Agilent MassHunter Workstation Software

Quantitative Analysis

Familiarization Guide

Agilent Technologies

Notices

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This guide is valid for the B.07.xx or later revision of the Agilent MassHunter Workstation Software - Quantitative Analysis program, until superseded.

If you have comments about this guide, please send an e-mail to feedback_lcms@agilent.com.

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In this Guide...

The *Familiarization Guide* presents step-by-step exercises to help you learn to use the Quantitative Analysis program. You can do these exercises with the demo batches DrugsOfAbuse and LC-QTOF Pesticide, shipped with the system in the **Data** folder of your installation disk, or with data you acquire.

1 Set Up and Quantitate a Batch of Acquired MRM Data Files

In this exercise, you set up a batch table, a quantitation method, and target compounds, using acquired data files, analyze the batch, and save the results. This chapter is applicable for uses of the Agilent 6400 Series Triple Quad LC/MS system and the Agilent 7000 Series Triple Quad GC/MS system.

2 Set Up and Quantitate a Batch of Acquired Q-TOF Data Files

In this exercise, you set up a batch table, a quantitation method, and a target compound, using acquired data files, analyze the batch, and save the results.

3 Review Quantitation Results

In this exercise, you inspect the sample and compound data in a batch file, customize layouts, and export your batch results to a Microsoft Excel file.

4 Use Tools to Evaluate Results

The tools in this exercise make it easier for you to evaluate and obtain more accurate quantitation results.

5 Work With Quantitation Reports

In this exercise, you generate reports using specified templates, then review these reports in Microsoft Excel.

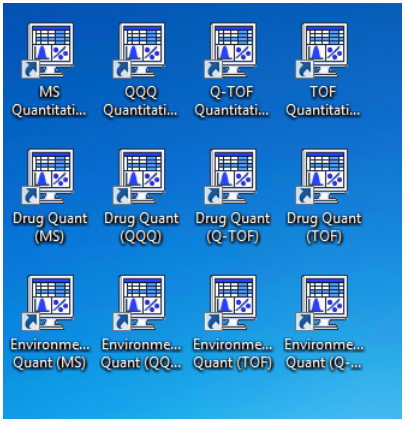
6 Reference

This section provides an overview of the special features of Quantitative Analysis.

Choosing the Correct Quantitative Analysis Icon

Depending on how the Quantitative Analysis program was installed, you may find several different icons on the desktop, each representing a different instrument type. When you start the Quantitative Analysis program from these icons, the default values and some of the features are customized to the selected instrument type.

When you click any of these icons, the full name of the installed program is displayed. Make sure you choose the icon that matches the type of data you want to analyze.



Before You Begin These Exercises

Be sure the data files you will be using as you complete the exercises in this document are on your PC.

- If the default MassHunter Quantitative Analysis Software Supplemental installation was completed, the data files needed for these exercises should be present in **MassHunter/Data/QuantExamples**.
- If these files were not already copied to your PC, copy the folder named **Data** from the **Supplemental** folder on your installation disk, in an uncompressed format, to any location on your hard disk.

Contents

Exercise 1
Set Up and Quantitate a Batch of Acquired MRM Data Files

In this exercise, you set up a quantitation method for a batch of acquired data files. You carry out the exercise with the **DrugsOfAbuse** data files on your installation disk and learn how to perform the following tasks:

- Set up a Batch Table containing unknown sample and calibration data files for drugs of abuse: amphetamine, cocaine, methamphetamine, and MDMA.
- Set up a new quantitation method based on the calibration standard of the highest concentration.
- Set up target compounds.
  - View the MRM transitions and chromatographic parameters for the compounds in the data file.
  - Set up an internal standard for each of the compounds.
- Set up quantitation for the method.
  - Create levels from calibration samples.
  - Set up qualifier ions and the calibration curve.
- Quantitate the batch and save the results.

Each exercise 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...

Make sure that you have copied the **DrugsOfAbuse** folder from the **Supplemental/Data/Quant Examples/QQQ** folder of the installation disk to a folder on your system. If the default MassHunter Quantitative Analysis Software Supplemental installation has been completed, then the data files needed for these exercises should be present in **MassHunter/Data/QuantExamples**.

Task 1. Set Up a New Batch

In this task, you set up a Batch Table containing data files for three unknown samples and several calibration samples of drugs of abuse: amphetamine, cocaine, methamphetamine, and MDMA.

Steps	Detailed instructions	Comments
1 Create a new batch to hold samples. • Select all of the data files from the **DrugsOfAbuse** folder. • Name the batch file, ***iii*_test_01**, where the letters "*iii*" are your initials.	**a** To start the Quantitative Analysis program, click the **Quantitative Analysis (QQQ)** icon on your desktop.  When you first use the program, the default layout appears, as shown in Figure 1.	• You can also access the program by clicking **Programs > Agilent > MassHunter Workstation > Quantitative Analysis (QQQ)** from the **Start** menu. • Different features are available when you are working with QQQ data.

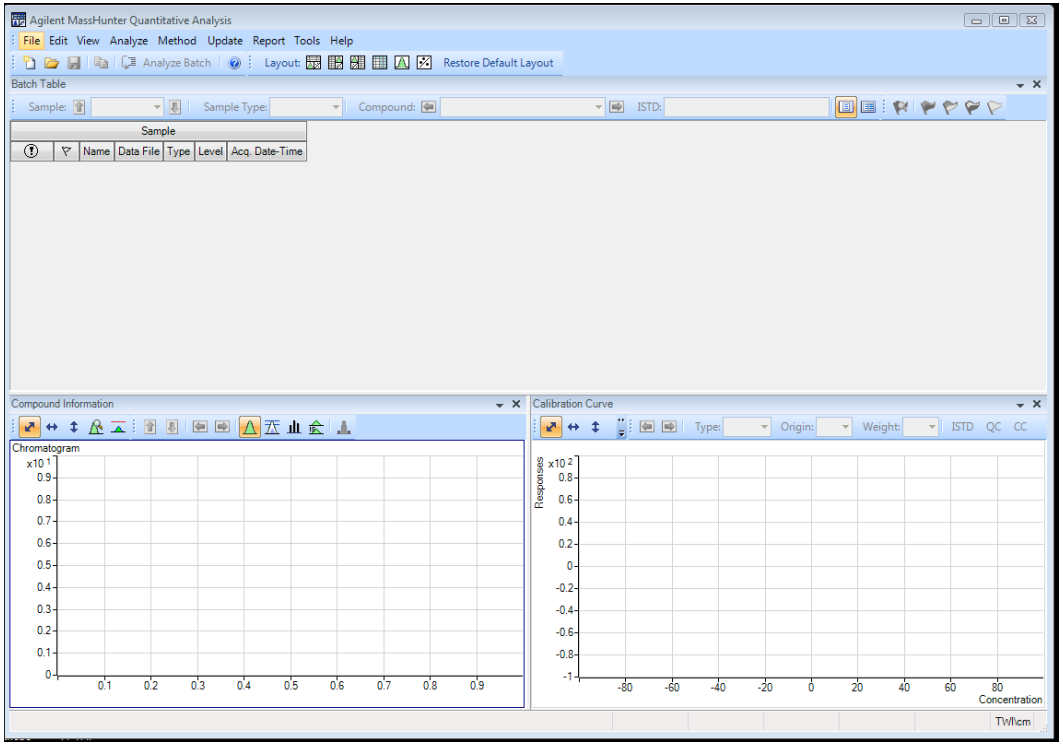
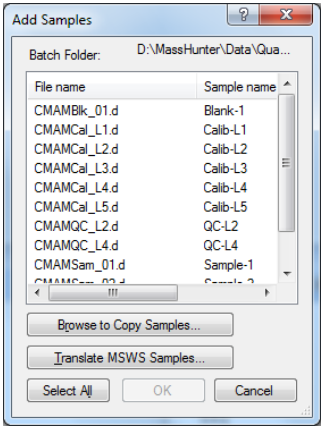


Figure 1 Default layout

Steps	Detailed instructions	Comments
	b Click **File > New Batch**. The system opens the **New Batch** dialog box. **c** Navigate to the folder ***Your Directory*\DrugsOfAbuse\.** **d** Type the batch file name ***iii*_Test_01** and click **Open**.	• If the default layout is not present, click **Restore Default Layout** on the toolbar before creating a new batch. Restore Default Layout
2 Add all the samples in the **DrugsOfAbuse** folder to the batch.	**a** **All Samples** should be selected. Click **OK** to add them to the batch. The **Batch Table** is no longer empty. It now contains the calibration, QC, and unknown samples. See Figure 2. 	• Note that only three of the files are unknown samples, one is a blank, five are calibration files at different calibration levels, and two are QC samples.

Steps	Detailed instructions	Comments

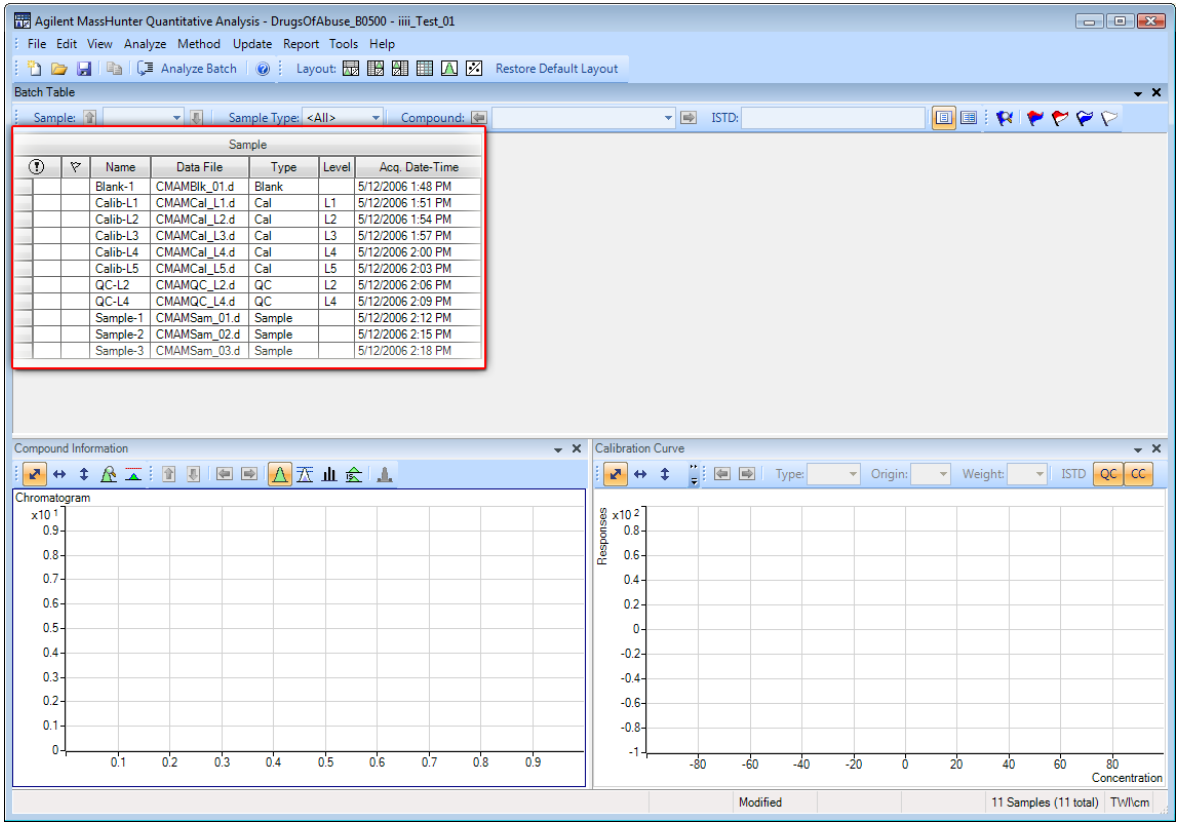
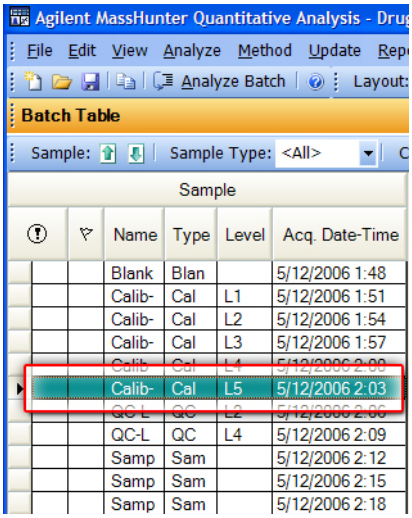


Figure 2 Batch Table containing Drugs of Abuse samples before quantitation

Task 2. Set Up a New Method for the Batch

This task shows you how to set up a new quantitation method based on the calibration data file with the highest concentration of sample.

Steps	Detailed instructions	Comments
1 Create a new method from acquired MRM data.	**a** Use the cursor to highlight the calibration standard that has the highest concentration level, as shown in the figure below.	• Using a sample with strong signals for the compounds, such as a high-concentration calibration sample, lets the program create a method with the appropriate retention times and qualifier ratios.



Steps	Detailed instructions	Comments
	b Click **Method > Edit** to switch to method editing mode. The **Method Tasks** appear in the column to the left of the view, as shown in Figure 3.	• Note that Figure 3 shows the default layout for method editing. • If the default layout is not present, click **Restore Default Layout** on the toolbar before creating a new method in the next step. Restore Default Layout

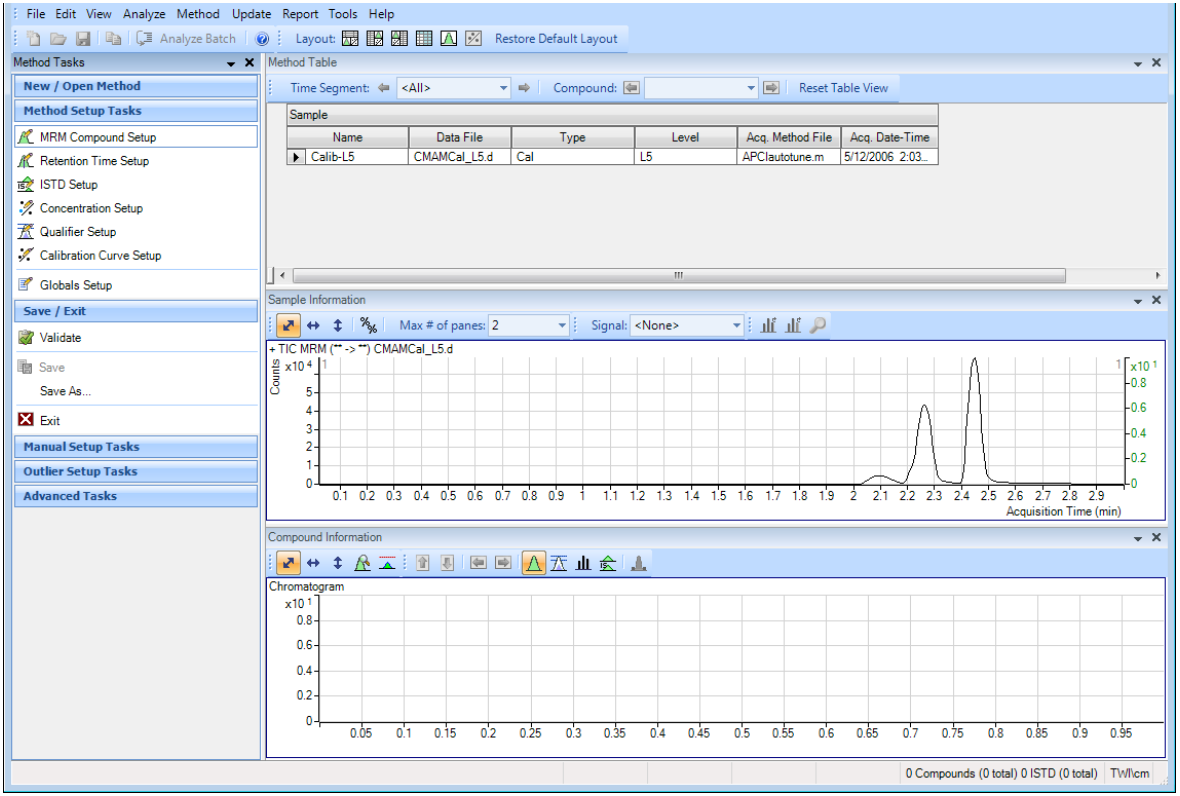
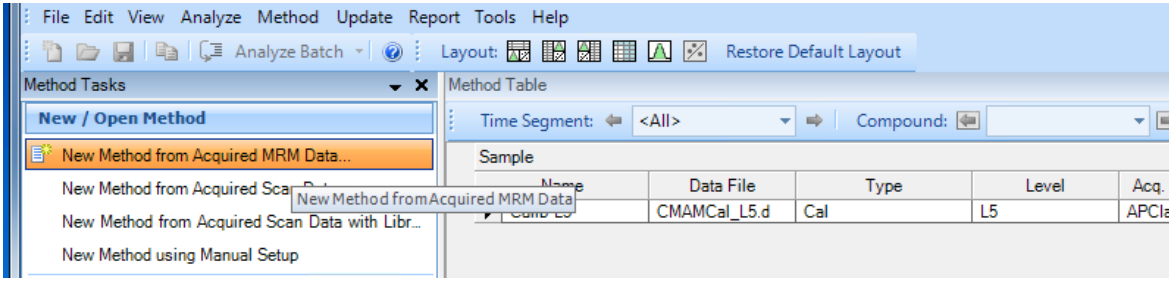


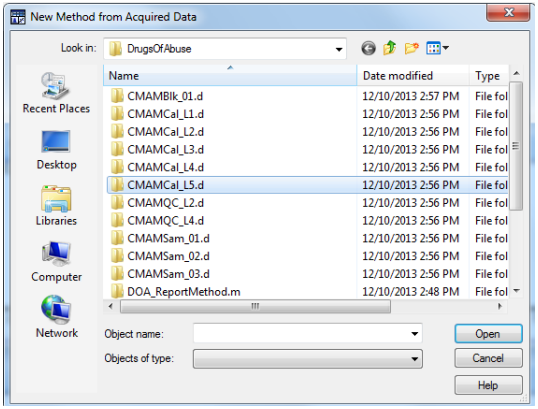
Figure 3 Method Edit mode

Steps	Detailed instructions	Comments
	c Under **Method Tasks** in the sidebar to the left of the **Method Table**, click **New/Open Method > New Method from Acquired MRM Data**.	• You can also click **Method > New > New Method from Acquired MRM Data.**



d Select **No** to the prompt **Would you like to apply this method to the batch?** The system displays a **New method From Acquired Data** dialog box.

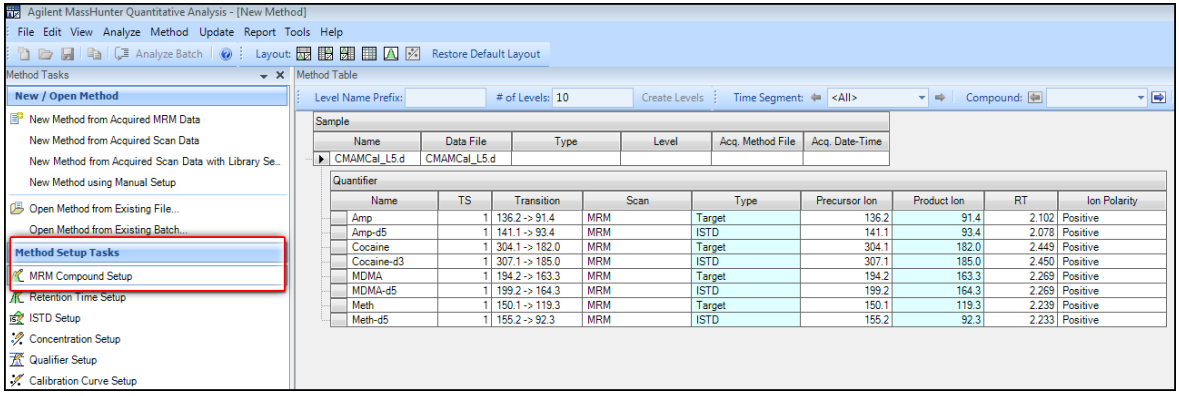
e Click **CMAMCal_L5.d** and click **Open** to import acquisition method information.



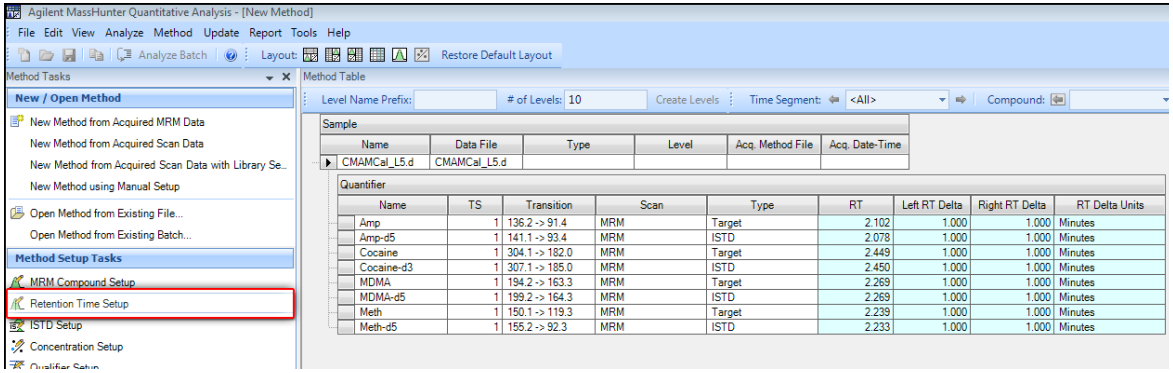
Task 3. Set Up Target Compounds

With this task, you learn to inspect the MRM transitions and the RT data for the new quantitation method, which you can change for individual target compounds. You also learn to set up an ISTD compound for each target compound.

Steps	Detailed instructions	Comments
1 Check the new quantitation method created from the imported acquisition method for MRM transitions.	**a** Under **Method Tasks** in the sidebar to the left of the **Method Table** window, click **Method Setup Tasks > MRM Compound Setup.**	• The compound names associated with MRM transitions are entered in the acquisition method. By default, the largest signal is chosen as the quantifier ion.



Steps	Detailed instructions	Comments
	b To inspect the imported retention time data, click **Method Setup Tasks > Retention Time Setup**.	• You can modify data fields in blue for individual compounds.

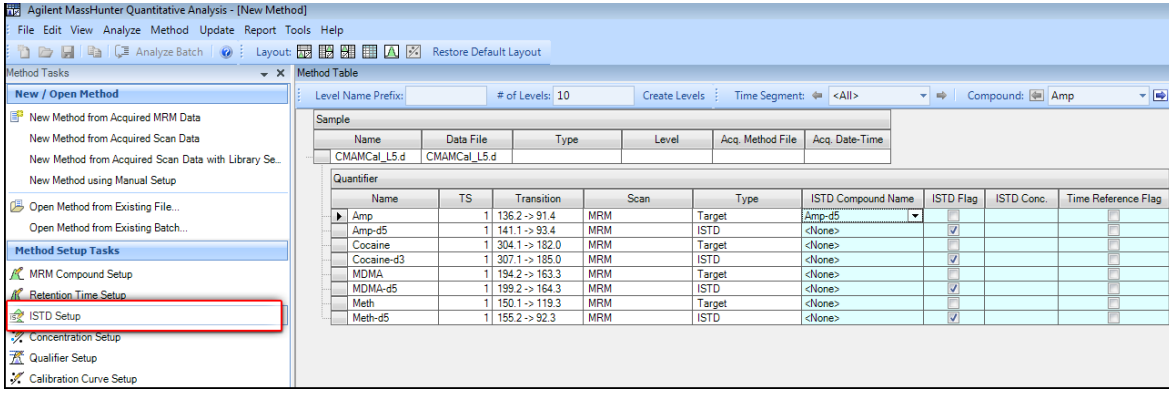


2 Set up ISTD compounds.
- Assign the corresponding deuterated compound as the internal standard (ISTD) for each target compound.

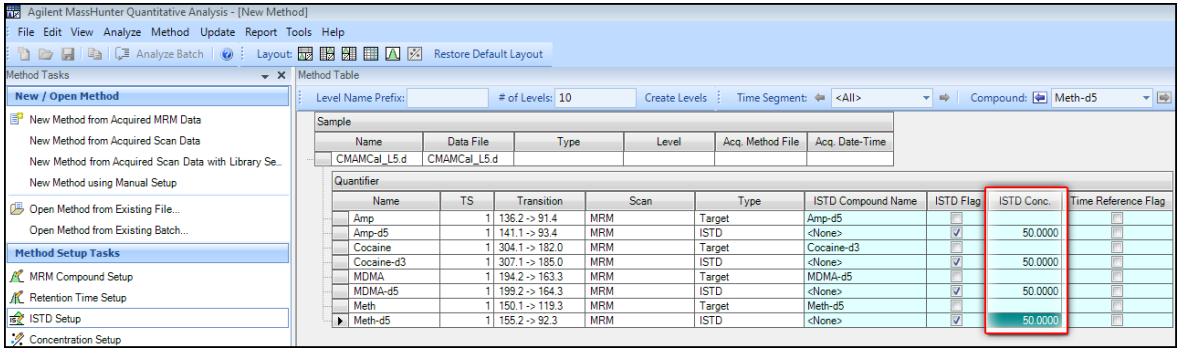
a Click **Method Setup Tasks > ISTD Setup**.

b For each target compound row, click the down arrow in the **ISTD Compound Name** cell.

