Agilent

MassHunter Quantitative Analysis LC/Q-TOF Screener
for High-Throughput Data Analysis

Method Development Using All Ions and Q-RAI Data

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Notices

Document Identification

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Agilent Technologies, Inc.
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Santa Clara, CA 95051

Software Revision

This guide is valid for the C.01.00 revision or higher of the MassHunter Quantitative Analysis LC/Q-TOF Screener program and compatible MassHunter Quantitative Analysis 10.1 and higher, programs, until superseded.

Instrument Manufacturing

Manufactured by Agilent Technologies Singapore Pte. Ltd.
No. 1 Yishun Avenue 7, Singapore 768923

Operating Temperature

Operating Temperature: 15 °C to 35 °C
Storage Temperature: –40 °C to 70 °C

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WARNING

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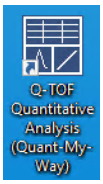
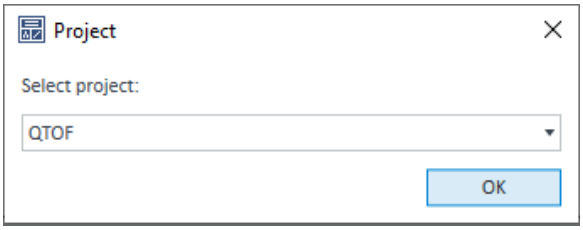
Contents

1 Create a Batch of Samples

1 Open MassHunter Q-TOF Quantitative Analysis 11.

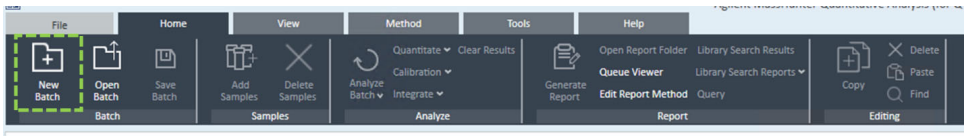


2 Select the Project containing the data and PCDL(s).



3 Create a new batch of data.

a Select **New Batch**.

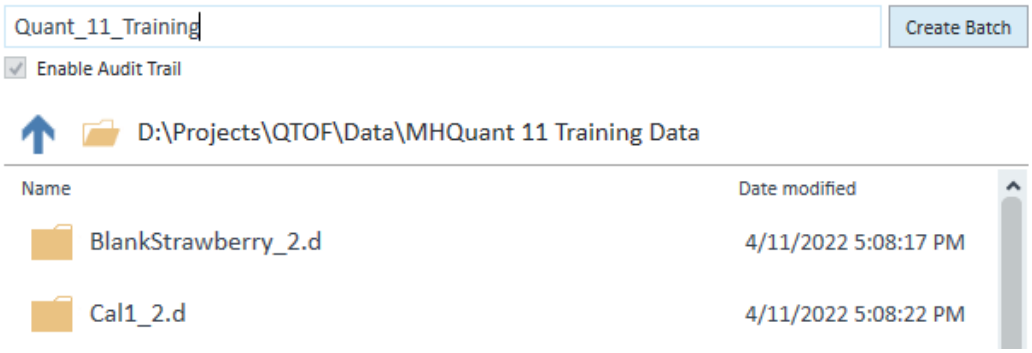


b Navigate to the folder that contains the data files (X:\Projects\QTOF\Data\MHQuant 11 Training Data).

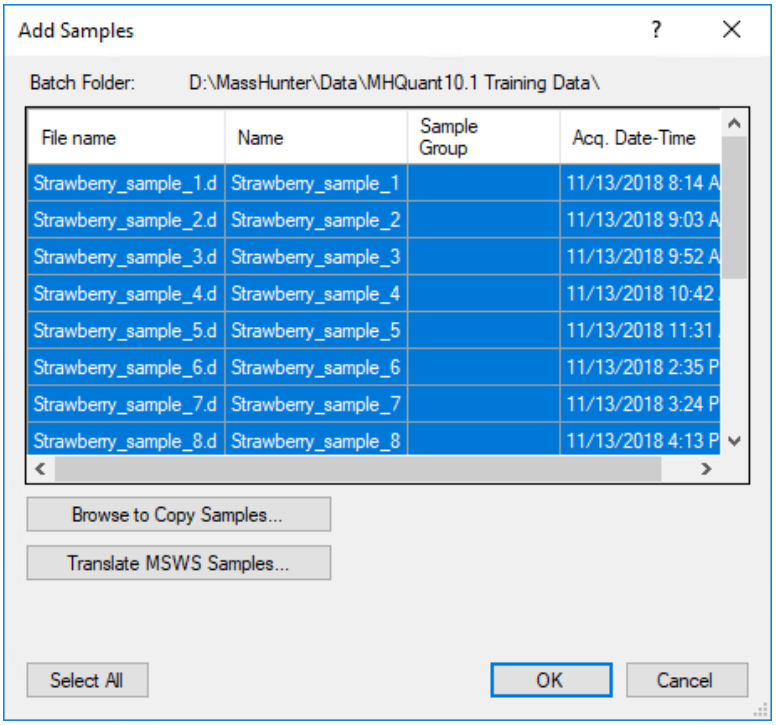
MassHunter Quantitative 11 Training data is included in the download. Store the data in the appropriate folder under `Projects`.

c Enter a batch file name.

The example displayed is `Quant_11_Training`.

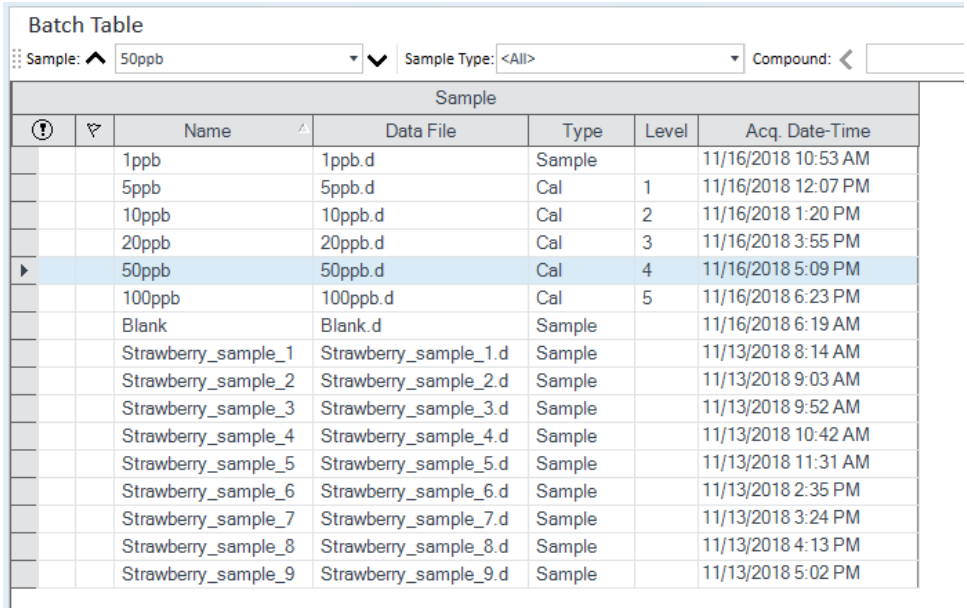


d Select **Create Batch**.
The **Add Samples** window automatically displays. By default, all samples in the folder are already highlighted.



4 Select **OK** to add samples.

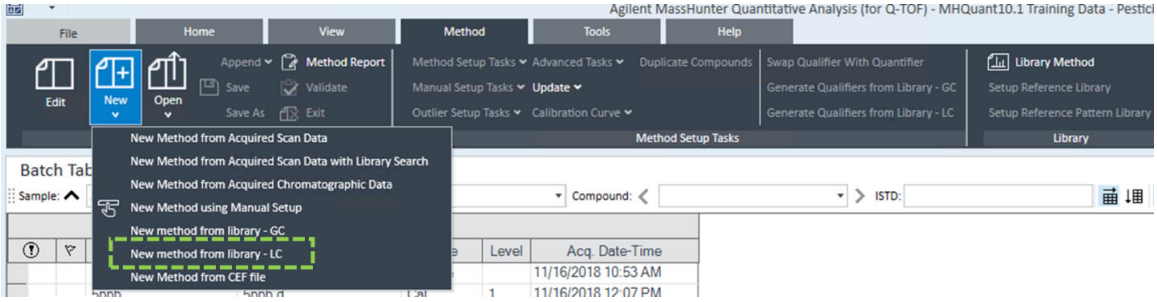
The data displays in the **Batch Table**.



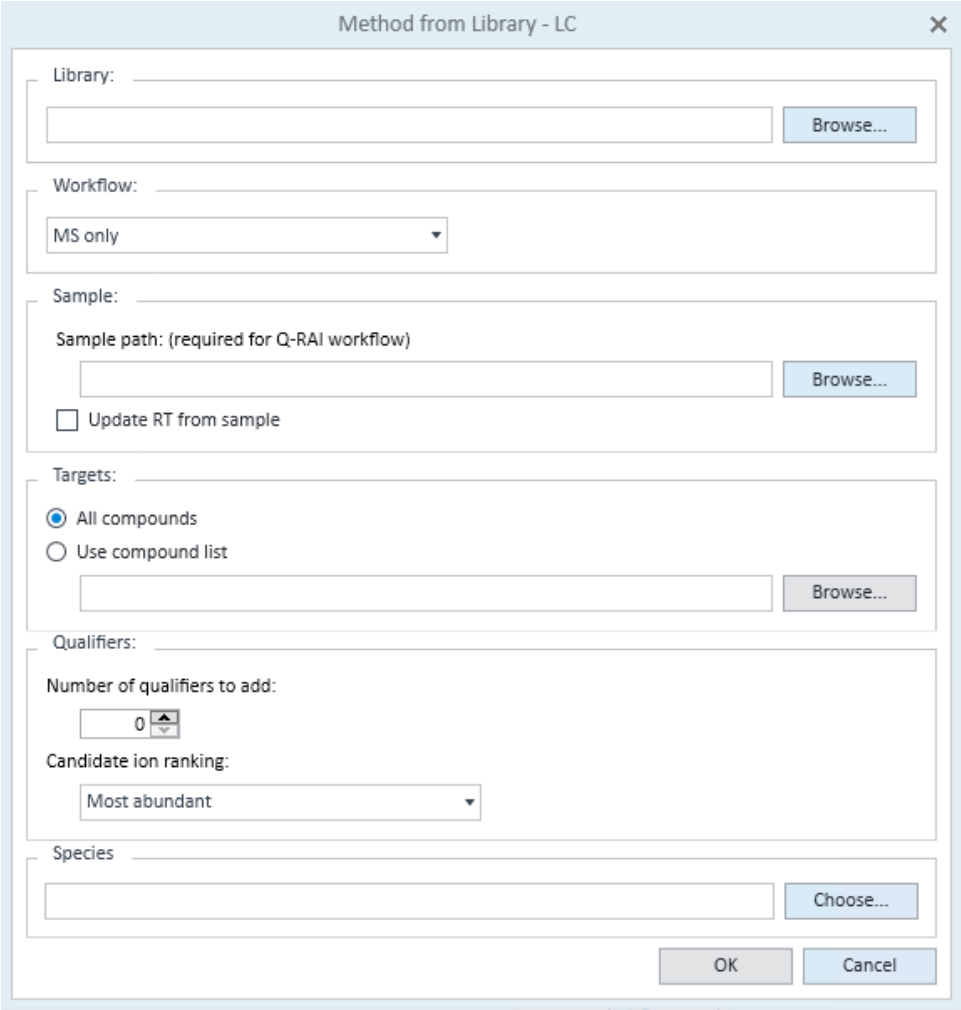
2 Build a Targeted Analysis Method from PCDL

Create a new method from PCDL

1 Within MassHunter, select the **Method** tab from the toolbar, then select **New** > **New method from Library – LC**.



The **Method from Library – LC** window displays.



2 In the **Library** panel, select **Browse...** to navigate to and select the PCDL containing the target and/or suspect analytes for the method.
   - If there is no RT in PCDL, the RT in the method will be left blank.
   - If there are no spectra, only a precursor ion will populate in the method. There will be no fragments.
   - If the method is intended for simultaneous quantitation and screening, Agilent recommends a separate Target PCDL and Suspect PCDL. Targets are analytes with a calibration curve, and suspects are analytes without a calibration curve.
3 Within the **Workflow** panel, choose the acquisition mode of the data to be analyzed.
   - MS scan only
   - All Ions
   - Q-RAI (Quadrupole resolved All-Ions)
   - Targeted MSMS
4 Within the **Sample Path** panel, select **Browse...** to navigate to and select an example data file to help build the method.

   This is needed for Q-RAI to establish what quadrupole bands were used. For **All Ions**, it helps define optimal CE for analysis based on the CE used in acquisition.

   For **All Ions**, if there is a mismatch between CE in the acquisition and PCDL (i.e. 15 for the acquisition method, but 10, 20, or 40 for PCDL), no fragments will be inputted, so leave this blank and manually add the CE in the method editor.
5 Within the **Targets** panel, select a radio button to determine what analytes are used to create a method.
   - Select **All Compounds** to create a method using all the analytes in the PCDL.
   - Select **Use Compound List** to navigate to and select a subset of analytes using a compound list *.txt file.
6 Within the **Qualifiers** panel, enter the number of qualifiers to add to the method according to their ranking.

   It is best to begin by selecting more fragments, then filter down to the best fragments in the analysis method optimization steps.
7 Within the **Qualifiers** panel, use the drop-down menu to choose how to rank candidate ions for selection.

Candidate Ion Ranking	Meaning
Most abundance	All candidate ions in the component spectrum are ranked by ion abundance.
Weighted	All candidate ions in the component spectrum are ranked by mass weight factor (MZ × MZ × Abundance).
Highest mass	The candidate ions in the component spectrum are ranked by m/z value.
Monoisotopic	Only works for GC data. It uses Monoisotopic field of library instead of Mass field.

   Candidate ion ranking offers control over the ion selection process while creating a new Quantitative Analysis method from library. For LC data, it is mapped to the qualifiers only.

