



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

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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Analysis LC/Q-TOF Screener program and compatible MassHunter Quantitative Analysis 10.1 and higher, programs, until superseded.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
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Operating Temperature

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

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1

Create a Batch of Samples

- 1 Open MassHunter Q-TOF Quantitative Analysis 11.

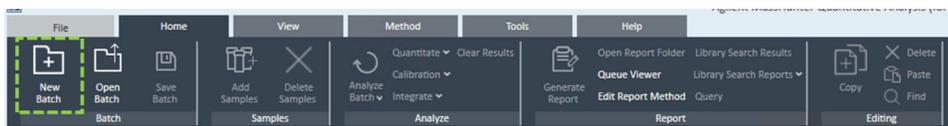


- 2 Select the Project containing the data and PCDL(s).



- 3 Create a new batch of data.

a Select **New Batch**.

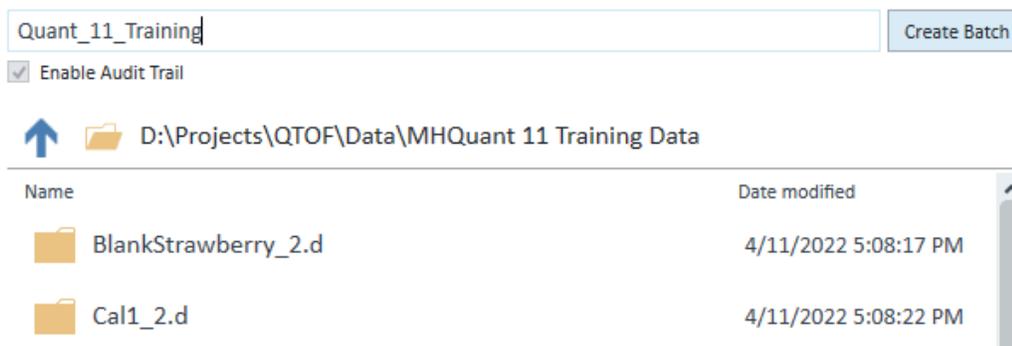


- b** Navigate to the folder that contains the data files (X:\Projects\QTOF\Data\MHQuant 11 Training Data).

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

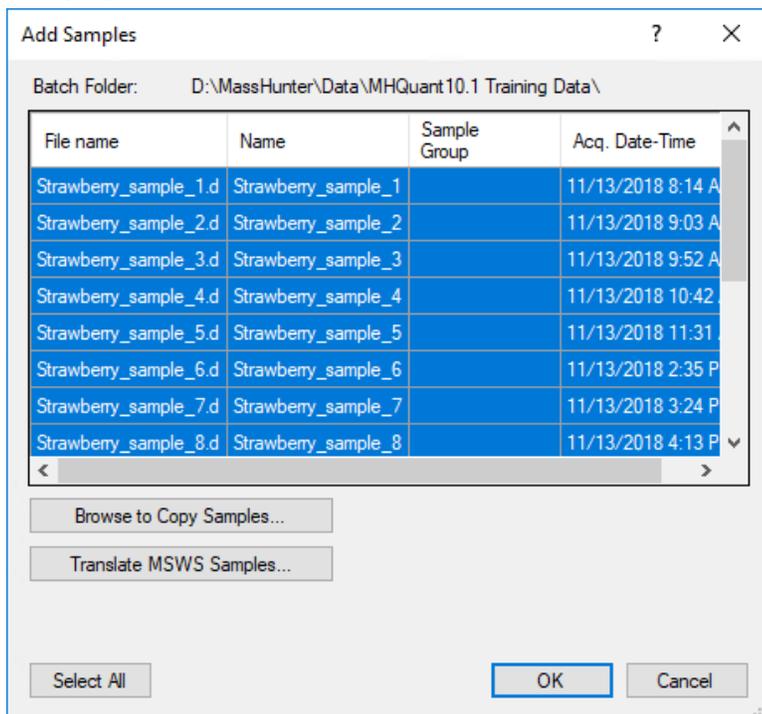
- c** Enter a batch file name.

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.

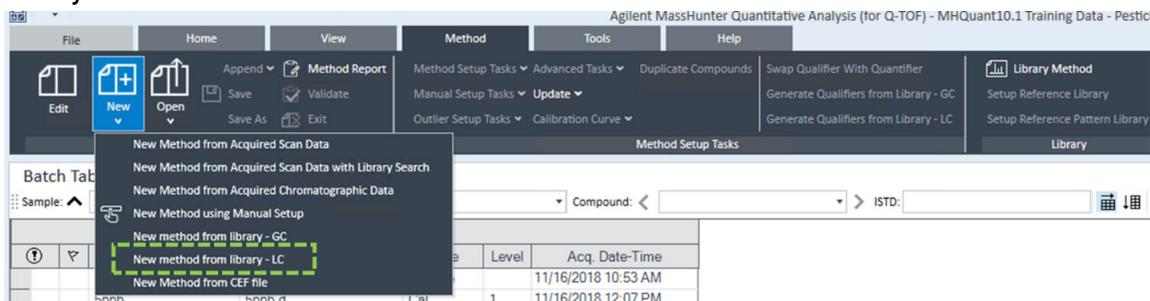
The data displays in the **Batch Table**.

Batch Table						
Sample: 50ppb		Sample Type: <All>		Compound: <		
Sample						
?	▼	Name ▲	Data File	Type	Level	Acq. Date-Time
		1ppb	1ppb.d	Sample		11/16/2018 10:53 AM
		5ppb	5ppb.d	Cal	1	11/16/2018 12:07 PM
		10ppb	10ppb.d	Cal	2	11/16/2018 1:20 PM
		20ppb	20ppb.d	Cal	3	11/16/2018 3:55 PM
		50ppb	50ppb.d	Cal	4	11/16/2018 5:09 PM
		100ppb	100ppb.d	Cal	5	11/16/2018 6:23 PM
		Blank	Blank.d	Sample		11/16/2018 6:19 AM
		Strawberry_sample_1	Strawberry_sample_1.d	Sample		11/13/2018 8:14 AM
		Strawberry_sample_2	Strawberry_sample_2.d	Sample		11/13/2018 9:03 AM
		Strawberry_sample_3	Strawberry_sample_3.d	Sample		11/13/2018 9:52 AM
		Strawberry_sample_4	Strawberry_sample_4.d	Sample		11/13/2018 10:42 AM
		Strawberry_sample_5	Strawberry_sample_5.d	Sample		11/13/2018 11:31 AM
		Strawberry_sample_6	Strawberry_sample_6.d	Sample		11/13/2018 2:35 PM
		Strawberry_sample_7	Strawberry_sample_7.d	Sample		11/13/2018 3:24 PM
		Strawberry_sample_8	Strawberry_sample_8.d	Sample		11/13/2018 4:13 PM
		Strawberry_sample_9	Strawberry_sample_9.d	Sample		11/13/2018 5:02 PM

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.

The 'Method from Library - LC' dialog box is shown. It contains the following fields and options:

- Library:** A text input field with a 'Browse...' button.
- Workflow:** A dropdown menu set to 'MS only'.
- Sample:** A section with a 'Sample path: (required for Q-RAI workflow)' text input field and a 'Browse...' button. Below it is an unchecked checkbox labeled 'Update RT from sample'.
- Targets:** Two radio buttons: 'All compounds' (selected) and 'Use compound list'. Below is a text input field and a 'Browse...' button.
- Qualifiers:** A section with a 'Number of qualifiers to add:' spin box set to '0'. Below it is a 'Candidate ion ranking:' dropdown menu set to 'Most abundant'.
- Species:** A text input field and a 'Choose...' button.

At the bottom of the dialog are 'OK' and 'Cancel' buttons.

- 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 ($MZ \times MZ \times Abundance$).
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.

Build a Targeted Analysis Method from PCDL

Create a new method from PCDL

- 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+NH₄ 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).

Method from Library - LC

Library:

Workflow:

Sample:

Update RT from sample

Targets: All compounds Use compound list

Qualifiers:

Species

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.

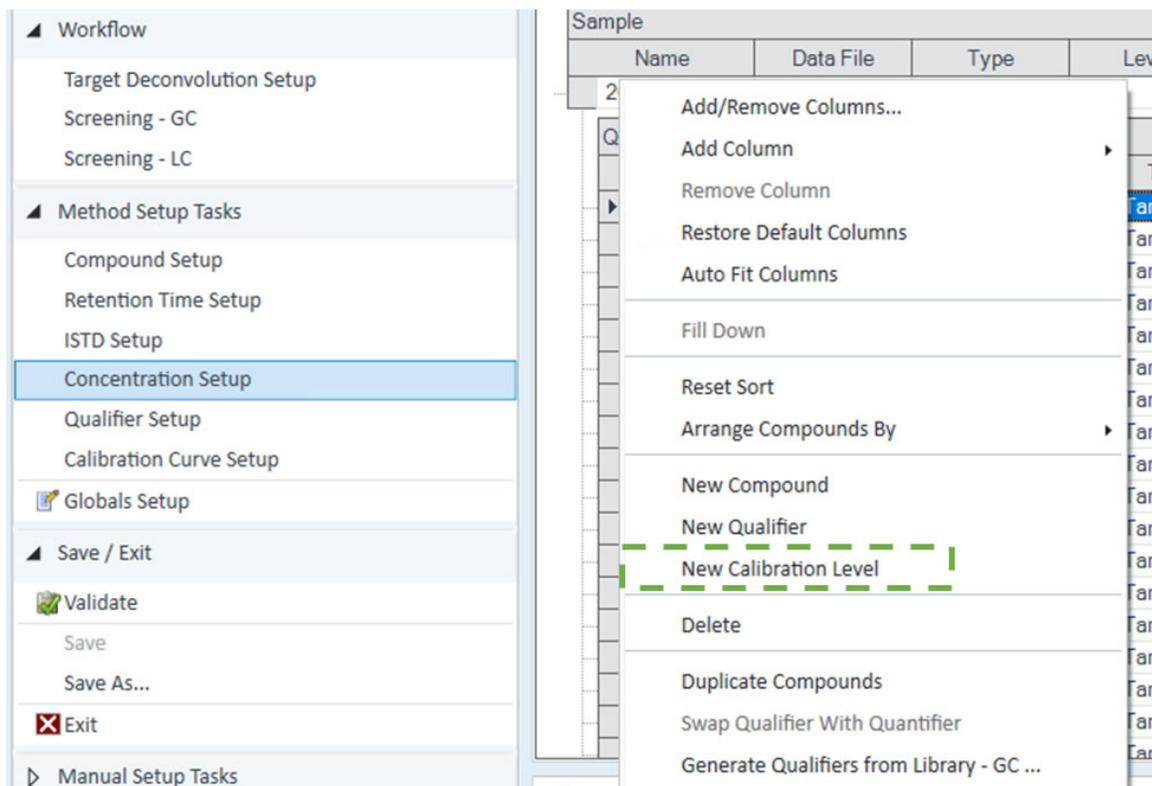
c In the new column, label all analytes **Targets**.

