Agilent

MassHunter Quantitative Analysis for GC/MSD

Familiarization Guide

Notices

Manual Part Number

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CAUTION

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In This Guide

This Familiarization Guide presents step-by-step exercises to help you learn to use the Quantitative Analysis program. You can do these exercises with batch directories located in the VoaDemoBatches folder. See **Step 2** on page 4.

1 "Set Up a New Method from Acquired Scan Data"

In this exercise, you set up a method using scan data that was previously generated from a single quad instrument.

2 "Review Quantitation Results"

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

3 "Compounds at a Glance"

In this exercise, you inspect the Compounds at a Glance feature and learn how it can help you save time with your reviews.

4 "Outliers and Quantitation Messages"

In this exercise, you review results for your batch using the Batch Table Outlier indicators and Quantitation Message features.

5 "Generate Quantitation Reports"

In this exercise, you generate report methods using one or more report templates, how to generate a report, and then review these reports in Microsoft Excel.

Before You Begin These Exercises

1 Make sure the demo files are copied to your PC.

To complete these exercises, copy the following batch folders to your MassHunter data directory on your PC.

- VoaDemo
- VoaSampleData

These two folders are found in the **VoaDemoBatches** folder. This folder is installed on your PC by one of the following:

- The MassHunter Supplemental disk installation program
- The GC/MS Software Information and Manuals (G1701-60172) installation program

These files **may have been automatically copied to your PC** during the initial MassHunter software installation. Check your MassHunter default directory (**MassHunter/Data/QuantExamples/MS/VoaDemoBatches**) to see if the batch folders are located there.

If they are not already on your PC, use one of the two programs listed above to install these files on your PC. Then copy the folders named **VoaDemo** and **VoaSampleData** from their installed location to the MassHunter Data directory, for example **/MassHunter/GCMS/1/Data**.

2 Review more information.

Accompanying your hardware and software is a comprehensive collection of manuals, videos, user applications, and method development tools. These are located on the:

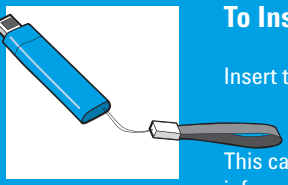
- MassHunter software installation disks
- GC/MS Software Information USB (G1701-60172)

If you haven't already done so, take a look at what is included in these libraries. They contain a vast amount of valuable information.

To Install Your Hardware Library

Insert the disk into your DVD drive and follow the prompts.

This can be installed by anyone who has authority to copy information onto the receiving computer.



To Install Your Software Library

Insert the memory stick into a USB port and follow the prompts.

This can be installed by anyone who has authority to copy information onto the receiving computer.

Choosing Quantitative Analysis Desktop Icons

Quantitative Analysis B.09.00 offers desktop icons for the Classic user interface and the Quant-My-Way user interface. The Classic user interface has a look and feel similar to the user interface offered in Quantitative Analysis B.08.00, with tools and options located in a menu bar. The Quant-My-Way user interface has a modern ribbon, with tools and options located on tabs and ribbons instead of in a menu bar. You can select to install the Classic user interface desktop icons, the Quant-My-Way user interface desktop icons, or a mix of both.

This *Familiarization Guide* follows the Classic user interface. However, where the Quant-My-Way user interface navigation differs, those steps are included and highlighted in blue.

Contents

1 Set Up a New Method from Acquired Scan Data

In this exercise, you create a quantitation method using previously acquired scan data. MassHunter analyzes a data file, identifies compound names, the target ion, qualifier ions and ratios, and retention times using search ID parameters that you specify, and, along with other default parameters, uses this information to fill in initial values for the quantitation method. This greatly reduces the time required for method creation.

Other methods exist for creating a quantitation method from scan data, but this method demonstrates most features in the Method Editor that assist with MassHunter familiarization. All of the method editor parameters discussed in this chapter also apply to SIM quantitation methods. In fact, this scan method can be easily turned into a SIM method as you will later see. This exercise ends with a overview on creating SIM methods.

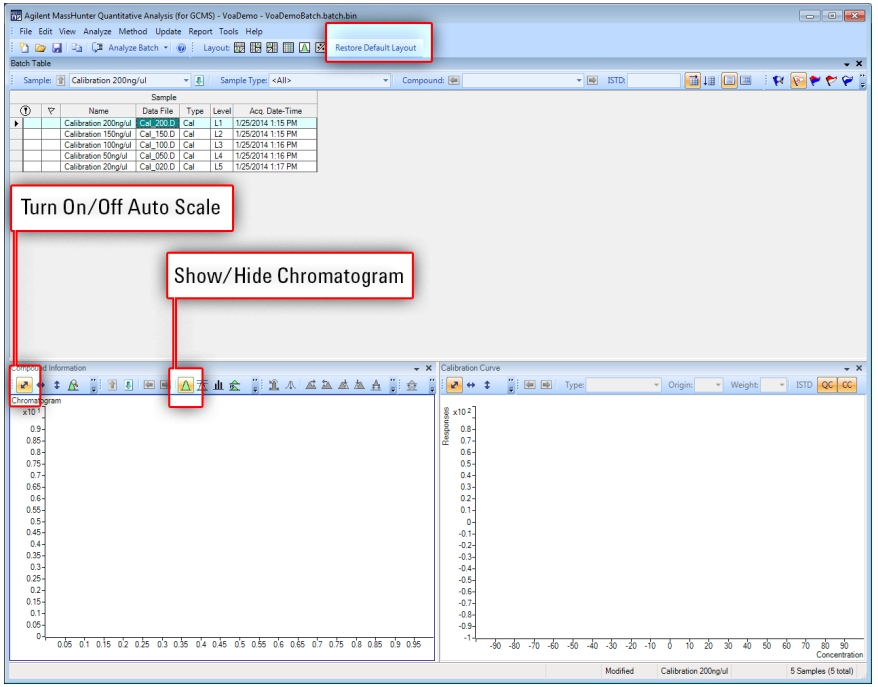
Task 1. Create a Batch of Calibration Samples

While completing this task, you will set up a method using scan data that was previously generated from a single quad instrument.

1 Start **Quantitative Analysis**.

a Use the **MS Quantitative Analysis** desktop icon to open MassHunter Quantitative Analysis. This starts the program for MSD single quad data analysis.

b Click **Restore Default Layout**, and unselect all icons in the **Compound Information** toolbar except the **Turn On/Off Auto Scale** and **Show/Hide Chromatogram** icons. Your screen should look similar to the one shown here.

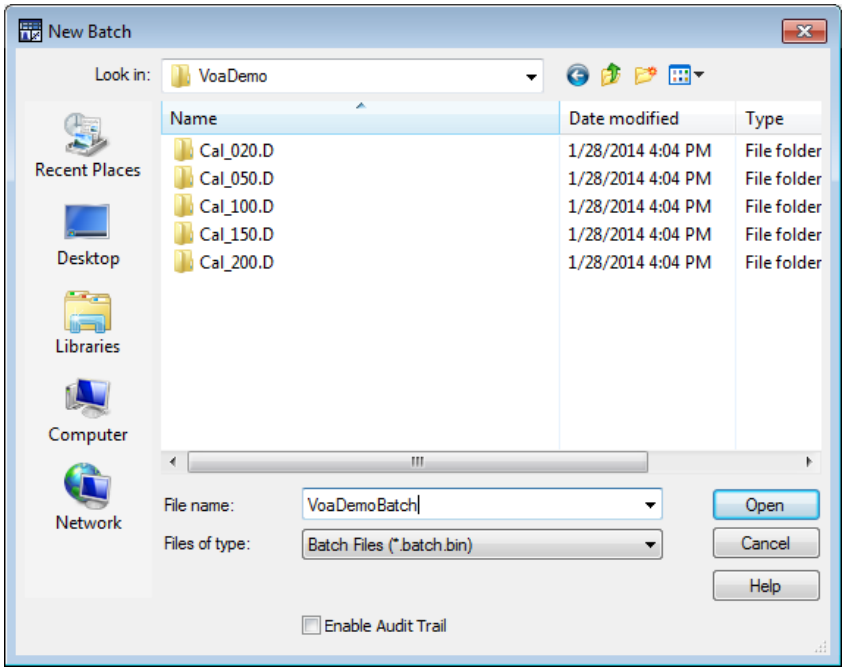


2 Navigate to the batch containing the data files you wish to use.

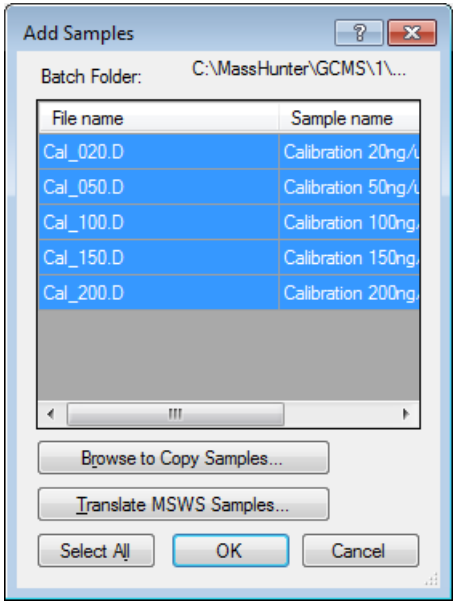
a Select **File > New Batch**.

b Use the **Look in** drop-down list to navigate to the directory where the batch data files are stored. In this case: **C: > MassHunter > GCMS > 1 > Data > VoaDemo**.

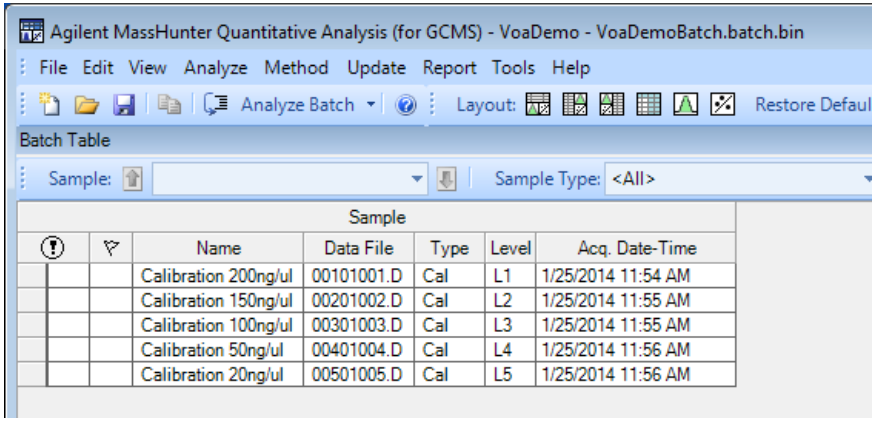
3 Type the file name **VoaDemoBatch** for this batch, and click **Open**.



4 Select the files for the batch. You may select individual sample data files or accept the default and add all the files. For this example, because we will be using all of these files to create a calibration curve, click **OK** to accept the default and add all the selected samples to this batch.



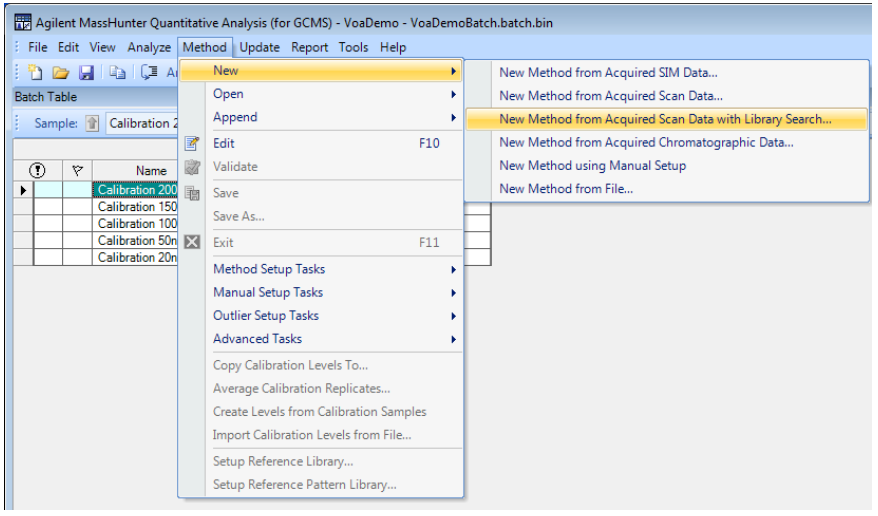
5 Review the **Batch Table**. The **Name**, **Data File**, **Type**, **Level**, **and Acq. Date-Time** are automatically included in the **Batch Table**.



Task 2. Add Calibration Compounds to the Method

The procedure we are using requires a library containing the compounds in your calibration sample. If you do not have access to an extensive library such as NIST or Wiley, use the alternate process **New Method from Acquired Scan Data** that will follow the procedure here in general although it will not identify the compound by name.

1 Select **Method > New > New Method from Acquired Scan Data with Library Search**.



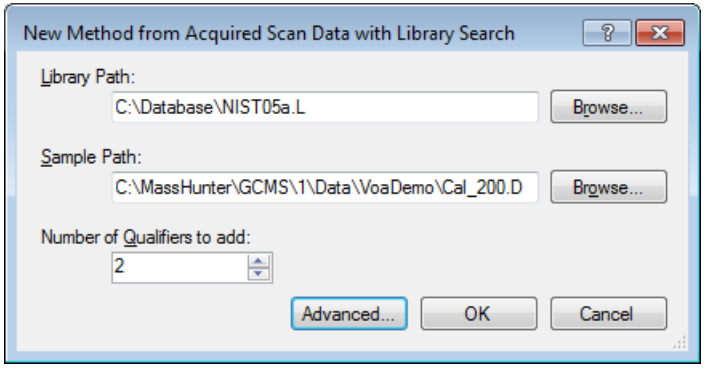
2 Browse to and select the library you want to use. Here we are using the NIST05 library.

3 Browse to and select the sample data file you want to use, and click **Open**. Here we are using CAL_200.

Remember, this should be a data file with high concentrations of the calibration compounds and internal standards of interest.

4 Enter the maximum number of qualifiers to include for each compound. Here we are specifying 2 qualifiers for each compound. If MassHunter cannot find the maximum qualifiers, it will show whatever it does find.

5 Click **Advanced** to display the **Scan Analysis Parameters** dialog box.

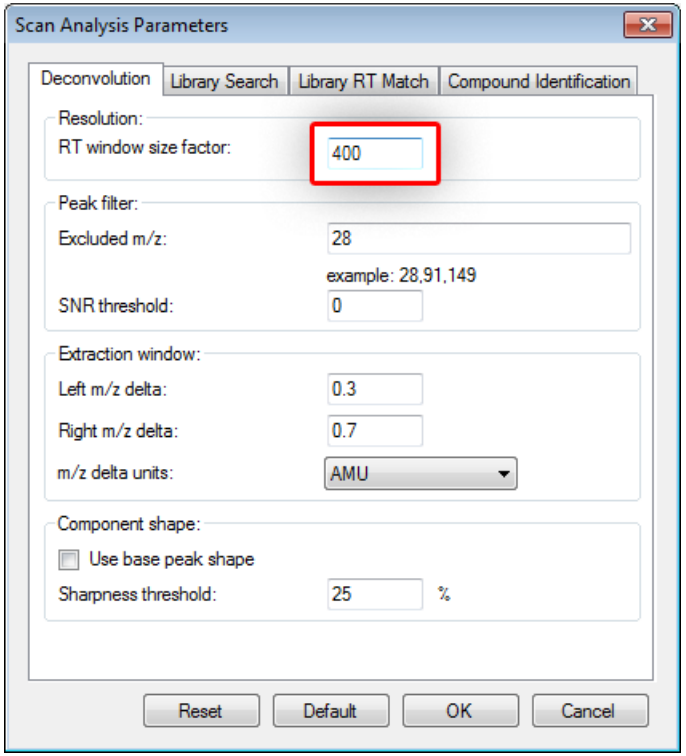


6 Click **Default** to return parameters on all tabs to their default values.

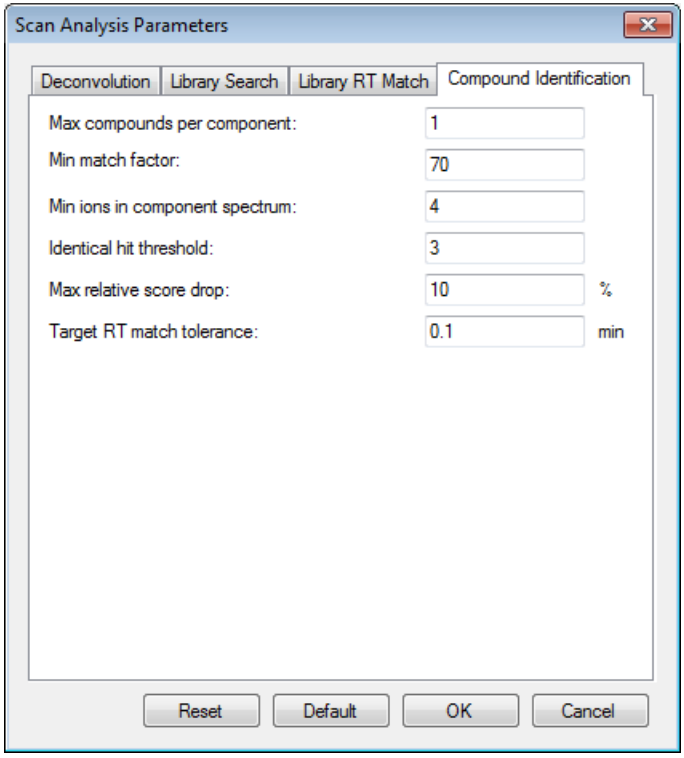
7 On the **Deconvolution** tab, set the **RT window size factor** to **400**.

The larger this number is, the fewer the number of compounds that will be found by deconvolution. Since the peaks in this data were chromatographically optimized, we want to reduce the number of compounds found by deconvolution.

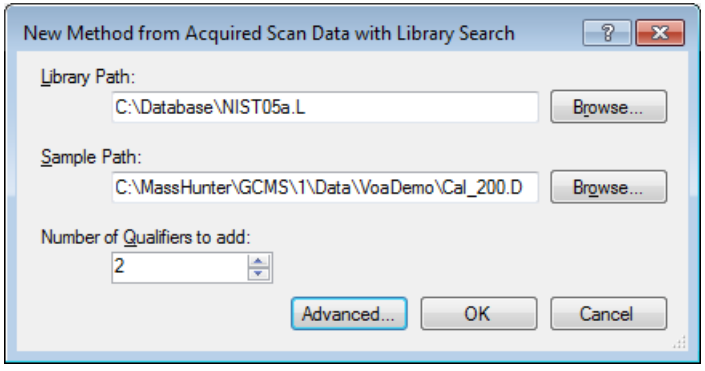
In this case, the default RT window size factor of 100 would identify 74 compounds, which is too many for this method. Increasing this number to 400 will reduce the number of compounds identified to 38, which is closer to the actual number of calibration compounds in the sample.



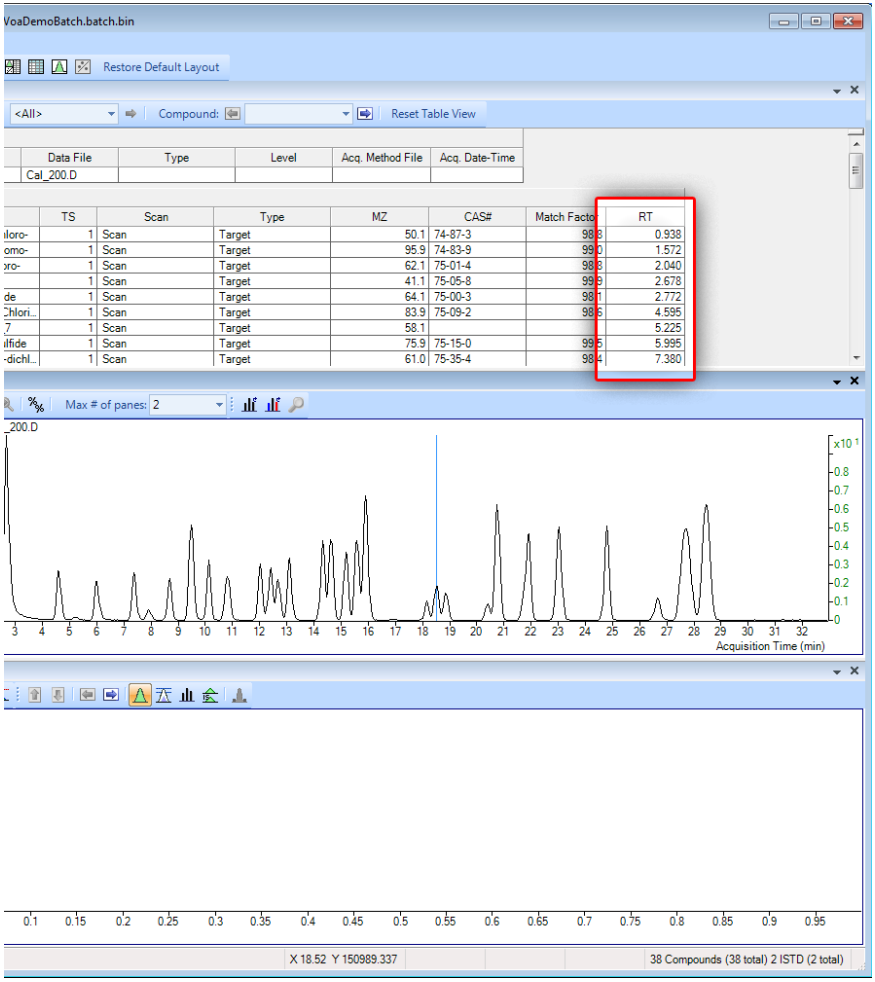
8 On the **Compound Identification** tab, change the **Min Match Factor** to **70**, then click **OK** to close the **Scan Analysis Parameters** dialog.



9 Click **OK** to close the **New Method from Acquired Scan Data with Library Search** dialog box and return to the **Method Editor**.



10 Review the **Method Table**. MassHunter processes the calibration compounds based on the scan analysis parameters you entered and displays the calibration target compounds and ISTDs in the **Method Editor** view of the **Method Table**, sorted by retention time (RT).



Task 3. Set Up the Compounds and Qualifiers in the Method Table

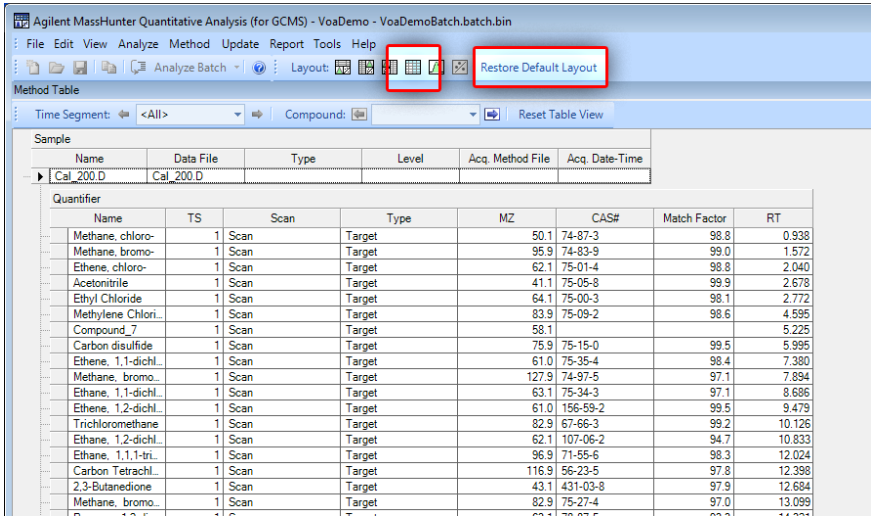
In this task, you will:

- Review the list of compounds and their qualifiers identified by MassHunter
- Edit the compound information if the compound or ISTD was misidentified
- Revise the quantifiers and qualifiers
- Check the retention time window then specify the ISTD for each calibration compound
- Assign quantifiers to an ISTD
- Setup concentration levels.
- Setup calibration curve.

1 Remove the **Compound Information** window and **Sample Information** window from the display by clicking the **Maximize Table** icon.

