



MassHunter Data Acquisition for 6400 Series Triple Quad LC/MS

Familiarization Guide



Notices

Manual Part Number

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Software Revision

This guide is valid for the 10.0 revision or higher of the Data Acquisition for 6400 Series Triple Quad LC/MS program and compatible Data Acquisition for 6400 Series Triple Quad LC/MS programs, until superseded.

Software Manufacturing



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In This Book

Use the exercises in this guide to learn how to use the Agilent Triple Quad LC/MS with the MassHunter Data Acquisition software Revision 10.0. You can do these exercises with the demo data files (**Test example**), shipped with the system (in the Data folder of your Qualitative Analysis installation disk), or with data you acquire.

- Exercise 1** Learn how to determine the best acquisition settings for analyzing your compounds of interest. These instructions help you understand not only how to set up a worklist to optimize instrument parameters for best sensitivity in acquisition, but also how to use the Agilent MassHunter Qualitative Analysis program to identify parameter values producing optimum signal response. You can also learn about the Qualitative Analysis program by using the *Qualitative Analysis Familiarization Guide*, the Qualitative Analysis *online Help*, or the *Qualitative Analysis eFamiliarization Guide*.
- Exercise 2** 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.
- Exercise 3** Learn how to create a triggered Dynamic MRM method.
- Exercise 4** 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.

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.

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1

Before you begin

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Before you begin, you need to check that your system is ready.

Prepare your system

1 Check that

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

If these actions have not yet been done, contact your Agilent representative.

2 Copy the data files to your PC.

Copy the folder **Test example** in the **Data** folder from the Qualitative Analysis Installation Disk to any location on your hard disk. This folder contains all the data files needed for this exercise.

NOTE

Do not re-use the *Test example* data files already on your system unless you know that you copied them from the originals on the disk and you are the only one using them. Data files that are already on the system may contain processed results, leading to different behavior during the exercises in this guide.

Prepare to acquire data

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

Parts List

The exercise in this guide uses this equipment and materials:

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

Table 1 Zorbax column

Triple Quadrupole	Column Description	Particle Size	Pore Size	Part Number
6410B, 6420, 6430, 6460, 6470, 6490, and 6495	RRHD Eclipse Plus C18.2.1 mm x 50 mm	1.8 μm	95 Å	959757-902

1 Prepare the LC solvents.

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

2 Prepare the sample.

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

3 Set up the LC column.

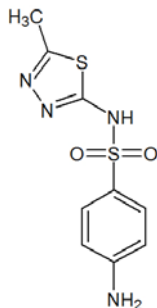
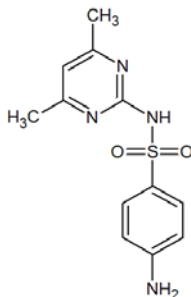
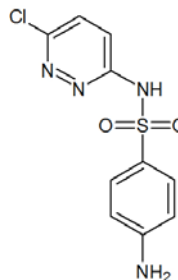
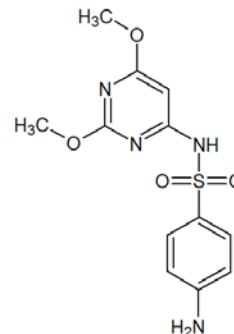
- Use the column from Table 1. Other columns and instrument parameters may be used in these exercises, but some parameters may need adjustment, and the results will differ.

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

Before you begin

Prepare to acquire data

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

Sulfamethizole**Sulfamethazine****Sulfachloropyridazine****Sulfadimethoxine****NOTE**

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

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

2

Exercise 1 - Develop an acquisition method

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- Task 2. Determine precursor ion masses 16
- Task 3. Find optimum fragmentor voltage for maximum response 19
- Task 4. Determine product ion masses 29
- Task 5. Find optimum collision energy for MRM acquisition 36

These tasks show you how to manually select the acquisition parameters including using the Qualitative Analysis program to analyze the data files. You can instead use the automated process to select some of the acquisition parameters. See “**Exercise 4 – Optimize Acquisition parameters**” on page 65 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 compounds.

Steps	Detailed Instructions	Comments
1 Enter LC parameters appropriate for the test mix. See Table 2 .	a Double-click the Data Acquisition icon. b On the toolbar, set Context to Acquisition . c Enter the LC parameters for your sample. An example set of parameters is listed in Table 2 .	The Data Acquisition window appears. See Figure 1 on page 13.

Exercise 1 - Develop an acquisition method

Task 1. Enter acquisition parameters and acquire data

Table 2 LC parameters for test 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 with an AJS source 40 °C for other instruments

Exercise 1 - Develop an acquisition method

Task 1. Enter acquisition parameters and acquire data

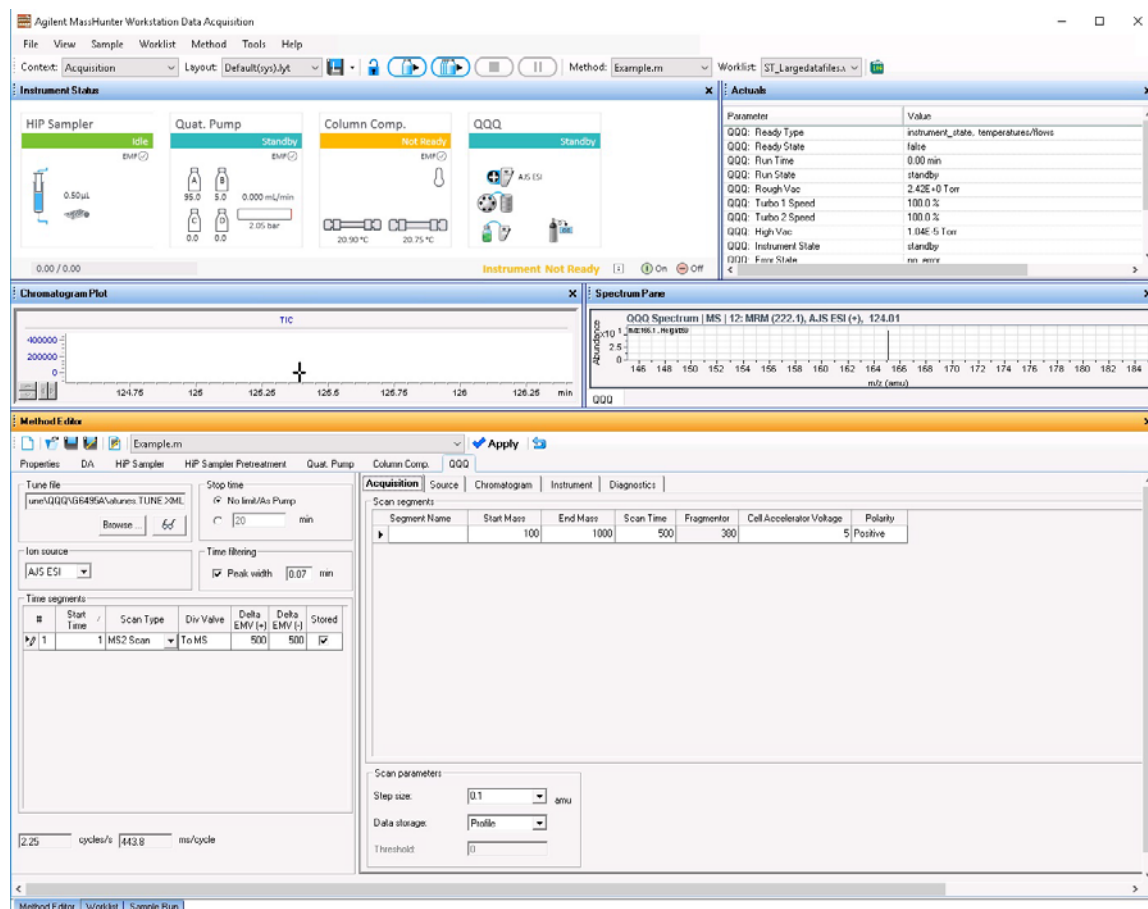


Figure 1. MassHunter Data Acquisition window

Steps	Detailed Instructions	Comments
2 Enter MS parameters appropriate for your sample and save the method as <i>iiiMS2Scantest.m</i>, where <i>iii</i> are your initials. See Table 3 on page 14.	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. An example is listed in Table 3. These parameters are in either the Acquisition or the Source tabs. d Save the method as <i>iiiMS2Scantest.m</i>, where <i>iii</i> are your initials.	

Table 3 MS parameters for test 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	350 °C 250 °C for 6490 and 6495
Gas Flow	12 L/min 14 L/min for 6490 and 6495	10 L/min 14 L/min for 6490 and 6495
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 where it comes from the Tune file)	100 V (not adjustable on 6490 nor 6495 where it comes from the Tune file)

Exercise 1 - Develop an acquisition method

Task 1. Enter acquisition parameters and acquire data

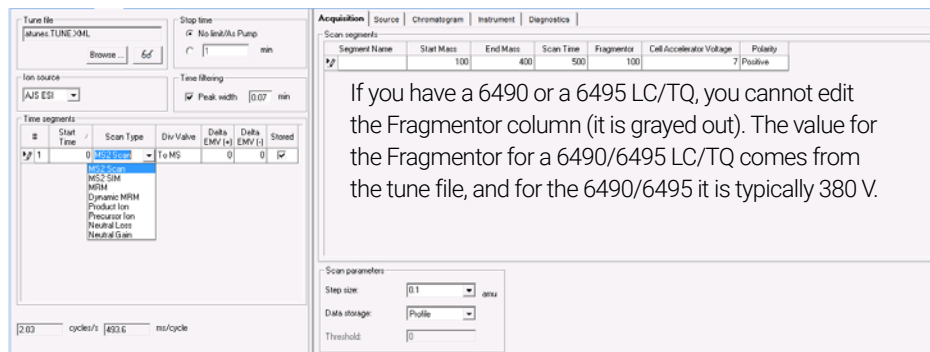


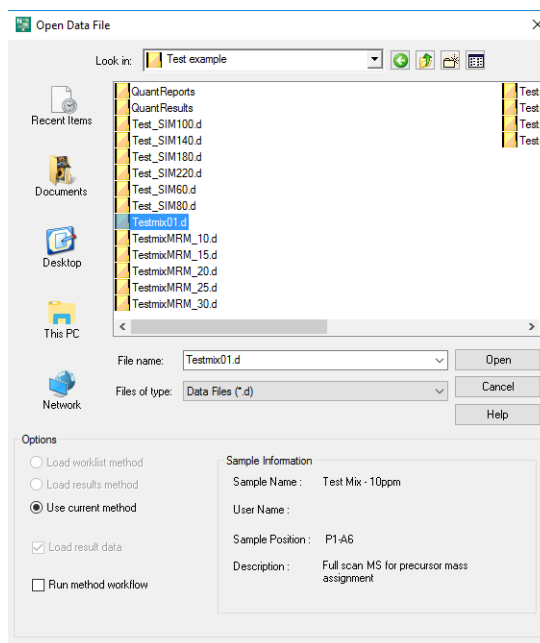
Figure 2. Select **Scan Type** of MS2 Scan in the QQQ tab

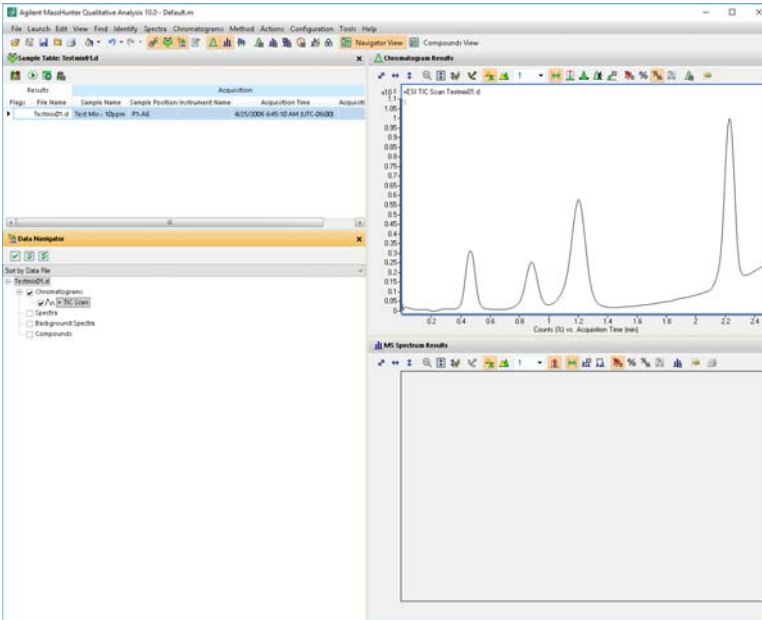
Steps	Detailed Instructions	Comments
3 Acquire data (optional). - Set up a one-line worklist with the method you just created. - Name the data file iiisulfamix01.d, where iii are your initials. - Designate a directory path to hold your data files and method.	a If necessary, click View > Worklist to display 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 Click the Sample Position tab. g Select the Autosampler, Well-plate or Vial Tray. h In the graphic, select a single position. Click OK. i In the Worklist window, mark the check box to the left of the sample.	- 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.
	j Click Start Worklist Run on the main toolbar, Run Worklist on the Worklist toolbar, or click Worklist > Run.	

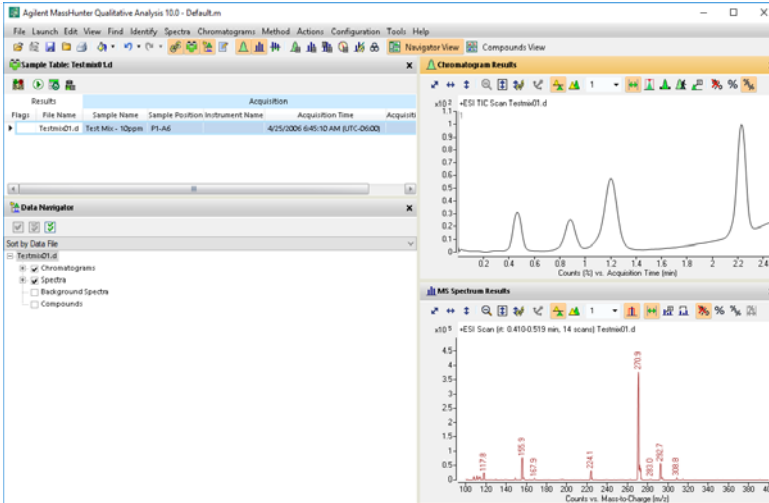
Task 2. Determine precursor ion masses

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

Steps	Detailed Instructions	Comments
1 Open the acquired data file. In the Qualitative Analysis program, open the example file, testmix01.d or the data file you created in "Task 1. Enter acquisition parameters and acquire data" on page 11.	a Double-click the Qualitative Analysis icon.  The program displays the Open Data File dialog box.	- When you open the Test example folder after installation, the Load result data (lower left corner) check box is grayed out. - If you see the check box marked, this means that the data file(s) already contains results. <i>Clear this check box before opening the file.</i>



Steps	Detailed Instructions	Comments
	b Do one of the following: - Select the example data file Testmix01.d, and click Open. - Select the data file you created in "Task 1. Enter acquisition parameters and acquire data" on page 11, and click Open. By default, the system displays the Total Ion Chromatogram (TIC).	- The figure below shows the default layout. - The Qualitative Analysis program displays a newly opened data file with the same layout and display settings used for the previous data file. Therefore, you MUST make sure to return to the default settings for this exercise.
		Before you begin, make sure that all previous settings are returned to their default values: - Click Navigator View on the toolbar. - Restore default layouts - Click Configuration > Window Layouts > Load Default Layout. - Make sure the method is default.m. (see title bar) - Click Method > Open. - Select default.m, and click Open.

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><i>m/z</i></th></tr> </thead> <tbody> <tr> <td>Compound1</td><td>2.23</td><td>311.0</td></tr> <tr> <td>Compound2</td><td>0.88</td><td>284.9</td></tr> <tr> <td>Compound3</td><td>1.2</td><td>279.0</td></tr> <tr> <td>Compound4</td><td>0.47</td><td>270.9</td></tr> </tbody> </table> - Close the data file after finding the precursor ion masses.	Compound	RT	<i>m/z</i>	Compound1	2.23	311.0	Compound2	0.88	284.9	Compound3	1.2	279.0	Compound4	0.47	270.9	a In the Chromatogram Results window, make sure that Range Select on the toolbar  is on. b Click the left mouse button and drag the cursor across the first peak to produce a shaded region, as in the figure below. c Right-click the shaded area, and click Extract MS Spectrum from the shortcut menu.	- The system displays an averaged spectrum across the peak in the MS Spectrum Results window. - The precursor mass of the first compound, Compound4, is determined to be <i>m/z</i> 270.9. - To obtain a single scan, double-click the apex of the peak.
Compound	RT	<i>m/z</i>															
Compound1	2.23	311.0															
Compound2	0.88	284.9															
Compound3	1.2	279.0															
Compound4	0.47	270.9															
																	
	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 <i>M</i> + 23 and <i>M</i> + 39 masses respectively. Seeing these masses along with the <i>M</i> + <i>H</i> can make for an easy confirmation of which ion is the pseudo-molecular ion (<i>M</i> + <i>H</i>)⁺.															

