

# MassHunter MRM/dMRM/tMRM Database

# **Familiarization Guide**

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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.





#### **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  $\mu$ L 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. Task 1. Create an MRM method

# 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			etailed Instructions	Comments			
1	In the Data Acquisition program, open the default method and save as: <i>iii</i> CheckoutMix_MRM.m, where <i>iii</i> are your initials.	a b c d	Start the Data Acquisition program. Open the <b>default.m</b> method. Click <b>Method &gt; Save As</b> . Type <i>iii</i> CheckoutMix_MRM.m, where <i>iii</i> 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 b c d f	In the Method Editor window, click the <b>HiP Sampler</b> tab. Enter the parameters from Figure 1 on page 65 Click the <b>Binary Pump</b> tab. Enter the parameters from Figure 2 on page 66. Click the <b>Column Comp.</b> tab. Enter the parameters from Figure 3 on page 67.	•	If you have a different LC model, the LC parameters can be different.		

Task 1. Create an MRM method

Steps		Detailed Instructions	Comments				
3	Set the source parameters and change the method to an MRM method.	<ul> <li>The values listed here are for non-iFunnel instruments for use with the Checkout Mix. For databases that support iFunnel instruments, refer to the <i>Method Setup Guide</i> for iFunnel settings.</li> <li>a In the Method Editor window, click the <b>QQQ</b> tab.</li> <li>b Click the Source tab.</li> <li>c For Gas Temp, type 250.</li> <li>d For Gas Flow, type 7.</li> <li>e For Nebulizer, type 40.</li> <li>f For Sheath Gas Temp, type 325.</li> <li>g For Sheath Gas Flow, type 11.</li> <li>h For Capillary, type 3500.</li> <li>i For Nozzle voltage, type 0.</li> <li>j Click the Acquisition tab.</li> <li>k In the Time segments on the left side of the QQQ tab, select MRM as the Scan Type for the first Time segment.</li> </ul>	<ul> <li>The Scan segments table always has to have at least one row. You manually remove this row after importing transitions from the Database Browser.</li> </ul>				
4	Open the <b>CheckoutMix_TriggeredMRM_B0</b> <b>600</b> in Database Browser, from the <b>QQQ Acquisition</b> tab.	<ul> <li>a Right-click the Scan segments table and click Import from Database Browser. The Database Browser opens. In the Database Browser, click File &gt; Open Database.</li> <li>b Select the CheckoutMix_TriggeredMRM_B0600 database in the \MassHunter\Databases\Product Database x.xx.xx\Example database folder.</li> <li>c Click OK.</li> </ul>					

Task 1. Create an MRM method



Task 1. Create an MRM method

Steps	Detailed Instructions	Comments				
<ul> <li>5 Select primary transitions.</li> <li>See "Primary and Secondary Transitions for Triggered MRM" on page 68 for a list of the Primary transitions.</li> <li>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.</li> <li>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.</li> </ul>	<ul> <li>a Click the Compound Name column header to sort the compounds by Compound Name.</li> <li>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.</li> <li>c To quickly mark only the primary transitions for the Checkout Mix:</li> <li>Under Search Compounds, mark the CAS check box. In the Search Text text box, type the CAS numbers for the Checkout Mix.</li> <li>Under Select Transitions, select Primary transitions and click Select Primary. See next page for filter examples.</li> <li>d Review the transitions in the table. Clear the check box next to any transitions that you do not want to include.</li> </ul>	<ul> <li>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.</li> <li>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).</li> <li>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 <i>Method Setup Guide</i> for the more details on choosing the most selective transitions for your analysis.</li> </ul>				

Task 1. Create an MRM method

#### 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.

20

10

5

15

4

60

V

V

**V** 

7

V

V

V

**V** 

Close

Import

Task 1. Create an MRM method

ep	os			Det	tailed Instru	uctions		Comments						
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 b c ns, d vith e	Click the <b>A</b> Click the <b>Im</b> Review the If needed, s transitions positive MF the selectio Click the <b>Im</b>	Only the transitions that you marked are added to the Import List. Removal of the negative MRM transition for compounds that als have a positive MRM transition ensures that one compound nam is associated with only one polarity. One compound cannot have both negative and positive polarity transitions.								
Data File	base Browser e Edit View												• 🔀	
Gear	rch/Filter Import List													
-	Compound Name	Formula	MW	Polarity	Species	Precursor	Product	Frag	CE	Primary	Trigger	RT	F.A.	
	Aminocarb	C11H16N2O2		Positive		209.1	137.2	105	24					
	Aminocarb	C11H16N2O2		Positive		209.1	152.2	105	12	<b>V</b>	<b>V</b>			
	Atrazine	C8H14CIN5		Positive		216.1	68	125	40	<b>V</b>			=	
	Atrazine	C8H14CIN5		Positive		216.1	174.1	125	16	<b>V</b>				
	Carbofuran	C12H15NO3		Positive		222.1	123.1	80	30	<b>V</b>				
	Carbofuran	C12H15NO3		Positive		222.1	165.1	80	20	<b>V</b>				
	Diazinon (Dimpylate	C12H21N2O3PS		Positive		305.1	97	105	40					
							1							
	Diazinon (Dimpylate	C12H21N2O3PS		Positive		305.1	169.1	105	32	V	<b>V</b>			
	Diazinon (Dimpylate Dimethoate	C12H21N2O3PS C5H12NO3PS2		Positive Positive		305.1 230	169.1 125	105 70	32	V				
	Diazinon (Dimpylate Dimethoate Dimethoate	C12H21N2O3PS C5H12NO3PS2 C5H12NO3PS2		Positive Positive Positive		305.1 230 230	169.1 125 198.8	105 70 70	32 16 0	<b>V</b>				
	Diazinon (Dimpylate Dimethoate Dimethoate Imazalil (Enilconazo	C12H21N2O3PS C5H12NO3PS2 C5H12NO3PS2 C14H14Cl2N2O		Positive Positive Positive Positive		305.1 230 230 297.1	169.1 125 198.8 159	105 70 70 115	32 16 0 20	V V V				
	Diazinon (Dimpylate Dimethoate Dimethoate Imazalil (Enilconazo Imazalil (Enilconazo	C12H21N2O3PS C5H12NO3PS2 C5H12NO3PS2 C14H14CI2N2O C14H14CI2N2O C14H14CI2N2O		Positive Positive Positive Positive Positive		305.1 230 230 297.1 297.1	169.1 125 198.8 159 201	105 70 70 115 115	32 16 0 20 15					

262.1

331

331

278.1

278.1

418

217.1

99

126.9

134.2

210.1

140

120

80

80

70

70

140

Positive

Positive

Positive

Positive

Positive

Positive

Positive

III

lmazapyr

lmazapyr

Malathion

Malathion

Metazachlor

Metazachlor

Metosulam

C13H15N3O3

C10H19O6PS2

C10H19O6PS2

C14H16CIN3O

C14H16CIN3O

C14H13CI2N5O4

Task 1. Create an MRM method

Steps			etailed Instructions	C	Comments			
7	Review the MRM transitions in the Data Acquisition program.	a b c	Delete the original compound in the Scan segments table. Sort the table by the Compound Name. Review the transitions for each compound.	•	If a red box appears in the Scan segments table, you click the <b>Apply</b> 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.			

Method Editor ×												
🗄 📄 💕 💾 🛃   🎅   CheckoutMix_MRM.m	•	✔ Apply 🛛 🔄										
Properties DA [000]												
Tune file Stop time ( No limit/As Pump	Chromatogram Instrument Diagnostics											
Browse 6d C 1 min	Compound Group	Compound Name 7	ISTD?	Precursor Ion	MS1 Res	Product Ion	MS2 Res	Dwell	Fragmentor	Collision Energy	Cell Accelerator Voltage	Polarity
- Ion source - Time filtering	•	Aminocarb		209.1	Unit	152.2	Unit	20	105	12	2	Positive
AISESI T		Aminocarb		209.1	Unit	137.2	Unit	20	105	24	2	Positive
		Atrazine	Г	216.1	Unit	174.1	Unit	20	125	16	3	Positive
Time segments		Atrazine	Π	216.1	Unit	68	Unit	20	125	40	3	Positive
# Start / Scan Type Div Valve Delta Delta Stored		Carbofuran		222.1	Unit	165.1	Unit	20	80	20	2	Positive
▶ 1 0 MRM To MS 0 0 V		Carbofuran		222.1	Unit	123.1	Unit	20	80	30	2	Positive
		Diazinon (Dimpylate)		305.1	Unit	169.1	Unit	20	105	32	2	Positive
		Diazinon (Dimpylate)		305.1	Unit	97	Unit	20	105	40	2	Positive
		Dimethoate		230	Unit	198.8	Unit	20	70	0	5	Positive
		Dimethoate		230	Unit	125	Unit	20	70	16	5	Positive
		Imazalii (Enilconazole		297.1	Unit	201	Unit	20	115	15	2	Positive
		Imazalil (Enilconazole		297.1	Unit	159	Unit	20	115	20	2	Positive
		Imazapyr		262.1	Unit	217.1	Unit	20	120	20	3	Positive
		Imazapyr		262.1	Unit	69.1	Unit	20	120	40	3	Positive
		Malathion		331	Unit	126.9	Unit	20	80	5	2	Positive
		Malathion	Γ	331	Unit	99	Unit	20	80	10	2	Positive
		Metazachlor		278.1	Unit	210.1	Unit	20	70	4	5	Positive
1.52 cycles/s 658.0 ms/cycle		Metazachlor	Π	278.1	Unit	134.2	Unit	20	70	15	5	Positive
<i>ϵ</i>		III										F

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.

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

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

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

S	teps	Detailed Instructions	Comments				
<u>S</u> 2	teps Find compounds using the Find Compound by MRM algorithm. • Open the data file CheckoutMix_MRM.d.	Detailed Instructions         Start the Qualitative Analysis program. If it is not running, double-click the Qualitative Analysis B.07.00 icon         Qualitative Analysis B.07.00 icon         ()       ()         ()<	<ul> <li>Comments</li> <li>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 &gt; User Interface Configuration. Then, you mark the Unit Mass check box and the MS/MS (QQQ, Q-TOF) check box. Then, click OK.</li> <li>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.</li> </ul>				
		Explorer window. n In the Find Compounds section, click Find by MRM.					
		<ul> <li>In the Method Editor Window, click the Group transitions by compound name option.</li> </ul>					
		<ul> <li>p Click the Peak area option for Detect most abundant peak by.</li> </ul>					



q Click Find > Find Compounds by MRM.