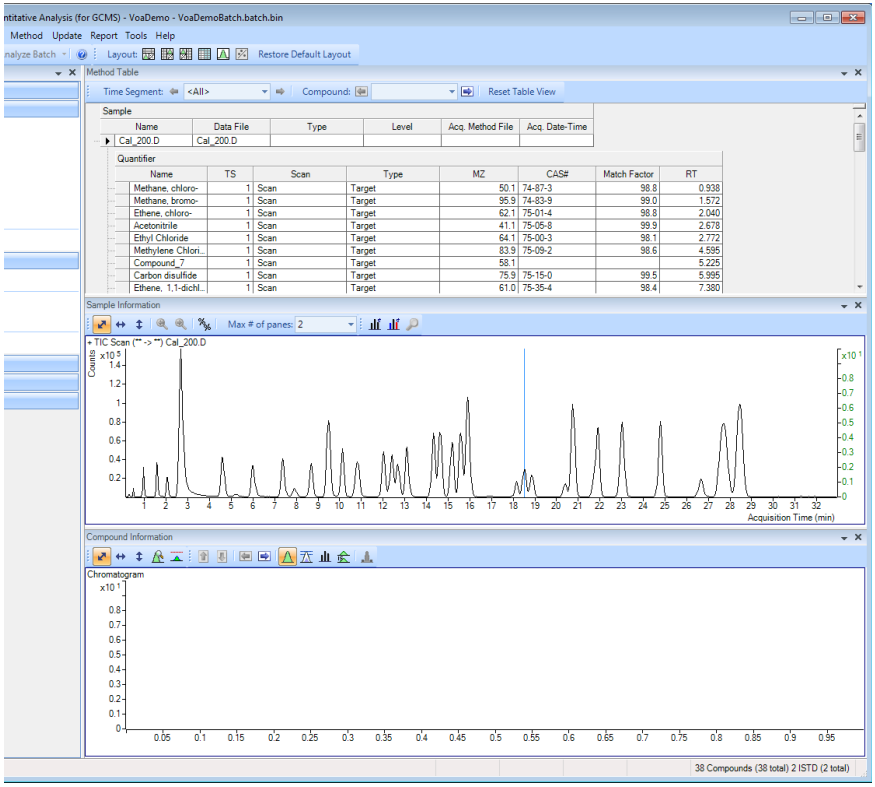
On the **View** tab, select **Maximize Pane** > **Maximize Table**.

This displays a view of the **Method Table** only. This view allows you to see all the compounds in the table, if screen resolution permits. The NIST library found 40 compounds including one not identified.

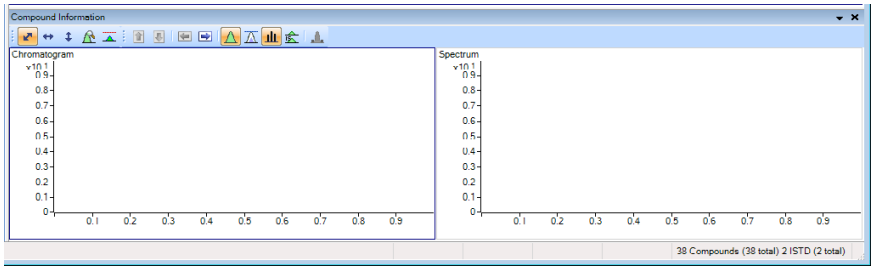


2 Click **Restore Default Layout** to return to the previous view.

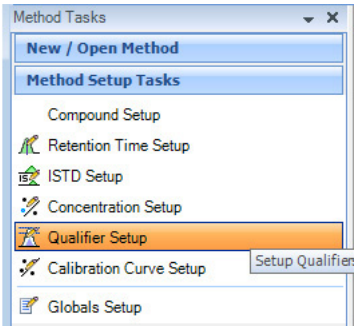
3 Review the default layout. Here you can see the **Method Tasks** area, **Method Table**, **Sample Information** window, and **Compound Information** window.



4 Click the **Show/Hide Spectrum** icon in the **Compound Information** toolbar to display the **Spectrum** pane to the right of the **Chromatogram** pane. The **Method Editor** views should now be identical to those here.

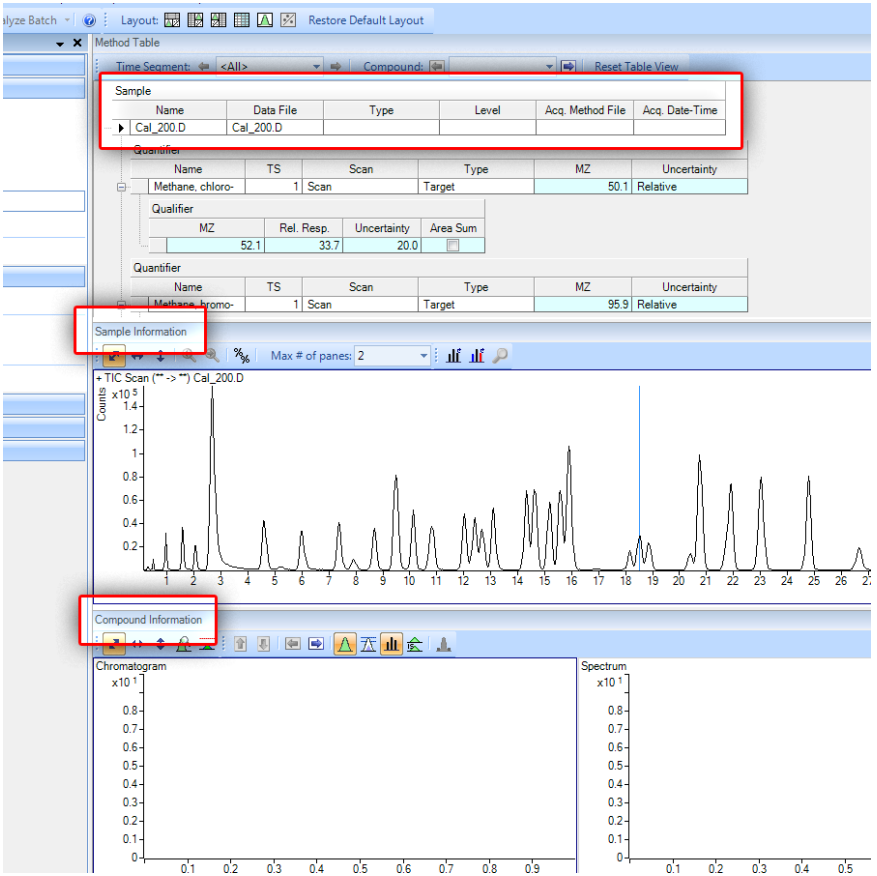


5 In the **Method Tasks** area, select **Method Setup Tasks > Qualifier Setup** to edit compound parameters that are misidentified.



6 Notice that the sample **Cal_200.D** is selected. The shaded aqua entries are the parameters that relate the qualifier to the target (quantifier) compound.

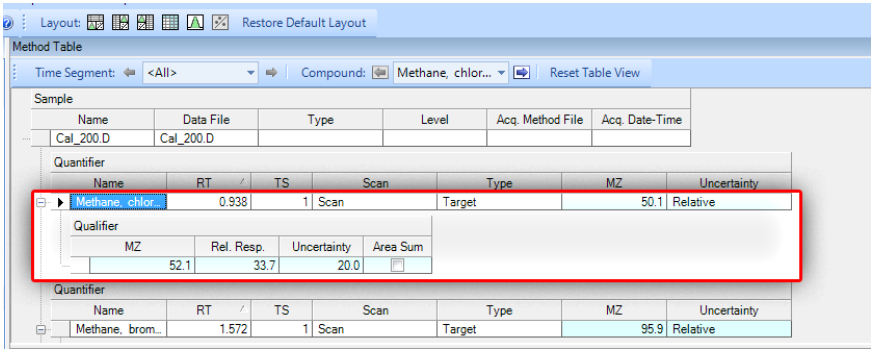
A filled triangle indicates that this sample is selected. The **Sample Information** window displays the chromatogram for this sample. The Compound Information window is blank since no compound in the **Method Table** is selected.



7 Click in the **methane, chloromethane** quantifier name field.

A filled triangle indicates that this compound is selected. When this compound is selected, its peak is highlighted in the **Sample Information Chromatogram** pane and is also displayed in the **Chromatogram** and **Spectrum** pane.

8 Look at the compound's qualifiers. The compound's qualifiers are shown in the **Method Table** directly below the **Quantifier** entry. For the quantifier we are using, you can see that MassHunter selected only a single qualifier.

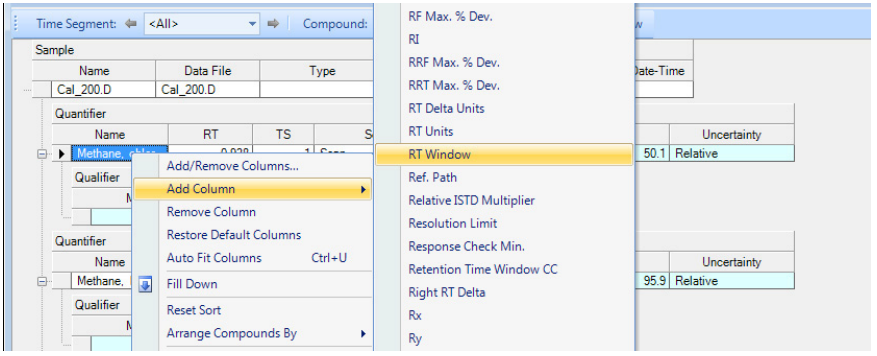


9 Add **Retention Time** to the **Method Table**. Since these are known compounds that were added to the calibration sample, we need to verify each name entry, the target ion, the qualifier ions, and the relative response of each qualifier ion.

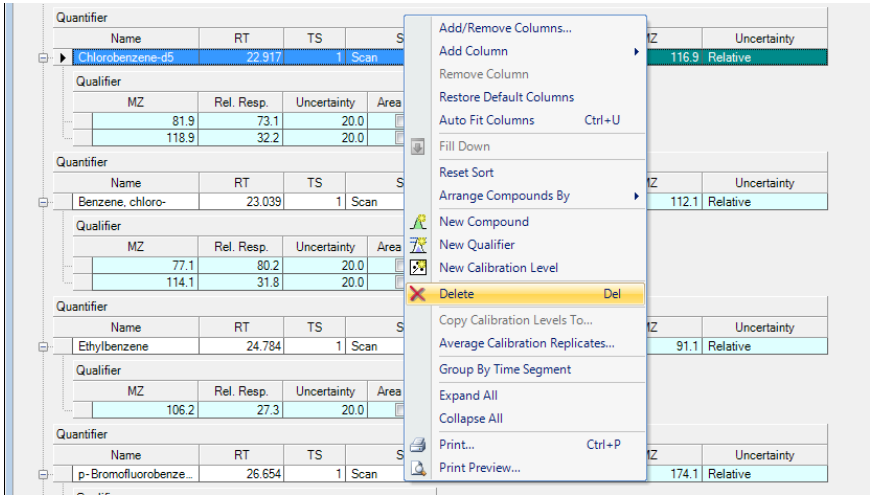
Click the **Maximize Table** icon .

On the **View** tab, select **Maximize Pane** > **Maximize Table**.

10 Right-click any quantifier entry and select **Add Column > RT** to add the **Retention Time (RT)** column to the **Method Table**.



11 For this example, we know that the compounds Acetonitrile at RT 2.678, Toluene-D8 at RT 21.71, and Chlorobenzene-d5 at RT 22.91 were not added calibration compounds, so delete them by first selecting the compound and then pressing the **Delete** key.

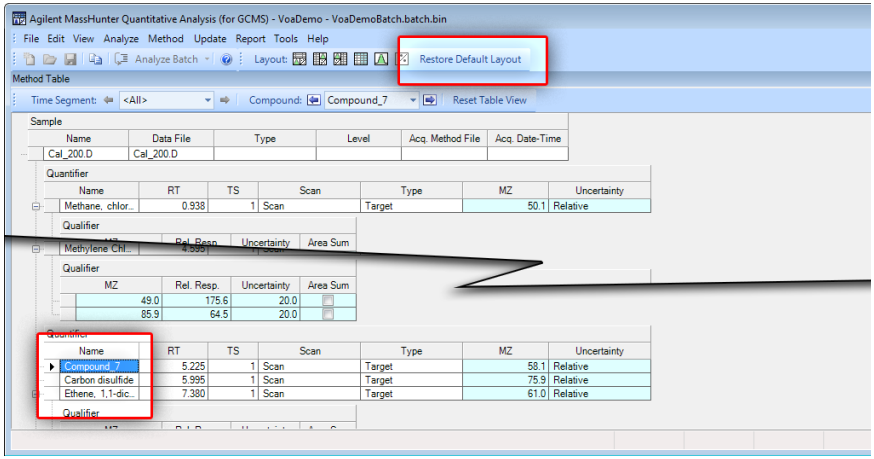


12 Use the **Library Search** feature to try to identify the unknown compound.

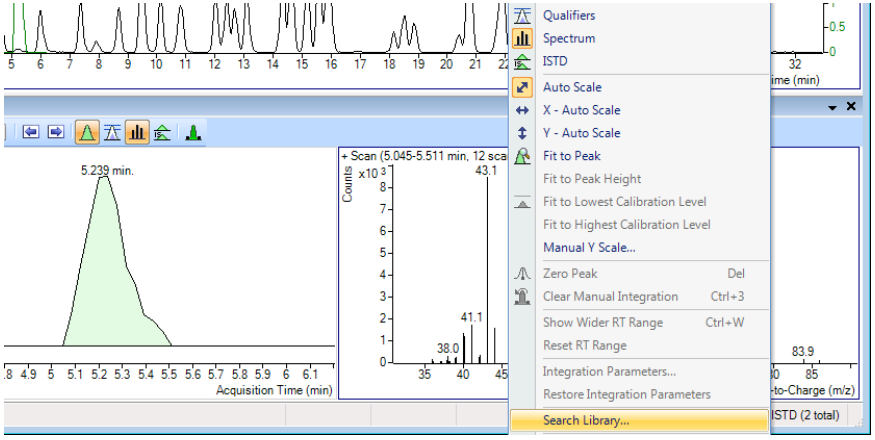
MassHunter could not identify the compound it gave the placeholder name Compound_7. We were expecting Acetone here from our calibration sample.

a Select **Compound_7**.

b Click **Restore Default Layout**.



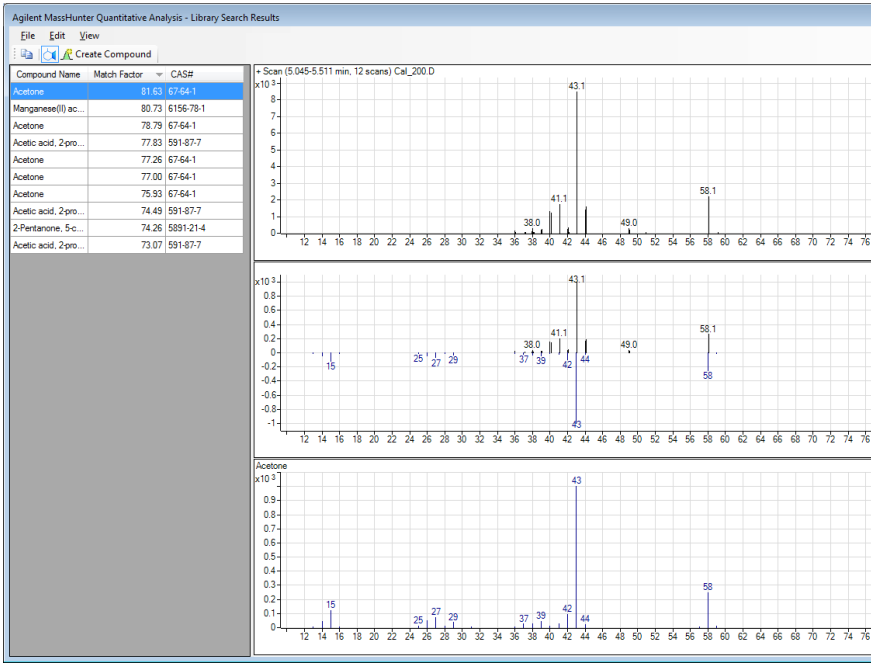
c Right-click inside the **Compound Information** window **Spectrum** pane, and select **Search Library**.



d When prompted, select your library. Here we are using the NIST05 library.

e Click **Open** to display the **Library Search Results** window.

This compound was identified as **Acetone**, and we see that the acetone spectrum (lower graph) has a target peak at 43 m/z and a qualifier peak at 58 m/z. The upper graph shows the spectrum in our calibration sample, and the center graph compares both showing a good match.



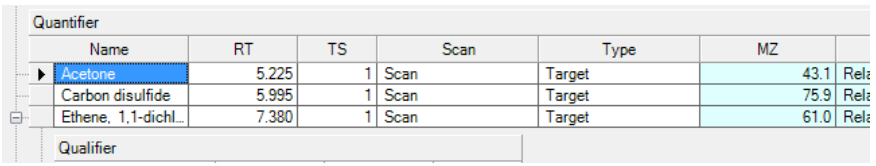
f Record the **Match Factor** value of 81.63, and CAS# of 67-64-1 for future use.

g Close the **Library Search Results** window.

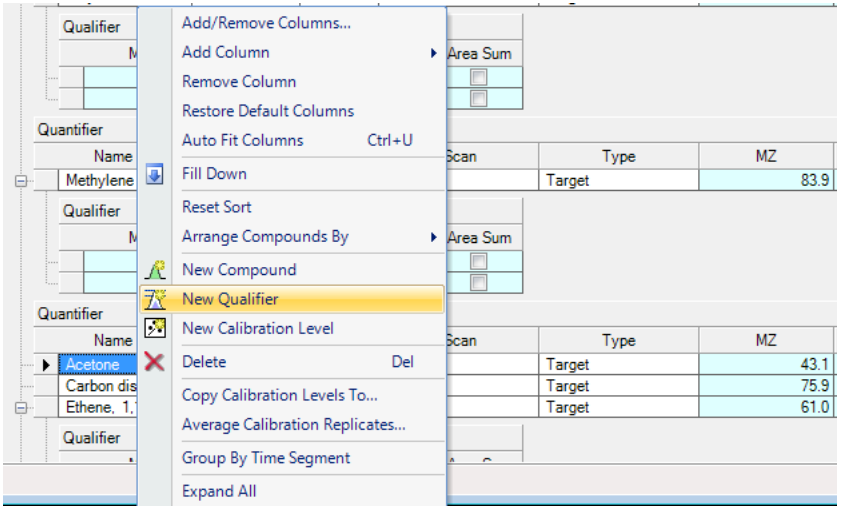
h Click **Maximize Table** .

On the **View** tab, select **Maximize Pane** > **Maximize Table**.

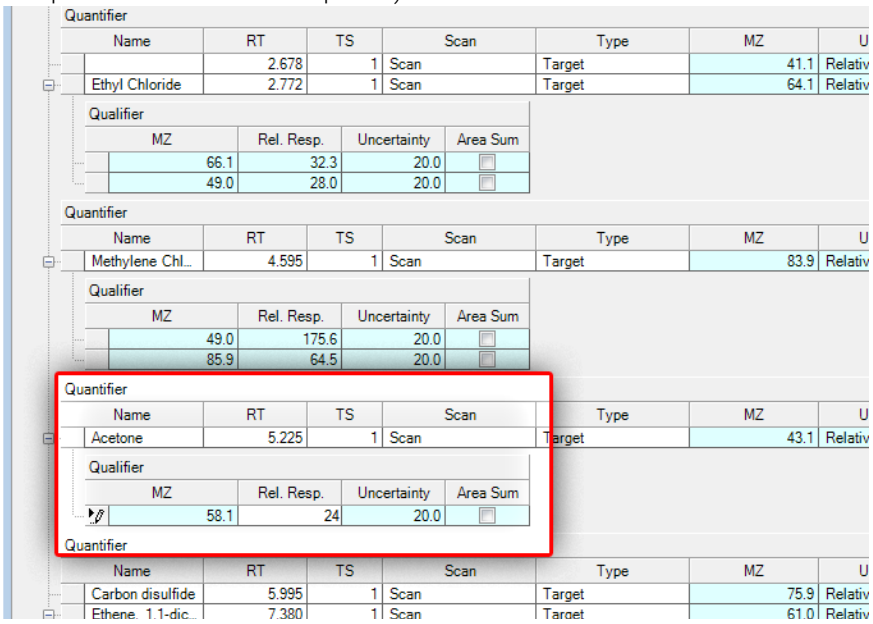
13 Change the Compound_7 **Name** parameter to **Acetone**, and change the Acetone **MZ** to 43.1. The 58.1 identified as the quantifier is actually the qualifier.



14 Add **Acetone** as a qualifier by right-clicking **Acetone** and selecting **New Qualifier** from the context menu. The qualifier is entered below the acetone quantifier.



15 Enter **58.1** as the qualifier **MZ** and **24** as the **Rel. Resp.** (= 2.2/9 from the samples 58.1 vs 43.1 ion response).

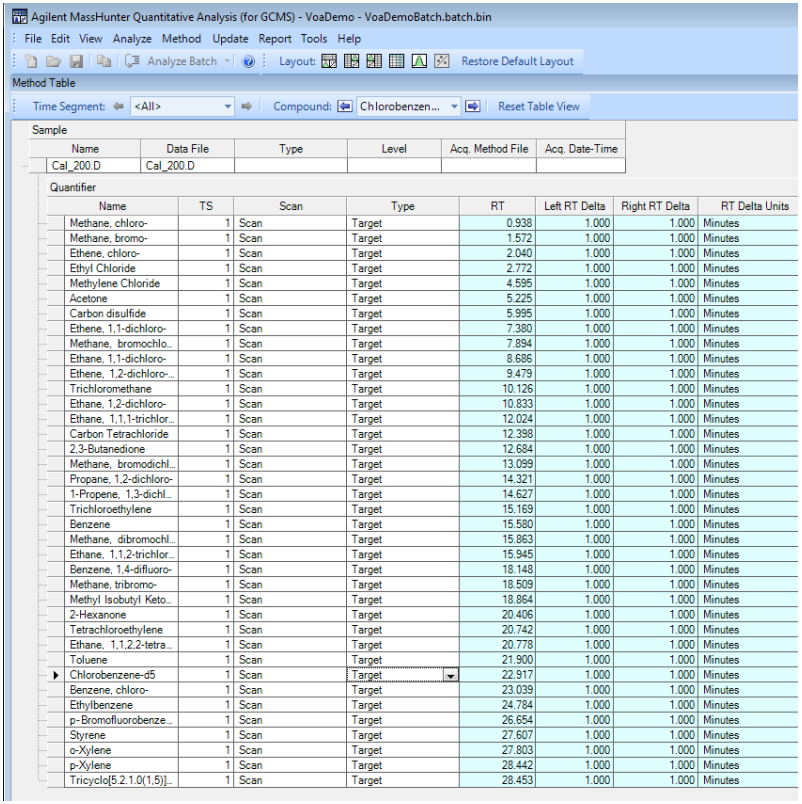


Continue to review all compounds and their qualifiers identified in the **Method Table**.

16 Review the Retention Time.

a Click **Restore Default Layout**, then, from the **Method Tasks** area, select **Method Setup Tasks > Retention Time Setup**.

b Click **Maximize Table** to view all compounds sorted by **RT**.



By default, the Left RT delta and Right RT Delta create a window 2 minutes wide centered around the RT specified here. Edit this window size and RT if necessary.

17 Identify the ISTDs added to the sample. No ISTDs are yet assigned to compounds.

a The sample data file contains Bromochloromethane, 1-4 Difluorobenzene, and Chlorobenzene-d5 ISTDs.

Select **Method > Method Setup Tasks > ISTD Setup** to access the ISTD Setup without exiting the **Maximum Table** view.

b For these three ISTDs, check the **ISTD Flag**, and note that checking this sets the **Type** to **ISTD**. Clearing the check box requires manually setting the **Type** to **Target**.