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”** on page 29. You can do the Qualitative Analysis part of this task by using the data files that were shipped with the software.

Steps

Detailed Instructions

Comments

- 1 Set up six methods for six different fragmentor voltages.
 - Change to a SIM experiment.
 - Use 60, 80, 100, 140, 180 and 220 volts as the fragmentor voltages for the six methods.
 - Save the methods as *iiiMS2SIMxxx.m*, where *iii* are your initials and *xxx* is the voltage.

- a In the **Scan Type** dropdown list, click **MS2 SIM**.

The screenshot shows the 'Properties' tab of the MassHunter software. The 'Tune file' is set to 'atunes.tune.xml'. The 'Ion source' is 'AJS ESI'. The 'Stop time' is set to 'No limit/As Pump'. The 'Time filtering' is set to 'Peak width' with a value of '0.07 min'. The 'Time segments' table shows a single segment with 'Scan Type' set to 'MS2 SIM'.

#	Start Time	Scan Type	Div Valve	Delta EMV (+)	Delta EMV (-)	Stored
1	0	MS2 SIM	To MS	0	0	☒

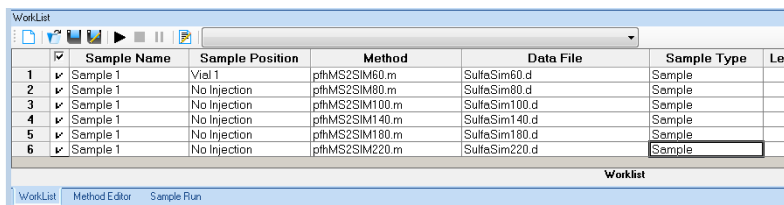
Exercise 1 - Develop an acquisition method

Task 3. Find optimum fragmentor voltage for maximum response

Steps	Detailed Instructions	Comments
	a On the Acquisition tab, enter the Compound Name and Mass (precursor ion mass) for Compound1 (311 m/z). b Right-click anywhere in the Scan segments section, and click Add Row. c Type the Compound Name and the Mass for Compound2 (285 m/z). d Repeat step b and step c for Compound3 (279 m/z) and Compound4 (271 m/z). e Save the method as <i>iii</i>MS2SIM140.m, where <i>iii</i> are your initials. f Change the fragmentor voltage to 60, and save the method as <i>iii</i>MS2SIM060, where <i>iii</i> are your initials. g Repeat step f for voltages 80, 100, 180 and 220. Save the methods as <i>iii</i>MS2SIM080, <i>iii</i>MS2SIM100, <i>iii</i>MS2SIM180 and <i>iii</i>MS2SIM220, where <i>iii</i> are your initials.	- With the MS2SIM Scan Type set, a different set of columns appears in the Acquisition window. - The Instrument Control and Data Acquisition program creates a SIM experiment for each compound mass, starting with a default fragmentor voltage of 140. See the example below. - The Fragmentor column is grayed out if the instrument type is a 6490 or 6495.

Acquisition	Source	Chromatogram	Instrument	Diagnostics						
Scan segments										
Compound Group	Compound Name	ISTD?	Mass	MS2 Res	Dwell	Fragmentor	Cell Accelerator Voltage	Polarity		
►	Compound1	☐	311	Unit	200	140	7	Positive		
	Compound2	☐	285	Unit	200	140	7	Positive		
	Compound3	☐	279	Unit	200	140	7	Positive		
	Compound4	☐	271	Unit	200	140	7	Positive		

Steps	Detailed Instructions	Comments
2 Set up and run the worklist (optional). - Set up six samples with Sample Name Compound Mix to inject 1 μL from vials 1 - 6 or the ones you choose. - Save the method as <code>iiiTest_SIMxxx.d</code>, where "iii" are your initials.	a Click Worklist 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 check box to the left of the Sample Name for each of the six samples..	- The Qualitative Analysis program should be open. If not, see "Double-click the Qualitative Analysis icon." on page 16. - By default, the Method Editor is a floating window. The window is docked for these images. See the <i>online Help</i> for more information on floating and docking windows.

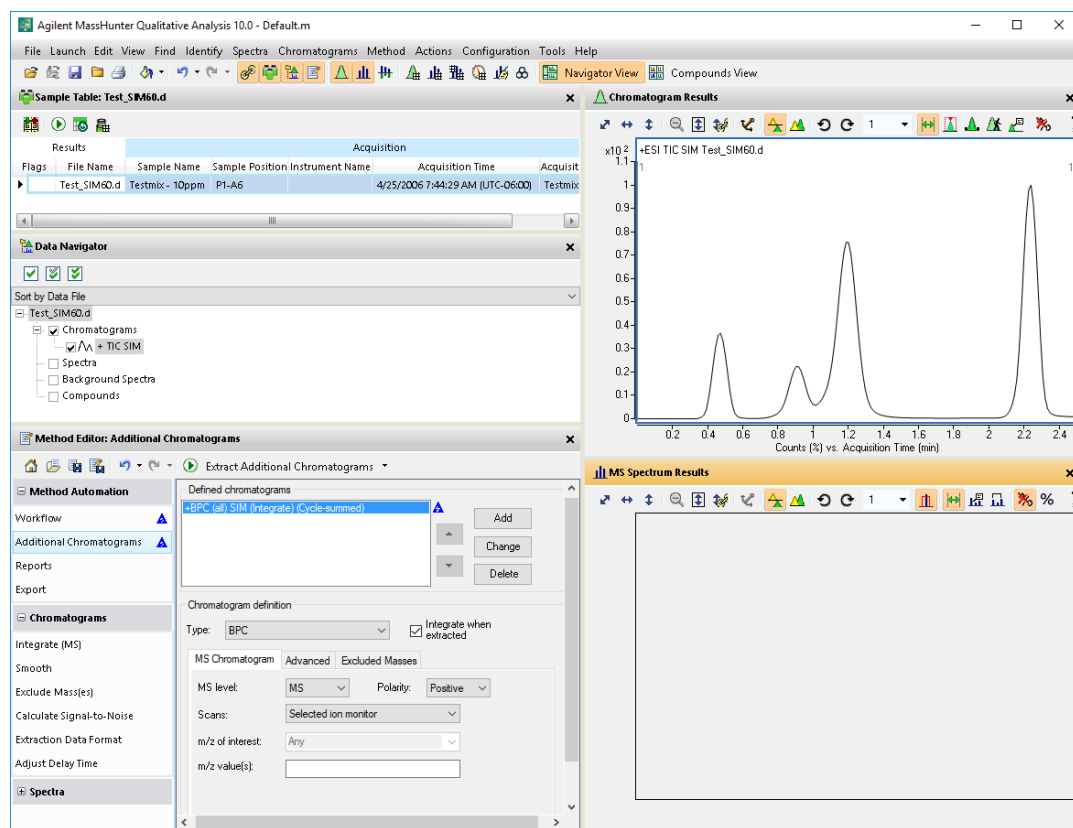


	Sample Name	Sample Position	Method	Data File	Sample Type	Level
1	Sample 1	Vial 1	pftMS2SIM60.m	SultaSim60.d	Sample	
2	Sample 1	No Injection	pftMS2SIM80.m	SultaSim80.d	Sample	
3	Sample 1	No Injection	pftMS2SIM100.m	SultaSim100.d	Sample	
4	Sample 1	No Injection	pftMS2SIM140.m	SultaSim140.d	Sample	
5	Sample 1	No Injection	pftMS2SIM180.m	SultaSim180.d	Sample	
6	Sample 1	No Injection	pftMS2SIM220.m	SultaSim220.d	Sample	

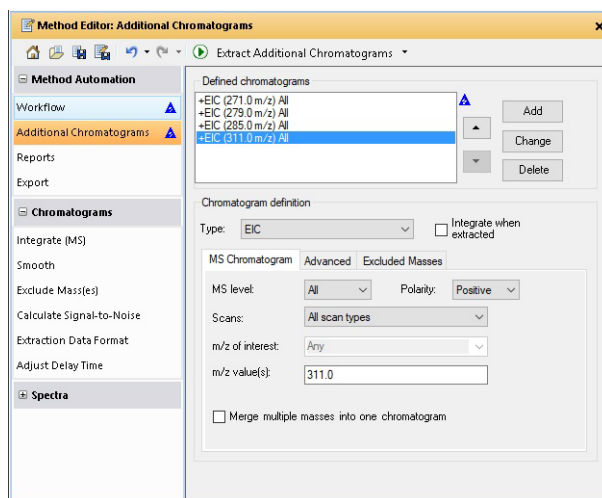
- h** Start the worklist.
- Click **Worklist > Run**.
 - Click  on the main toolbar.
 - Click  on the worklist toolbar.
- Note that the program only runs those samples that are marked with a checkmark.
 - You can also run the worklist in locked mode by clicking the lock button on the main toolbar (the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



Steps	Detailed Instructions	Comments
3 Set up a qualitative method to view the EIC data automatically. - Open the data file Test_SIM60.d. - In the Method Editor, add in the EICs corresponding to the precursor ion masses of 271, 279, 285, and 311. - Save the method as <i>iiiExercise1</i>, where "iii" are your initials.	a Click File > Open Data File . The system displays the Open Data File dialog box b Select Test_SIM60.d , and click Open . c Click Method > Method Editor or View > Method Editor . The system displays the Method Editor window.	- The Qualitative Analysis program should be open. If not, see "Double-click the Qualitative Analysis icon." on page 16. - By default, the Method Editor is a docked window. See the <i>online Help</i> for more information on floating and docking windows.

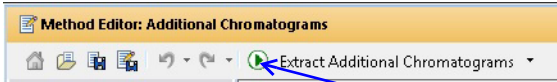
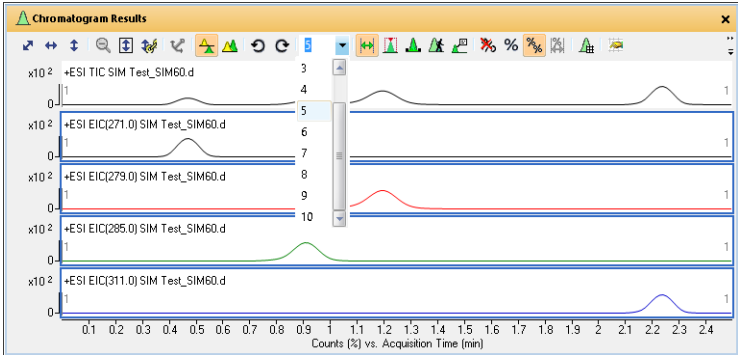


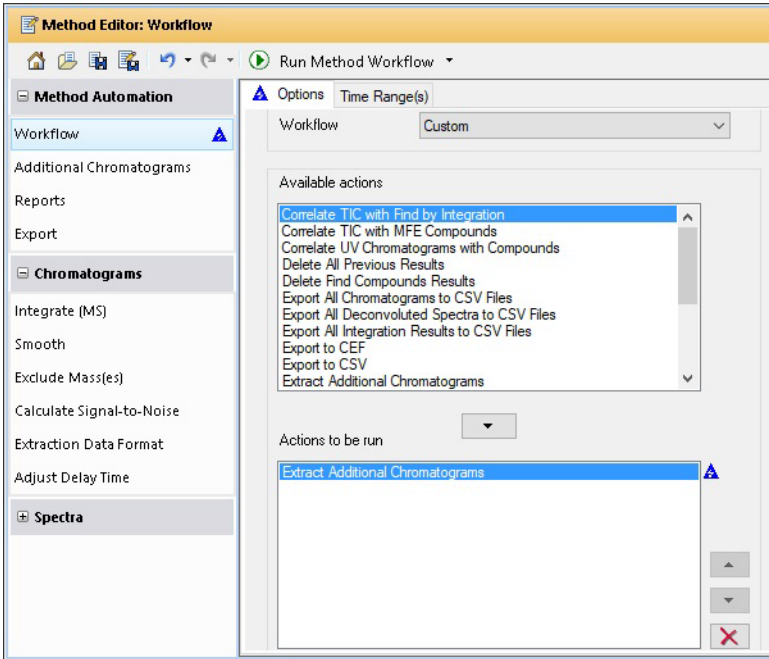
Steps	Detailed Instructions	Comments
	d If necessary, click Additional Chromatograms in the Method Automation section of the Method Explorer. e To delete the base peak chromatogram, click Delete in the Method Editor window. f Select EIC for the Chromatogram Definition Type. g Clear the Integrate when extracted check box. h On the MS Chromatogram tab, make sure MS Level is set to All and Scans is set to All scan types. i Type 271 as the m/z value. j Click Add. k Repeat step i and step 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 <code>iiiExercise1.m</code>. n Click Save.	- The default Method Editor list selection after installation is Method Automation > Workflows. - When you are defining chromatograms, instead of deleting the Defined chromatogram, you can select EIC. Then, you enter the <i>m/z</i> value and click Change.



Exercise 1 - Develop an acquisition method

Task 3. Find optimum fragmentor voltage for maximum response

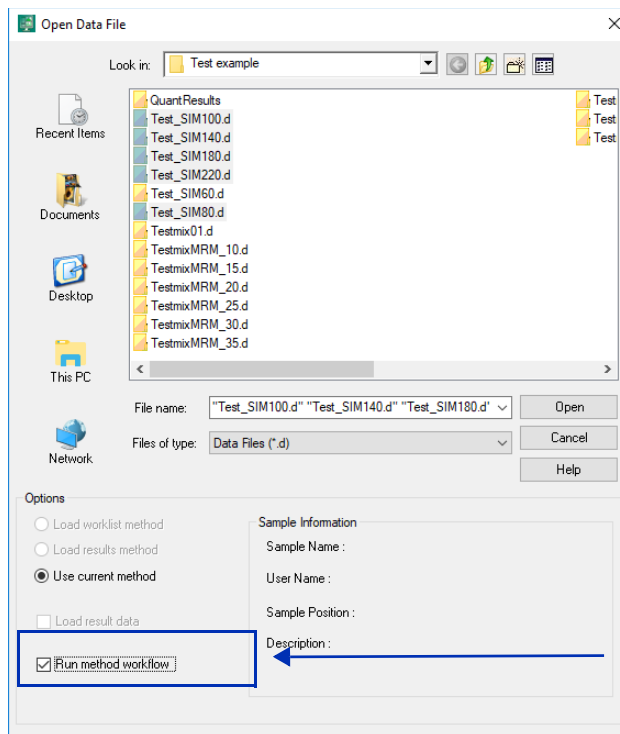
Steps	Detailed Instructions	Comments
4 Extract the chromatogram for the data file and view the results. Make sure you can see all five chromatograms, the TIC and four EICs.	a Click Run on the Method Editor toolbar to run the Extract Additional Chromatogram command.  b To see the TIC and four EICs, click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

Steps	Detailed Instructions	Comments
5 Extract the remaining ion chromatograms automatically. - Extract Defined Chromatograms should be the default action for Assign File Open Actions. - Open the remaining data files, Test_SIM80.d through Test_SIM220.d. - Close the Method Explorer.	a Select Workflow from the Method Automation section in the Method Editor. b Select Custom for the Workflow. c Make sure that Actions to be run list only contains Extract Additional Chromatograms.	The Qualitative Analysis Method Editor lets you define actions to be performed automatically upon opening a data file(s).
		
	d Click File > Open Data File. The system displays the Open Data File dialog box. e Select the data files to be opened, Test_SIM80.d through Test_SIM220.d. f Mark the Run method workflow check box (lower left corner).	

