16365	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	165.7	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		Sulfamethazole	☐	271	Unit	155.8	Unit	☒	☒	30000	0.37		1	100	10	7	Positive		Sulfamethazole	☐	271	Unit	177.8	Unit	☐	☐					100	10	7	Positive		Sulfamethazole	☐	271	Unit	115	Unit	☐	☐					100	10	7	Positive		Sulfamethazole	☐	271	Unit	91.3	Unit	☒	☐		0.37		1	100	10	7	Positive		Sulfamethazole	☐	271	Unit	80.1	Unit	☐	☐					100	10	7	Positive
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- 2 Save the method to a new method name, **iiiSulfas_TriggerOpt.m**, where **iii** are your initials.
 - a Click **Method > Save Method**.
 - b Type **iiiSulfas_TriggerOpt.m**.
 - c Click **Save**.

Exercise 3 – Create a Triggered Dynamic MRM acquisition method

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

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 on the Acquisition tab. c Click between Primaries only and All transitions if the Dynamic MRM Statistics information is not updating. Then, click All transitions. 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.



Exercise 3 – Create a Triggered Dynamic MRM acquisition method

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

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.	

Dynamic MRM Update Options

Select MassHunter QQQ data file or Quant report folder:

Method options

☒ Add new compound/transition

Peak abundance threshold:

Cycle time: ms

Retention time options

☒ Update retention time

☒ Update retention time window

RT window threshold: Minutes

Scale factor:

Trigger options

☒ Update threshold

Height percent:

Scale factor:

☒ Update trigger window

☒ Retention time ☐ FWHM

Absolute value: min

Percent value:

Scale factor:

Exercise 4 – Optimize Acquisition parameters

Task 1. Use Optimizer to optimize acquisition parameters

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.

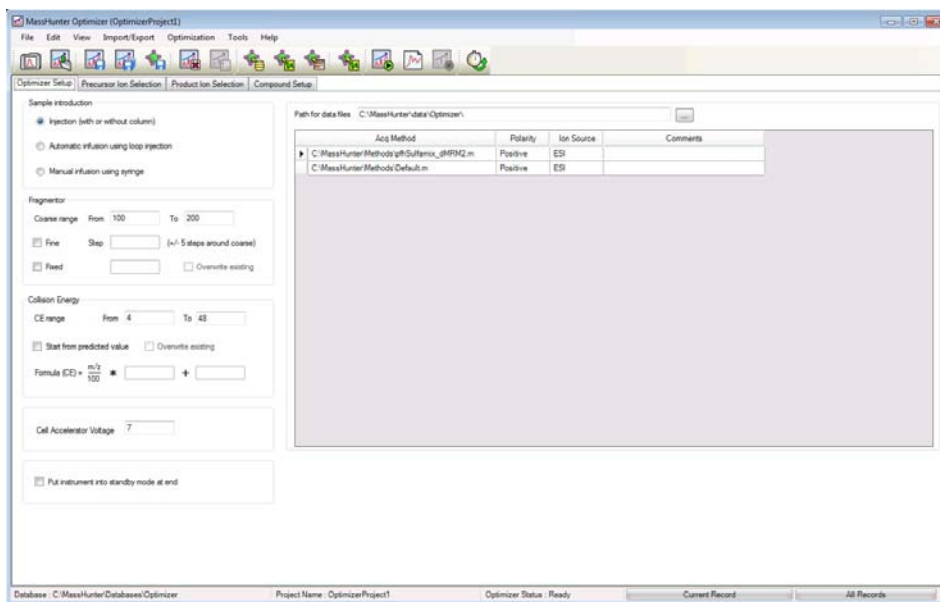
To do this task, you first need to create the method **iiiSulfamix MRM_10.m** in **“Task 5. Find optimum collision energy for MRM acquisition”** on page 30. You do not need to acquire the data file.

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.

Exercise 4 – Optimize Acquisition parameters

Task 1. Use Optimizer to optimize acquisition parameters

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.