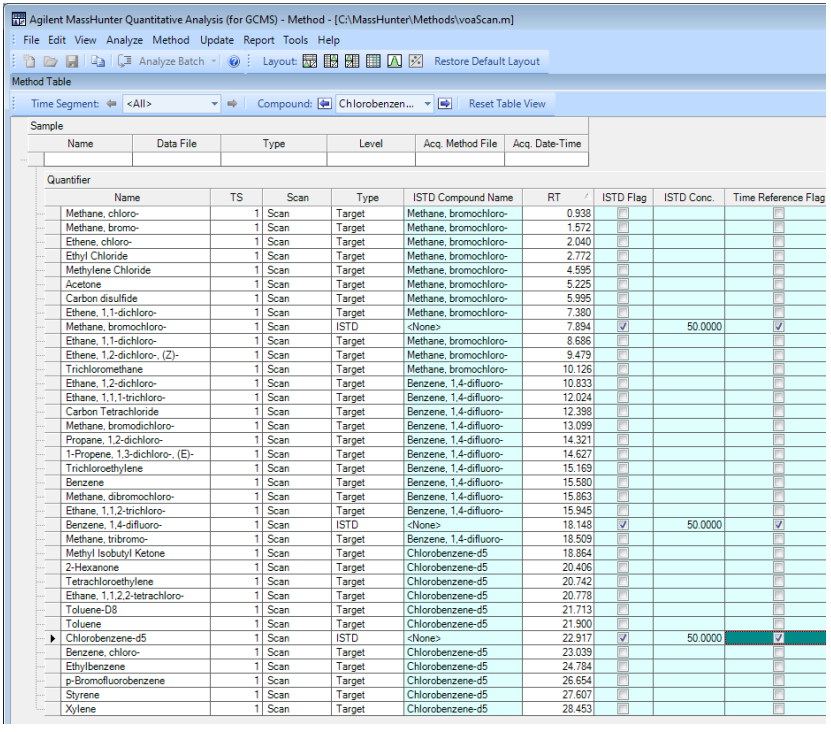
c For these three ISTDs, also check the **Time Reference Flag**. This specifies that the actual-to-expected time of the ISTD is used as a multiplier of the RT of all target compounds assigned to the ISTD.

18 Assign the **Methane, Bromo-chloromethane** ISTD to the calibration compounds in the RT range of 0.9 to 10.2. To do this, right-click the ISTD **Compound Name** for the first compound, and select **Fill Down** from to copy this ISTD to all compounds below it. When you are using the Fill Down option, you must change the ISTD **Compound Name** to **<none>** for overwritten ISTDs.

19 Assign the **1-4 Difluorobenzene** ISTD to the calibration compounds in the RT range of 10.8 to 18.5.

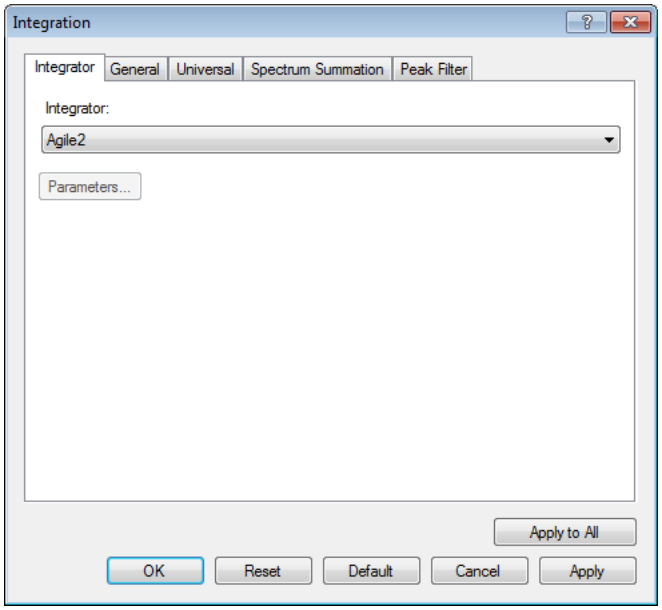
20 Assign the **Chlorobenzene-d5** ISTD to the calibration compounds in the RT range of 18.8 to 29.

Your screen should now look like this.



21 Select **Method > Advanced Tasks > Integration Parameters Setup**. By default, the **Integrator** is set to **Agile2**. This is a parameter-less integrator that is recommended for MS-MS data. Since we are integrating GC/MS single quad data we will select a more suitable integrator.

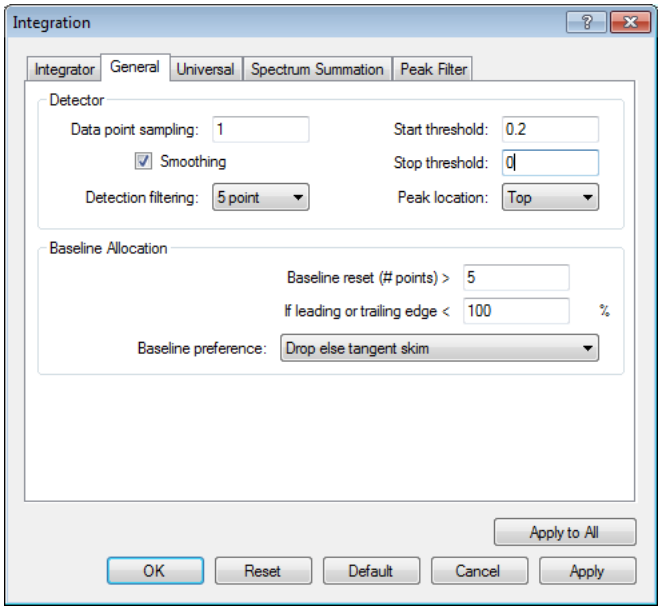
In the **Int.** column for the first compound in the **Method Table**, click the selection box. The **Integration** dialog box is displayed. **Agile2** is shown as the **Integrator** in our example.



22 Select **General** from the **Integrator** drop-down list. This integrator is similar to the Genie ChemStation integrator optimized for GC/MS integration.

23 Click **Parameters** to open the **General** tab.

24 Edit the settings to match the dialog below.



25 Click **Apply to All** and this integrator, with these parameter settings, is copied to every compound in the table. Click **OK** to close the dialog box.

Task 4. Add a Calibration Curve

1 Setup concentration levels for calibration compounds.

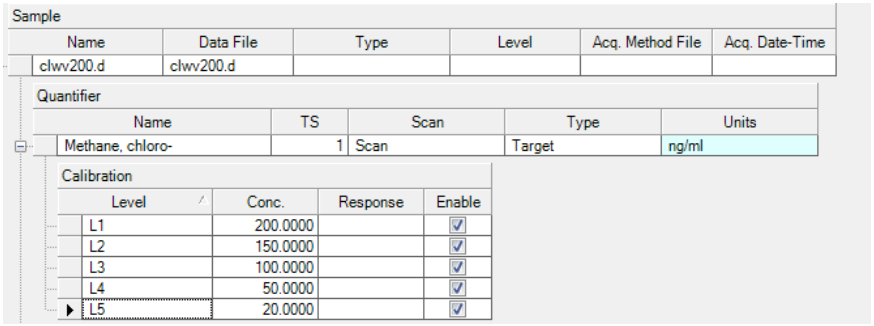
a Select **Method > Method Setup Tasks > Concentration Setup**.

b Select the first target compound in the table, then right-click and select **New Calibration Level**. A **Calibration** table with a single level is created below the **Quantifier** table.

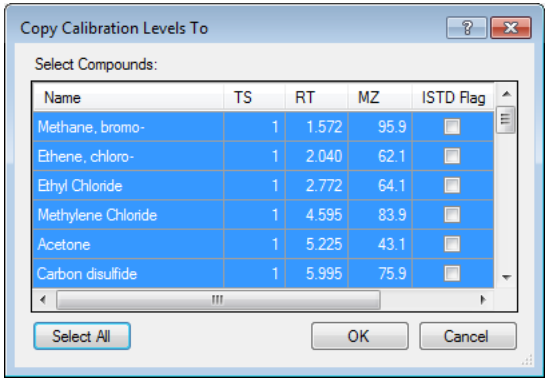
c Add four more levels to this table.

d In the **Level** column, add the names **L1**, **L2**, **L3**, **L4**, and **L5**.

e In the **Concentration** column, add the numbers 200, 150, 100, 50, and 20. Refer to the following figure.



2 Right-click in the **Calibration** table, and select **Copy Calibration Levels To**.

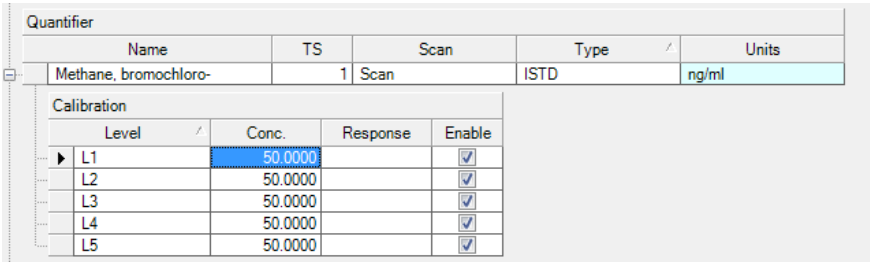


3 Click **Select All**, and then click **OK** to copy the **Calibration** table to all target compounds.

4 Setup concentration levels for the three internal standards.

a In the **Quantifier** table, click the **Type** column header to sort the table. The three ISTDs go to the top of the **Method Table**. Note that levels were not added to the ISTDs.

b Add a **Calibration** table with five levels to the first ISTD. To do so, select the ISTD, then right-click and select **New Calibration Level**. Repeat the process four more times. Label the levels L1 through L5, as before, and specify a concentration of 50 for all levels.



c Repeat this process to add an identical **Calibration** table to the other two ISTDs.

5 Setup the calibration curve.

a Select **Method > Method Setup Tasks > Calibration Curve Setup**.

b Select the first compound in the table.

c From the **CF** drop-down list, select **Average of Response Factors**. This works well for our data.

d Right-click on this last entry, and select **Fill Down**. The default curve fit (CF) parameters displayed are

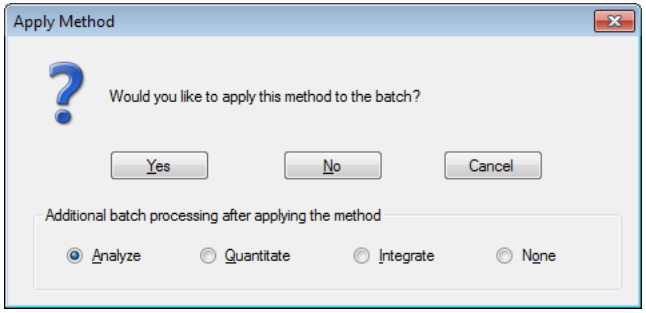
- **Ignore** under the **CF Origin** column
- **None** under the **CF Weight factor** column

Keep these settings for our example.

6 Select **Method > Exit**.

7 Select **Analyze** to analyze the entire batch after applying this new method to the batch.

8 Click **Yes** to begin analysis using this method. You will exit the **Method Editor** view and enter the batch analysis view.



Since the Data Acquisition sequence specified that the Response Factors of the compounds in each calibration sample are to replace the response factors in the quantitation method, this analysis of the batch will populate the calibration tables with the compound responses.

9 Review the calibration curve.

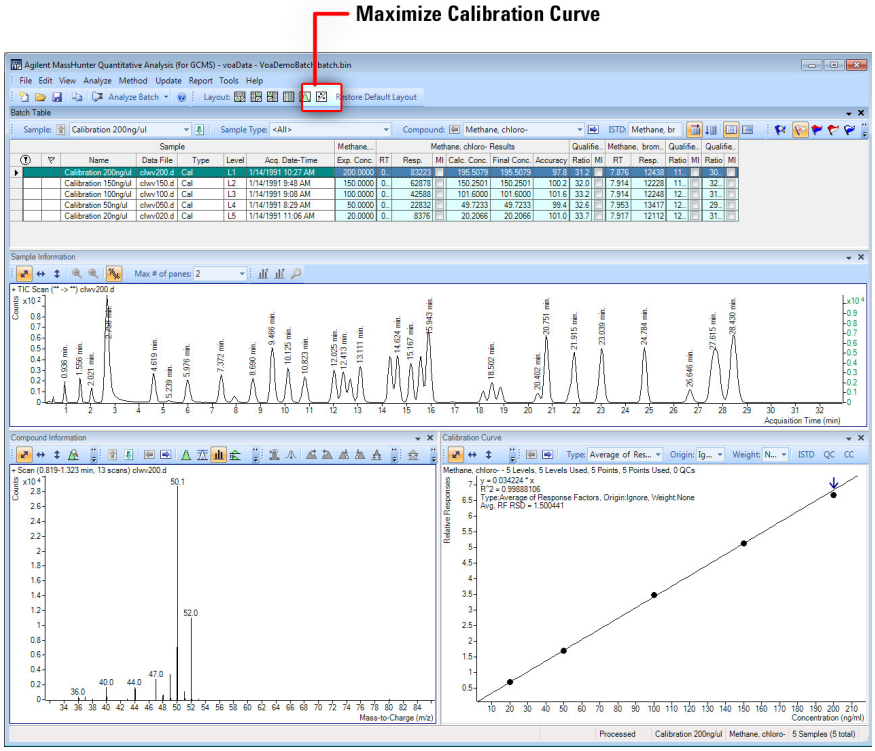
a Select the first sample in the table and notice that the **Calibration Curve** window shows the curve created by the 5 concentration levels.

b Right-click the **Sample** column header in the **Batch Table**, and select **Auto Fit Columns**.

10 Click the **Maximize Calibration Curve** icon.

On the **View** tab, select **Maximize Pane** > **Maximize Calibration Curve**.

The black line represents the curve fit (CF) that we previously applied to all compounds in the method. Its parameters are in the upper left part of the plot and are also in black. The first line of information identifies the compound name, the number of levels and the points used in the CF equation.



11 Click the right arrow icon in the **Calibration Curve** toolbar, several times, to select the **Ethyl Chloride** compound.

The L5 level calibration point is not located on the curve. Let's see if we can assign a different CF that will allow all 5 points to be included on the curve.

12 Explore the Curve Fit Assistant.

a Right-click in the **Calibration Curve** window, and select **Curve Fit Assistant**. This opens the **Curve Fit** table. The first line in the table should be selected. The colored line represents the curve for the CF

selected in the **Curve Fit** table. Its parameters are located below the currently assigned CF and they are colored the same as the curve.

b In the **Curve Fit** table, click the **# of Disabled Points** funnel icon, and set the column filter to **0**. All the selections remaining in the table pass through all 5 points.

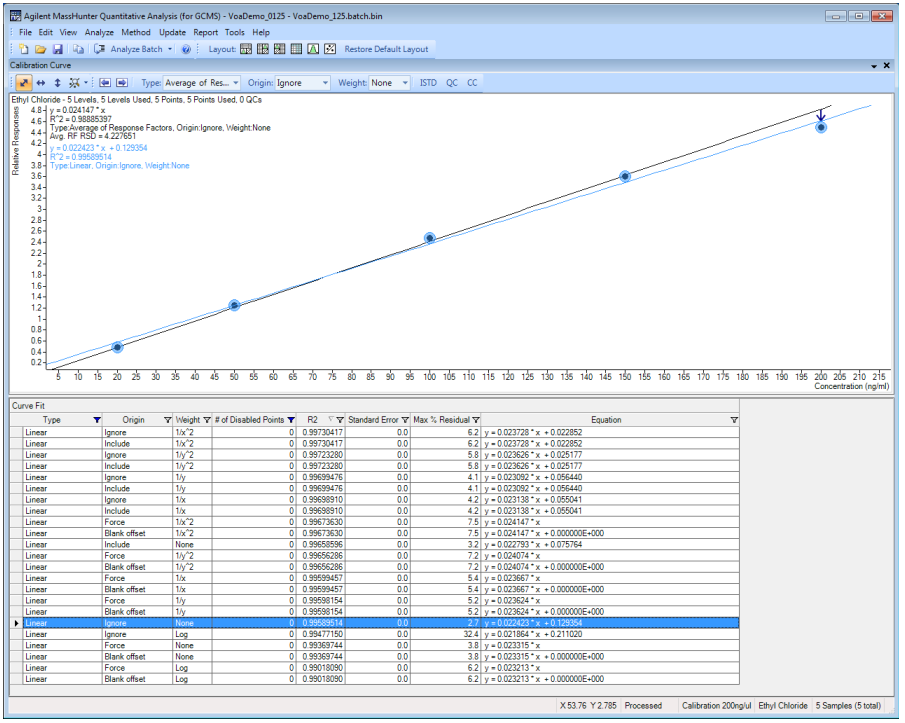
c Sort on the **R2** column so that the first CF in the table has the value closest to 1.0. Select this line to see how the curve goes nicely through all 5 points. This is a quadratic with weighting and we want to see if something simpler will work.

d Click the **Type** funnel icon, and set the column filter to **Linear**. We are skipping the simple Average of Response Factors since it isn't a very good fit at higher concentrations.

e Select various rows, and observe the colored curve. A simple linear curve with ignore origin and equal weighting is a good fit.

f Select **Ignore** from the **Origin** drop-down list, and select **None** from the **Weight** drop-down list.

g With this line in the **Curve Fit** table selected (filled triangle icon), right-click in the **Calibration Curve** window, and select **Accept Assistant Curve**.



h Click the **Go to Next Compound** arrow icon in the **Calibration Curve** toolbar.

i Repeat this curve fit review process until you are satisfied with the curve fit for all calibration compounds.

j Right-click and select **Curve Fit Assistant** to exit the assistant.

13 Review the curve fit changes in the **Method Editor**.

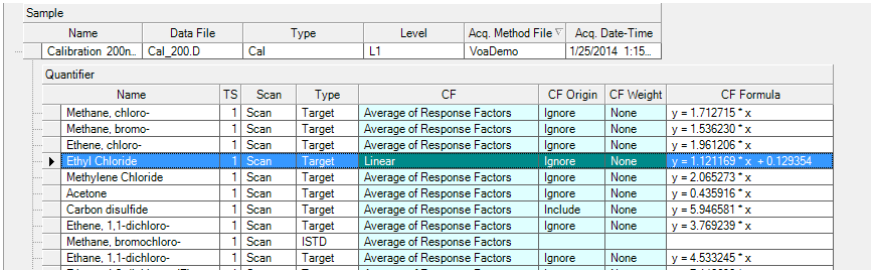
a Select **Method > Edit** to enter the **Method Editor** view.

b Select **Method > Method Setup Tasks > Calibration Curve Setup**.

c Right-click the **Quantifier** table label, and select **Add/Remove Columns**.

d Add the **CF Formula** column to view the CF equation (selected). The Curve Fit Assistant replaced the Average of Response Factors originally

specified by this method with the Linear CF parameters and CF Formula highlighted below.



Sample

Name	Data File	Type	Level	Acq. Method File	Acq. Date-Time
Calibration 200n..	Cal_200.D	Cal	L1	VoaDemo	1/25/2014 1:15..

Quantifier

Name	TS	Scan	Type	CF	CF Origin	CF Weight	CF Formula
Methane, chloro-	1	Scan	Target	Average of Response Factors	Ignore	None	y = 1.712715 * x
Methane, bromo-	1	Scan	Target	Average of Response Factors	Ignore	None	y = 1.536230 * x
Ethene, chloro-	1	Scan	Target	Average of Response Factors	Ignore	None	y = 1.961206 * x
Ethyl Chloride	1	Scan	Target	Linear	Ignore	None	y = 1.121169 * x + 0.129354
Methylene Chloride	1	Scan	Target	Average of Response Factors	Ignore	None	y = 2.065273 * x
Acetone	1	Scan	Target	Average of Response Factors	Ignore	None	y = 0.435916 * x
Carbon disulfide	1	Scan	Target	Average of Response Factors	Include	None	y = 5.946581 * x
Ethene, 1,1-dichloro-	1	Scan	Target	Average of Response Factors	Ignore	None	y = 3.769239 * x
Methane, bromochloro-	1	Scan	ISTD	Average of Response Factors			
Ethane, 1,1-dichloro-	1	Scan	Target	Average of Response Factors	Ignore	None	y = 4.533245 * x

e Select **Method > Save As**. Navigate to where you wish to save the method. Give the method a name. We used **Voa** for our example.

f Select **Method > Exit** to enter the batch analysis view.

g Save the batch and exit MassHunter.

Task 5. Add a New Compound to a Method

Once your method contains calibration compound data, you must add new compounds to the method by using an append function or by adding the individual compound to the method manually. The method append functions are similar to what we have covered in previous tasks and are covered in online help. The following describes how to manually add a calibration compound to your method in.

1 Access the method.

   a Select **File > Open Batch**, and select the **VOADemo.Batch.Bin** file. This batch data contains the new calibration compound in all calibration samples.

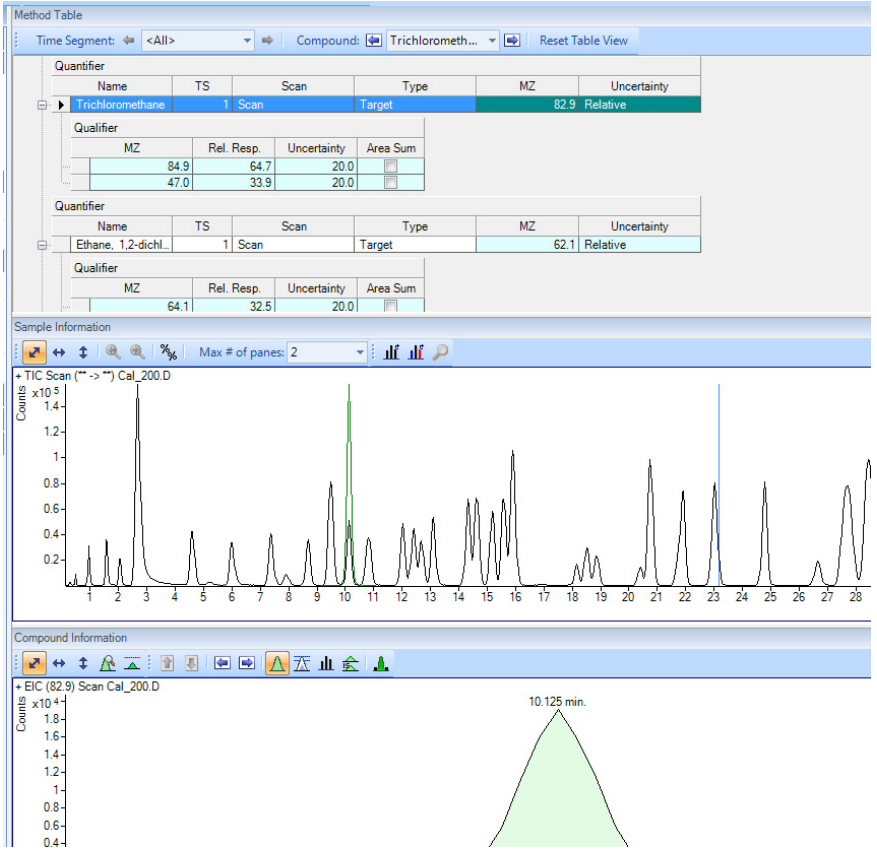
   b In the **Batch Table**, select the **CAL_200** sample, and then select **Method > Edit** to access the **Method Editor**.

2 Prepare the method data for this demonstration

   a Select **Method > Setup Tasks > Qualifier Setup**, and select the compound **Trichloromethane**. We will delete this compound from the method and then add it back into the method manually to demonstrate this task.

   b Before you delete this compound, note the target and qualifier parameters. Also note the compound at RT 10.125 minutes is highlighted in the **Sample Information** and **Compound Information** windows. This is the peak we will be using in this task.

c Right-click the compound selected in the **Quantifier** table, and select **Delete**.



3 Add the compound to the method.

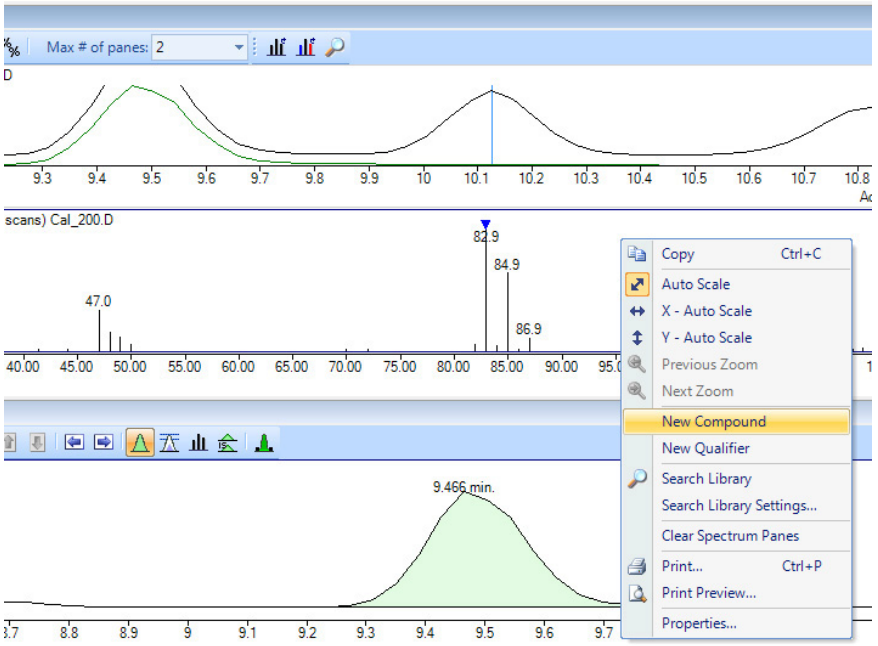
a In the **Sample Information** window, zoom (right-click and drag) between 9 and 11 minutes.

b Click at the peak apex to display a line running through the apex.

c Right-click in the window, and select **Extract Spectrum**. Examine the spectrum, and notice that the ion at **82.9** m/z is the target compound and the qualifiers are **84.9** m/z and **47.0** m/z based on abundance.

d Click on the ion line at **82.9** m/z. A blue triangle at the top of this line indicates the ion is selected. Right-click, and select **New Compound**. The compound is added to the **Quantifier** table in RT order.

e Add **Trichloromethane** to the blank **Name** cell of the **Quantifier** table. Keep this compound selected in the **Method Table** while you add the qualifiers in the next steps.



