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

Familiarization Guide

This guide shows how to use the Agilent 7000 Series 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).

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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 7890 Series GC with split/splitless inlet and automatic liquid sampler.
   - The 7000 Series 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 µm x 0.25 µm 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

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/µL.
   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/µL.
   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/µL.
   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 7000 Series

Task 1. Set the inlet and injection parameters

Steps	Detailed instructions	Comments
1 Set up the inlet, injection source, and enable the 7000 Series.	**a** Double-click the **Data Acquisition** icon on the windows desktop. **b** Click the **Inlet and Injection Parameters** icon. **c** Select **GC** for the sample inlet and the installed ALS for the injection source. **d** Select the **Use MS** check box.	• The **Data Acquisition** window shown in Figure 1 is displayed. • The **Inlet and Injection Parameters** dialog box shown in Figure 2 is displayed.

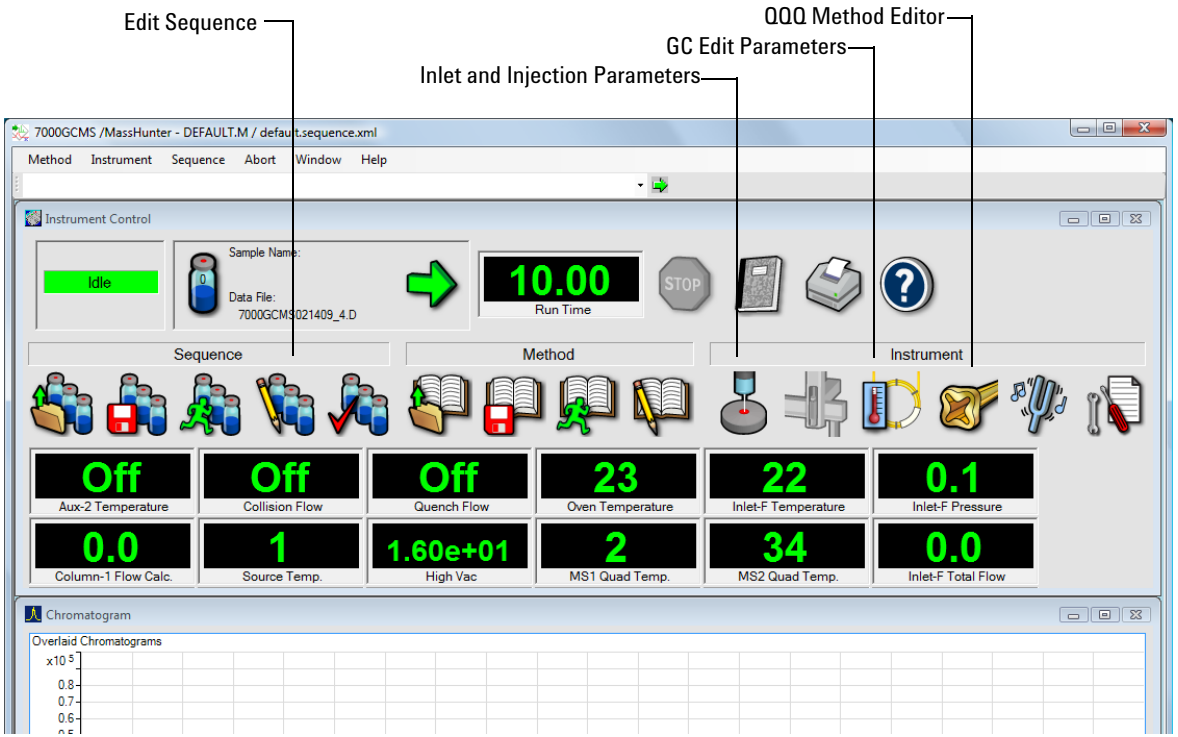


Figure 1 Agilent MassHunter Workstation Software – Data Acquisition window

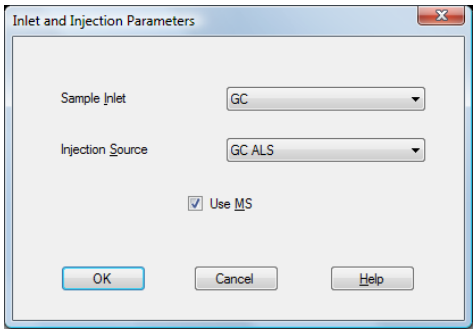


Figure 2 Inlet and Injection Parameters

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.	**a** Click the **GC Edit Parameters** icon (Figure 1).	• The **GC edit parameters** window shown in Figure 3 on page 8 is displayed.
	b Select the **CFT Settings** icon then select column **1** in the **Description** column.	• With the window selected, mouse over the icons to identify the icon from the tool tip.
	c Select control mode **On** and then select **Flow** mode. Enter 1.2 mL/min for the initial **Value** and the **Post Run** value.	
	d Select the collision cell **N2 EPC module** in the **Description** column and clear control **On**.	• The collision cell gas flow is off for this method.
	e Select the collision cell **He EPC module** in the **Description** column and clear control **On**.	
	f Select the **Inlets** icon then the **SSL** tab and enter the inlet parameters listed in Table 1.	
	g Select the **Oven** icon and enter the oven parameters listed in Table 1.	
	h Select the **ALS** icon then the **Front Injector** tab and enter the injector parameters listed in Table 1.	• If your ALS is attached to the **Back Inlet** select the **Back Injector** tab.
	i Select the **Aux Heaters** icon, enable, and set the temperature to 134 °C.	
	j Select the **OK** button.	• The GC parameters are downloaded to the GC and the window closes.

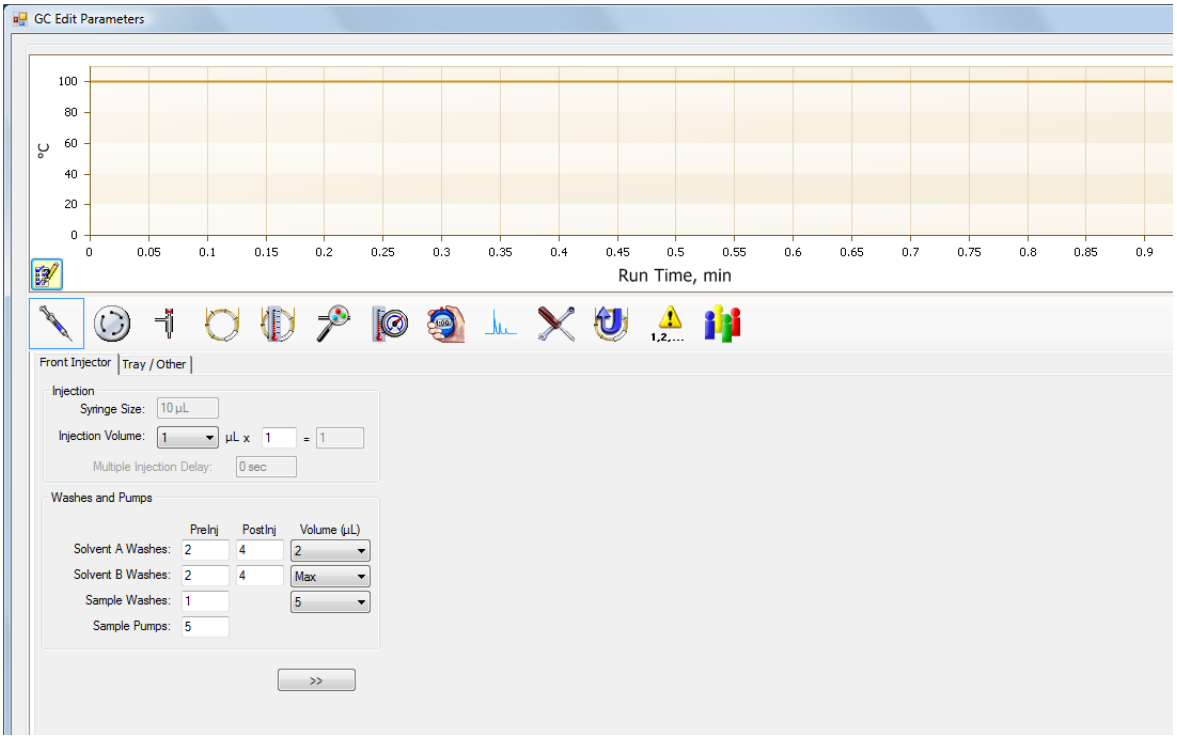


Figure 3 GC Edit Parameters window

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

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

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 7000 Series parameters for scanning the precursor ions and save to the method.