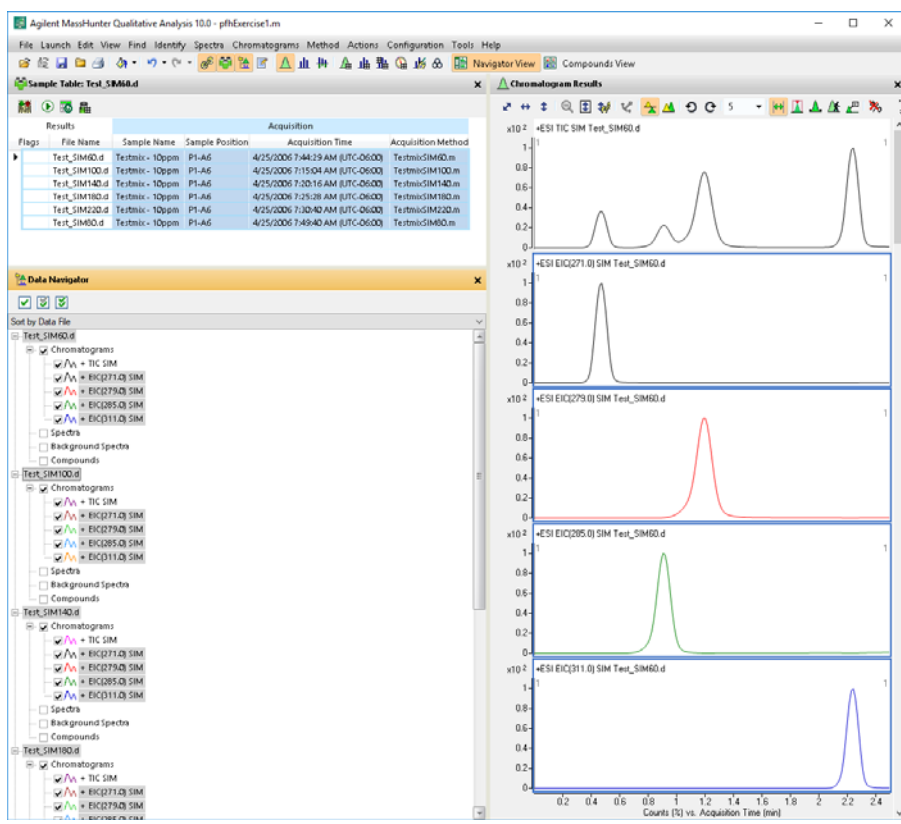
Steps

Detailed Instructions

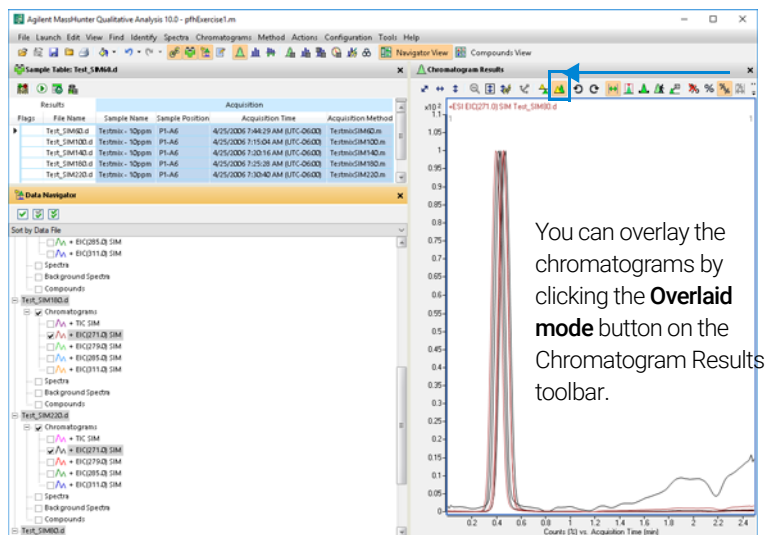
Comments



Steps	Detailed Instructions	Comments
	g Click Open. The Qualitative Analysis program displays all the EICs for the data selected data file. h Select all data files in the Sample Table window. i 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.



Steps	Detailed Instructions	Comments
6 Select the fragmentor voltage that produces the maximum response for each of the precursor ions. Close the data files after you determine the optimum voltage.	a In the Data Navigator window, highlight the EICs for 271.0 m/z. b Click Show only the highlighted items  in the Data Navigator window. Only the 271 m/z check boxes are now marked. c Look at the relative intensities of each peak to determine which fragmentor voltage setting will be best to use for the 271 precursor.	- You press the Ctrl key to be able to select multiple objects from the Data Navigator window. - You press the Shift key to be able to select a group of objects. - A fragmentor voltage of 100 should be sufficient for each precursor ion. - You can now determine the product ions that are available for the multiple-reaction monitoring experiments to maximize sensitivity for the analysis



- d Repeat **step a** through **step c** for the other three base peaks or precursor ions.
- e Click **File > Close All**.
- f Click **No** in the Save dialog box.
- Click **Method > Save** or **Method > Save As** to save the method changes.
 - Click an EIC in the Data Navigator window to change which chromatogram is labeled in the Chromatogram Results window. When the chromatogram label color matches the color of the chromatogram that has the highest intensity, you use the Fragmentor voltage that was used for that file.

Task 4. Determine product ion masses

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

Steps	Detailed Instructions	Comments
1 Set up three product ion acquisition methods and acquire data. - Use the MS parameters in the example below, but change the Fragmentor voltage to the optimum voltage you determined in the previous task. - Save methods as <i>iiiTestmixPI_xx.m</i>, where <i>iii</i> are your initials and <i>xx</i> 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 MS parameters, making sure the Collision Energy is set to 15 and the Fragmentor voltage is set to the optimum voltage determined in Task 3. d Save the method as <i>iiiTestmixPI_15.m</i> . e Repeat step c and step d for collision energies of 30 and 45.	- When you change the Scan Type in the Time Segments table, the Scan segments table is reset. If you want to copy the Scan segments to the new Scan segments table, highlight all of the lines in the Scan segments table and then right-click the Scan segments table and click Copy. After you select a new Scan Type, right-click the Scan segments table and click Paste from Clipboard. - You cannot copy and paste the Scan segments table between all Scan Types.

The screenshot displays the MassHunter software interface for developing an acquisition method. The 'Acquisition' tab is active, showing the 'Scan segments' table and 'Scan parameters'.

Scan segments table:

Segment Name	Precursor Ion	MS2 From	MS2 To	Scan Time	Fragmentor	Collision Energy	Cell Accelerator Voltage	Polarity
Compound1	311	50	320	250	140	15		7 Positive
Compound2	265	50	320	250	80	15		7 Positive
Compound3	279	50	320	250	140	15		7 Positive
Compound4	271	50	320	250	80	15		7 Positive

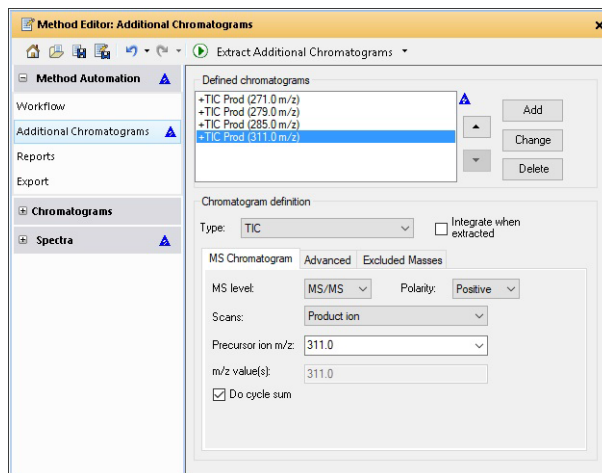
Scan parameters:

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

At the bottom left, the acquisition rate is shown as 1.1 cycles/s and 913.1 ms/cycle.

Steps	Detailed Instructions	Comments
2 Set up and run the worklist (optional). Specify the data files as iiiTestmixPI_xx.d, where <i>iii</i> are your initials and <i>xx</i> is the collision energy.	a Click the Worklist tab. b Add three samples to the worklist for collision energies 15, 30, and 45. c Mark the check box to the left of the Sample Name for each sample you are adding. d Start the worklist. - Click Worklist > Run. - Click  on the main toolbar. - Click  on the worklist toolbar.	- 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. Find optimum fragmentor voltage for maximum response” on page 19 to set up the worklist.

Steps	Detailed Instructions	Comments
3 Set up a qualitative method to integrate and extract product ion spectra. - Use the data files TestmixPI_xx.d, where xx is the collision energy, or your own data files, iiiTestmixPI_xx.d. - Open Method Explorer and Method Editor. - Use TICs set up for MS/MS, product ion and each of the precursor ions 271, 279, 285, 311. - Make sure the MS/MS integrator has been selected and the maximum number of peaks has been limited to the largest 100 peaks. - Add the ability to integrate and extract peak spectra to the file actions run upon data opening. - Save the changes to the current method.	a Click Open Data File on the toolbar. b Select TestmixPI_15.d. c Clear the Run method workflow check box, and click Open. d Make sure the Method Editor window is displayed; otherwise, click Method Editor.  e In the Method Automation section in the Method Editor window, select Additional Chromatograms. f Delete any existing chromatograms in the Defined chromatograms list. g Select TIC from the Type list in the Chromatogram definition section. h For MS level, select MS/MS. i Mark the Do cycle sum check box. j For Scans, select Product ion. k For Precursor ion m/z, type 271. l Click Add. m Repeat step j and step k for each ion.	The Qualitative Analysis program should already be open and contain iiiExercise1.m as the method.



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

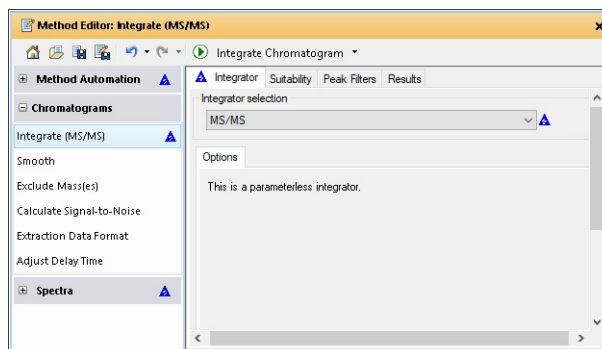


Figure 3. Integrate (MS/MS) > Integrator Tab

- p Click the **Peak Filters** tab. Make sure that the **Limit (by height) to the largest** check box is marked and set to the value 1.00 as shown below.

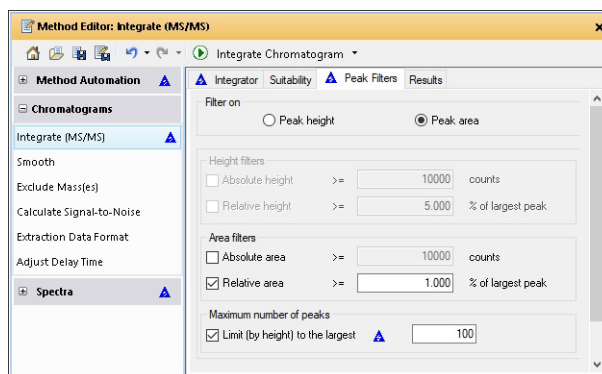


Figure 4. Integrate (MS/MS) > Peak Filters tab

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

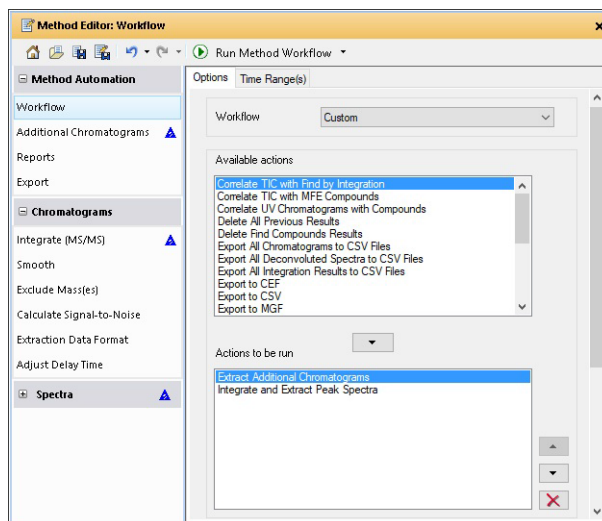


Figure 5. Method Automation > Workflow tab

- s To apply the changes to the current method, *Exercise1.m*, click **Save Method** .

You can also click **Method > Save**.

Steps	Detailed Instructions	Comments
4 Run the qualitative analysis workflow on the current data file.	a On the Method Editor toolbar, click Run . b Close the Method Editor window.	- 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.

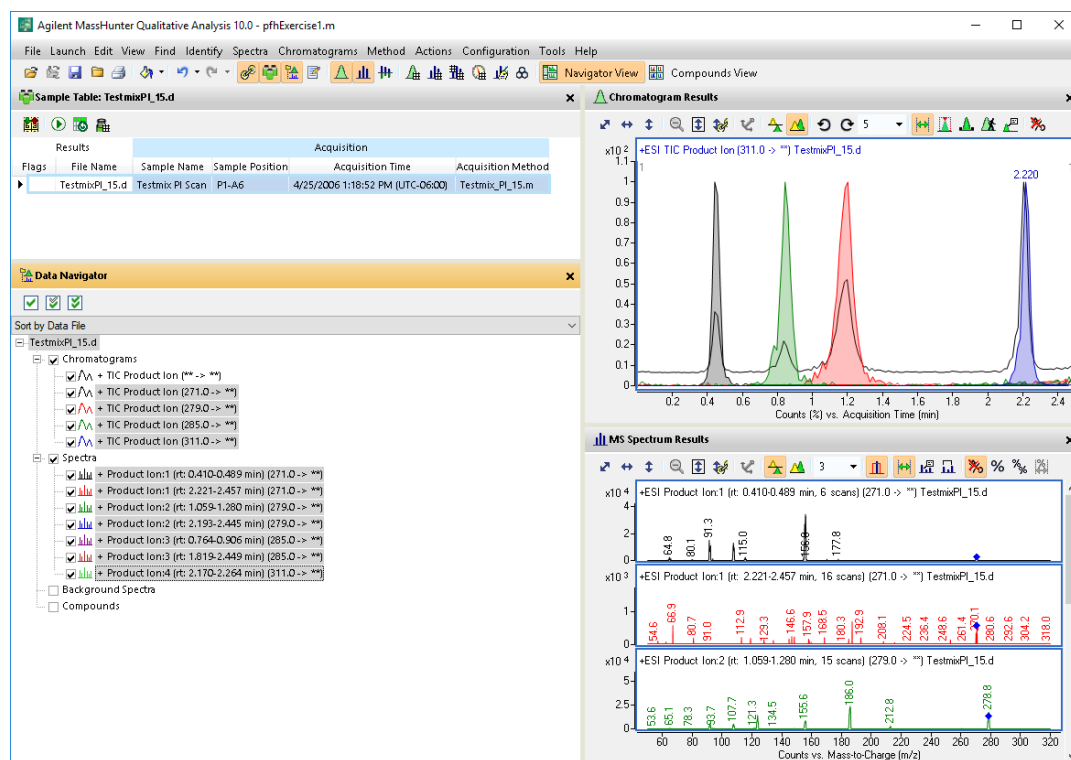


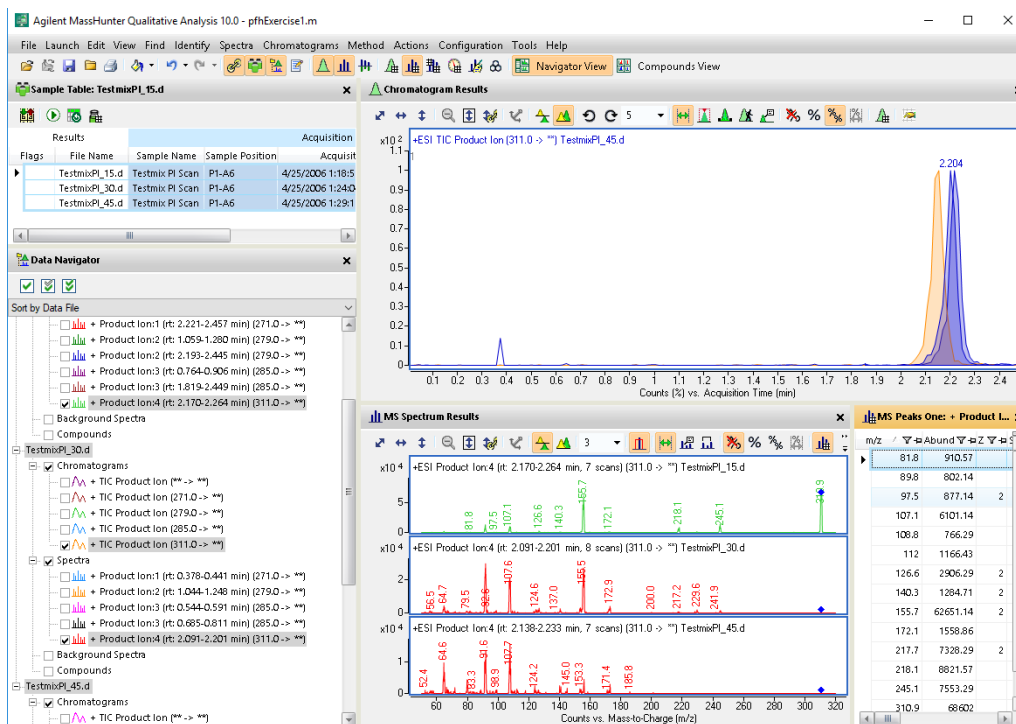
Figure 6. Results for integration and extraction of peak spectra.

5 Run the workflow on the remaining product ion data files.	a Click File > Open Data File. The system displays the Open Data File dialog box. b Hold the Ctrl key and select the two data files TestmixPI_30.d, and TestmixPI_45.d. 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 Analysis method first extracts the product ion chromatograms for each precursor ion. - Next, it integrates each total ion chromatogram and extracts peak spectra from each integrated peak.
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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 Sample Table window, select all data files. b In the Data Navigator, select the TICs and spectra for the 271 m/z precursor ion. c Click Show only the highlighted items  on the Data Navigator toolbar. d Click View > MS Spectrum Peak List 1. e Examine the spectra to see which fragment ions are produced at which collision energies. f Repeat steps b to e 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 Compound4 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.