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

Steps	Detailed Instructions	Comments			
<ul> <li>3 Review the results of the Find Compounds by MRM algorithm.</li> <li>Make sure that the primary ions are found for each compound.</li> <li>Switch to the Compound Details view.</li> <li>It is not possible to edit the retention times of compounds which are identified.</li> <li>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.</li> </ul>	<ul> <li>a Close the Method Explorer and Method Editor windows.</li> <li>b Click View &gt; MS Spectrum Peak List 1.</li> <li>c Click Compound Details View to change the view. See the figures that follow.</li> <li>d Click or use the arrow keys to move through the Compound Table to review one compound a a time.</li> <li>e Review each compound. Verify that the primary transitions for each compound were found.</li> <li>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.</li> </ul>	<ul> <li>You can also print a Compound Report to review results. You click File &gt; Print &gt; Compound Report. The Compound Report sorts the compounds by retention time.</li> <li>In the Chromatogram Results window, you can see the abundances for each transition.</li> <li>In Compound Details View, click each compound, or use the and buttons in the compound list window to review the results.</li> <li>In the Navigator view, you need to select a compound and click Edit &gt; Show &gt; Only Highlighted to show only that compounds in the Data Navienteenview of the select a</li> </ul>			
	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	the 💽 or 💽 buttons in the Compound List window.			

file" on page 22.

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

Steps				Deta	iled Ir	struct	tions				Com	mer	nts			
B Aglant MassHunter Qualitative Analys File Edit View Find Identify Metho B → D → C+ - E	ois 8.07.00 - Detaultum od Wizards Configuration Tool ⊙ 🕜 🚱 🛆 🕼 🕼 🕪]	s Help	avigator View 🔛 Compour	nd Details View												
Compound List			Cc	ompound Details View						X Conpound l	dentification Results: Cpd	1: Metosularn				
開 Automatically Show Columns 間	1919 9 9 8 8 8	a 🛪 🚳 🗄 L								Automatic	ally Show Columns   🛗	4154	8 8 8 8	4 X 💌		
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8 Cod 2: Pyraclostrobin	2 Checkoud/kx_MRM.d	Pyraclostrobin	100							Dest +	• Name • Formula • r	miz / 44 Mass	er Mass (Tgt) er De	if (pprt) + Score (Tgt) +	RT 🗢 RT (Tg) 👄 RT Diff 👄 Score (	RT) & Species & Flags & Notes #
S Cpd 4: Dispinon (Dimpylate)	4 Checkout/kx_MRM.d Disz	inon (Dimpyfate)	100							P	Metoculam				6.267	
2 Cpd 5: Imazalil (Enilconazole)	5 Checkoud/kc_MRM.d Imaza	Al (Enilconazole)	100													
R Cod 7: Imazapir	7 Checkout/kx MFM.d	Instantor	100							-						
E Cpd 8 Dimethoate	8 Checkout/Kx_MRM.d	Dimethosis	100							_						
E Cpd 9: Metoxuron	9 Checkout/Kx_MRM.d	Metoxuron	100							_						
E Cpd 11 Abazine	11 Checkoud/kx_NRM.d	Atrazine	100			-	+ +			-						
8 Cpd 12 Aminocarb	12 Checkoud/kx_NRM.d	Aminocarb	100							_						
Cpd 13: Thiabendazole	13 Checkoud/kx_MRM.d 14 Checkoud/kx_MRM.d	Molicate	100			_	-			-						
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				Counts v	s. Acquisition Time (mir)								140	Counts vs. Mass-to	Charge (m/z)	*

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



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

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

stopo	Detailed Instructions	Comments
	Time Segment: de (All)	▼ ■ Barat Tabla Viau
	Samle	. Est fabre view
	Name Data File Type	Level Acq. Method File Acq. Date-Time
	CheckoutMix_M CheckoutMix_MRM.d	
	Quantifier	
	Aminocarb 1 209 1 -> 137 2 MRM	Target
	Atrazine 1 216.1 -> 174.1 MRM	Target
	Carboturan 1 222.1 -> 123.1 MRM     Diazinon (Dimpv 1 305.1 -> 169.1 MRM	Target
	Dimethoate     1 230.0 -> 198.8     MRM	Target
	Imazalii (Enilcon 1 29/.1 -> 159.0 MRM     Imazapyr 1 262.1 -> 217.1 MRM	Target
	Malathion 1 331.0 -> 126.9 MRM	Target
	Metazachlor 1 278.1 -> 134.2 MRM     Metasulam 1 418.0 -> 175.0 MRM	Target Target
	Metosuron 1 229.0 -> 72.1 MRM	Target
	Molinate     1 188.0 -> 83.2 MRM     Dyraclostrobin     1 388 1 -> 193.8 MRM	Target
		Target
<ul> <li>Qualifier Setup, and Calibration Curve Setup.</li> <li>Add calibration level 1 with a concentration of 100.</li> <li>Set the Uncertainty to Relative for all qualifiers.</li> <li>Set the Curve Fit to Linear.</li> <li>Set the Curve Fit Origin to Force.</li> <li>Set the Curve Fit Weight to None.</li> </ul>	<ul> <li>Method Setup Tasks section in the Method Tasks pane.</li> <li>b Select the first compound in the table.</li> <li>c Right-click the compound row and click New Calibration Level from the shortcut menu.</li> <li>d In the Level column, type 1. In the Conc. column, type 100.</li> <li>e Right-click in the Level box and click Copy Calibration Levels To.</li> <li>f Click Select All. Click OK.</li> <li>g Select Qualifier Setup in the Method Setup Tasks section.</li> <li>h Verify that the Uncertainty is Relative.</li> <li>i Select Calibration Curve Setup in the Method Setup Tasks section.</li> <li>j Set Curve Fit to Linear for all compounds</li> </ul>	<ul> <li>Quantitative Analysis program for additional help on these tasks.</li> <li>After you select the option for the first compound in the Method Table, you can right-click the optior and click Fill Down from the shortcut menu.</li> </ul>



- 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.
- 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		
Review qualifier ratios	<ul> <li>p Select Qualifier Setup in the M Setup Tasks section.</li> <li>q Right-click the Method Table ar Expand All</li> </ul>	ethod d click	
	r Click View > Window Layout > Restore Default Layout.	•	
	s Click View > Sample Informatic close the Sample Information v	<b>on</b> to <i>v</i> indow.	
	t Click the Show/Hide Qualifiers in the toolbar in the Compound Information window.	button	
	u Click on each compound and vertication of the result	erify alifier e	
	spectrum pane.	n the	



Steps		Detailed Instructions	Comments
4	Verify method and then save the method and apply the method to the batch.	<ul> <li>a Click Method &gt; Validate.</li> <li>b Click OK on the message box. Fix any errors, if necessary.</li> <li>c Click Method &gt; Save As.</li> <li>d Type Checkout_MRM_to_DMRM.</li> <li>e Click the Save button.</li> <li>f Click Method &gt; Exit.</li> <li>g For the additional batch processing option, select None.</li> <li>h Click Yes to apply the method to the batch.</li> </ul>	Apply Method
5	Analyze and save the batch.	<ul> <li>a In the Batch Table window, select Cal as the Type.</li> <li>b Click Analyze &gt; Analyze Batch.</li> <li>c Click File &gt; Save Batch.</li> </ul>	
6	Review the batch to resolve errors or messages that are indicated in the Batch Table.	<ul><li>Resolve isomers.</li><li>Check qualifier ratios.</li><li>Resolve errors and messages</li></ul>	
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		D	Detailed Instructions		omments
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 b c d f	See "Task 1. Create a batch file from an existing MRM data file" on page 22. Click File > Save. Click Report > Generate. The system displays the Report dialog box. Select a Template file. Select the Report folder. This folder name is used in the next task. Click OK.	•	For this report, you do not need to print the report. You need to click <b>Advanced</b> to select a different printer. If you don't want to print this report, click <b>Advanced</b> instead.
		Re	port Template file: C.\MassHunter\Report Templates\Quart\en-US\Letter\ESTD\Results_NoGraphics\Quart\en-US\Letter\ESTD\Results_NoGraphics\Quart	nt Rep	

C. Massi la lei d'epoir Templates	s/Quant/en-US/Letter/ESTD/Results_NoGrap	hics\QuantRep
Report folder:		
C:\MassHunter\Data\Official\Qua	antReports\CheckoutMix_MRM	
Dutput:		
PDF to screen		
Print to default printer		
Report mode:		
Batch report		
Single sample report for select	ed samples	
Batch report     Single sample report for select	ed samples	

Task 2. Print a report in the Quantitative Analysis program

Steps		D	etailed 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 b	See "Task 1. Create a batch file from an existing MRM data file" on page 22. Click <b>File &gt; Save</b> .	
		C	Click <b>Report &gt; Generate</b> . The	
			Generate Report dialog box opens.	
		d	Under <b>Report method</b> , click <b>New</b> . The	
			Report Method Edit program opens.	
		e	Click <b>Add Template</b> . 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 <b>Open</b> .	

A				·	U
ame	Date modified	Туре	Size		
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Env Results withGraphics.report.xml	5/19/2015 5:42 PM	XML Document	1 KB		
Env TPH Validation.report.xml	5/19/2015 5:42 PM	XML Document	1 KB		
Gen ByCompound.report.xml	5/19/2015 5:42 PM	XML Document	1 KB		
Gen_BySample.report.xml	5/19/2015 5:42 PM	XML Document	1 KB		
Gen_BySample_withSN.report.xml	5/19/2015 5:42 PM	XML Document	1 KB		
Gen_Calibration.report.xml	5/19/2015 5:42 PM	XML Document	1 KB		
Gen_Complete.report.xml	5/19/2015 5:42 PM	XML Document	1 KB		
Gen_LIMsIntegration.report.xml	5/19/2015 5:42 PM	XML Document	1 KB		
Gen_ResultsQualifierRatio.report.xml	5/19/2015 5:42 PM	XML Document	1 KB		
Gen_ResultsSummary.report.xml	5/19/2015 5:42 PM	XML Document	1 KB		
Gen_Samples.report.xml	5/19/2015 5:42 PM	XML Document	1 KB		
LIMsIntegration_ExportResults.report.xm	5/19/2015 5:42 PM	XML Document	1 KB		

Task 2. Print a report in the Quantitative Analysis program

Steps	Detailed Instructions	Comments
	문화 Report Method Edit (Quantitative Analysis) File Edit Tools 한 같은 것 같은 것 같은 것 같이 한 것 같이 한 것 Templates Republic Graphics settings	
	Implate     Ference      Template	pot mode Destination file Publish format Language Page S ch

- h In the Report Method Edit program,
  - click Results.
- i Click Yes.