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).

Quantifier					
Name	TS	Transition	Scan	Type	
Methamidop...	1	142.0086	Scan	Target	ng

Calibration			
Level	Conc.	Response	Enable
1	5.0000		<input checked="" type="checkbox"/>
2	10.0000		<input checked="" type="checkbox"/>
3	20.0000		<input checked="" type="checkbox"/>
4	50.0000		<input checked="" type="checkbox"/>
5	100.0000		<input checked="" type="checkbox"/>

Build a Targeted Analysis Method from PCDL

Add Calibration Curves for Target Analytes

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

The screenshot shows a software window with a table of calibration levels. The table has columns for 'Level' and 'Conc.'. Level 3 is selected. A context menu is open over the table, and the option 'Copy Calibration Levels To...' is highlighted with a dashed green box.

Level	Conc.
1	5.0
2	10.0
3	20.0
4	50.0
5	100.0

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

The screenshot shows a dialog box titled 'Copy Calibration Levels To'. It contains a table with columns: Name, TS, RT, Transition, ISTD Flag, Cmpd. Group, and User Defined. All rows are selected. The 'Select All' button is highlighted.

Name	TS	RT	Transition	ISTD Flag	Cmpd. Group	User Defined
Methomyl	1	3.143	163.0536	<input type="checkbox"/>		Targets
Fenuron (N,N-Dimethyl-N-phenylurea)	1	3.857	165.1022	<input type="checkbox"/>		Targets
IPC / Propham	1	6.216	180.1019	<input type="checkbox"/>		Targets
Acephate	1	2.816	184.0192	<input type="checkbox"/>		Targets
Fuberidazole	1	3.783	185.0709	<input type="checkbox"/>		Targets
Molinate	1	8.667	188.1104	<input type="checkbox"/>		Targets
Propamocarb	1	2.809	189.1598	<input type="checkbox"/>		Targets

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

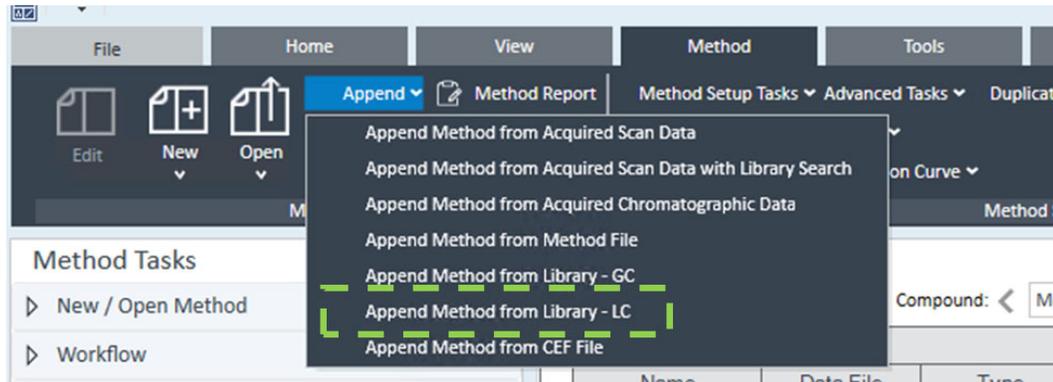
- 8 Make the following selections, as appropriate.

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

Quantifier	Name	TS	Transition	Scan	Type	CF	CF Origin	CF Weight	User Defined
	Methamidop...	1	142.0086	Scan	Target	Linear	Ignore	1/x	Targets
	Methomyl	1	163.0536	Scan	Target	Linear	Ignore	1/x	Targets
	Fenuron (N...	1	165.1022	Scan	Target	Linear	Ignore	1/x	Targets
	IPC / Propha...	1	180.1019	Scan	Target	Linear	Ignore	1/x	Targets

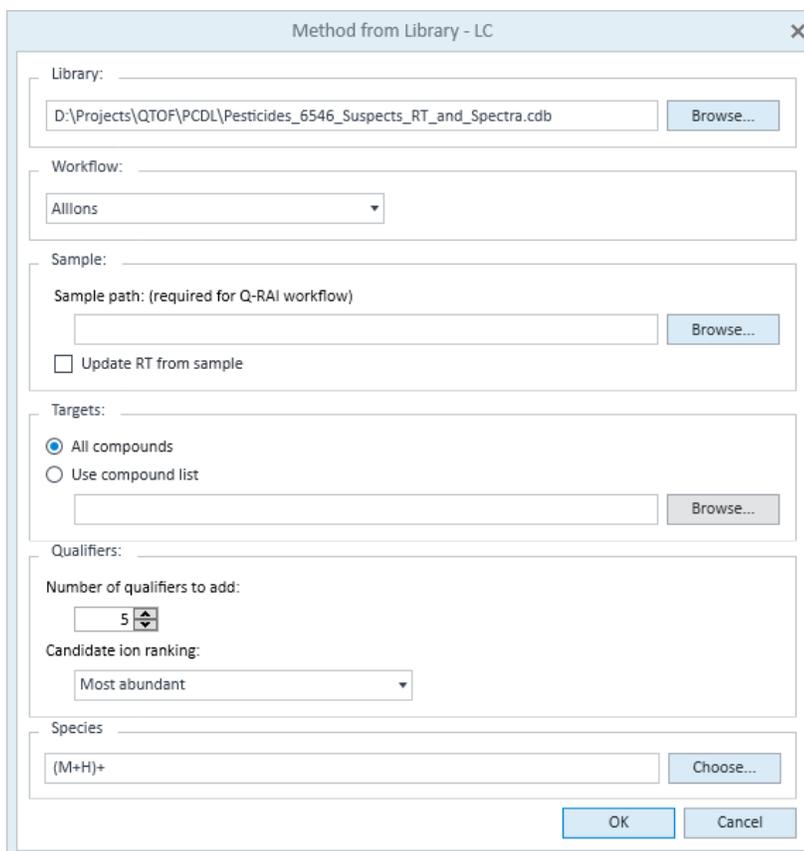
Append the method to add the suspect analytes

- 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.

A screenshot of the 'Method from Library - LC' dialog box. The 'Library' field contains 'D:\Projects\QTOF\PCDL\Pesticides_6546_Suspects_RT_and_Spectra.cdb'. The 'Workflow' dropdown is set to 'Allions'. The 'Sample' section has an empty 'Sample path' field and the 'Update RT from sample' checkbox is unchecked. Under 'Targets', the 'All compounds' radio button is selected. The 'Qualifiers' section has 'Number of qualifiers to add' set to 5 and 'Candidate ion ranking' set to 'Most abundant'. The 'Species' field contains '(M+H)+'. 'OK' and 'Cancel' buttons are at the bottom.

Build a Targeted Analysis Method from PCDL

Add Calibration Curves for Target 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	Q	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
Acinathrin (...)	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

