MassHunter Quantitative Analysis for GC/MSD

Familiarization Guide

Agilent Technologies

Notices

Manual Part Number

G3335-90200

Edition

First edition, February 2014

Printed in USA

Agilent Technologies, Inc.
5301 Stevens Creek Boulevard
Santa Clara, CA 95051 USA

Warranty

The material contained in this document is provided "as is," and is subject to being changed, without notice, in future editions. Further, to the maximum extent permitted by applicable law, Agilent disclaims all warranties, either express or implied, with regard to this manual and any information contained herein, including but not limited to the implied warranties of merchantability and fitness for a particular purpose. Agilent shall not be liable for errors or for incidental or consequential damages in connection with the furnishing, use, or performance of this document or of any information contained herein. Should Agilent and the user have a separate written agreement with warranty terms covering the material in this document that conflict with these terms, the warranty terms in the separate agreement shall control.

Technology Licenses

The hardware and/or software described in this document are furnished under a license and may be used or copied only in accordance with the terms of such license.

Restricted Rights Legend

If software is for use in the performance of a U.S. Government prime contract or subcontract, Software is delivered and licensed as "Commercial computer software" as defined in DFAR 252.227-7014 (June 1995), or as a "commercial item" as defined in FAR 2.101(a) or as "Restricted computer software" as defined in FAR 52.227-19 (June 1987) or any equivalent agency regulation or contract clause. Use, duplication or disclosure of Software is subject to Agilent Technologies' standard commercial license terms, and non-DOD Departments and Agencies of the U.S. Government will receive no greater than Restricted Rights as defined in FAR 52.227-19(c)(1-2) (June 1987). U.S. Government users will receive no greater than Limited Rights as defined in FAR 52.227-14 (June 1987) or DFAR 252.227-7015 (b)(2) (November 1995), as applicable in any technical data.

Safety Notices

CAUTION

A **CAUTION** notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in damage to the product or loss of important data. Do not proceed beyond a **CAUTION** notice until the indicated conditions are fully understood and met.

WARNING

A WARNING notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in personal injury or death. Do not proceed beyond a WARNING notice until the indicated conditions are fully understood and met.

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 "Where to Find More Information" 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 will learn how to 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 will learn how to review results for your batch using the Batch Table Outlier indicators and Quantitation Message features.

5 Generate Quantitation Reports

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

Before You Begin These Exercises:

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

To complete these exercises you will need to copy these 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:

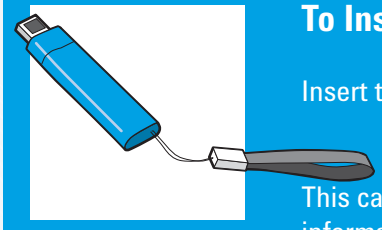
- MassHunter software installation disks
- GC/MS Software Information USB (G1701-60172)

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 Disk 1 into your DVD drive and follow the prompts.

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



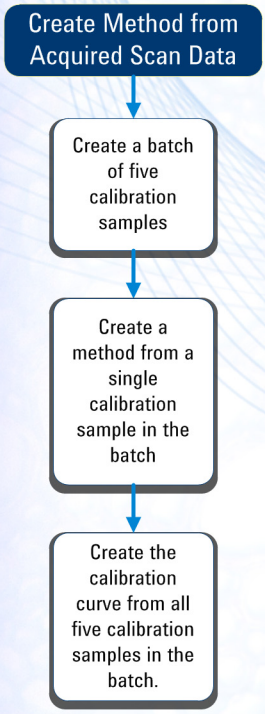
To Install Your Software Library

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.

Contents

1
Set Up a New Method from Acquired Scan Data



Agilent Technologies

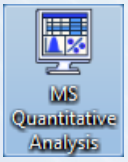
In this exercise you will create a quantitation method using previously acquired scan data. MassHunter analyzes a data file, and using search ID parameters that you specify, identifies compound names, the target ion, qualifier ions and ratios, retention times, and along with other default parameters, 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.

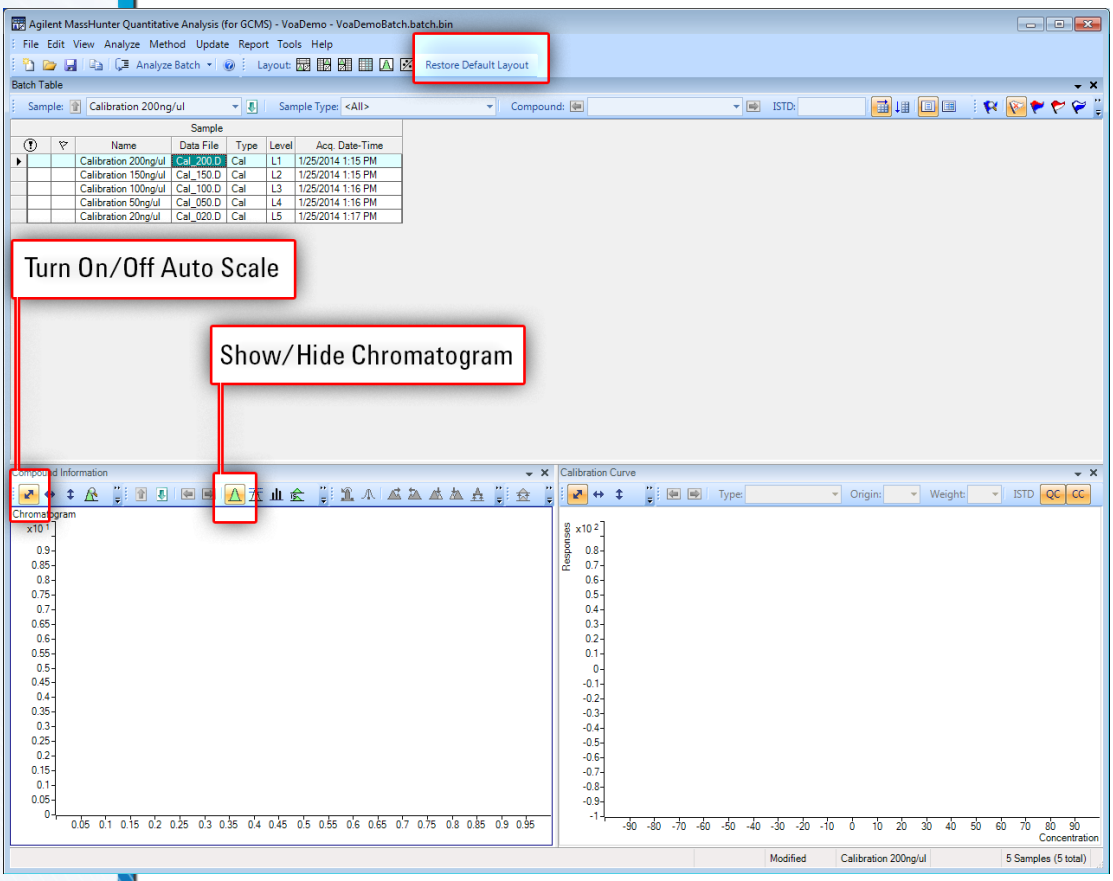
While completing this task, you will set up a method using scan data that was previously generated from a single quad instrument.

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.



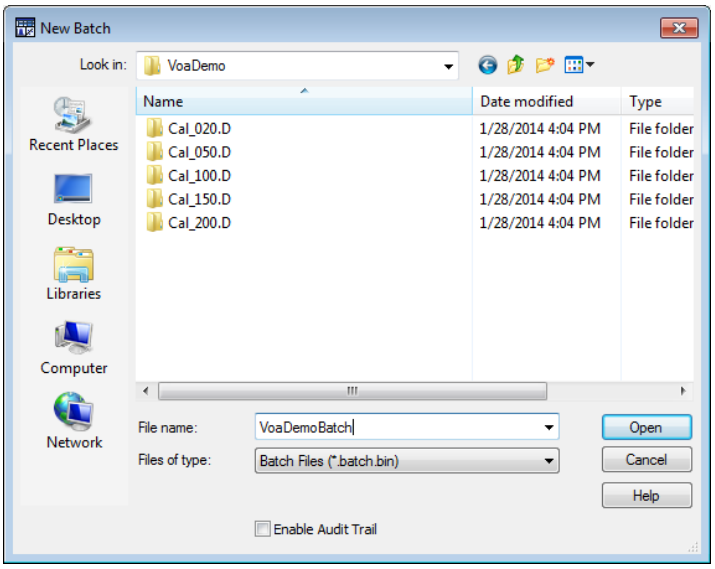
2. Navigate to the batch containing the data files you wish to use.

a Select **File > New Batch**. This displays the New Batch dialog.

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

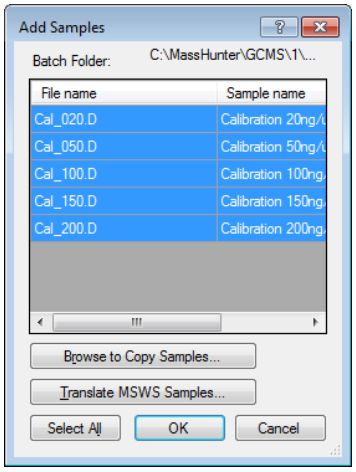
3. Assign the batch a name.

Type the File name **VoaDemoBatch** for this batch, and press **Open**.



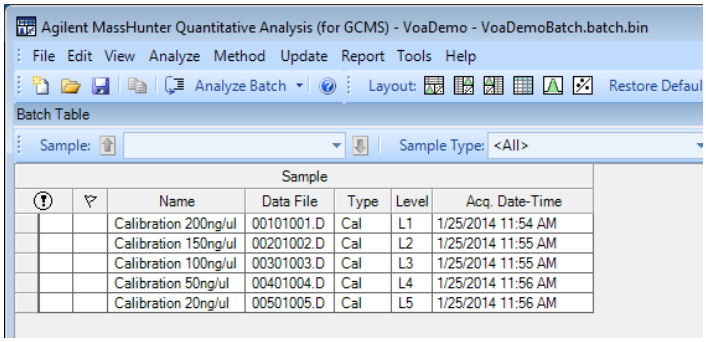
4. Select the files for this batch.

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.

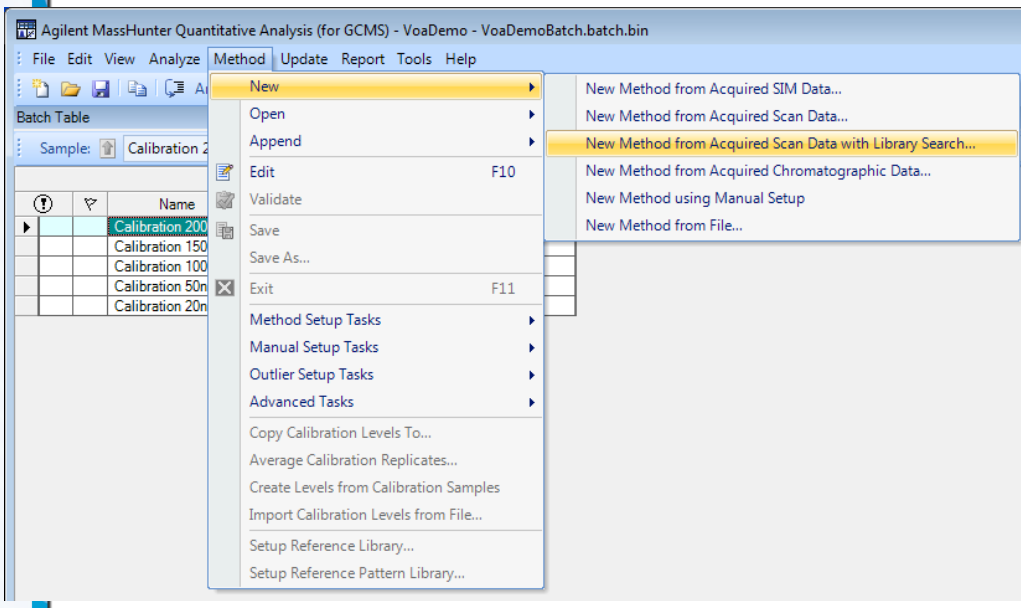


Sample						
		Name	Data File	Type	Level	Acq. Date-Time
		Calibration 200ng/ul	00101001.D	Cal	L1	1/25/2014 11:54 AM
		Calibration 150ng/ul	00201002.D	Cal	L2	1/25/2014 11:55 AM
		Calibration 100ng/ul	00301003.D	Cal	L3	1/25/2014 11:55 AM
		Calibration 50ng/ul	00401004.D	Cal	L4	1/25/2014 11:56 AM
		Calibration 20ng/ul	00501005.D	Cal	L5	1/25/2014 11:56 AM

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. Select the Library you want to use.

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

3. Select the data file you want to use.

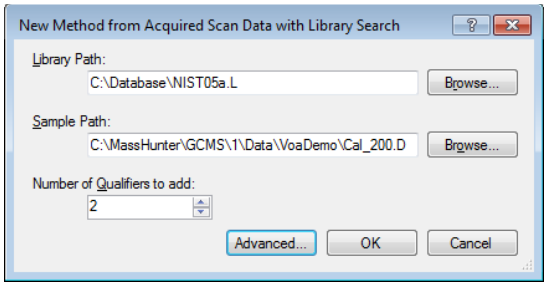
Browse to the sample data file you want to use and press **Open**. Here we are using **CAL_200.**

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

4. Enter the number of qualifiers to add.

a Enter the maximum number of qualifiers you wish 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.

b Click **Advanced** to display the Scan Analysis Parameters dialog.



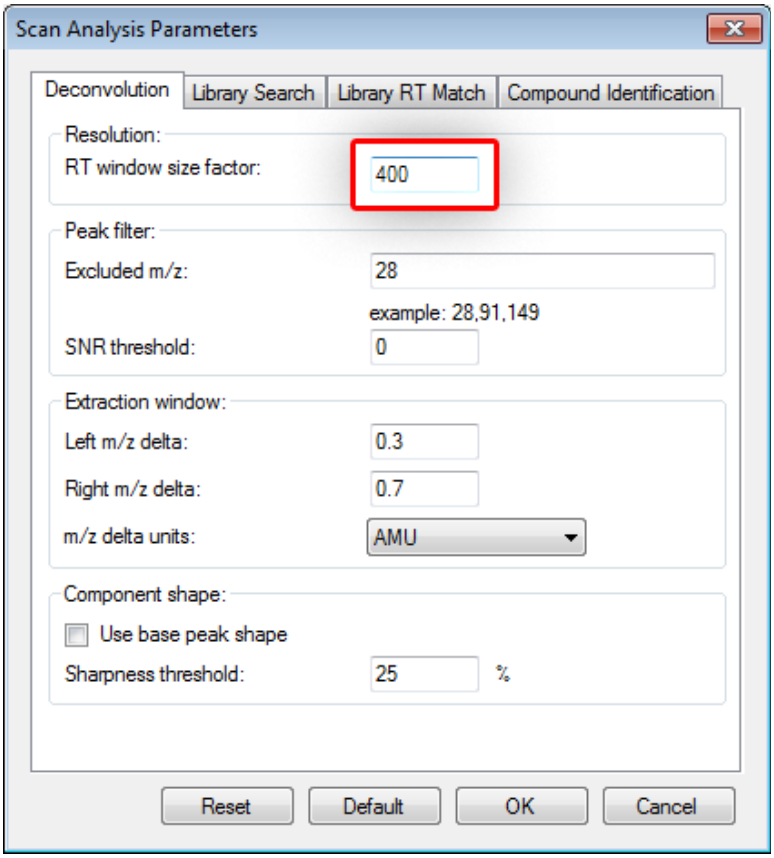
5. Set the RT window size factor.

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

b On the Deconvolution tab set the RT window size factor to 400.

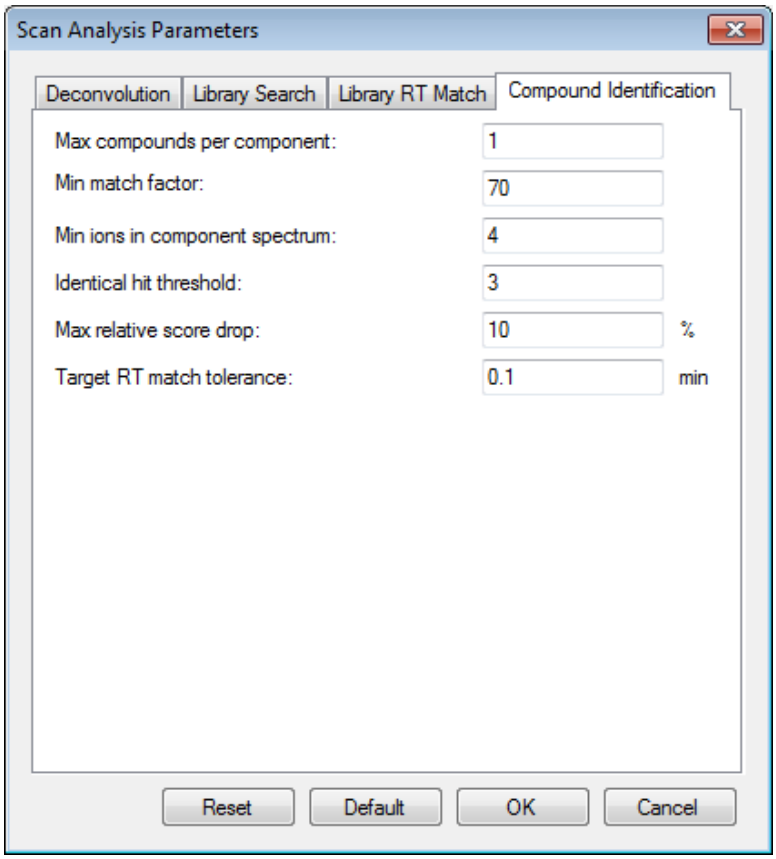
The larger this number is, the fewer the number of compounds that will be found by deconvolution. Since the peaks in this data were chromatographically optimized, we want to reduce the number of compounds found by deconvolution.

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.



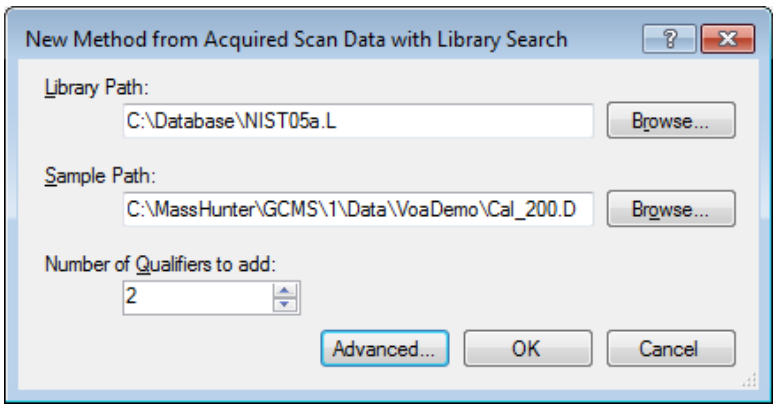
6. Change the minimum match factor to 70.

On the Compound Identification tab, change the minimum match factor to 70, then click **OK** to close the Scan Analysis Parameters dialog.



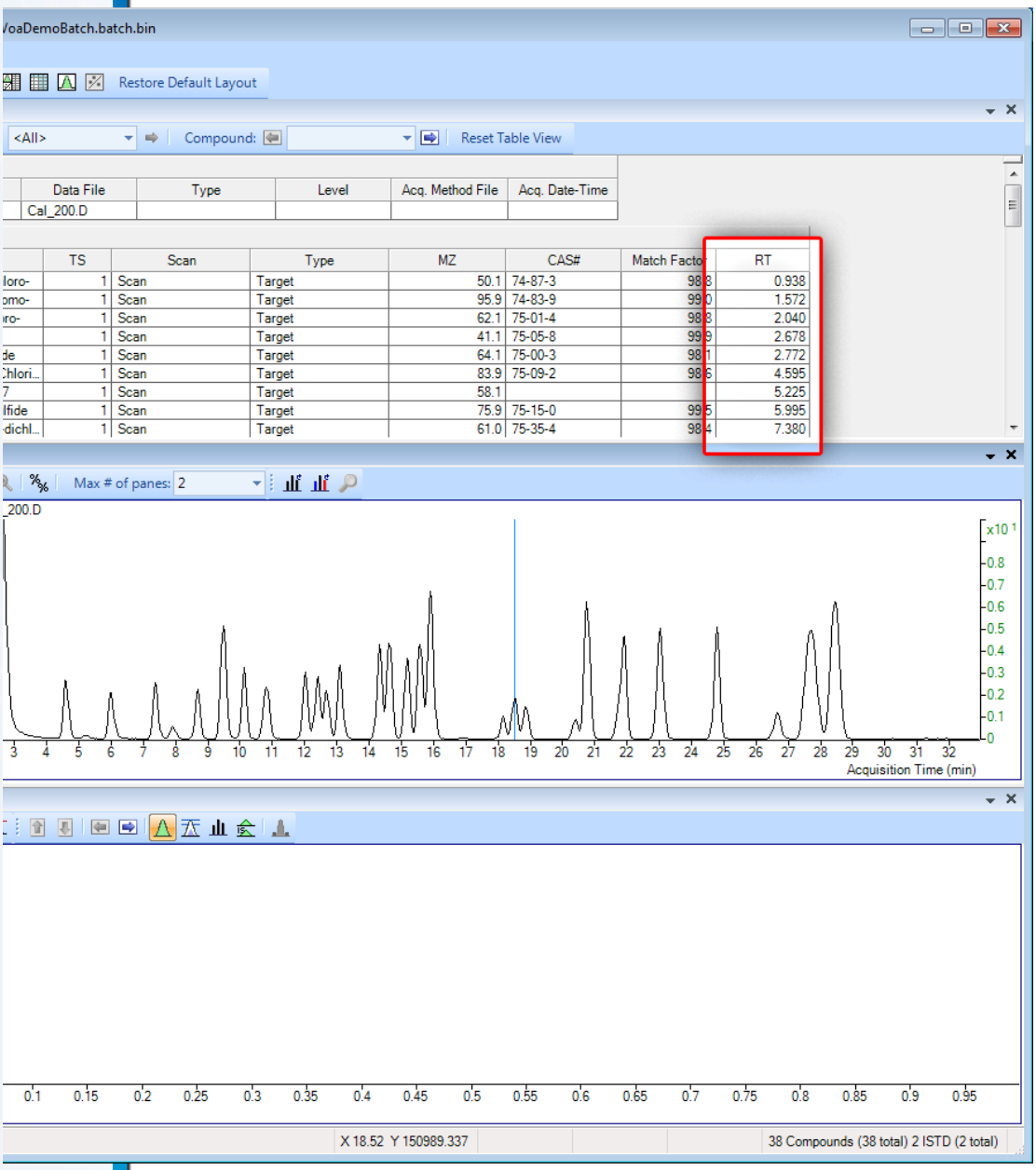
7. Return to the Method Editor view.

Click **OK** to close the New Method from Acquired Scan Data with Library Search dialog.



8. 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.



Task 3. Set up the compounds and qualifiers in the Method Table.

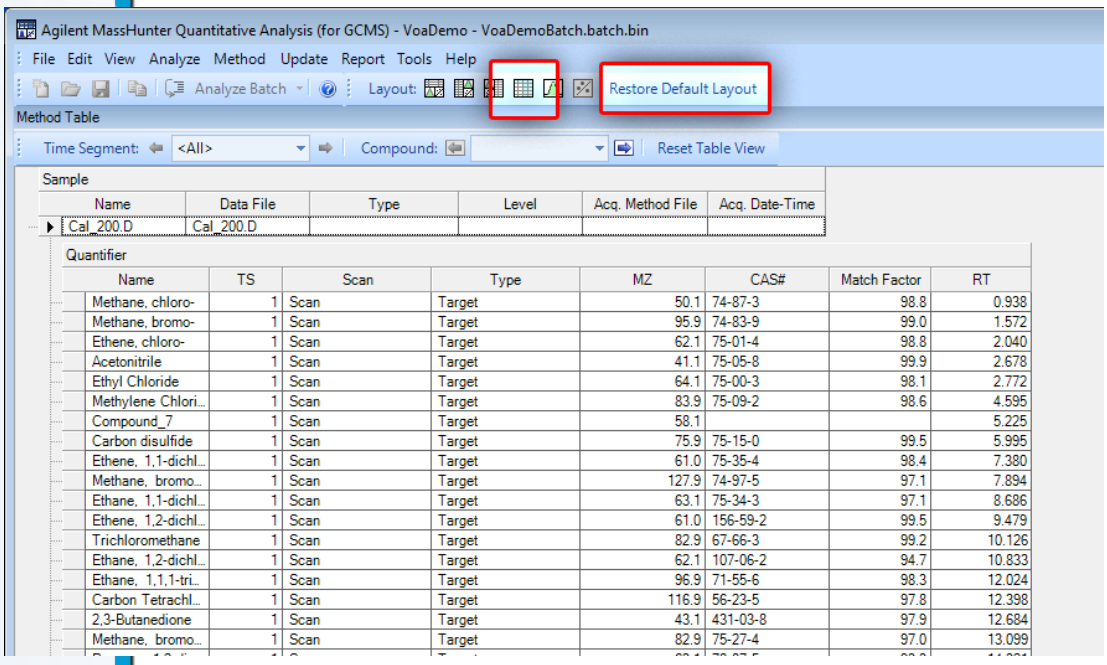
In this task you will:

- 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 and sample information from the display.

Click the **Maximize Table** icon  .

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.



