

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

# **Familiarization Guide**

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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).



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  $\mu m$  x 0.25  $\mu m$  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  $10 \text{ ng/}\mu\text{L}$ .
  - **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/ $\mu$ 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/ $\mu$ 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.

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



Figure 1 Agilent MassHunter Workstation Software – Data Acquisition window

Task 1. Set the inlet and injection parameters

Inlet and Injection Parame	ters	X
Sample <u>I</u> nlet	GC	
Injection <u>S</u> ource	GC ALS	
	Use MS	
ОК	Cancel	Help

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

**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	Не
Mode	Splitless
Heater	<b>On</b> 300 °C

Task 2. Enter GC acquisition parameters

Parameter	Value
Pressure	<b>On</b> Value automatically set with CFT Setting column flow
Septum Purge Flow	<b>On</b> 3 mL/min
Gas Saver	<b>On</b> 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 (Prelnj)	0 min

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 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 <i>iii</i> paul1_MS_Scan.M, where <i>iii</i> are your initials.	b	Click the QQQ Method Editor icon (Figure 1). 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. Set the Time Filtering to a Peak width	•	The <b>QQQ Method Editor</b> window shown in Figure 4 opens.	
		d	of 0.7 seconds. In the <b>Time segment</b> section, enter a start <b>Time</b> of 3.0 and select a <b>Scan</b> <b>Type</b> of <b>MS1Scan</b> from the drop-down list. Enter 0 for <b>Delta EMV</b> and select <b>Data stored</b> .	•	The 7000 Series starts collecting data at 4 minutes due to the <b>Solvent delay</b> setting.	
		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 h	Click OK to close the window. From the main window select Method > Save Method As and save the method as <i>iii</i> paul1_MS_Scan.M, where <i>iii</i> are your initials.			

Task 3. Create acquisition method for finding precursor ions

Q Method Editor						
une file	🔲 Run time 1	min	Instrument Chrom	natogram		
tunes.tune xml	Solvent delay 4	min Scan seg	gments Segment name	Start mass	End mass	Scan time (ms)
n source	Time filtering		scan	50		50arr time (ms)
lon source: El	Peak width 0.7	sec				
Source temp.: 250 C	Detector setting					
lectron energy:	─ Gain					
Tune setting     -70 eV						
○ Fixed eV						
Variable by time segment						
me segments						
Time Scan type Electron ene	rgy Delta EMV Data stored					
→ 1 0.00 MS1 Scan -	0					
		•		III		
		- Scan par	rameters			
		Step size	ze 0.1	▼ amu		
	1.9 cycle/s 517 ms/cy		ld 100			
	_	Profil	le data			
	Display Timed Ev	/ents				

Figure 4 000 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		
<ul> <li>4 Acquire data (optional).</li> <li>Name the data file <i>iii</i>first_trial3_scan.D, where <i>iii</i> are your initials.</li> <li>Designate a directory path to hold your data files and method.</li> </ul>	<ul> <li>a Click the Start Run (green arrow) icon.</li> <li>b In the Data Path enter the directory to save the data file that is acquired by this run.</li> <li>c In the Front Inlet section, enter <i>iii</i>first_trial3_scan.D for the Data File Name, where <i>iii</i> are your initials.</li> <li>d Enter the Vial location number in the auto sampler tray.</li> <li>e In the Method Sections to Run section, select Data Acquisition.</li> </ul>	• The <b>Start Run</b> dialog box shown in Figure 5 is displayed.		
	f Click the OK and Run Method button.	<ul> <li>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.</li> </ul>		

Task 4. Acquire precursor ion scan data (Optional)

Current Method Injection	-		MS Connected to:	
Front	Rear (	🔵 Dual	Front Inlet	Rear Inlet
	Operator Name:	PaulZ		
	Data <u>P</u> ath:	C:\MassHunter\GC	MS\3\DATA\	Browse
Front Inlet			Rear Inlet	
Data <u>Fi</u> le Name:	first_trial3_scan.D	Browse.	Data File Name: 7000GCMS	6021409_4.D Browse
Sample <u>N</u> ame:			Sample Name:	
Misc. Inf <u>o</u> :			Misc. Info:	
Expected Barcode:			Expected Barcode:	
Sample Amount:	0		Sample Amount:	0
Multiplier:	1		Multiplier:	1
<u>V</u> ial Number:			Vial Number: 1	
Tray Name:	Agilent ALS	-	Tray Name: Agilent AL	5 👻
Injection Volume:			Injection Volume:	
Curren	t Method 1 µL		Current Method	<b>0</b> μL
Overrid	de using μL		Override using	1 μL
Sample Amount: Enter a	number			