Exercise 4 – Optimize Acquisition parameters

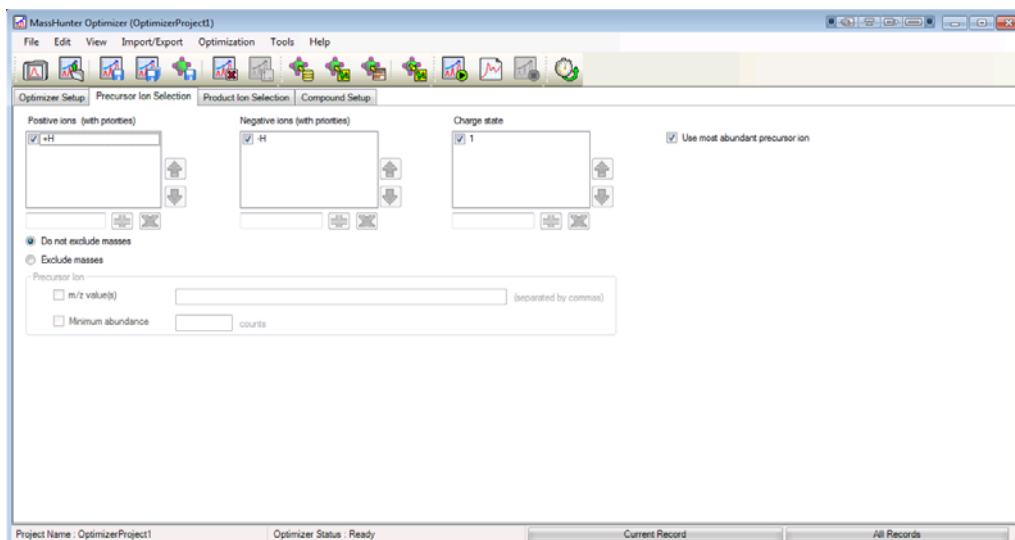
Task 1. Use Optimizer to optimize acquisition parameters

Steps	Detailed Instructions	Comments
2 Set the optimization parameters.	a On the Optimizer Setup tab, set the Sample introduction method to Injection (with or without column). b 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. c Set the range for ramping the Collision Energy from 0 to 40 V. d Select a Path for data files to store the optimization run data. e Right-click the table on the right and select Add Method from the shortcut menu. f Click the button on the right side of the Acq Method cell to open the Open Method dialog box. g Select the method created in the previous exercise iiiSulfamix MRM_10.m and click OK. The Polarity and Ion Source will be filled in from the values set in the selected method. h Check to make sure that the Ion Source from the method matches the physical configuration of your instrument. i Repeat step e to step h 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 the MassHunter Qualitative Analysis programs. • 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.

Exercise 4 – Optimize Acquisition parameters

Task 1. Use Optimizer to optimize acquisition parameters

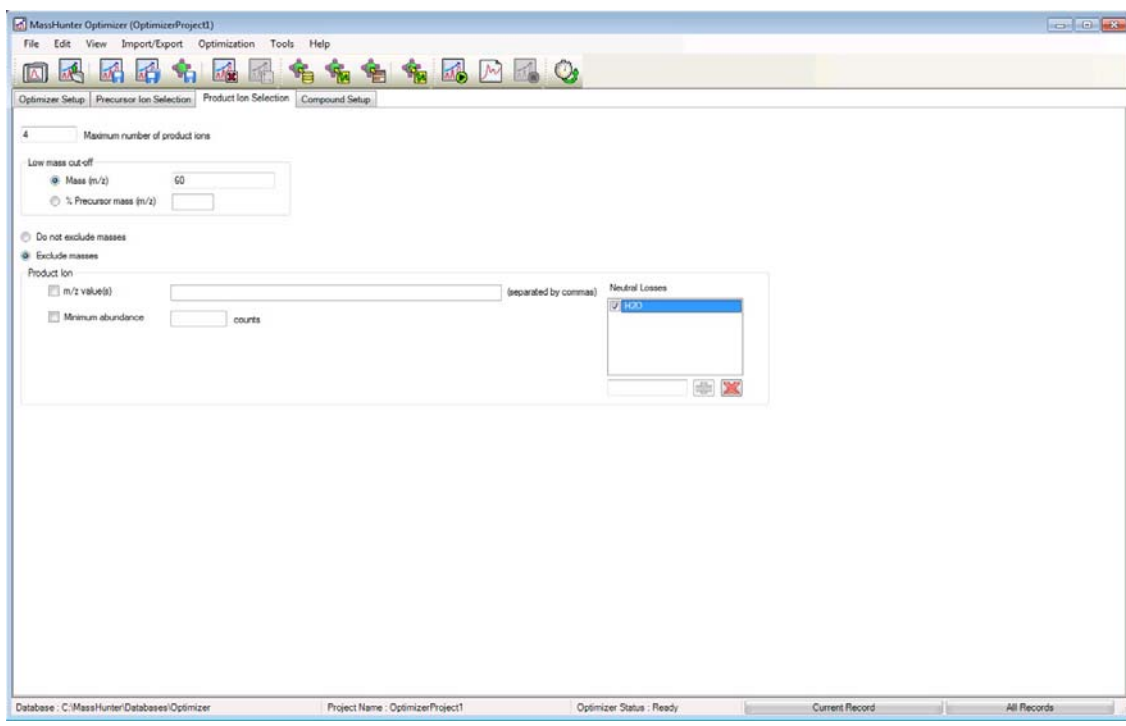
Steps	Detailed Instructions	Comments
3 Select the precursor ions	a On the Precursor Ion Selection tab, select the Positive ions +H adduct. b Select the Charge state of 1. c Set the search priority of the precursor ions. d (optional) To exclude certain masses from consideration, click Exclude masses at 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 arrows 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 H₂O).