4 Display the compound's spectrum and add its qualifiers.

a To once again display the spectrum for **Trichloromethane**, click at the peak apex to display a line running through the apex.

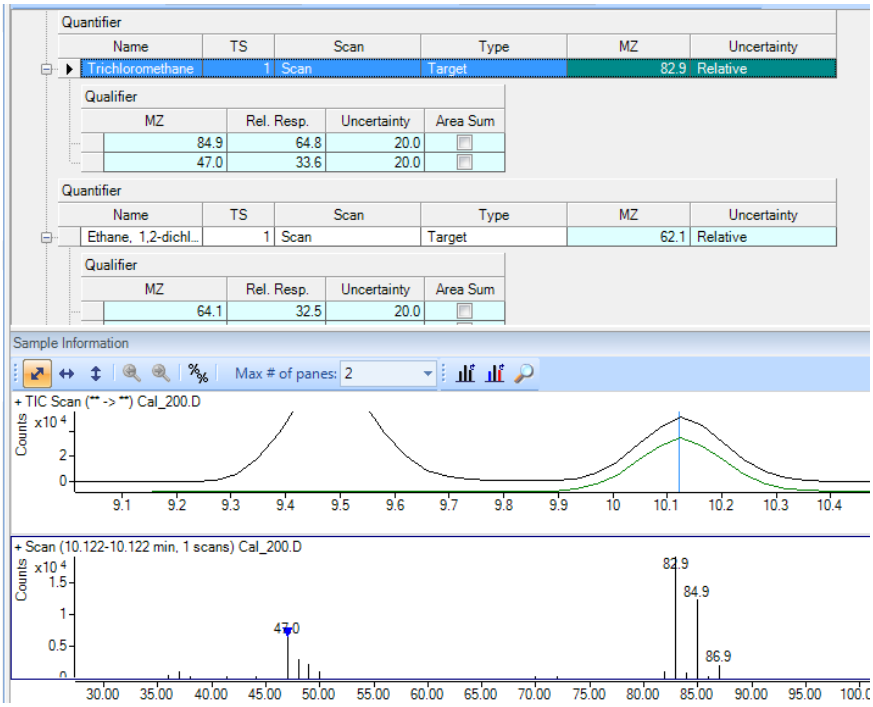
b Right-click, and select **Extract Spectrum**.

c Select the ion at **84.9** m/z (blue triangle) in the spectrum pane, and select **New Qualifier** from the context menu. The ion is added to the **Qualifier** table.

5 Add the second qualifier.

a Add the ion **47.0** m/z to the **Qualifier** table using the same procedure.

b Observe the **Trichloromethane** entry in the **Method Table**. The qualifiers added have their relative response calculated and added to the table. A default Relative Uncertainty of 20% was also added for you.



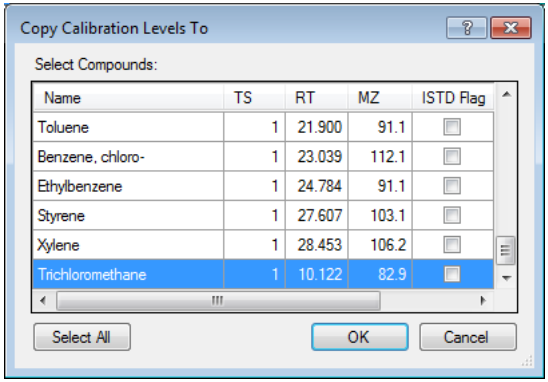
6 Review and update method parameters for this compound.

a In **Method Setup Tasks**, click on every task and notice where the MassHunter default needs to be revised. For instance, the ISTD needs to be assigned for this new compound.

b Select **Method Setup Tasks > Concentration Setup**.

c Right-click an adjacent compound in the **Method Table**, and select **Copy Calibration Levels To**.

d Select **Trichloromethane**, and then click **OK**.



When this batch is analyzed, the copied compound's responses are replaced with those from the Trichloromethane samples in the batch.

Task 6. Convert a Scan Method to a SIM Method

When using single quad data, you can create a SIM method from a scan method by changing a single method parameter and making a sequence table calibration run that replaces all Response Factors for target compounds and Qualifier Ratios.

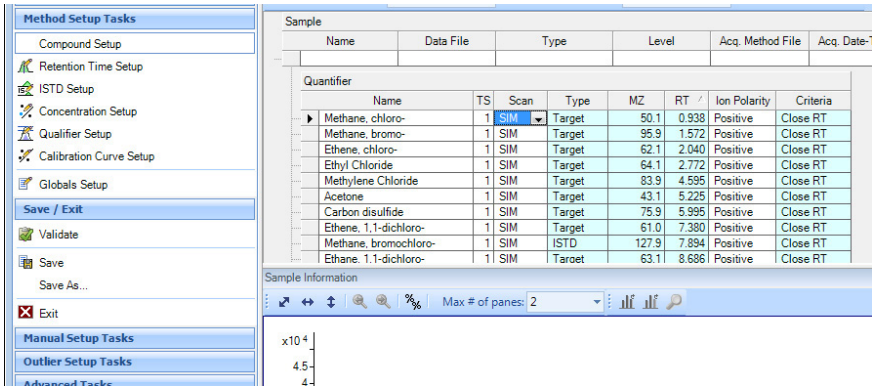
1 Edit the method.

a Select **Method > Open > Open Method From Existing File**, and select the method to convert. Here we are using **VoaDemo.M**.

b In **Method Setup Tasks**, click **Compound Setup**.

c In the table's first compound's **Scan** column, select **SIM** from the drop-down list.

d Right-click, and select **Fill Down** to copy **SIM** to all compounds in the table.



2 Select **Method > Save As**, and save this method to the same method location that you are using for SIM data acquisition.

3 Create a sequence that replaces the method response factors and qualifier ratios.

a Create a **Sequence** table that runs a SIM data acquisition for the calibration level samples that are used by your quantitation method's calibration curve.

b Set the method for the **Sequence** table to the SIM acquisition method that also contains the quantitation method created here.

c Set the **Sequence** table to replace the response factors for all target compounds and qualifier ratios.

4 Review the calibration curves.

a In MassHunter Quantitative Analysis, open the batch and **Analyze** the calibration data.

b Review the calibration curves using the curve fit assistant.

c When your review of the method parameters is complete, save the quantitation method back to the SIM data acquisition file.

The method is now ready to process SIM data acquisition samples for these compounds.

Creating SIM Methods

Creating a SIM quantitation method is similar to creating a scan quantitation method. An overview of the SIM method creation procedures follows.

From acquired SIM data

During the process of creating a quantitative analysis SIM method, you must run the SIM calibration samples and acquire the response data needed to calculate the calibration curves, as discussed in **"Task 4. Add a Calibration Curve"** on page 30. This presents an opportunity for automating the process. In MassHunter data acquisition, the SIM method created in the **Single Quadrupole MS Method Editor** contains the time segments, ion m/z, and optional compound name. MassHunter can use this data acquired for the calibration batch to populate this compound information in the quantitation method when you select **Method > New Method > New Method from Acquired SIM Data**. It can also use all the calibration samples in the batch to create the method's calibration tables and calculate the calibration curves based on its default curve types. After that is completed, the rest of the process involving setting the quantitation method parameters is similar to tasks presented in this exercise for Scan method.

One compound at a time

The procedure for adding a single compound to a SIM method is similar to the scan procedure covered in **"Task 5. Add a New Compound to a Method"** on page 37.

From a SIM method

New compounds can be added to an existing SIM method. To do this you would create an acquisition method and sequence containing only the new compounds. This would create a **Batch Table** in Quantitative Analysis containing the calibration data for the new compounds. You would then use the **Method > Append > Append Method from Acquired SIM Data** to automate the process of adding the new compounds to an existing quantitation method.

From a Scan method

The procedure for converting a scan method to a SIM method was presented in the previous task **"Task 6. Convert a Scan Method to a SIM Method"** on page 42.

This concludes the exercise on creating a new method from acquired scan data. Continue reading the next section for information on reviewing your quantitation results.

2 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, and export your data to Microsoft Excel.

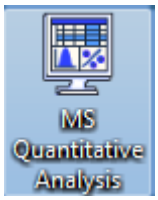
The **VoaSampleData** batch is used in this exercise. This data is located on the Agilent GC/MS Software Information memory stick along with the 5973/5975 and 5977 GC/MS Instrument User Information.

Copy this data folder to the **MassHunter\GCMS\1\data** folder on your PC.

Task 1. Navigate the Batch Table Results

This task shows you how to browse 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.

1 To start the **Quantitative Analysis** program, double-click the **MS Quantitative Analysis** icon on your desktop.



2 Click **Restore Default Layout** on the toolbar.

On the **Home** tab, click **Restore Default Layout**.

3 Right-click the **Sample** column header in the **Batch Table**, and select **Restore Default Columns**.

4 Click **Open Batch**  on the toolbar.

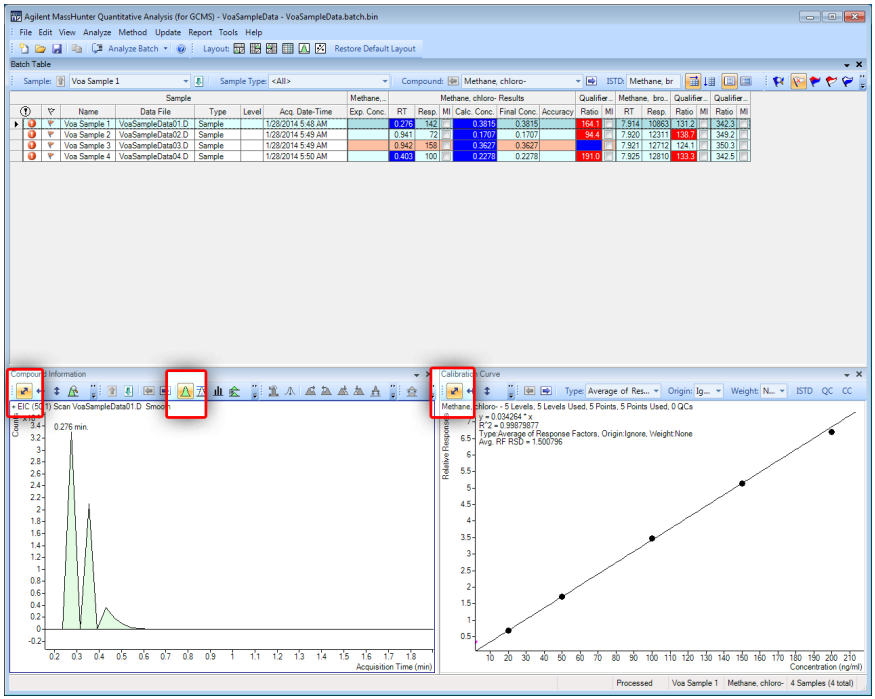
On the **Home** tab, click **Open Batch**.

5 Navigate to the directory **MassHunter\GCMS\1\data\VoaSampleData** and double-click **VoaSampleData.batch.bin**. The main view that appears should look like the one below. This is the default layout and contains the default column settings.

The default layout is set at the factory and cannot be changed. If you want to create your own layout, see **"Task 2. Change the Main Window Layout"** on page 50.

6 In the **Compound Information** toolbar, select the **Turn on/off Autoscale** and **Show/Hide Chromatogram** icons.

7 In the **Calibration Curve** toolbar, select the **Turn on/off Autoscale** icon.



8 Select **View > Sample Information** to display a chromatogram of the sample currently selected in the **Batch Table**. The selected sample is noted by a filled triangle in the far left column of the table.

On the **View** tab, select **Panes > Sample Information**.

9 Ensure that the **Turn on/off Autoscale** and **Normalize Each** are the only icons selected in the **Sample Information** window.

10 Use the **Next Sample** icon in the **Batch Table** standard toolbar to review the chromatogram of each sample.

11 Review how compounds are simultaneously displayed in the **Batch Table**, **Sample Information** window, and **Compound Information** window.

a Select the **Voa Sample 3** sample in the **Batch Table**.

b Right-click in the **Sample Information** chromatogram, and select **Compound**. This will highlight the compound peak in the chromatogram

for the compound selected from the **Batch Table** or **Compound Information** window.

c Use the **Go to Next Compound** icon in the **Batch Table** toolbar to move through every calibration compound in the method while observing:

- the compound's results columns in the **Batch Table**
- the compound's highlighted location in the **Sample Information** window
- the compound's peak in the **Compound Information** window

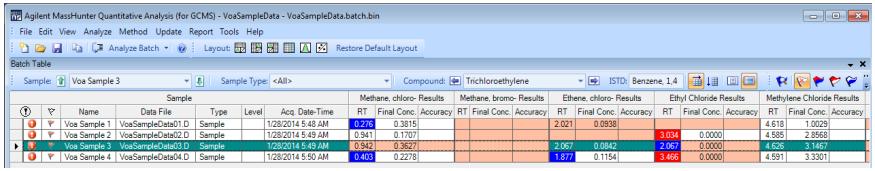
d Use the **Go to Next Compound** icon in the **Compound Information** toolbar to move through calibration compounds like done in the previous step.

12 Display the results for all calibration compounds in the **Batch Table**.

a Click the **Display Multiple Compounds/Samples in Batch Table View** icon in the toolbar to display the quantitation results for all target compounds. You can also click **View > Batch Table Layout > Multiple Compounds/Samples View**.

On the **View** tab, select **Batch Table Layout > Multiple Compounds/Sample View**.

b Note the difference in **RT** in the **Compound Information** window for each compound.



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

On the **View** tab, select **Batch Table Layout > Single Compound/Sample View**.

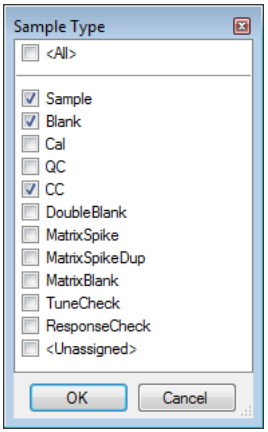
d If necessary, click the **Compound** drop-down list, and select **Cocaine**.

A different set of columns is displayed when you are in the **Multiple Compounds/Samples View** mode versus the **Single Compound View** mode. If you add a column to the table when you are in **Multiple Compounds/Samples View** mode, that change is not automatically made in the **Single Compound/Sample View** mode.

13 Filter the samples displayed in the **Batch Table** by sample type.

a Click the **Sample Type** drop-down list. The **Sample Type** dialog box is displayed.

b Clear the **<All>** check box, and then select the **Sample**, **Blank**, and **CC** sample types.



c Click **OK** to close the dialog, and apply this sample filter.

The **Batch Table** now only displays the Sample, Blank, and CC sample types. Other sample types included with the batch are hidden.

Task 2. Change the Main Window Layout

This task shows you how to rearrange your main window using the toolbar layout icons, add qualifier, spectrum, and ISTS panes to the Compound Information window, save and retrieve custom layouts for the main window, and export data from the batch table to Excel.

1 Use layout icons on the toolbar to position the **Batch Table**, **Compound Information** window, and **Calibration Curve** window.

Use the **Preset Layouts** on the **View** tab to position the **Batch Table**, **Compound Information** window, and **Calibration Curve** window.

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**, then 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 in the toolbar  .

2 Use layout icons on the toolbar to maximize each individual window.

Use the **Maximum Pane** option on the **View** tab to maximize each individual window.

a Click the **Maximize Table** icon in the toolbar  .

b Click the **Maximize Compound Information** icon in the toolbar  .

c Click the **Maximize Calibration Curve** icon on the toolbar  .

d To return to the default layout, click **Restore Default Layout** on the toolbar.

3 Add panes to the **Compound Information** window.

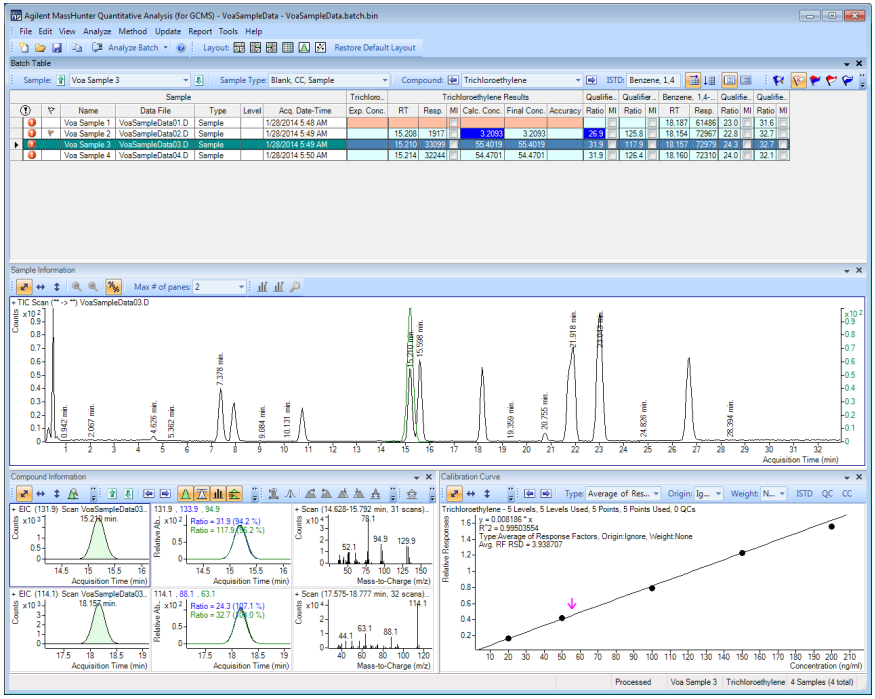
a In the **Batch Table**, select **Voa Sample 3**.

b Select **Trichloroethylene** from the **Compound** drop-down list in the **Batch Table** header.

c In the **Compound Information** toolbar, click the **Show/Hide Qualifiers** icon  .

d Click the **Show/Hide Spectrum** icon  .

e Click the **Show/Hide ISTD** icon  . The layout and results look like those in the following figure.



4 Save the default layout without the calibration curve.

a Close the **Calibration Curve** window.

b Click **View > Window Layout > Save Layout**.

On the **View** tab, select **Load/Save Layout > Save Layout**.

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

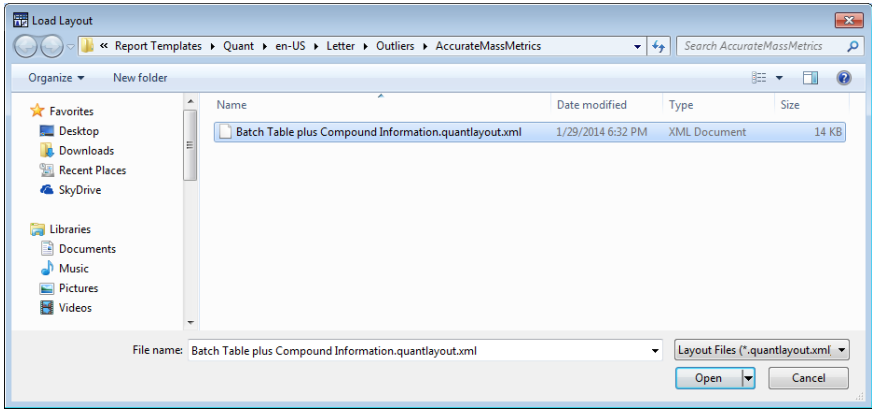
5 Load the newly created layout.

a Click **Restore Default Layout** on the toolbar.

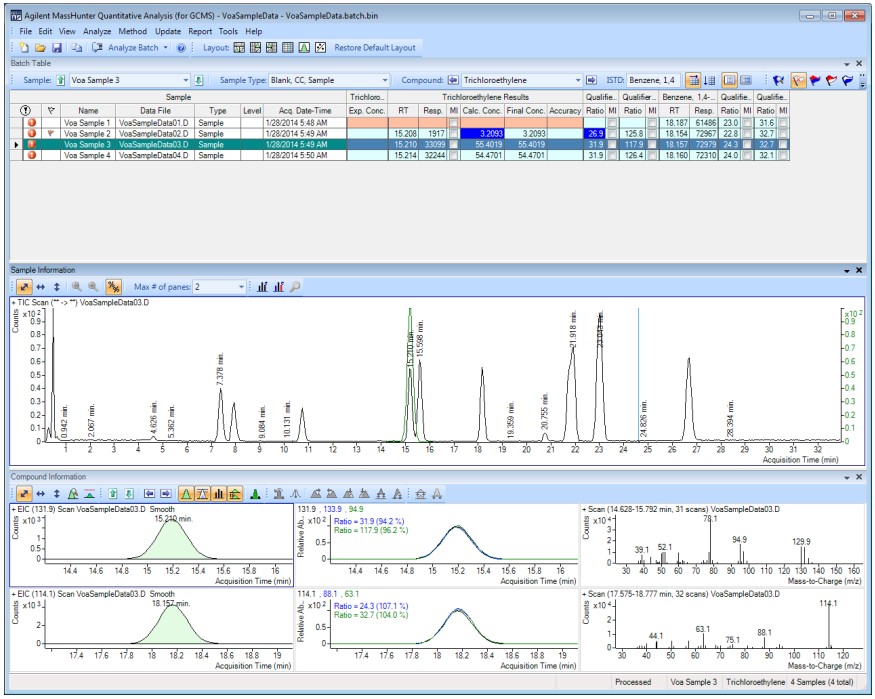
On the **Home** tab, click **Restore Default Layout**.

b Click **View > Window Layout > Load Layout**.

On the **View** tab, select **Load/Save Layout > Load Layout**.



c Select **Batch Table plus Compound Information**, and click **Open**. The main window layout without the **Calibration Curve** window is displayed.



6 Create a custom window layout.

a Click **Restore Default Layout** on the main window toolbar.

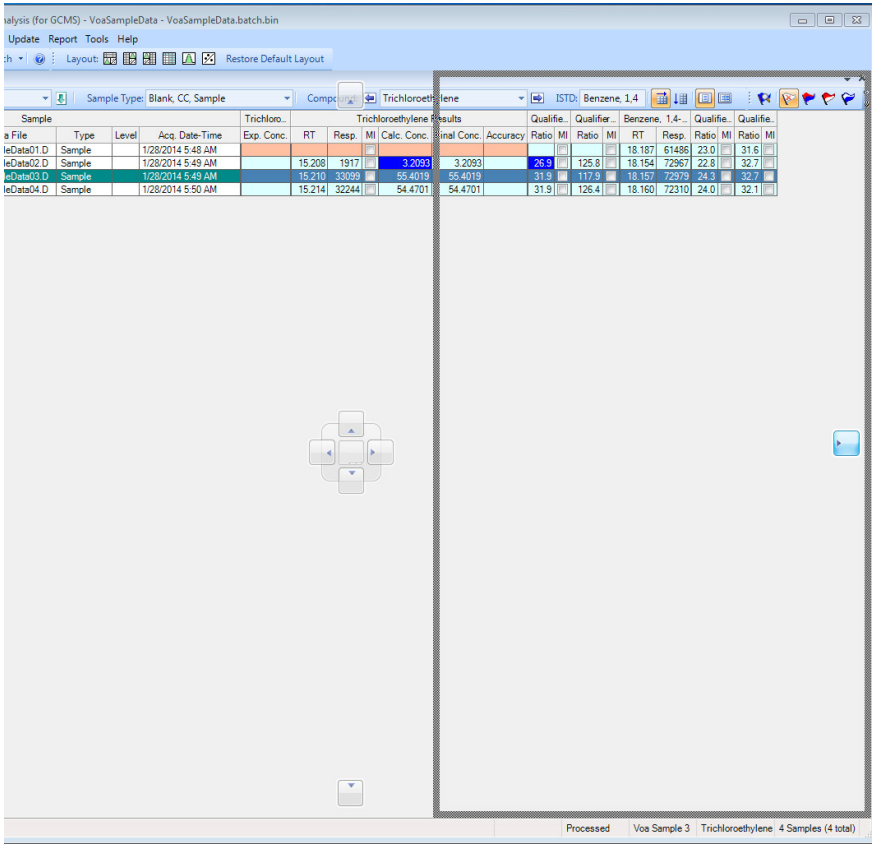
On the **Home** tab, click **Restore Default Layout**.

b To disconnect **Calibration Curve** window from the main window, double-click the title bar, or click the down arrow next to the pane's close icon, and select **Floating**.

c To disconnect the **Compound Information** window from the main window, double-click the title bar, or click the down arrow next to the pane's close icon, and select **Floating**.

