Agilent 6410 Triple Quad LC/MS

Familiarization Guide

Agilent Technologies

Notices

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Software Revision

This guide is valid for the B.01.03 or later revision of the Agilent MassHunter software for the Agilent 6410 Triple Quad LC/MS, until superseded.

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In this Guide...

The Familiarization Guide presents step-by-step exercises to help you learn to use the Agilent Triple Quad LC/MS. You can do these exercises with the demo data files, SulfaDrugs (Exercise 1) and DrugsOfAbuse (Exercises 2 through 5), shipped with the system (in the **Data** folder of your software CD), or with data you acquire.

1 Develop an acquisition method for the Agilent Triple Quad LC/MS

In this exercise, you learn how to determine optimum Agilent Triple Quad MS acquisition settings for analyzing your compounds of interest, including the fragmentor voltage and collision energy.

2 Set up and quantitate a batch of acquired datafiles

In this exercise, you set up a batch table, a quantitation method, and target compounds, using acquired datafiles. Finally, you 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 three new tools to evaluate results

The new 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, review reports in Microsoft Excel and customize the report template.

Before you start...

This guide assumes that the Agilent MassHunter Workstation Acquisition and Quantitative Analysis applications have been installed, and the LC modules and the 6410 Triple Quad LC/MS have been configured. Also, the performance has been verified, and the system has been turned on. If these actions have not yet been done, see the *Agilent 6410 Triple Quad LC/MS System Installation Guide*.

The exercises in this guide use this equipment and materials:

- Agilent 1100 LC modules: well-plate sampler, binary pump, thermostatted column compartment, DAD
- Zorbax, SB-C18 2.1mm x 30mm, 3.5um, 100Å, p/n 873700-902.
- A 10-µL sulfa mix sample, prepared as directed in Exercise 1.

Contents

Contents

Exercise 1
Develop an acquisition method for the Agilent Triple Quad LC/MS

With this exercise, you learn how to determine the best Agilent Triple Quad LC/MS acquisition settings for analyzing your compounds of interest. These instructions help you understand not only how to set up a worklist to optimize instrument parameters for best sensitivity in acquisition, but also how to use the Agilent MassHunter Qualitative Analysis program to identify parameter values producing optimum signal response.

In this exercise, you create an acquisition method for a mixture of four sulfa drugs, optimizing both the fragmentor and collision energy voltages to maximize sensitivity. One of the ways to optimize parameters is to create a worklist, or sequence, of data file acquisitions, each using a different method. This exercise uses this protocol for method development.

Another way to develop a method is to use the manual tune capability to optimize various parameters, including collision energy, to obtain the optimal signal response for each multiple reaction monitoring (MRM) transition. A third technique has you set up an acquisition method that directs the instrument to make multiple injections of the sample from an autosampler vial and acquire the data to a single data file. The method contains multiple time segments, one for each injection, with an incremental change made to a particular parameter (e.g., collision energy) in each segment.

This exercise uses the first protocol for method development.

NOTE

See the *Agilent Triple Quad LC/MS Concepts Guide* to learn more about how the triple quadrupole mass spectrometer works and why the fragmentor and collision energy voltages are important. For background information, see Chapter 3, Agilent Triple Quad MS and Sensitivity, in the *Concepts Guide*. See the Online Help for detailed information on how the program works.

Each exercise is presented in a table with three columns:

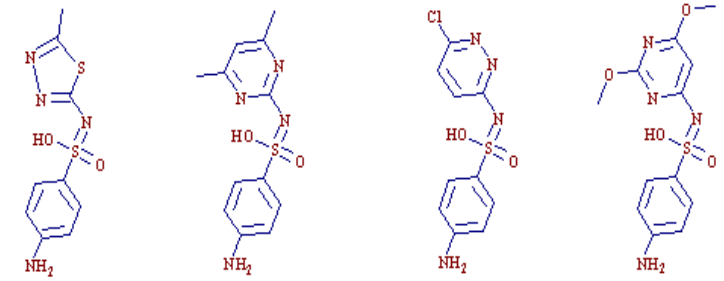
- Steps – Use these general instructions to proceed on your own to explore the program.
- Detailed Instructions – Use these if you need help or prefer to use a step-by-step learning process.
- Comments – Read these to learn tips and additional information about each step in the exercise.

Before you begin...

For this exercise you analyze a mixture of four sulfonamide compounds.

If you do not intend to perform data acquisition but want to learn how to use the Qualitative Analysis software for method development, you can skip this section, which gives instructions on how to prepare the demo sample. You then perform only those instructions that show you how to use the Qualitative Analysis software with the sulfa drug data files shipped with the system.

The Electrospray LC Demo Sample (P/N 59987-20033) contains five ampoules with 100 ng/µL each of sulfamethizole $(M+H)^+$ = 271, sulfamethazine $(M+H)^+$ = 279, sulfachloropyridazine $(M+H)^+$ = 285, and sulfadimethoxine $(M+H)^+$ = 311.



NOTE

Determining optimal parameter values for acquiring sample compound data requires that the Agilent Triple Quad instrument already be tuned on the Tuning Mix calibrant ions. Before proceeding with this exercise, make sure you have used Checktune or Autotune to verify that calibrant ions each have the proper mass assignment, peak width, and signal intensity.

See the *Agilent Triple Quad LC/MS Quick Start Guide* or Online Help for instructions on tuning the instrument.

1 Prepare the LC solvent.

In 1-liter reservoirs of HPLC-grade water and acetonitrile (ACN), add 1 ml of 5M NH_4HCO_2 (Ammonium Formate) each to make 5mM NH_4HCO_2 in H2O and ACN and use for the A and B channels, respectively.

2 Prepare the sample.

a Add 10 µL sulfa mix from one of the ampoules (500 µL) to 990 µL of solvent A in an Eppendorf vial so that the final concentration is 1 ng/µL.

b Place a sample vial containing an injectable amount of the prepared sample in the autosampler.

3 Set up the LC column.

Use this Agilent column or equivalent: Zorbax, SB-C18 2.1mm x 30mm, 3.5um, 100Å, p/n 873700-902.

4 Set the column temperature.

Agilent suggests a column temperature of 40°C, but this exercise can run at room temperature.

5 Copy the data files to a directory on your PC.

Copy the folder named **SulfaDrugs** in the **Data** folder on your software CD to any location on your hard disk. This folder contains all the data files needed for this exercise.

NOTE

Do not re-use the sulfa drug data files already on your system unless you know that you copied them from the originals on the CD and you are the only one using them.

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

Task 1. Enter acquisition parameters and acquire data

In this exercise, you enter the conditions for the analysis of the sulfa drug mix.

Steps	Detailed Instructions	Comments
1 Enter LC parameters appropriate for sulfa drug mix. See Table 1.	**a** Double-click the **Agilent Data Acquisition** icon. **b** Make sure that Acquisition appears as the selection in the **Context** text box. If Tune is the selection, select **Acquisition** from the **Context** dropdown menu. **c** Enter the LC parameters listed in the Table 1.	• The Agilent MassHunter Workstation Data Acquisition window appears. See Figure 1.

Table 1 LC parameters for sulfa drug mix

Parameter	Value
PUMP	
• Flowrate	800 µL/min
• Solvent A	5 mM NH_4HCO_2 in H_2O
• Solvent B	5 mM NH_4HCO_2 in ACN
• Gradient (min - %B)	0 min - 13% 1.80 min - 60% 2.50 min - 60%
• Stop Time	2.50 min
• Post Time	2.50 min
INJECTOR	
• Inj. Vol.	1 µL
• Injection	Standard
• Draw Position	3.0 mm
UV DETECTOR	
• Ch A	254 nm (4 nm BW on DAD)

Table 1 LC parameters for sulfa drug mix

Parameter	Value
• REF A (DAD only)	400 nm (80 nm BW)
COL THERM	
• Temp	40°C

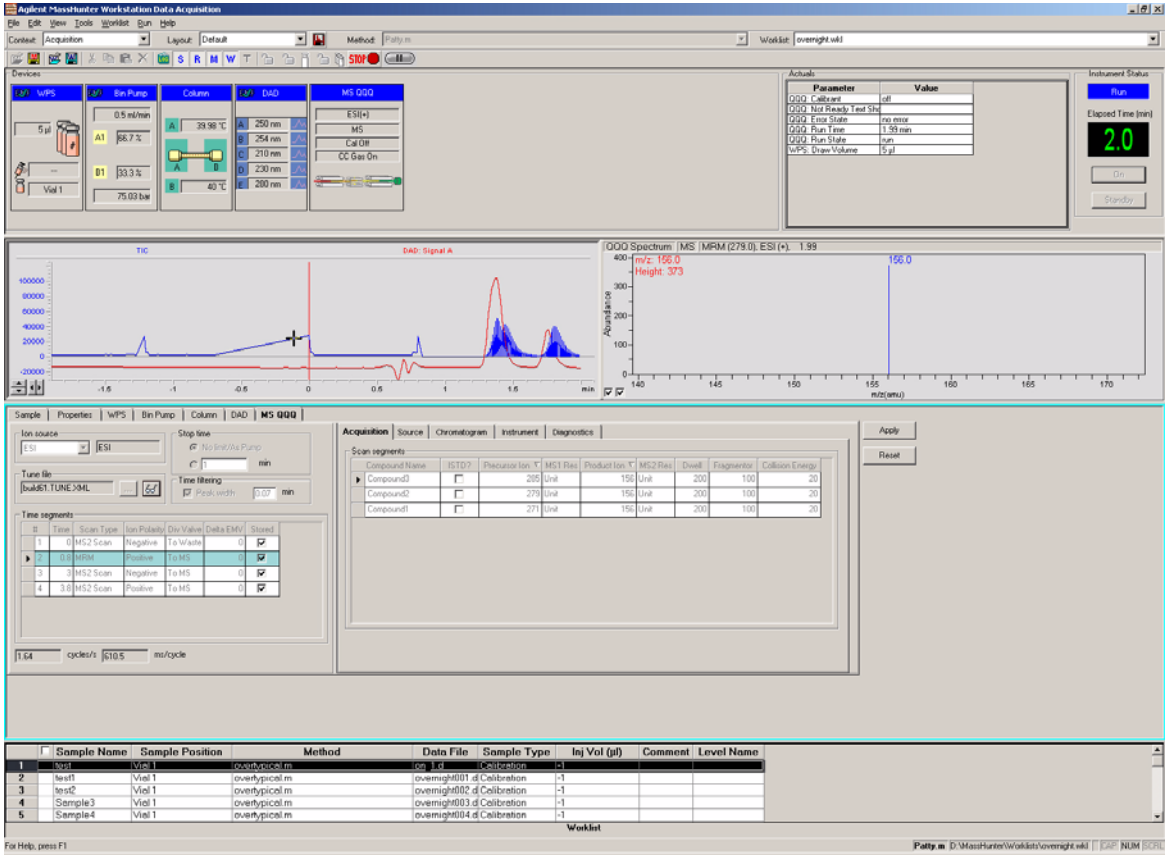


Figure 1 Agilent MassHunter Workstation Data Acquisition window

Steps	Detailed Instructions	Comments
2 Enter MS parameters appropriate for sulfa drug mix and save the method as ***iii*MS2Scantest.m**, where ***iii*** are your initials. See Table 2.	**a** Click the **MS QQQ** tab. **b** Click the **Scan Type** cell, and select **MS2Scan** from the list. **c** Enter the other MS parameters as listed in Table 2. These parameters are in either the Acquisition or the Source tabs. **d** Save the method as ***iii*MS2Scantest.m**, where ***iii*** are your initials.	

Table 2 MS parameters for sulfa drug mix

Parameter	Value
• Source	ESI (+ polarity)
• Gas Temp	350°C
• Scan Type	MS2Scan
• Nebulizer	50 psi
• Dry Gas	12 L/min
• Range	100 to 400

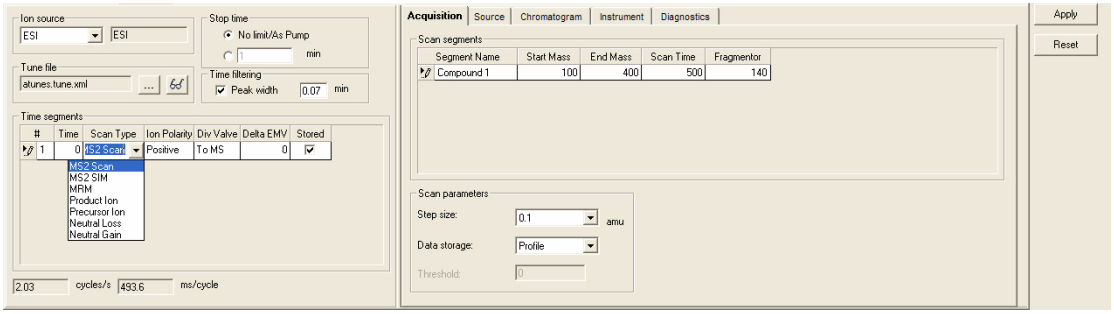
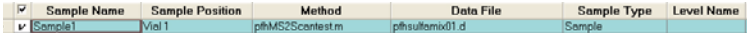


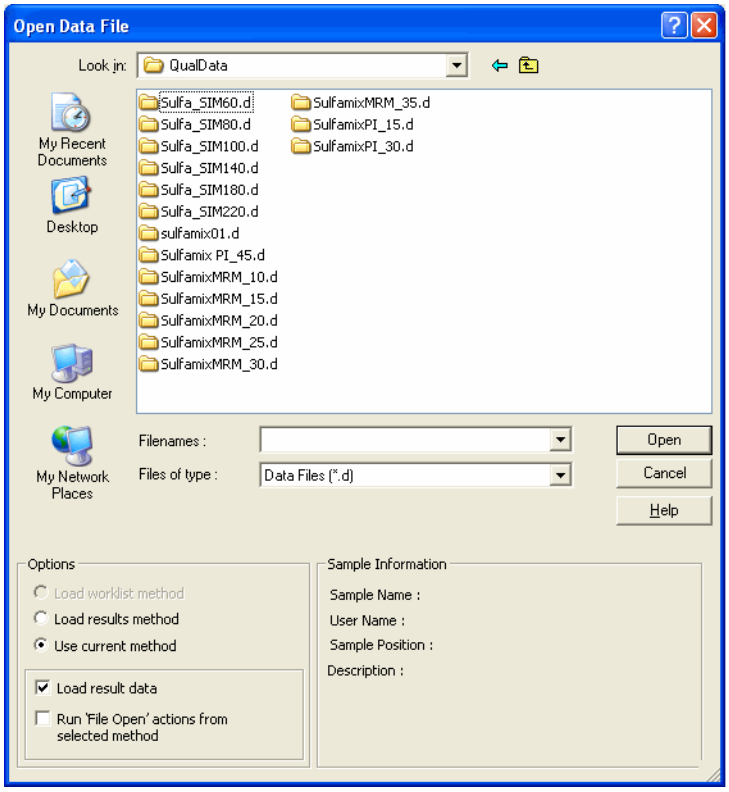
Figure 2 Select Scan Type of MS2 Scan in the Acquisition tab

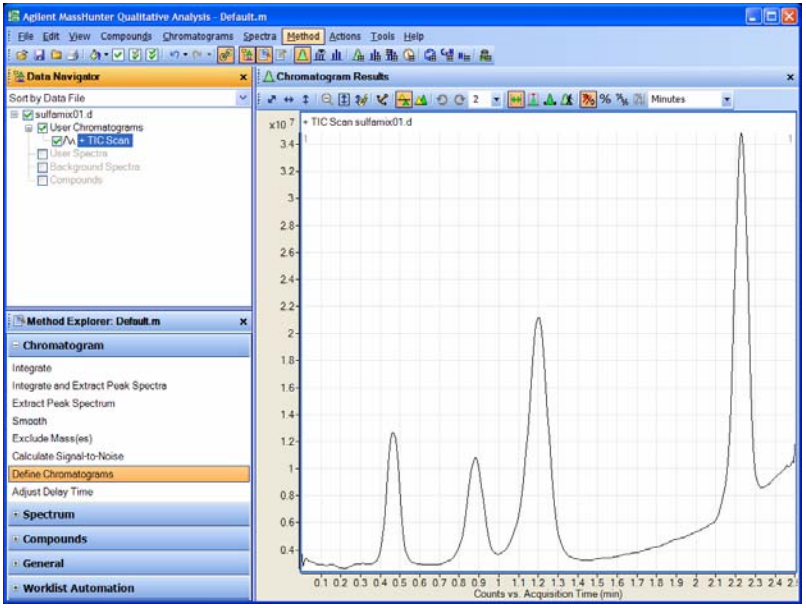
Steps	Detailed Instructions	Comments
3 Acquire data (optional). • Set up a one-line worklist with the method you just created. • Name the data file ***iii*sulfamix01.d**, where ***iii*** are your initials. • Designate a directory path to hold your data files and method.	**a** If necessary, click the **Worklist** icon to bring up the worklist. **b** Select **Worklist > Worklist Run Parameters.** Verify that the parameters are set properly. Click **OK**. **c** Type `iiiMS2Scantest.m` and `iiisulfamix01.d` as the method name and data file name, respectively. **d** Click **OK.** **e** At the bottom left portion of the Acquisition window, where you can find the worklist, mark the check box to the left of the sample as shown below.  **f** Click the **Run** icon.	• You have just acquired a full scan MS data file to see what ions are being formed from the sample. • This step is optional because you can perform the next step with an example data file that comes with the software. If you prefer, you can create your own data file as described in this step.

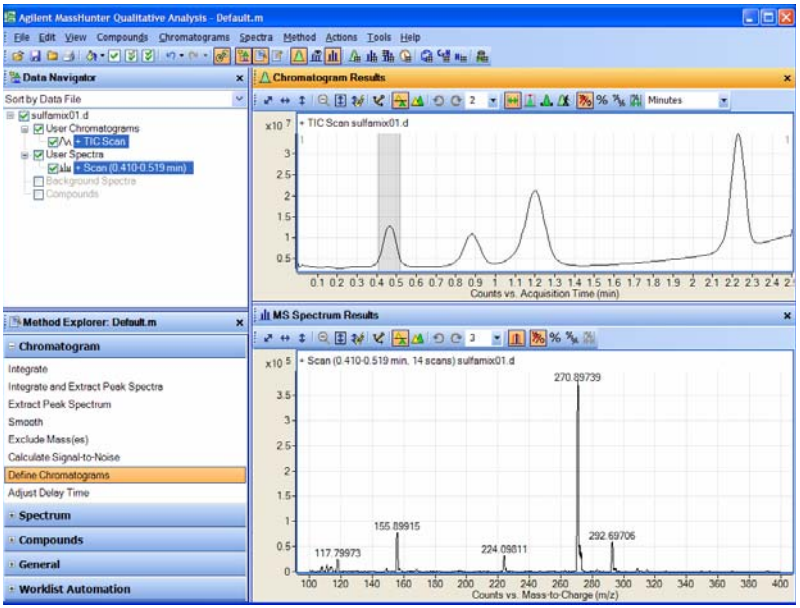
Task 2. Determine precursor ion masses

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

Steps	Detailed Instructions	Comments
1 Open the acquired data file. • In the Agilent MassHunter Qualitative Analysis program, open either the example file, **sulfamix01.d,** or the data file you created in "Task 1. Enter acquisition parameters and acquire data" on page 11.	**a** Double-click the **Agilent MassHunter Qualitative Analysis** icon. The system displays the Open Data File dialog box.	• When you open the sulfa drug directory after installation, the **Load result data** (lower left corner) check box is grayed out. • If you see the check box marked, this means that the data file(s) already contains results. ***Clear this check box before opening the file***.

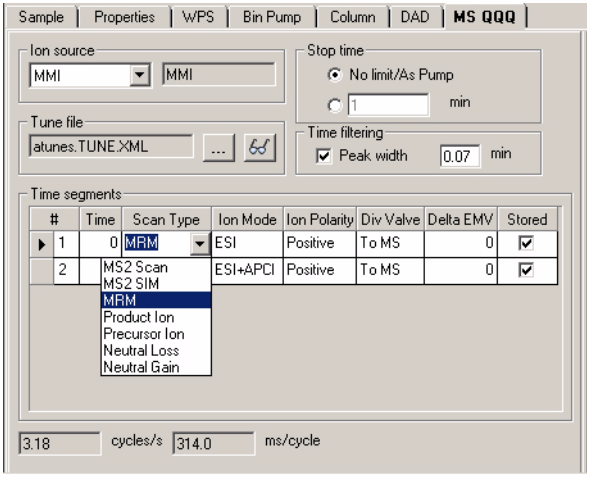


Steps	Detailed Instructions	Comments
	b Do one of the following: • Select the example data file **sulfamix01.d**, and click **Open**. • Select the data file you created in "Task 1. Enter acquisition parameters and acquire data" on page 11, and click **Open**. By default, the system displays the Total Ion Chromatogram (TIC).	• The figure below shows the default layout. This is what you want to see. • The Qualitative Analysis program displays a newly opened data file with the same layout and display settings used for the previous data file. Therefore, you MUST make sure to return to the default settings for this exercise.
Before you begin, make sure that all previous settings are returned to their default values: • Restore default layouts ◦ Click **View**, and select **Window Layouts**. ◦ Select **Restore Default Layout**. • Make sure the method is **default.m**. (see title bar) ◦ Select **Method > Open**. ◦ Select **default.m**, and click **Open**. • Return display options to default settings. ◦ Select **Tools > Plot Display Options...** ◦ Click **Default**, and then **OK**.		

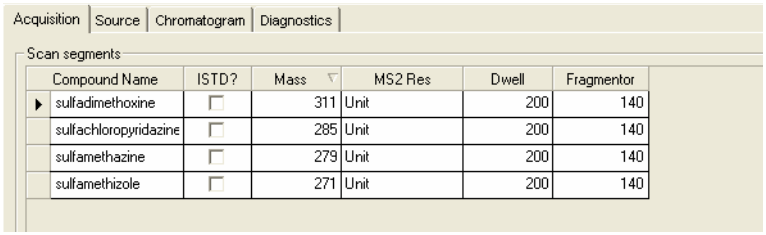
Steps	Detailed Instructions	Comments
2 Determine precursor ion masses for all four peaks. • You have determined them correctly if you find the values are similar to those shown in this table: **Compound** / RT / *m/z* Sulfamethizole 0.47 270.9 Sulfachloropyridazine 0.88 284.9 Sulfamethazine 1.20 279.0 Sulfadimethoxine 2.23 311.0 • Close the data file after finding the precursor ion masses.	**a** In the Chromatogram Results window, make sure that the **Range Select** icon in the toolbar is On. **b** Click the left mouse button and drag the cursor across the first peak to produce a shaded region, as in the figure below. **c** Right-click inside the shaded region, and select **Extract Spectrum** from the shortcut menu.  **d** Repeat step a through step c for the other compounds. The precursor ion masses should match those in the table in step 2. **e** Select **File > Close Data File**. **f** When asked if you want to save the results, click **No**.	• The system displays an averaged spectrum across the peak in the Spectrum Preview window. • The precursor mass of the first compound, sulfamethizole, is determined to be m/z 270.9. • To obtain a single scan, double-click the apex of the peak. • Some compounds form sodium (Na) and/or potassium (K) adducts as well, corresponding to M + 23 and M + 39 masses respectively. Seeing these masses along with the M + H can make for an easy confirmation of which ion is the pseudo-molecular ion (M + H)+.

