Agilent

MassHunter MRM/dMRM/tMRM Database

Familiarization Guide

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)

Supported MassHunter Programs

Make sure these MassHunter programs are installed.

- MassHunter Data Acquisition

  - for Ultivo LC/TQ, 1.2 or later

  - for 6470B, 10.1 or later

  - for 6495C, 10.0 SR1 or later
- MassHunter Quantitative Analysis 10.2 or later.
- MassHunter Qualitative Analysis 10.0 or later.

Familiarization files

These Familiarization files are included on the MRM Database Familiarization media (p/n G1736-10001, available on SubscribeNet) and are installed on your computer when the content of the Familiarization media is installed:

- Checkout Mix Databases:
  - CheckoutMix_TriggeredMRM_1.2_Ultivo
  - CheckoutMix_TriggeredMRM_10_6400_Series
- Checkout Mix methods:
  - CheckoutMix_MRM_6470B and CheckoutMix_MRM_6470B_WithCpds.m - for use with non-iFunnel based 6400 Series Triple Quad instruments
  - CheckoutMix_MRM_6495C and CheckoutMix_MRM_6495C_WithCpds.m - for use with iFunnel based 6400 Series Triple Quad instruments
  - CheckoutMix_MRM_Ultivo and CheckoutMix_MRM_Ultivo_WithCpds.m - for use with Ultivo Triple Quad instruments

Note that the respective dMRM and tMRM Checkout Mix methods for the 6470B and Ultivo instruments are also 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: This dataset was acquired on a 6470B instrument, and can be used to familiarize yourself with the data analysis workflow regardless of your LC/TQ model.
- 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.

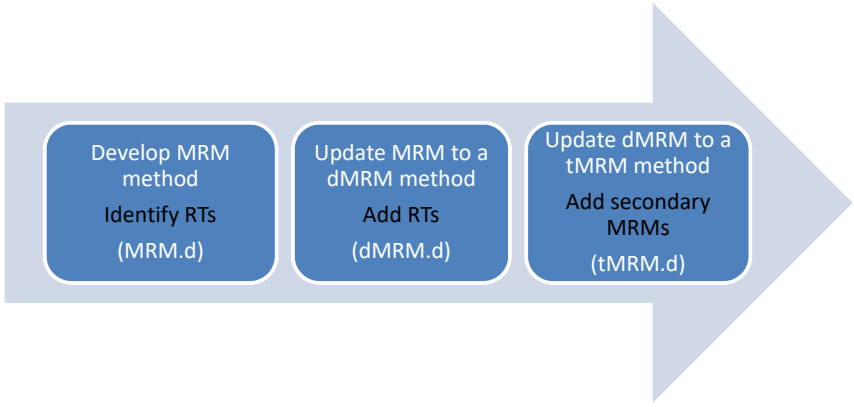


Figure 1. MRM to dMRM to tMRM Method Development Workflow for single standard mix

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. For an Ultivo system, use the **Convert to dMRM** command in the Method Navigator. For either system, 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.

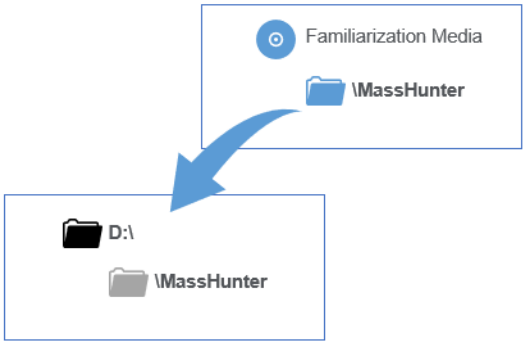
For ease of use, optimize no more than 50 compounds at a time in each **MRM -> dMRM -> tMRM** workflow.

Before You Begin

To do the exercises in this guide, you need to install the Familiarization Checkout example data, methods, and report.

To install the Familiarization files

- From the Familiarization media, copy the **\MassHunter** folder into the root folder of your MassHunter drive. By default, the folder is **D:**.



Two databases are provided — one for Ultivo and another for the 6470B. The latter can be imported correctly to all non-Ultivo 6400 instruments.

Three LC/MS methods are provided — for Ultivo, 6470B, and 6495C. These methods include ion source conditions.

To prepare to run the Checkout Mix

1 Make sure that you have these required parts and reagents:
   - Glacial acetic acid
   - 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, Infinity I, or Infinity II LC system is properly installed and verified.
3 If you have an Agilent 1260 Infinity I or Infinity II Binary Pump, bypass the mixer and damper. Refer to your respective user manual for details.
4 Check that the Agilent 6400 Series Triple Quad or Ultivo Triple Quad LC/MS System is properly installed and verified.
5 Make sure that the supported MassHunter programs are properly installed. See **"Supported MassHunter Programs"** on page 2.
6 To use a system configuration that is different from the one described in **"To run the Checkout Mix"** on page 8, 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:
   - QQQ Acquisition method info
   - Sampler settings
   - Pump settings
   - Column compartment settings

Refer to **"Primary and Secondary Transitions for Triggered MRM"** on page 65 for MS/MS transitions and their compound-dependent settings.

The three sets of example methods use the following instruments:

- Agilent 6470B Triple Quad LC/MS
- Agilent 6495C Triple Quad LC/MS
- Agilent Ultivo Triple Quad LC/MS

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). Use Working Solution 1 for non-iFunnel systems with an ESI source.

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 provided checkout method uses the LC system configuration listed in the next table. If your system deviates from this configuration, adjust the method as needed. Check the *Method Setup Guide*, if available with your database, or the *Quick Start Guide* for more information.

Column	**ZORBAX LC Column, Eclipse Plus C18, 2.1 mm × 100 mm, 1.8 µm (p/n 959758-902)**
Autosampler	1290 Infinity II Multisampler, G7167B
Pump	1290 Infinity II High Speed Pump, G7120A. If you use a 1260 Infinity I/II binary pump, configure the damper and mixer to be bypassed. Refer to the respective LC user manual for details.
Column Compartment	1290 Infinity II Multicolumn Thermostat, G7116B

5 Load the method.
   - If you have a non-iFunnel 6400 Series Triple Quad LC/MS system, open **CheckoutMix_MRM_6470B_WithCmpds.m**.
   - If you have an iFunnel 6400 Series Triple Quad LC/MS system, open **CheckoutMix_MRM_6495C_WithCmpds.m**.
   - If you have an Ultivo Triple Quad LC/MS system, open **CheckoutMix_MRM_Ultivo_WithCmpds.m**.

6 After loading the method, if your instrument model is different than the 6470A or 6495C, then the tune location may appear in red. Click the **Browse** folder and point to the correct tune folder for your instrument. Then click the most recent tune file, which should be **atunes.TUNE.XML**.

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

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

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

   a Extend your **Stop time**.

   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 appropriate MRM checkout method for your LC/TQ and save as: *iii***CheckoutMix_MRM.m**, where *iii* are your initials.	**a** Start the Data Acquisition program. **b** If you have a non-iFunnel 6400 Series Triple Quad LC/MS system, open the **CheckoutMix_MRM_6470B.m** method. If you have an iFunnel 6400 Series Triple Quad LC/MS system, open the **CheckoutMix_MRM_6495C.m** method. If you have an Ultivo Triple Quad LC/MS system, open the **CheckoutMix_MRM_Ultivo.m** method. **c** Click **Method > Save As**. **d** Type *iii***CheckoutMix_MRM.M**, where *iii* are your initials.	
2 Set the LC parameters according to the table on the next page.	**a** In the Method Editor window, click the tab for the configured autosampler. **b** Enter the parameters. **c** Click the tab for the configured pump. **d** Enter the parameters. **e** Click the **Column Comp.** tab. **f** Enter the parameters.	

Steps	Detailed Instructions	Comments

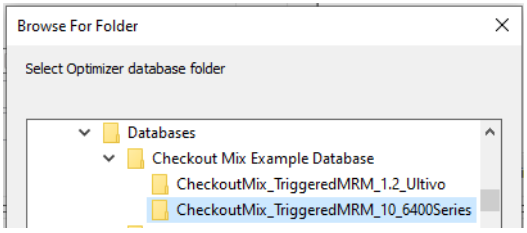
LC Parameters			
Column	Agilent ZORBAX LC Column, Eclipse Plus C18, 2.1 mm x 100 mm, 1.8 µm (p/n 959758-902)		
Column temperature	35 °C		
Injection volume	5 µL (0.5 µL for Funnel-based instruments)		
Needle Wash	5 seconds in 50:50 methanol/water		
Mobile phase	A= 5mM acetic acid in water B= Acetonitrile		
Flow rate	0.4 mL/min		
Timetable	Time (min)	A (%)	B (%)
	0.00	95.00	5.00
	12.00	5.00	95.00
Stoptime	12.00 min		
Posttime	3.00 min		

3 Set the source parameters.

Set the appropriate source parameters for your LC/TQ model according to the table below.

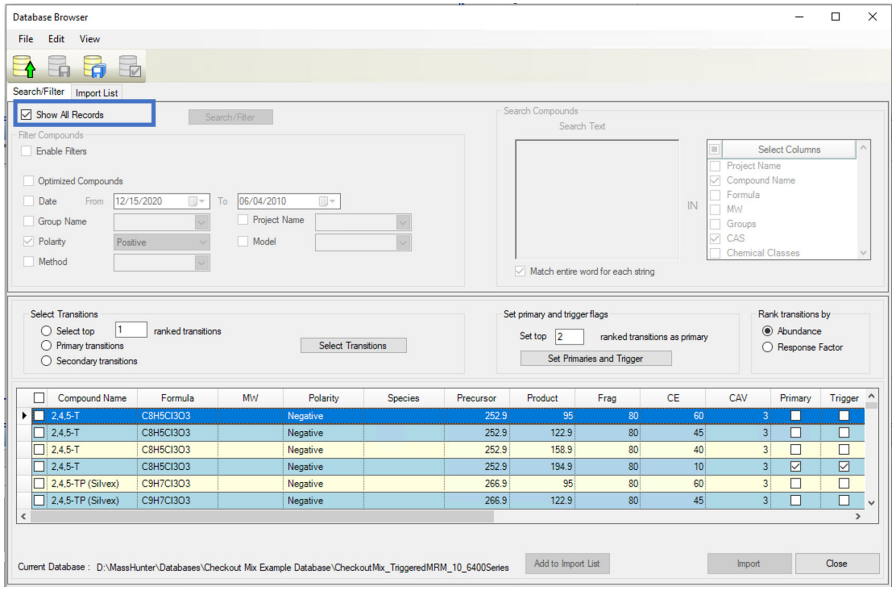
- The Scan segments table always must have at least one row. You manually remove this row after importing transitions from the Database Browser.

Source Parameters		
Instrument Model	**Ultivo and non-iFunnel 6400 Series**	**iFunnel 6400 Series**
Ion source	Agilent Jet Stream ESI	Agilent Jet Stream ESI
Gas temperature	250 °C	150 °C
Drying gas (nitrogen)	7 L/min	15 L/min
Nebulizer gas (nitrogen)	40 psi	30 psi
Sheath gas (nitrogen)	325 °C	300 °C
Sheath flow	11 L/min	12 L/min
Capillary voltage	3500/-3000 V	3500/-3500 V
Nozzle voltage	0/-1500 V	300/-500 V
iFunnel High/Low Pressure RF	NA	150/60 V (Positive) 90/60 V (Negative)

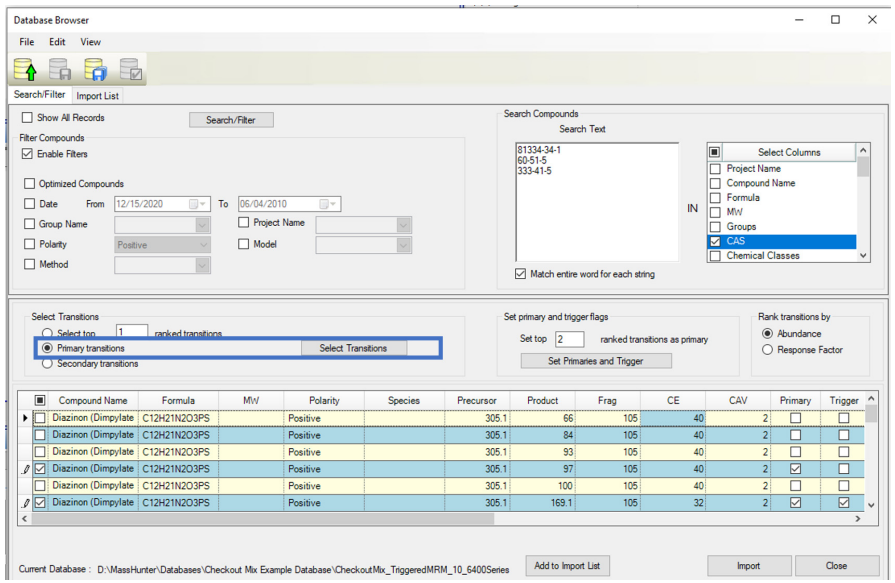
Steps	Detailed Instructions	Comments
4 Open the appropriate database in Database Browser, from the **QQQ** tab.	**a** For 6400 Series systems, right-click the Scan segments table and click **Import from Database Browser**. For Ultivo, click **Compound Browser.** For either system, the Database Browser opens. In the Database Browser, click **File > Open Database**. **b** For 6400 Series systems, select the **CheckoutMix_TriggeredMRM_10_6400 Series** database in the **\MassHunter\Databases\Checkout Mix Example Database** folder. For Ultivo, select the **CheckoutMix_TriggeredMRM_1.2_Ultivo** database. **c** Click **OK**. **d** Save the method with a new name. Do not overwrite the provided example method.	
		