Steps	Detailed instructions	Comments
3 Enter MS parameters appropriate for the Benzodiazepine mix and save the method as ***iii*paul1_MS_Scan.M**, where ***iii*** are your initials.	**a** Click the **QQQ Method Editor** icon (Figure 1). **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**. **c** Set the **Time Filtering** to a **Peak width** of 0.7 seconds. **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**. **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**. **f** In the **Scan parameters** section, enter a **Step size** of 0.1 amu and a **Threshold** of 100. **g** Click **OK** to close the window. **h** From the main window select **Method > Save Method As** and save the method as ***iii*paul1_MS_Scan.M**, where ***iii*** are your initials.	• The **QQQ Method Editor** window shown in Figure 4 opens. • The 7000 Series starts collecting data at 4 minutes due to the **Solvent delay** setting.

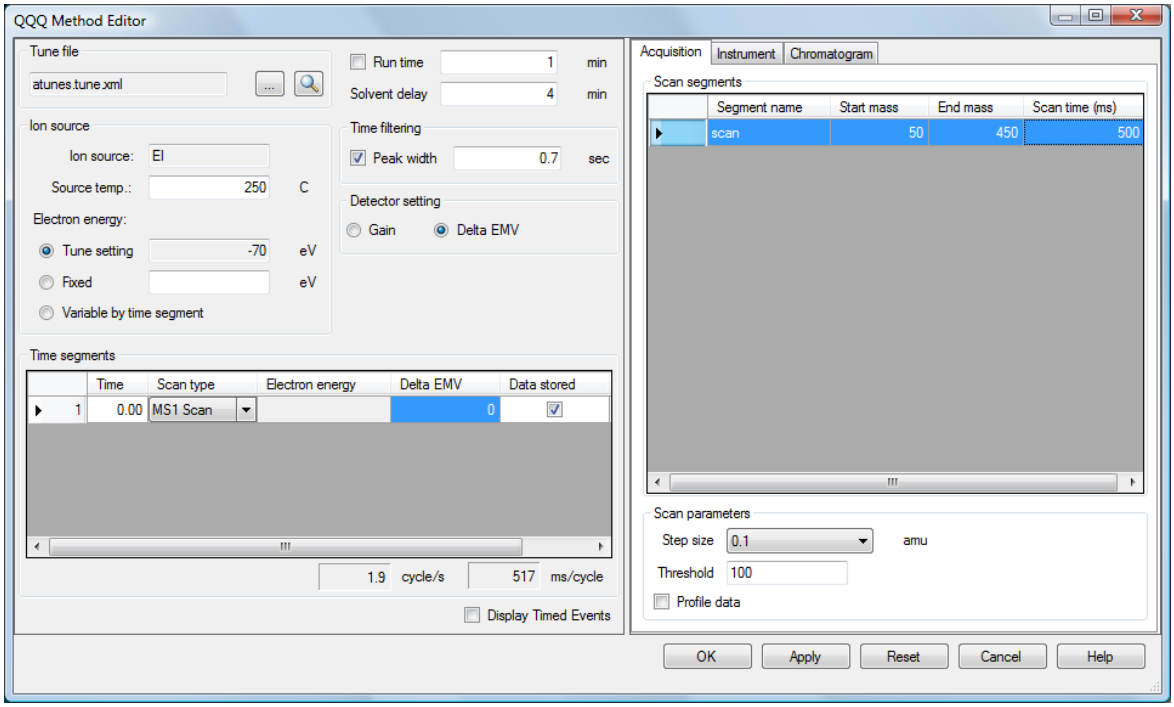


Figure 4 QQQ Method Editor

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
4 Acquire data (optional). • Name the data file ***iii*first_trial3_scan.D**, where ***iii*** are your initials. • Designate a directory path to hold your data files and method.	**a** Click the **Start Run** (green arrow) icon. **b** In the **Data Path** enter the directory to save the data file that is acquired by this run. **c** In the **Front Inlet** section, enter ***iii*first_trial3_scan.D** for the **Data File Name**, where ***iii*** are your initials. **d** Enter the **Vial** location number in the auto sampler tray. **e** In the **Method Sections to Run** section, select **Data Acquisition**. **f** Click the **OK and Run Method** button.	• The **Start Run** dialog box shown in Figure 5 is displayed. • The method is sent to the GC and the 7000 Series. When the instruments are ready the sample is injected and the data is collected and sent to the data directory specified.