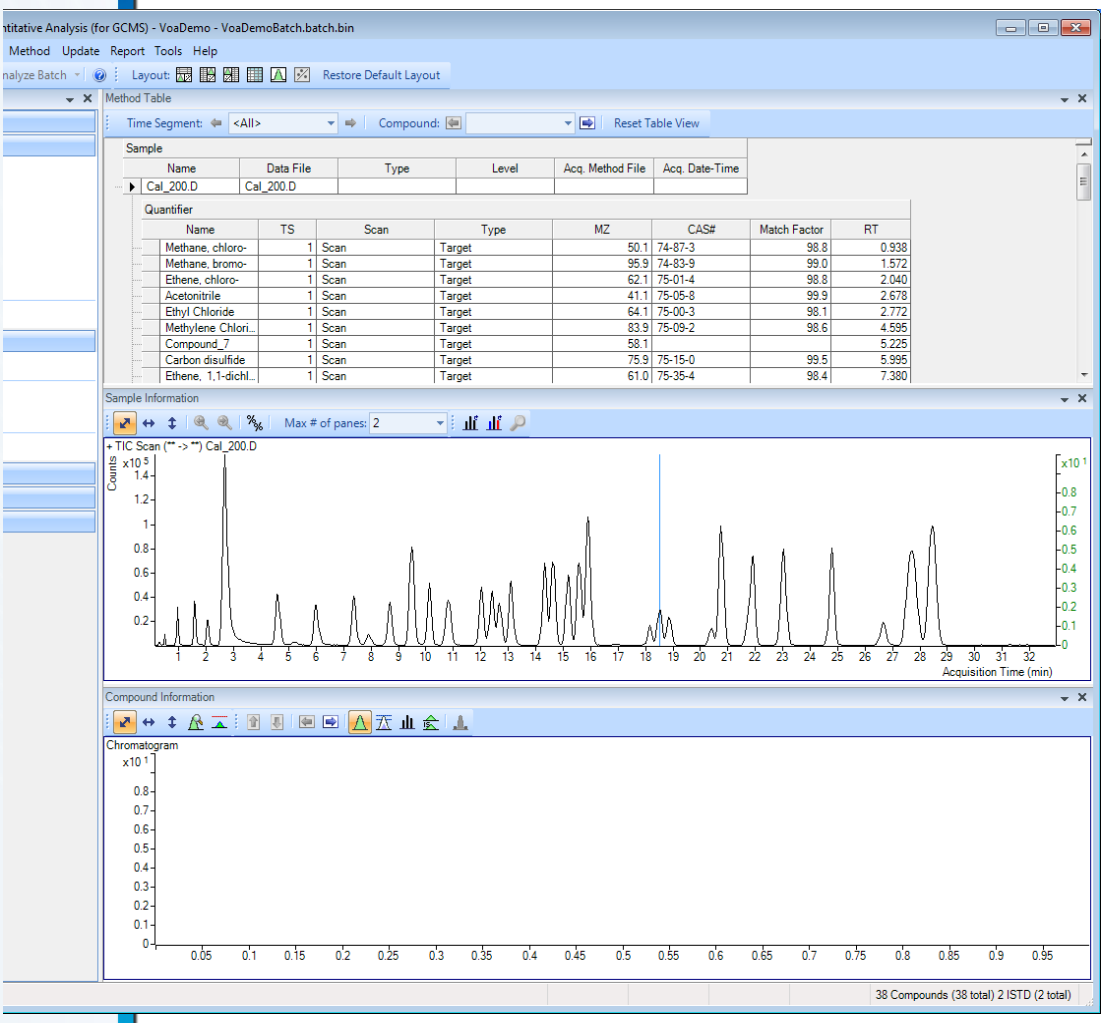
Name	TS	Scan	Type	MZ	CAS#	Match Factor	RT
Methane, chloro-	1	Scan	Target	50.1	74-87-3	98.8	0.938
Methane, bromo-	1	Scan	Target	95.9	74-83-9	99.0	1.572
Ethene, chloro-	1	Scan	Target	62.1	75-01-4	98.8	2.040
Acetonitrile	1	Scan	Target	41.1	75-05-8	99.9	2.678
Ethyl Chloride	1	Scan	Target	64.1	75-00-3	98.1	2.772
Methylene Chlori...	1	Scan	Target	83.9	75-09-2	98.6	4.595
Compound_7	1	Scan	Target	58.1			5.225
Carbon disulfide	1	Scan	Target	75.9	75-15-0	99.5	5.995
Ethene, 1,1-dichl...	1	Scan	Target	61.0	75-35-4	98.4	7.380
Methane, bromo...	1	Scan	Target	127.9	74-97-5	97.1	7.894
Ethane, 1,1-dichl...	1	Scan	Target	63.1	75-34-3	97.1	8.686
Ethene, 1,2-dichl...	1	Scan	Target	61.0	156-59-2	99.5	9.479
Trichloromethane	1	Scan	Target	82.9	67-66-3	99.2	10.126
Ethane, 1,2-dichl...	1	Scan	Target	62.1	107-06-2	94.7	10.833
Ethane, 1,1,1-tri...	1	Scan	Target	96.9	71-55-6	98.3	12.024
Carbon Tetrachl...	1	Scan	Target	116.9	56-23-5	97.8	12.398
2,3-Butanedione	1	Scan	Target	43.1	431-03-8	97.9	12.684
Methane, bromo...	1	Scan	Target	82.9	75-27-4	97.0	13.099

2. Restore the previous view.

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

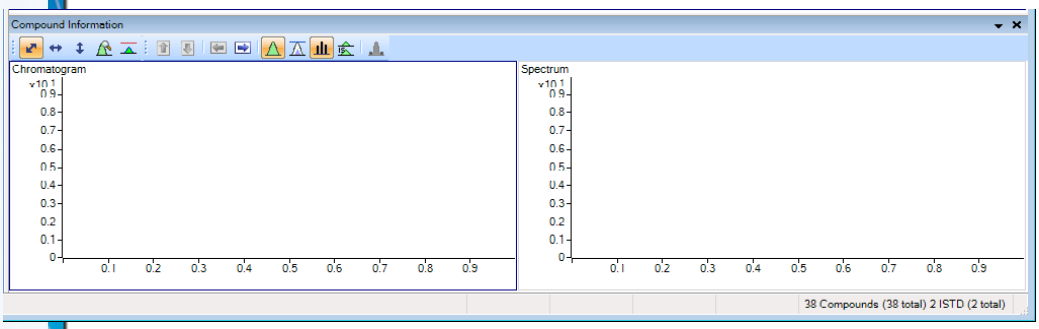
3. Review the default layout.

Here you can see the Method tasks area, the Method Table, the Sample Information window, and the Compound Information window.



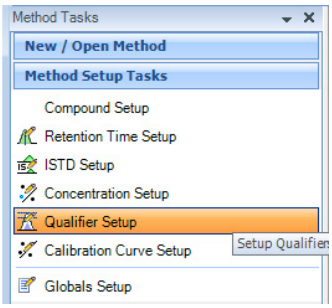
4. Display the spectrum pane.

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. Edit the compound information.

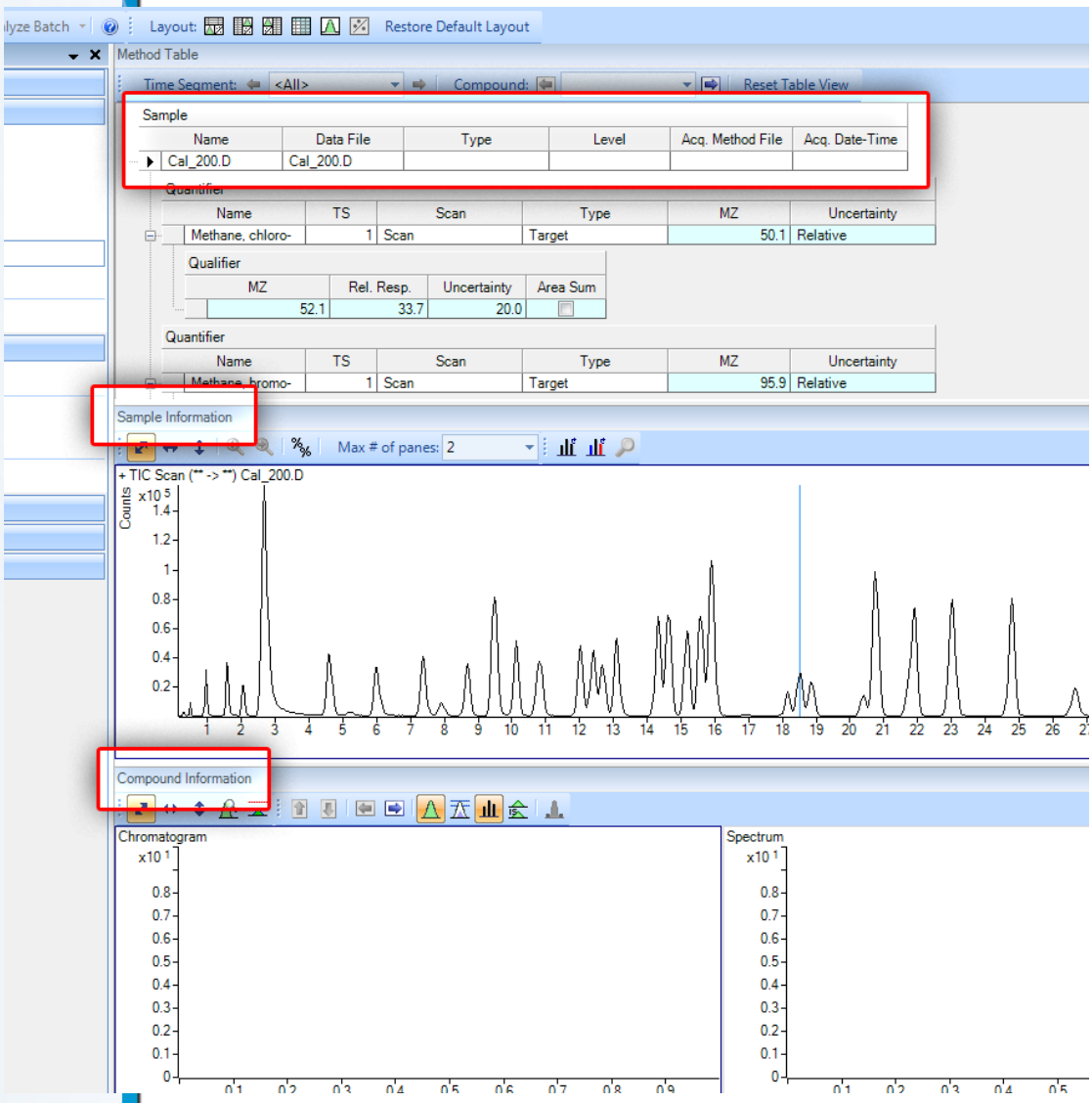
In the **Method Tasks** area select **Method Setup Tasks > Qualifier Setup** to edit compound parameters that are misidentified.



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.

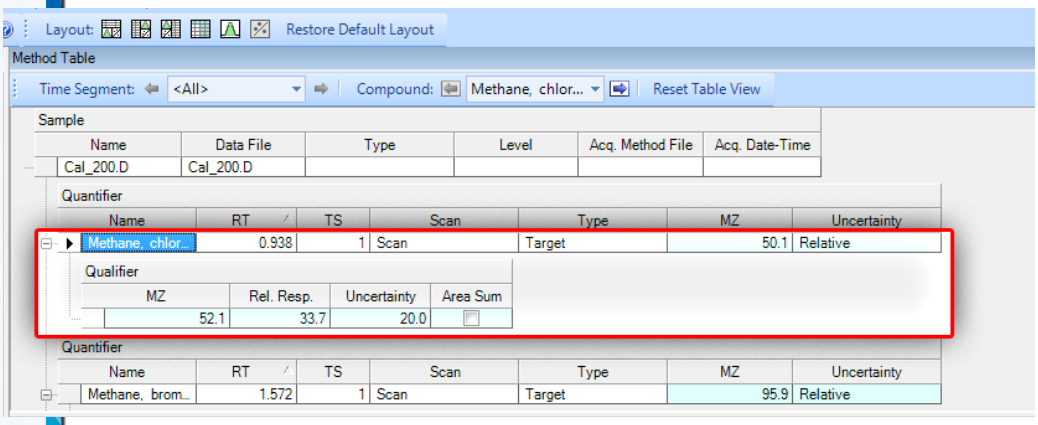


7. Click in the methane, chloromethane quantifier name field.

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.

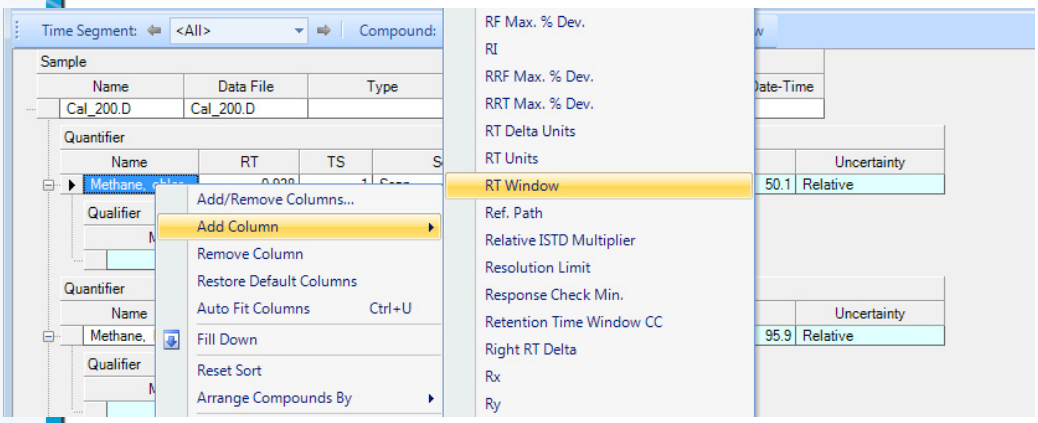
The compound's qualifiers are shown in the Method table directly below the Quantifier entry. For the quantifier we are using, you can see that MassHunter selected only a single qualifier.



9. Add Retention Time to the Method table.

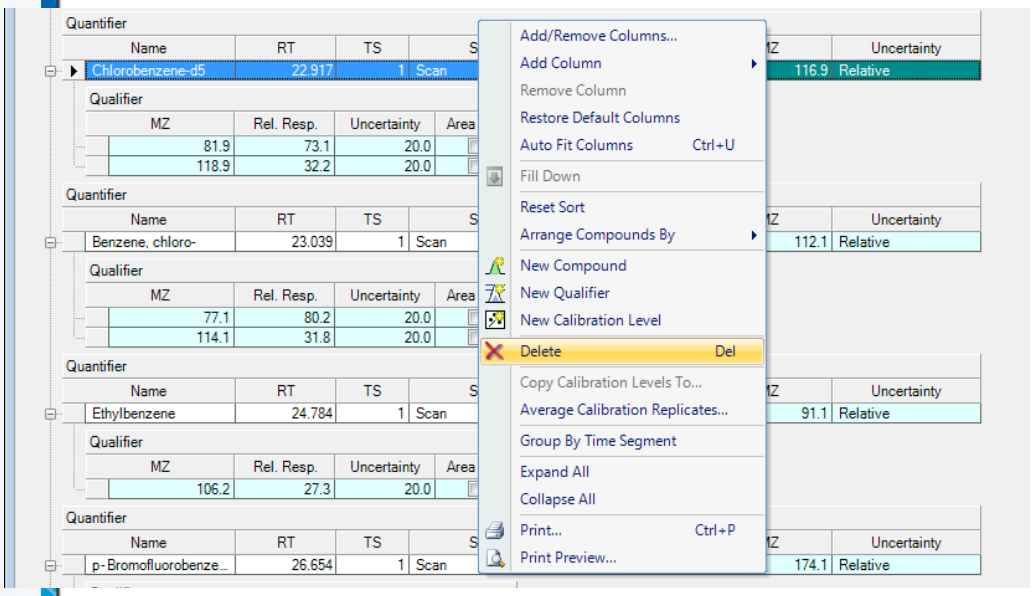
Since these are known compounds that were added to the calibration sample, we need to verify each name entry, the target ion, the qualifier ions, and the relative response of each qualifier ion.

Click the **Maximize Table** icon  then right click any quantifier entry and select **Add Column > RT** to add the Retention Time (RT) column to the Method table.



10. Delete unwanted compounds.

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 clicking the **Delete** key.

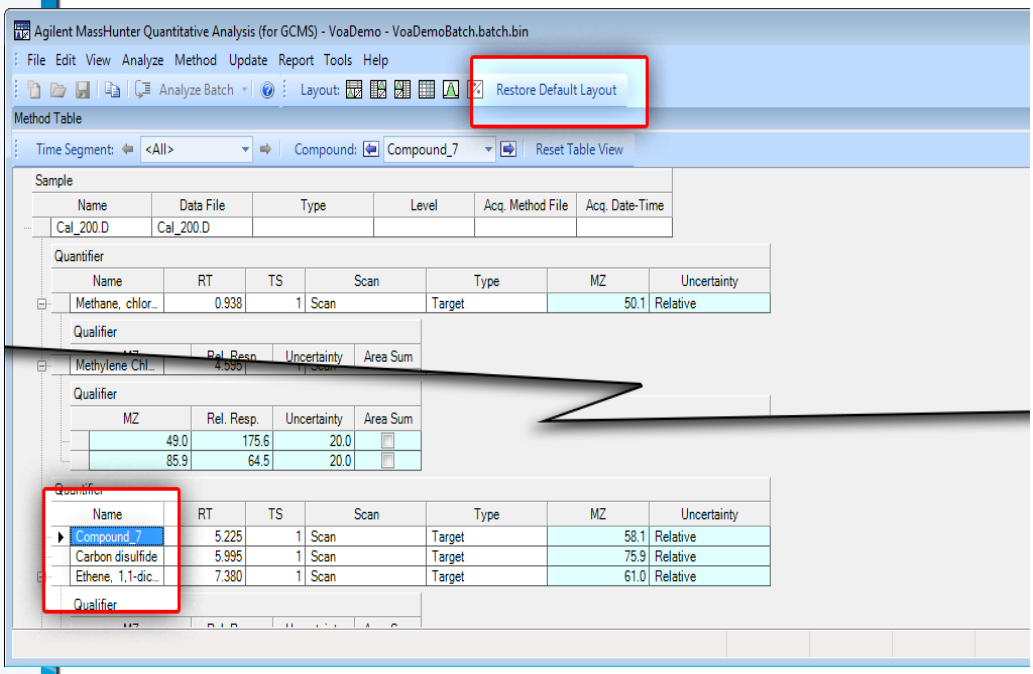


11. Use the Library Search feature to try to identify the unknown compound.

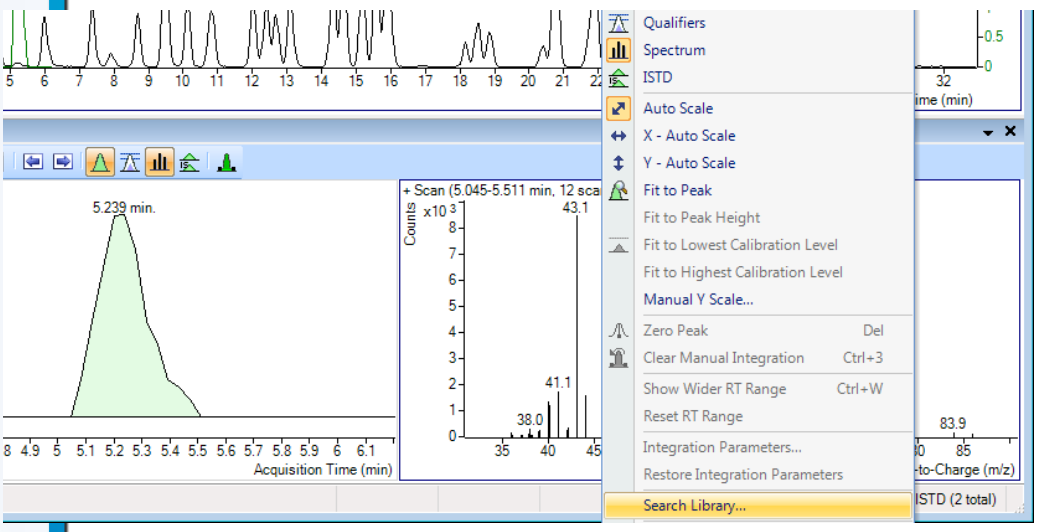
MassHunter could not identify the compound it gave the placeholder name Compound_7. We were expecting Acetone here from our calibration sample.

a Select Compound_7.

b Click **Restore Default Layout**,.



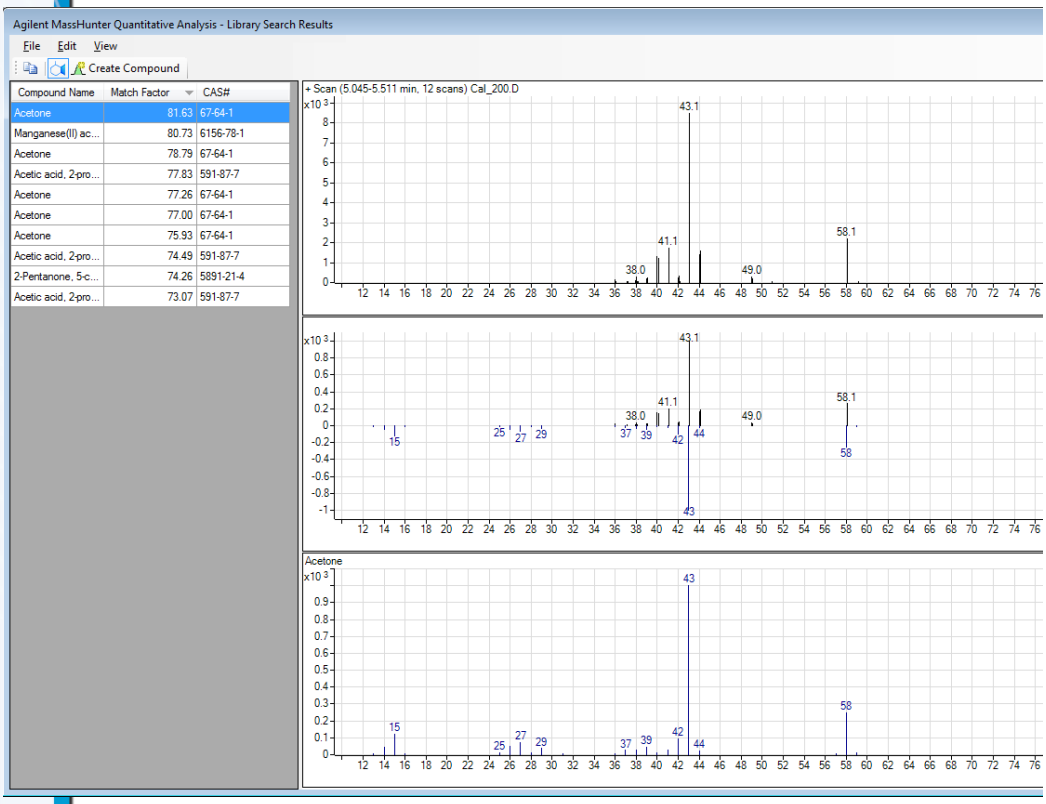
c Right-mouse click inside the Compound Information window Spectrum pane and select **Search Library** from the context menu.



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.

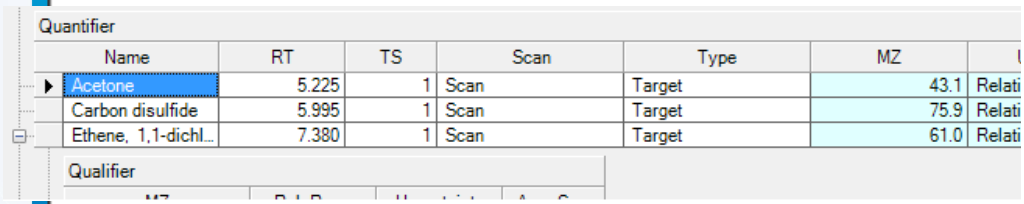


f Record the Match Factor value of 81.63, and CAS# of 67-64-1 for future use.

g Close the **Library Search Results** window, then click **Maximize Table**  .

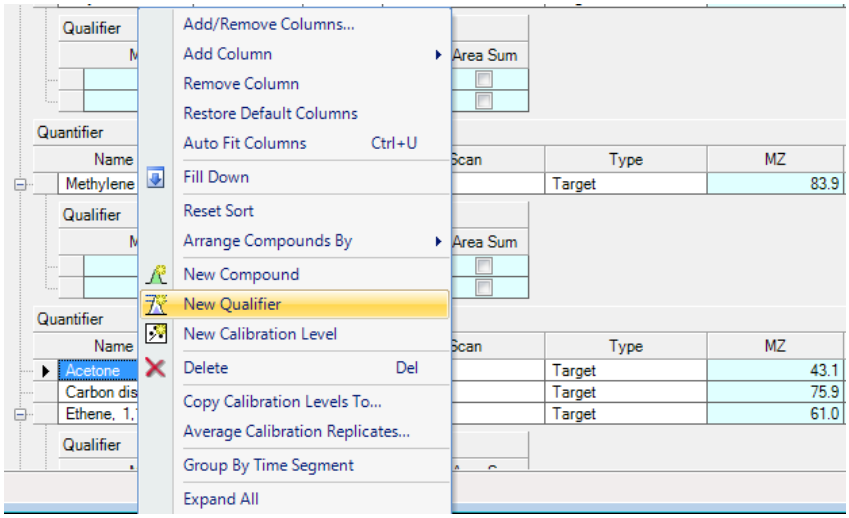
12. Change the unknown Compound_7 name to Acetone.

Change the Compound_7 **Name** parameter to **Acetone** and change the acetone m/z to 43.1. The 58.1 identified as the quantifier is actually the qualifier.



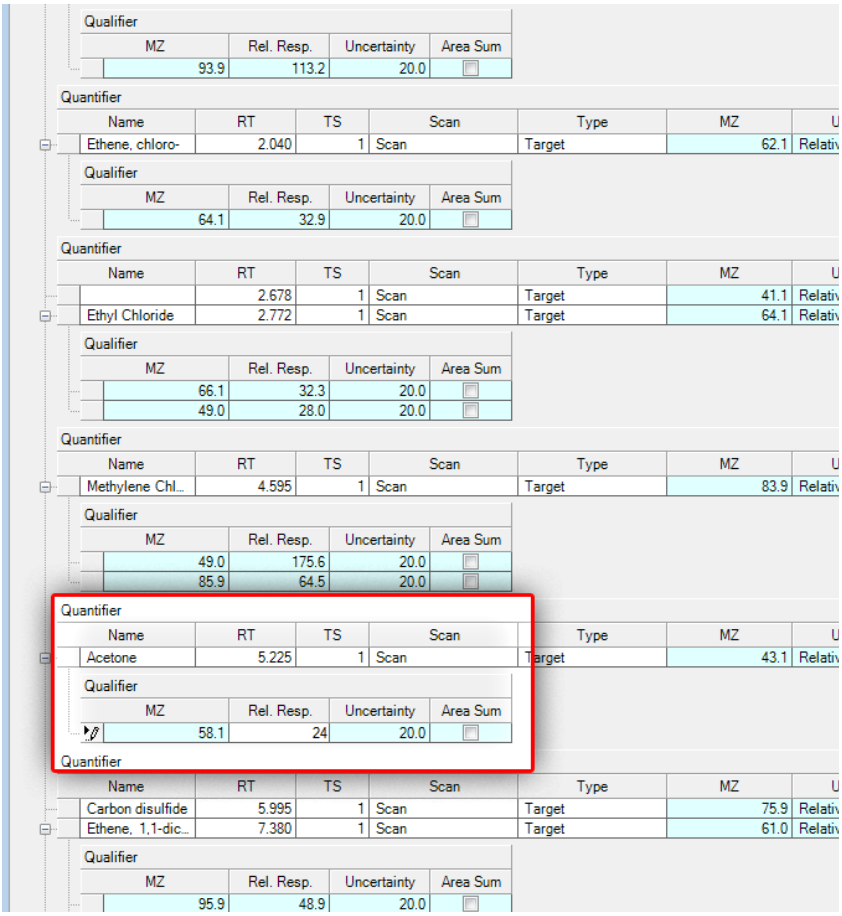
13. Add Acetone as a qualifier.