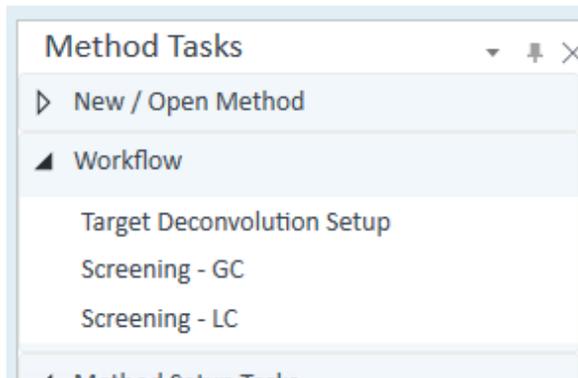
Create an LC Screener Method

LC Screener Workflows

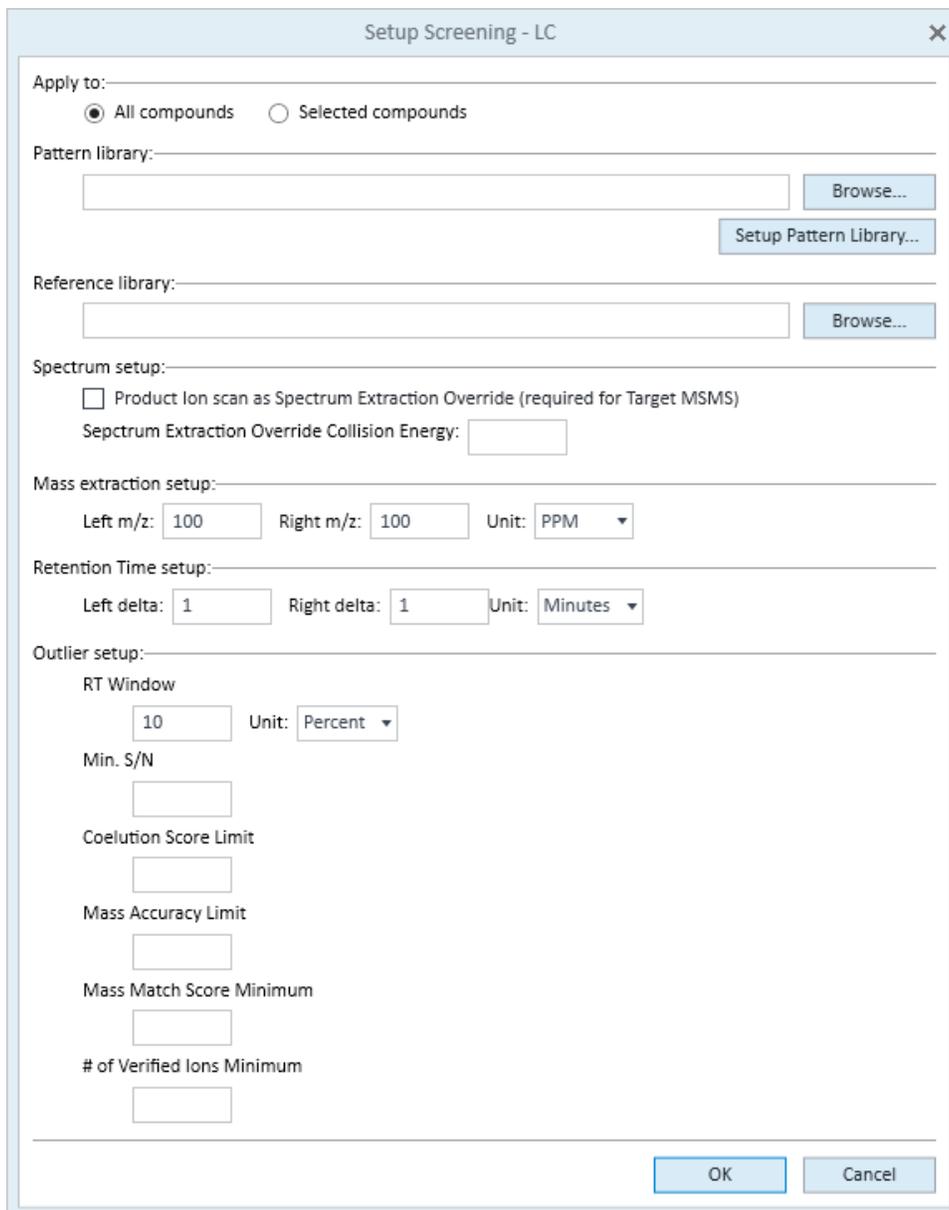
Build an LC screening method by adding parameters for extracting the analytes, analyzing the data, and flagging outliers.

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

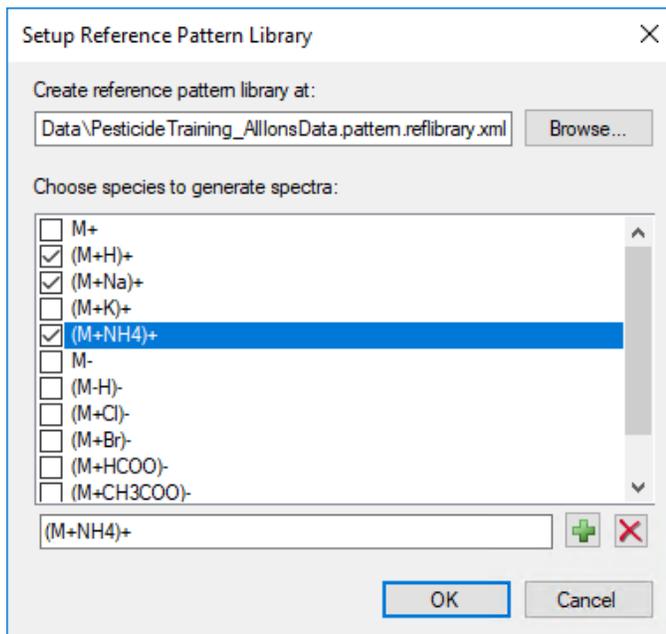
- 1 Within Method Tasks, select **Workflow > Screening - LC**.



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

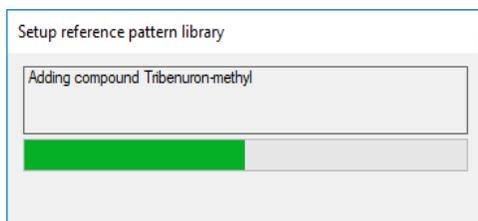
A screenshot of the 'Setup Screening - LC' dialog box. The dialog has a title bar with 'Setup Screening - LC' and a close button. The main area contains several sections with input fields and buttons. The 'Apply to:' section has two radio buttons: 'All compounds' (selected) and 'Selected compounds'. The 'Pattern library:' section has a text box and a 'Browse...' button, with a 'Setup Pattern Library...' button below it. The 'Reference library:' section has a text box and a 'Browse...' button. The 'Spectrum setup:' section has a checkbox for 'Product Ion scan as Spectrum Extraction Override (required for Target MSMS)' and a text box for 'Spectrum Extraction Override Collision Energy:'. The 'Mass extraction setup:' section has text boxes for 'Left m/z: 100', 'Right m/z: 100', and a dropdown for 'Unit: PPM'. The 'Retention Time setup:' section has text boxes for 'Left delta: 1', 'Right delta: 1', and a dropdown for 'Unit: Minutes'. The 'Outlier setup:' section has a text box for 'RT Window: 10' and a dropdown for 'Unit: Percent', followed by text boxes for 'Min. S/N', 'Coelution Score Limit', 'Mass Accuracy Limit', 'Mass Match Score Minimum', and '# of Verified Ions Minimum'. At the bottom right are 'OK' and 'Cancel' buttons.

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

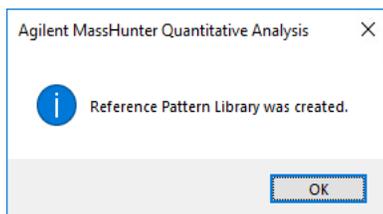


- 3 Select **OK**.

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**.

Setup Screening - LC

Apply to: All compounds Selected compounds

Pattern library: D:\Projects\QTOF\Data\MHQuant 11 Training Data\Demo.pattern.reflibrary.xml

Reference library:

Spectrum setup: Product Ion scan as Spectrum Extraction Override (required for Target MSMS)
Spectrum Extraction Override Collision Energy:

Mass extraction setup: Left m/z: Right m/z: Unit:

Retention Time setup: Left delta: Right delta: Unit:

Outlier setup:

RT Window: Unit:

Min. S/N:

Coelution Score Limit:

Mass Accuracy Limit:

Mass Match Score Minimum:

of Verified Ions Minimum:

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

Create an LC Screener Method

LC Screener Workflows

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

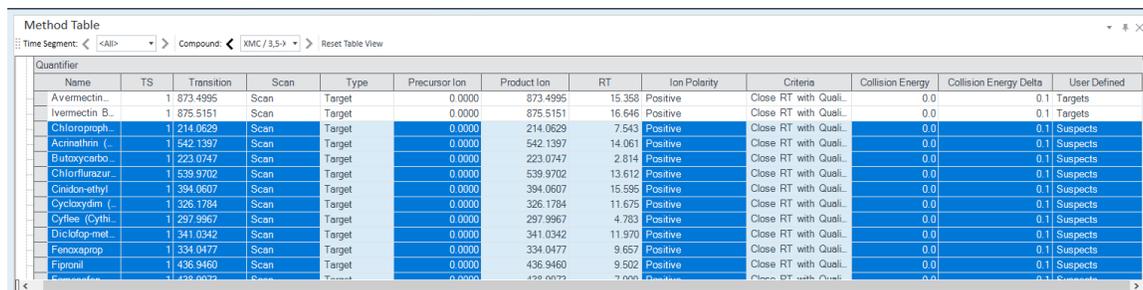
Parameter	Setting
Left delta	0.4
Right delta	0.4
Unit	Minutes

- 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.



The screenshot shows the 'Method Table' window with a table of compounds. The table has columns for Name, TS, Transition, Scan, Type, Precursor Ion, Product Ion, RT, Ion Polarity, Criteria, Collision Energy, Collision Energy Delta, and User Defined. The 'User Defined' column shows 'Targets' for Ivermectin B. and 'Suspects' for Chloroproth., Acimathrin, Butoxycarbo., Chlorfluazur., Cindori-ethyl, Cycloxydim, Gylise (Cyt), Diclifop-met., Fenoxaprop, Fipronil, and Fenoxaprop. The 'Criteria' column for all rows is 'Close RT with Quali..'. The 'Collision Energy' and 'Collision Energy Delta' are both 0.0 for all rows.

