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

Familiarization Guide

Notices

Manual Part Number

G7003-90010

Edition

First edition, January 2019

Printed in USA

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5301 Stevens Creek Boulevard
Santa Clara, CA 95051

Warranty

Technology Licenses

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Contents

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 shipped with MassHunter (See the **Reference material** on page 6).

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, "Triple Quad and Sensitivity", in the *Concepts Guide*. See the online Help for detailed information on how the program works.

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.

Reference material

Available with the installation of the MassHunter Software User Information, the following documentation is delivered to you as part of your MassHunter Software.

- *MassHunter Qualitative Analysis Familiarization Guide for GC/MS* for an introduction to many Qualitative Analysis program features that are not covered in this guide including the Qualitative Analysis Navigator program.

- *Qualitative Analysis Training Videos* for those seeking visual and audio lessons presenting a comprehensive use of the MassHunter Qualitative Analysis Navigator and MassHunter Qualitative Workflows.

- *Online Help* for detailed information on how Qualitative Analysis works.

- *Demo data files and a library file* that allow you to perform all the analysis steps demonstrated here using your own installation of MassHunter without acquiring compound data or owning a library license. These files are located in the **TripleQuadData\Benzodiazepine\Data** folder.

- *Quick Start Guide* explains what documentation is included in the applications and what information is in each document.

Available on the 7000 Series Triple Quad GC/MS installation of the User Manuals and Tools DVD, the following documentation is delivered to you with your instrument.

- *Concepts Guide* to learn more about how the 7000 Series Triple Quad GC/MS System works.

- *Quick Start Guide* explains what documentation is included in the applications and what information is in each document.

- *Hardware manuals* to learn how to operate and perform maintenance on the 7000 Series Triple Quad GC/MS.

Prepare your system

1   Check that:

- The Data Acquisition, Qualitative Analysis, and Quantitative Analysis programs are installed.
- Your system uses an Agilent 8890 or 7890 Series GC with a 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   If needed, copy the *Demo data files and library* noted in **Reference material** on page 6 to any location on your hard disk. The data file and library file are needed for this exercise if you are not acquiring data or you do not have a library of the compounds used in this example.

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, part number B-033.
- Acetonitrile for sample dilution
- Sample vials

Procedure

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 five 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 three 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

Set up the inlet, injection source, and enable the 7000 Series.

1   Double-click the Data Acquisition icon on the windows desktop.
    The **Data Acquisition** window shown in **Figure 1** on page 10 is displayed.

2   Click the **Inlet and Injection Parameters** icon.
    The **Inlet and Injection Parameters** dialog box shown in **Figure 2** on page 10 is displayed.

3   Select **GC** for the sample inlet and the installed ALS for the injection source.

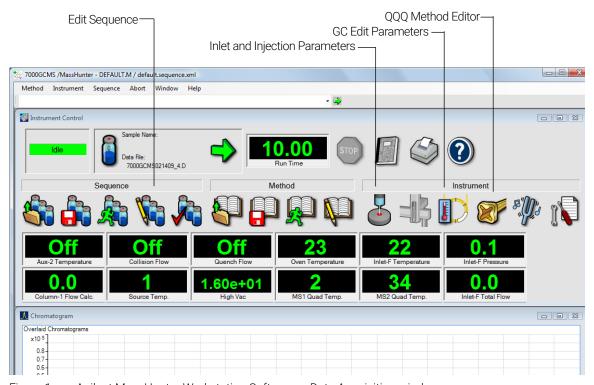4   Select the **Use MS** check box.

Figure 1.   Agilent MassHunter Workstation Software – Data Acquisition window
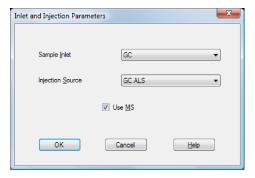
Figure 2.   Inlet and Injection Parameters

Task 2. Enter GC acquisition parameters

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

Procedure

Enter GC parameters appropriate for the Benzodiazepine mix. See **Table 1** on page 12.

1 Click the **GC Edit Parameters** icon (**Figure 1** on page 10).
   The GC edit parameters window shown in **Figure 3** on page 12 is displayed.

2 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.

3 Select control mode **On** and then select **Flow** mode. Enter **1.2 mL/min** for the initial **Value** and the **Post Run** value.