- g Click **Close Data File** on the main toolbar, and click **Close** when the dialog box containing the list of data files pops up.
- The product ions appear to be:
Compound1 -311.0 > 155.7
Compound2 -285.0 > 155.7
Compound3 -279.0 > 185.7
Compound4 -271.0 > 155.7

Task 5. Find optimum collision energy for MRM acquisition

In this task, you set up MRM acquisition methods 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 <code>iiiTestmix_MRM_xx.m</code>, where <i>iii</i> are your initials and <i>xx</i> is the collision energy.	a Click the QQQ tab. b Set Scan Type to MRM . c Enter MS parameters for your sample except for the collision energy value. d In the collision energy column, type 10 for each compound. e Save the method as <code>iiiTestmix_MRM_10.m</code> . f Repeat step d and step e for collision energies of 15, 20, 25, 30 and 35. Save the methods as <code>iiiTestmix_MRM_xx.m</code> , where <i>iii</i> are your initials and <i>xx</i> is the collision energy.	Because the largest peaks in the example files were produced with a collision energy of 15 in the previous exercise, you would look at only those collision energies to either side of 15.

Tune file
plumes.TUNE.XML
Browse ... *60f*

Ion source
ESI

Stop time
☒ No limit/As Pump
2.0 min

Time filtering
☒ Peak width 0.00 min

Time segments

#	Start Time	Scan Type	Div Valve	Delta EMV (+)	Delta EMV (-)	Stored
1	0	MRM	To MS	10	0	☒

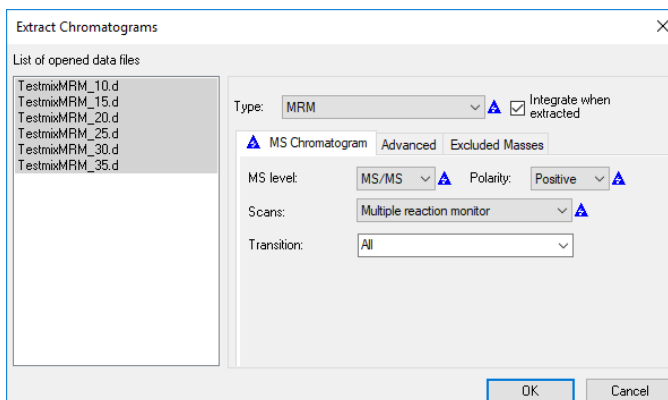
4.67 cycles/s 214.0 ms/cycle

Acquisition | Source | Chromatogram | Instrument | Diagnostics

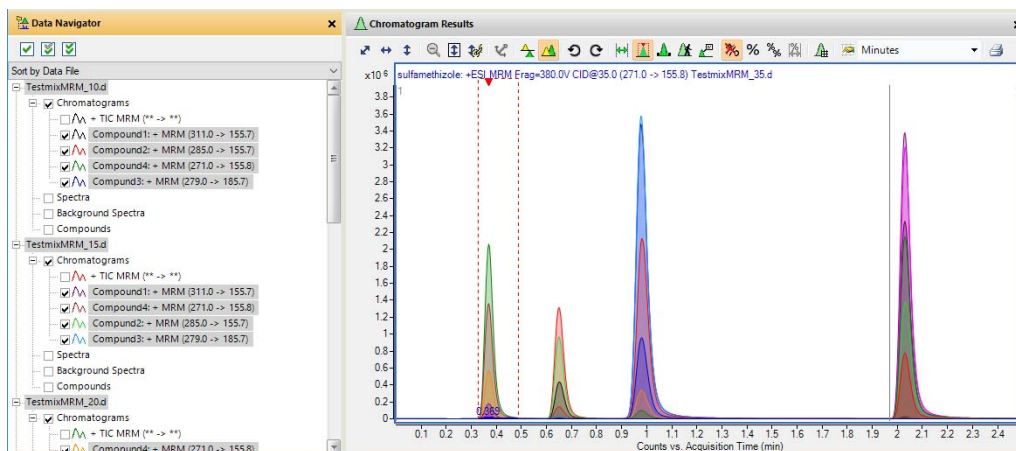
Scan segments

Compound Group	Compound Name	ISTD?	Precursor Ion	MS1 Res	Product Ion	MS2 Res	Dwell	Fragmentor	Collision Energy	Cell Accelerator Voltage	Polarity
▶	Compound1	☐	311	Unit	155.7	Unit	50	100	10	7	Positive
	Compound2	☐	205	Unit	155.7	Unit	50	100	10	7	Positive
	Compound3	☐	279	Unit	105.7	Unit	50	100	10	7	Positive
	Compound4	☐	271	Unit	155.8	Unit	50	100	10	7	Positive

Steps	Detailed Instructions	Comments
2 Set up and run the worklist (optional). Specify the data files as <i>iiiTestmixMRM_xx.d</i>, where <i>iii</i> are your initials and <i>xx</i> is the collision energy.	a Click the Worklist tab. 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 sample you are adding. d Start the worklist. - Click Worklist > Run. - Click  on the main toolbar. - Click  on the worklist toolbar.	- 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. Find optimum fragmentor voltage for maximum response" on page 19 to set up the worklist.
3 Compare the compound transition intensities at different collision energies. - Open the MRM data files: TestmixMRM_10.d TestmixMRM_15.d TestmixMRM_20.d TestmixMRM_25.d TestmixMRM_30.d TestmixMRM_35.d - Set the MRM chromatogram extraction parameters as shown at right for all transitions. - Disable the TICs for clarity and examine the peak intensities. - Compare the intensities of each compound transition obtained at one collision energy with the same compound transition obtained at another collision energy. (Do this in Overlaid Mode with all the MRM chromatograms.) - Close the data files but don't save results. - Refer to Table 4 on page 38 for optimal method settings for each compound.	a Open the Qualitative Analysis program. b Clear the Run method workflow check box. c Select the MRM data files and click Open . 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 open this dialog box.



Steps	Detailed Instructions	Comments
	h Click Overlaid Mode . 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 for the example files are as shown in **Table 4**.

- j** Click **Close Data File** on 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 test mixture. Consider doing at least one more run with those settings.

Table 4 Compounds and Collision Energy

Compounds	MRM Transition	Collision Energy (V)
Compound1	311.0 > 155.7	15
Compound2	285.0 > 155.7	10
Compound3	279.0 > 185.7	15
Compound4	271.0 > 155.8	10

3

Exercise 2 – Develop a Dynamic MRM method

- Task 1. Create a batch file from an existing MRM data file 39
- Task 2. Print a report in the Quantitative Analysis program 42
- Task 3. Create a Dynamic MRM method using Update dMRM 43
- Task 4. Create a Dynamic MRM method from an MRM method 45

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

Task 1, Task 2, and Task 3 are the main tasks.

Task 4 tells you how to easily create a Dynamic MRM method from an existing MRM method.

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.

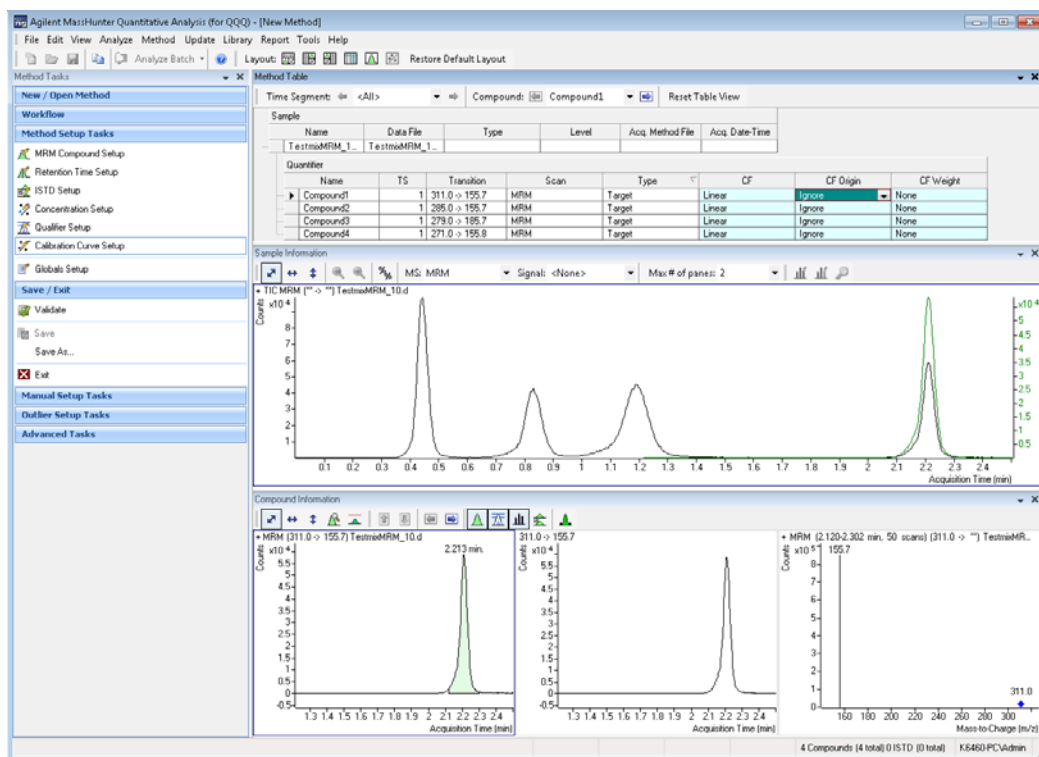
Steps	Detailed Instructions	Comments
1 Open the Quantitative Analysis program and create a batch file with one sample file, TestmixMRM_10.d. Copy the data file TestmixMRM_10.d from the installation media to the \MassHunter\Data\MRM_to_DMRM folder.	a Double-click the QQQ Quantitative Analysis icon. b Click File > New Batch . c Navigate to the \MassHunter\Data\MRM_to_DMRM folder. d Type MRM_to_DMRM in the File name text box. e Click Open . f If the Add Samples dialog box does not open, click File > Add Samples . g Select the file TestmixMRM_10.d . h Click OK .	The file TestmixMRM_10.d is on the installation disk in the \Support\Data folder. Copy this entire folder to the \MassHunter\Data\MRM_to_DMRM folder.

Steps	Detailed Instructions	Comments
2 Create a method for that batch using MRM data.	- Click Method > New > New Method from Acquired MRM Data. - Select the TestmixMRM_10.d data file. - Click Open.	
3 Set the Concentration Setup, Qualifier Setup, and Calibration Curve Setup. - Add calibration level 1 with a concentration of 10000. - Set the Uncertainty to Relative for all qualifiers. - Set the Curve Fit to Linear. - Set the Curve Fit Origin to Include. - Set the Curve Fit Weight to None.	- Select Concentration Setup in the Manual Setup Tasks section in the Method Tasks pane. - Select the first compound in the table. - Right-click the compound row and click New Calibration Level from the shortcut menu. - Enter A1 in the Level column and 1.0 in the Conc. column. - Right-click in the Level box and click Copy Calibration Levels To. - Click Select All. Click OK. - Select Qualifier Setup in the Manual Setup Tasks section in the Method Tasks pane. - Verify that the Uncertainty is Relative. - Select Calibration Curve Setup in the Manual Setup Tasks section in the Method Tasks pane. - Set Curve Fit to Linear for all compounds. - Set CF Origin to Include for all compounds. - 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.

Steps

Detailed Instructions

Comments



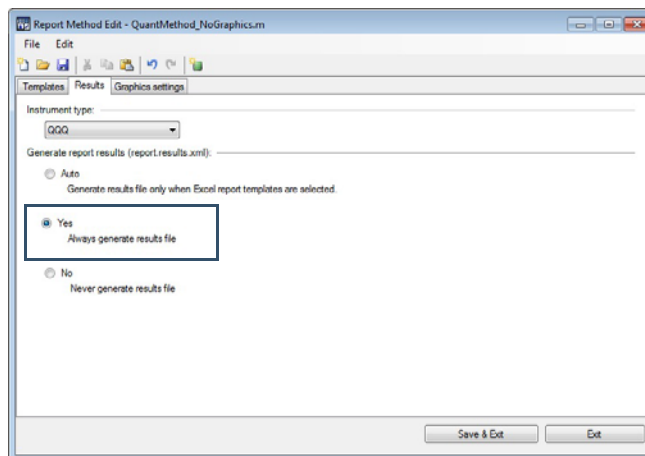
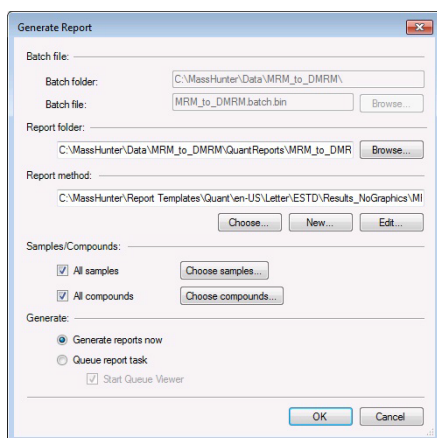
- 4 Verify method and then save the method and apply the method to the batch.
 - a Click **Method > Validate**.
 - b Click **OK** on the message box. Fix any errors, if necessary.
 - c Click **Method > Save As**.
 - d Enter **MRM_to_DMRM**.
 - e Click **Save**.
 - f Click **Method > Exit**.
 - g (optional)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

Task 2. Print a report in the Quantitative Analysis program

In this task, you print a report using the **MRM_to_DMRM.xltx** 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.

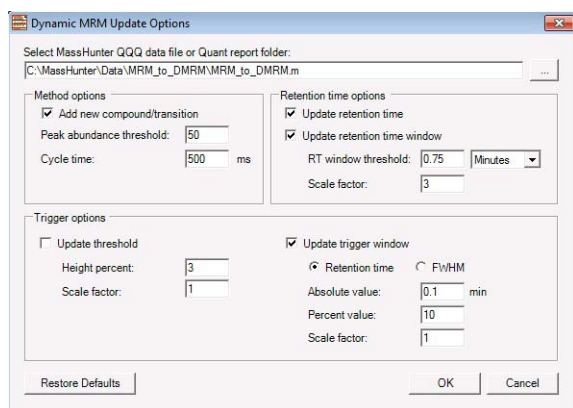
Steps	Detailed Instructions	Comments
1 Print a report using the template MRM_to_DMRM.xltx.	- Click File > Save Batch. - Click Report > Generate. The system displays the Generate Report dialog box. - Select the Report folder. This folder name will be used in the next task. - Select the Report method. - Click Edit. The Report Method Edit dialog box opens. - Click the Results tab. Click Yes Always generate results file. - Click Save & Exit. - Mark All samples. - Mark All compounds. - Click OK.	- Copy the MRM_to_DMRM.xltx template from the \Support\Data folder on the installation disk. - For this report, you do not need to print the report. - If you have not created a Report Method, see the <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.
2 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.	



Task 3. Create a Dynamic MRM method using Update dMRM

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

Steps	Detailed Instructions	Comments
1 Open the method <i>iiiTestmix_MRM_10.m</i> and save it to a new name with the format <i>iiiTestmix_dMRM.m</i> , where <i>iii</i> are your initials.	- Click File > Open > Method. - Select the <i>iiiTestmix_MRM_10.m</i> method. Click OK. - Click Method > Save As. - Type the new method name with the format <i>iiiTestmix_dMRM.m</i>.	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>iiiTestmix_MRM_10.d</i>. - Mark Add new compound/transition. - Mark Update retention time. - Mark Update trigger window. - Click OK.	- The Dynamic MRM Update tool automatically sets the Scan type to Dynamic MRM. - You can select either a data file that was acquired with a Scan Type of MRM or a Quant Report folder as the input to this dialog box. The Scan segments are created from one of these two input sources.



You can update the compounds in the Scan segments table by using a QQQ data file or a Quantitative analysis report folder.

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 42 to learn how to always generate the results file.

Steps		Detailed Instructions		Comments																																																																																
Tune file: tunes.TUNE.XML Browse... Load Stop time: ☒ No limit/As Pump ☐ 2.0 min Ion source: ESI Time filtering: ☒ Peak width: 0.03 min Time segments: <table border="1"> <thead> <tr> <th>#</th> <th>Start Time</th> <th>Scan Type</th> <th>Div Valve</th> <th>Delta EMV (+)</th> <th>Delta EMV (-)</th> <th>Stored</th> </tr> </thead> <tbody> <tr> <td>1</td> <td>0</td> <td>Dynamic MRM</td> <td>To MS</td> <td>18</td> <td>0</td> <td>☒</td> </tr> </tbody> </table> cycles/s: ms/cycle:		#	Start Time	Scan Type	Div Valve	Delta EMV (+)	Delta EMV (-)	Stored	1	0	Dynamic MRM	To MS	18	0	☒	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>MS1 Res</th> <th>Product Ion</th> <th>MS2 Res</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> </tr> </thead> <tbody> <tr> <td></td> <td>Compound1</td> <td>☐</td> <td>311</td> <td>Unit</td> <td>155.7</td> <td>Unit</td> <td>2.03</td> <td>0.75</td> <td>100</td> <td>10</td> <td></td> <td>7 Positive</td> </tr> <tr> <td></td> <td>Compound2</td> <td>☐</td> <td>285</td> <td>Unit</td> <td>155.7</td> <td>Unit</td> <td>0.85</td> <td>0.75</td> <td>100</td> <td>10</td> <td></td> <td>7 Positive</td> </tr> <tr> <td></td> <td>Compound3</td> <td>☐</td> <td>279</td> <td>Unit</td> <td>165.7</td> <td>Unit</td> <td>0.98</td> <td>0.75</td> <td>100</td> <td>10</td> <td></td> <td>7 Positive</td> </tr> <tr> <td></td> <td>Compound4</td> <td>☐</td> <td>271</td> <td>Unit</td> <td>155.0</td> <td>Unit</td> <td>0.37</td> <td>0.75</td> <td>100</td> <td>10</td> <td></td> <td>7 Positive</td> </tr> </tbody> </table> Dynamic MRM Parameters: Cycle Time: 500 ms Triggered MRM: ☐ Triggered Repeats: 3				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		Compound1	☐	311	Unit	155.7	Unit	2.03	0.75	100	10		7 Positive		Compound2	☐	285	Unit	155.7	Unit	0.85	0.75	100	10		7 Positive		Compound3	☐	279	Unit	165.7	Unit	0.98	0.75	100	10		7 Positive		Compound4	☐	271	Unit	155.0	Unit	0.37	0.75	100	10		7 Positive
#	Start Time	Scan Type	Div Valve	Delta EMV (+)	Delta EMV (-)	Stored																																																																														
1	0	Dynamic MRM	To MS	18	0	☒																																																																														
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	Compound3	☐	279	Unit	165.7	Unit	0.98	0.75	100	10		7 Positive																																																																								
	Compound4	☐	271	Unit	155.0	Unit	0.37	0.75	100	10		7 Positive																																																																								

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

- h Verify that each row has a **Compound Name**. A blank Compound Name is not allowed.
- i Click **Method > Save**.