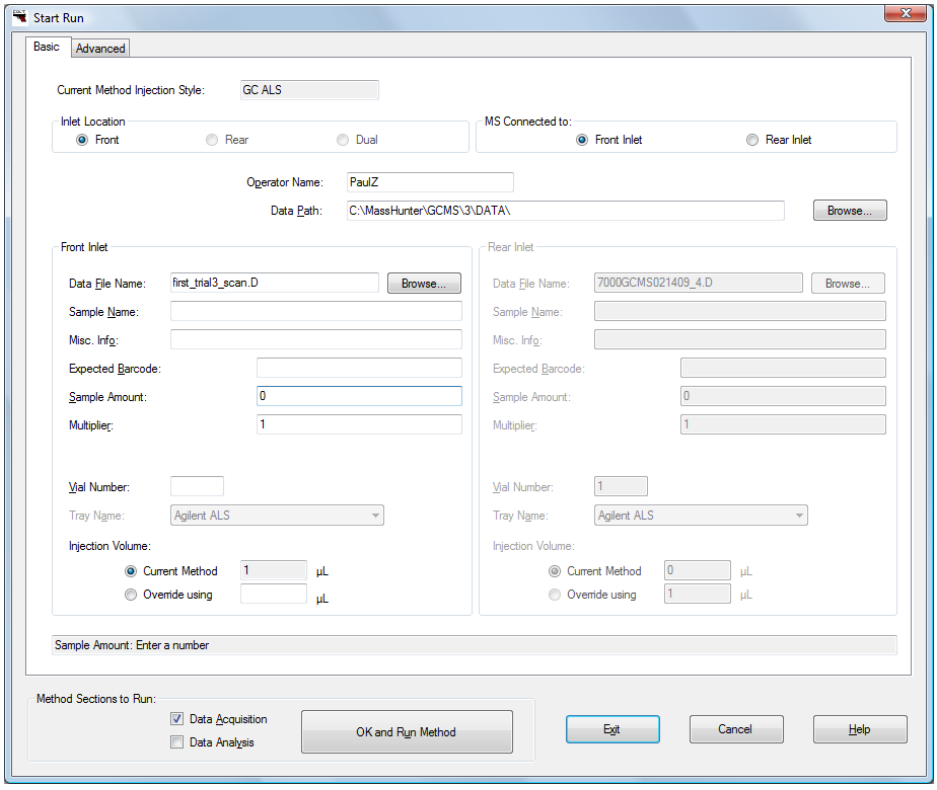


Figure 5 Start Run dialog box

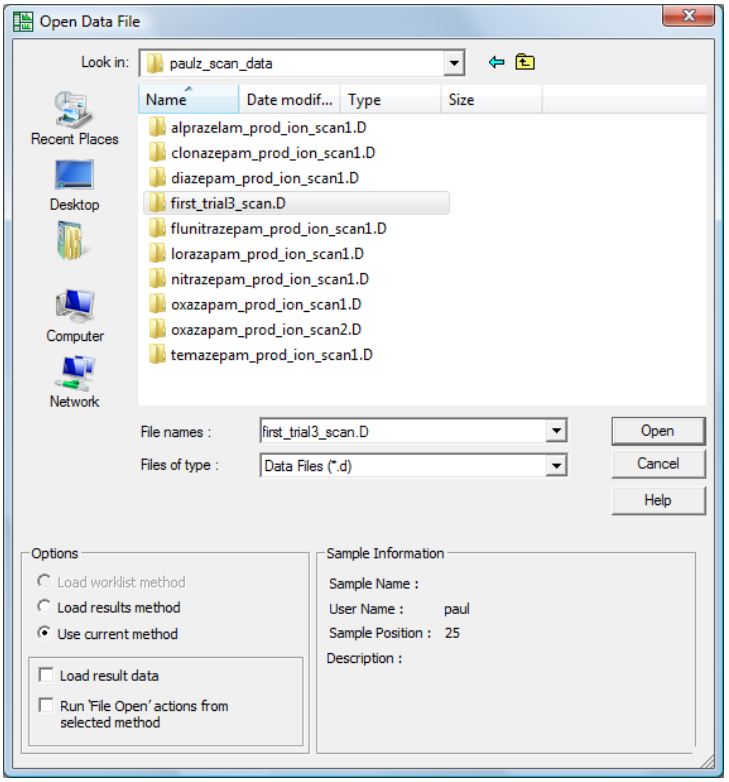
Task 5. Determine precursor ion masses

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

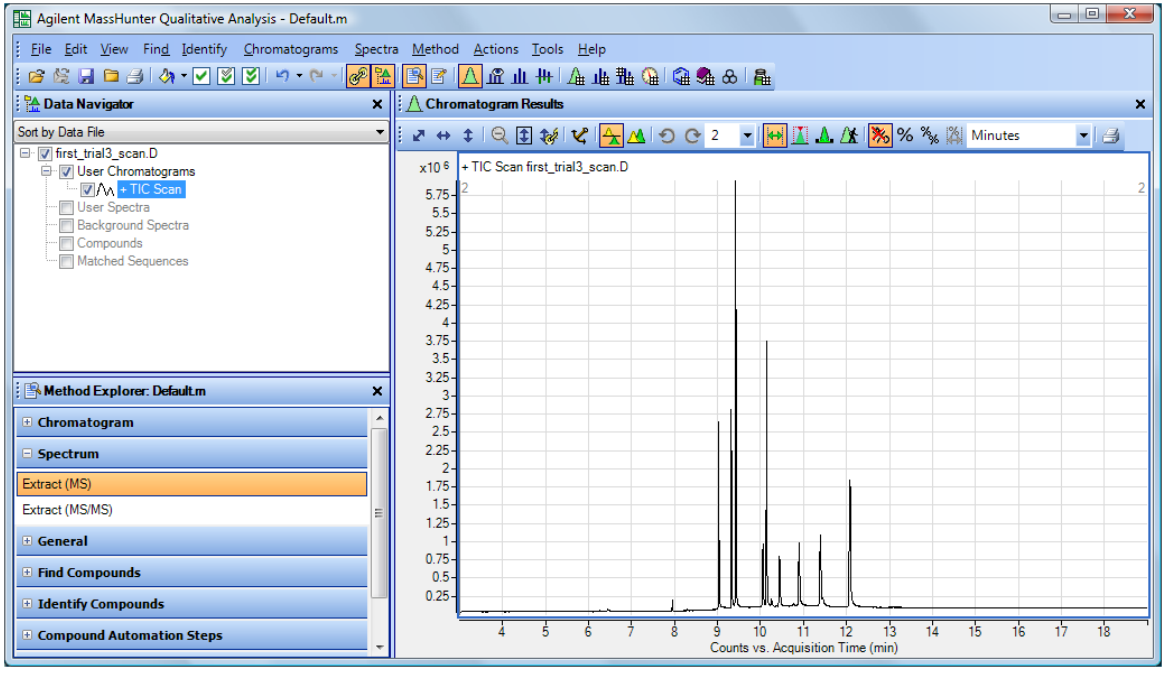
Steps	Detailed instructions	Comments
5 Open the acquired data file. • In the Qualitative Analysis program, open either the example file, **first_trial3_scan.D**, or the data file you created in "Task 1. Set the inlet and injection parameters" on page 5.	**a** Double-click the **Qualitative Analysis** icon. The program displays the **Open Data File** dialog box.	• 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**.



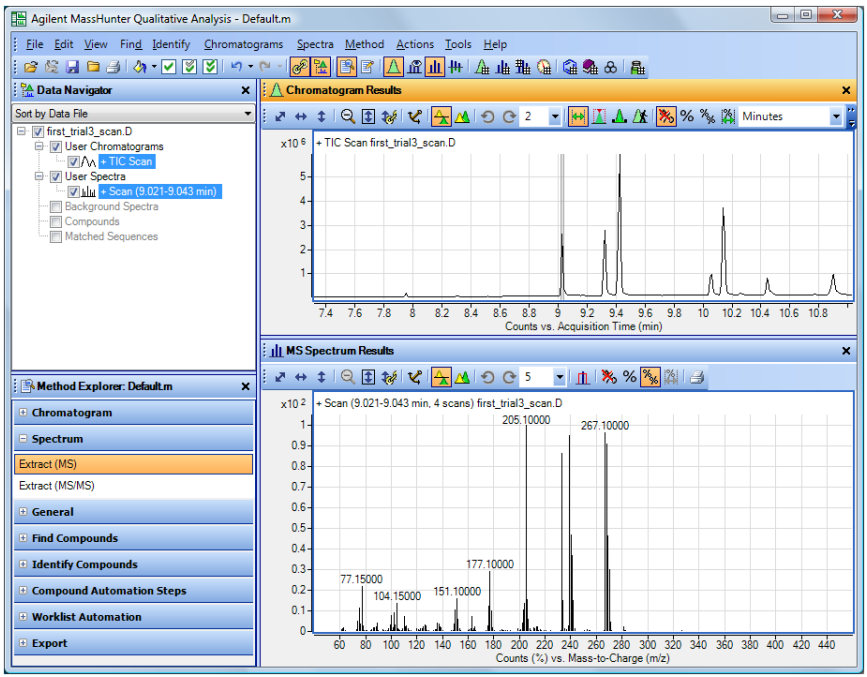
Steps	Detailed instructions	Comments
	b Do one of the following: • Select the example data file **first_trial3_scan.D**, and click **Open**. • Select the data file you created in "Task 4. Acquire precursor ion scan data (Optional)" on page 13, and click **Open**. By default, the system displays the Total Ion Chromatogram (TIC).	• 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.



Steps	Detailed instructions	Comments

• Determine precursor ion masses for all eight peaks.
  • 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.
	c In the **Chromatogram Results** window, the **Range Select** icon in the toolbar is selected.
	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.
	e Right-click inside the shaded area, and select **Extract MS Spectrum** from the shortcut menu.
	f Repeat **step d** through **step e** for the other compounds.
	The precursor ion masses should match those in the table in step 2.
	g Click **File > Close Data File**.
	h When asked if you want to save the results, click **No**.
		• 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.

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



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 7000 Series 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
6 Enter MS parameters appropriate for finding the Oxazepam product ion and save the method as ***iii*paul1_Oxazepam_prod_ion_Scan2.M**, where ***iii*** are your initials. Create additional methods for the other 7 compounds using the data from the previous task.	**a** From the Data Acquisition workstation open the ***iii*paul1_MS_Scan.M** method, where ***iii*** are your initials. **b** Click the **QQQ Method Editor** icon. **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**. **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**. **e** Set the **MS1 resolution** to **Wide**. **f** In the **Scan parameters** section, enter a **Step size** of 0.1 amu and select **Profile data**. **g** In the **Scan segments** section, right-click a cell and select **New Scan Segment** from the context menu. **h** Repeat the above step three times.	• 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 7000 Series.