4 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.

5 Select the collision cell **He EPC** module in the **Description** column and clear control **On**.

6 Select the **Inlets** icon then the **SSL** tab and enter the inlet parameters listed in **Table 1** on page 12.

7 Select the **Oven** icon and enter the oven parameters listed in **Table 1** on page 12.

8 Select the **ALS** icon then the **Front Injector** tab and enter the injector parameters listed in **Table 1** on page 12. If your ALS is attached to the Back Inlet select the Back Injector tab.

9 Select the **Aux Heaters** icon, enable, and set the temperature to 134 °C.

10 Select the **OK** button. The GC parameters are downloaded to the GC and the window closes.
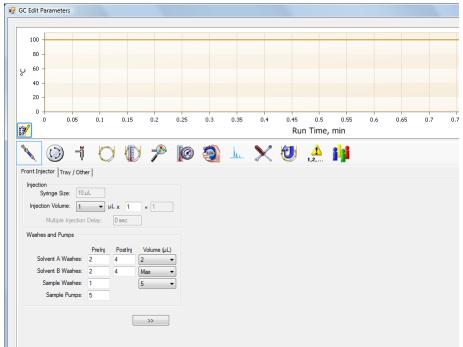
Figure 3.   GC Edit Parameters window

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
Pressure	**On** Value automatically set with CFT Setting column flow
Septum Purge Flow	**On** 3 mL/min

Table 1   GC parameters for data acquisition method (continued)

Parameter	Value
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
Dwell Time (PostInj)	0 min
Solvent Wash Draw Speed	300 µL/min
Solvent Wash Dispense Speed	6000 µL/min

Table 1   GC parameters for data acquisition method (continued)

Parameter	Value
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 task, 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.

Procedure

Enter MS parameters appropriate for the Benzodiazepine mix, and save the method as **iiipaul1_MS_Scan.M**, where **iii** are your initials.

1 Click the **QQQ Method Editor icon** (Figure 1 on page 10**).**
The **QQQ Method Editor** window shown in **Figure 4** on page 16 opens.

2 Set the **Source temperature** to 250 °C, set the **Electron energy** to **Tune Setting**, the **Solvent delay** to four minutes, and the **Detector setting** to **Delta EMV**.

3 Set the **Time Filtering** to a **Peak width** of 0.7 seconds.

4 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**. The 7000 Series starts collecting data at four minutes due to the **Solvent delay** setting.

5 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**.

6 In the **Scan parameters** section, enter a **Step size** of 0.1 amu and a **Threshold** of 100.

7 Click **OK** to close the window.

8 From the main window select
Method > Save Method As and save the method as *iii*paul1_MS_Scan.M,
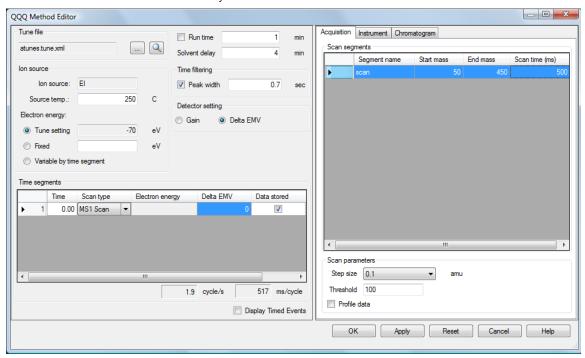where *iii* are your initials.

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.

Procedure

To acquire data, first name the data file *iii*first_trial3_scan.D, where *iii* are your initials, then designate a directory path to hold your data files and method:

1   Click the **Start Run (green arrow)** icon.
    The Start Run dialog box shown in **Figure 5** on page 18 is displayed.

2   In the **Data Path** enter the directory to save the data file that is acquired by this run.

3   In the **Front Inlet** section, enter *iii*first_trial3_scan.D for the **Data File Name**, where *iii* are your initials.

