

Agilent MassHunter Workstation Software – Data Acquisition for 7000A Triple Quad GC/MS

Familiarization Guide

Before you begin 3

Prepare your system 3

Prepare the samples required for data acquisition 3

Exercise – Develop an acquisition method for the 7000A 5

Task 1. Set the inlet and injection parameters 5

Task 2. Enter GC acquisition parameters 7

Task 3. Create acquisition method for finding precursor ions 11

Task 4. Acquire precursor ion scan data (Optional) 13

Task 5. Determine precursor ion masses 15

Task 6. Create acquisition methods for finding product ion masses 18

Task 7. Acquire scan data for finding product ions (Optional) 21

Task 8. Determine the product ions 23

Task 9. Create an MRM method 26

Exercise – Create a quantitative analysis method 28

Task 10. Acquire MRM data (Optional) 28

Task 11. Create a quantitative analysis batch 31

Task 12. Create an MRM quantitative analysis method 33

Task 13. Quantitate a batch of unknown samples 42

This guide shows how to use the Agilent 7000A Triple Quad GC/MS to acquire and analyze sample data. If you want to skip the data acquisition steps in this guide, use the demo data files located in the Benzodiazepine directory shipped with the system (in the **Benzodiazepine\Data** folder of your Data Acquisition installation disk).



Agilent Technologies

In this guide, you learn how to determine the best acquisition settings for analyzing your compounds of interest. These instructions help you understand not only how to set up a method to optimize instrument parameters for best sensitivity in acquisition, but also how to use the 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* and the Quantitative Analysis program by using the *Quantitative Analysis Familiarization Guide*.

See the *Concepts Guide* to learn more about how the triple quadrupole mass spectrometer works and why the collision energy voltage is important. For background information, see Chapter 3, “Agilent Triple Quad MS and Sensitivity”, in the *Concepts Guide*. See the online Help for detailed information on how the program works.

Each task is presented in a table with three columns:

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

Before you begin

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

Prepare your system

- 1 Check that:
 - The Data Acquisition, Qualitative Analysis, and Quantitative Analysis programs are installed.
 - Your system uses an Agilent 7890A GC with split/splitless inlet and automatic liquid sampler.
 - The 7000A Triple Quad GC/MS is configured.
 - The performance is verified.
 - The system is turned on.
 - The Agilent HP-5MS 5% Phenyl Methyl Siloxane: 30 m x 250 μ m x 0.25 μ m column is installed.
- 2 Configure the GC for the Agilent HP-5MS column.
- 3 Copy the data files to your PC.
- 4 Copy the data files in the **Benzodiazepine\Data** folder on your Data Acquisition installation disk to any location on your hard disk. This folder contains the data files needed for this exercise.

Do not re-use the Benzodiazepine 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. Do not use these sample data files to look at sample information or print a report.

Prepare the samples required for data acquisition

If you do not intend to acquire data but want to learn how to use the Qualitative and Quantitative Analysis programs for method development, you can skip this section. You can learn how to use the Qualitative and Quantitative Analysis programs with the Benzodiazepine data files shipped with the system.

Materials required for sample preparation:

- A 1-mL ampoule of a Benzodiazepine mix sample, Agilent part number B-033.
- Acetonitrile for sample dilution
- Sample vials

Before you begin

Prepare the samples required for data acquisition

- 1** Prepare the Qualitative Analysis samples.
 - a** Add a quantity from the Benzodiazepine ampoule to acetonitrile in a glass vial or tube and dilute twice so that the final concentration is 10 ng/ μ L.
 - b** Prepare 10 ALS sample vials using the solution obtained above.
- 2** Prepare the Quantitative Analysis calibration samples.
 - a** Add a quantity from the Benzodiazepine ampoule to acetonitrile in an Eppendorf vial and dilute to final concentrations of 1, 0.5, 0.25, 0.125, and 0.0625 ng/ μ L.
 - b** Prepare 5 ALS sample vials for the five concentration level solutions obtained above. Label the vials BenzoL1 through BenzoL5.
- 3** Prepare the Quantitative Analysis unknown samples.
 - a** Add a quantity from the Benzodiazepine ampoule to acetonitrile in a glass vial or tube and dilute to final concentrations of 0.4, 0.2, and 0.1 ng/ μ L.
 - b** Prepare 3 ALS sample vials for the solutions obtained above. Label the vials BenzoSample01 through BenzoSample03.

Before proceeding with this exercise, autotune the instrument. See the *Operators Manual* or online Help for instructions on tuning the instrument.

Exercise – Develop an acquisition method for the 7000A

Task 1. Set the inlet and injection parameters

Steps	Detailed instructions	Comments
1 Set up the inlet, injection source, and enable the 7000A.	<ol style="list-style-type: none"> Double-click the Data Acquisition icon on the windows desktop. Click the Inlet and Injection Parameters icon. Select GC for the sample inlet and the installed ALS for the injection source. Select the Use MS check box. 	<ul style="list-style-type: none"> The Data Acquisition window shown in Figure 1 is displayed. The Inlet and Injection Parameters dialog box shown in Figure 2 is displayed.

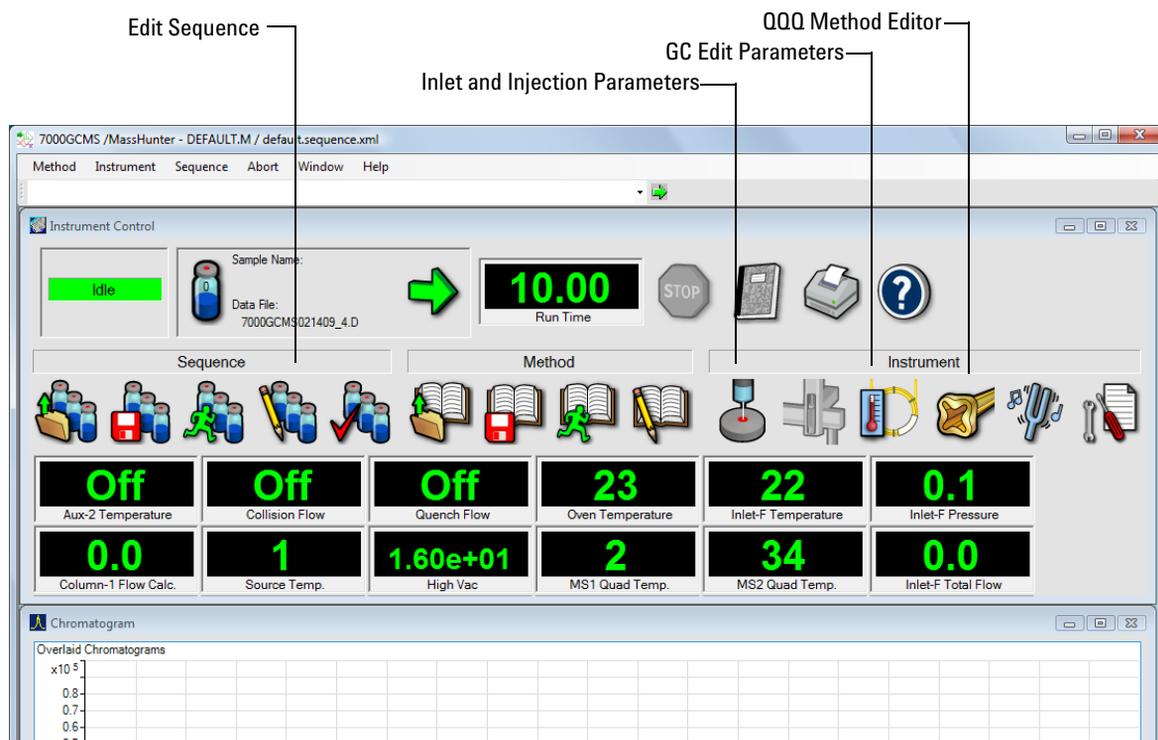


Figure 1 Agilent MassHunter Workstation Software – Data Acquisition window

Exercise – Develop an acquisition method for the 7000A

Task 1. Set the inlet and injection parameters

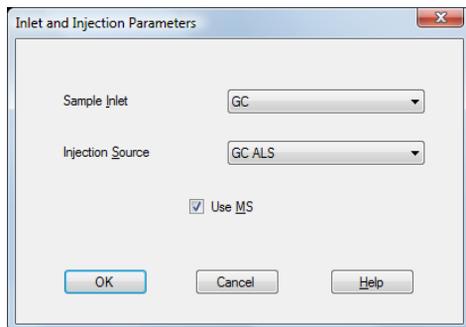


Figure 2 Inlet and Injection Parameters

Task 2. Enter GC acquisition parameters

In this exercise, you enter the GC conditions for the analysis.

Steps	Detailed instructions	Comments
2 Enter GC parameters appropriate for the Benzodiazepine mix. See Table 1.	<p>a Click the GC Edit Parameters icon (Figure 1).</p> <p>b Select the CFT Settings icon then select column 1 in the Description column.</p> <p>c Select control mode On and then select Flow mode. Enter 1.2 mL/min for the initial Value and the Post Run value.</p> <p>d Select the collision cell N2 EPC module in the Description column and clear control On.</p> <p>e Select the collision cell He EPC module in the Description column and clear control On.</p> <p>f Select the Inlets icon then the SSL tab and enter the inlet parameters listed in Table 1.</p> <p>g Select the Oven icon and enter the oven parameters listed in Table 1.</p> <p>h Select the ALS icon then the Front Injector tab and enter the injector parameters listed in Table 1.</p> <p>i Select the Aux Heaters icon, enable, and set the temperature to 134 °C.</p> <p>j Select the OK button.</p>	<ul style="list-style-type: none"> The GC edit parameters window shown in Figure 3 on page 8 is displayed. With the window selected, mouse over the icons to identify the icon from the tool tip. The collision cell gas flow is off for this method. If your ALS is attached to the Back Inlet select the Back Injector tab. The GC parameters are downloaded to the GC and the window closes.