Exercise 4 – Optimize Acquisition parameters

Task 1. Use Optimizer to optimize acquisition parameters

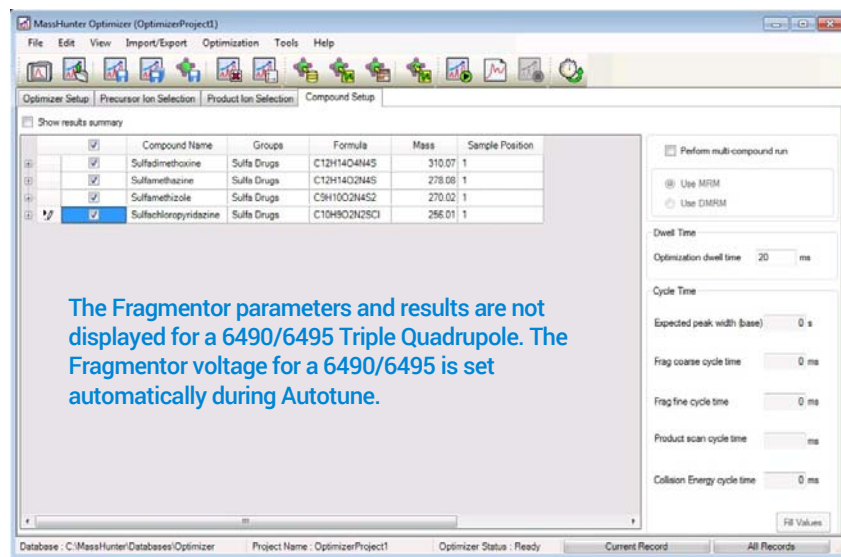
Steps	Detailed Instructions	Comments
4 Select the product ions	a On the Product Ion Selection tab, enter a Low mass cut-off value. Select Mass (m/z) of 60 m/z. b To exclude certain masses from consideration, click Exclude masses option at the bottom of the screen. Enter the m/z Values to exclude separated by commas and/or enter a Minimum abundance value in counts. c If desired, you can also enter Neutral Losses to exclude, for example H₂O. 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.



Exercise 4 – Optimize Acquisition parameters

Task 1. Use Optimizer to optimize acquisition parameters

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 $C_{10}H_9O_2N_4Cl$ • Sulfadimethoxine $C_{12}H_{14}O_4N_4S$	a On the Compound Setup tab, clear the Show results summary check box above the table while you set up the compound list. b Right-click the table and select Add Compound from the shortcut menu to add a row to the end of the table. c Enter Sulfamethizole as the Compound Name . d Enter Sulfa drugs as the group name in the Groups column. e Enter C9H10O2N4S2 as the Formula of the compound. The mass is calculated. f Enter the Sample Position for the new compound. g (optional) Enter an Optimization dwell time value to set longer or shorter cycle times. h Repeat the steps above to add the other three sulfa drugs to the table. i Mark the Select columns for the compounds (rows) to use for optimization. j 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.