📅 Report Method Edit (Quantitative Analysis)	Œ	
<u>Eile Edit T</u> ools		
🔁 🗁 🛃 🕺 🖏 🥙 🖤 📷		
Templates Results Graphics settings		
Instrument type:		
QQQ 🗸		
Generate report results (report results xm): Auto Generate results file only when Excel report templates are selected. () [Yes] Moves operate results file		
No Never generate results file		

Task 2. Print a report in the Quantitative Analysis program

Steps De	tailed Instructions	Comments		
j k I m	Click File > Save Method As. The Save As dialog box opens. Navigate to the example data folder. For the File name, type CheckoutMix_MRM_Report. Click Save.			
	Save As			×
	🗲 💭 🗢 🛄 « MassHunter 🕨 Data 🕨 Pesticides tMRM Database B.06.00	Example data	• Search Example data	Q
	Organize 💌 New folder			0
	Downloads     Recent Places     CheckoutMix_DMRM.d     CheckoutMix_MRM.d     CheckoutMix_MRM.d     CheckoutMix_TMRM.d     CheckoutMix_TMRM.d     CheckoutMix_TMRM.d     QuantResults     Videos     FORGRAMS (C)     DATA (D)	Date modified         Type           2/29/2016 2:09 PM         File folder           2/29/2016 2:09 PM         File folder           2/29/2016 2:09 PM         File folder           2/29/2016 2:09 PM         File folder	Size	
	File name: CheckoutMix_MRM_Report			-
	Save as type: Methods (*.m)			-
	Hide Folders		Save Cance	4

- 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.

Generate Report	
Batch file:	
Batch folder:	D:\MassHunter\Data\Pesticides tMRM Database B.06.00\
Batch file:	CheckMix_MRM.batch.bin Browse
Report folder:	
D:\MassHunter\Data	Pesticides tMRM Database B.06.00\Example da Browse
Report method:	
D:\MassHunter\Data	Pesticides tMRM Database B.06.00\Example data\QuantResults
	Choose New Edit
Samples/Compounds:	
All samples	Choose samples
All compounds	Choose compounds
Generate:	
Generate reports reports	now
Queue report task	c
🗸 Start Queu	le Viewer
	OK Cancel

# 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 meth CheckoutMix to a new nam <i>iii</i> CheckoutM <i>iii</i> are your ini	hod _ <b>MRM.m</b> and save it e with the format <b>ix_DMRM.m</b> , where tials.	<ul> <li>a In the Data Acquisition program, click Method &gt; Open.</li> <li>b Select the CheckoutMix_MRM.m method. Click OK.</li> <li>c Click Method &gt; Save As.</li> <li>d Type the new method name with the format <i>iii</i>CheckoutMix_DMRM.m.</li> </ul>	<ul> <li>In this example, the batch is in the \MassHunter\Data\Product Database x.xx.xx\Example data folder.</li> <li>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.</li> </ul>	

Task 3. Create a dMRM method using Update dMRM

Steps	Detailed Instructions	Comments
<ul> <li>2 Update the method to change from an MRM method via a Dynamic MRM method with the same compounds.</li> <li>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.</li> <li>Do not manually change the Scan Type to Dynamic MRM. If you do, the existing Scan segments table is cleared.</li> </ul>	<ul> <li>a Click the Acquisition tab in the QQQ tab in the Method Editor window.</li> <li>b Right-click the Scan segments table and click Update DMRM Method. The MRM Update Options dialog box opens.</li> <li>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.</li> <li>d Under Method Options, check Add new compound/transition.</li> <li>e For Peak abundance threshold, enter 50.</li> <li>f For Cycle time, enter 500 ms.</li> <li>g Under Retention time options, select Update retention time and Update retention time dupdate retention time to the drop-down list, select 1. From the drop-down list. Select 1. From the drop down down check box.&lt;</li></ul>	<ul> <li>The Update MRM Method tool automatically sets the Scan type to Dynamic MRM.</li> <li>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.</li> <li>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.</li> <li>A 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.</li> <li>The dwell times for MRM transitions will depend on the number of overlapping peaks and their respective peak widths.</li> <li>The method is now updated with the transitions, parameters, and retention times in the Quantitative Analysis renort</li> </ul>

Task 3. Create a dMRM method using Update dMRM

:\MassHunter\Data\Pesticides	tMRM Data	abase B	06.00\Example data\QuantRep	orts\Checko	ut Mix_MRM
Method options			Retention time options		
Add new compound/trans	ition		Update retention time		
Peak abundance threshold:	50		Update retention time with the second sec	indow	
Cycle time:	500	ms	RT window threshold:	1	Minutes 🔹
			Scale factor:	2	
Trigger options					
Update threshold			Update trigger window		
Height percent:	10	]	Retention time	© FWHM	
Scale factor:	1		Absolute value:	0.5	min
			Percent value:	0	
			Scale factor:	1	

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.

Task 3. Create a dMRM method using Update dMRM

#### Steps

**Detailed Instructions** 

#### Comments

Method Editor													
🗈 🔰 🖬 🚺 🍺 CheckoutMix_dMRM.m	•	✔ Apply 🛛 🔄											
Properties DA QQQ	,												
Tune file Stop time C No limit/As Pump C Stop time	Acquisition Source	Chromatogram In:	strument	t Diagnostics	1		l'one co	Bet Time	Dalta Bat		Collision	Cell Accelerator	
Browse 66	Compound Group	Compound Name /	ISTD?	Precursor Ion V	MS1 Res	Product Ion V	MS2 Res	(min)	Time	Fragmentor	Energy	Voltage	Polarity
- Ion source - Time filtering		Aminocarb		209.1	Unit	152.2	Unit	2.01	1	105	12	2	Positive
		Aminocarb		209.1	Unit	137.2	Unit	2.01	1	105	24	2	Positive
j≪ Peak width  0.07 min		Atrazine		216.1	Unit	174.1	Unit	6.1	1	125	16	3	Positive
Time segments		Atrazine		216.1	Unit	68	Unit	6.1	1	125	40	3	Positive
# Start / Scan Type Div Valve Delta Delta Stored		Carbofuran		222.1	Unit	165.1	Unit	5.73	1	80	20	2	Positive
1 Dupamic MBM To MS 0 0 F		Carbofuran		222.1	Unit	123.1	Unit	5.73	1	80	30	2	Positive
		Diazinon (Dimpylate		305.1	Unit	169.1	Unit	9.42	1	105	32	2	Positive
		Diazinon (Dimpylate		305.1	Unit	97	Unit	9.42	1	105	40	2	Positive
		Dimethoate		230	Unit	198.8	Unit	3.85	1	70	0	5	Positive
		Dimethoate		230	Unit	125	Unit	3.85	1	70	16	5	Positive
		Imazalil (Enilconazol	П	297.1	Unit	201	Unit	4.64	1	115	15	2	Positive
		Imazalil (Enilconazol		297.1	Unit	159	Unit	4.64	1	115	20	2	Positive
		Imazapyr		262.1	Unit	217.1	Unit	2.81	1	120	20	3	Positive
		Imazapyr		262.1	Unit	69.1	Unit	2.81	1	120	40	3	Positive
		Malathion		331	Unit	126.9	Unit	8.35	1	80	5	2	Positive
		kd statkisn	-	201	116a	00	i na	30.0	1	00	10		Davilia
cycles/s ms/cycle	Dynamic MRM Parameter Cycle Time 500	ers Total MRMs = 2 ms	8 Max	Concurrent MRN	ls = 6 Min/	'Max Dwell = 81.	56 ms/248.7	"3 ms		Triggere	d MRM	Repeats	3
•		III											•

- 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.

St	eps	Detailed Instructions	Comments
1	Start the Dynamic MRM Viewer dialog box.	• Right-click the Scan segments table and click <b>Edit DMRM Method</b> .	
2	Review each compound in the Dynamic MRM Viewer dialog box.	<ul> <li>a Click each compound in the table.</li> <li>b Verify in the table that two transitions are shown for each compound.</li> <li>c Examine the graphic to review how many concurrent MRMs are being acquired with that compound.</li> <li>d Adjust the cycle time so that all criteria for Minimum Dwell Time, and for good integration are met.</li> <li>e Click Close.</li> </ul>	• 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.