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 inst	ructions		Comments					
<ul> <li>5 Open the acquired data file.</li> <li>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.</li> </ul>	icon. 🟬 The progra	a Double-click the Qualitative Analysis icon.				<ul> <li>When you open a data file, the Load result data (lower left corner check box is grayed out if the resu data was not saved. If you see the check box selected, this means tha the data file(s) already contains results. Clear this check box before opening the file.</li> </ul>			
<ul> <li>Before you begin, make sure that all previous settings are returned to their default values:</li> <li>Restore default layouts</li> <li>Click View &gt; Window Layouts &gt; Restore Default Layout.</li> </ul>	Copen Data F Look in Recent Places	I: Daulz_scan	_data Date modif Type n_prod_ion_scan1.D m_prod_ion_scan1.D	▼ Size	← €				
<ul> <li>Make sure the method is default.m. (see title bar)</li> <li>Click Method &gt; Open.</li> <li>Select default.m, and click Open.</li> <li>Return display options to default settings.</li> <li>Click Tools &gt; Plot Display Options</li> </ul>	Desktop Disktop Computer	diazepam_prod_ion_scanl.D     desktop     district_trial3_scan.D     first_trial3_scan.D     lorazapam_prod_ion_scanl.1     lorazapam_prod_ion_scanl.D     lorazapam_prod_ion_scanl.D     loxazapam_prod_ion_scanl.D     loxazapam_prod_ion_scanl.D							
<ul> <li>Click <b>Default</b>, and then <b>OK</b>.</li> </ul>		File names : Files of type :	first_trial3_scan.D Data Files (*.d)		• •	Open Cancel Help			
	Options C Load worki C Use curren Load result Load result Run 'File O selected m	is method t method t data ipen' actions from	Sample Info Sample Nan User Name Sample Pos Description	ne: : paul ition:25					

Task 5. Determine precursor ion masses

Steps	Detailed instructions	Comments		
	<ul> <li>b Do one of the following:</li> <li>Select the example data file first_trial3_scan.D, and click Open.</li> <li>Select the data file you created in "Task 4. Acquire precursor ion scan data (Optional)" on page 13, and click Open.</li> <li>By default, the system displays the Total Ion Chromatogram (TIC).</li> </ul>	<ul> <li>The figure below shows the defaul layout. This is what you want to see.</li> <li>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.</li> </ul>		



Task 5. Determine precursor ion masses

Steps				Detailed instructions	Comments
<ul> <li>Determine precursor ion masses for all eight peaks.</li> <li>You have determined them correctly if you find the values are similar to those shown in this table.</li> <li>Close the data file after finding the precursor ion masses.</li> </ul>			em values wn in finding	<ul> <li>c In the Chromatogram Results window the Range Select icon in the toolbar is selected.</li> <li>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.</li> <li>e Right-click inside the shaded area, and select Extract MS Spectrum from the</li> </ul>	<ul> <li>An averaged spectrum from the highlighted area inside the peak i</li> </ul>
<b>Реак</b> 1 2 3 4 5 6 7	<b>Compound</b> Oxazepam Lorazepam Diazepam Temazepam Flunitrazepam Nitrazepam Clonazepam	<b>RT</b> 9.0 9.3 9.4 10.0 10.1 10.9 11.4	<b>m/z</b> 239.1 274.1 283.2 271.1 312.2 253.1 314.1	shortcut menu. Repeat <b>step d</b> through <b>step e</b> for the other compounds. The precursor ion masses should match those in the table in step 2. Click <b>File &gt; Close Data File</b> . When asked if you want to save the results, click <b>No</b> .	<ul> <li>displayed in the MS Spectrum Results window.</li> <li>The precursor mass of the first compound, Oxazepam, is determined to be m/z 239.1.</li> </ul>



8

Alprazolam

12.1

308.2

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 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			
<ul> <li>6 Enter MS parameters appropriate for finding the Oxazepam product ion and save the method as <i>iiip</i>aul1_Oxazepam_prod_ion_Sc an2.M, where <i>iii</i> are your initials.</li> <li>Create additional methods for the other 7 compounds using the data from the previous task.</li> </ul>	<ul> <li>a From the Data Acquisition workstation open the <i>iii</i>paul1_MS_Scan.M method, where <i>iii</i> are your initials.</li> <li>b Click the QQQ Method Editor icon.</li> <li>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.</li> <li>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.</li> <li>e Set the MS1 resolution to Wide.</li> <li>f In the Scan segments section, enter a Step size of 0.1 amu and select Profile data.</li> <li>g In the Scan segments section, right-click a cell and select New Scan Segment from the context menu.</li> <li>h Repeat the above step three times.</li> </ul>	<ul> <li>This method was previously saved in Task 3 and the GC acquisition parameters provide excellent compound separation.</li> <li>The QQQ Method Editor window opens.</li> <li>A new scan segment with all values from the first scan segment is created.</li> <li>A maximum of four scan segment is supported on the 7000 Series.</li> </ul>			

Task 6. Create acquisition methods for finding product ion masses

Steps	Detailed instructions	Comments
	<ul> <li>i Change the Collision energy to 15 for the second scan segment.</li> <li>j Change the Collision energy to 25 for the third scan segment.</li> <li>k Change the Collision energy to 35 for the fourth scan segment.</li> <li>I Click OK to close the window.</li> <li>m From the main window select Method &gt; Save Method As and save the method as <i>iiip</i>aul1_Oxazepam_prod_ion_Scan2.</li> </ul>	<ul> <li>A total of four scan segments, each with a different collision energy, is required for optimizing product ion sensitivity and selectivity.</li> </ul>
	<ul> <li>M, where <i>iii</i> are your initials.</li> <li>n Click the <b>QQQ Method Editor</b> icon.</li> <li>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.</li> </ul>	• 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_0xazepam_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