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

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

Steps	Detailed Instructions	Comments
1 Open the method <i>iiiTestmix_MRM_10.m</i> and save it to a new name with the format <i>iiiTestmix_dMRM2.m</i> , where <i>iii</i> are your initials.	- Click File > Open > Method. - Select <i>iiiTestmix_MRM_10.m</i>. - Click OK. - Click Method > Save As. - Type the new method name with the format <i>iiiTestmix_dMRM2.m</i>. - Click Save.	
2 Copy all compounds from the Scan segments table in the MRM method.	- In the Method Editor, click the Acquisition tab in the QQQ tab. - Select all of the rows in the Scan segments table. - 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.	- Select Dynamic MRM for the Scan Type. - Right-click the Scan segments table and click Paste from Clipboard. - 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.

Steps	Detailed Instructions	Comments
4	Enter the retention times and delete the original compound. a Type the value for the Ret. Time (min) for Compound1. b Type the value for the Ret. Time (min) for Compound2. c Type the value for the Ret. Time (min) for Compound3. d Type the value for the Ret. Time (min) for Compound4. 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: - Compound1: 2.03 minutes - Compound2: 0.65 minutes - Compound3: 0.98 minutes - Compound4: 0.37 minutes

The screenshot shows the MassHunter software interface. The 'Acquisition' tab is active, displaying the 'Scan segments' table. 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 of data for Compound1 through Compound4. A 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', 'Fill Column', 'Min: 0.0367', 'Max: 999.9', and 'Default: 1'. The 'Dynamic MRM Parameters' section at the bottom shows 'Cycle Time' set to 500 ms.

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
Compound1	Compound1	☐	320	Unit	200	Unit	0					ve
Compound2	Compound2	☐	311	Unit	155.7	Unit	0					ve
Compound3	Compound3	☐	205	Unit	155.7	Unit	0.65					ve
Compound4	Compound4	☐	279	Unit	185.7	Unit	0.98					ve
Compound4	Compound4	☐	271	Unit	155.8	Unit	0.37					ve

4

Exercise 3 – Create a Triggered Dynamic MRM acquisition method

- Task 1. Create a Triggered Dynamic MRM method from a Dynamic MRM method manually **47**
- Task 2. Add/Change compounds in an existing database **50**
- Task 3. Create a Triggered Dynamic MRM method from an existing database **60**

For this exercise you create a triggered MRM acquisition method either manually from a dynamic MRM method or by using the Optimizer program.

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.

Steps	Detailed Instructions	Comments
1 Open the method <i>iiiTestmix_dMRM2.m</i> , where <i>iii</i> are your initials.	- Click File > Open > Method. - Select the <i>iiiTestmix_dMRM2.m</i> method. - Click OK. - Click Method > Save As. - Type the new method name with the format <i>iiiTestmix_TriggeredDMRM.m</i>. - 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.

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

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

Scan segments

Compound Group	Compound Name	ISTD?	Precursor Ion	Product Ion	Primary	Trigger	Threshold	Ret Time (min)	Delta Ret Time	Fragmentor	Collision Energy	Trigger Entrance	Trigger Delay	Trigger Window	Polarity	MS1 Res	MS2 Res	Ce
1	Compound1	☐	311	245.1	☒	☒	1000	1.2	1	380	12	0	1	0	Positive	Unit	Unit	
1	Compound1	☐	311	173.1	☒	☐		1.2	1	380	24				Positive	Unit	Unit	
1	Compound1	☐	311	156	☐	☐				380	16				Positive	Unit	Unit	
1	Compound1	☐	311	108	☐	☐				380	24				Positive	Unit	Unit	
1	Compound2	☐	285	197	☒	☒	800	0.5	1	380	8	2	0	0	Positive	Unit	Unit	
1	Compound2	☐	285	156	☒	☐		0.5	1	380	8				Positive	Unit	Unit	
1	Compound2	☐	285	108	☐	☐				380	20				Positive	Unit	Unit	
2	Compound3	☐	279	186	☒	☒	900	0.8	1	380	12	0	0	0	Positive	Unit	Unit	
2	Compound3	☐	279	155.9	☒	☒	1000	0.8	1	380	12	0	0	0	Positive	Unit	Unit	
2	Compound3	☐	279	124.1	☐	☐				380	20				Positive	Unit	Unit	
2	Compound3	☐	279	124.1	☐	☐				380	24				Positive	Unit	Unit	
2	Compound4	☐	271	253.4	☒	☒	1100	0.3	1	380	0	0	0	0	Positive	Unit	Unit	
2	Compound4	☐	271	156	☒	☐		0.3	1	380	4				Positive	Unit	Unit	
2	Compound4	☐	271	108	☐	☐				380	20				Positive	Unit	Unit	

Dynamic MRM Parameters

Cycle Time ms

Triggered MRM

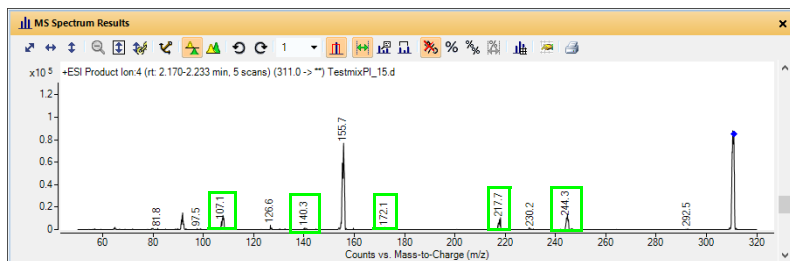
☒ Triggered Repeats

Task 2. Add/Change compounds in an existing database

You can also manually add compounds to a database and change 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>iiiTestmix_dMRM2.m</i> , where <i>iii</i> are your initials.	- Click File > Open > Method. - Select the <i>iiiTestmix_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. .	
3 Set parameters on the Optimizer Setup tab.	- Click the Optimizer Setup tab. - Click 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>iiiTestmix_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.	- Click 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.	- Click 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.

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	- Click the Compound Setup tab. - Click the Import/Export > Import from Acquisition Methods command. - Select the Testmix_dMRM2.m method and click Open. (optional) Right-click the tab and click Expand/Collapse All Rows. - Select one of the Product rows for one of the compounds. In this example, select the Product row 155.7 for Precursor 311. - Right-click the Product row and click Add Product Ion. In this example, you add 5 product ion rows. - Enter the Product in each of the product ion rows that were added. See “To determine product ions in the Qualitative Analysis program:” on page 52. - Add product ions for the other three compounds.	- For each compound, we are going to add additional transitions. - In the Qualitative Analysis program, you examine Product Ion data files which you acquired previously to determine additional transitions to add. See “Task 4. Determine product ion masses” on page 29. - You can use the arrow keys to move between rows in the Product table. - Some of the product ions were determined from the Veterinarian Drugs tMRM database. - See Table 5 on page 59 for values to use in the method.

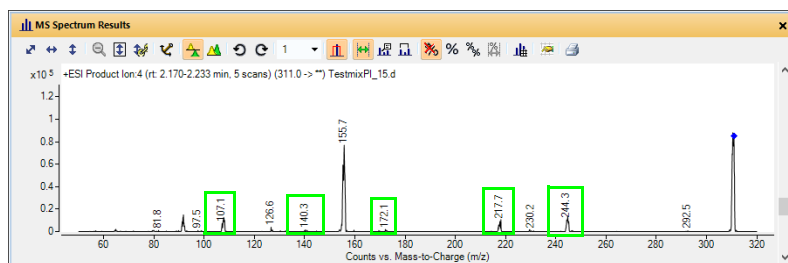


This product ion scan has a precursor mass of 311. You examine the MS spectrum to determine the product ions to add to the Product ion section of the Compound Setup table.

Optimizer Setup		Precursor Ion Selection		Product Ion Selection		Compound Setup		
☐ Show results summary								
☐	Compound Name		Groups		Formula		Mass	Seq
☐	Compound1							1
☐	Acq Method		Polarity		Ion Source		RT	RT Window
☐	D:\MassHunter\Met		Positive		ESI		2.03	1
☐	Precursor		Frag		Species		Abundance	
☐	311		100					
	Product		CE		Abundance		CAV	Primary
	155.7		10				7	☒
	245.1							☐
	218.1							☐
	172.1							☐
	107.1							☐

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

Steps	Detailed Instructions	Comments
To determine product ions in the Qualitative Analysis program:	- Open the TestmixPI_15.d from “Task 4. Determine product ion masses” on page 29. - Click Find > Find Compounds by Targeted MS/MS. - Close the Compound List window. - Select a compound in the Data Navigator window. For this example, click Cpd 4. - Click Autoscale Y-axis on the MS Spectrum Results toolbar. - Right-click and drag to zoom in on the MS spectrum.	- If possible, rearrange the windows on the screen so you can see the Optimizer program and the Qualitative Analysis program at the same time. - See Table 5 on page 59 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.

7 Set other parameters in the Compound Setup tab and start the optimization. - You cannot perform a multi-compound run. - You have to mark each row in the table to use.	- 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 on the Optimizer toolbar.	- You cannot perform a multi-compound run with the number of transitions that were added. If you mark this check box, then the Expected peak width (base) is automatically set to almost 80 seconds wide. If you clear this check box, then the Expected peak width is calculated to be around 9 seconds which is more appropriate. - See Table 5 on page 59 for values to use in the method.
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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: S491
Instrument Model: G6490A

Compound Name Formula Mass Sample Position
Compound2 1

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

Precursor Ion	Fragmentor	Product Ion	Collision Energy	Abundance
285	380	155.7	10	5725674
285	380	129.9	24	319469
285	380	107.9	24	5015922
285	380	91.9	24	7430199
285	380	79.8	48	1529346
285	380	64.8	48	6508111

Compound Name Formula Mass Sample Position
Compound1 1

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

Precursor Ion	Fragmentor	Product Ion	Collision Energy	Abundance
311	380	155.7	15	2325520
311	380	244.8	12	549481
311	380	229.7	20	125578
311	380	217.7	15	494965
311	380	172.9	24	414989
311	380	107.9	20	2291401
311	380	91.9	32	3102685
311	380	79.9	48	761344
311	380	64.8	48	2583686

Compound Name Formula Mass Sample Position
Compound3 1

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

Precursor Ion	Fragmentor	Product Ion	Collision Energy	Abundance
279	380	185.7	11	3730936
279	380	212.8	20	421592
279	380	155.9	12	1377529
279	380	123.9	24	2633948
279	380	107.9	24	2407737
279	380	91.9	24	3643700
279	380	79.8	48	1242301
279	380	64.9	48	3799952

Compound Name Formula Mass Sample Position
Compound4 1

Method Name
Polarity Positive Ion Source AJS ESI

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.	

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 5 on page 59 for values to use in the method.

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

Set primary and trigger flags: Set top 2 ranked transitions as primary

Rank transitions by: ☒ Abundance ☐ Response Factor

	Compound Name	Formula	MW	Polarity	Species	Precursor	Product	Frag	CE	CAV	Primary	Trigger
☒	Compound1			Positive		311	155.7	100	10	7	☒	☐
☒	Compound1			Positive		311	245.1	100			☐	☐
☒	Compound1			Positive		311	218.1	100			☐	☐
☒	Compound1			Positive		311	172.1	100			☐	☐
☒	Compound1			Positive		311	107.1	100			☐	☐
☒	Compound2			Positive		285	155.7	100	10	7	☒	☐
☒	Compound2			Positive		285	92.1	100			☐	☐
☒	Compound2			Positive		285	108.1	100			☐	☐
☒	Compound2			Positive		285	80.1	100			☐	☐
☒	Compound2			Positive		285	95.1	100			☐	☐
☒	Compound3			Positive		279	185.7	100	10	7	☒	☐
☒	Compound3			Positive		279	212.8	100			☐	☐
☐	Compound3			Positive		279	64.9	100			☐	☐
☒	Compound3			Positive		279	155.6	100			☐	☐
☒	Compound3			Positive		279	107.7	100			☐	☐
☒	Compound3			Positive		279	92.1	100			☐	☐
☒	Compound4			Positive		271	155.8	100	10	7	☒	☐
☒	Compound4			Positive		271	177.8	100			☐	☐
☒	Compound4			Positive		271	115.9	100			☐	☐
☒	Compound4			Positive		271	107.9	100			☐	☐
☒	Compound4			Positive		271	92	100			☐	☐
☒	Compound4			Positive		271	80	100			☐	☐
☐	Compound4			Positive		271	64.9	100			☐	☐

Steps	Detailed Instructions	Comments
12 In the Database Browser program, automatically select the Primary transitions and Trigger transition.	a In the Set top ranked transitions as primary box, enter 2. b Click 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 5 on page 59 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	MW	Polarity	Species	Precursor	Product	Flag	CAV	Primary	Trigger	RT	RT Wind
Compound1	C12H14O4N4S		Positive		311	155.7	100	7	☒	☒	2.03	
Compound1	C12H14O4N4S		Positive		311	245.1	100	7	☒	☐	2.03	
Compound1	C12H14O4N4S		Positive		311	218.1	100	7	☒	☐	2.03	
Compound1	C12H14O4N4S		Positive		311	172.1	100	7	☒	☐	2.03	
Compound1	C12H14O4N4S		Positive		311	107.1	100	7	☒	☐	2.03	
Compound1	C12H14O4N4S		Positive		311	91.9	100	7	☒	☐	2.03	
Compound2	C12H14O2N4S		Positive		205	155.7	100	7	☒	☒	0.65	
Compound2	C12H14O2N4S		Positive		205	92.1	100	7	☒	☐	0.65	
Compound2	C12H14O2N4S		Positive		205	108.1	100	7	☒	☐	0.65	
Compound2	C12H14O2N4S		Positive		205	80.1	100	7	☒	☐	0.65	
Compound2	C12H14O2N4S		Positive		205	65.1	100	7	☒	☐	0.65	
Compound3	C9H10O2N4S2		Positive		279	185.7	100	7	☒	☒	0.98	
Compound3	C9H10O2N4S2		Positive		279	212.8	100	7	☒	☐	0.98	
Compound3	C9H10O2N4S2		Positive		279	64.9	100	7	☒	☐	0.98	
Compound3	C9H10O2N4S2		Positive		279	155.6	100	7	☒	☐	0.98	
Compound3	C9H10O2N4S2		Positive		279	107.7	100	7	☒	☐	0.98	
Compound3	C9H10O2N4S2		Positive		279	92.1	100	7	☒	☐	0.98	
Compound3	C9H10O2N4S2		Positive		279	79.0	100	7	☒	☐	0.98	
Compound3	C9H10O2N4S2		Positive		279	123.9	100	7	☒	☐	0.98	
Compound4	C10H9O2N2SCl		Positive		271	195.8	100	7	☒	☒	0.37	
Compound4	C10H9O2N2SCl		Positive		271	177.0	100	7	☒	☐	0.37	
Compound4	C10H9O2N2SCl		Positive		271	115.9	100	7	☒	☐	0.37	
Compound4	C10H9O2N2SCl		Positive		271	107.9	100	7	☒	☐	0.37	
Compound4	C10H9O2N2SCl		Positive		271	92	100	7	☒	☐	0.37	
Compound4	C10H9O2N2SCl		Positive		271	80	100	7	☒	☐	0.37	
Compound4	C10H9O2N2SCl		Positive		271	64.9	100	7	☒	☐	0.37	