- Do not attempt to enter the ISTD name into the ISTD compound row.



Steps	Detailed instructions	Comments
	c Click the ISTD name associated with the target compound. **d** Type the ISTD concentration (**ISTD Conc.**) for each ISTD compound (50.0000 in this example).	

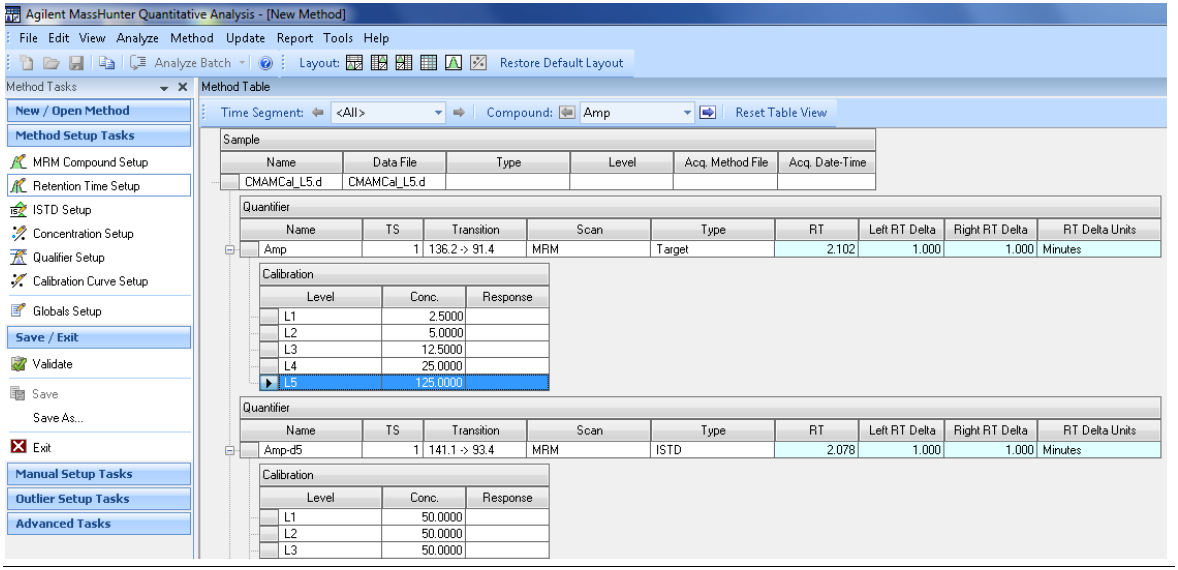


Task 4. Set Up Quantitation

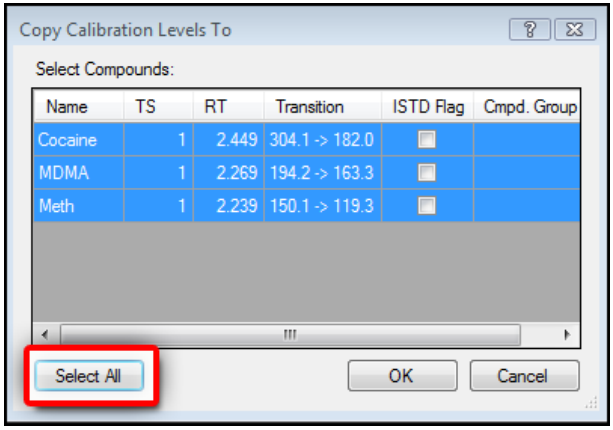
This task presents instructions for setting up the quantitation parameters for the method's:

- Calibration levels
- Qualifier ions
- Calibration curve fit

Steps	Detailed instructions	Comments
1 Create five calibration levels for the first compound.	**a** From the main menu select **Method>Create Levels from Calibration Samples**. The **Calibration** table opens under each Quantifier in the **Method Table.** **b** For one of the Quantifiers, change the default concentrations to the actual concentration for each level. • L1–2.5000 • L2–5.0000 • L3–12.5000 • L4–25.0000 • L5–125.0000	

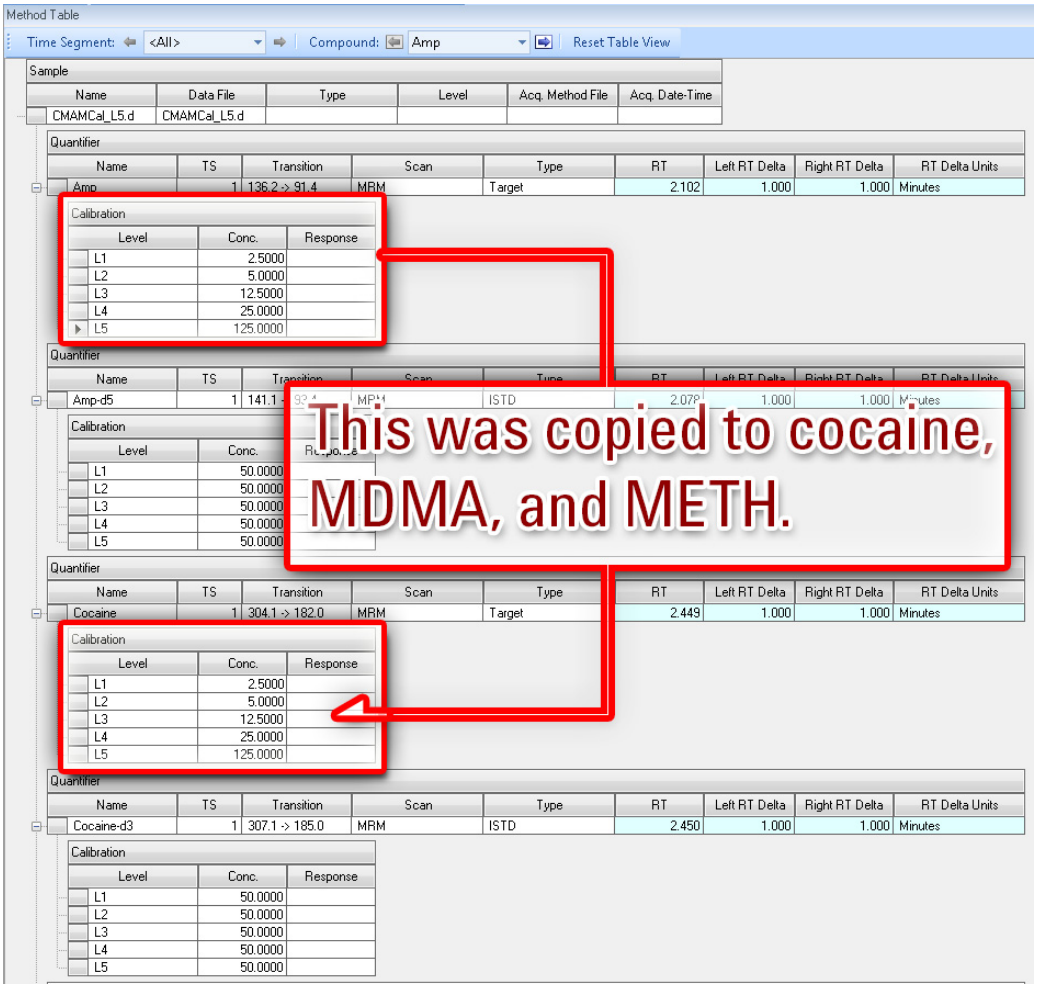


Steps	Detailed instructions	Comments
2 Copy the calibration levels and concentrations to the other compounds.	**a** Click **Method > Copy Calibration Levels To...** The system displays the **Copy Calibration Levels To** dialog box. **b** Click **Select All**, and then click **OK**.	

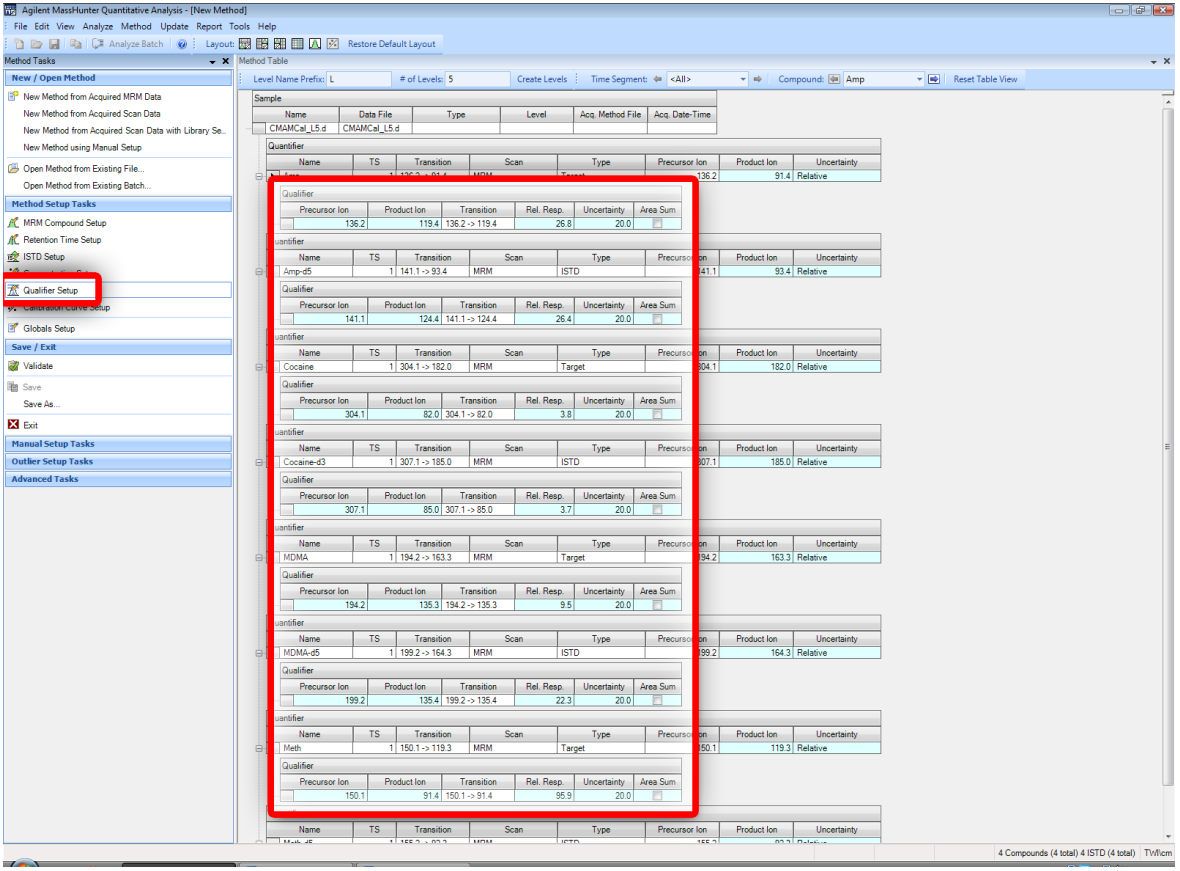


	c Close the **Compound Information** window and the **Sample Information** window in the lower half of the Quantitative Data Analysis main view.	

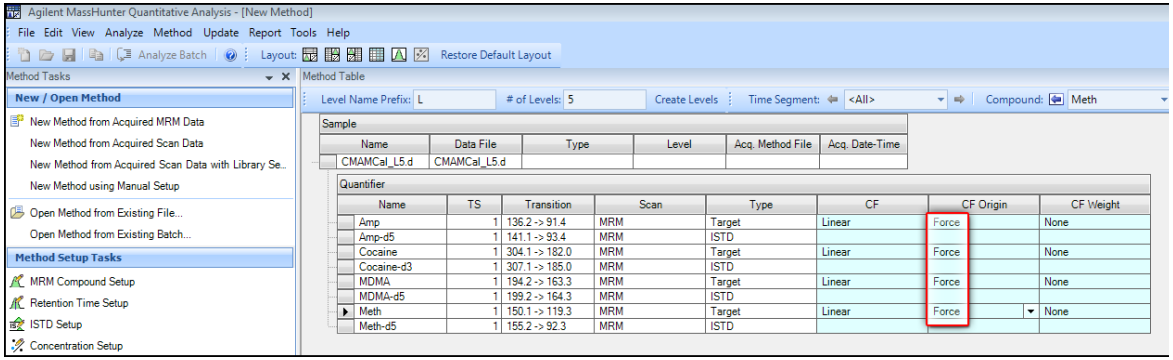
Steps	Detailed instructions	Comments
	d Browse the **Method Table** to compare the calibration concentration setup among the four target compounds, Amp, Cocaine, Meth, and MDMA.	



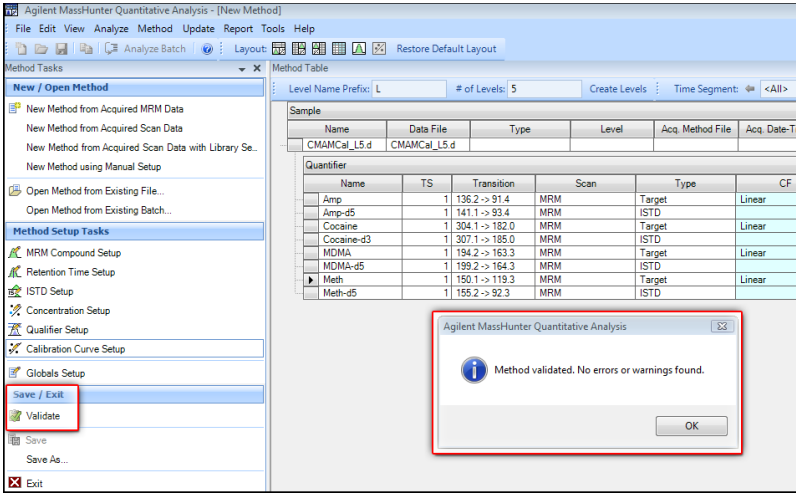
Steps	Detailed instructions	Comments
3 Set up qualifier ions and a calibration curve.	**a** Under **Method Setup Tasks,** click **Qualifier Setup**, and inspect the Qualifier setup parameters.	• The system automatically populates the qualifier setup parameters when it imports MRM acquisition information. • During method creation, additional MRM transitions besides the quantifier ion for a compound are assigned as qualifier ions.



Steps	Detailed instructions	Comments
	b Under **Method Setup Tasks,** click **Calibration Curve Setup**. **c** For each target compound, change the **CF Origin** to **Force**.	



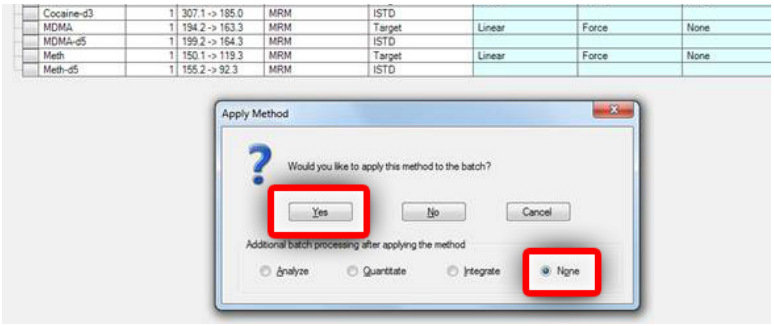
Steps	Detailed instructions	Comments
4 Validate the method.	**a** Under **Save/Exit**, click **Validate** to validate the method setup.	• You can view any validation errors that do occur at the bottom of the screen.



b After the validation message appears, click **OK.**

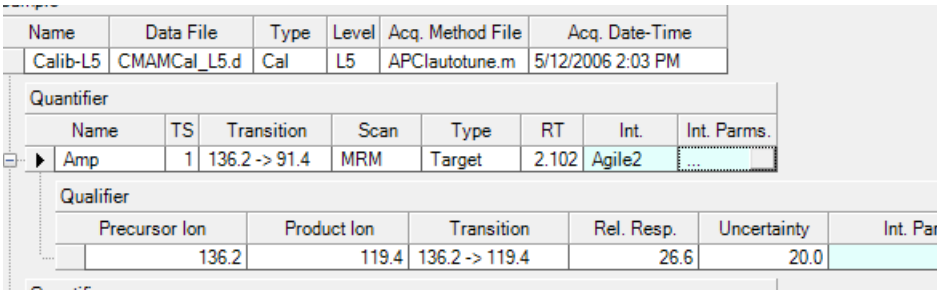
c Click **Save/Exit > Exit**.

d Select **None** under **Additional batch processing after applying the method,** and click **Yes** to the **Would you like to apply this method to the batch?** prompt.

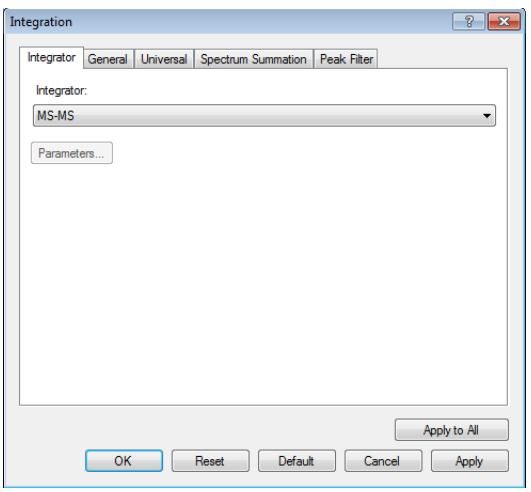


Task 5. Set the Integrator

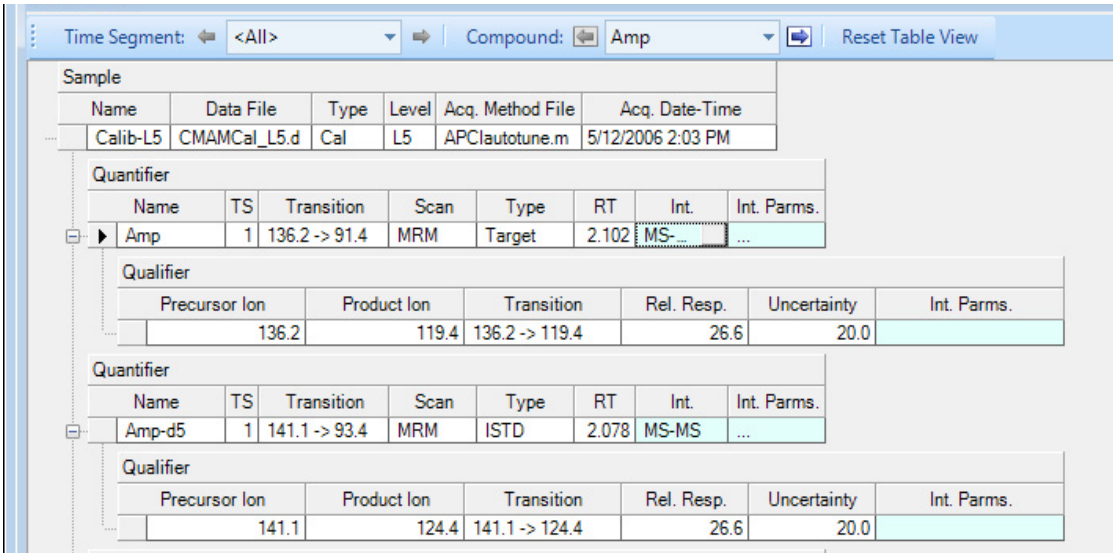
Steps	Detailed instructions	Comments
1 Change the default integrator to MS-MS.	**a** Click **Method >Edit** or press **F10.** **b** Click **Method > Advanced Tasks > Integration Parameters Setup**. **c** In the **Method Table**, click the box located on the right side of the **Int.** value.	The default and Agilent recommended integrator for MassHunter Quant is the Agile2 parameterless integrator. This task changes the default Agile2 integrator to the MS-MS integrator to demonstrate the procedure for changing the integrator for all compounds in a Quant method.



d Select **MS-MS** from the drop-down menu..



Steps	Detailed instructions	Comments
	e Click **Apply to All**. **f** Click **OK.**	

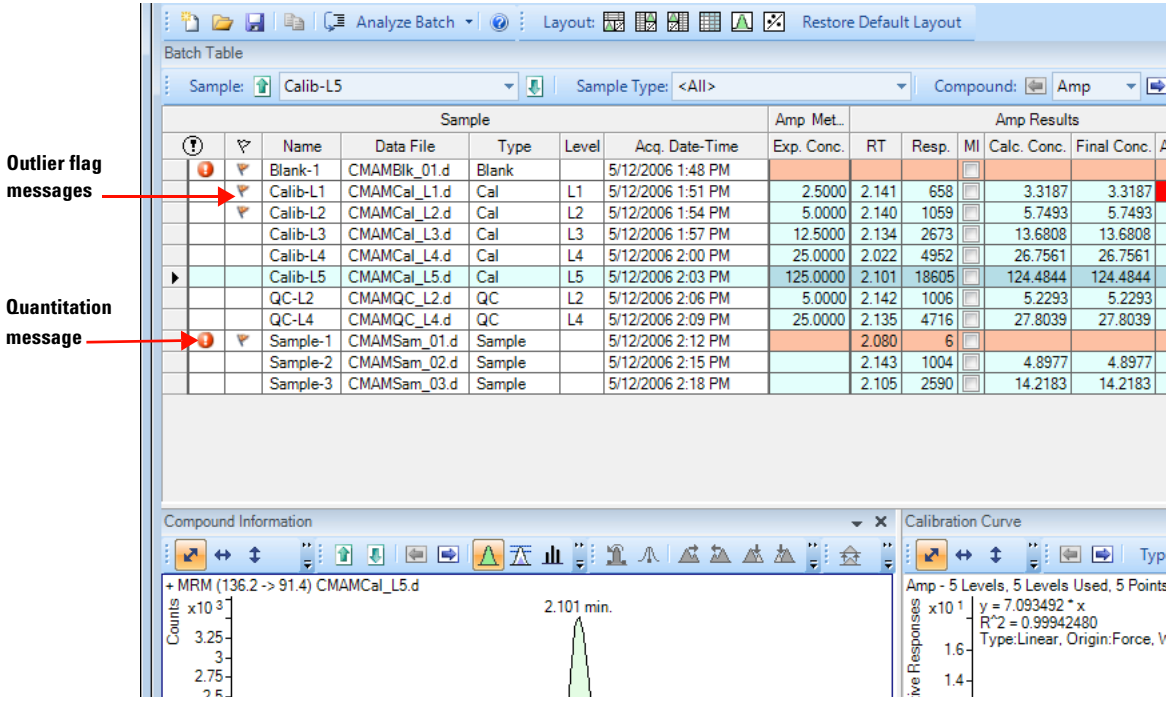


2 Save the method.	**a** Under **Save/Exit,** click **Exit**. **b** Select **None** under **Additional batch processing after applying the method,** and click **Yes** to the **Would you like to apply this method to the batch?** prompt.	

Task 6. Analyze and Save the Batch

In this exercise, you quantitate the batch and then save the results.

Steps	Detailed instructions	Comments
1 Analyze the batch, and inspect the results for each compound.	**a** Click the **Analyze Batch** icon (Analyze Batch) in the toolbar to start batch analysis. **b** Pass the cursor over the quantitation message for Sample 1. **c** Pass the cursor over the flags for the first two calibration standards.	• Note that two calibration standards contain outlier data. • Note that the program found no data for Amphetamine (Amp) in Sample-1.



2 Save the batch.	**a** Click **File > Save Batch**. **b** Click **File > Close Batch** to close the batch.	

Exercise 2
Set Up and Quantitate a Batch of Acquired Q-TOF Data Files

In this exercise, you set up a quantitation method for a batch of acquired Q-TOF data files. You carry out the exercise with the **LC-QTOF Pesticide** data files on your installation disk and learn how to perform the following tasks:

- Set up a Batch Table containing sample and calibration data files for the solvent.
- Set up a new quantitation method based on the calibration standard of the highest concentration.
- Set up a target compound.
  - View the product ion and chromatographic parameters for the solvent compound in the data file.
- Set up quantitation for the method.
  - Create levels from calibration samples.
  - Set up qualifier ions and the calibration curve.
- Quantitate the batch and save the results.

Each exercise 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...

Make sure that you have copied the **LC-QTOF Pesticide** folder from the **Supplemental/Data/Quant Examples/Q-TOF** folder of the installation disk to a folder on your system. If the default MassHunter Quantitative Analysis Software Supplemental installation has been completed, then the data files needed for these exercises should be present in **MassHunter/Data/QuantExamples**.