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



14. Enter the mz and relative response.

Enter 58.1 as the qualifier mz and 24 as the relative response (= 2.2/9 from the samples 58.1 vs 43.1 ion response).

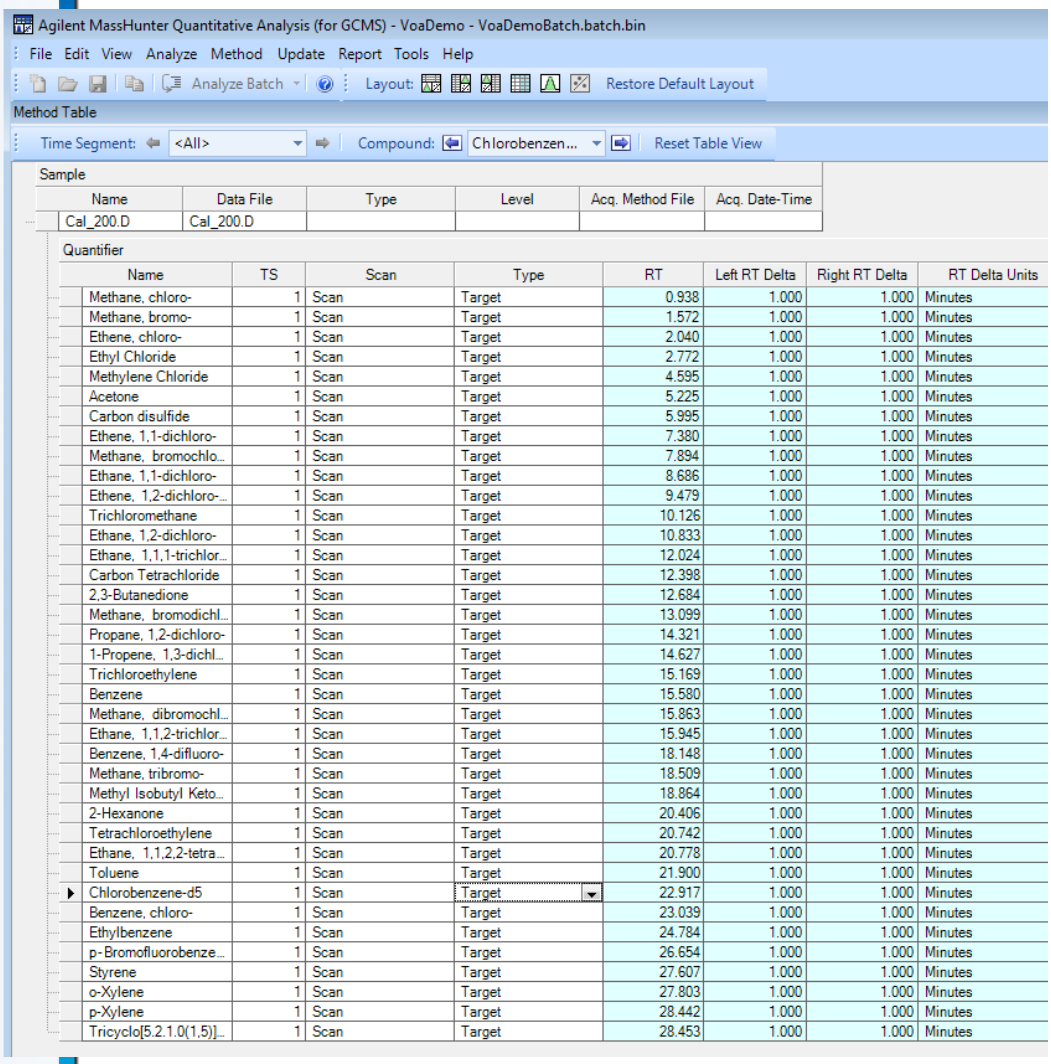


Continue to review all compounds and their qualifiers identified in the method table.

15. 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.



Name	TS	Scan	Type	RT	Left RT Delta	Right RT Delta	RT Delta Units
Methane, chloro-	1	Scan	Target	0.938	1.000	1.000	Minutes
Methane, bromo-	1	Scan	Target	1.572	1.000	1.000	Minutes
Ethene, chloro-	1	Scan	Target	2.040	1.000	1.000	Minutes
Ethyl Chloride	1	Scan	Target	2.772	1.000	1.000	Minutes
Methylene Chloride	1	Scan	Target	4.595	1.000	1.000	Minutes
Acetone	1	Scan	Target	5.225	1.000	1.000	Minutes
Carbon disulfide	1	Scan	Target	5.995	1.000	1.000	Minutes
Ethene, 1,1-dichloro-	1	Scan	Target	7.380	1.000	1.000	Minutes
Methane, bromochlo...	1	Scan	Target	7.894	1.000	1.000	Minutes
Ethane, 1,1-dichloro-	1	Scan	Target	8.686	1.000	1.000	Minutes
Ethene, 1,2-dichloro-...	1	Scan	Target	9.479	1.000	1.000	Minutes
Trichloromethane	1	Scan	Target	10.126	1.000	1.000	Minutes
Ethane, 1,2-dichloro-	1	Scan	Target	10.833	1.000	1.000	Minutes
Ethane, 1,1,1-trichlor...	1	Scan	Target	12.024	1.000	1.000	Minutes
Carbon Tetrachloride	1	Scan	Target	12.398	1.000	1.000	Minutes
2,3-Butanedione	1	Scan	Target	12.684	1.000	1.000	Minutes
Methane, bromodichl...	1	Scan	Target	13.099	1.000	1.000	Minutes
Propane, 1,2-dichloro-	1	Scan	Target	14.321	1.000	1.000	Minutes
1-Propene, 1,3-dichl...	1	Scan	Target	14.627	1.000	1.000	Minutes
Trichloroethylene	1	Scan	Target	15.169	1.000	1.000	Minutes
Benzene	1	Scan	Target	15.580	1.000	1.000	Minutes
Methane, dibromochl...	1	Scan	Target	15.863	1.000	1.000	Minutes
Ethane, 1,1,2-trichlor...	1	Scan	Target	15.945	1.000	1.000	Minutes
Benzene, 1,4-difluoro-	1	Scan	Target	18.148	1.000	1.000	Minutes
Methane, tribromo-	1	Scan	Target	18.509	1.000	1.000	Minutes
Methyl Isobutyl Keto...	1	Scan	Target	18.864	1.000	1.000	Minutes
2-Hexanone	1	Scan	Target	20.406	1.000	1.000	Minutes
Tetrachloroethylene	1	Scan	Target	20.742	1.000	1.000	Minutes
Ethane, 1,1,2,2-tetra...	1	Scan	Target	20.778	1.000	1.000	Minutes
Toluene	1	Scan	Target	21.900	1.000	1.000	Minutes
Chlorobenzene-d5	1	Scan	Target	22.917	1.000	1.000	Minutes
Benzene, chloro-	1	Scan	Target	23.039	1.000	1.000	Minutes
Ethylbenzene	1	Scan	Target	24.784	1.000	1.000	Minutes
p-Bromofluorobenze...	1	Scan	Target	26.654	1.000	1.000	Minutes
Styrene	1	Scan	Target	27.607	1.000	1.000	Minutes
o-Xylene	1	Scan	Target	27.803	1.000	1.000	Minutes
p-Xylene	1	Scan	Target	28.442	1.000	1.000	Minutes
Tricyclo[5.2.1.0(1,5)]...	1	Scan	Target	28.453	1.000	1.000	Minutes

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.

16. 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 checkbox requires manually setting the Type to Target.

17. Assign the Methane, Bromochloromethane ISTD to the calibration compounds in the RT range of 0.9 to 10.2.

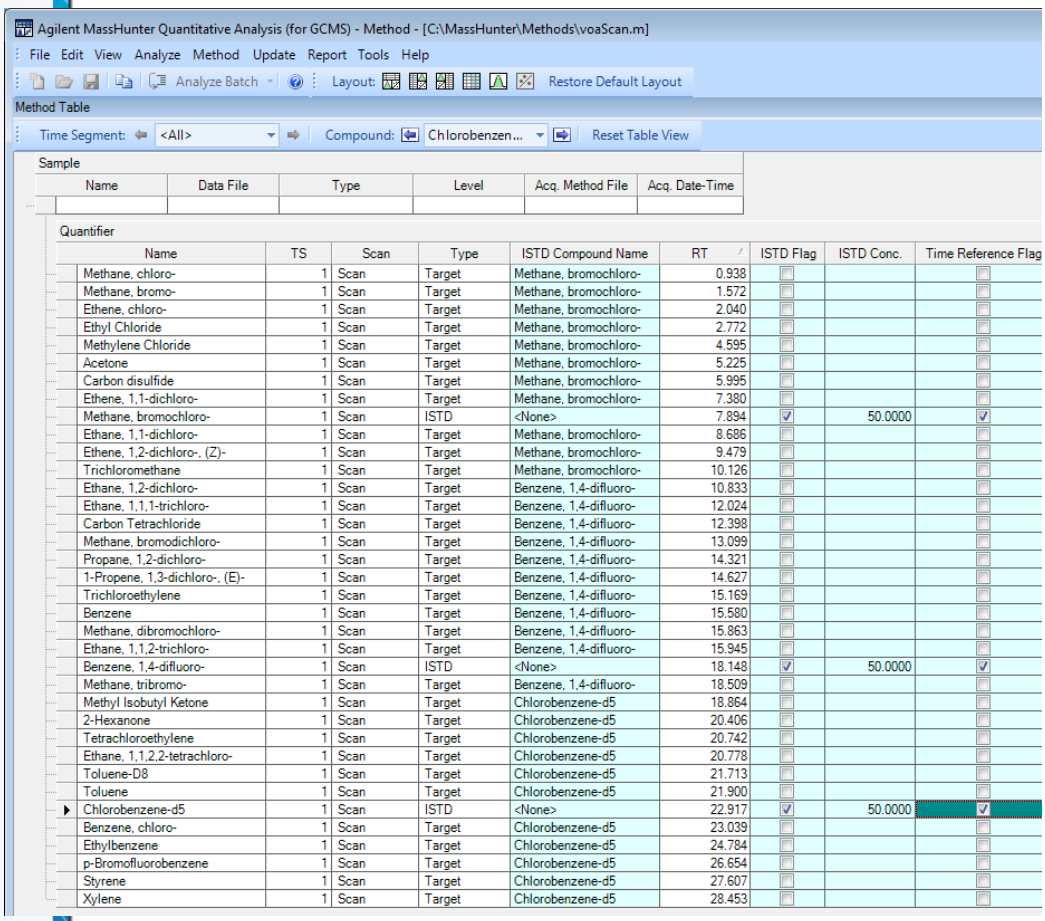
18. Assign the 1-4 Difluorobenzene ISTD to the calibration compounds in the RT range of 10.8 to 18.5.

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

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.

To do this, select the ISTD **Compound Name** for the first compound and then select **Fill Down** from the context menu 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.

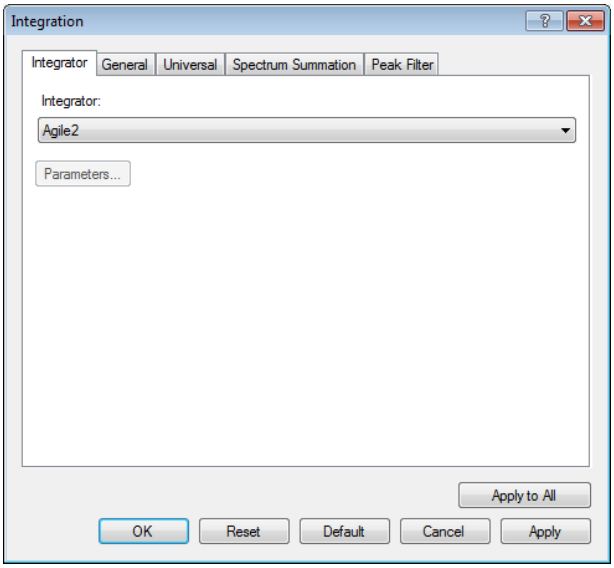
Your screen should now look like this.



20. Access the Integrator Parameters setup dialog.

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 is displayed. Agile 2 is shown as the integrator in our example.

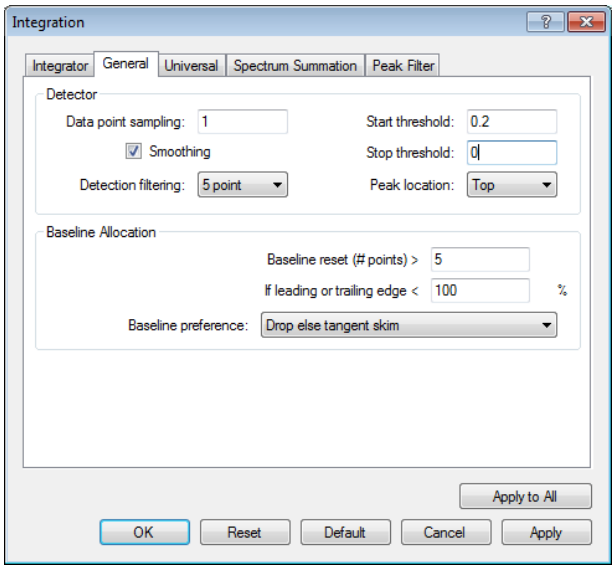


21. Setup the **General** integrator.

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

b Click **Parameters** to open the **General** tab on the integration dialog.

c Edit the settings to match the dialog below.



d 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.

Task 4. Add a calibration curve.

1. Setup concentration levels for calibration compounds.

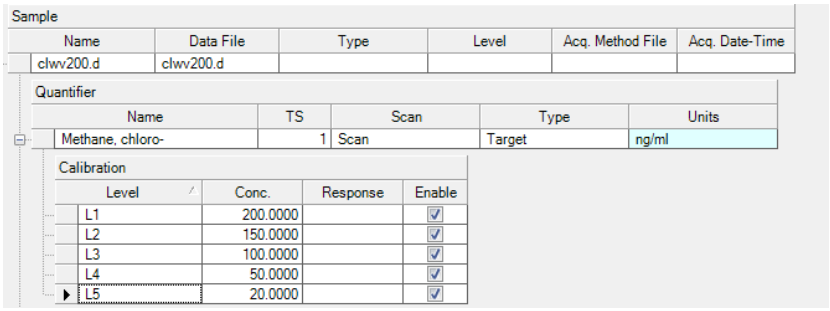
a Select **Method > Method Setup Tasks > Concentration Setup**.

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

c Add four more levels to this table.

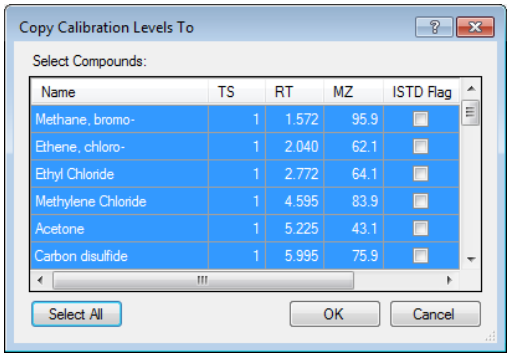
d In the **Level** column add the names L1, L2, L3, L4, and L5.

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



2. Copy the Calibration table to all target compounds.

a With your cursor in the Calibration table, right-click and select **Copy Calibration Levels To** from the context menu.



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

3. Setup concentration levels for the three internal standards.

a In the Quantifier table click **Type** 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.

Quantifier

Name	TS	Scan	Type	Units
Methane, bromochloro-	1	Scan	ISTD	ng/ml

Calibration

Level	Conc.	Response	Enable
L1	50.0000		☑
L2	50.0000		☑
L3	50.0000		☑
L4	50.0000		☑
L5	50.0000		☑

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

4. 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 dropdown select **Average of Response Factors**. This works well for our data.

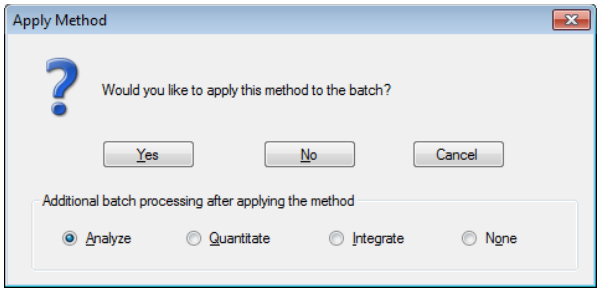
d With the cursor on this last entry, select **Fill Down** from the context menu. The default curve fit (CF) parameters displayed are, **Ignore** under the CF Origin column, and **None** under the CF Weight factor column. Keep these settings for our example.

5. Save the method.

a Select **Method > Exit**. The Apply Method dialog displays.

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

c 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 Quant method, this Analysis of the batch will populate the calibration tables with the compound responses.

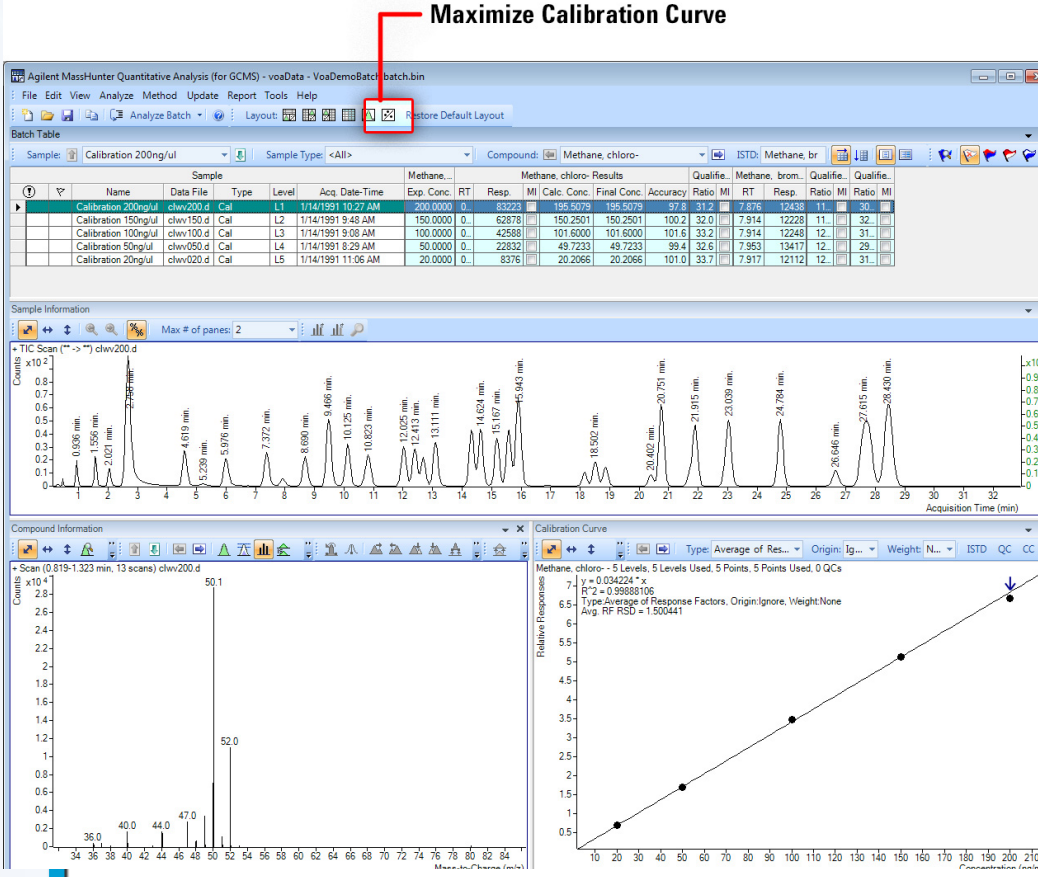
6. Review the calibration curve.

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

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

b Put your cursor over the word **Sample** in the Batch Table, then from the context menu, select **Auto Fit Columns**.

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.



8. 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.

9. Explore the Curve Fit Assistant.

a In the Calibration Curve window, right-click and select **Curve Fit Assistant**. This opens the Curve Fit parameter 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.

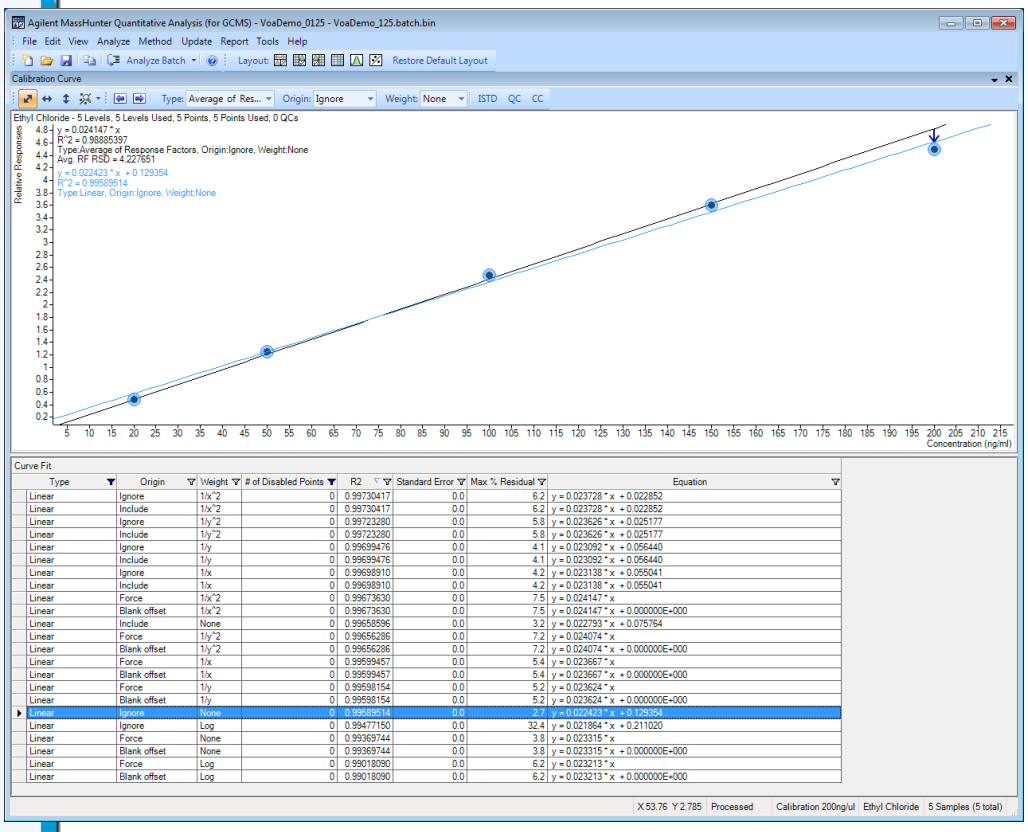
c Sort on the R2 column so that the first CF in the table has the value closest to 1.0. Select this line to see how the curve goes nicely through all 5 points. This is a quadratic with weighting and we want to see if something simpler will work.

d In the Curve Fit table click on the **TYPE** funnel icon and set the column filter to **Linea**r. We are skipping the simple Average of Response Factors since it isn't a very good fit at higher concentrations.

e In the Curve Fit table select various rows and observe the colored curve. A simple linear curve with ignore origin and equal weighting is a good fit.

f Select the **Linear** curve with **Ignore** Origin and **None** for weighting.

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



Type	Origin	Weight	# of Disabled Points	R2	Standard Error	Max % Residual	Equation
Linear	Ignore	1/x^2	0	0.99730417	0.0	6.2	y = 0.023728 * x + 0.022852
Linear	Include	1/x^2	0	0.99730417	0.0	6.2	y = 0.023728 * x + 0.022852
Linear	Ignore	1/y^2	0	0.99723280	0.0	5.8	y = 0.023626 * x + 0.025177
Linear	Include	1/y^2	0	0.99723280	0.0	5.8	y = 0.023626 * x + 0.025177
Linear	Ignore	1/y	0	0.99699476	0.0	4.1	y = 0.023092 * x + 0.056440
Linear	Include	1/y	0	0.99699476	0.0	4.1	y = 0.023092 * x + 0.056440
Linear	Ignore	1/x	0	0.99698910	0.0	4.2	y = 0.023138 * x + 0.055041
Linear	Include	1/x	0	0.99698910	0.0	4.2	y = 0.023138 * x + 0.055041
Linear	Force	1/x^2	0	0.99673630	0.0	7.5	y = 0.024147 * x
Linear	Blank offset	1/x^2	0	0.99673630	0.0	7.5	y = 0.024147 * x + 0.000000E+000
Linear	Include	None	0	0.99658596	0.0	3.2	y = 0.022793 * x + 0.075764
Linear	Force	1/y^2	0	0.99656286	0.0	7.2	y = 0.024074 * x
Linear	Blank offset	1/y^2	0	0.99656286	0.0	7.2	y = 0.024074 * x + 0.000000E+000
Linear	Force	1/x	0	0.99599457	0.0	5.4	y = 0.023667 * x
Linear	Blank offset	1/x	0	0.99599457	0.0	5.4	y = 0.023667 * x + 0.000000E+000
Linear	Force	1/y	0	0.99598154	0.0	5.2	y = 0.023624 * x
Linear	Blank offset	1/y	0	0.99598154	0.0	5.2	y = 0.023624 * x + 0.000000E+000
Linear	Ignore	None	0	0.99589514	0.0	2.7	y = 0.022423 * x + 0.129354
Linear	Ignore	Log	0	0.99477150	0.0	32.4	y = 0.021864 * x + 0.211020
Linear	Force	None	0	0.99369744	0.0	3.8	y = 0.023315 * x
Linear	Blank offset	None	0	0.99369744	0.0	3.8	y = 0.023315 * x + 0.000000E+000
Linear	Force	Log	0	0.99018090	0.0	6.2	y = 0.023213 * x
Linear	Blank offset	Log	0	0.99018090	0.0	6.2	y = 0.023213 * x + 0.000000E+000

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 Select **Curve Fit Assistant** from the context menu to exit the assistant.

10. 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 With the cursor positioned over the Quantifier table label, right-click and select **Add/Remove Columns** from the context menu. This displays the **Columns** dialog.

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.