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

Steps	Detailed Instructions	Comments
13 Review the Primary transitions and Trigger transitions. - For Compound1, select 311 <i>m/z</i> -> 155.7 <i>m/z</i> transition as the Primary and Trigger transition. - For Compound2, select 285 <i>m/z</i> -> 155.7 <i>m/z</i> transition as the Primary and Trigger transition. - For Compound3, select 279 <i>m/z</i> -> 185.7 <i>m/z</i> transition as the Primary and Trigger transition. - For Compound4, select 271 <i>m/z</i> -> 155.8 <i>m/z</i> 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 <i>m/z</i> for the Product Ion. A very abundant low <i>m/z</i> ion may be unsuitable as a Primary transition. - You can select two Primary transitions as triggers for a compound. - In the example below, all of the columns have values. The Fragmentor voltages and collision energy values were set in Optimizer. - See Table 5 on page 59 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

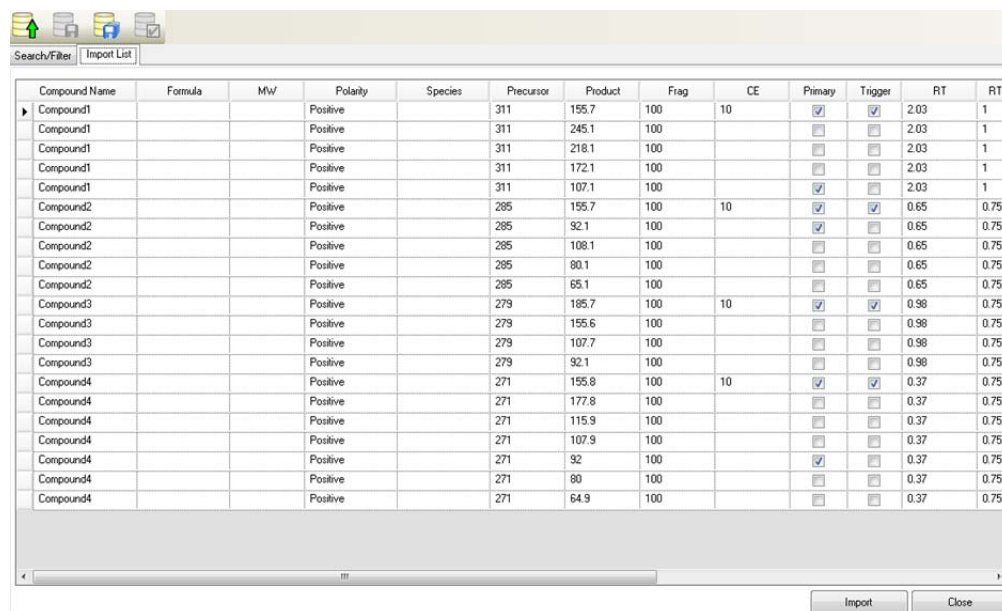
Rank transitions by

☒ Abundance

☐ Response Factor

	Compound Name	Formula	MW	Polarity	Species	Precursor	Product	Flag	CE	CAV	Primary	Trigger	RT	RT window	Abundance	R
☒	Compound1	C12H14O4N4S		Positive		311	155.7	100	15	7	☒	☒	2.03	1	3335528	
☒	Compound1	C12H14O4N4S		Positive		311	245.1	100	12	7	☐	☐	2.03	1	548481	
☒	Compound1	C12H14O4N4S		Positive		311	218.1	100	16	7	☐	☐	2.03	1	434965	
☒	Compound1	C12H14O4N4S		Positive		311	172.1	100	24	7	☐	☐	2.03	1	414589	
☒	Compound1	C12H14O4N4S		Positive		311	107.1	100	20	7	☒	☐	2.03	1	2291401	
☒	Compound1	C12H14O4N4S		Positive		311	91.9	100	32	7	☐	☐	2.03	1	2102895	
☒	Compound2	C12H14O2N4S		Positive		285	155.7	100	10	7	☒	☒	0.65	1	5723674	
☒	Compound2	C12H14O2N4S		Positive		285	92.1	100	24	7	☐	☐	0.65	1	4438199	
☒	Compound2	C12H14O2N4S		Positive		285	108.1	100	24	7	☒	☐	0.65	1	5015922	
☒	Compound2	C12H14O2N4S		Positive		285	80.1	100	48	7	☐	☐	0.65	1	1529348	
☒	Compound2	C12H14O2N4S		Positive		285	65.1	100	48	7	☐	☐	0.65	1	8581111	
☒	Compound3	C9H10O2N4S2		Positive		279	185.7	100	11	7	☒	☒	0.98	1	3720936	
☒	Compound3	C9H10O2N4S2		Positive		279	212.8	100	20	7	☐	☐	0.98	1	421592	
☒	Compound3	C9H10O2N4S2		Positive		279	64.9	100	48	7	☐	☐	0.98	1	179952	
☒	Compound3	C9H10O2N4S2		Positive		279	155.6	100	12	7	☐	☐	0.98	1	1377529	
☒	Compound3	C9H10O2N4S2		Positive		279	107.7	100	24	7	☐	☐	0.98	1	2407737	
☒	Compound3	C9H10O2N4S2		Positive		279	92.1	100	24	7	☐	☐	0.98	1	1643700	
☒	Compound3	C9H10O2N4S2		Positive		279	79.8	100	48	7	☐	☐	0.98	1	1242301	
☒	Compound3	C9H10O2N4S2		Positive		279	123.9	100	24	7	☒	☐	0.98	1	263848	
☒	Compound4	C10H9O2N2SCl		Positive		271	155.9	100	6	7	☒	☒	0.37	1	557854	
☒	Compound4	C10H9O2N2SCl		Positive		271	177.8	100	12	7	☐	☐	0.37	1	85336	
☒	Compound4	C10H9O2N2SCl		Positive		271	115.9	100	16	7	☐	☐	0.37	1	515222	
☒	Compound4	C10H9O2N2SCl		Positive		271	107.9	100	24	7	☐	☐	0.37	1	318252	
☒	Compound4	C10H9O2N2SCl		Positive		271	92	100	28	7	☒	☐	0.37	1	5152001	
☒	Compound4	C10H9O2N2SCl		Positive		271	80	100	40	7	☐	☐	0.37	1	253002	

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	RT1
Compound1			Positive		311	155.7	100	10	☒	☒	2.03	1
Compound1			Positive		311	245.1	100		☐	☐	2.03	1
Compound1			Positive		311	218.1	100		☐	☐	2.03	1
Compound1			Positive		311	172.1	100		☐	☐	2.03	1
Compound1			Positive		311	107.1	100		☒	☐	2.03	1
Compound2			Positive		285	155.7	100	10	☒	☒	0.65	0.75
Compound2			Positive		285	92.1	100		☒	☐	0.65	0.75
Compound2			Positive		285	108.1	100		☐	☐	0.65	0.75
Compound2			Positive		285	80.1	100		☐	☐	0.65	0.75
Compound2			Positive		285	65.1	100		☐	☐	0.65	0.75
Compound3			Positive		279	185.7	100	10	☒	☒	0.98	0.75
Compound3			Positive		279	155.6	100		☐	☐	0.98	0.75
Compound3			Positive		279	107.7	100		☐	☐	0.98	0.75
Compound3			Positive		279	92.1	100		☐	☐	0.98	0.75
Compound4			Positive		271	155.8	100	10	☒	☒	0.37	0.75
Compound4			Positive		271	177.8	100		☐	☐	0.37	0.75
Compound4			Positive		271	115.9	100		☐	☐	0.37	0.75
Compound4			Positive		271	107.9	100		☐	☐	0.37	0.75
Compound4			Positive		271	92	100		☒	☐	0.37	0.75
Compound4			Positive		271	80	100		☐	☐	0.37	0.75
Compound4			Positive		271	64.9	100		☐	☐	0.37	0.75

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 In the Compound Setup tab in Optimizer, review the compounds.	The compounds in Optimizer are overwritten by the compounds that you updated in the Database Browser program.
--	---	---

Replace

Similar record for the compound 'Compound1' is already present in current project.

Do you want to replace it?

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

Table 5 Information for TMRM method for test compounds

Compound	Precursor	Product Ion	Primary	Trigger	Threshold	Collision Energy
Compound1	311	155.7	Yes	Yes	500	16
Compound1	311	107.1	Yes	No		28
Compound1	311	245.1	No	No		16
Compound1	311	218.1	No	No		16
Compound1	311	172.1	No	No		32
Compound1	311	140.3	No	No		40
Compound2	285	155.7	Yes	Yes	500	12
Compound2	285	92.1	Yes	No		24
Compound2	285	108.1	No	No		24
Compound2	285	80.1	No	No		60
Compound2	285	65.1	No	No		60
Compound3	279	185.7	Yes	Yes	500	12
Compound3	279	92.1	Yes	No		32
Compound3	279	155.6	No	No		16
Compound3	279	107.7	No	No		28
Compound4	271	155.8	Yes	Yes	500	10
Compound4	271	91.3	Yes	No		40
Compound4	271	177.8	No	No		10
Compound4	271	115	No	No		20
Compound4	271	80.1	No	No		40
Compound4	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 your own custom database or 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. Make sure that the collision energies in your database are optimized, and the Primary and Trigger transitions are marked.

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
1	In the Data Acquisition program, you now import the updated compounds from your custom database. a Switch to the Data Acquisition program. b Open the <i>iiiTestmix_dMRM2.m</i> method. c In the QQQ tab, click the Acquisition tab. The Scan segments table contains four rows which are deleted later. d Right-click the Scan Segments table and click Import from Database Browser. The Database Browser program opens. e Mark the Show All Records check box. f Mark all of the transitions for the four 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.

Acquisition | Source | Chromatogram | Instrument | Diagnostics

Scan segments

Compound Group	Compound Name	ISTD?	Precursor Ion	Product Ion	Primary	Trigger	Threshold	Ret Time (min)	Delta Ret Time	Fragmentor	Collision Energy	Cell Accelerator Voltage	Polarity
Compound1	Compound1	☐	311	155.7	☒	☒	163665	2.03	1	100	15	7	Positive
Compound1	Compound1	☐	311	107.1	☒	☐		2.03	1	100	24	7	Positive
Compound1	Compound1	☐	311	245.1	☐	☐				100	12	7	Positive
Compound1	Compound1	☐	311	245	☐	☐				100	12	7	Positive
Compound1	Compound1	☐	311	173.1	☐	☐				100	24	7	Positive
Compound2	Compound2	☐	285	156	☒	☒	30000	0.65	1	100	10	7	Positive
Compound2	Compound2	☐	285	92.1	☒	☐		0.65	1	100	24	7	Positive
Compound2	Compound2	☐	285	197	☐	☐				100	8	7	Positive
Compound2	Compound2	☐	285	108	☐	☐				100	20	7	Positive
Compound2	Compound2	☐	285	80	☐	☐				100	48	7	Positive
Compound2	Compound2	☐	285	65.1	☐	☐				100	48	7	Positive
Compound3	Compound3	☐	279	186	☒	☒	20000	0.98	1	100	12	7	Positive
Compound3	Compound3	☐	279	92.1	☒	☐		0.98	1	100	24	7	Positive
Compound3	Compound3	☐	279	155.9	☐	☐				100	12	7	Positive
Compound3	Compound3	☐	279	107.7	☐	☐				100	20	7	Positive

Dynamic MRM Parameters
Cycle Time 500 ms

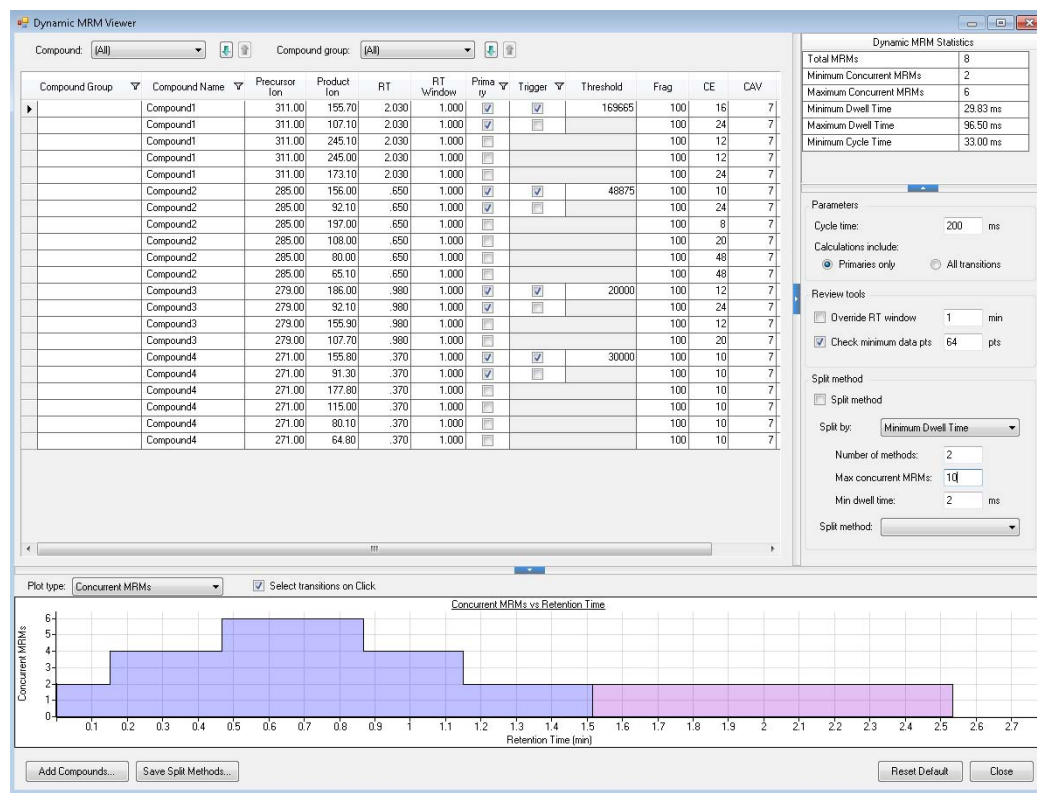
Triggered MRM
☒ Triggered Repeats 3

- | | | |
|---|---|---|
| 2 | Save the method to a new method name, <i>iiiTest_TriggerOpt.m</i> , where <i>iii</i> are your initials. | <p>a Click Method > Save Method.</p> <p>b Type <i>iiiTest_TriggerOpt.m</i>.</p> <p>c Click Save.</p> |
|---|---|---|

Exercise 3 – Create a Triggered Dynamic MRM acquisition method

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

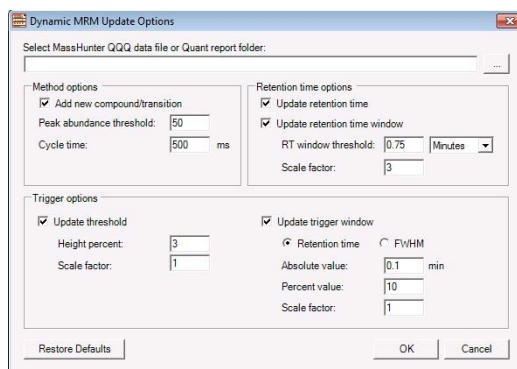
Steps	Detailed Instructions	Comments
3	Review the method in the Dynamic MRM Viewer dialog box. - Right-click the Scan segments table and click Edit DMRM Method. The Dynamic MRM Viewer dialog box is opened. - Type 200 for the Cycle time. This value is shown in the Acquisition tab. - Click between the Primaries only button and the All transitions button if the Dynamic MRM Statistics information is not updating. Then, click All transitions. - Click Close.	- The compounds in Optimizer were overwritten by the compounds that you updated in the Database Browser program. - You can change 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.	



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5

Exercise 4 – Optimize Acquisition parameters

Task 1. Use Optimizer to optimize acquisition parameters 65

Task 2. Use Source and iFunnel Optimizer to optimize acquisition parameters 72

For this exercise you optimize a mixture of 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 *iiiTestmix_MRM_10.m* in “**Task 5. Find optimum collision energy for MRM acquisition**” on page 36. You do not need to acquire the data file.

