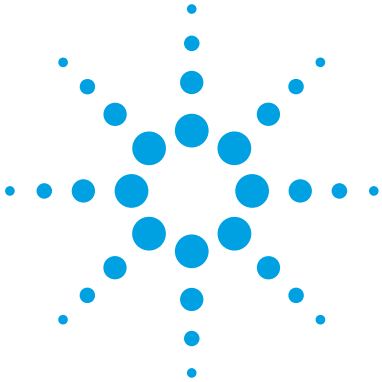


MassHunter MRM/dMRM/tMRM Database

Familiarization Guide

Agilent Technologies

Use the exercises in this guide to learn how to use your MassHunter MRM, dMRM, or tMRM Database with MassHunter Data Acquisition, Qualitative Analysis, and Quantitative Analysis programs. You use the example Checkout Mix data, method files, and database to learn how to find and identify compounds in a data file. The Checkout Mix data files, methods, and database are based on the Pesticides Checkout Test Mix, which contains a wide variety of compound classes.

As an optional step, you can separately purchase test mix and column to acquire your own data for use with this guide:

- LC TOF/QTOF/QQQ Pesticide Test Mixture (p/n 5190-0469)
- ZORBAX LC Column, Eclipse Plus C18, 2.1 mm × 100 mm, 1.8 µm (p/n 959758-902)

These Familiarization Files are included on the database installation media and are installed on your computer when the database is installed:

- Checkout Mix Database:
  - CheckoutMix_TriggeredMRM_B0600
- Checkout Mix methods:
  - CheckoutMix_MRM.m, also used to create dMRM method
  - CheckoutMix_DMRM.m, also used to create tMRM method
  - CheckoutMix_TMRM.m

  Note that the dMRM and tMRM Checkout Mix methods are included for reference only. These methods work only on an LC/MS system that produces the same retention times as the example data. Any retention time shifts will invalidate the retention time windows in these methods.
- Checkout Mix example Data:
  - CheckoutMix_MRM.d
  - CheckoutMix_DMRM.d
  - CheckoutMix_TMRM.d
- Checkout Mix example report

Workflow Overview

This Familiarization Guide uses example data from the Checkout Mix to illustrate the workflow and the familiarization exercises. Figure 1 summarizes the workflow, which includes incremental method development from MRM, over to dynamic MRM (dMRM) to triggered MRM (tMRM) methods, including identification of retention times (RT), trigger parameters, and secondary transitions.

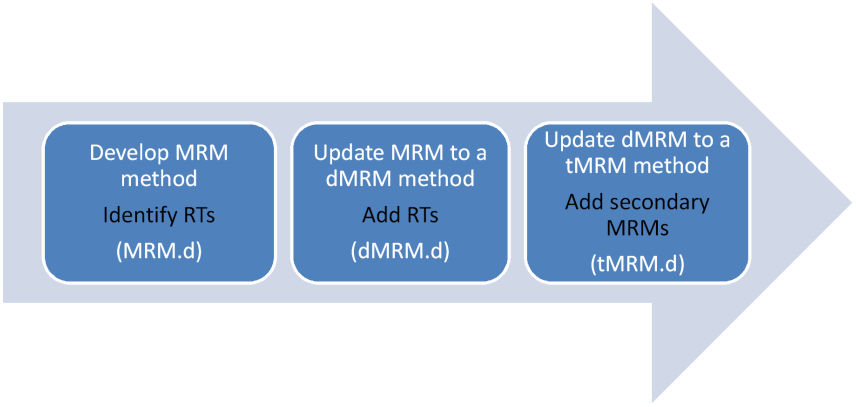


Figure 1 MRM to dMRM to tMRM Method Development Workflow for single standard mix

Single Standard Mix Workflow

You can use this complete workflow to create an MRM, dMRM, or tMRM method to analyze a single standard mix:

1. Use the database to create the MRM method for the primary transitions.
2. Establish the Retention Times, and then update the MRM method to a dMRM method using the **Update DMRM Method** command. Save as a dMRM method.
3. Check the dMRM editor for any overlaps in retention time. If needed, adjust the cycle time settings and/or the Retention Time windows.
4. Acquire data to make sure that the dMRM method is valid.
5. Update the dMRM method to a tMRM method with trigger parameters. Save as a tMRM method.
6. Add the secondary transitions.

After you have set up methods to analyze a single standard mix, you can adapt the same procedures for your unique multi-component analysis.

Multiple Standard Mix Workflow

Some analyses include multiple standard mixes.

To develop a method to analyze multiple compound mixes in one analytical run:

1. Create and optimize each dMRM or tMRM method for each standard mix separately. Use the same LC chromatographic method.
2. Combine these dMRM or tMRM methods. (Copy and paste transition tables of each dMRM or tMRM method into a single acquisition method.)
3. Re-optimize the parameters for overlapping dMRM or tMRM transitions for compounds that co-elute.

For ease of use, optimize no more than 50 compounds at a time in each **MRM -> dMRM -> tMRM** workflow.

Before You Begin

To do the exercises in this guide, you can use the Familiarization Checkout Mix example data files that are included with the database. Or you can acquire your own data.

The Checkout Mix Database and example data, methods and reports are installed when the complete database product is installed.

To prepare to run the Checkout Mix

1. Make sure that you have these required parts and reagents:
   - reagent-grade formic acid (p/n G2453-85060 or equivalent)
   - ZORBAX LC Column, Eclipse Plus C18, 2.1 mm × 100 mm, 1.8 µm (p/n 959758-902)
2. Check that the Agilent 1200 Series LC is properly installed and verified.
3. If you have a G1312B Agilent 1260 Infinity Binary Pump, bypass the mixer and damper. See "To bypass mixer and damper" on page 73 for details.
4. Check that the Agilent 6400 Series Triple Quadrupole LC/MS instrument is properly installed and verified.
5. Check that the following programs are properly installed:
   - MassHunter Data Acquisition B.06.00 or higher
   - MassHunter Quantitative Analysis B.07.01 or higher.
   - MassHunter Qualitative Analysis B.07.00 or higher
6. To use a system configuration that is different from the one described in "To run the Checkout Mix" on page 6, create or edit a method for your system configuration and the Checkout Mix method parameters. The Checkout Mix parameters are in the Checkout Mix acquisition method.

   Use the MassHunter Data Acquisition program to open and view the method. These data acquisition settings for the compounds are listed:
   - Acquisition method info
   - Sampler settings
   - Binary pump settings
   - Thermostatted column compartment settings

Refer to "Primary and Secondary Transitions for Triggered MRM" on page 68 for MS/MS transitions and their compound-dependent settings.

The configuration of the example methods is:

- Agilent 6400 Series Triple Quadrupole LC/MS
- Agilent G4226A HiP Sampler
- Agilent G4220A Binary Pump
- Agilent G1316C Column Compartment

To run the Checkout Mix

1 Do a check tune to verify that the instrument operates properly.

Change to the Tune context in the MassHunter Data Acquisition program and then click **Checktune** to verify the instrument is properly tuned. Do an Autotune if Checktune reports any failure.

2 Prepare the Checkout Mix.

The concentration of the Checkout Mix stock solution is 100 ppm for both positive and negative mixes. Only the positive mix is used in the *Familiarization Guide*. The negative mix is included for your convenience.

a Dilute 100 µL of the stock solution to 10.0 mL with acetonitrile to create Working Solution 1 (1 ppm).

b Take 1 mL of Working Solution 1 and dilute it to 10.0 mL with 10:90 acetonitrile:water to create Working Solution 2 (100ppb).

Use Working Solution 2 for systems with an Agilent Jet Stream source, or for systems with iFunnel optics.

c Transfer an aliquot of the Working Solution 2 to a standard 2 mL sample vial for analysis.

Do this separately for the positive and negative Checkout Mixes.

NOTE For some instrument configurations, this sample concentration is too high. If so, dilute the sample by a factor of 10 or more and inject the diluted sample, or simply inject 0.5 µL or less.

3 Prepare mobile phases A and B.

- A= 5 mM acetic acid in water (286 µL glacial acetic acid in 1 L water)
- B= 100% acetonitrile

These mobile phases are suitable for both positive and negative Checkout Mixes.

The examples in this guide were run in positive mode only.

4 Verify the system configuration.

The checkout method uses the system configuration listed in the next table. If your system deviates from this configuration, adjust the method as needed. Refer to the *Method Setup Guide* that is included on the installation media.

Column	ZORBAX LC Column, Eclipse Plus C18, 2.1 mm × 100 mm, 1.8 µm (p/n 959758-902)
Wellplate Sampler	HiP Sampler, G4226A
Pump	Binary Pump, G4220A. If you use a different binary pump, configure the damper and mixer to be bypassed. See "To bypass mixer and damper" on page 73.
Column Compartment	Column - SL, Model G1316C

5 Load the method CheckoutMix_MRM.m.

6 Check that the method is set up to make a 5 µL injection.

7 Click **Sample > Run** to do a single sample run, or create a worklist to make multiple injections.

8 If you do not see all the peaks after you process your data:

a Extend your **Stop time** in the method to 12 minutes.

b Run the test mix again.

This will not affect your results but will show if retention times are different on your system. There are a number of reasons your retention times can change from those determined by Agilent, such as different instrument delay volume, dead volumes or configuration.

Creating an MRM acquisition method from the database

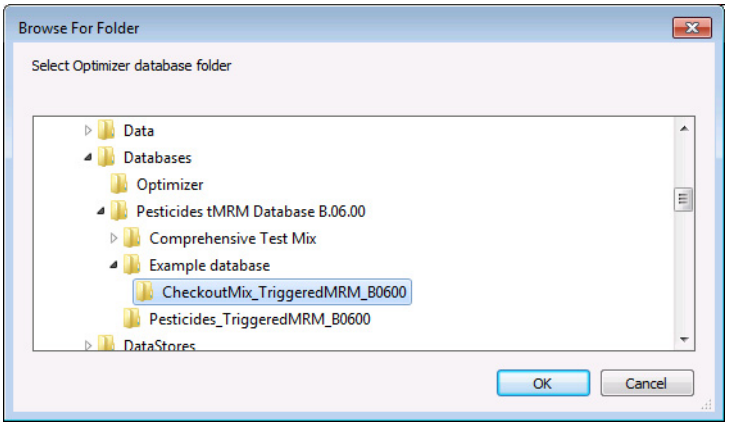
Task 1. Create an MRM method

MRM methods are simple to create and run. They are useful to analyze a small number of targeted compounds, each with quantifier and qualifier ions. You also create MRM methods as the first step to create both dMRM and tMRM methods.

An MRM data acquisition method contains settings such as compound names, ISTD (optional), MRM transitions, fragmentor voltages, and collision energies. With the MassHunter MRM/dMRM/tMRM Database, you can easily import all of these settings from the database to create an MRM method.

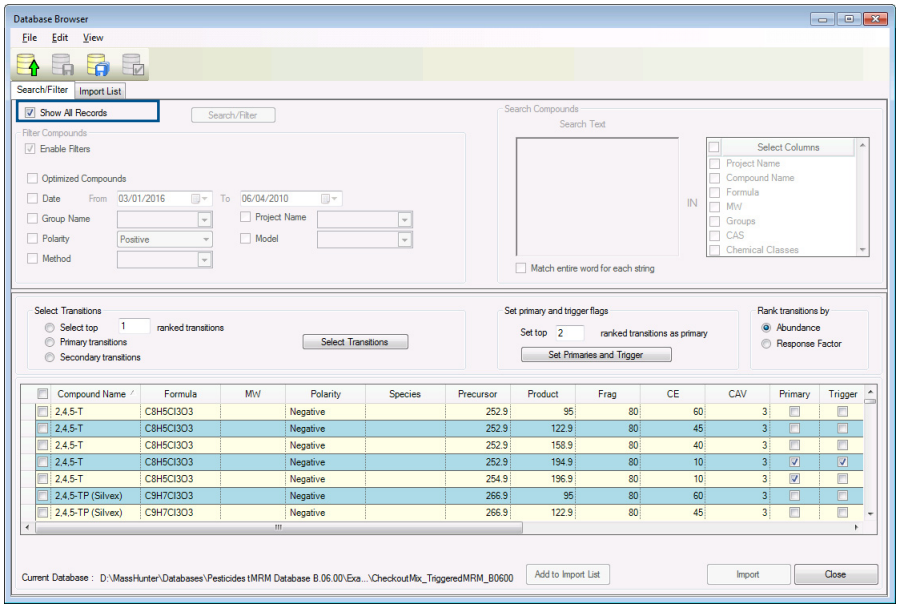
Steps	Detailed Instructions	Comments
1 In the Data Acquisition program, open the default method and save as: ***iii*CheckoutMix_MRM.m,** where *iii* are your initials.	**a** Start the Data Acquisition program. **b** Open the **default.m** method. **c** Click **Method > Save As**. **d** Type ***iii*CheckoutMix_MRM.m,** where *iii* are your initials.	• You can also use the **CheckoutMix_MRM.m** method in the **Example methods** folder.
2 Set the LC parameters according to "LC Parameters" on page 65. The following configuration was used to collect the included Checkout Mix data: • Agilent G4226A HiP Sampler • Agilent G4220A Binary Pump • Agilent G1316C Column Compartment	**a** In the Method Editor window, click the **HiP Sampler** tab. **b** Enter the parameters from Figure 1 on page 65 **c** Click the **Binary Pump** tab. **d** Enter the parameters from Figure 2 on page 66. **e** Click the **Column Comp.** tab. **f** Enter the parameters from Figure 3 on page 67.	• If you have a different LC model, the LC parameters can be different.

Steps	Detailed Instructions	Comments
3 Set the source parameters and change the method to an MRM method.	The values listed here are for non-iFunnel instruments for use with the Checkout Mix. For databases that support iFunnel instruments, refer to the *Method Setup Guide* for iFunnel settings. **a** In the Method Editor window, click the **QQQ** tab. **b** Click the **Source** tab. **c** For **Gas Temp,** type 250. **d** For **Gas Flow,** type 7. **e** For **Nebulizer,** type 40. **f** For **Sheath Gas Temp,** type 325. **g** For **Sheath Gas Flow,** type 11. **h** For **Capillary,** type 3500. **i** For **Nozzle** voltage, type 0. **j** Click the **Acquisition** tab. **k** In the Time segments on the left side of the QQQ tab, select **MRM** as the **Scan Type** for the first Time segment.	• The Scan segments table always has to have at least one row. You manually remove this row after importing transitions from the Database Browser.
4 Open the **CheckoutMix_TriggeredMRM_B0600** in Database Browser, from the **QQQ Acquisition** tab.	**a** Right-click the Scan segments table and click **Import from Database Browser**. The Database Browser opens. In the Database Browser, click **File > Open Database**. **b** Select the **CheckoutMix_TriggeredMRM_B0600** database in the **\MassHunter\Databases*Product* Database *x.xx.xx*\Example database** folder. **c** Click **OK**.	

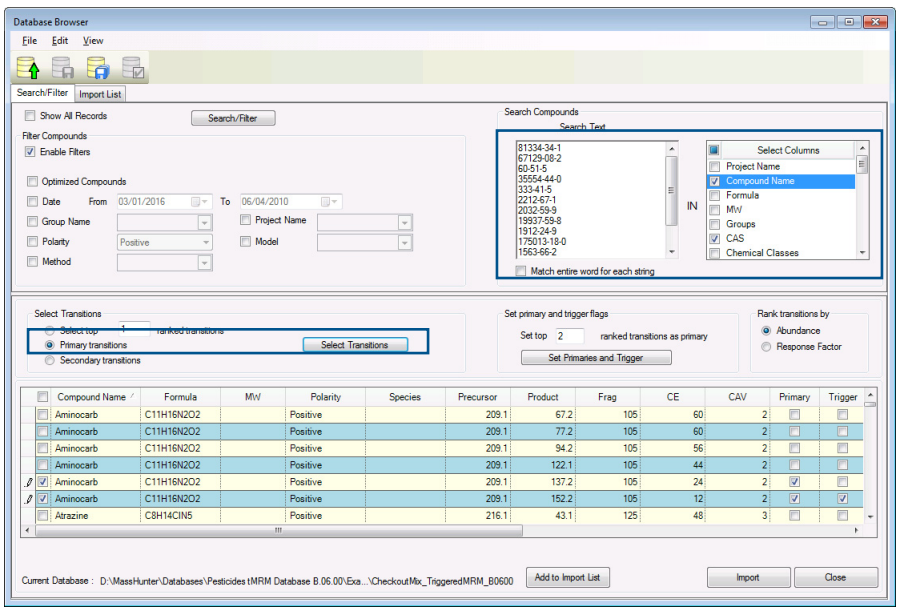
Steps	Detailed Instructions	Comments
		

Steps	Detailed Instructions	Comments
5 Select primary transitions. • See "Primary and Secondary Transitions for Triggered MRM" on page 68 for a list of the Primary transitions. • The secondary transition are added when you are creating the triggered MRM method. See "Task 1. Create a tMRM method from a dMRM method" on page 42. Note that in the example data file, when both polarities are available in the **CheckoutMix_TriggeredMRM_B0600** database, the analysis was run in positive mode only.	**a** Click the **Compound Name** column header to sort the compounds by Compound Name. **b** Mark the check boxes next to the primary transitions for each of the compounds in the "Primary and Secondary Transitions for Triggered MRM" on page 68. **c** To quickly mark only the primary transitions for the Checkout Mix: • Under **Search Compounds**, mark the **CAS** check box. In the **Search Text** text box, type the CAS numbers for the Checkout Mix. • Under **Select Transitions**, select Primary transitions and click **Select Primary**. See next page for filter examples. **d** Review the transitions in the table. Clear the check box next to any transitions that you do not want to include.	• Instead of individually marking each check box, you can use the search and filter function with the Select Transitions options to select a number of transitions according to the criteria you have specified. Refer to the help for the Database Browser Search Filter tab in the Optimizer Help. • The CAS number is a reliable item to use to filter the compounds. If you use the Compound Name, you have to spell the name exactly as it is written in the database; otherwise, you get too many random hits which you then have to remove from your import list. Also, you have to write the name or number as a vertical list (a new line for each name or number). • Qualifier and quantifier MRMs can have different precursor ion species but they cannot have different polarities. Compounds that contain halogens often have multiple precursors for the same compound in the database. Refer to the *Method Setup Guide* for the more details on choosing the most selective transitions for your analysis.