1. Access the Method.

2. Prepare the method data for this demonstration.

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 on-line help. The following describes how to manually add a calibration compound to your method in.

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 then select **Method > Edit** to access the method editor.

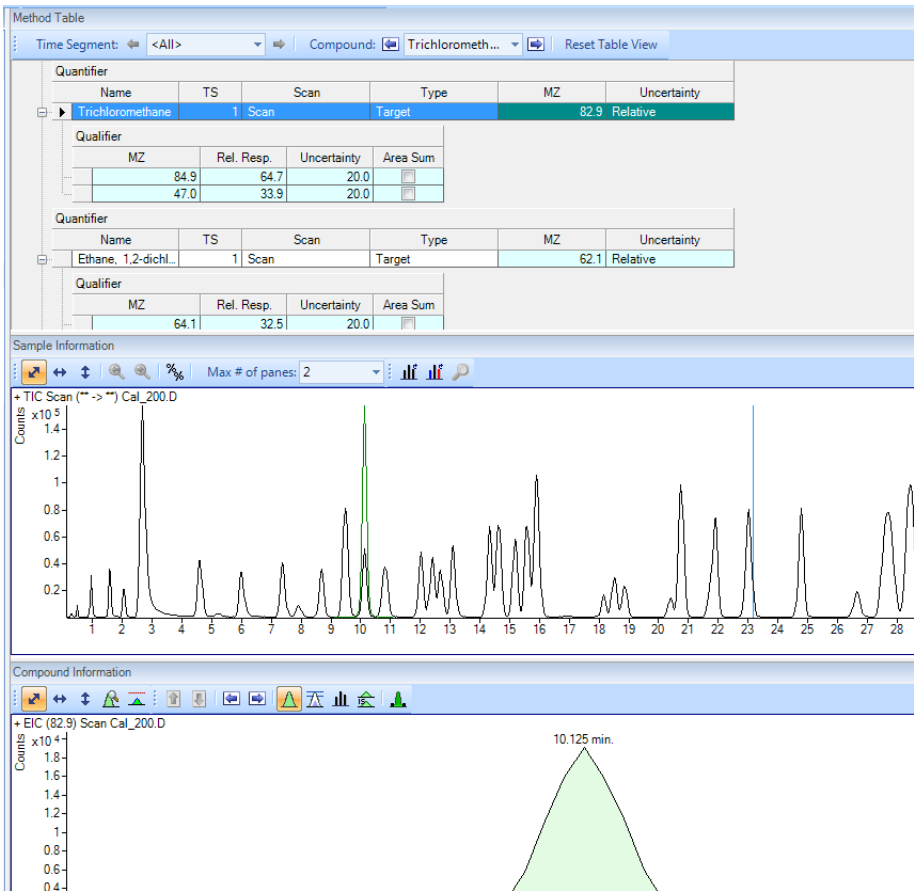
---

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 With the compound selected in the Quantifier table, from the context menu select **Delete**.



3. Add the Compound to the method.

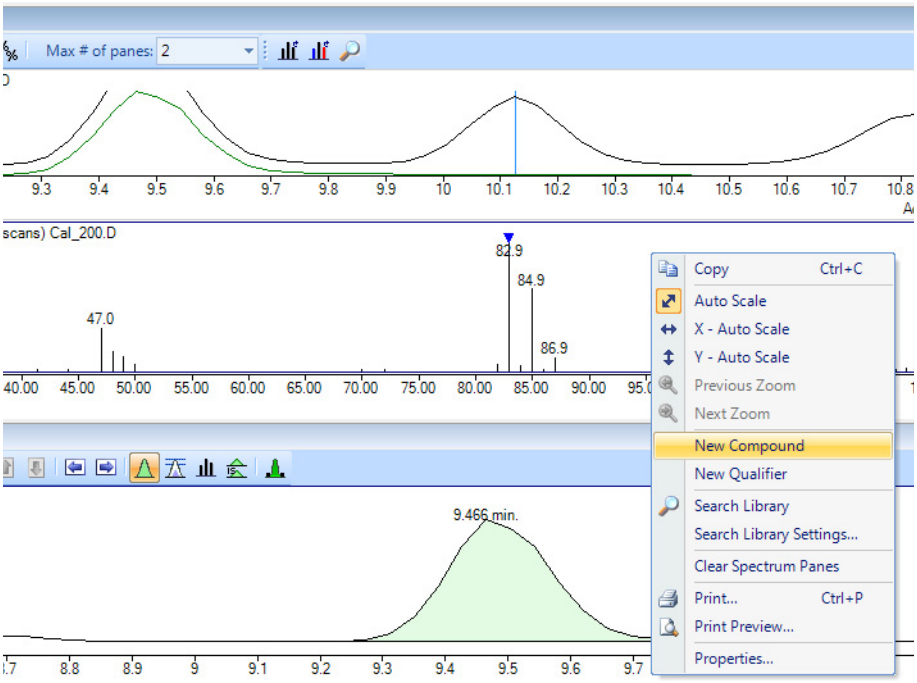
a In the Sample Information window zoom (right-mouse drag) between 9 and 11 minutes.

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

c From the context menu 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. From the context menu 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.

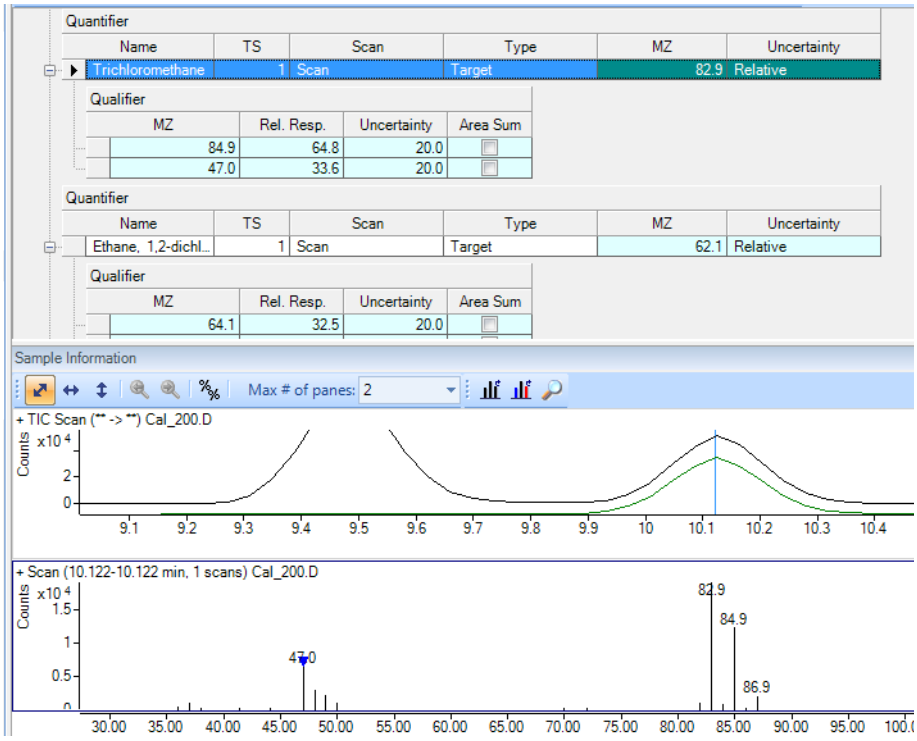
b From the context menu select **Extract Spectrum**.

c Select 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.



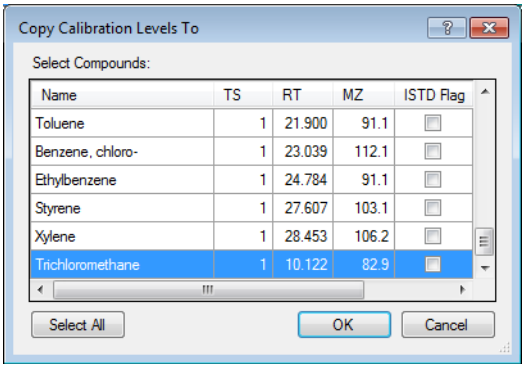
6. Review and update method parameters for this compound.

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 With an adjacent compound selected in the Method Table, from the context menu select **Copy Calibration Levels To** and from the dialog displayed select **Trichloromethane.**

d 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.

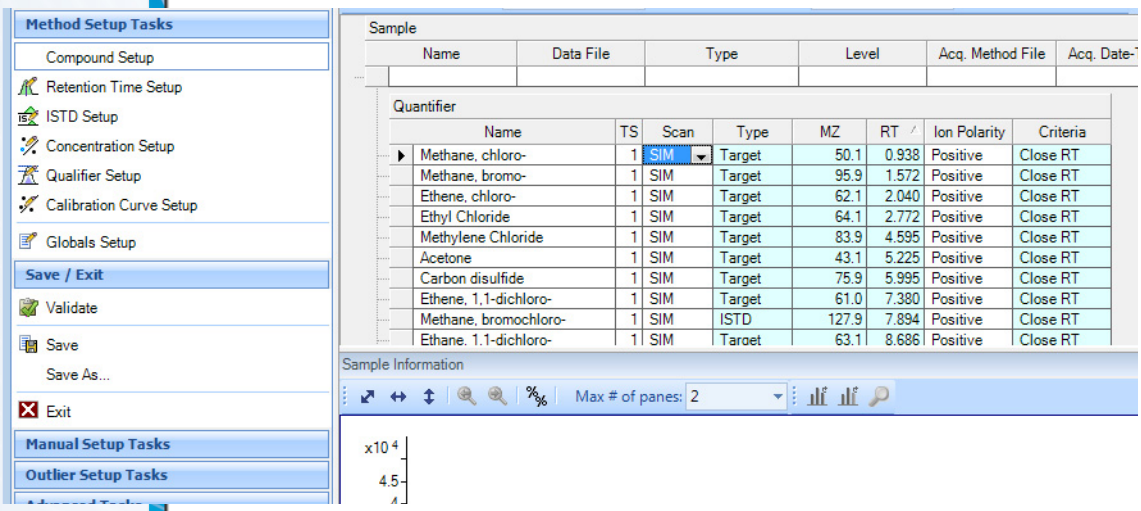
1. Edit the method.

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

b From Method Setup Tasks click **Compound Setup**.

c In the table's first compound's **Scan** column, select **SIM** from the dropdown.

d Select **Fill Down** from the context menu 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 quant method's calibration curve.

b Set the method for the sequence table to the SIM acquisition method that also contains the quant 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 Quant, 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 quant 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 27. 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 quant 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 32.

From a SIM Method

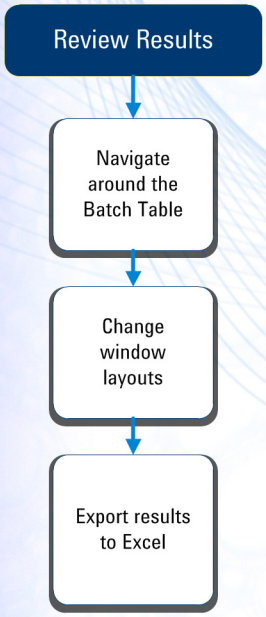
New compounds can be added to an existing SIM method. To do this you would create an acquisition method and sequence containing only the new compounds. This would create a batch table in Quant containing the calibration data for the new compounds. You would then use the **Method > Append > Append Method from Acquired SIM Data** to automate the process of adding the new compounds to an existing Quant 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 35.

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



Agilent Technologies

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.

1. Open the example batch file VoaSampleData.batch.bin.

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.

a To start the Quantitative Analysis program, click the **MS Quantitative Analysis** icon on your desktop  .

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

c With the cursor over the Sample label in the batch table, right-click and select **Restore Default Columns** from the context menu.

d Click **Open Batch**  on the toolbar, to display the **Open Batch** dialog box.

e 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 layouts." on page 42.

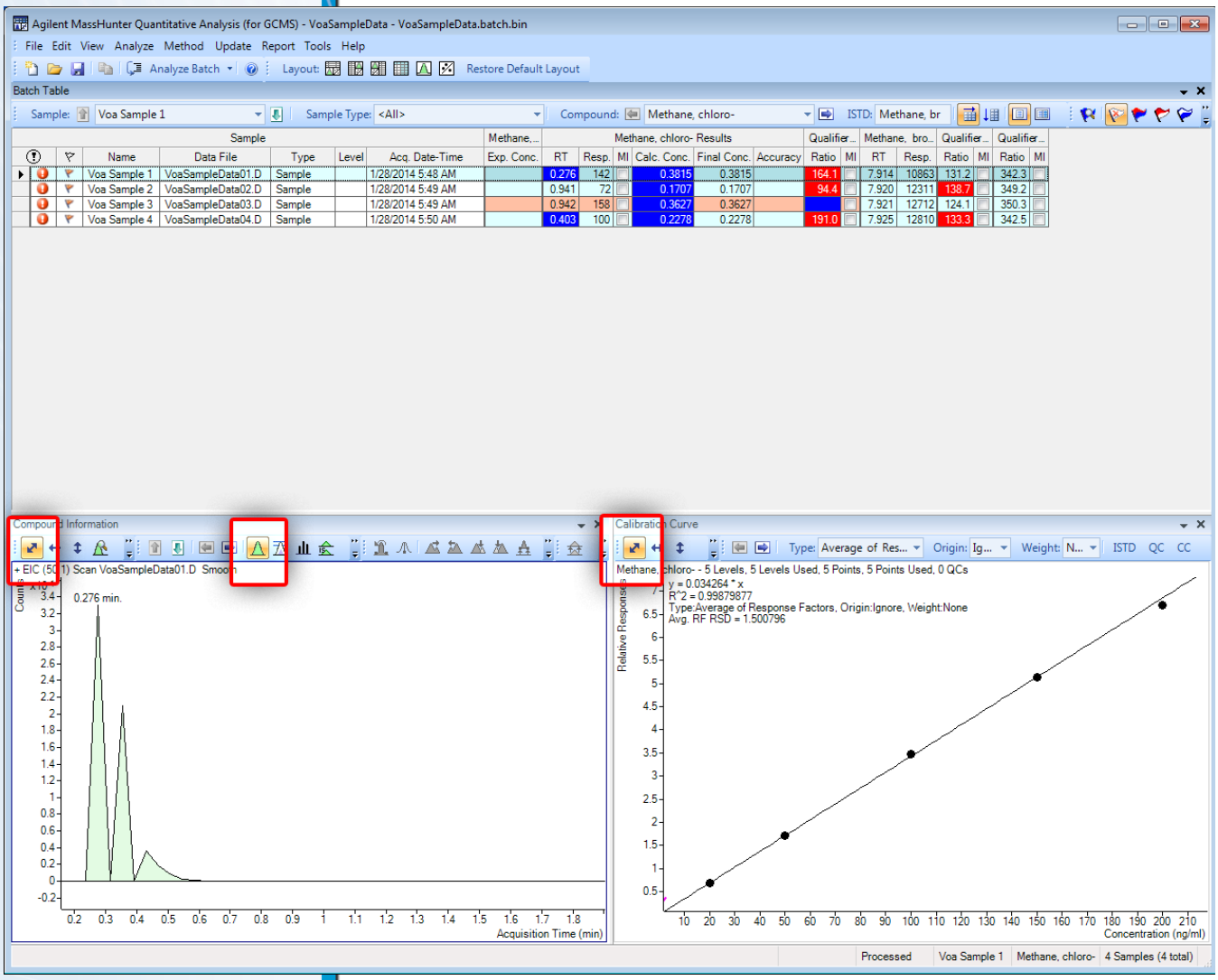
2. Highlight three icons.

a In the **Compound Information** window toolbar, highlight only two icons:

- **Turn on/off Autoscale**
- **Show/Hide Chromatogram**

b In the **Calibration Curve** window toolbar, highlight only one icon:

- **Turn on/off Autoscale**



3. Review the Sample Information window.

a 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.

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

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

4. Review how compounds are simultaneous displayed in the Batch Table, Sample Information window, and Compound Information window.

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

b With the cursor in the Sample Information chromatogram select **Compound** from the context menu. 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:

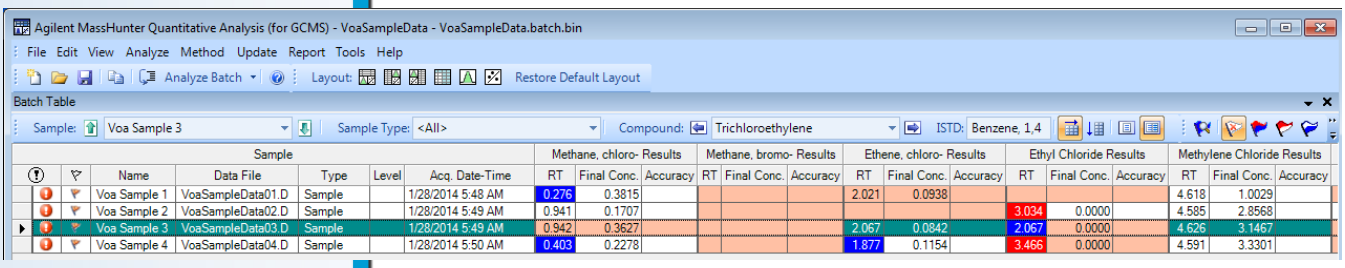
- the compound's results columns in the Batch Table
- the compound's highlighted location in the Sample Information window
- the compound's peak in the Compound Information window

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

5. 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**.

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



Sample						Methane, chloro- Results			Methane, bromo- Results			Ethene, chloro- Results			Ethyl Chloride Results			Methylene Chloride Results		
Name	Data File	Type	Level	Acq. Date-Time		RT	Final Conc.	Accuracy	RT	Final Conc.	Accuracy	RT	Final Conc.	Accuracy	RT	Final Conc.	Accuracy	RT	Final Conc.	Accuracy
Voa Sample 1	VoaSampleData01.D	Sample		1/28/2014 5:48 AM		0.276	0.3815					2.021	0.0938					4.618	1.0029	
Voa Sample 2	VoaSampleData02.D	Sample		1/28/2014 5:49 AM		0.941	0.1707								3.034	0.0000		4.585	2.8568	
Voa Sample 3	VoaSampleData03.D	Sample		1/28/2014 5:49 AM		0.942	0.3627					2.067	0.0842		2.067	0.0000		4.626	3.1467	
Voa Sample 4	VoaSampleData04.D	Sample		1/28/2014 5:50 AM		0.403	0.2278					1.877	0.1154		3.466	0.0000		4.591	3.3301	

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

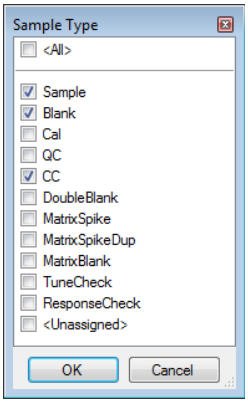
d If necessary, click the down arrow next to the **Compound** list, and click **Cocaine**.

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.

6. Filter the samples displayed in the batch table by sample type.

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

b Unselect **<All>**, 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 layouts.

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

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

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

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.

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  .

---

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

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

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

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

---

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

b Select **Trichloroethylene** from the Compound drop-down 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**.

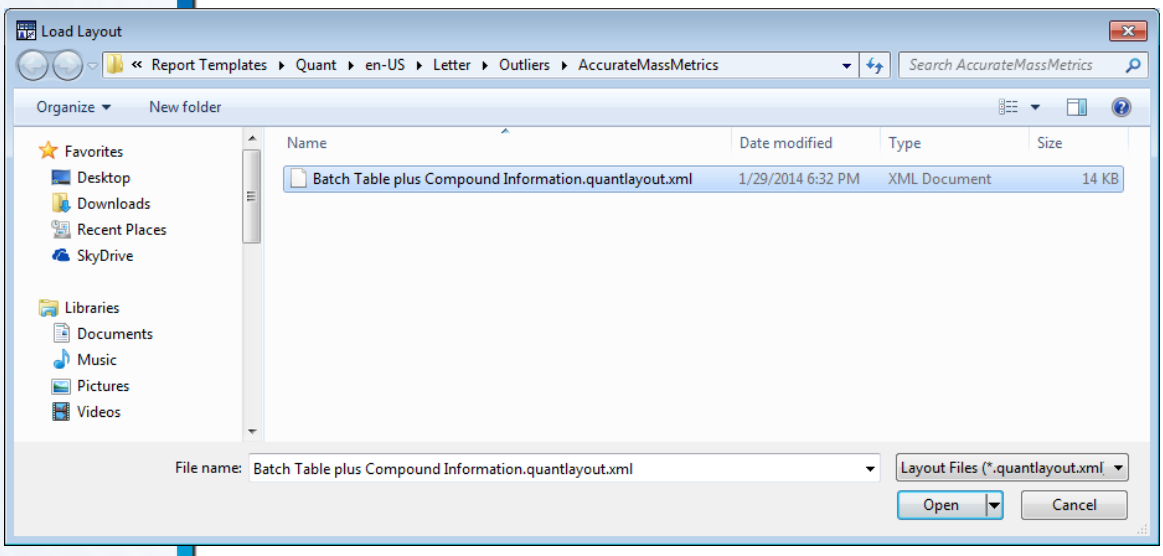
c The system displays the **Save Layout File** dialog box.

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

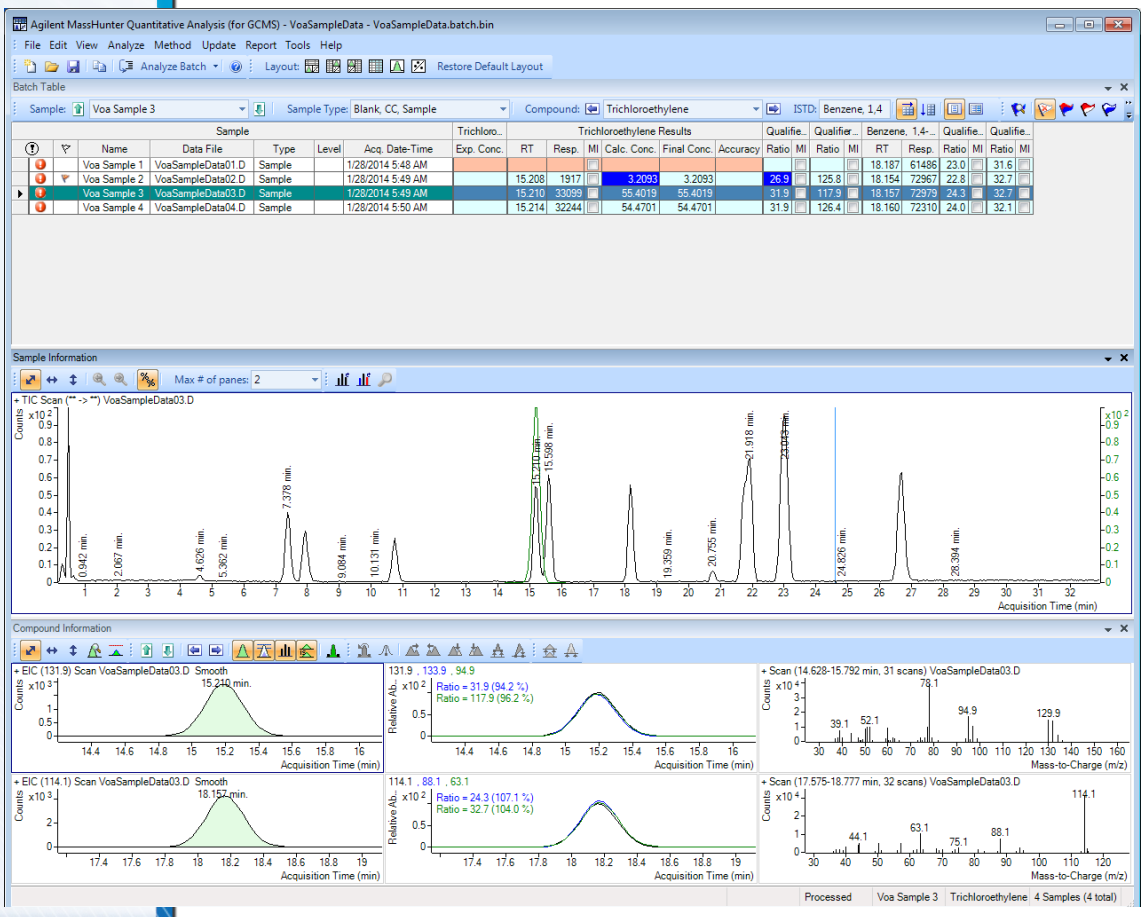
5. Load the newly created layout.

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

b Click **View > Window Layout > Load Layout**. The system displays the Load Layout dialog.



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



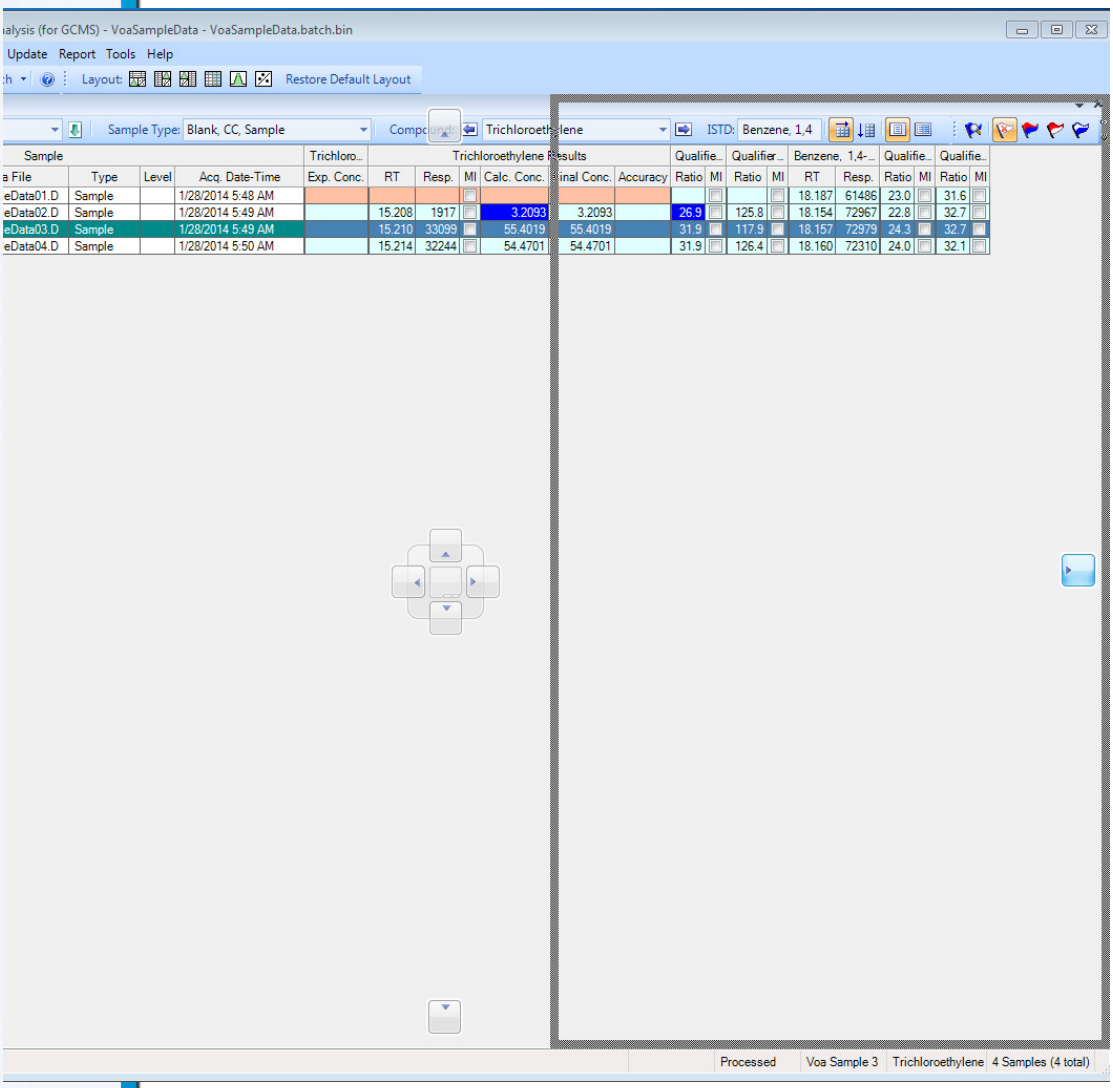
6. Create a custom window layout.