Steps	Detailed instructions	Comments
	i Change the **Collision energy** to 15 for the second scan segment. **j** Change the **Collision energy** to 25 for the third scan segment. **k** Change the **Collision energy** to 35 for the fourth scan segment. **l** Click **OK** to close the window. **m** From the main window select **Method > Save Method As** and save the method as ***iii*paul1_Oxazepam_prod_ion_Scan2.M**, where ***iii*** are your initials. **n** Click the **QQQ Method Editor** icon. **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.	• 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
*iii*paul1_Oxazepam_prod_ion_Scan2.M	Oxazepam	239.1	50	275	300	5, 15, 25, 35
*iii*paul1_Lorazepam_prod_ion_Scan1.M	Lorazepam	274.1	50	280	300	5, 15, 25, 35
*iii*paul1_diazepam_prod_ion_Scan1.M	Diazepam	283.1	50	290	300	5, 15, 25, 35
*iii*paul1_flunitrazepam_prod_ion_Scan1.M	Flunitrazepam	312.2	50	315	300	5, 15, 25, 35
*iii*paul1_temzepam_prod_ion_Scan1.M	Temazepam	271.1	50	280	300	5, 15, 25, 35
*iii*paul1_nitrazepam_prod_ion_Scan1.M	Nitrazepam	253.1	50	270	300	5, 15, 25, 35
*iii*paul1_clonazepam_prod_ion_Scan1.M	Clonazepam	314.1	50	320	300	5, 15, 25, 35
*iii*paul1_alprazelam_prod_ion_Scan1.M	Alprazolam	308.2	50	320	300	5, 15, 25, 35

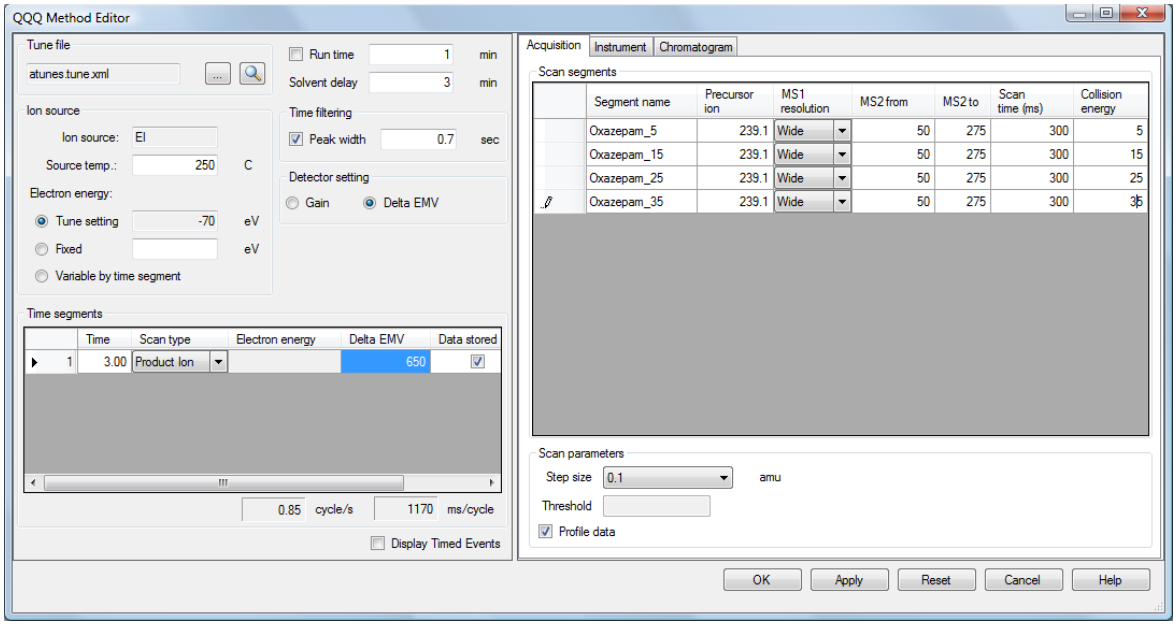


Figure 6 Oxazepam 7000 Series acquisition method for determining the product ion

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
7 Set up and run a sequence (optional). • Name the data files ***iiixx*_prod_ion_scan1.d**, where ***iii*** are your initials and ***xx*** is the compound name.	**a** Click the **Sequence Edit** icon to display the **Sequence Table**. **b** Use the drop-down list next to the **New Sample(s)** button in the **Sequence Table** toolbar and select **5 Samples**. **c** Enter a sample **Name**, **Vial** ALS location number, **Method File** name, and **Data File** name for each sample as shown in Figure 7. **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. **e** Click the **OK** button. **f** Select **Sequence > Save Sequence As** to save the sequence as **BenzoPI.Sequence.xml**. **g** Place eight sample vials in the specified location of the ALS tray. **h** Click the **Sequence Run** icon to display the **Start Sequence** dialog box. **i** If needed, add a sequence **Comment**, change the **Data File Directory** and click the **Run Sequence** button.	• This step is optional because you can use the six example data files in the next step. • The table now contains 8 sample lines.

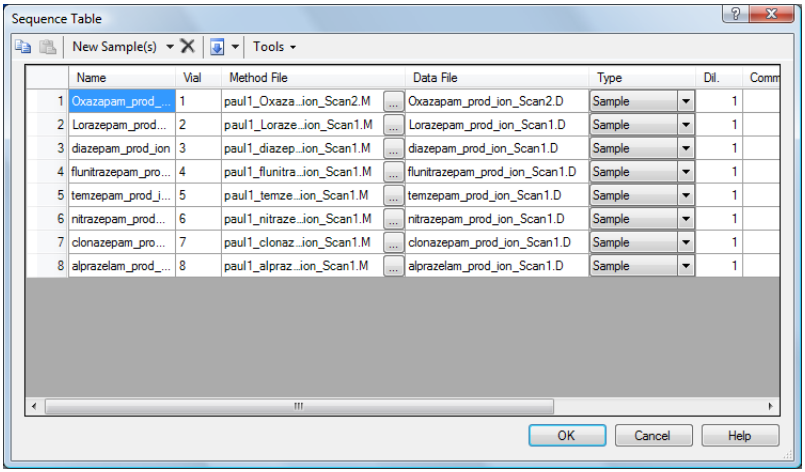


Figure 7 Sequence Table values

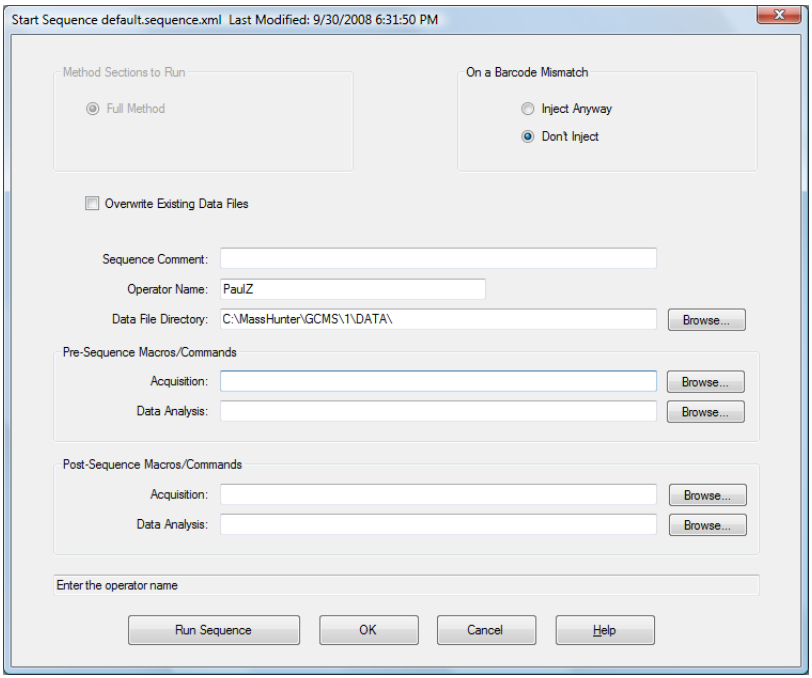


Figure 8 Start Sequence dialog box

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 Open the acquired data files in the Qualitative Analysis program.	**a** Double-click the **Qualitative Analysis** icon.  The program displays the **Open Data File** dialog box.	• 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***.
	b Do one of the following: • 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**.	• Use the **CTRL** key to select multiple data files.
	c In the **Data Navigator**, clear the check boxes for all compounds except **Oxazepam**.	• Only a single acquisition file at a time is displayed.
	d From the **MS Spectra Results** window toolbar, enter **5** from the drop-down menu.	• A maximum of 5 spectrum panes can be displayed.