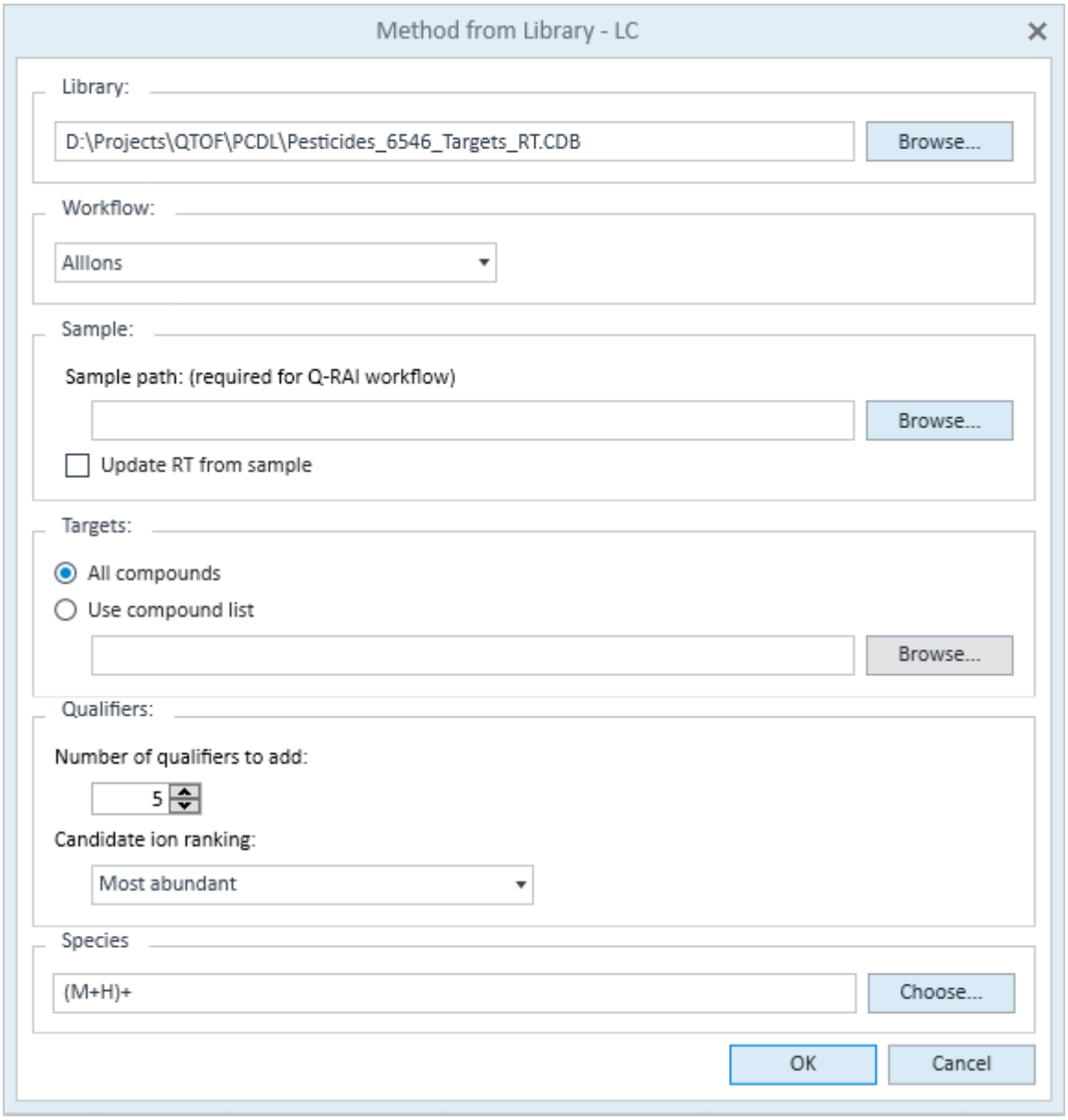
   If there is a selectivity issue, try most abundant first, followed by weighted.

8 In the **Species** panel, select **Choose...** to navigate to and select the ion species for the analysis method.

When left blank, the ion species defaults to what is in the PCDL. If the PCDL has spectra for multiple adduct types it will create multiple entries (duplicate compound names but with different precursor ions). If one ion species is selected (i.e. [M+H]+), all analytes will have that precursor. If the PCDL does not have spectra for the selected species, no fragments will be imported.

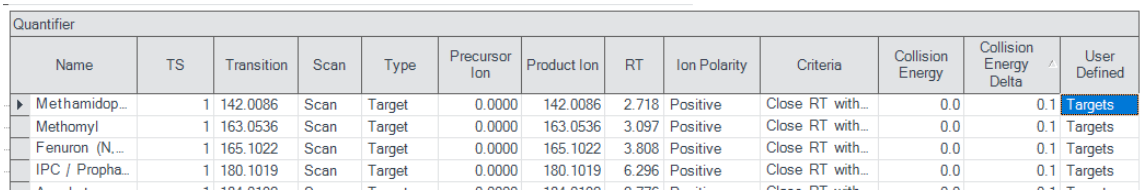
For a mixture of M+H and M+NH_4 ions in the method, either curate the PCDL with the preferred adduct only and leave the species selection blank, or separate analytes in two PCDLs (or use a compound list) and import the different adducts separately.

Here is an example of a completed Method builder dialog box for adding the Target analytes to the method using All Ions data (Target pesticide PCDL is located in the Training data folder).



9 Select **OK**.

A Quantifier window displays.



Quantifier

Name	TS	Transition	Scan	Type	Precursor Ion	Product Ion	RT	Ion Polarity	Criteria	Collision Energy	Collision Energy Delta	User Defined
Methamidop...	1	142.0086	Scan	Target	0.0000	142.0086	2.718	Positive	Close RT with...	0.0	0.1	Targets
Methomyl	1	163.0536	Scan	Target	0.0000	163.0536	3.097	Positive	Close RT with...	0.0	0.1	Targets
Fenuron (N,...	1	165.1022	Scan	Target	0.0000	165.1022	3.808	Positive	Close RT with...	0.0	0.1	Targets
IPC / Propha...	1	180.1019	Scan	Target	0.0000	180.1019	6.296	Positive	Close RT with...	0.0	0.1	Targets

10 Label the analytes as **Targets**.

a In the method editor, navigate to and select **Method Setup Tasks** > **Compound Setup**.

b Right-click, and select to **add a User Defined column**.

In MassHunter Q-TOF Quantitative Analysis 11, there are instances of User Defined columns numbered 1 through 9. However, in Compounds-at-a-Glance, there is only one available User Defined Column, and it is not numbered.

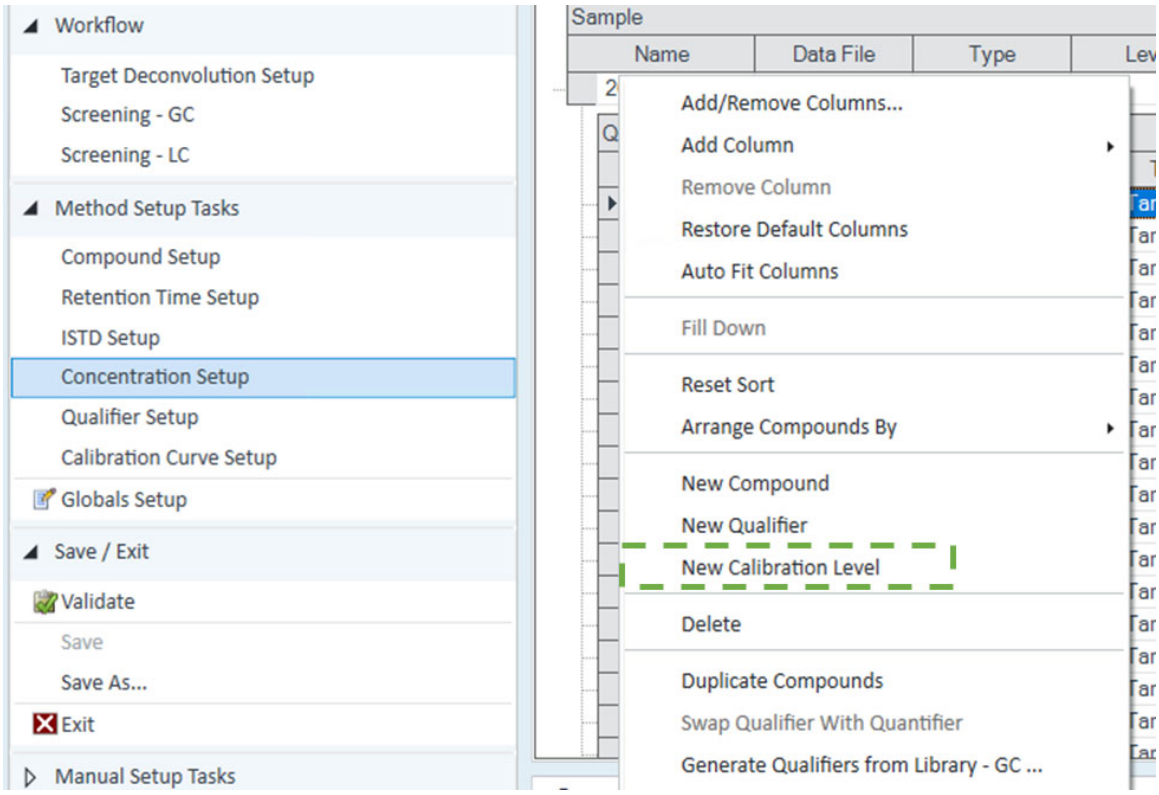
c In the new column, label all analytes **Targets**.

d (*Optional*) Label the first row as `Targets`, then right-click and select **fill down** to duplicate the entry down the column.

Add Calibration Curves for Target Analytes

Set concentration levels

1 Within MassHunter, select the **Method** tab from the toolbar, then select **Method Setup Tasks** > **Concentration Setup**.

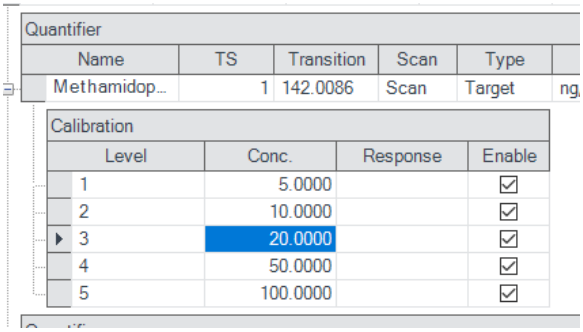


2 Right-click any target analyte, then select **New Calibration Level**.

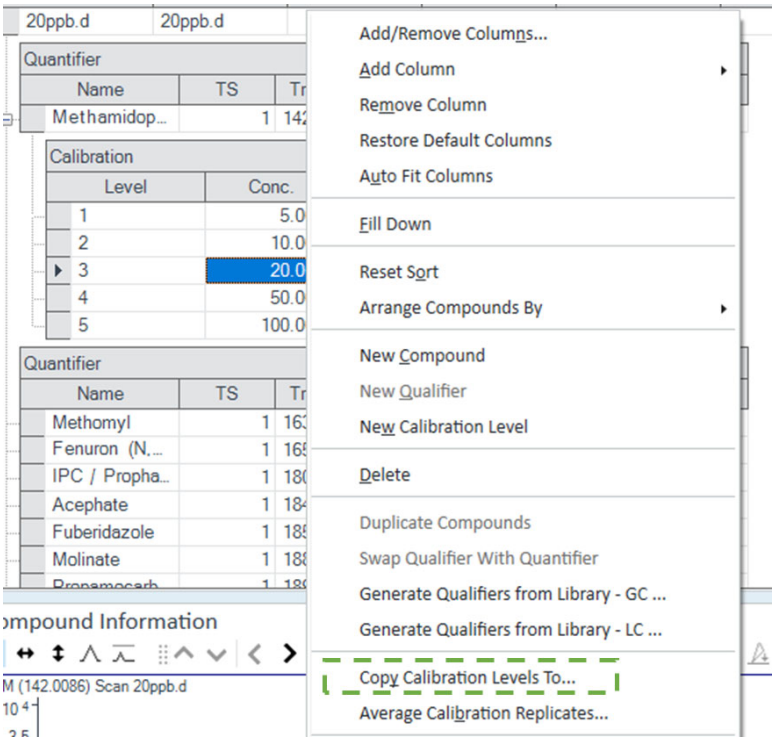
If Sample Type, Level, and Expected Concentration have already been set up in the Batch Table, select **Calibration Curve** > **Create Levels from Calibration Samples**, then skip to step 7 below.

3 Repeat step 2 four more times for the same analyte to create 5 levels under that analyte.

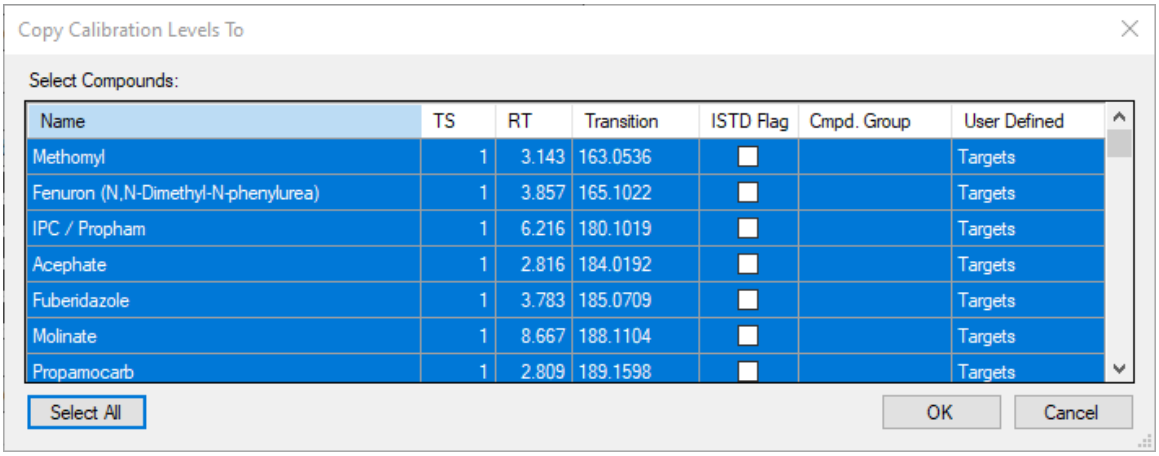
4 Add level numbers (1 to 5) and their respective concentrations (5, 10, 20, 50, and 100 ppb).