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

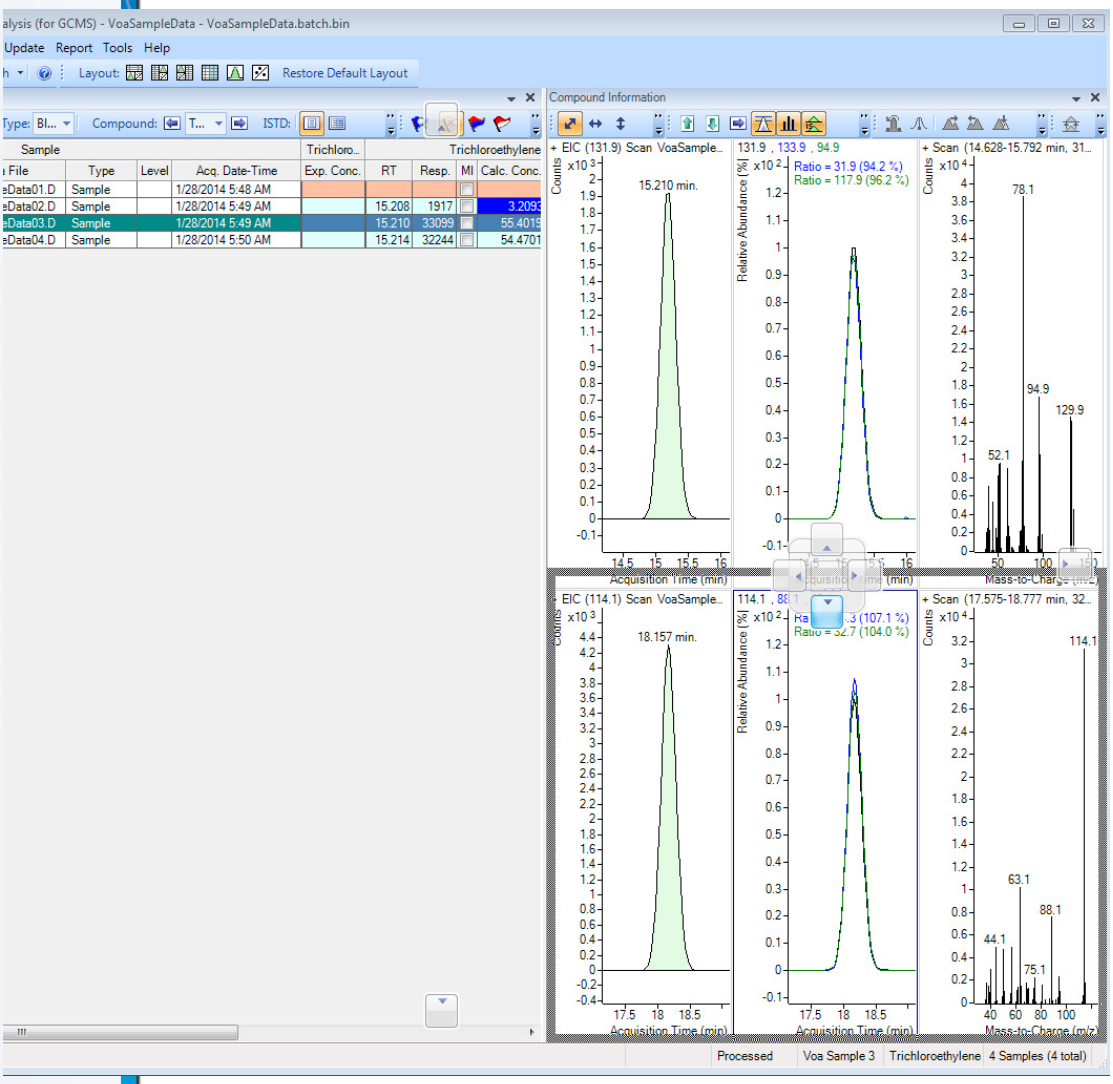
b Double-click inside the title bar of the **Calibration Curve** window, or click the down arrow next to the pane's close icon and select **Floating** to disconnect this window from the main window.

c Double-click inside the title bar of the **Compound Information** window, or click the down arrow next to the pane's close icon and select **Floating** to disconnect this window from the main window

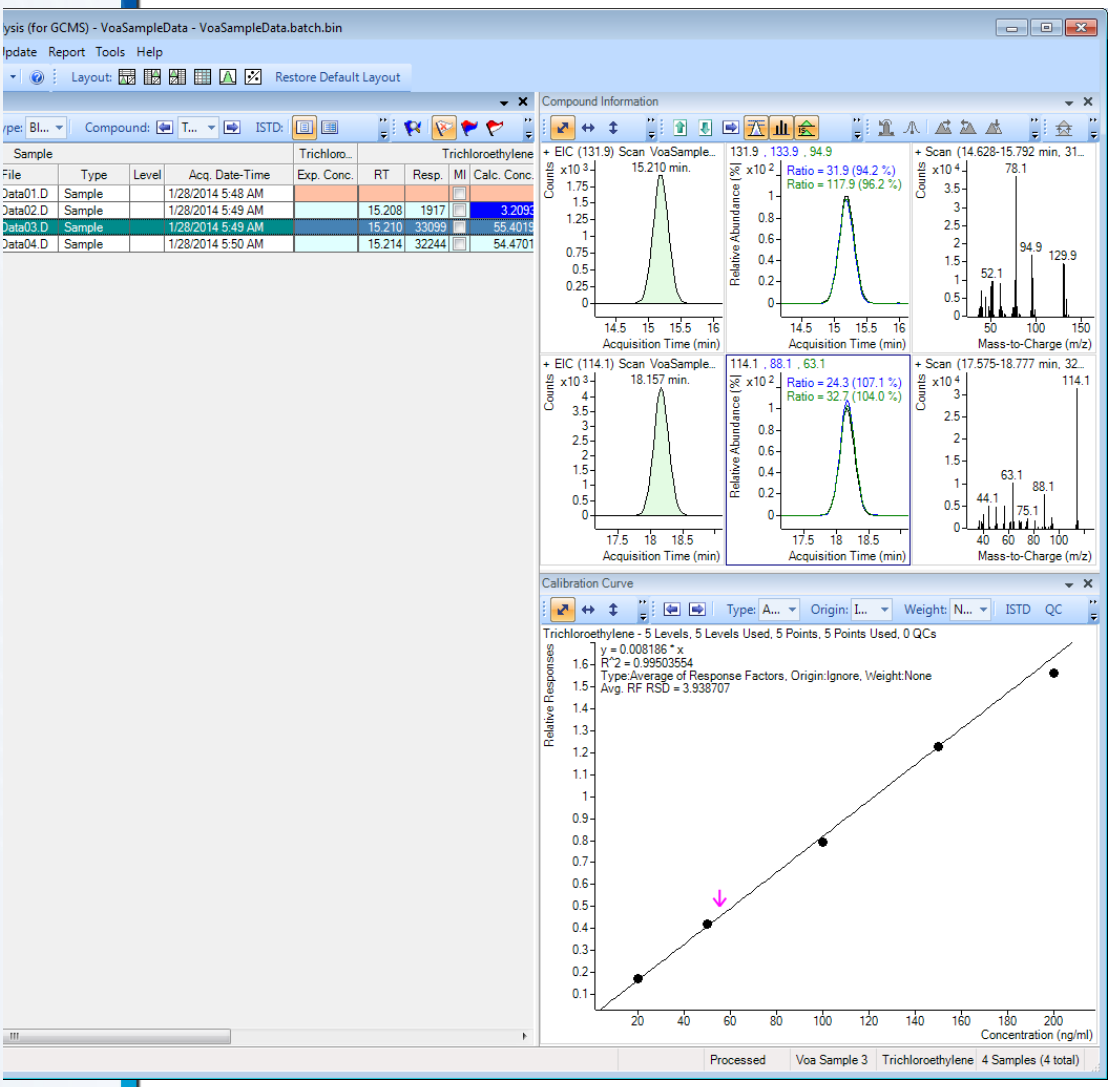
d Drag the **Compound Information** window by its title bar to the right side of the main window.Position the cursor over the right side anchor button, and when the anchor button turns blue and the outline of the main window appear as shown below, release the mouse button. The Compound Information window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

1. Load the Batch.

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

3. Access the Integration Parameter settings for the displayed compound.

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

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

a Click **Restore Default** **Layout**.

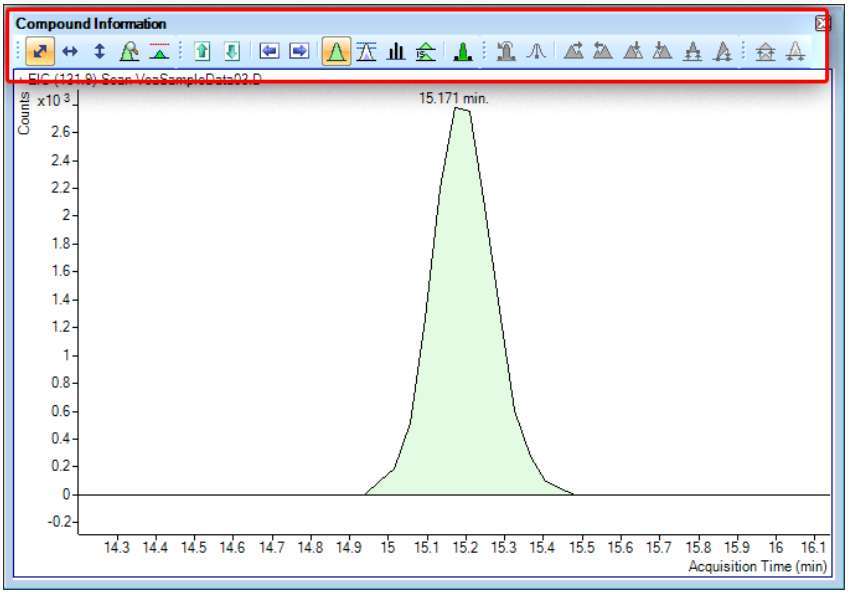
b Select the sample **Voa Sample 3** in the Batch Table.

c Select the compound **Trichloroethylene** in the Batch Table.

d In the Compound Information window context menu, select **Properties** to display the properties dialog, then click **Default**.

e Click the **Compound Information (2)** tab, click **Default** again, and then click **OK**.

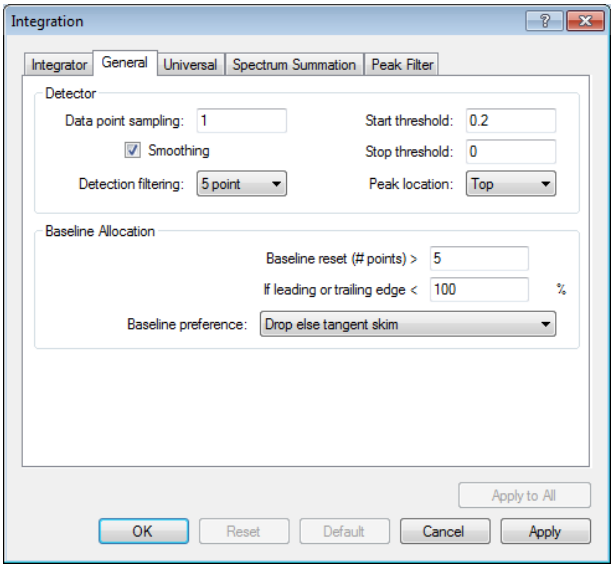
f Position the cursor over the border between the **Compound Information** and **Calibration Curve** windows and drag the border to the right until all icons are displayed in the Compound Information window as shown below.



---

a In the Compound Information window context menu, select **Integration Parameters** to display the Integration dialog, then click **Parameters**, to display the Integration dialog General tab showing the Parameters for the General Integrator.

b 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.



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

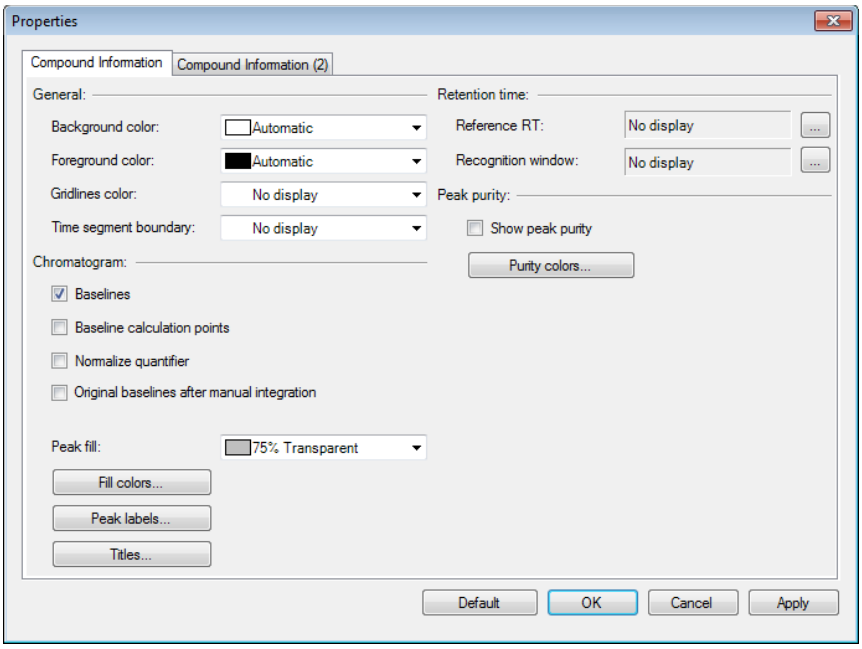
Task 4. Configure the settings in the Compound Information window.

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

1. Access the Properties dialog.

a In the Compound Information window context menu, select **Properties** to display the properties dialog.

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



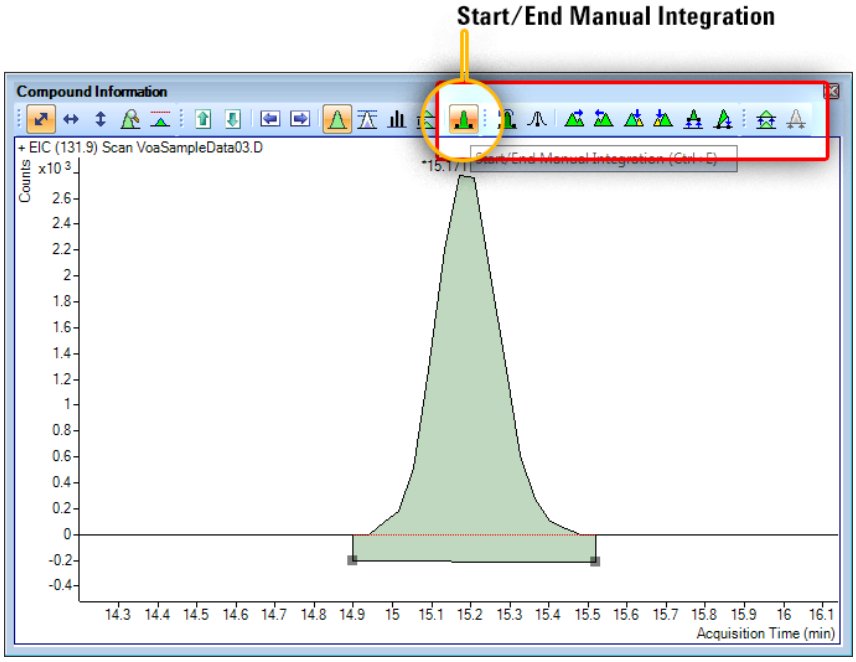
2. Select the **Original baselines after manual integration** and click **OK**.

3. In the Compound Information window 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.

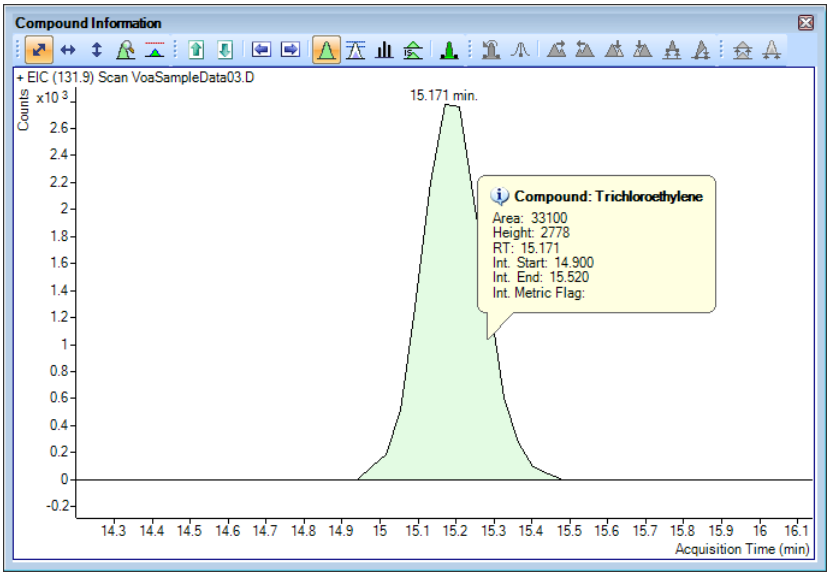
6. Click the **Zero Peak** icon, to the right of the last icon.

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.

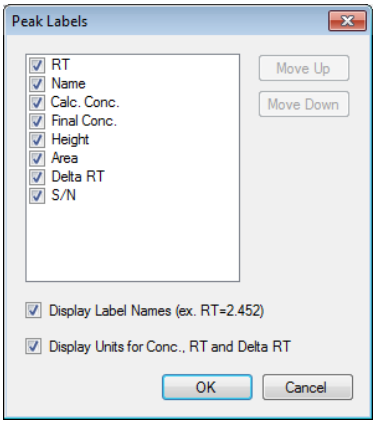
a Mouse over the peak to display the peak information as shown below. You may want some or all of this information to be displayed permanently as described below.



b In the Compound Information window context menu select **Properties** to display the properties dialog.

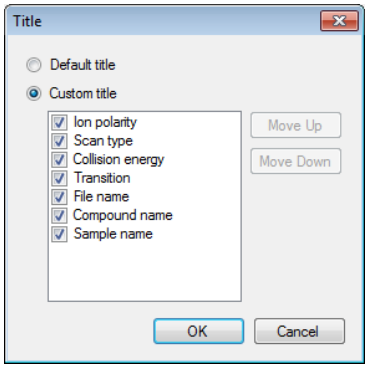
c In the Peak fill area click **Peak Labels** to display the Peak Labels dialog. Labels selected here will appear above the peak.

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

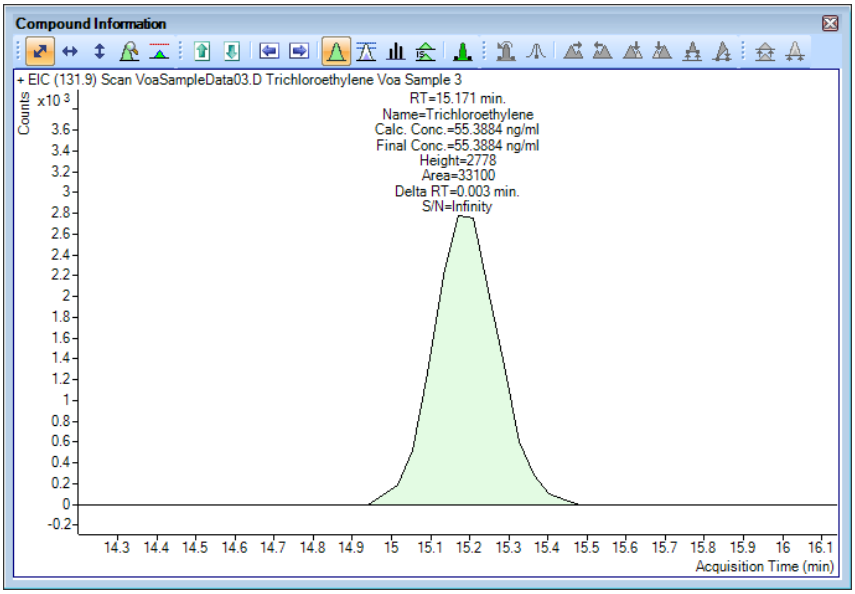


e In the Peak fill area click **Titles** to display the Titles dialog. The Title selected here will appear in the upper right part of the chromatogram.

f Select Custom title then select every label in this dialog. Click **OK**.


Title
Default title
Custom title
Ion polarity
Scan type
Collision energy
Transition
File name
Compound name
Sample name
Move Up
Move Down
OK
Cancel

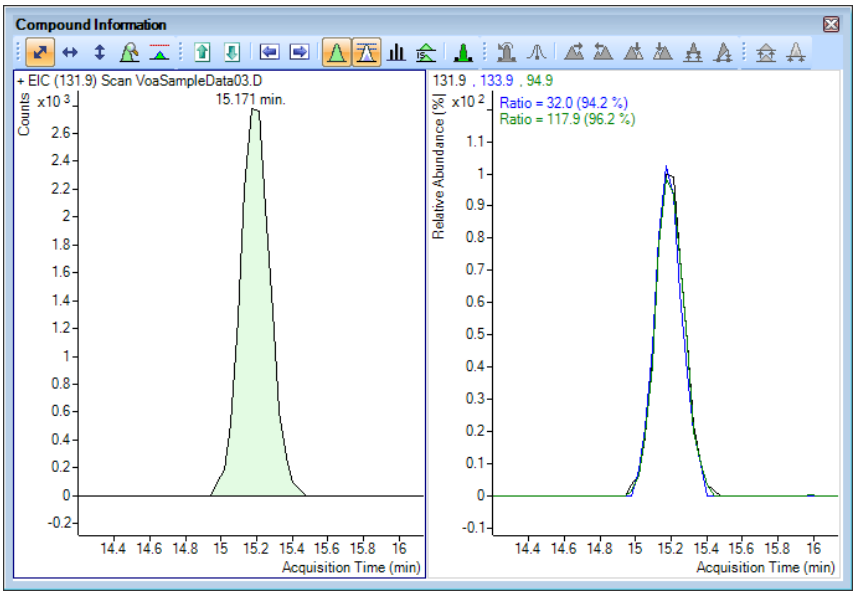
g Click **Apply** and observe the revised Title and new Peak Labels applied.



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

9. Setup the display of qualifier peaks.

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



b In the Compound Information window context menu select **Properties** to display the properties dialog.

c Click the **Compound Information (2)** tab. In the **Qualifiers** area, make **Normalize** unselected.

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.

---

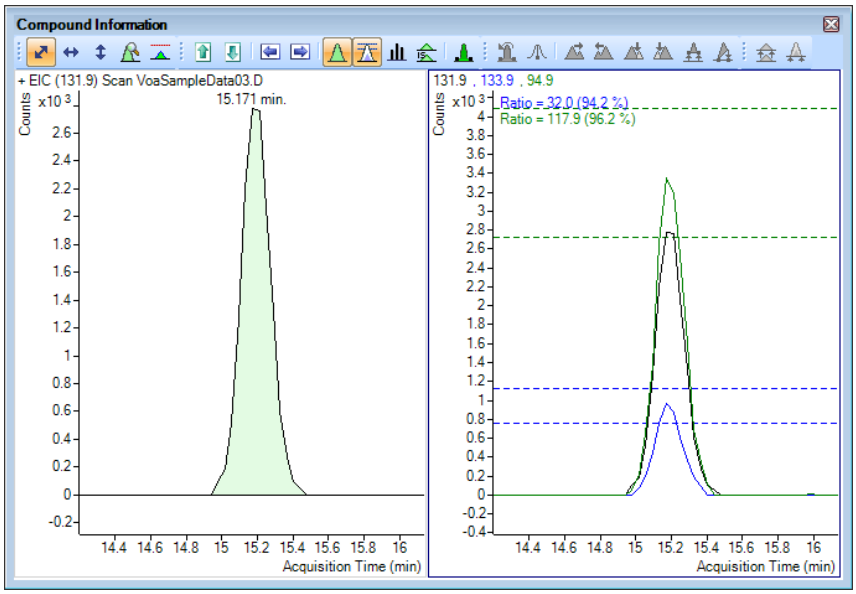
10. View the uncertainty band.

This step starts with the **Compound Information (2)** tab opened.

a For the **Uncertainty band** select the dashed line and click **Apply**.

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**.



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

Task 5. Export results to Excel.