Steps	Detailed instructions	Comments
	e In the **Chromatogram Results** window, the **Range Select** icon in the toolbar is selected.	
	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.	• For Oxazepam this peak is at about 9 minutes.
	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.	• 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.

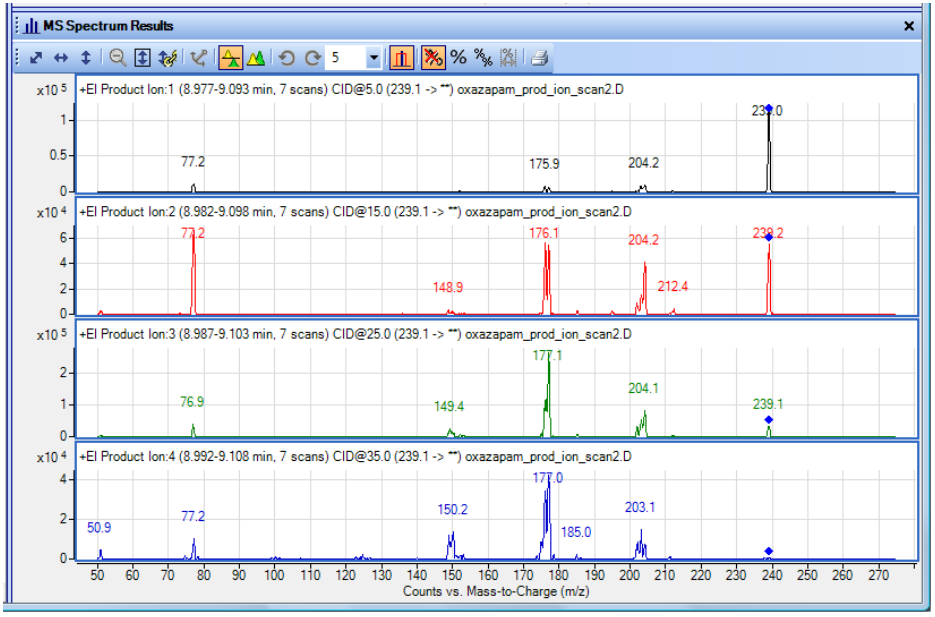


Figure 9 Extracted spectra for 4 collision energy scans showing product ion selected

Steps	Detailed instructions	Comments

• Determine product ion masses for all eight peaks.
  • 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.
	h The product ion for the first compound, **Oxazepam**, is determined to be m/z 177.1 at CID 25.
	i Clear the check box for the current data file in the **Data Navigator**.
	j Select the check box for the next data file.
	k Repeat **step f** through **step j** for the other compounds.
	The product ion masses should match those in Table 3.
	l Click **File > Close Data File**.
	m When asked if you want to save the results, click **No**.
		• 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.
		
		Here the best ion selection has a count of 100 compared to other ions with a count of 25 maximum in any other spectrum.

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

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.	**a** From the Data Acquisition workstation open the ***iii*paul1_MS_Scan.M** method, where ***iii*** are your initials. **b** Click the **QQQ Method Editor** icon. **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**. **d** In the **Scan segments** section, enter **Wide** for the **MS1 Resolution** and **MS2 Resolution**, and **50** for the **Dwell**. **e** In the **Scan segments** section, right-click a cell and select **New Scan Segment** from the context menu. **f** Repeat the above step seven times. **g** Fill in the rest of the **Scan Segments** section to match Figure 10 on page 27. **h** Click **OK** to close the window. **i** From the main window select **Method > Save Method As** and save the method as ***iii*paul1_benzo_mrm_1.M**, where ***iii*** are your initials.	• The GC acquisition parameters from this method are used unchanged. The 7000 Series method parameters are edited in this procedure to create the MRM method.

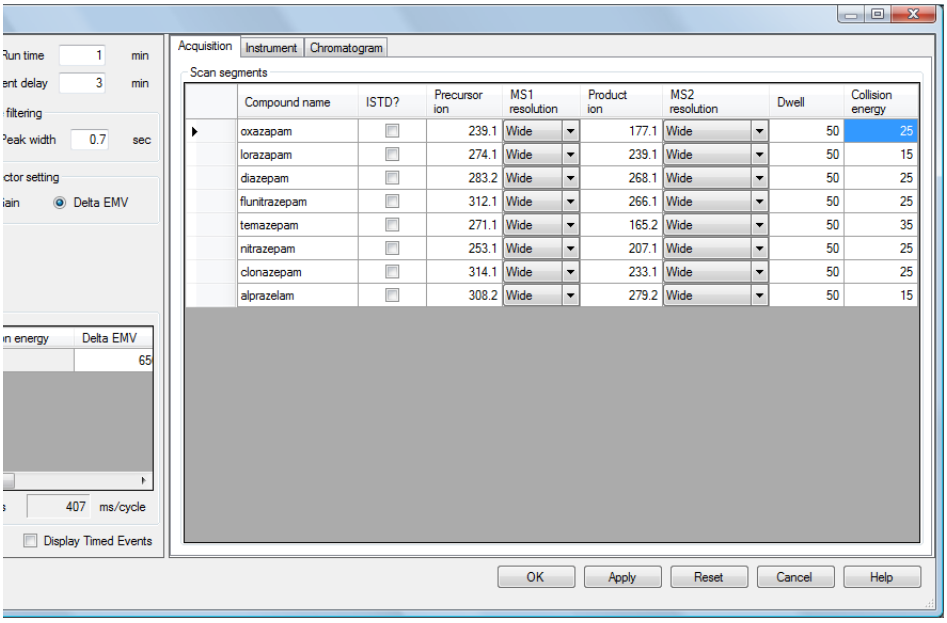


Figure 10 Scan section completed for an MRM method

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
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.	**a** From the Data Acquisition workstation load the ***iii*paul1_benzo_mrm_1.M** method. **b** Click the **Edit Sequence** icon to display the **Sequence Table**. **c** **Add** or **Delete** sample entries so that the **Sequence Table** contains five sample lines. **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. **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. **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. **g** Change _L1_ part of the **Data File** name to follow the pattern shown in Figure 11 on page 30. **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. **i** Click **OK** to close the window.	• 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.

Steps	Detailed instructions	Comments
	j Select **Sequence > Save Sequence As** to save the sequence as **BenzoCalibration.Sequence.xml**. **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. **l** Click the **Sequence Run** icon to display the **Start Sequence** dialog box. **m** If needed, add a sequence **Comment**, change the **Data File Directory** and click the **Run Sequence** button.	• The remaining steps are optional. These sample vials are prepared in "Prepare the samples required for data acquisition" on page 3.