4   Enter the **Vial** location number in the auto sampler tray.

5   In the **Method Sections to Run** section, select **Data Acquisition**.

6   Click the **OK** and **Run Method** button.

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.
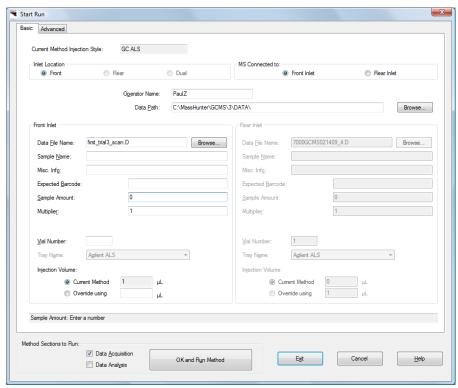

Figure 5.   Start Run dialog box

Task 5. Determine precursor ion masses

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

Procedure

Configure the Qualitative Analysis user interface for this task, open the data file in Qualitative analysis, and determine the 8 precursor ion masses.

1 Double-click the **Qualitative Analysis** icon.
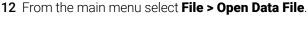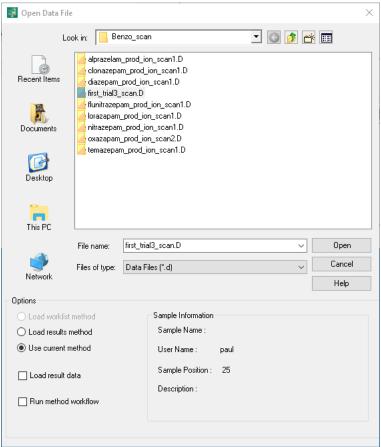
   The system displays the **Open Data File** dialog.

2 Click **Clear** to close the dialog without opening a data file.

3 From the main menu select **Configuration > Show Advanced Settings** if it is missing a check mark next to it.

4 From the main menu select **Configuration > Window Layouts > Load Default Layout**.

5 From the main menu select **Method > Open**.

6 Select default-GCMS.m and click **Open**.

7 From the main menu, select **Configuration > User Interface Configuration**.

   The User Configuration dialog opens. The settings selected here are based on the data file loaded and the default method previously selected. You may edit these if required.

8 Click **OK** to close the dialog.

9 Click **Navigator View** on the toolbar.

10 On the **Chromatogram Results** toolbar, click **List Mode**.

11 Click the down arrow next to **Maximum Number of List Panes** on the **Chromatogram Results** toolbar, and select 1.

12 From the main menu select **File > Open Data File**.
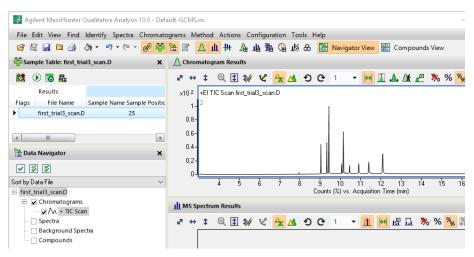
13 Do one of the following:

- Select the example data file **first_trial3_scan.D**.
- Select the data file you created in **Task 4. Acquire precursor ion scan data (Optional)** on page 17.

14 If selected, clear the **Load result data** check box.

The **Load result data** 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.
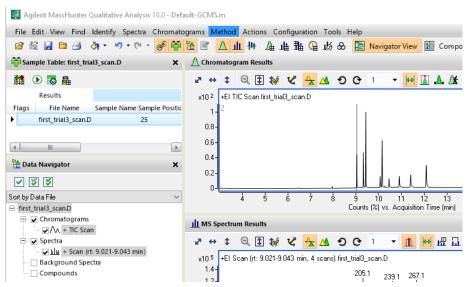
15 Click **Open**.

The figure below shows the default layout. This is what you want to see.

By default, the system displays the Total Ion Chromatogram (TIC).

16 In the **Chromatogram Results** window, the **Range Select** icon in the toolbar 〔↔〕 is selected.

17 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.

18 Right-click inside the shaded area, and select **Extract MS Spectrum** from the shortcut menu. An averaged spectrum from the highlighted area inside the peak is displayed in the **MS Spectrum Results** window.

19 Repeat **step 17** through **step 18** for the other compounds.

20 The precursor ion masses should match those in the table below. 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

21 Click **File > Close Data File**.

When asked if you want to save the results, click **No**.

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 15 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.

Procedure

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 seven compounds using the data from the previous task:

1   From the Data Acquisition workstation open the *iii***paul1_MS_Scan.M** method, where iii are your initials. This method was previously saved in Task 3 and the GC acquisition parameters provide excellent compound separation.