Task 3. Find optimum fragmentor voltage for maximum response

Task 3 shows you how to carry out the optimization for fragmentor voltage by creating selected ion-monitoring experiments for each compound within a method and setting up multiple methods with varying fragmentor voltages.

Steps	Detailed Instructions	Comments
1 Set up six methods for six different fragmentor voltages. • Change to a SIM experiment. • Use 60, 80, 100 and 140, 180 and 220 volts as the fragmentor voltages for the six methods. • Save the methods as ***iii*MS2SIM*xxx*.m**, where ***iii*** are your initials and ***xxx*** is the voltage.	**a** In the **Scan Type** dropdown list, select **MS2SIM**. 	

Steps	Detailed Instructions	Comments
	b In the **Acquisition** tab, enter the **Compound Name** and **Mass** (precursor ion mass) for sulfadimethoxine. **c** Click the arrow to the left of the compound name, and select **Add Row**. **d** Enter **Compound Name** and **Mass** for sulfachloropyridazine. **e** Repeat steps c and d for sulfamethazine and sulfamethizole. **f** Save the method as ***iii*MS2SIM140.m**, where ***iii*** are your initials. **g** Change the fragmentor voltage to 60, and save the method as ***iii*MS2SIM060**, where ***iii*** are your initials. **h** Repeat step g for voltages 80, 100, 180 and 220, saving the methods as ***iii*MS2SIM080, *iii*MS2SIM100, *iii*MS2SIM180 and *iii*MS2SIM220,** where ***iii*** are your initials.	• With the MS2SIM Scan Type set, a different set of columns appears in the Acquisition window. • The Instrument Control and Data Acquisition program creates a SIM experiment for each compound mass, starting with a default fragmentor voltage of 140. See the example below.

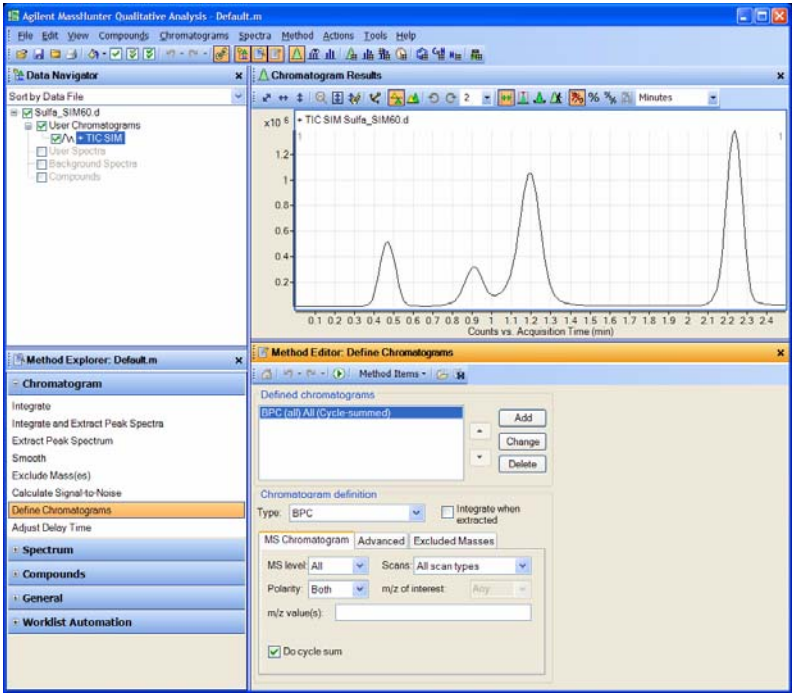


Steps	Detailed Instructions	Comments
2 Set up and run the worklist (optional). • Set up six samples with Sample Name SulfaDrugMix to inject 1ul from vials 1-6 or the ones you choose. • Specify the data files as ***iii*SulfaSIM*xxx*.d**, where ***iii*** are your initials and ***xxx*** is the voltage.	**a** Click the **Worklist** icon if necessary to make sure the worklist is visible. **b** Enter the information for the 60 voltage run. **c** Right-click the upper left corner of the worklist, and select **Add Multiple Samples**. **d** Add five more samples to the worklist for voltages 80-220, and click **OK**. **e** To set up the run, right-click the upper left corner, and select **Worklist Run Parameters**. **f** Enter the paths for the method and data files. **g** Click the tab to specify the sample vial locations, specify the locations and click **OK**. **h** Mark the checkbox to the left of the Sample Name for each of the six samples.	• This step is optional because you can use data files shipped with the system to perform many of the tasks in this exercise.

	✓	Sample Name	Sample Position	Acq Method	Data File	Sample Type
1	✓	SulfaDrugMix	Vial 1	MS2SIM060.m	d:\Sulfa_SIM060.d	Sample
2	✓	SulfaDrugMix	Vial 1	MS2SIM080.m	d:\Sulfa_SIM080.d	Sample
3	✓	SulfaDrugMix	Vial 1	MS2SIM100.m	d:\Sulfa_SIM100.d	Sample
4	✓	SulfaDrugMix	Vial 1	MS2SIM140.m	d:\Sulfa_SIM140.d	Sample
5	✓	SulfaDrugMix	Vial 1	MS2SIM180.m	d:\Sulfa_SIM180.d	Sample
6	✓	SulfaDrugMix	Vial 1	MS2SIM220.m	d:\Sulfa_SIM220.d	Sample

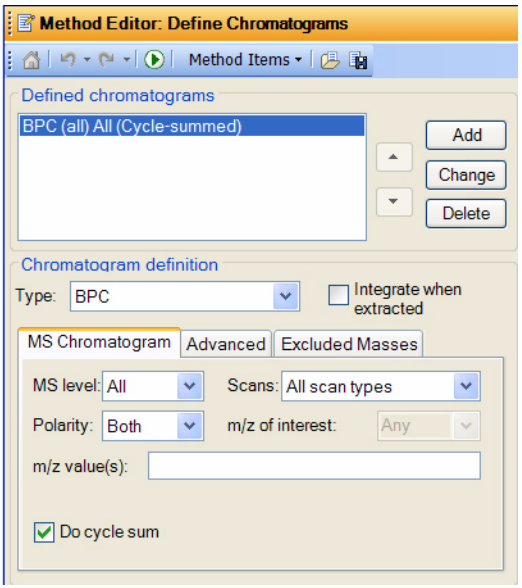
	i Select **Run > Worklist**.	• Note that the program only runs those samples that are enabled with a checkmark.

Steps	Detailed Instructions	Comments
3 Set up a qualitative method to view the EIC data automatically. • Open the data file **Sulfa_SIM60.d** or your own ***iii*Sulfa_SIM60.d**, where ***iii*** are your initials. • In the Method Editor, add in the EICs corresponding to the precursor ion masses of 271, 279, 285, and 311. • Save the method as *iii*Exercise1, where "*iii*" are your initials.	**a** Select **File > Open Data File**. The system displays the Open Data File dialog box **b** Select either **Sulfa_SIM60.d** or ***iii*Sulfa_SIM60.d**, and click **Open**. .	• The Qualitative Analysis program should be open. If not, see step 1 of Task 1 in this exercise.



c Select **Method > View Method Editor**. The system displays the Method Editor window. (See figure on next page.)

Steps	Detailed Instructions	Comments
	d If necessary, select **Define Chromatograms** from the Method Items pull-down menu.	• The default Method Editor list selection after installation is **Integrate**.



e To delete the BPC chromatogram, click **Delete**.

f For the Chromatogram Definition Type, select **EIC**.

g In the MS Chromatogram tab, make sure **MS Level** is set to **All** and **Scans** is set to **All Scan Types**.

h Clear the **Do cycle sum** check box.

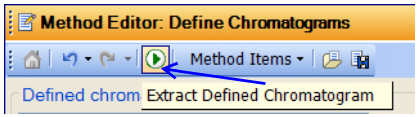
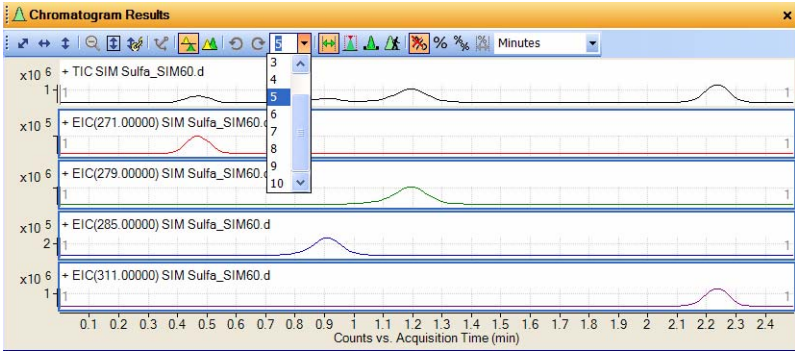
i Enter 271 as the **m/z value**.

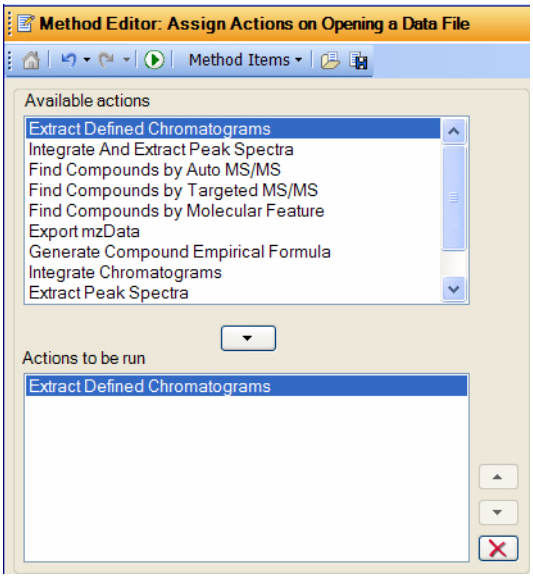
j Click **Add**.

k Repeat steps i and j for the other precursor ions, 279, 285 and 311.

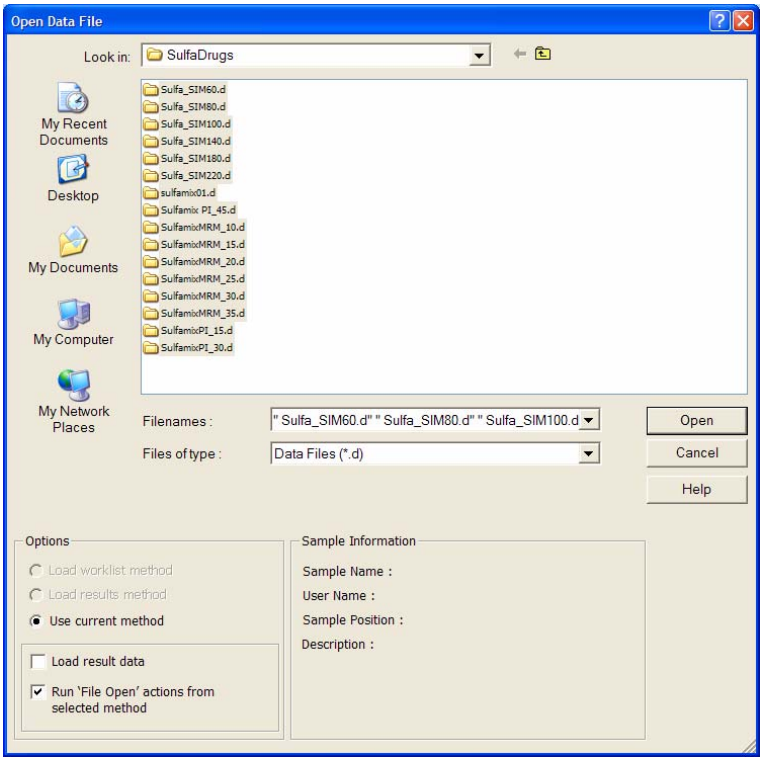
l Select **Method > Save As**.
The system opens the Save As dialog box.

m Save the method as ***iii*Exercise 1.m.**

Steps	Detailed Instructions	Comments
4 Extract the chromatogram for the data file and view the results. • Make sure you can see all five chromatograms, the TIC and four EICs.	**a** To apply the method settings to the data file, click the **Extract Defined Chromatogram** icon on the toolbar.  **b** To see the TIC and four EICs, click the down arrow to display a numerical list as shown in the example below. **c** Select **5** to view five chromatograms simultaneously. The system displays chromatogram results as shown below.  	

Steps	Detailed Instructions	Comments
5 Extract the remaining ion chromatograms automatically. • Extract Defined Chromatograms should be the default action for Assign File Open Actions. • Open the remaining data files, Sulfa_SIM80.d through Sulfa_SIM220.d. • Close the Method Explorer.	**a** Select **Assign File Open Actions** from the Method Editor dropdown menu. **b** Make sure that **Actions to be run** is set to **Extract Defined Chromatograms.**  **c** Select **File > Open Data File.** The system displays the Open Data File dialog box. **d** Select all the data files to be opened. **e** Mark the **Run 'File Open' actions from selected method** checkbox. (lower left corner)	• The Qualitative Analysis Method Editor lets you define actions to be performed automatically upon opening a data file(s).

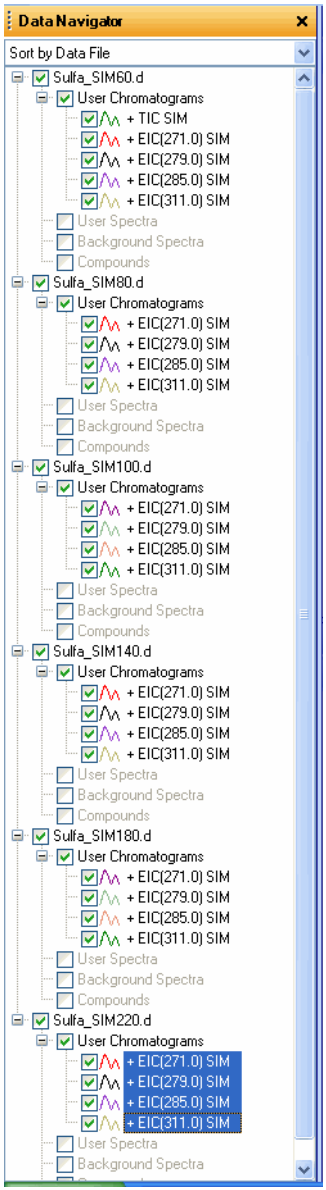
Steps	Detailed Instructions	Comments

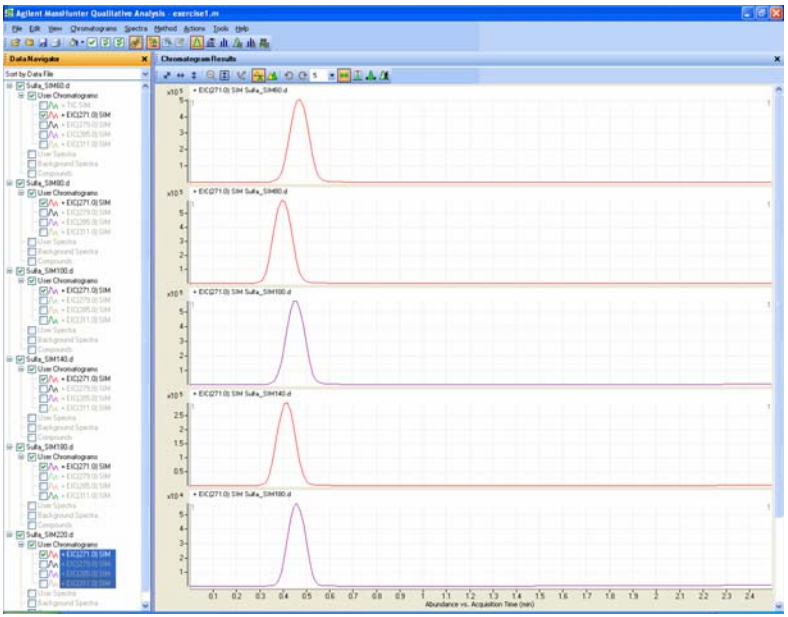


f Click **Open.**
The Qualitative Analysis program displays all the EICs for all the data files selected.

g To close the Method Explorer and Method Editor, click the **X** in the upper right corner of each window.

Steps	Detailed Instructions	Comments

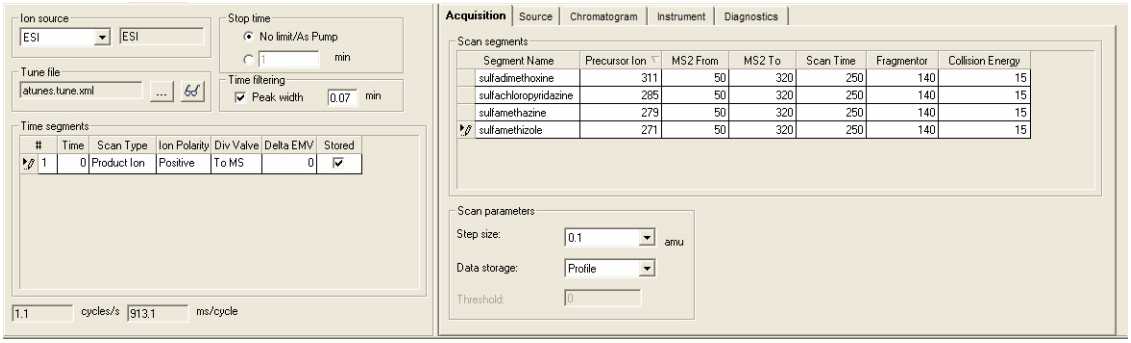


Steps	Detailed Instructions	Comments
6 Select the fragmentor voltage that produces the maximum response for each of the precursor ions. • Close the data files after you determine the optimum voltage.	**a** In the Data Navigator window, highlight the EICs for 271.0 m/z. **b** Click the **Show only the highlighted items** icon,  . Only the 271 m/z check boxes are now marked.  **c** Look at the relative intensities of each peak to determine which fragmentor voltage setting will be best to use for the 271 precursor. Or to overlay the peaks, you can click **Overlaid Mode**. **d** Repeat steps a-c for the other three base peaks or precursor ions. **e** Select **File > Close Data File**. **f** Click **Close** when the Close Data File dialog box appears.	• You press the **Ctrl** key to be able to select multiple files from the list You press the **Shift** key to be able to select a group of files. • A fragmentor voltage of 100 should be sufficient for each precursor ion. • You can now determine the product ions that are available for the multiple-reaction monitoring experiments to maximize sensitivity for the analysis.

Task 4. Determine product ion masses

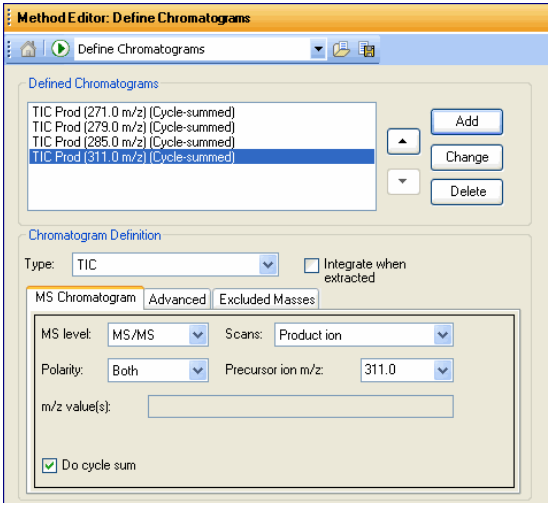
In this part of the method development, we will use three collision energies to determine the best fragment ions to use for the eventual Multiple Reaction Monitoring (MRMs).

Steps	Detailed Instructions	Comments
1 Set up three product ion acquisition methods and acquire data. • Use the MS parameters in the example below, but change the Fragmentor voltage to the optimum voltage you determined in the previous task. • Save methods as ***iii*Sulfamix PI_*xx*.m**, where ***iii*** are your initials and ***xx*** is the collision energy.	**a** Click the **MS QQQ** tab. **b** Select **ProductIon** in the **Scan Type** field to scan each precursor ion for all its product ions. **c** Enter all MS parameters as listed in the example below, making sure the **Collision Energy** is set to `15` and the Fragmentor voltage is set to the optimum voltage determined in Task 3. **d** Save the method as ***iii*Sulfamix PI_15.m**. **e** Repeat step c and step d for collision energies of 30 and 45.	

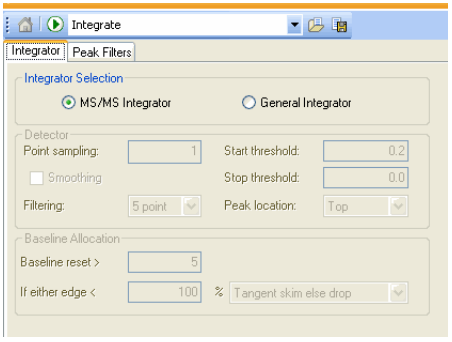


2 Set up and run the worklist (optional). • Specify the data files as ***iii*Sulfamix PI_*xx*.d**, where ***iii*** are your initials and ***xx*** is the collision energy.	**a** Scroll down if necessary to make sure the worklist is visible. **b** Add three samples to the worklist for collision energies 15, 30 and 45. **c** Mark the checkbox to the left of the Sample Name for each sample you are adding. **d** Select **Run > Worklist**.	• This step is optional because you can determine the product ion masses from the data files shipped with the system. • Use the instructions in Step 2 of Task 3 to set up the worklist.

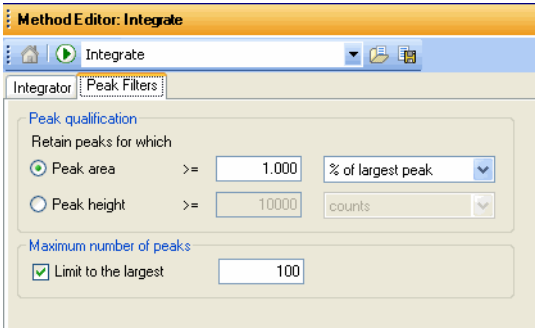
Steps	Detailed Instructions	Comments
3 Set up a qualitative method to integrate and extract product ion spectra. • Use the data files **SulfamixPI_xx.d,** where xx is the collision energy, or your own data files, ***iii*SulfamixPI_xx.d**. • Open Method Explorer and Method Editor. • Use TICs set up for MS/MS, product ion and each of the precursor ions 271, 279, 285, 311. • Make sure the MS/MS integrator has been selected and the maximum number of peaks has been limited to the largest 100 peaks. • Add the ability to integrate and extract peak spectra to the file actions run upon data opening. • Save the changes to the current method.	**a** Click the **Open Data File** icon in the toolbar. **b** Select **SulfamixPI_15.d**. **c** Make sure that the **Run File Open Actions from Specified Method** check box is clear, and click **Open**. **d** Make sure the Method Explorer and the Method Editor windows are displayed; otherwise, click the **Method Explorer** and then **Method Editor** icons. **e** Under Chromatograms in Method Explorer, click **Define Chromatograms.** **f** Delete any existing chromatograms in the Defined Chromatograms box. **g** Select **TIC** from the **Chromatogram Definition** list. **h** For **MS Level**, select **MS/MS**. **i** For **Scans**, select **Product ion**. **j** For **Precursor ion m/z**, enter `271`. **k** Click **Add**. **l** Repeat steps j and k for each ion.	• The Qualitative Analysis program should already be open and contain *iii*exercise 1.m as the method.