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

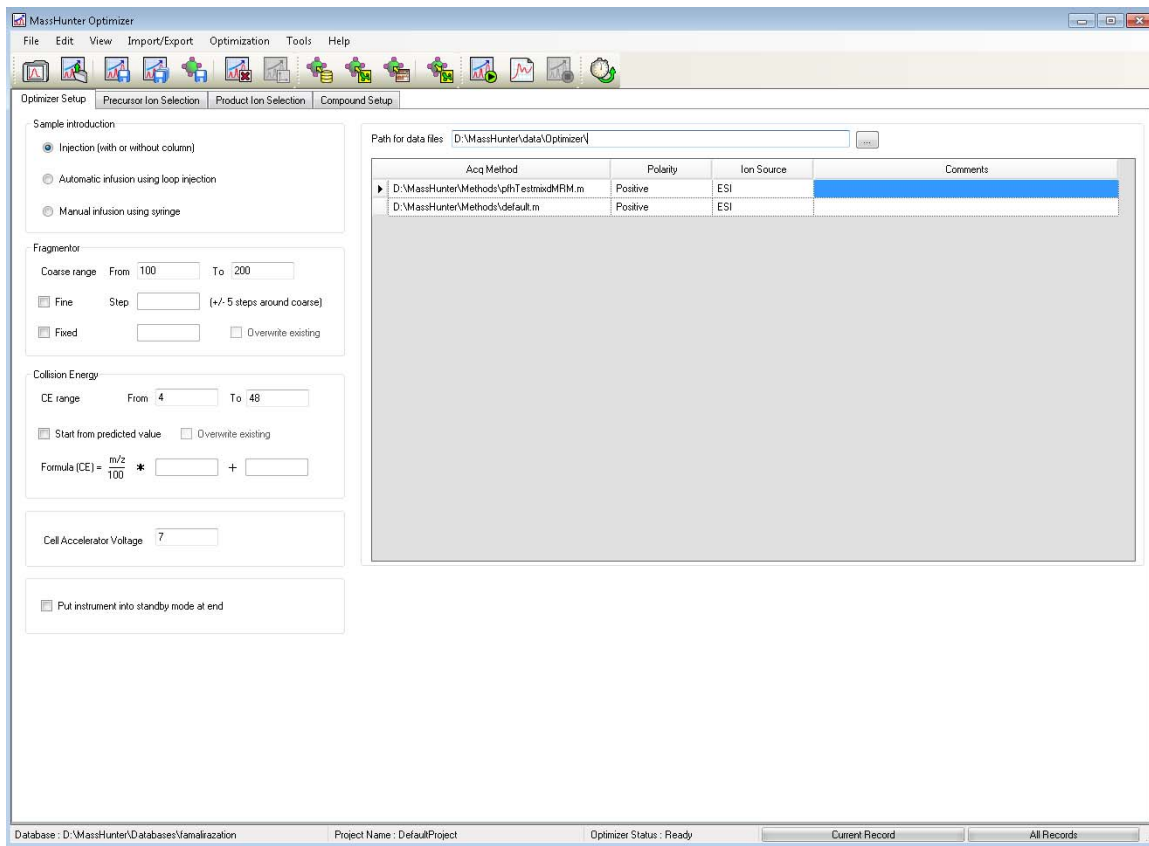
Steps

Detailed Instructions

Comments

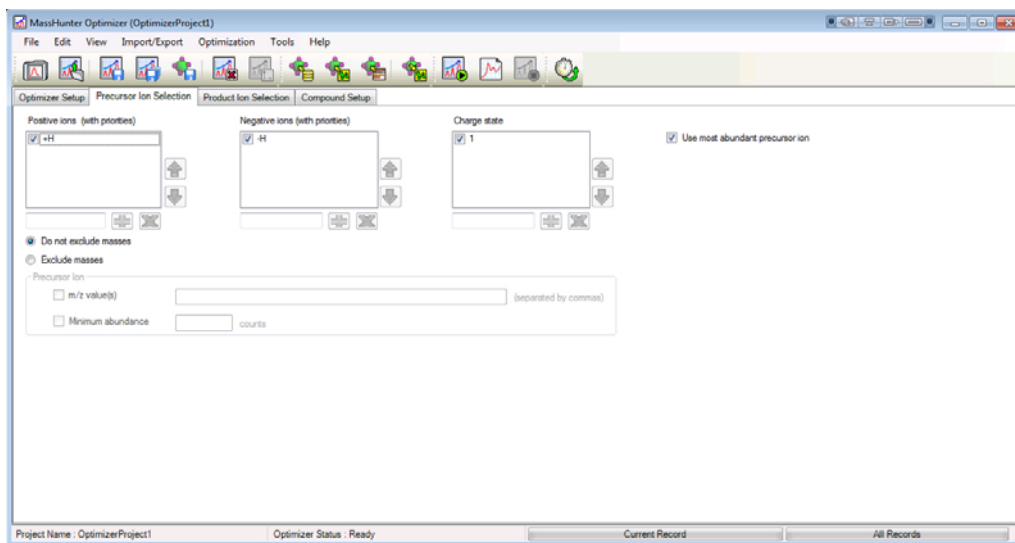
- 1 Start the MassHunter Optimizer program.

- Double-click the **Optimizer** icon. 

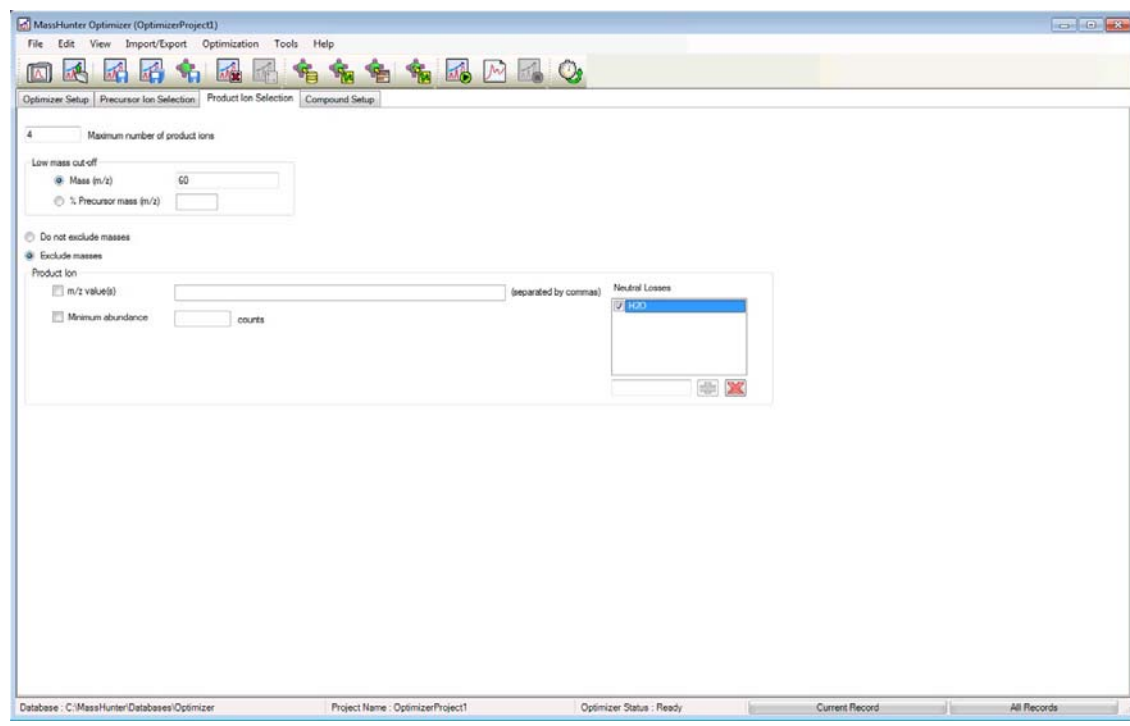


Steps	Detailed Instructions	Comments
2 Set the optimization parameters.	a Click the Optimizer Setup tab. b Set the Sample introduction method to Injection (with or without column). c Set the range for ramping the Collision Energy from 0 to 40 V. d Select a Path for data files to store the optimization 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 <i>iiiTestmix_MRM_10.m</i> 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 MassHunter Qualitative Analysis program.

Steps	Detailed Instructions	Comments
3 Select the precursor ions	a Click the Precursor Ion Selection tab. b Select the Positive ions +H adduct. c Select the Charge state of 1. d Set the search priority of the precursor ions. e (optional) To exclude certain masses from consideration, click Exclude masses at the bottom of the screen. Enter the m/z Values to exclude separated by commas and/or enter a Minimum abundance value in counts.	- Mark the Use most abundant precursor ion check box to use the most abundant precursor ion. - Clear the Use most abundant precursor ion check box and use the Up and Down arrow buttons to set the search order (ions at the top of the list are given more priority). - You can also enter Neutral Losses to exclude (for example H₂O).



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

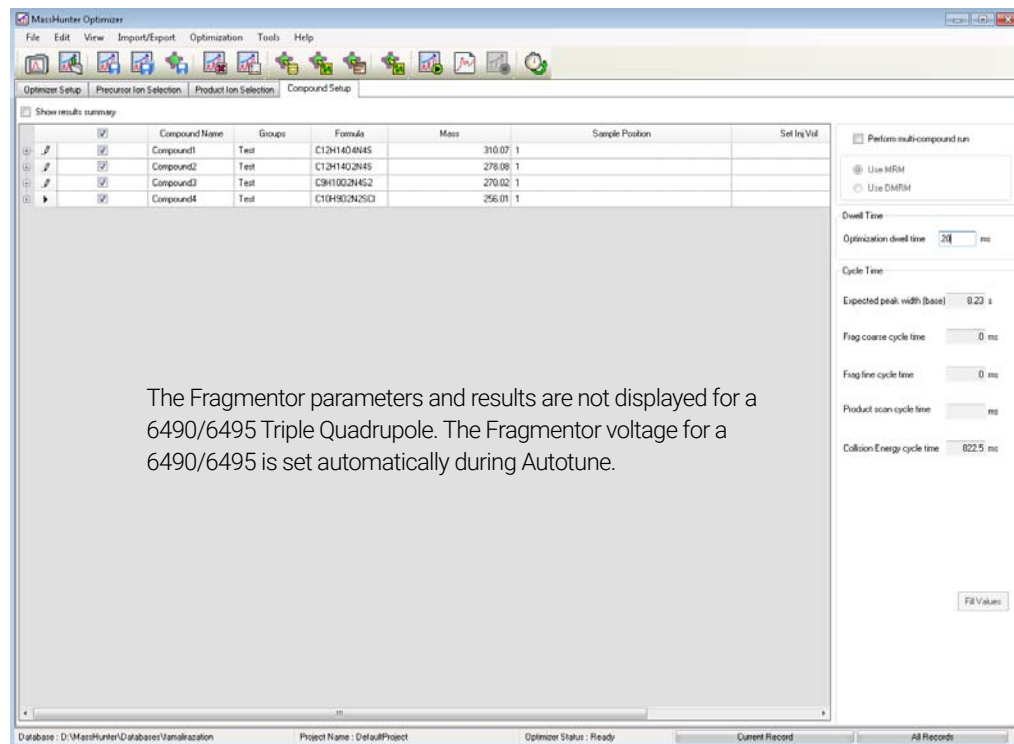


Steps	Detailed Instructions	Comments
5 Set up a compound list. The formula for the four compounds are: - Compound1 <chem>C12H14O4N4S</chem> - Compound2 <chem>C12H14O2N4S</chem> - Compound3 <chem>C9H10O2N4S2</chem> - Compound4 <chem>C10H9O2N4SCl</chem>	a Click the Compound Setup tab. b Clear the Show results summary check box above the table while you set up the compound list. c Right-click the table and select Add Compound from the shortcut menu to add a row to the end of the table. d Enter Compound1 as the Compound Name . e Enter Test as the group name in the Groups column. f Enter <chem>C12H14O4N4S</chem> as the Formula of the compound. The mass is calculated. g Enter the Sample Position for the new compound. h (optional) Enter an Optimization dwell time value to set longer or shorter cycle times. i Repeat the steps above to add the other three compounds to the table. j Mark the Select columns for the compounds (rows) to use for optimization. k Save the compound list to the database or to the current project.	- Compounds are global to all projects. Compound information such as name, group, formula, and mass in one project will be reflected in the entire database. - If no methods or ions are specified here, then optimization for the compound uses the methods from the Optimizer Setup tab and information from the Precursor Ion Selection and Product Ion Selection tabs to generate the ions. - You can also enter the monoisotopic mass in the Mass column instead of the Formula.

Steps

Detailed Instructions

Comments



6 Start the optimization process.

- Click **Start Optimization**  on the toolbar
- or
- Click **Ion Breakdown Profile**  on the toolbar.

7 Review results.

- Click the **Compound Setup** tab.
 - Mark the **Show results summary** check box above the table.
 - Review the following values for each transition ion in the Compound Table:
 - Fragmentor
 - Collision Energy
 - Review the printed optimization report.
- (optional) Use the MassHunter Workstation Qualitative Analysis program to look at the data.
 - See the [online Help](#) for Optimizer or the Optimizer *Quick Start Guide* to learn how to import optimization results to acquisition for MRM time segments.

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

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

To do this task, you first need to create the method *iiiTestmix_dMRM2.m* in “Task 4. Create a Dynamic MRM method from an MRM method” on page 45. 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 <i>iiiTestMix_dMRM2.m</i> method. Save this method to a new name.	- a Start the Data Acquisition program. Make sure Context is set to Acquisition. - b Load <i>iiiTestmix_dMRM2.m</i>. - c Save this method to the name <i>iiiTestmix_SourceOpt.m</i> - d 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.

Steps	Detailed Instructions	Comments
2 Edit the Source parameters.	- Click the QQQ tab. - Click the Source tab on the QQQ tab. - Change the parameters to the recommended starting parameters for the source optimization. These parameters are in the following image.	

Method Editor

Properties DA Sampler Sampler Pretreatment Binary Pump Column Comp. DAD **QQQ**

Tune file: atunes.tune.xml

Unit Only ☒ Browse ...

Ion source: AJS ESI

Stop time: ☒ No limit/As Pump

Time filtering: ☒ Peak width: 0.07 min

Time segments:

#	Start Time	Scan Type	Div Valve	Delta EMV (+)	Delta EMV (-)	Stored
1	0	Dynamic MRM	To MS	200	0	☒

cycles/s: ms/cycle:

Acquisition Source Chromatogram Instrument Diagnostics

Source parameters:

Gas Temp: 150 °C 150 °C

Gas Flow: 14 L/min 14.0 L/min

Nebulizer: 20 psi 20.0 psi

Sheath Gas Temp: 350 °C 350 °C

Sheath Gas Flow: 11 L/min 11.0 L/min

Capillary: Positive 3000 V Negative 3000 V 8792 nA

Nozzle Voltage: 300 V 300 V

Chamber Current: 0.37 µA

iFunnel parameters:

High Pressure RF: Positive 150 V Negative 90 V

Low Pressure RF: 60 V 40 V

Copy Paste

Paste to All Segments

3 Create template method.	- Click the Acquisition tab. - Select a single ion for each compound for the optimization. - Save the method	A transition for each compound is already included in the Dynamic MRM method.
---------------------------	---	---

Method Editor

Properties DA Sampler Sampler Pretreatment Binary Pump Column Comp. DAD **Acquisition** Source Chromatogram Instrument Diagnostics

Tune file: atunes.TUNE.XML

Unit Only ☒ Browse ...

Ion source: ESI

Stop time: ☒ No limit/As Pump

Time filtering: ☒ Peak width: 0.03 min

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:

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
Compound1		☐	311 Unit		155.7 Unit		2.03	0.75	100	10	7	Positive
Compound2		☐	285 Unit		155.7 Unit		0.85	0.75	100	10	7	Positive
Compound3		☐	279 Unit		165.7 Unit		0.90	0.75	100	10	7	Positive
Compound4		☐	271 Unit		155.8 Unit		0.37	0.75	100	10	7	Positive

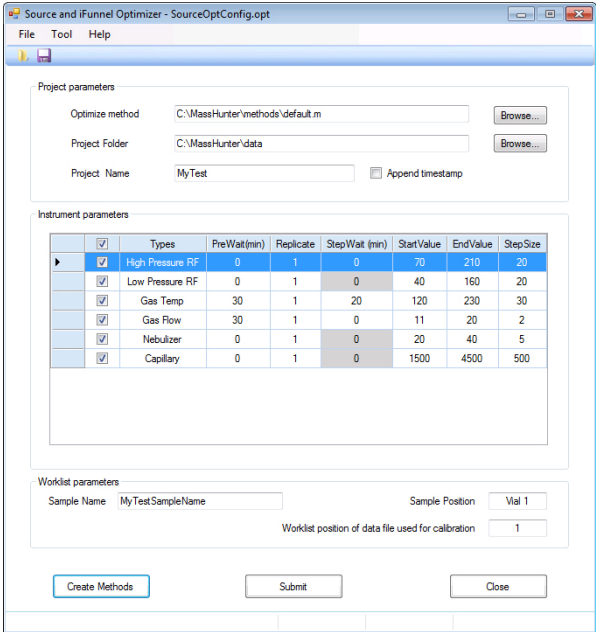
Dynamic MRM Parameters:

Cycle Time: 500 ms

Triggered MRM:

☐ Triggered Repeats: 3

Steps	Detailed Instructions	Comments
4	Start the Source and iFunnel Optimizer program. Double-click the Source Optimizer icon .	



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.

	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
☒	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

Steps	Detailed Instructions	Comments
5 Select the template method, iiiTestmix_SourceOpt.m	a Click Browse. The Browse For Folder dialog box is opened. b Select the iiiTestmix_SourceOpt.m method. Click OK. c If the ion source in the method different than the ion source in the Instrument parameters list, a warning message is opened. Click OK.	

Source and iFunnel Optimizer - SourceOptConfig.opt

File Tool Help

Project parameters

Optimize method: C:\MassHunter\Methods\gfmTest mix_SourceOpt.m **Browse...**

Project Folder: C:\MassHunter\data **Browse...**

Project Name: MyTest ☐ Append timestamp

Instrument parameters

	Type	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

Worklist parameters

Sample Name: MyTestSampleName Sample Position: Vial 1

Worklist position of data file used for calibration: 1

Create Methods **Submit** **Close**

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 included only for 6490 or a 6495 models.