Exercise – Develop an acquisition method for the 7000A

Task 2. Enter GC acquisition parameters

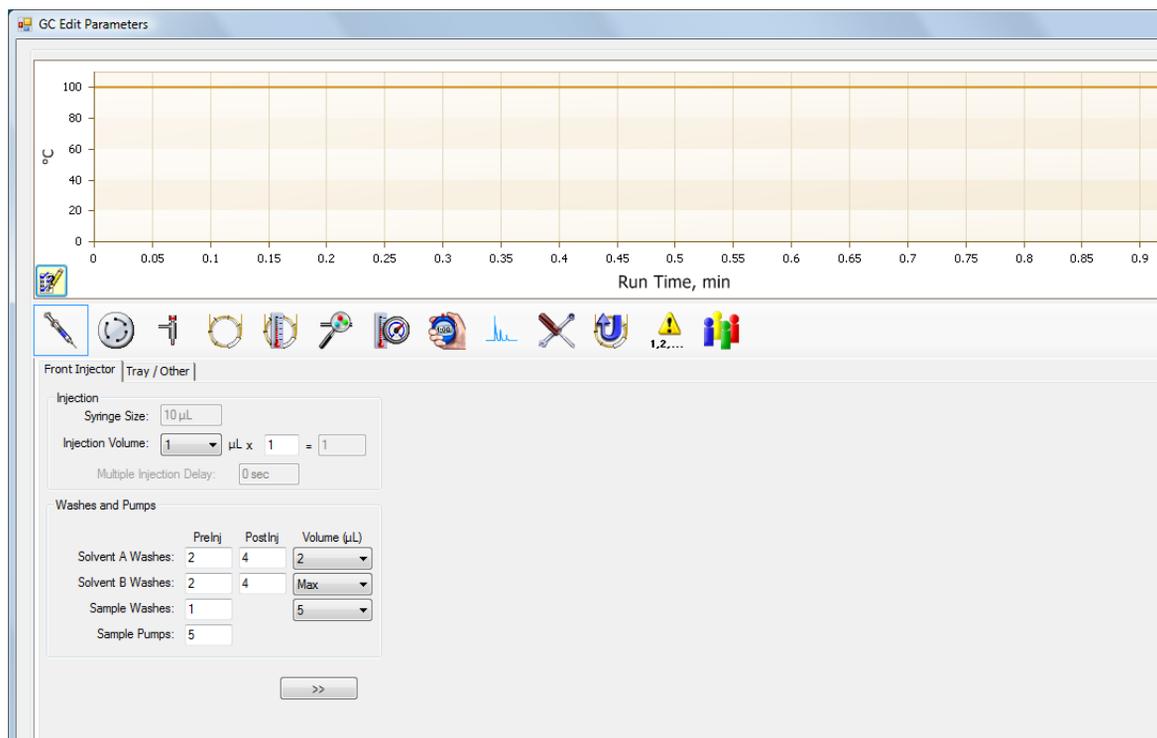


Figure 3 GC Edit Parameters window

Table 1 GC parameters for data acquisition method

Parameter	Value
Oven	
Equilibration Time	0.5 min
Oven Program	100 °C for 1 min, 25 °C/min to 300 °C, hold for 10 min
Run Time	19 min
Front SS Inlet	He
Mode	Splitless
Heater	On 300 °C

Exercise – Develop an acquisition method for the 7000A

Task 2. Enter GC acquisition parameters

Parameter	Value
Pressure	On Value automatically set with CFT Setting column flow
Septum Purge Flow	On 3 mL/min
Gas Saver	On 20 mL/min after 3 min
Purge Flow to Split Vent	50 mL/min at 1 min
Thermal Aux 2 {MSD Transfer Line}	
Heater	On
Temperature	134 °C
Column # 1	HP-5MS 5% Phenyl Methyl Siloxane: 30 m x 250 µm x 0.25 µm
In	Front SS Inlet He
Out	Vacuum
(Initial)	100 °C
Flow	1.2 mL/min
Flow Program	Off
Front Injector	
Syringe Size	10 µL
Injection Volume	1 µL
Solvent A Washes (PreInj)	2
Solvent A Washes (PostInj)	4
Solvent A Volume	2 µL
Solvent B Washes (PreInj)	2
Solvent B Washes (PostInj)	4
Solvent B Volume	Max
Sample Washes	1
Sample Wash Volume	5 µL
Sample Pumps	5
Dwell Time (PreInj)	0 min

Exercise – Develop an acquisition method for the 7000A

Task 2. Enter GC acquisition parameters

Parameter	Value
Dwell Time (PostInj)	0 min
Solvent Wash Draw Speed	300 µL/min
Solvent Wash Dispense Speed	6000 µL/min
Sample Wash Draw Speed	300 µL/min
Sample Wash Dispense Speed	6000 µL/min
Injection Dispense Speed	6000 µL/min
Viscosity Delay	7 sec
Sample Depth	Disabled
Collision cell EPC Module	
Nitrogen	Off
Helium	Off

Task 3. Create acquisition method for finding precursor ions

In this exercise, you start with the GC parameters entered to the method in Task 2, then enter the 7000A parameters for scanning the precursor ions and save to the method.

Steps	Detailed instructions	Comments
<p>3 Enter MS parameters appropriate for the Benzodiazepine mix and save the method as iiipaul1_MS_Scan.M, where iii are your initials.</p>	<p>a Click the QQQ Method Editor icon (Figure 1).</p> <p>b Set the Source temperature to 250 °C, set the Electron energy to Tune Setting, the Solvent delay to 4 minutes, and the Detector setting to Delta EMV.</p> <p>c Set the Time Filtering to a Peak width of 0.7 seconds.</p> <p>d In the Time segment section, enter a start Time of 3.0 and select a Scan Type of MS1Scan from the drop-down list. Enter 0 for Delta EMV and select Data stored.</p> <p>e In the Scan segments section, enter Scan for the Segment name, 50 for the Start mass, 450 for the End Mass, and 500 for the Scan time.</p> <p>f In the Scan parameters section, enter a Step size of 0.1 amu and a Threshold of 100.</p> <p>g Click OK to close the window.</p> <p>h From the main window select Method > Save Method As and save the method as iiipaul1_MS_Scan.M, where iii are your initials.</p>	<ul style="list-style-type: none"> • The QQQ Method Editor window shown in Figure 4 opens. • The 7000A starts collecting data at 4 minutes due to the Solvent delay setting.

Exercise – Develop an acquisition method for the 7000A

Task 3. Create acquisition method for finding precursor ions

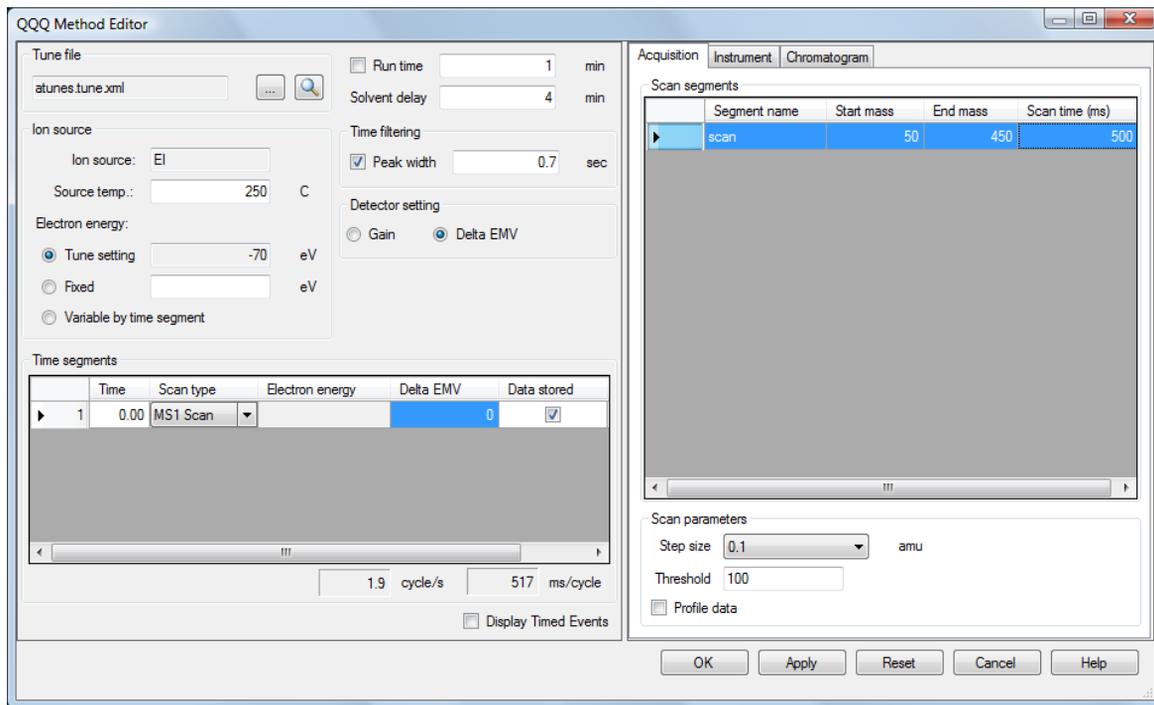


Figure 4 QQQ Method Editor

Task 4. Acquire precursor ion scan data (Optional)

In this task, you acquire the scan data using the method developed in the previous tasks. This task is optional because you can perform the next task with an example data file that comes with the program. If you prefer, you can create your own data file as described in this task.

Steps	Detailed instructions	Comments
<p>4 Acquire data (optional).</p> <ul style="list-style-type: none"> Name the data file iiifirst_trial3_scan.D, where iii are your initials. Designate a directory path to hold your data files and method. 	<p>a Click the Start Run (green arrow) icon.</p> <p>b In the Data Path enter the directory to save the data file that is acquired by this run.</p> <p>c In the Front Inlet section, enter iiifirst_trial3_scan.D for the Data File Name, where iii are your initials.</p> <p>d Enter the Vial location number in the auto sampler tray.</p> <p>e In the Method Sections to Run section, select Data Acquisition.</p> <p>f Click the OK and Run Method button.</p>	<ul style="list-style-type: none"> The Start Run dialog box shown in Figure 5 is displayed. The method is sent to the GC and the 7000A. When the instruments are ready the sample is injected and the data is collected and sent to the data directory specified.