5 Right-click any of the levels and select **Copy calibration levels to…**.



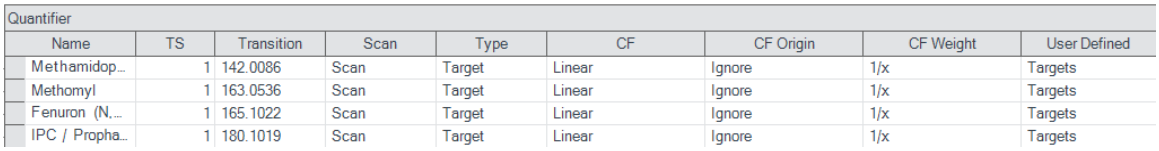
6 Click **Select All** to highlight all compounds, then select **OK**.



7 Under Method Setup Task, select **Calibration Curve Setup**.

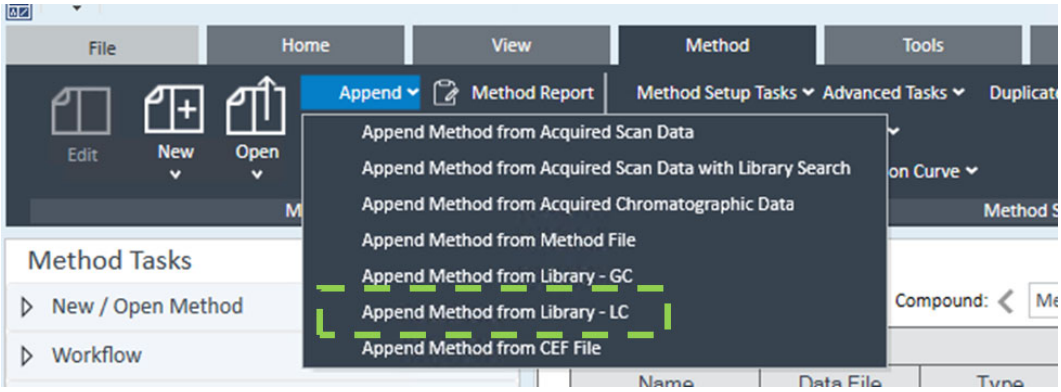
8 Make the following selections, as appropriate.

- Select Linear for CF
- Ignore for CF Weight
- 1/x for CF Weight.



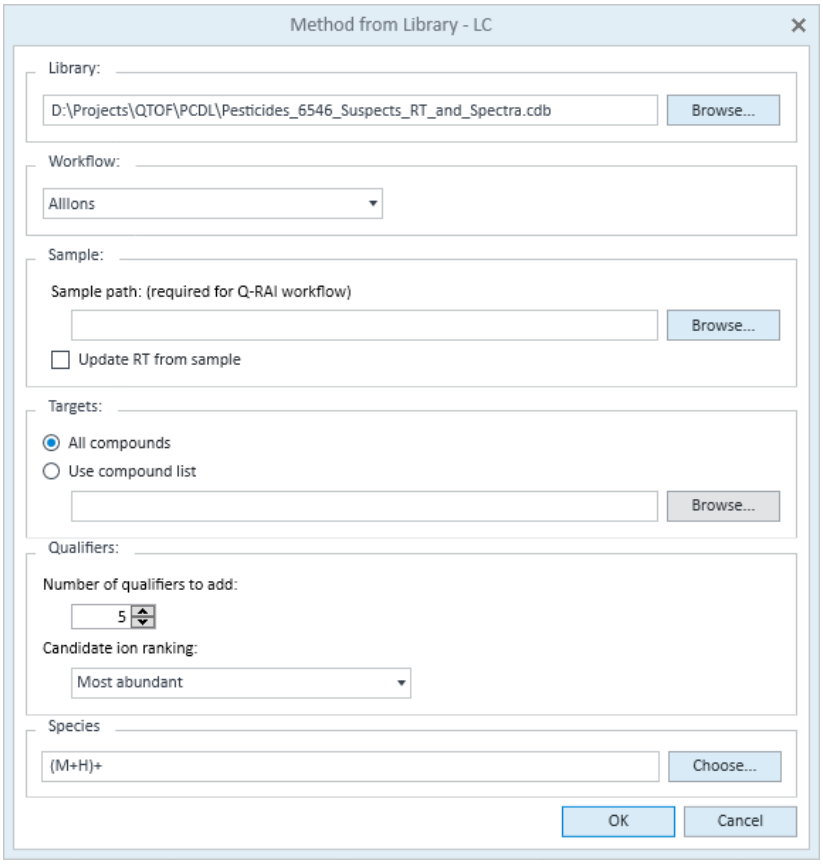
Append the method to add the suspect analytes

1 From the **Method** tab of MassHunter, select **Append** > **Append Method from Library-LC**.



2 Specify the appropriate selections in the **Method from Library - LC** window. Refer to steps 2 to 9 in **Create a new method from PCDL** for instructions. However, in step 8, navigate to and select **Suspect PCDL** rather than **Target PCDL**.

The method now contains targets and suspect analytes.



3 In the method editor, label the suspects in the user defined column.
The suspects will have a blank **User Defined** column. Enter `Suspects` in that column.

Quantifier

Name	TS	Transition	Scan	Type	Precursor Ion	Product Ion	RT	Ion Polarity	Criteria	Collision Energy	Collision Energy Delta	User Defined
Avermectin...	1	873.4995	Scan	Target	0.0000	873.4995	15.358	Positive	Close RT with...	0.0	0.1	Targets
Ivermectin B...	1	875.5151	Scan	Target	0.0000	875.5151	16.646	Positive	Close RT with...	0.0	0.1	Targets
Chloroproph...	1	214.0629	Scan	Target	0.0000	214.0629	7.543	Positive	Close RT with...	0.0	0.1	Suspects
Acrinathrin (...	1	542.1397	Scan	Target	0.0000	542.1397	14.061	Positive	Close RT with...	0.0	0.1	Suspects
Butoxycarbo...	1	223.0747	Scan	Target	0.0000	223.0747	2.814	Positive	Close RT with...	0.0	0.1	Suspects
Chlorflurazur...	1	539.9702	Scan	Target	0.0000	539.9702	13.612	Positive	Close RT with...	0.0	0.1	Suspects

4 In the **Criteria** column, specify **Close RT with Qualifiers** for Suspects and Target analytes.

See **PeakSelectionCriterion** below for an explanation of additional potential criteria.

PeakSelectionCriterion

PeakSelectionCriterion represents the algorithm employed to select the peak which is designated the **hit**, i.e. the PrimaryHitPeakID. The criteria affect the mechanism of ranking the Peak rows associated with this TargetCompound row. The most suitable Peak row is then chosen as the primary hit.

PeakSelectionCriterion has several possible values.

PeakSelectionCriterion	Value
GreatestResponse	GreatestResponse causes the peaks (such that the Peak row) that exhibit the greatest integrator-determined response to be designated the hit. For response, either height or area will be used as indicated by the value of the QuantitateByHeight column. This is the default value. There is only one primary hit. Other non-hit peaks are considered alternative hits.
Close RT	Close RT causes the ranking of the peaks such that the Peak row that exhibits the closest integrator-determined retention time, irrespective of quality and fidelity of qualifier signal ratios is designated the hit. Other non-hit peaks are considered alternative hits.
Close RT with Qualifiers	Close RT with Qualifiers causes the ranking of the peaks such that the Peak row that exhibits the best retention time among the Peak rows for which all qualifier ratio tolerances are satisfied is designated the hit. If there is no Qualified Peak row, this ranking algorithm will be equivalent to Close RT.
Greatest Q-Value	Greatest Q-Value causes the ranking of the peaks such that the Peak row that exhibits the nominal qualifier ratios that are best satisfied by the actual ratios. A correlation computation called QVALUE is performed to measure the fidelity of the observed qualifier ratios versus the nominal qualifier ratios. The definition of the QVALUE function is a well-defined statistic that yields an efficient measure of the **goodness of fit** of the observed qualifier ratios to the nominal qualifier ratios.

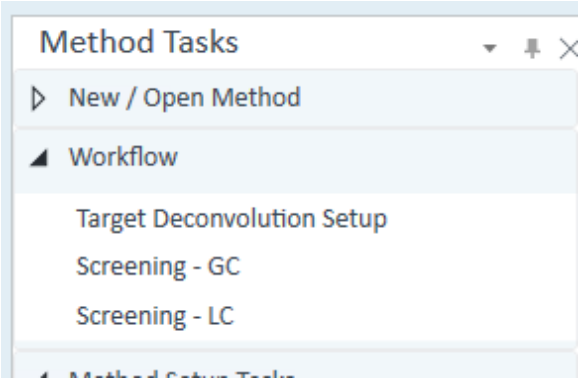
3 Create an LC Screener Method

LC Screener Workflows

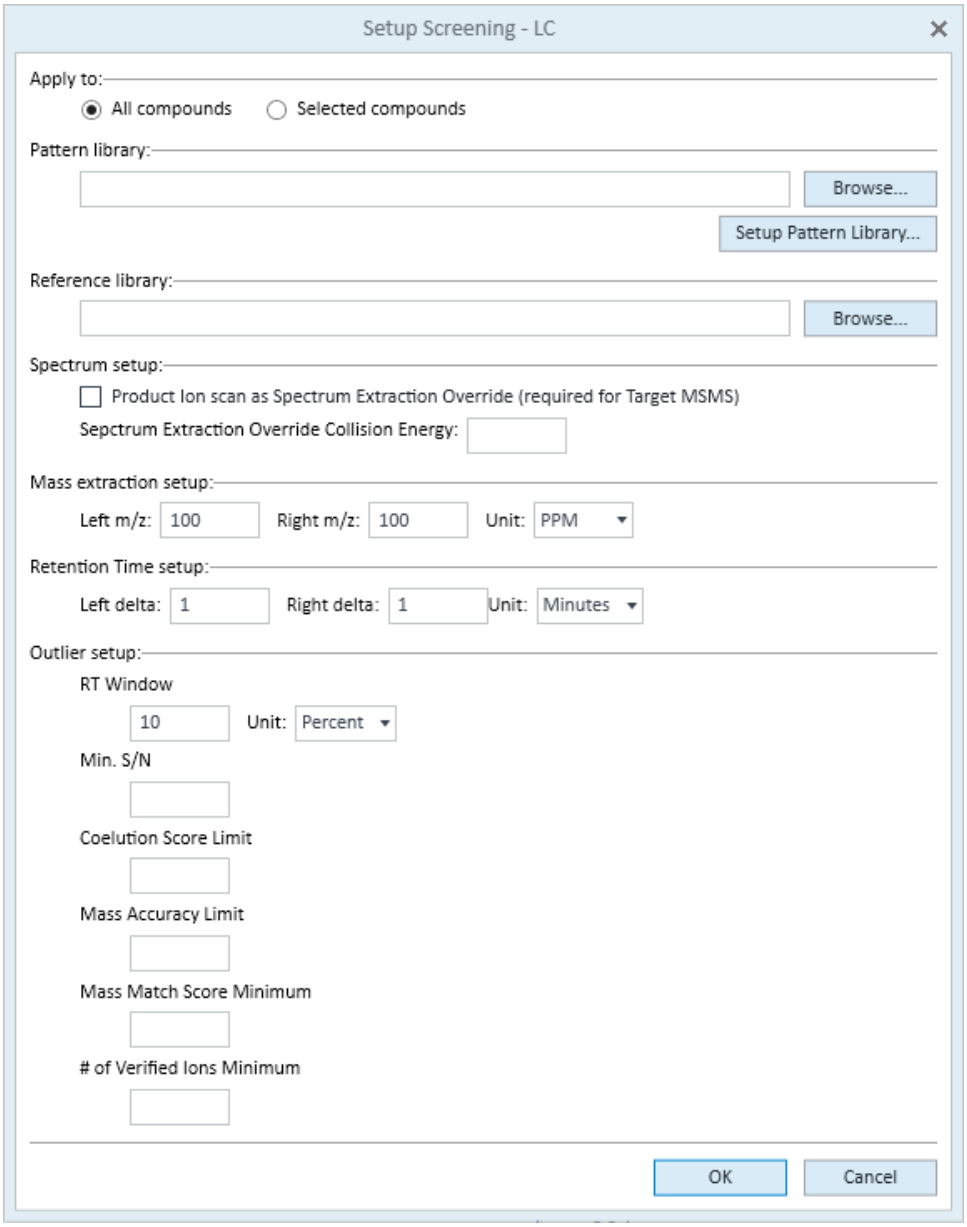
Build an LC screening method by adding parameters for extracting the analytes, analyzing the data, and flagging outliers.

For this application, there are different parameters for the Targets and Suspects. Other applications might have the same parameters.

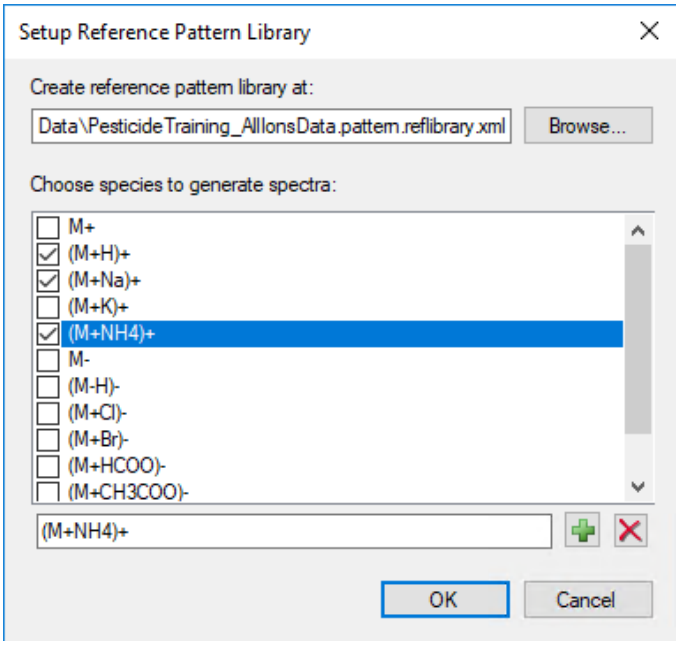
1 Within Method Tasks, select **Workflow** > **Screening - LC**.