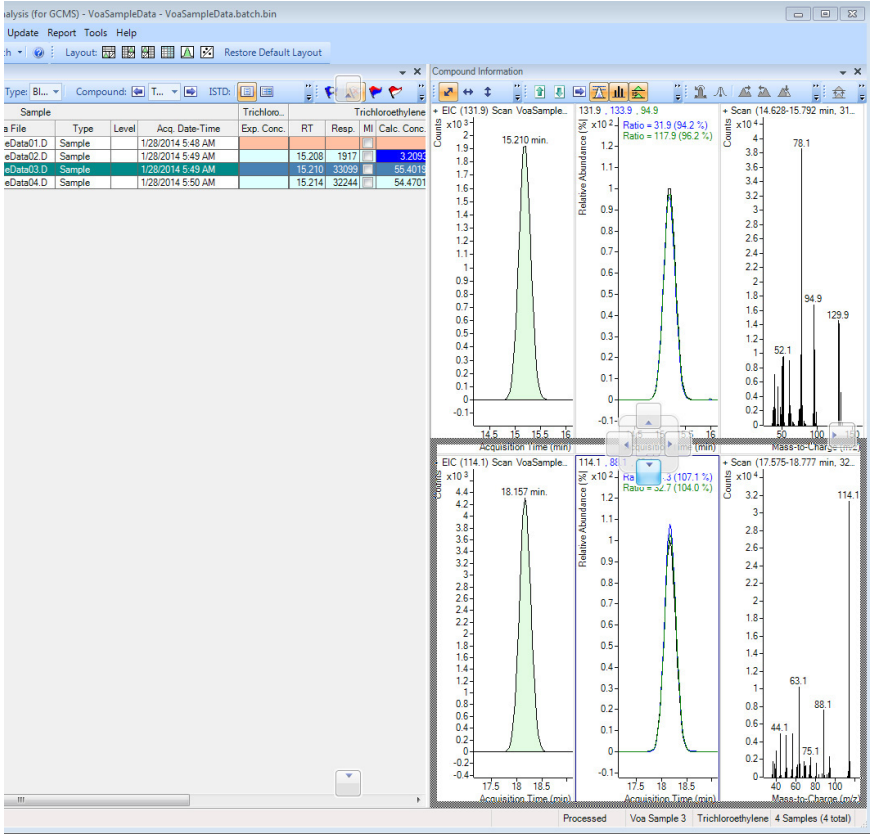
d Drag the **Compound Information** window by its title bar to the right side of the main window. Position the cursor over the right side anchor button, and when the anchor button turns blue and the outline of the main window appear as shown below, release the mouse button. The

Compound Information window is now anchored to the right side of the main window.

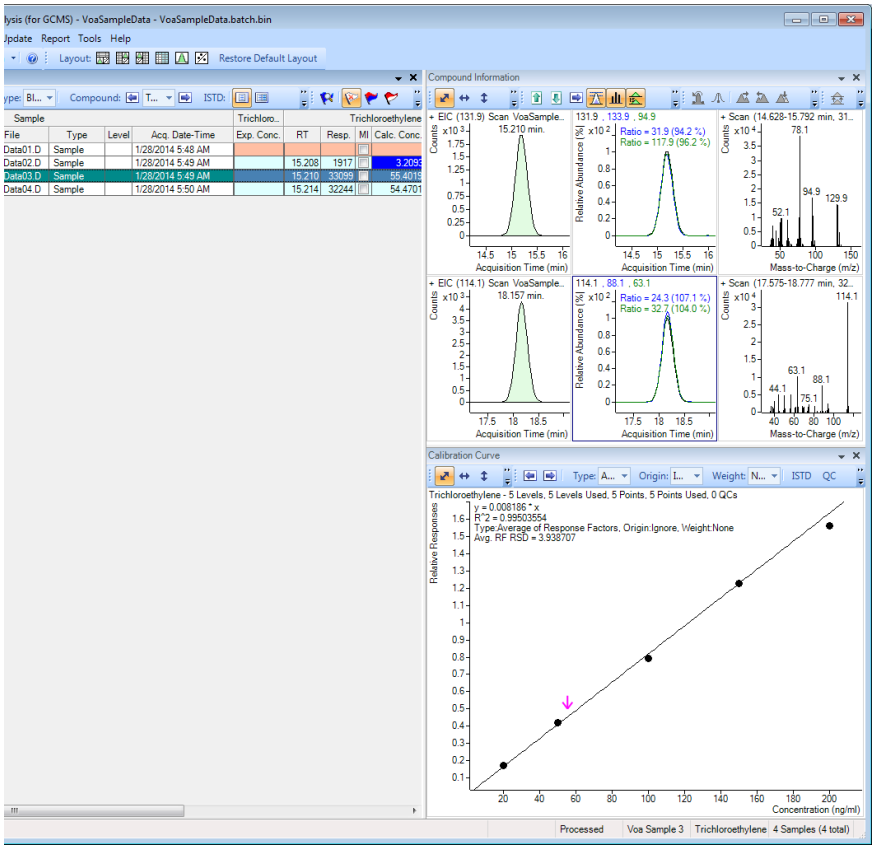


e Drag the **Calibration Curve** window by its title bar to the right side of the main window. Position the cursor over the center-bottom anchor button, and when the anchor button turns blue and the outline of the window appears as shown below, release the mouse button. The **Calibration**

Curve window is now anchored to the lower-right side of the main window.



The custom view is shown in the layout below.



f Click **Restore Default Layout** on the main window toolbar.

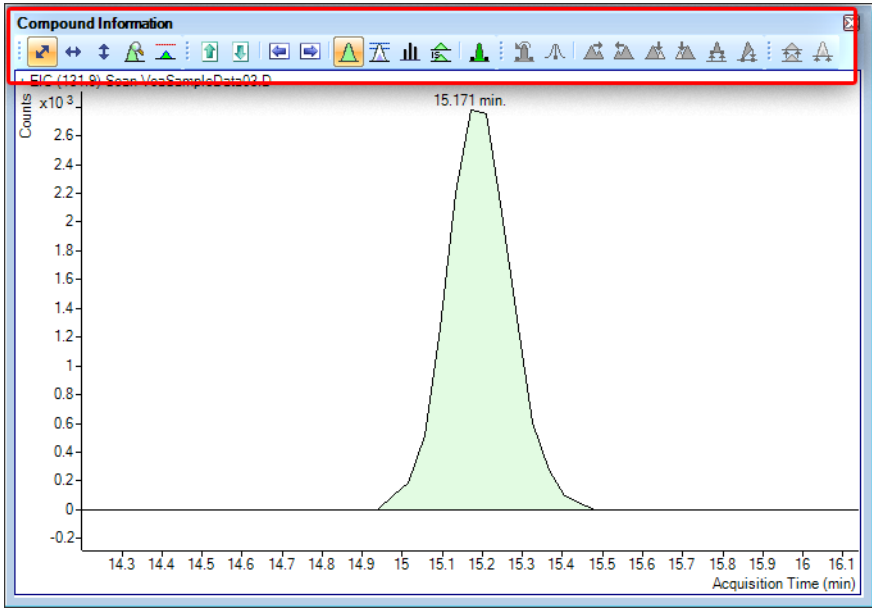
Task 3. Access Integration Parameters

This task shows you how to access the Integration Parameters from the **Compound Information** window.

1 Select **File > Open Batch**, and load **VoaSampleData.batch.bin**.

2 Set up the **Compound Information** window for this exercise.

- **a** Click **Restore Default Layout**.
- **b** Select the **Voa Sample 3** sample in the **Batch Table**.
- **c** Select the **Trichloroethylene** compound in the **Batch Table**.
- **d** Right-click in the **Compound Information** window, select **Properties**, and then click **Default** in the **Properties** window.
- **e** On the **Compound Information (2)** tab, click **Default**, and then click **OK**.
- **f** Position your cursor over the border between the **Compound Information** window and **Calibration Curve** window, and drag the border to the right until all icons are displayed in the **Compound Information** window as shown below.

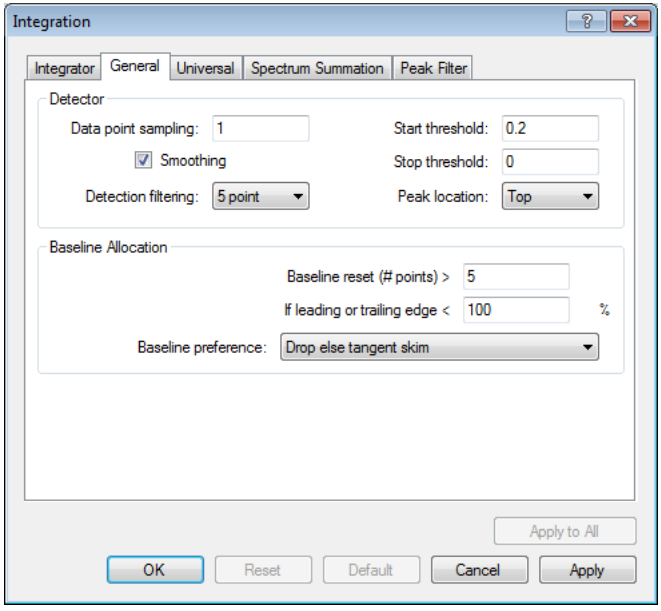


3 Access the Integration Parameter settings for the displayed compound.

a Right-click in the **Compound Information** window, select **Integration Parameters**.

b Click the **General** tab to display the parameters for the **General** integrator.

c The **General** integrator was set for all compounds when we created this method. Use this dialog to change the integrator settings for the selected compound.



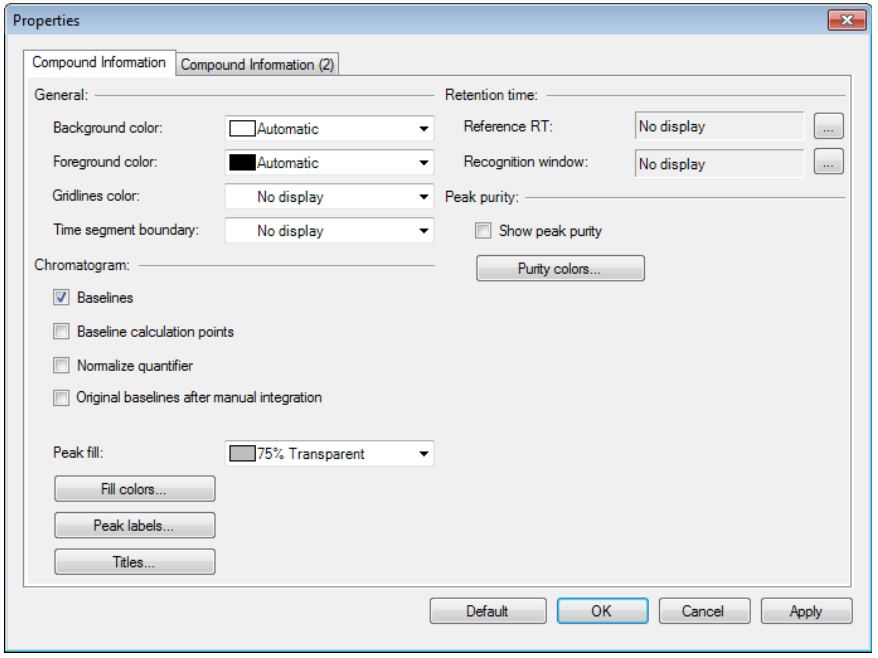
d Click **OK** to close the dialog box.

Task 4. Configure the Settings in the Compound Information Window

This task shows you how to set up the **Compound Information** window for reviewing integration results. It assumes that the defaults were set up in the previous task.

1 Right-click in the **Compound Information** window, and select **Properties**.

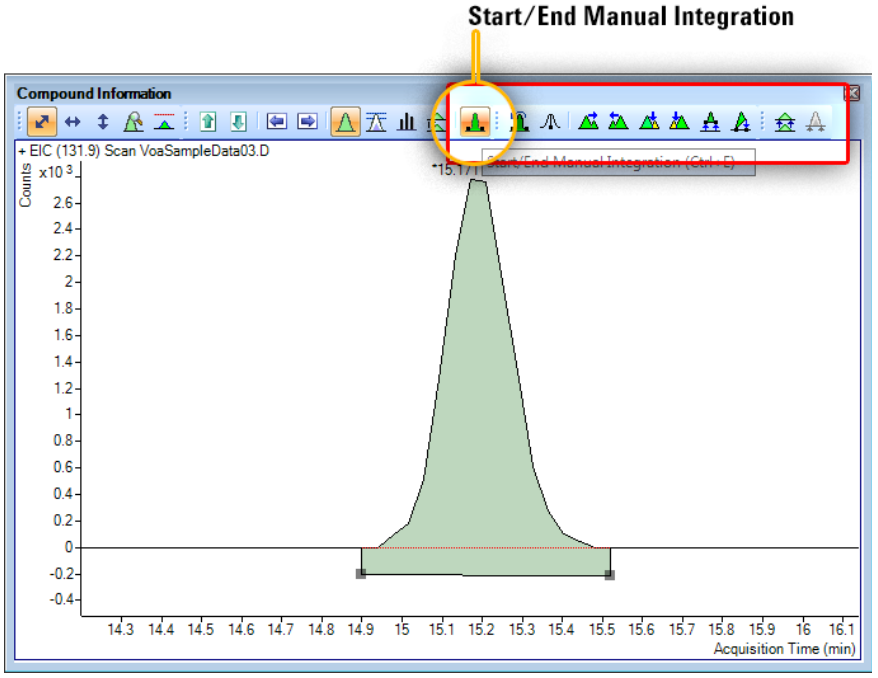
Notice that **Baselines** is a selected default parameter. This displays the baseline for the peak in the **Compound Information** window.



2 Select the **Original baselines after manual integration** and click **OK**.

3 In the **Compound Information** toolbar, select the **Start/End Manual Integration** icon. Observe that the icons to the right of this icon are now enabled. Mouse over these icons to view their names.

4 Drag the baseline endpoints down a bit and note the dotted red line. This is the path of the original baseline that we enabled above.



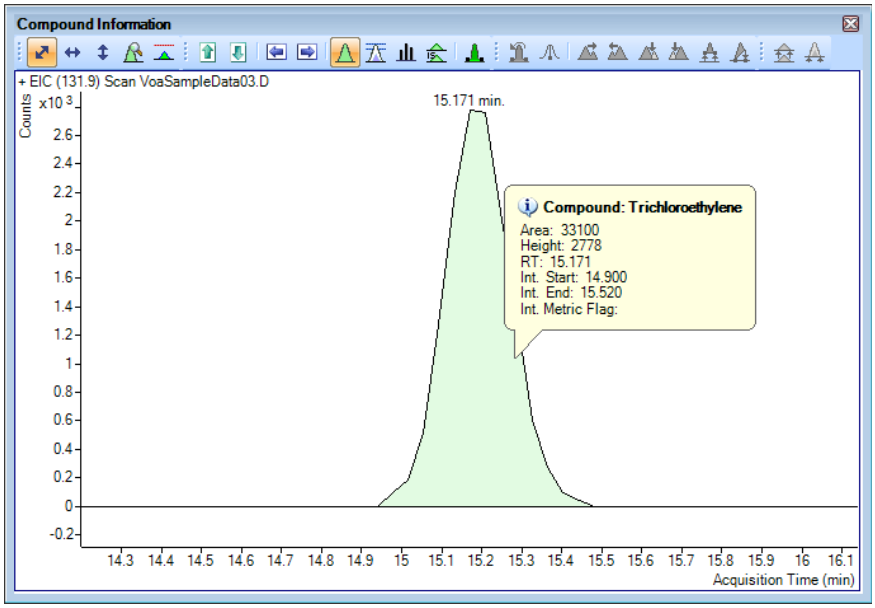
5 Click the **Clear manual Integration** icon. Observe the change made to the end points is removed.

6 Click the **Zero Peak** icon. Observe the baseline is a single vertical line with no area, effectively deleting the peak. Here again the dotted red line shows where the baseline was originally located.

7 Click the **Clear manual Integration** icon and the **Start/End Manual Integration** icon to restore the original peak.

8 Label the chromatogram.

a Mouse over the peak to display the peak information as shown below. You may want some or all of this information to be displayed permanently as described below.



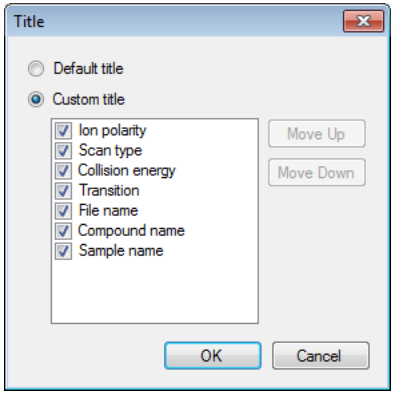
b Right-click in the **Compound Information** window, and select **Properties**.

c In the **Peak fill** area, click **Peak Labels**. Labels selected here will appear above the peak.

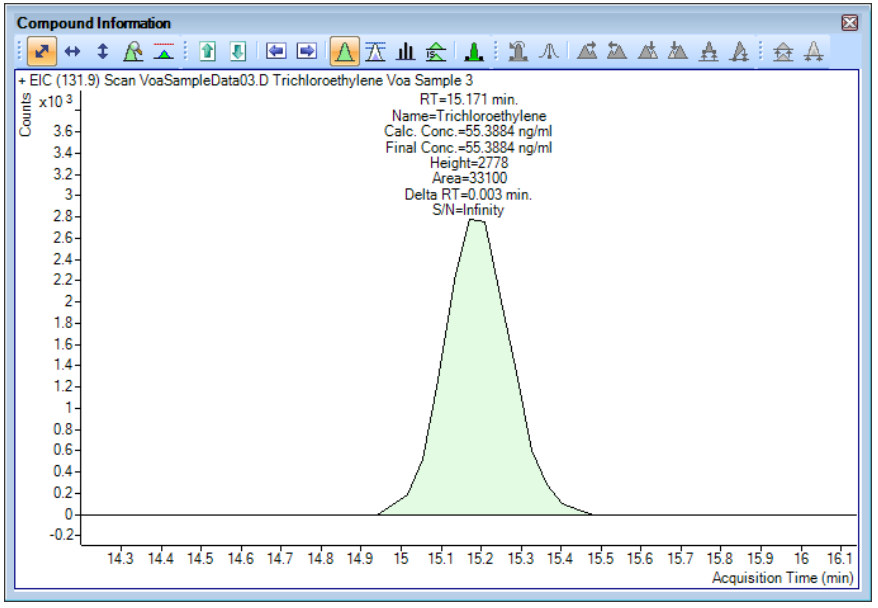
d Select every label and option in this dialog, and click **OK**.

e In the **Peak fill** area, click **Titles**. The title selected here will appear in the upper right part of the chromatogram.

f Select **Custom title**, and select every label in this window.



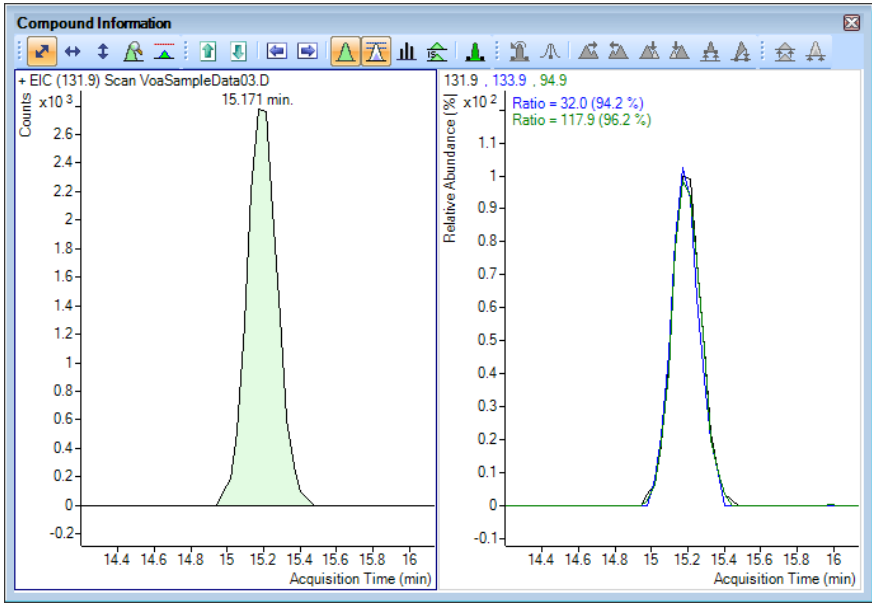
g Click **OK**, and then click **Apply**. Observe the revised title and new peak labels applied.



h Click **Default** to return your settings to the default view, and then click **OK**.

9 Set up the display of qualifier peaks.

a Click **Show/Hide Qualifiers** to display the qualifiers peak to the right of the target peak.



b Right-click in the Compound Information window, and select **Properties**.

c Click the **Compound Information (2)** tab. Under **Qualifiers**, clear the **Normalize qualifiers** check box.

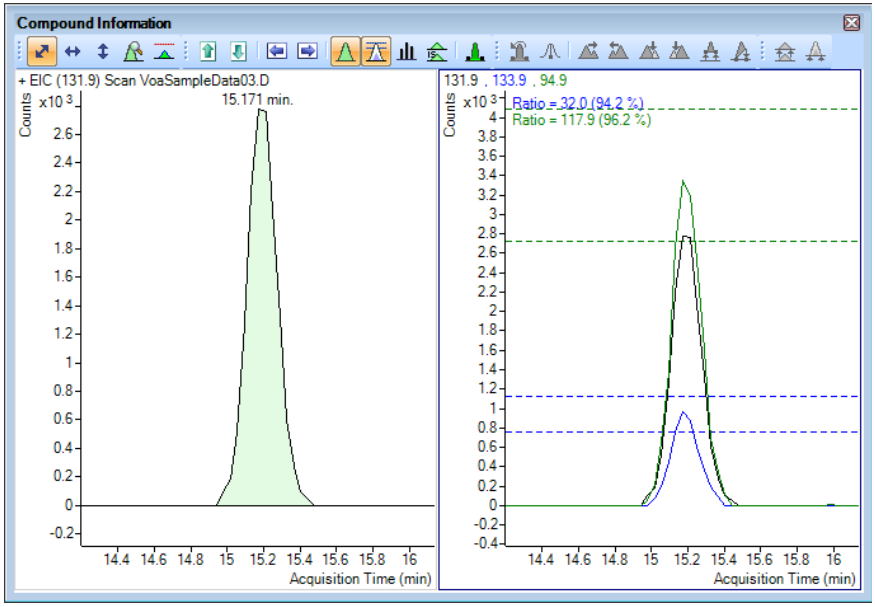
d Click **Apply** and observe the two qualifier peaks. The blue colored qualifier peak and its annotation is at 133.9 m/z. The green colored qualifier peak and its annotation is at 94.9 m/z. To see how these colors were set click **Qualifier Colors** and see the order of blue, green, then brown for the qualifier colors.

e Click **Cancel** to close this **Qualifiers Colors** dialog box.

f Select a dashed line from the **Uncertainty band** drop-down list.

Observe that the green colored qualifier has the green colored uncertainty band showing a near centered 96.2% of expected ratio. Likewise the blue colored qualifier shows a near centered 94.2% of expected ratio. This is the default Response and ratio label setting of Ratio and percent of expected ratio.