Exercise – Develop an acquisition method for the 7000A

Task 4. Acquire precursor ion scan data (Optional)

The screenshot shows the 'Start Run' dialog box with the 'Advanced' tab selected. The 'Current Method Injection Style' is set to 'GC ALS'. Under 'Inlet Location', 'Front' is selected. 'MS Connected to:' is set to 'Front Inlet'. The 'Operator Name' is 'PaulZ' and the 'Data Path' is 'C:\MassHunter\GCMS\3\DATA\'. There are two columns for 'Front Inlet' and 'Rear Inlet' configurations. The 'Front Inlet' column has 'Data File Name' as 'first_trial3_scan.D', 'Sample Name' as an empty field, 'Misc. Info.' as an empty field, 'Expected Barcode' as an empty field, 'Sample Amount' as '0', and 'Multiplier' as '1'. The 'Rear Inlet' column has 'Data File Name' as '7000GCMS021409_4.D', 'Sample Name' as an empty field, 'Misc. Info.' as an empty field, 'Expected Barcode' as an empty field, 'Sample Amount' as '0', and 'Multiplier' as '1'. Both columns have 'Vial Number' as an empty field and 'Tray Name' as 'Agilent ALS'. Under 'Injection Volume', 'Current Method' is selected with a value of '1' μL for the front inlet and '0' μL for the rear inlet. At the bottom, 'Method Sections to Run:' has 'Data Acquisition' checked and 'Data Analysis' unchecked. Buttons for 'OK and Run Method', 'Exit', 'Cancel', and 'Help' are present.

Figure 5 Start Run dialog box

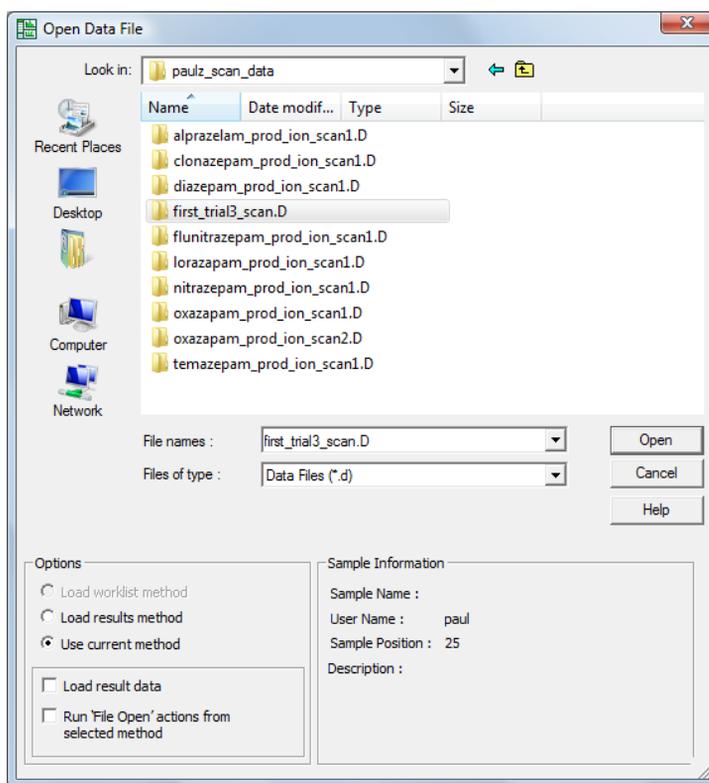
Task 5. Determine precursor ion masses

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

Steps	Detailed instructions	Comments
<p>5 Open the acquired data file.</p> <ul style="list-style-type: none"> In the Qualitative Analysis program, open either the example file, first_trial3_scan.D, or the data file you created in “Task 1. Set the inlet and injection parameters” on page 5. 	<p>a Double-click the Qualitative Analysis icon.  The program displays the Open Data File dialog box.</p>	<ul style="list-style-type: none"> When you open a data file, the Load result data (lower left corner) check box is grayed out if the result data was not saved. If you see the check box selected, this means that the data file(s) already contains results. Clear this check box before opening the file.

Before you begin, make sure that all previous settings are returned to their default values:

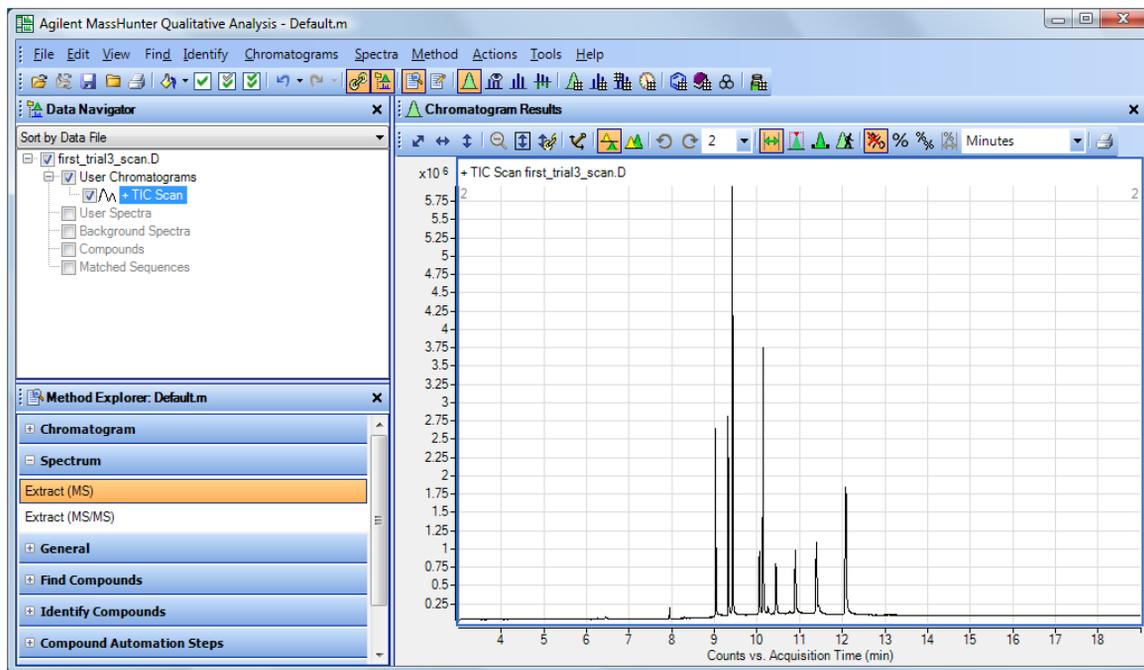
- Restore default layouts
 - Click **View > Window Layouts > Restore Default Layout.**
- Make sure the method is **default.m.** (see title bar)
 - Click **Method > Open.**
 - Select **default.m**, and click **Open.**
- Return display options to default settings.
 - Click **Tools > Plot Display Options...**
 - Click **Default**, and then **OK.**



Exercise – Develop an acquisition method for the 7000A

Task 5. Determine precursor ion masses

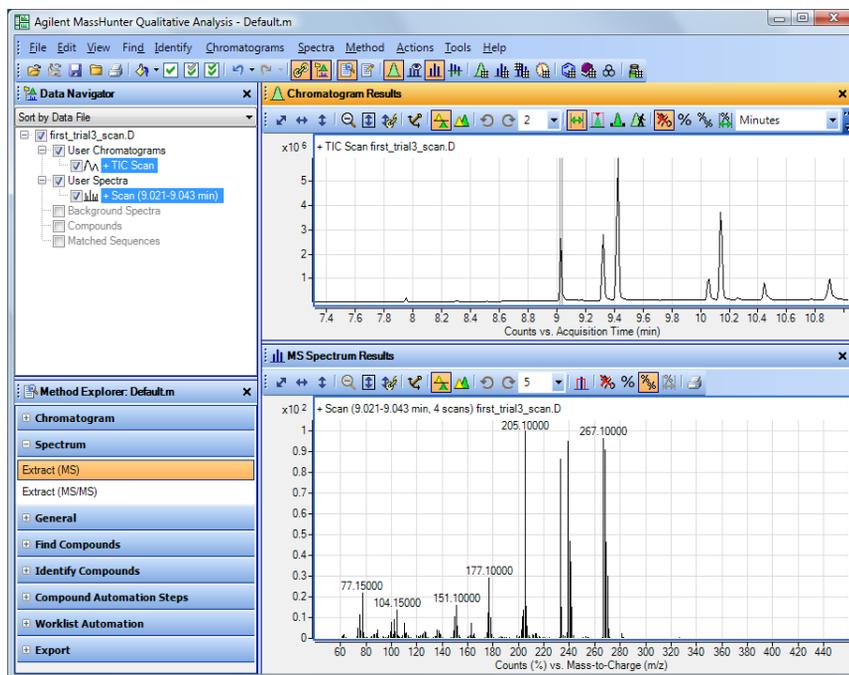
Steps	Detailed instructions	Comments
	<p>b Do one of the following:</p> <ul style="list-style-type: none">• Select the example data file first_trial3_scan.D, and click Open.• Select the data file you created in “Task 4. Acquire precursor ion scan data (Optional)” on page 13, and click Open. <p>By default, the system displays the Total Ion Chromatogram (TIC).</p>	<ul style="list-style-type: none">• The figure below shows the default layout. This is what you want to see.• 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.



Exercise – Develop an acquisition method for the 7000A

Task 5. Determine precursor ion masses

Steps	Detailed instructions	Comments																																				
<ul style="list-style-type: none"> Determine precursor ion masses for all eight peaks. <ul style="list-style-type: none"> You have determined them correctly if you find the values are similar to those shown in this table. Close the data file after finding the precursor ion masses. 	<p>c In the Chromatogram Results window, the Range Select icon in the toolbar  is selected.</p> <p>d Click the left mouse button and drag the cursor across the first peak at RT of about 9.0, to produce a shaded region, as shown below.</p> <p>e Right-click inside the shaded area, and select Extract MS Spectrum from the shortcut menu.</p> <p>f Repeat step d through step e for the other compounds. The precursor ion masses should match those in the table in step 2.</p> <p>g Click File > Close Data File.</p> <p>h When asked if you want to save the results, click No.</p>	<ul style="list-style-type: none"> An averaged spectrum from the highlighted area inside the peak is displayed in the MS Spectrum Results window. The precursor mass of the first compound, Oxazepam, is determined to be m/z 239.1. 																																				
<table border="1"> <thead> <tr> <th>PEAK</th> <th>Compound</th> <th>RT</th> <th>m/z</th> </tr> </thead> <tbody> <tr> <td>1</td> <td>Oxazepam</td> <td>9.0</td> <td>239.1</td> </tr> <tr> <td>2</td> <td>Lorazepam</td> <td>9.3</td> <td>274.1</td> </tr> <tr> <td>3</td> <td>Diazepam</td> <td>9.4</td> <td>283.2</td> </tr> <tr> <td>4</td> <td>Temazepam</td> <td>10.0</td> <td>271.1</td> </tr> <tr> <td>5</td> <td>Flunitrazepam</td> <td>10.1</td> <td>312.2</td> </tr> <tr> <td>6</td> <td>Nitrazepam</td> <td>10.9</td> <td>253.1</td> </tr> <tr> <td>7</td> <td>Clonazepam</td> <td>11.4</td> <td>314.1</td> </tr> <tr> <td>8</td> <td>Alprazolam</td> <td>12.1</td> <td>308.2</td> </tr> </tbody> </table>	PEAK	Compound	RT	m/z	1	Oxazepam	9.0	239.1	2	Lorazepam	9.3	274.1	3	Diazepam	9.4	283.2	4	Temazepam	10.0	271.1	5	Flunitrazepam	10.1	312.2	6	Nitrazepam	10.9	253.1	7	Clonazepam	11.4	314.1	8	Alprazolam	12.1	308.2		
PEAK	Compound	RT	m/z																																			
1	Oxazepam	9.0	239.1																																			
2	Lorazepam	9.3	274.1																																			
3	Diazepam	9.4	283.2																																			
4	Temazepam	10.0	271.1																																			
5	Flunitrazepam	10.1	312.2																																			
6	Nitrazepam	10.9	253.1																																			
7	Clonazepam	11.4	314.1																																			
8	Alprazolam	12.1	308.2																																			