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 Quali..	0.0	0.0	0.1	Targets
Ivermectin B...	1 875.5151	Scan	Target	0.0000	875.5151	16.646	Positive	Close RT with Quali..	0.0	0.0	0.1	Targets
Chloroproth...	1 214.0629	Scan	Target	0.0000	214.0629	7.543	Positive	Close RT with Quali..	0.0	0.0	0.1	Suspects
Acimathrin (...)	1 542.1397	Scan	Target	0.0000	542.1397	14.061	Positive	Close RT with Quali..	0.0	0.0	0.1	Suspects
Butoxycarbo...	1 223.0747	Scan	Target	0.0000	223.0747	2.814	Positive	Close RT with Quali..	0.0	0.0	0.1	Suspects
Chlorfluazur...	1 539.9702	Scan	Target	0.0000	539.9702	13.612	Positive	Close RT with Quali..	0.0	0.0	0.1	Suspects
Cindori-ethyl	1 394.0607	Scan	Target	0.0000	394.0607	15.595	Positive	Close RT with Quali..	0.0	0.0	0.1	Suspects
Cycloxydim (...)	1 326.1784	Scan	Target	0.0000	326.1784	11.675	Positive	Close RT with Quali..	0.0	0.0	0.1	Suspects
Gylise (Cyt)	1 297.5967	Scan	Target	0.0000	297.5967	4.783	Positive	Close RT with Quali..	0.0	0.0	0.1	Suspects
Diclifop-met...	1 341.0342	Scan	Target	0.0000	341.0342	11.970	Positive	Close RT with Quali..	0.0	0.0	0.1	Suspects
Fenoxaprop	1 334.0477	Scan	Target	0.0000	334.0477	9.657	Positive	Close RT with Quali..	0.0	0.0	0.1	Suspects
Fipronil	1 436.9460	Scan	Target	0.0000	436.9460	9.502	Positive	Close RT with Quali..	0.0	0.0	0.1	Suspects
Fenoxaprop...	1 438.2022	Scan	Target	0.0000	438.2022	7.000	Positive	Close RT with Quali..	0.0	0.0	0.1	Suspects

- 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**.

Setup Screening - LC

Apply to: All compounds Selected compounds

Pattern library:

Reference library:

Spectrum setup: Product Ion scan as Spectrum Extraction Override (required for Target MSMS)
Spectrum Extraction Override Collision Energy:

Mass extraction setup: Left m/z: Right m/z: Unit:

Retention Time setup: Left delta: Right delta: Unit:

Outlier setup:

RT Window: Unit:

Min. S/N:

Coelution Score Limit:

Mass Accuracy Limit:

Mass Match Score Minimum:

of Verified Ions Minimum:

Enable SureMass

- 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.

The screenshot displays the software interface for configuring a method. On the left, a navigation pane shows the following menu items:

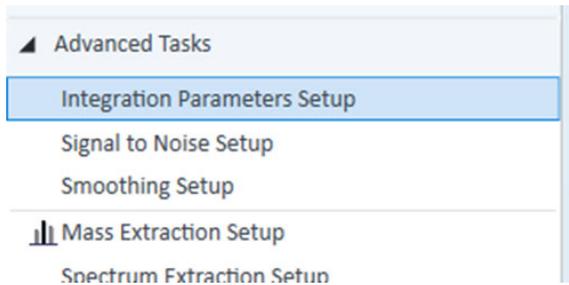
- Target Deconvolution Setup
- Screening - GC
- Screening - LC
- Method Setup Tasks (expanded)
- Compound Setup
- Retention Time Setup
- ISTD Setup
- Concentration Setup
- Qualifier Setup
- Calibration Curve Setup
- Globals Setup (selected)
- Save / Exit
- Validate
- Save
- Save As...
- Exit

The main window shows the 'Global Setup' configuration for a method named '20ppb.d'. The 'Globals' section is expanded, showing the following settings:

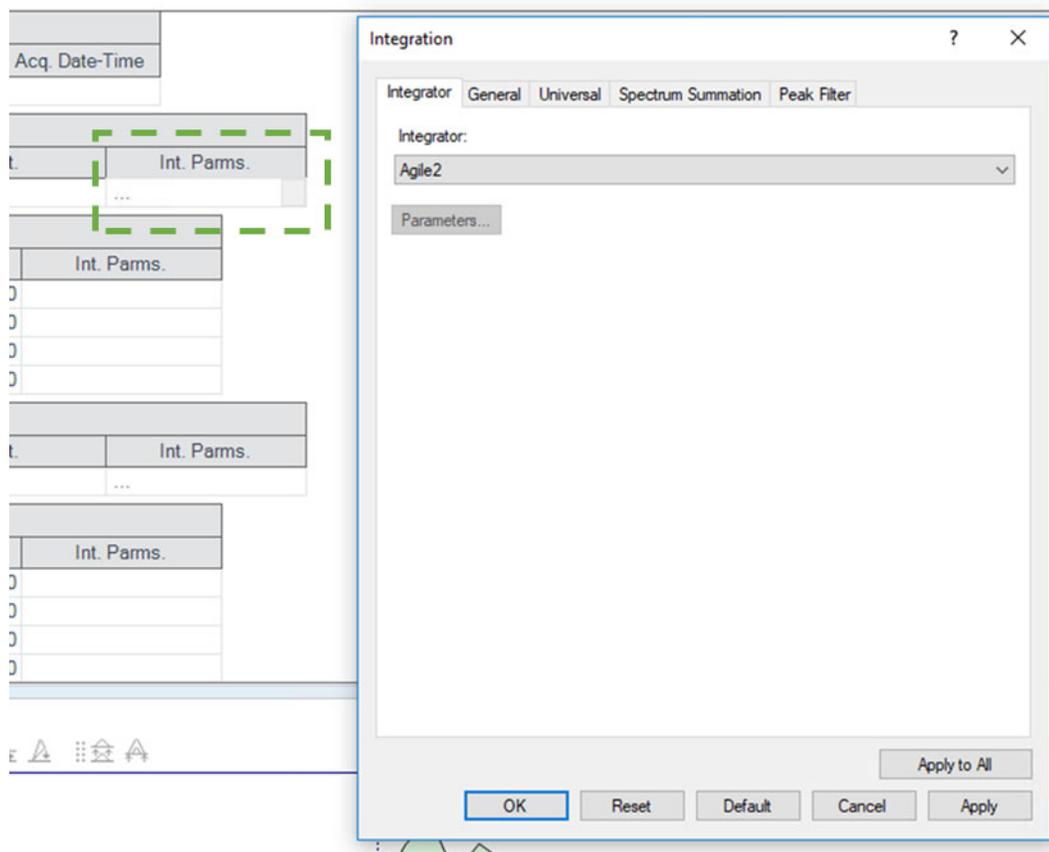
Parameter	Value	Checkbox
Apply Multiplier to Matrix Spike		<input checked="" type="checkbox"/>
Apply Multiplier to Surrogate		<input checked="" type="checkbox"/>
Apply Multiplier to Target		<input checked="" type="checkbox"/>
Bracketing Type	None	
Correlation Window	0.100	
Ignore Peaks Not Found		<input type="checkbox"/>
Library Method		
Non Reference Window	70.000	
Non Reference Window Type	Percent	
Reference Library		
Reference Pattern Library	D:\MassHunter\Data\MH_ata.pattern.reflibrary.xml	
Reference Window	80.000	
Reference Window Type	Percent	
Relative ISTD		<input type="checkbox"/>
Standard Addition		<input type="checkbox"/>
SureMass		<input checked="" type="checkbox"/>
Use Profile Data		<input type="checkbox"/>

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**.

The screenshot shows the 'Integration' dialog box with the 'Peak Filter' tab selected. The 'Peak Threshold' section has five radio button options: 'Peak Area (counts)' (selected), 'Peak Height (counts)', 'Peak Area (%)', 'Peak Height (%)', and 'Signal to Noise'. The 'Peak Area (counts)' option has a value of '1000' in its input field. The 'Peak Height (counts)' option has a value of '10000'. The 'Peak Area (%)' option has a value of '5'. The 'Peak Height (%)' option has a value of '5'. The 'Signal to Noise' option has a value of '3'. The 'Maximum number of peaks' section has a checkbox labeled 'Limit to the largest' which is unchecked, and an input field with the value '100'. At the bottom of the dialog, there are buttons for 'OK', 'Reset', 'Default', 'Cancel', 'Apply', and 'Apply to All'.

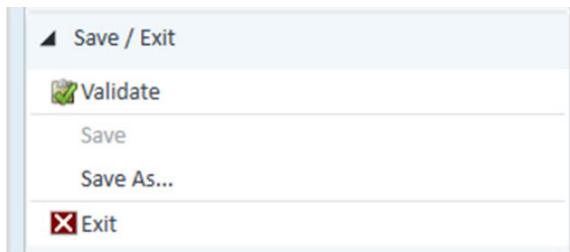
- 4 Select **Apply to All**, then select **OK**.

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.

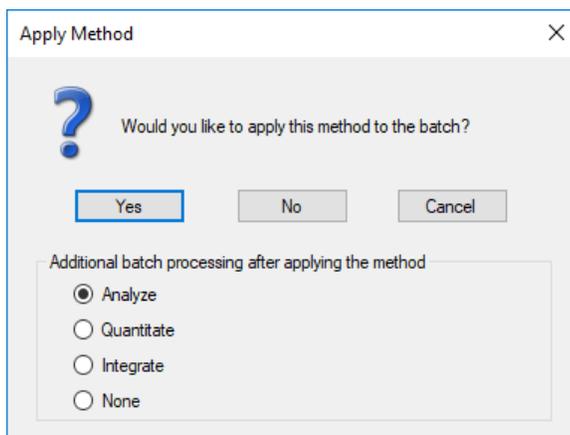
4

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.