Steps	Detailed Instructions	Comments

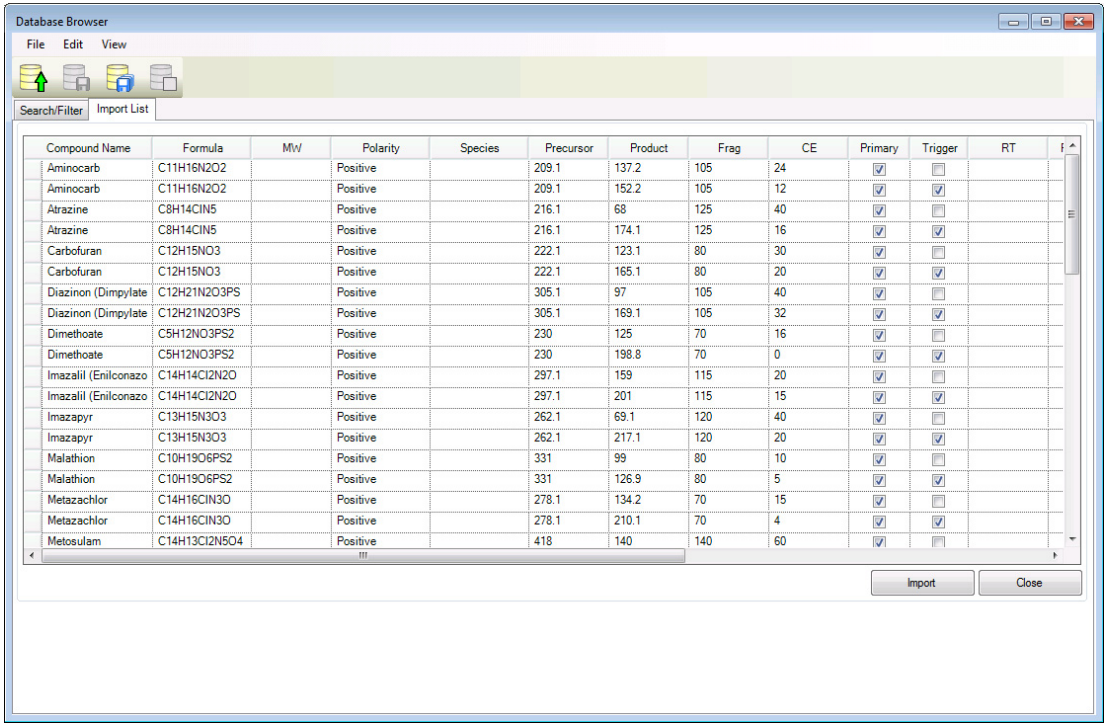


If you mark the Show All Records check box, then all compounds in the database are shown in the table. You scroll through the compounds and mark the Primaries for the compounds you are using.



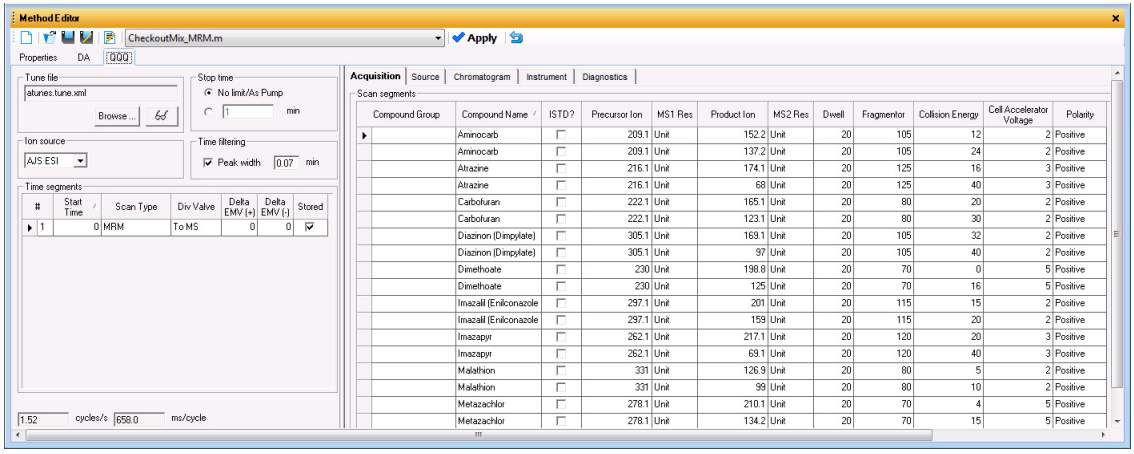
If you clear the Show All Records check box, you can limit the compounds that are shown in the table. In this example, the CAS check box is marked in the Search Compounds group and a list of CAS numbers was typed in the Search Text. Each CAS number was typed on a separate line. Only the compounds with one of those CAS numbers is shown in the table. You can then click the Primary transitions button and click Select Transitions. Then, all of the Primary transitions for the selected compounds are marked.

Steps	Detailed Instructions	Comments
6 Import transitions to the Data Acquisition program. For compounds that have both negative and positive transitions, remove any negative MRM transition for any compounds with positive MRM transitions.	**a** Click the **Add to Import List** button. **b** Click the **Import List** tab. **c** Review the Import List table. **d** If needed, select all negative MRM transitions for any compounds with positive MRM transition. Right-click the selection, and then click **Remove**. **e** Click the **Import** button.	• Only the transitions that you marked are added to the Import List. • Removal of the negative MRM transition for compounds that also have a positive MRM transition ensures that one compound name is associated with only one polarity. One compound cannot have both negative and positive polarity transitions.



Compound Name	Formula	MW	Polarity	Species	Precursor	Product	Frag	CE	Primary	Trigger	RT
Aminocarb	C11H16N2O2		Positive		209.1	137.2	105	24	☑	☐	
Aminocarb	C11H16N2O2		Positive		209.1	152.2	105	12	☑	☑	
Atrazine	C8H14ClN5		Positive		216.1	68	125	40	☑	☐	
Atrazine	C8H14ClN5		Positive		216.1	174.1	125	16	☑	☑	
Carbofuran	C12H15NO3		Positive		222.1	123.1	80	30	☑	☐	
Carbofuran	C12H15NO3		Positive		222.1	165.1	80	20	☑	☑	
Diazinon (Dimpylate	C12H21N2O3PS		Positive		305.1	97	105	40	☑	☐	
Diazinon (Dimpylate	C12H21N2O3PS		Positive		305.1	169.1	105	32	☑	☑	
Dimethoate	C5H12NO3PS2		Positive		230	125	70	16	☑	☐	
Dimethoate	C5H12NO3PS2		Positive		230	198.8	70	0	☑	☑	
Imazalil (Enilconazo	C14H14Cl2N2O		Positive		297.1	159	115	20	☑	☐	
Imazalil (Enilconazo	C14H14Cl2N2O		Positive		297.1	201	115	15	☑	☑	
Imazapyr	C13H15N3O3		Positive		262.1	69.1	120	40	☑	☐	
Imazapyr	C13H15N3O3		Positive		262.1	217.1	120	20	☑	☑	
Malathion	C10H19O6PS2		Positive		331	99	80	10	☑	☐	
Malathion	C10H19O6PS2		Positive		331	126.9	80	5	☑	☑	
Metazachlor	C14H16ClN3O		Positive		278.1	134.2	70	15	☑	☐	
Metazachlor	C14H16ClN3O		Positive		278.1	210.1	70	4	☑	☑	
Metosulam	C14H13Cl2N5O4		Positive		418	140	140	60	☑	☐	

Steps	Detailed Instructions	Comments
7 Review the MRM transitions in the Data Acquisition program.	**a** Delete the original compound in the **Scan segments** table. **b** Sort the table by the **Compound Name**. **c** Review the transitions for each compound.	• If a red box appears in the Scan segments table, you click the **Apply** button in the toolbar. If the red box does not clear, the value is not valid. • The example method may not exactly match the transitions in the database. Use the transitions in the database if you find a discrepancy.



8 In the Data Acquisition program, save the method.	• Click the **Method > Save** command.	

Task 2. Acquire, inspect, and analyze MRM data in Qualitative Analysis

After you acquire the MRM data file, you examine the data file in the Qualitative Analysis program to verify that the transitions were acquired.

To identify isomeric compounds during routine LC/MS, an authentic sample of each isomer is injected, and its retention time is determined under the chromatographic conditions used for the analysis.

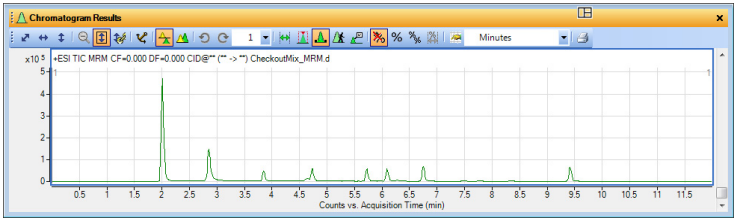
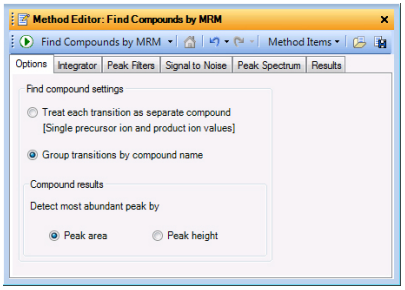
The retention time is needed for identification when the MS/MS spectra, and hence MRM transitions, of these isomers are very similar. The Checkout Mix (p/n 5190-0469) does not contain isomers, and the retention time is not required for identification of the compounds in the Checkout mix.

The elution order of the compounds in the Checkout Mix were determined using the Eclipse Plus C18 column and mobile phases specified in the "To run the Checkout Mix" on page 6. The expected elution order is:

- Aminocarb
- Imazapyr
- Thiabendazole
- Dimethoate
- Imazalil (Enilconazole)
- Metoxuron
- Carbofuran
- Atrazine
- Metosulam
- Metazachlor
- Molinate
- Malathion
- Pyraclostrobin
- Diazinon (Dimpylate)

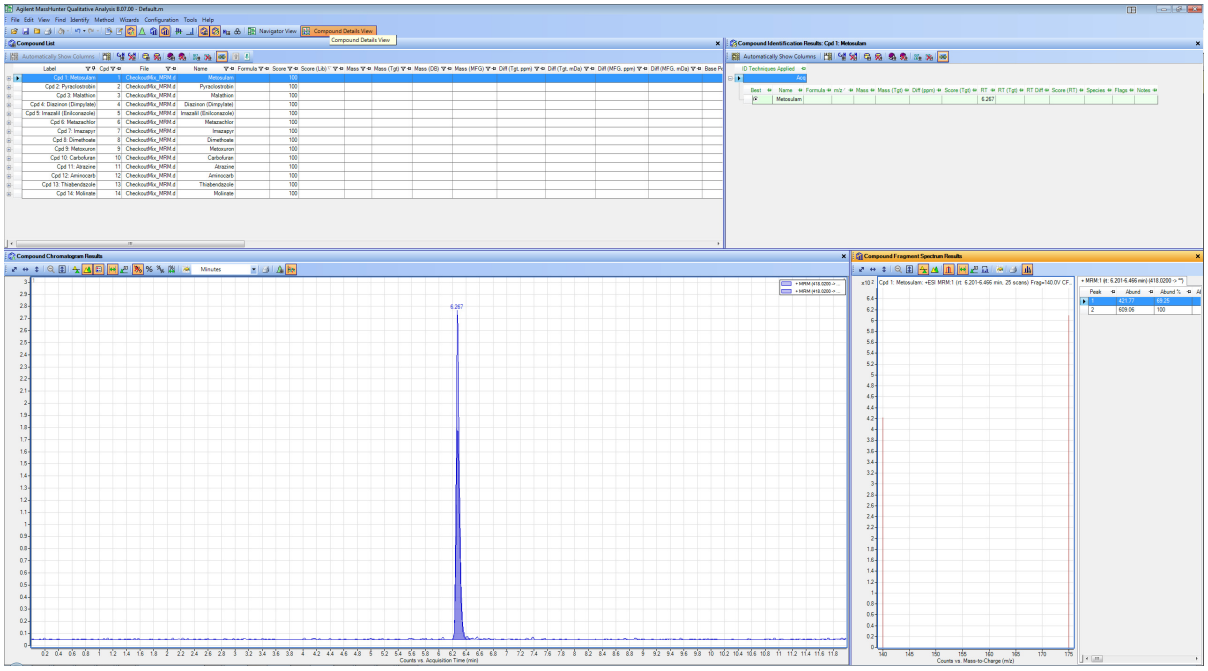
Depending on the delay volume, the compounds Pyraclostrobin and Diazinon can co-elute, separate slightly, or reverse elution order.

Steps	Detailed Instructions	Comments
Do this step if you want to acquire data with the Checkout Mix. Otherwise, continue at step 2. **1** Acquire data. • Set up a one-line worklist with the method you just created. • Name the data file **CheckoutMix_MRM.d**. • Designate a directory path to hold your data files and method.	**a** If necessary, click **View > Worklist** to display the Worklist window. **b** Click **Worklist > Worklist Run Parameters**. Verify that the parameters are set properly. Click **OK**. **c** Click **Worklist > Add Multiple Samples**. **d** Type `CheckoutMix_MRM.d` as the data file name **e** Select **CheckoutMix_MRM.m** as the method name. **f** Click the **Sample Position** tab. **g** Select the Autosampler, Well-plate or Vial Tray. **h** In the graphic, select a single position. Click **OK.** **i** In the Worklist window, mark the check box to the left of the sample. **j** Click the **Start Worklist Run** icon in the main toolbar, the **Run Worklist** icon in the Worklist toolbar, or click the **Worklist > Run** command.	• The Worklist window is tabbed with the Method Editor window by default. Click the **Worklist** tab at the bottom left corner of the program to show the Worklist window. • See also "To run the Checkout Mix" on page 6. • Make sure your dwell time for all transitions gives an appropriate cycle time. This criterion determines how many transitions you can put within one cycle. • For peaks that are 5 seconds wide, use a cycle time of 500 ms to give 10 points across the peak. For 50 compounds with 2 transitions each, use a 2 ms dwell time to give 5.5 ms total per transition.

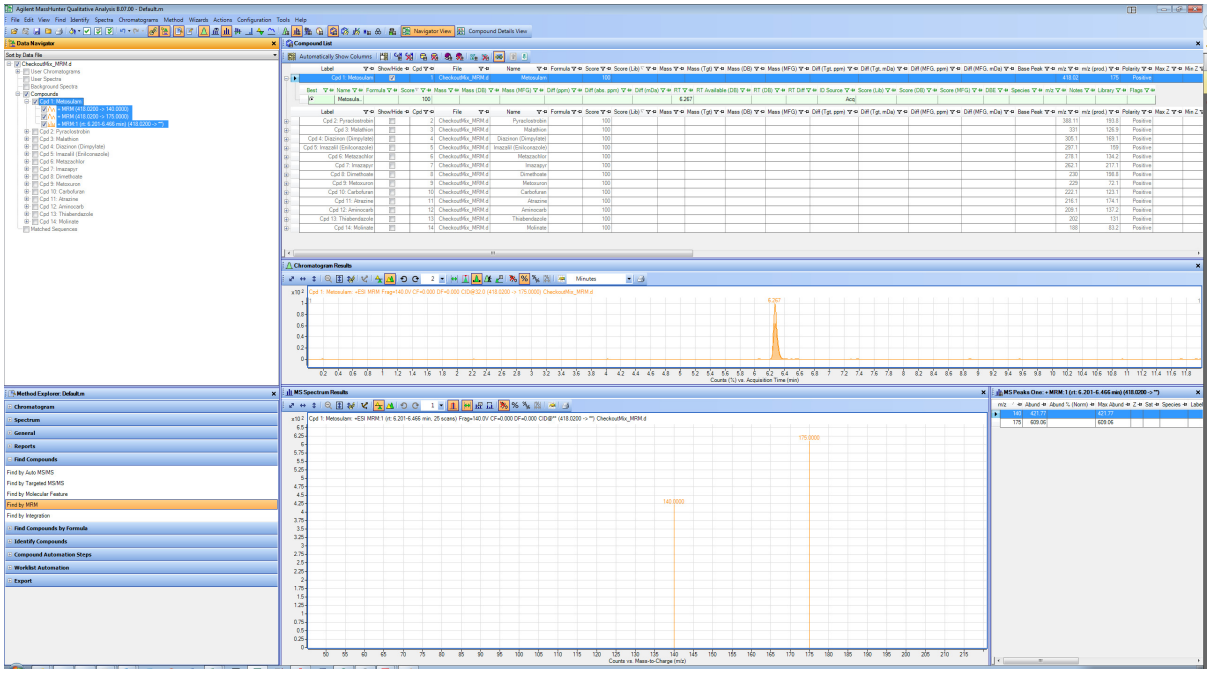
Steps	Detailed Instructions	Comments
2 Find compounds using the Find Compound by MRM algorithm. • Open the data file **CheckoutMix_MRM.d**.	Start the Qualitative Analysis program. If it is not running, double-click the **Qualitative Analysis B.07.00** icon ( ). **k** Click **File > Open Data File**. The system displays the **Open Data File** dialog box **l** From the folder **\MassHunter\Data*Product Database x.xx.xx*\Example Data** folder, select **CheckoutMix_MRM.d**, and click **Open**. **m** If needed, click **Method > Method Explorer** or **View > Method Explorer**. The system displays the Method Explorer window. **n** In the Find Compounds section, click **Find by MRM**. **o** In the Method Editor Window, click the **Group transitions by compound name** option. **p** Click the **Peak area** option for **Detect most abundant peak by**.	• If the Find by MRM section is not available, you need to modify the options available in the User Interface Configuration dialog box. You click **Configuration > User Interface Configuration**. Then, you mark the **Unit Mass** check box and the **MS/MS (QQQ, Q-TOF)** check box. Then, click **OK**. • You can also use the example MRM data file that was installed to the **Example Data** folder. If the file is not on your computer, install it from the installation media.
	 	
	q Click **Find > Find Compounds by MRM**.	

Steps	Detailed Instructions	Comments
3 Review the results of the Find Compounds by MRM algorithm. • Make sure that the primary ions are found for each compound. • Switch to the Compound Details view. • It is not possible to edit the retention times of compounds which are identified. NOTE: The retention times for pairs of isomers that have identical MRMs are listed under the Retention Time of the compound that is most abundant.	**a** Close the **Method Explorer** and **Method Editor** windows. **b** Click **View > MS Spectrum Peak List 1**. **c** Click **Compound Details View** to change the view. See the figures that follow. **d** Click or use the arrow keys to move through the **Compound Table** to review one compound a a time. **e** Review each compound. Verify that the primary transitions for each compound were found. Qualitative Analysis is the best program to do a quick review of the MRM compound information and to check the chromatography of multiple data files. NOTE: You can manually edit these retention times in the Quantitative Analysis program. See "Task 1. Create a batch file from an existing MRM data file" on page 22.	• You can also print a Compound Report to review results. You click **File > Print > Compound Report**. The Compound Report sorts the compounds by retention time. • In the Chromatogram Results window, you can see the abundances for each transition. • In Compound Details View, click each compound, or use the  and  buttons in the compound list window to review the results. • In the Navigator view, you need to select a compound and click **Edit > Show > Only Highlighted** to show only that compound. You can switch compounds in the Data Navigator window, or you can click the  or  buttons in the Compound List window.