Exercise – Develop an acquisition method for the 7000A

Task 6. Create acquisition methods for finding product ion masses

Task 6. Create acquisition methods for finding product ion masses

In this part of the method development, we will use four collision energies to determine the best product ion to use for Multiple Reaction Monitoring (MRMs). We start with the method previously saved in “Task 3. Create acquisition method for finding precursor ions” on page 11 and change the 7000A part of the acquisition method to fragment the previously identified precursor ions and scan for product ions at four different collision energies.

Steps	Detailed instructions	Comments
<p>6 Enter MS parameters appropriate for finding the Oxazepam product ion and save the method as iiipaul1_Oxazepam_prod_ion_Scan2.M, where iii are your initials.</p> <p>Create additional methods for the other 7 compounds using the data from the previous task.</p>	<p>a From the Data Acquisition workstation open the iiipaul1_MS_Scan.M method, where iii are your initials.</p> <p>b Click the QQQ Method Editor icon.</p> <p>c In the Time segment section, enter a start Time of 3.0, select a Scan Type of Product Ion from the drop-down list, enter 650 for the Delta EMV, and select Data stored.</p> <p>d In the Scan segments section, enter Oxazepam for the Segment name, 239.1 for the Precursor ion, 50 for MS2 from, 275 for MS2 to, 300 for Scan time (ms), and 5 for the Collision energy.</p> <p>e Set the MS1 resolution to Wide.</p> <p>f In the Scan parameters section, enter a Step size of 0.1 amu and select Profile data.</p> <p>g In the Scan segments section, right-click a cell and select New Scan Segment from the context menu.</p> <p>h Repeat the above step three times.</p>	<ul style="list-style-type: none">• This method was previously saved in Task 3 and the GC acquisition parameters provide excellent compound separation.• The QQQ Method Editor window opens.• A new scan segment with all values from the first scan segment is created.• A maximum of four scan segments is supported on the 7000A.

Exercise – Develop an acquisition method for the 7000A
Task 6. Create acquisition methods for finding product ion masses

Steps	Detailed instructions	Comments
	<p>i Change the Collision energy to 15 for the second scan segment.</p> <p>j Change the Collision energy to 25 for the third scan segment.</p> <p>k Change the Collision energy to 35 for the fourth scan segment.</p> <p>l Click OK to close the window.</p> <p>m From the main window select Method > Save Method As and save the method as iiiipaul1_Oxazepam_prod_ion_Scan2.M, where iii are your initials.</p> <p>n Click the QQQ Method Editor icon.</p> <p>o For each compound shown in Table 2, create a new method by repeating the above steps substituting the values from Table 2 and saving each method to the table named method.</p>	<ul style="list-style-type: none"> • A total of four scan segments, each with a different collision energy, is required for optimizing product ion sensitivity and selectivity. • Eight compound specific methods are created.

Table 2 Required methods and changed values for finding product ions

Method	Segment Name	Precursor Ion	MS2 from	MS2 to	Scan time	Collision energy
iiiipaul1_Oxazepam_prod_ion_Scan2.M	Oxazepam	239.1	50	275	300	5, 15, 25, 35
iiiipaul1_Lorazepam_prod_ion_Scan1.M	Lorazepam	274.1	50	280	300	5, 15, 25, 35
iiiipaul1_diazepam_prod_ion_Scan1.M	Diazepam	283.1	50	290	300	5, 15, 25, 35
iiiipaul1_flunitrazepam_prod_ion_Scan1.M	Flunitrazepam	312.2	50	315	300	5, 15, 25, 35
iiiipaul1_temzepam_prod_ion_Scan1.M	Temazepam	271.1	50	280	300	5, 15, 25, 35
iiiipaul1_nitrazepam_prod_ion_Scan1.M	Nitrazepam	253.1	50	270	300	5, 15, 25, 35
iiiipaul1_clonazepam_prod_ion_Scan1.M	Clonazepam	314.1	50	320	300	5, 15, 25, 35
iiiipaul1_alprazolam_prod_ion_Scan1.M	Alprazolam	308.2	50	320	300	5, 15, 25, 35

Exercise – Develop an acquisition method for the 7000A
Task 6. Create acquisition methods for finding product ion masses

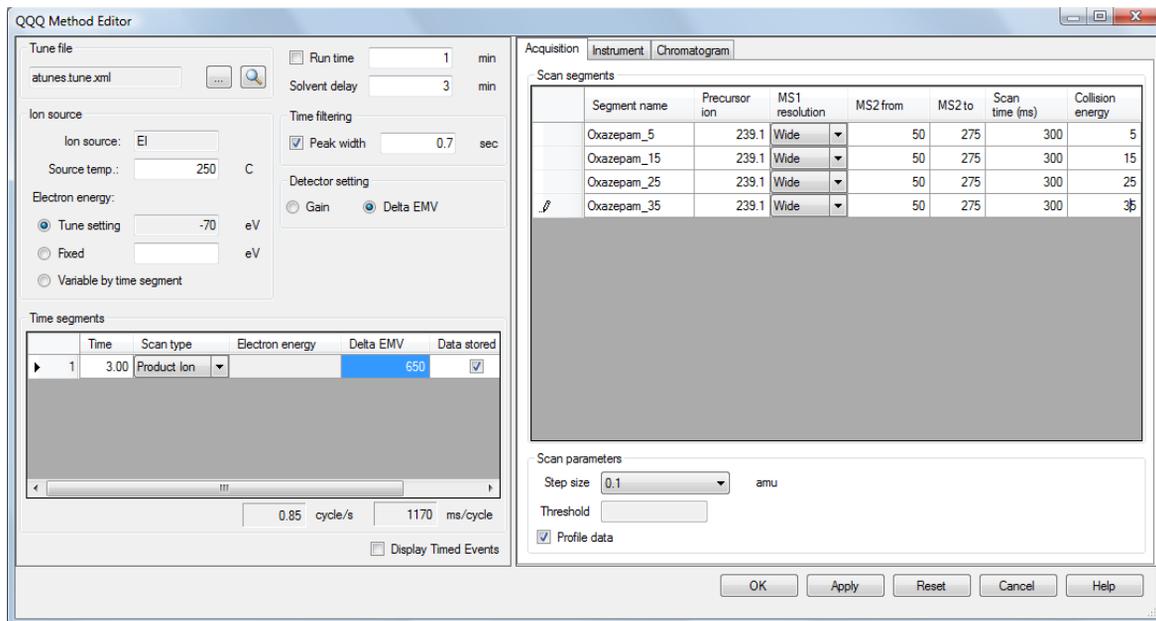


Figure 6 Oxazepam 7000A acquisition method for determining the product ion

Task 7. Acquire scan data for finding product ions (Optional)

In this task, you create a sequence to acquire data for finding the product ions of the Benzodiazepine compounds using the eight compound specific methods developed in the previous task.

Steps	Detailed instructions	Comments
<p>7 Set up and run a sequence (optional).</p> <ul style="list-style-type: none"> Name the data files iiixx_prod_ion_scan1.d, where iiix are your initials and xx is the compound name. 	<p>a Click the Sequence Edit icon to display the Sequence Table.</p> <p>b Use the drop-down list next to the New Sample(s) button in the Sequence Table toolbar and select 5 Samples.</p> <p>c Enter a sample Name, Vial ALS location number, Method File name, and Data File name for each sample as shown in Figure 7.</p> <p>d Use the toolbar's Fill down drop-down list to copy the Type column Sample value and the Dil. column 1 value to all samples.</p> <p>e Click the OK button.</p> <p>f Select Sequence > Save Sequence As to save the sequence as BenzoPI.Sequence.xml.</p> <p>g Place eight sample vials in the specified location of the ALS tray.</p> <p>h Click the Sequence Run icon to display the Start Sequence dialog box.</p> <p>i If needed, add a sequence Comment, change the Data File Directory and click the Run Sequence button.</p>	<ul style="list-style-type: none"> This step is optional because you can use the six example data files in the next step. The table now contains 8 sample lines.

Exercise – Develop an acquisition method for the 7000A
Task 7. Acquire scan data for finding product ions (Optional)

Name	Val	Method File	Data File	Type	Dil.	Comm
Oxazepam_prod...	1	paul1_Oxaza_ion_Scan2.M	Oxazepam_prod_ion_Scan2.D	Sample	1	
Lorazepam_prod...	2	paul1_Loraze_ion_Scan1.M	Lorazepam_prod_ion_Scan1.D	Sample	1	
diazepam_prod_ion	3	paul1_diazep_ion_Scan1.M	diazepam_prod_ion_Scan1.D	Sample	1	
flunitrazepam_pro...	4	paul1_flunitra_ion_Scan1.M	flunitrazepam_prod_ion_Scan1.D	Sample	1	
temzepam_prod_i...	5	paul1_temze_ion_Scan1.M	temzepam_prod_ion_Scan1.D	Sample	1	
nitrazepam_prod...	6	paul1_nitraze_ion_Scan1.M	nitrazepam_prod_ion_Scan1.D	Sample	1	
clonazepam_pro...	7	paul1_clonaz_ion_Scan1.M	clonazepam_prod_ion_Scan1.D	Sample	1	
alprazolam_prod...	8	paul1_alpraz_ion_Scan1.M	alprazolam_prod_ion_Scan1.D	Sample	1	

Figure 7 Sequence Table values

Start Sequence default.sequence.xml Last Modified: 9/30/2008 6:31:50 PM

Method Sections to Run: Full Method

On a Barcode Mismatch: Inject Anyway, Don't Inject

Overwrite Existing Data Files

Sequence Comment: _____

Operator Name: PaulZ

Data File Directory: C:\MassHunter\GCMS\1\DATA\ Browse...