Exercise 4 – Optimize Acquisition parameters

Task 1. Use Optimizer to optimize acquisition parameters

Steps	Detailed Instructions	Comments
6 Start the optimization process.	- Click Start Optimization () on the toolbar - or - Click Ion Breakdown Profile () on the toolbar.	
7 Review results.	a On the Compound Setup tab, mark the Show results summary check box above the table. b Review the following values for each transition ion in the Compound Table: • Fragmentor • Collision Energy c Review the printed optimization report.	• (optional) Use the MassHunter Workstation Qualitative Analysis programs to look at the data. • See the <i>online Help</i> for Optimizer or the <i>Optimizer Quick Start Guide</i> to learn how to import optimization results to acquisition for MRM time segments.

Exercise 4 – Optimize Acquisition parameters

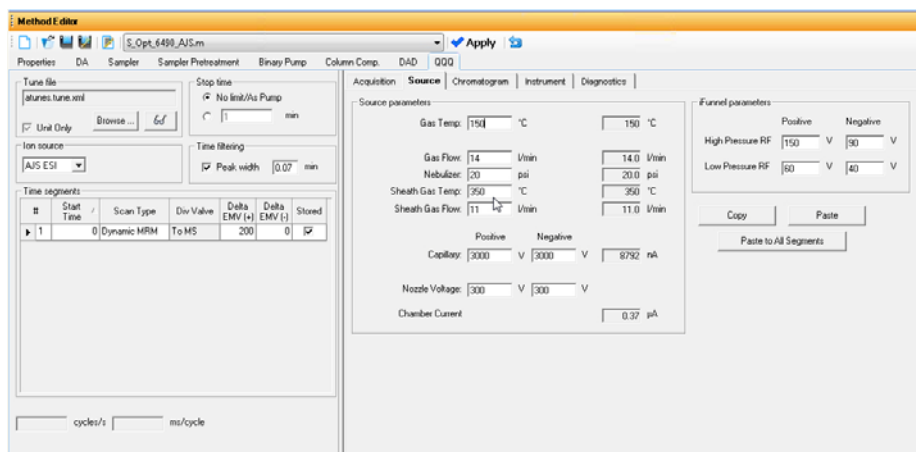
Task 2. Use Source and iFunnel Optimizer to optimize acquisition parameters

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 **iiiSulfamix_dMRM2.m** in “**Task 4. Create a Dynamic MRM method from an MRM method**” on page 40. 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, select Acquisition from the Context dropdown menu. c Load the iiiSulfamix_dMRM2.m method. d Save this method to the name iiiSulfamix_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 On the QQQ tab, click Source. b Modify the parameters to the recommended starting parameters for source optimization. These parameters are shown in the following image.	



Exercise 4 – Optimize Acquisition parameters

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

Steps	Detailed Instructions	Comments
3 Create template method.	a On the Acquisition tab, select a single ion for each compound for the optimization. b Save the method.	A transition for each compound is already included in the Dynamic MRM method.

The screenshot shows the 'Acquisition' tab in the software. The 'Scan segments' table is as follows:

Compound Group	Compound Name	ISTD?	Precursor Ion	MS1 Res	Product Ion	MS2 Res	Ret Time (min)	Delta Ret Time	Fragmentor	Collision Energy	Cell Accelerator Voltage	Polarity
	sulfachloropyridazine	☐	295	Unit	155.7	Unit	0.65	1	380	10	7	Positive
	sulfachlorothiazine	☐	311	Unit	155.7	Unit	2.03	1	380	15	7	Positive
	sulfamethazine	☐	279	Unit	185.7	Unit	0.98	1	380	15	7	Positive
	sulfamethizole	☐	271	Unit	155.0	Unit	0.37	1	300	10	7	Positive

The 'Dynamic MRM Parameters' section shows:

- Cycle Time: 200 ms
- Total MRMs: 4
- Max Concurrent MRMs: 3
- Min/Max Dwell: 85.78 ms/199.20 ms
- Triggered MRM: ☐ Triggered, Repeats: 3

- 4 Start the Source and iFunnel Optimizer program.
- Double-click the **Source Optimizer** icon.