Steps	Detailed Instructions	Comments
5 Select primary transitions corresponding to the basic checkout mix compounds. • See **"Primary and Secondary Transitions for Triggered MRM"** on page 65 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 44. Note that in the example data file, when both polarities are available in the **CheckoutMix_TriggeredMRM_1.2_Ultivo** database, the analysis was run in positive mode only. The CAS numbers for the basic checkout mix (positive ion compounds) are: 2032-59-9 1912-24-9 1563-66-2 333-41-5 60-51-5 35554-44-0 81334-34-1 121-75-5 139528-85-1 19937-59-8 2212-67-1 175013-18-0 148-79-8	**a** Click the **Compound Name** column header to sort the compounds by Compound Name. **b** Ensure the correct check boxes next to the primary transitions are marked according to the **"Primary and Secondary Transitions for Triggered MRM"** on page 65. **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. **d** Under **Select Transitions**, select Primary transitions and click **Select Primary**. See next page for filter examples. **e** 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. If a *Method Setup Guide* is available for your database, refer to the guide for more details on choosing the most selective transitions for your analysis.

Steps	Detailed Instructions	Comments

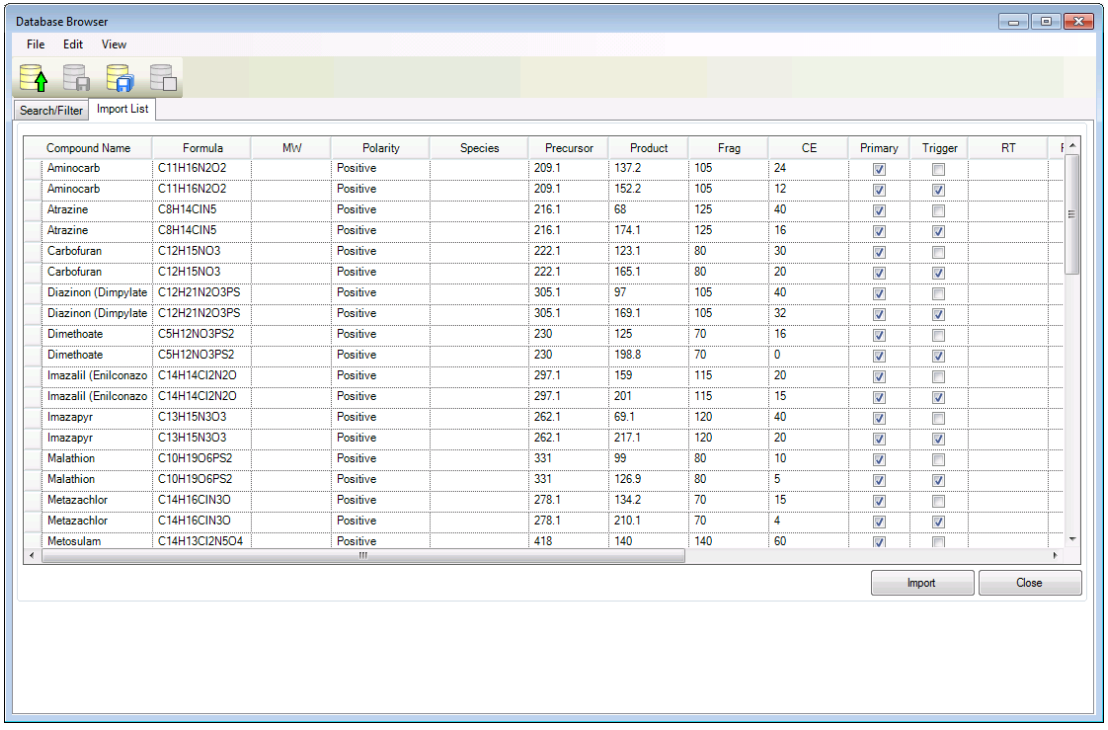


If you mark the Show All Records check box, then all compounds in the database are shown in the table. You scroll through the compounds and mark the Primaries for the compounds you are using.



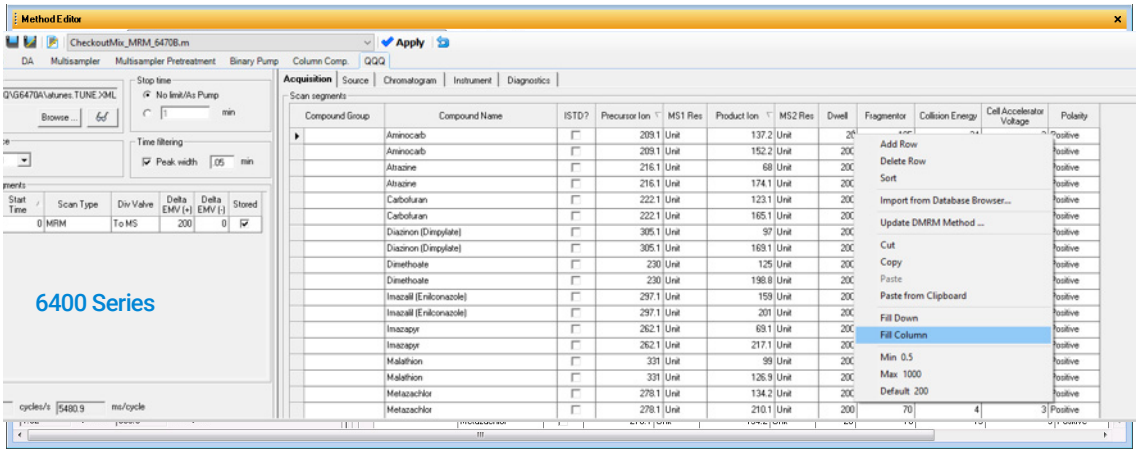
If you clear the Show All Records check box, you can limit the compounds that are shown in the table. In this example, the CAS check box is marked in the Search Compounds group and a list of CAS numbers was typed in the Search Text. Each CAS number was typed on a separate line. Only the compounds with 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.	**a** Click the **Add to Import List** button. **b** Click the **Import List** tab. **c** Review the Import List table. **d** 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.

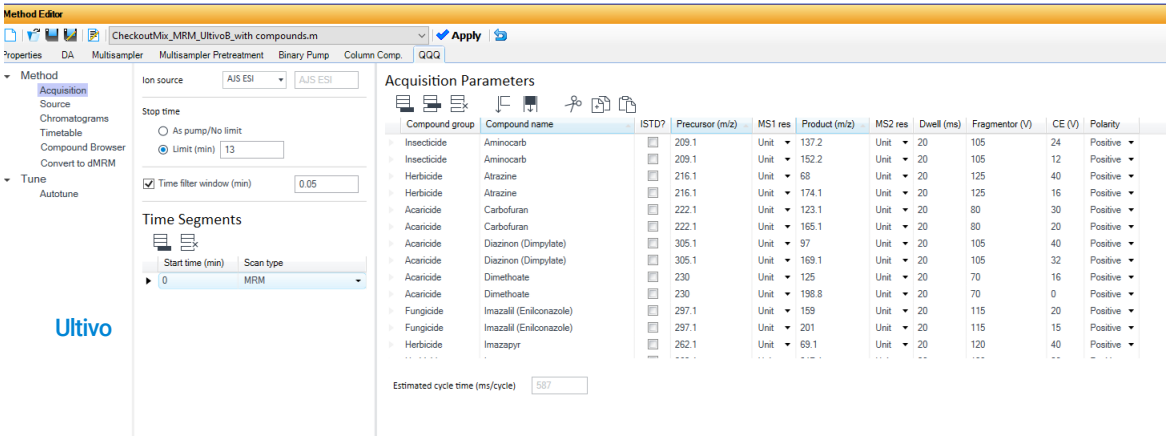


Compound Name	Formula	MW	Polarity	Species	Precursor	Product	Frag	CE	Primary	Trigger	RT
Aminocarb	C11H16N2O2		Positive		209.1	137.2	105	24	☑	☐	
Aminocarb	C11H16N2O2		Positive		209.1	152.2	105	12	☑	☑	
Atrazine	C8H14ClN5		Positive		216.1	68	125	40	☑	☐	
Atrazine	C8H14ClN5		Positive		216.1	174.1	125	16	☑	☑	
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. In the provided method, this compound has been named "delete this compound after DB import". **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 Change the dwell times.	**a** In the Scan segments table, type **20** in the column labeled **Dwell**. Type it in the cell for the first compound (Aminocarb). **b** Right-click the cell and select **Fill column** so that all compounds have a dwell time of 20 ms.	• 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 example, 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



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

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.

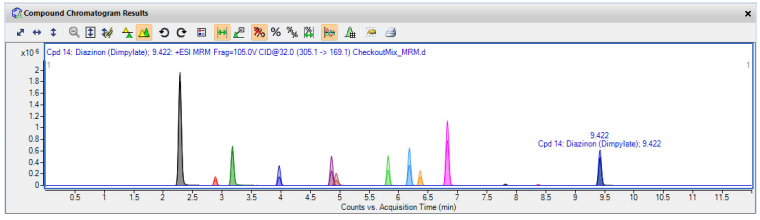
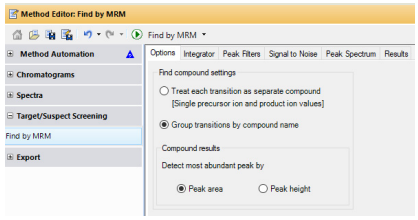
The retention time is needed for identification when the MS/MS spectra, and hence MRM transitions, of these isomers are very similar. The Checkout Mix (p/n 5190-0469) does not contain isomers, and the retention time is not required for identification of the compounds in the Checkout Mix.

The elution order of the compounds in the Checkout Mix was determined using the Eclipse Plus C18 column and mobile phases specified in the **"To run the Checkout Mix"** on page 8. The expected elution order is:

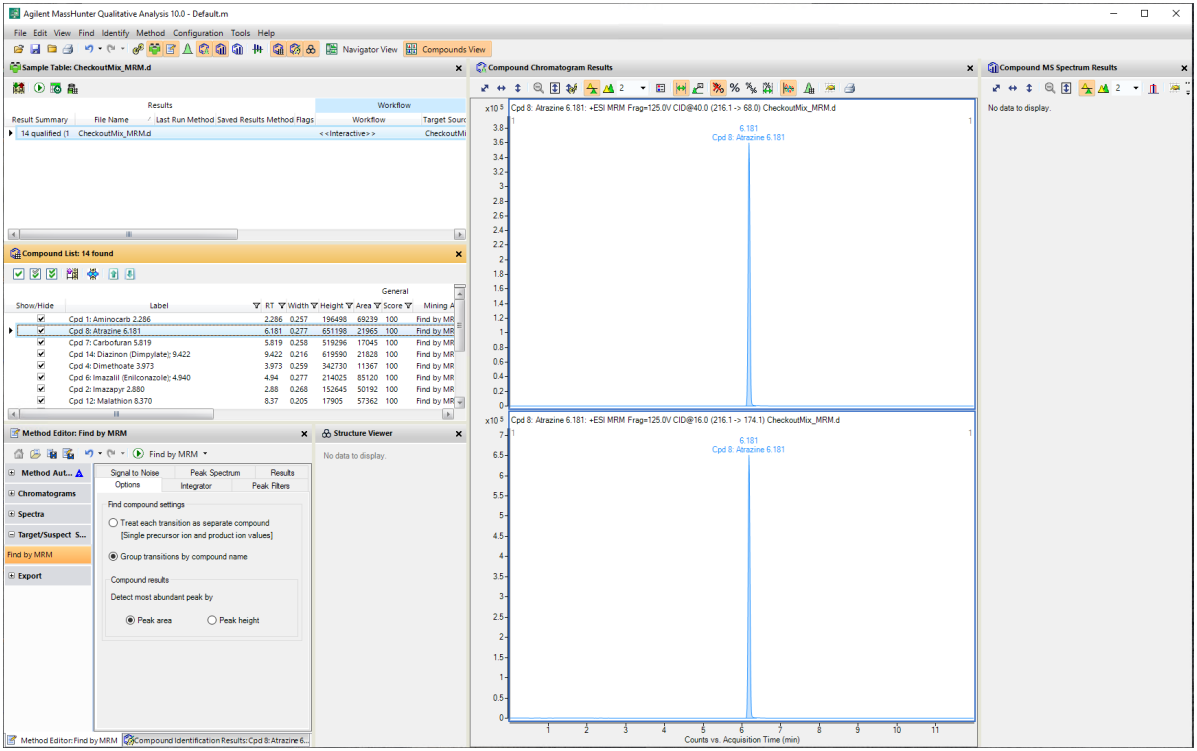
- Aminocarb
- Imazapyr
- Thiabendazole
- Dimethoate
- Metoxuron
- Imazalil (Enilconazole)
- Carbofuran
- Atrazine
- Metosulam
- Metazachlor
- Molinate
- Malathion
- Diazinon (Dimpylate)
- Pyraclostrobin

Depending on the delay volume, the compounds Pyraclostrobin and Diazinon can co-elute, separate slightly, or reverse elution order. Imazalil and Metoxuron are also very close in retention time and may 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, different than the path used for the example methods and data.	**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** In the Data File Settings tab, under the File Naming section, type `CheckoutMix_MRM`. **d** Click **Worklist > Add Multiple Samples**. **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 **Worklist > Run**.	• 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 8.