2   Click the **QQQ Method Editor** icon. The **QQQ Method Editor** window opens.

3   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**.

4   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**.

5   Set the **MS1** resolution to **Wide**.

6   In the **Scan parameters** section, enter a **Step size** of 0.1 amu, and select **Profile data**.

7   In the **Scan segments** section, right-click a cell, and select **New Scan Segment** from the context menu. A new scan segment with all values from the first scan segment is created.

8   Repeat the above step three times. A maximum of four scan segments is supported on the 7000 Series.

9   Change the **Collision energy** to 15 for the second scan segment. A total of four scan segments, each with a different collision energy, is required for optimizing product ion sensitivity and selectivity.

10 Change the **Collision energy** to 25 for the third scan segment.

11 Change the **Collision energy** to 35 for the fourth scan segment.

12 Click **OK** to close the window.

13 From the main window select
Method > Save Method As and save the method as
iiipaul1_Oxazepam_prod_ion_Scan2.M, where **iii** are your initials.

14 Click the **QQQ Method Editor** icon.

15 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. 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
iiipaul1_Oxazepam_prod_ion_Scan2.M	Oxazepam	239.1	50	275	300	5, 15, 25, 35
iiipaul1_Lorazepam_prod_ion_Scan1.M	Lorazepam	274.1	50	280	300	5, 15, 25, 35
iiipaul1_diazepam_prod_ion_Scan1.M	Diazepam	283.1	50	290	300	5, 15, 25, 35
iiipaul1_flunitrazepam_prod_ion_Scan1.M	Flunitrazepam	312.2	50	315	300	5, 15, 25, 35
iiipaul1_temzepam_prod_ion_Scan1.M	Temazepam	271.1	50	280	300	5, 15, 25, 35
iiipaul1_nitrazepam_prod_ion_Scan1.M	Nitrazepam	253.1	50	270	300	5, 15, 25, 35
iiipaul1_clonazepam_prod_ion_Scan1.M	Clonazepam	314.1	50	320	300	5, 15, 25, 35
iiipaul1_alprazelam_prod_ion_Scan1.M	Alprazolam	308.2	50	320	300	5, 15, 25, 35
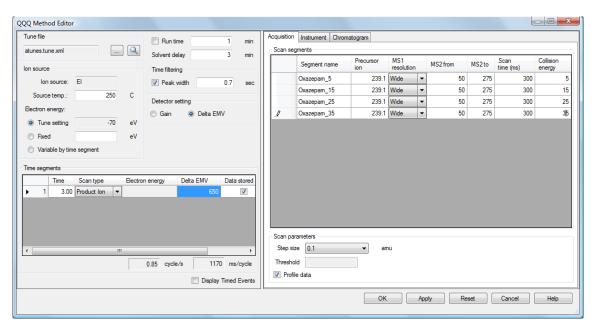
Figure 6.   Oxazepam 7000 Series acquisition method for determining the product ion

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.

Procedure

To set up and run a sequence, name the data files *iii***xx_prod_ion_scan1.d**, where *iii* are your initials and *xx* is the compound name:

1 Click the **Sequence Edit** icon to display the **Sequence Table**.

2 Use the drop-down list next to the **New Sample(s)** button in the **Sequence Table** toolbar and select **5 Samples**. This step is optional because you can use the six example data files in the next step. The table now contains eight sample lines.

3 Enter a sample **Name**, **Vial ALS** location number, **Method File** name, and **Data File** name for each sample as shown in **Figure 7**.

4 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.

5 Click the **OK** button.

6 Select **Sequence > Save Sequence As** to save the sequence as **BenzoPI.Sequence.xml**.

7 Place eight sample vials in the specified location of the ALS tray.

8 Click the **Sequence Run** icon to display the **Start Sequence** dialog box.

9 If needed, add a sequence **Comment**, change the **Data File Directory**, and click the **Run Sequence** button.
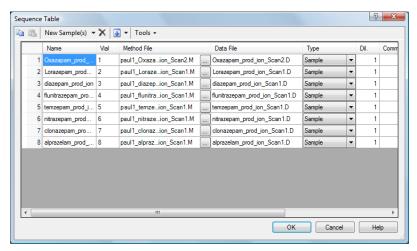
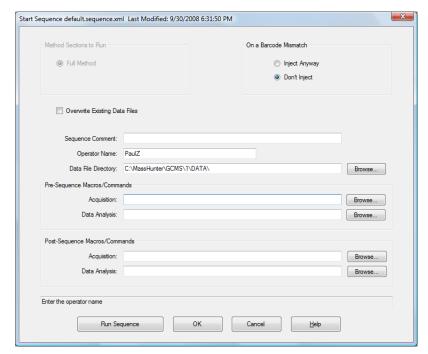Figure 7.   Sequence Table values