The **Setup Screening – LC** screen opens to display default parameters.

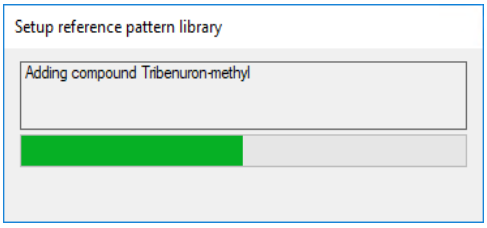


2 To build a pattern reference library, select **Setup Pattern Library…**, then click to check **(M+H)+**, **(M+Na)+**, and **(M+NH4)+** adducts.

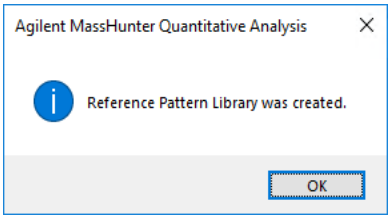


3 Select **OK**.

The Library editor initiates and a pop-up progress window displays.

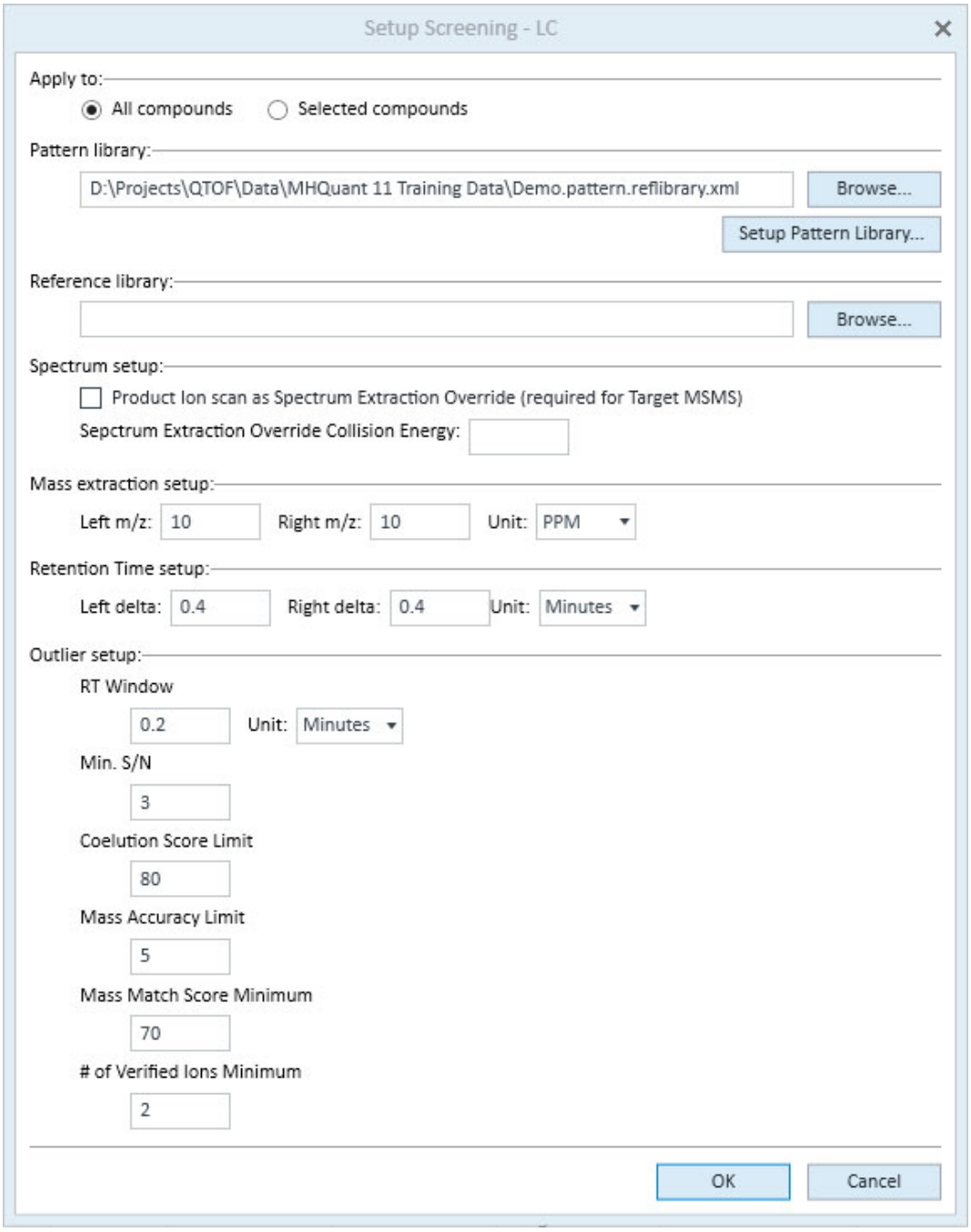


4 Select **OK** when the reference pattern library is complete.



NOTE If a pattern reference library is not set up, the isotope abundance visualization (denoted by red boxes) will not appear in the LC Screener Tool.

5 On the **Setup Screening – LC** screen, use the radio button to select **All Compounds**.



These are the parameters for the Target analytes.

6 Under **Mass extraction setup**, enter or select the following settings.

Parameter	Setting
Left m/z	10
Right m/z	10
Unit	PPM

7 Under **Retention Time setup**, enter or select the following settings.

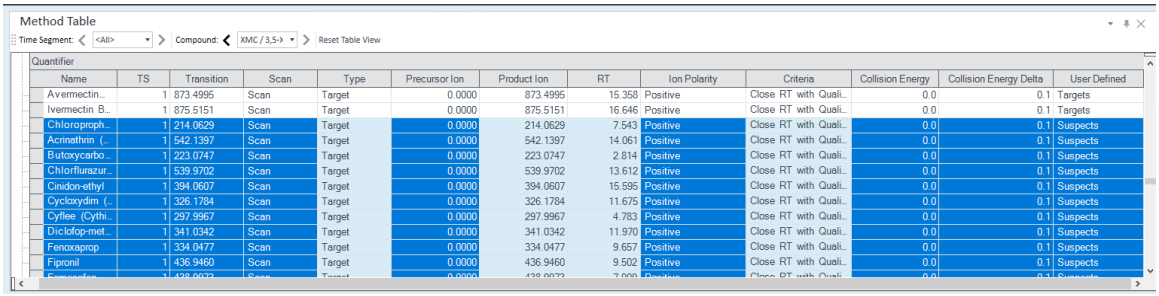
Parameter	Setting
Left delta	0.4
Right delta	0.4
Unit	Minutes

8 Under **Outlier setup**, enter or select the following settings.

Parameter	Setting
RT Window	0.2
RT Window Unit	Minutes
Min. S/N	3
Coelution Score Limit	80
Mass Accuracy Limit	5
Mass Match Score Minimum	70
# of Verified Ions Minimum	2

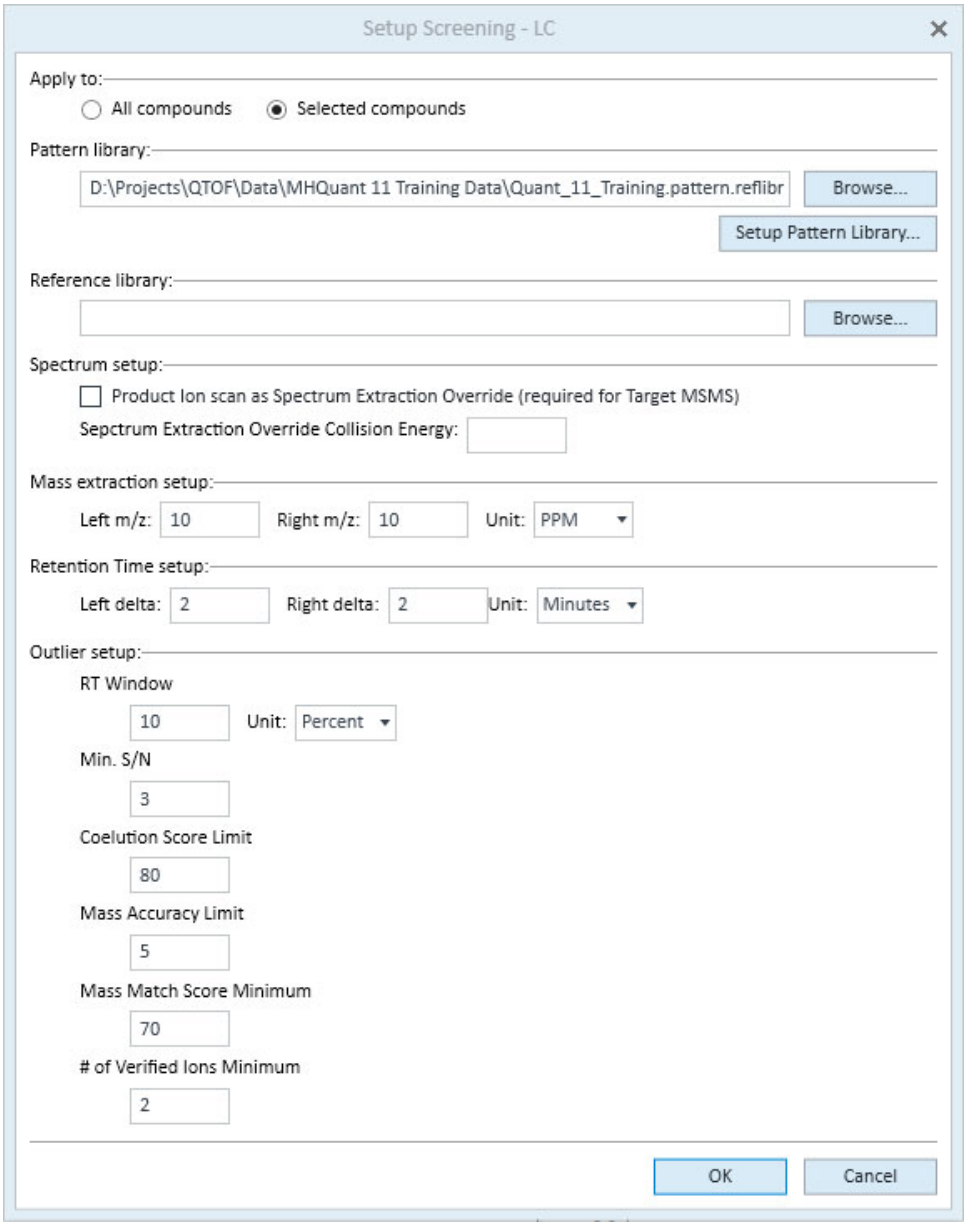
9 Select **OK** once all settings have been entered.

10 Adjust the suspect parameters if the extraction or outlier parameters differs between targets and suspects. Press **Ctrl + Shift** to select all Suspects in the compound table.



11 Within Method Tasks, select **Workflow** > **Screening - LC**.

12 Fill in the following parameters in the **Setup Screening - LC** window. The differences between targets and suspects are the selection of **Selected compounds** (under **Apply to:**) and the **Retention Time setup**.



Enable SureMass

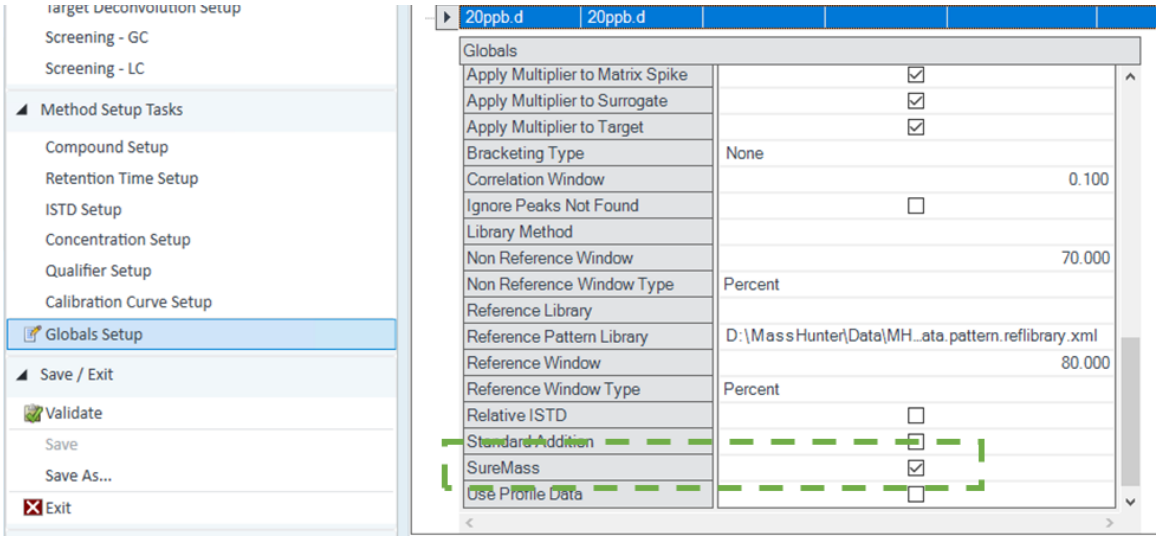
1 Convert the data to SureMass at the time of acquisition. It can be converted in one of two ways.

- Select **Acq 10.1** > **Method** > **Post run script** > **SCP_SureMassConversion**(){**MH_Acq_Scripts.exe**}.
- Select **Acq 11** > **Method** > **DA Tab** > **Check SureMass box**.