Task 1. Set Up a New Batch

In this task, you set up a Batch Table containing data files for calibration samples of the solvent. Many of the tasks in this section are similar to the tasks in Exercise 1.

Steps	Detailed instructions	Comments
1 Create a new batch to hold samples. • Select all of the data files from the **LC-QTOF Pesticide** folder. • Name the batch file, ***iii*_test_01**, where "*iii*" are your initials.	**a** To start the Quantitative Analysis program, click the **Quantitative Analysis (Q-TOF)** icon on your desktop. When you first use the program, the default layout appears, as shown in Figure 4.	• You can also access the program by clicking **Programs > Agilent > MassHunter Workstation > Quantitative Analysis (Q-TOF)** from the Start menu.

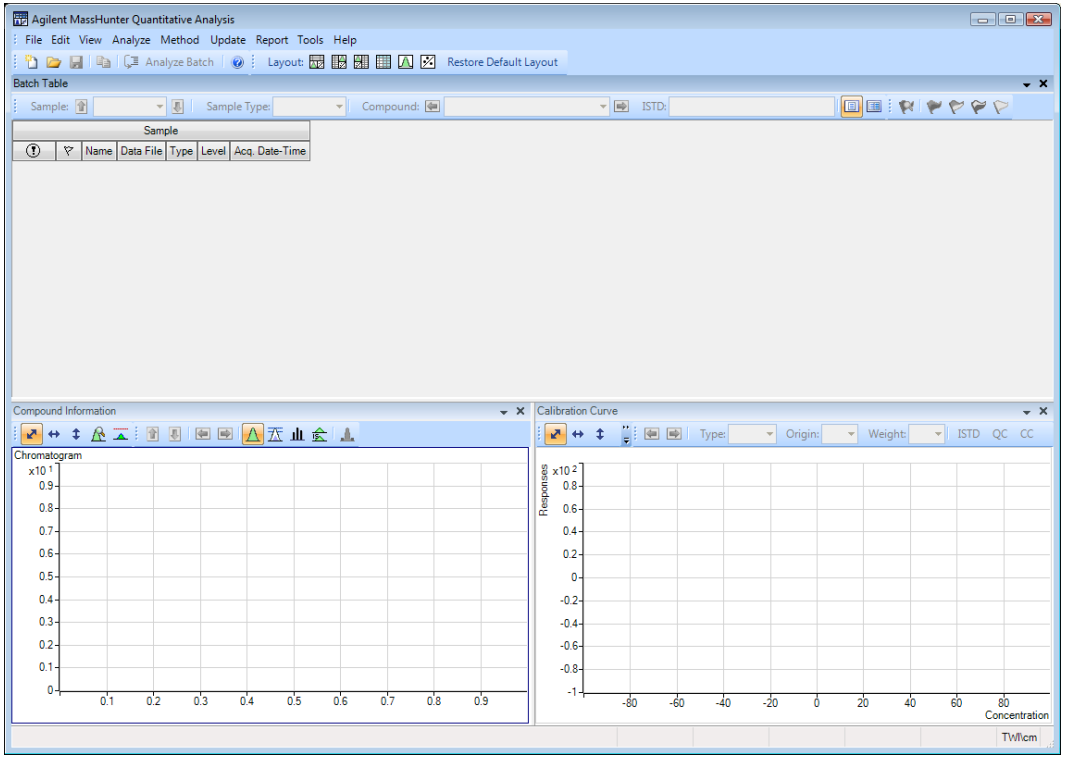
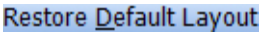
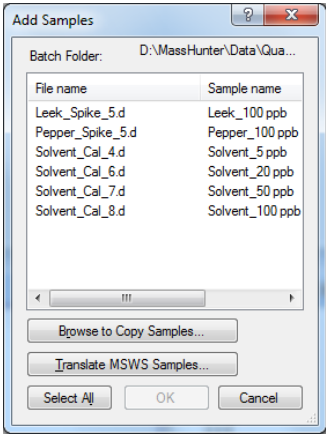


Figure 4 Default layout

Steps	Detailed instructions	Comments
	b Click **File > New Batch**. The system opens the **New Batch** dialog box. **c** Navigate to the folder \ ***Your Directory*** **LC-QTOF Pesticide**\. **d** Type the batch file name ***iii*_Test_01** and click **Open**.	• If the default layout is not present, click **Restore Default Layout** on the toolbar before creating a new batch. 
2 Add all the samples in the **LC-QTOF Pesticide** folder to the batch.	**a** The system displays the **Add Samples** dialog box. **All Samples** should be selected. Click **OK** to add them to the batch. The **Batch Table** is no longer empty. It now contains the samples. See Figure 5. 	• Note that there are four calibration samples.

Steps	Detailed instructions	Comments

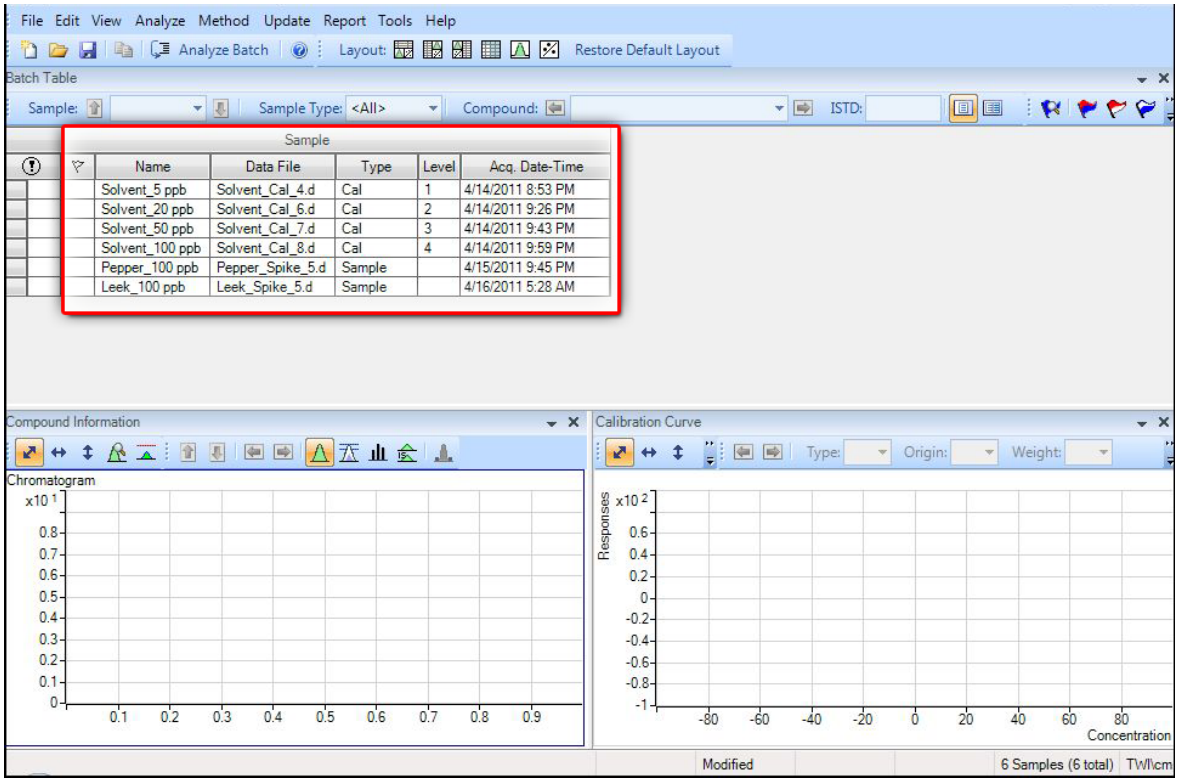


Figure 5 Batch Table containing samples before quantitation

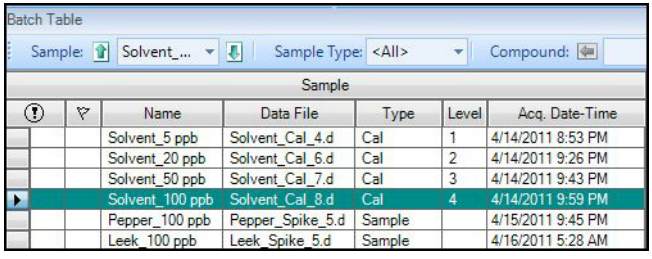
Task 2. Set Up a New Method for the Batch

This task shows you how to set up a new quantitation method based on a batch containing calibration sample data files. In this task we will use a single calibration sample and extract from it the necessary data to add a calibration compound to the method.

The procedure described in Task 2 is a manual one. There is also an automated procedure in MassHunter that allows you to create a quantitation method that adds a large number of calibration compounds in a single step using acquired scan data with a library search. In the automated method, MassHunter analyzes a data file, and using search ID parameters that you specify, identifies compound names, the target ion, qualifier ions and ratios, and retention times. Then it uses this information along with other default parameters to fill in initial values for the quantitation method. This automated method greatly reduces the time required for method creation.

Additionally, you can add compounds found in Qualitative Data Analyses by transferring the data from Qual to Quant using CEF files. Refer to your online Help for more details.

Steps	Detailed instructions	Comments
1 Create a new method from acquired Q-TOF data.	**a** Use the cursor to highlight the calibration standard that has the highest concentration level, as shown in the figure below.	• Using a sample with strong signals for the compounds, such as a high-concentration calibration sample, lets the program create a method with the appropriate retention times and qualifier ratios.



		Name	Data File	Type	Level	Acq. Date-Time
		Solvent_5 ppb	Solvent_Cal_4.d	Cal	1	4/14/2011 8:53 PM
		Solvent_20 ppb	Solvent_Cal_6.d	Cal	2	4/14/2011 9:26 PM
		Solvent_50 ppb	Solvent_Cal_7.d	Cal	3	4/14/2011 9:43 PM
▶		Solvent_100 ppb	Solvent_Cal_8.d	Cal	4	4/14/2011 9:59 PM
		Pepper_100 ppb	Pepper_Spike_5.d	Sample		4/15/2011 9:45 PM
		Leek_100 ppb	Leek_Spike_5.d	Sample		4/16/2011 5:28 AM

Steps	Detailed instructions	Comments
	b Click **Method > Edit** to switch to method editing mode. The **Method Tasks** appear in the column to the left of the view, as shown in Figure 6.	• Note that Figure 6 shows the default layout for method editing. • If the default layout is not present, click **Restore Default Layout** on the toolbar before creating a new method in the next step. Restore Default Layout

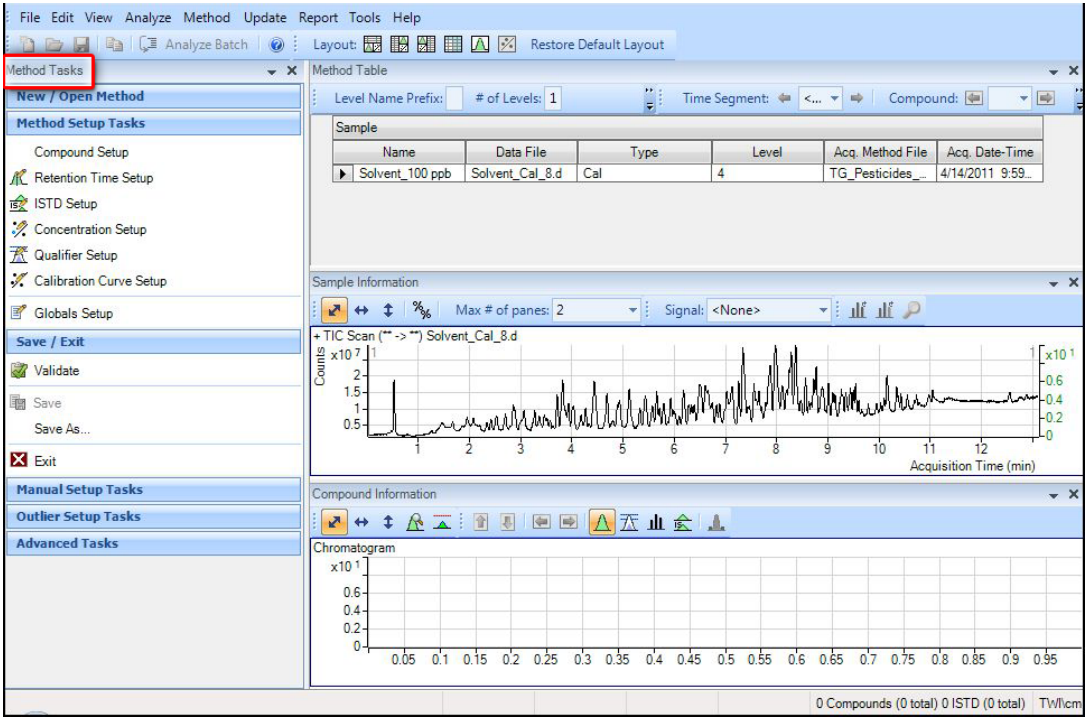
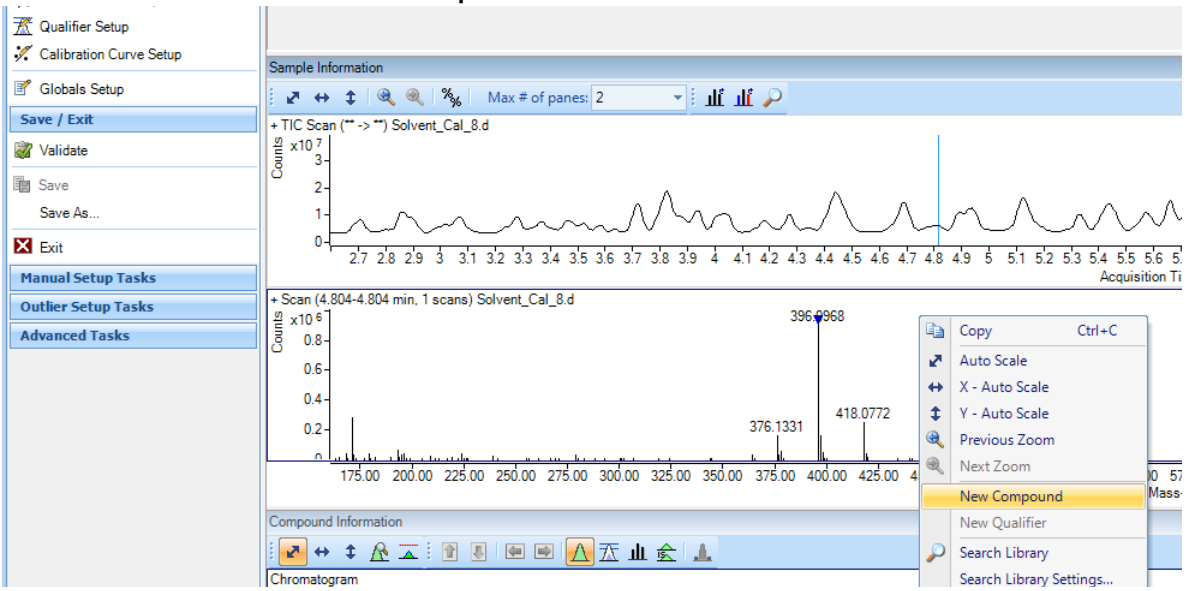
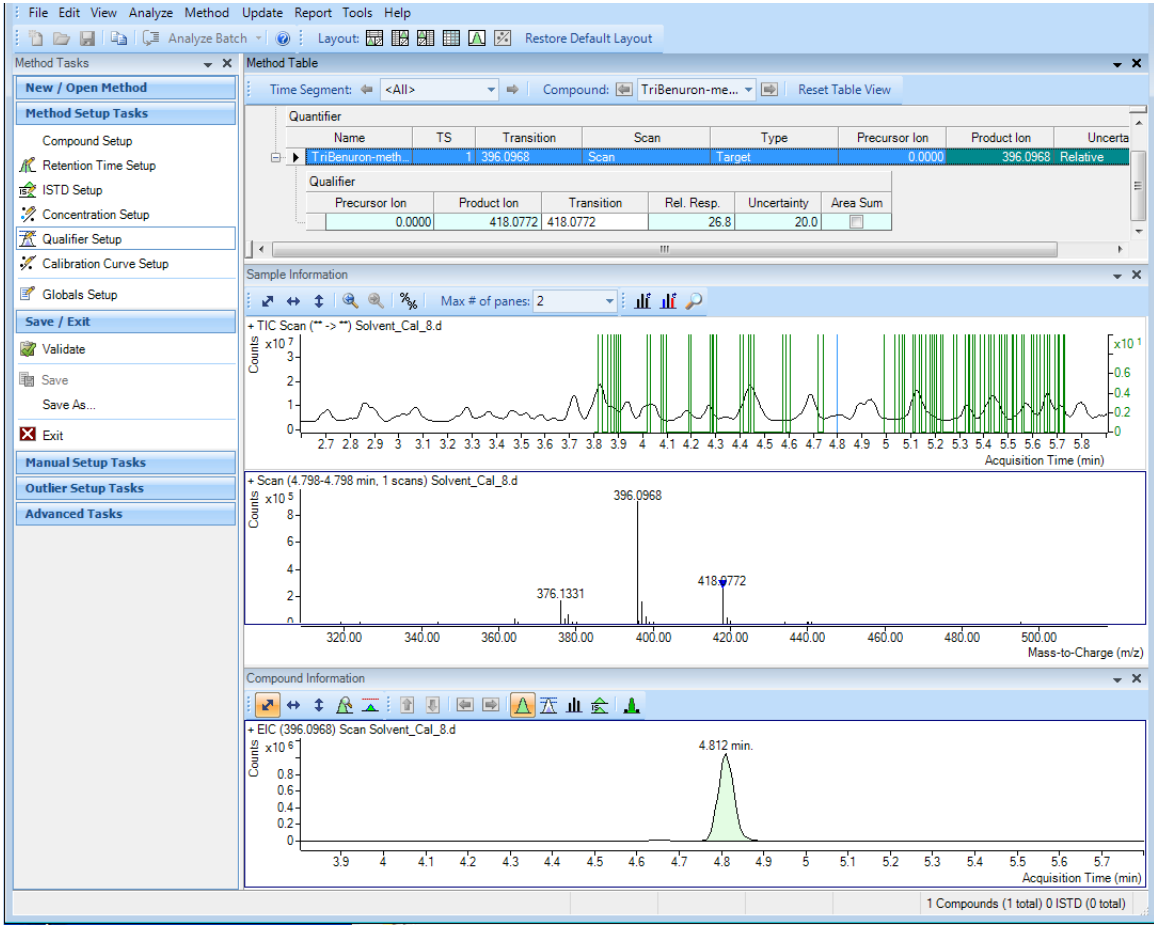


Figure 6 Method Edit mode

Steps	Detailed instructions	Comments
	c In the **Sample Information** window, click the middle of the peak at approximately 4.82 on the x-axis. Right-click and click **Extract Spectrum**. **d** Click the largest ion, **396.0966.** Right-click that location and click **New Compound**.	• To accurately select the ion, hold down the right mouse button while hovering over the spectra and zoom in on the range around the ion you are trying to select.



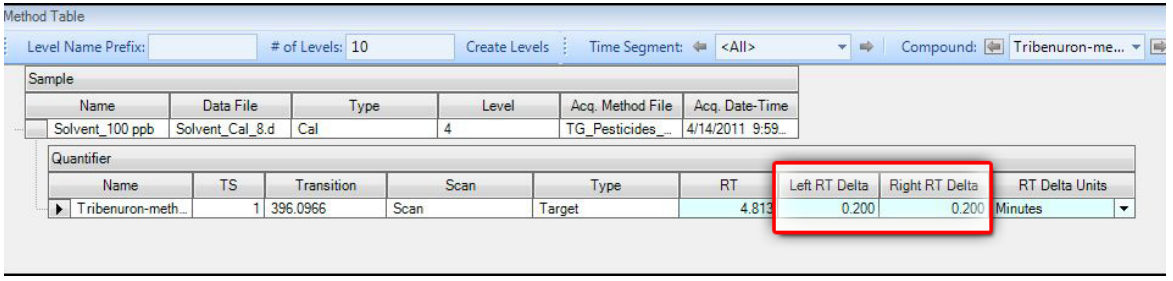
Steps	Detailed instructions	Comments
	e Type `Tribenuron-methyl` as the **Name** in the **Method Table**. Keep this compound selected in the Method table while you add the qualifier in the next step.	• You can select more than one qualifier ion. • A blue triangle indicates the selected *m/z* in the spectrum.
	f To once again display the spectrum for **Tribenuron-methyl**, click at the peak apex to display a line running through the apex.	
	g Click **418.0776** to select that ion (blue filled triangle).Right-click that location and click **New Qualifier**.	The qualifier is added to the Method Table as shown.



Task 3. Set Up Target Compounds

With this task, you learn to inspect the product ions and the RT data for the new quantitation method, which you can change for individual target compounds.

Steps	Detailed instructions	Comments
1 Check the new quantitation method created from the **Sample Information** window for the product ion.	**a** To inspect the retention time set from the spectrum, click **Method Setup Tasks > Retention Time Setup**. **b** In the **Left RT Delta** column, enter `0.2`. **c** In the **Right RT Delta** column, enter `0.2`.	• You can modify data fields in blue for individual compounds.

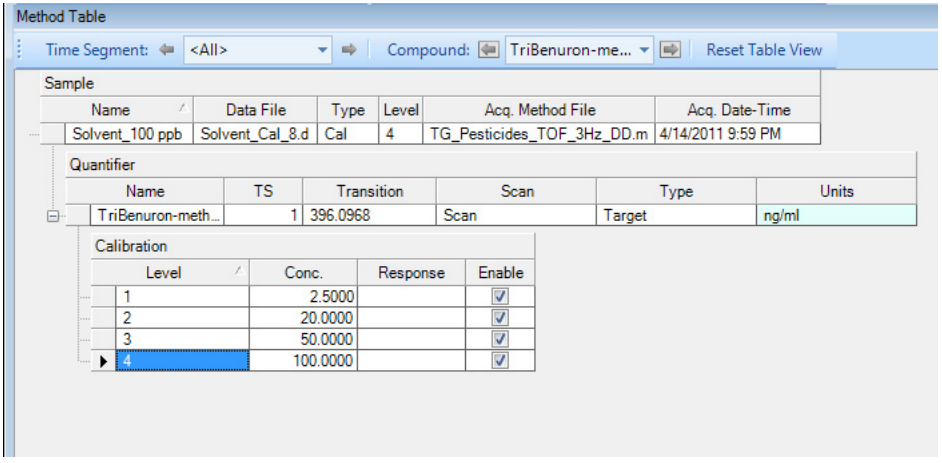


Task 4. Set Up Quantitation

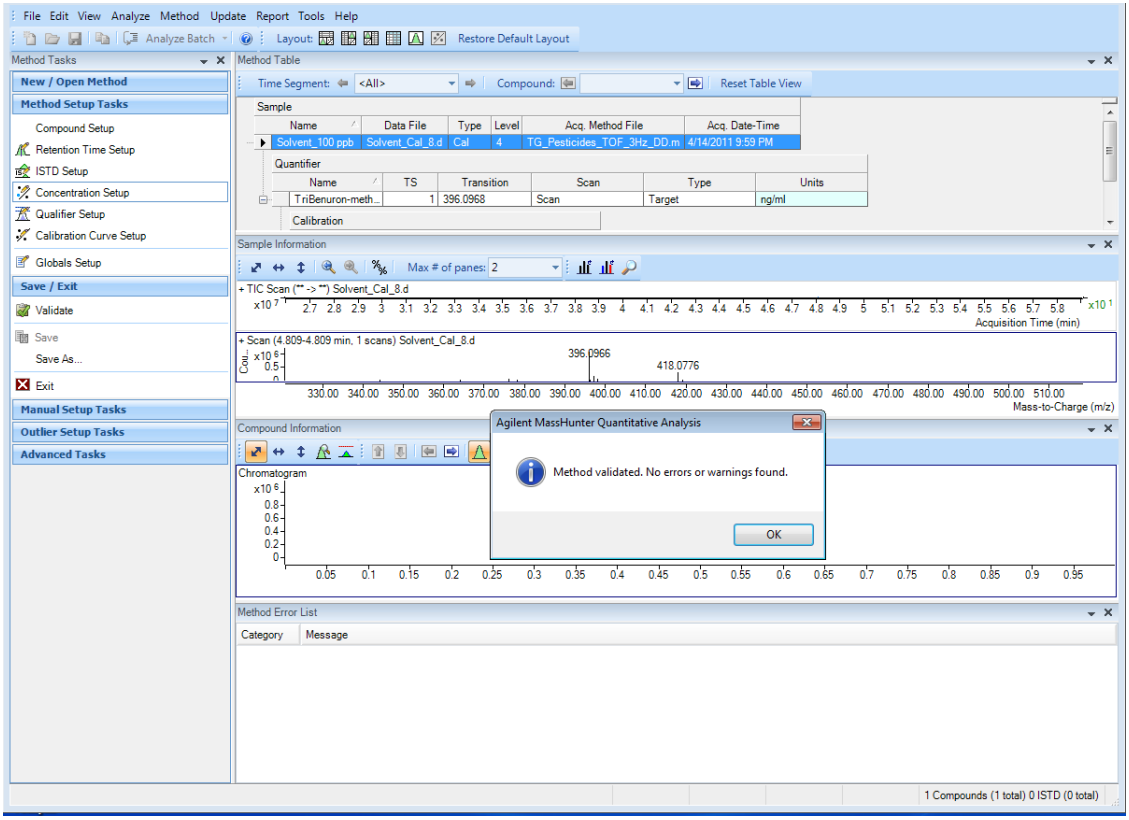
This task presents instructions for setting up the quantitation parameters for the method's:

- Calibration levels
- Qualifier ions
- Calibration curve fit

Steps	Detailed instructions	Comments
1 Create four calibration levels.	**a** From the main menu select **Method>Create Levels from Calibration Samples**. The **Calibration** table opens under each Quantifier in the **Method Table.** **b** For one of the Quantifiers, change the default concentrations to the actual concentration for each level. • L1–2.5000 • L2–20.0000 • L3–50.0000 • L4–100.0000	



Steps	Detailed instructions	Comments
2 Validate and save the method.	**a** Click **Save/Exit > Validate** to validate the method setup.	• You can view any validation errors that do occur at the bottom of the screen.