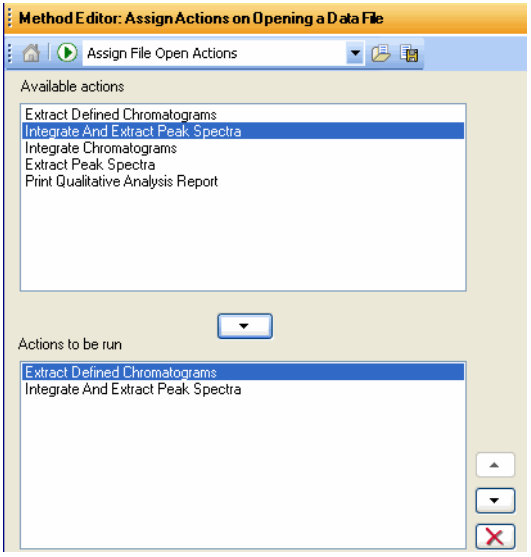
Steps	Detailed Instructions	Comments
	m From the Method Editor drop-down list, select **Integrate**. **n** Select the **MS/MS Integrator**, if necessary.	



	o Click the **Peak Filters** tab. Make sure that the **Limit to the largest** check box is marked and set to the value `100` (peaks) as shown below.	



	p Click **General** in Method Explorer, and then click **Assign File Open Actions**. **q** Select **Integrate and extract peak spectra** from the Available actions list and click  to add this to **Actions to be run**.	

Steps	Detailed Instructions	Comments
	 **r** To apply the changes to the current method, *iii*exercise1.m, click the **Save Method** icon.	
4 Run the qualitative method on the current data file.	• In the Method Editor: Assign Actions on Opening a Data File window, click the **Action** button.	• The Qual method first extracts the product ion chromatograms for each precursor ion in the data file. • Next, it finds the largest peak in the total ion chromatograms, and integrates and extracts peak spectra from under each integrated peak. • See Figure 3 on page 32 on the next page.

Steps	Detailed Instructions	Comments

Figure 3 Results for integration and extraction of peak spectra.

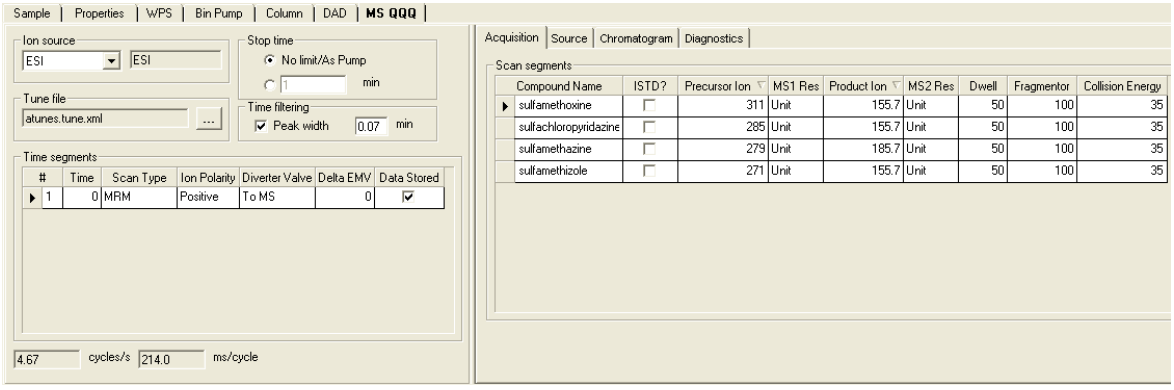
Steps	Detailed Instructions	Comments
5 Run the qualitative method on the remaining product ion data files. • Use either the example files, **Sulfamix PI_xx.d**, or the data files you acquired in step 2.	**a** Select **File > Open Data File.** The system displays the Open Data File dialog box. **b** Hold the **Ctrl** key and do one of these: • Select the two data files **Sulfamix PI_30.d,** and **Sulfamix PI_45.d**. • Select the data files you acquired in step 2. **c** Mark the **Run actions from method** checkbox in the Open Data File dialog box, and click **Open**.	• After the data files open, the Qual method first extracts the product ion chromatograms for each precursor ion. • Next, it finds the largest peak in the total ion chromatograms, and integrates and extracts peak spectra from under each integrated peak. • See the figure in step 6.

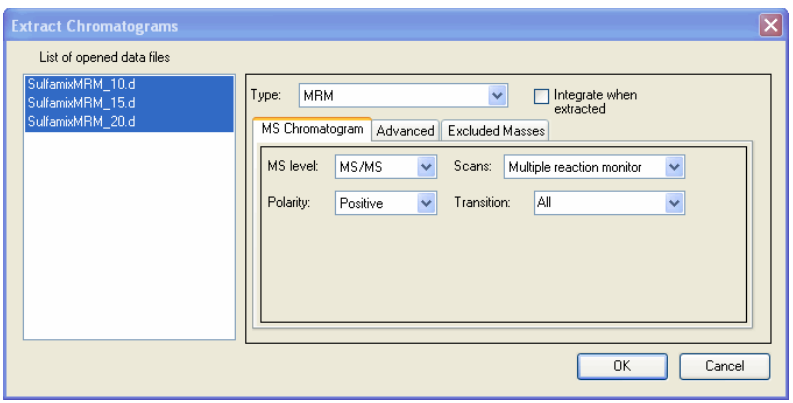
Steps	Detailed Instructions	Comments
6 Identify product ions. • View each set of TICs and spectra individually (e.g., 271 m/z first). • Close the data files.	**a** In the Data Navigator, select the TICs and spectra for the 271 m/z precursor ion. **b** Click the **Show only the highlighted items** icon,  . **c** Examine the spectra to see which fragment ions are produced at which collision energies. **d** Repeat steps a-c until all the product ions are identified.	• The m/z 155.7 product ion is the most abundant of any product ion and the highest signal is recorded at 15 V. This means that a good choice for the MRM for sulfamethizole would be 271.0 > 155.7 when the collision energy is around 15 V.
	e Click the **Close Data File** icon, and click **Close** when the dialog box containing the list of data files pops up.	• The product ions appear to be: Sulfamethizole-271.0 > 155.7 Sulfamethazine-279.0 > 185.8 Sulfachloropyridazine-285.0 > 155.7 Sultadimethozine-311 > 155.7

Task 5. Find optimum collision energy for MRM acquisition

In this task, you set up MRM acquisition methods for the sulfa drugs for different collision energies. By examining the spectra and comparing peak intensities, you determine the optimal collision energy settings for the compounds.

Steps	Detailed Instructions	Comments
1 Set up three MRM acquisition methods. • Use all the MS parameters in the example below except for the collision energy value. • Use collision energies of 10, 15 and 20. • Save methods as ***iii*Sulfamix MRM_*xx*.m**, where ***iii*** are your initials and ***xx*** is the collision energy.	**a** Click the **MS QQQ** tab. **b** Set **Scan Type** to **MRM**. **c** Enter all MS parameters shown in the example below except for the collision energy value. **d** In the collision energy column, type `10` for each compound. **e** Save the method as ***iii*Sulfamix MRM_10.m**. **f** Repeat step d and step e for collision energies of 15, 20, saving the methods as ***iii*Sulfamix MRM_*xx*.m**, where ***iii*** are your initials and ***xx*** is the collision energy.	• Because the largest peaks were produced with a collision energy of 15 in the previous exercise, you will look at only those collision energies to either side of 15.



Steps	Detailed Instructions	Comments
2 Set up and run the worklist (optional). • Specify the data files as ***iii*Sulfamix MRM_*xx*.d**, where ***iii*** are your initials and ***xx*** is the collision energy.	**a** Click the **Worklist** icon if necessary to make sure the worklist is visible. **b** Add three samples to the worklist for collision energies 10, 15, 20. **c** Mark the checkbox to the left of the Sample Name for each of the three samples. **d** Select **Run > Worklist**.	• This step is optional because you can use the six example data files in the next step.
3 Compare the compound transition intensities at different collision energies. • Open all the MRM data files. • Set the MRM chromatogram extraction parameters as shown at right for all transitions. • Disable the TICs for clarity and examine the peak intensities. • Compare the intensities of each compound transition obtained at one collision energy with the same compound transition obtained at another collision energy. (Do this in Overlaid Mode with all the MRM chromatograms.) • Close the data files but don't save results. • Refer to Table 3 on page 38 for optimal method settings for each compound.	**a** Open the **Qualitative Analysis** software. **b** Clear the **Run 'File Open' actions...** check box. **c** Open all the MRM data files in the Qualitative Analysis program. **d** Right-click the Chromatogram Results window, and select **Extract Chromatograms** from the shortcut menu. **e** To select all data files, click the last file while holding down the **Shift** key. **f** Enter the parameters as listed in the example below, and click **OK**. **g** Clear the TIC check boxes to make the MRM chromatograms easier to view. 	• Why a spectrum for MRM? It's a feature of the program to show spectra even for MRM experiments and can be quite handy for comparing relative intensities of product ions generated from the same precursor.

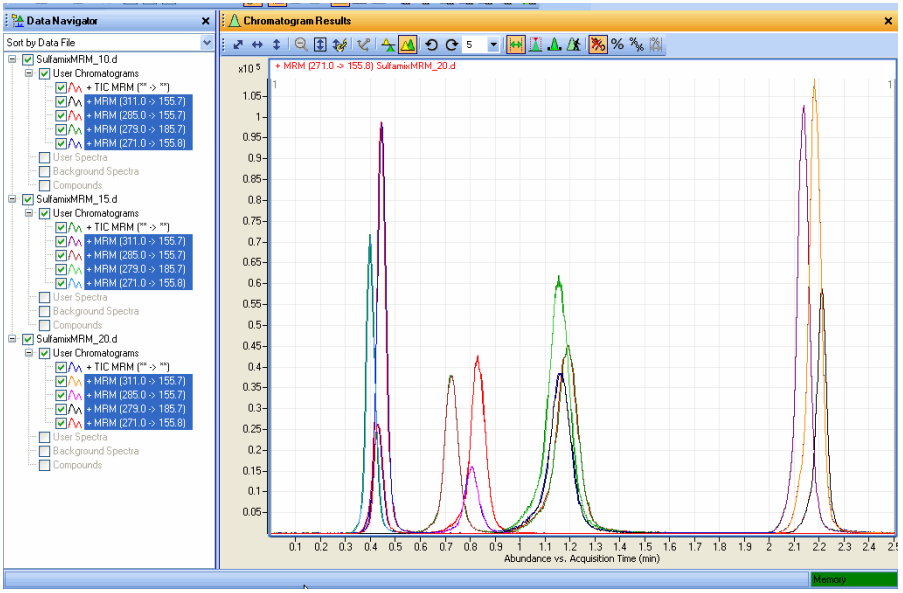
Steps	Detailed Instructions	Comments
	h Click the **Overlaid Mode** icon,  . **i** Compare peak intensities for each compound transition in each data file in the Chromatogram Results window.	• Compare the colors shown in Chromatogram Results with the color next to the MRM transition name in Navigator.
		
	Unless you decide to acquire MRMs at lower collision energies, you should find that the optimal method settings are as shown in Table 3. **j** Click the **Close Data File** icon, and click **Close** when the Close Data File dialog box appears.	• You now have all the information you need to do an MRM acquisition experiment of the sulfa drug mixture. Consider doing at least one more run with those settings.

Table 3

Compound Energy	MRM Transition	Fragmentor	Collision
Sulfamethizole	271.0 > 155.8	100 V	10
Sulfamethazine	279.0 > 185.7	100	15
Sulfachloropyradizine	285.0 > 155.7	100	10
Sulfadimethoxine	311.0 > 155.7	100	20

Exercise 2
Set up and quantitate a batch of acquired 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 software CD 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.
  - Enter the concentration of the highest concentration calibration standard and the dilution pattern.
  - Set up qualifier ions and the calibration curve.
- Automatically 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 **Data** folder of the software CD to a folder on your system.

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 "*iii*" are your initials.	**a** To start the Quantitative Analysis program, click the **Quantitative Analysis** icon on your Desktop. When you first use the program, the default layout appears, as shown in Figure 4 below.	• You can also access the program by selecting **Programs > Agilent > MassHunter Workstation > Quantitative Analysis** from the Start menu.

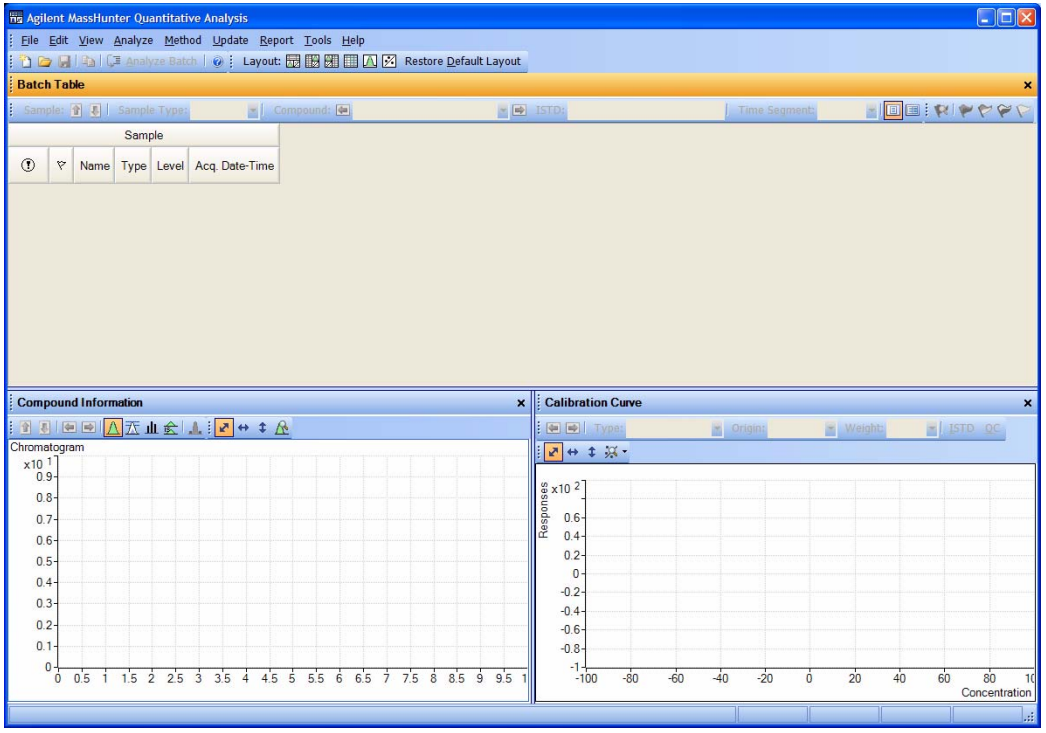
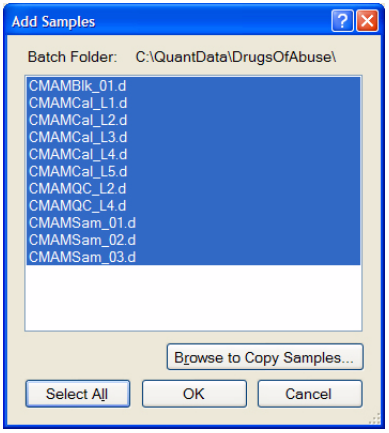


Figure 4 Default layout

Steps	Detailed Instructions	Comments
	b Select **File > New Batch**. The system opens the New Batch dialog box. **c** Navigate to the folder ***Your Directory*\DrugsOfAbuse\.** **d** Enter the batch filename ***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 DrugsOfAbuse folder to the batch.	**a** Select **File > Add Samples**: The system displays the Add Sample dialog box. **b** Click **Select All** to select all samples, and then 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 5 on the next page. 	• 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

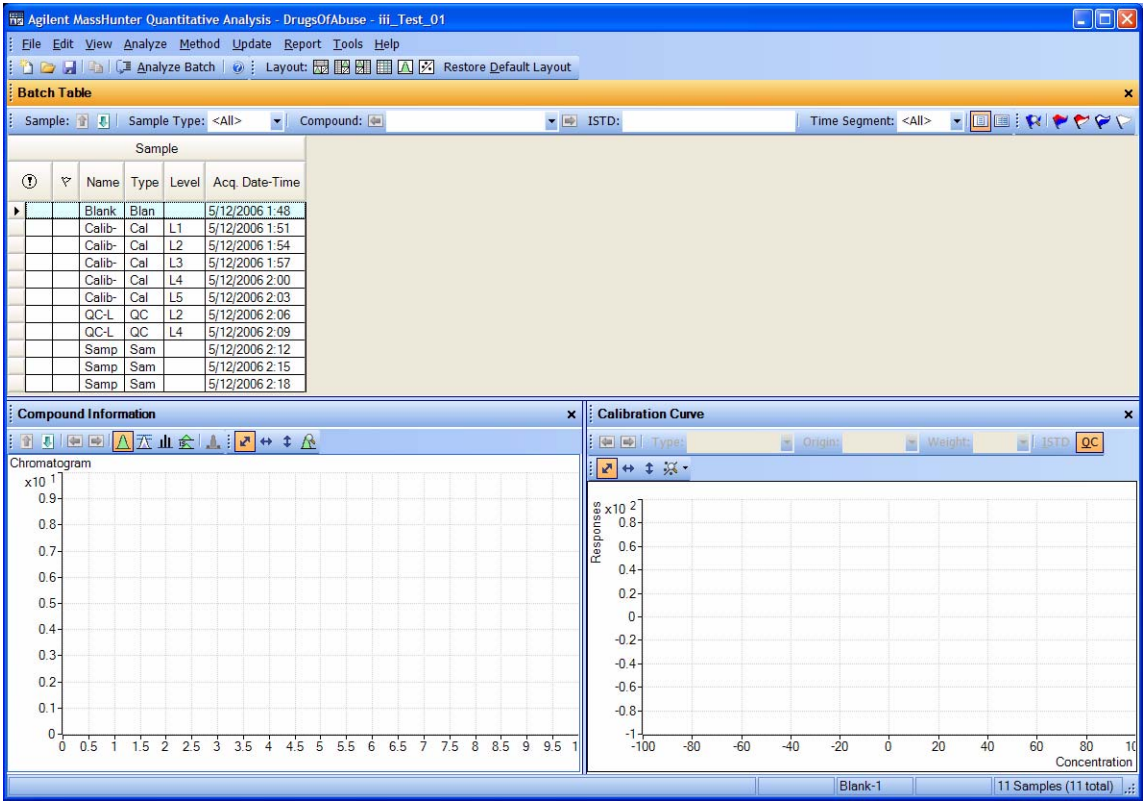
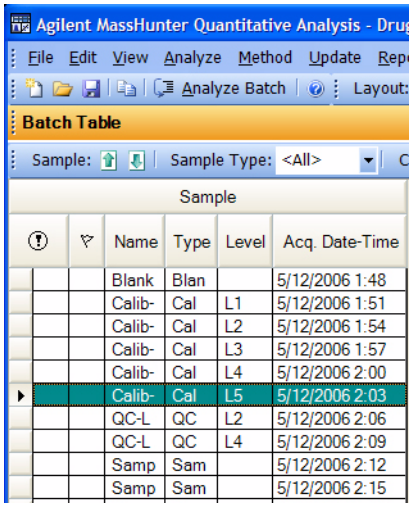
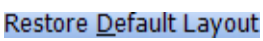


Figure 5 Batch table containing Drugs of Abuse samples before quantitation

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. • Use the calibration data file with the highest signal.	**a** Use the mouse 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.
		
	b Select **Method > Edit** to switch to method editing mode. The Method Edit 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. 