The screenshot shows the 'Source and iFunnel Optimizer - SourceOptConfig.opt' window. The 'Instrument parameters' table is as follows:

	Types	PreVal (min)	Replicate	StepVal (min)	Start Value	End Value	Step Size
☒	High Pressure RF	0	1	0	40	160	20
☒	Low Pressure RF	30	1	20	120	230	30
☒	Gas Temp	30	1	0	11	20	2
☒	Gas Flow	0	1	0	20	40	5
☒	Nebulizer	0	1	0	1500	4500	500
☒	Capillary	0	1	0			

The 'Workflow parameters' section shows:

- Sample Name: MyTestSampleName
- Sample Position: Val 1
- Workflow position of data file used for calibration: 1

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.

Exercise 4 – Optimize Acquisition parameters

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

Steps	Detailed Instructions	Comments
5 Select the template method, iiiSulfamix_SourceOpt.m .	a Click Browse =. The Browse For Folder dialog box is opened. b Select the iiiSulfamix_SourceOpt.m method. Click OK. 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.	

Types	PreWait (min)	Replicate	StepWait (min)	Start Value	End Value	Step Size
☒ 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 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.

- | | | |
|---|---|--|
| <p>6 Change the order of the rows in the Instrument parameters table to the following:</p> <ul style="list-style-type: none"> • Ion Funnel parameters • Sheath Gas temperature and flow • Gas temperature and flow • Nebulizer • Capillary • Nozzle Voltage | <p>a If necessary, select the row that shows the High Pressure RF parameter.</p> <p>b Drag this row to the top of the table. Both of the Ion Funnel parameters are moved together.</p> <p>c Verify that the order of the rows in your table is as indicated.</p> | <ul style="list-style-type: none"> • 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. |
|---|---|--|

Exercise 4 – Optimize Acquisition parameters

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

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 Funnel parameters.

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**.

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.

Exercise 4 – Optimize Acquisition parameters

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

Steps	Detailed Instructions	Comments
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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**.

- Select the **Project Folder**. In this example, select *MassHunter\Data*.
 - Enter a **Project Name**.
 - (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 **Submit**.

Project parameters

Optimize method: C:\MassHunter\Methods\ipfmSulfamix_SourceOpt.m

Project Folder: C:\MassHunter\data

Project Name: MyTest ☒ Append timestamp

11 Set the **Worklist parameters**.

- Type the **Sample Name**.
 - Type the **Sample Position**.
 - 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.

Worklist parameters

Sample Name: oufas

Sample Position: 31

Worklist position of data file used for calibration: 1

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

- Click **Create Methods**.
 - 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.

Exercise 4 – Optimize Acquisition parameters

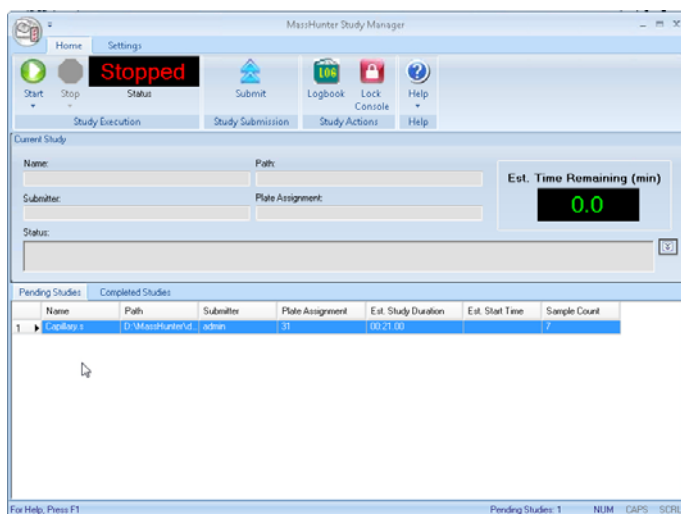
Task 2. Use Source and iFunnel Optimizer to optimize acquisition parameters

Steps	Detailed Instructions	Comments
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Project created: D:\MassHunter\data\S_Opt_vcp_2012912_1 6 methods 6 injections Estimated time: 21 minutes