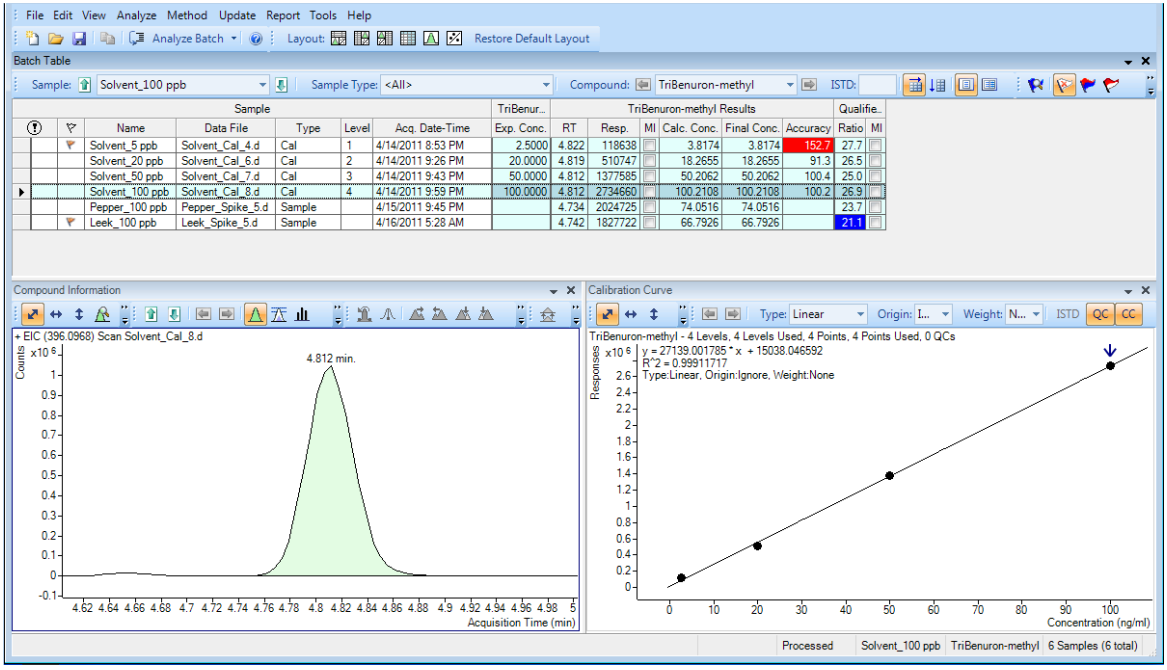
b After the validation message appears, click **OK.**

c Under **Save/Exit,** click **Exit,** then select **None** under **Additional batch processing after applying the method,** and click **Yes** to the **Would you like to apply this method to the batch?** prompt.

Task 5. Analyze and Save the Batch

In this exercise, you automatically quantitate the batch and then save the results.

Steps	Detailed instructions	Comments
1 Analyze the batch, and inspect the results for each compound. • Examine the Quantitation Message(s), which identify samples with no quantitated signals.	Click the **Analyze Batch** icon Analyze Batch in the toolbar to start batch analysis.	



2 Save the batch.	**a** Click **File > Save Batch**. **b** Click **File > Close Batch** to close the batch.	

Exercise 3
Review Quantitation Results

The tasks in this exercise show you how to inspect the sample and compound data in a batch file, customize result layouts, export your data to Microsoft Excel, and preview and print the data.

Use the **DrugsOfAbuse** batch in this exercise. Similar tasks can be performed with Triple Quad data files, Q-TOF data files, and TOF data files.

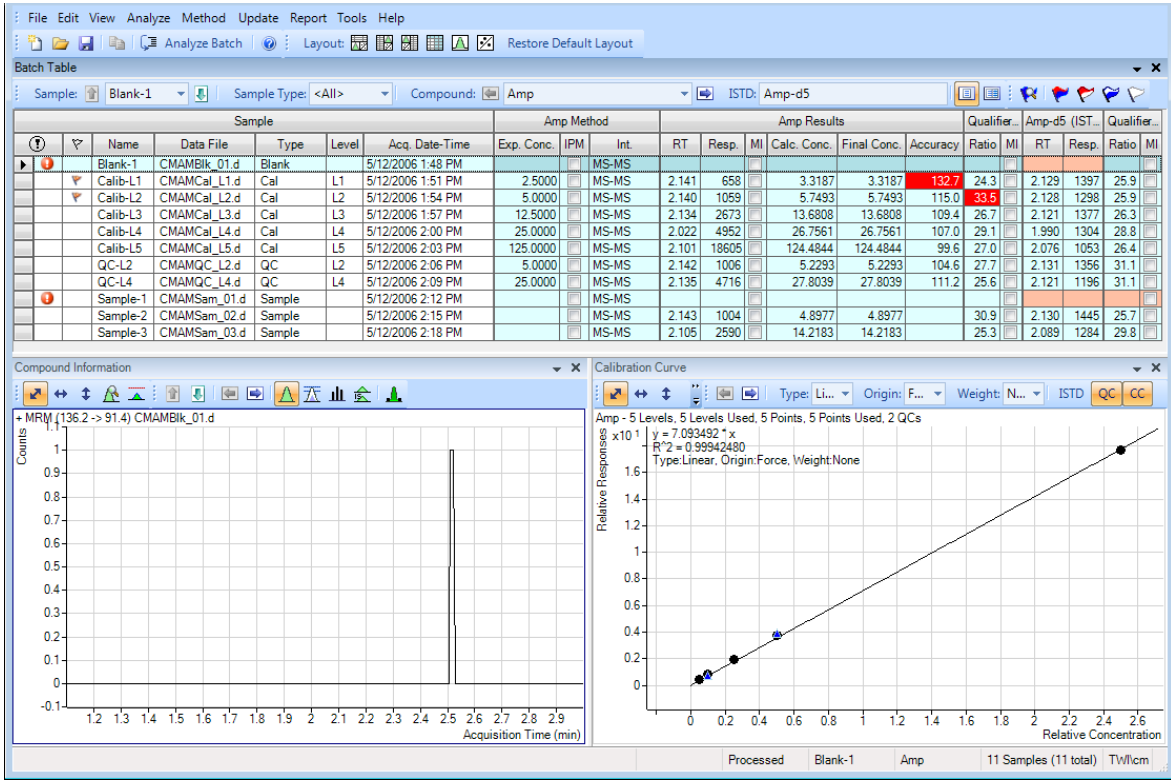
Each exercise is presented in a table with three columns:

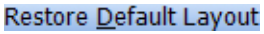
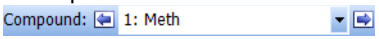
- Steps – Use these general instructions to proceed on your own to explore the program.
- Detailed Instructions – Use these if you need help or prefer to use a step-by-step learning process.
- Comments – Read these to learn tips and additional information about each step in the exercise.

Task 1. Navigate the Batch Table Results

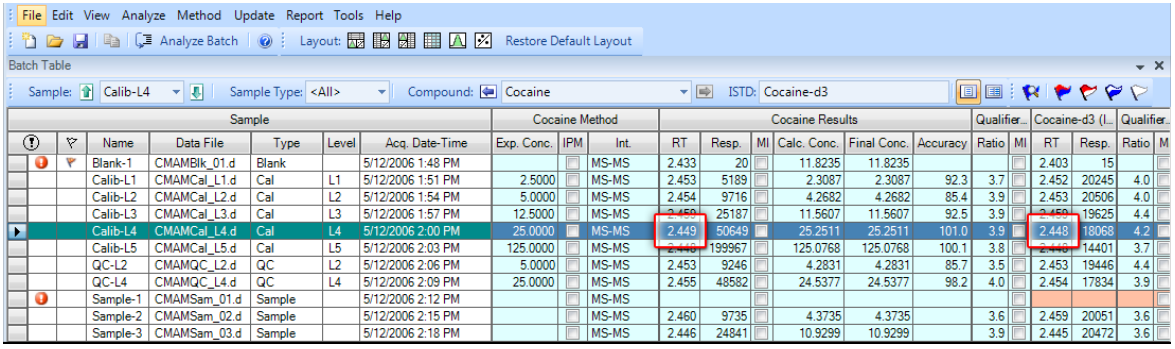
This task shows you how to scroll through your samples and compounds, while observing changes in the Batch Table and compound information data. It also shows you how to display various sample types.

Steps	Detailed instructions	Comments
1 Open the batch file ***iii*_Test_01.batch.bin**, created in Exercise 1.	**a** To start the Quantitative Analysis program, click the **Quantitative Analysis** icon on your desktop . **b** Click **Open Batch** on the toolbar to display the **Open Batch** dialog box. **c** Navigate to \ ***Your Directory*** \ **DrugsOfAbuse** and click ***iii*_Test_01.batch.bin**	• The main view that appears should look like the one below. This is the default layout and contains the default column settings.



Steps	Detailed instructions	Comments
2 (Optional) If you see a different layout than the one in the figure on the previous page... • If fewer than three windows are present in the main view, or they are in a different arrangement, restore the default layout. • If the column settings are different, restore the default column settings. • If panes other than the Chromatogram pane are present in the **Compound Information** window, hide the other panes.	• To restore the default layout, click **Restore Default Layout** on the toolbar before scrolling from sample to sample.  • To restore the default column settings, right-click anywhere in the **Batch Table** window and click **Restore Default Columns**. • To hide extra panes, click the highlighted icons other than the Show/Hide Chromatogram icon  in the Compound Information toolbar.	• The default layout is set at the factory and cannot be changed. If you want to create your own layout, see "Task 2. Change Result Window Layouts" on page 49.
3 Scroll from sample to sample until you reach the end of the **Batch Table**, and then return to Cal-L5.	**a** Click the **Next Sample** arrow  in the Batch Table Standard toolbar until the system displays the desired sample. Inspect the changes in the **Compound Information** window. **b** To return to Cal-L5, click the **Previous Sample** icon  in the Batch Table Standard toolbar. **c** Select any cell in the row for sample **Calib_L4** in the **Batch Table** window to view the changes.	• Note the linkage between the highlighted data file in the **Batch Table** and the chromatogram in the **Compound Information** window. • Note the changes in the **Batch Table** and **Compound Information** of amphetamine for each sample.
4 Scroll from compound to compound through all four compounds.	**a** Click the **Next Compound** or **Previous Compound** arrow in the toolbar until the system displays the desired compound.  **b** Inspect the changes in the **Batch Table**, **Compound Information,** and **Calibration Curve** windows. **c** Click the down arrow next to the **Compound** list. **d** Click **Cocaine**.	

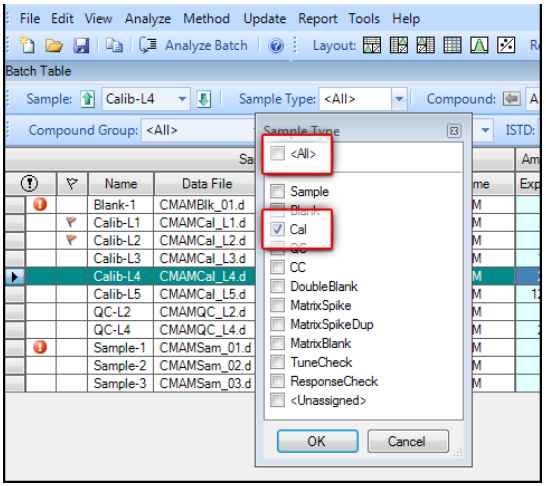
Steps	Detailed instructions	Comments
5 Examine results for multiple compounds. • View the RT for each compound for the Cal-L4 sample. • After reviewing the results for all the compounds, return to viewing the cocaine results.	**a** Click the **Display Multiple Compounds/Samples in Batch Table View** icon in the toolbar to display the quantitation results for all target compounds. You can also click **View > Batch Table Layout > Multiple Compounds/Samples View**. **b** Click the Cal-L4 cell, and note the difference in **RT** in the **Compound Information** window for each compound.	A different set of columns is displayed when you are in **Multiple Compounds/Samples View** mode versus **Single Compound View** mode. If you add a column to the table when you are in **Multiple Compounds/Samples View** mode, that change is not automatically made in the **Single Compound/Sample View** mode.



Sample						Cocaine Method			Cocaine Results						Qualifier...		Cocaine-d3 (I...		Qualifier...	
		Name	Data File	Type	Level	Acq. Date-Time	Exp. Conc.	IPM	Int.	RT	Resp.	MI	Calc. Conc.	Final Conc.	Accuracy	Ratio	MI	RT	Resp.	Ratio	M
		Blank-1	CMAMBlk_01.d	Blank		5/12/2006 1:48 PM			MS-MS	2.433	20		11.8235	11.8235				2.403	15		
		Calib-L1	CMAMCal_L1.d	Cal	L1	5/12/2006 1:51 PM	2.5000		MS-MS	2.453	5189		2.3087	2.3087	92.3	3.7		2.452	20245	4.0	
		Calib-L2	CMAMCal_L2.d	Cal	L2	5/12/2006 1:54 PM	5.0000		MS-MS	2.454	9716		4.2682	4.2682	85.4	3.9		2.453	20506	4.0	
		Calib-L3	CMAMCal_L3.d	Cal	L3	5/12/2006 1:57 PM	12.5000		MS-MS	2.450	25187		11.5607	11.5607	92.5	3.9		2.450	19625	4.4	
▶		Calib-L4	CMAMCal_L4.d	Cal	L4	5/12/2006 2:00 PM	25.0000		MS-MS	2.449	50649		25.2511	25.2511	101.0	3.9		2.448	18068	4.2	
		Calib-L5	CMAMCal_L5.d	Cal	L5	5/12/2006 2:03 PM	125.0000		MS-MS	2.448	199967		125.0768	125.0768	100.1	3.8		2.448	14401	3.7	
		QC-L2	CMAMQC_L2.d	QC	L2	5/12/2006 2:06 PM	5.0000		MS-MS	2.453	9246		4.2831	4.2831	85.7	3.5		2.453	19446	4.4	
		QC-L4	CMAMQC_L4.d	QC	L4	5/12/2006 2:09 PM	25.0000		MS-MS	2.455	48582		24.5377	24.5377	98.2	4.0		2.454	17834	3.9	
		Sample-1	CMAMSam_01.d	Sample		5/12/2006 2:12 PM			MS-MS												
		Sample-2	CMAMSam_02.d	Sample		5/12/2006 2:15 PM			MS-MS	2.460	9735		4.3735	4.3735		3.6		2.459	20051	3.6	
		Sample-3	CMAMSam_03.d	Sample		5/12/2006 2:18 PM			MS-MS	2.446	24841		10.9299	10.9299		3.9		2.445	20472	3.6	

c To return to the display of detailed quantitation results for the selected target compound, click the **Display Single Compound/Sample in Batch Table** icon in the toolbar.

d If necessary, click the down arrow next to the **Compound** list, and click **Cocaine**.

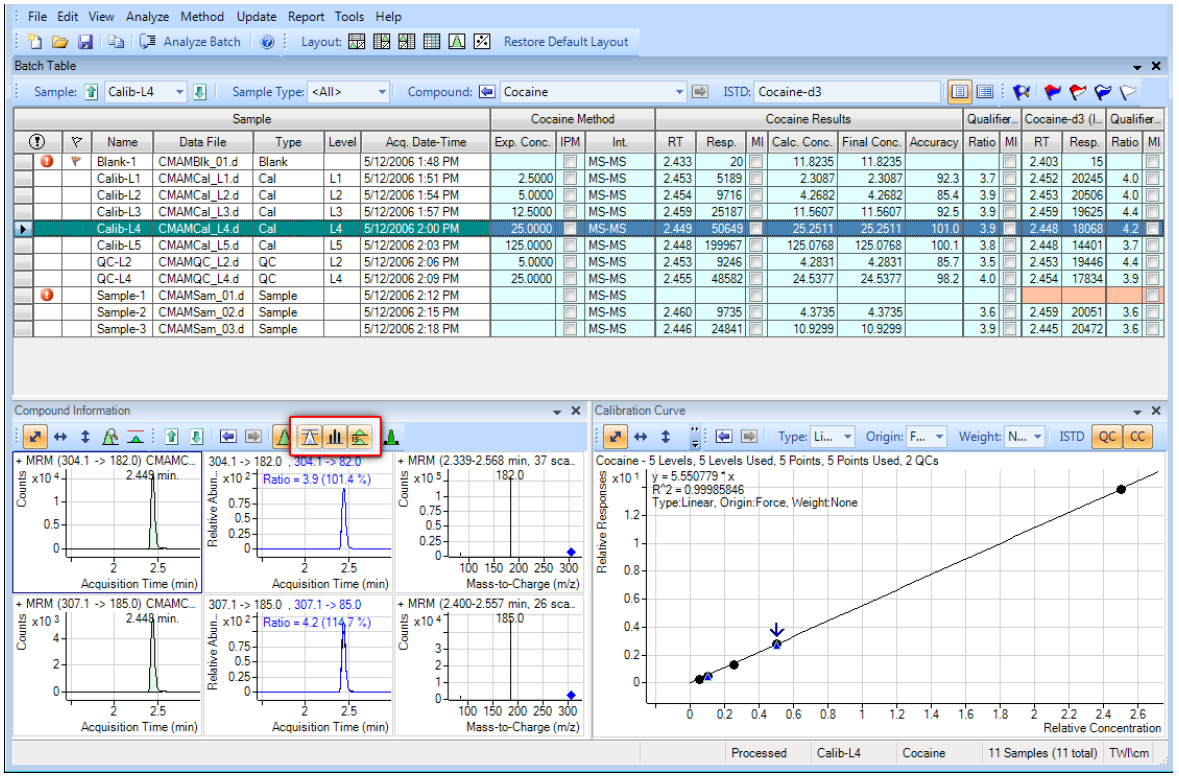
Steps	Detailed instructions	Comments
6 View selected sample types. • Display only the calibration standards. • Then display all sample types.	**a** Click the down arrow in the **Sample Type** drop down list. The **Sample Type** dialog box is displayed. **b** Clear the **<All>** check box and mark the **Cal** check box.  **c** Click **OK**. The **Batch Table** should contain only the **Cal** standards for cocaine. **d** Click the down arrow in the **Sample Type** drop down list. **e** Click **<All>**, and then click **OK**. The system marks all the check boxes and displays all sample types.	

Task 2. Change Result Window Layouts

This task shows you how to customize your layout using the toolbar icons and how to recreate the default layout.

Steps	Detailed instructions	Comments
1 Use layout icons on the toolbar to position the **Batch Table**, **Compound Information**, and **Calibration Curve** windows.	**a** Click the **Layout – Table Left** icon in the toolbar  . **b** Click the **Layout – Table Right** icon in the toolbar  . **c** Click the **Layout – Table Top** icon  .	
2 Use layout icons on the toolbar to maximize each individual window:	**a** Click the **Maximize Table** icon in the toolbar  . **b** Click the **Maximize Compound Information** icon in the toolbar  . **c** Click the **Maximize Calibration Curve** icon in the toolbar  . **d** To return to the default layout, click the **Restore Default Layout** icon on the toolbar.	
3 Change the panes in the **Compound Information** window for Cal-L4.	**a** In the **Batch Table**, select the **Cal-L4** row. **b** In the Compound Information toolbar, click the **Show/Hide Qualifiers** icon  . **c** Click the **Show/Hide Spectrum** icon  . **d** Click the **Show/Hide ISTD** icon  . The layout and results look like those in the following figure.	• This step assumes that you started this task with just the Chromatogram pane in the **Compound Information** window. • Changing the layout changes only the position and visibility of the six panes. The panes in the **Compound Information** window are not affected by changing the layout.

Steps	Detailed instructions	Comments

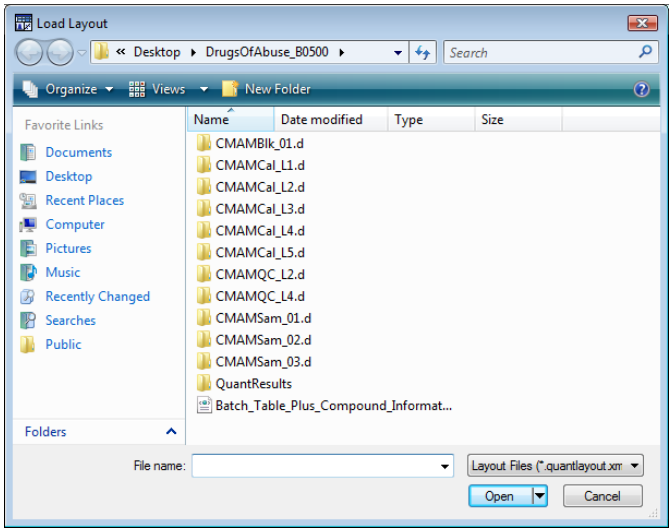


4 Save the default layout without the calibration curve.

a Close the **Calibration Curve** window.

b Click **View > Window Layout > Save Layout**.
The system displays the **Save Layout File** dialog box.

c Name the layout file **Batch Table plus Compound Information,** and click **Save**.

Steps	Detailed instructions	Comments
5 Load the newly created layout.	**a** Click **Restore Default Layout** on the toolbar. **b** Click **View > Window Layout > Load Layout**. The system displays the **Load Layout** dialog box.  **c** Click **Batch Table plus Compound Information** and click **Open**. The results window should now look like Figure 7.	

Steps	Detailed instructions	Comments

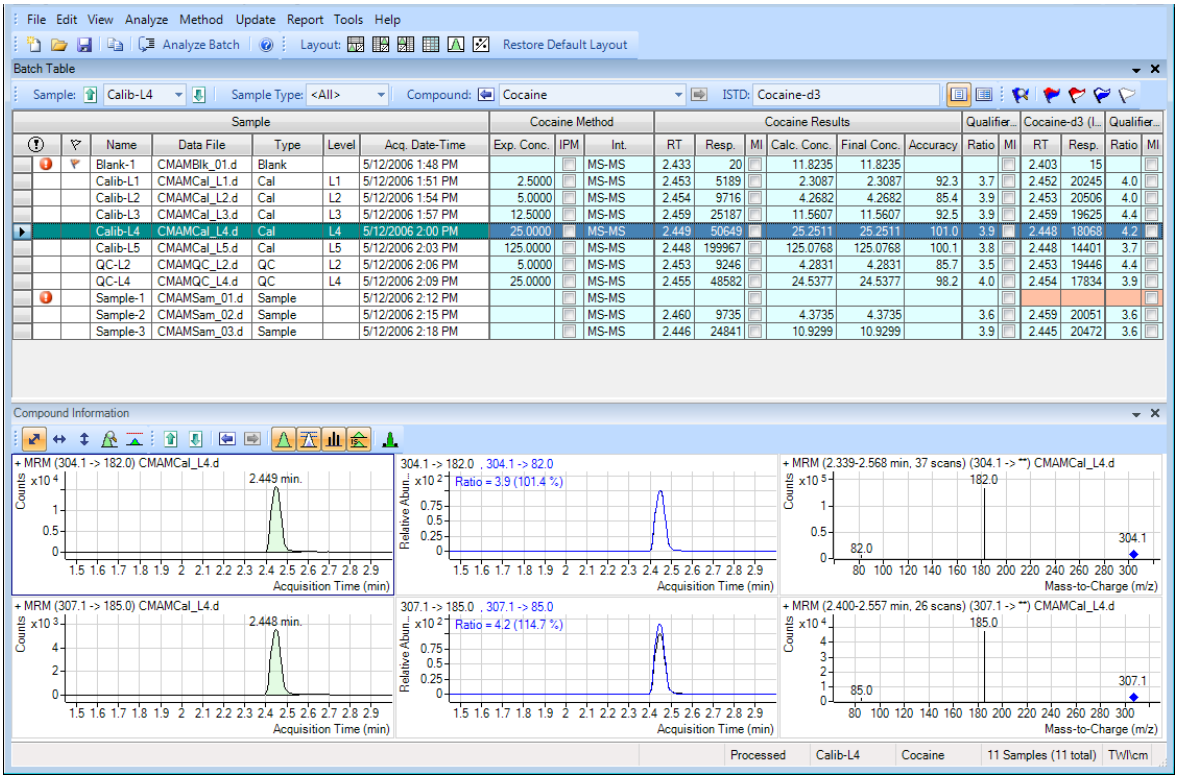
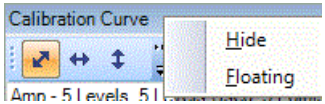


Figure 7 Results window

Steps	Detailed instructions	Comments
6 Create the layout as shown in Figure 8 on page 54.	**a** Restore the default layout (click **Restore Default Layout** on the toolbar). **b** Right-click inside the title bar of the **Calibration Curve** window, and then mark the **Floating** check box.  **c** Right-click the title bar of the **Compound Information** window, and then mark the **Floating** check box. **d** Resize the windows to match the layout in Figure 8.	• More than the **Batch Table** is on the left.