Steps	Detailed Instructions	Comments

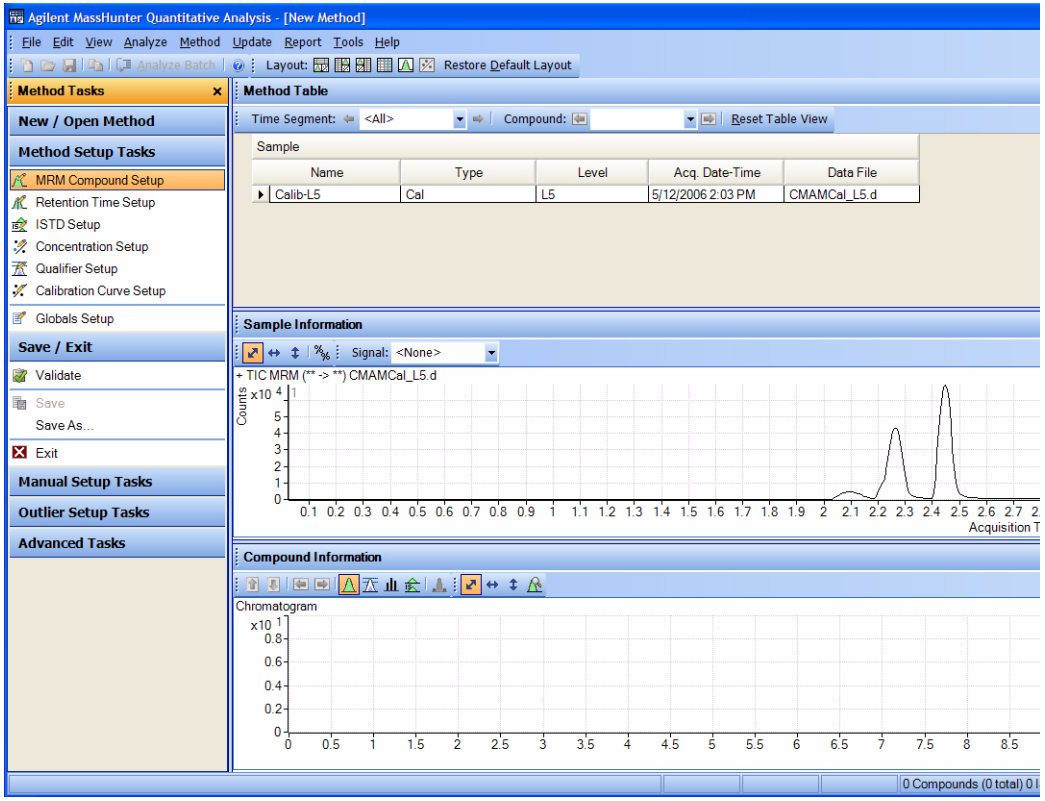
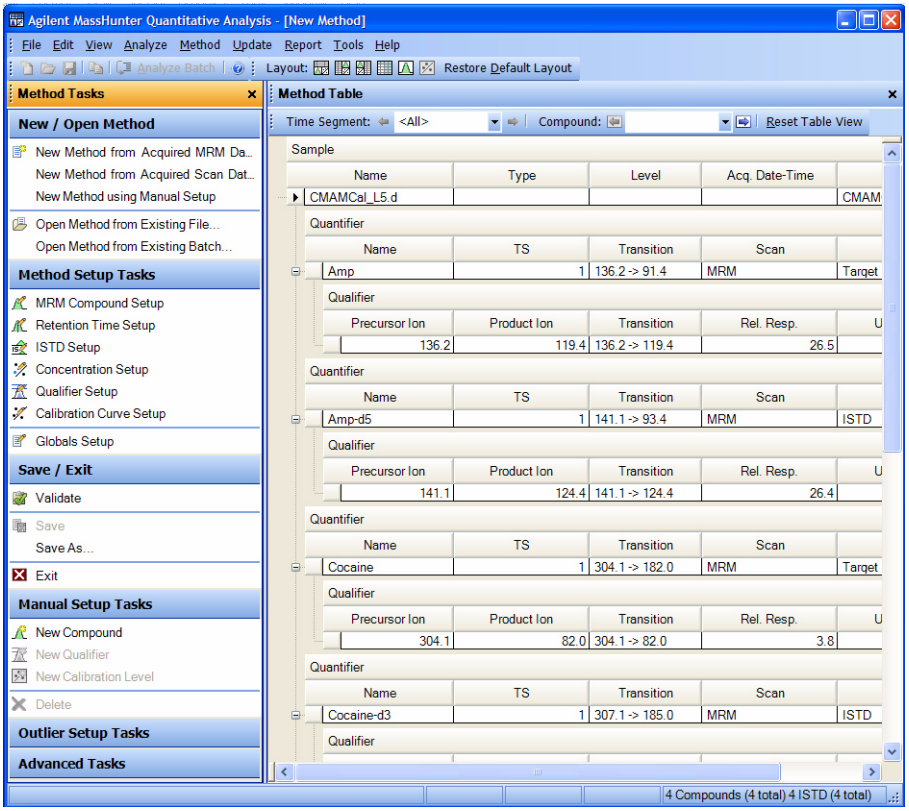


Figure 6 Method Edit mode

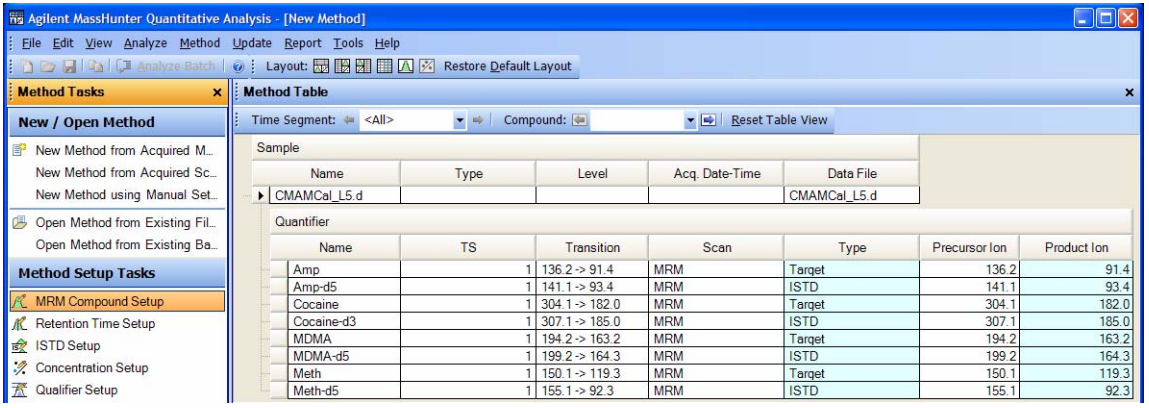
Steps	Detailed Instructions	Comments
	c Under Method Tasks in the sidebar to the left of the View, select **New/Open Method > New Method from Acquired MRM Data**. The system displays a *Please select a sample folder...* dialog box. **d** Select **CMAMCal_L5.d** and click **Open** to import acquisition method information.	• You can also select **Method > New > New Method from Acquired MRM Data.** • The figure below shows the default layout for the level 5 calibration standard.



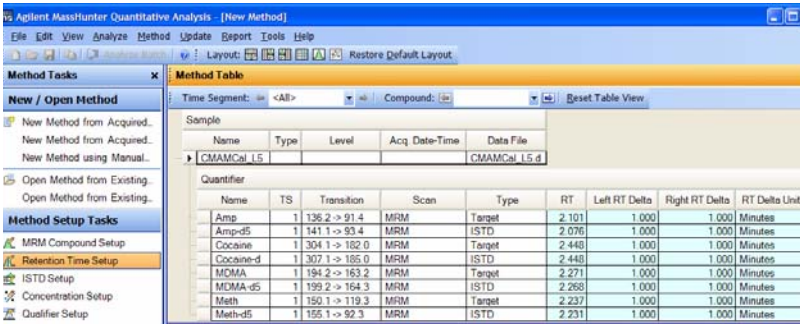
Task 3. Set up target compounds

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 View, 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.



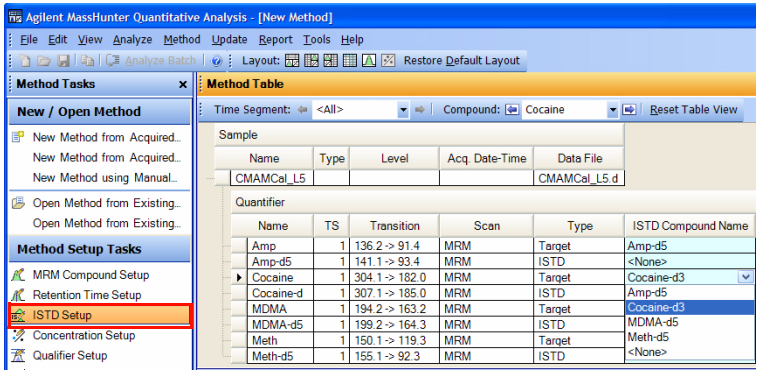
Steps	Detailed Instructions	Comments
	b To inspect the imported retention time data, click **Method Setup Tasks > Retention Time Setup**.	• You can modify data fields in blue for individual compounds.



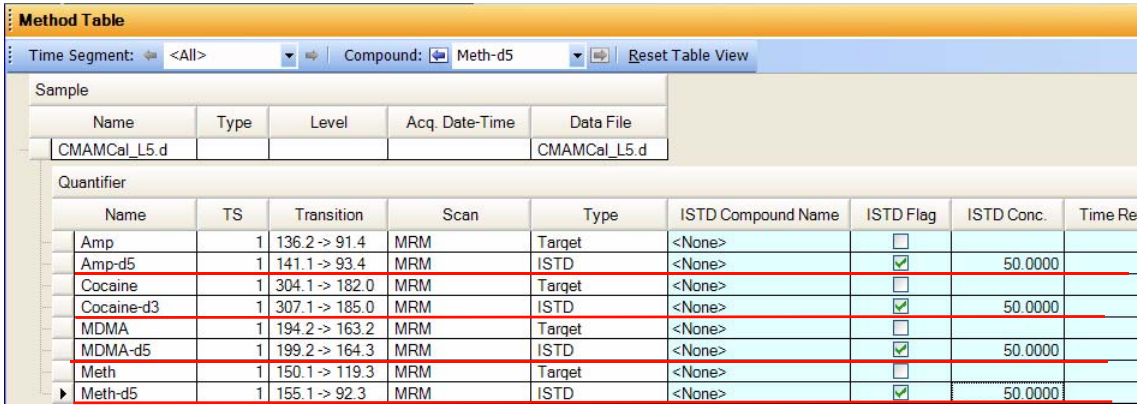
2 Set up ISTD compounds.
- Assign the corresponding deuterated compound as the internal standard (ISTD) for each target compound.

a Select **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 Select the ISTD name associated with the target compound. **d** Enter the ISTD Conc (Concentration) for each ISTD compound.	



Method Table

Time Segment: <All> Compound: Meth-d5 Reset Table View

Sample

Name	Type	Level	Acq. Date-Time	Data File
CMAMCal_L5.d				CMAMCal_L5.d

Quantifier

Name	TS	Transition	Scan	Type	ISTD Compound Name	ISTD Flag	ISTD Conc.	Time Re
Amp	1	136.2 -> 91.4	MRM	Target	<None>	☐		
Amp-d5	1	141.1 -> 93.4	MRM	ISTD	<None>	☑	50.0000	
Cocaine	1	304.1 -> 182.0	MRM	Target	<None>	☐		
Cocaine-d3	1	307.1 -> 185.0	MRM	ISTD	<None>	☑	50.0000	
MDMA	1	194.2 -> 163.2	MRM	Target	<None>	☐		
MDMA-d5	1	199.2 -> 164.3	MRM	ISTD	<None>	☑	50.0000	
Meth	1	150.1 -> 119.3	MRM	Target	<None>	☐		
Meth-d5	1	155.1 -> 92.3	MRM	ISTD	<None>	☑	50.0000	

Task 4. Set up quantitation

This task presents instructions for setting up the quantitation parameters for the method.

- Calibration levels
- Qualifier ions
- Calibration curve fit

Steps	Detailed Instructions	Comments
1 Create five calibration levels for each compound. • Set the highest concentration for amphetamine of 125. • Set a Dilution Pattern of 1:5:2:2.5:2 for amphetamine. • Compare the concentrations for the five levels with the Dilution Pattern.	**a** Select **Method Setup Tasks > Concentration Setup**, and type `125` in the **Dil. High Conc**. column for amphetamine (Amp). **b** Type `1:5:2:2.5:2` in the **Dil. Pattern** column for Amp. **c** Make sure **Level Name Prefix** is **L** and **# of Levels** is **5** in the **Serial Dilution** toolbar.	

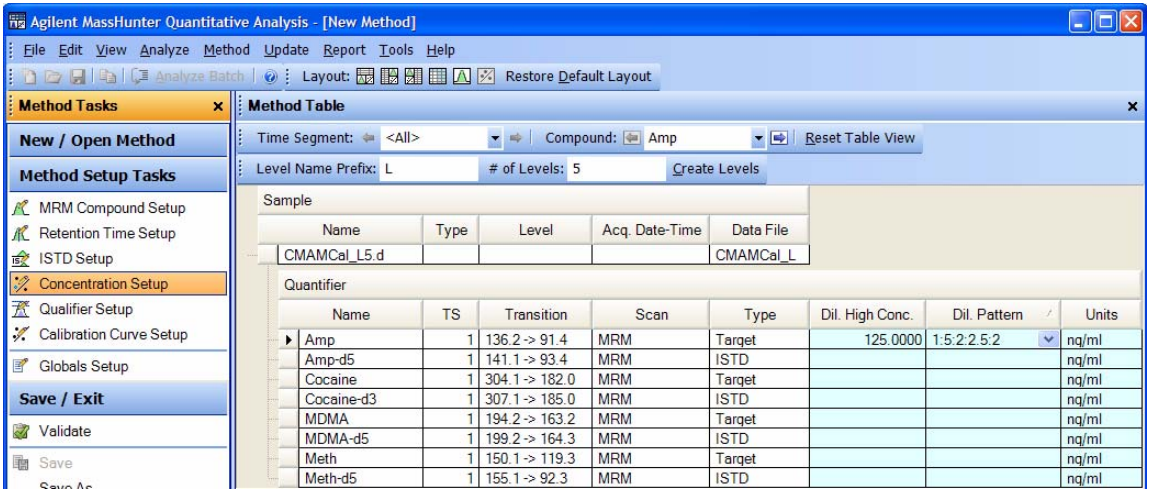
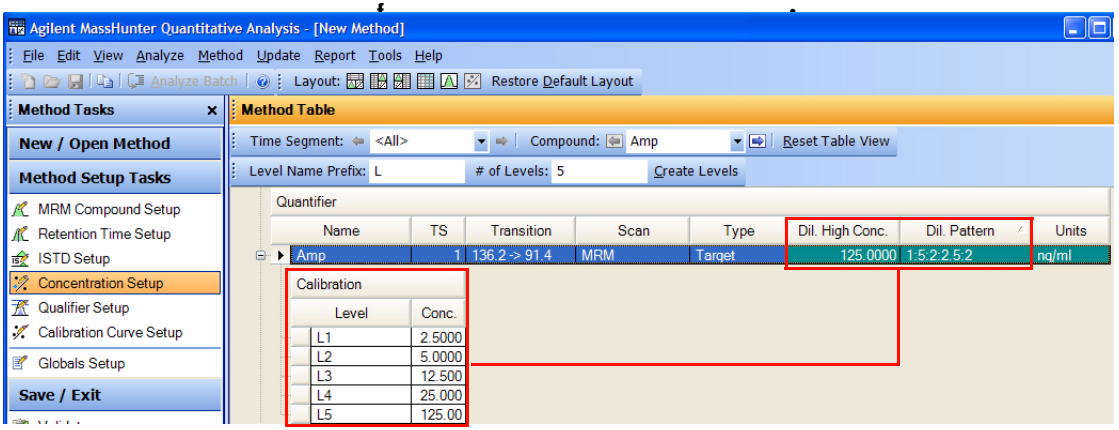


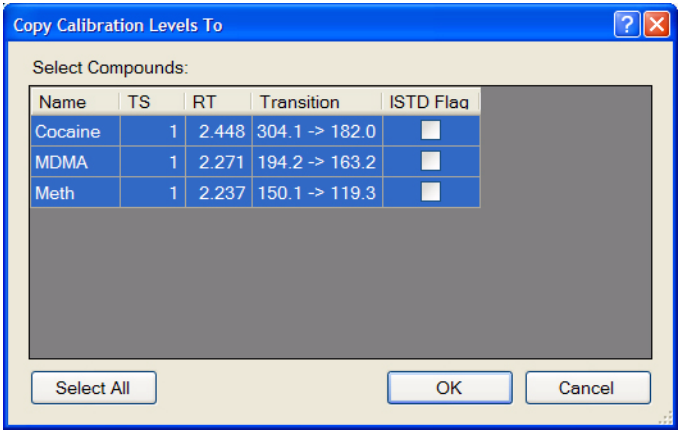
Figure 7 Creating five calibration levels for first compound

Steps	Detailed Instructions	Comments
	d Click **Create Levels**. **e** Compare the newly created calibration levels with Dilution High Concentration and Dilution Pattern.	• After you create the calibration table for amphetamine, you tell the program to copy this table to the other target compounds in step 2.

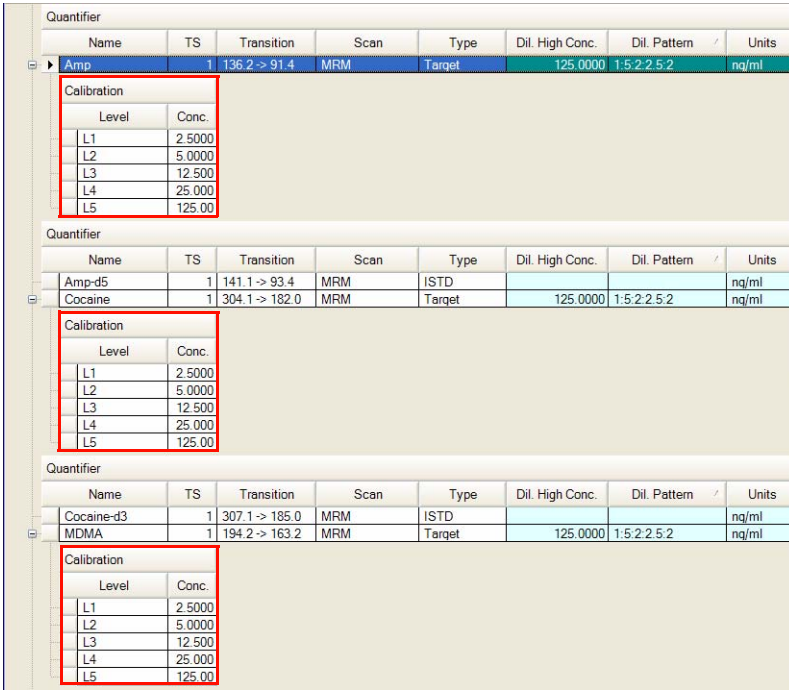


2 Copy the calibration levels and concentrations to the other compounds.
- Close the Compound Information window.
- Compare the calibration setup for the four compounds.

a Select **Method > Copy Calibration Levels To...**
The system displays the Copy Calibration Levels dialog box.
b Click **Select All**, and then click **OK**.



Steps	Detailed Instructions	Comments
	c Close the **Compound Information** window and the Sample Information window in the lower half of the Quantitative Data Analysis main view. **d** Browse the Method Table to compare the calibration concentration setup among the four target compounds, Amp, Cocaine, Meth and MDMA	



Quantifier

Name	TS	Transition	Scan	Type	Dil. High Conc.	Dil. Pattern	Units
Amp	1	136.2 -> 91.4	MRM	Target	125.0000	1:5:2:2.5:2	ng/ml

Calibration

Level	Conc.
L1	2.5000
L2	5.0000
L3	12.500
L4	25.000
L5	125.00

Quantifier

Name	TS	Transition	Scan	Type	Dil. High Conc.	Dil. Pattern	Units
Amp-d5	1	141.1 -> 93.4	MRM	ISTD			ng/ml
Cocaine	1	304.1 -> 182.0	MRM	Target	125.0000	1:5:2:2.5:2	ng/ml

Calibration

Level	Conc.
L1	2.5000
L2	5.0000
L3	12.500
L4	25.000
L5	125.00

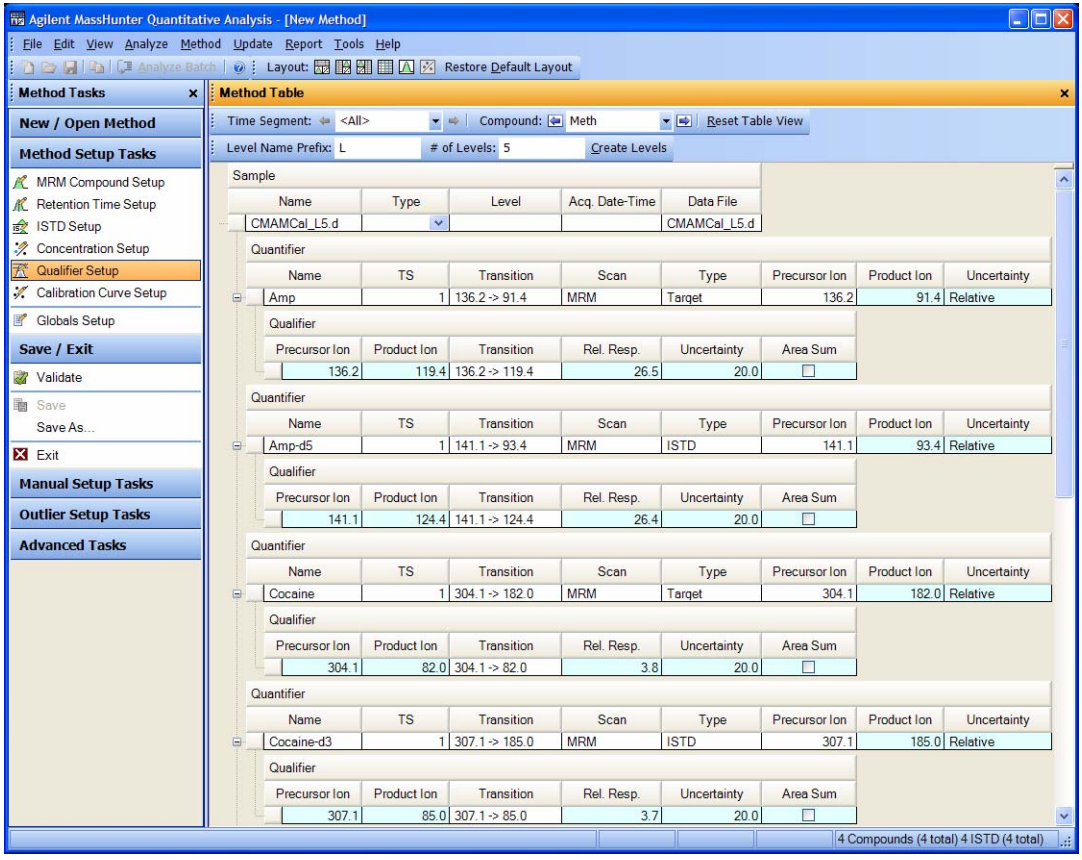
Quantifier

Name	TS	Transition	Scan	Type	Dil. High Conc.	Dil. Pattern	Units
Cocaine-d3	1	307.1 -> 185.0	MRM	ISTD			ng/ml
MDMA	1	194.2 -> 163.2	MRM	Target	125.0000	1:5:2:2.5:2	ng/ml

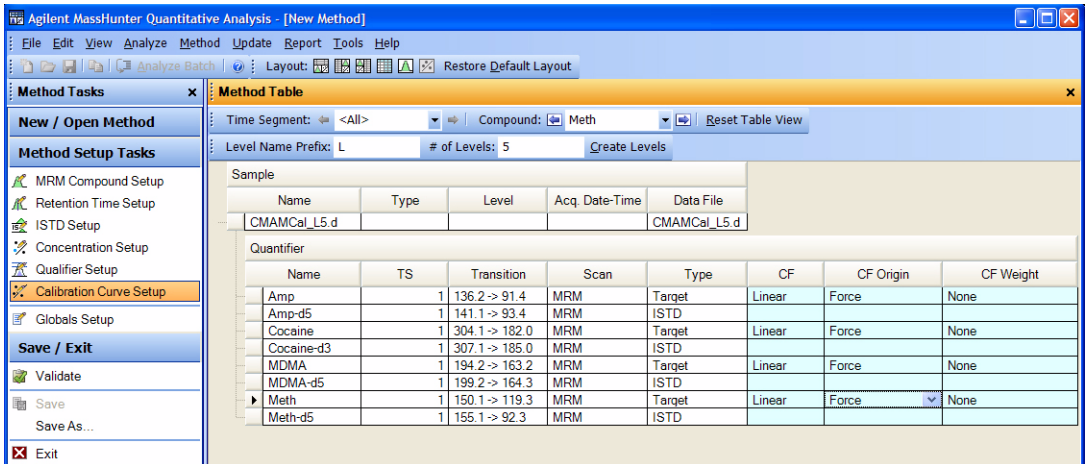
Calibration

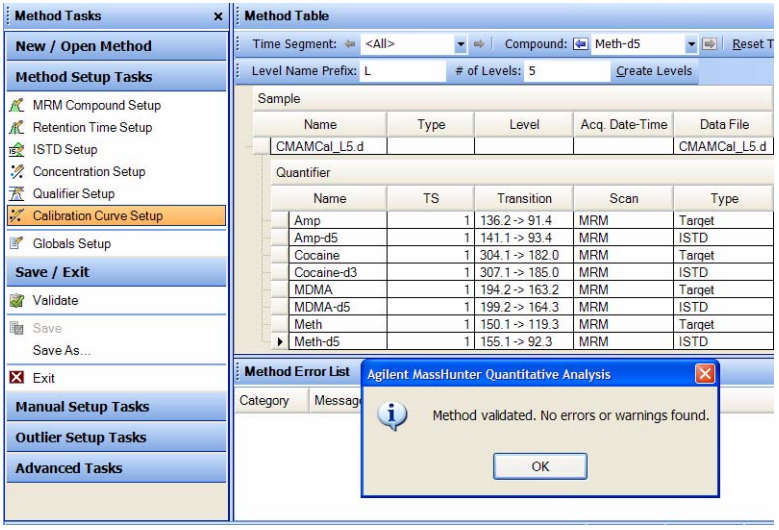
Level	Conc.
L1	2.5000
L2	5.0000
L3	12.500
L4	25.000
L5	125.00

Steps	Detailed Instructions	Comments
3 Set up qualifier ions and a calibration curve. • Review the Qualifier setup parameters. • Change the default curve origin from Linear to Force.	**a** Select **Method Tasks > 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 Select **Method Tasks > Calibration Curve Setup**, **c** For each target compound change the **CF Origin** to **Force**.	