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

mamic MRM Viewer mpound: (All) ompound Group 7	• 8	) C													
ompound: (All)	• 4 1	. C												-	-
ompound Group 7		Lompou	nd aroun: [i	(AII)	-								Dynamic MRN	1 Statistics	
ompound Group	- Contract and	)	in group. (									Total MRMs		28	
	Compound Name Y	Precursor	Product	RT	RT	Frag	CE	CAV	Average		~	Minimum Conc	urrent MRMs	2	
	Aminoopth	100 209 10	162.20	2.010	1 000	200	12	2	161 70			Maximum Con	Current MIKIMS	6	2
	Aminocarb	203.10	137.20	2.010	1.000	380	24	2	151.78			Maximum Dwei	l Time	02.1	2 ms
	Atrazine	216 10	174 10	6 100	1.000	380	16	2	96.06			Minimum Cuck	a Time	19.2	8 me
	Atrazine	216.10	68.00	6 100	1.000	380	40	3	96.06			- Minimum Cych	e mine	13.2	01113
	Carbofuran	222.10	165.10	5.730	1.000	380	20	2	110.02		-				
	Carbofuran	222.10	123.10	5.730	1.000	380	30	2	110.02		-				
	Diazinon (Dimpylate)	305.10	169.10	9,420	1.000	380	32	2	123.93			Parameters			
	Diazinon (Dimpylate)	305.10	97.00	9.420	1.000	380	40	2	123.93			Cycle time:		500	ms
	Dimethoate	230.00	198.80	3.850	1.000	380	0	5	110.08			Calculatio	abuda.		
	Dimethoate	230.00	125.00	3.850	1.000	380	16	5	110.08			Calculations in	iciude:	All 1	
	Imazalil (Enilconazol	297.10	201.00	4.640	1.000	380	15	2	110.05			• Primane	s only	<ul> <li>Mitransi</li> </ul>	10115
	Imazalil (Enilconazol	297.10	159.00	4.640	1.000	380	20	2	110.05			Review tools			
	Imazapyr	262.10	217.10	2.810	1.000	380	20	3	110.02						
	Imazapyr	262.10	69.10	2.810	1.000	380	40	3	110.02			Override I	R1 window	1	min
	Malathion	331.00	126.90	8.350	1.000	380	5	2	186.47			Check min	nimum data pts	64	pts
	Malathion	331.00	99.00	8.350	1.000	380	10	2	186.47						
	Metazachlor	278.10	210.10	6.760	1.000	380	4	5	151.62			Split method			
	Metazachlor	278.10	134.20	6.760	1.000	380	15	5	151.62			Colt math	ad		
	Metosulam	418.02	175.00	6.270	1.000	380	32	3	96.02			Opic mouri	00		
	Metosulam	418.02	140.00	6.270	1.000	380	60	3	96.02			Split by:	Max Concu	ment MRMs	•
	Metoxuron	229.00	72.10	4.740	1.000	380	16	3	144.83					-	
	Metoxuron	229.00	46.10	4.740	1.000	380	12	3	144.83			Number	of methods:	2	
	Molinate	188.00	126.10	7.790	1.000	380	25	2	186.45			Max con	current MRMs	10	
	Molinate	188.00	83.20	7.790	1.000	380	16	2	186.45			Min dwel	I time:	2	ms
	Pyraclostrobin	388.11	193.80	9.420	1.000	380	8	2	123.93					-	
	Pyraclostrobin	388.11	163.10	9.420	1.000	380	20	2	123.93			Split method			-
	Thiabendazole	202.00	1/5.00	2.860	1.000	380	24	2	144.80		-				
						2011									
ype: Concurrent MF	RMs 👻	Select trans	nsitions on Cli	ck											
6.				1	Conc	urrent MRN	Is vs Ret	ention Time	1						
5-															
4-						_									
3-															
2-			U												
1-															
0-				_		-	-	_	-						
1 1	.5 2 2.5	3	3.5	4	4.5	5 Pot	5.5 ention Til	6 me (min)	6.5	7 7.5	8	8.5 9	9.5	10	10.5
						1101	ana di Ti	ine (milli)					~		
dd Compounds	Save Splt Methods												Reset Det	fault	Close

The default setting of 500 ms is

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.
 a Type the Cycle Time, if necessary.
 b Save the method.
 When you close the Dynamic MRM Viewer, changes made to the cycle

time in the Dynamic MRM Viewer

recommended for most analysis are *not* entered into the acquisition containing more than 15 compounds. method.

3 Once a cycle time is determined

for good integration, set the Cycle

Time in the QQQ > Acquisition tab.
# **Creating a Dynamic MRM acquisition method**

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

iteps	Detaile	d Instruc	S	iments										
Method Editor	•	✔ Apply 🔄												×
Properties DA [QQQ] Tune file Stop time atunes.tune.sml No limit/As Pump	Acquisition Source	Chromatogram Int	strument	Diagnostics	1		1	Det Time	D-I- D-I		College	C. L. L. L. L.		
Browse 6d C 1 min	Compound Group	Compound Name /	ISTD?	Precursor Ion 7	MS1 Res	Product Ion V	MS2 Res	(min)	Time	Fragmentor	Energy	Voltage	Polarity	
Ion source		Aminocarb		209.1	Unit	152.2	Unit	2.01	1	105	12	2	Positive	
ALS ESL T		Aminocarb		209.1	Unit	137.2	Unit	2.01	1	105	24	2	Positive	_
preserved in 10.07 mill		Atrazine		216.1	Unit	174.1	Unit	6.1	1	125	16	3	Positive	
Time segments		Atrazine		216.1	Unit	68	Unit	6.1	1	125	40	3	Positive	
# Start / Scan Type Div Valve Delta Delta Stored		Carbofuran		222.1	Unit	165.1	Unit	5.73	1	80	20	2	Positive	
▶ 1 0 Dynamic MRM To MS 0 0 V		Carbofuran		222.1	Unit	123.1	Unit	5.73	1	80	30	2	Positive	_
		Diazinon (Dimpylate		305.1	Unit	169.1	Unit	9.42	1	105	32	2	Positive	_
		Diazinon (Dimpylate		305.1	Unit	97	Unit	9.42	1	105	40	2	Positive	
		Dimethoate		230	Unit	198.8	Unit	3.85	1	70	0	5	Positive	
		Dimethoate		230	Unit	125	Unit	3.85	1	70	16	5	Positive	_
		Imazali (Enilconazol		297.1	Unit	201	Unit	4.64	1	115	15	2	Positive	
		Imazali (Enilconazol		297.1	Unit	159	Unit	4.64	1	115	20	2	Positive	_
		Imazapyr		262.1	Unit	217.1	Unit	2.81	1	120	20	3	Positive	_
		Imazapyr		262.1	Unit	69.1	Unit	2.81	1	120	40	3	Positive	
		Malathion	Ľ	331	Unit	126.9	Unit	8.35	1	80	5	2	Positive	_
cycles/s ms/cycle	Cycle Time 500	ns Total MRMs = 2	B Max C	oncurrent MRM	ls = 6 Min.	/Max Dwell = 81.	56 ms/248.3	73 ms		Triggere	d MRM	Repeats	3	

Task 5. Acquire dMRM data and inspect in Qualitative Analysis

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

#### **Creating a Dynamic MRM acquisition method**

Method Editor: Find Compounds by MRM

 Treat each transition as separate compound [Single precursor ion and product ion values]
 Group transitions by compound name

Find compound settings

Compound results

Detect most abundant peak by
 Peak area

😥 Find Compounds by MRM 🔹 🚮 🔄 🕈 🖓 🖬

Options Integrator Peak Filters Signal to Noise Peak Spectrum Results

Peak height

Task 5. Acquire dMRM data and inspect in Qualitative Analysis

Steps Detailed Instructions	Comments				
<ul> <li>2 Find compounds using the Find Compound by MRM algorithm in the Qualitative Analysis program.</li> <li>Popen the data file CheckoutMix_DMRM.d.</li> <li>b Click File &gt; Open Data File. The system displays the "Open Data File" dialog box</li> <li>c Select CheckoutMix_DMRM.d, and click Open.</li> <li>d Click Method &gt; Method Explorer or View &gt; Method Explorer. The system displays the Method Explorer window.</li> <li>e In the Find Compounds section, click Find by MRM.</li> <li>f In the Method Editor window, click the Group transitions by compound name option.</li> <li>g Click the Peak area option for Detect</li> </ul>	<ul> <li>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 &gt; User Interface Configuration. Then, you mark the Unit Mass check box and the MS/MS (QQQ, Q-TOF) check box. Then, click OK.</li> <li>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.</li> </ul>				

most abundant peak by.

h Click Find > Find Compounds by

2 22 24 26 28 3 32 34 36 38 4 42 44 46 45 5 25 4 56 58 6 42 66 65 7 72 74 76 78 8 62 84 86 88 9 92 94 96 Courts vs. Accustor Ture (main)

MRM.

H

• 🕘



A Chromatogram Results

iư ↔ \$ | Q 🔢 \$# 14 🖌 🛆 | O O 1 💌 🕶 🔟 🗘 🎊 🖉 🧞 % % 🕅 🚈 Minutes

x10 5 +ESI TIC MRM CF=0.000 DF=0.000 (\*\* -> \*\*) CheckoutMix\_DMRM.d

×

## **Creating a Dynamic MRM acquisition method**

Task 5. Acquire dMRM data and inspect in Qualitative Analysis

Steps	Detailed Instructions	Comments
<ul> <li>3 Review the results of the Find Compounds by MRM algorithm.</li> <li>Make sure that the transitions are found for each compound.</li> <li>These transitions become the primary transitions if you create a tMRM method.</li> <li>It is not possible to edit the retention times of compounds which are identified.</li> </ul>	<ul> <li>a Close the Method Explorer and Method Editor windows.</li> <li>b Click Compound Details View to change the view. See the figure that follows.</li> <li>c Click or use the arrow keys to move through the Compound Table to review one compound a a time.</li> <li>d Verify that the transitions for each compound were found.</li> <li>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.</li> </ul>	<ul> <li>You can also print a Compound Report to review results. You click File &gt; Print &gt; Compound Report. The Compound Report sorts the compounds by retention time.</li> </ul>
Rigitent Manshfunter Qualitative Analysis 8.07.00 - Defaultrm		Œ



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 EntranceUse this parameter to shift the acquisition of secondary ions towards apex of<br/>peak. When the signal for the designated primary MRM transitions cross the<br/>triggering Threshold, the Trigger Entrance Delay postpones triggering for a<br/>user-defined number of cycles, which moves the acquisition of secondary MRM<br/>transitions closer to the apex of the peak.
  - Trigger DelayUse this parameter to spread acquisition of secondary ion across the peak.<br/>Once the triggering Threshold is met, the trigger delay defines the number of<br/>cycles to skip between triggers, which spreads the acquisition of secondary<br/>MRM transitions across a peak. This function can be combined with the<br/>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 MRMUse this parameter to define the number of secondary transition cycles that<br/>are acquired. This parameter applies to the whole triggered MRM method, not<br/>to individual compounds.