Steps	Detailed Instructions	Comments



Steps	Detailed Instructions	Comments



Creating a Dynamic MRM acquisition method

To create dMRM methods, retention times (RT) and RT windows are added to MRM methods. dMRM methods are very useful for targeted analysis of a large number of compounds, each with quantifier and qualifier ions. The creation of dMRM method from an MRM method is the second step in the tMRM method creation workflow.

The process to create methods that contain large numbers of standards is described in Figure 1. The figure shows an example of 150 standards. You can update an existing MRM method to a Dynamic MRM (dMRM) method using the MRM Update Options dialog box if you have an MRM data file. You can either specify the data file directly in this dialog box or you can create a report in the Quantitative Analysis program and specify the report file.

For the MassHunter MRM/dMRM/tMRM Database, Agilent recommends that you create a Quantitative Analysis report to specify in "Task 3. Create a dMRM method using Update dMRM" on page 31.

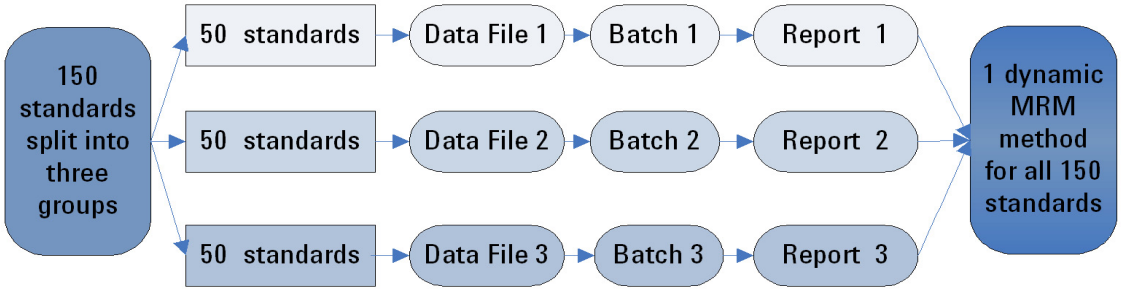


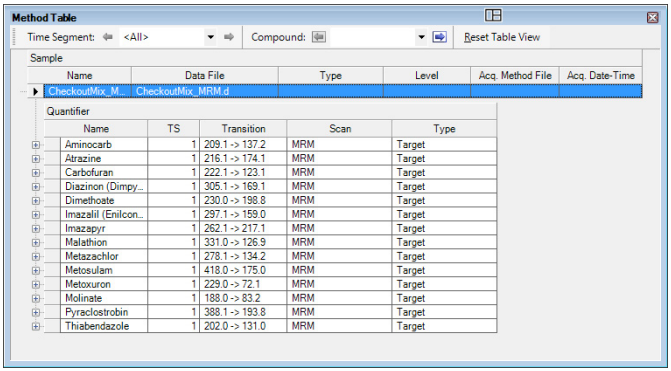
Figure 1 Example process for analyses that have more than 50 compounds

Task 1. Create a batch file from an existing MRM data file

In this exercise, you create a batch and a method from an existing MRM data file.

Steps	Detailed Instructions	Comments
1 Open the Quantitative Analysis program and create a batch file with one sample file, **CheckoutMix_MRM.d**.	**a** Double-click the **QQQ Quantitative Analysis B.07.00** icon. **b** Click **File > New Batch**. **c** Navigate to installed data in the folder **\MassHunter\Data*Product* Database *x.xx.xx*\Example data**. **d** Type `CheckoutMix_MRM` in the **File Name** text box. **e** Click **Open**. **f** To add samples, select the file **CheckoutMix_MRM.d**. **g** Click **OK**.	• The file **CheckoutMix_MRM.d** is installed in the folder **\MassHunter\Data*Product* Database *x.xx.xx*\Example data** folder. • You can also use the **CheckoutMix_MRM.d** file that you created if you ran the Checkout Mix in the previous exercise. Your results can vary slightly.
2 Create a method for that batch using MRM data.	**a** Click **Method > New > New Method from Acquired MRM data**. **b** Select the **CheckoutMix_MRM.d** data file, click **Open**. **c** Right-click the Method Table and click **Collapse All**. **d** Click **View > Window Layout > Table Top**. **e** Close the Sample Information window.	• You can change which windows are displayed when you use the View menu. • You can open or close a single window. • You can also load a layout which already has specific windows displayed in specific locations. • You can also load or save layouts. See the online Help in the Quantitative Analysis program for more information.

Steps	Detailed Instructions	Comments



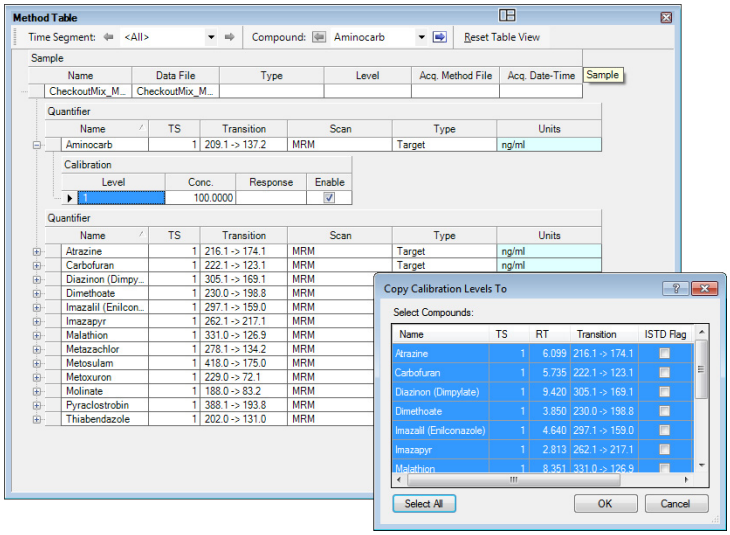
3 Set the Concentration Setup, Qualifier Setup, and Calibration Curve Setup.

- Add calibration level 1 with a concentration of 100.
- Set the **Uncertainty** to Relative for all qualifiers.
- Set the **Curve Fit** to Linear.
- Set the **Curve Fit Origin** to **Force**.
- Set the **Curve Fit Weight** to None.

a Select **Concentration Setup** in the Method Setup Tasks section in the Method Tasks pane.
b Select the first compound in the table.
c Right-click the compound row and click **New Calibration Level** from the shortcut menu.
d In the **Level** column, type `1`. In the **Conc.** column, type `100`.
e Right-click in the Level box and click **Copy Calibration Levels To**.
f Click **Select All**. Click **OK**.
g Select **Qualifier Setup** in the Method Setup Tasks section.
h Verify that the **Uncertainty** is Relative.
i Select **Calibration Curve Setup** in the Method Setup Tasks section.
j Set **Curve Fit** to **Linear** for all compounds.
k Set **CF Origin** to **Force** for all compounds.
l Set **CF Weight** to **None** for all compounds.

- Refer to the online Help in the Quantitative Analysis program for additional help on these tasks.
- After you select the option for the first compound in the Method Table, you can right-click the option and click **Fill Down** from the shortcut menu.

Steps	Detailed Instructions	Comments



- Verify retention time elution order:
  - Aminocarb
  - Imazapyr
  - Thiabendazole
  - Dimethoate
  - Imazalil (Enilconazole)
  - Metoxuron
  - Carbofuran
  - Atrazine
  - Metosulam
  - Metazachlor
  - Molinate
  - Malathion
  - Pyraclostrobin
  - Diazinon (Dimpylate)

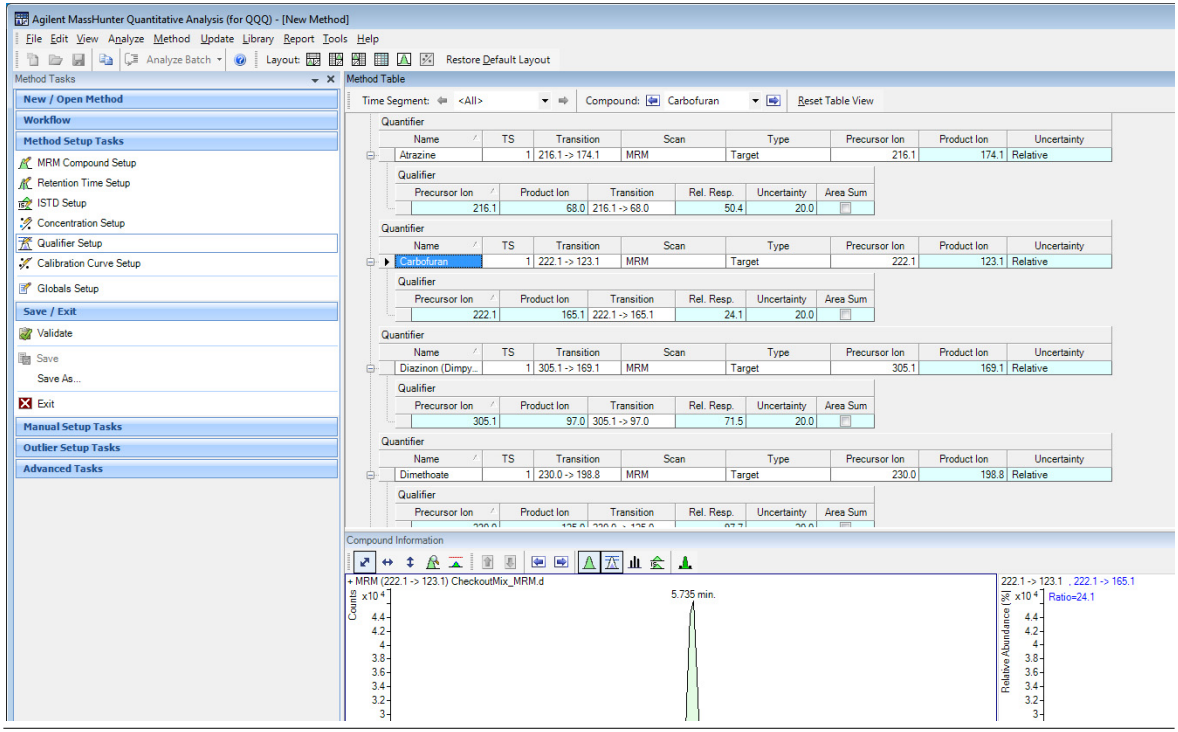
m Select **Retention Time Setup** in the Method Setup Tasks section.

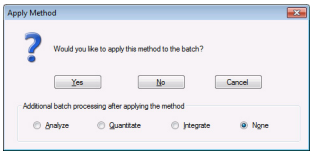
n (optional) Enter 2 for the **Left RT Delta** and **Right RT Delta** for each compound to compensate for potential RT drift.

o Verify the retention time order of the analytes is the same as shown in the figure below. At this time, if your sample contains isomeric compounds, you need to resolve any retention time issues for the isomeric compounds by changing the RT value in the Method Table.

- If you increase the retention time window to cover the complete run, then all compounds that share the same precursor and product ion are seen. In these cases, the automatic processing always picks the more abundant peak.
- Depending on the delay volume, the compounds Pyraclostrobin and Diazinon can co-elute, separate slightly, or reverse elution order.

Steps	Detailed Instructions	Comments
• Review qualifier ratios	**p** Select **Qualifier Setup** in the Method Setup Tasks section. **q** Right-click the Method Table and click **Expand All**. **r** Click **View > Window Layout > Restore Default Layout**. **s** Click **View > Sample Information** to close the Sample Information window. **t** Click the Show/Hide Qualifiers button in the toolbar in the Compound Information window. **u** Click on each compound and verify that the Rel. Resp. for each Qualifier matches the value shown in the Compound Information window in the spectrum pane.	



Steps	Detailed Instructions	Comments
4 Verify method and then save the method and apply the method to the batch.	**a** Click **Method > Validate.** **b** Click **OK** on the message box. Fix any errors, if necessary. **c** Click **Method > Save As**. **d** Type `Checkout_MRM_to_DMRM`. **e** Click the **Save** button. **f** Click **Method > Exit**. **g** For the additional batch processing option, select **None**. **h** Click **Yes** to apply the method to the batch.	
5 Analyze and save the batch.	**a** In the Batch Table window, select **Cal** as the **Type**. **b** Click **Analyze > Analyze Batch.** **c** Click **File > Save Batch**.	
6 Review the batch to resolve errors or messages that are indicated in the Batch Table.	• Resolve isomers. • Check qualifier ratios. • Resolve errors and messages	
7 Save the batch again.	• Click **File > Save Batch**.	

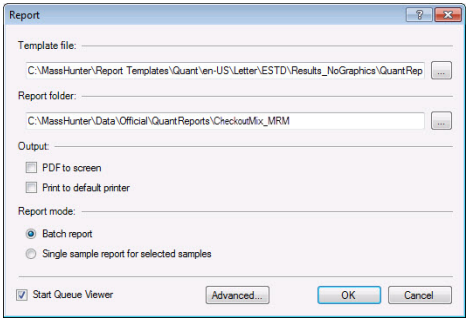
Task 2. Print a report in the Quantitative Analysis program

In this task, you create the template file **report.results.xml** that you use to update the MRM method to a dMRM method. You can use any report template, but the quickest report to create is a summary report without graphics.

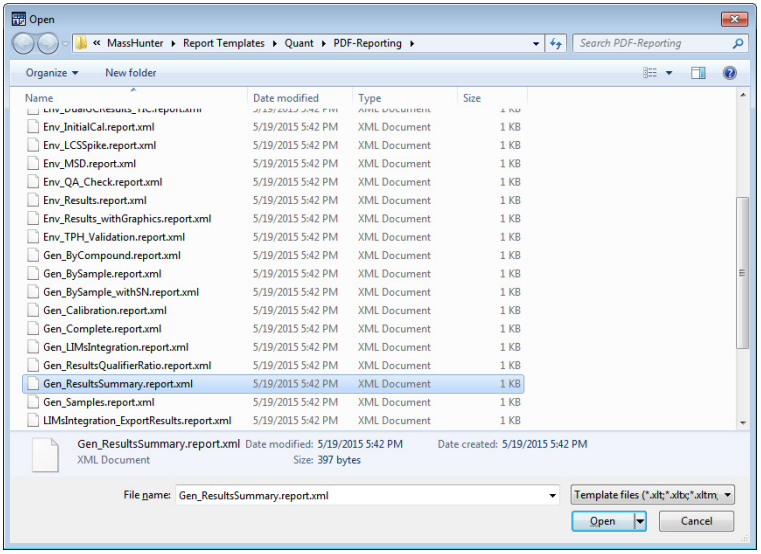
You can use either a Quantitative Analysis report or a data file to create a dMRM method, but the Quantitative Analysis report is recommended. If you use a data file and an error is generated, then none of the compounds in that data file are included in the dMRM method.

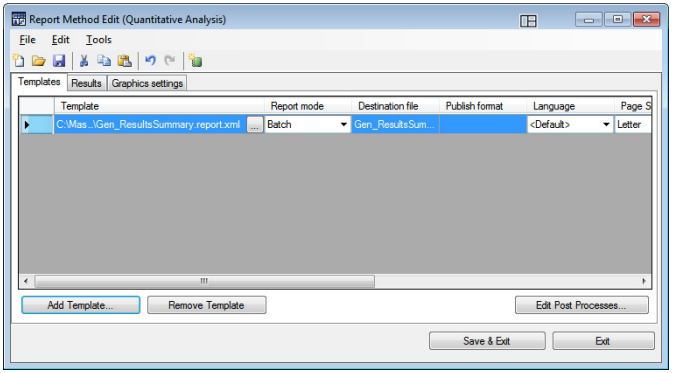
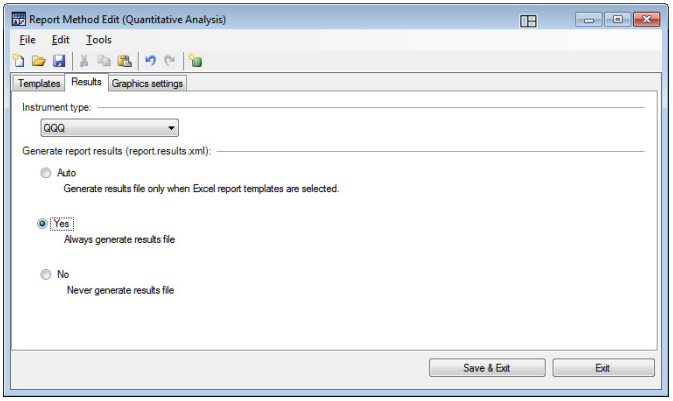
In this task, you:

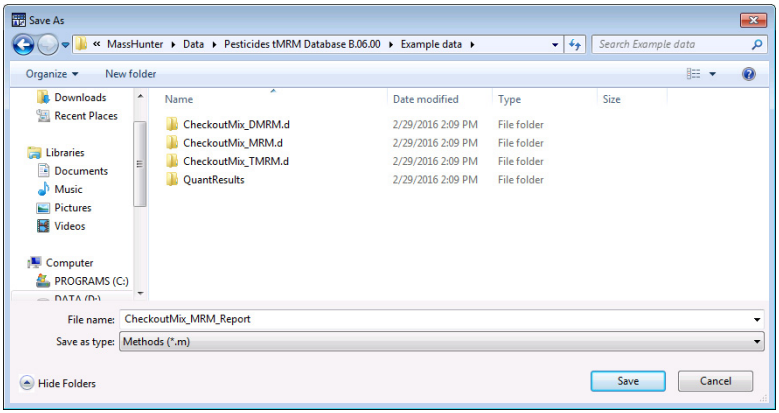
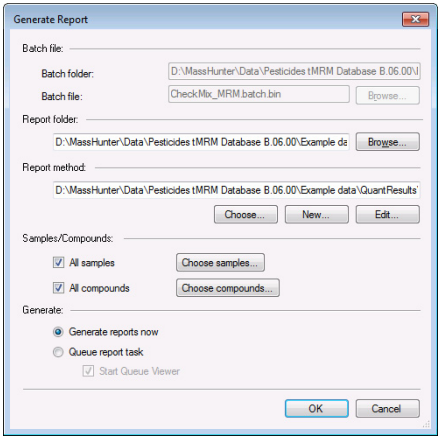
- Manually generate a report for a data file.
- Remove all errors in the manually generated quantitation method.