Task 6. Create acquisition methods for finding product ion masses

QQQ Method Editor									
Tune file atunes tune xml Q	Run time 1 min		n Instrument Chrom	atogram					
	Solvent delay 3 min Time filtering		Segment name	Precursor ion	MS1 resolution	MS2 from	MS2 to	Scan time (ms)	Collision energy
Ion source; El	Peak width 0.7 sec		Oxazepam_5	239.1	Wide 💌	50	275	300	5
Source temp.: 250 C			Oxazepam_15	239.1	Wide 💌	50	275	300	15
· · · · · · · · · · · · · · · · · · ·	Detector setting		Oxazepam_25	239.1	Wide 🔻	50	275	300	25
Electron energy:     O Tune setting -70 eV	Gain Olda EMV	1	Oxazepam_35	239.1	Wide 💌	50	275	300	3 5
Fixed     eV     Variable by time segment Time segments     Time Scan type Electron     1 3.00 Product Ion     v	650	Step s		■ an	14				
0	0.85 cycle/s 1170 ms/cycle	Thresh							
				ОК	App	oly Re	set	Cancel	Help

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			
<ul> <li>7 Set up and run a sequence (optional).</li> <li>Name the data files <i>iiixx_prod_ion_scan1.d</i>, where <i>iii</i> are your initials and <i>xx</i> is the compound name.</li> </ul>	<ul> <li>a Click the Sequence Edit icon to display the Sequence Table.</li> <li>b Use the drop-down list next to the New Sample(s) button in the Sequence Table toolbar and select 5 Samples.</li> <li>c Enter a sample Name, Vial ALS location number, Method File name, and Data File name for each sample as shown in Figure 7.</li> <li>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.</li> <li>e Click the OK button.</li> <li>f Select Sequence &gt; Save Sequence As to save the sequence as BenzoPI.Sequence.xml.</li> <li>g Place eight sample vials in the specified location of the ALS tray.</li> <li>h Click the Sequence dialog box.</li> <li>i If needed, add a sequence Comment, change the Data File Directory and click the Run Sequence button.</li> </ul>	<ul> <li>This step is optional because you can use the six example data files in the next step.</li> <li>The table now contains 8 sample lines.</li> </ul>			

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

	Name	Vial	Method File	Data File	Туре		Dil.	Со
1	Oxazapam_prod	1	paul1_Oxazaion_Scan2.M	 Oxazapam_prod_ion_Scan2.D	Sample	-	1	
2	Lorazepam_prod	2	paul1_Lorazeion_Scan1.M	 Lorazepam_prod_ion_Scan1.D	Sample	-	1	
3	diazepam_prod_ion	3	paul1_diazepion_Scan1.M	 diazepam_prod_ion_Scan1.D	Sample	-	1	
4	flunitrazepam_pro	4	paul1_flunitraion_Scan1.M	 flunitrazepam_prod_ion_Scan1.D	Sample	-	1	
5	temzepam_prod_i	5	paul1_temzeion_Scan1.M	 temzepam_prod_ion_Scan1.D	Sample	•	1	
6	nitrazepam_prod	6	paul1_nitrazeion_Scan1.M	 nitrazepam_prod_ion_Scan1.D	Sample	-	1	
7	clonazepam_pro	7	paul1_clonazion_Scan1.M	 clonazepam_prod_ion_Scan1.D	Sample	-	1	
8	alprazelam_prod	8	paul1_alprazion_Scan1.M	 alprazelam_prod_ion_Scan1.D	Sample	-	1	
			"					

# Figure 7 Sequence Table values

Method Sections to Run			On a Barcode M	ismatch	
(a) Full Method			🔘 In	ject Anyway	
0			-	on't Inject	
				one ngoot	
Overwrite Existing Da	ta Files				
Sequence Comment:					
	0.17				
Operator Name:	PaulZ				
Data File Directory:	C:\MassHunter\GCMS\1	\DATA\			Browse
Pre-Sequence Macros/Comm	ands				
Acquisition:					Browse
Data Analysis:					Browse
Data Malyon.					Diowac
Post-Sequence Macros/Comr	nands				
Acquisition:					Browse
Data Analysis:					
Data Analysis:					Browse
nter the operator name					
nter the operator hame					

**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.

S	eps	De	etailed instructions	Comments			
8	Open the acquired data files in the Qualitative Analysis program.	а	Double-click the <b>Qualitative Analysis</b> icon.	•	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. <i>Clear this check box</i> <i>before opening the file</i> .		
		b	<ul> <li>Do one of the following:</li> <li>Select the eight example data files and click <b>Open</b>.</li> <li>Select all the data files you acquired in "Task 7. Acquire scan data for finding product ions (Optional)" on page 21, and click <b>Open</b>.</li> </ul>	•	Use the <b>CTRL</b> key to select multiple data files.		
		C	In the <b>Data Navigator</b> , clear the check boxes for all compounds except <b>Oxazepam</b> .	•	Only a single acquisition file at a time is displayed.		
		d	From the <b>MS Spectra Results</b> window toolbar, enter <b>5</b> from the drop-down menu.	•	A maximum of 5 spectrum panes can be displayed.		