Conversion can be performed after acquisition in Quant 10.1 or higher by selecting **Quant** > **Tools** > **Convert Samples** > **MassHunter TOF/Q-TOF tab**. However, Agilent recommends converting data at the time of acquisition whenever possible.

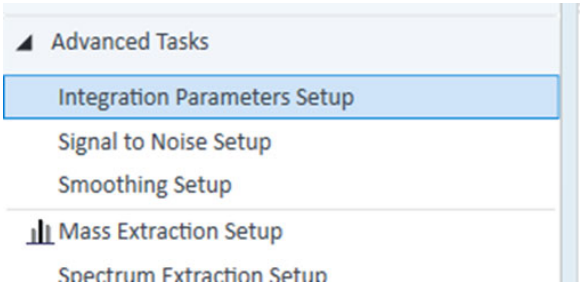
The data represented in this guide has already been converted.

2 Enable SureMass analysis. Select **Method Setup Tasks** > **Global Setup**, then click to check the SureMass box.

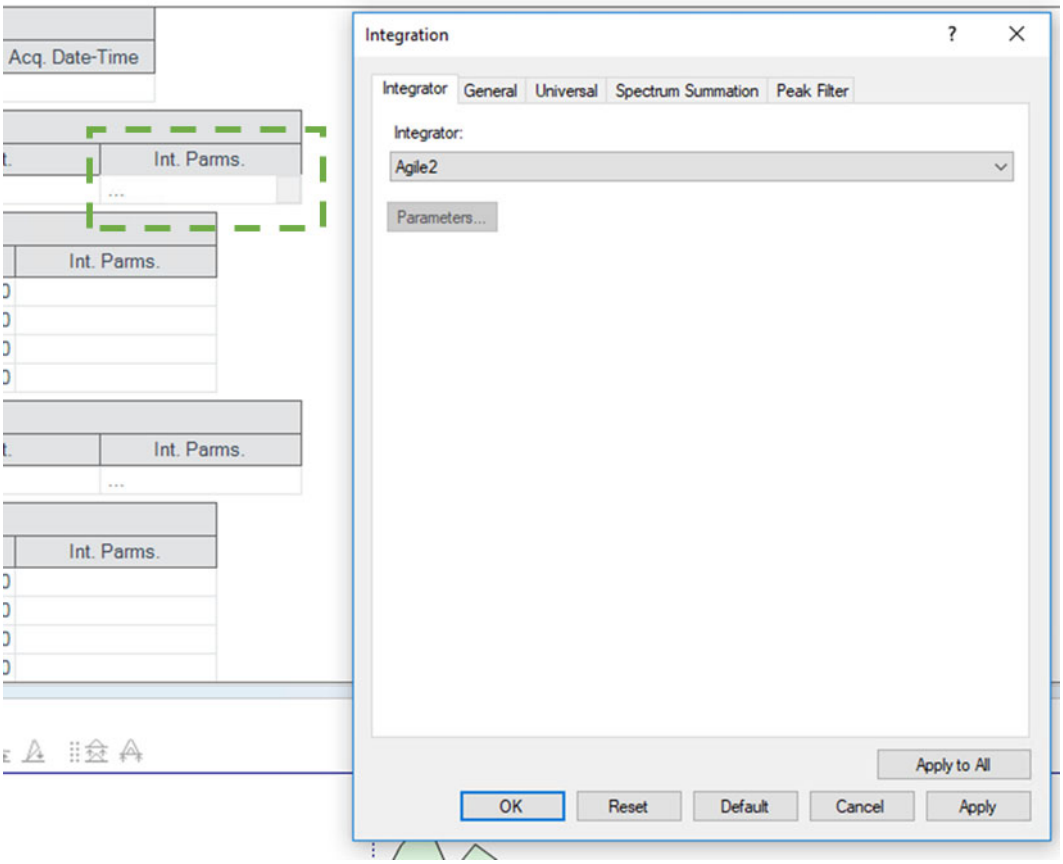


Add Peak Filtering

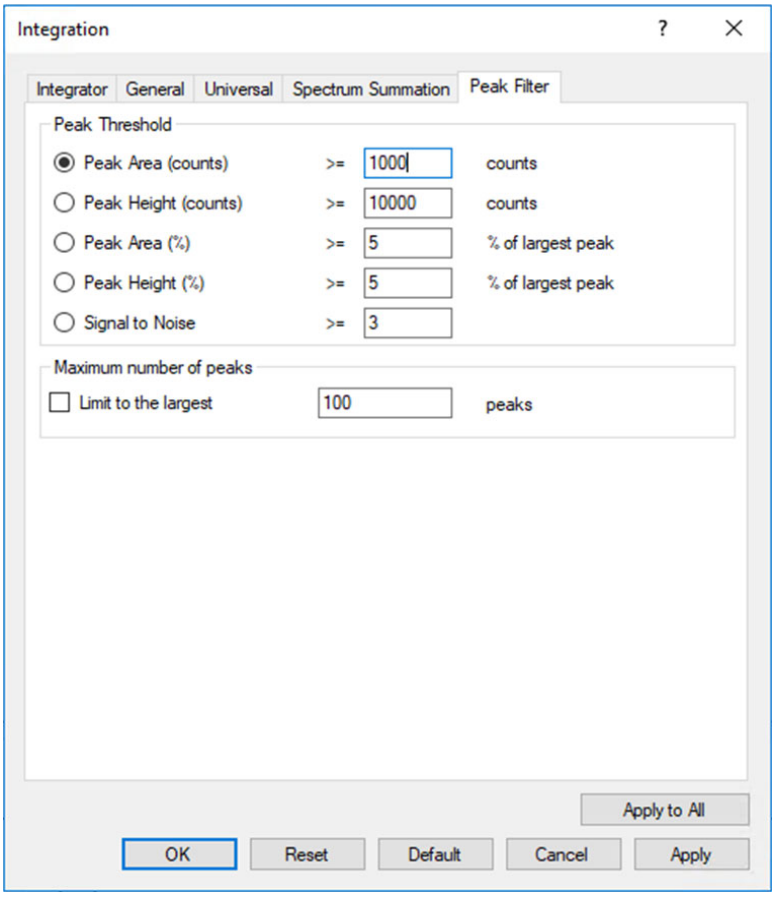
1 To adjust integration thresholding, select **Advanced Tasks** > **Integration Parameters Setup**.



2 Double-click **Integration Parameters** to open the Integration box.



3 Select the **Peak Filter** tab, then select **Peak Area**, and adjust counts to **1000**.

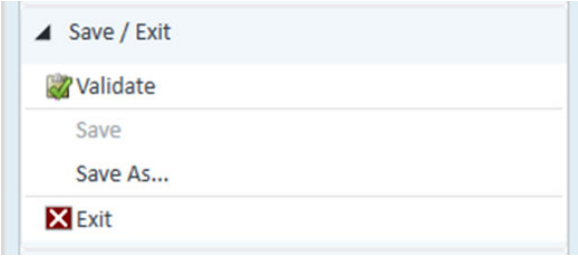


4 Select **Apply to All**, then select **OK**.

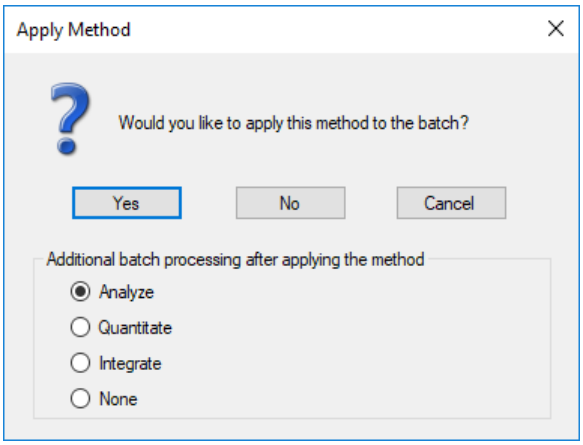
If this step is skipped, there may be a significant number of analytes that need review with the LC screener. These are false positives and this step removes a lot of analysis work.

4 Apply the LC Screener Method to Data

1 Select **Exit**.



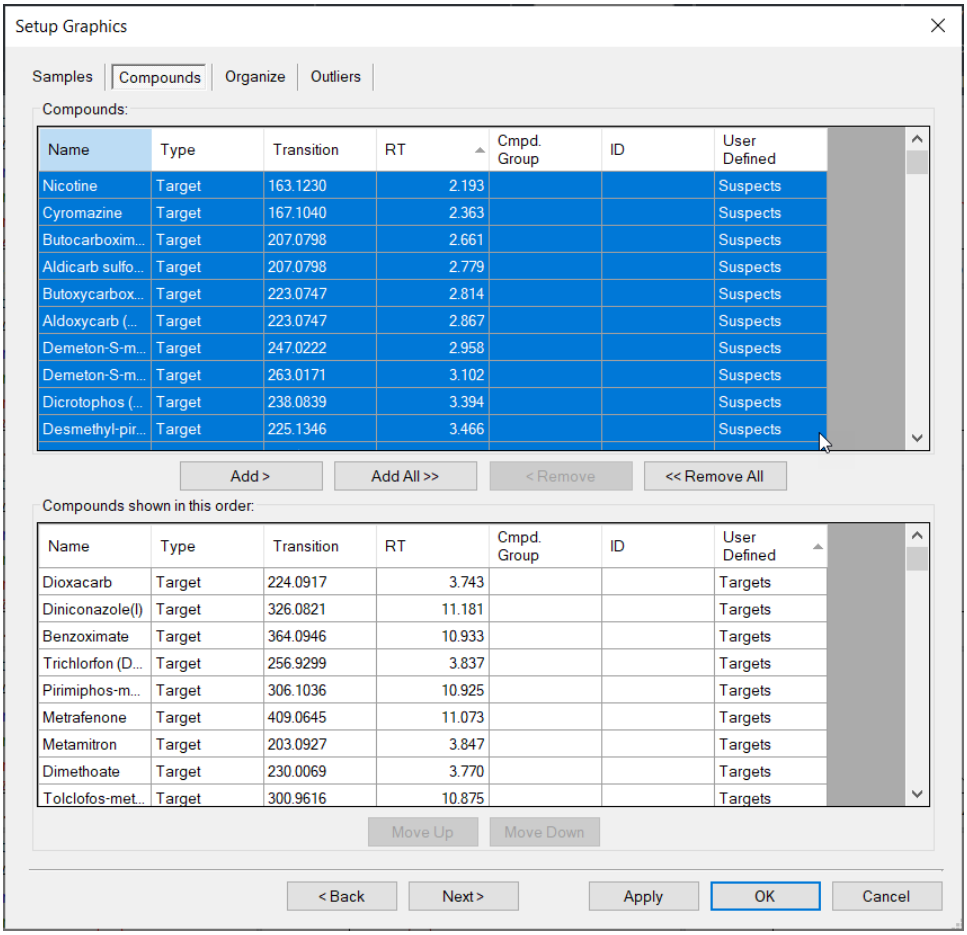
2 Apply the method to the batch and Analyze the data.



5 Analyze Data Using the LC Screener

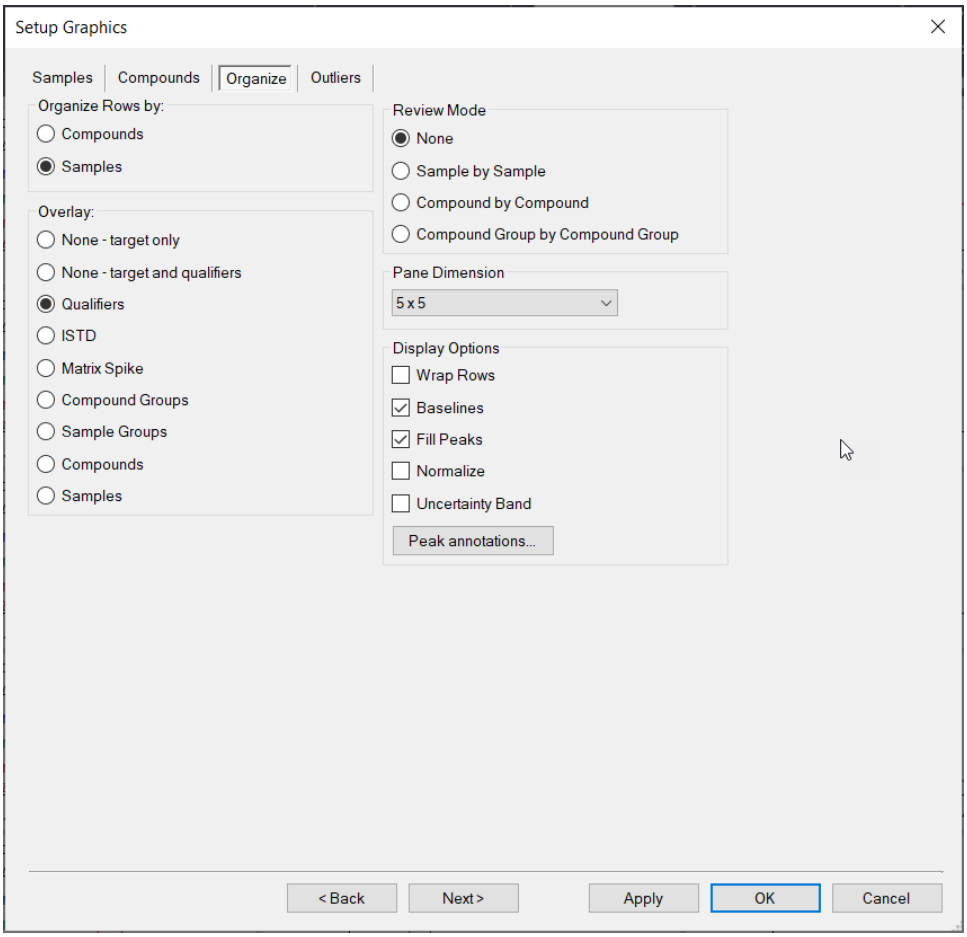
Update Calibrators

1 From the **View Tab**, select **Compounds-at-a-Glance**.
2 Select **Setup Layout**.
3 Remove all samples that are not Type **Cal**.
4 Select the **Compounds** tab.