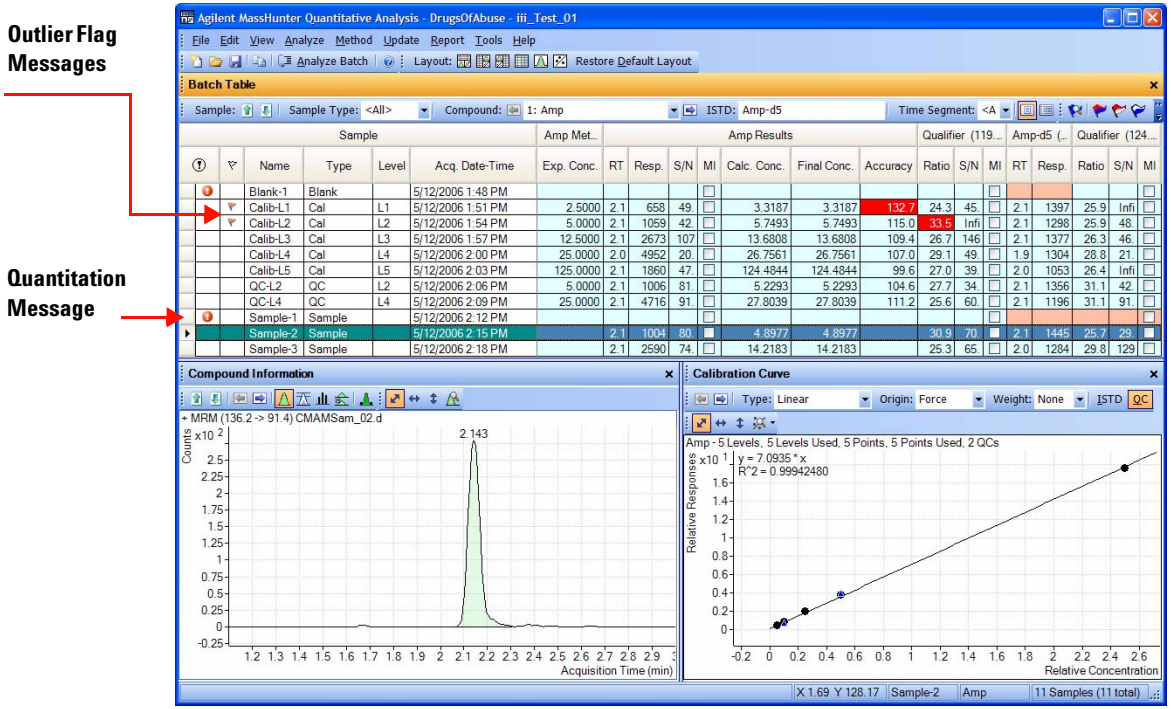


Steps	Detailed Instructions	Comments
4 Validate and save the method.	**a** Select **Save/Exit > Validate** to validate the method setup.	• You can view any validation errors that do occur at the bottom of the screen.
		
	b After the validation message appears, click **OK.**	
	c Select **Save/Exit > Exit**, 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. • Examine the outlier flag messages.	**a** Click the **Analyze Batch** icon (Analyze Batch) in the toolbar to start batch analysis. **b** Pass the mouse cursor over the quantitation message for Sample 1. **c** Pass the mouse cursor over the flags for the first 2 calibration standards.	• Note that the program found no data for Amphetamine (Amp) in Sample-1. • Note that two calibration standards contain outlier data.



2 Save the batch.	**a** Select **File > Save Batch**. **b** Select **File > Close** 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.

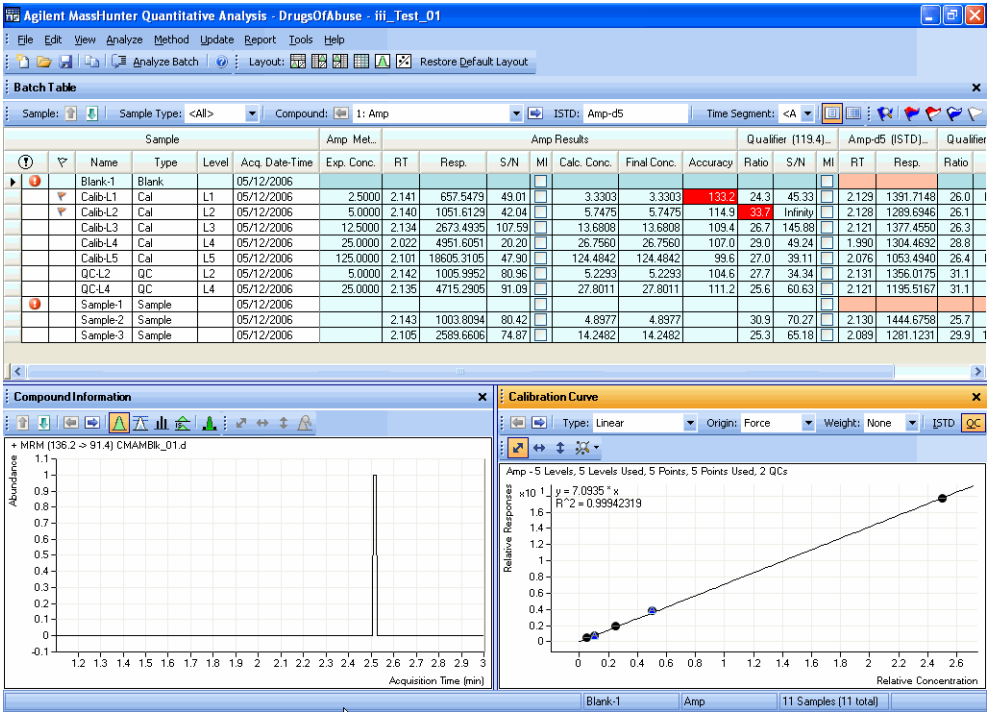
Each exercise is presented in a table with three columns:

- 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.

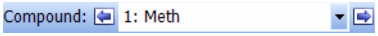
Task 1. Navigate the Batch Table results

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

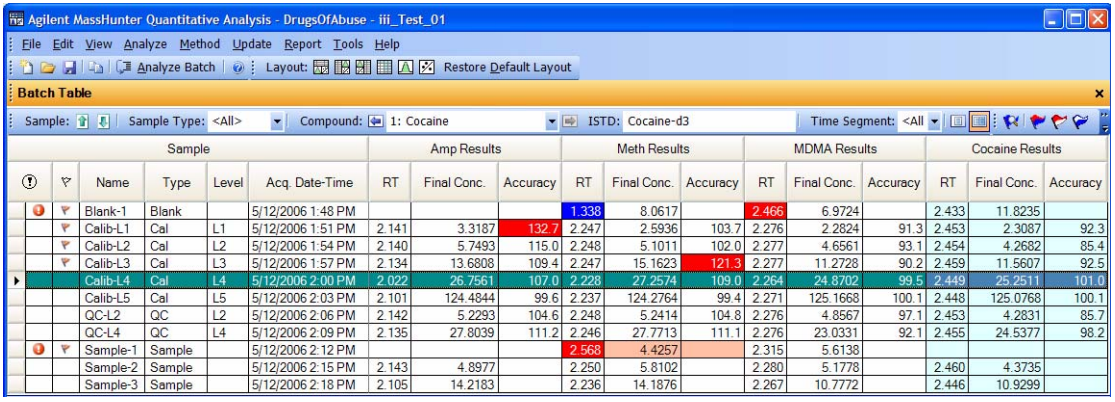
Steps	Detailed Instructions	Comments
1 Open the batch file ***iii*_Test_01.batch.xml**., created in Exercise 2.	**a** To start the Quantitative Analysis program, click the **Quantitative Analysis** icon on your Desktop . **b** Click **Open Batch** on the toolbar to display the Open Batch dialog box. **c** Navigate to \ ***Your Directory*\ DrugsOfAbuse** and select ***iii*_Test_01.batch.xml.**	• 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 than those you see in the figure above, 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. Restore Default Layout • To restore the default column settings, right-click anywhere in the Batch Table window and select **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 63.
3 Scroll from sample to sample until you reach the end of the Batch Table, and then return to Cal-L5. • Use the Next Sample and Previous Sample arrows on the toolbar  . • Note the changes in the Batch Table and Compound Information of amphetamine for each sample. • Select sample Calib L4 in the Batch Table to view the Batch Table and Compound Information changes.	**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.

Steps	Detailed Instructions	Comments
4 Scroll from compound to compound through all four compounds. • Use the Next Compound and Previous Compound arrows on the toolbar.  • Review the differences in the Batch Table, Compound Information and Calibration Curve windows between the compounds. • Select Cocaine from the list.	**a** Click the **Next Compound** or **Previous compound** arrow in the toolbar until the system displays the desired compound. **b** Inspect the changes in Batch Table, Compound Information and Calibration Curve windows. **c** Click the down arrow next to the Compound list. **d** Select **Cocaine**.	

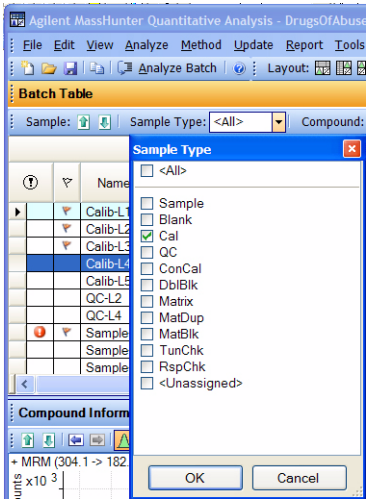
Steps	Detailed Instructions	Comments
5 Examine results for multiple compounds. • View the RT for each compound for the Cal-L4 sample. • After reviewing the results for all the compounds, return to viewing the cocaine results.	**a** Click the **Multiple Compound View** icon in the toolbar to display the quantitation results for all target compounds. You can also the **View > Batch Table Layout > Multiple Compound View** menu item. **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 Compound View mode versus Single Compound View mode. If you add a column to the table when you are Multiple Compound View mode, that change is not automatically made in the Single Compound View mode.



Sample						Amp Results			Meth Results			MDMA Results			Cocaine Results		
		Name	Type	Level	Acq. Date-Time	RT	Final Conc.	Accuracy	RT	Final Conc.	Accuracy	RT	Final Conc.	Accuracy	RT	Final Conc.	Accuracy
		Blank-1	Blank		5/12/2006 1:48 PM				1.338	8.0617		2.466	6.9724		2.433	11.8235	
		Calib-L1	Cal	L1	5/12/2006 1:51 PM	2.141	3.3187	132.7	2.247	2.5936	103.7	2.276	2.2824	91.3	2.453	2.3087	92.3
		Calib-L2	Cal	L2	5/12/2006 1:54 PM	2.140	5.7493	115.0	2.248	5.1011	102.0	2.277	4.6561	93.1	2.454	4.2682	85.4
		Calib-L3	Cal	L3	5/12/2006 1:57 PM	2.134	13.6808	109.4	2.247	15.1623	121.3	2.277	11.2728	90.2	2.459	11.5607	92.5
▸		Calib-L4	Cal	L4	5/12/2006 2:00 PM	2.022	26.7561	107.0	2.228	27.2574	109.0	2.264	24.8702	99.5	2.449	25.2511	101.0
		Calib-L5	Cal	L5	5/12/2006 2:03 PM	2.101	124.4844	99.6	2.237	124.2764	99.4	2.271	125.1668	100.1	2.448	125.0768	100.1
		QC-L2	QC	L2	5/12/2006 2:06 PM	2.142	5.2293	104.6	2.248	5.2414	104.8	2.276	4.8567	97.1	2.453	4.2831	85.7
		QC-L4	QC	L4	5/12/2006 2:09 PM	2.135	27.8039	111.2	2.246	27.7713	111.1	2.276	23.0331	92.1	2.455	24.5377	98.2
		Sample-1	Sample		5/12/2006 2:12 PM				2.568	4.4257		2.315	5.6138				
		Sample-2	Sample		5/12/2006 2:15 PM	2.143	4.8977		2.250	5.8102		2.280	5.1778		2.460	4.3735	
		Sample-3	Sample		5/12/2006 2:18 PM	2.105	14.2183		2.236	14.1876		2.267	10.7772		2.446	10.9299	

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

d If necessary, click the down arrow next to the Compound list, and select **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** dropdown 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** dropdown list. **e** Click **<All>**, 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. • The default layout is called Table Top because the Batch table is at the top of the main view. • Change the layout to Table Left, then to Table Right. • Return to the Table Top layout.	**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: • Table • Compound Information • Calibration Curve • Return to the default layout.	**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. • Show qualifiers. • Show spectra. • Show ISTD chromatogram, qualifiers and spectra.	**a** In the Batch Table, select the **Cal-L4** row. **a** In the Compound Information toolbar, click the **Show/Hide Qualifiers** icon  . **b** Click the **Show/Hide Spectrum** icon  . **c** Click the **Show/Hide ISTD** icon  . The layout and results look like those in the figure on the next page.	• 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

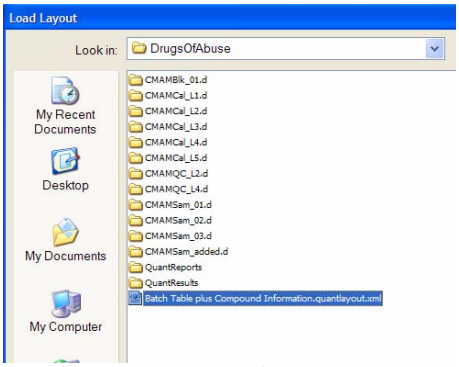
4 Save the default layout without the calibration curve.

- Save the new layout as **Batch Table plus Compound Information** in the **DrugsOfAbuse** folder.

a Close the **Calibration Curve** window.

b Select **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. • Restore the default layout. • Load the layout **Batch Table plus Compound Information**.	**a** Click **Restore Default Layout** on the toolbar. **b** Select **View > Window Layout > Load Layout**. The system displays the Load Layout dialog box.  **c** Select **Batch Table plus Compound Information** and click **Open**. The results window should now look like Figure 8 on the next page	

Steps	Detailed Instructions	Comments

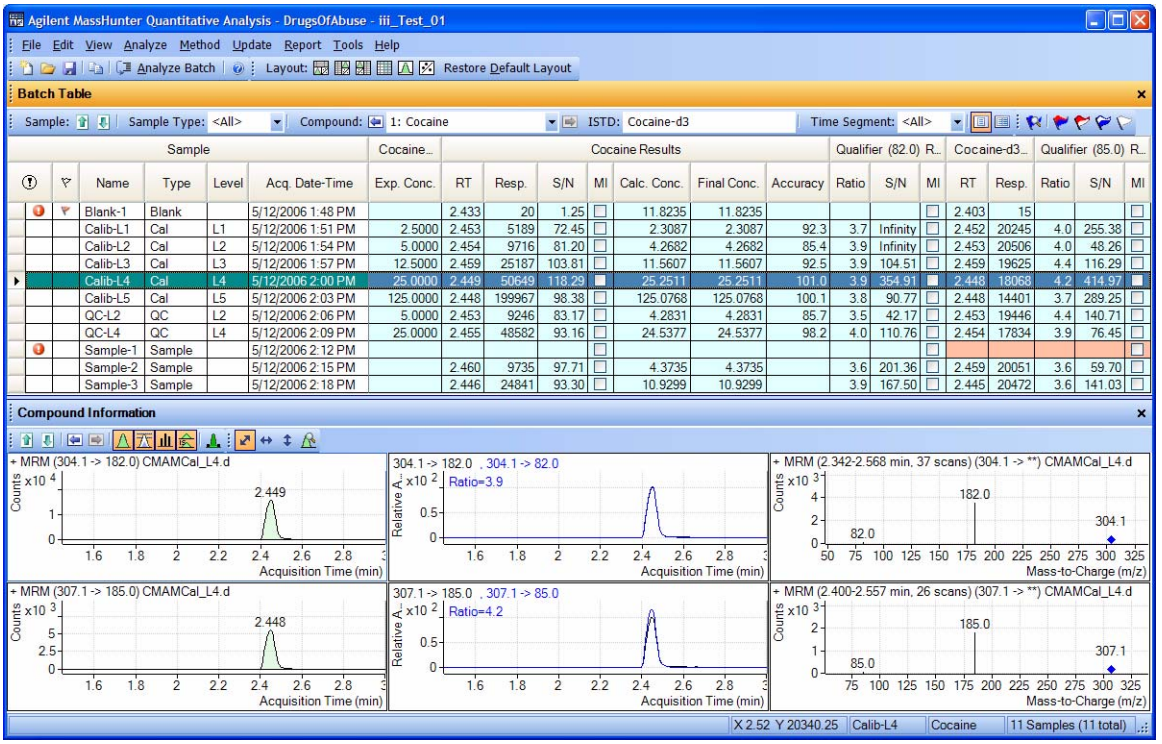
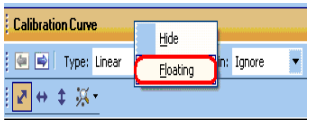


Figure 8 Results window

Steps	Detailed Instructions	Comments
6 Create the layout as shown in Figure 9 on page 67, with the calibration curve and compound information windows floating. Hint: More than the Batch Table is on the left.	**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 9.

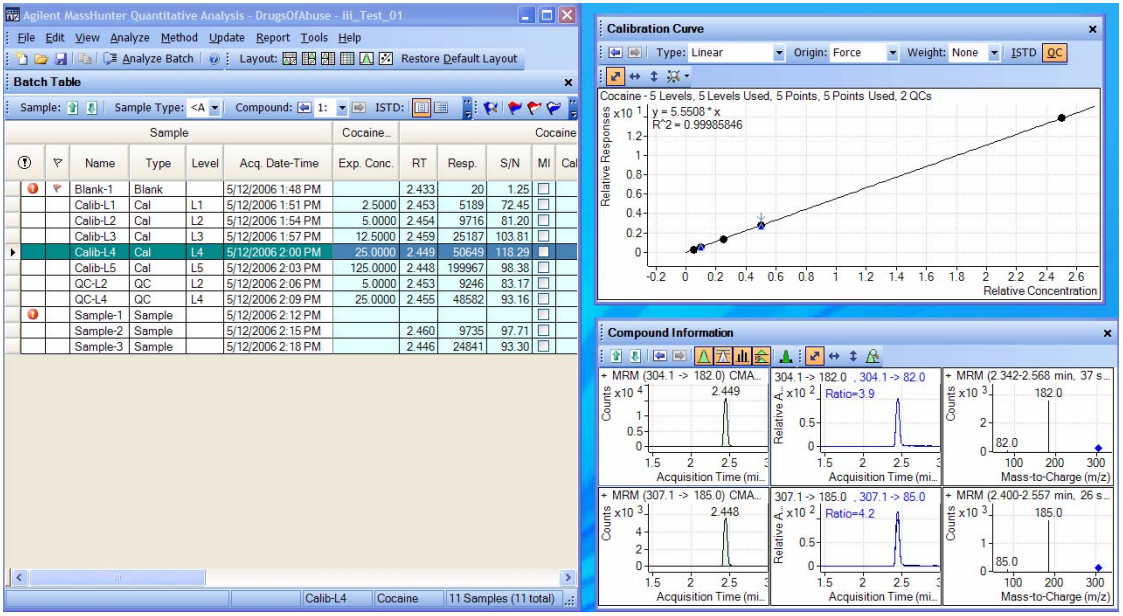


Figure 9 Display with Calibration Curve and Compound Information windows floating

Steps	Detailed Instructions	Comments
	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 10.	

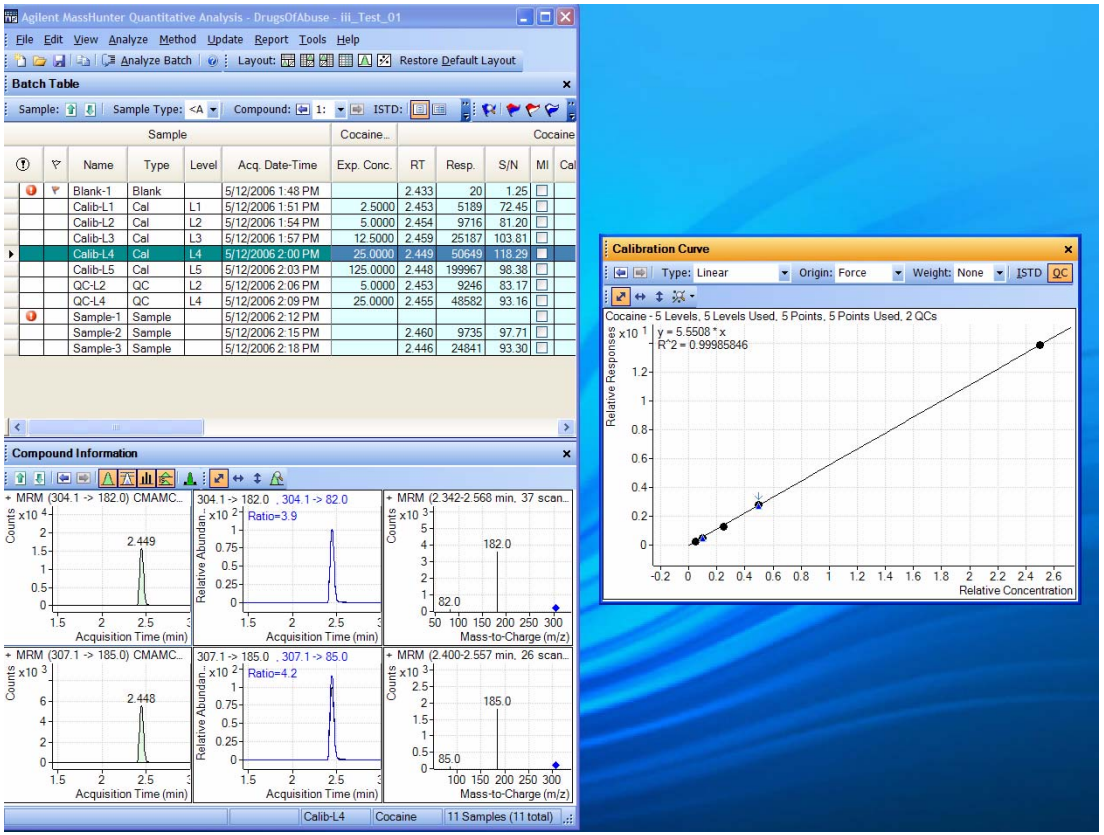


Figure 10 Resized window

Steps	Detailed Instructions	Comments
	g Right-click inside the title bar of the Calibration Curve window, and clear the **Floating** checkbox. **h** Move the Compound Information window so that the layout corresponds to the one pictured at the start of the task.	
7 Recreate (do not restore) the default layout. • In this step you learn to recreate layouts without using the layout icons or **Restore Default Layout**.	**a** Maximize the program main View.	• You must 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, you can right-click the title bar of the Calibration Curve window and drag it to the right. A gray rectangle is drawn that 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**. • Specify My Documents as the destination directory. • Use ***iii*_Test_01.xls** as the export file name, where "*iii*" are your initials.	**a** To make the Batch Table window active, click the title bar of the **Batch Table** window. **b** Select **File > Export > Export Table**. **c** Select **My Documents** as the destination directory. **d** Enter ***iii*_Test_01.xls** as the export file name. **e** Click **Save**.	