1. Export the batch table results.

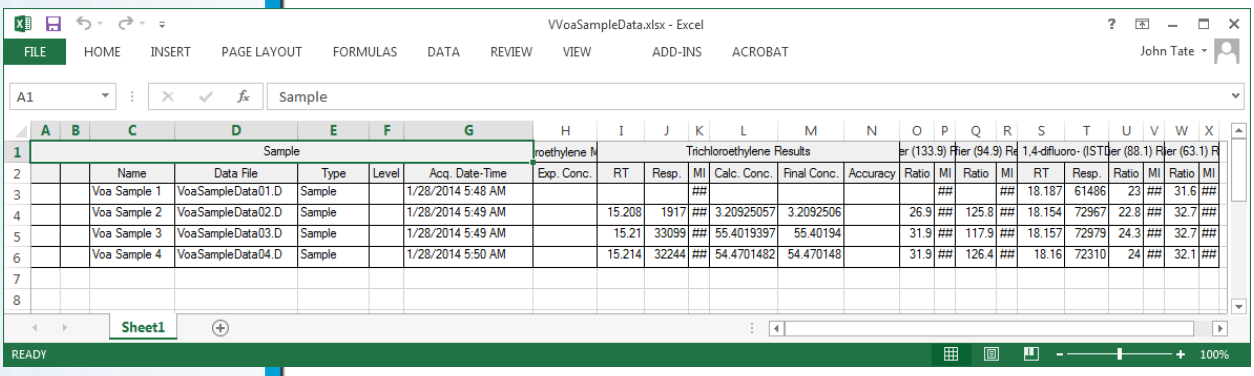
a To make the Batch Table window active, click the title bar of the Batch Table window.

b Click **File > Export > Export Table**.

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

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

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



	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X
1			Sample					roethylene M	Trichloroethylene Results						er (133.9) R	ier (94.9) Re	1,4-difluoro- (IST		er (88.1) R	ier (63.1) R				
2			Name	Data File	Type	Level	Acq. Date-Time	Exp. Conc.	RT	Resp.	MI	Calc. Conc.	Final Conc.	Accuracy	Ratio	MI	Ratio	MI	RT	Resp.	Ratio	MI	Ratio	MI
3			Voa Sample 1	VoaSampleData01.D	Sample		1/28/2014 5:48 AM				##					##		##	18.187	61486	23	##	31.6	##
4			Voa Sample 2	VoaSampleData02.D	Sample		1/28/2014 5:49 AM		15.208	1917	##	3.20925057	3.2092506		26.9	##	125.8	##	18.154	72967	22.8	##	32.7	##
5			Voa Sample 3	VoaSampleData03.D	Sample		1/28/2014 5:49 AM		15.21	33099	##	55.4019397	55.40194		31.9	##	117.9	##	18.157	72979	24.3	##	32.7	##
6			Voa Sample 4	VoaSampleData04.D	Sample		1/28/2014 5:50 AM		15.214	32244	##	54.4701482	54.470148		31.9	##	126.4	##	18.16	72310	24	##	32.1	##
7																								
8																								

3
Compounds at a Glance

Agilent Technologies

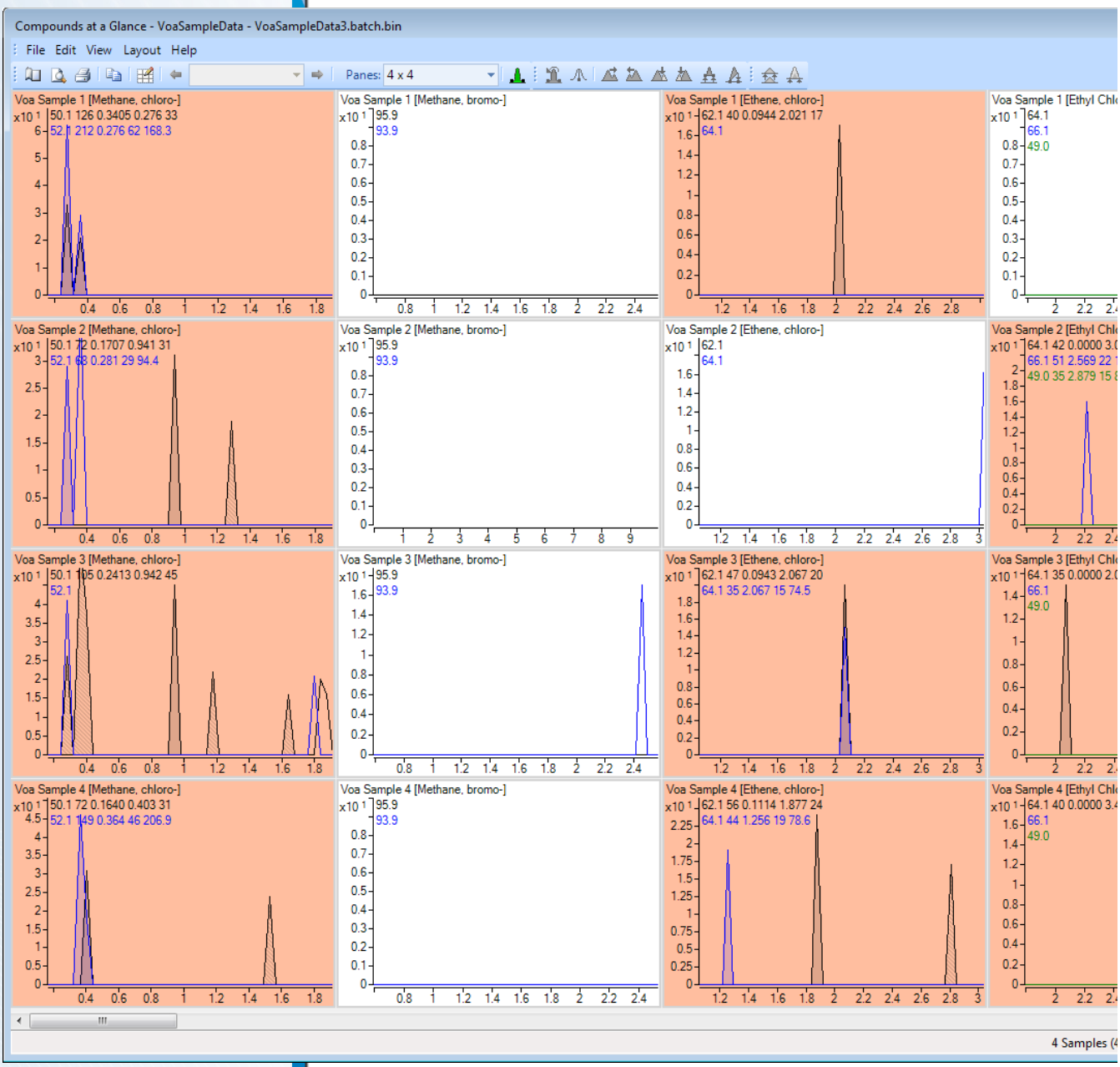
Task 1. Review the Compounds at a Glance view.

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. Open the Compounds at a Glance window.

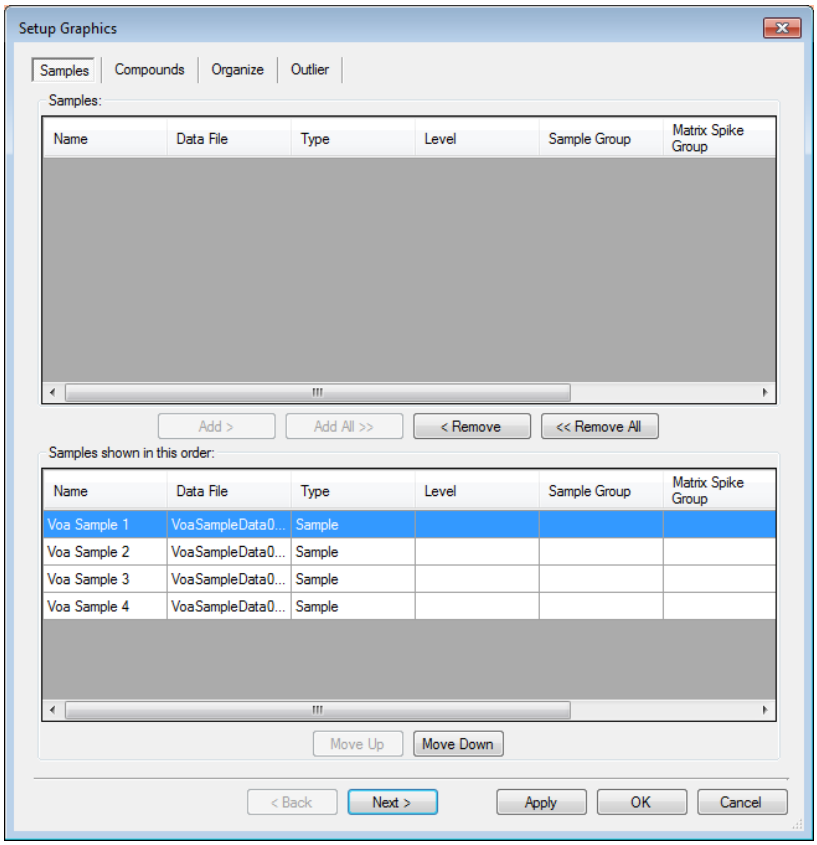
   a Select **File > Open Batch** and load VoaSampleData.batch.bin.

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



2. Display the Setup Graphics window Samples tab.

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



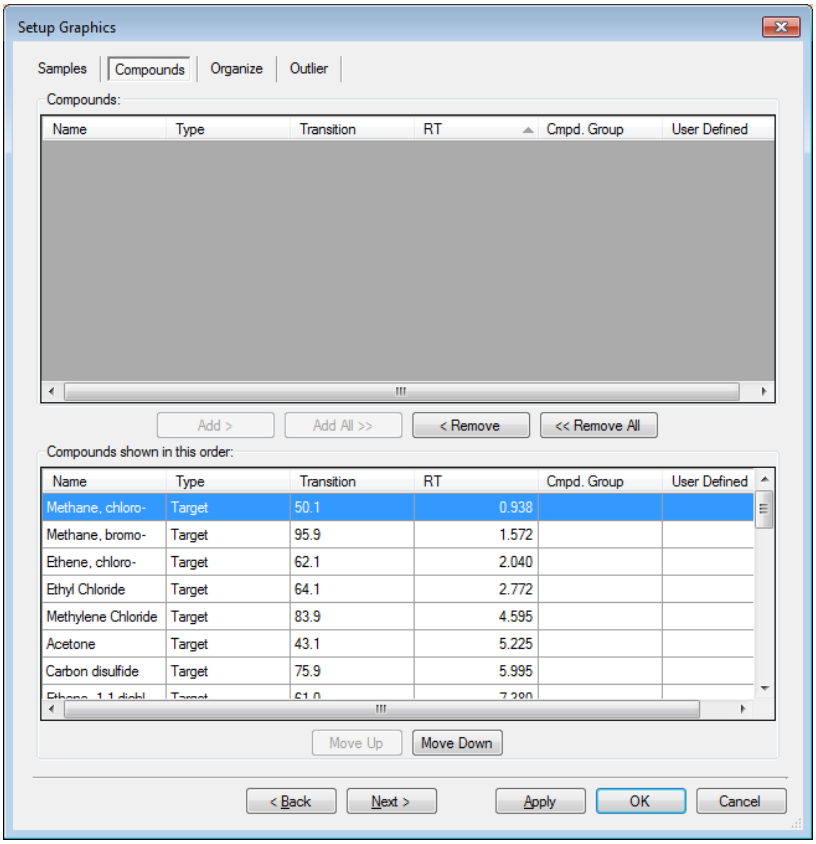
b Arrange the desktop so that the Compounds at a Glance and Setup Graphics windows are both visible.

c 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.

3. Display the Compounds tab.

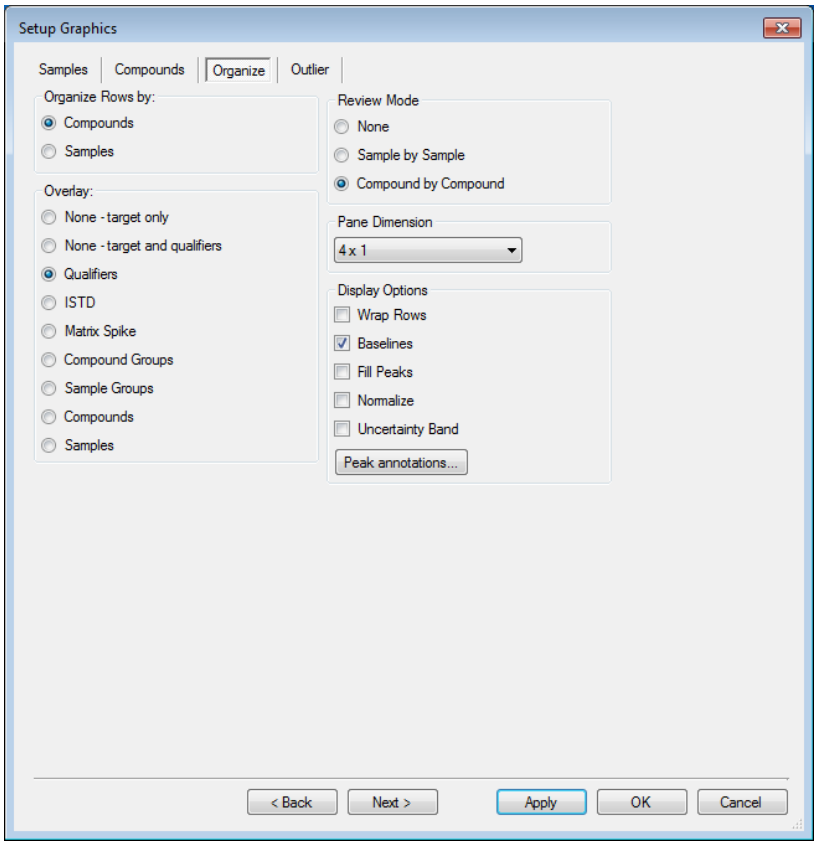
a Click **Next** to display the Compounds tab.

b 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.



4. Display the Organize tab.

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



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

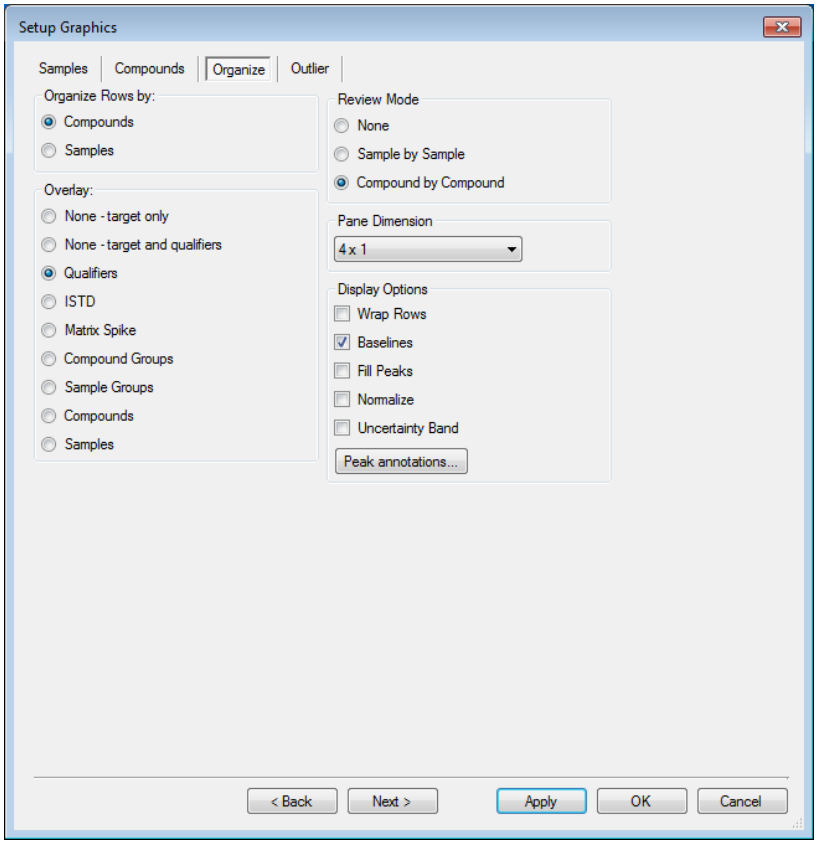
Each column now displays a single sample and the single row displays the selected compound found in all four samples.

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

The reviewer button 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.

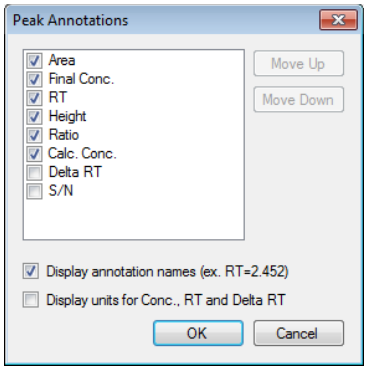
d In the Pane Dimension area, select the drop-down, mouse over the upper left square, and continue mouse overing down until the panes displays 4X1 and click.

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



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

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

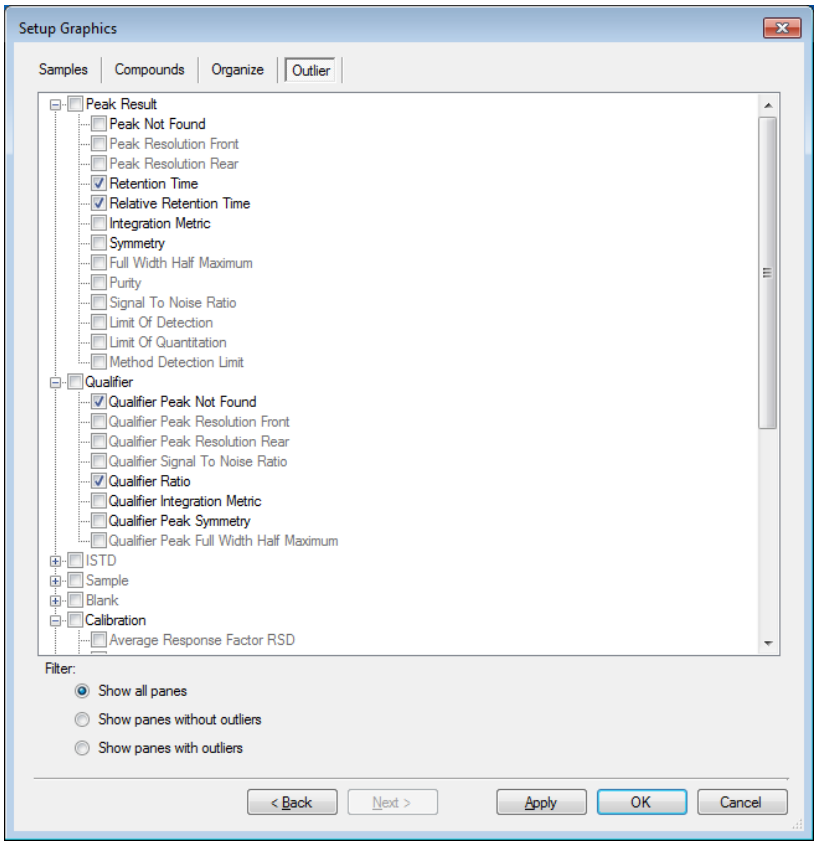


5. Display the Outlier tab.

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

b Make the selections shown in this example:

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



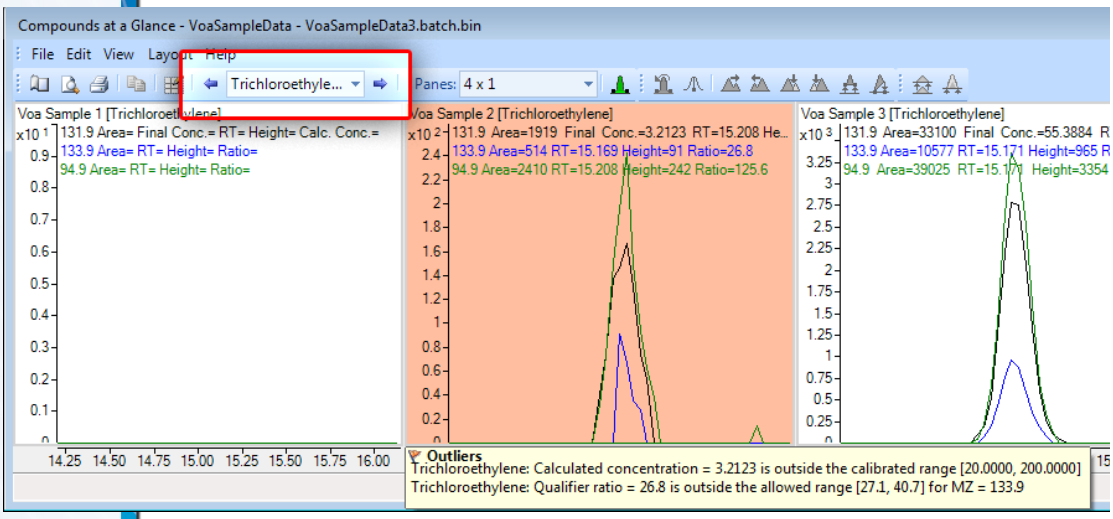
c Click **OK** to close the Setup Graphics window, and notice the highlighted pane in the Compounds at a Glance window.

See "Task 1. Review the outliers." on page 72 for details on these settings.

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

   a 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.



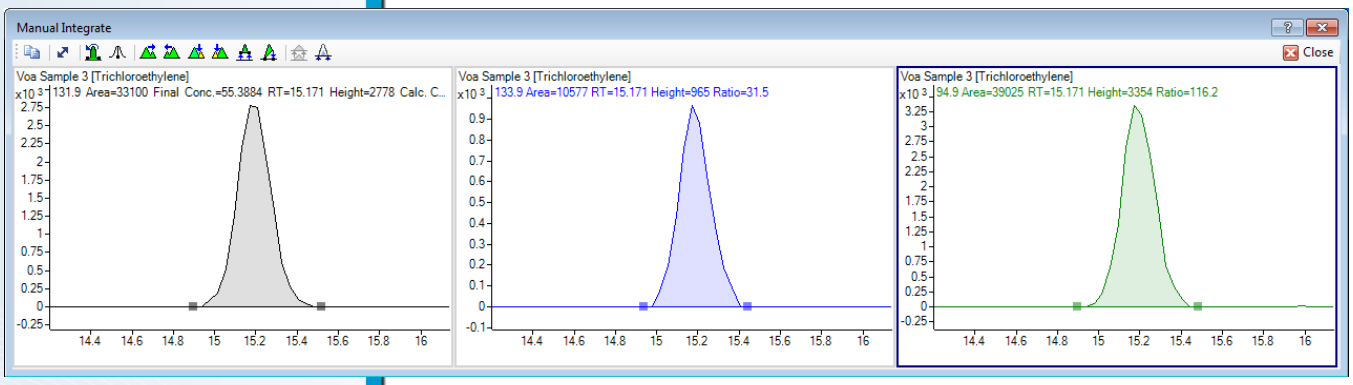
7. See how **Link All X-Axis** works.

   a Right click in any pane to display the context menu 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.

8. Open a Manual Integrate window with three panes.

   a 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.



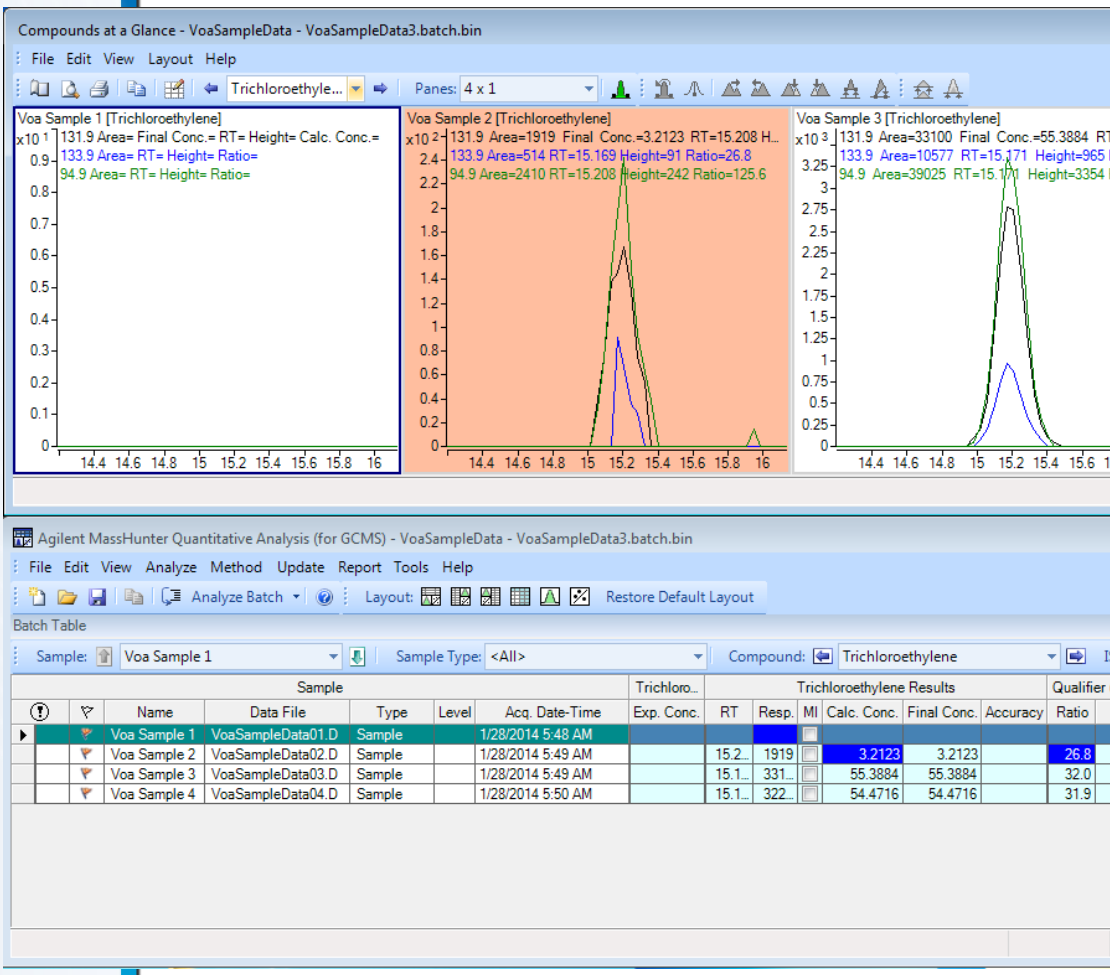
   b Close the Manual Integrate window and review the remaining compounds by clicking the **Next** or **Previous** arrow icon on the Review parameter shown the compound name currently being reviewed.