Steps	Detailed Instructions	Comments
1 (*For Quantitative Analysis B.05.02*) Print a report. Use a template that creates a summary report without graphics for fastest report creation.	**a** See "Task 1. Create a batch file from an existing MRM data file" on page 22. **b** Click **File > Save**. **c** Click **Report > Generate**. The system displays the Report dialog box. **d** Select a **Template** file. **e** Select the **Report** folder. This folder name is used in the next task. **f** Click **OK**.	• For this report, you do not need to print the report. You need to click **Advanced** to select a different printer. If you don't want to print this report, click **Advanced** instead.
		

Steps	Detailed Instructions	Comments
1 (*For Quantitative Analysis B.07.01 and higher*) Print a report. Use a template that creates a summary report for fastest report creation.	**a** See "Task 1. Create a batch file from an existing MRM data file" on page 22. **b** Click **File > Save**. **c** Click **Report > Generate**. The Generate Report dialog box opens. **d** Under **Report method**, click **New**. The Report Method Edit program opens. **e** Click **Add Template**. The Open dialog box opens. **f** Navigate to the folder **MassHunter\Report Templates\ Quant\PDF-Reporting** **g** Select a simple report, such as **Gen_ResultsSummary.report.xml**. Click **Open**.	



Steps	Detailed Instructions	Comments
		
	h In the Report Method Edit program, click **Results**. **i** Click **Yes**.	
		

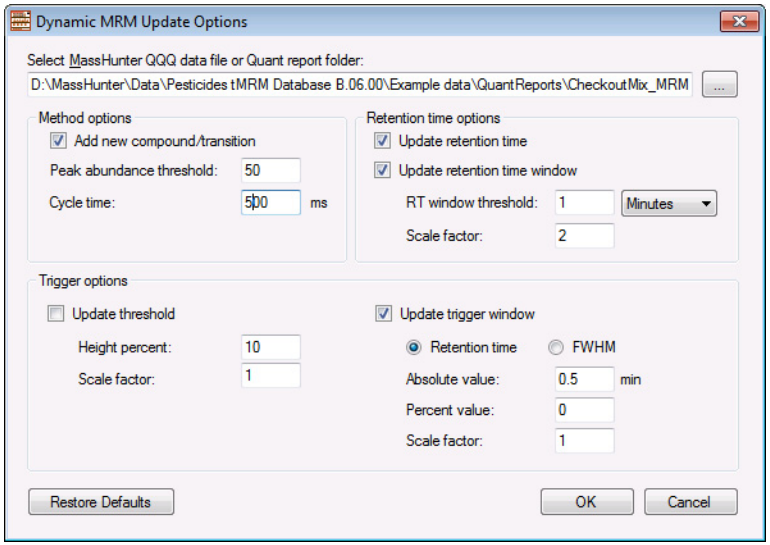
Steps	Detailed Instructions	Comments
	j Click **File > Save Method As**. The Save As dialog box opens. **k** Navigate to the example data folder. **l** For the **File name**, type `CheckoutMix_MRM_Report`. **m** Click **Save**.	
		
	n In the Report Method Edit program, click **File > Exit**. **o** Click **Generate reports now**. **p** Click **OK**.	The report.results.xml file is in the **\MassHunter\Data*Product* Database *x.xx.xx*\Example data\QuantReports\CheckoutMix_MRM** folder.
		

Task 3. Create a dMRM method using Update dMRM

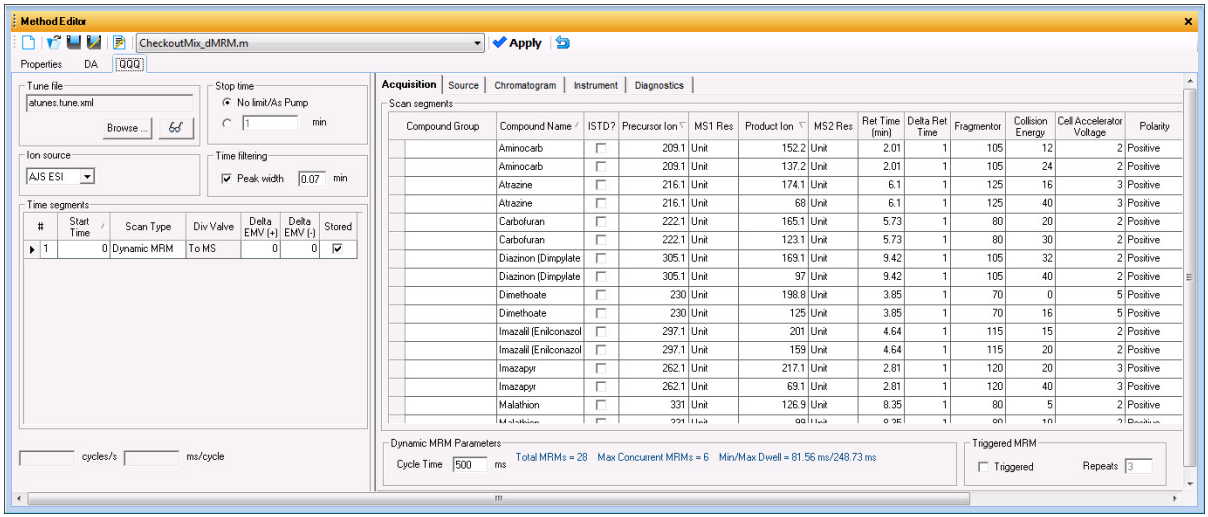
You can create a dMRM method from an MRM data file or a Quantitative Analysis report. You use the Update MRM Method dialog box.

Steps	Detailed Instructions	Comments
1 Open the method **CheckoutMix_MRM.m** and save it to a new name with the format ***iii*CheckoutMix_DMRM.m**, where ***iii*** are your initials.	**a** In the Data Acquisition program, click **Method > Open**. **b** Select the **CheckoutMix_MRM.m** method. Click **OK**. **c** Click **Method > Save As**. **d** Type the new method name with the format ***iii*CheckoutMix_DMRM.m.**	• In this example, the batch is in the **\MassHunter\Data*Product* Database *x.xx.xx**Example data*** folder. • The LC conditions must be the same as those used to acquire the MRM data files so that the retention times will be the same.

Steps	Detailed Instructions	Comments
2 Update the method to change from an MRM method to a Dynamic MRM method with the same compounds. If you have isomers in the data file, specify a report instead of a data file for the source of the update, to ensure that you identify the isomers correctly. Do not manually change the Scan Type to Dynamic MRM. If you do, the existing Scan segments table is cleared.	**a** Click the **Acquisition** tab in the QQQ tab in the Method Editor window. **b** Right-click the **Scan segments** table and click **Update DMRM Method**. The MRM Update Options dialog box opens. **c** Select the folder containing the *report.results.xml* file. The name of this folder is shown in the Report dialog box in the Quantitative Analysis program. By default, this file is in a folder in the QuantReports folder. The QuantReports folder is in the same folder as the Batch. By default, the folder has the same name as the Batch. **d** Under **Method Options**, check **Add new compound/transition.** **e** For **Peak abundance threshold**, enter 50. **f** For **Cycle time**, enter 500 ms. **g** Under **Retention time options**, select **Update retention time** and **Update retention time window**. **h** For **RT window threshold**, select 1. From the drop-down list, select **Minutes**. **i** For **Scale factor**, select **2**. **j** Under **Trigger options**, clear the **Update threshold** check box. **k** Mark the **Update trigger window** check box. **l** For **Absolute value (mins)**, select **0.5**. **m** Review other parameters. **n** Click **OK**.	• The Update MRM Method tool automatically sets the Scan type to **Dynamic MRM**. • You can select either a data file that was acquired with a **Scan Type** of **MRM** or a Quant Report folder as the input to this dialog box. It is recommended to use the Quant Report. • The Delta Retention Time is scaled to the peak width found for that compound. A scale factor of 2 creates a retention time window that is 2 times the peak width (baseline to baseline). Choose a larger factor if you want to acquire more data points for the transition. • A Delta Retention Time or Retention Time Window of 1 minute is chosen for this method. A large delta retention time is recommended for early eluting compounds, which tend to have a high background. This ensures sufficient baseline for the peak integration. The automatic calculation which provides a smaller delta retention time is recommend for later eluters. • The dwell times for MRM transitions will depend on the number of overlapping peaks and their respective peak widths. • The method is now updated with the transitions, parameters, and retention times in the Quantitative Analysis report.

Steps	Detailed Instructions	Comments
		**You can update the compounds in the Scan segments table by using a QQQ data file or a Quantitative Analysis report folder.** **If you select a QQQ data file and an error is generated, none of the compounds is updated. In case of an error, manually create the report and select the report folder in this location instead of the QQQ data file. See** "Task 2. Print a report in the Quantitative Analysis program" **on page 27.** **Note that all transitions must be detected in each data file, or the MassHunter Quantitative Analysis program will generate an error when you update the method.** **Note that the cycle time in the MRM Update Options dialog box is applied only the first time the method is created using the Update Method function. After that, the cycle time must be manually typed into the QQQ > Acquisition tab.** **When you close the method viewer, changes made to the cycle time in the viewer are not entered into the acquisition method.**

Steps	Detailed Instructions	Comments



o Review the results of updating the MRM method to a dMRM method and then click **Close**.

p Verify that each row has a **Compound Name**. A blank Compound Name is not allowed.

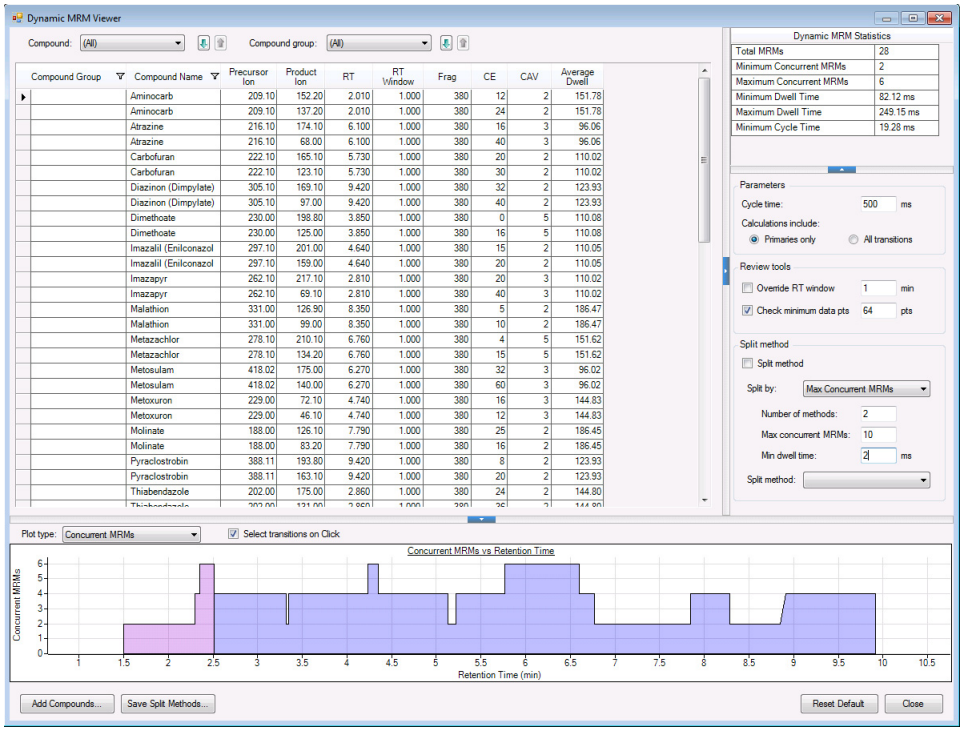
q Click **Method > Save**.

Task 4. Check dMRM acquisition method setup in Dynamic MRM Viewer

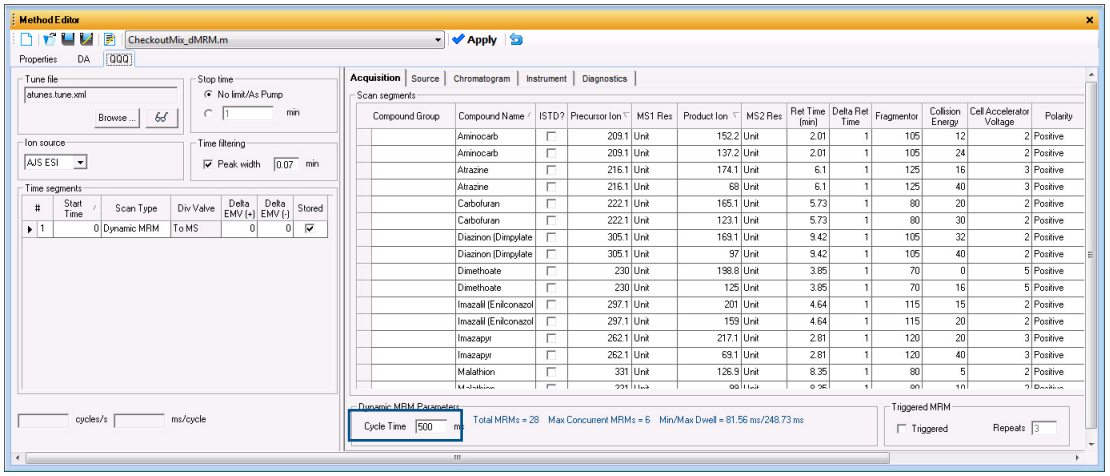
The Dynamic MRM Viewer provides a powerful display to show you important details of your method. The maximum and minimum Dwell times in milliseconds are shown in the table.

- A dwell time of 5 ms or more is recommended to acquire data for dMRM. If cycle time and concurrent MRMs reduce the dwell time to below this value, the minimum cycle time and minimum dwell time on the right are highlighted. Increase the cycle time or extend the LC run can correct the minimum dwell time problem.
- At a minimum, for good quantitative results, peaks must have at least 10 data points. In an example of a 5 second peak width, a cycle time of 500 ms barely provides this.

Steps	Detailed Instructions	Comments
1 Start the Dynamic MRM Viewer dialog box.	• Right-click the Scan segments table and click **Edit DMRM Method**.	
2 Review each compound in the Dynamic MRM Viewer dialog box.	**a** Click each compound in the table. **b** Verify in the table that two transitions are shown for each compound. **c** Examine the graphic to review how many concurrent MRMs are being acquired with that compound. **d** Adjust the cycle time so that all criteria for **Minimum Dwell Time**, and for good integration are met. **e** Click **Close**.	• To use the Agile integrator, 64 data points are required in the retention time window. Either increase the Delta Ret Time for the transition(s) with less than 64 points, or decrease the cycle time. As a general rule, set the retention time factor based on reproducibility of the chromatography.

Steps	Detailed Instructions	Comments
		
		• When you change the cycle time in the Dynamic MRM viewer, you immediately see its effects on the **Minimum Dwell Time** and **Maximum Dwell Time**.
3 Once a cycle time is determined for good integration, set the Cycle Time in the QQQ > Acquisition tab.	**a** Type the **Cycle Time**, if necessary. **b** Save the method. The default setting of 500 ms is recommended for most analysis containing more than 15 compounds.	• When you close the Dynamic MRM Viewer, changes made to the cycle time in the Dynamic MRM Viewer are *not* entered into the acquisition method.

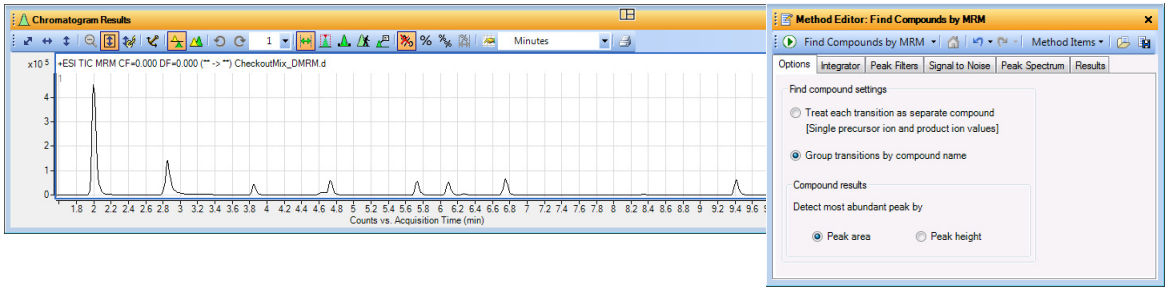
Steps	Detailed Instructions	Comments



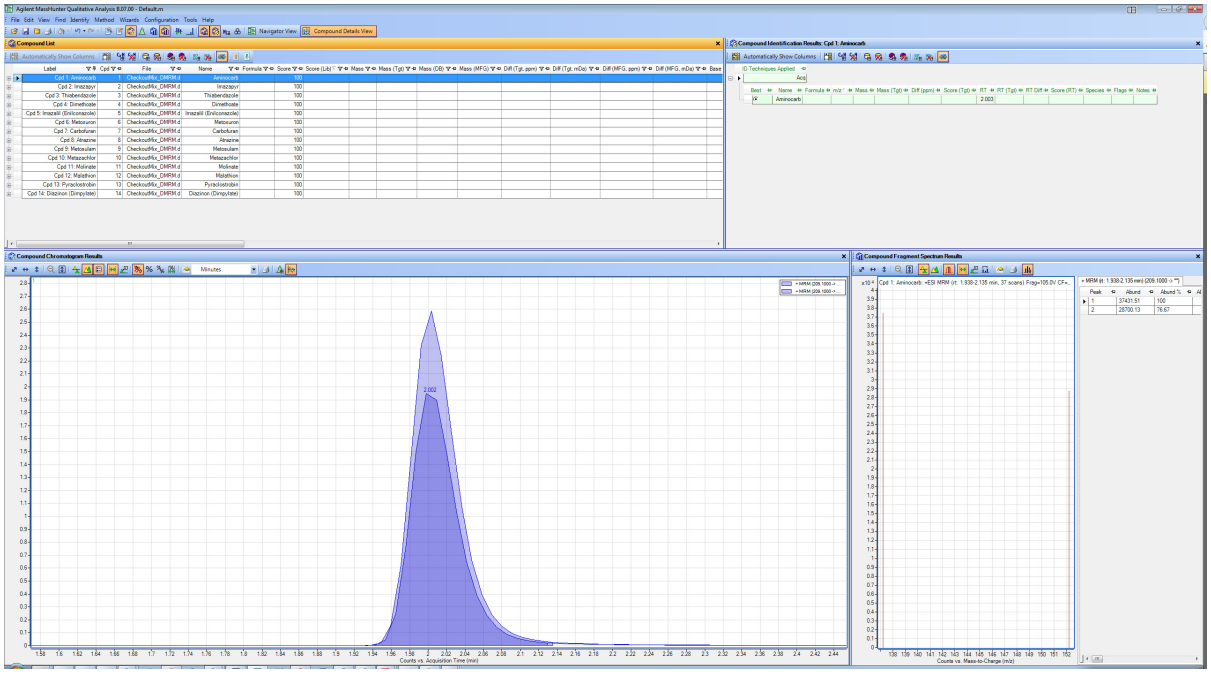
Task 5. Acquire dMRM data and inspect in Qualitative Analysis

After you acquire the dMRM data file, you examine the data file in the Qualitative Analysis program to verify that the transitions were acquired.