**Task 8. Determine the product ions** 

Steps	Detailed instructions	Comments		
	<ul> <li>In the Chromatogram Results window, the Range Select icon in the toolbar</li> <li>is selected.</li> <li>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.</li> </ul>	<ul> <li>For Oxazepam this peak is at about 9 minutes.</li> </ul>		
	<b>g</b> Right-click inside the shaded area, and select <b>Extract MS Spectrum</b> from the shortcut menu. The spectrum for this peak is shown in Figure 9.	<ul> <li>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.</li> </ul>		





Steps	Detailed instructions	Comments
<ul> <li>Determine product ion masses for all eight peaks.</li> <li>You have determined them correctly if you find the values are similar to those shown in Table 3.</li> </ul>	<ul> <li>h The product ion for the first compound, Oxazepam, is determined to be m/z 177.1 at CID 25.</li> <li>i Clear the check box for the current data file in the Data Navigator.</li> <li>j Select the check box for the next data</li> </ul>	• 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.
<ul> <li>Close the data file after finding the precursor ion masses.</li> </ul>	<ul> <li>file.</li> <li>k Repeat step f through step j for the other compounds. The product ion masses should match those in Table 3.</li> <li>I Click File &gt; Close Data File.</li> <li>m When asked if you want to save the results, click No.</li> </ul>	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 lons found for the Benzodiazepine compounds

Compound	Data File	Precursor Ion	Product Ion	<b>Collision Energy</b>
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

# Task 9. Create an MRM method

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

St	eps	Detailed instructions	Comments
9	Open the Data Acquisition program and create an MRM method using the data from Table 3 on page 25.	<ul> <li>a From the Data Acquisition workstation open the <i>iii</i>paul1_MS_Scan.M method, where <i>iii</i> are your initials.</li> <li>b Click the QQQ Method Editor icon.</li> <li>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.</li> <li>d In the Scan segments section, enter Wide for the MS1 Resolution and MS2 Resolution, and 50 for the Dwell.</li> <li>e In the Scan segments section, right-click a cell and select New Scan Segment from the context menu.</li> <li>f Repeat the above step seven times.</li> <li>g Fill in the rest of the Scan Segments section to match Figure 10 on page 27 h Click OK to close the window.</li> <li>i From the main window select Method &gt; Save Method As and save the method as <i>iiip</i>aul1_benzo_mrm_1.M, where <i>iii</i> are your initials.</li> </ul>	from this method are used unchanged. The 7000 Series method parameters are edited in this procedure to create the MRM method.

Task 9. Create an MRM method

	Acquisition	indication: Onionia	logram								
nt delay 3 min filtering		Compound name	ISTD?	Precursor ion	MS1 resolution		Product ion	MS2 resolution		Dwell	Collision energy
eak width 0.7 sec	•	oxazapam		239.1	Wide	-	177.1	Wide	•	50	25
		lorazapam		274.1	Wide	-	239.1	Wide	-	50	15
tor setting		diazepam		283.2	Wide	-	268.1	Wide	-	50	25
ain 💿 Delta EMV		flunitrazepam		312.1	Wide	-	266.1	Wide	-	50	25
		temazepam		271.1	Wide	-	165.2	Wide	-	50	35
		nitrazepam		253.1	Wide	-	207.1	Wide	-	50	25
		clonazepam		314.1	Wide	-	233.1	Wide	-	50	25
		alprazelam		308.2	Wide	-	279.2	Wide	-	50	15
65											
407 ms/cycle											

Figure 10 Scan section completed for an MRM 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.

Task 10. Acquire MRM data (Optional)

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	<ul> <li>a From the Data Acquisition workstation load the <i>iii</i>paul1_benzo_mrm_1.M method.</li> <li>b Click the Edit Sequence icon to display the Sequence Table.</li> </ul>	<ul> <li>This method was created in "Task 9. Create an MRM method" on page 26.</li> </ul>
required to create a quantitative analysis method in the Quantitative Analysis program.	<ul> <li>the Sequence Table.</li> <li>c Add or Delete sample entries so that the Sequence Table contains five sample lines.</li> <li>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.</li> <li>e Use the Fill Increment drop-down in the Sequence Table toolbar to copy</li> </ul>	<ul> <li>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.</li> <li>The Vial location incremented in this way assumes that the vials are placed in consecutive locations in the sampler tray.</li> </ul>
	<ul> <li>and increment the values listed for the first line sample values for Name, Vial, and Level to the other four samples.</li> <li>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.</li> </ul>	
	<ul> <li>g Change _L1_ part of the Data File name to follow the pattern shown in Figure 11 on page 30.</li> <li>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.</li> <li>i Click OK to close the window.</li> </ul>	

Task 10. Acquire MRM data (Optional)

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

	Name	Vial	Method File		Data File	Туре		Level	Dil.	Commer
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	



# 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
<b>11</b> Open the Quantitative Analysis program and create a batch to analyze data.	a Double-click the <b>QQQ Quantitative</b> <b>Analysis</b> icon on your window's desktop.	The Quantitative Analysis Workstation opens.
Name the batch and assign a batch directory.	<ul> <li>b From the main menu, select File &gt; 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.</li> </ul>	<ul> <li>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.</li> </ul>
<ul> <li>Add samples to the batch.</li> </ul>	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. Batch Folder: C:\MassHunter\GCMS\1\DAT paul1_benzo_L1_19Nov08.D paul1_benzo_L3_19Nov08.D paul1_benzo_L4_19Nov08.D paul1_benzo_L5_19Nov08.D paul1_benzo_L5_19Nov08.D paul1_benzo_L4_19Nov08.D paul1_benzo_L5_19Nov08.D	<ul> <li>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.</li> <li>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.</li> </ul>