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

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

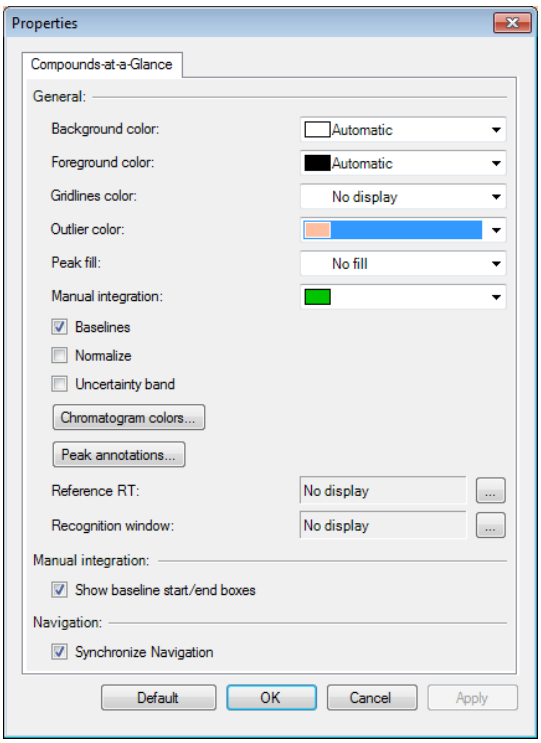
a Use the Compound at a Glance window from the previous step for this procedure.

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

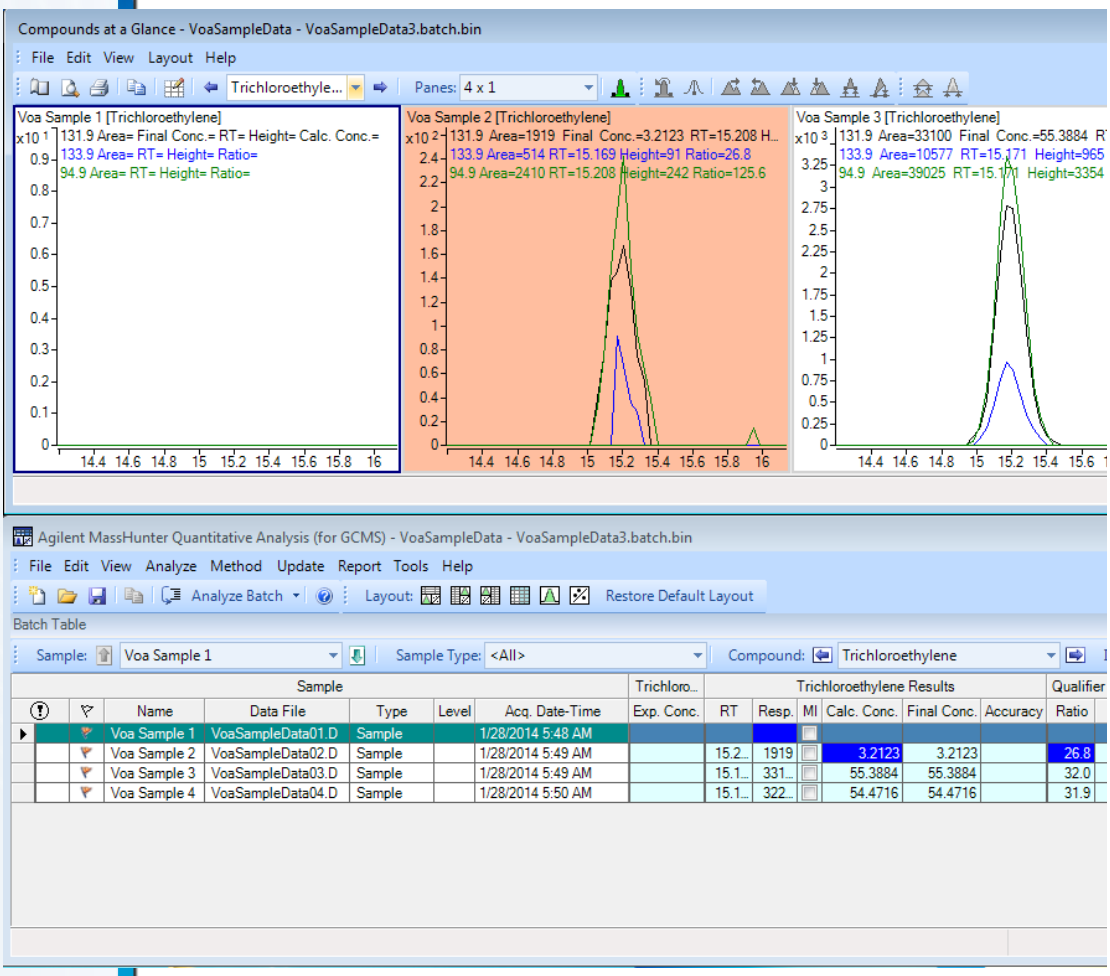


c From the Compounds at a Glance window context menu select **Properties** to display the Properties dialog.

d From the Navigation section select **Synchronize Navigation** and click **OK** to close the dialog.



e Select a compound in the Batch table and the same compound is selected in the Batch at a Glance table. Selecting the compound in the Compound at a Glance window changes the selection in the Batch table



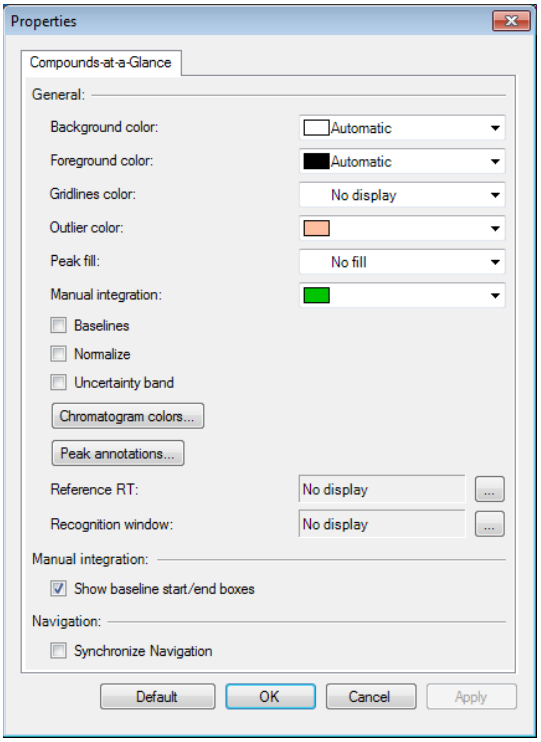
Task 2. Display Properties for the Compound at a Glance window.

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. Access the Properties dialog.

a In the Compounds at a Glance window context menu select **Properties** to display the Properties dialog.

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



Notice that **Show baseline start/end boxes** is a selected default parameter. This is only shown when manual integration is enabled.

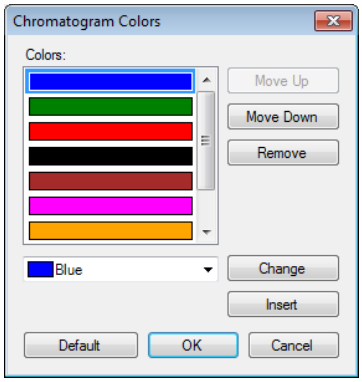
The default outlier color is salmon. If an outlier exists for a sample's compound, that chromatogram has a salmon background.

2. View the uncertainty band, set peak colors, and peak labels.

a In the manual integration area, enable the **Uncertainty band**.

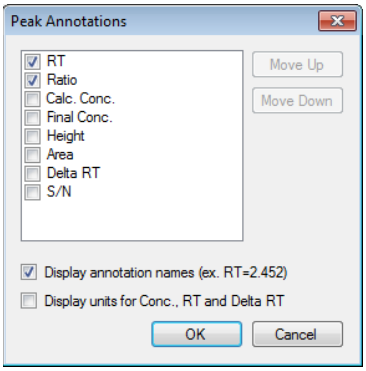
b Click **Chromatogram colors** and observe the default order of black, blue, green, etc.

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

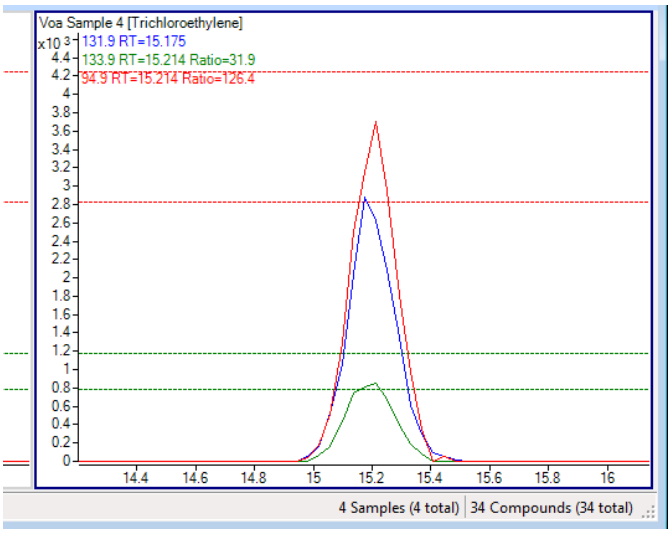


These settings will make the target compound peak and labels blue, the first qualifier peak, labels and uncertainty band green, and the second qualifier peak, labels and uncertainty band red.

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



e Click **OK** to close the Properties dialog and observe the edits to the display.



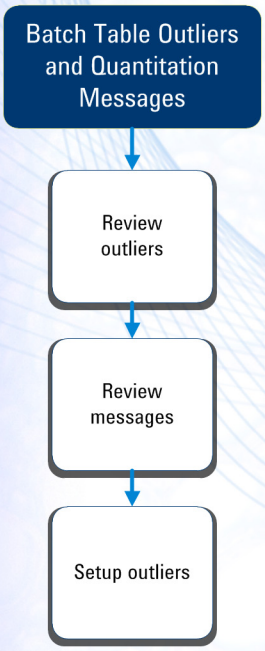
Observe that the target peak and peak label is blue as specified.

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



Agilent Technologies

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 View.

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.

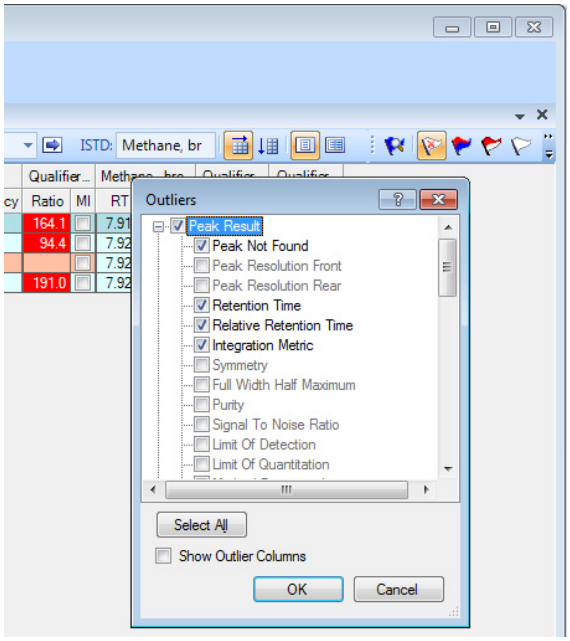
This task shows how outliers are displayed for a compound in the Batch Table and how you can filter results with outliers flags. In this example, we set this filter to only display the outliers MassHunter sets up by default.

1. Initialize the default outliers.

a Select **File > Open Batch**, navigate to and double-click the **VoaSampleData** batch file.

b Click the **Select Outliers**  icon 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 view.



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 standard toolbar. The outliers for the batch are found and displayed.

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

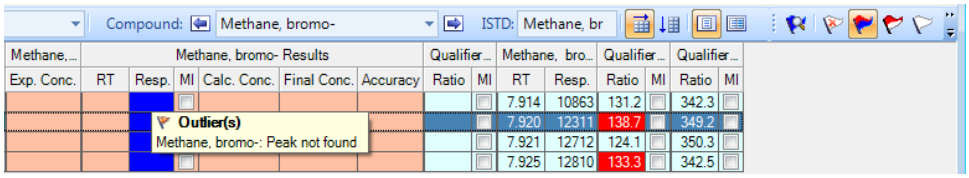
c Select chloromethane in the batch table and note there are outliers denoted by red and blue highlighted cells.

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

e Click the **Display rows that have High/Low outliers**  icon 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.

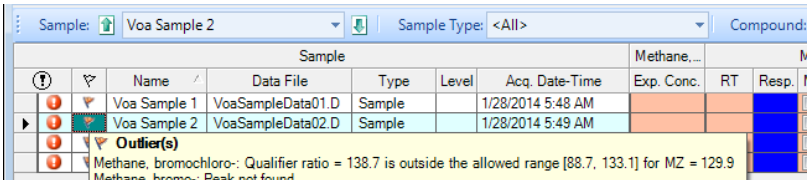
g Mouse over the blue cell to display the outliers message.



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.



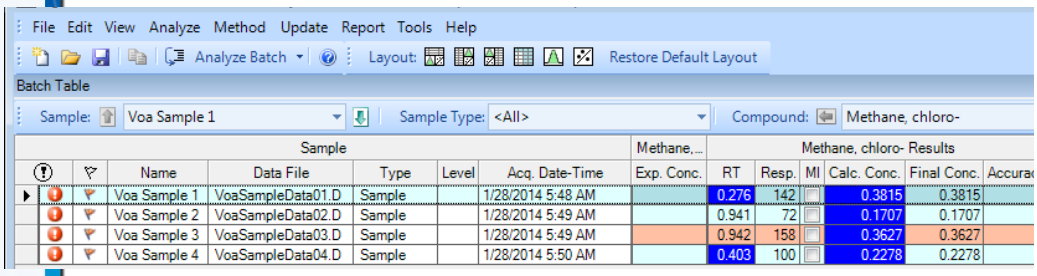
3. Deselect the **Peak not found** outliers.

a Click the **Select Outliers**  icon to bring up the **Outliers** dialog. In the Outliers dialog deselect the default outlier **Peak not found** in the Peak Result group.

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.

b Click the **Turn off outlier filter**  icon to display all samples.

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

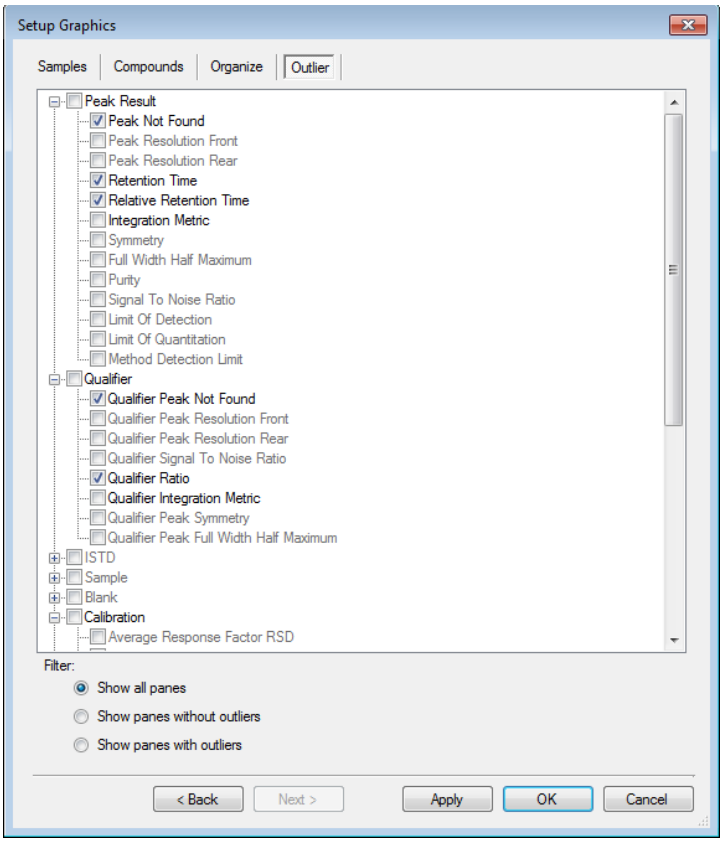


Sample						Methane,...	Methane, chloro- Results					
Name	Data File	Type	Level	Acq. Date-Time	Exp. Conc.	RT	Resp.	MI	Calc. Conc.	Final Conc.	Accura
Voa Sample 1	VoaSampleData01.D	Sample		1/28/2014 5:48 AM		0.276	142		0.3815	0.3815	
Voa Sample 2	VoaSampleData02.D	Sample		1/28/2014 5:49 AM		0.941	72		0.1707	0.1707	
Voa Sample 3	VoaSampleData03.D	Sample		1/28/2014 5:49 AM		0.942	158		0.3627	0.3627	
Voa Sample 4	VoaSampleData04.D	Sample		1/28/2014 5:50 AM		0.403	100		0.2278	0.2278	

4. Setup the outliers display in the Compounds-at-a-Glance view.

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

b Select **Layout > Setup Layout** then 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 view are setup in the Setup Graphics dialog Outlier tab.

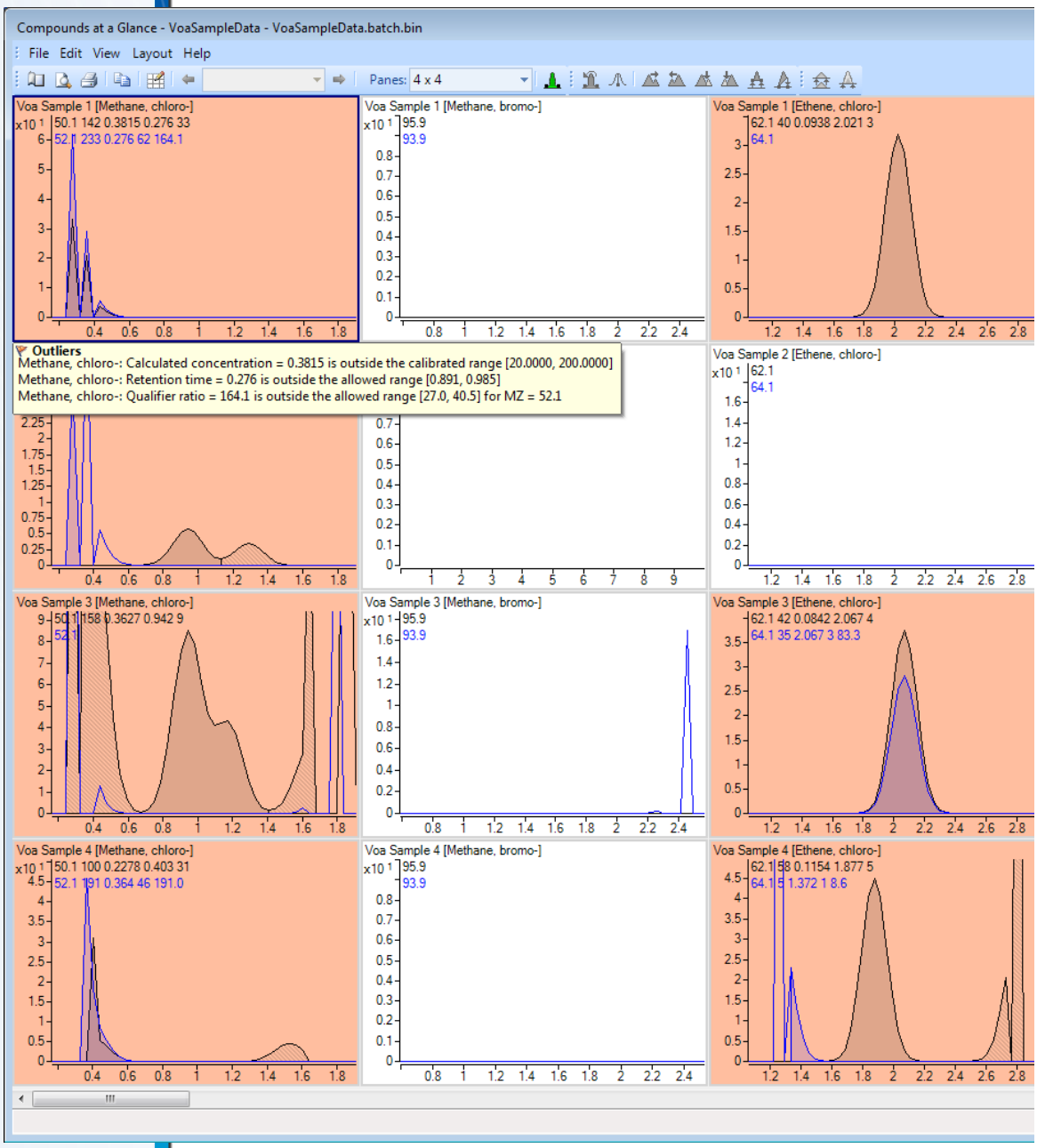


c As done previously with the Batch Table outliers settings, select the defaults indicated in bold but uncheck **Peak Not Found**. Also uncheck **Integration Metric** and **Qualifier Integration Metric** do not function with the General Integrator.

d For the filter, select **Show all panes**.

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

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

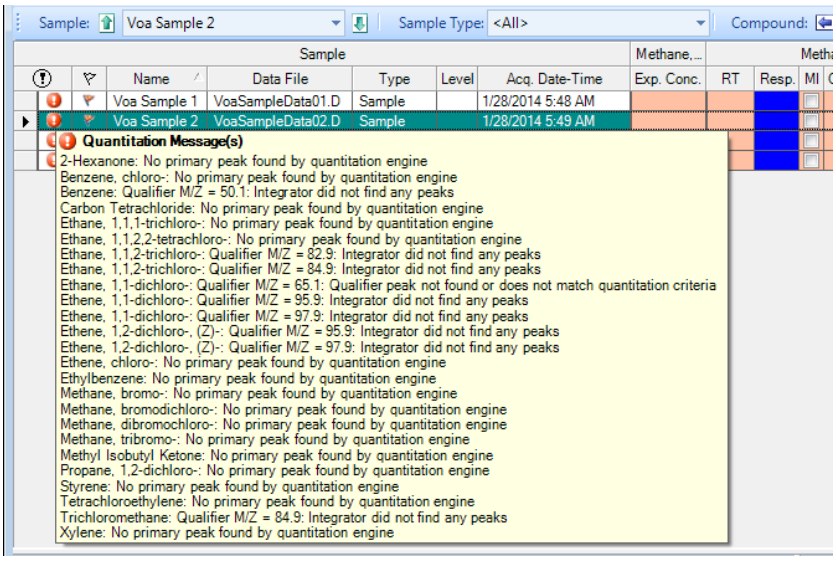


Task 2. Review Quantitation messages.

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.

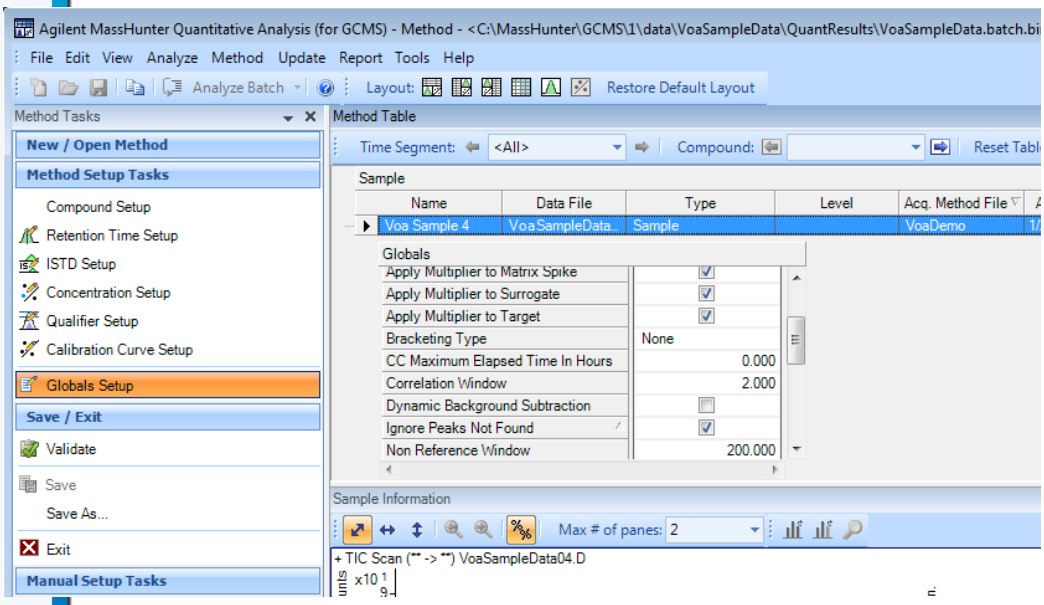
For the **Voa Sample 2**, mouse-over the **Quantitation Message Summary** icon (exclamation point inside a filled red circle) to display the Quantitation Messages. The messages in our example are all due to calibration compounds not found in the sample.



2. Suppress quantitation messages in the batch table that involve missing peaks.

a Select **Method > Edit**.

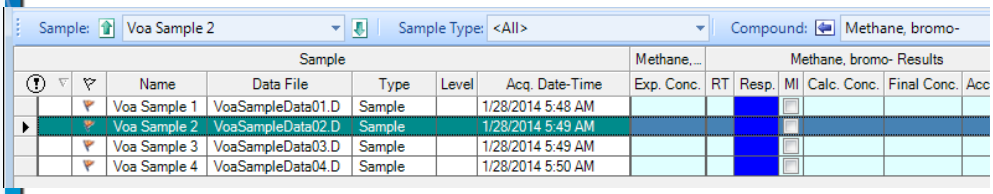
b Select **Globals Setup** from the Method Tasks area and then select **Ignore Peaks Not Found** in the Method Table.



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. Setup outliers.

1. Load VoaSampleData2.batch.bin.

2. Edit the acceptable range for the RT outlier.

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.

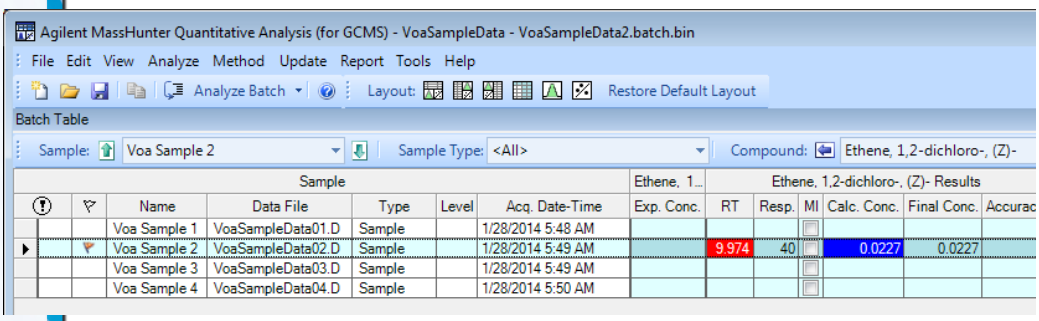
Select **File > Open Batch** and load the VoaSampleData2.batch.bin file.

---

a Select **Ethene, 1,2-dichloro** from the Compound dropdown located in the Batch Table header.