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 10.0** icon, or that of a later version.  (). **a** Click **File > Open Data File**. The system displays the Open Data File dialog box. **b** From the folder **\MassHunter\Data\Checkout Mix Example Data** folder, select **CheckoutMix_MRM.d**, and click **Open**. **c** Click the **Compounds View** tab at the top of the Qualitative Analysis screen. **d** If needed, click **View > Method Editor**. The system displays the Method Editor window. **e** In the Method Automation section, click **Workflow**, and ensure the Workflow is set to **Target/Suspect Screening** and that Compound Mining is set to **Find by MRM**. **f** In the Method Editor Window, in the Target/Suspect Screening section, click **Find by MRM**. Click the **Group transitions by compound name** option. **g** Click the **Peak area** option for **Detect most abundant peak by Peak area**.	• You can also use the example MRM data file that was installed to the **Checkout Mix Example Data** folder. If the file is not on your computer, install it from the installation media.
	 	
	h Click **Find > Find 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. • You cannot 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** Click **View > Compound List**. **b** Click or use the arrow keys to move through the **Compound Table** to review one compound at a time. See the figures that follow. **c** 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 23.	• You can also print a Compound Report to review results. You click **File > Print > Workflow Report**. The Compound Report sorts the compounds by retention time. • In the Compound Chromatogram Results window, you can see the abundances for each transition. • In Compound List, click each compound, or use the  and  buttons in the compound list window to review the results.



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 32.

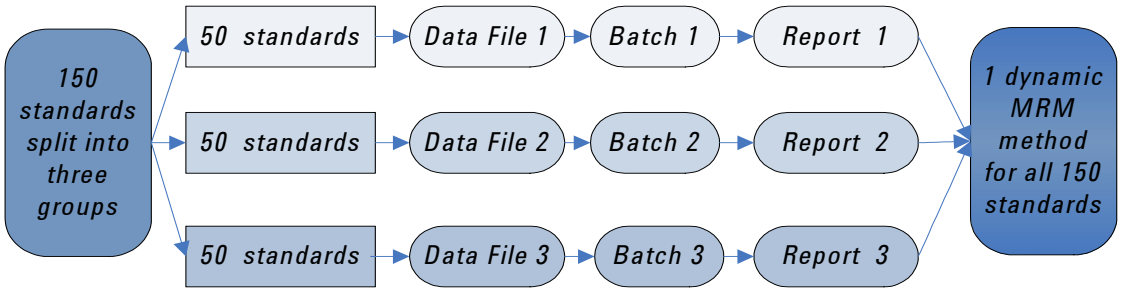


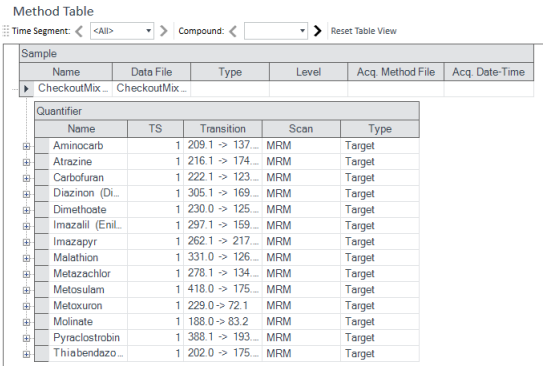
Figure 1. Example process for analyses that have more than 50 compounds

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 (Quant-My-Way) icon. (). **b** Click **New Batch**. **c** Navigate to installed data in the folder **\MassHunter\Data\Checkout Mix 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\Checkout Mix Example Data** folder. • You can also use the Checkout Mix data 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 > Preset Layouts > 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



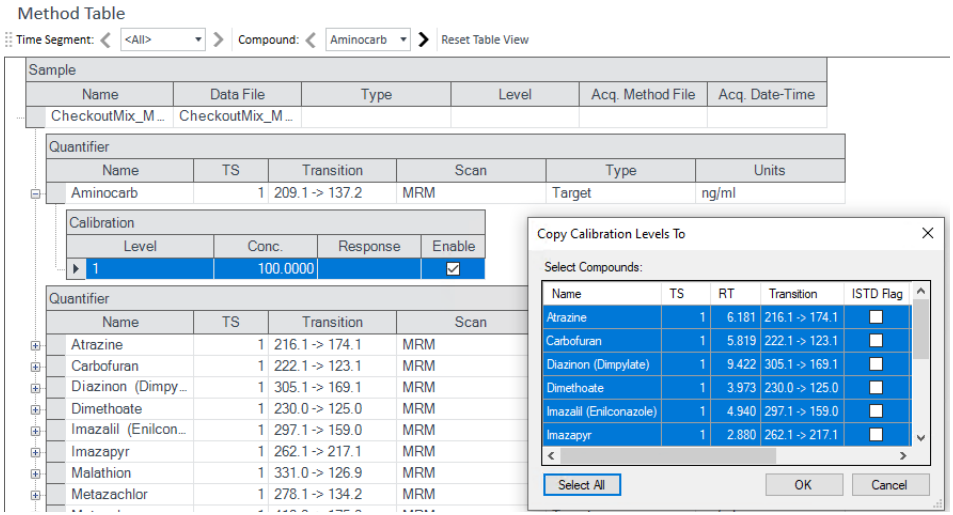
3 Set the Concentration Setup, Qualifier Setup, and Calibration Curve Setup.

- 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
  - Metoxuron
  - Imazalil (Enilconazole)
  - Carbofuran
  - Atrazine
  - Metosulam
  - Metazachlor
  - Molinate
  - Malathion
  - Diazinon (Dimpylate)
  - Pyraclostrobin

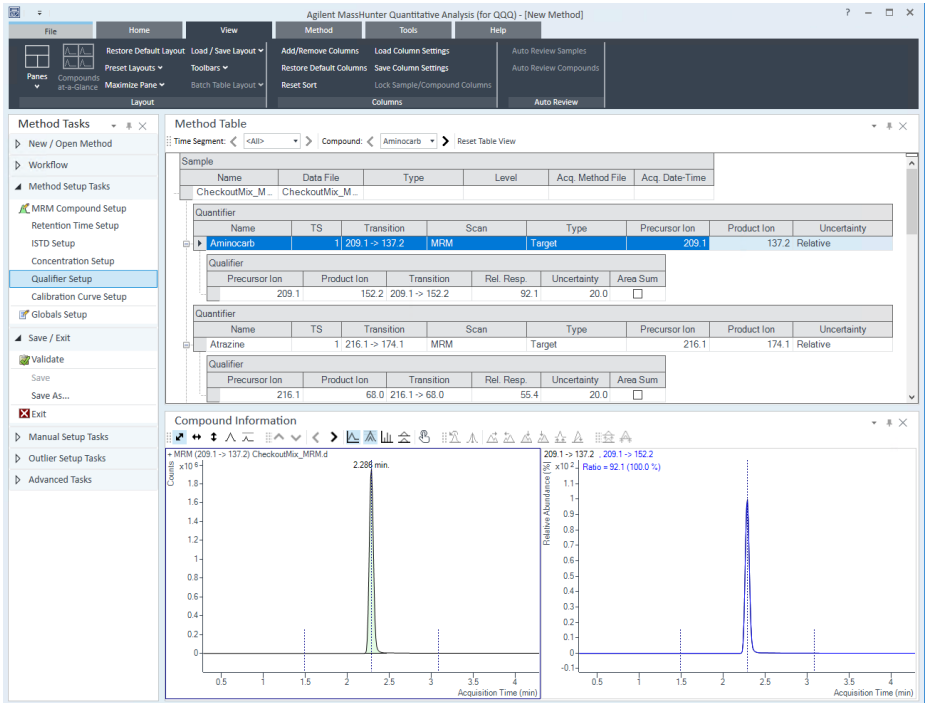
m Select **Retention Time Setup** in the Method Setup Tasks section.

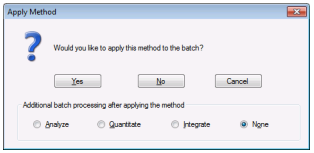
n (optional) Enter 2 for the **Left RT Delta** and **Right RT Delta** for each compound to compensate for potential RT drift.

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 > Restore Default Layout**. **s** Click **View > Panes > 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** under the Save/Exit section in Method tasks (left panel)**.** **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**. Select **Level** as **1**. **b** Click **Home > Analyze Batch > 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

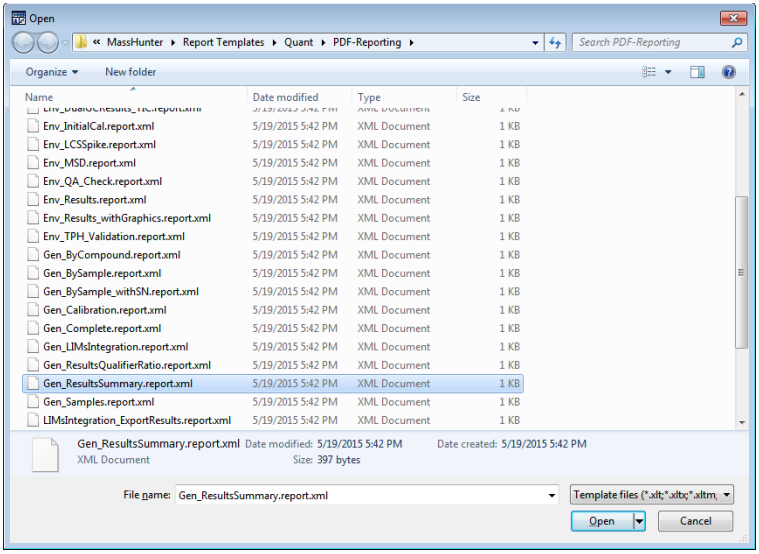
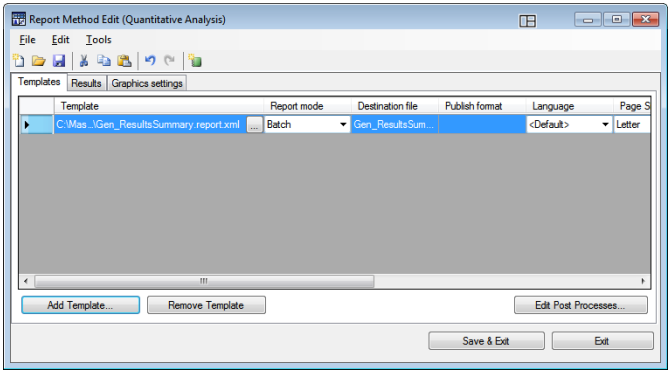
In this task, you create the template file **report.results.xml** that you use to update the MRM method to a dMRM method. You can use any report template, but the quickest report to create is a summary report without graphics.

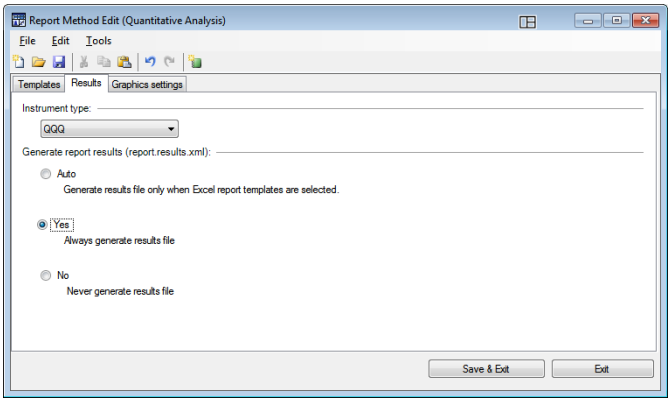
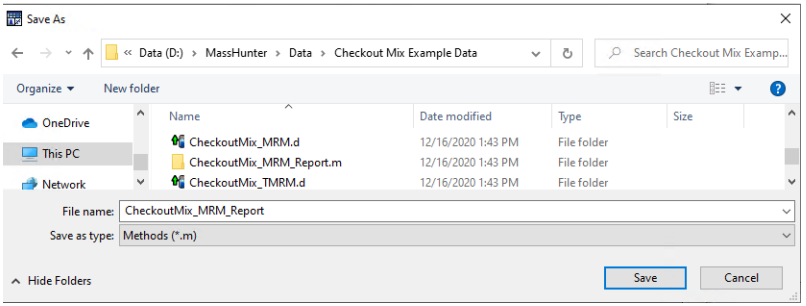
You can use either a Quantitative Analysis report or a data file to create a dMRM method, but the Quantitative Analysis report is recommended. If you use a data file and an error is generated, then none of the compounds in that data file are included in the dMRM method.