Task 11. Create a quantitative analysis batch

teps		Detailed	d instruction	5	Co	omments
		e fine sort the v f If ne colur Batc	contains the cessary, char on the <b>Name</b> ralues shown cessary, add mns to the <b>S</b> a <b>h Table</b> . Ente	n. The <b>Batch Table</b> 5 samples. ge the <b>Type</b> to <b>Cal</b> , column, and enter in the <b>Level</b> column. the <b>Dil</b> and <b>Amt</b> <b>imple</b> section of the r a value of 1 for all es in these columns.	•	The batch now requires a data analysis method to automatically analyze the sample.
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Steps	Detailed instru	uctions	Comments			
<b>I2</b> Create a Data Analysis method.	<ul> <li>a From the main menu, select Method &gt; New &gt; New Method from Acquired MRM Data to display the New Method from Acquired Data dialog box.</li> <li>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.</li> <li>The sample data so acquisition method.</li> <li>The sample data so acquisition method.</li> <li>The sample data so acquisition method.</li> <li>The program uses file to create much analysis method, ti user with the method can be manually.</li> </ul>					
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Task 12. Create an MRM quantitative analysis method

iteps	Detailed in	structions		Comme	ents					
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I Retention Time Setup	paul1_benzo_L5 paul1_ben	zo_L5								
छ्रि ISTD Setup	Quantifier									
: Concentration Setup	Name TS	Transition	Scan	Туре	Precursor Ion	Product Ion	RT			
T Qualifier Setup	- Palprazolam	2 308.2 -> 279.2	MRM	Target	308.2	279.2	12.078			
Calibration Curve Setup	clonazepam diazepam	2 314.1 -> 268.2 2 283.2 -> 238.1	MRM	Target Target	314.1 283.2	268.2	11.395			
	flunitrazepam	2 312.1 -> 266.1	MRM	Target	312.1	266.1	10.15			
Globals Setup	lorazapam	2 274.1 -> 239.1	MRM	Target	274.1	239.1	9.33			
Save / Exit	nitrazepam	2 253.1 -> 152.1	MRM	Target	253.1	152.1	10.89			
Validate	oxazepam temazepam	2 239.1 -> 177.1 2 271.1 -> 77.1	MRM	Target Target	239.1 271.1	177.1	12.07			
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8 Compounds (8 total) 0 ISTD (0 total) TW/I2\jmt ..:

Advanced Tasks

Steps	Detailed instructions	Comments			
Create a calibration table.	<ul> <li>d In the Method Tasks pane, click Method Setup Tasks, then click Concentration Setup.</li> <li>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.</li> </ul>	<ul> <li>The Method Table columns change to assist with entering the concentration levels.</li> <li>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.</li> </ul>			

	0	Layou ethod Ta	t: 🔤 😥 😥 🔲	1 🕰 🖗	Restore <u>D</u> efau	ılt Layout				
Method Tasks 20 New / Open Method Method Setup Tasks MRM Compound Setup	× Me	ethod Ta	able		Restore <u>D</u> efau	It Layout				
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			flunitrazepam	-	312.1 -> 266.1	MRM	Target			ng/ml
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Save / Exit			nitrazepam	_	253.1 -> 152.1	MRM	Target			ng/ml
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teps			Detailed instr	uction	15		Comn	nents		
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K Retention Time Setup			aul1_benzo_L5_19N	ov08 .D	paul1_benzo_L	5_19Nov08 .D				
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K Exit			clonazepam		314.1 -> 268.2	MRM	Target			ng/ml
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		1	flunitrazepam	<b>   </b>	312.1->200.1	MIRIM	Target			ng/ml

Task 12. Create an MRM quantitative analysis method

compounds.

	mments	Co		s	uction	etailed instr	Steps	
Calibration Levels To is displayed. Use this to calibration table from the ompound to selected or al apounds.	lick the <b>PPY</b>	right-cl lect <b>Cc</b>	Table, and se I Level	Select the f <b>Quantifier</b> compound <b>Calibration</b> shortcut m	Copy the calibration table to other quantifiers.			
	? x			То	n Levels <sup>-</sup>	Copy Calibratio		
					inds:	Select Compou		
	<u>^</u>	ISTD Flag	Transition	RT	TS	Name		
			314.1 -> 268.2		2	clonazepam		
			283.2 -> 238.1		2	diazepam		
	=		312.1 -> 266.1		2	flunitrazepam		
			274.1 -> 239.1		2	lorazapam		
			253.1 -> 152.1		2	nitrazepam		
			239.1 -> 177.1 271.1 -> 77.1		2	oxazepam		
				10.034	2	temazepam		
	Cancel	)K			J	Select All		
2								
	This calibrat the <b>Quantifi</b>					Click the <b>S</b> compounds		

the **OK** button.