Steps	Detailed instructions	Comments

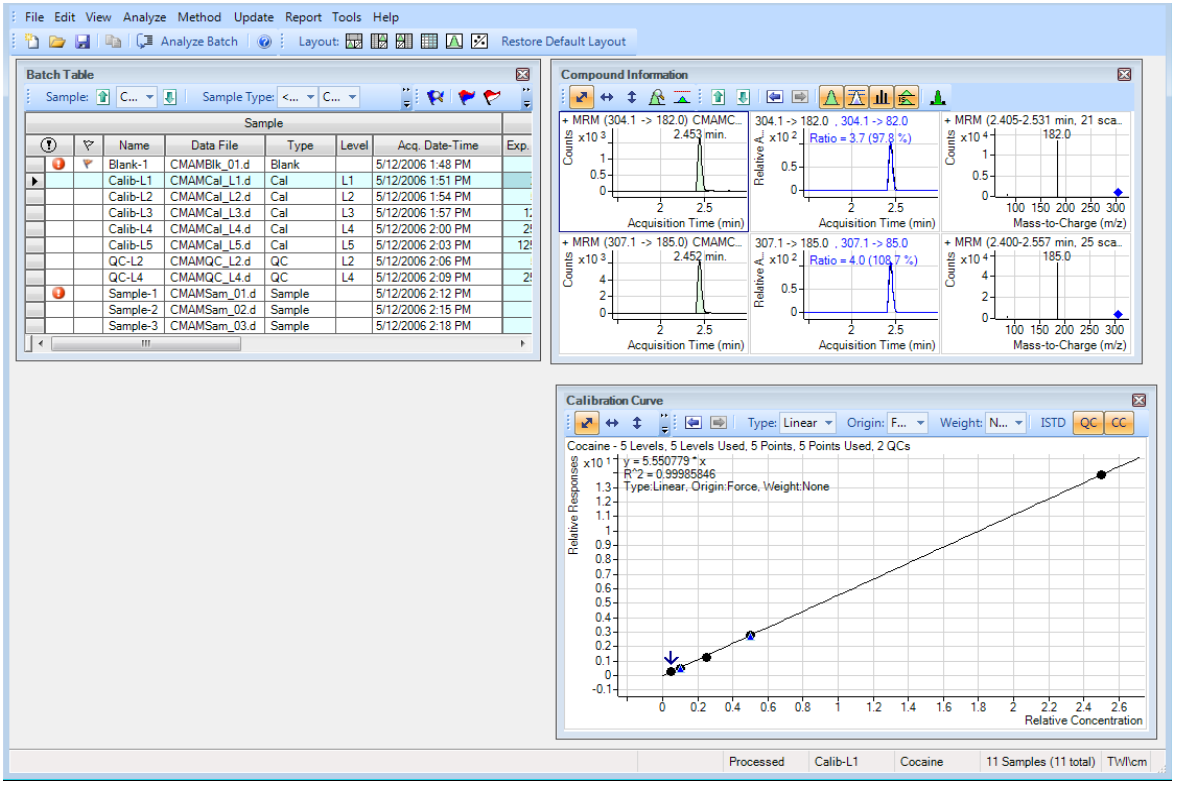


Figure 8 Display with Calibration Curve and Compound Information windows floating

e Right-click inside the title bar of the **Compound Information** window, and then clear the **Floating** check box.

f Resize the windows to match the layout in Figure 9.

Steps	Detailed instructions	Comments

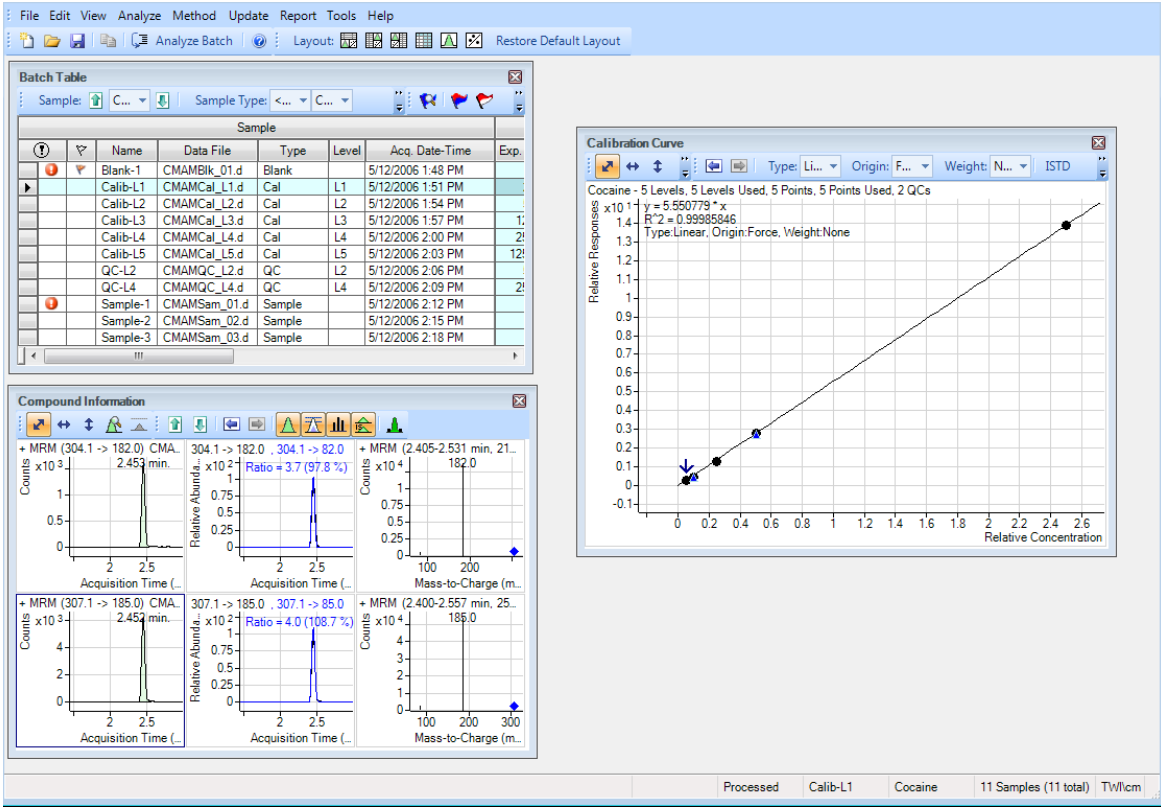


Figure 9 Resized window

g Right-click inside the title bar of the **Calibration Curve** window, and clear the **Floating** check box.

h Move the **Compound Information** window so that the layout corresponds to the one pictured at the start of the task.

Steps	Detailed instructions	Comments
7 Recreate (do not restore) the default layout.	**a** Maximize the program main view. • Anchor the **Calibration Curve** window first, and then the **Compound Information** window, to recreate the default layout. • If after anchoring the two windows, the calibration curve is on the left side, right-click the title bar of the **Calibration Curve** window and drag it to the right. A gray rectangle shows where this window will be placed within the main view. • Drag the calibration curve to the bottom right corner of the main view.	

Task 3. Export and Print Results

This exercise shows you how to export your data to a Microsoft Excel file and how to preview and print your Batch Table and compound information data.

Steps	Detailed instructions	Comments
1 Export the batch file ***iii*_Test_01**.	**a** To make the **Batch Table** window active, click the title bar of the **Batch Table** window. **b** Click **File > Export > Export Table**. **c** Select **My Documents** as the destination directory. **d** Type `iii_Test_01.xlsx` as the export file name. **e** Click **Save**. The Excel file **My Documents*iii*_Test_01.xlsx** opens automatically.	• ***iii*** = User initials

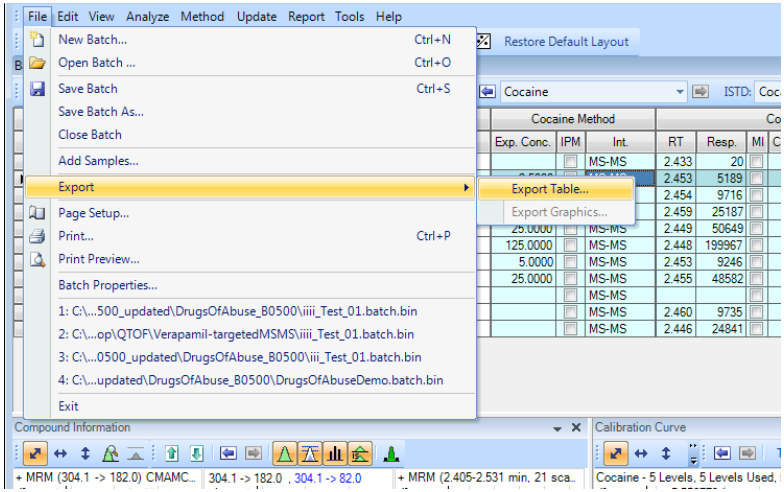
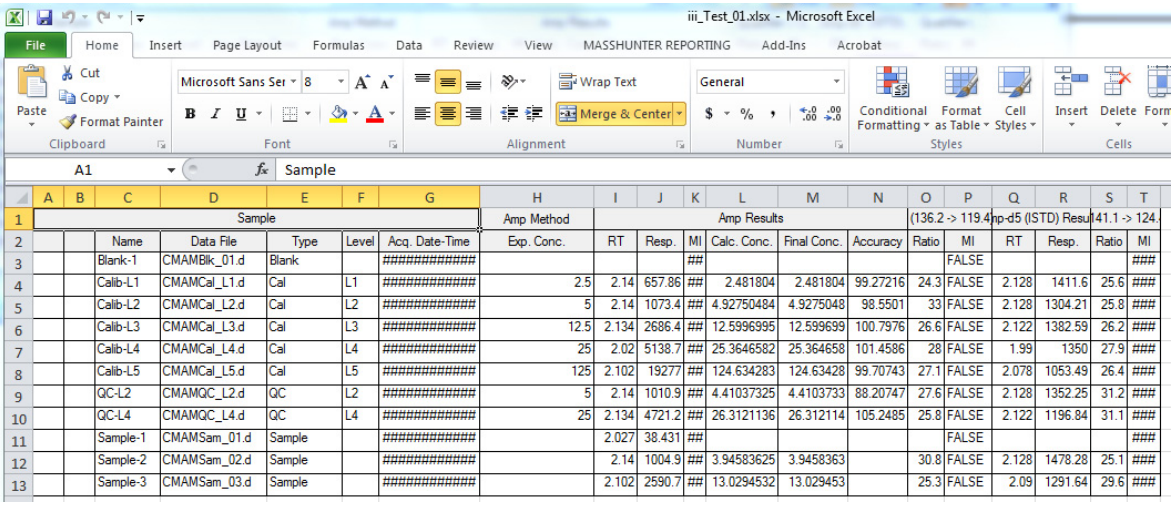


Figure 10 Export results

2 View the batch results as they appear in Excel; then exit Excel.	**a** Note what is exported and what is not. **b** Close Excel when you are finished.	

Steps	Detailed instructions	Comments



	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T
1			Sample					Amp Method	Amp Results						(136.2 -> 119.4	np-d5 (ISTD) Resul			141.1 -> 124.	
2			Name	Data File	Type	Level	Acq. Date-Time	Exp. Conc.	RT	Resp.	MI	Calc. Conc.	Final Conc.	Accuracy	Ratio	MI	RT	Resp.	Ratio	MI
3			Blank-1	CMAMBlk_01.d	Blank		##########				##					FALSE				
4			Calib-L1	CMAMCal_L1.d	Cal	L1	##########	2.5	2.14	657.86	##	2.481804	2.481804	99.27216	24.3	FALSE	2.128	1411.6	25.6	###
5			Calib-L2	CMAMCal_L2.d	Cal	L2	##########	5	2.14	1073.4	##	4.92750484	4.9275048	98.5501	33	FALSE	2.128	1304.21	25.8	###
6			Calib-L3	CMAMCal_L3.d	Cal	L3	##########	12.5	2.134	2686.4	##	12.5996995	12.599699	100.7976	26.6	FALSE	2.122	1382.59	26.2	###
7			Calib-L4	CMAMCal_L4.d	Cal	L4	##########	25	2.02	5138.7	##	25.3646582	25.364658	101.4586	28	FALSE	1.99	1350	27.9	###
8			Calib-L5	CMAMCal_L5.d	Cal	L5	##########	125	2.102	19277	##	124.634283	124.63428	99.70743	27.1	FALSE	2.078	1053.49	26.4	###
9			QC-L2	CMAMQC_L2.d	QC	L2	##########	5	2.14	1010.9	##	4.41037325	4.4103733	88.20747	27.6	FALSE	2.128	1352.25	31.2	###
10			QC-L4	CMAMQC_L4.d	QC	L4	##########	25	2.134	4721.2	##	26.3121136	26.312114	105.2485	25.8	FALSE	2.122	1196.84	31.1	###
11			Sample-1	CMAMSam_01.d	Sample		##########		2.027	38.431	##					FALSE				###
12			Sample-2	CMAMSam_02.d	Sample		##########		2.14	1004.9	##	3.94583625	3.9458363		30.8	FALSE	2.128	1478.28	25.1	###
13			Sample-3	CMAMSam_03.d	Sample		##########		2.102	2590.7	##	13.0294532	13.029453		25.3	FALSE	2.09	1291.64	29.6	###

Figure 11 Batch table in Excel

3 Preview printouts for Batch Table and Compound Information data.

a In Excel, click **File > Print.**
b Inspect the **Print Preview** window to make sure it looks the way you want it.
c Click **File > Print**.
d Repeat steps a-e for the compound information.
e If you are not moving on to Exercise 4, click **File > Save Batch**.
f Click **File > Exit**.

You can also print the **Batch Table** from the **Print Preview** program by clicking the **File > Print** menu item in the **Print Preview** program.

Exercise 4
Use Three Tools to Evaluate Results

In this exercise, you will use three tools to help you evaluate and obtain more accurate quantitation results:

- Curvefit Assistant, which calculates all combinations of curves and presents results with an equation and confidence band
- Parameterless integrator, so you don't have to figure out the parameters to change to improve the integration
- Outlier messages to help you easily detect result values that are out of the specified range

The DrugsOfAbuse batch is used in this exercise. The same tasks can be performed with Triple Quad data files, Q-TOF data files, and TOF data files.

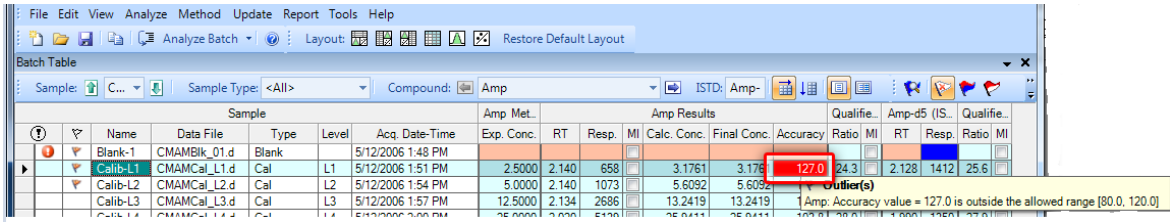
Each exercise is presented in a table with three columns:

- 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.

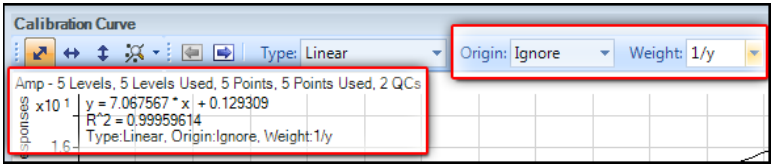
Task 1. Adjust the Calibration Curve Fit

This task shows you how to find the accuracy outlier for a compound, adjust its curve fit, and reanalyze the batch.

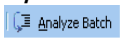
Steps	Detailed instructions	Comments
1 If necessary, open the batch file ***iii*_Test_01.batch.bin**.	**a** To start the Quantitative Analysis program, click the **Quantitative Analysis (QQQ)** icon on your desktop. **b** Click **Open Batch** on the toolbar to display the **Open Batch** dialog box. **c** Navigate to ***Your Directory*****DrugsOfAbuse** and click ***iii*_Test_01.batch.bin.**	• You can also access the program by clicking **Programs > Agilent > MassHunter Workstation > Quantitative Analysis (QQQ)** from the Start menu. • If the default layout is not present, click **Restore Default Layout** on the toolbar before opening the batch. Restore Default Layout
2 Find the accuracy outlier for amphetamine, and change the curve fit.	**a** Make sure the **Batch Table** is set to single compound display mode, and the displayed target compound is **Amp**. See boxed portions of the illustration below. 	
	b Point to the cell in the **Calib-L1** row and the **Accuracy** column to display the Outlier message as shown below.	• Cells containing outliers can be in red (high) or blue (low).



Steps	Detailed instructions	Comments
	c In the **Calibration Curve** window, set **Origin** to **Ignore**, and **Weight** to **1/y.** The program displays a new curve fit formula and R^2 value.	Curve Fit Origin • **Force** – Forces the curve fit line to go through the origin point (X=0, Y=0). • **Ignore** – Does not force the curve fit line to use the origin point (X=0, Y=0). Curve Fit Weight • **None** – Gives equal weight to all data points. • **1/Y** – Applies the formula 1/Y to the data points. This formula reduces the influence of high Y values while boosting the influence of low Y values.
3 Analyze the batch and inspect the results in the **Batch Table**.	**a** Click the **Analyze Batch** icon in the toolbar Analyze Batch to analyze the batch. **b** Inspect the results in the **Batch Table** after batch analysis.	
4 Find accuracy outliers, if any, for other compounds.	**a** Click **Next Compound** in the Batch Table toolbar to view individual compounds, such as Cocaine, MDMA, and Met. **b** Examine the quantitation results, especially the values in the **Accuracy** column.	• Note that the Accuracy value for the Calib-L3 standard for methamphetamine is out of the specified range.



Accuracy
96.6
97.1
102.5
103.8
99.2
86.7
108.0

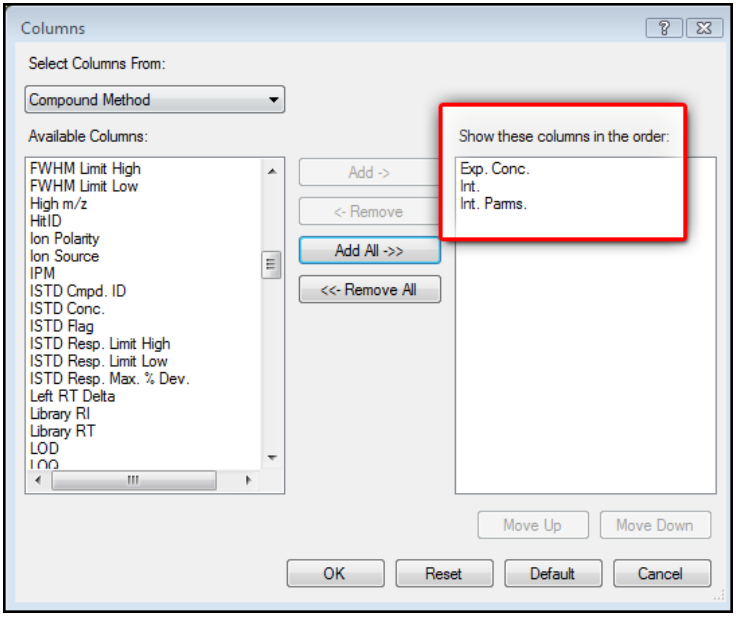
Steps	Detailed instructions	Comments
5 Change the curve fit for methamphetamine, and analyze the batch.	**a** In the **Calibration Curve Fit** window, set **Origin** to **Ignore**, and **Weight** to **1/y**. The Quantitative Analysis program displays a revised curve fit formula and R2 value. **b** Click **Analyze Batch** in the main toolbar  to analyze the batch. The **Batch Table** displays the new results after batch analysis.	

Task 2. Integrate Without Parameters

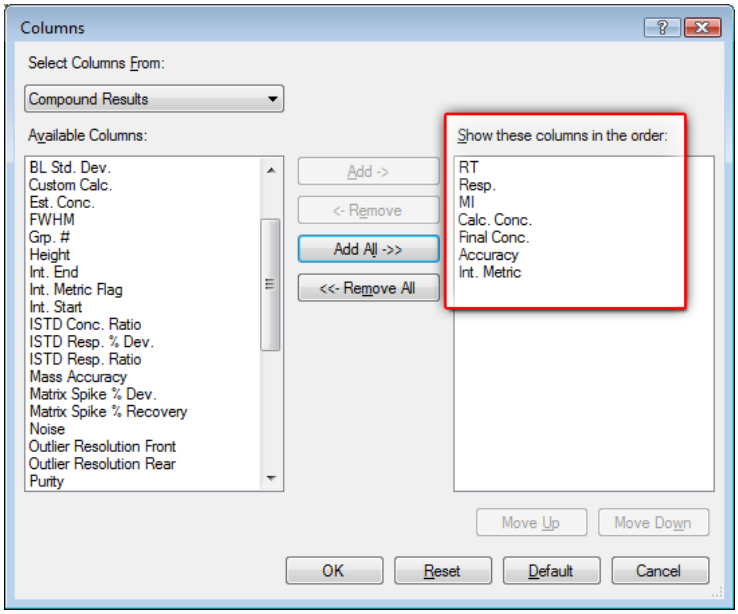
This task shows you how to inspect data for proper integration. You learn how to perform the following tasks:

- Add integration columns to the Batch Table
- View default integration values
- Closely examine the chromatogram, looking for such details as:
  - Outlier messages
  - Baseline parameters
  - Peak labels

Steps	Detailed instructions	Comments
1 Add integration columns to the Batch Table.	**a** Right-click anywhere in the **Batch Table**, and click **Add/Remove Columns**. The system displays the **Columns** dialog box. **b** From the **Select Columns From** drop-down list, select **Compound Method**. **c** From the **Available Columns** list, select **Int.** (Integrator Type) and **Int. Parms.** (Integrator Parameters) and click **Add**. The Quantitative Analysis program moves the selected columns to the **Show these columns in the order** list.	• This task assumes that the batch, ***iii*_Test_01**, is already open. If it is not, see step 1 in Task 1.



Steps	Detailed instructions	Comments
	d From the **Select Columns From** drop-down list, select **Compound Results.** **e** From the **Available Columns** list, select **Int. Metric** (Integrator Metric) and click **Add**. The system moves the selected column to the **Show these columns in the order** list. **f** Click **OK**.	