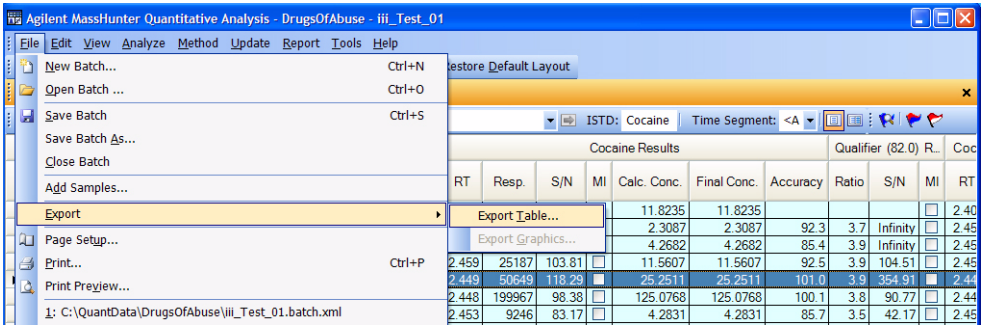
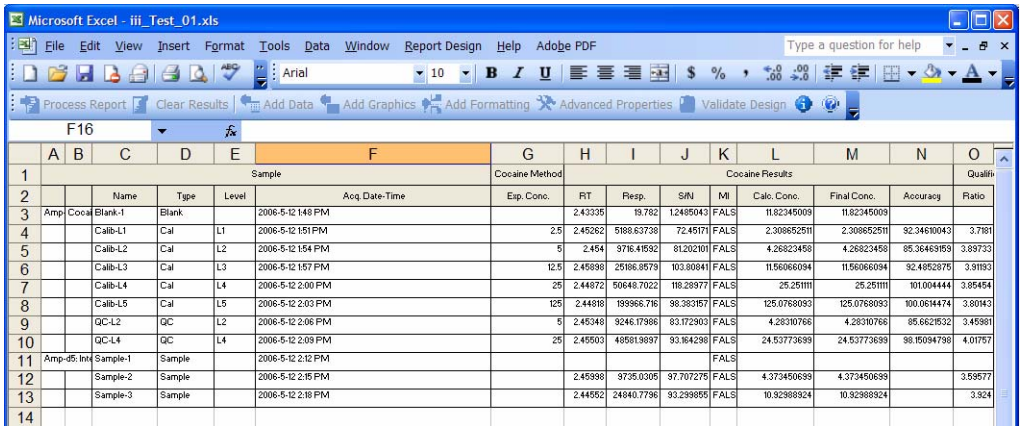


Figure 11 Export results

2 View the batch results as they appear in Excel; then exit Excel. • Note what is exported and what is not.	**a** Start Microsoft Excel. **b** Open **My Documents*iii*_Test_01.xls.** **c** Note what is exported and what is not. **d** 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
1	Sample						Cocaine Method	Cocaine Results							Qualifi
2			Name	Type	Level	Acq. Date-Time	Exp. Conc.	RT	Resp.	S/N	MI	Calc. Conc.	Final Conc.	Accuracy	Ratio
3	Amp	Coca	Blank-1	Blank		2006-5-12 1:48 PM		2.43335	19.782	1.2485043	FALS	11.82345009	11.82345009		
4			Calib-L1	Cal	L1	2006-5-12 1:51 PM	2.5	2.45262	5188.63738	72.45171	FALS	2.308852511	2.308852511	92.34610043	3.7181
5			Calib-L2	Cal	L2	2006-5-12 1:54 PM	5	2.454	9716.41592	81.202101	FALS	4.26823458	4.26823458	85.36469159	3.89733
6			Calib-L3	Cal	L3	2006-5-12 1:57 PM	12.5	2.45898	25186.8579	103.80841	FALS	11.56066094	11.56066094	92.4852875	3.91193
7			Calib-L4	Cal	L4	2006-5-12 2:00 PM	25	2.44872	50648.7022	118.28977	FALS	25.251111	25.251111	101.004444	3.85454
8			Calib-L5	Cal	L5	2006-5-12 2:03 PM	125	2.44818	199366.716	98.383157	FALS	125.0768093	125.0768093	100.0614474	3.80143
9			QC-L2	QC	L2	2006-5-12 2:06 PM	5	2.45348	9246.17986	83.172903	FALS	4.28310766	4.28310766	85.6621532	3.45981
10			QC-L4	QC	L4	2006-5-12 2:09 PM	25	2.45503	48581.9897	93.164298	FALS	24.53773699	24.53773699	98.15094798	4.01757
11	Amp-d5: Int		Sample-1	Sample		2006-5-12 2:12 PM					FALS				
12			Sample-2	Sample		2006-5-12 2:15 PM		2.45998	9735.0305	97.707275	FALS	4.373450699	4.373450699		3.59577
13			Sample-3	Sample		2006-5-12 2:18 PM		2.44552	24840.7796	93.299855	FALS	10.92988924	10.92988924		3.924
14															

Figure 12 Batch table in Excel

3 Preview printouts for Batch Table and Compound Information data.

- Print the Batch Table and Compound Information.
- Save and exit the batch if you are not going to perform Exercise 4 right away.

a Click inside the title bar of the Batch Table window, and select **File > Print Preview**.

b Inspect the display of the Batch Table in the **Print Preview** window to make sure it looks the way you want it.

c Close the **Print Preview** window.

d When the Batch Table is satisfactory, select **File > Print**.

e Repeat steps a-d for the Compound Information.

f If you are not moving on to Exercise 4, select **File > Save Batch**.

g Select **File > Exit**.

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

Exercise 4
Use three new tools to evaluate results

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

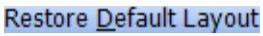
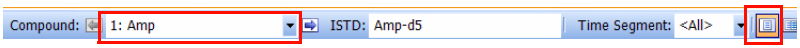
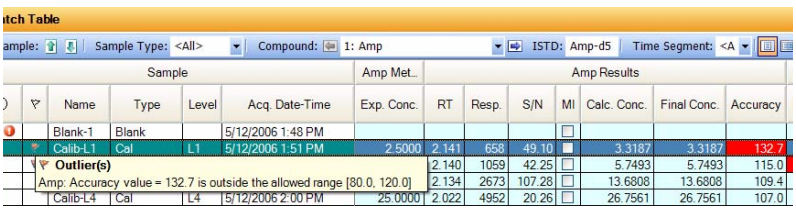
- Curvefit Assistant, which calculates all combinations of curves and presents results with an equation and confidence band
- Parameter-less integrator so you don't have to figure out the parameters to change to improve the integration
- Outlier messages to help you easily detect result values that are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

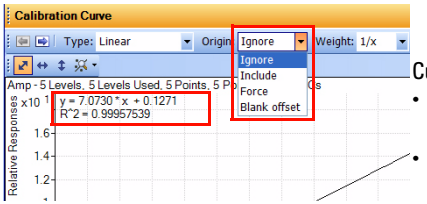
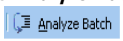
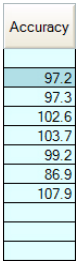
Each exercise is presented in a table with three columns:

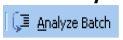
- Steps – Use these general instructions to proceed on your own to explore the program.
- Detailed Instructions – Use these if you need help or prefer to use a step-by-step learning process.
- Comments – Read these to learn tips and additional information about each step in the exercise.

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 re-analyze the batch.

Steps	Detailed Instructions	Comments
1 If necessary, open the batch file ***iii*_Test_01.batch.xml**. If the batch is already open, skip to step 2.	**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 select ***iii*_Test_01.batch.xml.**	• You can also access the program by selecting **Programs > Agilent > MassHunter Workstation > Quantitative Analysis** from the Start menu**.** • If the default layout is not present, click **Restore Default Layout** on the toolbar before opening the batch. 
2 Find the accuracy outlier for amphetamine, and change the curve fit. • Set **Origin** to **Ignore**, and **Weight** to **1/y**.	**a** Make sure the Batch Table is set to single compound display mode, and the displayed target compound is Amp. See circled 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 R2 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  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.

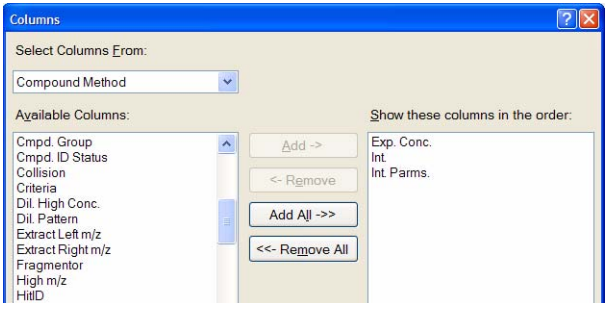
Steps	Detailed Instructions	Comments
5 Change the curve fit for methamphetamine, and analyze the batch.	**a** In the Calibration Curve Fit window, set **Origin** to **Ignore**, and **Weight** to **1/y**. MassHunter displays a revised curve fit formula and R2 value. **b** Click **Analyze Batch** in the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

Task 2. Integrate without parameters

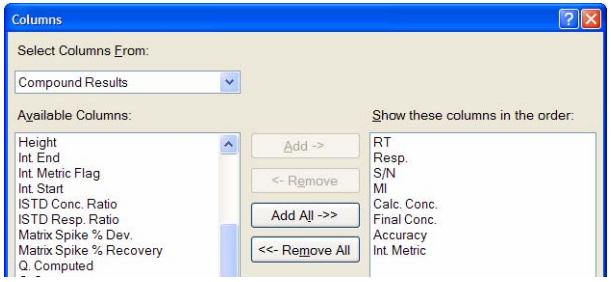
This section shows you how to inspect data for proper integration. You learn how to perform the following tasks.

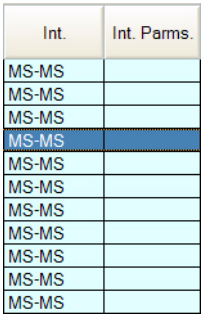
- Add integration columns to the Batch Table
- View default integration values
- Closely examine the chromatogram, looking for such details as:
  - outlier messages
  - baseline parameters
  - peak labels

Steps	Detailed Instructions	Comments
1 Add integration columns to the Batch Table. • Add the Integrator Type and Integrator Parameters columns from the Compound Method list. • Add the Integrator Metric column to the Batch Table from the Compound Results list.	**a** Right-click anywhere in the Batch Table, and select **Add/Remove Columns**. The system displays the Columns dialog box. **b** Select **Compound Method** from the **Select Columns From** dropdown list. **c** Select **Int.** (Integrator Type) and **Int. Parms.** (Integrator Parameters) from the **Available Columns** list, 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 Select **Compound Results** from the **Select Columns From** dropdown list. **e** Select **Int. Metric** (Integrator Metric) from the **Available Columns** list, and click **Add**. The system moves the selected column to the **Show these columns in the order** list. **f** Click **OK**.	
2 View the default integration values for amphetamine. • View the Int. type and Int. Parms. columns • View the Int. Metric column.	**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 default integrator used is the MS-MS integrator, which does not need you to enter parameters. That is why the Int. Parms column is blank.

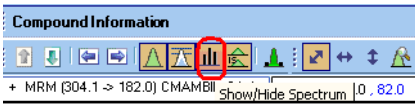


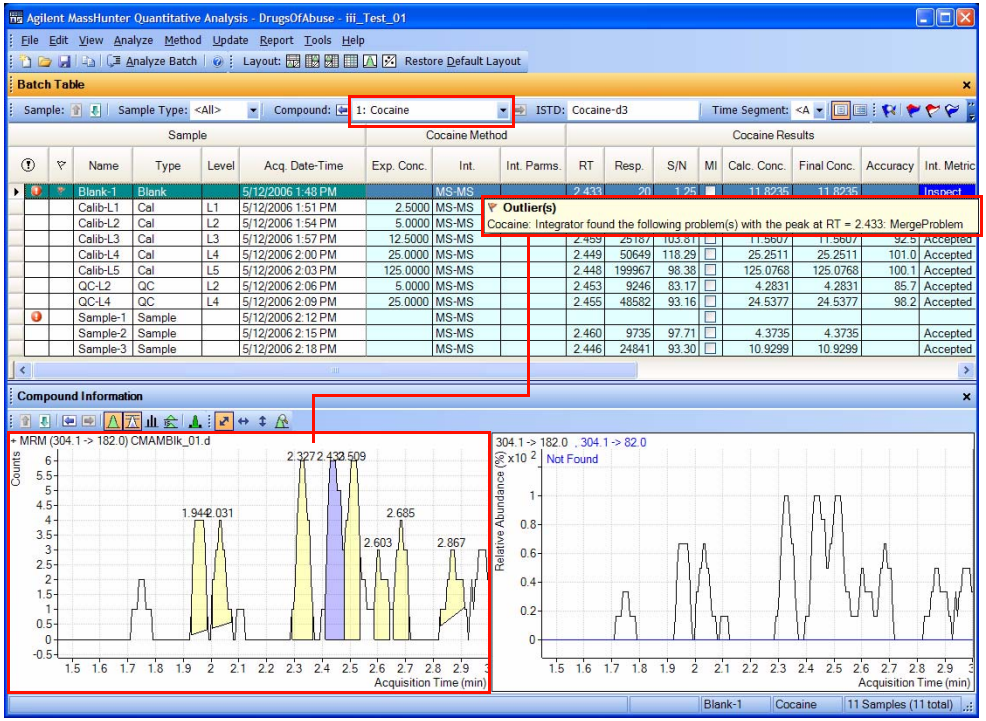


Steps	Detailed Instructions	Comments
	c Examine the default values in the Int. Metric column in the Batch Table.	• These values reflect the default integration quality metric used for the target compound Amp .

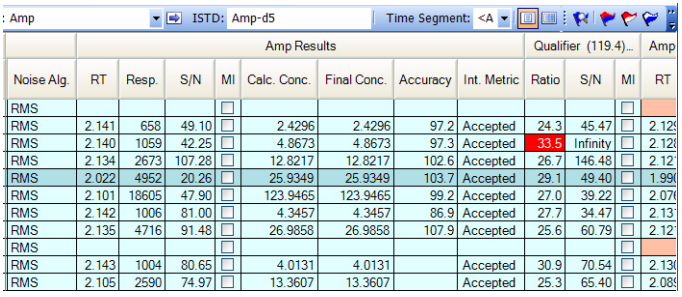
1: Amp ISTD: Amp-d5 Time Segment: <All>

Amp Method			Amp Results							
Sample Exp. Conc.	Int.	Int. Parms.	RT	Resp.	S/N	MI	Calc. Conc.	Final Conc.	Accuracy	Int. Metric
	MS-MS					☐				
2.5000	MS-MS		2.141	658	49.10	☐	2.4296	2.4296	97.2	Accepted
5.0000	MS-MS		2.140	1059	42.25	☐	4.8673	4.8673	97.3	Accepted
12.5000	MS-MS		2.134	2673	107.28	☐	12.8217	12.8217	102.6	Accepted
25.0000	MS-MS		2.022	4952	20.26	☐	25.9349	25.9349	103.7	Accepted
125.0000	MS-MS		2.101	18605	47.90	☐	123.9465	123.9465	99.2	Accepted
5.0000	MS-MS		2.142	1006	81.00	☐	4.3457	4.3457	86.9	Accepted
25.0000	MS-MS		2.135	4716	91.48	☐	26.9858	26.9858	107.9	Accepted
	MS-MS					☐				
	MS-MS		2.143	1004	80.65	☐	4.0131	4.0131		Accepted
	MS-MS		2.105	2590	74.97	☐	13.3607	13.3607		Accepted

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

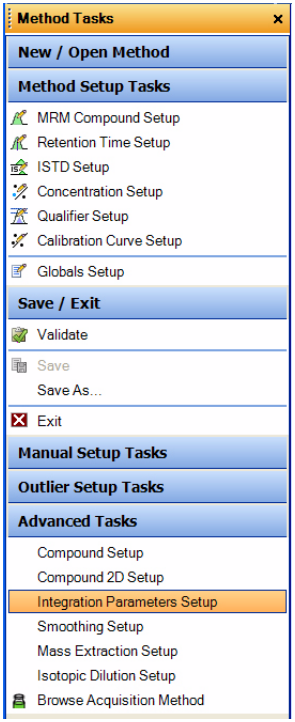


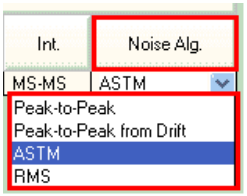
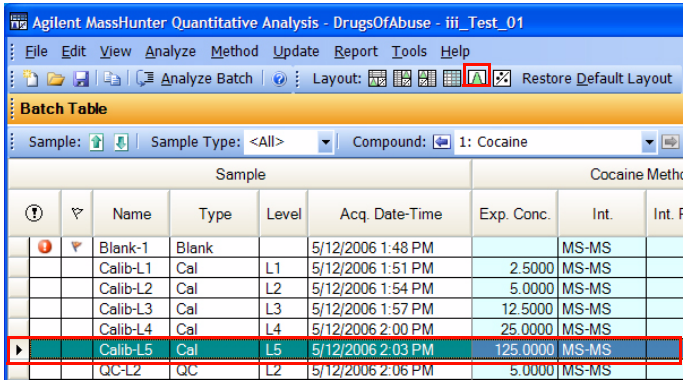
Steps	Detailed Instructions	Comments
	f Click the **Next Compound** icon in the Batch Table Standard tool bar or the Previous Compound icon in the Batch Table Standard toolbar until the system displays the compound MDMA. **g** Select the **Blank-1** row, and point to the Int. Metric column. The system displays any outlier message for that data, as well as the integrated chromatogram for MDMA.	• The outlier messages 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. • Add the Noise Algorithm column from the Compound Method list. • View the values in the Noise Alg. and S/N columns for amphetamine.	**a** Right-click anywhere in the Batch Table, and select **Add/Remove Columns**. The system displays the Columns dialog box. **b** Select **Compound Method** from the **Select Columns From** dropdown list. **c** Select **Noise Alg**. (Noise Algorithm Type) from the **Available Columns** list, 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 tool bar until the system displays the compound Amp. **f** Examine the values in the **Noise Alg.** and **S/N** (signal-to-noise ratio) columns.	



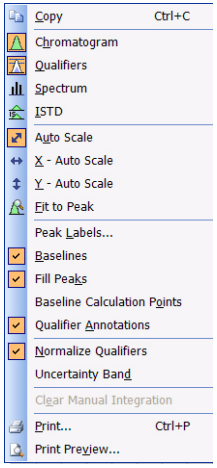
Amp ISTD: Amp-d5 Time Segment: <A

	Amp Results								Qualifier (119.4)...			Amp
Noise Alg.	RT	Resp.	S/N	MI	Calc. Conc.	Final Conc.	Accuracy	Int. Metric	Ratio	S/N	MI	RT
RMS												
RMS	2.141	658	49.10		2.4296	2.4296	97.2	Accepted	24.3	45.47		2.12
RMS	2.140	1059	42.25		4.8673	4.8673	97.3	Accepted	33.5	Infinity		2.12
RMS	2.134	2673	107.28		12.8217	12.8217	102.6	Accepted	26.7	146.48		2.12
RMS	2.022	4952	20.26		25.9349	25.9349	103.7	Accepted	29.1	49.40		1.99
RMS	2.101	18605	47.90		123.9465	123.9465	99.2	Accepted	27.0	39.22		2.07
RMS	2.142	1006	81.00		4.3457	4.3457	86.9	Accepted	27.7	34.47		2.13
RMS	2.135	4716	91.48		26.9858	26.9858	107.9	Accepted	25.6	60.79		2.12
RMS												
RMS	2.143	1004	80.65		4.0131	4.0131		Accepted	30.9	70.54		2.13
RMS	2.105	2590	74.97		13.3607	13.3607		Accepted	25.3	65.40		2.08

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** Select **Method > Edit** to switch to method editing mode. **b** Select **Method Tasks > Advanced Tasks > Integrator Parameters Setup**. The system displays the integrator parameters in the Method Table.  **c** Click the **Noise Alg.** column for Amp in the Method Table. A list of available Noise Algorithms appears. **d** Select **ASTM**.	

Steps	Detailed Instructions	Comments
	 **e** Select **Method Tasks > Save/Exit > Exit**. **f** Click **No** to the exit prompt **Would you like to apply this method to the batch?** The system displays Batch Analysis mode.	
6 Turn the baseline (highest concentration standard) off and then back on for amphetamine. • Make sure that only the Compound Information pane is visible in the window. • 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. 	• Notice that the baseline is drawn in for the quantifier chromatogram as the default setting.

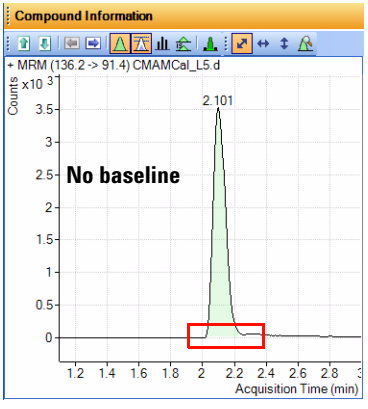
Steps	Detailed Instructions	Comments
	b Right-click either of the chromatograms to bring up the shortcut menu.	• Notice that the baseline disappears after deselecting it in the shortcut menu.

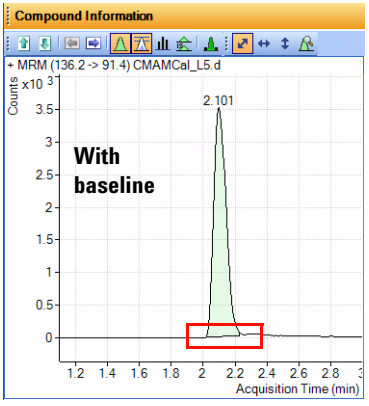


c Clear the **Baselines** checkbox in the shortcut menu.