Steps	Detailed Instructions	Comments
6 Change the order of the rows in the Instrument parameters table to the following: • Ion Funnel parameters • Sheath Gas temperature and flow • Gas temperature and flow • Nebulizer • Capillary • Nozzle Voltage	a If necessary, select the row that shows the High Pressure RF parameter. b Drag this row to the top of the table. Both of the Ion Funnel parameters are moved together. c Verify that the order of the rows in your table is as indicated.	• The order of the parameters in the Instrument parameters table is the order that the parameters are optimized. You want to optimize the parameters that have the greatest effect on the source optimization first. The Ion Funnel parameters have the greatest effect, so you move those parameters to the top of the list. • By default, the parameters are in the optimized list.

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.
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Steps	Detailed Instructions	Comments
8 Save the Instrument parameters.	- Click File > Save As (*.opt). - Enter the name for this set of instrument parameters. - Click OK.	
9 Change the Instrument parameters table to only change one parameter for this task. This task only optimizes the Capillary voltage.	- Mark the check box next to the Capillary. - Clear the check boxes next to all of the other parameters.	For this example, you optimize the Capillary voltage. Usually, you optimize the parameters in the order specified in the Instrument Parameters table.

Instrument parameters

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

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

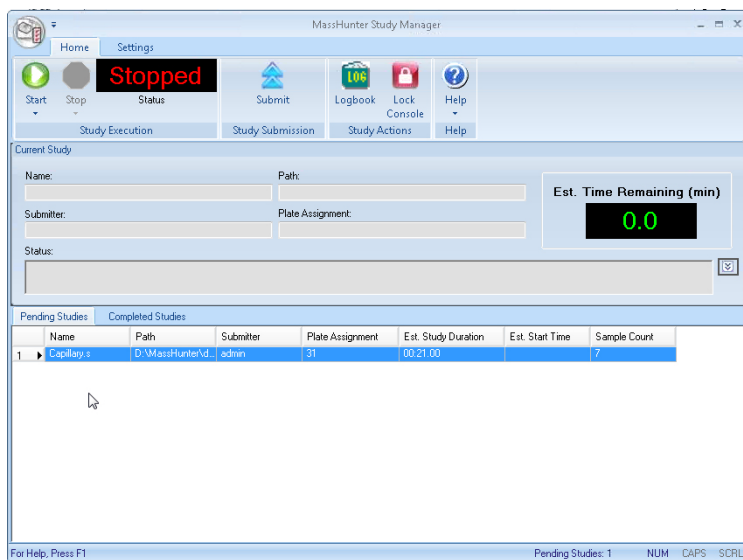
10 Set the Project Folder and the Project Name .	- Select the Project Folder. In this example, select \\MassHunter\\Data - Enter a Project Name. (optional) Mark the Append timestamp check box.	If you mark the Append timestamp check box, then a time stamp is automatically added to the Project Name when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

Project parameters

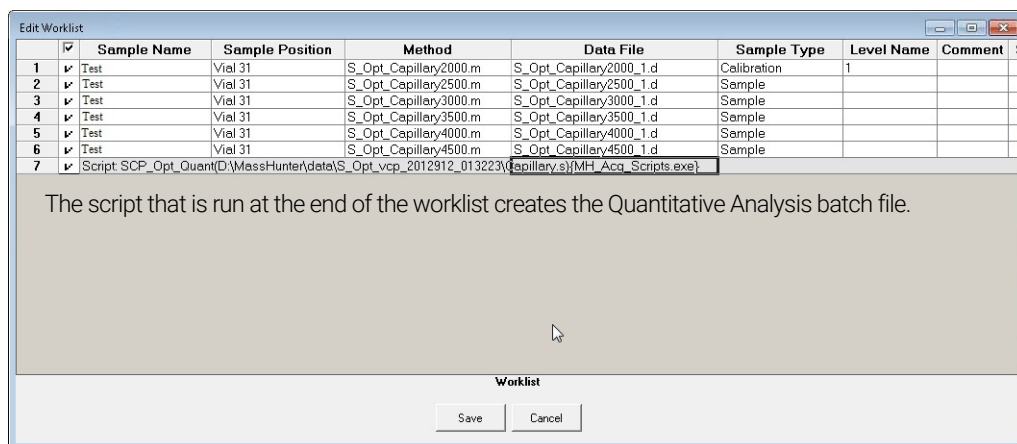
Optimize method	C:\MassHunter\Methods\pftTest mix_SourceOpt.m	Browse...
Project Folder	C:\MassHunter\data	Browse...
Project Name	MyTest	☒ Append timestamp

Steps	Detailed Instructions	Comments
11 Set the Worklist parameters.	- a Type the Sample Name. - b Type the Sample Position. - c Type the Worklist position of data file used for calibration.	• If you mark the Append timestamp check box, then a time stamp is automatically added to the Project Name when you click Submit. • For each parameter that is optimized, a batch file is created for Quantitative Analysis. One of the injections is considered 100% of the starting value. The value of Worklist position of data file used for calibration states which data file to use. If you enter 1, then the data file from the first row is used.
Worklist parameters Sample Name Test Sample Position 31 Worklist position of data file used for calibration 1		
12 Create the methods and submit the study to the Study Manager.	- a Click Create Methods. - b Click Submit.	• When you click Create Methods, a message at the bottom of the main window states how many Methods were created, how many Injections are involved, and the Estimated time. The Estimated time is only an approximation.
Project created: D:\MassHunter\data\S_Opt_vcp_2012912_I 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.		

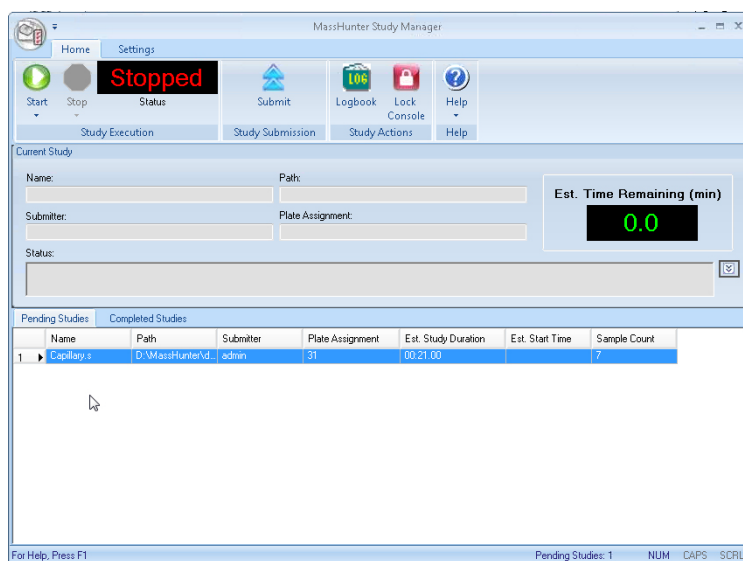
Steps	Detailed Instructions	Comments
13 Review the study (or studies) submitted to Study Manager.	- Open the Study Manager program. - Select a row in the Pending Studies table. - Right-click the row and click Edit Worklist From Study. - Review the worklist in the Edit Worklist dialog box. Click Save.	- A study is submitted for each parameter that you marked in the Instrument parameters table. - Only one study is created for the High Pressure RF and Low Pressure RF parameters.



The name of the study is the Instrument parameter that is being optimized. A separate study is added for each parameter that is being optimized. You can examine or edit the worklist for the study. You right-click the line in the Pending Studies table and click Edit Worklist from Study.



Steps	Detailed Instructions	Comments
14 Change the Study Manager parameters to run a standby script when the study completes and then start the Study Manager.	a Click the Settings tab in the Ribbon. b Mark the Enable standby script execution on idle or error check box. c Click the "... " button to select the script to run. d Select SCP_InstrumentStandby and click OK. e Enter 1 for the Wait for time. f Click Start if necessary.	When the Study Manager is not running a study for the time specified, the script you select is run.

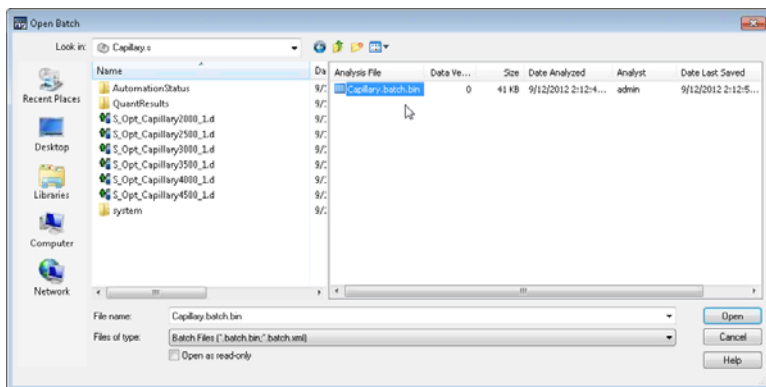


The name of the study is the Instrument parameter that is being optimized. A separate study is added for each parameter that is being optimized.

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

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.
--	--

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 Capillary.batch.bin and click Open.	



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

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

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

17 Review the Batch Table.

- 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 online Help for the Quantitative Analysis program to learn how to do these tasks.
 - In this case, all four compounds optimize at the same setting. Often, different compounds have different optimal settings, and you have to compromise.

Batch Table																						
Sampler: sulfis		Sample Type: <All>		Compound: sulfamethazine		ISTD:																
Sample					sulfamethazole Results				sulfachloropyridazine Results				sulfamethazine Results				sulfadimethoxine Results					
①	▼	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
		sulfis	S_Opt_Capillary2000_1.d	Cal	1	9/12/2012 1:36 PM	0.365	100.0000	100.0	2355920	0.649	100.0000	100.0	1745.206	0.977	100.0000	100.0	5145261	2.025	100.0000	100.0	3693772
	▼	sulfis	S_Opt_Capillary2500_1.d	Sample		9/12/2012 1:42 PM	0.365	82.9676		1952236	0.646	85.3296		1485063	0.970	73.9517		4538509	2.021	99.2632		3687022
	▼	sulfis	S_Opt_Capillary3000_1.d	Sample		9/12/2012 1:48 PM	0.365	81.0429		1908346	0.646	82.1921		1434503	0.977	69.8619		4793407	2.026	97.4503		3656568
	▼	sulfis	S_Opt_Capillary3500_1.d	Sample		9/12/2012 1:54 PM	0.365	73.6758		1735779	0.649	73.5906		1204520	0.977	62.5007		3045934	2.025	88.9432		3201004
	▼	sulfis	S_Opt_Capillary4000_1.d	Sample		9/12/2012 2:00 PM	0.365	72.4241		1706291	0.646	71.1249		1241346	0.974	61.3445		3769965	2.025	87.5352		3223895
	▼	sulfis	S_Opt_Capillary4500_1.d	Sample		9/12/2012 2:07 PM	0.365	69.3667		1634258	0.649	67.9868		1186792	0.977	58.4264		3908633	2.026	83.3787		3076480

Steps	Detailed Instructions	Comments
18 (optional) Review the data files in the Qualitative Analysis program.	a Start the Qualitative Analysis program. b Open all of the data files in the study. c Click Compounds View on the toolbar. d Select all of the data files in the Sample Table window. e Click Find > Find by MRM. f Select all of the data files and click Find. g Click  on the Compound List toolbar to remove empty columns from the Compound List window. h Click the Label header in the Compound List window to sort the table by the Label. i Examine the results in the Compound List window. Compare the Area from each file for each compound. j Close the Qualitative Analysis program.	- By default, the program selects different colors for different transitions. - Three out of four compounds have the highest area count when the capillary voltage was set to 3000. - Compound 1 - 47385 for capillary voltage 2000 - Compound 2 - 63099 for capillary voltage 3000 - Compound 3 - 23286 for capillary voltage 3000 - Compound 4 - 28320 for capillary voltage 3000

Compound List: 28 found											
General											
Show/Hide	Label	RT	Width	Height	Area	Score	Mining Algorithm	ID Source	CE	File	m/z
☒	Cpd 1: Compound3 1.236	1.236	0.293	9081	38101	100	Find by MRM	Acq	15	iiiTestmix_SourceOpt_Capillary1500_1.d	279
☒	Cpd 1: Compound3 0.813	0.813	0.422	8919	47385	100	Find by MRM	Acq	15	iiiTestmix_SourceOpt_Capillary2000_1.d	279
☒	Cpd 1: Compound3 0.799	0.799	0.432	9160	46590	100	Find by MRM	Acq	15	iiiTestmix_SourceOpt_Capillary2500_1.d	279
☒	Cpd 1: Compound3 0.813	0.813	0.375	9042	45956	100	Find by MRM	Acq	15	iiiTestmix_SourceOpt_Capillary3000_1.d	279
☒	Cpd 1: Compound3 0.813	0.813	0.451	8384	45422	100	Find by MRM	Acq	15	iiiTestmix_SourceOpt_Capillary3500_1.d	279
☒	Cpd 1: Compound3 0.813	0.813	0.268	7513	39464	100	Find by MRM	Acq	15	iiiTestmix_SourceOpt_Capillary4000_1.d	279
☒	Cpd 1: Compound3 0.813	0.813	0.366	6947	36200	100	Find by MRM	Acq	15	iiiTestmix_SourceOpt_Capillary4500_1.d	279
☒	Cpd 2: Compound4 1.526	1.526	0.363	125936	53708	100	Find by MRM	Acq	10	iiiTestmix_SourceOpt_Capillary1500_1.d	271
☒	Cpd 2: Compound4 0.980	0.98	0.492	101592	62575	100	Find by MRM	Acq	10	iiiTestmix_SourceOpt_Capillary2000_1.d	271
☒	Cpd 2: Compound4 0.953	0.953	0.492	107533	62804	100	Find by MRM	Acq	10	iiiTestmix_SourceOpt_Capillary2500_1.d	271
☒	Cpd 2: Compound4 0.966	0.966	0.477	107116	63099	100	Find by MRM	Acq	10	iiiTestmix_SourceOpt_Capillary3000_1.d	271
☒	Cpd 2: Compound4 0.953	0.953	0.546	99899	61210	100	Find by MRM	Acq	10	iiiTestmix_SourceOpt_Capillary3500_1.d	271
☒	Cpd 2: Compound4 0.966	0.966	0.546	90587	55342	100	Find by MRM	Acq	10	iiiTestmix_SourceOpt_Capillary4000_1.d	271
☒	Cpd 2: Compound4 0.966	0.966	0.461	85705	50448	100	Find by MRM	Acq	10	iiiTestmix_SourceOpt_Capillary4500_1.d	271
☒	Cpd 3: Compound2 2.148	2.148	0.341	481014	17574	100	Find by MRM	Acq	10	iiiTestmix_SourceOpt_Capillary1500_1.d	285
☒	Cpd 3: Compound2 1.957	1.957	0.382	506372	23133	100	Find by MRM	Acq	10	iiiTestmix_SourceOpt_Capillary2000_1.d	285
☒	Cpd 3: Compound2 1.943	1.943	0.382	504904	23222	100	Find by MRM	Acq	10	iiiTestmix_SourceOpt_Capillary2500_1.d	285
☒	Cpd 3: Compound2 1.957	1.957	0.396	503263	23286	100	Find by MRM	Acq	10	iiiTestmix_SourceOpt_Capillary3000_1.d	285
☒	Cpd 3: Compound2 1.943	1.943	0.382	483978	22306	100	Find by MRM	Acq	10	iiiTestmix_SourceOpt_Capillary3500_1.d	285
☒	Cpd 3: Compound2 1.957	1.957	0.382	442968	20296	100	Find by MRM	Acq	10	iiiTestmix_SourceOpt_Capillary4000_1.d	285
☒	Cpd 3: Compound2 1.957	1.957	0.382	418712	18903	100	Find by MRM	Acq	10	iiiTestmix_SourceOpt_Capillary4500_1.d	285
☒	Cpd 4: Compound1 2.664	2.664	0.244	40104	14718	100	Find by MRM	Acq	15	iiiTestmix_SourceOpt_Capillary1500_1.d	311
☒	Cpd 4: Compound1 2.636	2.636	0.246	76720	27595	100	Find by MRM	Acq	15	iiiTestmix_SourceOpt_Capillary2000_1.d	311
☒	Cpd 4: Compound1 2.636	2.636	0.26	75912	27360	100	Find by MRM	Acq	15	iiiTestmix_SourceOpt_Capillary2500_1.d	311
☒	Cpd 4: Compound1 2.636	2.636	0.246	78261	28320	100	Find by MRM	Acq	15	iiiTestmix_SourceOpt_Capillary3000_1.d	311
☒	Cpd 4: Compound1 2.623	2.623	0.341	72981	26675	100	Find by MRM	Acq	15	iiiTestmix_SourceOpt_Capillary3500_1.d	311
☒	Cpd 4: Compound1 2.636	2.636	0.252	67524	24226	100	Find by MRM	Acq	15	iiiTestmix_SourceOpt_Capillary4000_1.d	311
☒	Cpd 4: Compound1 2.636	2.636	0.222	62629	22592	100	Find by MRM	Acq	15	iiiTestmix_SourceOpt_Capillary4500_1.d	311

In This Book

The exercises in this guide help you learn to use the Agilent MassHunter Data Acquisition program with your Agilent 6400 Series Triple Quad LC/MS. In this guide, you learn how to develop an acquisition method.

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

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