Figure 8.   Start Sequence dialog box

Task 8. Determine the product ions

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

Procedure

Open the acquired data files in the Qualitative Analysis program and determine product ion masses for all eight peaks.

1   Double-click the **Qualitative Analysis** icon.

    The program displays the **Open Data File** dialog. 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.***

2   Do one of the following:
    • Select the eight example data files and click **Open**. Use the **CTRL** key to select multiple data files.
    • Select all the data files you acquired in **Task 7. Acquire scan data for finding product ions (Optional)** on page 26, and click **Open**.

3   In the **Data Navigator**, clear the check boxes for all compounds except **Oxazepam**. Only a single acquisition file at a time is displayed.

4   From the **MS Spectra Results** window toolbar, enter **5** from the drop-down menu. A maximum of five spectrum panes can be displayed.

5   In the **Chromatogram Results** window, the **Range Select** icon in the toolbar is selected.

6   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 nine minutes.

7   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

8   The product ion for the first compound, **Oxazepam**, is determined to be *m/z* 177.1 at CID 25.

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.

9   Determine product ion masses for the other seven peaks.

You have determined them correctly if you find the values are similar to those shown in **Table 3** on page 30. Close the data file after finding the precursor ion masses.

10 Clear the check box for the current data file in the **Data Navigator**.

11 Select the check box for the next data file.

12 Repeat **steps 6** through **step 11** for the other compounds.

13 Click **File > Close Data File**.
When asked if you want to save the results, click **No**.

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 task, you create an MRM method that finds any Benzo compound in a sample.

Procedure

Open the Data Acquisition program and create an MRM method using the data from **Table 3** on page 30:

1   From the Data Acquisition workstation open the *iii*paul1_MS_Scan.M **method**, 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.

2   Click the **QQQ Method Editor** icon.

3   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**.

4   In the **Scan segments** section, enter **Wide** for the **MS1 Resolution** and **MS2 Resolution**, and **50** for the **Dwell**.

5   In the **Scan segments** section, right-click a cell and select **New Scan Segment** from the context menu.

6   Repeat the above step seven times.

7   Fill in the rest of the **Scan Segments** section to match **Figure 10** on page 32.

8   Click **OK** to close the window.

9   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.
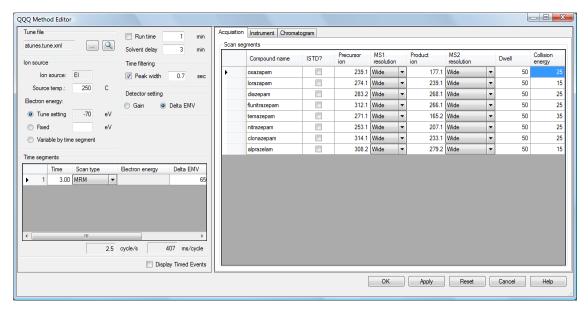
Figure 10.   Scan section completed for an MRM method

Exercise – Create a quantitative analysis method

Task 10. Acquire MRM data (Optional)

In this task, 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.

Procedure

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.

1   From the Data Acquisition workstation load the *iii*paul1_benzo_mrm_1.M method. This method was created in **Task 9. Create an MRM method** on page 31.

2   Click the **Edit Sequence** icon to display the **Sequence Table**.

3   Add or Delete sample entries so that the **Sequence Table** contains five sample lines. 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.

4   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 34.

5   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. The Vial location incremented in this way assumes that the vials are placed in consecutive locations in the sampler tray.

6 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.

7 Change **_L1_** part of the **Data File** name to follow the pattern shown in **Figure 11** on page 34.

8 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 34.

9 Click **OK** to close the window.

10 Select **Sequence > Save Sequence As** to save the sequence as **BenzoCalibration**.Sequence.xml.

11 Place the five sample vials containing known concentrations and the three samples containing the unknown concentrations in the specified locations of the ALS tray.