Task 1. Create a tMRM method from a dMRM method

# 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 you open t CheckoutN You can op created or	a Acquisition program, he dMRM method: <b>/ix_DMRM.m</b> en the method that you the example method.	<ul> <li>a Switch to the Data Acquisition program.</li> <li>b Open the CheckoutMix_DMRM.m method.</li> </ul>	<ul> <li>An example</li> <li>CheckoutMix_DMRM.m method can be found in the Example</li> <li>methods folder and also on the installation media.</li> </ul>					
2 Change the method an secondary Database E	e method to a tMRM d start to import the transitions from the Browser.	<ul> <li>a In the Method Editor window, click the 000 &gt; Acquisition tab.</li> <li>b Mark the Triggered check box under Triggered MRM.</li> <li>c Manually mark the Triggers shown in the "Primary and Secondary Transitions for Triggered MRM" on page 68.</li> <li>d Type 3 for Repeats.</li> <li>e Right-click the Scan Segments table and click Import from Database Browser. The Database Browser opens.</li> </ul>	<ul> <li>The triggering information is loaded from the Database Browser even if the <b>Triggered</b> check box is clear. This includes the trigger Threshold values if the Trigger MRM Threshold column has a value.</li> <li>Later in this section, we replace the values manually with the values shown "Primary and Secondary Transitions for Triggered MRM" on page 68.</li> </ul>					

Acquisiti	n Source Chromatog	am I	nstrument	Diagnos	stics														
C Scan seg	ments																		
Compo Grou	und 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	0	0	0.5	
	Aminocarb		209.1	Unit	137.2	Unit	7			2.01	1	105	24	2	Positive				
	Atrazine		216.1	Unit	174.1	Unit	~	7	2010	6.1	1	125	16	3	Positive	0	0	0.5	
	Atrazine		216.1	Unit	68	Unit	~			6.1	1	125	40	3	Positive				
•	Carbofuran		222.1	Unit	165.1	Unit	~	<b>V</b>	728	5.73	1	80	20	2	Positive	0	0	0.5	
	Carbofuran		222.1	Unit	123.1	Unit	~			5.73	1	80	30	2	Positive				
	Diazinon (Dimpylate		305.1	Unit	169.1	Unit	•		2788	9.42	1	105	32	2	Positive	0	0	0.5	
	Diazinon (Dimpylate		305.1	Unit	97	Unit	~			9.42	1	105	40	2	Positive				
	Dimethoate	Г	230	Unit	198.8	Unit	~	<b>v</b>	1547	3.85	1	70	0	5	Positive	0	0	0.5	
	Dimethoate		230	Unit	125	Unit	~			3.85	1	70	16	5	Positive				
	Imazalil (Enilconazo		297.1	Unit	201	Unit	~	<b>V</b>	443	4.64	1	115	15	2	Positive	0	0	0.5	
	Imazalil (Enilconazo		297.1	Unit	159	Unit	~			4.64	1	115	20	2	Positive				
	Imazapyr		262.1	Unit	217.1	Unit	~	<b>V</b>	249	2.81	1	120	20	3	Positive	0	0	0.5	
	Imazapyr		262.1	Unit	69.1	Unit	~			2.81	1	120	40	3	Positive				
	Malathion	Г	331	Unit	126.9	Unit	~	<b>V</b>	175	8.35	1	80	5	2	Positive	0	0	0.5	
	ki statkion	-	201	1143	00	1169	17	-	1	90.0	- 1	on	10	2	Davitina	1 1			-
Dynamic Cycle T	MRM Parameters me 500 ms Primar	1RMs =	28 Max Cor	current	MRMs = 6	Min/M	ax Dwell =	81.56 ms/:	248.73 ms,			Friggered MRN	и I	Repeats 3					
	, Thina,	only -	rotal minima -	- 20 M	lax concure	ank ton no	18 - 0 Mi	IV MOX DVM	51 - 01.50			,		interne la					

Task 1. Create a tMRM method from a dMRM method

Steps	Detailed Instructions	Comments				
3 Open the CheckoutMix_TriggeredMRM_B0 600 database in the Database Browser.	<ul> <li>a In the Database Browser, click File &gt; Open Database.</li> <li>b Select the CheckoutMix_TriggeredMRM_B0600 database in the folder \MassHunter\Databases\Product Database x.xx.xx\Example database.</li> <li>a Click OK</li> </ul>	If the transitions in the example method do not match the transitions in the database, use the transitions in the database.				

Browse For Folder	
Select Optimizer database folder	
Data	
4 📔 Databases	
Dptimizer	
a 🌗 Pesticides tMRM Database B.06.00	
Description of the second s	
Example database	
CheckoutMix_TriggeredMRM_B0600	
Pesticides_TriggeredMRM_B0600	
DataStores	
	OK Cancel

- 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.

Task 1. Create a tMRM method from a dMRM method

	Search Correct and		
	Sauch Company		
	Search Companyede		
	Search Compounds		
	Jearch Compounda		
	Search Te	xt	
e v Select Transitions	67129-08-2 60-51-6 33554-44-0 33514-7 2032-59-9 19937-59-8 1912-24-9 175013-18-0 1563-6-2 ■ Match entre word Set primary and trigger fla Set top 2 ■ Set Primaries	for each string	Project Name Compound Name Formula MrV Groups Chemical Classes Rank transitions by @ Abundance @ Response Factor
Polarity Species Pr	ecursor Product	Frag CE	CAV Primary Trigger
ositive	209.1 67.2	105 60	2
isitive	209.1 77.2	105 60	2 🔲 🗖
sitive	209.1 94.2	105 56	2
sitive	209.1 122.1	105 44	2 📃 🔲
ositive	209.1 137.2	105 24	2 🔽 🔲
sitive	209.1 152.2	105 12	2 🔽 🕅
			,
	Polarity Species Pri astitive astative	image: state in the s	E         E         F0515         F           Image: September 2005         September 2005         E         IN         Image: September 2005           Image: September 2005         I

#### 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.

Task 1. Create a tMRM method from a dMRM method

NOTE				• Qua com tran whil metl the l	lifier an pound sitions. e you d hod to " pest pol	d quan cannot If you evelop ' <i>compo</i>	itifier id contai want to a metl oundna	ons mus in both r o includ hod, you	et hav negat e botl i mus	e the s ive and h polar t renar	ame d posi rities ne th	pola tive for o e co
				or "	method _neg″ f	arity an all oth rom the	nd tran er tran e rema	sitions a sitions f sitions f	s and are fo for the mpou	d " <i>com</i> und fo e comp ind na	r a co r a co oound me.	<i>dnai</i> mpo . The
latabase Browser Eile Edit View				<ul> <li>To e supe conf conf have</li> </ul>	nsure g erfluous firmatio firmatio e intens	jood sig s secon n must n shou e ion p	gnal/n ndary tr t be rer ild be a neaks.	oise rat ransitior noved. { s uniqu	ios fo ns wh Secon e as p	r the s ich are idary ti iossibl	econo e not r ransit e to a	dary requ ions part
Search/Filter Import List												
Compound Name	Formula	MW	Polarity	Species	Precursor	Product	Frag	CE	Primary	Trigger	BT	F 🔺
Aminocarb (	C11H16N2D2				209.1	67.2	105	60				
Aminocarb (	C11H16N2D2				209.1	77.2	105	60				=
Aminocarb (	C11H16N2O2				209.1	94.2	105	56				
Aminocarb (	C11H16N2D2				209.1	122.1	105	44				
Atrazine	C8H14CIN5				216.1	43.1	125	48				
Atrazine	C8H14CIN5				216.1	62.1	125	56				
Atrazine (	C8H14CIN5				216.1	79	125	24				
Atrazine	C8H14CIN5				216.1	104	125	28				
Atrazine	C8H14CIN5				216.1	132	125	20				
Atrazine	C8H14CIN5				216.1	145.9	125	20				
Carbofuran	C12H15N03				222.1	55.2	80	24				
Carbofuran	C12H15NO3				222.1	78	80	50				
Carbofuran	C12H15NO3				222.1	124	80	20				
Carbofuran	C12H15N03				222.1	137	80	16				
Carbofuran	C12H15N03				222.1	166	80	4				
Carbofuran	C12H15N03				222.1	207	80	12				
Diazinon (Dimpylate 0	C12H21N2D3PS				305.1	66	105	40				
Diazinon (Dimpylate (	C12H21N2O3PS				305.1	84	105	40				
Diazinon (Dimpylate )	C12H21N2D3PS		III		305.1	93	105	40				*
										Import	Close	

Task 1. Create a tMRM method from a dMRM method

Ste	ps					D	Detailed Instructions									Comments					
6 Review the secondary transitions in the Data Acquisition program.							In th by th Revi tran	ne Ac he <b>Co</b> iew t sitior	quisi ompo he pr 1s for	tion t <b>und f</b> imary <sup>•</sup> each	ab, s <b>Name</b> r and n com	ort th e. secc npour	ne tabl ondary nd.	e	• If a seg but doe vali	red bo ments ton in es not d.	ox app table the to clear,	ears i , you c olbar. the va	in the Scan click the <b>Ap</b> If the red b alue is not		
-Sr	an segment:	<del>،</del> ۱		1	-														- 1		
	Compound	Compound Name /	ISTD?	Precursor Ion 🗸	MS1 Res	Product Ion V	MS2 Res	Primary 🗸	Trigger	Threshold	Ret Time	Delta Ret	Fragmentor	Collision Energy	Cell Accelerator Voltage	Polarity	Trigger Entrance	Trigger ▲			
,		Aminocarb		209.1	Unit	152.2	Unit	•	•	6937	2.01	1	105	12	2	Positive	0				
		Aminocarb		209.1	Unit	137.2	Unit	~			2.01	1	105	24	2	Positive					
		Aminocarb		209.1	Unit	122.1	Unit						105	44	2	Positive					
		Aminocarb		209.1	Unit	94.2	Unit						105	56	2	Positive					
		Aminocarb		209.1	Unit	77.2	Unit						105	60	2	Positive					
		Aminocarb		209.1	Unit	67.2	Unit						105	60	2	Positive					
		Atrazine		216.1	Unit	174.1	Unit	•	~	2010	6.1	1	125	16	3	Positive	0				
		Atrazine		216.1	Unit	68	Unit	•			6.1	1	125	40	3	Positive					
		Atrazine		216.1	Unit	145.9	Unit						125	20	3	Positive					
F		Atrazine		216.1	Unit	132	Unit		Г				125	20	3	Positive					
F		Atrazine		216.1	Unit	104	Unit						125	28	3	Positive					
F		Atrazine		216.1	Unit	79	Unit						125	24	3	Positive					
F		Atrazine		216.1	Unit	62.1	Unit		Г				125	56	3	Positive					
F		Atrazine		216.1	Unit	43.1	Unit						125	48	3	Positive					
F		Carbofuran		222.1	linit	165.1	Unit	<b></b>		728	5.73	1	80	20	2	Positive	0	•			

 Dynamic MRM Parameters
 Total MRMs = 93
 Max Concurrent MRMs = 22
 Min/Max Dwell = 21.36 ms/248.71 ms

 Cycle Time
 500
 ms
 Primary Dnly - Total MRMs = 28
 Max Concurrent MRMs = 6
 Min/Max Dwell = 81.56

7 Enter the Trigger Entrance Delay, Trigger Delay and Trigger Window values.

a Sort the table by the Trigger column.