Setup Graphics

Samples | **Compounds** | Organize | Outliers

Compounds:

Name	Type	Transition	RT	Cmpd. Group	ID	User Defined
Nicotine	Target	163.1230	2.193			Suspects
Cyromazine	Target	167.1040	2.363			Suspects
Butocarboxim...	Target	207.0798	2.661			Suspects
Aldicarb sulfo...	Target	207.0798	2.779			Suspects
Butoxycarbox...	Target	223.0747	2.814			Suspects
Aldoxycarb (...)	Target	223.0747	2.867			Suspects
Demeton-S-m...	Target	247.0222	2.958			Suspects
Demeton-S-m...	Target	263.0171	3.102			Suspects
Dicrotophos (...)	Target	238.0839	3.394			Suspects
Desmethyl-pir...	Target	225.1346	3.466			Suspects

Add > Add All >> < Remove << Remove All

Compounds shown in this order:

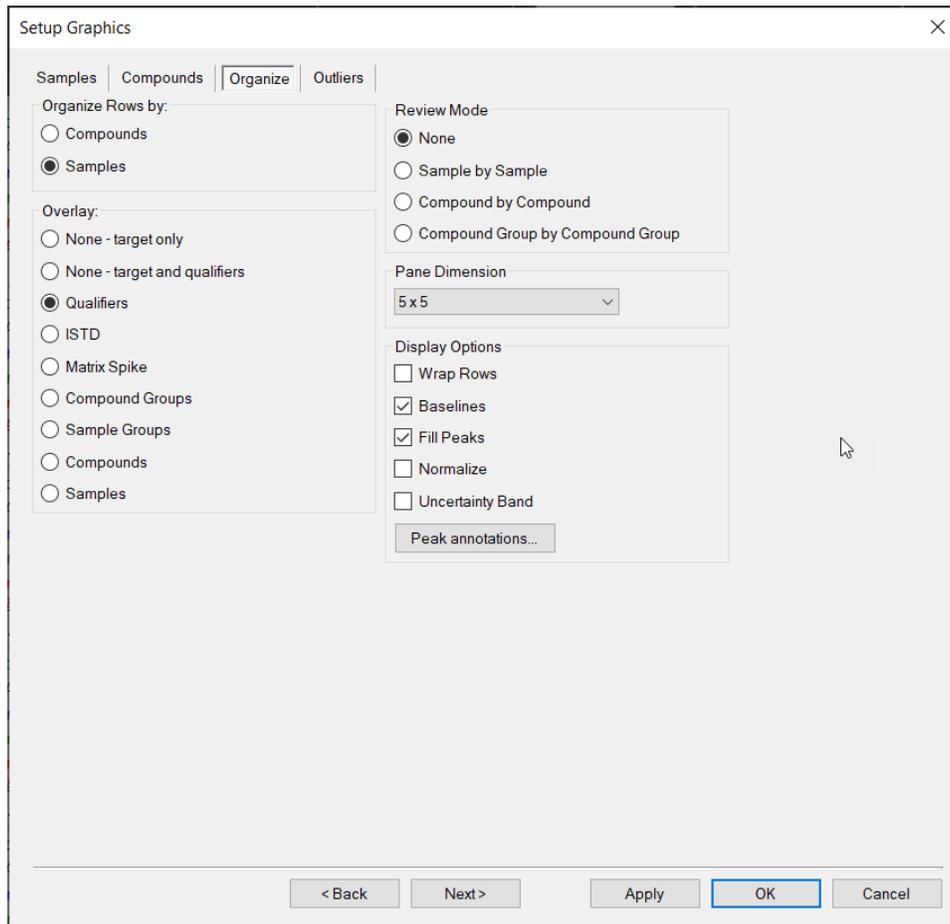
Name	Type	Transition	RT	Cmpd. Group	ID	User Defined
Dioxacarb	Target	224.0917	3.743			Targets
Diniconazole(I)	Target	326.0821	11.181			Targets
Benzoximate	Target	364.0946	10.933			Targets
Trichlorfon (D...	Target	256.9299	3.837			Targets
Pirimiphos-m...	Target	306.1036	10.925			Targets
Metrafenone	Target	409.0645	11.073			Targets
Metamitron	Target	203.0927	3.847			Targets
Dimethoate	Target	230.0069	3.770			Targets
Tolclofos-met...	Target	300.9616	10.875			Targets

Move Up Move Down

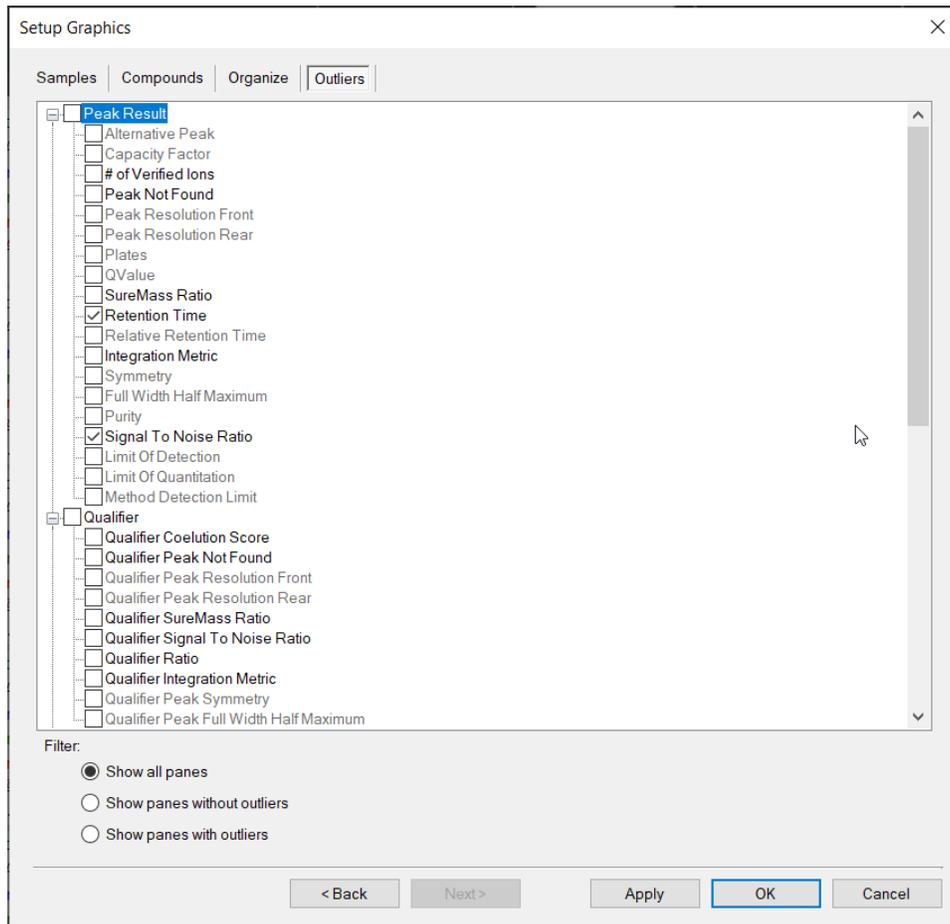
< Back Next > Apply OK Cancel

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

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



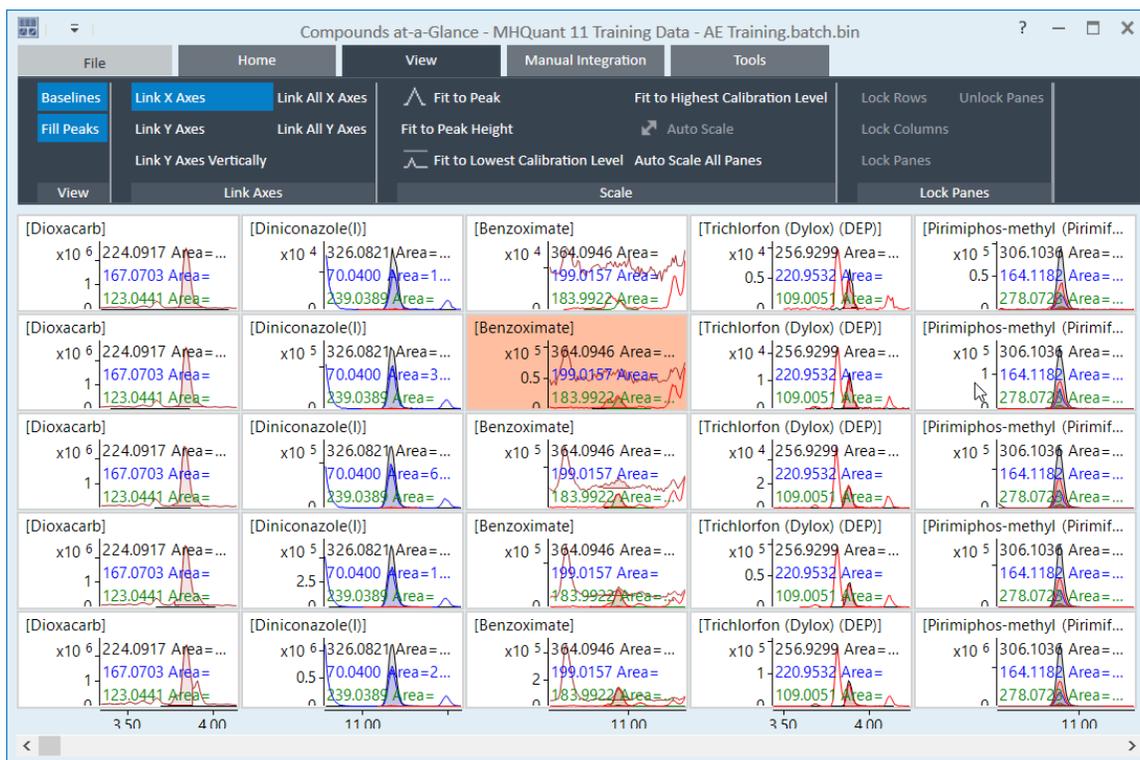
8 Select **Retention Time** and **Signal To Noise Ratio** on the **Outliers** tab, then select **OK**.