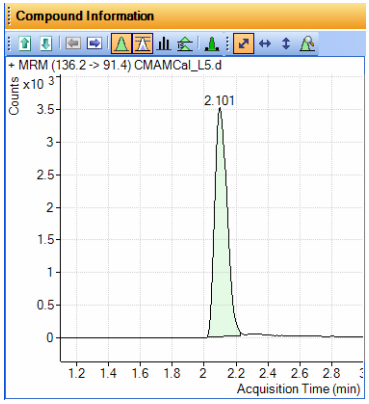
d Right-click either of the two chromatograms, and mark the **Baselines** checkbox in the shortcut menu.

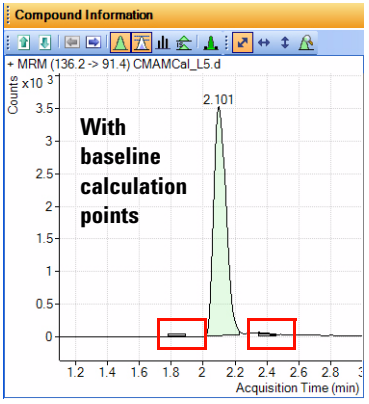
e Compare the chromatograms with and without a drawn baseline.



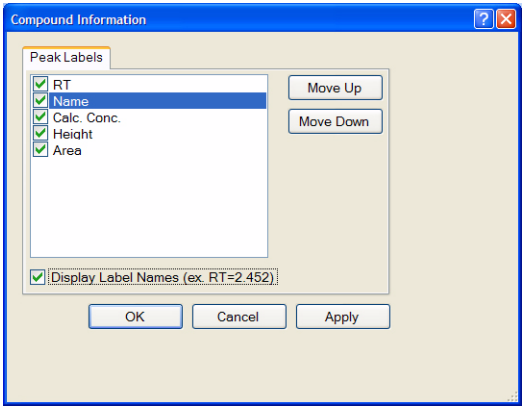


Steps	Detailed Instructions	Comments
7 Inspect the calculation points for the baseline for amphetamine.	**a** Right-click either of the two chromatograms, and mark the **Baseline Calculation Points** checkbox in the shortcut menu. You can now see where the baseline starts and stops. **b** Right-click either of the two chromatograms, and clear the **Baseline Calculation Points** checkbox in the shortcut menu. **c** Compare the chromatograms with and without Baseline Calculation Points.	

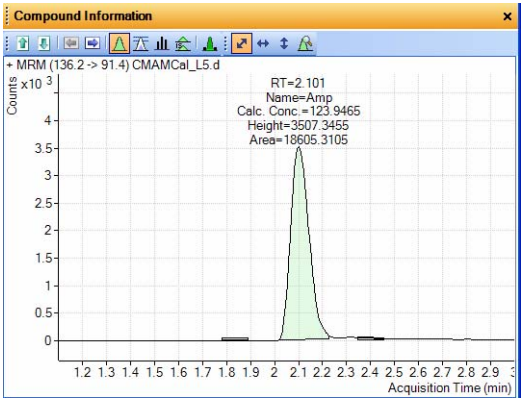


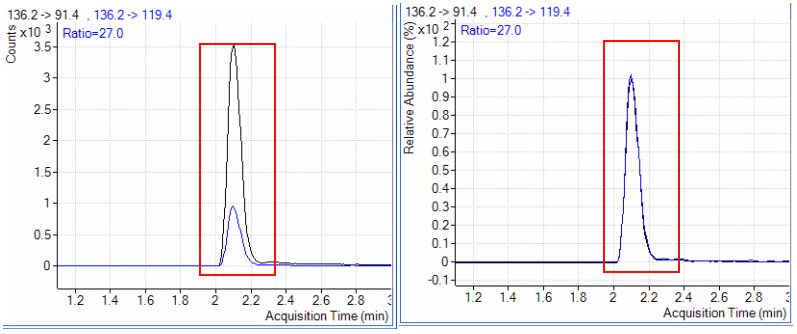


Steps	Detailed Instructions	Comments
8 Display the peak labels for amphetamine. • Display those found in the figure on the next page. • Then display the original retention time peak label.	**a** Right-click either of the two chromatograms, and select **Peak Labels** from the shortcut menu. The system displays the Compound Information dialog box. **b** Mark all the **Peak Labels** check boxes, and the **Display Label Names** check box, and click **OK**.	

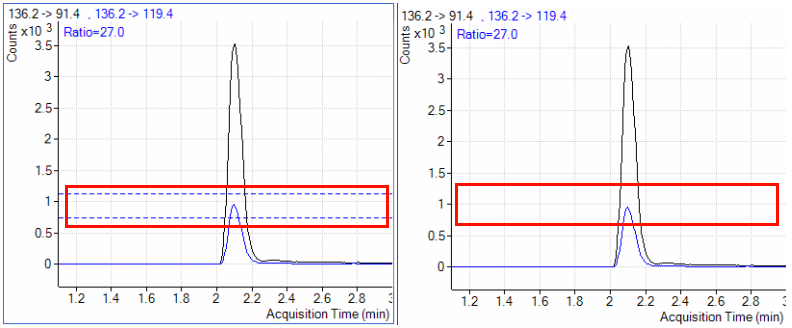


The peak labels should now match those shown in the example below.



Steps	Detailed Instructions	Comments
	c Right-click either of the two chromatograms, and select **Peak Labels** from the shortcut menu. The system displays the Compound Information dialog box. **d** Clear all the **Peak Labels** checkboxes except RT (retention time). Clear the **Display Label Names** checkbox, and click **OK**.	
9 Display the qualifier chromatogram before and after normalization.	**a** Right-click either of the two chromatograms, and mark the **Normalize Qualifiers** checkbox in the shortcut menu. The two peaks now converge and appear as one peak. **b** Right-click in the Compound Information window, and clear the **Normalize Qualifiers** checkbox in the shortcut menu. **c** Compare the qualifier chromatogram with and without normalization. 	• Notice that the default setting displays the qualifier peak overlaid on the quantifier peak before normalization.

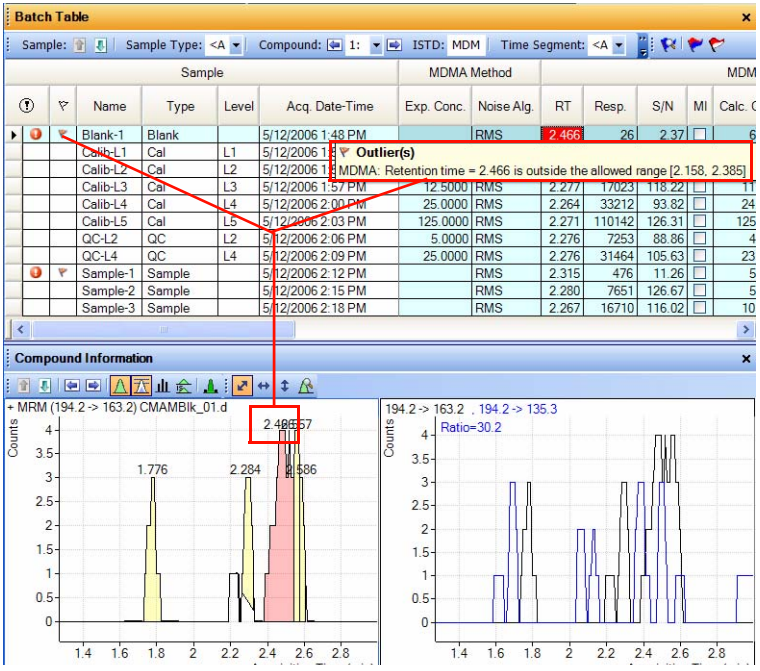
Steps	Detailed Instructions	Comments
10 View the uncertainty band.	**a** Right-click either of the two chromatograms, and mark the **Uncertainty Band** check box in the shortcut menu. The uncertainty band appears in the qualifier chromatogram. **b** Right-click either of the two chromatograms, and clear the **Uncertainty Band** checkbox in the shortcut menu. **c** Compare the qualifier chromatogram with and without 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.	**a** Right-click the Batch Table, and select **Add/Remove Columns**. **b** Select **Int.** and **Int. Parms.** (Compound Methods) from the right-hand list. **c** Click **Remove**, then **OK**.	



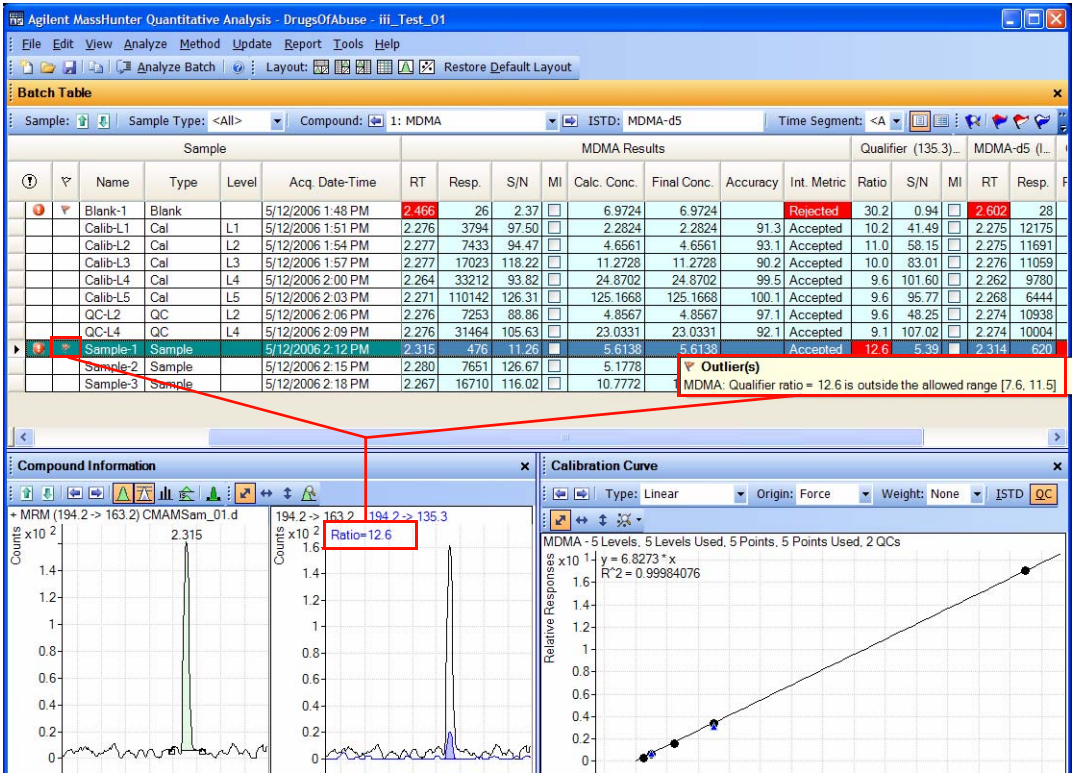
Task 3. Detect outliers

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.	

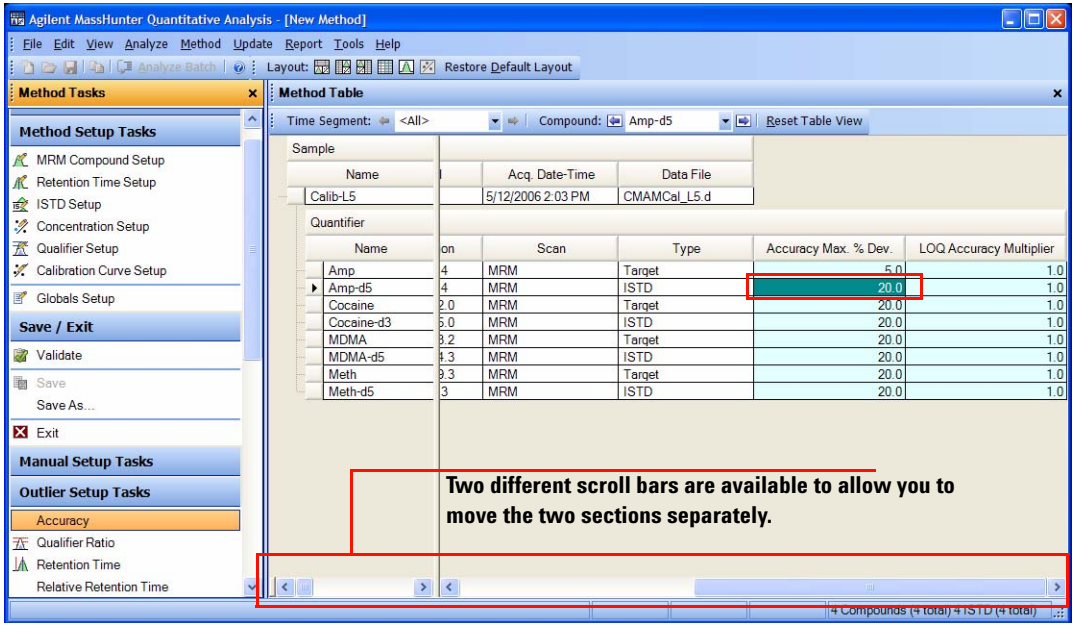


Steps	Detailed Instructions	Comments
	c Examine the outlier information in the Qualifier ... Results > Ratio column for Sample 1, as shown in the example below.	



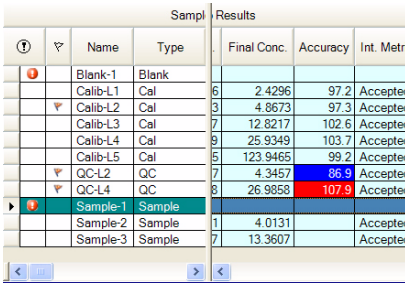
2 Change the accuracy range for amphetamine in the method, and re-analyze the batch. • Set the accuracy maximum percent deviation (**Accuracy Max % Dev**): to **5%**.	**a** Click the **Previous Compound** icon in the toolbar until the system displays the compound **Amp**. **b** Select the **Calib-L5** row in the table. **c** Select **Method > Edit** to switch to method editing mode. **d** Select **Method Tasks > 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



f Select **Method Tasks > Exit,** and click **Yes** in the confirmation prompt, to exit the method and apply the method to the batch.

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  : • Check for samples with high outliers • Check for samples with both high and low outliers • Display all samples again. • Hide the outlier flags for Accuracy and RT for Amp. • Show these outlier flags again	**a** Click the **Display samples that have High outliers**  icon on the toolbar to display only samples with high outliers. **b** Click the **Display samples that have High/Low outliers**  icon on the toolbar to display only samples with low outliers. **c** Click the **Display samples that have High/Low outliers**  icon again to display all the samples. **d** Click the **Select Outliers**  icon to bring up the Outliers dialog box. **e** Clear the **Accuracy** and **Retention Time** check boxes, and click **OK**. **f** Click the **Select Outliers**  icon to bring up the Outliers dialog box. **g** 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
Work with quantitation reports

This exercise helps you learn how to do these tasks:

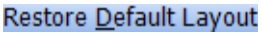
- Generate reports using specified templates
- Review the reports, looking for outliers
- Customize reports by adding columns and changing the report header

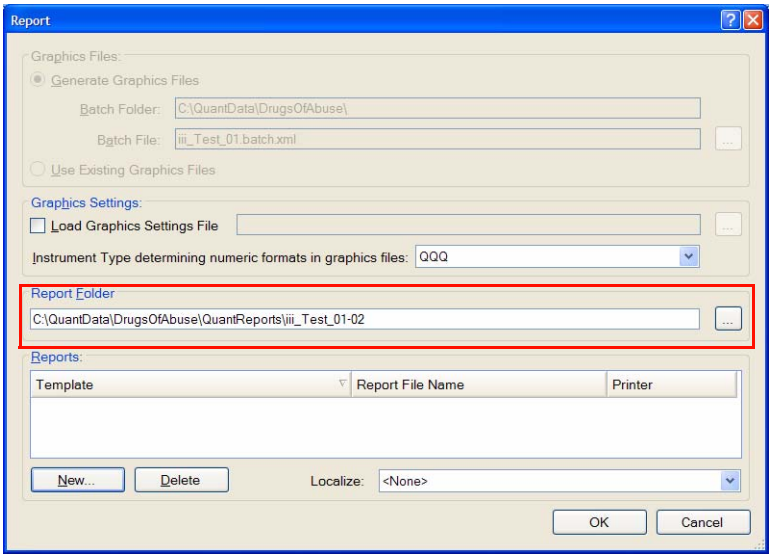
Each exercise is presented in a table with three columns:

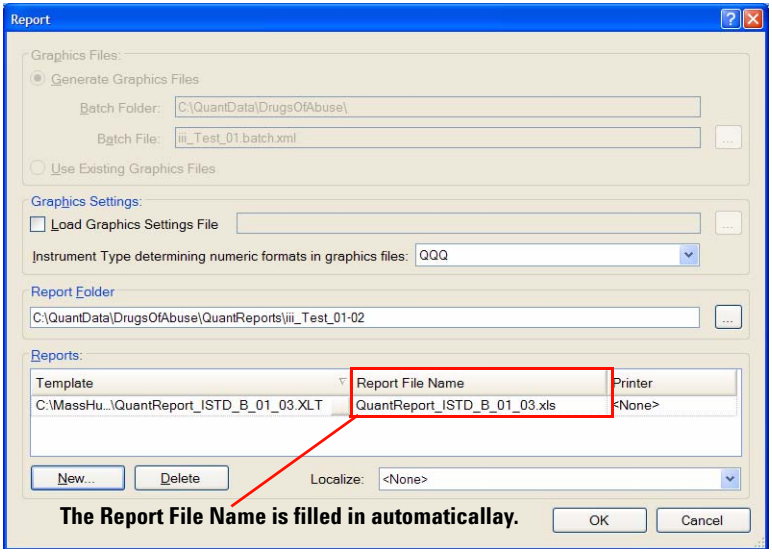
- Steps – Use these general instructions to proceed on your own to explore the program.
- Detailed Instructions – Use these if you need help or prefer to use a step-by-step learning process.
- Comments – Read these to learn tips and additional information about each step in the exercise.

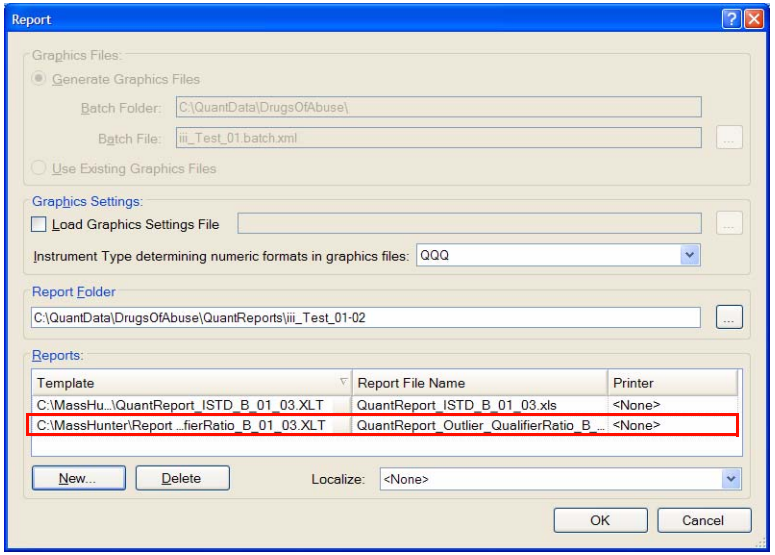
Task 1. Generate quantitation reports

In this task, you generate ISTD and Qualifier Ratio reports using the corresponding templates.

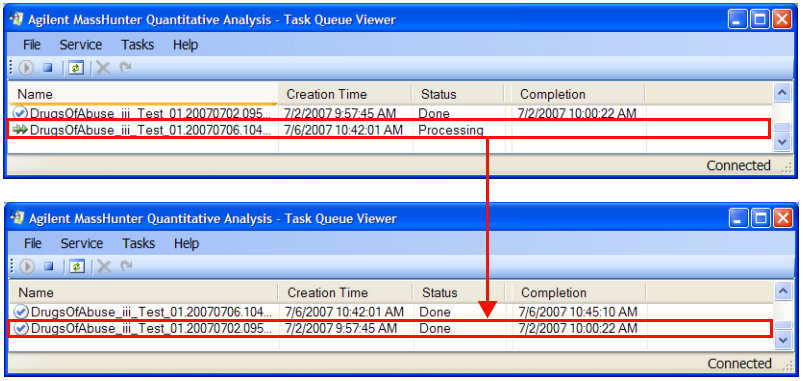
Steps	Detailed Instructions	Comments
1 If necessary, open the batch file ***iii*_Test_01.batch.xml**. If the batch is already open, skip to step 2.	**a** To start the Quantitative Analysis program, click the **Quantitative Analysis** icon on your Desktop.  **b** Click **Open Batch**  on the toolbar to display the Open Batch dialog box. **c** Navigate to ***Your Directory*****DrugsOfAbuse** and select ***iii*_Test_01.batch.xml.**	• You can also access the program by selecting **Programs > Agilent > MassHunter Workstation > Quantitative Analysis** from the Start menu**.** • If the default layout is not present, click **Restore Default Layout** on the toolbar before opening the batch. 
2 Verify the default destination directory for reports. • The destination directory should be ***Your Directory****DrugsofAbuse****QuantReports**. • The default filename is ***iii*_Test_01**, where "*iii*" are your initials.	**a** Select **Report > Generate**. The system displays the Report dialog box. **b** Specify the default destination directory for saving Excel reports in the **Report Folder** text box; for example, ***Your Directory*****DrugsOfAbuse****QuantReports*****iii*_Test_01**.	You can also specify the **Instrument Type determining numeric formats in graphics files**. This value is used to determine how many decimal places to show in the graphics.

Steps	Detailed Instructions	Comments
		
3 Add an ISTD template. • Add the template, Quantreport_ISTD_B_01_03.xlt. • Make sure the report name is *TemplateName*.xls, where *TemplateName* is the exact name of the template.	**a** Click **New** in the Report dialog box. The system displays the Open dialog box. **b** Select **Quantreport_ISTD_B_01_03.xlt** and click **Open**. The program adds the template to the Template field in the Reports pane. **c** In the Report File Name field in the Reports pane, verify that the report file name is **QuantReport_ISTD_B_01_03.xls**.	• Note that the **B_01_03** designation corresponds to the Quantitative Analysis software release, which will change over time. Therefore, the default report file name may change correspondingly.

Steps	Detailed Instructions	Comments
		
4 Add a Qualifier Ratio template. • Add the template, Quantreport_Outlier_Qualifier ratio_B_01_03.xlt. • Make sure the report name is *TemplateName*.xls, where *TemplateName* is the exact name of the template.	**a** Click **New** in the Report dialog box. The system displays the Open dialog box. **b** Switch to the **Outliers** directory. **c** Select **Quantreport_Outlier_Qualifier ratio_B_01_03.xlt** and click **Open**. **d** In the Report File Name field in the Reports pane, verify that the report file name is **Quantreport_Outlier_Qualifier Ratio_B_01_03.xls**.	