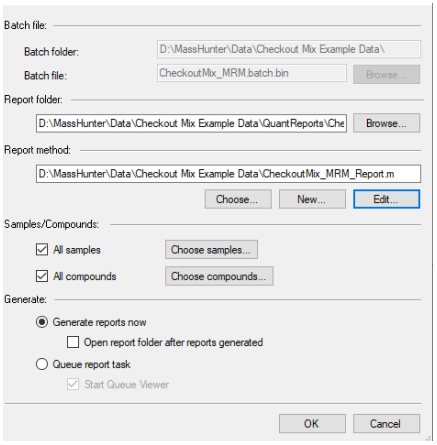
In this task, you:

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

Steps	Detailed Instructions	Comments
1 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 23. **b** Click **File > Save**. **c** Click **Home > Generate Report**. 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
		
		

Steps	Detailed Instructions	Comments
	h In the Report Method Edit program, click **Results**. **i** Click **Yes**.	
		
	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 **Save and Exit**. **o** Click **Generate reports now**. **p** Click **OK**.	The report.results.xml file is in the **\MassHunter\Data\Checkout Mix Example Data \QuantReports\CheckoutMix_MRM** folder.

Steps	Detailed Instructions	Comments
		

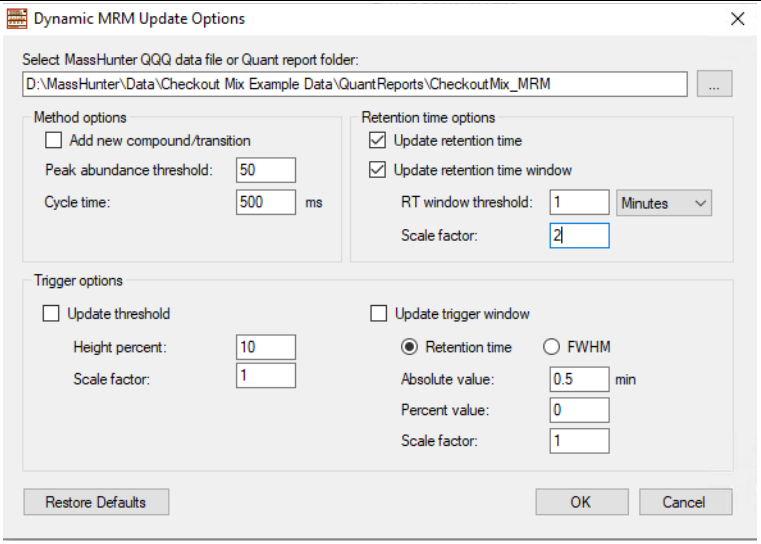
Task 3. Create a dMRM method using Update dMRM

You can create a dMRM method from an MRM data file or a Quantitative Analysis report. You use the Update DMRM Method dialog box. For Ultivo, this is Convert DMRM.

Steps	Detailed Instructions	Comments
1 Open the MRM method **CheckoutMix_MRM.m** you created on **page 10** 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 MRM method you created in step 1 on **page 10**. Click **OK**. **c** Click **Method > Save As**. **d** Type the new method name with the format *iii***CheckoutMix_DMRM.m.**	• 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** For 6400 Series, right-click the **Scan segments** table and click **Update DMRM Method**. The MRM Update Options dialog box opens. For Ultivo, click **Convert to dMRM.** Then click **Update 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.	• 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. Agilent recommends 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



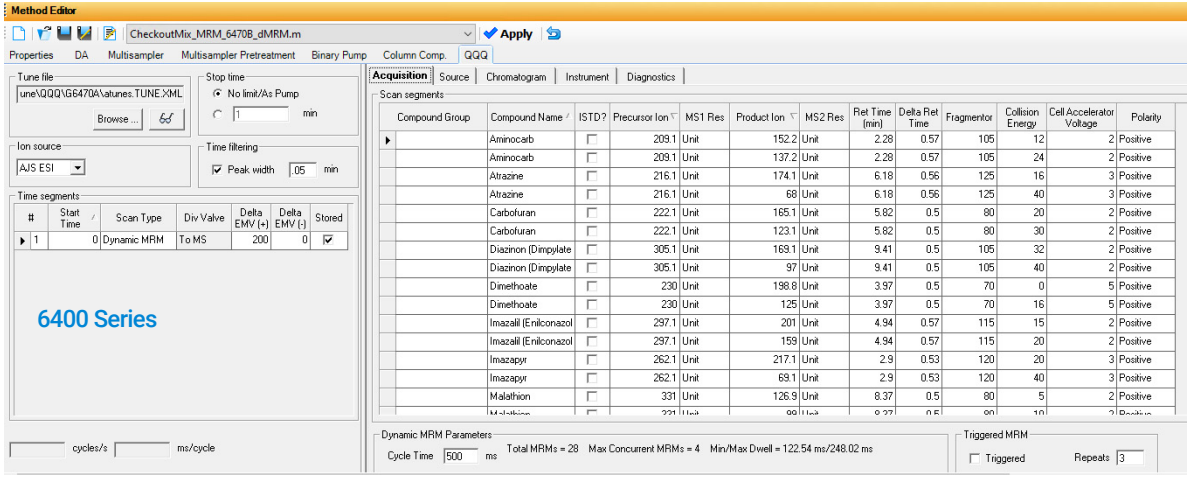
You can update the compounds in the Scan segments table by using a QQQ data file or a Quantitative Analysis report folder.

If you select a QQQ data file and an error is generated, 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 28. The folder path of the quant report in the image is for the provided example report. Instead use the location of the report you created in Task 2.

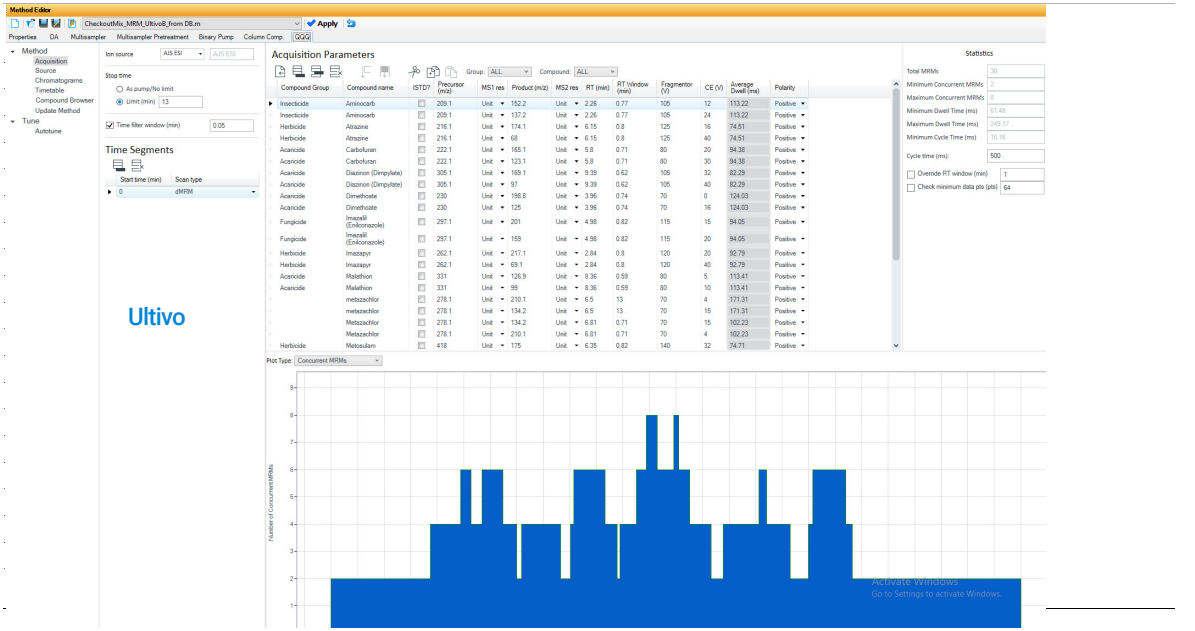
Note that all transitions must be detected in each data file, or the program will generate an error when you update the method.

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**.



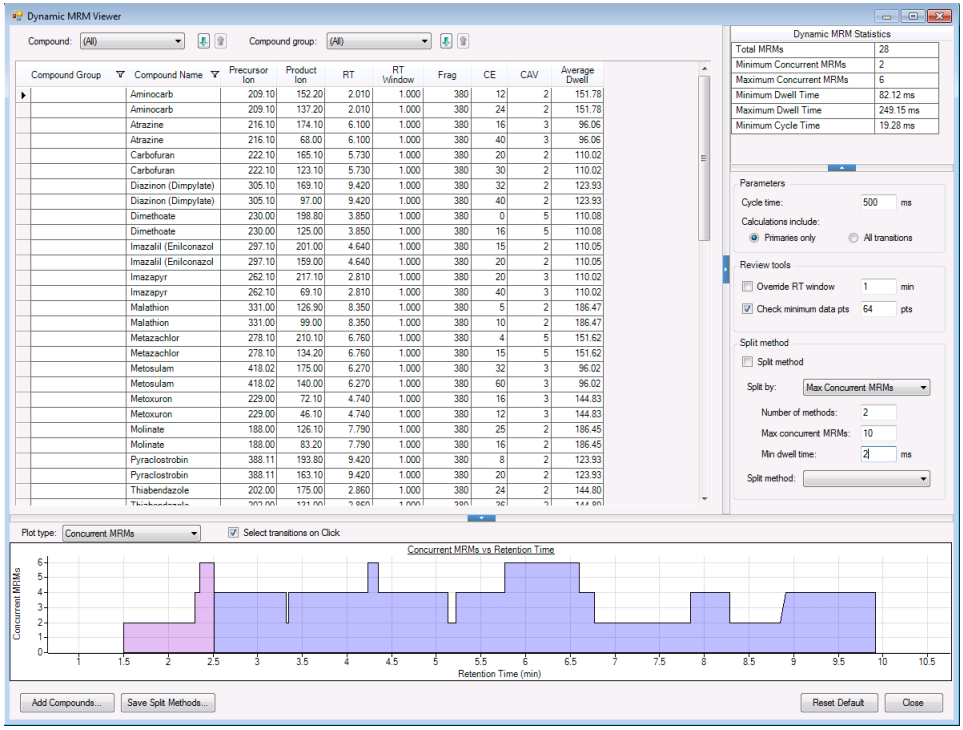
Task 4. Check dMRM acquisition method setup in Dynamic MRM Viewer

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 dMRM data for this particular analysis. If cycle time and concurrent MRMs reduce the dwell time below this value, the minimum cycle time and minimum dwell time on the right will be highlighted.If the minimum dwell time is below the specification of the instrument, a warning will be provided.
- For some newer Agilent LC/TQ models, the dwell time can be as low as 0.5 ms. Lower dwell times allow for faster cycle times at the cost of data points across a chromatographic peak and chromatographic peak reproducibility.
- For good quantitative results, make sure you have 10 data points across the chromatographic peak.

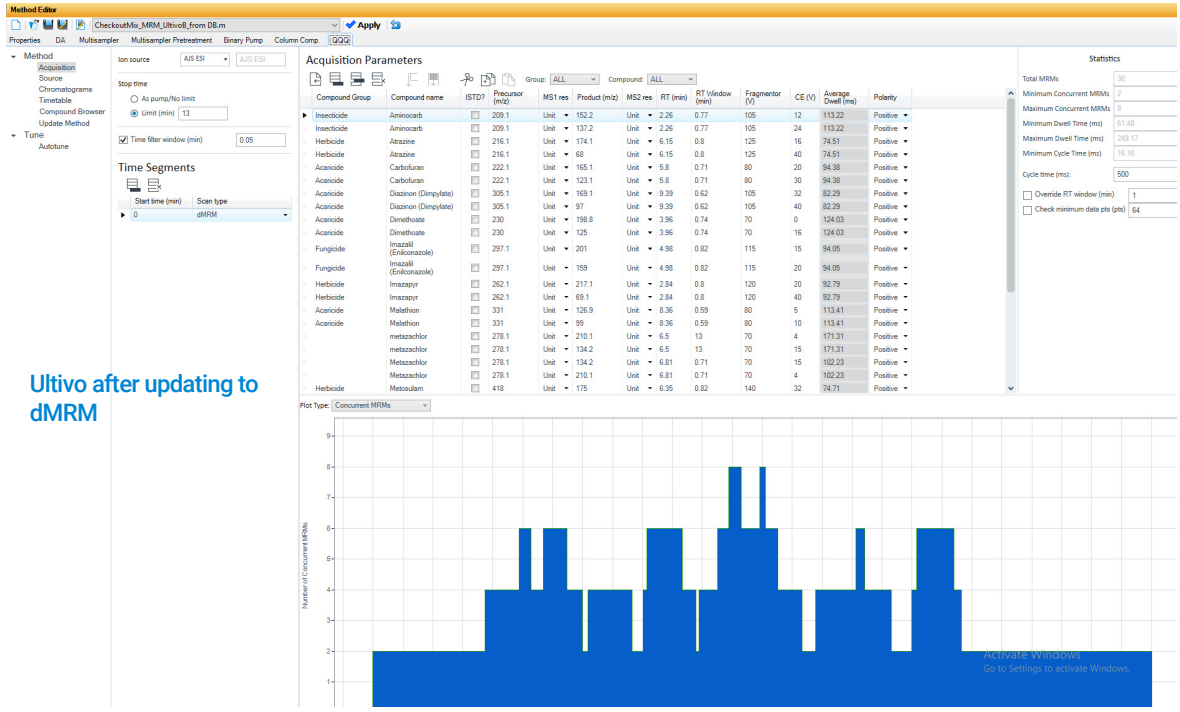
Steps	Detailed Instructions	Comments
1 Start the Dynamic MRM Viewer dialog box. Note: If you have an Ultivo system, the Dynamic MRM Viewer is displayed automatically, so you can skip this step.	• Right-click the Scan segments table and click **Edit DMRM Method**.	
2 Review each compound in the Dynamic 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. • 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**.