Also note that the default qualifier setting for **Fill peaks** is to Fill out of limits qualifier peaks.



g Click **OK** to accept these settings for the **Compound Information** window.

Task 5. Export Results to Excel

1 To make the **Batch Table** window active, click the title bar of the **Batch Table** window.

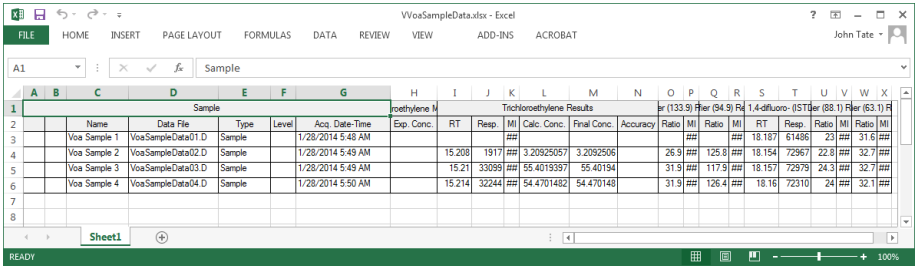
2 Click **File > Export > Export Table**.

Right-click in the **Batch Table**, and select **Export Table**.

3 Select **Documents** as the destination directory and **Excel Files (*.xlsx)** as the Save as type.

4 Type **VoaSampleDataBatch** as the export file name.

5 Click **Save**. The Excel file opens.



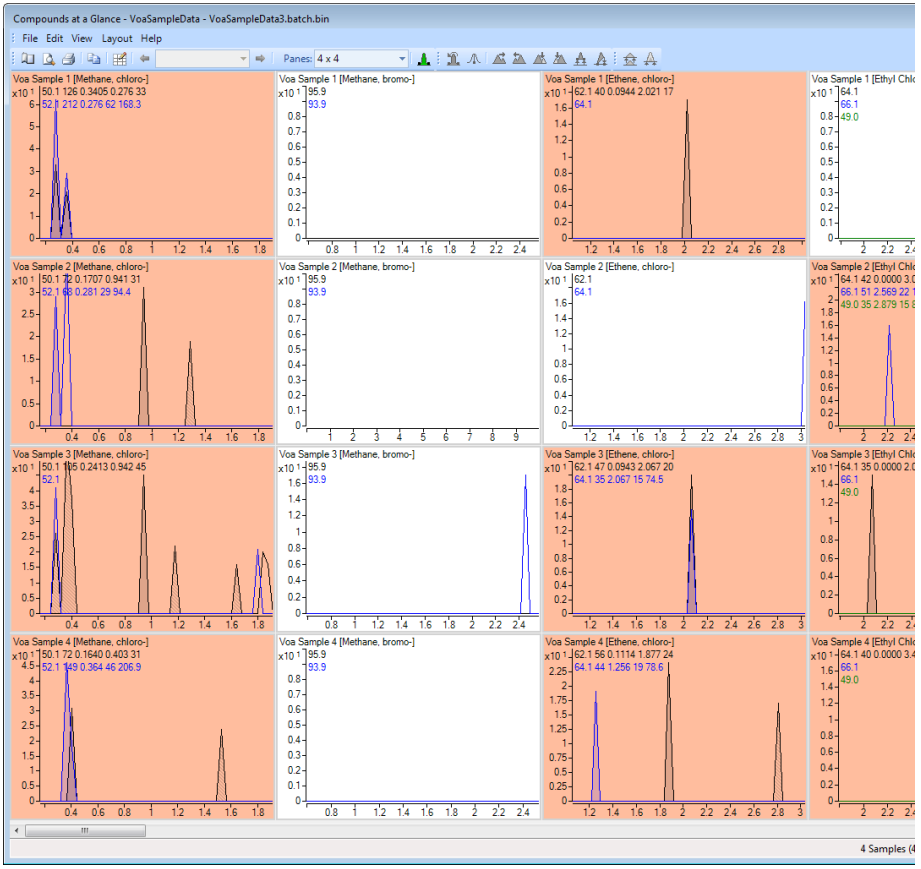
3 Compounds at a Glance

Task 1. Review the Compounds at a Glance Window

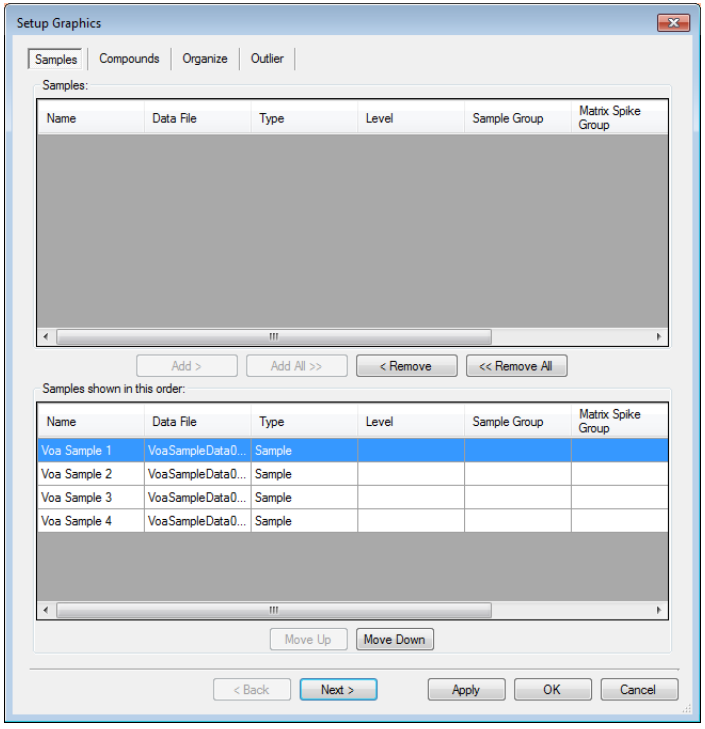
In the **Compounds at a Glance** window, you can review all or selected compound chromatograms in a batch by compound name or by sample. The compound peak can be overlaid with qualifiers, ISTDs, a matrix spike, all compounds or compound groups, and all samples or sample groups. All compounds can be manually integrated from this window. Configured outlier results can also be identified on each compound peak.

1 Select **File > Open Batch**, and load **VoaSampleData.batch.bin**.

2 Select **View > Compounds at a Glance**. The window layout is defaulted to its last configured settings.

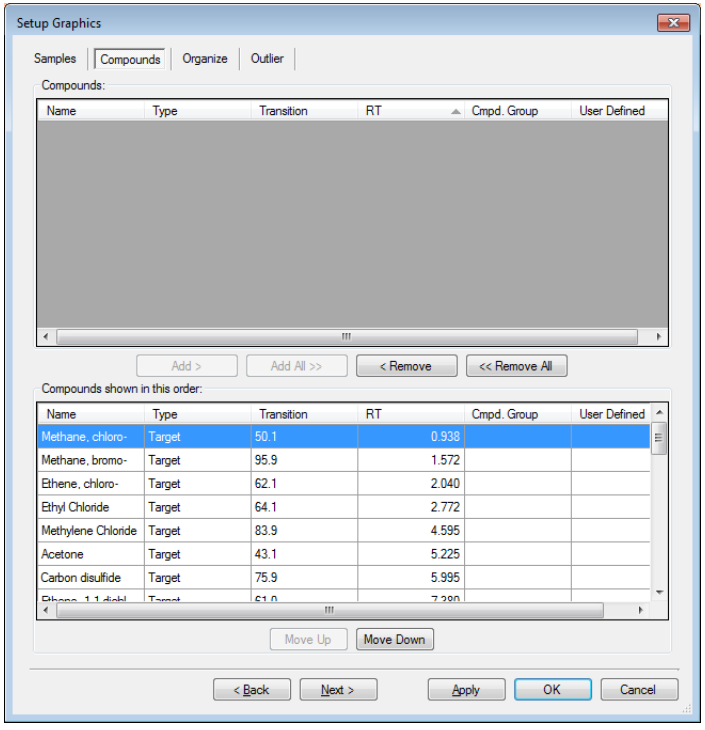


3 Click the **Setup Layout** icon to display the **Setup Graphics** window **Samples** tab.



4 Arrange the desktop so that the **Compounds at a Glance** and **Setup Graphics** windows are both visible.

5 In the lower pane of the **Setup Graphics** window, select samples to remove from the **Compounds at a Glance** window and click **Remove**. In our example, we are using all four samples, so do not remove samples from the lower pane.



6 Click **Next** to display the **Compounds** tab.

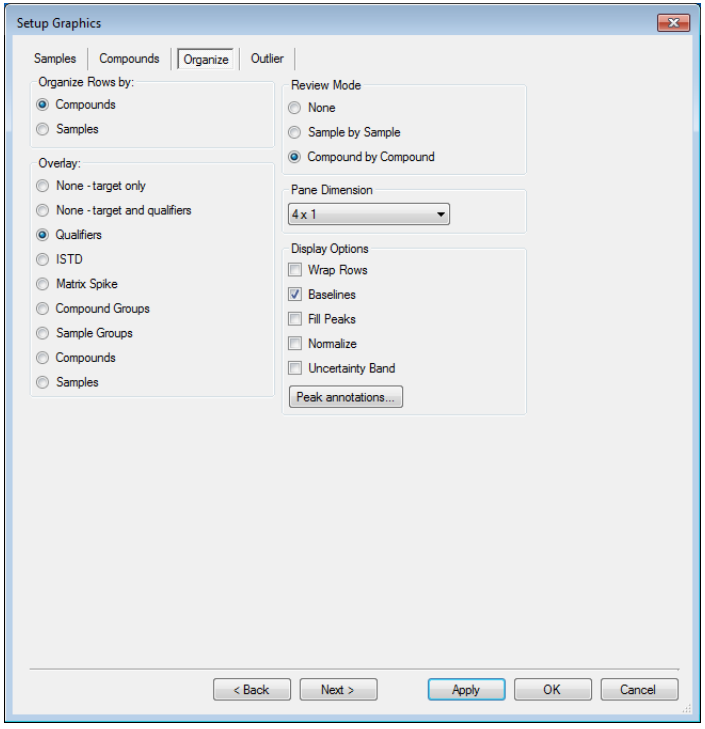
7 In the lower pane of the **Setup Graphics** window, select compounds to remove from the **Compounds at a Glance** window, and click **Remove**. In our example, we are reviewing all compounds, so do not remove compounds from the lower pane.

8 Click **Next** to display the **Organize** tab.

9 In the **Organize Rows by** area, select **Compounds**. Click **Apply** to see the changes in the **Compounds at a Glance** window.

Each column now displays a single sample and the single row displays the selected compound found in all four samples.

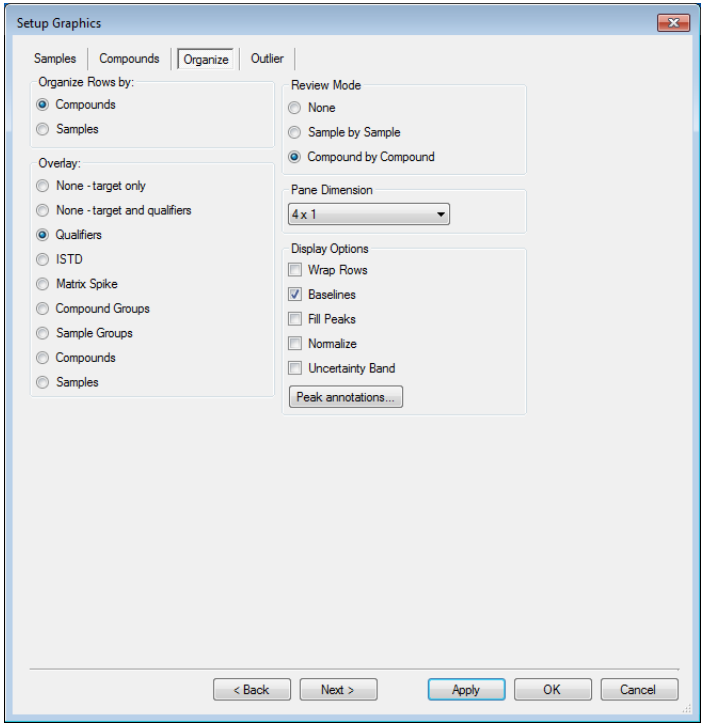
10 In the **Review Mode** area, select **Compound by Compound**. Click **Apply** to see the changes in the **Compounds at a Glance** window.



The reviewer mode in the toolbar of the **Compounds at a Glance** window is now enabled. In this review mode, since **Organize Rows by Samples** was previously selected, a single named compound is displayed in a column and each row in the column displays that compound result in the included sample.

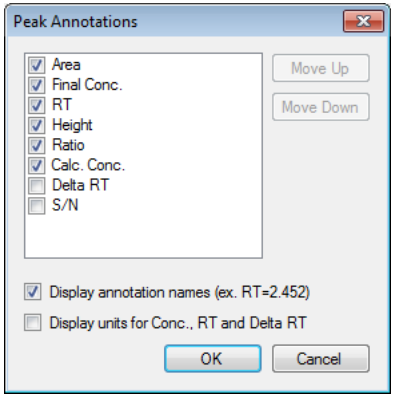
11 In the **Pane Dimension** area, select the drop-down, and mouse over the squares until the pane displays **4X1**. Click **Apply**.

The window now displays the results of the selected compound in all 4 samples containing a single compound.



12 In the **Display Options** area, select **Baselines**. Click **Apply** to see the changes in the **Compounds at a Glance** window.

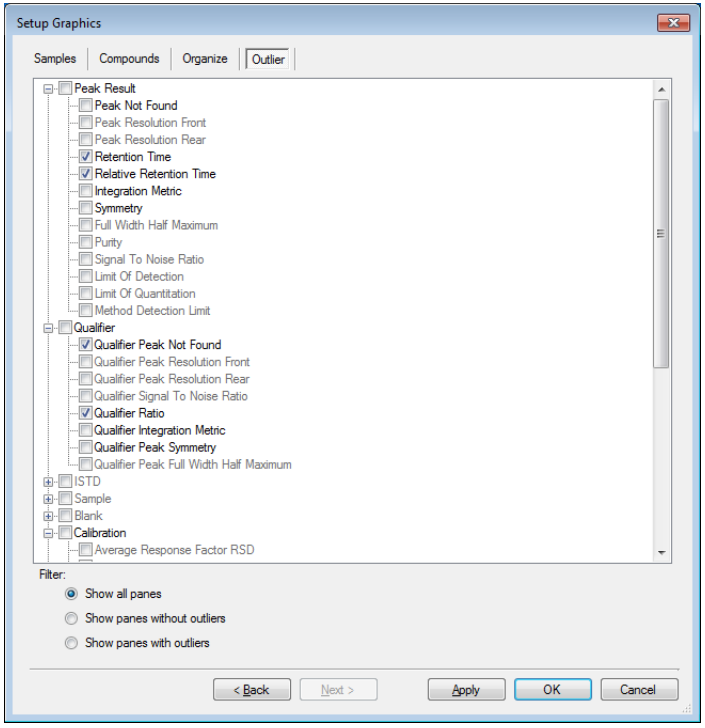
13 In the **Display Options** area, click **Peak annotations**. Click **Apply** to see the changes in the **Compounds at a Glance** window.



14 Click **Next** to display the **Outlier** tab.

15 Make the selections shown in this example:

- **Peak Result** - **Retention Time** and **Relative Retention Time**
- **Qualifier**- **Qualifier Peak Not Found** and **Qualifier Ratio**
- **Calibration** - **Calibration Range**



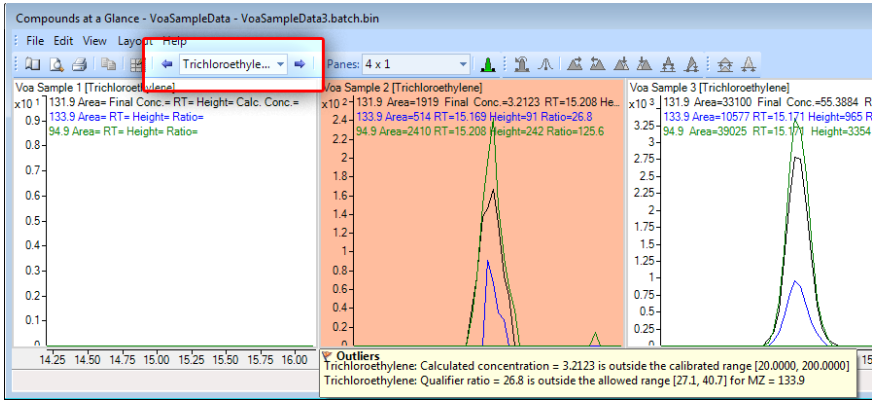
16 Click **OK** to close the **Setup Graphics** window, and notice the highlighted pane in the **Compounds at a Glance** window.

See **"Task 1. Review the Outliers"** on page 78 for details on these settings.

17 In the **Compounds at a Glance** window, from the **Review** drop-down, select the compound **Trichloroethylene**.

18 Mouse over the salmon highlighted pane in the **Compounds at a Glance** window to see outlier messages for the analyzed results.

Here we see that the calculated concentration is outside the calibration range and the qualifier ratio is also outside its allowable range.

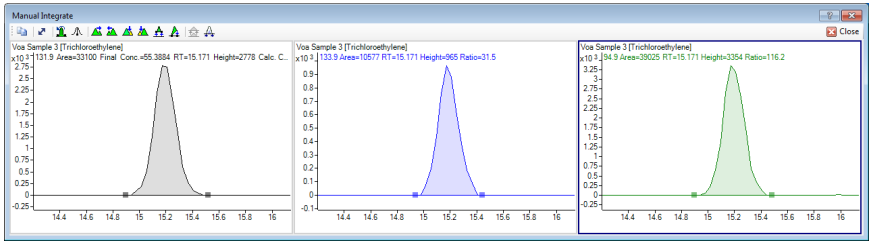


19 See how **Link All X-Axis** works.

a Right-click in any pane, and select **Link All X-Axis**.

b To see how the **Link All X-Axis** function works, left-click and drag the RT scale, and observe the peaks move in all panes. Zoom in on a region in one chromatogram and the same area is zoomed in the other chromatograms.

20 Double-click the pane for **Voa Sample 3** to open a **Manual Integrate** window with three panes. The first pane contains the target pane and the other two panes contain its qualifiers.



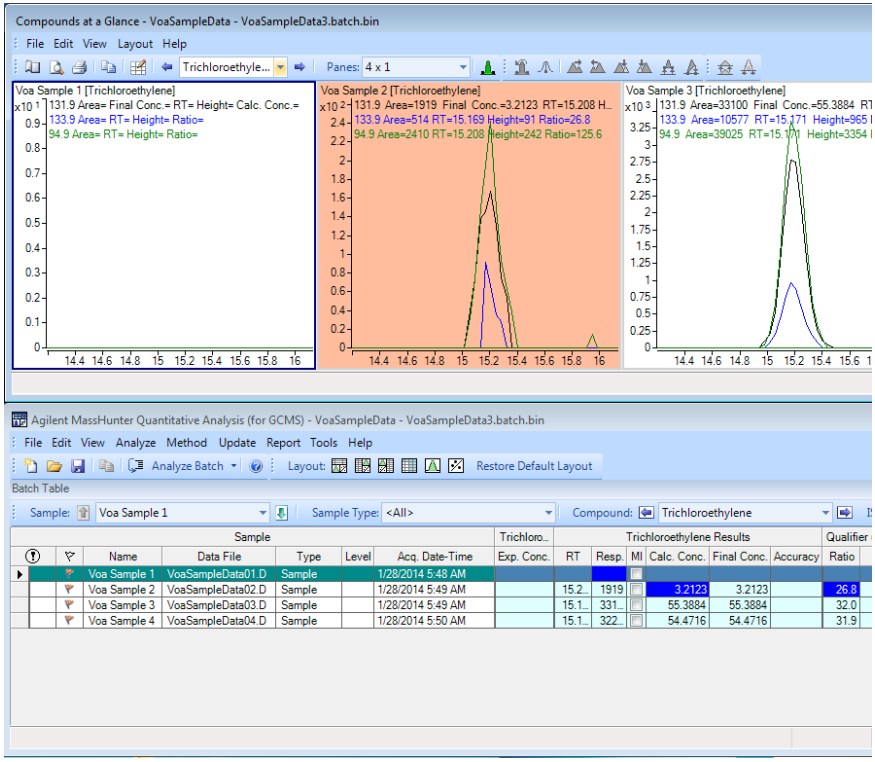
21 Close the **Manual Integrate** window and review the remaining compounds by clicking the **Next** or **Previous** arrow icon on the **Review** parameter shown the compound name currently being reviewed.

22 When finished you can print the **Compounds at a Glance** window. Use **Page Setup** to set the page properties.

23 Synchronize sample and compounds in the **Batch Table** with the **Compounds at a Glance** window.

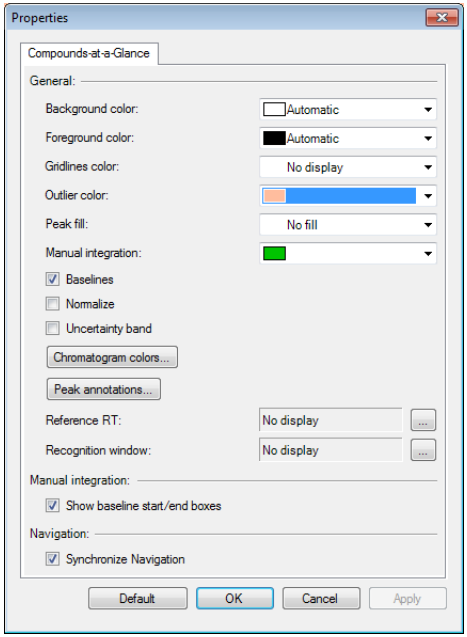
a Use the **Compounds at a Glance** window from the previous steps for this procedure.

b Size the **Compounds at a Glance** window and the **Quantitative Analysis** window to the same width, and place them vertically as shown below.

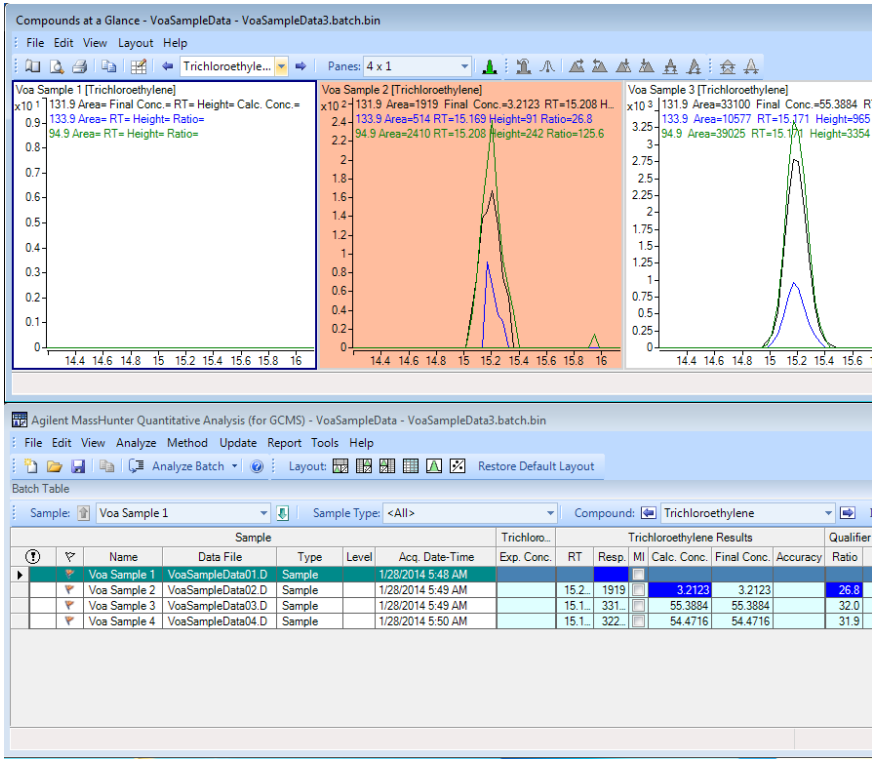


c Right-click in the **Compounds at a Glance** window, and select **Properties**.

d Under **Navigation**, select **Synchronize Navigation**, and click **OK** to close the dialog box.



e Select a compound in the **Batch Table** and the same compound is selected in the **Compounds at a Glance** window. Selecting the compound in the **Compounds at a Glance** window changes the selection in the **Batch Table**.