Pre-Sequence Macros/Commands: Acquisition: _____ Browse..., Data Analysis: _____ Browse...

Post-Sequence Macros/Commands: Acquisition: _____ Browse..., Data Analysis: _____ Browse...

Enter the operator name: _____

Run Sequence OK Cancel Help

Figure 8 Start Sequence dialog box

Task 8. Determine the product ions

In this exercise, you determine the product ion for each compound in the compound specific acquired data file.

Steps	Detailed instructions	Comments
8	<p>Open the acquired data files in the Qualitative Analysis program.</p> <p>a Double-click the Qualitative Analysis icon.  The program displays the Open Data File dialog box.</p> <p>b Do one of the following:</p> <ul style="list-style-type: none"> Select the eight example data files and click Open. Select all the data files you acquired in “Task 7. Acquire scan data for finding product ions (Optional)” on page 21, and click Open. <p>c In the Data Navigator, clear the check boxes for all compounds except Oxazepam.</p> <p>d From the MS Spectra Results window toolbar, enter 5 from the drop-down menu.</p>	<ul style="list-style-type: none"> When you open a data file, the Load result data (lower left corner) check box is grayed out if result data was not saved. If you see the check box selected, this means that the data file(s) already contains results. Clear this check box before opening the file. Use the CTRL key to select multiple data files. Only a single acquisition file at a time is displayed. A maximum of 5 spectrum panes can be displayed.

Exercise – Develop an acquisition method for the 7000A

Task 8. Determine the product ions

Steps	Detailed instructions	Comments
	<p>e In the Chromatogram Results window, the Range Select icon in the toolbar  is selected.</p> <p>f Click the left mouse button and drag the cursor across the prominent peak corresponding to the RT of this compound, to produce a shaded region.</p> <p>g Right-click inside the shaded area, and select Extract MS Spectrum from the shortcut menu. The spectrum for this peak is shown in Figure 9.</p>	<ul style="list-style-type: none">• For Oxazepam this peak is at about 9 minutes.• An averaged spectrum from the highlighted area inside the peak is displayed in the MS Spectrum Results window for each of the four collision energy scan segments in the acquisition method.

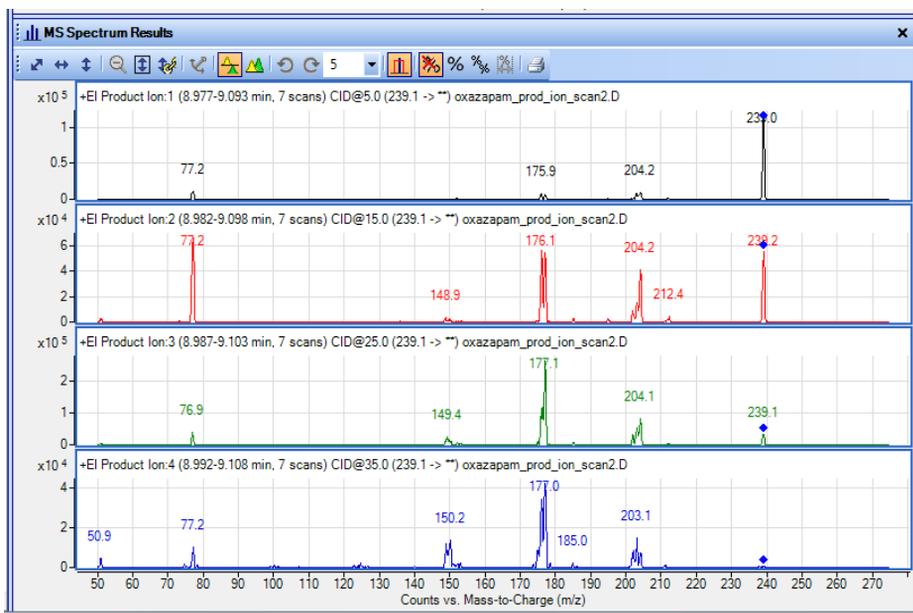


Figure 9 Extracted spectra for 4 collision energy scans showing product ion selected

Steps	Detailed instructions	Comments
<ul style="list-style-type: none"> Determine product ion masses for all eight peaks. <ul style="list-style-type: none"> You have determined them correctly if you find the values are similar to those shown in Table 3. Close the data file after finding the precursor ion masses. 	<p>h The product ion for the first compound, Oxazepam, is determined to be m/z 177.1 at CID 25.</p> <p>i Clear the check box for the current data file in the Data Navigator.</p> <p>j Select the check box for the next data file.</p> <p>k Repeat step f through step j for the other compounds. The product ion masses should match those in Table 3.</p> <p>l Click File > Close Data File.</p> <p>m When asked if you want to save the results, click No.</p>	<ul style="list-style-type: none"> Examine each spectrum to find the most selective ion. Look for the highest count of a mass closest to the precursor ion with minimum interference from adjacent ions. <p>Here the best ion selection has a count of 100 compared to other ions with a count of 25 maximum in any other spectrum.</p>

Table 3 Product Ions found for the Benzodiazepine compounds

Compound	Data File	Precursor Ion	Product Ion	Collision Energy
Oxazepam	Oxazepam_prod_ion_scan2.d	239.1	177.1	25
Lorazepam	Lorazepam_prod_ion_scan1.d	274.1	239.1	15
Diazepam	Diazepam_prod_ion_scan1.d	283.1	238.1	25
Flunitrazepam	Flunitrazepam_prod_ion_scan1.d	312.2	266.1	25
Temazepam	Temazepam_prod_ion_scan1.d	274.1	77.1	35
Nitrazepam	Nitrazepam_prod_ion_scan1.d	253.1	152.1	25
Clonazepam	Clonazepam_prod_ion_scan1.d	314.1	268.2	25
Alprazolam	Alprazolam_prod_ion_scan1.d	308.2	279.2	15

Exercise – Develop an acquisition method for the 7000A

Task 9. Create an MRM method

Task 9. Create an MRM method

In this exercise, you create an MRM method that finds any Benzo compound in a sample.

Steps	Detailed instructions	Comments
9 Open the Data Acquisition program and create an MRM method using the data from Table 3 on page 25.	<p>a From the Data Acquisition workstation open the iiipaul1_MS_Scan.M method, where iii are your initials.</p> <p>b Click the QOQ Method Editor icon.</p> <p>c In the Time segment section, enter a start Time of 3.0, select a Scan Type of MRM from the drop-down list, enter 650 for the Delta EMV, and select Data stored.</p> <p>d In the Scan segments section, enter Wide for the MS1 Resolution and MS2 Resolution, and 50 for the Dwell.</p> <p>e In the Scan segments section, right-click a cell and select New Scan Segment from the context menu.</p> <p>f Repeat the above step seven times.</p> <p>g Fill in the rest of the Scan Segments section to match Figure 10 on page 27.</p> <p>h Click OK to close the window.</p> <p>i From the main window select Method > Save Method As and save the method as iiipaul1_benzo_mrm_1.M, where iii are your initials.</p>	<ul style="list-style-type: none">• The GC acquisition parameters from this method are used unchanged. The 7000A method parameters are edited in this procedure to create the MRM method.

Exercise – Develop an acquisition method for the 7000A
Task 9. Create an MRM method

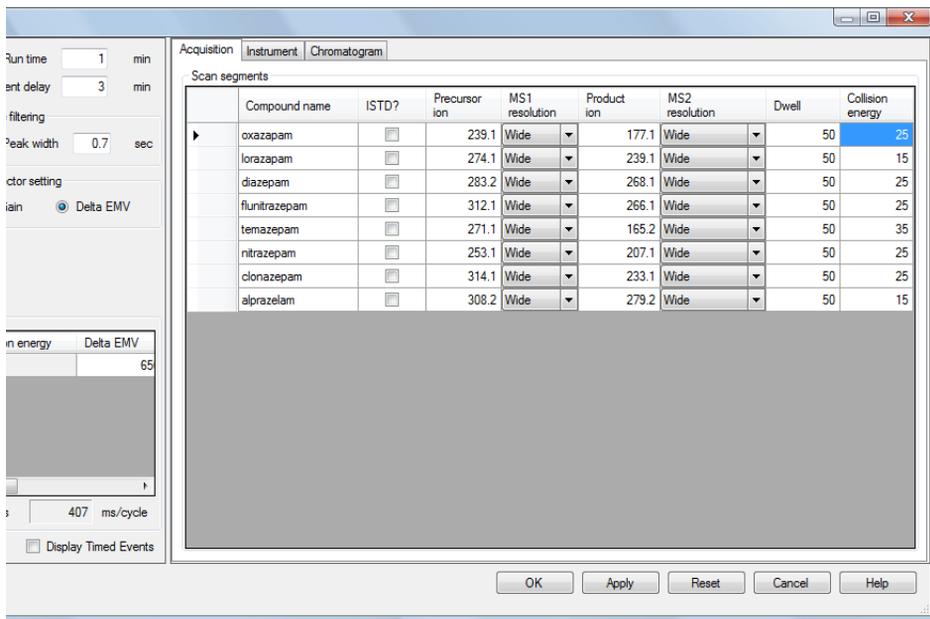


Figure 10 Scan section completed for an MRM method

Exercise – Create a quantitative analysis method

Task 10. Acquire MRM data (Optional)

Exercise – Create a quantitative analysis method

Task 10. Acquire MRM data (Optional)

In this exercise, you create a sequence to acquire calibration data used for the quantitative analysis of MRM-acquired samples containing Benzo compounds. Additionally there are 3 samples that contain concentrations of the Benzo compounds to demonstrate the quantitation of unknown samples. The data acquisition portion of the program is optional for the Benzo calibration samples since example data files are included on the program disk.