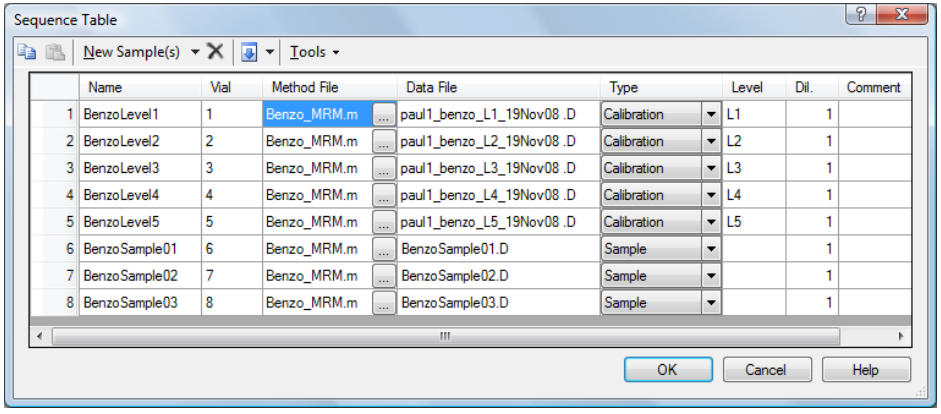
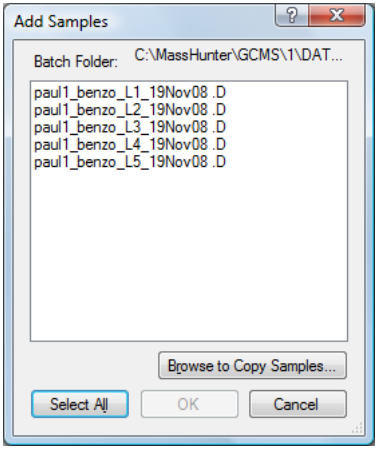


Figure 11 Sequence for Benzo calibration samples and unknowns

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
11 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 5 calibration files, and enter the batch name **BenzoCalibrationData** and click the **Open** button.	• 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.
• Add samples to the batch.	**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**. 	• 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.

Steps	Detailed instructions	Comments
	d Click the **OK** button. The **Batch Table** now contains the 5 samples. **e** If necessary, change the **Type** to **Cal**, sort on the **Name** column, and enter the values shown in the **Level** column. **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.	• The batch now requires a data analysis method to automatically analyze the sample.

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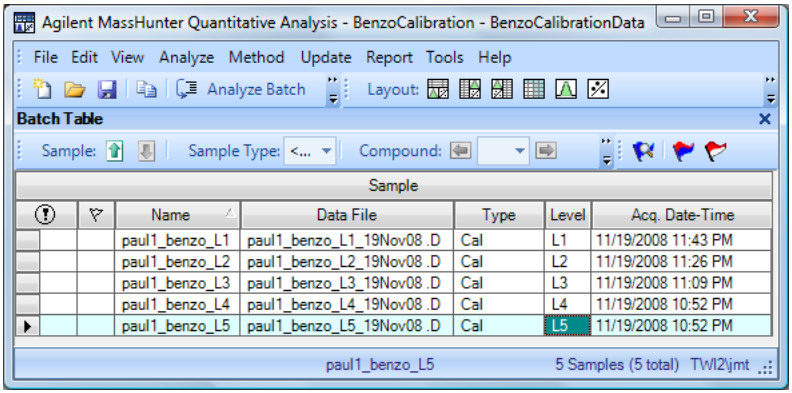
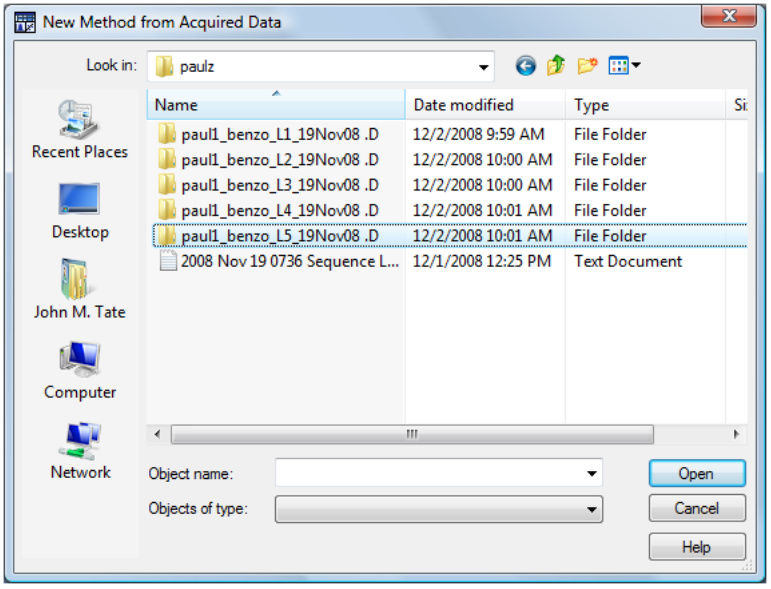
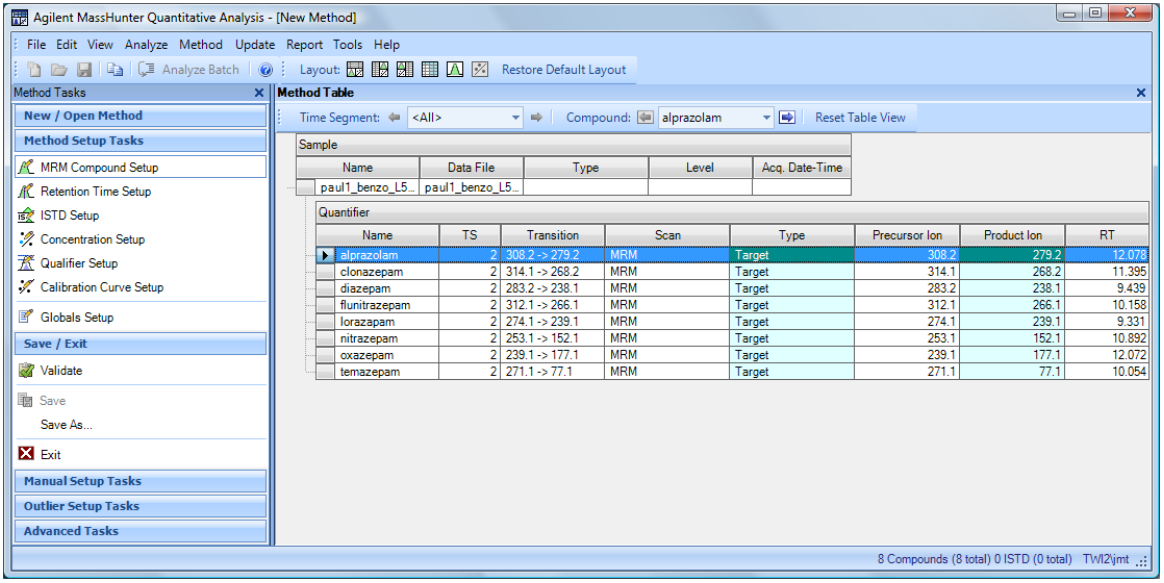