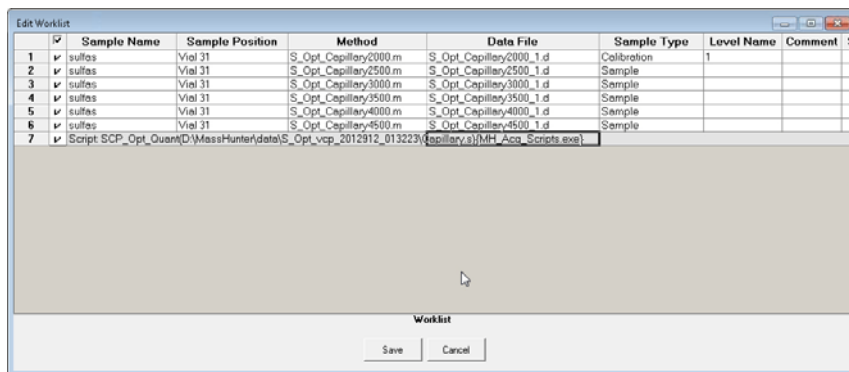
The estimated time includes the Stoptime for the method plus one minute per injection. It does not consider the Posttime specified in the method. Also, it does not include the PreWait nor the StepWait that you entered in the Instrument parameters table.

- 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.

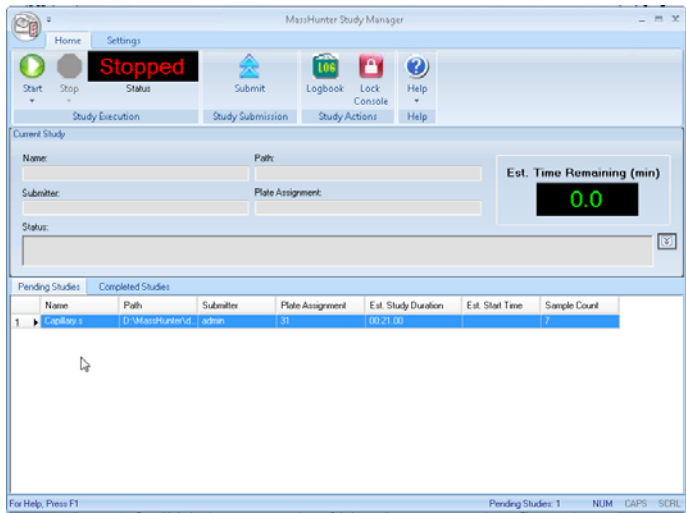


The script that is run at the end of the worklist creates the Quantitative Analysis batch file.

Exercise 4 – Optimize Acquisition parameters

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

Steps	Detailed Instructions	Comments
14 Modify the Study Manager parameters to run a standby script when the study completes and then start the Study Manager.	- a On the Settings tab, mark the Enable standby script execution on idle or error check box. - b Click "... " to select the script to run. - c Select SCP_InstrumentStandby and click OK. - d Enter 1 for the Wait for time. - e Click Start if needed.	• 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.

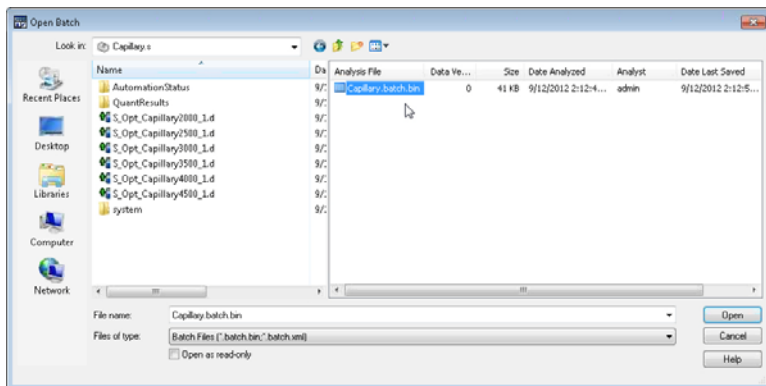
You can examine or edit the worklist for the study. You right-click the line in the Pending Studies table and click Edit Worklist from Study.

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.
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Exercise 4 – Optimize Acquisition parameters

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

Steps	Detailed Instructions	Comments
16 Open the data in the Quantitative Analysis program.	- Start the Quantitative Analysis program. - Click File > Open Batch. - Navigate to the location of the study. - Select the Batch file named <i>Capillary.batch.bin</i> 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.