Steps	Detailed instructions	Comments
<p>10 Open the Data Acquisition program and use the MRM method created in the last task to acquire calibration data. This data is required to create a quantitative analysis method in the Quantitative Analysis program.</p>	<p>a From the Data Acquisition workstation load the iiiipaul1_benzo_mrm_1.M method.</p> <p>b Click the Edit Sequence icon to display the Sequence Table.</p> <p>c Add or Delete sample entries so that the Sequence Table contains five sample lines.</p> <p>d For the first sample enter BenzoLevel1 for the Name, enter the ALS vial location for the Vial, select Benzo_MRM.M for the Method File, enter paul1_benzo_L1_19Nov08.D for the Data File, select Cal from the drop-down list for the Type, enter L1 for the Level, and enter 1 for the Dilution. See Figure 11 on page 30.</p> <p>e Use the Fill Increment drop-down in the Sequence Table toolbar to copy and increment the values listed for the first line sample values for Name, Vial, and Level to the other four samples.</p> <p>f Use the Fill drop-down in the Sequence Table toolbar to copy the values listed for the first line sample values for Method File, Data File, Type, and Dilution to the other four samples.</p> <p>g Change _L1_ part of the Data File name to follow the pattern shown in Figure 11 on page 30.</p> <p>h Add three additional lines to the sample table and fill in the values for these samples so that the completed table resembles Figure 11 on page 30.</p> <p>i Click OK to close the window.</p>	<ul style="list-style-type: none"> • This method was created in “Task 9. Create an MRM method” on page 26. • To delete consecutive sample entries, click in the left sample number column of the first sample to delete, hold down the Shift key and click the last sample number column in the table. With the samples to delete highlighted, press the Delete key on your computer. • The Vial location incremented in this way assumes that the vials are placed in consecutive locations in the sampler tray.

Exercise – Create a quantitative analysis method

Task 10. Acquire MRM data (Optional)

Steps	Detailed instructions	Comments
	<p>j Select Sequence > Save Sequence As to save the sequence as BenzoCalibration.Sequence.xml.</p> <p>k Place the five sample vials containing known concentrations and the 3 samples containing the unknown concentrations in the specified locations of the ALS tray.</p> <p>l Click the Sequence Run icon to display the Start Sequence dialog box.</p> <p>m If needed, add a sequence Comment, change the Data File Directory and click the Run Sequence button.</p>	<ul style="list-style-type: none">• The remaining steps are optional. These sample vials are prepared in “Prepare the samples required for data acquisition” on page 3.

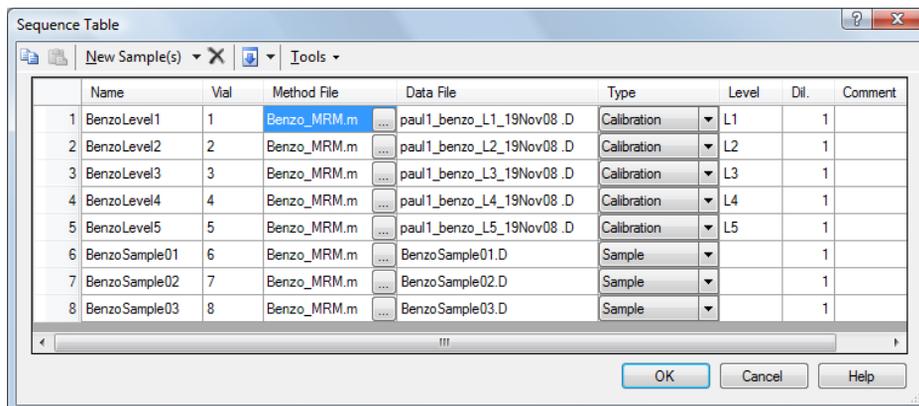
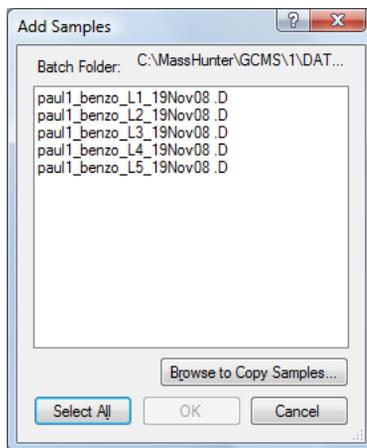


Figure 11 Sequence for Benzo calibration samples and unknowns

Task 11. Create a quantitative analysis batch

In this exercise, you create a batch that is used to create a quantitative method using data acquired from the five calibration samples ran in the last task.

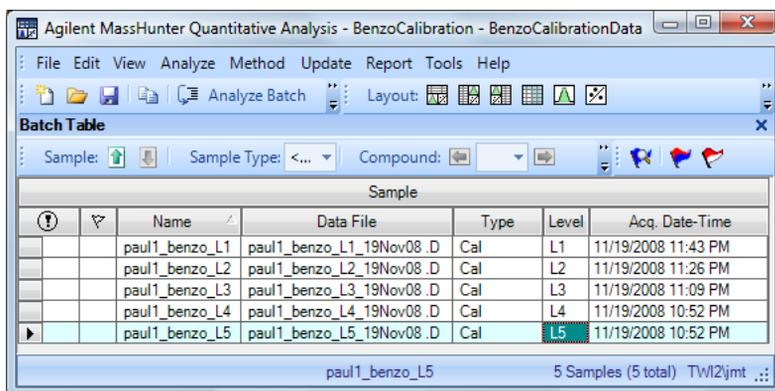
Steps	Detailed instructions	Comments
<p>11 Open the Quantitative Analysis program and create a batch to analyze data. Name the batch and assign a batch directory.</p> <ul style="list-style-type: none"> Add samples to the batch. 	<p>a Double-click the QQQ Quantitative Analysis icon on your window's desktop.</p> <p>b From the main menu, select File > New batch, use the Look In drop-down list to navigate to the directory containing the 5 calibration files, and enter the batch name BenzoCalibrationData and click the Open button.</p> <p>c From the main menu, select File > Add samples and select the 5 data files paul1_benzo_L1_19Nov08.D through paul1_benzo_L5_19Nov08.D.</p>	<ul style="list-style-type: none"> The Quantitative Analysis Workstation opens. This is the directory selected for the data files in "Task 10. Acquire MRM data (Optional)" on page 28. If you skipped this data acquisition task you can substitute the 5 sample data files included on the program disk. Just copy these sample data files to this directory. The 5 calibration files are need to create the data analysis calibration curve and one of these files is used for extracting MRM data for the method. The Type for all 5 samples is Cal and the Level should range from L1 to L5. The Type and Level are taken from the acquisition sequence Table 11 on page 30.



Exercise – Create a quantitative analysis method

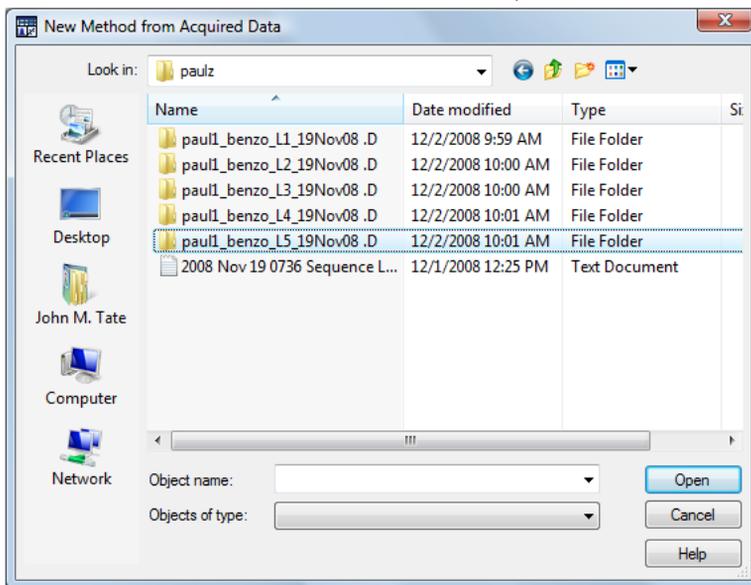
Task 11. Create a quantitative analysis batch

Steps	Detailed instructions	Comments
	<p>d Click the OK button. The Batch Table now contains the 5 samples.</p> <p>e If necessary, change the Type to Cal, sort on the Name column, and enter the values shown in the Level column.</p> <p>f If necessary, add the Dil and Amt columns to the Sample section of the Batch Table. Enter a value of 1 for all Dil and Amt entries in these columns.</p>	<ul style="list-style-type: none">• The batch now requires a data analysis method to automatically analyze the sample.



Task 12. Create an MRM quantitative analysis method

Steps	Detailed instructions	Comments
12 Create a Data Analysis method.	<p>a From the main menu, select Method > New > New Method from Acquired MRM Data to display the New Method from Acquired Data dialog box.</p> <p>b Use the Look In drop-down list to select the batch directory. Click the paul1_benzo_L5_19Nov08.D data file, then click the Open button.</p>	<ul style="list-style-type: none"> • The sample data stored in the acquisition method created in “Task 9. Create an MRM method” on page 26 is used to automatically fill in parts of the data analysis method. • The program uses this sample data file to create much of the data analysis method, then presents the user with the method editor so that the method can be finished manually.