Steps	Detailed Instructions	Comments
Do this step if you want to acquire data with the Checkout Mix. Otherwise, continue at step 2. **1** Acquire data. • Set up a one-line worklist with the method you just created. • Name the data file **CheckoutMix_DMRM.d**. • Designate a directory path to hold your data files and method.	**a** If necessary, click **View > Worklist** to display the Worklist window. **b** Click **Worklist > Worklist Run Parameters.** Verify that the parameters are set properly. Click **OK**. **c** Click **Worklist > Add Multiple Samples**. **d** Type `CheckoutMix_DMRM.d` as the data file name **e** Select **CheckoutMix_DMRM.d** as the method name. **f** Click the **Sample Position** tab. **g** Select the Autosampler, Well-plate or Vial Tray. **h** In the graphic, select a single position. Click **OK.** **i** In the Worklist window, mark the check box to the left of the sample. **j** Click the **Start Worklist Run** icon in the main toolbar, the **Run Worklist** icon in the Worklist toolbar or click the **Worklist > Run** command.	• The Worklist window is tabbed with the Method Editor window by default. Click the **Worklist** tab to show the Worklist window. • See also "To run the Checkout Mix" on page 6.

Steps	Detailed Instructions	Comments
2 Find compounds using the Find Compound by MRM algorithm in the Qualitative Analysis program. • Open the data file **CheckoutMix_DMRM.d**.	Start the Qualitative Analysis program. If it is not running, double-click the **Qualitative Analysis** **a** icon,  . **b** Click **File > Open Data File**. The system displays the "Open Data File" dialog box **c** Select **CheckoutMix_DMRM.d**, and click **Open**. **d** Click **Method > Method Explorer** or **View > Method Explorer**. The system displays the Method Explorer window. **e** In the Find Compounds section, click **Find by MRM**. **f** In the Method Editor window, click the **Group transitions by compound name** option. **g** Click the **Peak area** option for **Detect most abundant peak by**.	• If the Find by MRM section is not available, you need to modify the options available in the User Interface Configuration dialog box. You click **Configuration > User Interface Configuration**. Then, you mark the **Unit Mass** check box and the **MS/MS (QQQ, Q-TOF)** check box. Then, click **OK**. • You can also use the example dMRM data file in the **Example Data** folder. If the data file is not on your computer, install it from the installation media.
		
	h Click **Find > Find Compounds by MRM**.	

Steps	Detailed Instructions	Comments
3 Review the results of the Find Compounds by MRM algorithm. • Make sure that the transitions are found for each compound. • These transitions become the primary transitions if you create a tMRM method. • It is not possible to edit the retention times of compounds which are identified.	**a** Close the **Method Explorer** and **Method Editor** windows. **b** Click **Compound Details View** to change the view. See the figure that follows. **c** Click or use the arrow keys to move through the **Compound Table** to review one compound a a time. **d** Verify that the transitions for each compound were found. Qualitative Analysis is the best program to do a quick review of the MRM compound information and to check the chromatography of multiple data files.	• You can also print a Compound Report to review results. You click **File > Print > Compound Report**. The Compound Report sorts the compounds by retention time.



Creating a Triggered MRM Method

To create tMRM methods, trigger parameters and secondary transitions are added to dMRM methods. tMRM provides further confirmation, especially for those compounds that share the same primary transitions.

The creation of a tMRM method from a dMRM method is the last step in the tMRM method creation workflow.

During method development, the trigger parameters Threshold, Trigger Entrance Delay, Trigger Delay and Trigger Window are first created in the method for standards in solvent. These trigger parameters need to be checked when standards are diluted in a complex matrix.

Triggering parameters and their function

Trigger Entrance Use this parameter to shift the acquisition of secondary ions towards apex of peak. When the signal for the designated primary MRM transitions cross the triggering Threshold, the Trigger Entrance Delay postpones triggering for a user-defined number of cycles, which moves the acquisition of secondary MRM transitions closer to the apex of the peak.

Trigger Delay Use this parameter to spread acquisition of secondary ion across the peak. Once the triggering Threshold is met, the trigger delay defines the number of cycles to skip between triggers, which spreads the acquisition of secondary MRM transitions across a peak. This function can be combined with the Trigger Entrance Delay function.

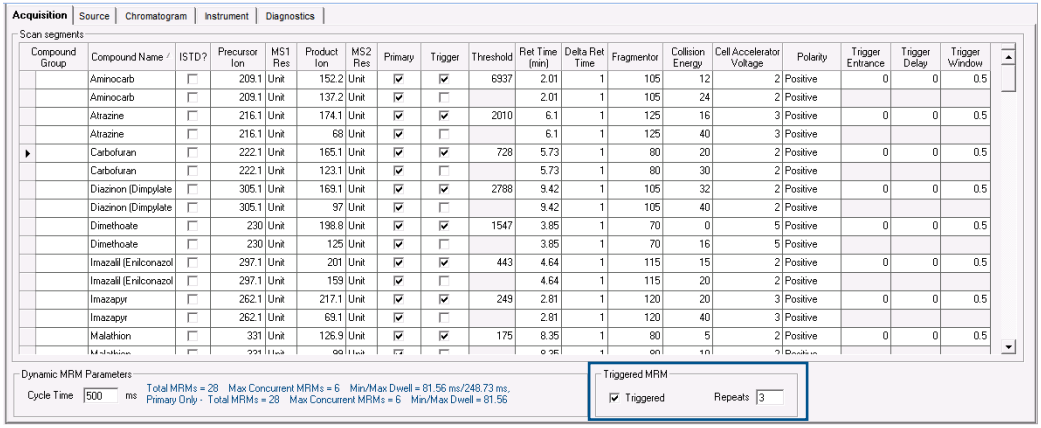
Trigger Window Use this parameter to confine the activation of all triggering functions to a user-defined window around the expected retention time for a particular peak. This function increases triggering specificity based on the target compounds and known retention times for a particular tMRM method.

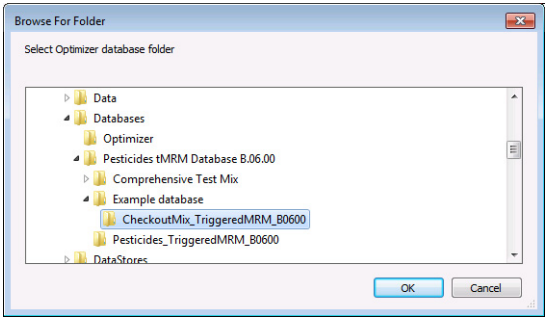
Triggered MRM Repeats Use this parameter to define the number of secondary transition cycles that are acquired. This parameter applies to the whole triggered MRM method, not to individual compounds.

Task 1. Create a tMRM method from a dMRM method

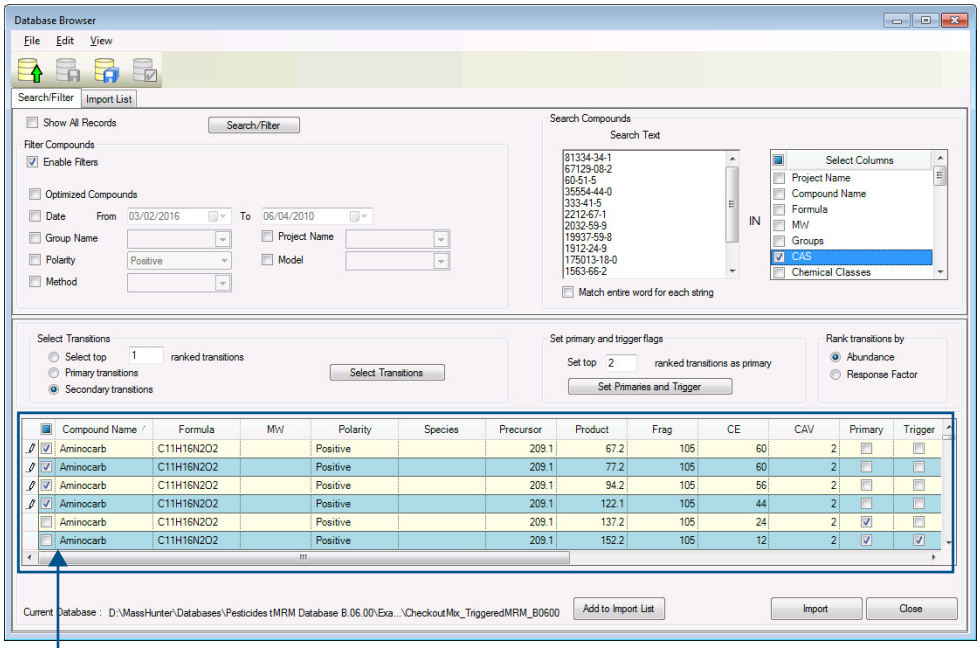
If you have a dMRM method, you can change it to a tMRM method.

Steps	Detailed Instructions	Comments
1 In the Data Acquisition program, you open the dMRM method: **CheckoutMix_DMRM.m** You can open the method that you created or the example method.	**a** Switch to the Data Acquisition program. **b** Open the **CheckoutMix_DMRM.m** method.	• An example **CheckoutMix_DMRM.m** method can be found in the **Example methods** folder and also on the installation media.
2 Change the method to a tMRM method and start to import the secondary transitions from the Database Browser.	**a** In the Method Editor window, click the **QQQ** > **Acquisition** tab. **b** Mark the **Triggered** check box under Triggered MRM. **c** Manually mark the Triggers shown in the "Primary and Secondary Transitions for Triggered MRM" on page 68. **d** Type 3 for **Repeats**. **e** Right-click the Scan Segments table and click **Import from Database Browser**. The Database Browser opens.	• The triggering information is loaded from the Database Browser even if the **Triggered** check box is clear. This includes the trigger Threshold values if the Trigger MRM Threshold column has a value. • Later in this section, we replace the values manually with the values shown "Primary and Secondary Transitions for Triggered MRM" on page 68.



Steps	Detailed Instructions	Comments
3 Open the **CheckoutMix_TriggeredMRM_B0600** database in the Database Browser.	**a** In the Database Browser, click **File > Open Database**. **b** Select the **CheckoutMix_TriggeredMRM_B0600** database in the folder **\MassHunter\Databases*Product Database x.xx.xx*\Example database**. **c** Click **OK**.	If the transitions in the example method do not match the transitions in the database, use the transitions in the database.
		
4 Select secondary transitions.	**a** Click **Abundance** under Rank transitions by. **b** Click the **Secondary transitions** option under Select Transitions. **c** Click the **Compound Name** column header to sort the compounds by Compound Name. **d** Mark the check boxes next to the secondary transitions for each of the compounds in the dMRM method. See "Primary and Secondary Transitions for Triggered MRM" on page 68. **e** Review the transitions in the table. Clear the check box next to any secondary transition that you do not want to include.	• The *Aminocarb* compound has two primary transitions and four secondary transitions. • You can also clear the **Show All Records** check box. Then, you can search for each compound in the database by writing on separate lines the full name or CAS number of each compound in the **Search Text** list, mark the **Compound Name** or **CAS** check box, and then click **Search Filter**. • Once you have the list of desired compounds, click the **Secondary transitions** button and then click **Select Transitions**. All of the secondary transitions for the compounds in the table are marked.

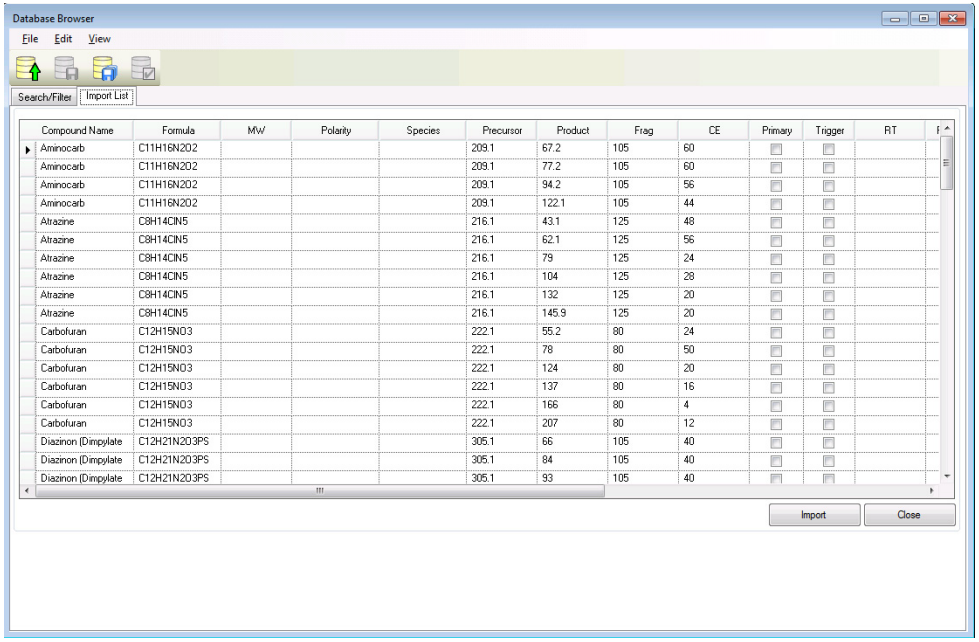
Steps	Detailed Instructions	Comments



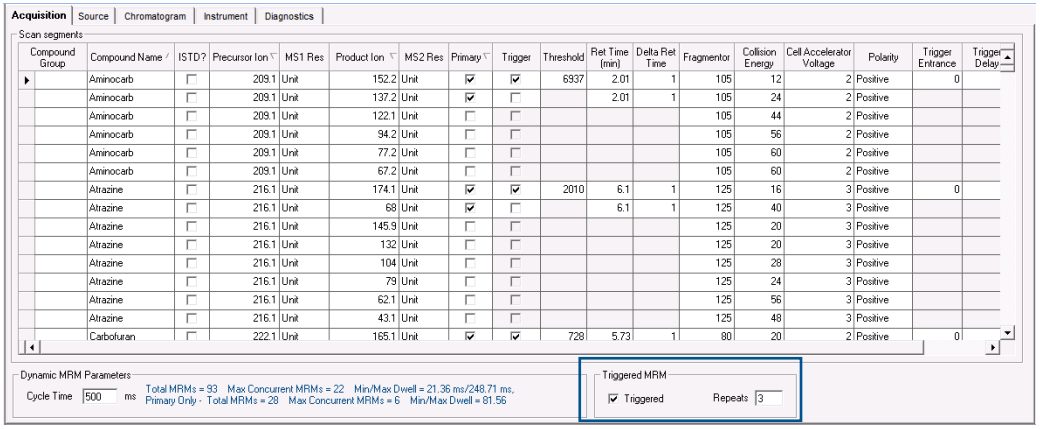
Do not mark the two Primary transitions.

5	Import secondary transitions to the Data Acquisition program. (If you are using these steps to customize your own method, remove negative MRM transitions from any compound with positive MRM transitions.)	**a** Click the **Add to Import List** button. **b** Click the **Import List** tab. **c** Review the Import List table. **d** Select all negative MRM transitions for any compounds with positive MRM transition. Right-click the selection, and then click **Remove**. **e** Click the **Import** button.	• The compound Aminocarb has four secondary transitions. • Only the transitions that you marked are added to the Import List. • All transitions that have the same **Compound Name** are part of the same compound.

Steps	Detailed Instructions	Comments
NOTE	• Qualifier and quantifier ions must have the same polarity, so one compound cannot contain both negative and positive polarity transitions. If you want to include both polarities for one compound while you develop a method, you must rename the compounds in the method to "*compoundname*_pos" and "*compoundname*_neg". When the best polarity and transitions are found for a compound, remove from the method all other transitions for the compound. Then remove "_pos" or "_neg" from the remaining compound name. • To ensure good signal/noise ratios for the secondary transitions, the superfluous secondary transitions which are not required for confirmation must be removed. Secondary transitions required for confirmation should be as unique as possible to a particular analysis or have intense ion peaks.	

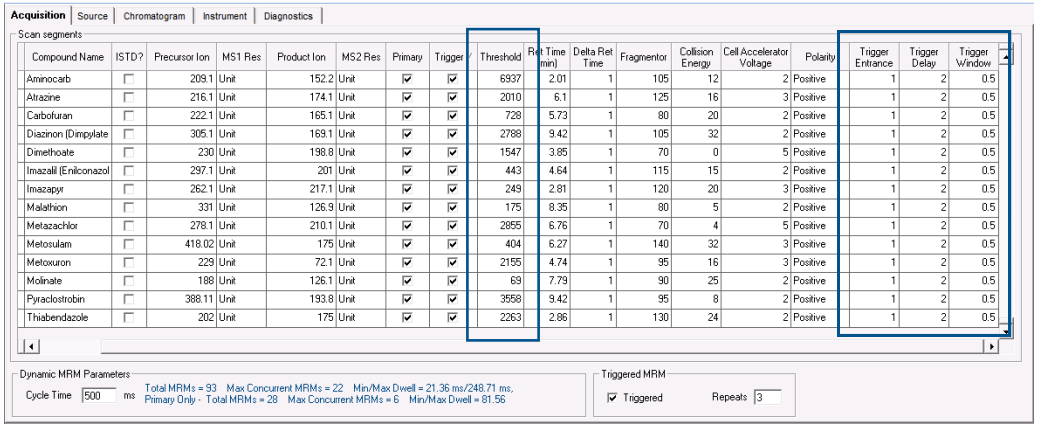


Steps	Detailed Instructions	Comments
6 Review the secondary transitions in the Data Acquisition program.	**a** In the Acquisition tab, sort the table by the **Compound Name**. **b** Review the primary and secondary transitions for each compound.	• If a red box appears in the Scan segments table, you click the **Apply** button in the toolbar. If the red box does not clear, the value is not valid.