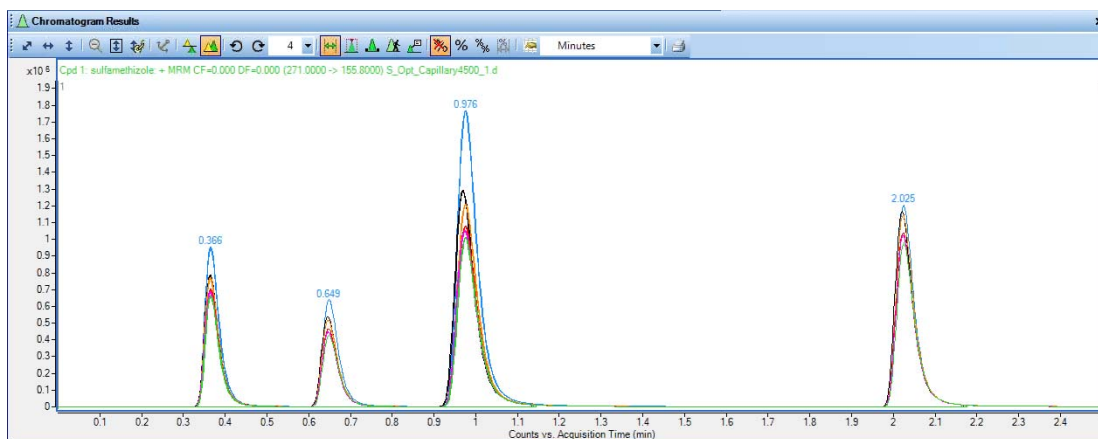
17 Review the Batch Table.	- Switch to Multiple Compound View. - Add the Area column to the table. - For each compound, right-click the Final Conc. column and click Plot this column. - Examine the Area column and the Final Conc. graph to determine the best capillary voltage. - Close the Quantitative Analysis program.	- Refer to the <i>online Help</i> 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.
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Batch Table																					
Sample: <i>sulfas</i>					Sample Type: <All>		Compound: <i>sulfamethazine</i>		ISTD:												
Sample					sulfamethazole Results				sulfachloropyridazine Results				sulfamethazine Results				sulfadimethoxine Results				
	Name	Data File	Type	Level	Acq. Date/Time	RT	Final Conc.	Accuracy	Area	RT	Final Conc.	Accuracy	Area	RT	Final Conc.	Accuracy	Area	RT	Final Conc.	Accuracy	Area
	<i>sulfas</i>	<i>S_Opt_Capillary2000_1.d</i>	Cal	1	9/12/2012 1:36 PM	0.365	100.0000	100.0	2365070	0.649	100.0000	100.0	1745306	0.977	100.0000	100.0	6145561	2.025	100.0000	100.0	3600776
	<i>sulfas</i>	<i>S_Opt_Capillary2000_1.d</i>	Sample		9/12/2012 1:42 PM	0.365	87.8676		1962936	0.646	85.3296		1489363	0.970	73.8517		4538998	2.021	98.3833		3967020
	<i>sulfas</i>	<i>S_Opt_Capillary3000_1.d</i>	Sample		9/12/2012 1:48 PM	0.365	81.0429		1903346	0.646	82.1821		1434003	0.977	69.8619		4233407	2.025	97.4203		3596360
	<i>sulfas</i>	<i>S_Opt_Capillary3000_1.d</i>	Sample		9/12/2012 1:54 PM	0.365	73.6766		1799779	0.649	73.6966		1264520	0.970	62.5607		3845934	2.025	88.9432		3261804
	<i>sulfas</i>	<i>S_Opt_Capillary4000_1.d</i>	Sample		9/12/2012 2:00 PM	0.365	72.4241		1706291	0.646	71.1248		1241346	0.974	61.3445		3769965	2.025	87.5352		3223852
	<i>sulfas</i>	<i>S_Opt_Capillary4000_1.d</i>	Sample		9/12/2012 2:07 PM	0.365	68.3667		1634768	0.649	67.8968		1186752	0.977	58.4264		3590633	2.025	83.3787		3076487

Exercise 4 – Optimize Acquisition parameters

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

Steps	Detailed Instructions	Comments
18 (optional) Review the data files in the Qualitative Navigator program.	a Start the Qualitative Navigator 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 Find. 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 Navigator 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.



In This Book

The exercises in this guide help you learn to use the Agilent Workstation Data Acquisition program with your Agilent Triple Quad LC/MS. In this guide, you learn how to develop an acquisition method.

In this guide, you acquire data and then analyze the results using the MassHunter Workstation Qualitative Analysis programs.

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



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