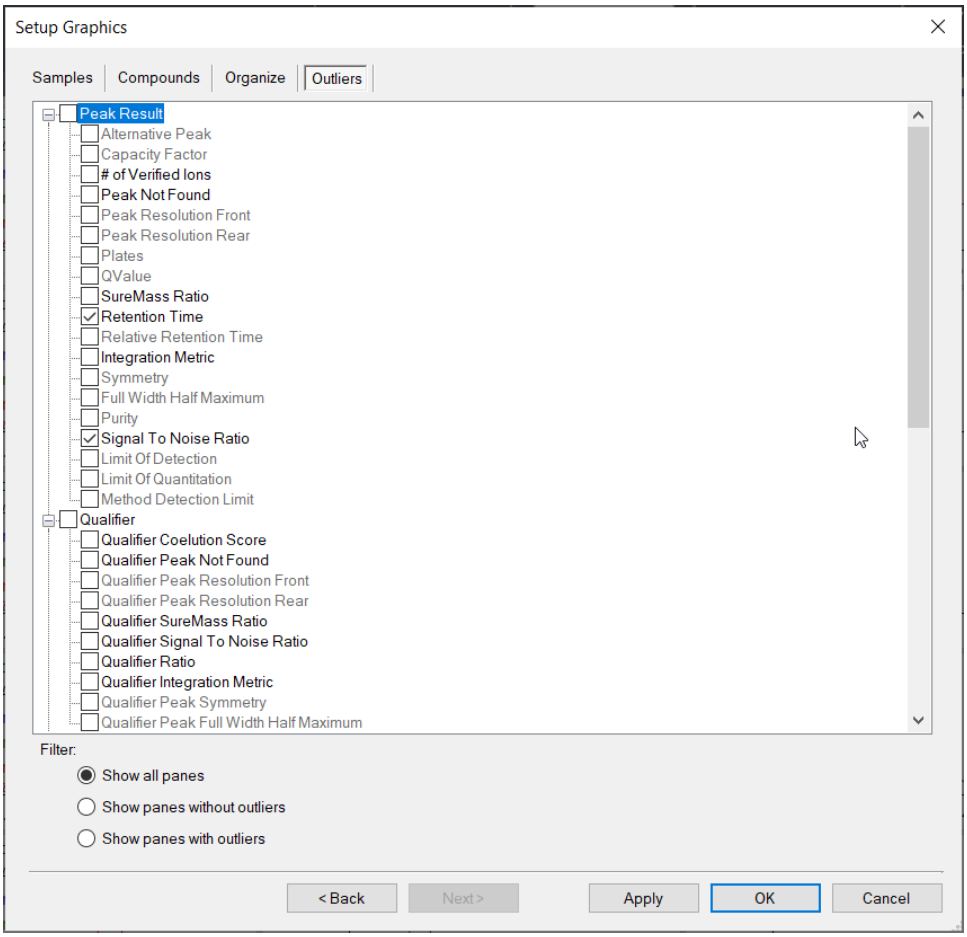


5 Sort the list by **User Defined**.
6 Remove **Suspects** to focus on **Targets**.

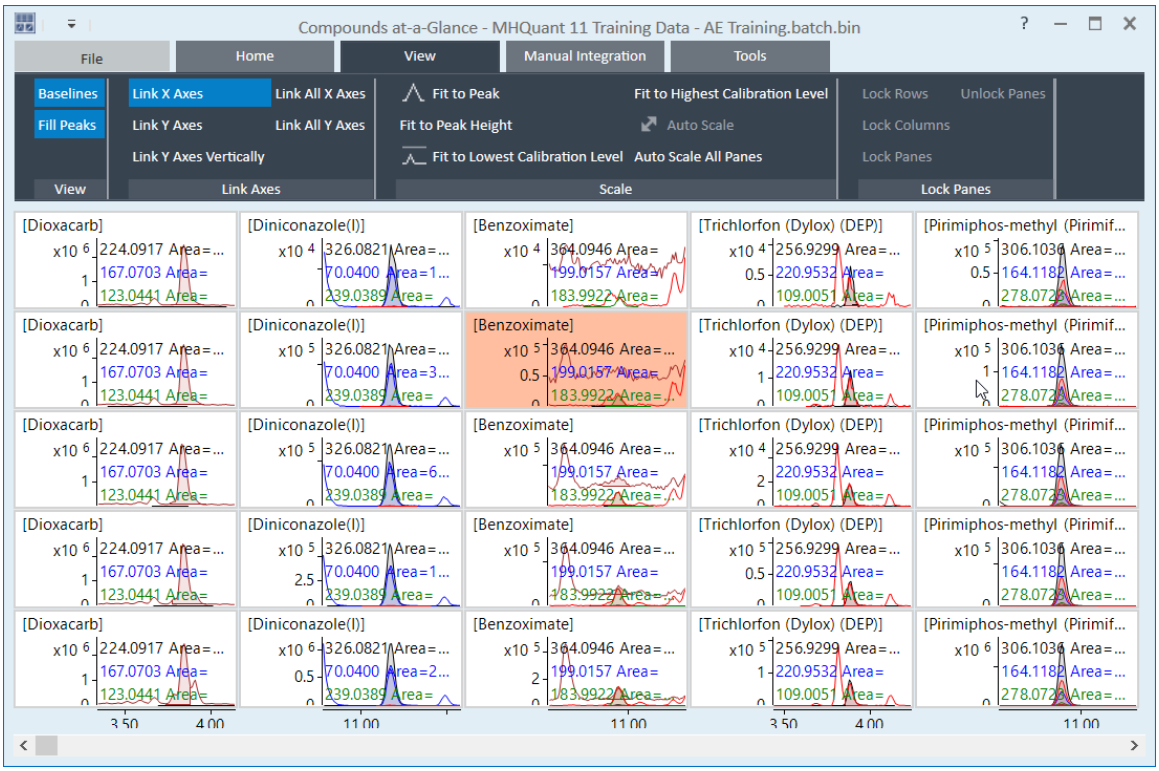
7 Select **Fill Peaks** on the **Organize** tab.



8 Select **Retention Time** and **Signal To Noise Ratio** on the **Outliers** tab, then select **OK**.



9 On the **View** ribbon, select **Link X Axes**.

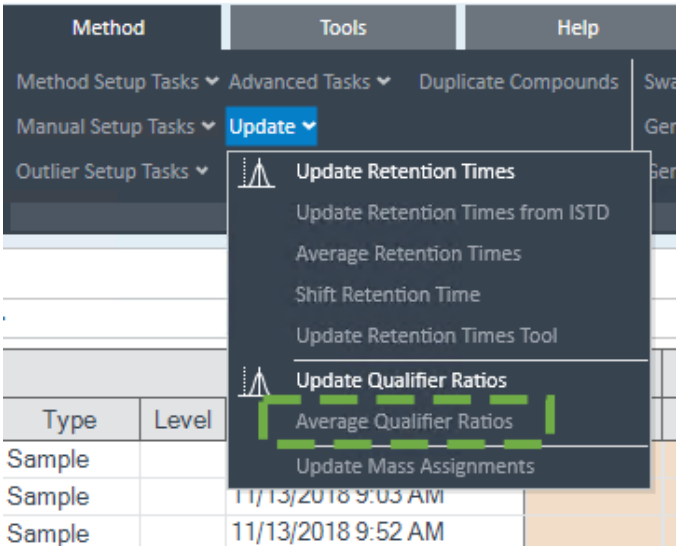


10 Review and correct integrations.

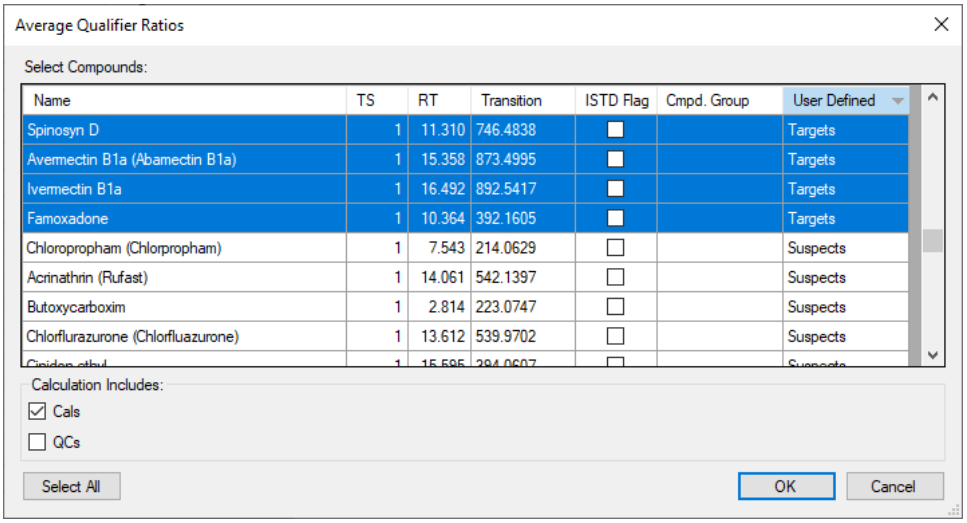
Update Target Qualifier Ratios

1 On the Method ribbon, select **Edit**.

2 Select **Update** > **Average Qualifier Ratios**.



3 Select the **User Defined** heading to sort the table by that column.



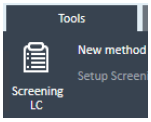
4 Highlight all Targets.

5 Select **Cals** under **Calculation Includes:**, then select **OK**.

6 Perform Rapid Bulk Data Review

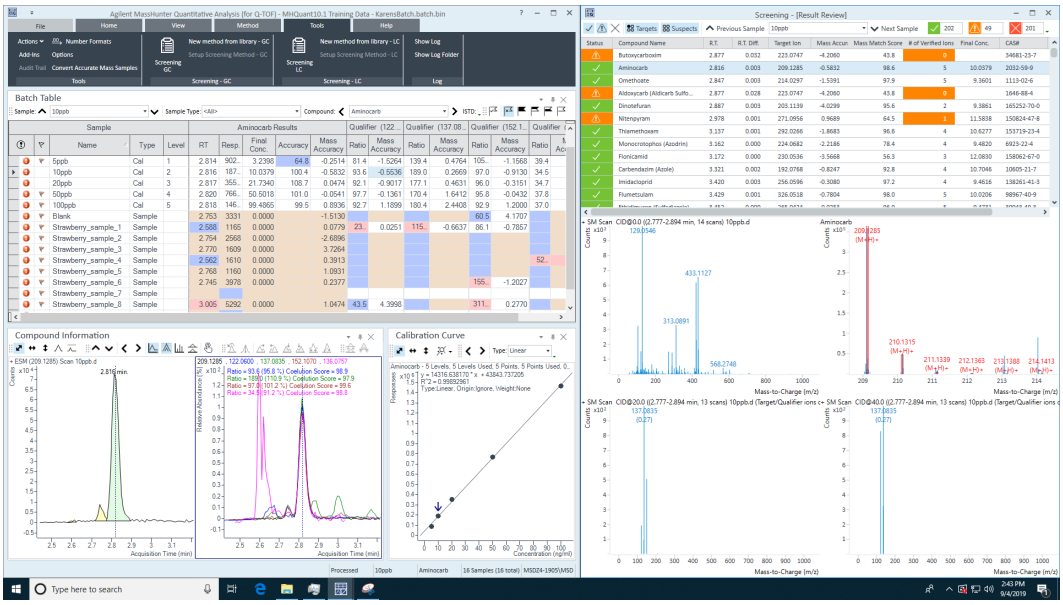
After analyzing the batch, perform data analysis in the traditional manner using Quant software (similar to MRM methods). However, when evaluating hundreds of analytes this can be time consuming and inefficient. Use the LC Screener for rapid data review based on the outlier parameters stated above.

1 Select **Tools** > **Screening LC** to navigate to the LC Screener.



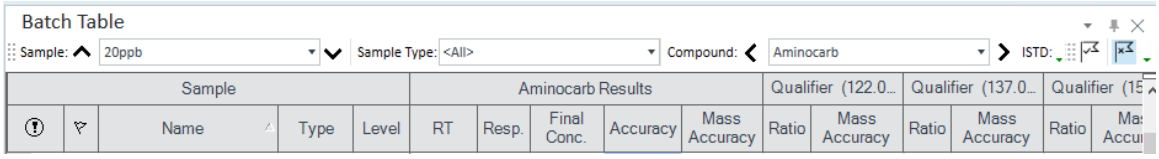
The LC Screener displays.

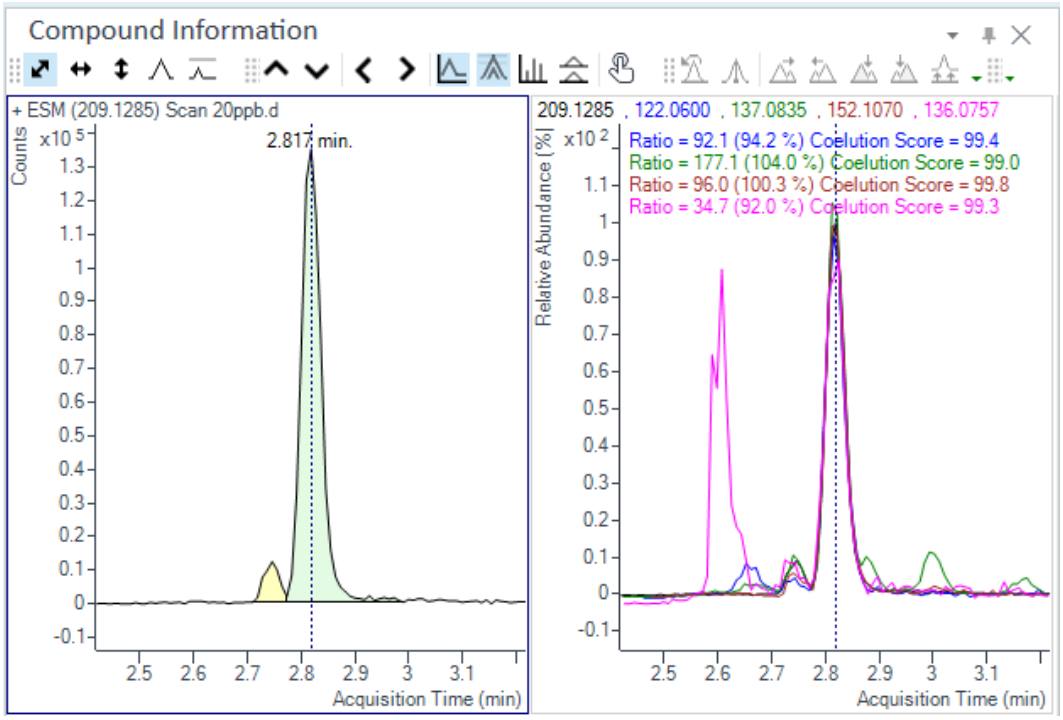
2 Adjust the screens as necessary. If only one monitor is available, Agilent recommends the split-screen feature in the classic Quant-My-Way UI screener tool, as shown below. Drag the Screener to the right side of the window until it automatically fills that space.