Steps	Detailed instructions	Comments				
Calibration Curve Setup	<ul> <li>i In the Method Tasks pane, click Method Setup Tasks, then click Calibration Curve Setup.</li> <li>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.</li> </ul>	<ul> <li>The Method Table is displayed with the CF, CF Origin, and CF Weight columns highlighted.</li> <li>The Benzo compounds in this example will have a CF of Quadratic, a CF Origin of Ignore, and a CF Weight of None.</li> </ul>				

📅 Agilent MassHunter Quantitative Ana	ysis ·	- [N	ew Me	ethod]								- 0 <b>X</b>
: <u>F</u> ile <u>E</u> dit <u>V</u> iew <u>A</u> nalyze <u>M</u> ethod <u>U</u>	pdat	e j	<u>R</u> eport	: <u>T</u> ools <u>H</u> elp								
🚹 🗁 📕 🗈 💭 Analyze Batch			Layo	out: 🙀 💆 🕅		Restore Defau	It Layout					
Method Tasks	×	M	ethod	Table			-					×
New / Open Method	-		Leve	l Name Prefix: L		# of Levels:	5	Create Le	evels			
Method Setup Tasks		┢	Sam	ple								
K MRM Compound Setup				Name		Data	File	1	Гуре	Leve	1	Acq. Date-Tim
K Retention Time Setup				paul1_benzo_L5_19No	v08 .D	paul1_benzo_L5	_19Nov08 .D					
ाई ISTD Setup			0	Quantifier	_						_	
Concentration Setup				Name	TS	Transition	Scan	Туре	CF		(	CF Origin
Cualifier Setup			-	alprazolam	_	308.2 -> 279.2	MRM	Target	Quadratic		Ignore	_
				clonazepam	2	314.1 -> 268.2	MRM	Target	Linear		Ignore	
ϔ Calibration Curve Setup				diazepam	2	283.2 -> 238.1	MRM	Target	Linear		Ignore	
Globals Setup			-	flunitrazepam Iorazapam		312.1 -> 266.1 274.1 -> 239.1	MRM	Target Target	Linear Linear		Ignore Ignore	
				nitrazepam	_	253.1 -> 152.1	MRM	Target	Linear		lanore	
Save / Exit			-	oxazepam		239.1 -> 177.1	MRM	Target	Linear		Ignore	
🖓 Validate				temazepam		271.1 -> 77.1	MRM	Target	Linear		Ignore	
In Save												
Save As												
X Exit												
Manual Setup Tasks												
Outlier Setup Tasks	-	h	•									Þ
		0-1						8 (	Compounds (8 to	tal) 0 ISTI	D (0 tota	al) TWI2\imt .:
	_	_	_									.,

Steps	Detailed instructions	Comments			
• Select an Integrator.	<ul> <li>k In the Method Tasks pane, click Advanced Tasks, then click Integration Parameters Setup.</li> <li>I 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.</li> </ul>	<ul> <li>You can select from several integrators with MassHunter.</li> <li>Use the MS-MS (GC) integrator which is optimized for MRM data with GC use. This is a parameter-less integrator.</li> </ul>			
	Integrator General Universal Peak Filter Integrator: MS-MS (GC) Parameters OK Reset Default	Apply to All       Cancel			
	<ul> <li>m Click the Apply to All button to set the Integrator for all quantifier peaks to this value.</li> <li>n Click OK to close this dialog box.</li> </ul>	<ul> <li>This integrator is used for integrating all peaks.</li> </ul>			
• Exit the method editor and save the method.	<ul> <li>In the Method Tasks pane, click Save/Exit, then click Exit.</li> <li>Agilent MassHunter Quantitative Analysis</li> <li>Would you like to apply this method to the batching</li> <li>Yes</li> <li>No</li> </ul>				