Steps	Detailed Instructions	Comments



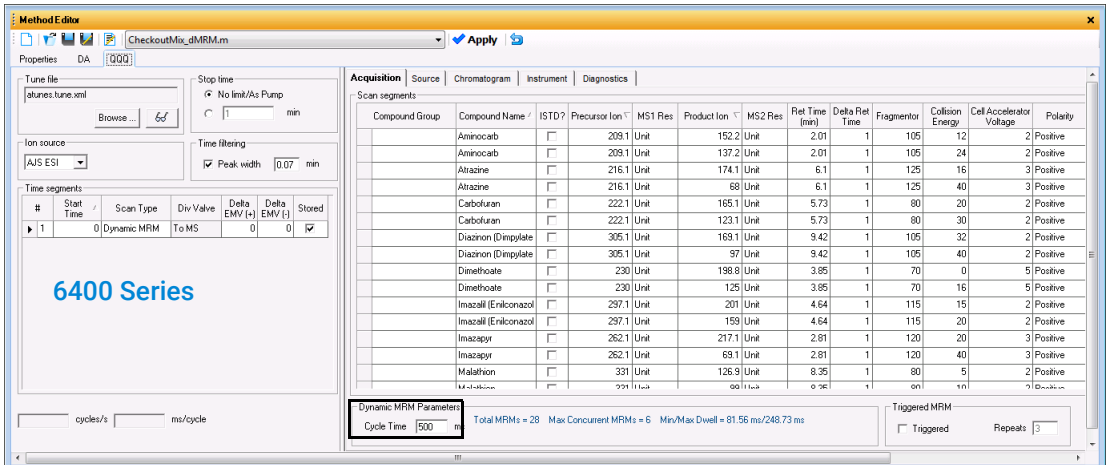
6400 Series

Steps	Detailed Instructions	Comments



Ultivo after updating to dMRM

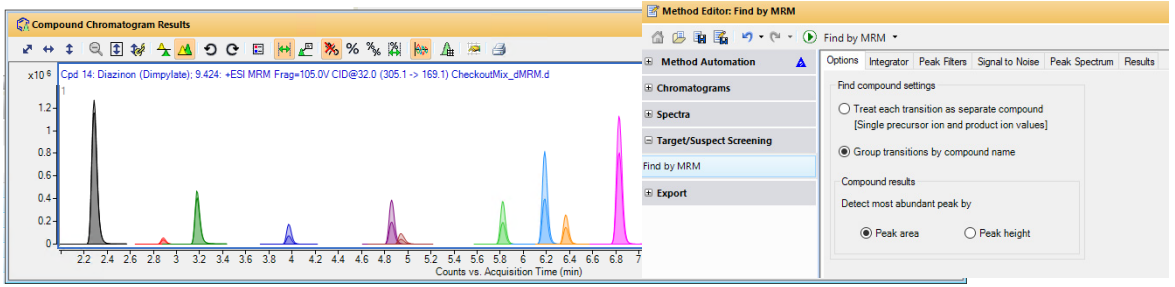
Steps	Detailed Instructions	Comments
3 Once a cycle time is determined for good integration, set the Cycle Time in the QQQ > Acquisition tab.	**a** Type the **Cycle Time**, if necessary. **b** Save the method. The default setting of 500 ms is recommended for most analysis containing more than 15 compounds.	• When you close the Dynamic MRM Viewer, unless you have an Ultivo system, changes made to the cycle time in the Dynamic MRM Viewer are *not* entered into the acquisition method.



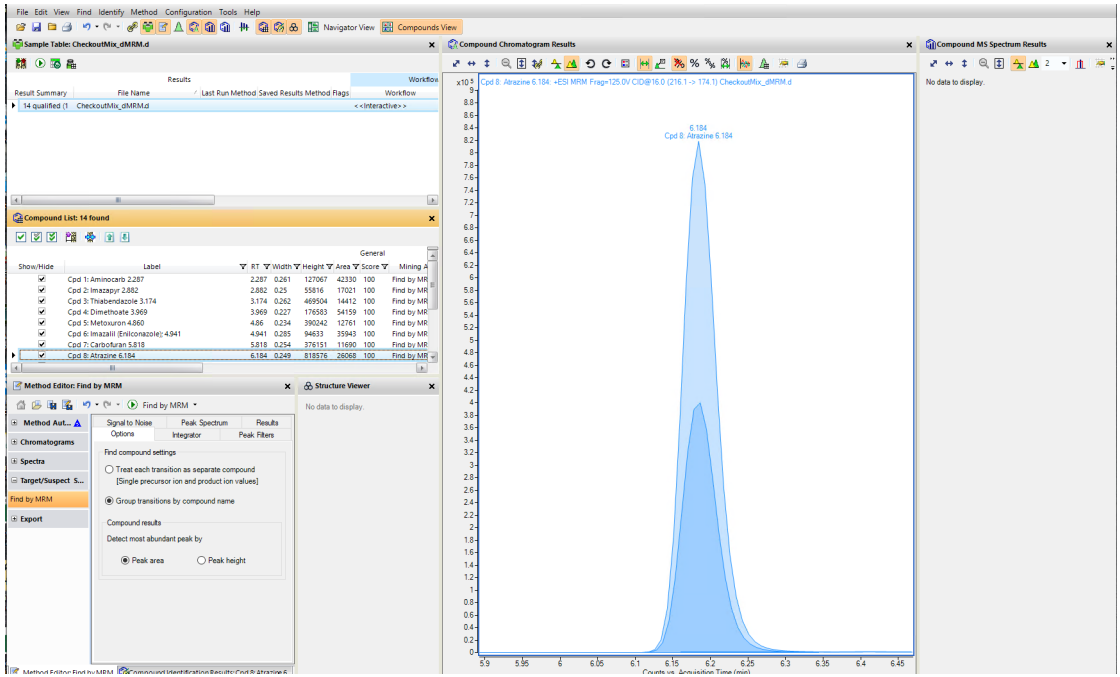
Task 5. Acquire dMRM data and inspect in Qualitative Analysis

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. In the Data File Settings tab, under the File Naming section, type `CheckoutMix_DMRM`. Click **OK**. **c** Click **Worklist > Add Multiple Samples**. **d** Select the DMRM method you created in step 1 on **page 32** as the method name. **e** Click the **Sample Position** tab. **f** Select the Autosampler, Well-plate or Vial Tray. **g** In the graphic, select a single position. Click OK. **h** In the Worklist window, mark the check box to the left of the sample. **i** Click the Start Worklist Run icon in the main toolbar, the **Run Worklist** icon in the Worklist toolbar or click **Worklist > Run**.	• 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 8.

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 10.0** icon,  **a** Click **File > Open Data File**. The system displays the "Open Data File" dialog box. **b** Select **CheckoutMix_DMRM.d**, and click Open. **c** Click the **Compounds View** tab at the top of the Qualitative Analysis user interface. **d** If needed, click **View > Method Editor**. The system displays the Method Editor window. **e** In the Method Automation section, click **Workflow**, and ensure the Workflow is set to Target/Suspect Screening and that Compound Mining is set to Find by MRM. **f** In the Method Editor window, in the Target/Suspect Screening section, click **Find by MRM**. Click the **Group transitions by compound name** option. **g** Click the Peak area option for **Detect most abundant peak by Peak area**.	• You can also use the example dMRM data file in the **Checkout Mix 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. • You cannot edit the retention times of compounds which are identified.	**a** Click **View > Compound List**. **b** Click or use the arrow keys to move through the **Compound Table** to review one compound a a time. See the figure that follows. **c** 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 > Workflow 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.

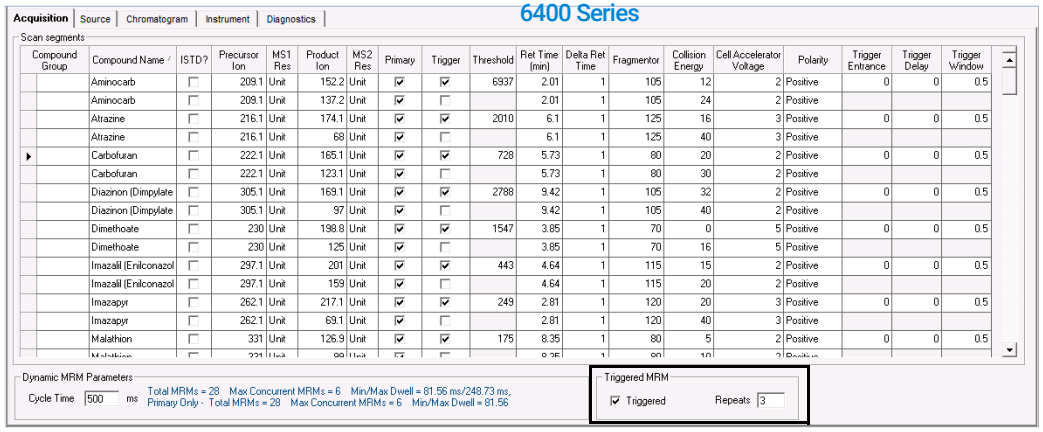
Triggered MRM Repeats Use this parameter to define the number of secondary transition cycles that are acquired. This parameter applies to the whole triggered MRM method, not to individual compounds.

Task 1. Create a tMRM method from a dMRM method

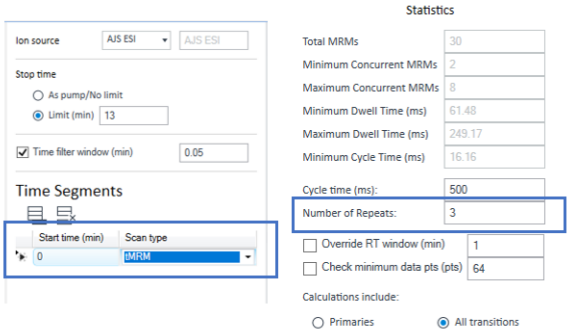
If you have a dMRM method, you can change it to a tMRM method.

Steps	Detailed Instructions	Comments
1 In the Data Acquisition program, you open the dMRM method. You can open the method that you created in step 1 on **page 32** or the example method.	**a** Switch to the Data Acquisition program. **b** Open the DMRM method that you created in step 1 on **page 32**.	• Example **CheckoutMix_DMRM.m** methods for the 6470B and Ultivo instruments can be found in the **Checkout Mix 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** For 6400 Series, mark the **Triggered** check box under Triggered MRM. For Ultivo, under Time Segments, change the Scan type to **tMRM.** **c** Manually mark the Triggers shown in the **"Primary and Secondary Transitions for Triggered MRM"** on page 66. **d** Type 3 for **Repeats**. **e** For 6400 Series systems, right-click the Scan Segments table and click **Import from Database Browser**. The Database Browser opens. For Ultivo, click **Compound Browser** in the left pane. The Database Browser opens.	• For 6400 Series systems, 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 66.