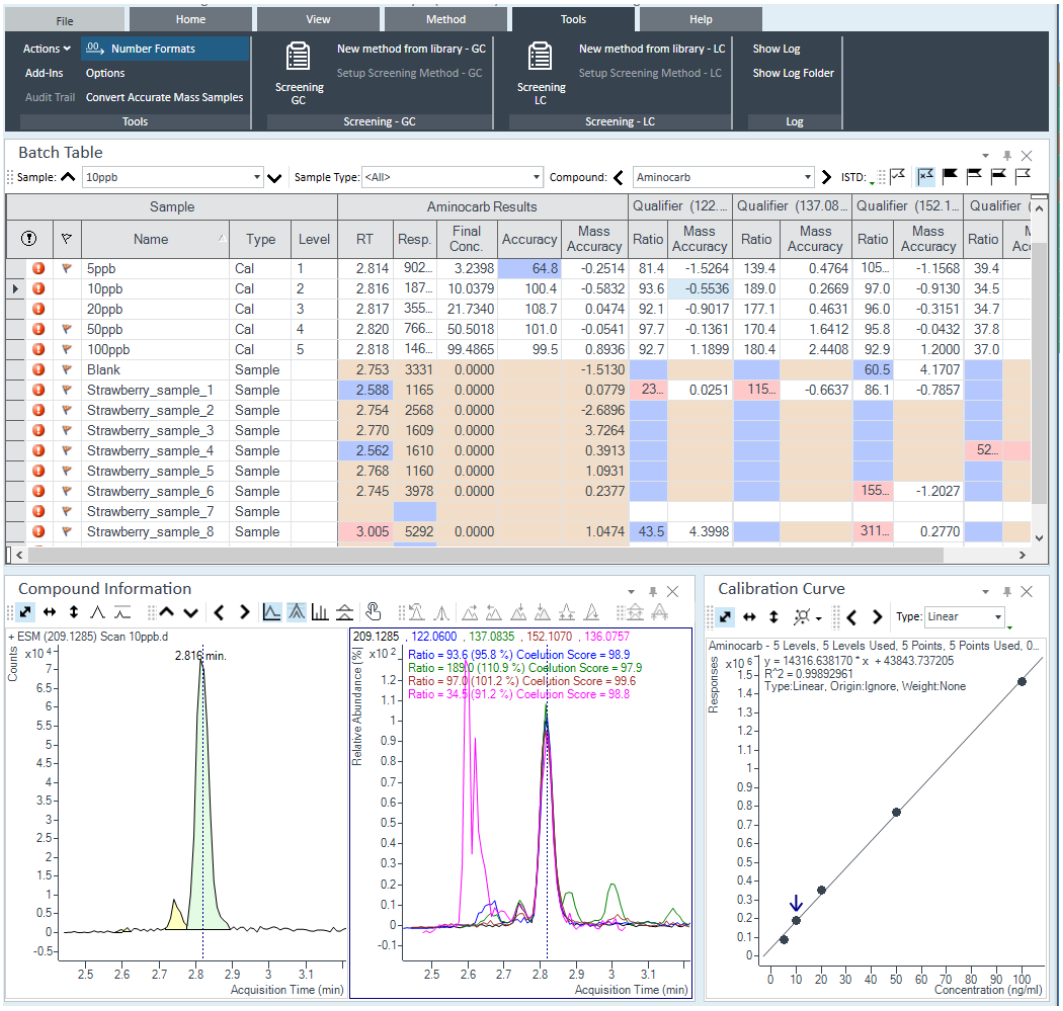
3 Adjust the information displayed in the Quant-My-Way user interface. This maximizes the pertinent information displayed in the Quant-My-Way UI and complements the LC screener information. Agilent recommends the following adjustments.

- Delete unnecessary columns.
- Add the mass accuracy column for the fragment ions.
- Add the fragment chromatograms to the **Compound Information**.
- Add the co-elution score to that plot. Right-click in the qualifier window, then select **Properties** > **Compound Information (2)**, then select **Show co-elution score**.

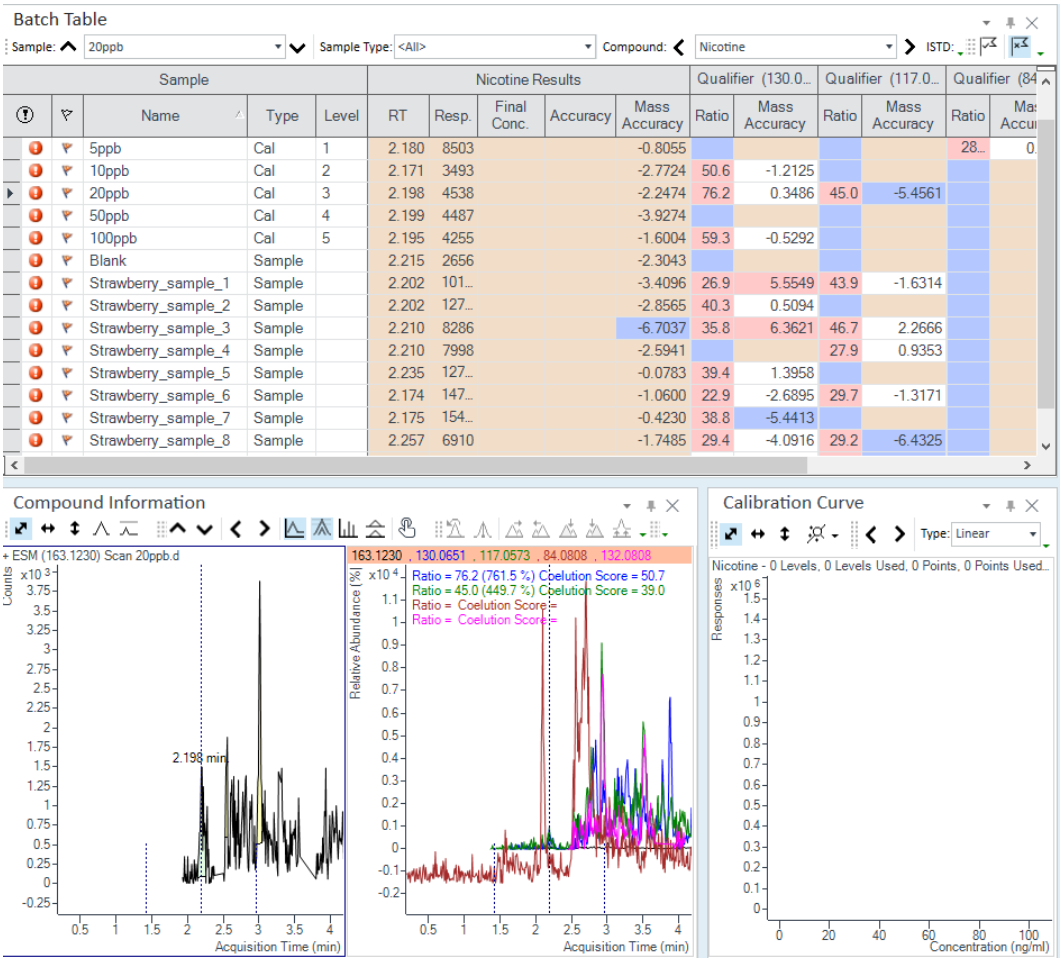




A Target analyte in the Quant-My-Way UI will have signal for the calibration spiked samples.



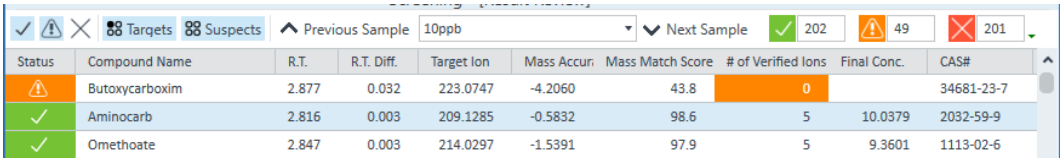
A suspect analyte does not have a signal appear in calibrators.



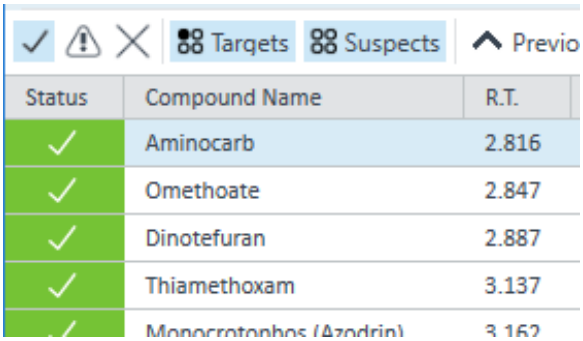
The Screener displays compounds in a sample and assigns them a status based on the outlier parameters set in the method. The green check means it passes all criteria, the orange means that the data needs review because there is an outlier in the criteria, and the red means that it is negative.

The Screener has five main screens. The top panel contains all the analytes and their status. When an analyte is selected in the Screener, it will display that analyte in the Quant-My-Way UI automatically. The middle left is an averaged spectrum of the full san (CE = 0) at the analytes RT, the middle right is the isotopic profile of the real data (blue) compared to the theoretical (red boxes). If a Pattern Reference Library was not created in the **LC Screener Workflows step 2**, this will not appear. The lower left and right are extracted precursor and fragment ions at different CE.

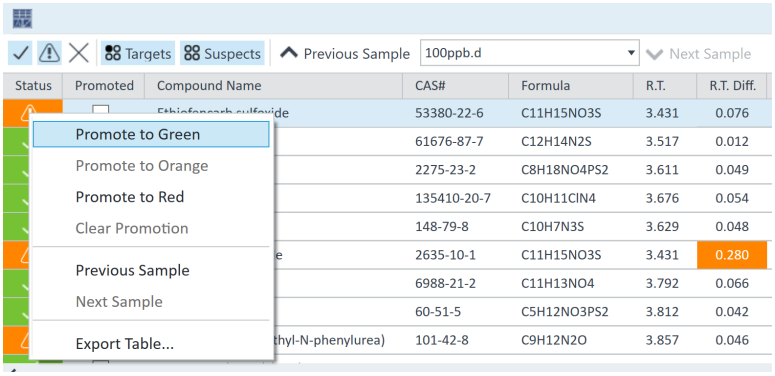
The columns in the screener can be moved around by dragging and dropping them in the table. These can also be used to sort data. When an analyte is flagged for review, the aspect of the data that is an outlier is also highlighted in orange.



The top bar allows for filtering based on if the analyte is positive or needs review (or negative). It also allows filtering based on Targets and Suspects. Targets are defined by this software as having a calibration curve or not.



A new feature available in Quant 11 allows for promotion of compounds to green, orange, or red through right-clicking. Right-click on the desired compound, then select **Promote to Green**, **Promote to Orange**, or **Promote to Red**. To clear the promotion, right-click the compound, then select **Clear Promotion**. This is useful when using either the ScreeningLC_Detailed or ScreeningLC_Summary PDF Report Builder templates. Only Green compounds are reported with these templates.



7 Optimize Methods with the Update Retention Time Tool

This analysis method is not optimized for fragments or qualifiers. Fragments from the PCDL were added, and it is assumed that in a complex matrix, one or more fragments will work well.

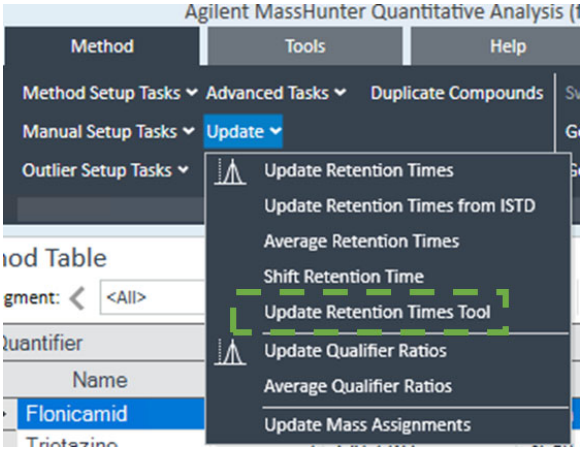
For routine deployment of a method, Agilent recommends that an R&D scientist optimize the method, keeping the best fragment(s) for a given method and matrix and removing all others. An optimized method is faster to process and easier to analyze than a method with many fragments. To optimize the method, delete fragments that are not working from a spiked sample.

For target analytes, a standard is required. Suspect analytes (where no standard exists) should have more than one fragment in the method.

The Retention Time Tool in the method editor has been updated to make visualizing the fragments easier.