ps		Deta	iled instructions		<ul> <li>Comments</li> <li>The method is applied to all samples in the Batch Table.</li> <li>Selecting Analyze Batch takes the compound responses in each calibration sample and uses them to create the CurveFit equations fo each compound.</li> <li>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.</li> </ul>				
Apply the da the batch.	ata analysis method to	q C	lick the <b>Yes</b> button. lick the <b>Analyze Batcl</b> uantitate all samples i						
Review the e each compo	calibration curve for bund.	ri A s Ir a a	n the calibration curve ght-click and select <b>C</b> issistant from the sho n the <b>CurveFit</b> table, se <b>Type</b> of <b>Quadratic</b> , Igu nd use a maximum of <b>Disabled Points</b> .	u <b>rve Fit</b> rt-cut menu. elect a line with nore the <b>Origin</b> ,					
	📆 Agilent MassHunter Qua	antitative	Analysis - BenzoCalibration - I	BenzoCalibrationData					
	Edit View Analyze	e Method	d Update Report Tools He	elp					
	🗄 🛅 🗁 🛃 🖬 💭 /	Analyze Ba	atch 🛛 🕢 🕴 Layout: 🔣 📕	🛛 🕅 🛄 🛕 📝 Res	store Default Layou	t			
	Calibration Curve	Analyze Ba	atch 🕜 Eayout: 🛺 f	Res	store Default Layou	t			
	Calibration Curve	itic	▼ Origin: Ignore ▼ \	Weight: None 🔻 IST					
	Calibration Curve	tic Is Used, 5 + 12 9892 + 12 9892	Origin: Ignore     Normal Science (Construction)     Source (Cons	Weight: None VIST		↔ ‡ ¾ ~ 0 850 900 950 1000 1050			
	Calibration Curve	tic Is Used, 5 + 12 9892 + 12 9892	Origin: Ignore     Normal Science (Construction)     Source (Cons	Weight: None V IST		+			
	Calibration Curve	ttic Is Used, 5 + 12,9892 + 12,9892 0 150 20	Origin: Ignore     Normal States of the second	Weight: None  IST	D QC CC : 2				
	Calibration Curve         Image: Type: Quadratic constraints         lorazapam - 5 Levels, 5 Level         g x10 3 ↓ y = 0.5111 * x ^ 2         g x10 5 ↓ y = 0.5111 * x ^ 2         g x10 5 ↓ y = 0.5111 * x ^ 2         g x10 5 ↓ y = 0.5111 * x ^ 2         g x10 5 ↓ y = 0.5111 * x ^ 2         g x10 5 ↓ y = 0.5111 * x ^ 2         g x10 5 ↓ y = 0.5111 * x ^ 2         g x10 5 ↓ y = 0.5111 * x ^ 2         g x10 5 ↓ y = 0.5111 * x ^ 2         g x10 5 ↓ y = 0.5111 * x ^ 2         g x10 5 ↓ y = 0.5111 * x ^ 2         g x 10 5 ↓ y = 0.5111 * x ^ 2         g x 10 5 ↓ y = 0.5111 * x ^ 2         g x 10 5 ↓ y = 0.5111 * x ^ 2         g x 10 5 ↓ y = 0.5111 * x ^ 2         g x 10 5 ↓ y = 0.5111 * x ^ 2         g x 10 5 ↓ y = 0.5111 * x ^ 2         g x 10 5 ↓ y = 0.5111 * x ^ 2         g x 10 5 ↓ y = 0.5111 * x ^ 2         g x 10 5 ↓ y = 0.511 * x ^ 2         g x 10 5 ↓ y = 0.511 * x ^ 2         g x 10 5 ↓ y = 0.511 * y = 0.5	ttic +Is Used, 5 + 12,9892 + 12,9982 +		Weight:         None         IST           Image: Standard Error         V	D QC CC : 2				
	Calibration Curve         Image: Colspan=1 to the second s	tic Is Used, 5 + 12,9892 + 12,9892 + 12,9892 ↓ 12,998 ↓ 12,998		Weight:         None         IST           50         500         550         600         60           50         500         550         600         60           50         500         550         600         60           50         500         550         600         60           50         500         550         600         60           50         500         550         600         60           50         500         550         600         60           50         500         550         600         60	D QC CC : 2 2 50 700 750 80 Max % Residual ♥ 7.4 7.8	↔ ‡ ¾ - 0 850 900 950 1000 1050 Concentration (ng/n y = 0.5186 *x ^2 + 3.7063 *. y = 0.5111 *x ^2 + 12.9892.			
	Calibration Curve	ttic Is Used, 5 + 12,9892 + 12,9892 + 12,9892 0 150 20 Weight ⊽ Log None		Weight:         None         IST           50         500         550         600         60           50         500         550         600         60           VY         Standard Error         Y         93056         2905.7           90055         3102.7         83778         3171.5	D QC CC : 2 50 700 750 80 Max % Residual ¥ 7.4 7.8 8.1				
	Calibration Curve         Image: marked colspan="2">Type: Quadrati         lorazapam - 5 Levels, 5 Level         g x103 - y = 0.5111 * x <sup>2</sup> 2         g x103 - y = 0.5111 * x <sup>2</sup> 2         g x103 - y = 0.5111 * x <sup>2</sup> 2         g x103 - y = 0.5111 * x <sup>2</sup> 2         g x - 0.511 + x <sup>2</sup> 2         g x - 0.50 + 0.00         mark - 0.50 + 0.00         mark - 0.50 + 0.00         G quadratic Ignore         Quadratic Ignore         Quadratic Ignore         Quadratic Ignore         Quadratic Force	ttic Is Used, 5 + 12,9892 + 12,9892 + 12,9892 + 12,9892 Use of the second secon		Weight: None ▼ 157 50 500 550 600 68 V V Standard Error V 93056 2905.7 93055 3107.7 85778 3171.5 83445 3663.2	D QC CC : 2 50 700 750 80 Max % Residual ♥ 7.4 7.8 8.1 10.1				
	Calibration Curve           Image: CurveFit           Type           Image: CurveFit           Type           Quadratic           Image: CurveFit           Quadratic           Image: CurveFit           Quadratic           Image: CurveFit           Quadratic           Image: CurveFit           Image: CurveFit           Type           Quadratic           Image: CurveFit           Quadratic           Image: CurveFit	ttic Is Used, 5 + 12,9892 + 12,9892 + 12,9892 0 150 20 Weight ⊽ Log None	Vrigin: Ignore     V Points, 5 Points Used, 0 QCs     x + 4765.7909     x + 4765.7909     x + 4765.7909     v + 4765.790	Weight:         None         IST           50         500         550         600         60           50         500         550         600         60           VY         Standard Error         Y         93056         2905.7           90055         3102.7         83778         3171.5	D QC CC : ₽ D QC				
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	Calibration Curve         Image: Second se	ttic Is Used, 5 + 12,9892 + 12,9892 + 12,9892 + 12,9892 Use of the second secon	▼ Origin: Ignore         ▼           Points, 5 Points Used, 0 QCs           1x + 4765.7909           1x + 4765.7909 <t< td=""><td>Weight:         None         IST           50         500         550         600         60           50         500         550         600         60           50         500         550         600         60           50         500         550         600         60           50         500         550         600         60           50         500         50         3107.15         58748         3167.15           83445         3663.2         3171.55         3445         3663.2         3131.55           83445         3663.2         31657.2         79361         3657.2         77321         3273.7</td><td>D QC CC : 2 D QC</td><td></td></t<>	Weight:         None         IST           50         500         550         600         60           50         500         550         600         60           50         500         550         600         60           50         500         550         600         60           50         500         550         600         60           50         500         50         3107.15         58748         3167.15           83445         3663.2         3171.55         3445         3663.2         3131.55           83445         3663.2         31657.2         79361         3657.2         77321         3273.7	D QC CC : 2 D QC				
	Calibration Curve           Image: CurveFit           Type           Image: CurveFit           Type           Quadratic           Imate: Calibration           Quadratic           Quadratic           Quadratic           Quadratic           Quadratic           Quadratic           Quadratic	ttic Is Used, 5 + 12,9892 + 12,9892 + 12,9892 + 12,9892 12,9892 Use of the second sec	♥ Origin: Ignore         ♥           Points, 5 Points Used, 0 QCs         *           * + 4765,7909         *           * + 4765,7909         *           * + 4765,7909         *           * of Disabled Points         R2           0         0.999           0         0.999           0         0.999           0         0.999           0         0.999           0         0.999           0         0.999           0         0.999           0         0.999           0         0.999           0         0.999           0         0.999           0         0.999           0         0.999           0         0.999           0         0.999           0         0.999           0         0.999	Weight:         None         IST           50         500         550         600         61           50         500         550         600         61           7         V         Standard Error         Y           93056         2905.7         3107.7         85778         3171.5           83445         3663.2         283445         3663.2         79361         3657.2           79361         3657.2         79361         3657.2         79361         3657.2         793214         2123.1	D QC CC : ₽ D QC				
	Calibration Curve Calibration Curve CurveFit Curve	ttic Is Used, 5 + 12,9892 + 12,9892 + 12,9892 + 12,9892 Use of the second secon	▼ Origin: Ignore         ▲           Points. 5 Points Used, 0 QCs           * x + 4765.7909           * x + 4765.7909           * x + 4765.7909           * x + 4765.7909           * 4765.7909           * 0 0 250 300 350 400 40           # of Disabled Points ▼ R2           0 0.999	Weight:         None         IST           50         500         550         600         60           50         500         550         600         60           50         500         550         600         60           50         500         550         600         60           50         500         550         600         60           50         500         50         3107.15         58748         3167.15           83445         3663.2         3171.55         3445         3663.2         3131.55           83445         3663.2         31657.2         79361         3657.2         77321         3273.7	D QC CC : 2 D QC				