The remaining steps are optional. These sample vials are prepared in **Prepare the samples required for data acquisition** on page 7

12 Click the **Sequence Run** icon to display the **Start Sequence** dialog box.

13 If needed, add a sequence **Comment**, change the **Data File Directory** and click the **Run Sequence** button.
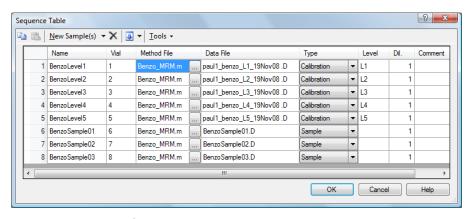
	Name	Vial	Method File	Data File	Type		Level	Dil.	Comment
1	BenzoLevel1	1	Benzo_MRM.m	paul1_benzo_L1_19Nov08 .D	Calibration	▼	L1	1	
2	BenzoLevel2	2	Benzo_MRM.m	paul1_benzo_L2_19Nov08 .D	Calibration	▼	L2	1	
3	BenzoLevel3	3	Benzo_MRM.m	paul1_benzo_L3_19Nov08 .D	Calibration	▼	L3	1	
4	BenzoLevel4	4	Benzo_MRM.m	paul1_benzo_L4_19Nov08 .D	Calibration	▼	L4	1	
5	BenzoLevel5	5	Benzo_MRM.m	paul1_benzo_L5_19Nov08 .D	Calibration	▼	L5	1	
6	BenzoSample01	6	Benzo_MRM.m	BenzoSample01.D	Sample	▼		1	
7	BenzoSample02	7	Benzo_MRM.m	BenzoSample02.D	Sample	▼		1	
8	BenzoSample03	8	Benzo_MRM.m	BenzoSample03.D	Sample	▼		1	

Figure 11.   Sequence for Benzo calibration samples and unknowns

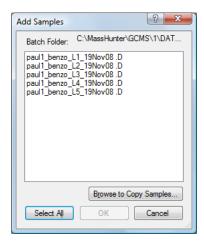# Task 11. Create a quantitative analysis batch

In this task, 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.

1   Double-click the **QQQ Quantitative Analysis icon on your window's desktop.** The Quantitative Analysis Workstation opens.

2   **Name the batch and assign a batch directory:** From the main menu, select **File > New batch,** use the **Look In** drop-down list to navigate to the directory containing the five calibration files, and enter the batch name **BenzoCalibrationData** and click the **Open** button.

This is the directory selected for the data files in "**Task 10. Acquire MRM data (Optional)** on page 33. If you skipped this data acquisition task you can substitute the five sample data files included on the program disk. Just copy these sample data files to this directory.

3   **Add samples to the batch:** 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 five 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.
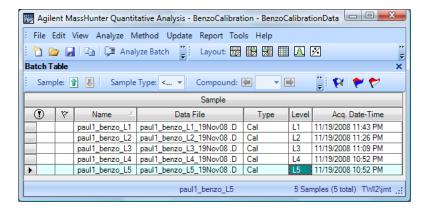
The **Type** for all five samples is **Cal** and the **Level** should range from **L1** to **L5**. The **Type** and **Level** are taken from the acquisition sequence **Table 11** on page 34.

4 Click the **OK** button. The Batch Table now contains the five samples.

The batch now requires a data analysis method to automatically analyze the sample.

5 If necessary, change the **Type** to **Cal**, sort on the **Name** column, and enter the values shown in the **Level** column.

6 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.

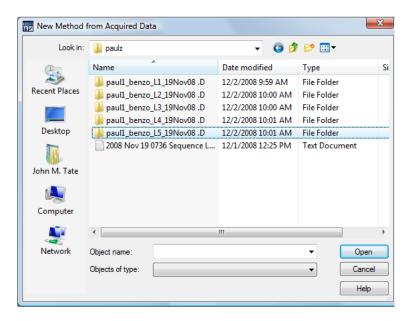# Task 12. Create an MRM quantitative analysis method

Step 1   Create a Data Analysis method.

1   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 31 is used to automatically fill in parts of the data analysis method.