Analyze Data Using the LC Screener

Update Target Qualifier Ratios

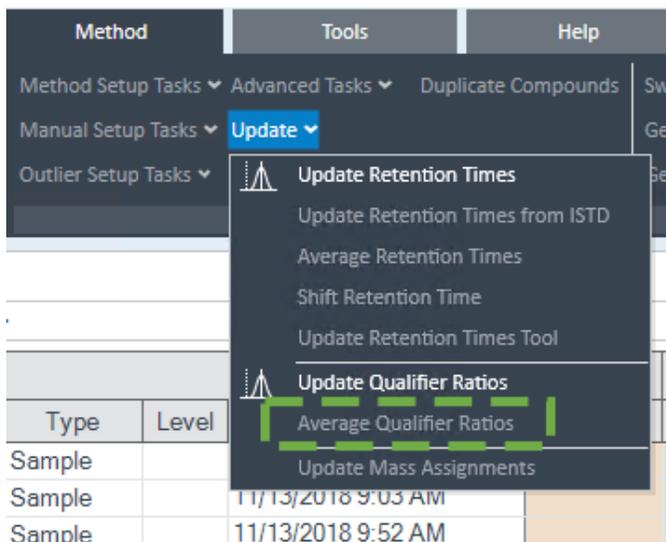
- On the **View** ribbon, select **Link X Axes**.



- Review and correct integrations.

Update Target Qualifier Ratios

- On the Method ribbon, select **Edit**.
- Select **Update > Average Qualifier Ratios**.



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

Average Qualifier Ratios

Select Compounds:

Name	TS	RT	Transition	ISTD Flag	Cmpd. Group	User Defined
Spinosyn D	1	11.310	746.4838	<input type="checkbox"/>		Targets
Avermectin B1a (Abamectin B1a)	1	15.358	873.4995	<input type="checkbox"/>		Targets
Ivermectin B1a	1	16.492	892.5417	<input type="checkbox"/>		Targets
Famoxadone	1	10.364	392.1605	<input type="checkbox"/>		Targets
Chloroprotham (Chloroprotham)	1	7.543	214.0629	<input type="checkbox"/>		Suspects
Acinathrin (Rufast)	1	14.061	542.1397	<input type="checkbox"/>		Suspects
Butoxycarboxim	1	2.814	223.0747	<input type="checkbox"/>		Suspects
Chlorfluazurone (Chlorfluazurone)	1	13.612	539.9702	<input type="checkbox"/>		Suspects
Chlorfluazurone	1	15.595	284.0607	<input type="checkbox"/>		Suspects

Calculation Includes:

Cals

QCs

Select All OK Cancel

- 4 Highlight all Targets.

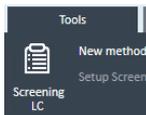
- 5 Select **Cals** under **Calculation Includes**; then select **OK**.

6

Perform Rapid Bulk Data Review

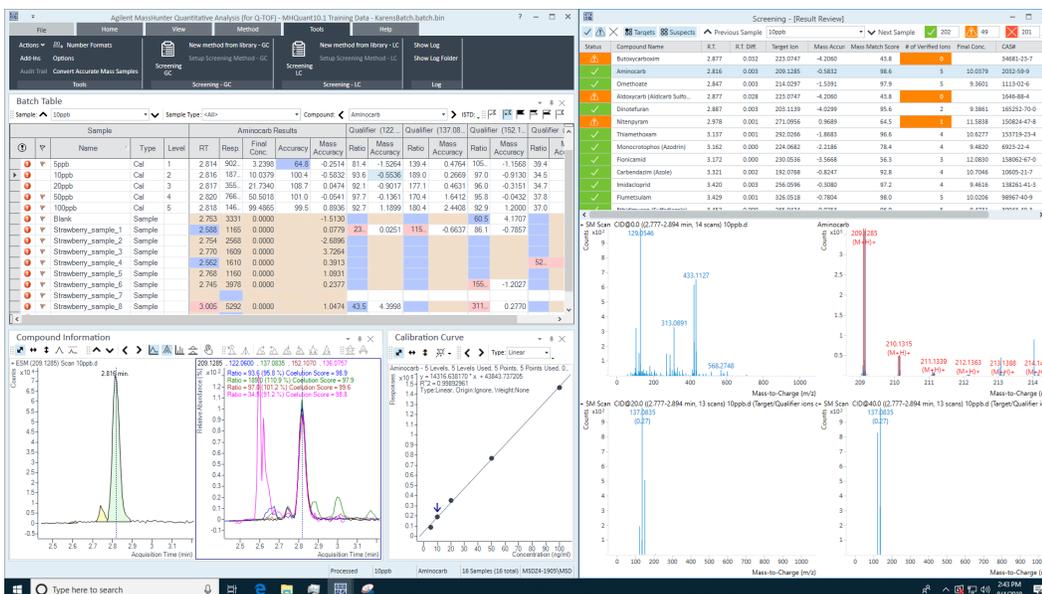
After analyzing the batch, perform data analysis in the traditional manner using Quant software (similar to MRM methods). However, when evaluating hundreds of analytes this can be time consuming and inefficient. Use the LC Screener for rapid data review based on the outlier parameters stated above.

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



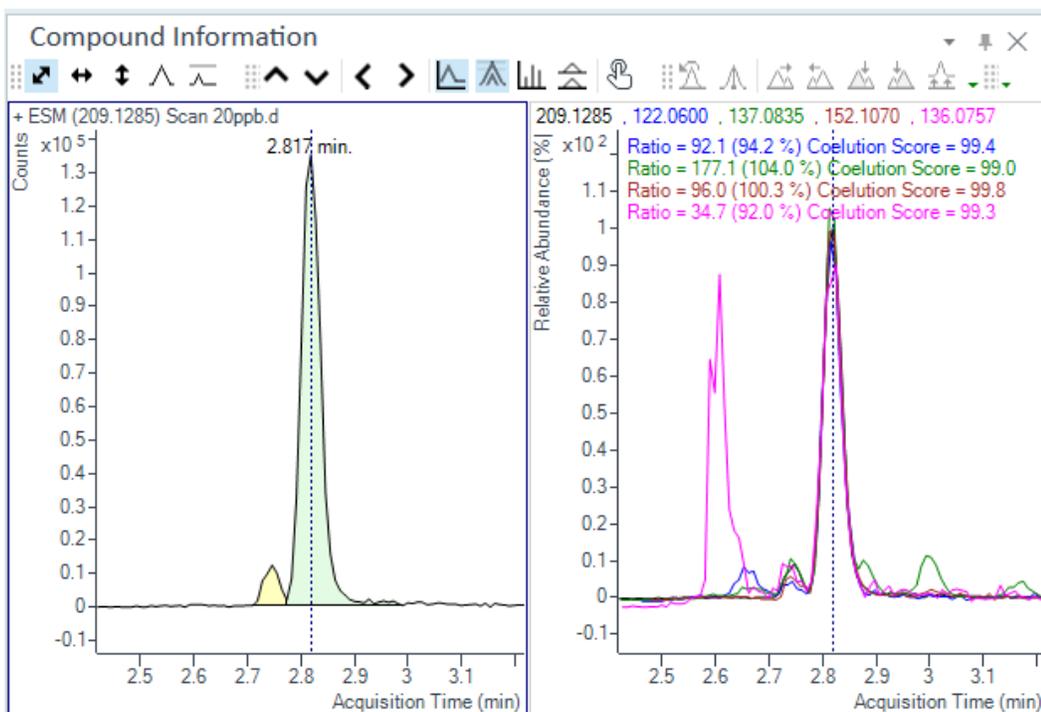
The LC Screener displays.

- 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.

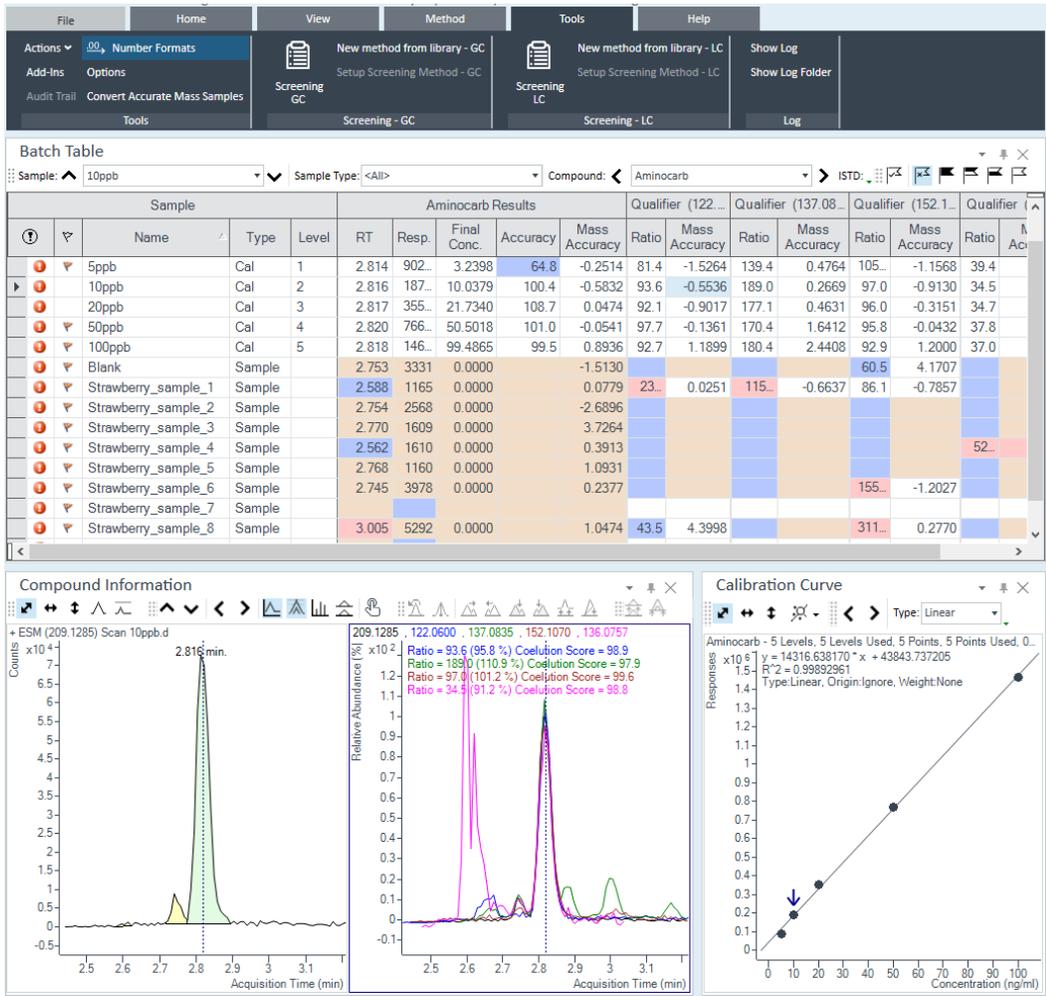


- 3 Adjust the information displayed in the Quant-My-Way user interface. This maximizes the pertinent information displayed in the Quant-My-Way UI and complements the LC screener information. Agilent recommends the following adjustments.
- Delete unnecessary columns.
 - Add the mass accuracy column for 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**.