Steps	Detailed instructions	Comments
	<ul> <li>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.</li> <li>In the Batch Table toolbar, click the right arrow to select the next calibrated compound and repeat steps</li> </ul>	<ul> <li>These CurveFits were originally selected non-graphically in step j.</li> <li>The Calibration Curve pane only displays the calibration data for one compound at a time.</li> </ul>
	<ul> <li>q through s.</li> <li>v Repeat the above step until all eight calibrated compounds are reviewed and corrected if necessary.</li> </ul>	
	w Click the Analyze Batch button to quantitate all samples in the batch.	<ul> <li>This step is only necessary if you changed any CurveFit equation during the review above.</li> </ul>
Save the Data Analysis method Agilent MassHunter Quantitative Analysis - I Elle Edit View Analyze Method Update Carlot Setup Tasks Save / Exit Validate Save Save As X Exit Save MethodAs Manual Setup Tasks Outlier Setup Tasks Advanced Tasks	<ul> <li>x From the main menu select Method &gt; Edit to display the Method Editor.</li> <li>y In the Method Tasks pane, click Save / Exit, then click Save As.</li> <li>z Enter BenzoDA for the method name and click the Save button.</li> </ul>	

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.	a Double-click the <b>QQQ Quantitative</b> <b>Analysis</b> icon on your window's desktop.	• The Quantitative Analysis Workstation opens.
<ul> <li>Name the batch and assign a batch directory.</li> <li>Add samples to the batch.</li> </ul>	<ul> <li>b From the main menu, select File &gt; 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.</li> <li>c From the main menu, select File &gt; Add</li> </ul>	<ul> <li>This is the directory included on the program disk. Just copy these sample data files to this directory.</li> </ul>
	samples and select the samples BenzoSample01.D through BenzoSample03.D.	
	<ul> <li>d Click the OK button. The Batch Table now contains the 3 unknown samples.</li> <li>e If necessary, change the Type to</li> </ul>	<ul> <li>The batch now requires a data analysis method to automatically analyze the sample.</li> </ul>
	<ul> <li>Sample.</li> <li>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.</li> </ul>	
	<ul> <li>g In the main menu select Method &gt;</li> <li>Open &gt; Open and Apply from existing file to display the Open Method File dialog box.</li> </ul>	
	h Enter the BenzoDA.quantmethod.xml for the File name and click the Open button.	• The method is applied to the batch.
	i Click the <b>Analyze Batch</b> button to quantitate all samples in the batch.	
	j Review the sample results for each compound in the method.	• Print one or more reports.

Task 13. Quantitate a batch of unknown samples

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