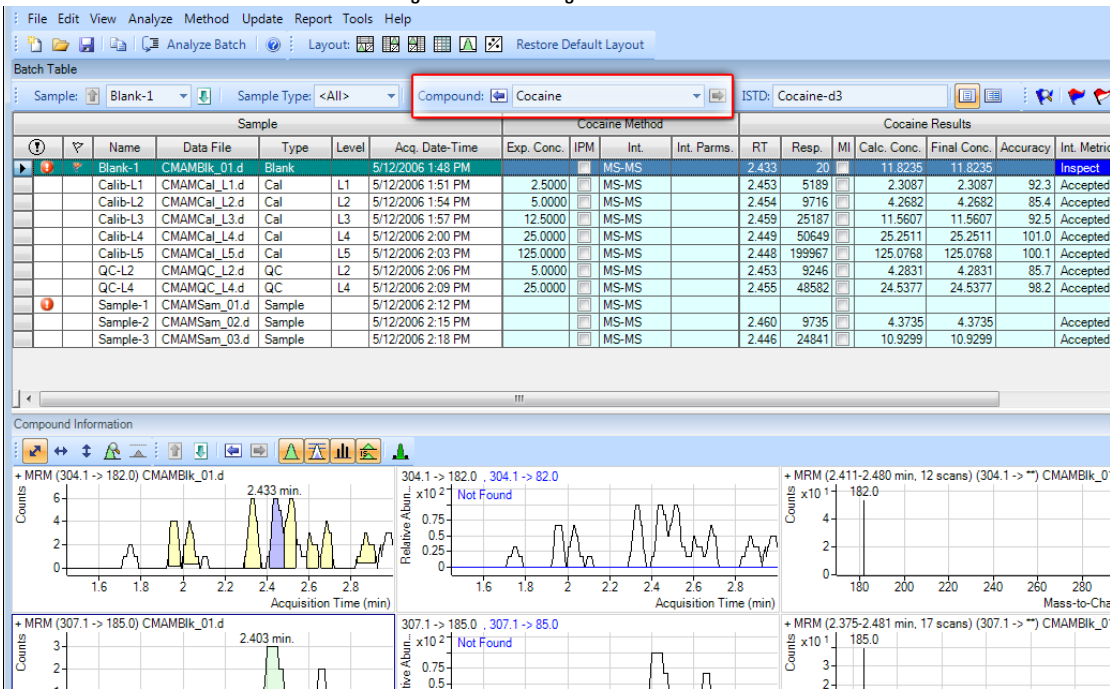
Steps	Detailed instructions	Comments
2 View the default integration values for amphetamine.	**a** Click **Previous Compound** in the Batch Table toolbar to view amphetamine (**Amp**). **b** Examine the default values in the **Int.** and **Int. Parms** columns in the **Batch Table.**	• Note that the integrator used is the MS-MS integrator, which does not need you to enter parameters. That is why the **Int. Parms** column is blank.

Int.	Int. Parms.
MS-MS	
MS-MS	
MS-MS	
MS-MS	
MS-MS	
MS-MS	
MS-MS	
MS-MS	
MS-MS	
MS-MS	

Amp Method			Amp Results						
Exp. Conc.	Int.	Int. Parms.	RT	Resp.	MI	Calc. Conc.	Final Conc.	Accuracy	Int. Metric
	MS-MS								
2.5000	MS-MS		2.141	658		2.4161	2.4161	96.6	Accepted
5.0000	MS-MS		2.140	1059		4.8556	4.8556	97.1	Accepted
12.5000	MS-MS		2.134	2673		12.8162	12.8162	102.5	Accepted
25.0000	MS-MS		2.022	4952		25.9394	25.9394	103.8	Accepted
125.0000	MS-MS		2.101	18605		124.0262	124.0262	99.2	Accepted
5.0000	MS-MS		2.142	1006		4.3336	4.3336	86.7	Accepted
25.0000	MS-MS		2.135	4716		26.9911	26.9911	108.0	Accepted
	MS-MS								
	MS-MS		2.143	1004		4.0008	4.0008		Accepted
	MS-MS		2.105	2590		13.3556	13.3556		Accepted

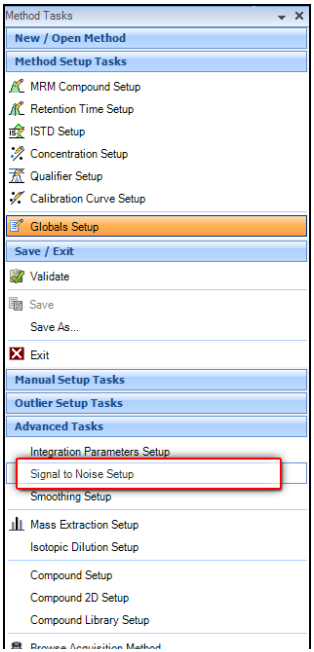
	c Examine the default values in the **Int. Metric** column in the **Batch Table**.	• These values reflect the default integration quality metric used for the target compound Amp.

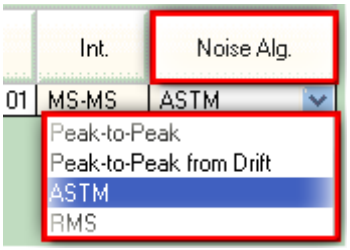
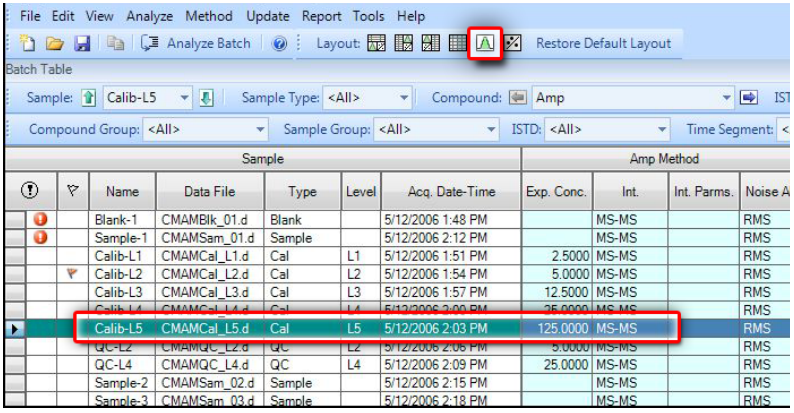
Steps	Detailed instructions	Comments
3 View integration problems for cocaine and MDMA. • Look for outlier messages at the intersection of the **Int. Metric** column and the **Blank-1** sample.	**a** Close the **Calibration Curve** window. **b** Enlarge the chromatogram portion of Compound Information toolbar so that only the quantifier and qualifier chromatograms appear. Click the **Show/Hide Spectrum** icon.  **c** Also click the **Show/Hide ISTD** icon. **d** Click the **Next Compound** icon in the Batch Table toolbar until the system displays the compound **Cocaine**. **e** Select the **Blank-1** row, and mouse over the word **Inspect** in the **Int. Metric** column for that row. The system displays any outlier message for that data, as well as the integrated chromatogram for cocaine.	



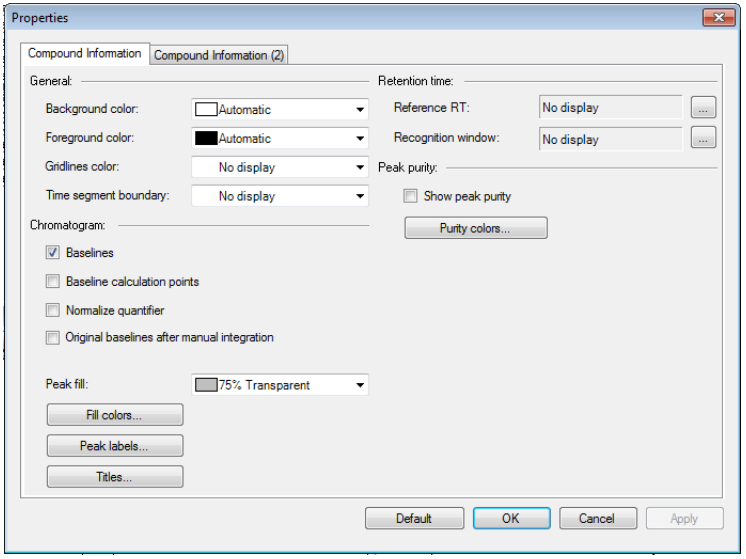
Steps	Detailed instructions	Comments
	f Click the **Next Compound** icon in the Batch Table Standard toolbar or the Previous Compound icon in the Batch Table Standard toolbar until the system displays the compound MDMA. **g** Select the **Blank-1** row, and point to the **Int. Metric** column. The system displays any outlier message for that data, as well as the integrated chromatogram for MDMA.	• The outlier message reads "MDMA: Integrator found the following problems with the peak at RT = 2.4664: Interference Problem." • Note that these colors appear for the integration metric: Green - Accepted Blue - Inspect Red - Rejected • These colors are also reflected in the peak colors.
4 Change the noise algorithm.	**a** Right-click anywhere in the **Batch Table,** and click **Add/Remove Columns**. The system displays the **Columns** dialog box. **b** From the **Select Columns From** drop-down list, select **Compound Method** **c** From the **Available Columns** list, select **Noise Alg**. (Noise Algorithm Type) and click **Add**. The system moves the selected column to the **Show these columns in the order** list. **d** Click **OK**. **e** Click the **Previous Compound** icon in the Batch Table toolbar until the system displays the compound Amp. **f** Examine the values in the **Noise Alg.** and **S/N** (signal-to-noise ratio) columns.	

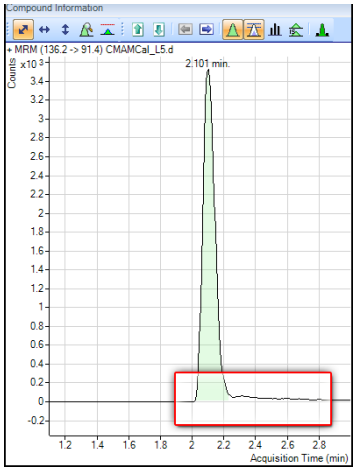
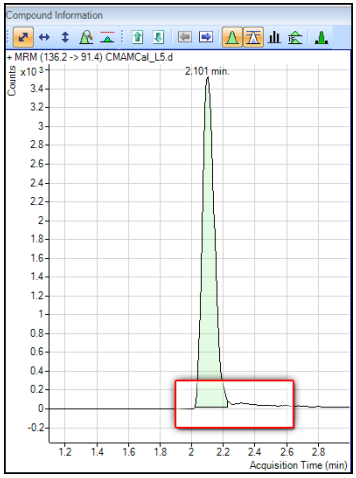
Amp Method				Amp Results							Qualifier...		Amp-d5 (IST...			
Exp. Conc.	Int.	Int. Parms.	Noise Alg.	RT	Resp.	MI	Calc. Conc.	Final Conc.	Accuracy	Int. Metric	Ratio	MI	RT	Resp.	Ratio	MI
	MS-MS		RMS													
2.5000	MS-MS		RMS	2.141	658		2.1151	2.1151	84.6	Accepted	24.3		2.129	1397	25.9	
5.0000	MS-MS		RMS	2.140	1059		4.5770	4.5770	91.5	Accepted	33.5		2.128	1298	25.9	
12.5000	MS-MS		RMS	2.134	2673		12.6107	12.6107	100.9	Accepted	26.7		2.121	1377	26.3	
25.0000	MS-MS		RMS	2.022	4952		25.8545	25.8545	103.4	Accepted	29.1		1.990	1304	28.8	
125.0000	MS-MS		RMS	2.101	18605		124.8426	124.8426	99.9	Accepted	27.0		2.076	1053	26.4	
5.0000	MS-MS		RMS	2.142	1006		4.0502	4.0502	81.0	Accepted	27.7		2.131	1356	31.1	
25.0000	MS-MS		RMS	2.135	4716		26.9159	26.9159	107.7	Accepted	25.6		2.121	1196	31.1	
	MS-MS		RMS													
	MS-MS		RMS	2.143	1004		3.7144	3.7144		Accepted	30.9		2.130	1445	25.7	
	MS-MS		RMS	2.105	2590		13.1551	13.1551		Accepted	25.3		2.089	1284	29.8	

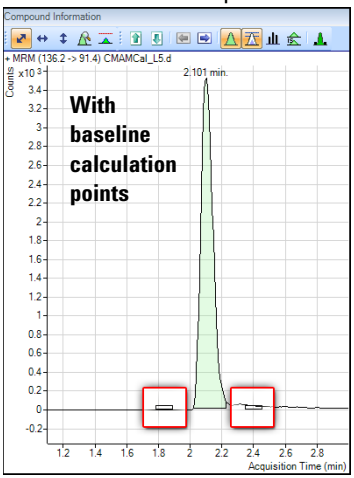
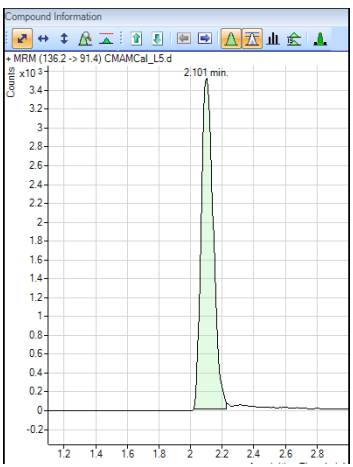
Steps	Detailed instructions	Comments
5 Practice changing the noise algorithm from RSM to ASTM for amphetamine in the method. • Exit, but don't save, the method.	**a** Click **Method > Edit** to switch to method editing mode. **b** In the Method Tasks column, click **Advanced Tasks > Signal to Noise Setup**. The system displays the integrator parameters in the **Method Table**.  **c** In the **Method Table,** click the drop down arrow in the **Noise Alg.** column for Amp. A list of available noise algorithms appears. **d** Click **ASTM**.	

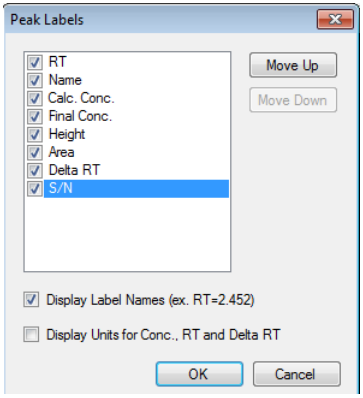
Steps	Detailed instructions	Comments
		
	e Under **Method Tasks/Save/Exit,** click **Exit**. **f** At the **Would you like to apply this method to the batch?** prompt, click **No.** The system displays Batch Analysis mode.	
6 Turn off the baseline (highest concentration standard) and then back on for amphetamine. • Compare the two chromatograms: one with the baseline on and the other with it off.	**a** Select sample **Calib-L5** (if it is not already selected), and click the **Maximize Compound Information** icon in the toolbar. Make sure that only the Compound Information pane is visible in the window. 	• Notice that the baseline is drawn in for the quantifier chromatogram as the default setting.

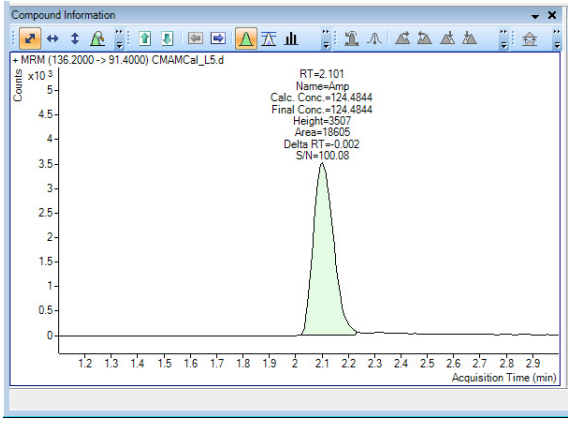
Steps	Detailed instructions	Comments
	b Right-click either of the chromatograms to open the shortcut menu. **c** Click **Properties** at the bottom of the shortcut menu to open the **Properties** dialog box.	



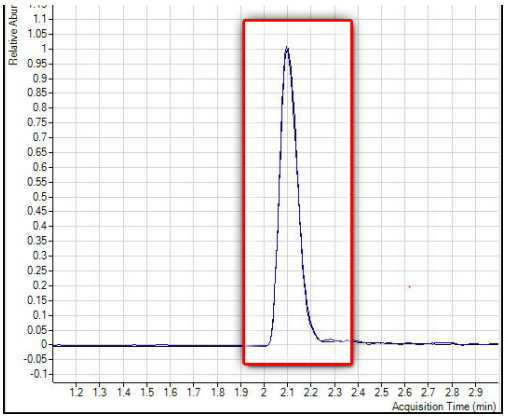
Steps	Detailed instructions	Comments
	d Clear the **Baselines** check box in the **Properties** dialog box. **e** Click the **Apply** button and observe the peak without the baseline.	• Notice that the baseline disappears after clearing the baseline check box. 
	f Mark the **Baselines** check box in the **Properties** dialog box. **g** Click the **Apply** button and observe the peak with the baseline drawn.	

Steps	Detailed instructions	Comments
7 Inspect the calculation points for the baseline for amphetamine.	**a** Mark the **Baseline Calculation Points** check box in the **Properties** dialog box. **b** Click **Apply** and observe where the baseline starts and stops.  **c** Clear the **Baseline Calculation Points** check box in the **Properties** dialog box. **d** Click **Apply** and observe the chromatograms. **e** Compare the chromatograms with and without Baseline Calculation Points.  	

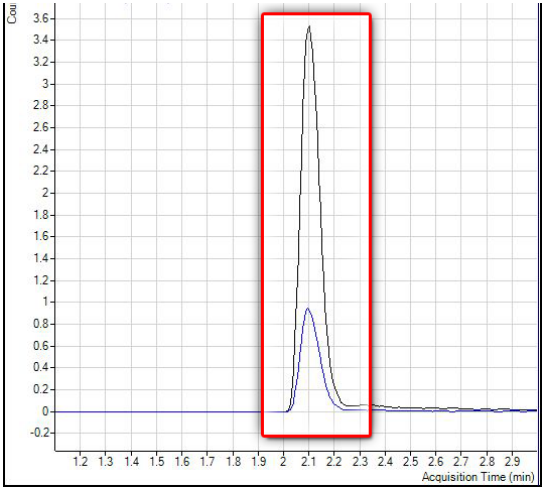
Steps	Detailed instructions	Comments
8 Display the peak labels for amphetamine.	**a** From the **Properties** dialog box, click **Peak Labels.** The system displays the **Peak Label dialog** box. **b** Mark all the **Peak Labels** check boxes and the **Display Label Names** check box. **c** Click **OK**. 	

Steps	Detailed instructions	Comments
	d Click the **Apply** button in the **Properties** dialog box. The peak labels should now match those shown in the example below.	
		
	e Click **Peak Labels** in the **Properties** dialog box. The system displays the **Peak Labels** dialog box. **f** Clear all the **Peak Labels** check boxes except RT (retention time). Clear the **Display Label Names** check box, and click **OK**. **g** Click **Apply** in the **Properties** dialog box and observe the change in Peak Labels.	

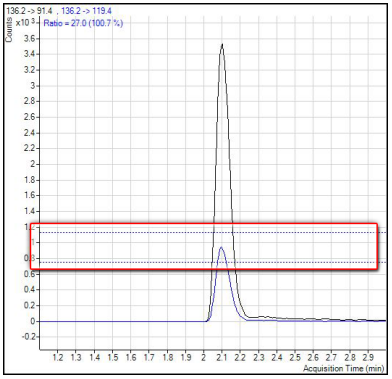
Steps	Detailed instructions	Comments
9 Display the qualifier chromatogram on the right-side before and after normalization.	**a** Click the **Compound Information (2)** tab. In the **Qualifiers** area, mark the **Normalize** check box. **b** Click **Apply** and observe that the two peaks now converge and appear as one peak.	• For B.04.01 and later revision: Notice that the default setting displays the normalized qualifier peak overlaid on the quantifier peak.

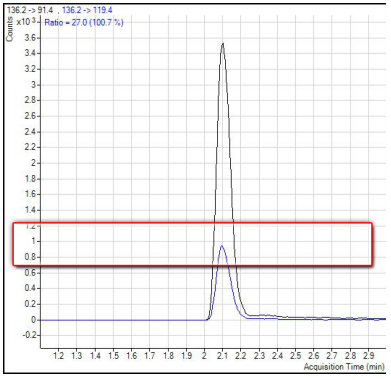


c Clear the **Normalize Qualifiers** check box of the **Properties** dialog box.
d Click **Apply** to display the qualifier second quantifier peaks again.



Steps	Detailed instructions	Comments
10 View the uncertainty band.	**a** Select the type of uncertainty band you would like to display from the drop-down menu in the **Uncertainty Band** field of the **Properties** dialog box. Click **Apply** and the uncertainty band appears in the qualifier chromatogram. **b** Select **No display** from the **Uncertainty Band** drop-down menu of the **Properties** dialog box. Click **Apply** to remove the uncertainty band from the qualifier chromatogram. **c** Click **OK** to close the **Properties** dialog box. **d** Compare the qualifier chromatogram with and without the **Uncertainty band**.	• Uncertainty band - a dashed band that shows the upper and lower boundaries for the qualifier abundance
11 Remove the **Int.** and **Int. Parms.** columns from the **Batch Table**.	**a** Click the **Restore Default Layout** button. **b** Right-click the **Compound Method** section of the **Batch Table**, and click **Add/Remove Columns**. **c** From the right-hand list, select **Int.** and **Int. Parms.** (Compound Methods). **d** Click **Remove**, and then **OK**.	

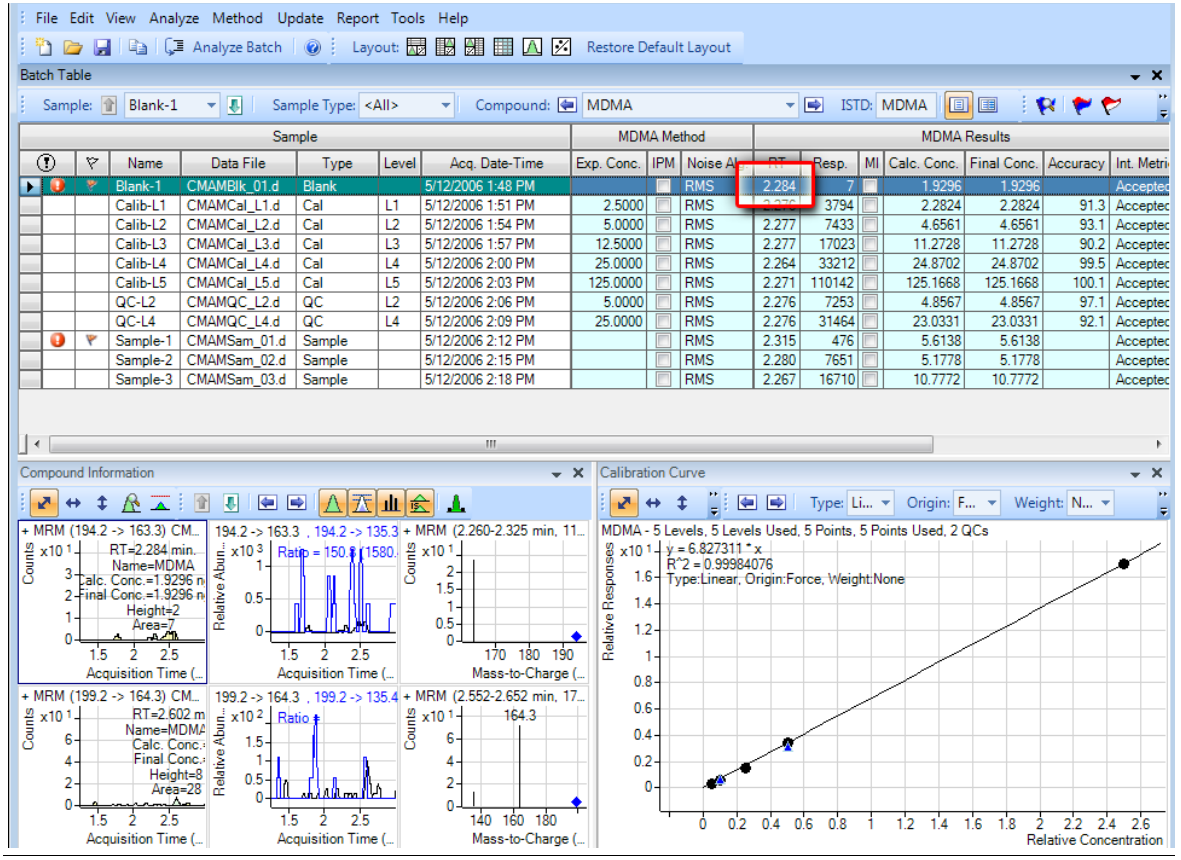




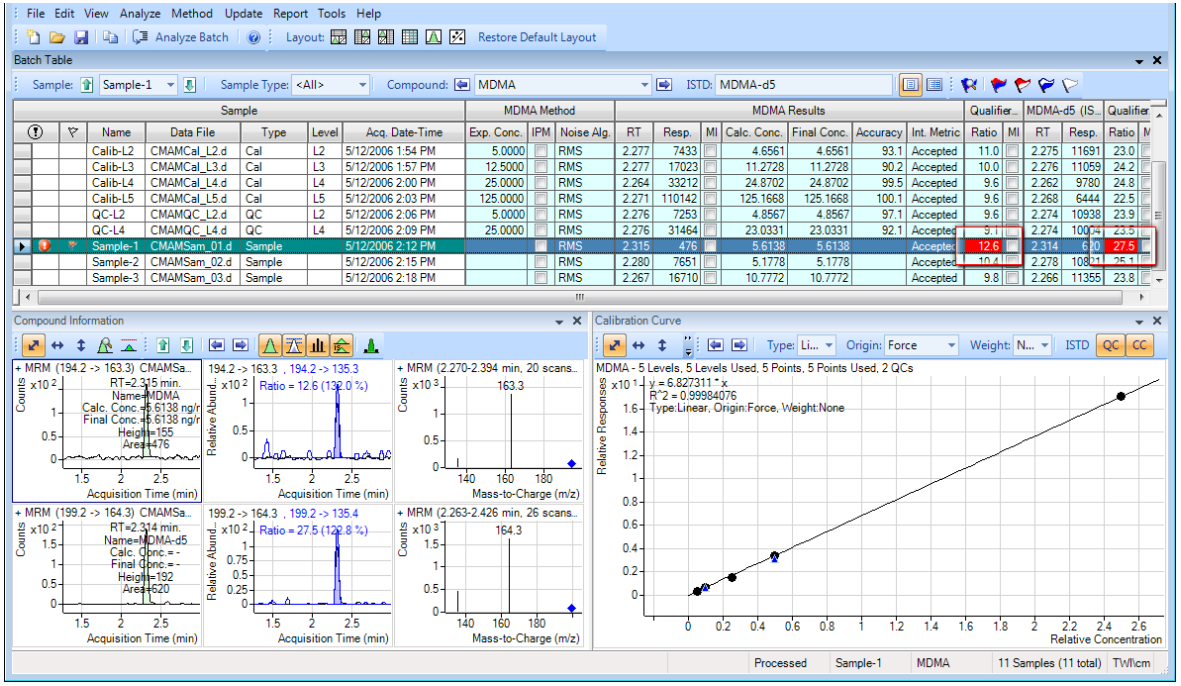
Task 3. Detect Outliers

This task shows you how to fine-tune the accuracy range for a compound and hide and show results with outlier flags.