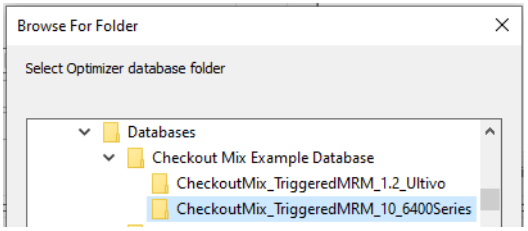
6400 Series



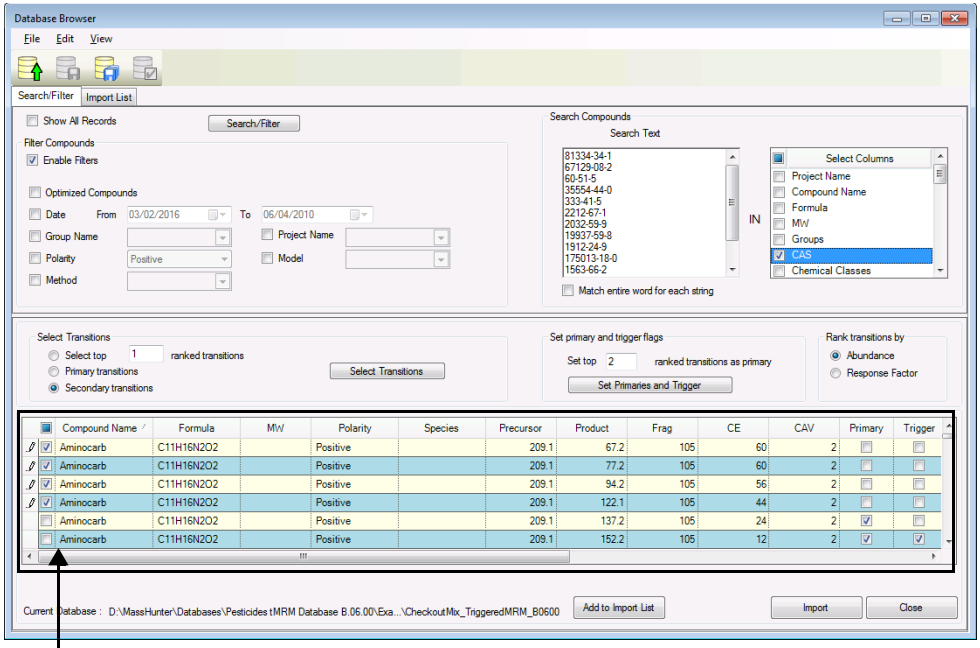
Steps	Detailed Instructions	Comments



3 For 6400 Series, open the **Checkout_Mix_TriggeredMRM_10_6400Series** database. For Ultivo, open the **CheckoutMix_TriggeredMRM_1.2_Ultivo** database in the Database Browser.	**a** In the Database Browser, click **File > Open Database**. **b** Select the appropriate database in the folder **\MassHunter\Databases\Checkout Mix Example Database**. **c** Click **OK**.	



Steps	Detailed Instructions	Comments
4 Select secondary transitions. The CAS numbers are: 2032-59-9 1912-24-9 1563-66-2 333-41-5 60-51-5 35554-44-0 81334-34-1 121-75-5 139528-85-1 19937-59-8 2212-67-1 175013-18-0 148-79-8	**a** Click the **Secondary transitions** option under Select Transitions. **b** Click the **Compound Name** column header to sort the compounds by Compound Name. **c** 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 66. **d** 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**. • To speed this step, you can copy in the entire list of CAS numbers shown in the first column of this table. • 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.

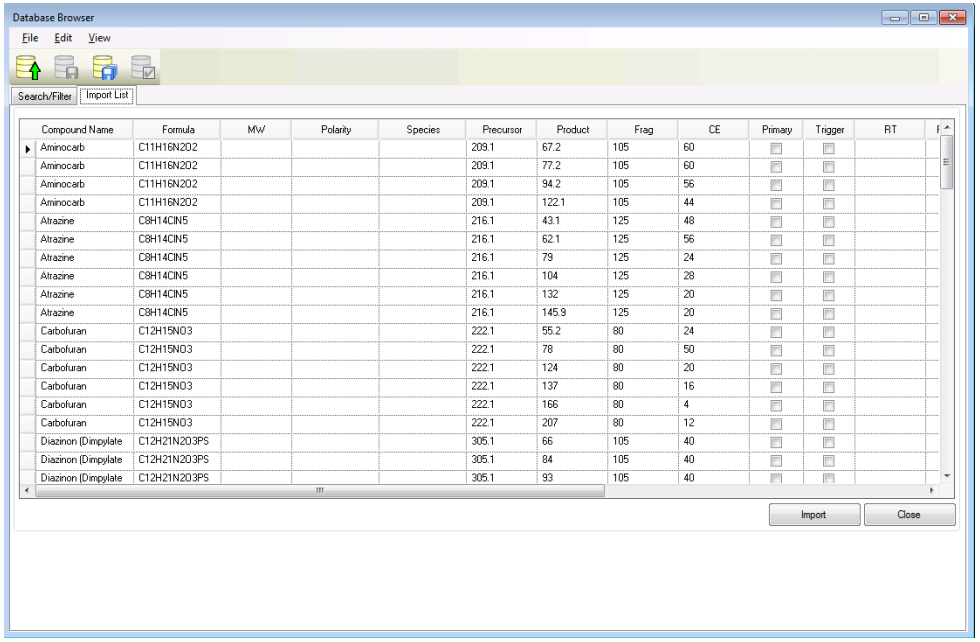


Do not mark the two Primary transitions.

Steps	Detailed Instructions	Comments
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.

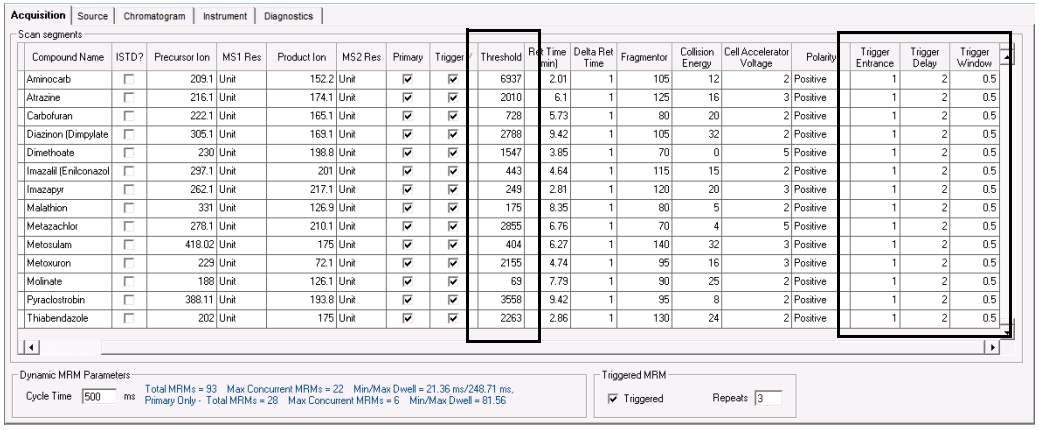
NOTE

- 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**, **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**.	• See the QQQ Concepts guide or the online Help for more information on these values.
8 Update the **Trigger Threshold.**	**a** In the MRM Update Options dialog box, 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 33. • You can also enter the Threshold directly.

Steps	Detailed Instructions	Comments



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

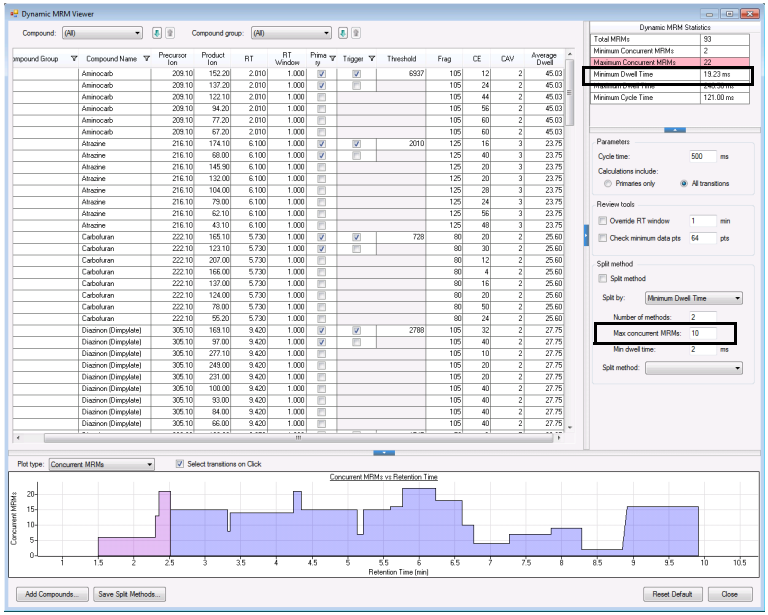
a Click the **Method > Save Method As** command.
b Type ***iii*CheckoutMix_TMRM.m**.
c Click the **Save** button.

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

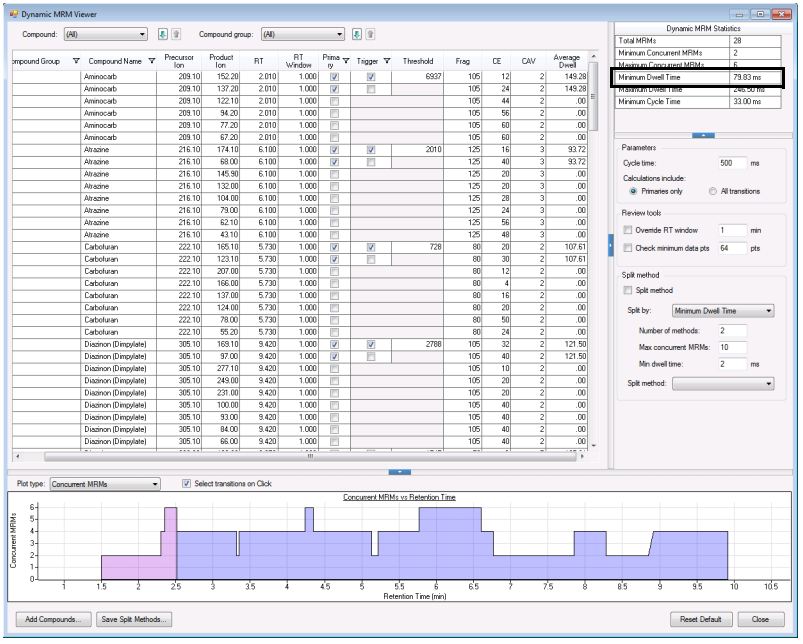
a Right-click the Scan segments table and click **Edit DMRM Method**. The Dynamic MRM Viewer dialog box is opened. If you have an Ultivo system, you can skip this step because the Dynamic MRM Viewer is displayed automatically.
b Switch between the **Primaries only** button and the **All transitions** 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.
- While newer Agilent instruments enable a **Dwell Time** of 0.5 ms, a **Dwell Time** of 5 ms per transition is recommended for this particular analysis.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 for the 6400 Series 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 for the 6400 Series, the Primaries only option is clicked. The Minimum Dwell Time is 79.83 ms.



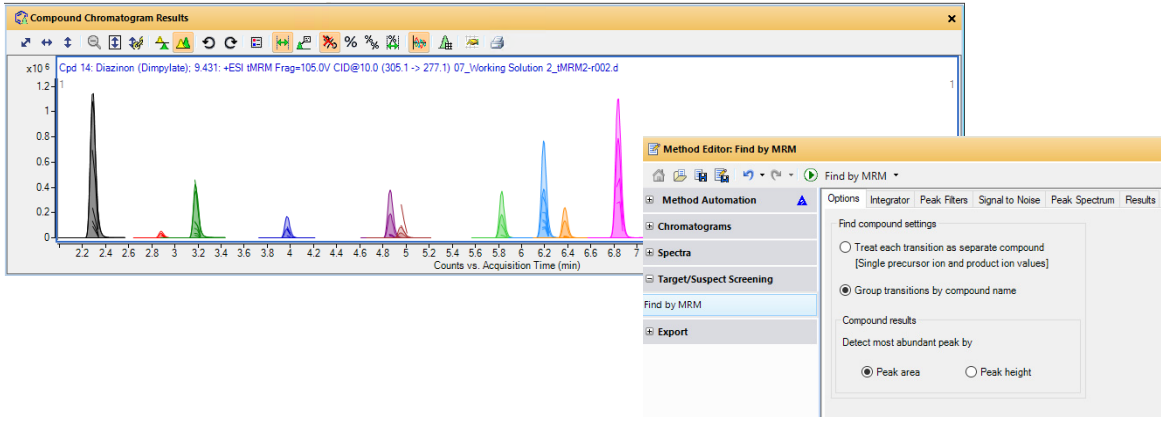
Steps	Detailed Instructions	Comments
11 Adjust the cycle time.	**a** See **step 10** on **page 50** for details.	• The cycle time can be optimized for each analysis. The default cycle time is 500 ms. For methods that contain more than 15 compounds, the cycle time usually needs to be at least 500 ms. 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. In the Data File Settings tab, under the File Naming section, type `CheckoutMix_tMRM_`. Click **OK**. **c** Click **Worklist > Add Multiple Samples**. **d** Select the tMRM method you just created in the previous task as the method name. **e** Click the **Sample Position** tab. **f** Select the Autosampler, Well-plate or Vial Tray. **g** In the graphic, select a single position. Click OK. **h** In the Worklist window, mark the check box to the left of the sample. **i** Click the Start Worklist Run icon in the main toolbar, the **Run Worklist** icon in the Worklist toolbar or click **Worklist > Run**.	• 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 8.