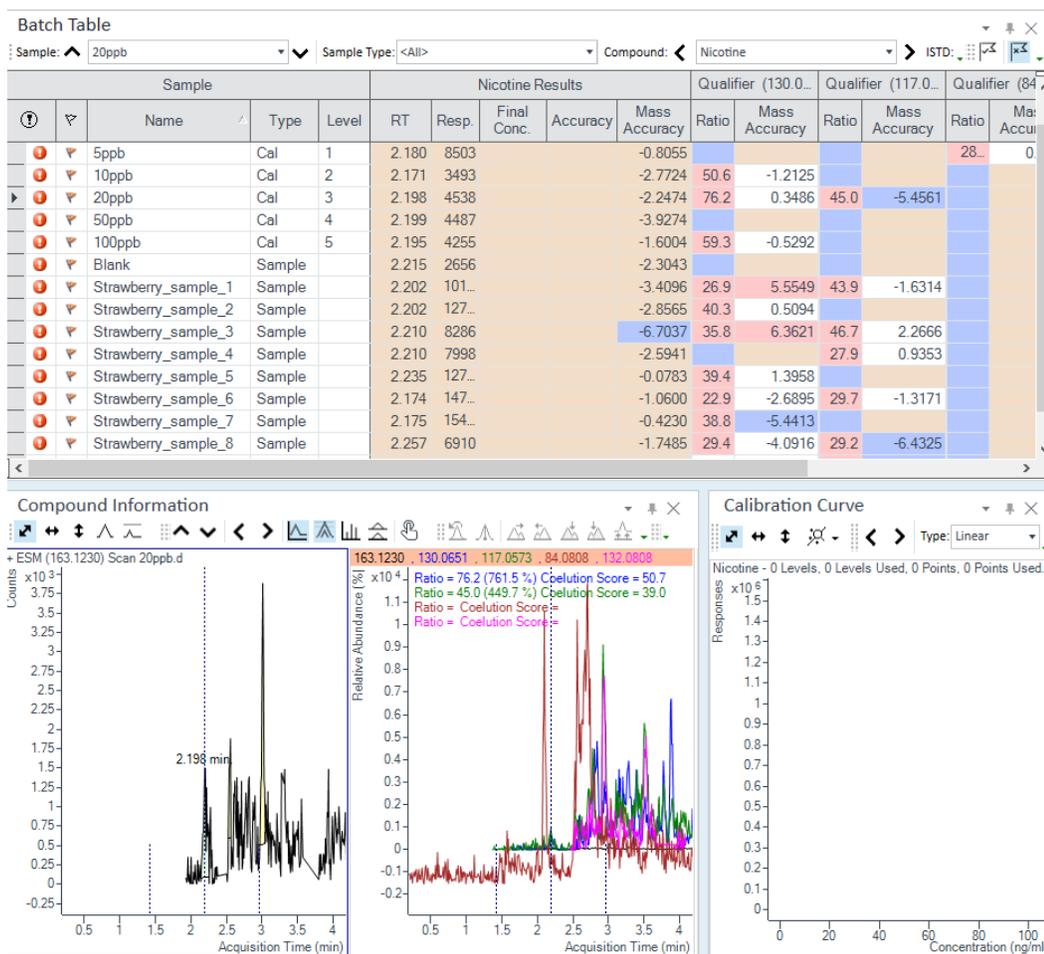
Batch Table															
Sample: 20ppb		Sample Type: <All>			Compound: Aminocarb			ISTD:							
Sample				Aminocarb Results					Qualifier (122.0...		Qualifier (137.0...		Qualifier (15...		
?	▼	Name	Type	Level	RT	Resp.	Final Conc.	Accuracy	Mass Accuracy	Ratio	Mass Accuracy	Ratio	Mass Accuracy	Ratio	Mass Accuracy



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 scan (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.

Status	Compound Name	R.T.	R.T. Diff.	Target Ion	Mass Accur.	Mass Match Score	# of Verified Ions	Final Conc.	CAS#
⚠	Butoxycarboxim	2.877	0.032	223.0747	-4.2060	43.8	0		34681-23-7
✓	Aminocarb	2.816	0.003	209.1285	-0.5832	98.6	5	10.0379	2032-59-9
✓	Omethoate	2.847	0.003	214.0297	-1.5391	97.9	5	9.3601	1113-02-6

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.

Status	Compound Name	R.T.
✓	Aminocarb	2.816
✓	Omethoate	2.847
✓	Dinotefuran	2.887
✓	Thiamethoxam	3.137
✓	Monocrotophos (Azodrin)	3.162

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.

Status	Promoted	Compound Name	CAS#	Formula	R.T.	R.T. Diff.
✓	<input type="checkbox"/>	Ethionazek sulfide	53380-22-6	C11H15NO3S	3.431	0.076
✓	<input type="checkbox"/>		61676-87-7	C12H14N2S	3.517	0.012
✓	<input type="checkbox"/>		2275-23-2	C8H18NO4P5S2	3.611	0.049
✓	<input type="checkbox"/>		135410-20-7	C10H11ClN4	3.676	0.054
✓	<input type="checkbox"/>		148-79-8	C10H7N3S	3.629	0.048
✓	<input type="checkbox"/>		2635-10-1	C11H15NO3S	3.431	0.280
✓	<input type="checkbox"/>		6988-21-2	C11H13NO4	3.792	0.066
✓	<input type="checkbox"/>		60-51-5	C5H12NO3P5S2	3.812	0.042
✓	<input type="checkbox"/>	thyl-N-phenylurea)	101-42-8	C9H12N2O	3.857	0.046

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.

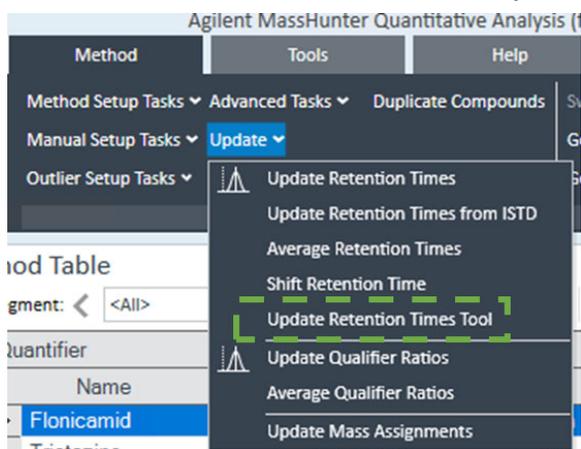
For routine deployment of a method, Agilent recommends that an R&D scientist optimize the method, keeping the best fragment(s) for a given method and matrix and removing all others. An optimized method is faster to process and easier to analyze than a method with many fragments. To optimize the method, 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**.

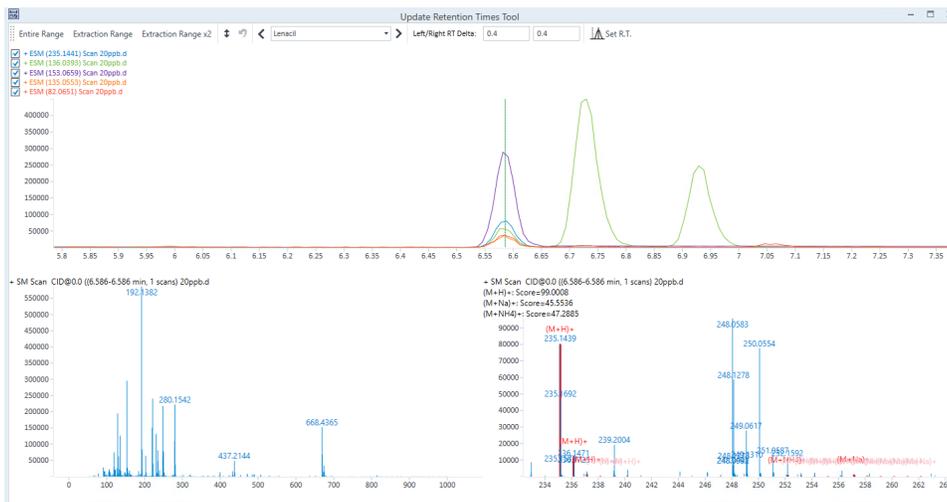


The Retention Times Tool displays in a new window. It shows the extracted chromatograms for the precursor and fragment ions in the top panel. The lower left panel has the full spectrum at the selected RT and the isotopic pattern in the lower right panel with the mass match score and theoretical isotopes.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

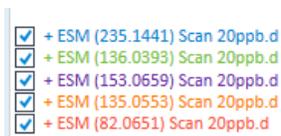
Optimize Methods with the Update Retention Time Tool

Delete fragments to optimize the method

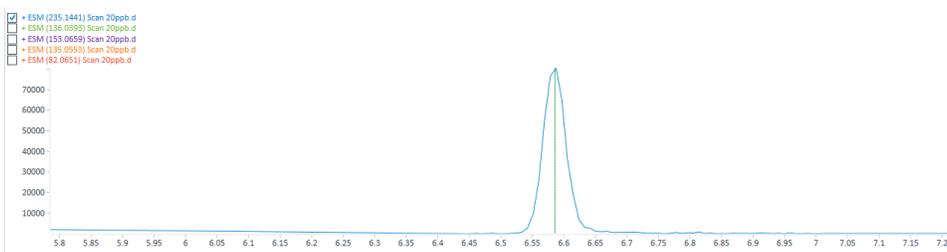
The top bar allows selection of the RT range displayed, the analyte, and the RT. It updates in the method. Lenacil is selected in this example.