Triggered MRM

✓ Triggered

Repeats 3

- **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.

Task 1. Create a tMRM method from a dMRM method

040					[	Detai	led	Instruc	tio	IS	Comments						
quisition Source	Chro	matogram   Ins	strument	Diagnostics													
can segments		I		1					1								
Compound Name	ISTD?	Precursor Ion	MS1 Res	Product Ion	MS2 Res	Primary	Trigger	Threshold F	Ret Time	Delta Ret	Fragmentor	Collision	Cell Accelerator Po	plarity Trigger	Trigger	Trigger	
Aminocarb	Г	209.1	Unit	152.2	Unit		•	6937	2.0	1 1	105	12	2 Posit	ive	1	2 0.5	5
Atrazine	Г	216.1	Unit	174.1	Unit	<b>V</b>	V	2010	6.	1 1	125	16	3 Posit	ive	1	2 0.5	5
Carbofuran	Г	222.1	Unit	165.1	Unit	<b>v</b>	V	728	5.7	3 1	80	20	2 Posit	ive	1	2 0.5	5
Diazinon (Dimovlate	Г	305.1	Unit	169.1	Unit		V	2788	9.4	2 1	105	32	2 Posit	ive	1	2 0.5	5
Dimethoate		230	Unit	198.8	Unit	V		1547	38	5 1	70	0	5 Posit	ive	1	2 05	5
Imazalil (Enilconazo		297.1	Unit	201	Unit	R R		443	4.6	4 1	115	15	2 Posit	ive	1	2 05	
Imazanır		262.1	Unit	217.1	Unit	17		249	2.8	1 1	120	20	2 Posit	ive	1	2 0.5	
Malathion		331	Unit	126.9	Linit	E E		175	8.0	5 1	80	5	2 Porit	ive	1	2 0.5	-
Metazachlor		270.1	Unit	210.1	Unit			2055	6.5	c 1	70	4	E Post	ive	1	2 0.0	
Metazacriio		410.02	Unit	210.1	Unit			2000	0.7	7 1	140		O Posk	ive	1	2 0.5	
Metosulam		418.02	Unit	1/5	Unit		M	404	6.2		140	32	3 Posit	ive .	-	2 0.5	-
Metoxuron		229	Unit	72.1	Unit	V		2155	4.7	4 1	35	16	3 Posit	ive	1	2 0.8	
Molinate		188	Unit	126.1	Unit	~	V	69	7.7	9 1	90	25	2 Posit	ive	1	2 0.5	
Pyraclostrobin		388.11	Unit	193.8	Unit			3558	9.4	2 1	95	8	2 Posit	ive	1	2 0.5	5
Thiabendazole		202	Unit	175	Unit	•		2263	2.8	6 1	130	24	2 Posit	ive	1	2 0.5	5_1
																1	
ynamic MRM Param	eters									- Tri	gered MRM-						
Cycle Time 500	ms	Total MHMs = 9. Primary Only - To	3 Max Coni otal MRMs =	28 Max Concu	2 Min/Ma rrent MRMs	× Dwell = 2 = 6 Min/	21.36 ms/ Max Dwe	248.71 ms, II = 81.56		F	Triggered	B	epeats 3				
where <i>ii</i>	<i>i</i> are	e your ir	nitials		1, I (	Cli	ick t	he Sav	e b	utton.		<b></b> .					
Review t MRM Vi	he r ewe	nethod r dialog	in the ı box.	Dynam	ica I	a Riy an Dy op Sv bu	ght- d cli nan ene vitch tton	click th ick <b>Edit</b> nic MRI d. n betwe and th	e S M V een ie A	can s /IRM /iewe the P /II tra /IRM	egmen Metho r dialog rimari nsitior Statist	its tal od. Th g box ies or is but tics	ble • ne is is hly tton	Inspect Statistic You can see how maximu change	the C cs in t mod v the im <b>Dv</b> d.	lynam he up ify the minim <b>vell T</b> i	nic MRM oper right co e <b>Cycle tim</b> num and <b>imes</b> are

Task 1. Create a tMRM method from a dMRM method

#### 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.

- -

Compound	(AD	•		Compound on	(AD									Dynamic MRM 9	Statistics
														Total MRM:	28
10		c	Precursor	Product	07	BT	Prima				~	CAN	Avelage ^	Minimum Concurrent MRMs	2
mpound uroup		Compound Name V	lon	Ion	10	Window	ry *	ingger v	Inteshold	Frag	UE .	CAV	Direl	Maximum Concurrent MBMs	6
		Aminocarb	209.10	152.20	2.010	1.000	1	V	6937	105	12	2	149.28	Minimum Dwell Time	79.83 ms
		Aminocarb	209.10	137.20	2.010	1.000	1			105	24	2	149.28	Maxmum Dwei Time	245.50 ms
		Aminocarb	209.10	122.10	2.010	1.000				105	44	2	.00	Minimum Cycle Time	33.00 ms
		Aminocarb	209.10	94.20	2.010	1.000				105	56	2	.00		
		Aminocarb	209.10	77.20	2.010	1.000				105	60	2	.00		
		Aminocarb	209.10	67.20	2.010	1.000				105	60	2	.00	-	
		Atrazine	216.10	174.10	6.100	1.000		2	2010	125	16	3	93.72	Parameters	
		Atrazine	216.10	68.00	6.100	1.000	1			125	40	3	93.72	Cycle time:	500 ms
		Atrazine	216.10	145.90	6.100	1.000				125	20	3	.00	Calculations include:	
		Atrazine	216.10	132.00	6.100	1.000				125	20	3	.00	Primarian only	Altransitions
		Altrazine	216.10	104.00	6.100	1.000				125	28	3	.00	e material	- reconstant
		Atrazine	216.10	79.00	6.100	1.000				125	24	3	.00	Review tools	
		Atrazine	216.10	62.10	6.100	1.000				125	56	3	.00		
		Atrazine	216.10	43.10	6.100	1.000				125	48	3	.00	Cvende RT window	i min
		Carboluran	222.10	165.10	5.730	1.000	<b>V</b>	<b>V</b>	728	80	20	2	107.61	Check minimum data pts	64 pts
		Carboluran	222.10	123.10	5.730	1.000				80	30	2	107.61		
		Carboluran	222.10	207.00	5.730	1.000				80	12	2	.00	Snik method	
		Carbofuran	222.10	166.00	5.730	1.000				80	4	2	.00		
		Carbofuran	222.10	137.00	5.730	1.000	[[]]			80	16	2	.00	Splt method	
		Carboluran	222.10	124.00	5.730	1.000				80	20	2	.00	Split by: Minimum Dwa	Time •
		Carboluran	222.10	78.00	5.730	1.000	100			80	50	2	.00	opic of .	
		Carboluran	222.10	55.20	5.730	1.000	1			80	24	2	.00	Number of methods:	2
		Diszinon (Dimpylate)	305.10	169.10	9.420	1.000		<b>V</b>	2788	105	32	2	121.50	Man and a MDMan	10
		Diszinon (Dimpylate)	305.10	97.00	9.420	1.000		8		105	40	2	121.50	Max concurers minims.	10
		Diazinon (Dimovlate)	305.10	277.10	9.420	1.000	[11]			105	10	2	.00	Min dwell time:	2 ms
		Diazinon (Dimovlate)	305.10	249.00	9.420	1.000	[[7]]			105	20	2	.00	Solt method.	
		Diazinon (Dimpulate)	305.10	231.00	9.420	1.000	[77]			105	20	2	.00	Spik metridu.	•
		Disginon (Dimpulate)	305 10	100.00	9.420	1.000				105	40	2	00		
		Diszinon (Dimpulate)	305.10	93.00	9.420	1.000				105	40	2	00		
		Distinger (Dimpulate)	305.10	84.00	9.420	1,000				105	40	2	00		
		Distince (Dimpulate)	205.10	00.33	9.420	1,000	(==)			105	40	2	00		
		Distantion (Dimpyrand)	363.10		0.420	1.000				100		-			
Plot type: Cor	ocurre	nt MRMs -	) V Se	lect transitions	i on Click		ß	incurrent MRM	s vs Retention T	ine					
4-							_								
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0-		10	20	1	16	-	10		e'e /	ć.	4		e ).	0.5 0.05	10 105
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And Compos		Save Spic Method	····											Hese Dela	Close

Task 1. Create a tMRM method from a dMRM method

Steps	Detailed Instructions	Comments		
<b>10</b> Adjust the cycle time.	<b>a</b> 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

# 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.	<ul> <li>a If necessary, click View &gt; Worklist to display the Worklist window.</li> <li>b Click Worklist &gt; Worklist Run Parameters. Verify that the parameters are set properly. Click OK.</li> <li>c Click Worklist &gt; Add Multiple</li> </ul>	<ul> <li>The Worklist window is tabbed with the Method Editor window by default. Click the Worklist tab to show the Worklist window.</li> <li>This step is optional because you can perform the next step with an</li> </ul>
<ul> <li>Name the data file CheckoutMix_TMRM.d.</li> <li>Designate a directory path to hold your data files and method.</li> </ul>	<ul> <li>Samples.</li> <li>d Type CheckoutMix_TMRM.d as the data file name</li> <li>e Select CheckoutMix_TMRM.m as the method name.</li> <li>f Click the Sample Position tab.</li> <li>g Select the Autosampler, Well-plate or Vial Tray.</li> </ul>	<ul> <li>example data file that comes with the program. If you prefer, you can create your own data file as described in this step.</li> <li>See also "To run the Checkout Mix" on page 6.</li> </ul>
	<ul> <li>h In the graphic, select a single position. Click OK.</li> <li>i In the Worklist window, mark the check box to the left of the sample.</li> <li>j Click the Start Worklist Run icon in the main toolbar, the Run Worklist icon in the Worklist toolbar or click the Worklist &gt; Run command.</li> </ul>	