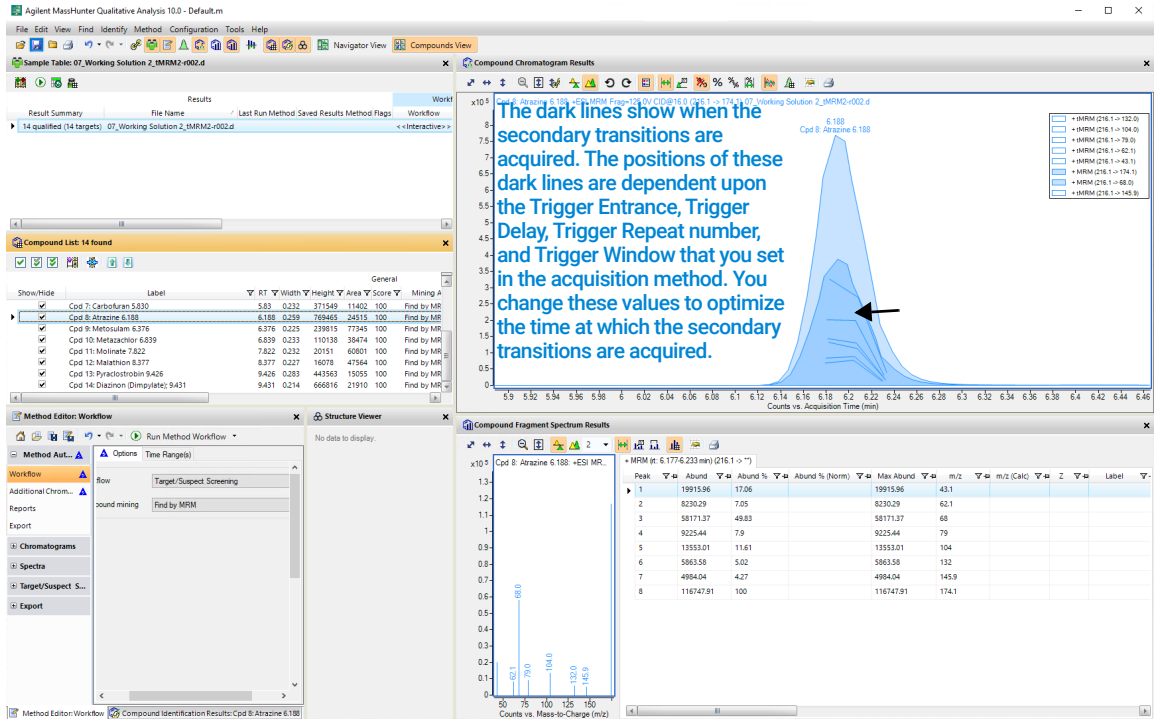
Steps	Detailed Instructions	Comments
2 Find compounds using the Find Compound by MRM algorithm. • Open the data file **CheckoutMix_TMRM.d**.	**a** Start the Qualitative Analysis 10.0 program. **b** Click File > Open Data File. The system displays the "Open Data File" dialog box. **c** Click the **Compounds View** tab at the top of the Qualitative Analysis screen. **d** Select the tMRM method you created in step 9 on page 49, and click **Open**. **e** Click the **Compounds View** tab at the top of the Qualitative Analysis screen. **f** If needed, click **View > Method Editor**. The system displays the Method Editor window. **g** In the Method Automation section, click **Workflow**, and ensure the Workflow is set to Target/Suspect Screening and that Compound Mining is set to Find by MRM. **h** In the Method Editor window, in the Target/Suspect Screening section, click **Find by MRM**. Click the Group transitions by compound name option. **i** Click the Peak area option for **Detect most abundant peak by**.	• 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 **Checkout Mix Example Data** folder.If this file is not on your computer, install it from the installation media.



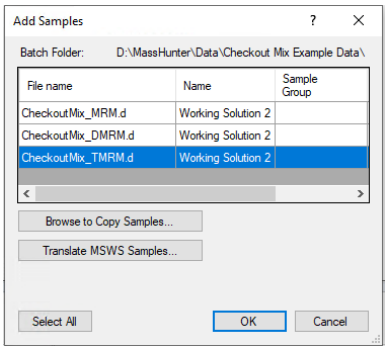
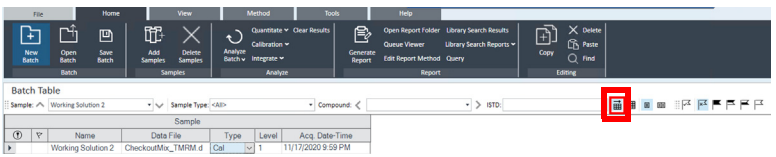
j Click **Find > Find Compounds by MRM**.

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 Compound MS Spectrum Results window. **b** In the Compound Chromatogram Results window, click the **Overlaid mode** button and the **Show Legend in Overlaid** mode button. **c** Click **View > Compound Fragment Spectrum Results**. **d** In the Compound Fragment Spectrum Results window, click the **Spectrum Peak List** button. **e** 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 > Workflow 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 57.
	f Select **Cpd1:Aminocarb**. You click this compound in the Compound List. **g** 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 Compound Chromatogram Results window, you can see lines which indicate the abundances for each Secondary transition.
	h Continue checking each compound to verify that the Primary and Secondary transitions were acquired. See **"Primary and Secondary Transitions for Triggered MRM"** on page 66 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.

Steps	Detailed Instructions	Comments



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

Steps	Detailed Instructions	Comments
1 Start the Quantitative Analysis program.	**a** Click the QQQ Quantitative Analysis (Quant My Way) icon. ().	
2 Set up a batch and add the TMRM data file. • Add the data file **CheckoutMix_TMRM.d**.	**a** Click **New Batch**. **b** Navigate to the location of the TMRM data file. **c** Type `CheckoutMix_TMRM` for the **Batch file** name. **d** Click **Create Batch**. 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

Method Table

Time Segment: <All> Compound: Aminocarb Reset Table View

Sample

Name	Data File	Type	Level	Acq. Method File	Acq. Dat
CheckoutMix_TMRM.d	CheckoutMix_TMRM.d				

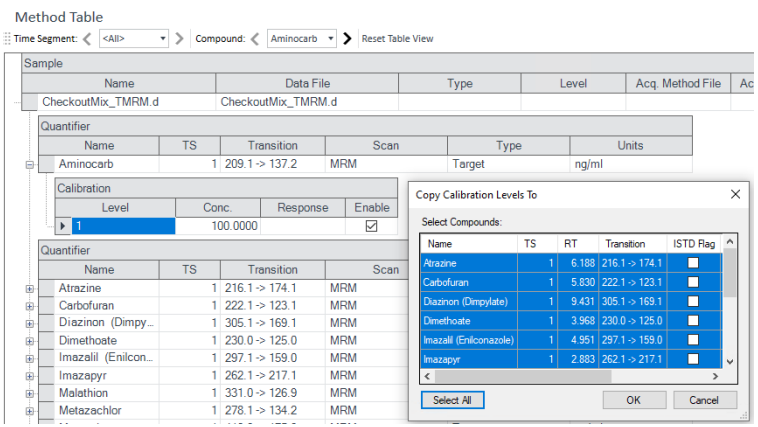
Quantifier

Name	TS	Transition	Scan	Type	Precursor Ion	Product Ion
Aminocarb	1	209.1 -> 137.2	MRM	Target	209.1	137.2
Atrazine	1	216.1 -> 174.1	MRM	Target	216.1	174.1
Carbofuran	1	222.1 -> 123.1	MRM	Target	222.1	123.1
Diazinon (Dimpy...	1	305.1 -> 169.1	MRM	Target	305.1	169.1
Dimethoate	1	230.0 -> 125.0	MRM	Target	230.0	125.0
Imazalil (Enilcon...	1	297.1 -> 159.0	MRM	Target	297.1	159.0
Imazapyr	1	262.1 -> 217.1	MRM	Target	262.1	217.1
Malathion	1	331.0 -> 126.9	MRM	Target	331.0	126.9
Metazachlor	1	278.1 -> 134.2	MRM	Target	278.1	134.2
Metosulam	1	418.0 -> 175.0	MRM	Target	418.0	175.0
Metoxuron	1	229.0 -> 72.1	MRM	Target	229.0	72.1
Molinate	1	188.0 -> 83.2	MRM	Target	188.0	83.2
Pyraclostrobin	1	388.1 -> 193.8	MRM	Target	388.1	193.8
Thiabendazole	1	202.0 -> 175.0	MRM	Target	202.0	175.0

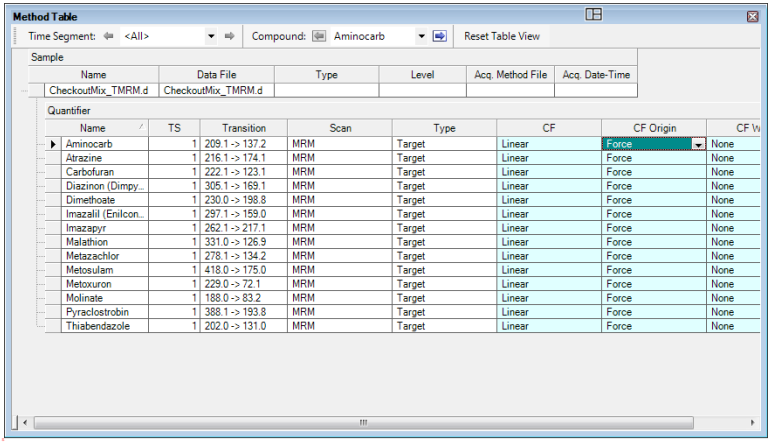
4 Set the Concentration Setup.
- Add calibration level 1 with a concentration of 100.

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 1 0 0.
e Right-click in the Level box and click **Copy Calibration Levels To**.
f Click **Select All**. Click **OK**.

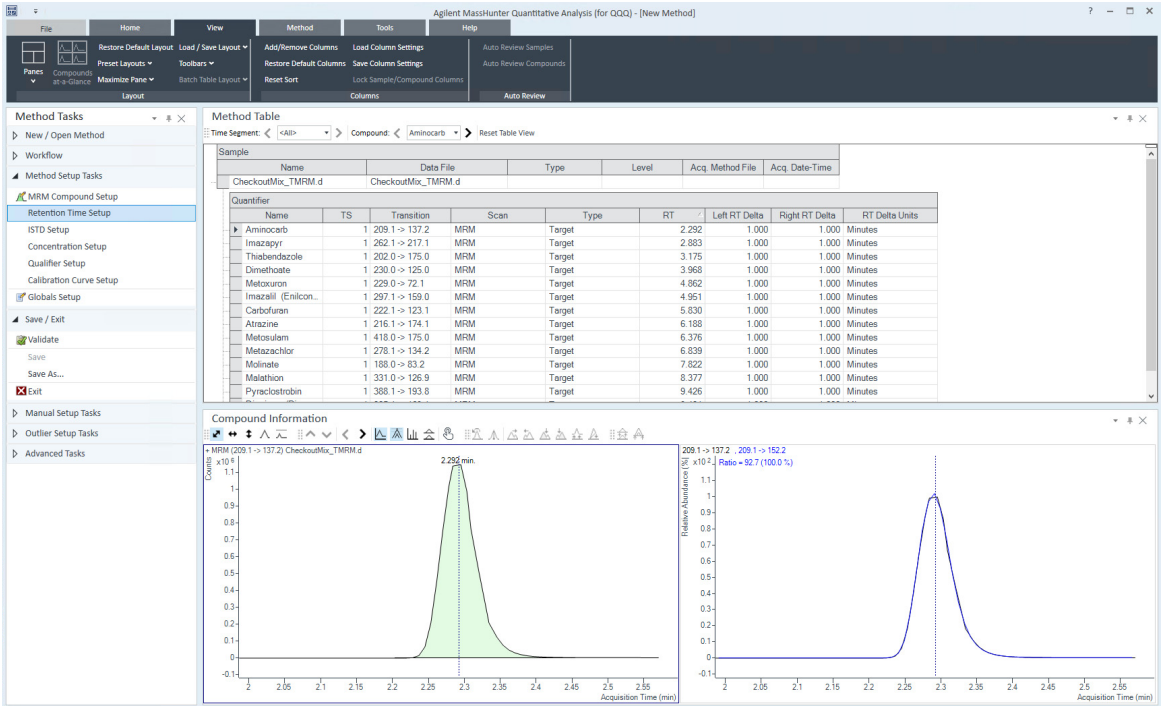
- Refer to the online Help in the Quantitative Analysis program for additional help on these tasks.