Task 12. Create an MRM quantitative analysis method

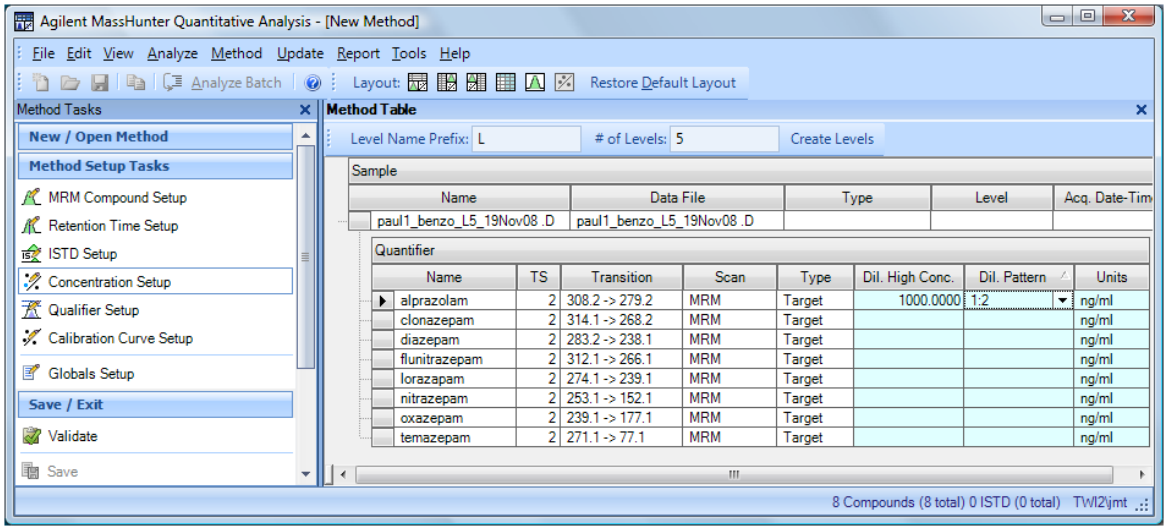
Steps	Detailed instructions	Comments
12 Create a Data Analysis method.	**a** From the main menu, select **Method > New > New Method from Acquired MRM Data** to display the **New Method from Acquired Data** dialog box.	• The sample data stored in the acquisition method created in "Task 9. Create an MRM method" on page 26 is used to automatically fill in parts of the data analysis method.
	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.	• 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.



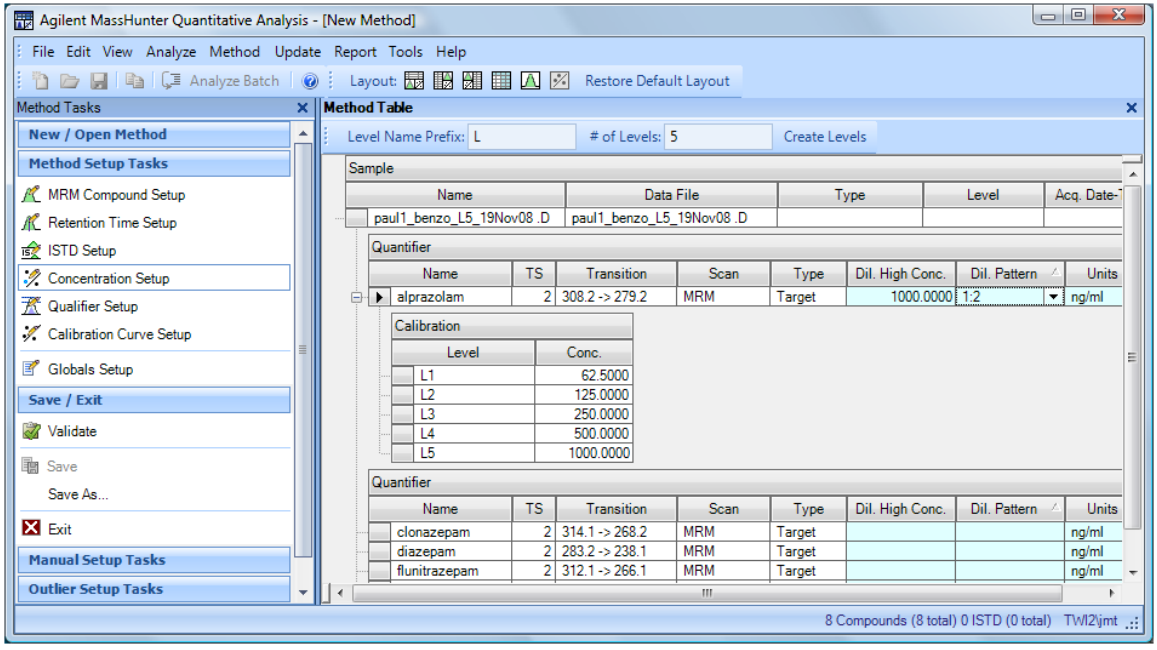
Steps	Detailed instructions	Comments
	c In the **Method Tasks** pane, click **Method Setup Tasks**, then click **MRM Compound Setup** to display the **Method Table**.	• Review the eight compounds that were originally included in the acquisition method.



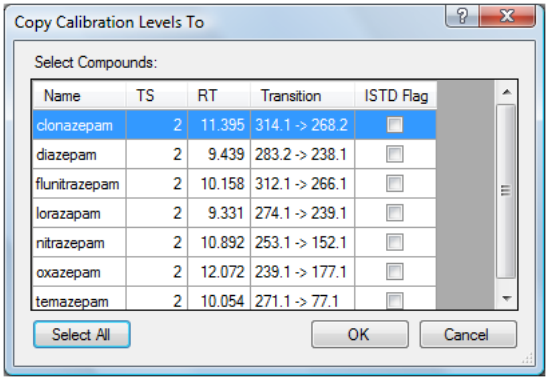
Steps	Detailed instructions	Comments
• Create a calibration table.	**d** In the **Method Tasks** pane, click **Method Setup Tasks**, then click **Concentration Setup**. **e** In the **Dil. High Conc.** column for the first compound in the table enter **1000.0** then select **1:2** from the drop-down list for the **Dil. Pattern** column.	• 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.



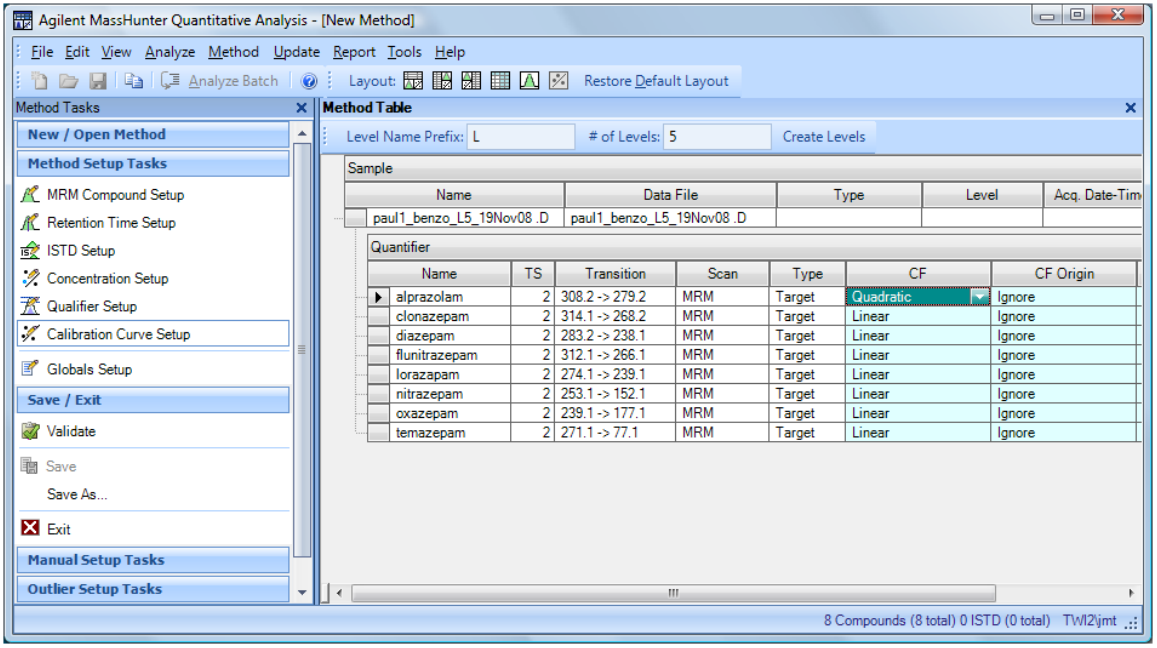
Steps	Detailed instructions	Comments
	f In the **Method Table** toolbar, enter **L** for a **Level Name Prefix**, and **5** for the **# of Levels** then click the **Create Level** button.	• The calibration table containing a concentration entry for each of the 5 calibration levels is shown below for the first compound in the method table.