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

Steps	Detailed Instructions	Comments		
<ul> <li>2 Find compounds using the Find Compound by MRM algorithm.</li> <li>• Open the data file CheckoutMix_TMRM.d.</li> </ul>	<ul> <li>a Start the Qualitative Analysis program.</li> <li>b Click File &gt; Open Data File. The system displays the "Open Data File" dialog box</li> <li>c Select CheckoutMix_TMRM.d, and click Open.</li> <li>d Click Method &gt; Method Explorer or View &gt; Method Explorer. The system displays the Method Explorer window.</li> <li>e In the Find Compounds section, click Find by MRM.</li> <li>f In the Method Editor window, click the Group transitions by compound name option.</li> <li>g Click the Peak area option for Detect most abundant peak by.</li> </ul>	<ul> <li>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 &gt; User Interface Configuration. Then, you mark the Unit Mass check box and the MS/MS (QQQ, Q-TOF) check box, and click OK.</li> <li>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.</li> <li>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.</li> </ul>		



h Click Find > Find Compounds by MRM.

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

Steps	Detailed Instructions	Comments
<ul> <li>3 Review the results of the Find Compounds by MRM algorithm.</li> <li>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.</li> </ul>	<ul> <li>a Close the Method Explorer and Method Editor windows.</li> <li>b Click the Compound Details View button.</li> <li>c Close the Compound MS Spectrum Results window.</li> <li>d In the Compound Chromatogram Results window, click the Overlaid mode button and the Show Legend in Overlaid mode button.</li> <li>e In the Compound Fragment Spectrum Results window, click the Spectrum Peak List button.</li> <li>f Review each compound. Verify that the primaries and secondaries for each compound were found.</li> </ul>	<ul> <li>You can also print a Compound Report to review results. You click File &gt; Print &gt; Compound Report. The Compound Report sorts the compounds by retention time.</li> <li>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.</li> <li>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.</li> <li>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.</li> </ul>
	<ul> <li>g Select Cpd1:Aminocarb. You click this compound in the Compound List.</li> <li>h In the Compound Fragment Spectrum Results window, verify that these transition are all found: <ul> <li>209.1 -&gt; 67.2 (Secondary)</li> <li>209.1 -&gt; 77.2 (Secondary)</li> <li>209.1 -&gt; 94.2 (Secondary)</li> <li>209.1 -&gt; 122.1 (Secondary)</li> <li>209.1 -&gt; 137.2 (Primary)</li> <li>209.1 -&gt; 155.2 (Primary)</li> </ul> </li> </ul>	<ul> <li>In the MS Peaks One table, you check the abundance for each Primary and Secondary transition.</li> <li>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.</li> <li>In the Compound Chromatogram Results window, you can see lines which indicate the abundances for each Secondary transition.</li> </ul>

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

Steps	Detailed Instructions	Comments
	<ul> <li>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.</li> </ul>	<ul> <li>In the Compound Fragment Spectrum Results table, you can check the abundance for each Primary and Secondary.</li> <li>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.</li> </ul>

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Carrowshild	aco vev (20. Contorna rear ven	N Concerned Martification Bomby Cold 2 Anisotration
1 Antonia in the Second Columns 1 1 6 6 6 1 1 1 1 1 1 1 1 1 1 1 1 1 1		10 Linearchild See Character 110 State 20 Caracter 20 Linear 20
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One         Intellight         Table         Table <thtable< th="">         Table         Table         &lt;</thtable<>	The dark lines show when the so acquired. The positions of these upon the Trigger Entrance, Trigger number, and Trigger Window the acquisition method. You change the time at which the secondary	dark lines are dependent er Delay, Trigger Repeat at you set in the these values to optimize transitions are acquired.

Agilent MassHunter Qualitative Analysis 8.07.00 - Default.r

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

St	teps	Detailed Instructions	Comments
1	Start the Quantitative Analysis program.	• Click the QQQ Quantitative Analysis icon.	
2	<ul> <li>Set up a batch and add the TMRM data file.</li> <li>Add the data file CheckoutMix_TMRM.d.</li> </ul>	<ul> <li>a Click File &gt; New Batch.</li> <li>b Navigate to the location of the TMRM data file.</li> <li>c Type CheckoutMix_TMRM for the Batch file name.</li> <li>d Click Open. The Add Sample window opens.</li> <li>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.</li> <li>f Select the CheckoutMix_TMRM.d data file and click OK.</li> <li>g Check that Flat Table (shown in red in the next figure) is selected. Then select Cal as the Type.</li> <li>h Type 1 for the Level.</li> </ul>	Add Samples       ? **         Batch Folder:       D:\MassHunter\Data\Pesti         File name       Sample name         CheckoutMix_DMRM.d       SuL x 10 ppb Pest         CheckoutMix_DMRM.d       SuL x 10 ppb Pest         CheckoutMix_TMRM.d       SuL x 10 ppb Pest         CheckoutMix_TMRM.d       SuL x 10 ppb Pest         CheckoutMix_TMRM.d       SuL x 10 ppb Pest         Browse to Copy Samples       Translate MSWS Samples         Select All       OK       Cancel
3	Set up the TMRM method.	Agilent MassHunter Quantitative Analysis (for QQQ) - Example data - CheckoutMi     File Edit View Analyze Method Update Library Report Tools Hep     Date Tools     Sample: Analyze Batch · · · Layout: D · · · · · · · · · · · · · · · · · ·	MRM.batch.bin Restore Default Layout Impound: Im
		<ul> <li>c Click Open.</li> <li>d Right-click the Method Table window and click Collapse All.</li> </ul>	

	Detailed Instructions						ents
	Metho	d Table					
	Time Segment: 🖛 <all></all>			▼ ⇒ Comp	ound: 💷	▼ ➡ R	eset Table View
	Sa	mole					
	_	Name		Data File	Туре	Level	Acq. Method File Acq. Date-Time
	· ··· •	CheckoutMix_TMRM.d	Checko	utMix_TMRM.d		][	
		Quantifier					
		Name 🛆	TS	Transition	Scan	Туре	
	۲	Aminocarb	1	209.1 -> 137.2	MRM	Target	
		Atrazine	1	216.1 -> 174.1	MRM	Target	
	•	Diazinon (Dimny	1	3051->1691	MRM	Target	
		Dimethoate	1	230.0 -> 198.8	MRM	Target	
	۲	Imazalil (Enilcon	1	297.1 -> 159.0	MRM	Target	
		- Imazapyr Malathion	1	262.1 -> 217.1	MRM	l arget	
	(U) (+)	Metazachlor	1	278.1 -> 134.2	MRM	Target	
		Metosulam	1	418.0 -> 175.0	MRM	Target	
	Ð	Metoxuron	1	229.0 -> 72.1	MRM	Target	
	(±)	Pyraclostrobin	1	388 1 -> 193 8	MRM	Target	
	Ū.	Thiabendazole	1	202.0 -> 131.0	MRM	Target	_
Set the Concentration Setup. • Add calibration level 1 with a	a S N	elect <b>Conco</b> Aethod Setu	entrat ip Tas	tion Setu sks sectio	<b>p</b> in the	• Ref Qua	er to the online Help in the antitative Analysis program f
<ul> <li>Set the Concentration Setup.</li> <li>Add calibration level 1 with a concentration of 100.</li> </ul>	a S N N	elect <b>Conc</b> o Nethod Setu Nethod Task	entrat ip Tas is par	<b>tion Setu</b> sks sectione.	p in the on in the	• Ref Qua add	er to the online Help in the antitative Analysis program f litional help on these tasks.
<ul> <li>Set the Concentration Setup.</li> <li>Add calibration level 1 with a concentration of 100.</li> </ul>	a S N b S	elect <b>Conc</b> o Aethod Setu Aethod Task elect the fii	entrat ip Tas is par ist co	<b>tion Setu</b> sks sectione. mpound	p in the on in the in the table	• Ref Qua add	er to the online Help in the antitative Analysis program f litional help on these tasks.
<ul> <li>Set the Concentration Setup.</li> <li>Add calibration level 1 with a concentration of 100.</li> </ul>	a S M b S c R c s	elect <b>Conc</b> a Aethod Setu Aethod Task elect the fin ight-click th lick <b>New C</b> a hortcut men	entrat ip Tas is par ist co ne co alibra nu.	tion Setu sks sectione. mpound mpound stion Lev	<b>p</b> in the on in the in the table row and <b>el</b> from the	• Ref Qua add e.	er to the online Help in the antitative Analysis program f litional help on these tasks.
<ul> <li>Set the Concentration Setup.</li> <li>Add calibration level 1 with a concentration of 100.</li> </ul>	a S N b S c R c s d lr	elect Conce Aethod Setu Aethod Task elect the fin ight-click th lick New Ca hortcut men the Level	entrat ip Tas is par rst co ne col alibra nu. colun n typ	tion Setu sks sectione. mpound mpound ntion Lev	<b>p</b> in the on in the in the table row and <b>el</b> from the 1. In the	• Ref Qua add e.	er to the online Help in the antitative Analysis program f litional help on these tasks.
<ul> <li>Set the Concentration Setup.</li> <li>Add calibration level 1 with a concentration of 100.</li> </ul>	a S N b S c R c s d Ir	elect Conce Aethod Setu Aethod Task elect the fin ight-click th lick <b>New C</b> a hortcut men the <b>Level</b> conc. colum	entrat ip Tas is par rst co ne col alibra nu. colun n, typ	tion Setu sks sectione. mpound mpound ntion Lev nn, type 1 ne 100.	<b>p</b> in the on in the in the table row and <b>el</b> from the 1. In the	• Ref Qua add	er to the online Help in the antitative Analysis program f litional help on these tasks.
Set the Concentration Setup. • Add calibration level 1 with a concentration of 100.	a S M b S c R c s d In C e R	elect Conce Aethod Setu Aethod Task elect the fin light-click th lick <b>New C</b> a hortcut men the <b>Level</b> conc. colum light-click ir copy Calibra	entrat up Tas s par st co ne cou alibra nu. colun n, typ n the ation	tion Setu sks sectione. mpound mpound tion Lev nn, type 1 ne 100. Level box Level box	<b>p</b> in the on in the in the table row and <b>el</b> from the 1. In the c and click <b>b</b> .	• Ref Qua add e.	er to the online Help in the antitative Analysis program f litional help on these tasks.
<ul> <li>Set the Concentration Setup.</li> <li>Add calibration level 1 with a concentration of 100.</li> </ul>	a S M b S c R c s d Ir C e R f c	elect Conce Aethod Setu Aethod Task elect the fin light-click th lick New Ca hortcut men the Level Conc. colum light-click in copy Calibra	entrat up Tas as par rst co ne col alibra nu. colun n, typ n the ation	tion Setu sks sectione. mpound mpound tion Lev nn, type : te 100. Level box Levels To Levels To	p in the on in the row and el from the 1. In the ( and click ).	• Ref Qua add	er to the online Help in the antitative Analysis program f litional help on these tasks.