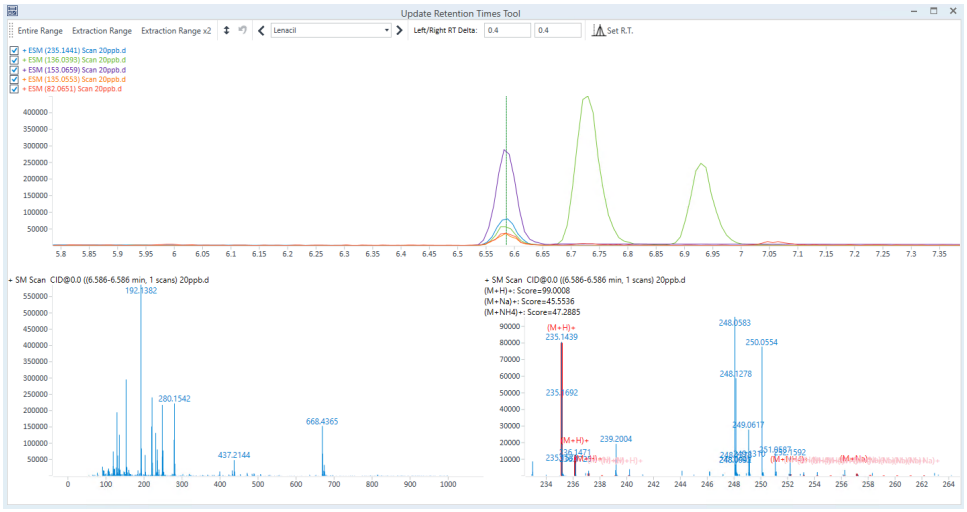
Delete fragments to optimize the method

1 In method editor window, select **Method** > **Update** > **Update Retention Times Tool**.

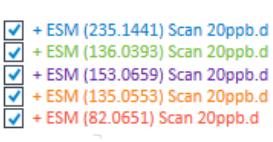


The Retention Times Tool displays in a new window. It shows the extracted chromatograms for the precursor and fragment ions in the top panel. The lower left panel has the full spectrum at the selected RT and the isotopic pattern in the lower right panel with the mass match score and theoretical isotopes. This is useful for adding confidence to the RT assignment of the analyte.

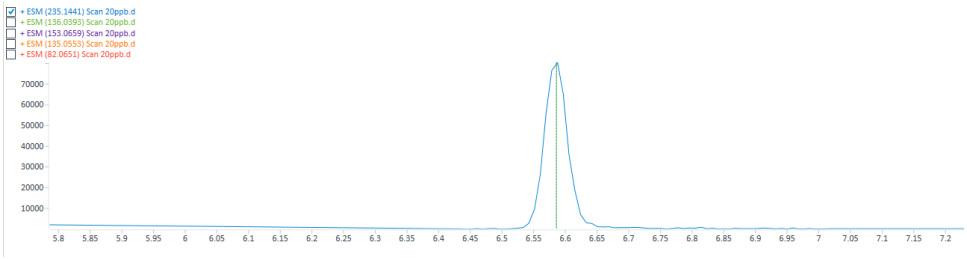
The top bar allows selection of the RT range displayed, the analyte, and the RT. It updates in the method. Lenacil is selected in this example.



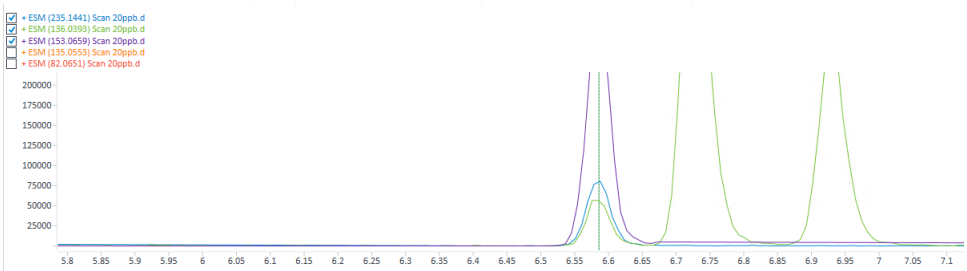
2 Use the top panel to select the ions displayed.



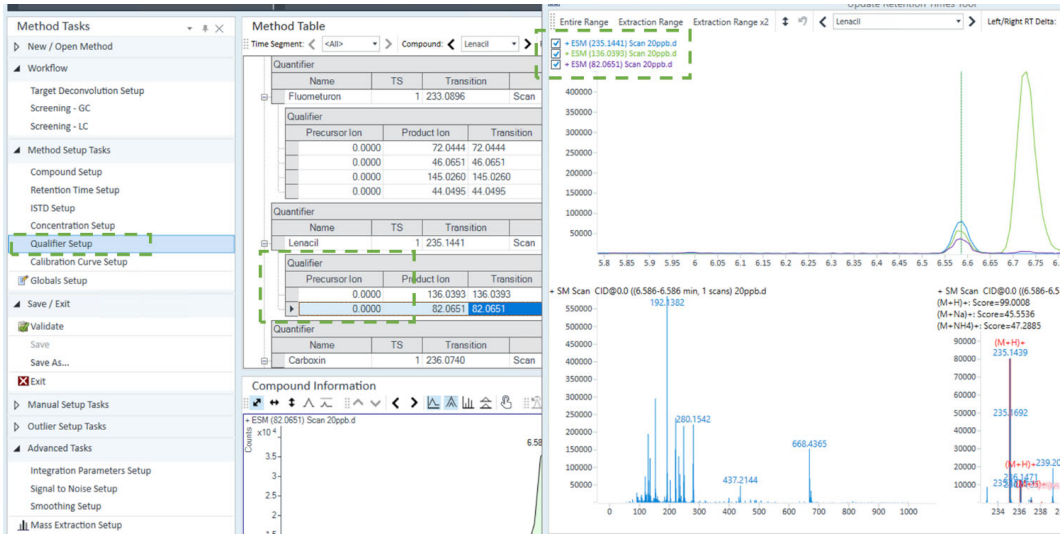
3 To display the precursor ion only, un-check all but the blue ion (precursor).



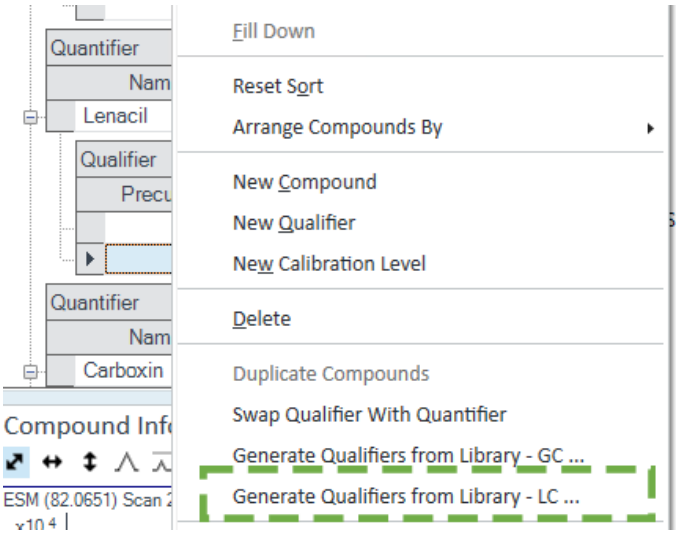
4 Select two fragments, such as green (m/z 136.0393) and purple (m/z 153.0659), to display. In this example, the purple ion has no nearby analytes and a stable signal. This is preferred to the green ion, which has nearby interferences.



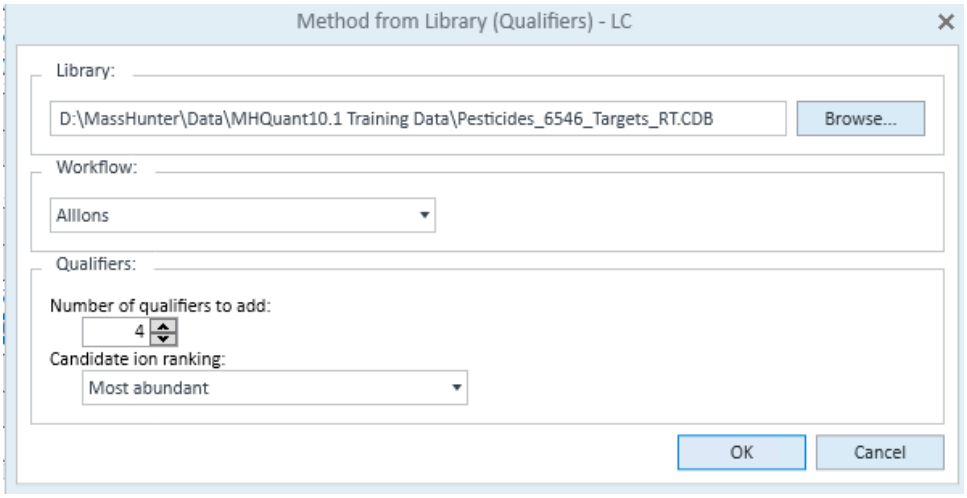
5 Under the Qualifier tab, delete the analytes that are not working well in the method.



6 If more fragments are needed, or if incorrect fragments are deleted, add them back in from the PCDL. Right-click the analyte and select **Generate Qualifiers from Library – LC**.



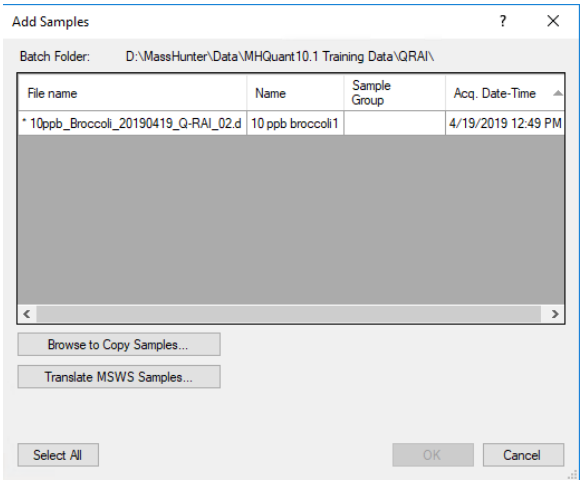
7 Add the PCDL to the table, then adjust the number of fragments.



8 Select **OK**.

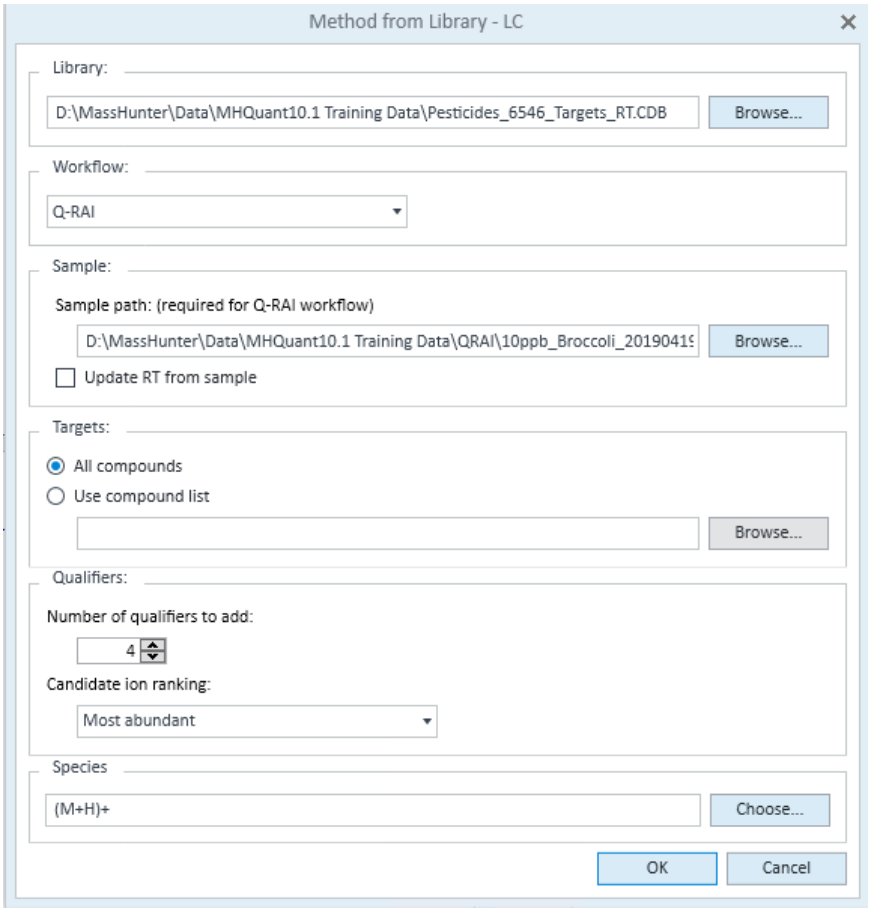
8 Build a Method with Q-RAI Data

1 Before starting, create a new batch and add samples as described in **Create a Batch of Samples**.

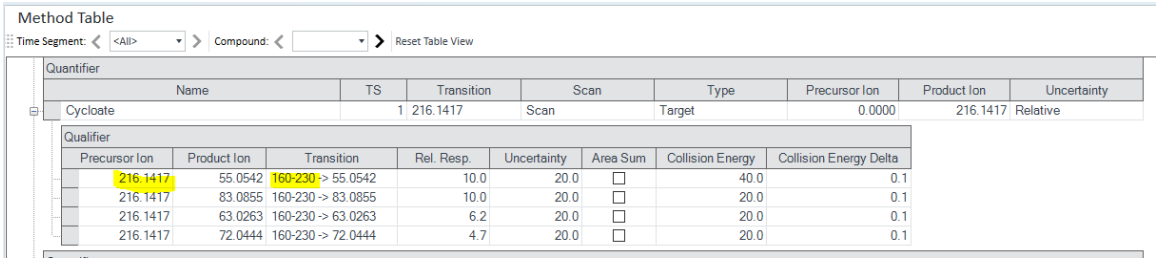


2 Build a targeted analysis method as described in **Build a Targeted Analysis Method from PCDL**.

The sample **Method from Library - LC** window below provides suggested parameters to set up the method. See **Build a Targeted Analysis Method from PCDL** for more information on selecting appropriate criteria.



The **Method Table** window below is an example of a Q-RAI data file. The **Precursor Ion** is imported from the PCLD, based on the species selected during method set up. The **Transition** shows the quadrupole bands set in the acquisition method.



Follow the above outlined sections to Create an LC Screener Method, apply it to the data, and analyze the data using the LC Screener.

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