Exercise – Create a quantitative analysis method

Task 12. Create an MRM quantitative analysis method

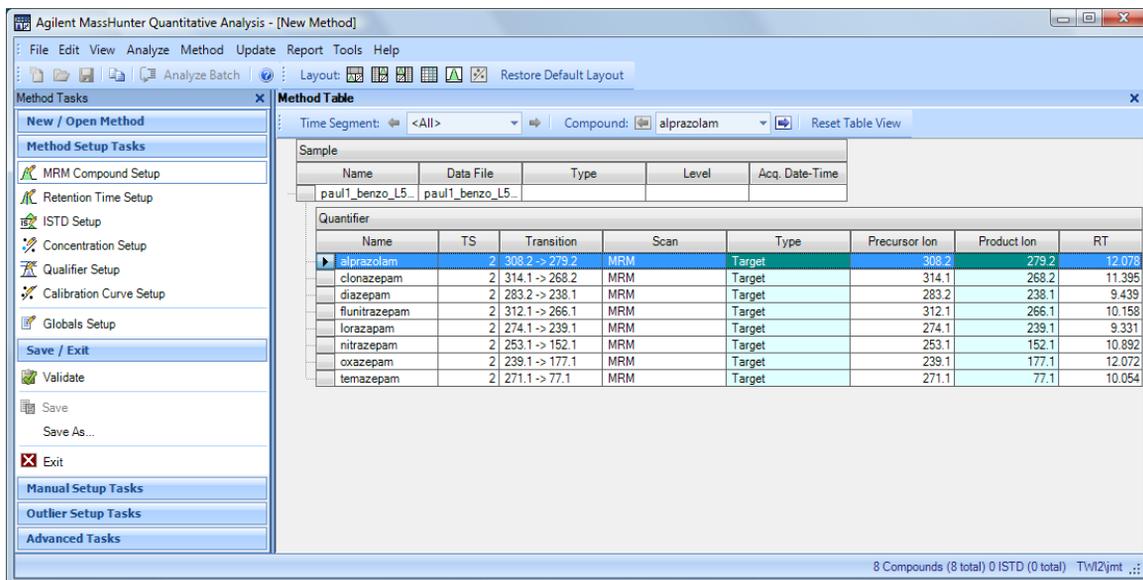
Steps

Detailed instructions

Comments

- c In the **Method Tasks** pane, click **Method Setup Tasks**, then click **MRM Compound Setup** to display the **Method Table**.

- Review the eight compounds that were originally included in the acquisition method.



Agilent MassHunter Quantitative Analysis - [New Method]

File Edit View Analyze Method Update Report Tools Help

Layout: [Icons] Restore Default Layout

Method Tasks

New / Open Method

Method Setup Tasks

MRM Compound Setup

Retention Time Setup

ISTD Setup

Concentration Setup

Qualifier Setup

Calibration Curve Setup

Globals Setup

Save / Exit

Validate

Save

Save As...

Exit

Manual Setup Tasks

Outlier Setup Tasks

Advanced Tasks

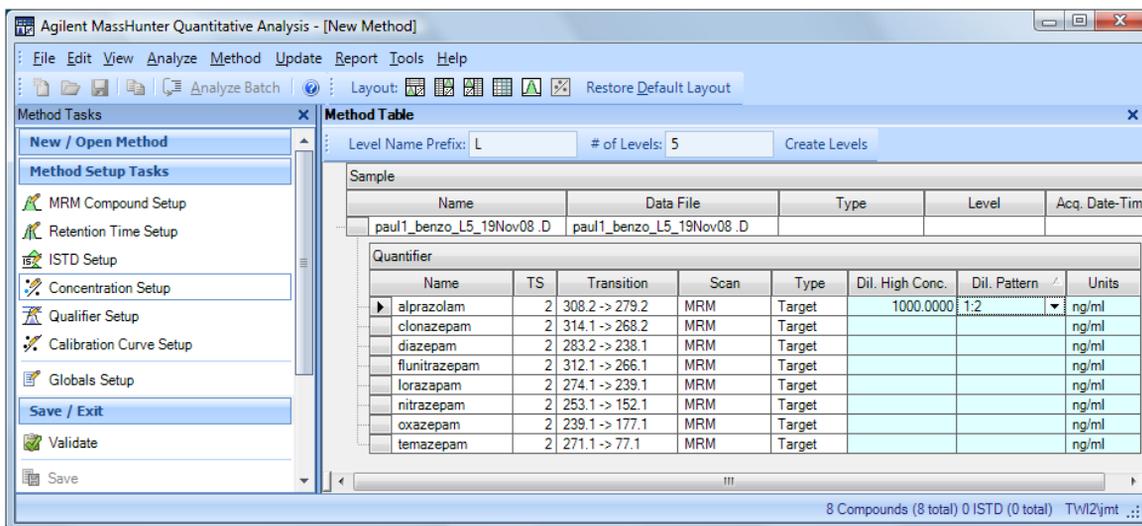
Time Segment: <All> Compound: alprazolam Reset Table View

Sample		Name	Data File	Type	Level	Acq. Date-Time	
paul1_benzo_L5_		paul1_benzo_L5_					
Quantifier							
Name	TS	Transition	Scan	Type	Precursor Ion	Product Ion	RT
alprazolam	2	308.2 -> 279.2	MRM	Target	308.2	279.2	12.078
clonazepam	2	314.1 -> 268.2	MRM	Target	314.1	268.2	11.395
diazepam	2	283.2 -> 238.1	MRM	Target	283.2	238.1	9.439
flunitrazepam	2	312.1 -> 266.1	MRM	Target	312.1	266.1	10.158
lorazepam	2	274.1 -> 239.1	MRM	Target	274.1	239.1	9.331
nitrazepam	2	253.1 -> 152.1	MRM	Target	253.1	152.1	10.892
oxazepam	2	239.1 -> 177.1	MRM	Target	239.1	177.1	12.072
temazepam	2	271.1 -> 77.1	MRM	Target	271.1	77.1	10.054

8 Compounds (8 total) 0 ISTD (0 total) TV12jmt ...

Exercise – Create a quantitative analysis method
Task 12. Create an MRM quantitative analysis method

Steps	Detailed instructions	Comments
<ul style="list-style-type: none"> Create a calibration table. 	<p>d In the Method Tasks pane, click Method Setup Tasks, then click Concentration Setup.</p> <p>e In the Dil. High Conc. column for the first compound in the table enter 1000.0 then select 1:2 from the drop-down list for the Dil. Pattern column.</p>	<ul style="list-style-type: none"> The Method Table columns change to assist with entering the concentration levels. The program uses the highest concentration for a calibration sample, the number of calibration levels, and a dilution pattern to automatically calculate the concentrations for the other levels.



Exercise – Create a quantitative analysis method

Task 12. Create an MRM quantitative analysis method

Steps

Detailed instructions

Comments

- f In the **Method Table** toolbar, enter **L** for a **Level Name Prefix**, and **5** for the **# of Levels** then click the **Create Level** button.

- The calibration table containing a concentration entry for each of the 5 calibration levels is shown below for the first compound in the method table.

The screenshot displays the Agilent MassHunter Quantitative Analysis interface. The 'Method Table' window is open, showing the following data:

Name	Data File	Type	Level	Acq. Date
paul1_benzo_L5_19Nov08.D	paul1_benzo_L5_19Nov08.D			

Quantifier table:

Name	TS	Transition	Scan	Type	Dil. High Conc.	Dil. Pattern	Units
alprazolam	2	308.2 -> 279.2	MRM	Target	1000.0000	1:2	ng/ml

Calibration table:

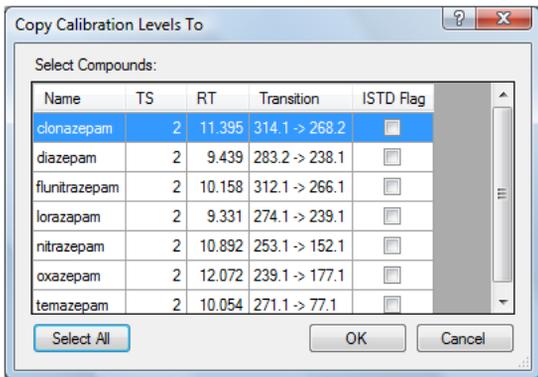
Level	Conc.
L1	62.5000
L2	125.0000
L3	250.0000
L4	500.0000
L5	1000.0000

Quantifier table (continued):

Name	TS	Transition	Scan	Type	Dil. High Conc.	Dil. Pattern	Units
clonazepam	2	314.1 -> 268.2	MRM	Target			ng/ml
diazepam	2	283.2 -> 238.1	MRM	Target			ng/ml
flunitrazepam	2	312.1 -> 266.1	MRM	Target			ng/ml

8 Compounds (8 total) 0 ISTD (0 total) TW12jmt

Exercise – Create a quantitative analysis method
Task 12. Create an MRM quantitative analysis method

Steps	Detailed instructions	Comments
<ul style="list-style-type: none"> Copy the calibration table to other quantifiers. 	<p>g Select the first compound in the Quantifier Table, right-click the compound and select Copy Calibration Levels To from the shortcut menu.</p>	<ul style="list-style-type: none"> The Copy Calibration Levels To dialog box is displayed. Use this to copy the calibration table from the current compound to selected or all other compounds.
		
	<p>h Click the Select All button to select all compounds in the dialog box then click the OK button.</p>	<ul style="list-style-type: none"> This calibration table is copied to the Quantifier table of all eight compounds.

Exercise – Create a quantitative analysis method

Task 12. Create an MRM quantitative analysis method

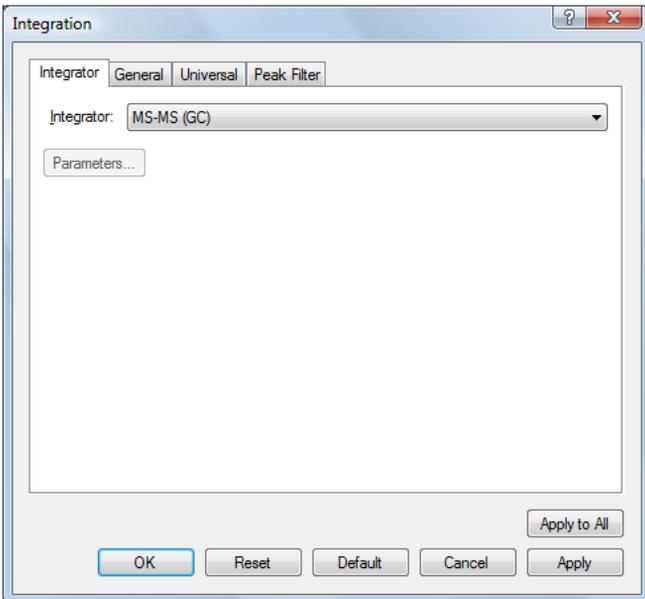
Steps	Detailed instructions	Comments
<ul style="list-style-type: none">Calibration Curve Setup	<ol style="list-style-type: none">In the Method Tasks pane, click Method Setup Tasks, then click Calibration Curve Setup.In the Quantifier table CF column of the first compound select Quadratic from the drop-down list. With this cell selected, right-click and select Fill Down from the short-cut menu.	<ul style="list-style-type: none">The Method Table is displayed with the CF, CF Origin, and CF Weight columns highlighted.The Benzo compounds in this example will have a CF of Quadratic, a CF Origin of Ignore, and a CF Weight of None.