2   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.
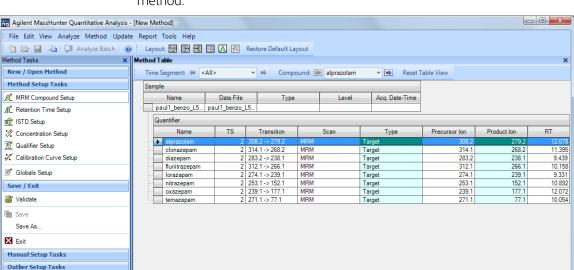
The program uses this sample data file to create much of the data analysis method, then presents the user with the method editor so that the method can be finished manually.

3   In the **Method Tasks** pane, click **Method Setup Tasks,** then click **MRM Compound Setup** to display the Method Table.
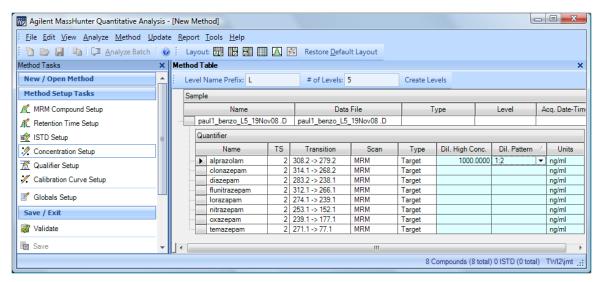
Review the eight compounds that were originally included in the acquisition method.
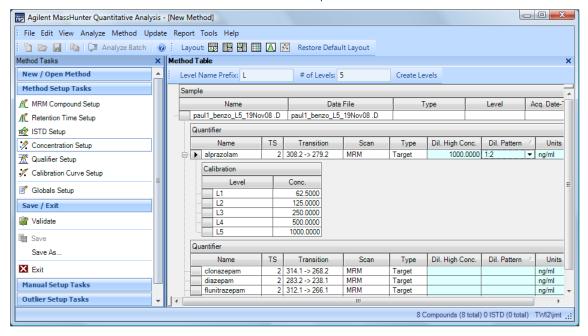

Step 2 Create a calibration table.

1 In the **Method Tasks** pane, click **Method Setup Tasks**, then click **Concentration Setup**. The Method Table columns change to assist with entering the concentration levels.

2 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 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.
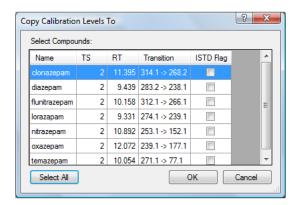
3 In the **Method Table** toolbar, enter **L** for a **Level Name Prefix**, and **5** for the **# of Levels** then click the **Create Level** button. The calibration table containing a concentration entry for each of the five calibration levels is shown below for the first compound in the method table.

Step 3 Copy the calibration table to other quantifiers.

1 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.
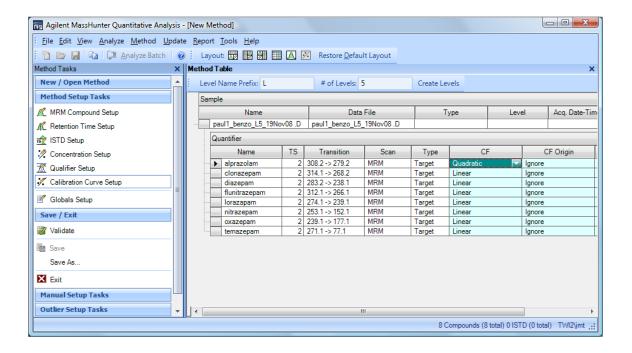

2 Click the **Select All** button to select all compounds in the dialog box, then click the **OK** button. This calibration table is copied to the Quantifier table of all eight compounds.

Step 4 Calibration Curve Setup

1 In the **Method Tasks** pane, click **Method Setup Tasks,** then click **Calibration Curve Setup**. The **Method Table** is displayed with the **CF**, **CF Origin**, and **CF Weight** columns highlighted.

2 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 Benzo compounds in this example will have a **CF** of **Quadratic**, a **CF Origin** of **Ignore**, and a **CF Weight** of **None**.
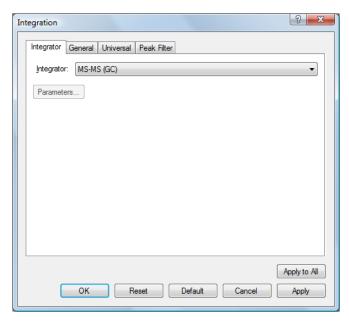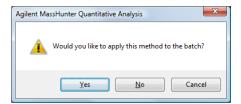
Step 5 Select an Integrator.