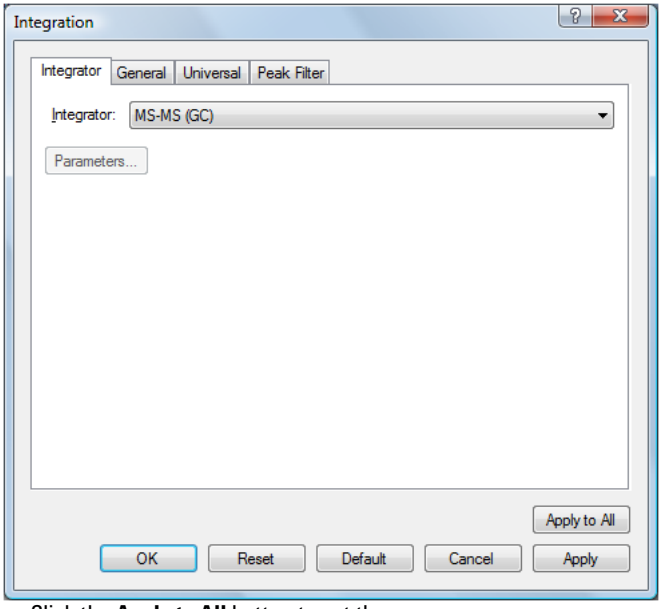
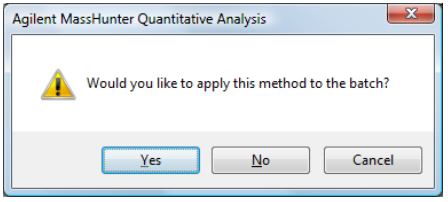


Steps	Detailed instructions	Comments
• Copy the calibration table to other quantifiers.	**g** Select the first compound in the **Quantifier Table**, right-click the compound and select **Copy Calibration Levels To** from the shortcut menu.	• The **Copy Calibration Levels To** dialog box is displayed. Use this to copy the calibration table from the current compound to selected or all other compounds.
	h Click the **Select All** button to select all compounds in the dialog box then click the **OK** button.	• This calibration table is copied to the **Quantifier** table of all eight compounds.

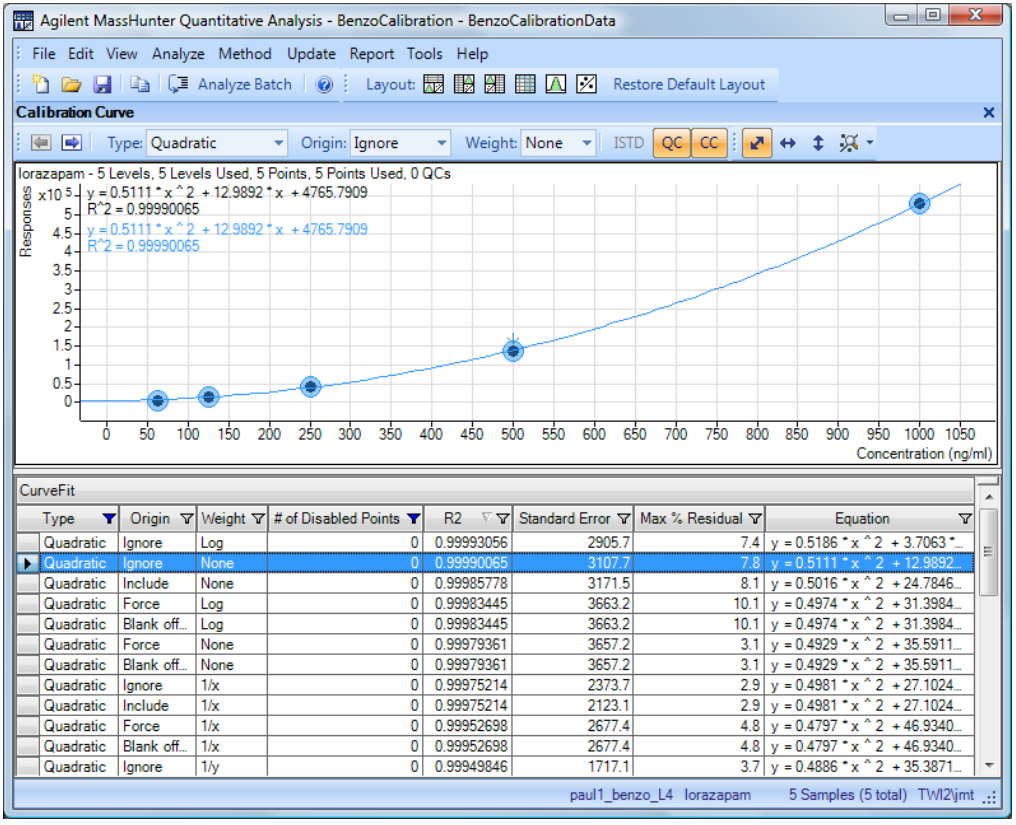


Steps	Detailed instructions	Comments
• Calibration Curve Setup	**i** In the **Method Tasks** pane, click **Method Setup Tasks**, then click **Calibration Curve Setup**. **j** In the **Quantifier** table **CF** column of the first compound select **Quadratic** from the drop-down list. With this cell selected, right-click and select **Fill Down** from the short-cut menu.	• 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**.

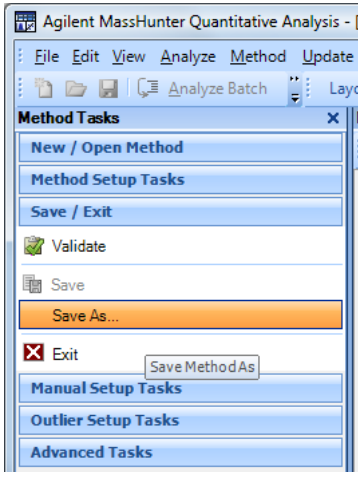


Steps	Detailed instructions	Comments
• Select an Integrator.	**k** In the **Method Tasks** pane, click **Advanced Tasks**, then click **Integration Parameters Setup**. **l** In the **Method Table** pane, for any **Quantifier** click the **Int.** column entry to display the **Integration** dialog box and select **MS-MS (GC)** from the **Integrator** drop-down list.	• 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.
		
	m Click the **Apply to All** button to set the **Integrator** for all quantifier peaks to this value. **n** Click **OK** to close this dialog box.	• This integrator is used for integrating all peaks.
• Exit the method editor and save the method.	**o** In the **Method Tasks** pane, click **Save/Exit**, then click **Exit**.	
		

Steps	Detailed instructions	Comments
• Apply the data analysis method to the batch.	**p** Click the **Yes** button. **q** Click the **Analyze Batch** button to quantitate all samples in the batch.	• 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.
• Review the calibration curve for each compound.	**r** In the calibration curve pane, right-click and select **Curve Fit Assistant** from the short-cut menu. **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**.	• 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.



Steps	Detailed instructions	Comments
	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. **u** In the **Batch Table** toolbar, click the right arrow to select the next calibrated compound and repeat steps **q** through **s**. **v** Repeat the above step until all eight calibrated compounds are reviewed and corrected if necessary.	• 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.
	w Click the **Analyze Batch** button to quantitate all samples in the batch.	• This step is only necessary if you changed any CurveFit equation during the review above.
• Save the Data Analysis method .	**x** From the main menu select **Method > Edit** to display the **Method Editor**. **y** In the **Method Tasks** pane, click **Save / Exit**, then click **Save As**. **z** Enter **BenzoDA** for the method name and click the **Save** button.	



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.	• The Quantitative Analysis Workstation opens. • This is the directory included on the program disk. Just copy these sample data files to this directory.
• 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.	• 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.	• The method is applied to the batch. • Print one or more reports.

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