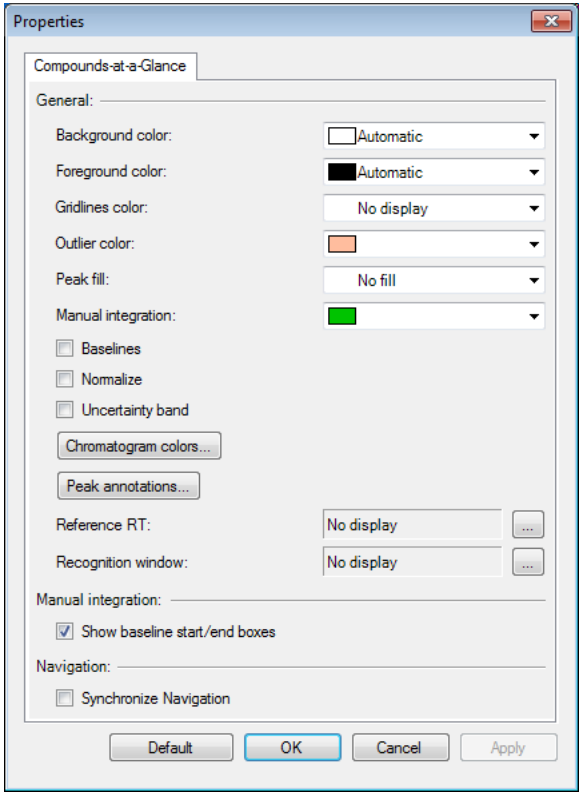


Task 2. Display Properties for the Compounds at a Glance Window

This task shows you how to setup the labels and colors for the **Compounds at a Glance** window for reviewing results. It assumes that the window was configured in the previous task.

1 Right-click in the **Compounds at a Glance** window, and select **Properties**.

2 Click **Default** to return the properties settings to their defaults.



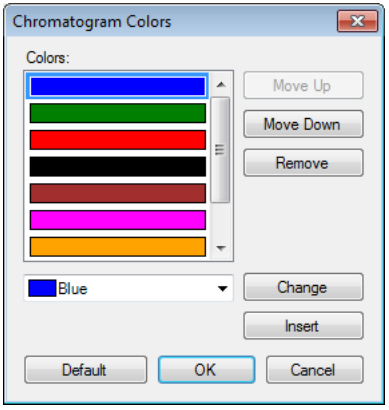
Notice that under **Manual integration**, **Show baseline start/end boxes** is selected. This is only shown when manual integration is enabled.

The default outlier color is salmon. If an outlier exists for a sample's compound, that chromatogram has a salmon background.

3 Under **General**, select **Uncertainty band**.

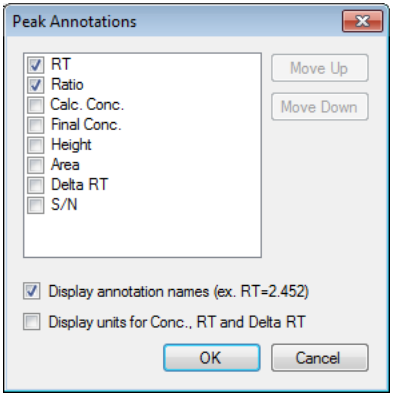
4 Click **Chromatogram colors**, and observe the default order of black, blue, green, etc.

5 With black selected at the top, click **Move Down** to make this order blue, green, red as shown below.

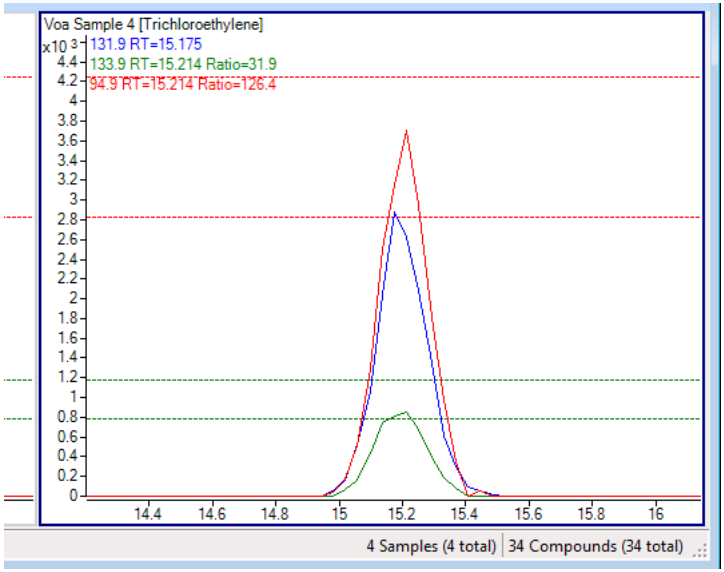


These settings will make the target compound peak and labels blue, the first qualifier peak, labels and uncertainty band green, and the second qualifier peak, labels and uncertainty band red. Click **OK**.

6 Click **Peak annotations** and select **RT** and **Ratio** as shown below. Click **OK**.



7 Click **OK** to close the **Properties**, and observe the edits to the display.



Observe that the target peak and peak label is blue as specified.

The green colored qualifier has the green colored uncertainty band with a ratio of 31.9 just making it inside the band.

The red colored qualifier shows a ratio of 126.4 nicely centered in the band.

The labels specified for **RT** and **Ratio** are color-coded to the peaks.

4 Outliers and Quantitation Messages

In this exercise, you will learn how to review results for your batch using the **Batch Table Outliers** indicators and **Quantitation Message** features. You will also review outliers settings for the **Compounds at a Glance** window.

Outliers allow you to setup ranges of parameters that represent acceptable results. Results outside these acceptable parameters are considered outliers. MassHunter monitors the outliers found in compounds present in every sample. It then presents these color-coded results graphically in tables.

Task 1. Review the Outliers

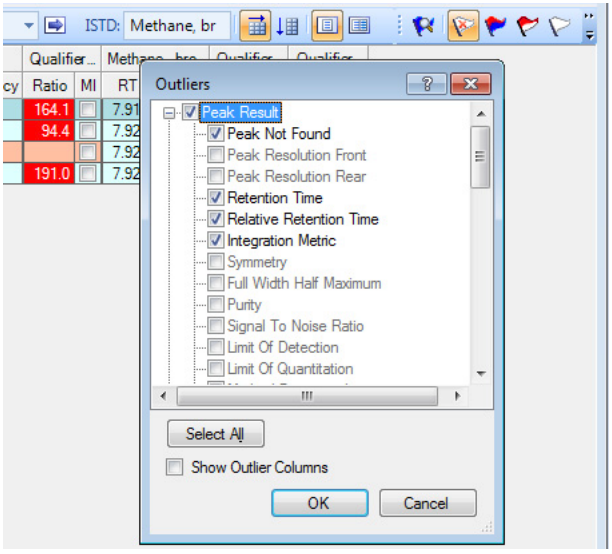
This task shows how outliers are displayed for a compound in the **Batch Table** and how you can filter results with outliers flags. In this example, we set this filter to only display the outliers MassHunter sets up by default.

1 Initialize the default outliers.

a Select **File > Open Batch**, then navigate to and open the **VoaSampleData** batch file.

b Click **Select Outliers** to bring up the **Outliers** dialog. In the **Outliers** dialog, select only the default outliers, those shown in bold, in the **Peak Result**, **Qualifier**, and **Calibration** groups.

Filter settings here apply only to the **Batch Table** and are not valid for the **Compounds at a Glance** window.



c Click **OK** to enable the display of default outliers.

2 Review the outliers displayed in the **Batch Table**.

a Click **Analyze Batch** in the main window toolbar. The outliers for the batch are found and displayed.

On the **Home** tab, click **Analyze Batch**.

b If not selected, click **Turn off outliers filter** to display all samples.

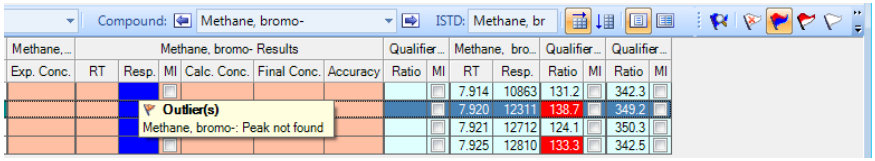
c Select **chloromethane** in the **Batch Table**, and note there are outliers denoted by red and blue highlighted cells.

d Click **Display rows that have no outliers** . All of the samples will be hidden because chloromethane had outliers in all 4 samples.

e Click **Display rows that have High/Low outliers** to display all 4 samples once again.

f Click **Next Compound** in the **Batch Table** toolbar to review the results for bromomethane. The salmon shading in the this target compound's **Method** and **Results** area indicates that no target compound was found. All four samples indicate an outliers in the target's **Resp.** column.

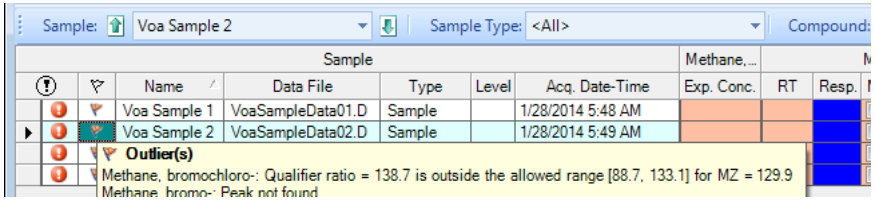
g Mouse over the blue cell to display the outliers message.



For the compound selected for **Voa Sample 2,** there are multiple outliers, one blue shaded cell in the targets **Result** area and one red shaded cell in the second qualifier's **Ratio** column.

View the outliers message in this sample's red shaded cell. The cell is shaded red because the ratio detected value of 138.7 is greater than the maximum value of 133.1 allowed in this range. If its value were lower than the lowest value of 88.7 allowed in this range it would be shaded blue.

h Mouse over the **Outliers Summary** icon (red filled flag) to display the outliers messages. Note from the previous step, there were two outliers in two different cells. They are summarized here.



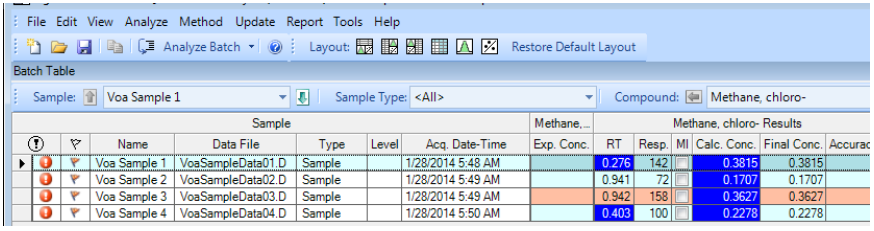
3 Click **Select Outliers** .

4 Under **Peak Result**, clear the **Peak not found** check box.

The **Batch Table** contains only two samples now that **Peak Not Found** is no longer an outlier. This is because our outlier filter is set to only show samples with outliers for the current compound. These outliers have no merit since a qualifier to target ratio cannot exist without a target compound.

5 Click **Turn off outlier filter** to display all samples.

6 Click **Next Compound** , repeatedly, in the **Batch Table** toolbar to review the results for each calibration compound in all four samples.



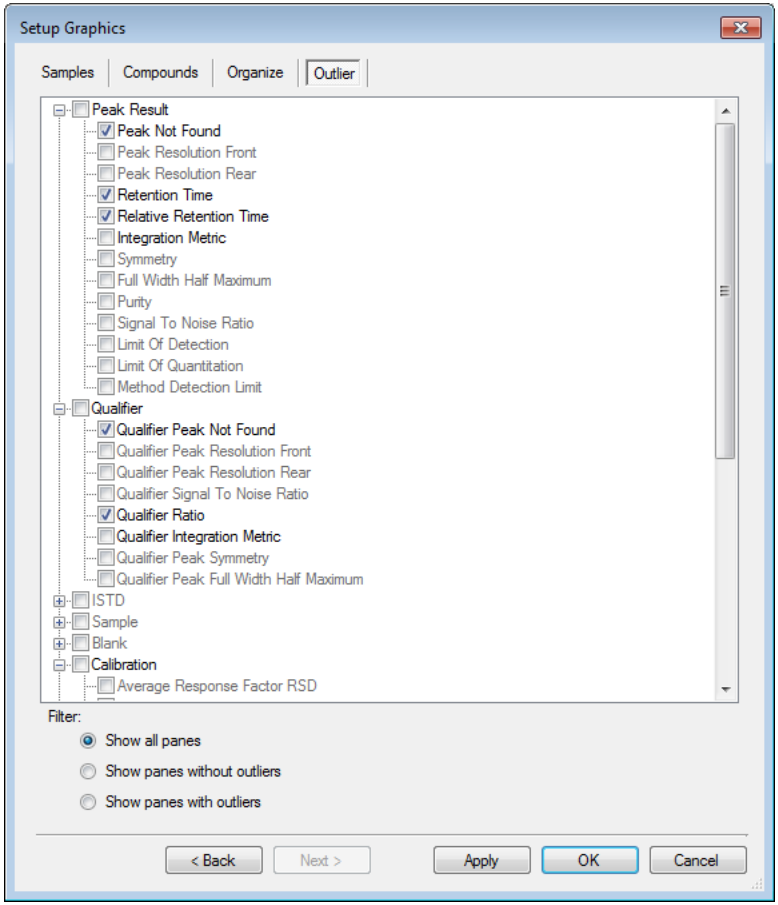
7 Set up the outliers display in the **Compounds at a Glance** window.

a In the main window, select **View > Compounds at a Glance**.

b Select **Layout > Setup Layout**.

On the **Home** table, click **Setup Layout**.

c Click the **Outlier** tab to display the **Setup Graphics Dialog** tab used to adjust the Outlier settings. Outliers displayed in the **Compounds at a Glance** window are set up in the **Setup Graphics** dialog box **Outlier** tab.

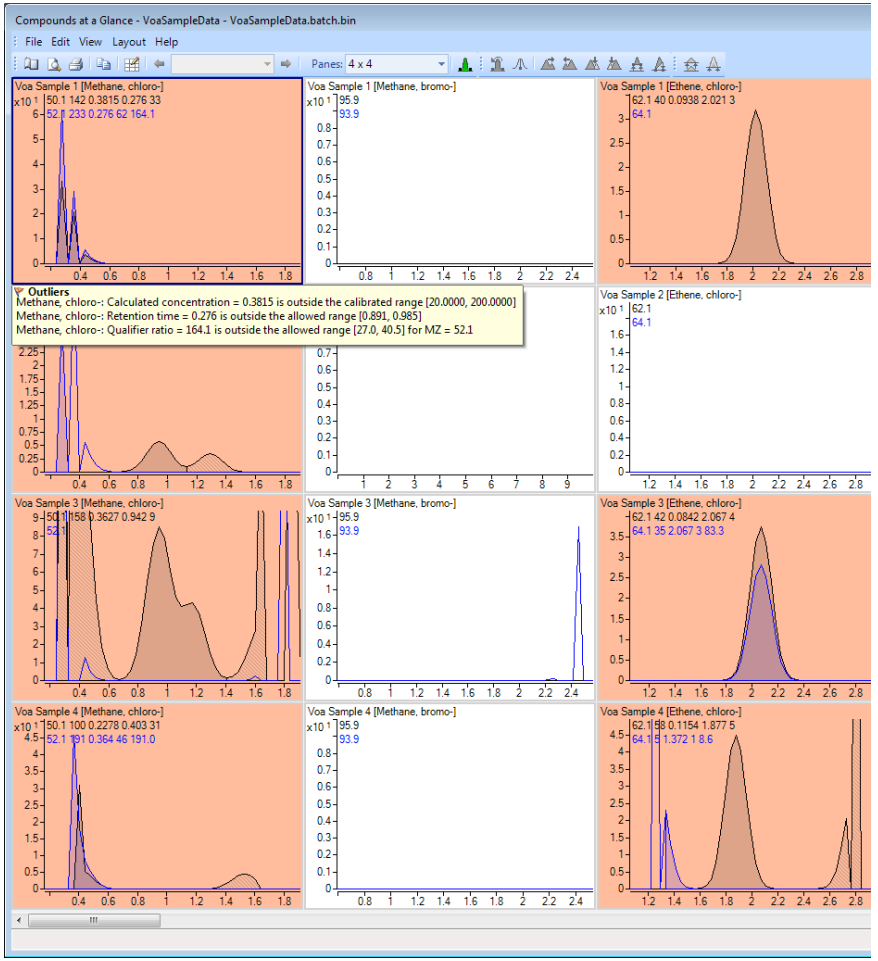


d As done previously with the **Batch Table** outliers settings, select the defaults indicated in bold, but clear the **Peak Not Found** check box. Also clear the **Integration Metric** and **Qualifier Integration Metric** check boxes, as they do not function with the **General** integrator.

e Under **Filter**, select **Show all panes**.

f Click **OK** to apply these settings to the view. The salmon-colored chromatograms represent a compound in a sample where an outlier exists.

g Mouse over the chromatogram to display an **Outlier Summary** for that sample's compound.

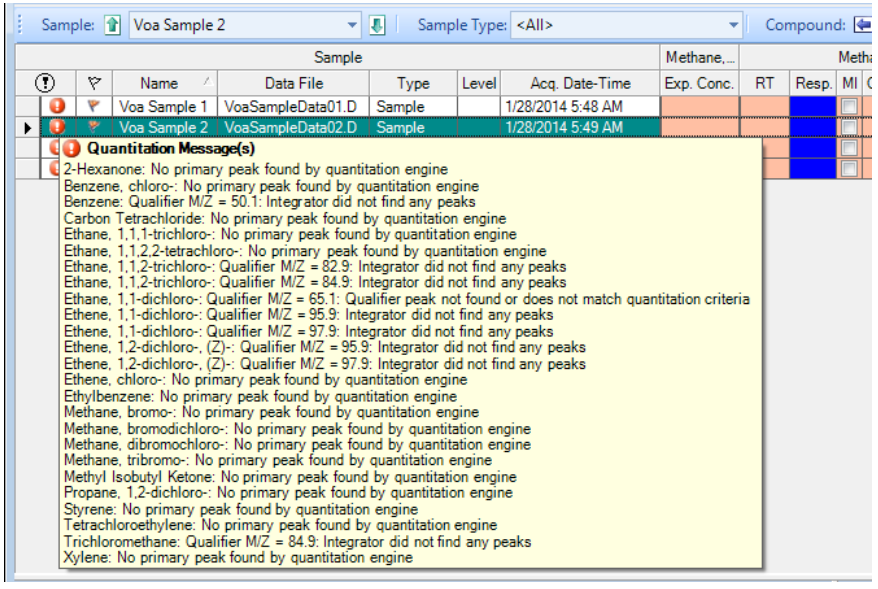


Task 2. Review Quantitation Messages

Quantitation messages are informational but not necessarily to identify an out of an acceptable range of values condition. A good example of a quantitation message is not finding a peak defined in your quantitative method in an unknown sample. We will examine how to suppress the peak not found message.

1 View quantitation messages for a single sample in the **Batch Table**.

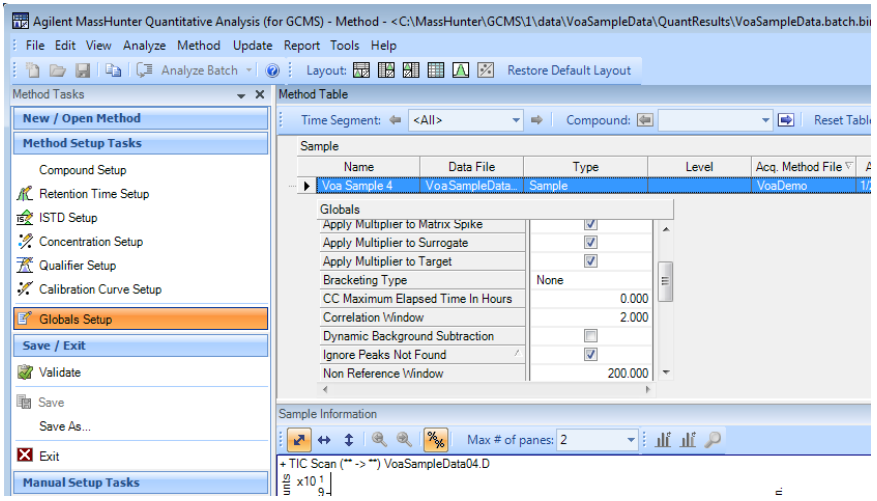
For the **Voa Sample 2**, mouse-over the **Quantitation Message Summary** icon (exclamation point inside a filled red circle) to display the **Quantitation Messages**. The messages in our example are all due to calibration compounds not found in the sample.



2 Suppress quantitation messages in the **Batch Table** that involve missing peaks.

a Select **Method > Edit**.

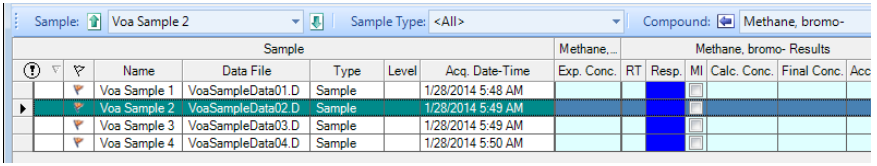
b Select **Globals Setup** from the **Method Tasks** area, and then select **Ignore Peaks Not Found** in the **Method Table**.



c Select **Method > Exit**. This displays the **Apply Method** dialog.

d Select **Analyze**, and click **Yes**. This runs the analysis with the revised method settings.

e Notice the absence of **Quantitation Message Summary** icons. Compare this to the previous messages in the **Quantitation Message Summary** icons for **Voa Sample 2** before **Ignore Peaks Not Found** was added to our method.



f Save the method as **VoaSampleData2.batch.bin**.

Task 3. Set Up Outliers

Previous tasks in this exercise discussed setting up outliers views using the MassHunter default outliers setups. This task looks at where the default outliers settings can be edited and reviews the setup of non-default outliers.

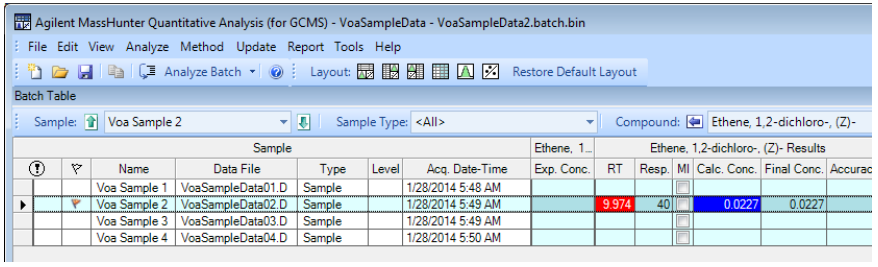
1 Select **File > Open Batch**, and load the **VoaSampleData2.batch.bin** file.

2 Edit the acceptable range for the RT outlier.

a Select **Ethene, 1,2-dichloro** from the **Compound** drop-down list in the **Batch Table** toolbar.

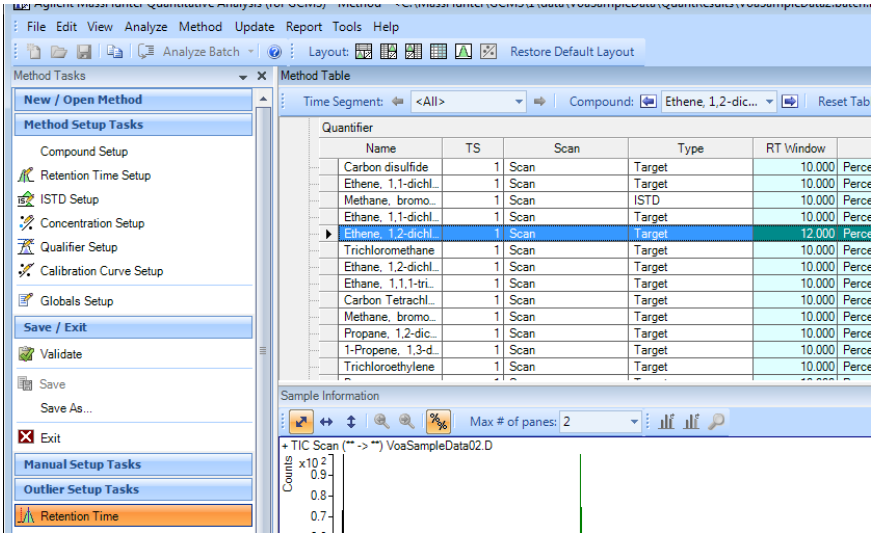
b Select the **Voa Sample 2** sample in the table.