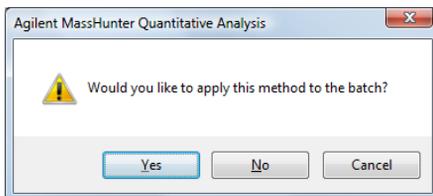
The screenshot shows the Agilent MassHunter Quantitative Analysis software interface. The main window is titled "Agilent MassHunter Quantitative Analysis - [New Method]". The "Method Tasks" pane on the left is expanded to "Method Setup Tasks", with "Calibration Curve Setup" selected. The "Method Table" pane on the right shows the "Sample" table and the "Quantifier" table. The "Sample" table has one row: "paul1_benzo_L5_19Nov08.D" with "Data File" "paul1_benzo_L5_19Nov08.D". The "Quantifier" table has 8 rows of compounds. The "CF" column for the first row, "alprazolam", is set to "Quadratic".

Sample						
Name	Data File	Type	Level	Acq. Date-Tim		
paul1_benzo_L5_19Nov08.D	paul1_benzo_L5_19Nov08.D					
Quantifier						
Name	TS	Transition	Scan	Type	CF	CF Origin
alprazolam	2	308.2 -> 279.2	MRM	Target	Quadratic	Ignore
clonazepam	2	314.1 -> 268.2	MRM	Target	Linear	Ignore
diazepam	2	283.2 -> 238.1	MRM	Target	Linear	Ignore
flunitrazepam	2	312.1 -> 266.1	MRM	Target	Linear	Ignore
lorazepam	2	274.1 -> 239.1	MRM	Target	Linear	Ignore
nitrazepam	2	253.1 -> 152.1	MRM	Target	Linear	Ignore
oxazepam	2	239.1 -> 177.1	MRM	Target	Linear	Ignore
temazepam	2	271.1 -> 77.1	MRM	Target	Linear	Ignore

8 Compounds (8 total) 0 ISTD (0 total) TWI2jmt ..:

Exercise – Create a quantitative analysis method
Task 12. Create an MRM quantitative analysis method

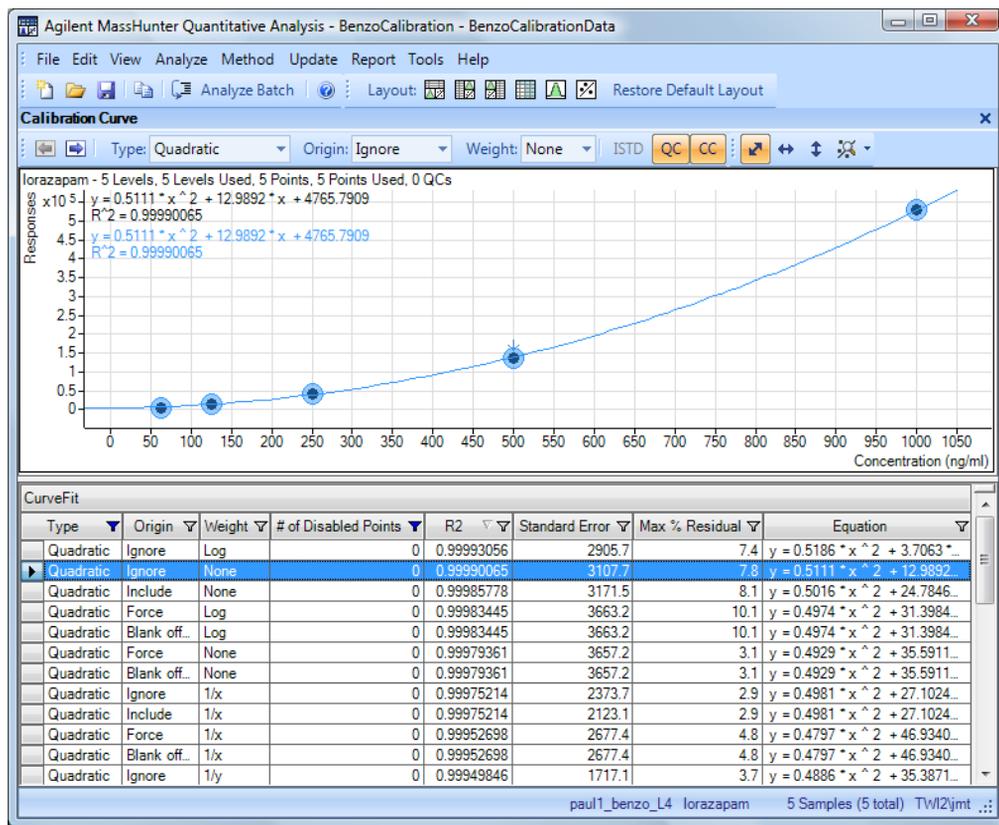
Steps	Detailed instructions	Comments
<ul style="list-style-type: none"> Select an Integrator. 	<p>k In the Method Tasks pane, click Advanced Tasks, then click Integration Parameters Setup.</p> <p>l In the Method Table pane, for any Quantifier click the Int. column entry to display the Integration dialog box and select MS-MS (GC) from the Integrator drop-down list.</p>	<ul style="list-style-type: none"> You can select from several integrators with MassHunter. Use the MS-MS (GC) integrator which is optimized for MRM data with GC use. This is a parameter-less integrator.
		
	<p>m Click the Apply to All button to set the Integrator for all quantifier peaks to this value.</p> <p>n Click OK to close this dialog box.</p>	<ul style="list-style-type: none"> This integrator is used for integrating all peaks.
<ul style="list-style-type: none"> Exit the method editor and save the method. 	<p>o In the Method Tasks pane, click Save/Exit, then click Exit.</p>	



Exercise – Create a quantitative analysis method

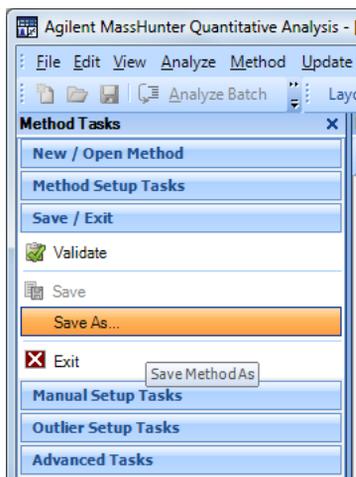
Task 12. Create an MRM quantitative analysis method

Steps	Detailed instructions	Comments
<ul style="list-style-type: none"> Apply the data analysis method to the batch. 	<p>p Click the Yes button.</p> <p>q Click the Analyze Batch button to quantitate all samples in the batch.</p>	<ul style="list-style-type: none"> The method is applied to all samples in the Batch Table. Selecting Analyze Batch takes the compound responses in each calibration sample and uses them to create the CurveFit equations for each compound.
<ul style="list-style-type: none"> Review the calibration curve for each compound. 	<p>r In the calibration curve pane, right-click and select Curve Fit Assistant from the short-cut menu.</p> <p>s In the CurveFit table, select a line with a Type of Quadratic, Ignore the Origin, and use a maximum of 0 for the # of Disabled Points.</p>	<ul style="list-style-type: none"> In the CurveFit table, click a filter icon next to the column name. Clicking the Type filter icon and selecting Quadratic allows only quadratic equations for this compound's calibration curve. Try this for the other criteria.



Exercise – Create a quantitative analysis method
Task 12. Create an MRM quantitative analysis method

Steps	Detailed instructions	Comments
	<p>t Review the curve fit for different equations by selecting a different line in the CurveFit table. If you find a better CurveFit, select it and in the graph area of the calibration curve, right-click and select Accept Assistant Curve from the shortcut menu.</p> <p>u In the Batch Table toolbar, click the right arrow to select the next calibrated compound and repeat steps q through s.</p> <p>v Repeat the above step until all eight calibrated compounds are reviewed and corrected if necessary.</p>	<ul style="list-style-type: none"> • These CurveFits were originally selected non-graphically in step j. • The Calibration Curve pane only displays the calibration data for one compound at a time.
	<p>w Click the Analyze Batch button to quantitate all samples in the batch.</p>	<ul style="list-style-type: none"> • This step is only necessary if you changed any CurveFit equation during the review above.
<ul style="list-style-type: none"> • Save the Data Analysis method 	<p>x From the main menu select Method > Edit to display the Method Editor.</p> <p>y In the Method Tasks pane, click Save / Exit, then click Save As.</p> <p>z Enter BenzoDA for the method name and click the Save button.</p>	



Exercise – Create a quantitative analysis method

Task 13. Quantitate a batch of unknown samples

Task 13. Quantitate a batch of unknown samples

In this exercise, you create a batch that is used to quantitate unknown Benzo samples using the quantitative method created in the last task. Sample data files are not provided for these unknown Benzo samples. These data files are only obtained by completing “Task 10. Acquire MRM data (Optional)” on page 28.

Steps	Detailed instructions	Comments
13 Open the Quantitative Analysis program and create a batch to analyze data. Name the batch and assign a batch directory.	a Double-click the QQQ Quantitative Analysis icon on your window's desktop. b From the main menu, select File > New batch , use the Look In drop-down list to navigate to the directory containing the 3 Benzo sample files, and enter the batch name BenzoSamples031709 and click the Open button.	<ul style="list-style-type: none">• The Quantitative Analysis Workstation opens.• This is the directory included on the program disk. Just copy these sample data files to this directory.
<ul style="list-style-type: none">• Add samples to the batch.	c From the main menu, select File > Add samples and select the samples BenzoSample01.D through BenzoSample03.D .	
	d Click the OK button. The Batch Table now contains the 3 unknown samples. e If necessary, change the Type to Sample . f If necessary, add the Dil and Amt columns to the Sample section of the Batch Table. Enter a value of 1 for all Dil and Amt entries in these columns.	<ul style="list-style-type: none">• The batch now requires a data analysis method to automatically analyze the sample.
	g In the main menu select Method > Open > Open and Apply from existing file to display the Open Method File dialog box. h Enter the BenzoDA.quantmethod.xml for the File name and click the Open button. i Click the Analyze Batch button to quantitate all samples in the batch. j Review the sample results for each compound in the method.	<ul style="list-style-type: none">• The method is applied to the batch.• Print one or more reports.

Exercise – Create a quantitative analysis method

Task 13. Quantitate a batch of unknown samples

www.agilent.com

© Agilent Technologies, Inc. 2009

Second Edition, June 2009

G6845-90001