Steps	Detailed instructions	Comments
1 View outlier information for MDMA.	**a** Click **Next Compound** in the Batch Table toolbar until the system displays the compound MDMA. **b** Select the **Blank-1** row, and point the cursor to the **RT** column, as shown in the example below.	

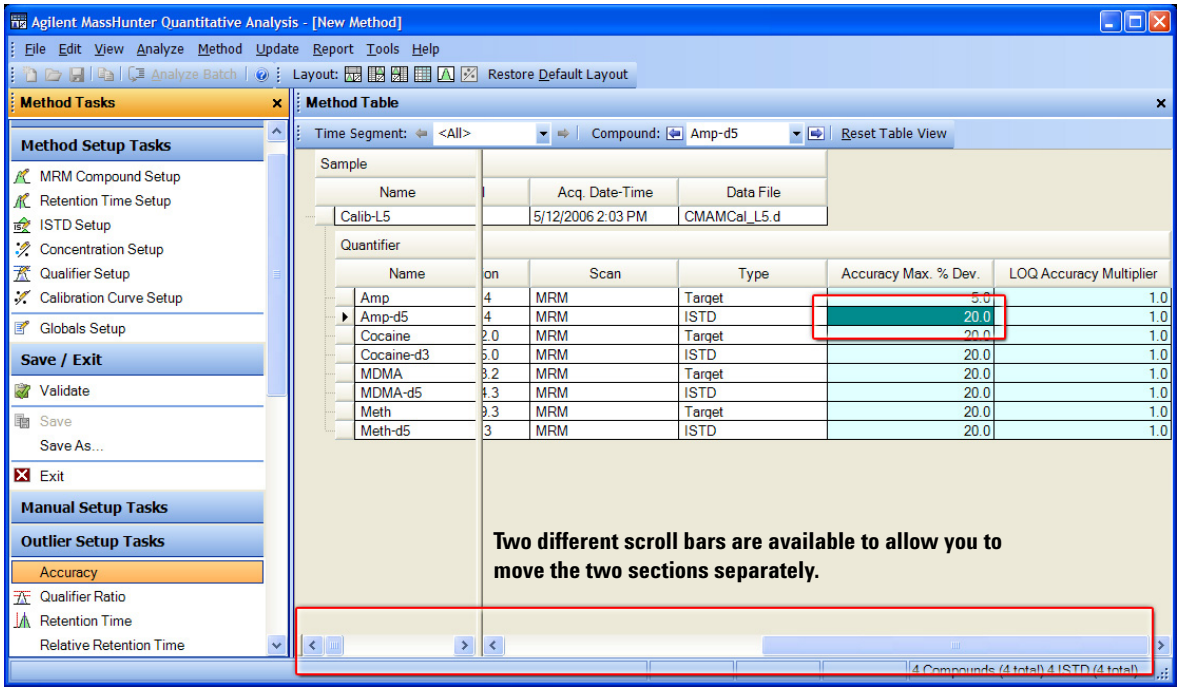


Steps	Detailed instructions	Comments
	c Examine the outlier information in the **Qualifier ... Results > Ratio** column for Sample 1, as shown in the example below.	



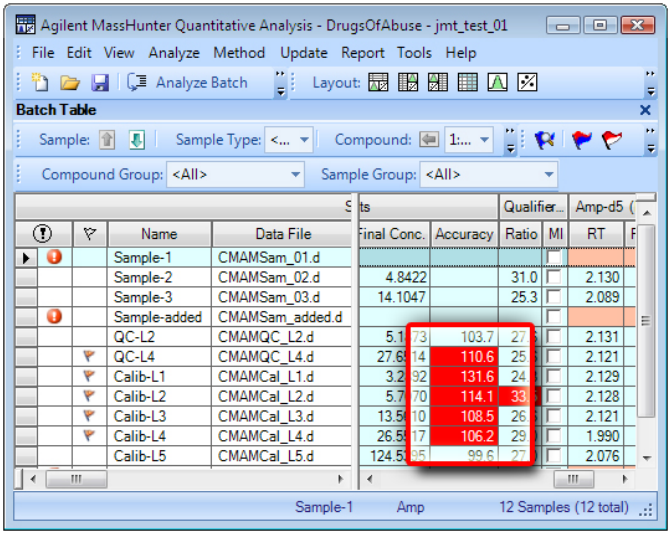
2 Change the accuracy range for amphetamine in the method, and reanalyze the batch.	**a** Click the **Previous Compound** icon in the toolbar until the system displays the compound **Amp**. **b** Select the **Calib-L5** row in the table. **c** Click **Method > Edit** to switch to method editing mode. **d** In the Method Tasks column, click **Outlier Setup Tasks > Accuracy**. **e** Set the **Accuracy Max % Dev** value to **5%** for **Amp**.	You can split the **Method Table** by dragging the small rectangle to the left of the scroll bar. In the example below, the rectangle next to the bottom scroll bar was used to split the **Method Table**. The information in the two sections is exactly the same. You can use these two panes to look at two sections of the table at the same time.

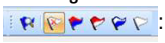
Steps	Detailed instructions	Comments



Steps	Detailed instructions	Comments
	f In the **Method Tasks** column, click **Save/Exit > Exit,** then select **None** under **Additional batch processing after applying the method,** and click **Yes** to the **Would you like to apply this method to the batch?** prompt. **g** Press F5 to analyze the batch. Red (high) and blue (low) outlier values now appear in the **Accuracy** column for Amp	You can also split the **Batch Table** into two sections. By default, the **Sample** columns are locked in position and only the other columns are scrolled. If you split the table into two sections, you can determine which columns appear in each section. You need to clear the **Lock Sample Columns** menu item in the Batch Table shortcut menu if you split the **Batch Table**.

.



Steps	Detailed instructions	Comments
3 Using the following set of outlier flag icons  :	**a** Click the **Display rows that have High outliers**  icon on the toolbar to display only samples with high outliers. **b** Click the **Turn off outlier filter**  icon to display all samples. **c** Click the **Display rows that have High/Low outliers**  icon on the toolbar to display only samples with low outliers. **d** Click the **Display rows that have High/Low outliers**  icon again to display all the samples. **e** Click the **Select Outliers**  icon to bring up the **Outliers** dialog box. **f** Clear the **Accuracy** and **Retention Time** check boxes, and click **OK**. **g** Click the **Select Outliers**  icon to bring up the **Outliers** dialog box. **h** Mark the **Accuracy** and **Retention Time** check boxes, and click **OK**.	• Note that to restore the **Batch Table** to view all data files, with and without outliers, simply click again on the icon you selected for filtering outliers.

Exercise 5
Generate Quantitation Reports

This exercise helps you learn how to do these tasks:

- Generate report methods using one or more report templates
- Generate a report

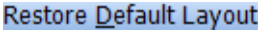
The **DrugsOfAbuse** batch is used in this exercise. The same tasks can be performed with Triple Quad data files, Q-TOF data files, and TOF data files.

Each exercise 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.

The report method you develop determines the report you create in MassHunter. Report methods are made of one or more report templates combined and edited to meet your reporting requirements. When developing a report method, you can use either Excel or PDF templates. PDF templates are included with this release and can generate reports 20 times faster than Excel templates. In addition, they have more options for scalability and performance.

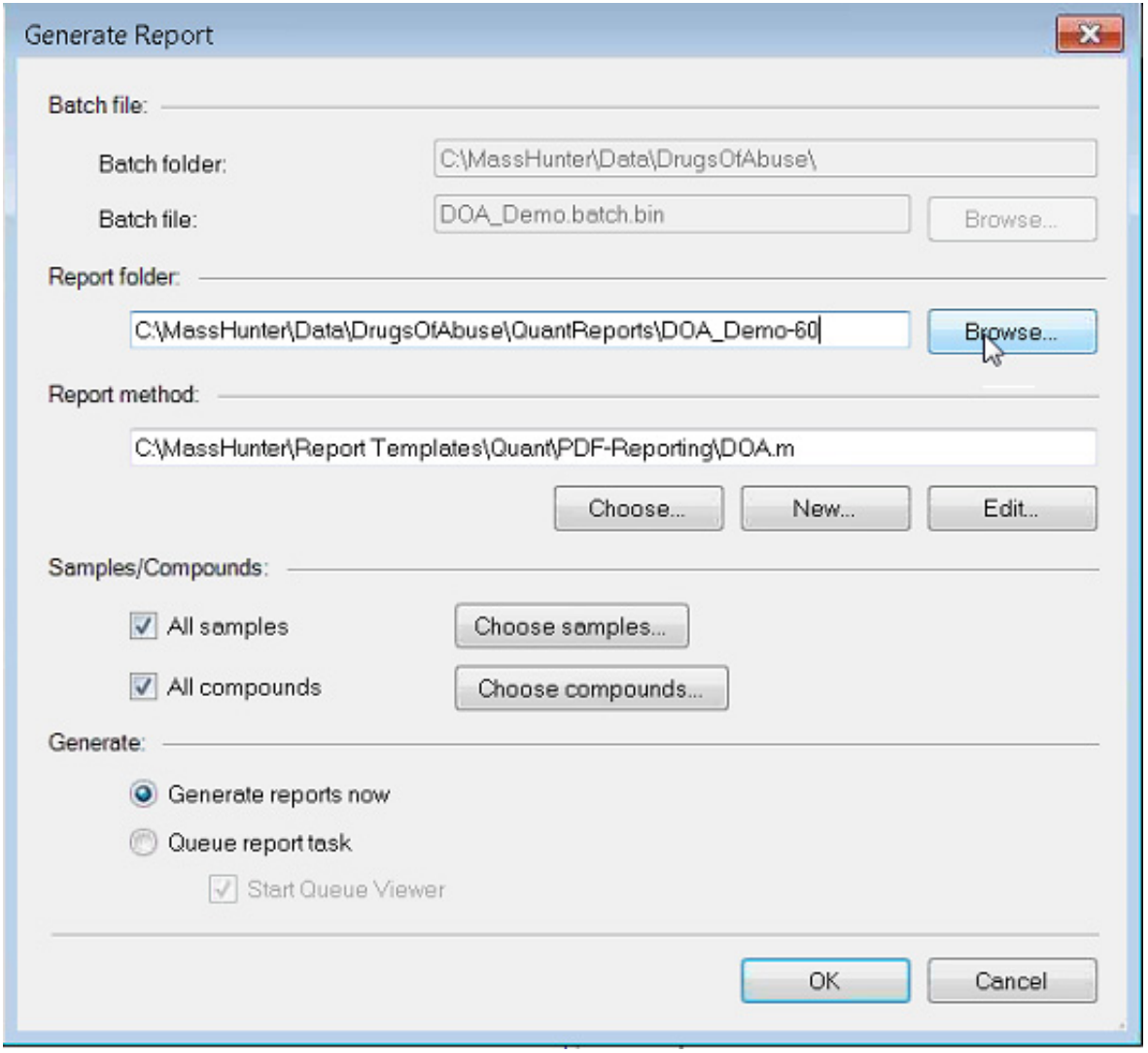
In this exercise, you will first develop a report method using PDF templates, and create single sample and batch reports using this same method.

Steps	Detailed instructions	Comments
1 Open the batch file ***iii*_Test_01.batch.bin**.	**a** To start the Quantitative Analysis program, click the **MS Quantitative Analysis (QQQ)** icon on your desktop. **b** Click **Open Batch**  on the toolbar to display the **Open Batch** dialog box. **c** Navigate to ***Your Directory***\ **DrugsOfAbuse** and click ***iii*_Test_01.batch.bin.**	• If the batch is already open, skip to step 2. • You can also access the program by clicking **Programs > Agilent > MassHunter Workstation > MS Quantitative Analysis (QQQ)** from the **Start** menu. • If the default layout is not present, click **Restore Default Layout** on the toolbar before opening the batch. 
2 Quantitate the samples for this batch and save your results.	**a** With the batch table open, click the **Analyze Batch** button on the tool bar to generate results. **b** Click **File>Save** to save the batch.	• Quantitative reports contain sample information generated during the batch. The reporting function will not work until sample results have been quantitated and saved. • If the batch is already quantitated, skip to step 3.

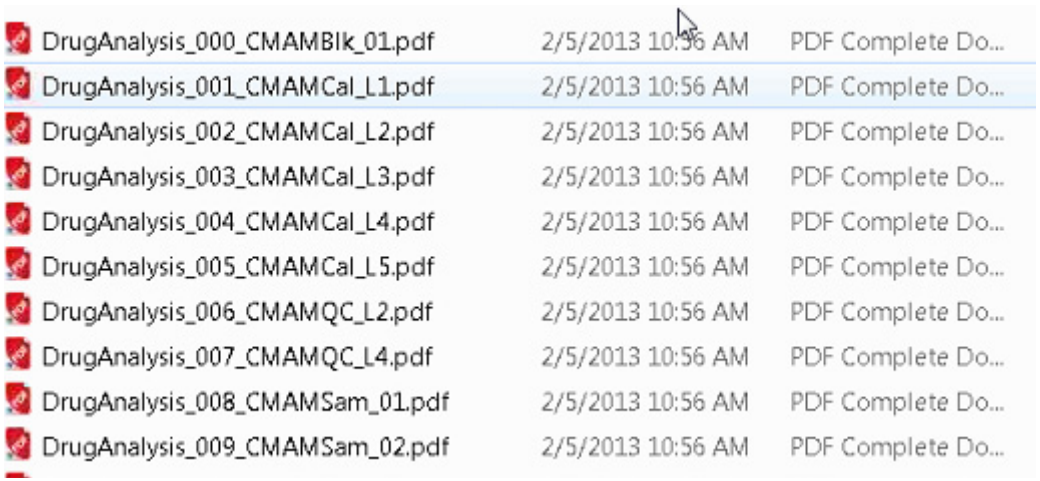
Steps	Detailed instructions	Comments
3 Create a PDF report method.	**a** Click **Report > Generate** from the toolbar. The system displays the **Generate Report** dialog box. **b** Accept the default **Report Folder** directory for this report. **c** Under the **Report Method** field, click the **New** button to create a new report method. **d** Click the **Add Template** button in the **Report Method Edit** dialog box to open the browser. **e** Navigate to the **MassHunter/Report Templates/Quant/PDF-Reporting** directory, select **DrugAnalysis.report.xml** and click **Open**. The program adds the template to the **Template** field in the **Report Method Edit** pane. **f** Repeat steps d and e to add **DrugAnalysis_DopingScreening.report .xml.**	• You may change the destination directory for saving the report in the **Report Folder** field; • The Report Method Edit feature of the software allows you to combine existing templates into a report method for developing an Excel or PDF report, or both. • The software defaults to the last report method used for the last report generated. Rather than generate a new report method, you can use the default method if appropriate, or select a different existing method. • To select an existing report method, click the **Choose** button under the **Report Method** field, and navigate to the folder to select your method.
4 Edit the report method to create single sample and batch PDF reports.	**a** In the **Report Method Edit** dialog box, on the **DrugAnalysis.report** template line, **Report Mode** field, select **Single Sample** from the drop down menu. **b** On the **DrugAnalysis _Doping Screening. report** template line, select **Batch** from the drop down menu in the **Report Mode** field. **c** Select your language from the drop down menu in the **Language** field. **d** Select a paper size from the drop down menu in the **Paper Size** field.	• The **Report Method Edit** dialog box allows you to edit certain features of the templates you choose to include in the report method. • The PDF reporting option allows you to create English, Chinese, or Japanese reports. Excel reports are provided in English only so this option will be grayed out. • In Excel reports, there are limits on your paper size. PDF reports provides a choice. • You can also select your **Publish Format**. In PDF reports, there is only one Publish Format; therefore, this field is grayed out for this example.

Steps	Detailed instructions	Comments
5 Select the way the system handles your report results.	**a** Select the **Results** tab of the **Report Method Edit** window. **b** Under the **Generate Reports results file** field, click **Auto.** **c** From the drop down menu of the Instrument field, select **QQQ.**	• It is recommended to use **Auto** in most cases. This limits the generation of an Excel file with the report to only those cases in which an Excel report is selected. PDF reports are quick and efficient when the generation of an Excel file is not necessary.
6 Set the graphic setting options for the method.	**a** Click the **Graphic Settings** tab to review the graphic settings. **b** Select the **Generate graphic file** checkbox to add graphics to your report. **c** Leave the default settings for the rest of the graphic setting fields.	• The **Graphic Settings** tab allows you to specify the appearance of the graphics in your report by editing the **Quantifier/Qualifier Overlay chromatogram**, **Spectra, Sample chromatogram, Calibration Curves** and **Fixed range graphic settings**. If you do not change the settings, the software will provide default settings appropriate for your data.
7 Save the report method.	**a** Click the save icon in the **Report Method Edit** window. **b** Name the report method **DOA.m.**	• You must save the method before you can close the window and generate a report.
8 Close the **Report Method Edit** window.	Click **Save & Exit** to close the **Report Method Edit** dialog box to return to the **Generate Report** window.	

Steps	Detailed instructions	Comments



Steps	Detailed instructions	Comments
9 Generate a report from the method	**a** Verify that the method you just created is in the **Report Method** field. **b** In the **Samples/Compounds** field, uncheck **All Samples**, to open the batch table. **c** Highlight one of the samples in the batch table window and click **OK**. **d** Click **All Compounds** to show all the compounds in the sample you have selected. **e** Select **Generate reports now** and click **OK** to generate the report.	• You can choose to show all the samples and all the compounds in the batch, or select specific samples or compounds in the batch table to show in your report. • PDF reports generate quickly so **Generate the report now** is the best option to obtain the report right away. If you are generating an Excel file along with the report, you can select **Queue report task** to view the progress of the report it is generating. • All reports generated are displayed in the viewer. The most recent display at the top of the list. • Reports are viewed or printed from the Excel or the PDF file you have created.



10 View the report.	Double-click on a file to open and display the report.	• Alternatively, you may open the report by selecting the file in Windows Explorer.

Reference

Ten Main Capabilities

Quantitative Analysis includes ten capabilities that help you integrate, quantitate, and review your data more easily and powerfully:

Batch-at-a-Glance: Batch Table Setup

- New batch — Creates a batch table in which you can operate on samples and compounds from a single view
- Analyze — Recreates the calibration curve and requantitates all samples using the method that is currently open
- Quantitate — Applies the existing calibration curve to the current batch, sample, or compound

  The granularity of applying quantitation allows you to quickly manipulate a particular signal.
- Integrate — Integrates signals to the current batch, sample, or compound

Method Editor

- MRM Setup — Presents a quantitation method in simple stepwise fashion
- Create method from acquired MRM data — Creates a quantitation method automatically from the acquisition method after requiring only the assignment of ISTD relationship and concentrations
- Create a method manually using the graphics in the Sample Information window
- Group by time segment — Organizes methods by compounds in ordered time segments
- Validate — Ensures that a quantitation method meets rigorous criteria
- Isotopic dilution — Supports adjustments from (Rx, Ry) Colby constant calculations

Calibration

- CurveFit assistant — Calculates all combinations of curves; picks disabled points; and presents results with an equation that is sortable by confidence band and custom filterable by R^2, standard error, and max % residual
- Dilution assistant — Calculates and creates calibration levels based on a default or specified serial dilution scheme

- Copy Cal levels — Copies calibration levels from one compound to other compounds
- Disable Cal points — Disables calibration points based on level, or individual compounds in tables, or interactively through graphs
- Curve fits — Supports curves by:
  - Type: Linear, Quadratic, First order ln, Second order ln, Average of Response Factors
  - Origin: Ignore, Include, Force, Blank Offset
  - Weight: None, 1/x, $1/x^2$, 1/y, $1/y^2$, Log, $1/SD^2$
- Replace curve — Creates calibration curves from existing calibration samples
- Average replicates — Averages replicate levels in the method calibration table.
- Import levels — Imports calibration levels and concentrations from a file
- Scale graphs — Provides graphs with the capability to be autoscalable by X, Y, X-log, and Y-log; and intelligent zooming to fit specified levels

Integrator

- Agile and Agile2 integrator — Provides a parameter-free integrator at all levels of signals that reduces manual integration efforts
- Integrator metrics — Generates metrics that characterize the signal's integration to accept, inspect, or reject the integration
- Signal-to-noise — Calculates signal-to-noise for peaks
- Graphics — Shows superior interaction with the graphing of a compound and the display of peak information

Batch-at-a-Glance: Results

- Navigation — Moves (previous, next, direct) between samples, compounds, time segments, and compound groups
- Compound views — Switches between the details of the current compound/sample or the summaries of multiple compounds/samples
- Batch Table views — Enables flat-table layouts or the capability to drill down to vertically or horizontally nested tables for details and compound table layout

- Window layout — Reorganizes the screen to its defaults, or saves or loads custom-window layouts
- AutoReview — Displays each sample automatically and interactively, allowing you to stop at any time for closer inspection
- Columns — Enables you to add, remove, reorder, save, load, restore, or reset columns
- Float pane — Floats any pane onto another monitor to enable dual-monitor presentations
- Export Table — Exports Batch-at-a-Glance tables directly to Excel files
- Export Graphics — Exports any graphic to a customized size in multiple formats
- Copy/Paste — Copies or pastes any graphic directly into Microsoft Office applications such as Word, PowerPoint, Excel, etc.
- Print/Preview — Prints or previews screen content in WYSIWYG format (what-you-see-is-what-you-get)
- Filter — Displays any combination of sample types
- Sort — Sorts any column that appears in a table

Compounds-at-a-Glance: Results

- Print/Preview — Prints or previews compound chromatograms.
- Copy/Copy Page — Copies selected compound chromatograms, or all compound chromatograms on the screen into Microsoft Office applications such as Word, PowerPoint, Excel, etc.
- Edit Compound Chromatograms — Manually integrate the data, or select zero-peak compounds.
- Views — Displays chromatogram details such as baselines, filled peaks.
- Adjust Axes — Link/Unlink X or Y axes, autoscale to fit the panes, fit to peaks or fit to calibration levels.
- Layout — Organize rows by compounds or samples, select chromatogram overlays, review sample by sample or compound by compound, set display options.
- Highlight — Compounds with outliers

Outlier Detection

- Manage — Sets up and selects specific outliers that can be detected and individually controlled
- Highlight — Highlights outlier values (high = red, low = blue) in the results table
- Filters — Lets you display the results of selected types of filters
- Outliers — Supports specific types of data for outlier detection
- Quantitation message — Warns you of samples that encountered serious problems during quantitation

Report

- Generate — Generates graphics and report results for importing and formatting for Excel XML
- Custom — Lets you customize the Excel template

Update

- Update/Average RT — Updates or calculates weighted averages of the compound's retention times
- Update Qualifier Ratios — Updates qualifier ratios based on the compound's current sample
- Update Mass Assignments — Updates mass assignments based on compounds current sample

Qualitative

- Sample Information — lets you display the chromatogram and extracted spectra for the current sample
- Chromatogram/Spectrum — Provides significant features that can be used to explore spectra for different types of signals

Quantitative Methods

The Method Editor lets you create a new quantitation method from an MRM acquisition data file (Figure 12), from SIM data, from an acquired Scan data file, or manually.