Note the red shaded RT cell for the selected sample's compound.



c Click **Method > Edit** to switch to method editing mode.

d In the **Method Tasks** window, click **Outlier Setup Tasks > Retention Time**. In the **Quantifier** table, note that the compound selected is the same compound selected in the **Batch Table**.

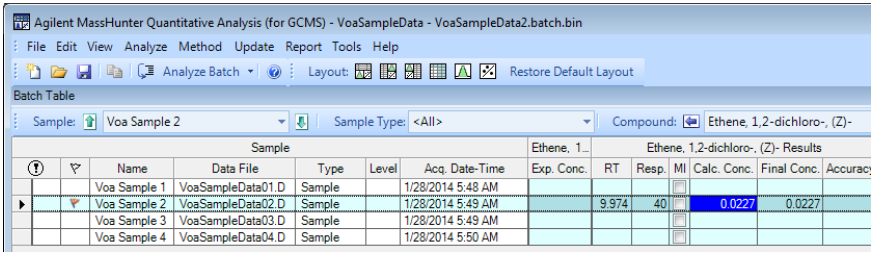


e Set the **RT Window** value to **12**.

f Select **Method > Exit**.

g Select **Analyze**, and click **Yes**. This runs the analysis with the revised method that increases the acceptable range for the **Retention Time**. You are returned to the **Batch Table**.

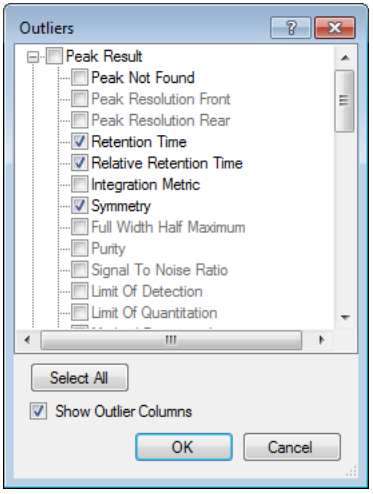
The outlier that was noted by the red shaded **RT** cell is now gone. This indicates that the change you made to the acceptable RT range now includes this result.



3 Allow a new outlier to be displayed in the **Batch Table**.

a Click **Select Outliers**  to bring up the **Outliers** dialog box.

b In the **Peak Result** group, select the **Symmetry** outlier.



c Select **Show Outlier Columns** to add the **Symmetry** column to the target compound results area in the **Batch Table**.

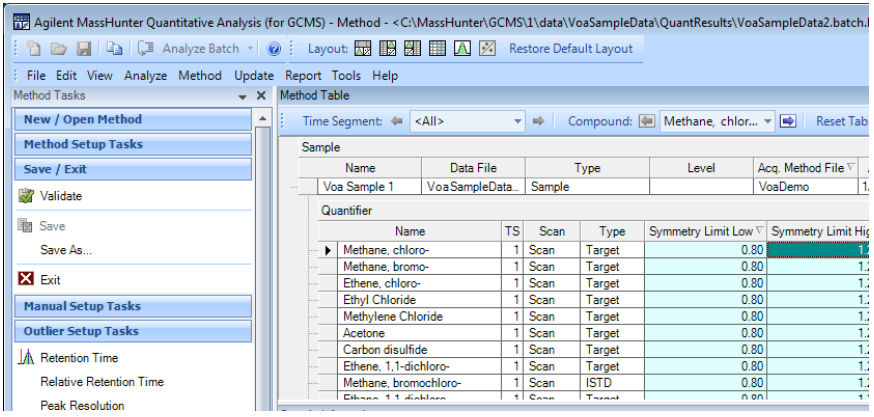
d Click **OK** to enable the display of **Symmetry** outliers.

e If not selected, click **Turn off outlier filter**  to display all samples.

4 Setup a new outlier in the method.

a Select **Method > Edit** to switch to method editing mode.

b In the **Method Tasks** window, click **Outlier Setup Tasks > Peak Symmetry**.

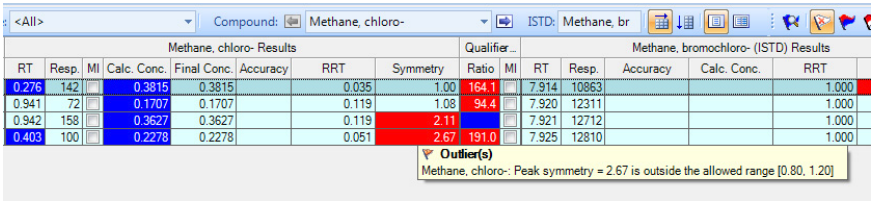


c Set the **Symmetry Limit Low** value to **0.80**.

d Set the **Symmetry Limit High** value to **1.20**.

e Select **Method > Exit**. This displays the **Apply Method** dialog.

f Select **Analyze**, and click **Yes**. This runs the analysis with the revised method and adds the **Symmetry** outlier to the method.



The new **Symmetry** outlier is detecting peak trailing of the chloromethane compound in two samples as noted by the red-shaded cells in the **Symmetry** column.

5 Generate Quantitation Reports

This exercise helps you learn how to do these tasks:

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

The **VoaSamples** batch is used in this exercise.

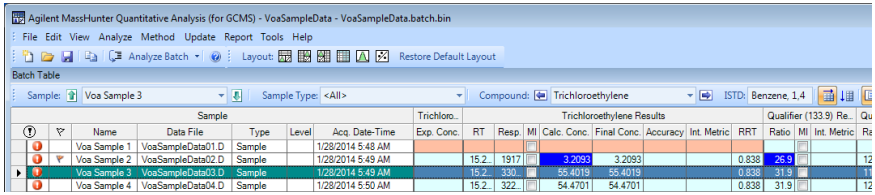
The report method you develop determines the report you create in MassHunter. Report methods are made of one or more report templates combined and edited to meet your reporting requirements. When developing a report method, you can use either Excel or PDF templates. PDF templates can generate reports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

Task 1. Develop a Report Method

In this exercise, you will develop a report method using PDF templates.

1 To start the **Quantitative Analysis** program, click the **MS Quantitative Analysis** icon on your desktop. You can also access the program by clicking **Windows Start > All Programs > Agilent > MassHunter Workstation > MS Quantitative Analysis**.

If the default layout is not present, click **Restore Default Layout** on the toolbar. On the **Home** tab, click **Restore Default Layout**.



2 Click **Open Batch**  on the toolbar.

On the **Home** tab, click **Open**.

3 Navigate to the directory containing the **VoaSamples** batch.

4 With the **Batch Table** open, click **Analyze Batch** on the toolbar to generate results.

On the **Home** tab, click **Analyze Batch**.

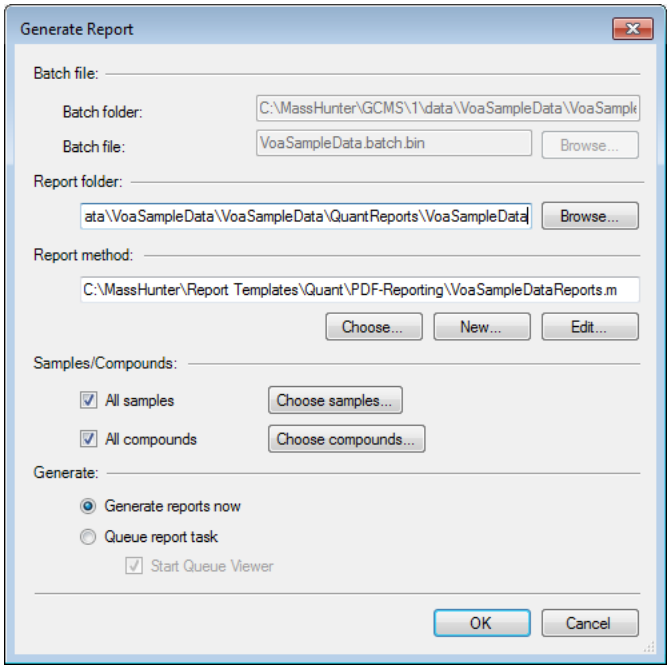
If the batch is already quantitated, skip to step 5.

5 Click **File > Save Batch** to save the batch. Quantitative reports contain sample information generated during the batch. The reporting function will not work until sample results have been quantitated and saved.

6 Create a PDF report method.

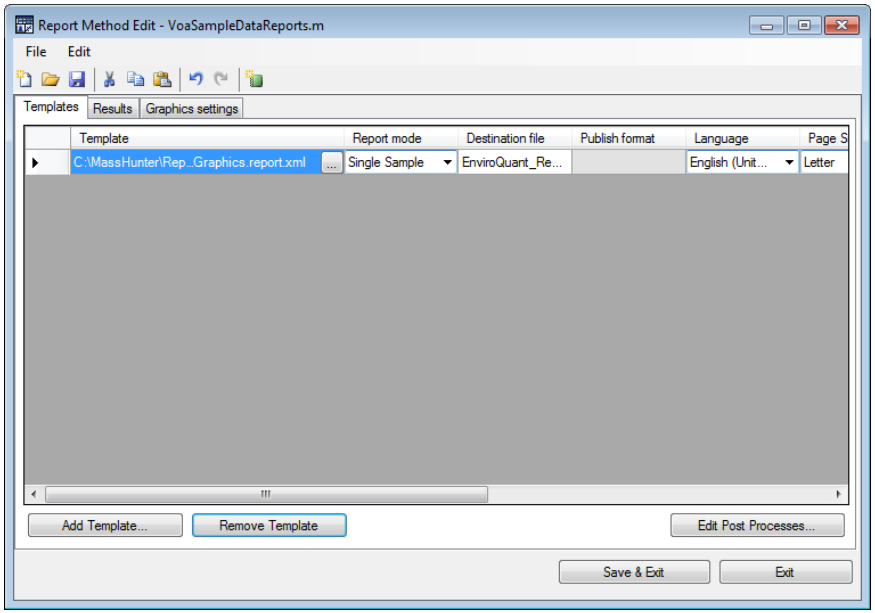
a Select **Report > Generate**.

On the **Home** tab, click **Generate Report**.



b Accept the default **Report Folder** directory for this report.

c Under the **Report Method** field, click **New** to create a new report method.



d Click **Add Template** in the **Report Method Edit** dialog box to open the browser.

e Navigate to the **MassHunter/Report Templates/Quant/PDF-Reporting** directory, select a template, and click **Open**. The program adds the template to the **Template** field in the **Report Method Edit** dialog box.

f Repeat steps **d** and **e** to add a second template.

You may change the destination directory for saving the report in the **Report Folder** field.

The **Report Method Edit** feature of the software allows you to combine existing templates into a report method for developing an Excel or PDF report, or both.

The software defaults to the last report method used for the last report generated. Rather than generate a new report method, you can use the default method if appropriate, or select a different existing method.

To select an existing report method, click **Choose** under the **Report Method** field, and navigate to the folder to select your method.

7 Edit the report method to create single sample and batch PDF reports.

The **Report Method Edit** dialog box allows you to edit certain features of the templates you choose to include in the report method.

The PDF reporting option allows you to create English, Chinese, Japanese, or Russian reports. Excel reports are provided in English only so this option will be grayed out.

In Excel reports, there are limits on your paper size. PDF reports provides a choice.

You can also select your **Publish Format**. In PDF reports, there is only one publish format; therefore, this field is grayed out for this example.

a In the **Report Method Edit** dialog box, on the first template line, in the **Report Mode** column, select **Single Sample** from the drop-down list.

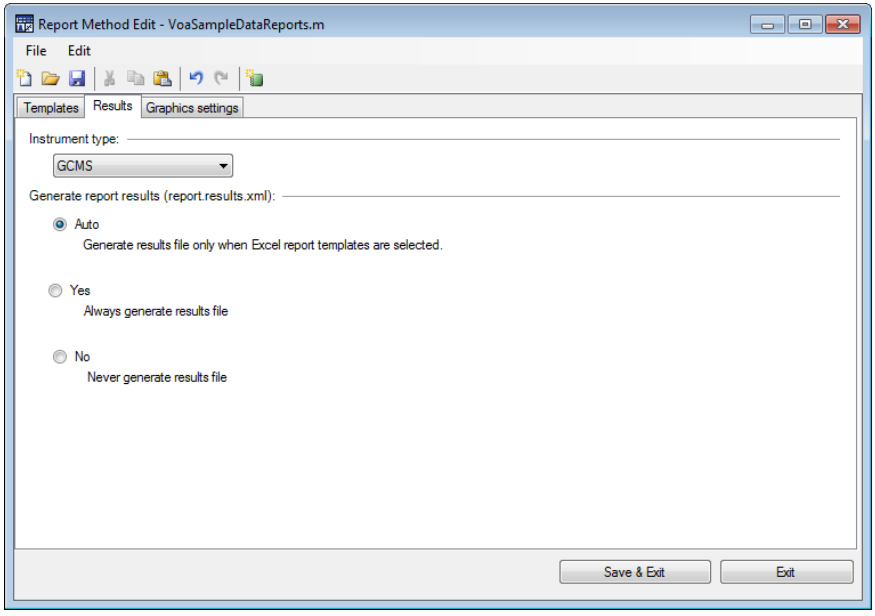
b On the second template line, select **Batch** from the drop-down list in the **Report Mode** column.

c In the **Language** column, select your language from the drop-down list.

d In the **Paper Size** field, select a paper size from the drop-down list.

8 Select the way the system handles your report results.

a On the **Results** tab, select **GCMS** from the **Instrument Type** drop-down list.

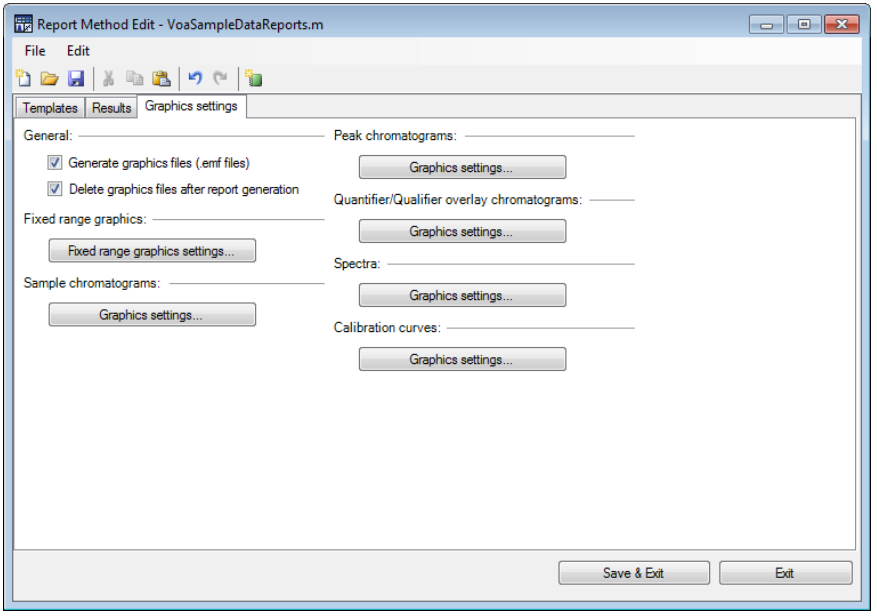


b Under **Generate report results**, select **Auto**.

Use **Auto** in most cases. This limits the generation of an Excel file with the report to only those cases in which an Excel report is selected. PDF reports are quick and efficient when the generation of an Excel file is not necessary.

9 Set the graphic setting options for the method.

a On the **Graphic Settings** tab, select **Generate graphic files** to add graphics to your report.



b Leave the default settings for the rest of the graphic setting fields.

The **Graphic Settings** tab allows you to specify the appearance of the graphics in your report by editing the **Quantifier/Qualifier Overlay chromatogram**, **Spectra**, **Sample chromatogram**, **Calibration Curves**, and **Fixed range graphic** settings. If you do not change the settings, the software will provide default settings appropriate for your data.

10 Click **Save** in the **Report Method Edit** window.

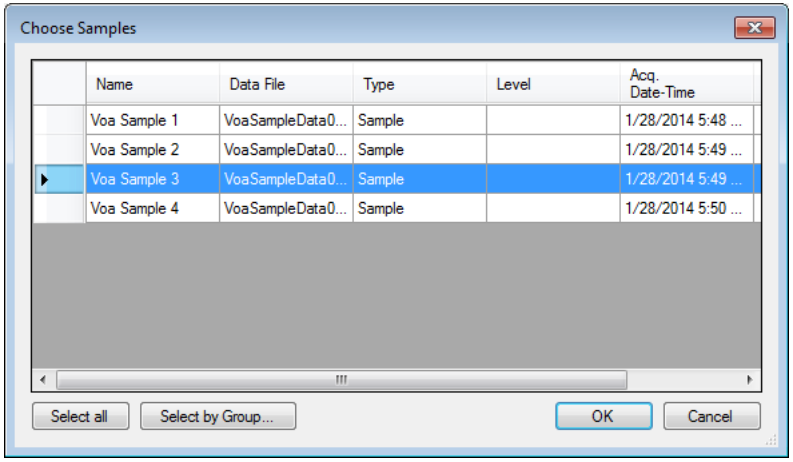
11 Name the report method **VoaSamples.m**.

12 Click **Save & Exit** to close the **Report Method Edit** dialog box and return to the **Generate Report** dialog box.

Task 2. Generate a report

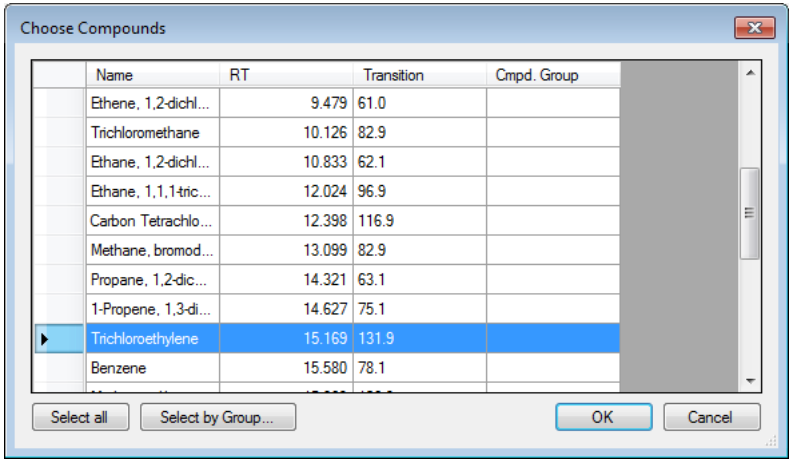
1 Verify that the method you just created is in the **Report Method** field.

2 In the **Samples/Compounds** field, click **Choose Samples** to open the **Choose Samples** dialog box.

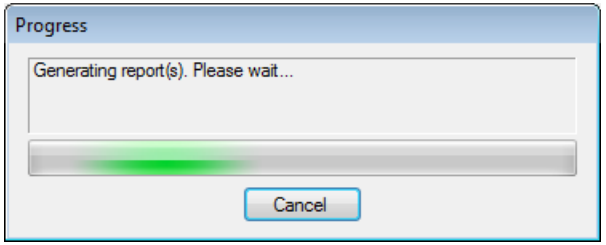


3 Select the samples to include in the report, and click **OK**.

4 Click **All Compounds**, select the compounds to include in the report, and click **OK**.



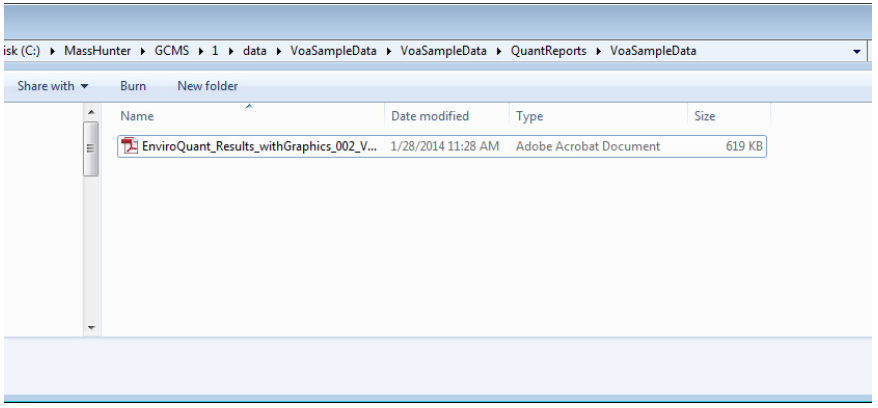
5 Select **Generate reports now,** and click **OK** to generate the report.



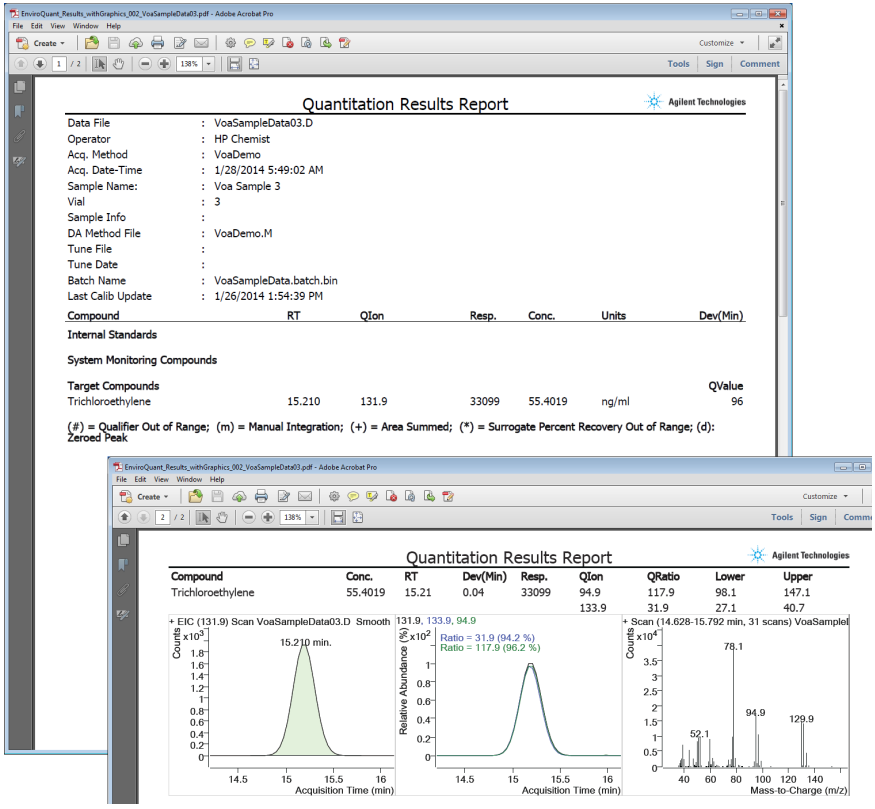
You can choose to show all the samples and all the compounds in the batch, or select specific samples or compounds in the batch table to show in your report.

PDF reports generate quickly, so **Generate the report now** is the best option to obtain the report right away. If you are generating an Excel file along with the report, you can select **Queue report task** to view the progress of the report it is generating.

All reports generated are accessed by selecting **Report > Open Report Folder**. Reports are viewed or printed from the Excel or the PDF file you have created.



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



Quantitation Results Report

Agilent Technologies

Data File	:	VoaSampleData03.D
Operator	:	HP Chemist
Acq. Method	:	VoaDemo
Acq. Date-Time	:	1/28/2014 5:49:02 AM
Sample Name:	:	Voa Sample 3
Vial	:	3
Sample Info	:	
DA Method File	:	VoaDemo.M
Tune File	:	
Tune Date	:	
Batch Name	:	VoaSampleData.batch.bin
Last Calib Update	:	1/26/2014 1:54:39 PM

Compound	RT	QIon	Resp.	Conc.	Units	Dev(Min)
Internal Standards						
System Monitoring Compounds						
Target Compounds						QValue
Trichloroethylene	15.210	131.9	33099	55.4019	ng/ml	96

(#) = Qualifier Out of Range; (m) = Manual Integration; (+) = Area Summed; (*) = Surrogate Percent Recovery Out of Range; (d): Zeroed Peak

Quantitation Results Report

Agilent Technologies

Compound	Conc.	RT	Dev(Min)	Resp.	QIon	QRatio	Lower	Upper
Trichloroethylene	55.4019	15.21	0.04	33099	94.9	117.9	98.1	147.1
					133.9	31.9	27.1	40.7

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