1   In the **Method Tasks** pane, click **Advanced Tasks,** then click **Integration Parameters Setup.** You can select from several integrators with MassHunter.

2   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. Use the MS-MS (GC) integrator which is optimized for MRM data with GC use. This is a parameter-less integrator.

3 Click the **Apply to All** button to set the **Integrator** for all quantifier peaks to this value. This integrator is used for integrating all peaks.

4 Click **OK** to close this dialog box.

Step 6 Exit the method editor and save the method.

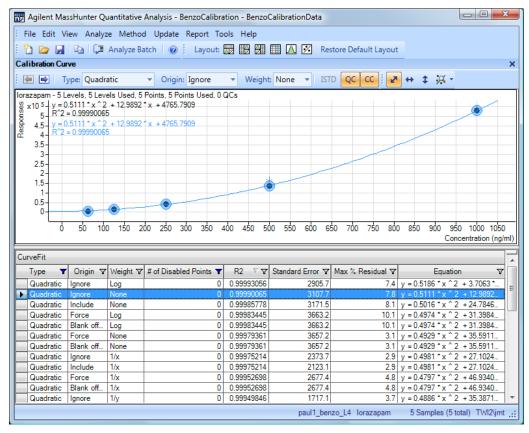In the **Method Tasks** pane, click **Save/Exit,** then click **Exit.**

Step 7 Apply the data analysis method to the batch.

1   Click the **Yes** button. The method is applied to all samples in the **Batch Table**.

2   Click the **Analyze Batch** button to quantitate all samples in the batch. Selecting **Analyze Batch** takes the compound responses in each calibration sample and uses them to create the CurveFit equations for each compound.
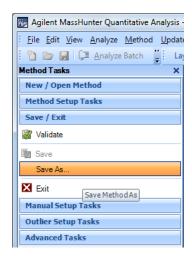
Step 8 Review the calibration curve for each compound.

1   In the calibration curve pane, right-click and select **Curve Fit Assistant** from the short-cut menu. 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.

2   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**.

3 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. These CurveFits were originally selected non graphically in **step 2**.

4 In the **Batch Table** toolbar, click the right arrow to select the next calibrated compound and repeat **step 2** in step 7 through **step 2** in step 8. The **Calibration Curve** pane only displays the calibration data for one compound at a time.

5 Repeat the above step until all eight calibrated compounds are reviewed and corrected if necessary.

6 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.

Step 9 Save the Data Analysis method.

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1 From the main menu select **Method > Edit** to display the Method Editor.

2 In the **Method Tasks** pane, click **Save / Exit,** then click **Save As.**

3 Enter **BenzoDA** for the method name and click the **Save** button.

Task 13. Quantitate a batch of unknown samples

In this task, 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 33.

Procedure

Open the Quantitative Analysis program and create a batch to analyze data:

1 Double-click the **QQQ Quantitative Analysis icon on your window's desktop.** The Quantitative Analysis Workstation opens.

2 **Name the batch and assign a batch directory:** From the main menu, select **File > New batch,** use the **Look In** drop-down list to navigate to the directory containing the three Benzo sample files, and enter the batch name **BenzoSamples031709** and click the **Open** button. This is the directory included on the program disk. Just copy these sample data files to this directory.

3 **Add samples to the batch:** From the main menu, select **File > Add samples** and select the samples **BenzoSample01.D** through **BenzoSample03.D**.

4 Click the **OK** button. The Batch Table now contains the three unknown samples. The batch now requires a data analysis method to automatically analyze the sample.

5 If necessary, change the **Type** to **Sample**.

6 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.

7 In the main menu select **Method > Open > Open and Apply from existing file** to display the **Open Method File** dialog box.

8 Enter the **BenzoDA**.quantmethod.xml for the **File name** and click the **Open** button. The method is applied to the batch.

9 Click the **Analyze Batch** button to quantitate all samples in the batch.

10 Review the sample results for each compound in the method. Print one or more reports.

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