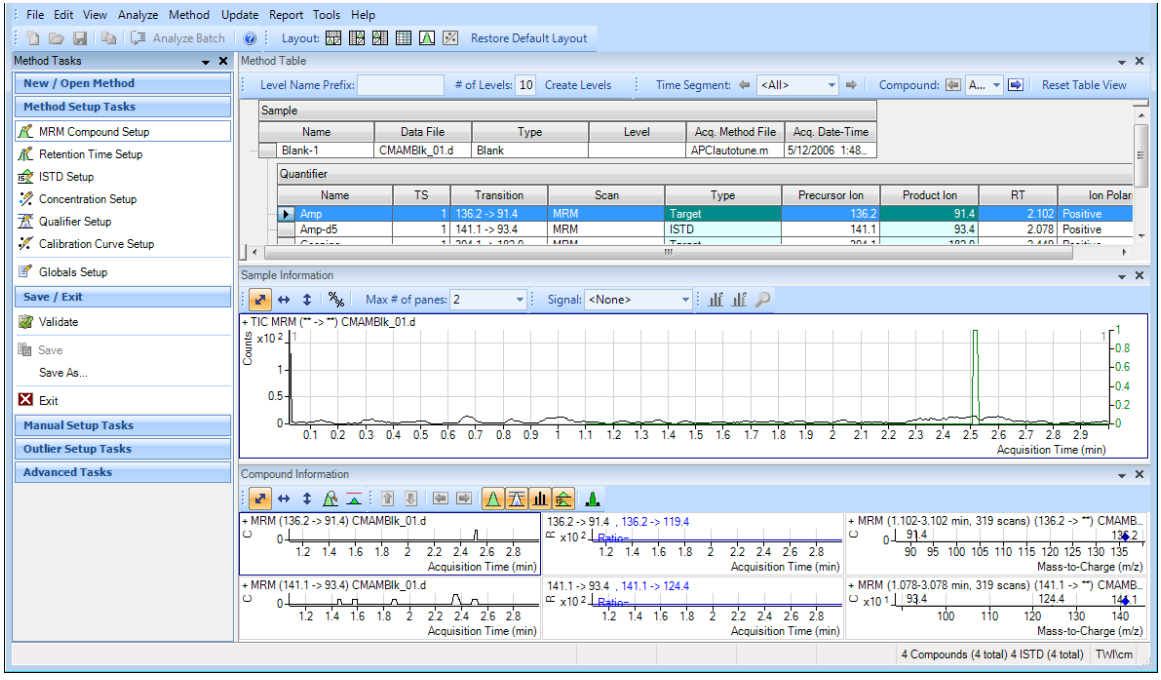


Figure 12 Quantitative view – Method Editor

A file selected from the Batch Table is used as a reference for developing the method settings. These settings are then used to generate the calibration curve and quantitate the standards, QCs, and samples.

Parameter-Free Integrator

What is the parameter-free integrator?

Agilent has developed a new peak integrator algorithm that works especially well for MS/MS data. The parameter-free integrator presents these advantages:

- Handles low-level noisy data by setting a peak's starting and ending points statistically
- Adjusts the threshold automatically
- Eliminates the need for manually reintegrating peaks for low-level MRM signals
- Identifies those peaks that appear reliable and those that should be discarded

Example of integration results

Figure 13 shows data at two extremes.

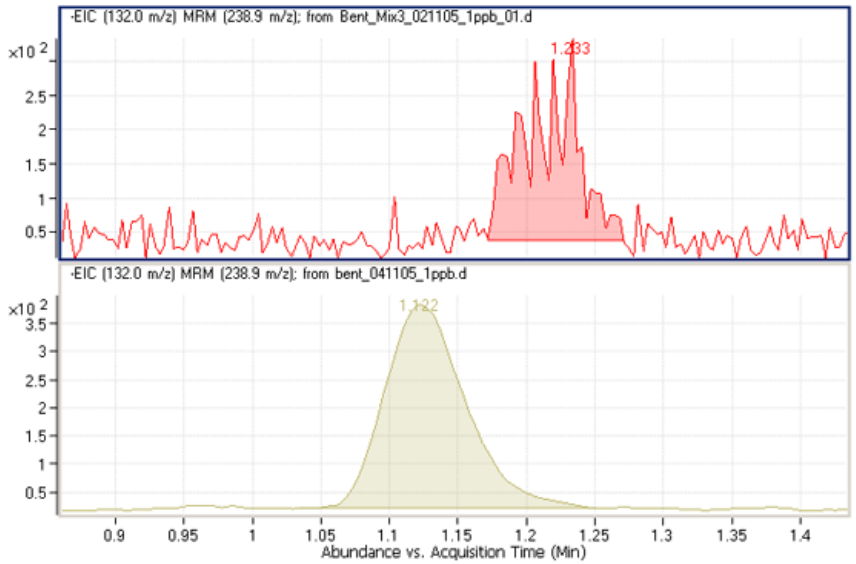


Figure 13 Parameter-free integrator – Data at two extremes

The lower chromatographic peak could be easily integrated since it is a nice Gaussian-shaped peak, but it would be difficult to define the baseline of the upper peak. In fact, many integrator algorithms might interpret these results as multiple peaks.

However, Agilent's new algorithm had no trouble defining the baseline and recognized this as a single peak. In fact, the new integrator algorithm would integrate this as a single peak even if the baseline were rising, instead of being flat, as shown.

Batch-at-a-Glance: Results

The integration results obtained from the analysis of amphetamine (Amp) are shown in Figure 14. This is a flat view of the **Batch Table**, **Compound Information**, and **Calibration Curve**.

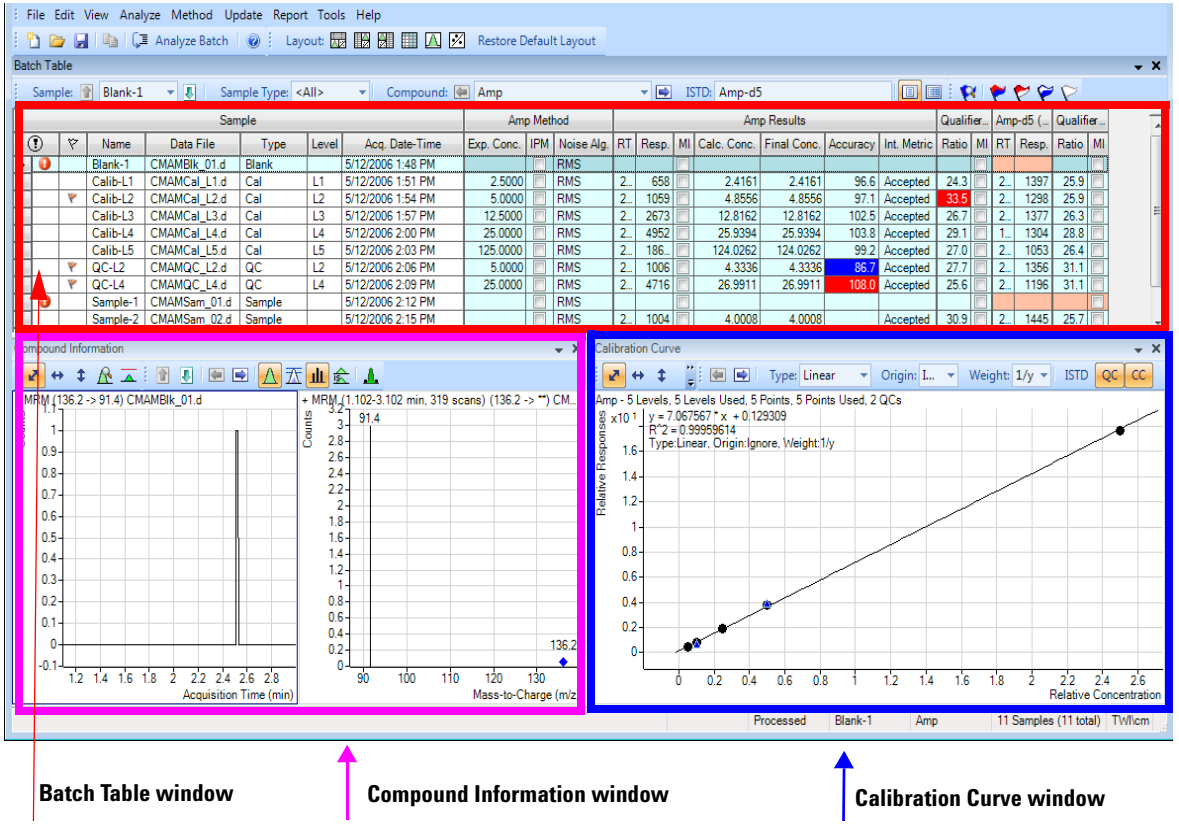


Figure 14 Amp results

- The **Batch Table** shows the integration results from applying the quantitation method to each data file. Colored highlights correspond to results that are lower (blue) or higher (red) than expected.
- The **Compound Information** window at the lower left displays the integrated chromatographic peaks.
- The **Calibration Curve** is shown at the lower right.

Compounds-at-a-Glance

The Compounds-at-a-Glance view shows specific compounds detected in each sample, as shown in Figure 15. This feature allows you to view the compound chromatograms and arrange them for easy data analysis. It is especially useful for food safety labs that look for compound trends within batches of samples.

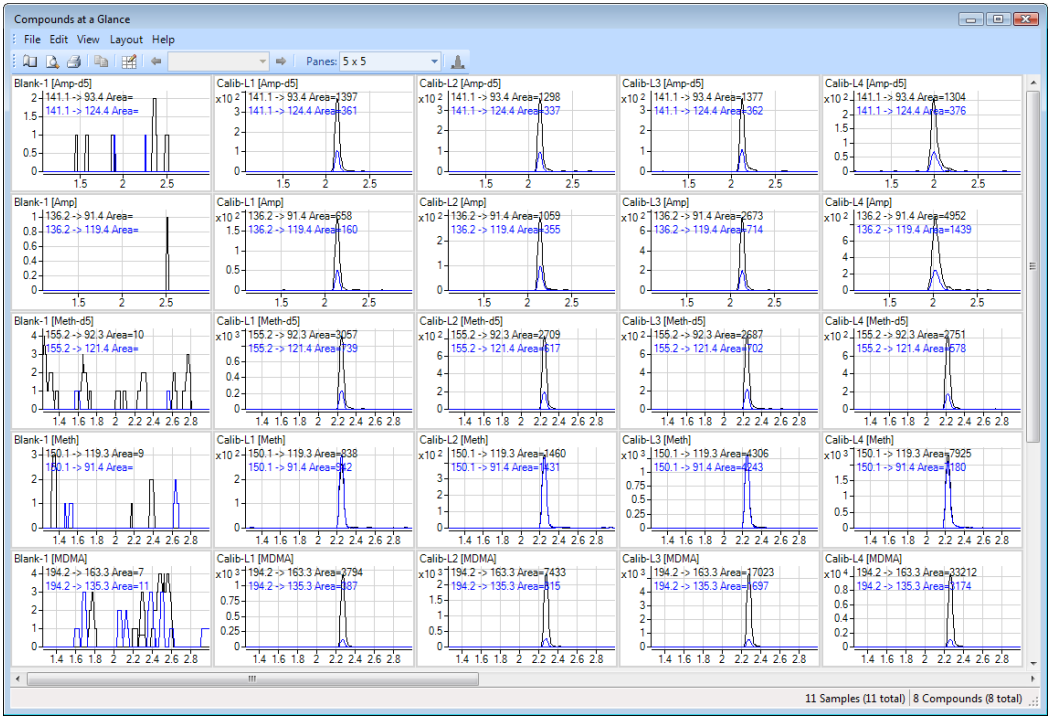


Figure 15 Compounds-at-a-Glance in Quantitative Analysis

The setup feature in the Compounds-at-a-Glance allows you to select the compounds and samples you would like included in the view. As shown in Figure 16 the different tabs at the top of the **Setup Graphics** box provide different options for selecting and arranging the chromatograms.

- The **Samples** tab lists all the samples included in the batch, and gives options for selecting all samples or specific samples.
- The **Compounds** tab lists the compounds detected in the batch. It allows you to choose the compounds you would like to view.

- The **Organize** tab allows you to specify the arrangement of the chromatograms, according to sample and compound. It provides overlay options for compounds, samples, and outliers. The tab gives choices for adjusting the chromatograms, such as displaying baselines or fill peaks to best illustrate compound detection trends.
- The **Outlier** tab provides options for showing outliers in the data.

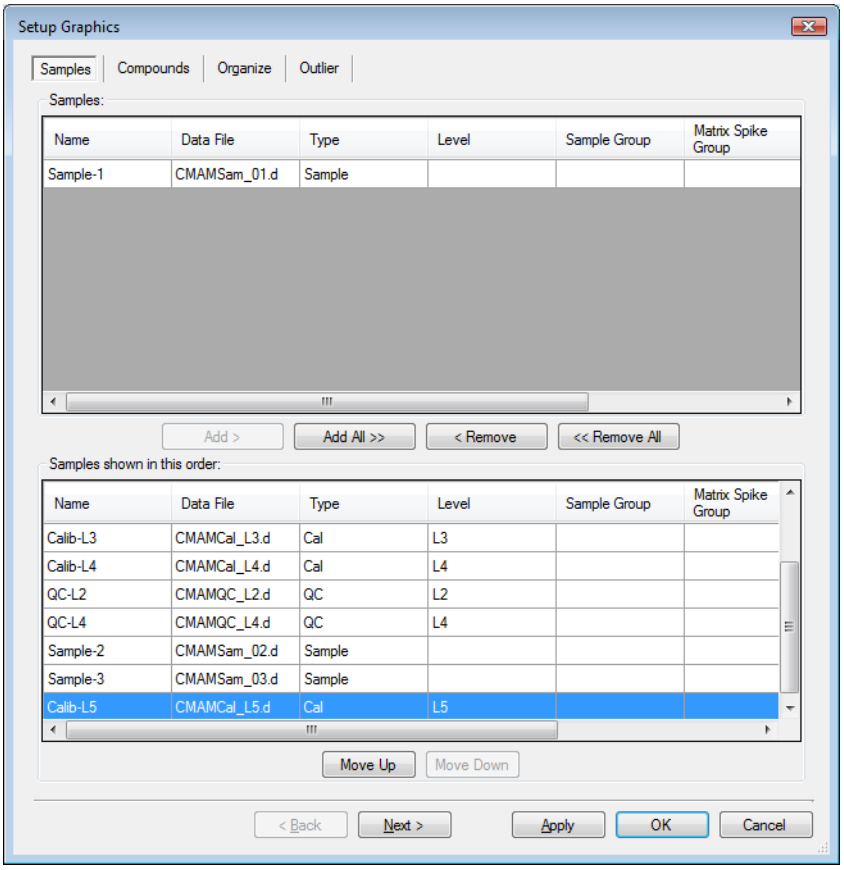


Figure 16 Setup options for Compounds-at-a-Glance

Compound Confirmation

The format shown in Figure 17 can be of value to certified drug-testing laboratories. It shows two sets of plots that can be obtained from a THC analysis.

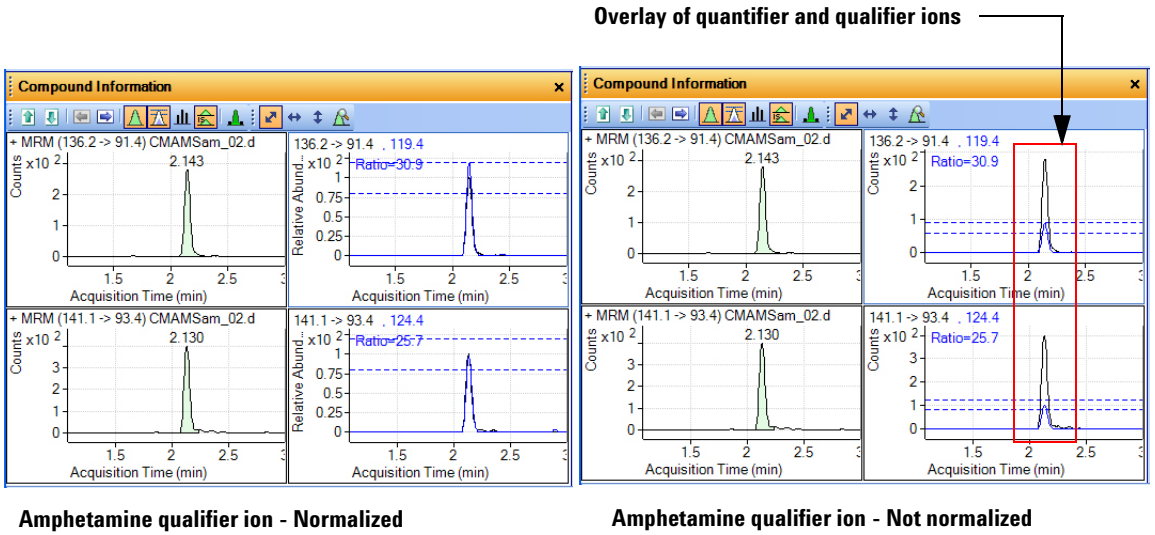


Figure 17 Amp in Quantitative Analysis

Two product ions must be acquired for confirmation: a quantifier ion and a qualifier ion. Typically, the quantifier ion that is used for quantitation is the most abundant of the two product ions.

To be able to confirm the presence of Amphetamine, the qualifier ion peak area must be at least a certain percentage of the quantifier ion, a number that is set in the quantitation method. In this example, 26.5% is used with a window of ± 20%. This means that the area of the qualifier ion must be in the range of 21.2 to 31.8% of the quantifier ion for the analyte Amp. The qualifier for the ISTD, or Amp-d5, also has a specific range that it must be in.

From the figure on the left, whether or not the qualifier ion falls within the accepted window is not easily determined because the size of the qualifier peak is normalized by a factor of 1/0.265. In the figure on the right, the acceptance window is centered at 26.5% of the quantifier ion peak and the

qualifier ion is drawn not normalized, or on the same scale as the quantifier. If the ion is not within the required acceptance window, then it is shaded blue, but is still transparent so as not to hide the quantifier ion. This makes it easier to confirm the presence of compounds visually.

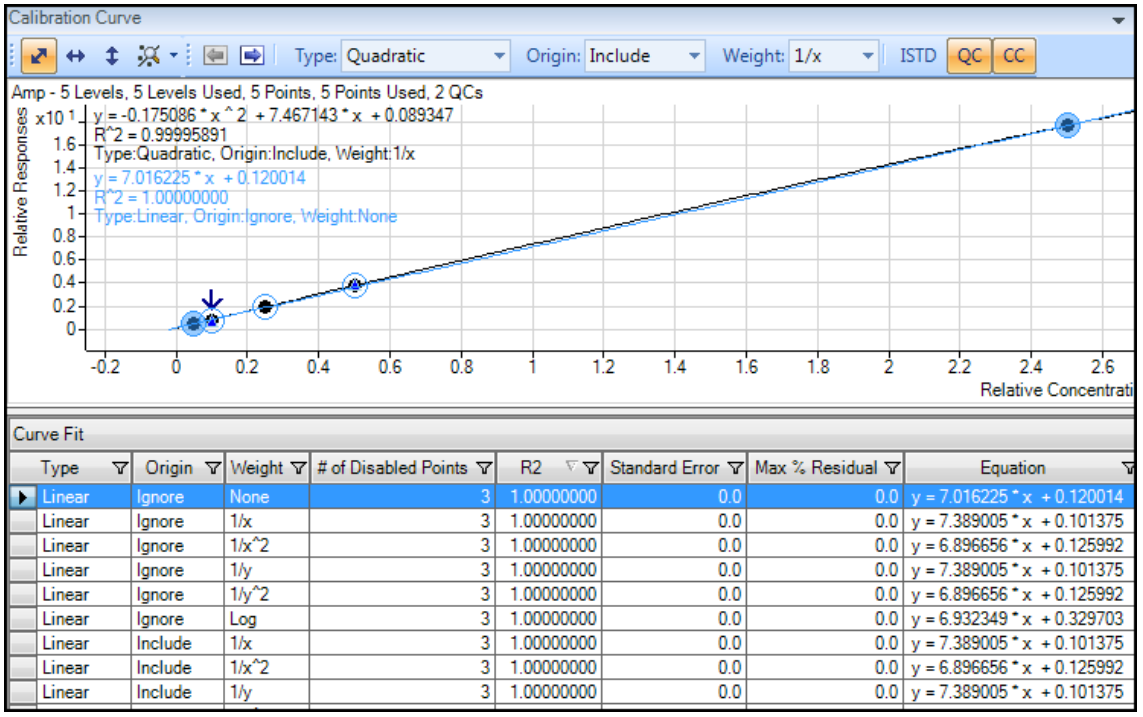
Compound Calibration

The Quantitative Analysis program contains several tools to help calibrate and quantitate compounds:

- CurveFit Assistant
- Cursor Pointer for Data Point Information
- Data Point Zooming

CurveFit Assistant

The CurveFit Assistant provides an analytical view of evaluating the possible curve fits (Figure 18).



Type	Origin	Weight	# of Disabled Points	R2	Standard Error	Max % Residual	Equation
Linear	Ignore	None	3	1.00000000	0.0	0.0	y = 7.016225 * x + 0.120014
Linear	Ignore	1/x	3	1.00000000	0.0	0.0	y = 7.389005 * x + 0.101375
Linear	Ignore	1/x^2	3	1.00000000	0.0	0.0	y = 6.896656 * x + 0.125992
Linear	Ignore	1/y	3	1.00000000	0.0	0.0	y = 7.389005 * x + 0.101375
Linear	Ignore	1/y^2	3	1.00000000	0.0	0.0	y = 6.896656 * x + 0.125992
Linear	Ignore	Log	3	1.00000000	0.0	0.0	y = 6.932349 * x + 0.329703
Linear	Include	1/x	3	1.00000000	0.0	0.0	y = 7.389005 * x + 0.101375
Linear	Include	1/x^2	3	1.00000000	0.0	0.0	y = 6.896656 * x + 0.125992
Linear	Include	1/y	3	1.00000000	0.0	0.0	y = 7.389005 * x + 0.101375

Figure 18 CurveFit Assistant

Note that the black line drawn through the data points uses Quadratic as the Fit, 1/x as the Weight, and Include as the Origin as shown at the top. Many other combinations of the curve settings are listed below the calibration curve, with the selected one highlighted in blue. The highlighted settings are also plotted in blue in the curve window.

You can find the best curve fit, for example, one that corresponds to the highest R^2 value, by ordering all of the possible results from the best to the worse R^2 values and then deciding how many data points to consider as being outliers.

For example, the first set of parameters in the list corresponds to a Linear Fit, Ignore Origin, and Equal Weight. The corresponding R^2 value is 0.9998001477, which is very good. The corresponding curve can be plotted by simply clicking this entry in the table.

Using these settings, data can be requantitated. Eliminating outliers is common as a standard operating procedure (SOP) in some laboratories.

Data point information

Overlapping data points are not unusual in a calibration curve, especially with triple quad MS data, where %RSD values are quite low (Figure 19). To help distinguish the data points from one another, the cursor can be moved over the data points to obtain more information about them.

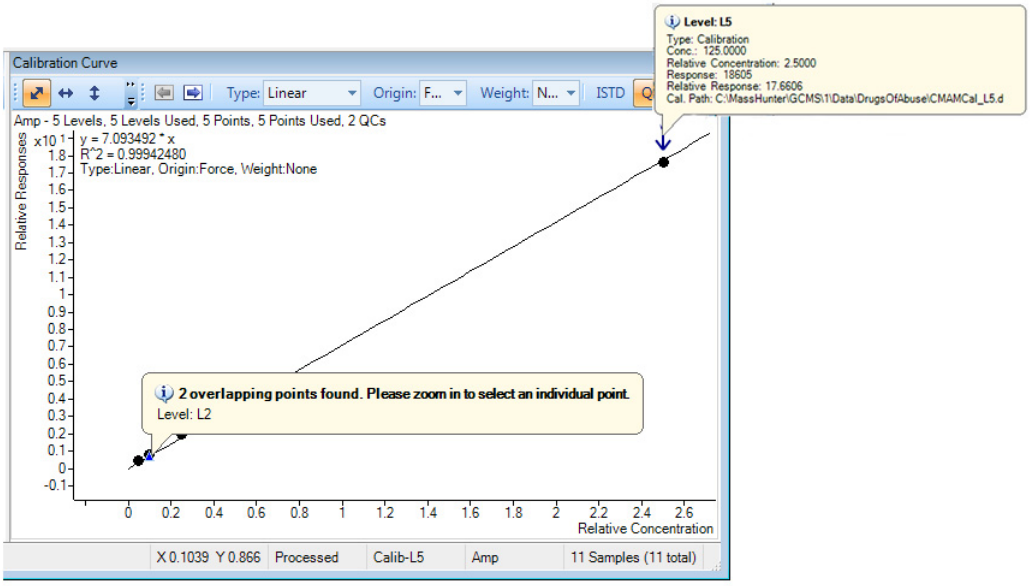


Figure 19 Amp results: Calibration data point information

This figure shows two examples of this type of information. The first example shows that the data points overlap and advised you to zoom in to see them separately. The second example shows information on the data point itself.

Data point zooming

You can zoom in on overlapping data points to see individual data points not visible in the visual presentation.

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In This Book

The Familiarization Guide presents exercises to help you use the Quantitative Analysis program. In this guide you learn:

- How to set up and quantitate a batch of Agilent Triple Quad LC/MS and GC/MS data files
- How to set up and quantitate a batch of Agilent Q-TOF LC/MS data files
- How to inspect your quantitation results and spot irregularities
- How to improve result accuracy
- How to generate and review quantitation reports

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