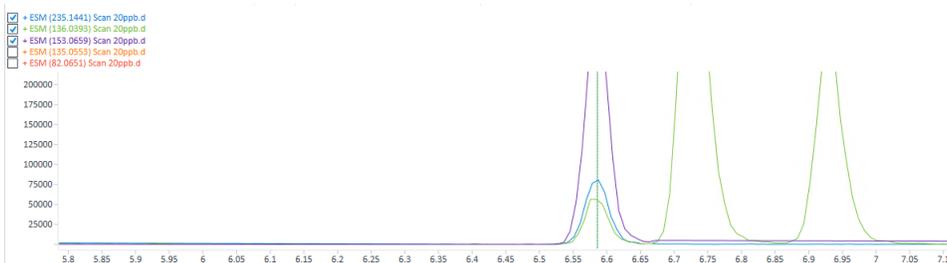
2 Use the top panel to select the ions displayed.



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



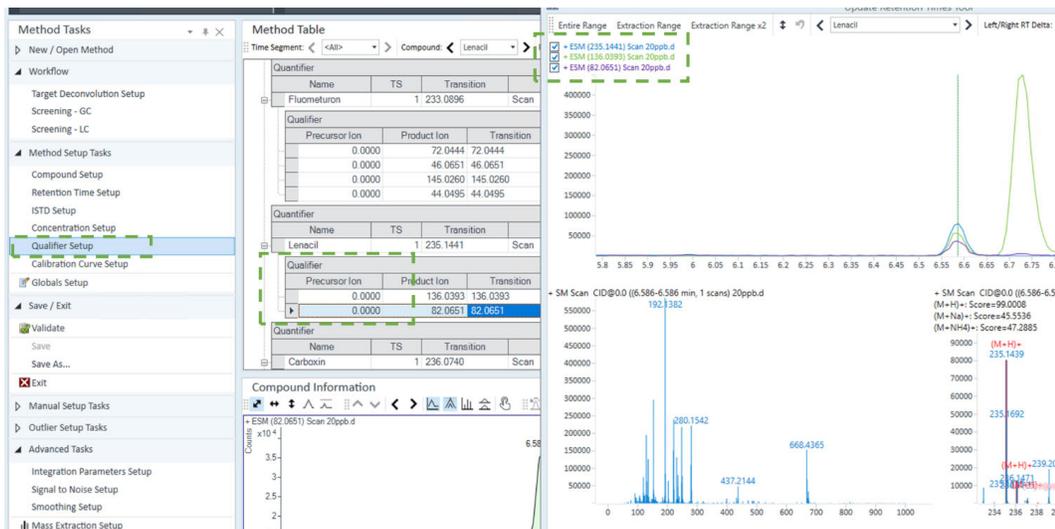
4 Select two fragments, such as green (m/z 136.0393) and purple (m/z 153.0659), to display. In this example, the purple ion has no nearby analytes and a stable signal. This is preferred to the green ion, which has nearby interferences.



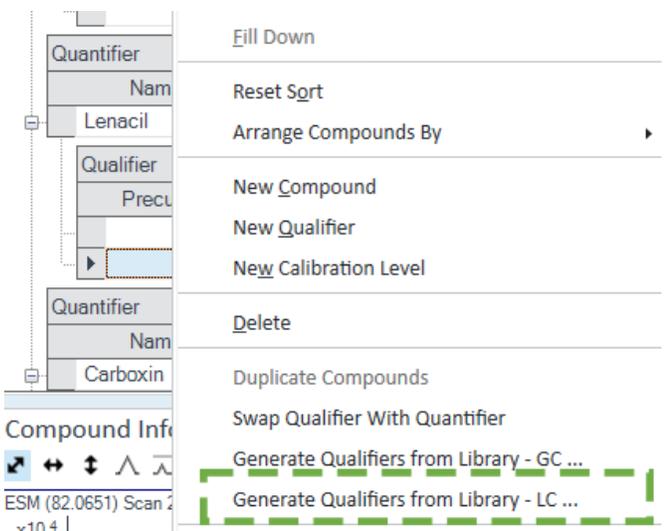
Optimize Methods with the Update Retention Time Tool

Delete fragments to optimize the method

- 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**.



Optimize Methods with the Update Retention Time Tool

Delete fragments to optimize the method

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

Method from Library (Qualifiers) - LC

Library:

Workflow:

Qualifiers:

Number of qualifiers to add:

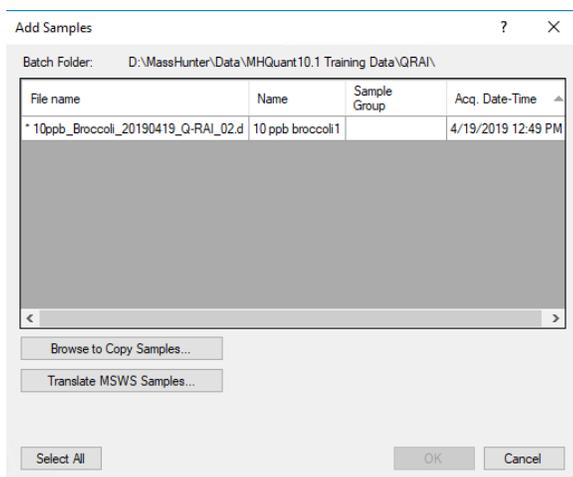
Candidate ion ranking:

- 8 Select **OK**.

8

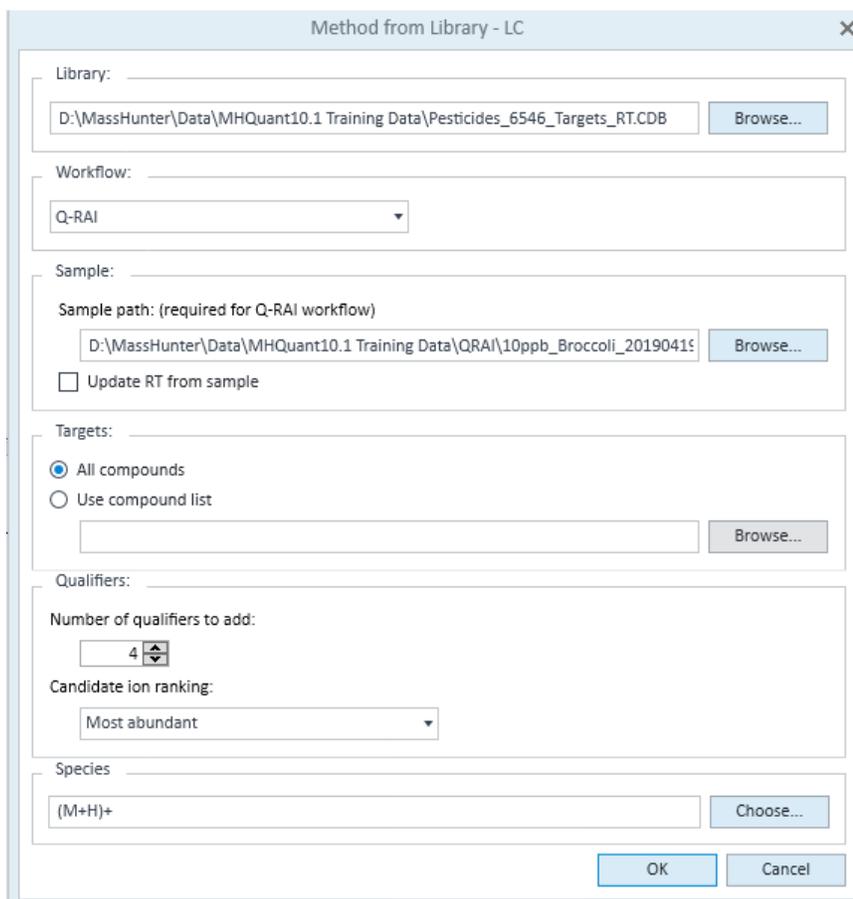
Build a Method with Q-RAI Data

- 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.

Method Table								
Time Segment: < <All> >		Compound: < >		Reset Table View				
Quantifier								
Name	TS	Transition	Scan	Type	Precursor Ion	Product Ion	Uncertainty	
Cycloate	1 216.1417	Scan	Target	0.0000	216.1417	Relative		
Qualifier								
Precursor Ion	Product Ion	Transition	Rel. Resp.	Uncertainty	Area Sum	Collision Energy	Collision Energy Delta	
216.1417	55.0542	160-230 -> 55.0542	10.0	20.0	<input type="checkbox"/>	40.0	0.1	
216.1417	83.0855	160-230 -> 83.0855	10.0	20.0	<input type="checkbox"/>	20.0	0.1	
216.1417	63.0263	160-230 -> 63.0263	6.2	20.0	<input type="checkbox"/>	20.0	0.1	
216.1417	72.0444	160-230 -> 72.0444	4.7	20.0	<input type="checkbox"/>	20.0	0.1	

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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