7 Enter the **Trigger Entrance Delay**, **Trigger Delay** and **Trigger Window** values.	**a** Sort the table by the Trigger column. **b** For each Trigger transition, type `1` for the **Trigger Entrance**. You can type `1`in the first row, right-click and select **Fill down**. **c** For each Trigger transition, type `2` for the **Trigger Delay**. **d** For each Trigger transition, type `0.5` for the **Trigger Window**. **e** For each Trigger transition, type the threshold from "Primary and Secondary Transitions for Triggered MRM" on page 68.	• See the QQQ Concepts guide or the online Help for more information on these values. • The trigger **Threshold** values are brought over automatically from the Database Browser if the Trigger MRM Threshold column has a value. This column is not visible by default in the Database Browser. • In the MRM Update Options dialog box, you can also select to update the Trigger Threshold from the MassHunter QQQ data file or Quant report folder. See "Task 3. Create a dMRM method using Update dMRM" on page 31. • You can also enter the Threshold directly.

Steps	Detailed Instructions	Comments



Acquisition | Source | Chromatogram | Instrument | Diagnostics

Scan segments

Compound Name	ISTD?	Precursor Ion	MS1 Res	Product Ion	MS2 Res	Primary	Trigger	Threshold	Ret Time (min)	Delta Ret Time	Fragmentor	Collision Energy	Cell Accelerator Voltage	Polarity	Trigger Entrance	Trigger Delay	Trigger Window
Aminocarb	☐	209.1	Unit	152.2	Unit	☑	☑	6937	2.01	1	105	12	2	Positive	1	2	0.5
Atrazine	☐	216.1	Unit	174.1	Unit	☑	☑	2010	6.1	1	125	16	3	Positive	1	2	0.5
Carbofuran	☐	222.1	Unit	165.1	Unit	☑	☑	728	5.73	1	80	20	2	Positive	1	2	0.5
Diazinon (Dimpylate	☐	305.1	Unit	169.1	Unit	☑	☑	2788	9.42	1	105	32	2	Positive	1	2	0.5
Dimethoate	☐	230	Unit	198.8	Unit	☑	☑	1547	3.85	1	70	0	5	Positive	1	2	0.5
Imazalil (Enilconazol	☐	297.1	Unit	201	Unit	☑	☑	443	4.64	1	115	15	2	Positive	1	2	0.5
Imazapyr	☐	262.1	Unit	217.1	Unit	☑	☑	249	2.81	1	120	20	3	Positive	1	2	0.5
Malathion	☐	331	Unit	126.9	Unit	☑	☑	175	8.35	1	80	5	2	Positive	1	2	0.5
Metazachlor	☐	278.1	Unit	210.1	Unit	☑	☑	2855	6.76	1	70	4	5	Positive	1	2	0.5
Metosulam	☐	418.02	Unit	175	Unit	☑	☑	404	6.27	1	140	32	3	Positive	1	2	0.5
Metoxuron	☐	229	Unit	72.1	Unit	☑	☑	2155	4.74	1	95	16	3	Positive	1	2	0.5
Molinate	☐	188	Unit	126.1	Unit	☑	☑	69	7.79	1	90	25	2	Positive	1	2	0.5
Pyraclostrobin	☐	388.11	Unit	193.8	Unit	☑	☑	3558	9.42	1	95	8	2	Positive	1	2	0.5
Thiabendazole	☐	202	Unit	175	Unit	☑	☑	2263	2.86	1	130	24	2	Positive	1	2	0.5

Dynamic MRM Parameters

Cycle Time 500 ms Total MRMs = 93 Max Concurrent MRMs = 22 Min/Max Dwell = 21.36 ms/248.71 ms,
Primary Only - Total MRMs = 28 Max Concurrent MRMs = 6 Min/Max Dwell = 81.56

Triggered MRM

☑ Triggered Repeats 3

8 In the Data Acquisition program, Save the method to a new method name, ***iii*CheckoutMix_TMRM.m**, where ***iii*** are your initials.

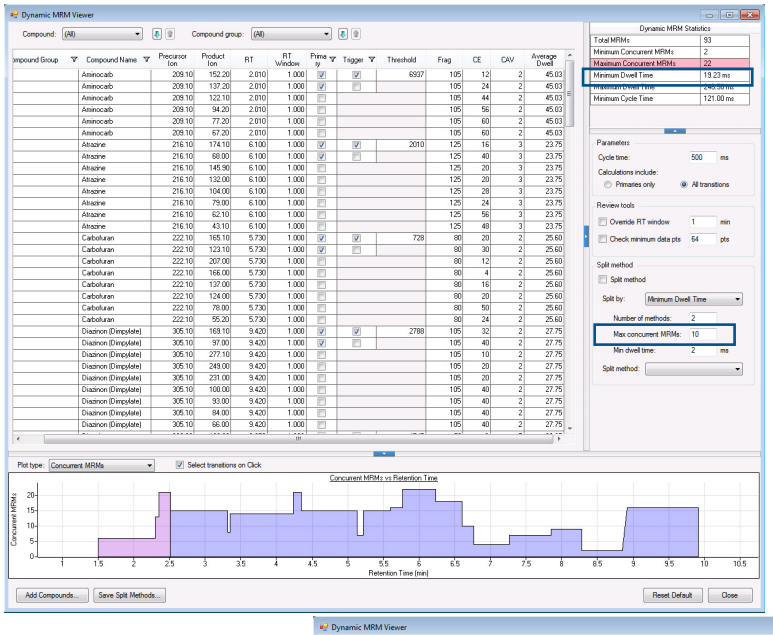
a Click the **Method > Save Method As** command.
b Type ***iii*CheckoutMix_TMRM.m**.
c Click the **Save** button.

9 Review the method in the Dynamic MRM Viewer dialog box.

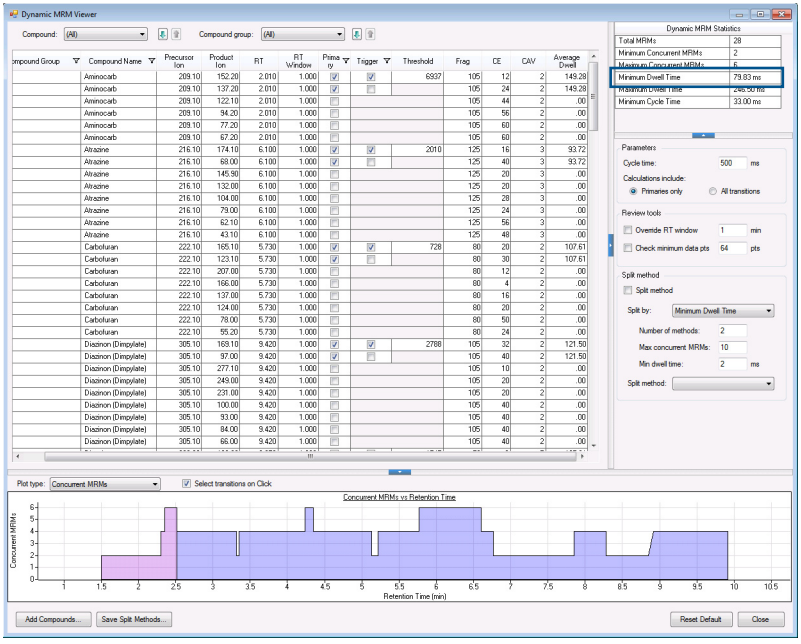
a Right-click the Scan segments table and click **Edit DMRM Method**. The Dynamic MRM Viewer dialog box is opened.
b Switch between the **Primaries only** button and the **All transitions** button if the **Dynamic MRM Statistics** information is not updating.

- Inspect the Dynamic MRM Statistics in the upper right corner. You can modify the **Cycle time** and see how the minimum and maximum **Dwell Times** are changed.
- A **Dwell Time** of 5 ms or higher per transition is fine.
- When you click **All transitions**, **Maximum Concurrent MRMs** value can change.
- If the minimum **Dwell Time** was lower than 5 ms, then you can change the **Cycle time** to a larger value to increase the **Dwell** time.

Steps	Detailed Instructions	Comments



The image to the left shows All transitions. If all of the secondary ions are acquired at the same time, then the Minimum Dwell time is 19.23 ms. However, in the image below, the Primaries only option is clicked. The Minimum Dwell Time is 79.83 ms.



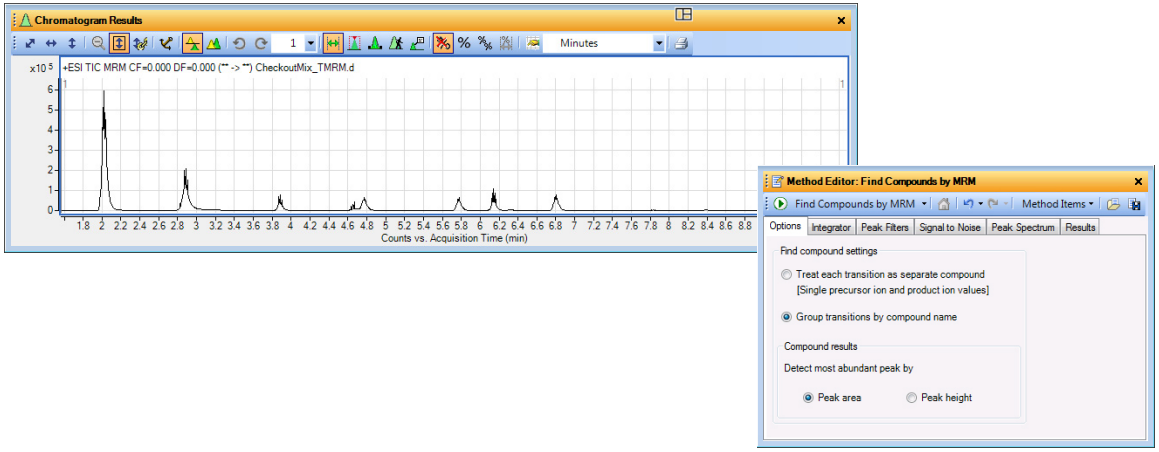
Steps	Detailed Instructions	Comments
10 Adjust the cycle time.	**a** See step 9 on page 47 for details.	• The cycle time can be optimized for each analysis. The default cycle time is 500 ms. For methods that contain more than 15 compounds, the cycle time usually needs to be at least 500 ms. Use the Dynamic MRM Viewer to see what the Minimum Dwell Time is and increase the cycle time so that the Minimum Dwell Time is at least 5.

Task 2. Acquire tMRM data and inspect data in Qualitative Analysis

After you acquire the tMRM data file, you examine the data file in the Qualitative Analysis program to verify that all of the Primary and Secondary transitions were acquired.

Steps	Detailed Instructions	Comments
Do this step if you want to acquire data with the Checkout Mix. Otherwise, continue at step 2. **1** Acquire data. • Set up a one-line worklist with the method you just created. • Name the data file **CheckoutMix_TMRM.d**. • Designate a directory path to hold your data files and method.	**a** If necessary, click **View > Worklist** to display the Worklist window. **b** Click **Worklist > Worklist Run Parameters.** Verify that the parameters are set properly. Click **OK**. **c** Click **Worklist > Add Multiple Samples**. **d** Type `CheckoutMix_TMRM.d` as the data file name **e** Select **CheckoutMix_TMRM.m** as the method name. **f** Click the **Sample Position** tab. **g** Select the Autosampler, Well-plate or Vial Tray. **h** In the graphic, select a single position. Click **OK.** **i** In the Worklist window, mark the check box to the left of the sample. **j** Click the **Start Worklist Run** icon in the main toolbar, the **Run Worklist** icon in the Worklist toolbar or click the **Worklist > Run** command.	• The Worklist window is tabbed with the Method Editor window by default. Click the **Worklist** tab to show the Worklist window. • This step is optional because you can perform the next step with an example data file that comes with the program. If you prefer, you can create your own data file as described in this step. • See also "To run the Checkout Mix" on page 6.

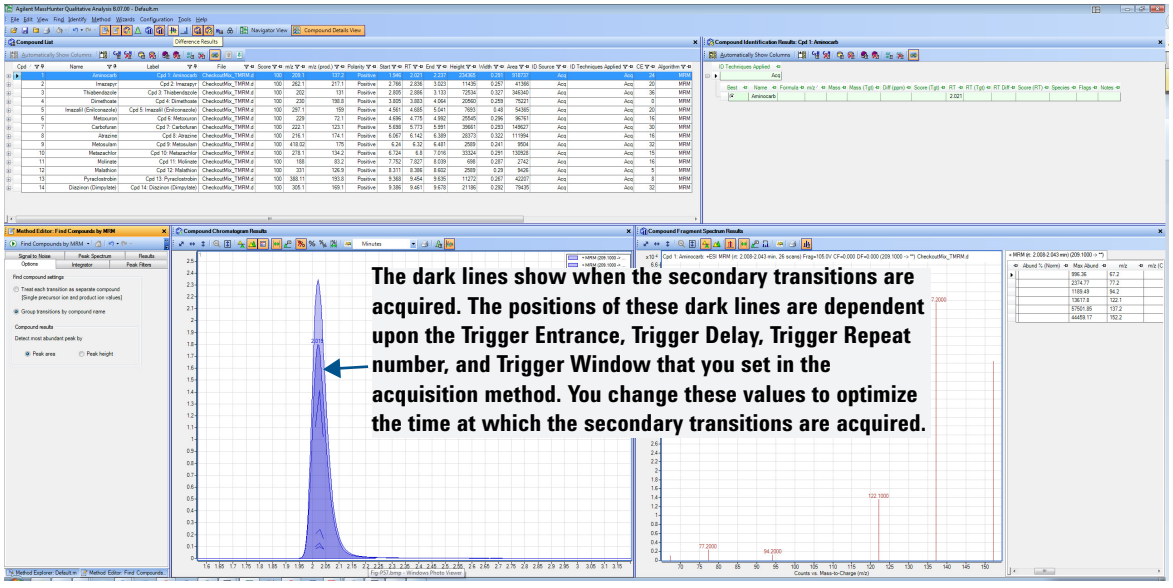
Steps	Detailed Instructions	Comments
2 Find compounds using the Find Compound by MRM algorithm. • Open the data file **CheckoutMix_TMRM.d**.	**a** Start the Qualitative Analysis program. **b** Click **File > Open Data File**. The system displays the "Open Data File" dialog box **c** Select **CheckoutMix_TMRM.d**, and click **Open**. **d** Click **Method > Method Explorer** or **View > Method Explorer**. The system displays the Method Explorer window. **e** In the Find Compounds section, click **Find by MRM**. **f** In the Method Editor window, click the **Group transitions by compound name** option. **g** Click the **Peak area** option for **Detect most abundant peak by**.	• If the Find by MRM section is not available, you need to modify the options available in the User Interface Configuration dialog box. You click **Configuration > User Interface Configuration**. Then, you mark the **Unit Mass** check box and the **MS/MS (QQQ, Q-TOF)** check box, and click **OK**. • The peaks in the TIC have a jagged appearance due to the triggering. This is the expected appearance. When the secondary transitions are acquired, the abundance in the TIC is increased immediately. • You can also use the example tMRM data file in the **Example Data** folder.If this file is not on your computer, install it from the installation media.



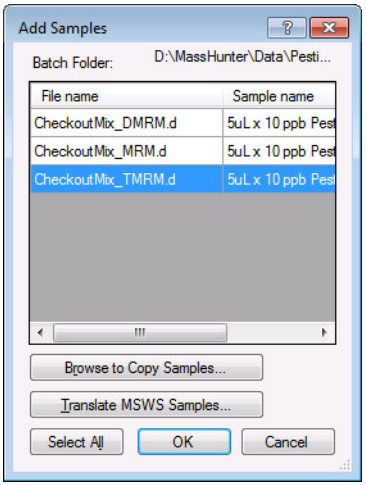
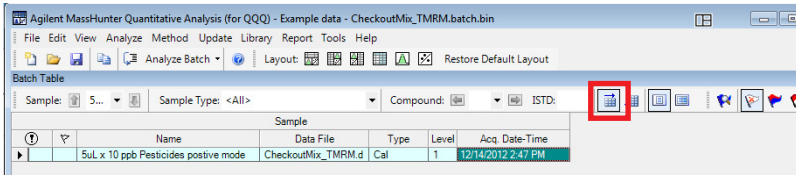
	h Click **Find > Find Compounds by MRM**.	

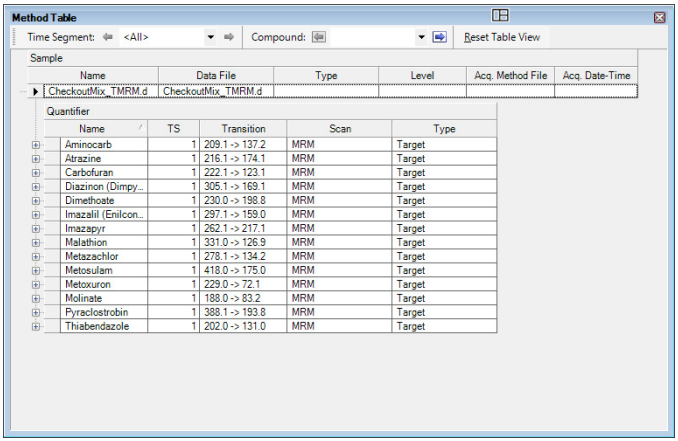
Steps	Detailed Instructions	Comments
3 Review the results of the Find Compounds by MRM algorithm. • Make sure that the primary ions are found for each compound. In the example data and example database, the compound Malathion does not have any secondary transitions.	**a** Close the **Method Explorer** and **Method Editor** windows. **b** Click the **Compound Details View** button. **c** Close the Compound MS Spectrum Results window. **d** In the Compound Chromatogram Results window, click the **Overlaid mode** button and the **Show Legend in Overlaid** mode button. **e** In the Compound Fragment Spectrum Results window, click the **Spectrum Peak List** button. **f** Review each compound. Verify that the primaries and secondaries for each compound were found.	• You can also print a Compound Report to review results. You click **File > Print > Compound Report**. The Compound Report sorts the compounds by retention time. • If you are using the Navigator View, then in the Data Navigator window, the primary transitions are labeled MRM and the secondary transitions are labeled tMRM. • If you are using the Compound Details View, then in the legend in the Compound Chromatogram Results window, the primary transitions are labeled MRM, and the secondary transitions are labeled tMRM. • The Retention Times of the isomers will not be resolved if they have unique transitions until "Task 3. Create a Reference Library in the Quantitative Analysis program" on page 54.
	g Select **Cpd1:Aminocarb**. You click this compound in the Compound List. **h** In the Compound Fragment Spectrum Results window, verify that these transition are all found: • 209.1 -> 67.2 (Secondary) • 209.1 -> 77.2 (Secondary) • 209.1 -> 94.2 (Secondary) • 209.1 -> 122.1 (Secondary) • 209.1 -> 137.2 (Primary) • 209.1 -> 155.2 (Primary)	• In the MS Peaks One table, you check the abundance for each Primary and Secondary transition. • In the Chromatogram Results window, you can click the Walk Chromatogram tool to review each of the spectra across a peak. You can determine when the Secondaries are acquired. • In the Compound Chromatogram Results window, you can see lines which indicate the abundances for each Secondary transition.