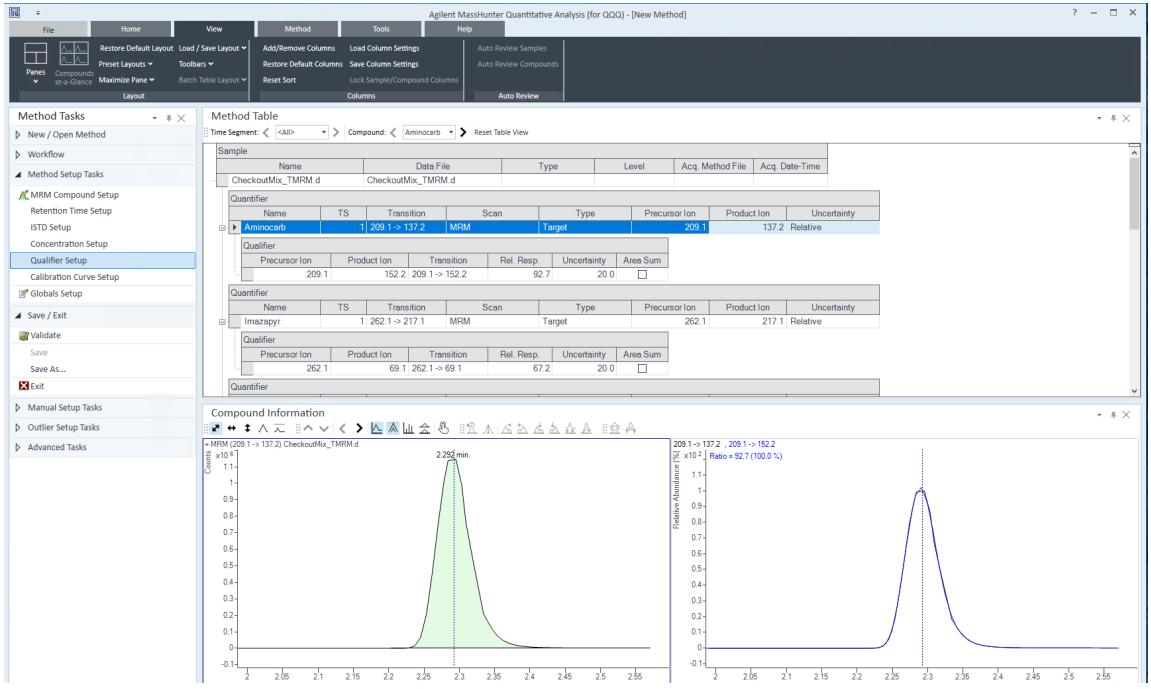
Steps	Detailed Instructions	Comments
5 Change the calibration curve. • Force the origin to be included.	**a** Select **Calibration Curve Setup** in the Method Setup Tasks section in the Method Tasks pane. **b** Set the **CF Origin** to **Force** for the first compound. **c** Right-click this value and 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.



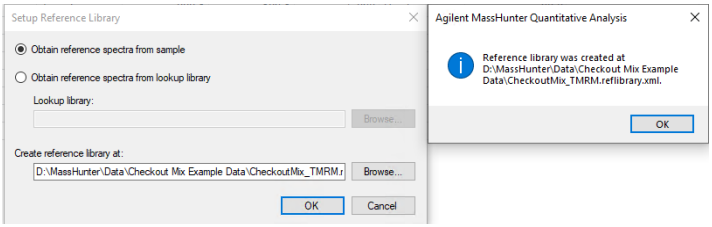
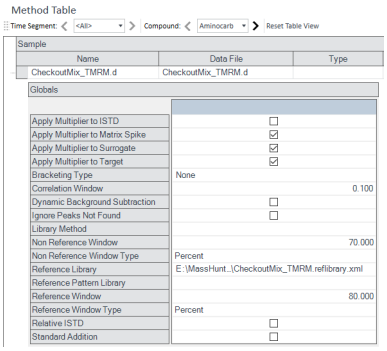
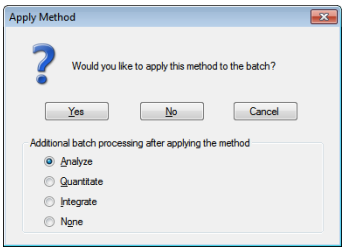
Steps	Detailed Instructions	Comments
6 Resolve the RTs. The RT elution order is: • Aminocarb • Imazapyr • Thiabendazole • Dimethoate • Metoxuron • Imazalil (Enilconazole) • Carbofuran • Atrazine • Metosulam • Metazachlor • Molinate • Malathion • Diazinon (Dimpylate) • Pyraclostrobin	**a** Select **Retention 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.

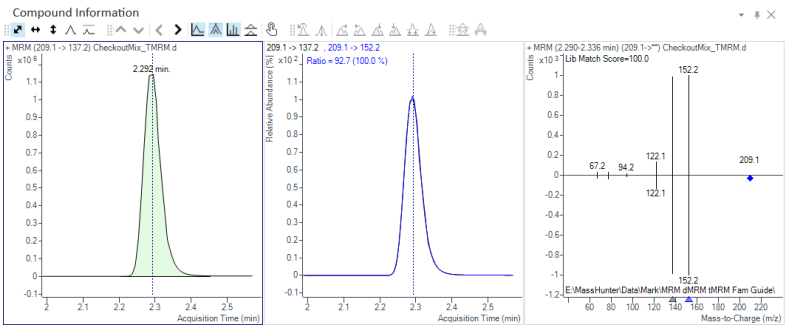


Steps	Detailed Instructions	Comments
7 Review qualifier ratios.	**c** Select **Qualifier Setup** in the Method Setup Tasks section. **d** Right-click the Method Table and click **Expand All**. **e** Click the Show/Hide Qualifiers button in the toolbar in the Compound Information window. **f** Click on 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.	



8 Set up the Reference Library.	**a** Click **Method > 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".

Steps	Detailed Instructions	Comments
		
	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.	
		
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.	
		

Steps	Detailed Instructions	Comments
10 Review the results, and save the batch.	**a** Inspect the results. **b** If the Compound Information window is not open, click **View > Panes > 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)**
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 > Update > Update Qualifier Ratios**. **b** Select the compounds for update and click **OK**. **c** Click **Method > Exit**. **d** Click **Yes** to apply the method to the batch. **e** Check qualifier ratios. **f** Resolve errors and messages. **g** 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/81334-34-1	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.

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.

Table 3. Primary and secondary positive transitions for Checkout Mix analytes

Compound Name	Primary?	Trigger	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Ret Time (min)	Delta RT (min)	CE
Aminocarb	Yes	Yes	209.1	Unit	152.2	Unit	2.01	0.88	12
Aminocarb	Yes		209.1	Unit	137.2	Unit	2.01	0.88	24
Aminocarb			209.1	Unit	122.1	Unit			44
Aminocarb			209.1	Unit	94.2	Unit			56
Aminocarb			209.1	Unit	77.2	Unit			60
Aminocarb			209.1	Unit	67.2	Unit			60
Atrazine	Yes	Yes	216.1	Unit	174.1	Unit	6.1	0.83	16
Atrazine	Yes		216.1	Unit	68	Unit	6.1	0.83	40
Atrazine			216.1	Unit	145.9	Unit			20
Atrazine			216.1	Unit	132	Unit			20
Atrazine			216.1	Unit	104	Unit			28
Atrazine			216.1	Unit	79	Unit			24
Atrazine			216.1	Unit	62.1	Unit			56
Atrazine			216.1	Unit	43.1	Unit			48
Carbofuran	Yes	Yes	222.1	Unit	165.1	Unit	6.83	0.68	20
Carbofuran	Yes		222.1	Unit	123.1	Unit	6.83	0.68	30
Carbofuran			222.1	Unit	207	Unit			12
Carbofuran			222.1	Unit	166	Unit			4
Carbofuran			222.1	Unit	137	Unit			16
Carbofuran			222.1	Unit	124	Unit			20

Table 3. Primary and secondary positive transitions for Checkout Mix analytes

Compound Name	Primary?	Trigger	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Ret Time (min)	Delta RT (min)	CE
Carbofuran			222.1	Unit	78	Unit			50
Carbofuran			222.1	Unit	55.2	Unit			24
Diazinon (Dimpylate)	Yes	Yes	305.1	Unit	169.1	Unit	10.4	1.04	32
Diazinon (Dimpylate)	Yes		305.1	Unit	97	Unit	10.4	1.04	40
Diazinon (Dimpylate)			305.1	Unit	277.1	Unit			10
Diazinon (Dimpylate)			305.1	Unit	249	Unit			20
Diazinon (Dimpylate)			305.1	Unit	231	Unit			20
Diazinon (Dimpylate)			305.1	Unit	100	Unit			40
Diazinon (Dimpylate)			305.1	Unit	93	Unit			40
Diazinon (Dimpylate)			305.1	Unit	84	Unit			40
Diazinon (Dimpylate)			305.1	Unit	66	Unit			40
Dimethoate	Yes		230	Unit	198.8	Unit	4.95	0.6	0
Dimethoate	Yes		230	Unit	125	Unit	4.95	0.6	16
Dimethoate			230	Unit	170.9	Unit			8
Dimethoate			230	Unit	156.9	Unit			16
Dimethoate			230	Unit	88	Unit			8
Dimethoate			230	Unit	79	Unit			32
Imazalil (Enilconazole)	Yes	Yes	297.1	Unit	201	Unit	6.23	0.99	15
Imazalil (Enilconazole)	Yes		297.1	Unit	159	Unit	6.23	0.99	20
Imazalil (Enilconazole)			297.1	Unit	133	Unit			12
Imazalil (Enilconazole)			297.1	Unit	105.1	Unit			36
Imazalil (Enilconazole)			297.1	Unit	93.1	Unit			20
Imazalil (Enilconazole)			297.1	Unit	77.1	Unit			60
Imazalil (Enilconazole)			297.1	Unit	69	Unit			60
Imazalil (Enilconazole)			297.1	Unit	41	Unit			36
Imazapyr	Yes	Yes	262.1	Unit	217.1	Unit	3.83	0.63	20
Imazapyr	Yes		262.1	Unit	69.1	Unit	3.83	0.63	40
Imazapyr			262.1	Unit	220.1	Unit			20

Table 3. Primary and secondary positive transitions for Checkout Mix analytes

Compound Name	Primary?	Trigger	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Ret Time (min)	Delta RT (min)	CE
Imazapyr			262.1	Unit	202.1	Unit			20
Imazapyr			262.1	Unit	149	Unit			20
Imazapyr			262.1	Unit	131	Unit			40
Imazapyr			262.1	Unit	86.1	Unit			20
Malathion	Yes	Yes	331	Unit	126.9	Unit	9.37	0.94	5
Malathion	Yes		331	Unit	99	Unit	9.37	0.94	10
Metazachlor	Yes	Yes	278.1	Unit	210.1	Unit	7.83	0.86	4
Metazachlor	Yes		278.1	Unit	134.2	Unit	7.83	0.86	15
Metazachlor			278.1	Unit	105.1	Unit			44
Metazachlor			278.1	Unit	79.1	Unit			60
Metosulam	Yes	Yes	418	Unit	175	Unit	7.36	0.79	32
Metosulam	Yes		418	Unit	140	Unit	7.36	0.79	60
Metosulam			418	Unit	354.2	Unit			20
Metosulam			418	Unit	238.2	Unit			16
Metosulam			418	Unit	190	Unit			20
Metosulam			418	Unit	77.2	Unit			60
Metoxuron	Yes	Yes	229	Unit	72.1	Unit	5.86	0.63	16
Metoxuron	Yes		229	Unit	46.1	Unit	5.86	0.63	12
Metoxuron			229	Unit	165.3	Unit			4
Metoxuron			229	Unit	156.1	Unit			24
Metoxuron			229	Unit	109	Unit			12
Metoxuron			229	Unit	80	Unit			44
Metoxuron			229	Unit	55.9	Unit			60
Molinate	Yes	Yes	188	Unit	126.1	Unit	8.81	0.88	25
Molinate	Yes		188	Unit	83.2	Unit	8.81	0.88	16
Molinate			188	Unit	98	Unit			12
Molinate			188	Unit	95.5	Unit			28
Molinate			188	Unit	81	Unit			20

Table 3. Primary and secondary positive transitions for Checkout Mix analytes

Compound Name	Primary?	Trigger	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Ret Time (min)	Delta RT (min)	CE
Molinate			188	Unit	70	Unit			16
Molinate			188	Unit	55.1	Unit			19
Pyraclostrobin	Yes	Yes	388.1	Unit	193.8	Unit	10.4	1.04	8
Pyraclostrobin	Yes		388.1	Unit	163.1	Unit	10.4	1.04	20
Pyraclostrobin			388.1	Unit	218.6	Unit			32
Pyraclostrobin			388.1	Unit	196.2	Unit			4
Pyraclostrobin			388.1	Unit	164.1	Unit			12
Pyraclostrobin			388.1	Unit	104.1	Unit			60
Pyraclostrobin			388.1	Unit	91.1	Unit			60
Thiabendazole	Yes	Yes	202	Unit	175	Unit	4.1	0.66	24
Thiabendazole	Yes		202	Unit	131	Unit	4.1	0.66	36
Thiabendazole			202	Unit	143.1	Unit			40
Thiabendazole			202	Unit	104.1	Unit			44
Thiabendazole			202	Unit	92.1	Unit			36
Thiabendazole			202	Unit	77	Unit			60
Thiabendazole			202	Unit	65	Unit			52
Thiabendazole			202	Unit	51	Unit			60

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In This Guide

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

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