Steps	Detailed Instructions	Comments
		
5 Generate the reports. • View the status of the report generation in the Task Queue Viewer.	**a** Click **OK** in the Report dialog box to generate the report. **b** Select **Report > Queue Viewer** to monitor the report generation process. The system displays the Task Queue Viewer dialog box. **c** Watch the progress of the report in the Status column.	

Steps	Detailed Instructions	Comments



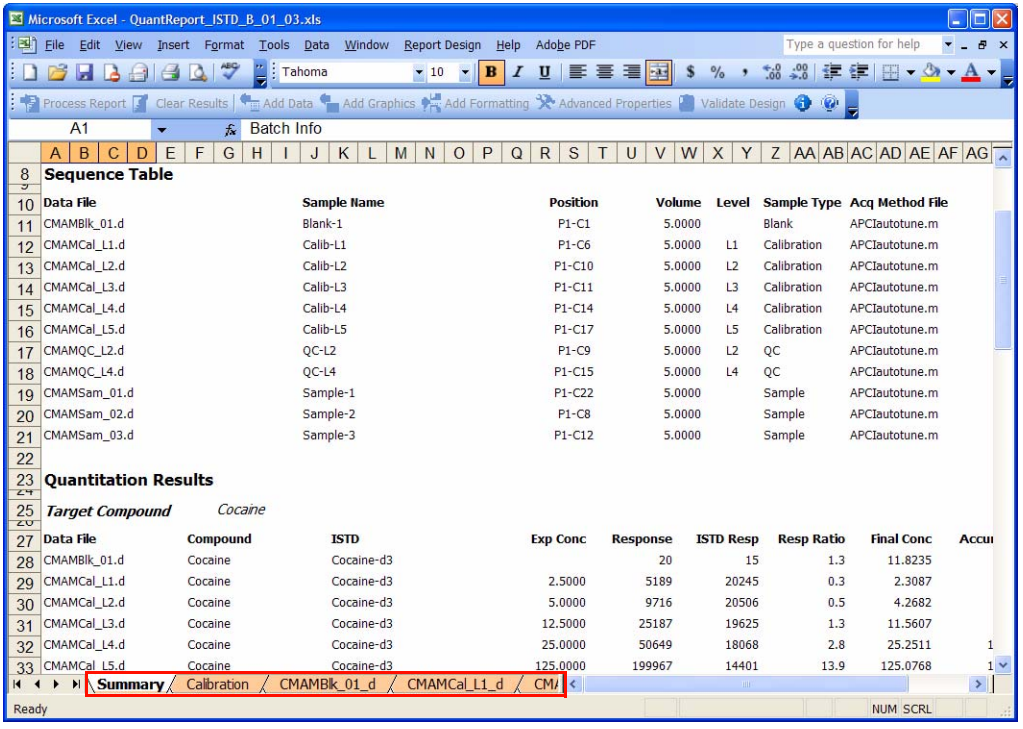
When the Status column says Done, the report is finished.

d Close the **Task Queue Viewer**.

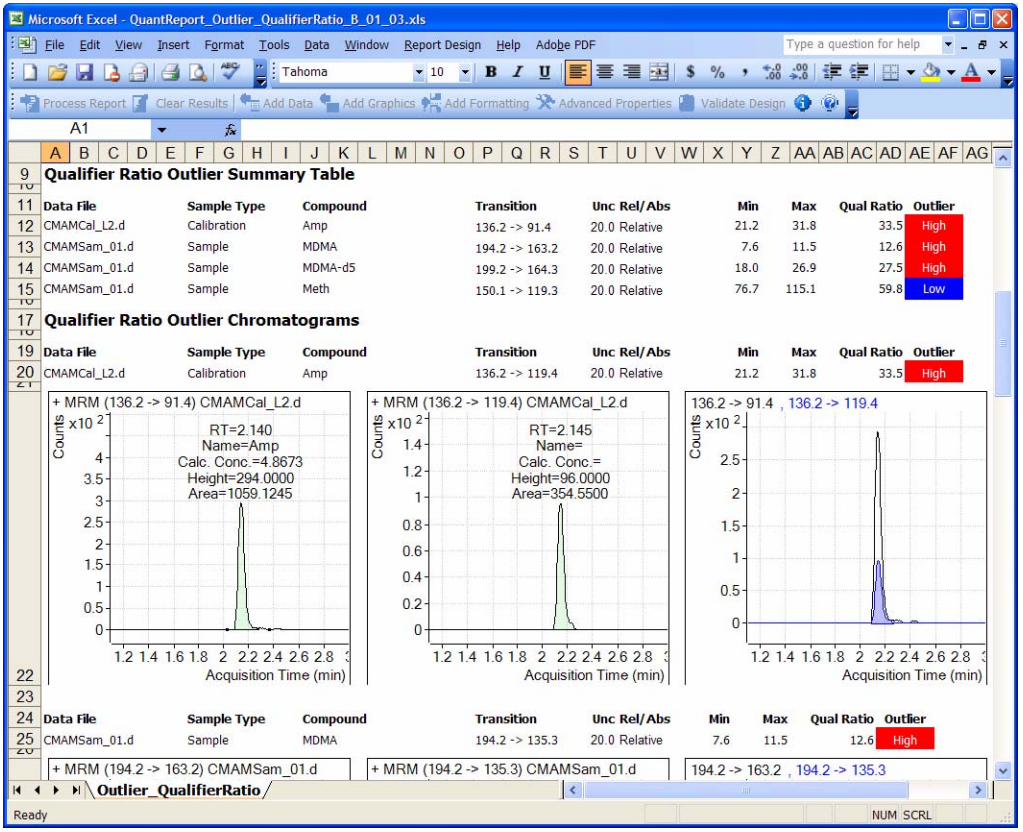
Task 2. Review the reports

In this task, you review the reports from the last task in Microsoft Excel and look for outliers.

Steps	Detailed Instructions	Comments
1 Review the ISTD report generated in the previous task to familiarize yourself with its organization. • View the organization of each worksheet.	**a** Go to the directory **C:*Your Directory*\DrugsOfAbuse\QuantReports\Test_01**. **b** Right-click **Quantreport_ISTD_B_01_03.xls**, and click **Open.** **c** Inspect the contents of each worksheet in the Excel file.	



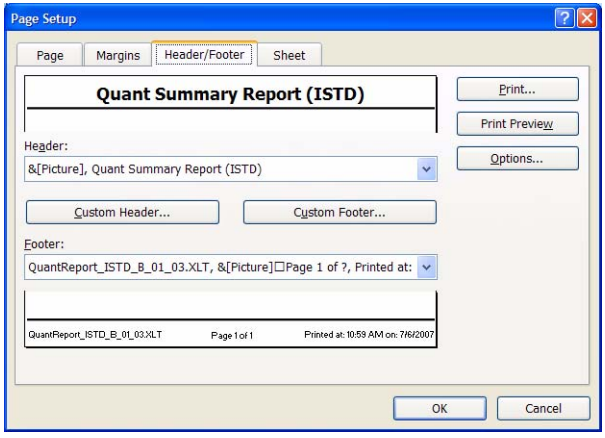
Steps	Detailed Instructions	Comments
2 Review the qualifier ratios in the Qualifier Ratio report, **Quantreport_Outlier_Qualifier ratio_B_01_03.xls**.	**a** Go to **C:\Quantdata\DrugsOfAbuse\QuantReports\Test_01.** **b** Right-click **Quantreport_Outlier_Qualifier Ratio_B_01_03.xls**, and select **Open.** **c** Examine the qualifier ratio outliers reported in the Excel file.	Only Qualifier Ratios that are either **High** or **Low** are included in the report.

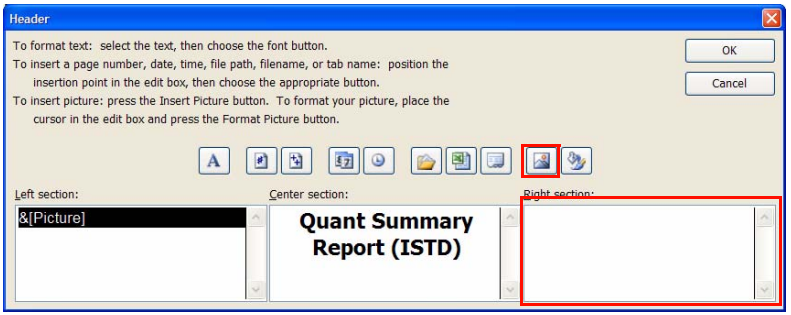
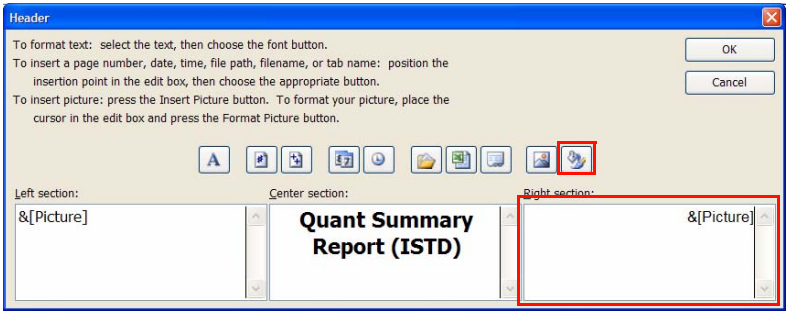


Task 3. Customize a report template

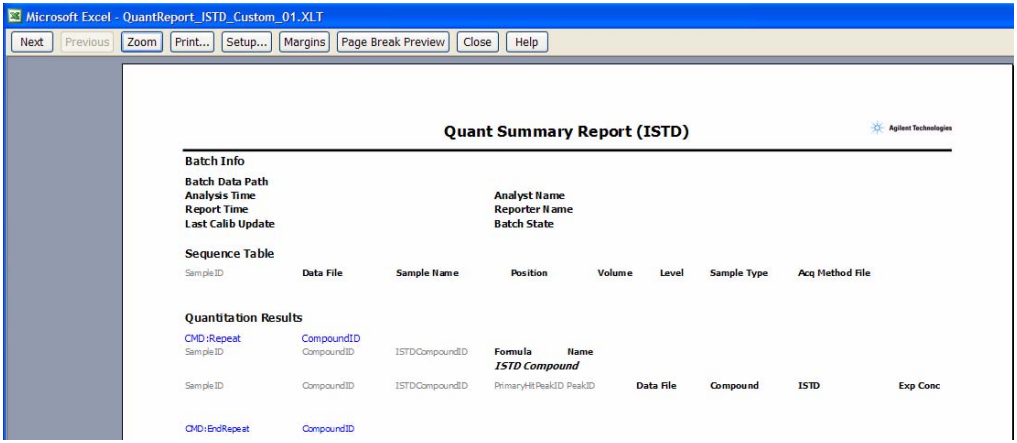
This task shows you how to:

- add a logo to a report header of the ISTD template
- add a column and change the font color
- generate and review the new report based on the customized template

Steps	Detailed Instructions	Comments
1 Modify the ISTD report template header. • Open Quantreport_ISTD_B_01_03.xlt. • Add the ASMS2006logo.bmp file to the header. • Look at a preview of the report.	**a** Go to the folder **\Report Templates\ Quant.** **b** Right-click Quantreport_ISTD_B_01_03.xlt, and select **Open** from the shortcut menu. **c** Select **View >Header and Footer** in the Excel window. The system displays the Page Setup dialog box. **d** Click the **Header/Footer** tab.  **e** Click **Custom Header.** The system opens the Header dialog box.	• You must open the Excel file in this way (right-click the file name, and select **Open**) to access an editable file.

Steps	Detailed Instructions	Comments
	f Move mouse cursor to the **Right section**, and click the **Insert Picture** icon. **g** If you are asked, click **Replace** on the message asking whether or not to replace the existing picture.	• The image Agilent_Logo.tif is in the directory Report Templates\Quant\Logo+Header+Footer. • You can use this image to learn how to include additional graphics in the header.
		
	h In the Insert Picture dialog box, select **Agilent_Logo.tif** and click **Insert**. You may also need to format the picture by clicking the **Format Picture** icon. In the Agilent templates, the Height is scaled to 80%.	
		
	i Click **OK** in the Header dialog box. **j** Click **OK** in the Page Setup dialog box.	

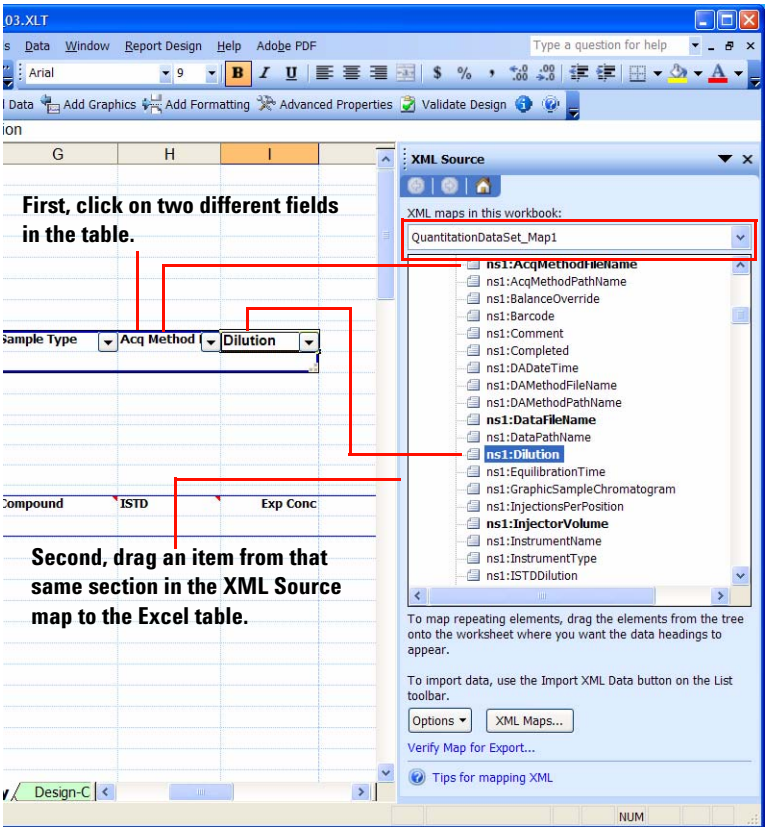
Steps	Detailed Instructions	Comments
	k Click the **Print Preview** icon to view the position of the logo on the page.	• When adding your own company logo, make sure that it is an appropriate size to fit in the header.



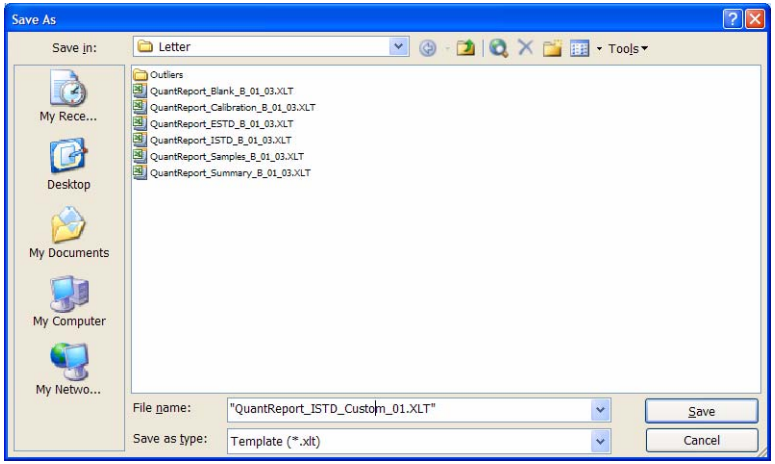
l Verify the display of the modified header, and click **Close**.

m If more than one design tab is part of the report, you need to make the change on each tab. For this report, you need to change the header on the Design-Summary tab, the Design-Calibration tab and the Design-Sample tab.

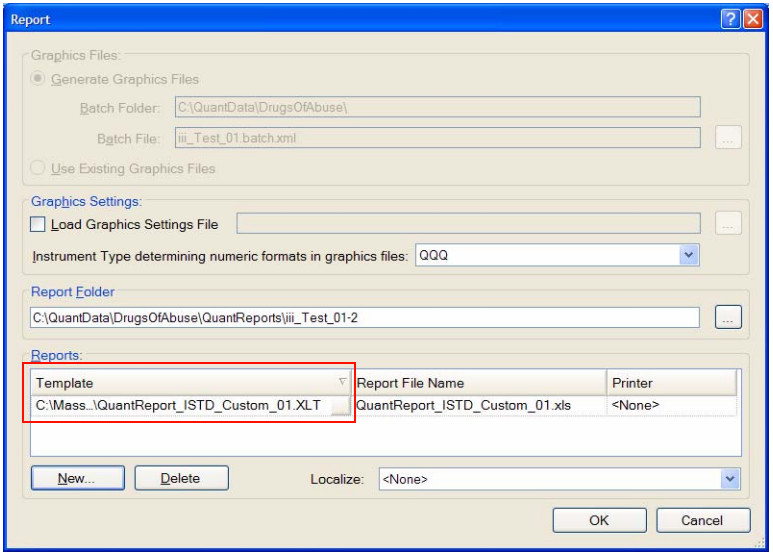
Steps	Detailed Instructions	Comments
2 Add a Dilution column. • Use QuantitationDataSet_ Map1to find the Dilution column. • Change the font color of this column name to **Automatic**.	**a** Select **Data > XML > XML Source**. The system displays the XML Source window on the right side of the Excel window. **b** Click on two different columns in the table that you are adding to. **c** Drag the element **Dilution** from the XML Source window, and drop it in the Sequence Table as shown in the example below.	• See the Online Help for definitions of each of the Quantitation DataSet_Map1 columns that you can add to the report. • You can use any of the XML maps that start QuantitationDataSet_Map. • If the cell you dragged the element to displays in red, it is because it is because it is not from the same section of the XML Source map as the table's element.



Steps	Detailed Instructions	Comments
	d Click the new column heading, **Dilution.** **e** Click the **Font Color** button on the toolbar. **f** Select **Automatic** from the Color dropdown menu.	
3 Save the new template. • Use the filename **Quantreport_ISTD_custom_01.xlt**. • Hint: The filename must be double-quoted.	**a** Select **File > Save As**. The system displays Save As dialog box. **b** Type the file name **Quantreport_ISTD_custom_01.xlt** in the File name text box. Make sure the filename is double quoted in the dialog box. **c** Click **Save** to close the Save As dialog box and save the modified template.	

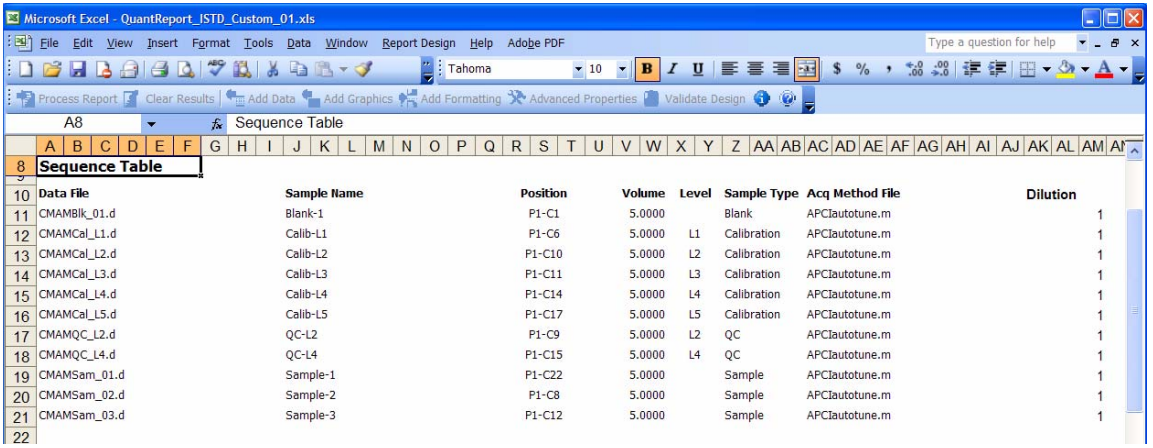


Steps	Detailed Instructions	Comments
4 Generate a new ISTD report in the folder, Test_01-1.	**a** To exit Excel, select **File > Exit**. **b** Select **Report > Generate.** The system opens the Report dialog box. **c** Change the Report Folder from \ Test_01 to **Test_01-1**. **d** Click **New**. **e** Select **Quantreport_ISTD_Custom_01.xlt**, and click **Open**.	• This step assumes that the program is still running. If not, see Task 1, step 1.



	f Click **OK** in the Report dialog box to begin generating the report. **g** Select **Report > Queue Viewer** to monitor the progress of report generation. The system displays the Task Queue Viewer dialog box.	

Steps	Detailed Instructions	Comments
5 Make sure that the Dilution column appears in the new ISTD report.	**a** Go to the directory**Quantdata****DrugsOfAbuse****QuantReports****Test_01-1.** **b** Double-click **Quantreport_ISTD_custom_01.xls**.	



	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	AA	AB	AC	AD	AE	AF	AG	AH	AI	AJ	AK	AL	AM
8	**Sequence Table**																																						
10	**Data File**						**Sample Name**								**Position**				**Volume**		**Level**		**Sample Type**			**Acq Method File**												**Dilution**	
11	CMAMBlk_01.d						Blank-1								P1-C1				5.0000				Blank			APCIautotune.m												1	
12	CMAMCal_L1.d						Calib-L1								P1-C6				5.0000		L1		Calibration			APCIautotune.m												1	
13	CMAMCal_L2.d						Calib-L2								P1-C10				5.0000		L2		Calibration			APCIautotune.m												1	
14	CMAMCal_L3.d						Calib-L3								P1-C11				5.0000		L3		Calibration			APCIautotune.m												1	
15	CMAMCal_L4.d						Calib-L4								P1-C14				5.0000		L4		Calibration			APCIautotune.m												1	
16	CMAMCal_L5.d						Calib-L5								P1-C17				5.0000		L5		Calibration			APCIautotune.m												1	
17	CMAMQC_L2.d						QC-L2								P1-C9				5.0000		L2		QC			APCIautotune.m												1	
18	CMAMQC_L4.d						QC-L4								P1-C15				5.0000		L4		QC			APCIautotune.m												1	
19	CMAMSam_01.d						Sample-1								P1-C22				5.0000				Sample			APCIautotune.m												1	
20	CMAMSam_02.d						Sample-2								P1-C8				5.0000				Sample			APCIautotune.m												1	
21	CMAMSam_03.d						Sample-3								P1-C12				5.0000				Sample			APCIautotune.m												1	
22																																							

	c Verify that the new column you added, Dilution, appears in the Excel spreadsheet. **d** Select **File > Exit.**	

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In This Book

The Familiarization Guide presents exercises to help you use the Agilent 6410 Triple Quad LC/MS system. In this guide you learn:

- How to develop an acquisition method
- How to set up and quantitate a batch of Agilent Triple Quad LC/MS data files
- How to inspect your quantitation results, and how to spot irregularities
- How to improve result accuracy
- How to generate and review quantitation reports

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