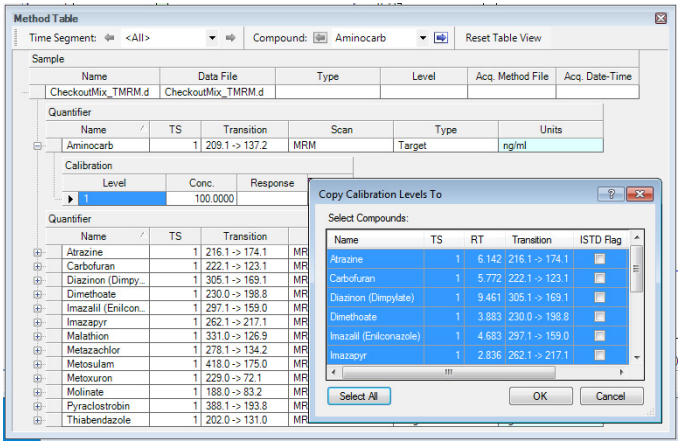
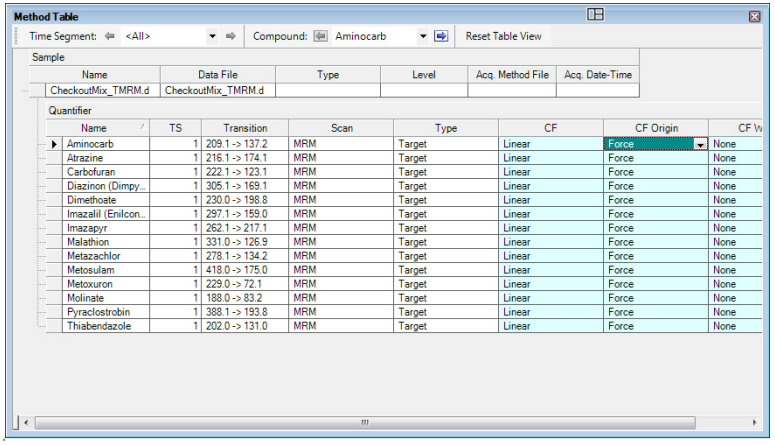
Steps	Detailed Instructions	Comments
	i Continue checking each compound to verify that the Primary and Secondary transitions were acquired. See "Primary and Secondary Transitions for Triggered MRM" on page 68 for a list of the transitions to verify.	• In the Compound Fragment Spectrum Results table, you can check the abundance for each Primary and Secondary. • If you are using the Navigator View, then in the Chromatogram Results window, you can click the **Walk Chromatogram** tool to review each of the spectra across a peak. You can determine when the Secondaries are acquired.



Task 3. Create a Reference Library in the Quantitative Analysis program

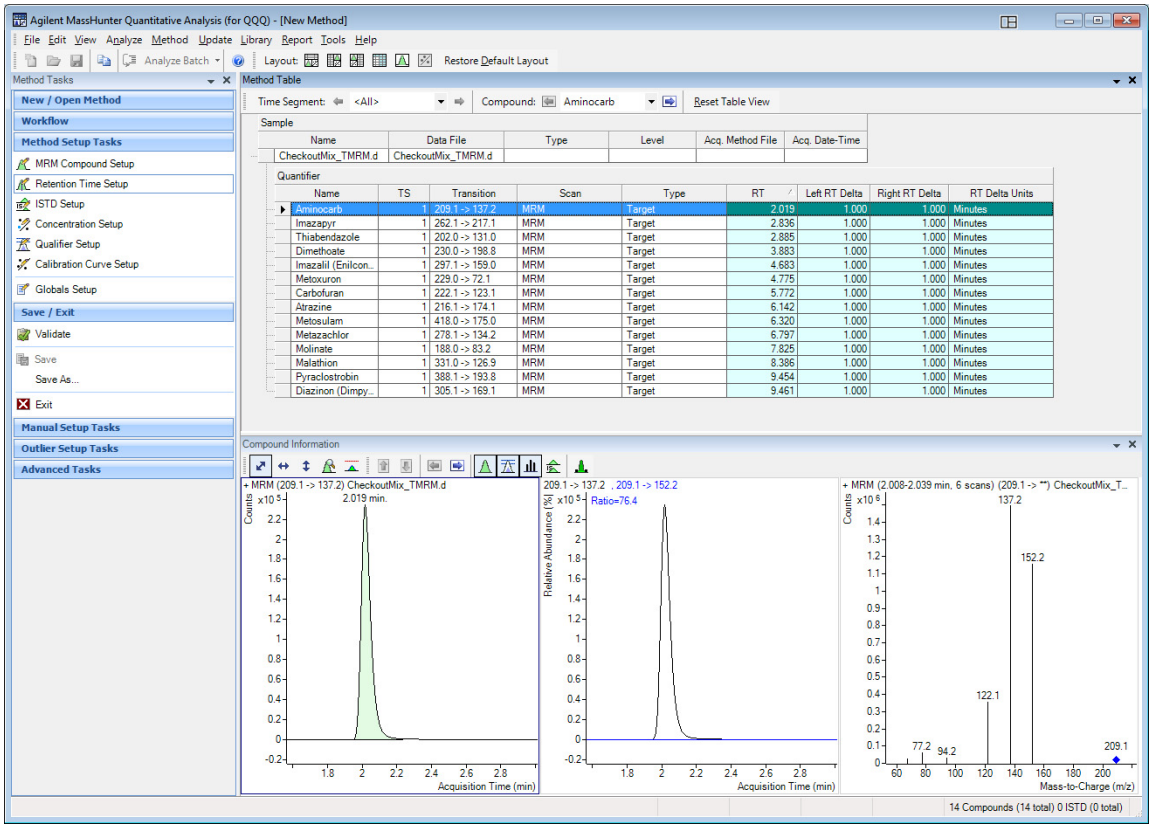
Steps	Detailed Instructions	Comments
1 Start the Quantitative Analysis program.	• Click the QQQ Quantitative Analysis icon.	
2 Set up a batch and add the TMRM data file. • Add the data file **CheckoutMix_TMRM.d**.	**a** Click **File > New Batch**. **b** Navigate to the location of the TMRM data file. **c** Type `CheckoutMix_TMRM` for the **Batch file** name. **d** Click **Open**. The Add Sample window opens. **e** If the data that you want to include in this batch are in a different folder, click **Browse to Copy Samples** to find your files. **f** Select the **CheckoutMix_TMRM.d** data file and click **OK**. **g** Check that **Flat Table** (shown in red in the next figure) is selected. Then select **Cal** as the **Type**. **h** Type `1` for the **Level**.	
		
3 Set up the TMRM method.	**a** Click **Method > New > New Method from Acquired MRM Data**. **b** Select the **CheckoutMix_TMRM.d** data file. **c** Click **Open**. **d** Right-click the Method Table window and click **Collapse All**.	• If you added more than one sample, then you select one of the calibration data files to create the method.

Steps	Detailed Instructions	Comments
		
4 Set the Concentration Setup. • Add calibration level 1 with a concentration of 100.	**a** Select **Concentration Setup** in the Method Setup Tasks section in the Method Tasks pane. **b** Select the first compound in the table. **c** Right-click the compound row and click **New Calibration Level** from the shortcut menu. **d** In the **Level** column, type `1`. In the **Conc.** column, type `100`. **e** Right-click in the Level box and click **Copy Calibration Levels To**. **f** Click **Select All**. Click **OK**.	• Refer to the online Help in the Quantitative Analysis program for additional help on these tasks.

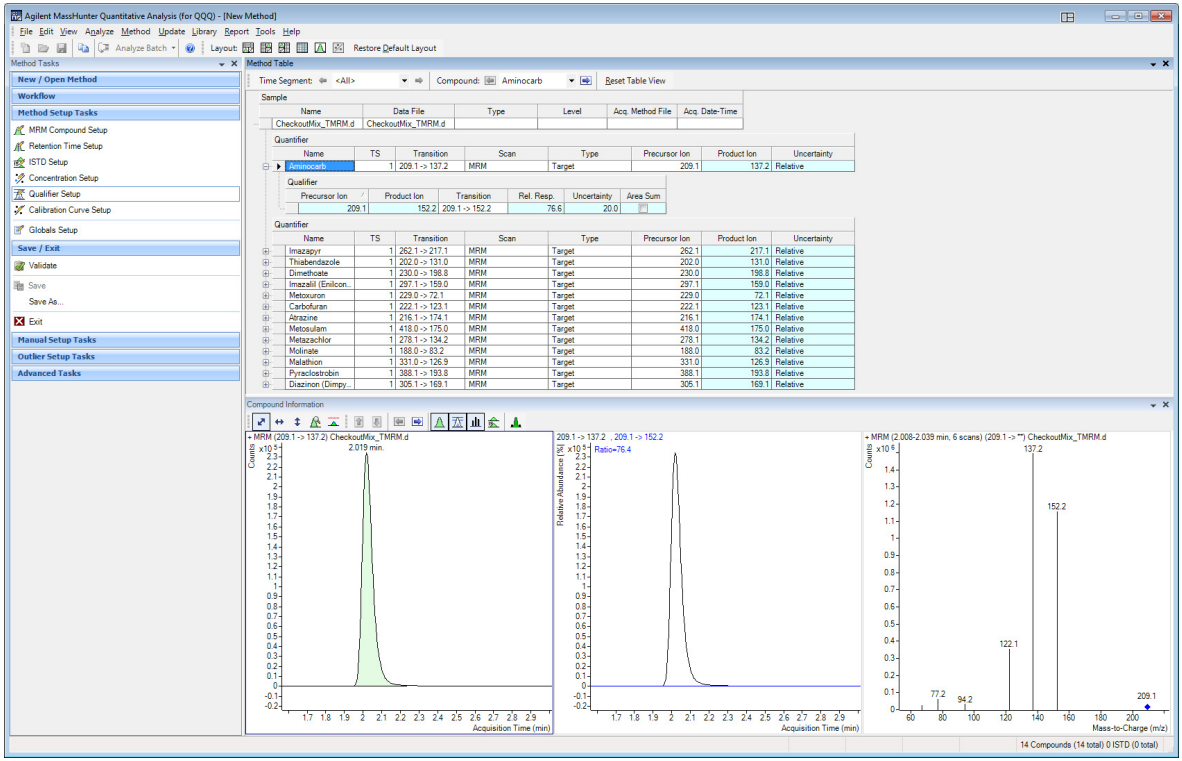
Steps	Detailed Instructions	Comments
		
5 Change the calibration curve. • Force the origin to be included.	**a** Select **Calibration Curve Setup** in the Method Setup Tasks section in the Method Tasks pane. **b** Set the **CF Origin** to **Force** for the first compound. **c** Right-click this value and click **Fill Down**.	• We only have one data file so we need to include the origin. • The Fill Down command copies the value of the current cell to all of the other rows in the table.
		

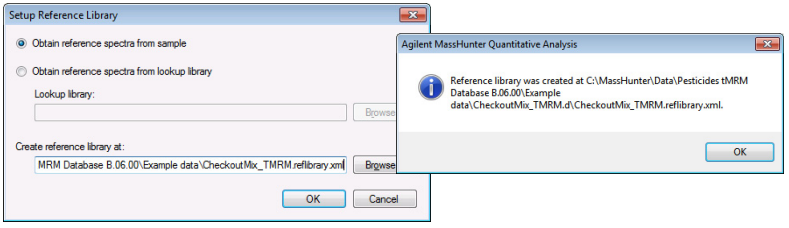
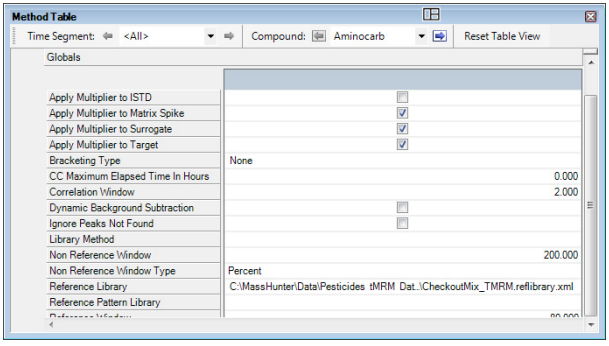
Steps	Detailed Instructions	Comments
6 Resolve the RTs. The RT elution order is: • Aminocarb • Imazapyr • Thiabendazole • Dimethoate • Imazalil (Enilconazole) • Metoxuron • Carbofuran • Atrazine • Metosulam • Metazachlor • Molinate • Malathion • Pyraclostrobin • Diazinon (Dimpylate)	**a** Select **Retention Time Setup** in the Method Setup Tasks section. **b** Verify the retention time order of the compounds is the same as shown in the figure below. Resolve any retention time issues for the compounds.	Depending on the delay volume, the compounds Pyraclostrobin and Diazinon can co-elute, separate slightly, or reverse elution order.

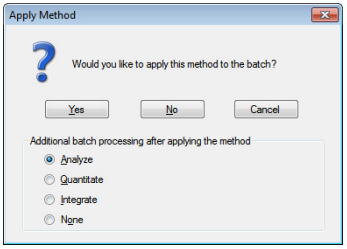
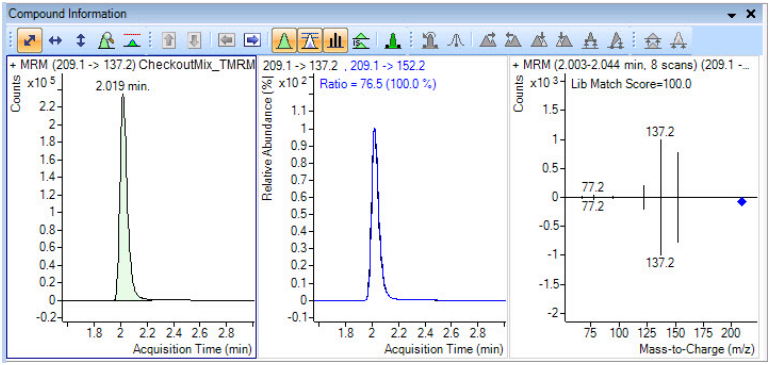
Steps	Detailed Instructions	Comments



Steps	Detailed Instructions	Comments
7 Review qualifier ratios	**c** Select **Qualifier Setup** in the Method Setup Tasks section. **d** Right-click the Method Table and click **Expand All**. **e** Click the Show/Hide Qualifiers button in the toolbar in the Compound Information window. **f** Click on each compound and verify that the Rel. Resp. for each Qualifier matches the value shown in the Compound Information window in the spectrum pane. **g** Click Method > Validate and fix any errors.	



Steps	Detailed Instructions	Comments
8 Set up the Reference Library.	**a** Click **Library > Setup Reference Library**. **b** Click **Obtain reference spectra from sample**. **c** Verify that **Create reference library at** is set to the folder you wish to use. **d** Click **OK**. **e** Click **OK** in the "Reference library was created" message.	• Refer to the online Help in the Quantitative Analysis program for information on doing library searches using the reference library. You can also watch the advanced video on "Batch-at-a-Glance - TMRM Library Reference Spectra".
		
	f Select **Globals Setup** in the Method Setup Tasks section in the Method Tasks pane. **g** Verify that the Reference Library is set to the Reference Library you just created.	
		

Steps	Detailed Instructions	Comments
9 Save the method and set additional batch processing to analyze.	**a** Click **Method > Save As**. **b** Type `Quant_Checkout_TMRM` in the **File name**. **c** Click **Save**. **d** Click **Method > Exit**. **e** Verify that **Additional batch processing after applying the method** is set to **Analyze**. **f** Click **Yes** to apply the method to the batch.	
		
10 Review the results, and save the batch.	**a** Inspect the results. **b** If the Compound Information window is not open, click **View > Compound Information**. **c** Click the Show/Hide Spectrum button. **d** Click **File > Save Batch**.	
	**The LibMatchScore is 100 because you are comparing against the same spectrum.** **The matching algorithm applies penalties when less than five peaks are present in the spectrum:** **75% (1 peak)** **88% (2 peaks)** **94% (3 peaks)** **97% (4 peaks)**	

Steps	Detailed Instructions	Comments
11 Inspect the Library Match Score. Review the batch to resolve errors or messages that are indicated in the Batch Table.	• Check library match scores • Check qualifier ratios. • Resolve errors and messages NOTE: The sample data included for this exercise does not contain isomers. But if your sample does, you would resolve isomers at this time.	If an error is reported for a compound qualifier ratio: **a** Click **Method > Edit**. **b** Click **Update > Update Qualifier Ratios**. **c** Select the compounds for update and click **OK**. **d** Click **Method > Exit**. **e** Click **Yes** to apply the method to the batch. **f** Check qualifier ratios. **g** Resolve errors and messages. **h** Click **File > Save Batch**.

Reference

Checkout Mix Content

The content of the Checkout Mix is listed here. In addition to standard MRM parameters, the retention time and retention window settings are listed for each compound. This allows longer dwell time, better signal stability, and higher data quality compared to traditional MRM method.