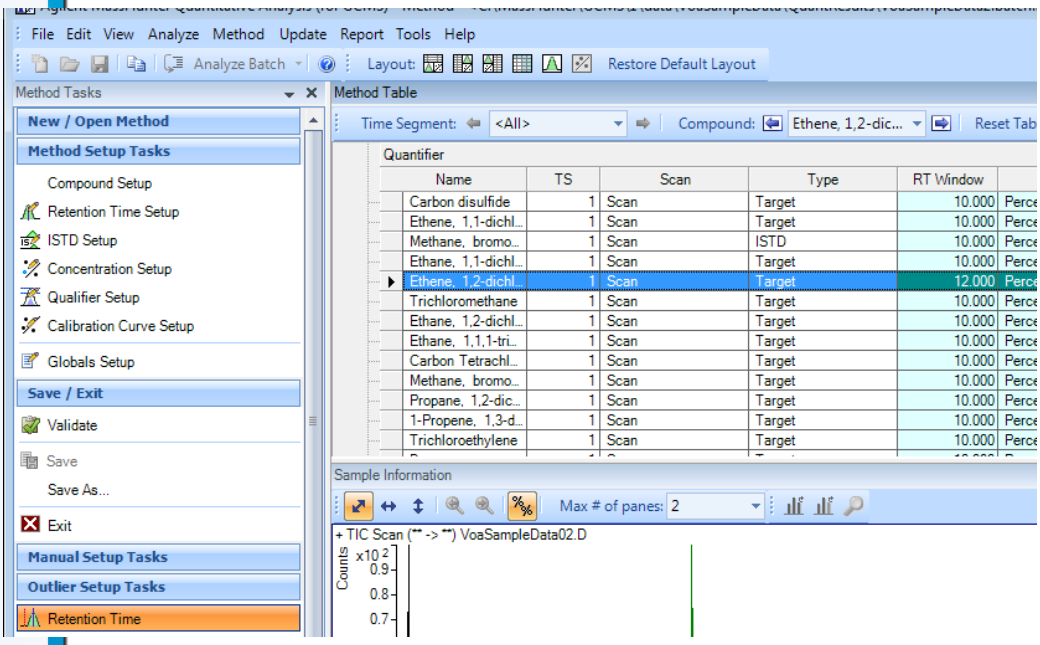
b Select the **Voa Sample 2** sample in the table.

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

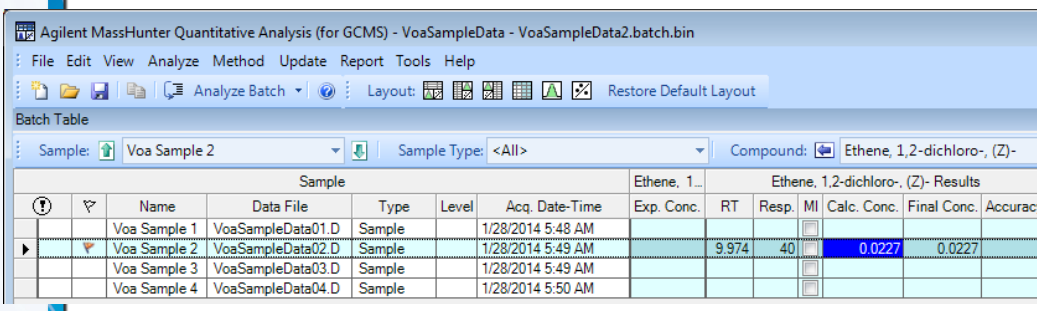


e Set the **RT Window** value to **12**.

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

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 view.

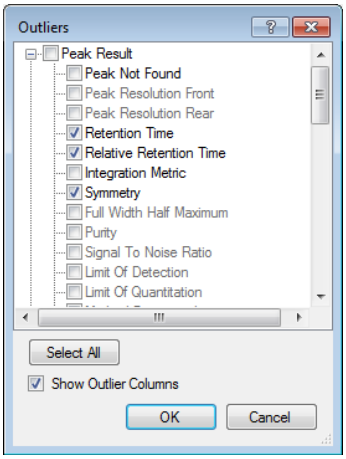
The outlier that was noted by the red shaded **RT** cell is now gone. This indicates that the change you made to the acceptable RT range now includes this result.



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

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

b In the Outliers dialog select the **Symmetry** outlier in the Peak Result group.



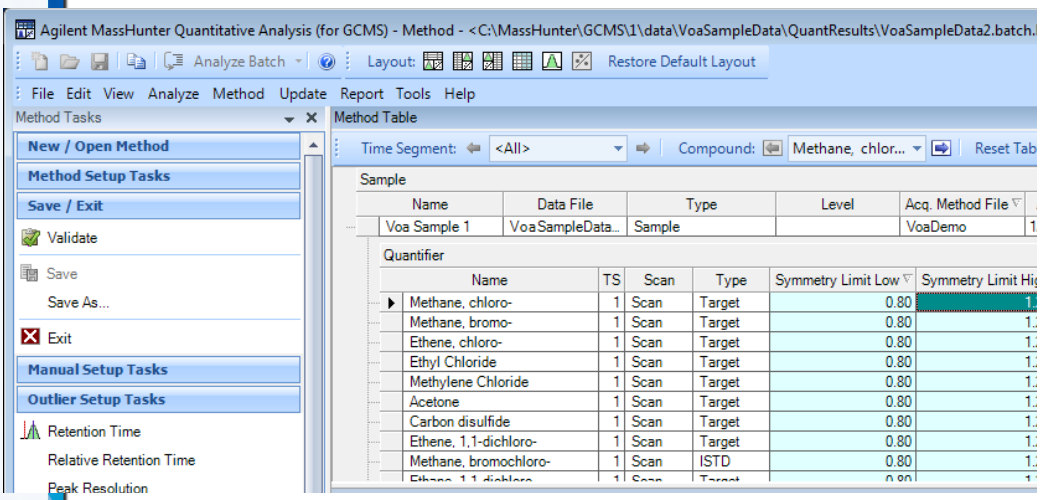
c Select **Show Outlier** Columns to add the Symmetry column to the target compound results area in the batch table.

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

e If not selected, click the **Turn off outlier filter** icon to display all samples. Setup a new outlier in the method.

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

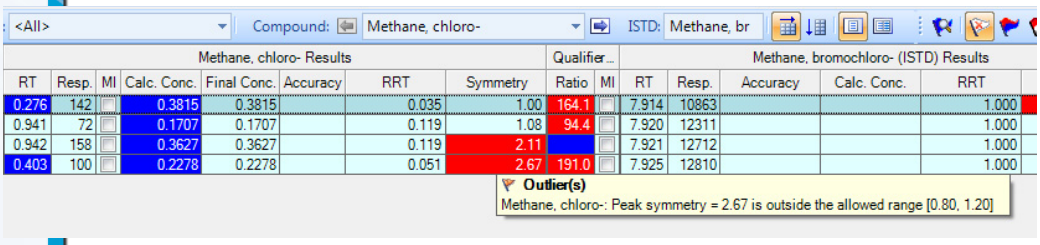
g In the Method Tasks column, click **Outlier Setup Tasks > Peak Symmetry**.



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

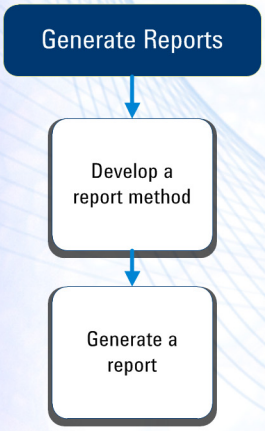
i Set the **Symmetry Limit High** value to **1.20.**

j Select **Method > Exit**. This displays the Apply Method dialog.Select **Analyze** for Additional batch processing after applying the method and click **Yes**. This runs the analysis with the revised method adds the Symmetry outlier to the method. The Batch Table view is entered.



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



Agilent Technologies

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.

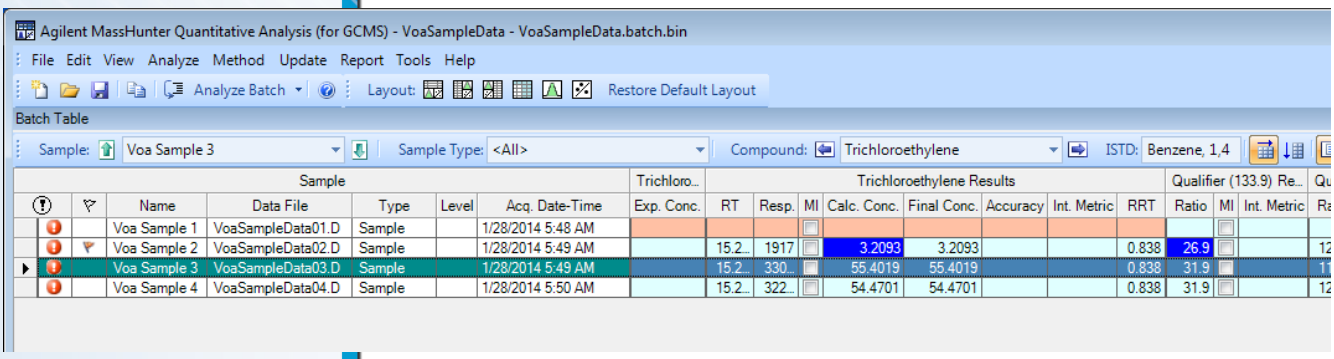
The report method you develop determines the report you create in MassHunter. Report methods are made of one or more report templates combined and edited to meet your reporting requirements. When developing a report method, you can use either Excel or PDF templates. PDF templates can generate reports 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. Open the batch file ***VoaSampleData*.batch.bin**.

a To start the Quantitative Analysis program, click the **MS Quantitative Analysis** icon on your desktop.

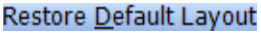


b Click **Open Batch**  on the toolbar to display the **Open Batch** dialog.

c Navigate to the directory containing the VoaSamples batch.

You can also access the program by clicking **Programs > Agilent > MassHunter Workstation > MS Quantitative Analysis** from the **Start** menu.

If the default layout is not present, click **Restore Default Layout** on the toolbar before opening the batch.



2. Quantitate the samples for this batch and save your results.

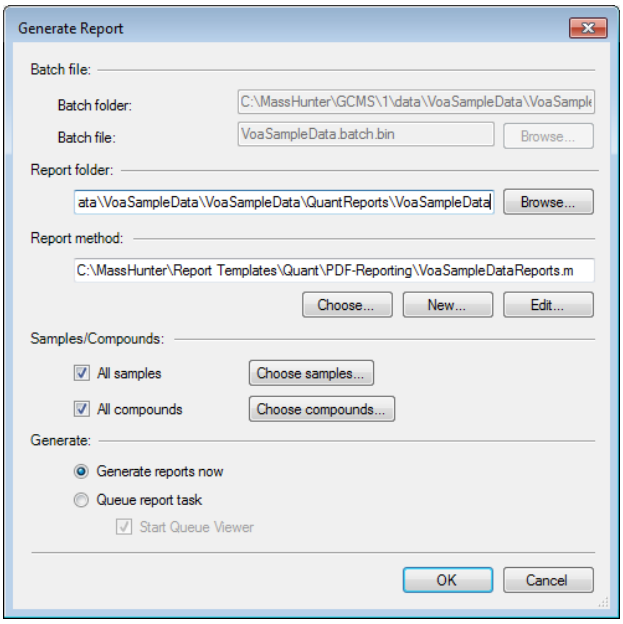
a With the batch table open, click the **Analyze Batch** button on the tool bar to generate results. If the batch is already quantitated, skip to step 3.

b Click **File>Save** to save the batch.

Quantitative reports contain sample information generated during the batch. The reporting function will not work until sample results have been quantitated and saved.

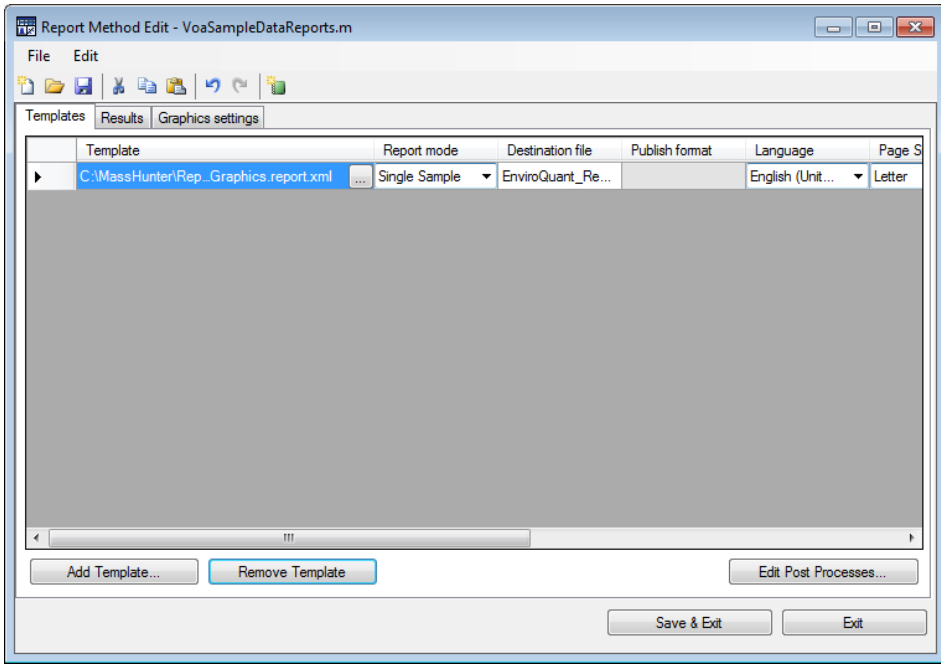
3. Create a PDF report method.

a Click **Report > Generate** from the toolbar. The system displays the **Generate Report** dialog box.



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

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



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

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

4. 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 the **Choose** button under the **Report Method** field, and navigate to the folder to select your method.

---

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

The **Report Method Edit** dialog 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, **Report Mode** field, select **Single Sample** from the drop down menu.

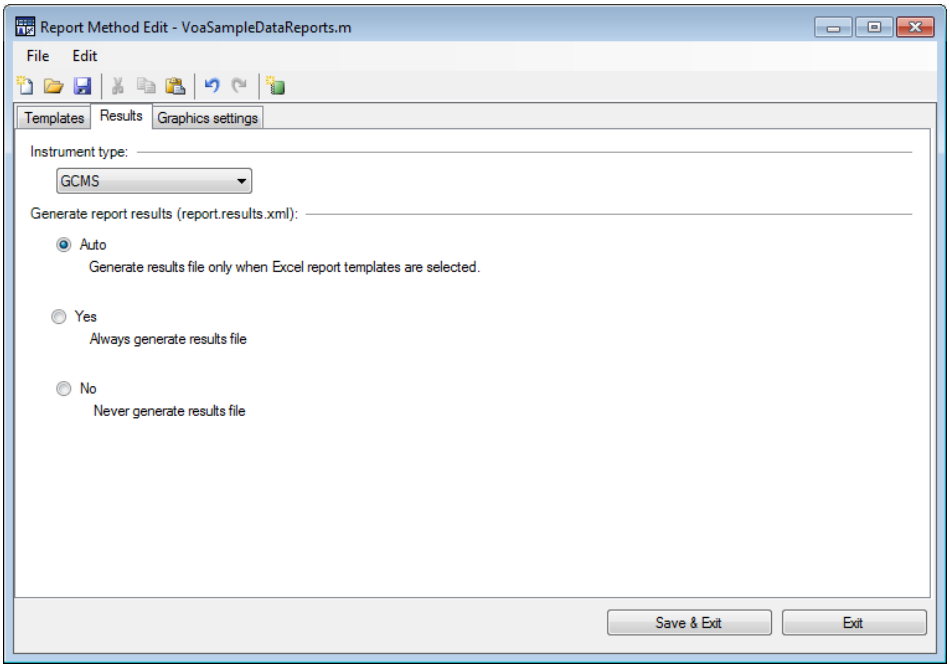
b On the second template line, select **Batch** from the drop down menu in the **Report Mode** field.

c Select your language from the drop down menu in the **Language** field.

d Select a paper size from the drop down menu in the **Paper Size** field.

6. Select the way the system handles your report results.

a Select the **Results** tab of the **Report Method Edit** window.



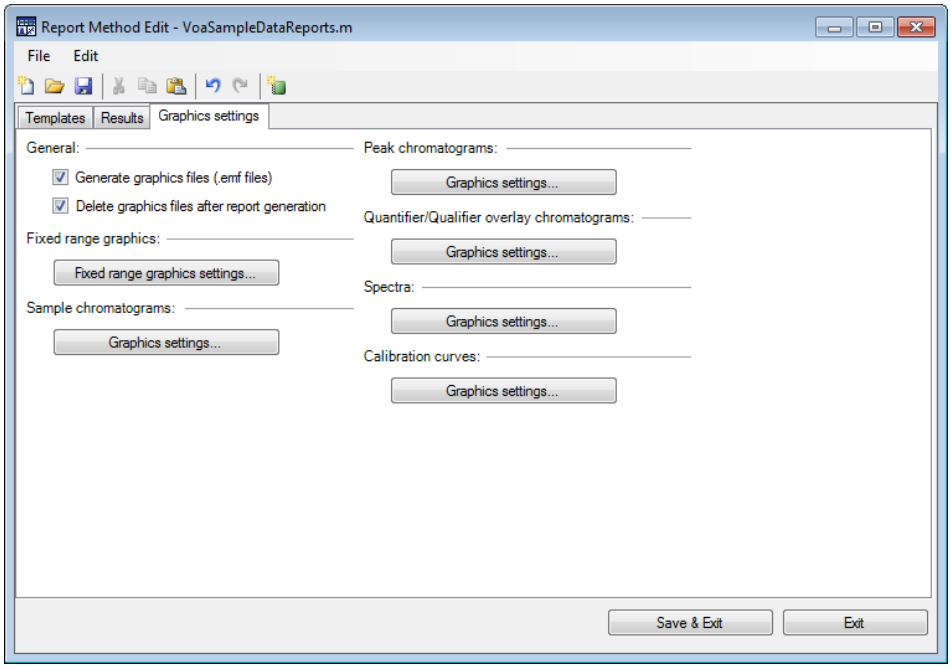
b Under the **Generate Reports results file** field, click **Auto.**

c From the drop down menu of the Instrument field, select **GCMS.**

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.

7. Set the graphic setting options for the method.

a Click the **Graphic Settings** tab to review the graphic settings.



b Select the **Generate graphic file** checkbox to add graphics to your report.

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

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

8. Save the report method.

You must save the method before you can close the window and generate a report.

a Click the save icon in the **Report Method Edit** window.

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

9. Close the **Report Method Edit** window.

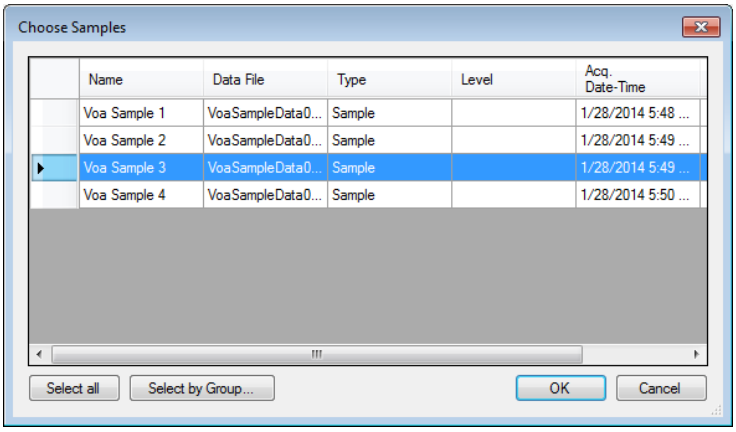
Click **Save & Exit** to close the **Report Method Edit** dialog to return to the **Generate Report** window.

Task 2. Generate a report.

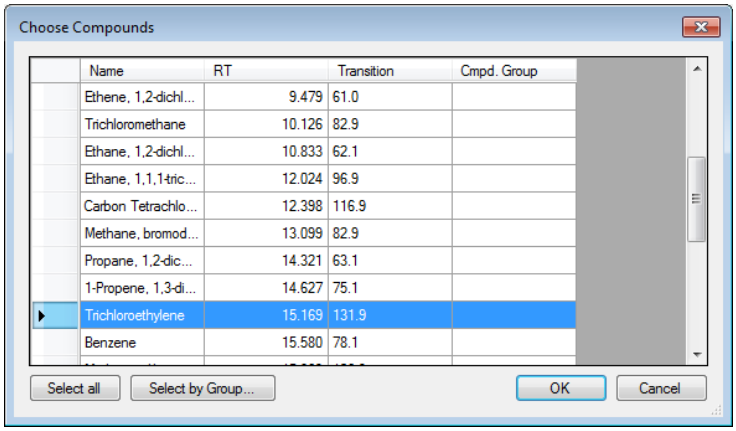
1. Generate a report from the method.

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

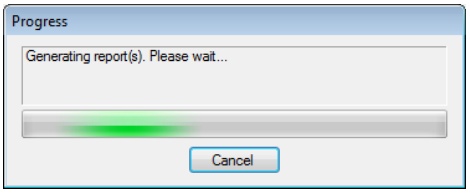
b In the **Samples/Compounds** field, click **Choose Samples** to open the Choose Samples dialog. Select the samples to include on the report.



c Click **All Compounds** and select the compounds to include in the report.



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

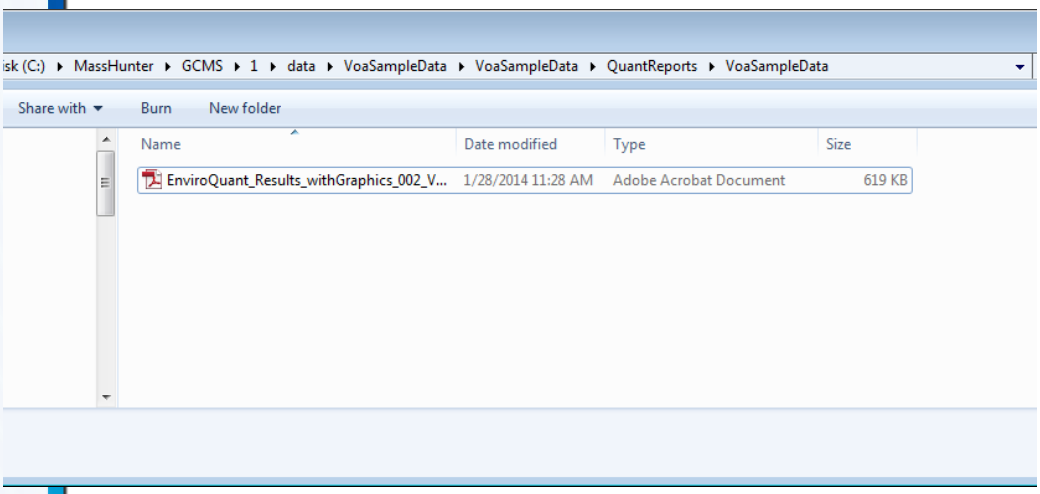


You can choose to show all the samples and all the compounds in the batch, or select specific samples or compounds in the batch table to show in your report.

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**.

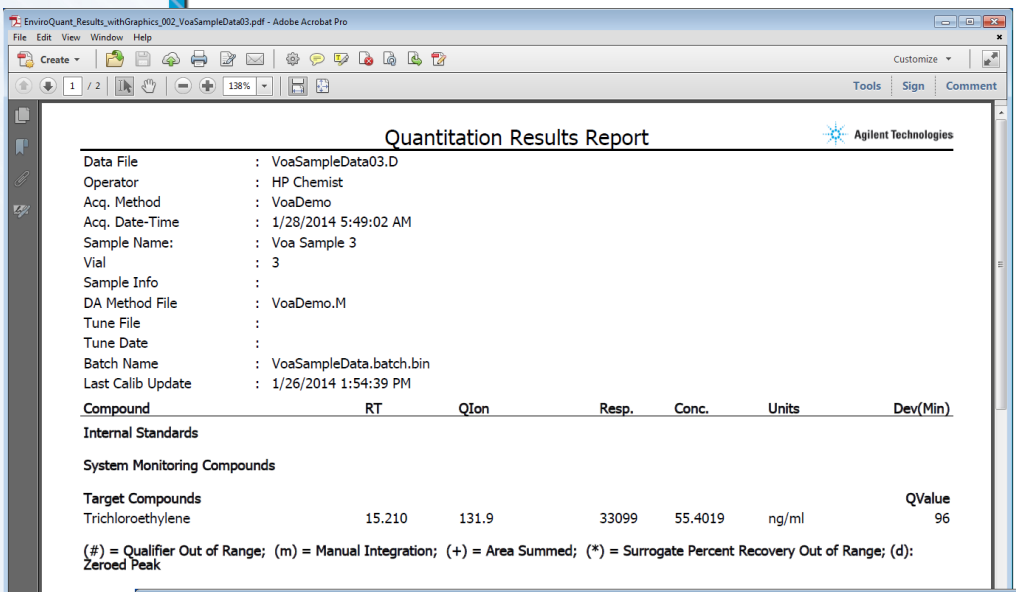
Reports are viewed or printed from the Excel or the PDF file you have created.

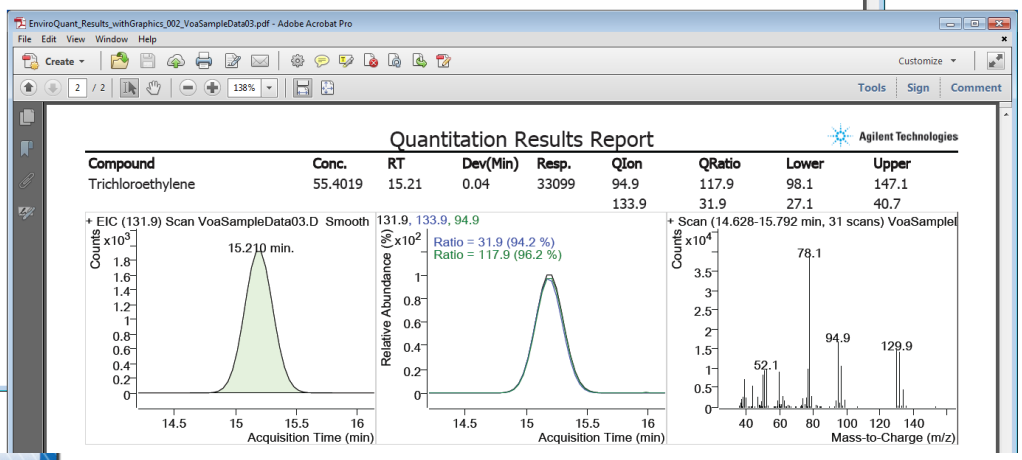


2. View the report.

Double-click on a file to open and display the report.

Alternatively, you may open the report by selecting the file in Windows Explorer.





Agilent Technologies

Printed in USA, February 2014