Table 1 Checkout Mix (p/n 5190-0469) Basic Compounds

#	Chemical Name/CAS #	Concentration / Units	Tolerance (+/-)	Formula	Mass
1	Aminocarb/2032-59-9	100.2 µg/mL	0.5 µg/mL	$C_{11}H_{16}N_2O_2$	208.1211777698
2	Atrazine/1912-24-9	100.4 µg/mL	0.5 µg/mL	$C_8H_{14}ClN_5$	215.0937731936
3	Carbofuran/1563-66-2	100.2 µg/mL	0.5 µg/mL	$C_{12}H_{15}NO_3$	221.1051933528
4	Diazinon (Dimpylate)/333-41-5	100.4 µg/mL	0.5 µg/mL	$C_{12}H_{21}N_2O_3PS$	304.1010497716
5	Dimethoate/60-51-5	100.2 µg/mL	0.5 µg/mL	$C_5H_{12}NO_3PS_2$	228.9996212071
6	Imazalil (Enilconazole)/35554-44-0	100.4 µg/mL	0.5 µg/mL	$C_{14}H_{14}Cl_2N_2O$	296.0483185037
7	Imazapyr/8331-34-1	100.2 µg/mL	0.5 µg/mL	$C_{13}H_{15}N_3O_3$	261.1113413676
8	Malathion/121-75-5	100.4 µg/mL	0.5 µg/mL	$C_{10}H_{19}O_6PS_2$	330.0360662899
9	Metazachlor/67129-08-2	100.2 µg/mL	0.5 µg/mL	$C_{14}H_{16}ClN_3O$	277.0981898649
10	Metosulam/139528-85-1	100.4 µg/mL	0.5 µg/mL	$C_{14}H_{13}Cl_2N_5O_4S$	417.0065300909
11	Metoxuron/19937-59-8	100.2 µg/mL	0.5 µg/mL	$C_{10}H_{13}ClN_2O_2$	228.0665553841
12	Molinate/2212-67-1	100.4 µg/mL	0.5 µg/mL	$C_9H_{17}NOS$	187.103084902
13	Pyraclostrobin/175013-18-0	100.2 µg/mL	0.5 µg/mL	$C_{19}H_{18}ClN_3O_4$	387.0985837956
14	Thiabendazole/148-79-8	100.4 µg/mL	0.5 µg/mL	$C_{10}H_7N_3S$	201.0360679755
	Acetonitrile	Solvent		C_2H_3N	41.0265

Table 2 Checkout Mix (p/n 5190-0469) Acidic Compounds

#	Chemical Name/CAS #	Concentration / Units	Tolerance (+/-)	Formula	Mass
1	Acifluorfen/50594-66-6	100.2 µg/mL	0.5 µg/mL	$C_{14}H_7ClF_3NO_5$	360.9964846522
2	2,4,5-T/93-76-5	100.4 µg/mL	0.5 µg/mL	$C_8H_5Cl_3O_3$	253.9304271564
3	Bentazone/25057-89-0	100.2 µg/mL	0.5 µg/mL	$C_{10}H_{12}N_2O_3S$	240.0568629945
4	Dinoseb (Subitex)/88-85-7	100.4 µg/mL	0.5 µg/mL	$C_{10}H_{12}N_2O_5$	240.0746215091
5	2,4,5-TP (Silvex) (Fenoprop)/93-72-1	100.2 µg/mL	0.5 µg/mL	$C_9H_7Cl_3O_3$	267.9460772202
6	Hexaflumuron/86479-06-3	100.4 µg/mL	0.5 µg/mL	$C_{16}H_8Cl_2F_6N_2O_3$	459.9816167569
	Acetonitrile	Solvent		C_2H_3N	41.0265

Note that Familiarization exercises use the positive test mix only (Basic Compounds). The negative checkout mix (Acid Compounds) is provided for your convenience only.

LC Parameters

Name: HiP Sampler	Model: G4226A
Auxiliary	
Draw Speed	100.0 µL/min
Eject Speed	100.0 µL/min
Draw Position Offset	0.0 mm
Wait Time After Drawing	2.0 s
Sample Flush Out Factor	5.0
Vial/Well bottom sensing	No
Injection	
Injection Mode	Standard injection
Injection Volume	5.00 µL
High throughput	
Automatic Delay Volume Reduction	No
Overlapped Injection	
Enable Overlapped Injection	No
Valve Switching	
Valve Movements	0
Valve Switch Time 1	
Switch Time 1 Enabled	No
Valve Switch Time 2	
Switch Time 2 Enabled	No
Valve Switch Time 3	
Switch Time 3 Enabled	No
Valve Switch Time 4	
Switch Time 4 Enabled	No
Stop Time	
Stoptime Mode	As pump/No limit
Post Time	
Posttime Mode	Off

Figure 1 HiP Sampler parameters

Name: Binary Pump **Model:** G4220A

Flow	0.400 mL/min
Use Solvent Types	Yes
Stroke Mode	Synchronized
Low Pressure Limit	0.00 bar
High Pressure Limit	600.00 bar
Max. Flow Ramp Up	100.000 mL/min²
Max. Flow Ramp Down	100.000 mL/min²
Expected Mixer	No check
Stroke A	
Automatic Stroke Calculation A	Yes
Compress A	
Compressibility Mode A	Compressibility Value Set
Compressibility A	45 10e-6/bar
Compress B	
Compressibility Mode B	Compressibility Value Set
Compressibility B	75 10e-6/bar
Stop Time	
Stoptime Mode	Time set
Stoptime	12.00 min
Post Time	
Posttime Mode	Time set
Posttime	3.00 min
Timetable	

Timetable

	Time	Function	Parameter
1	12.00 min	Change Solvent Composition	Solvent composition A: 5.00 % B:95.00 %
2	12.00 min	Change Flow	Flow: 0.4 mL/min
3	12.00 min	Change Max. Pressure Limit	Max. Pressure Limit: 600.00 bar

Solvent Composition

	Channel	Ch. 1 Solv.	Name 1	Ch2 Solv.	Name 2	Selected	Used	Percent
1	A	100.0 % H20 (migrated)	H20 w/ 5mM acetic acid	100.0 % H20 (migrated)		Ch. 1	Yes	95.00 %
2	B	100.0 % ACN (migrated)	ACN	100.0 % H20 (migrated)		Ch. 1	Yes	5.00 %

Figure 2 Binary Pump parameters

Name: Column Comp.	Model: G1316C
Ready when front door open	Yes
Left Temperature Control	
Temperature Control Mode	Temperature Set
Temperature	35.00 °C
Enable Analysis Left Temperature	
Enable Analysis Left Temperature On	Yes
Enable Analysis Left Temperature Value	0.80 °C
Right Temperature Control	
Right temperature Control Mode	Temperature Set
Right temperature	35.00 °C
Enable Analysis Right Temperature	
Enable Analysis Right Temperature On	Yes
Enable Analysis Right Temperature Value	0.80 °C
Stop Time	
Stoptime Mode	As pump/injector
Post Time	
Posttime Mode	Off

Figure 3 Column Comp. parameters

Primary and Secondary Transitions for Triggered MRM

The Primary and Secondary transitions for the Checkout Mix analytes in positive mode and their chromatographic-dependent settings are listed here. These values can differ from the values in the database. Retention times can also vary, depending on the LC model and system configuration.

If the transitions in the example method do not match those in the database, use the transitions in the database.

The acquisition method parameters for the negative ion test mix are in the test mix method **Acid Pesticides Test Mix_DMRM.m**.

Table 3 Primary and secondary positive transitions for Checkout Mix analytes

Compound Name	Primary?	Trigger	Threshold	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Ret Time (min)	Delta RT (min)	Frag	CE	CAV
Aminocarb	Yes	Yes	6937	209.1	Unit	152.2	Unit	2.01	0.88	105	12	2
Aminocarb	Yes			209.1	Unit	137.2	Unit	2.01	0.88	105	24	2
Aminocarb				209.1	Unit	122.1	Unit			105	44	2
Aminocarb				209.1	Unit	94.2	Unit			105	56	2
Aminocarb				209.1	Unit	77.2	Unit			105	60	2
Aminocarb				209.1	Unit	67.2	Unit			105	60	2
Atrazine	Yes	Yes	2010	216.1	Unit	174.1	Unit	6.1	0.83	125	16	3
Atrazine	Yes			216.1	Unit	68	Unit	6.1	0.83	125	40	3
Atrazine				216.1	Unit	145.9	Unit			125	20	3
Atrazine				216.1	Unit	132	Unit			125	20	3
Atrazine				216.1	Unit	104	Unit			125	28	3
Atrazine				216.1	Unit	79	Unit			125	24	3
Atrazine				216.1	Unit	62.1	Unit			125	56	3
Atrazine				216.1	Unit	43.1	Unit			125	48	3
Carbofuran	Yes	Yes	728	222.1	Unit	165.1	Unit	6.83	0.68	80	20	2
Carbofuran	Yes			222.1	Unit	123.1	Unit	6.83	0.68	80	30	2

Table 3 Primary and secondary positive transitions for Checkout Mix analytes

Compound Name	Primary?	Trig-ger	Thresh-old	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Ret Time (min)	Delta RT (min)	Frag	CE	CAV
Carbofuran				222.1	Unit	207	Unit			80	12	2
Carbofuran				222.1	Unit	166	Unit			80	4	2
Carbofuran				222.1	Unit	137	Unit			80	16	2
Carbofuran				222.1	Unit	124	Unit			80	20	2
Carbofuran				222.1	Unit	78	Unit			80	50	2
Carbofuran				222.1	Unit	55.2	Unit			80	24	2
Diazinon (Dimpylate)	Yes	Yes	2788	305.1	Unit	169.1	Unit	10.4	1.04	105	32	2
Diazinon (Dimpylate)	Yes			305.1	Unit	97	Unit	10.4	1.04	105	40	2
Diazinon (Dimpylate)				305.1	Unit	277.1	Unit			105	10	2
Diazinon (Dimpylate)				305.1	Unit	249	Unit			105	20	2
Diazinon (Dimpylate)				305.1	Unit	231	Unit			105	20	2
Diazinon (Dimpylate)				305.1	Unit	100	Unit			105	40	2
Diazinon (Dimpylate)				305.1	Unit	93	Unit			105	40	2
Diazinon (Dimpylate)				305.1	Unit	84	Unit			105	40	2
Diazinon (Dimpylate)				305.1	Unit	66	Unit			105	40	2
Dimethoate	Yes		1547	230	Unit	198.8	Unit	4.95	0.6	70	0	5
Dimethoate	Yes			230	Unit	125	Unit	4.95	0.6	70	16	5
Dimethoate				230	Unit	170.9	Unit			70	8	5
Dimethoate				230	Unit	156.9	Unit			70	16	5
Dimethoate				230	Unit	88	Unit			70	8	5
Dimethoate				230	Unit	79	Unit			70	32	5
Imazalil (Enilconazole)	Yes	Yes	443	297.1	Unit	201	Unit	6.23	0.99	115	15	2
Imazalil (Enilconazole)	Yes			297.1	Unit	159	Unit	6.23	0.99	115	20	2
Imazalil (Enilconazole)				297.1	Unit	133	Unit			115	12	2

Table 3 Primary and secondary positive transitions for Checkout Mix analytes

Compound Name	Primary?	Trig-ger	Thresh-old	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Ret Time (min)	Delta RT (min)	Frag	CE	CAV
Imazalil (Enilconazole)				297.1	Unit	105.1	Unit			115	36	2
Imazalil (Enilconazole)				297.1	Unit	93.1	Unit			115	20	2
Imazalil (Enilconazole)				297.1	Unit	77.1	Unit			115	60	2
Imazalil (Enilconazole)				297.1	Unit	69	Unit			115	60	2
Imazalil (Enilconazole)				297.1	Unit	41	Unit			115	36	2
Imazapyr	Yes	Yes	249	262.1	Unit	217.1	Unit	3.83	0.63	120	20	3
Imazapyr	Yes			262.1	Unit	69.1	Unit	3.83	0.63	120	40	3
Imazapyr				262.1	Unit	220.1	Unit			120	20	3
Imazapyr				262.1	Unit	202.1	Unit			120	20	3
Imazapyr				262.1	Unit	149	Unit			120	20	3
Imazapyr				262.1	Unit	131	Unit			120	40	3
Imazapyr				262.1	Unit	86.1	Unit			120	20	3
Malathion	Yes	Yes	175	331	Unit	126.9	Unit	9.37	0.94	80	5	2
Malathion	Yes			331	Unit	99	Unit	9.37	0.94	80	10	2
Metazachlor	Yes	Yes	2855	278.1	Unit	210.1	Unit	7.83	0.86	70	4	5
Metazachlor	Yes			278.1	Unit	134.2	Unit	7.83	0.86	70	15	5
Metazachlor				278.1	Unit	105.1	Unit			70	44	5
Metazachlor				278.1	Unit	79.1	Unit			70	60	5
Metosulam	Yes	Yes	404	418	Unit	175	Unit	7.36	0.79	140	32	3
Metosulam	Yes			418	Unit	140	Unit	7.36	0.79	140	60	3
Metosulam				418	Unit	354.2	Unit			140	20	3
Metosulam				418	Unit	238.2	Unit			140	16	3
Metosulam				418	Unit	190	Unit			140	20	3
Metosulam				418	Unit	77.2	Unit			140	60	3

Table 3 Primary and secondary positive transitions for Checkout Mix analytes

Compound Name	Primary?	Trig-ger	Thresh-old	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Ret Time (min)	Delta RT (min)	Frag	CE	CAV
Metoxuron	Yes	Yes	2155	229	Unit	72.1	Unit	5.86	0.63	95	16	3
Metoxuron	Yes			229	Unit	46.1	Unit	5.86	0.63	95	12	3
Metoxuron				229	Unit	165.3	Unit			95	4	3
Metoxuron				229	Unit	156.1	Unit			95	24	3
Metoxuron				229	Unit	109	Unit			95	12	3
Metoxuron				229	Unit	80	Unit			95	44	3
Metoxuron				229	Unit	55.9	Unit			95	60	3
Molinate	Yes	Yes	69	188	Unit	126.1	Unit	8.81	0.88	90	25	2
Molinate	Yes			188	Unit	83.2	Unit	8.81	0.88	90	16	2
Molinate				188	Unit	98	Unit			90	12	2
Molinate				188	Unit	95.5	Unit			90	28	2
Molinate				188	Unit	81	Unit			90	20	2
Molinate				188	Unit	70	Unit			90	16	2
Molinate				188	Unit	55.1	Unit			90	19	2
Pyraclostrobin	Yes	Yes	3558	388.1	Unit	193.8	Unit	10.4	1.04	95	8	2
Pyraclostrobin	Yes			388.1	Unit	163.1	Unit	10.4	1.04	95	20	2
Pyraclostrobin				388.1	Unit	218.6	Unit			95	32	2
Pyraclostrobin				388.1	Unit	196.2	Unit			95	4	2
Pyraclostrobin				388.1	Unit	164.1	Unit			95	12	2
Pyraclostrobin				388.1	Unit	104.1	Unit			95	60	2
Pyraclostrobin				388.1	Unit	91.1	Unit			95	60	2
Thiabendazole	Yes	Yes	2263	202	Unit	175	Unit	4.1	0.66	130	24	2
Thiabendazole	Yes			202	Unit	131	Unit	4.1	0.66	130	36	2
Thiabendazole				202	Unit	143.1	Unit			130	40	2

Table 3 Primary and secondary positive transitions for Checkout Mix analytes

Compound Name	Primary?	Trig-ger	Thresh-old	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Ret Time (min)	Delta RT (min)	Frag	CE	CAV
Thiabendazole				202	Unit	104.1	Unit			130	44	2
Thiabendazole				202	Unit	92.1	Unit			130	36	2
Thiabendazole				202	Unit	77	Unit			130	60	2
Thiabendazole				202	Unit	65	Unit			130	52	2
Thiabendazole				202	Unit	51	Unit			130	60	2

To bypass mixer and damper

You only need to bypass the mixer and damper if you have a G1312B Agilent 1260 Infinity Binary Pump.

The Binary Pump SL is delivered in standard configuration (damper and mixer connected). This step shows how to bypass the damper and mixer and convert the pump to low delay volume mode.

Configurations where only the damper or the mixer is disconnected while the other part is still in line are not supported by Agilent Technologies.

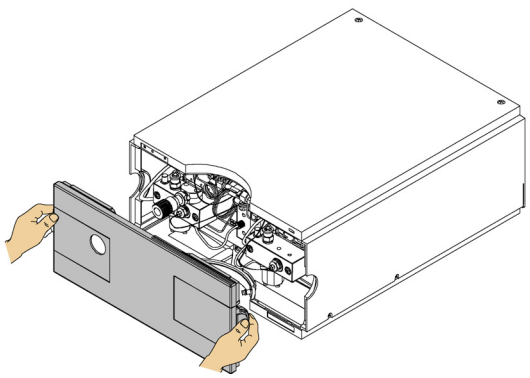
Tools required

- wrench, 1/4-inch x 5/16-inch (p/n 8710-0510)
- wrench, open end, 14-mm (p/n 8710-1924)
- hex driver, 1/4-inch, slitted (p/n 5023-0240)

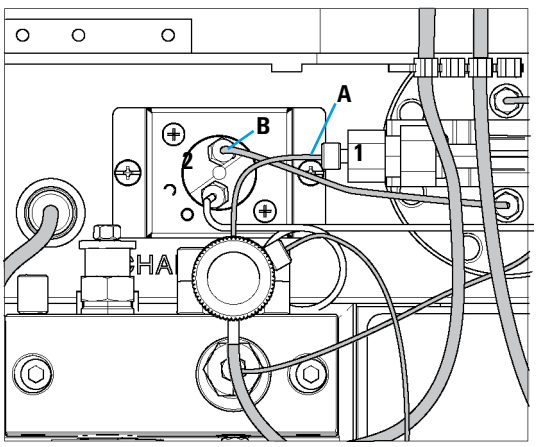
Preparations for this procedure

- Flush the system (water if buffers were used, otherwise IPA).
- Turn the flow off.

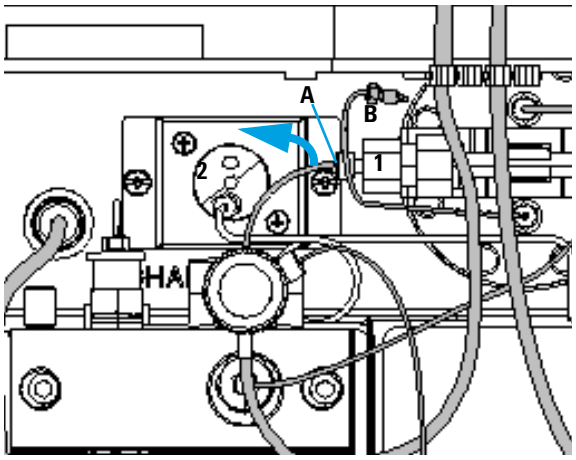
1 Remove the front cover by pressing the clip fastener on both sides of the cover.



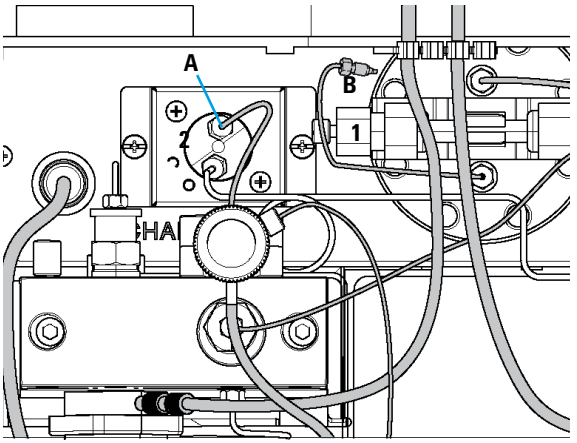
2 Use the 1/4 inch hex driver to remove fitting **B** from port *2* of the pressure sensor.



3 Fold capillary end **B** away. It remains unconnected. Disconnect fitting **A** from outlet **1** of the mixer.



4 Connect fitting **A** to port **2** of the pressure sensor. Seal port **1** of the mixer with a plastic blank nut.



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In This Guide

This Familiarization Guide describes how to use your MassHunter MRM/dMRM/tMRM Database.

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