Steps	Detailed Instructions	Comments		
<ul> <li>6 Resolve the RTs. The RT elution order is:</li> <li>Aminocarb</li> <li>Imazapyr</li> <li>Thiabendazole</li> <li>Dimethoate</li> <li>Imazalil (Enilconazole)</li> <li>Metoxuron</li> <li>Carbofuran</li> <li>Atrazine</li> <li>Metosulam</li> <li>Metazachlor</li> <li>Molinate</li> <li>Malathion</li> <li>Pyraclostrobin</li> <li>Diazinon (Dimpylate)</li> </ul>	<ul> <li>a Select Retention Time Setup in the Method Setup Tasks section.</li> <li>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.</li> </ul>	Depending on the delay volume, the compounds Pyraclostrobin and Diazinon can co-elute, separate slightly, or reverse elution order.		

teps	De	tailed Instructions		Comments	
Anilent MassHunter Quantitative Analysis (for	000) - [New Method]				
File Edit View Analyze Method Undate I	ibrary Report Tools Help				
		Restore Default Lavout			
lathad Taska		ag [24] Nestore <u>D</u> erault cayout			
					• •
New / Open Method	Time Segment: 🖤 <all></all>	<ul> <li>Compound: Aminocar</li> </ul>	o ▼ 📄 <u>R</u> eset lat	ble View	
Workflow	Sample				
Method Setup Tasks	Name Charlen Min, TMDM J. Ch	Data File I ype	Level Acq. M	ethod File Acq. Date-Time	
MRM Compound Setup	Checkoulmix_TMRM.d Cr	neckoutmix_TMRM.d			r -
Retention Time Setup	Quantifier		-		
ISTD Setup	Name	1 200 1 x 127 2 MPM	Target	RI Left RI Delta Right RI	1 000 Minutes
Concentration Setup	Imazapyr	1 262.1 -> 217.1 MRM	Target	2.836 1.000	1.000 Minutes
Qualifier Setup	Thiabendazole	1 202.0 -> 131.0 MRM	Target	2.885 1.000	1.000 Minutes
Calibration Curve Setun	Dimethoate	1 230.0 -> 198.8 MRM	Target	3.883 1.000	1.000 Minutes
campration curve octup	Metoxuron	1 229.0 -> 72.1 MRM	Target	4.775 1.000	1.000 Minutes
Globals Setup	Carbofuran	1 222.1 -> 123.1 MRM	Target	5.772 1.000	1.000 Minutes
ave / Exit	Atrazine	1 216.1 -> 174.1 MRM	Target	6.142 1.000	1.000 Minutes
? Validate	Metosulam	1 418.0 -> 1/5.0 MRM 1 278 1 -> 134 2 MRM	Target	6.320 1.000	1.000 Minutes
	Molinate	1 188.0 -> 83.2 MRM	Target	7.825 1.000	1.000 Minutes
Save	Malathion	1 331.0 -> 126.9 MRM	Target	8.386 1.000	1.000 Minutes
Save As	Pyraclostrobin Diszinos (Dimov	1 388.1 -> 193.8 MRM	Target	9.454 1.000	1.000 Minutes
Exit	Diazinon (Dimpy	1 303.1 22 103.1	Taiger	5.461	1.000 Minutes
Manual Setup Tasks					
)utlier Setup Tasks	Compound Information				- ×
Advanced Tasks					
Advanced tasks	+ MBM (209 1 -> 137 2) CheckoutMix	(TMRM d 2091->1372	209.1 -> 152.2	+ MRM (2 008-2 0	39 min 6 scans) (209 1 -> **) CheckoutMix T
	≅ x10 <sup>5</sup> - 2.019 min.	∞ x10 5 - Rati	p=76.4	월 x10 6	137.2
	8 2.2-	8 22-	8	0 14	
	2-	토 2-	l l	13-	
	19	Abur 1	1	1.2-	152.2
	10	1.0	10	1.1-	1011
	1.0-			1-	
	1.4-	1.4 -		0.9-	
	1.2-	1.2-		0.8-	
	1-	1-		0.7 -	
	0.8-	0.8-	{	0.6 -	
	0.6-	0.6-	11	0.5 -	
	0.4-	0.4-	11	0.4 -	122.1
	0.2-	0.2-		0.3-	
				0.2 -	
				0.1- 7	7.2 94.2 209.1
	-0.2-1.8 2 2.2	-0.2- 2.4 2.6 2.8 Acquisition Time (min)	1.8 2 2.2 2.4	4 2.6 2.8 60	80 100 120 140 160 180 200 Mass-to-Charge (m/z)
		Acquiation Time (min)		Ploqueruer rinne (min)	made to onlarge (mrz)

St	ieps	Detailed Instructions Comments	
7	Review qualifier ratios	c Select <b>Qualifier Setup</b> in the Method Setup Tasks section.	
		d Right-click the Method Table and click Expand All.	
		<ul> <li>Click the Show/Hide Qualifiers button in the toolbar in the Compound Information window.</li> </ul>	
		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.	<ul> <li>a Click Library &gt; Setup Reference Library.</li> <li>b Click Obtain reference spectra from sample.</li> <li>c Verify that Create reference library at is set to the folder you wish to use.</li> <li>d Click OK.</li> <li>e Click OK in the "Reference library was created" message.</li> </ul>	<ul> <li>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".</li> </ul>		



- 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.

ime Segment: 🖛 <all></all>	Compound: Aminocarb     View     Reset Table View
Globals	
Apply Multiplier to ISTD	
Apply Multiplier to Matrix Spike	
Apply Multiplier to Surrogate	
Apply Multiplier to Target	
Bracketing Type	None
CC Maximum Elapsed Time In Hours	0.00
Correlation Window	2.00
Dynamic Background Subtraction	
Ignore Peaks Not Found	
Library Method	
Non Reference Window	200.00
Non Reference Window Type	Percent
Reference Library	C:\MassHunter\Data\Pesticides tMRM Dat\CheckoutMix_TMRM.reflibrary.xm
Reference Pattern Library	

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

Steps	Detailed Instructions	Comments
9 Save the method and set additional batch processing to analyze.	<ul> <li>a Click Method &gt; Save As.</li> <li>b Type Quant_Checkout_The in the File name.</li> <li>c Click Save.</li> <li>d Click Method &gt; Exit.</li> <li>e Verify that Additional batch processing after applying the method is set to Analyze.</li> <li>f Click Yes to apply the method to batch.</li> </ul>	MRM ethod the
	Apply Method         Would you like to apply this method to the batch?         Yes       No         Cancel         Additional batch processing after applying the method         © protype         Quantitate         O friegrate         Ngne	
<b>10</b> Review the results, and save the batch.	<ul> <li>a Inspect the results.</li> <li>b If the Compound Information win is not open, click View &gt; Compo Information.</li> <li>c Click the Show/Hide Spectrum bid Click File &gt; Save Batch.</li> </ul>	idow <b>und</b> utton.
Compound Information           Image: Image	会 ▲ 201 -> 1522 + MRM (2003-2.044 min, 8 scans) (2(	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.	<ul> <li>Check library match scores</li> <li>Check qualifier ratios.</li> <li>Resolve errors and messages</li> <li>NOTE: The sample data included for this exercise does not contain isomers. But if your sample does, you would resolve isomers at this time.</li> </ul>	<ul> <li>If an error is reported for a compound qualifier ratio:</li> <li>a Click Method &gt; Edit.</li> <li>b Click Update &gt; Update Qualifier Ratios.</li> <li>c Select the compounds for update and click OK.</li> <li>d Click Method &gt; Exit.</li> <li>e Click Yes to apply the method to the batch.</li> <li>f Check qualifier ratios.</li> <li>g Resolve errors and messages.</li> <li>h Click File &gt; Save Batch.</li> </ul>			

# **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 <sub>11</sub> H <sub>16</sub> N <sub>2</sub> O <sub>2</sub>	208.1211777698
2	Atrazine/1912-24-9	100.4 µg/mL	0.5 µg/mL	$C_8H_{14}CIN_5$	215.0937731936
3	Carbofuran/1563-66-2	100.2 µg/mL	0.5 µg/mL	C <sub>12</sub> H <sub>15</sub> NO <sub>3</sub>	221.1051933528
4	Diazinon (Dimpylate)/333-41-5	100.4 µg/mL	0.5 µg/mL	C <sub>12</sub> H <sub>21</sub> N <sub>2</sub> O <sub>3</sub> PS	304.1010497716
5	Dimethoate/60-51-5	100.2 µg/mL	0.5 µg/mL	C <sub>5</sub> H <sub>12</sub> NO <sub>3</sub> PS <sub>2</sub>	228.9996212071
6	Imazalil (Enilconazole)/35554-44-0	100.4 µg/mL	0.5 µg/mL	$\mathrm{C_{14}H_{14}Cl_2N_2O}$	296.0483185037
7	lmazapyr/8331-34-1	100.2 µg/mL	0.5 µg/mL	C <sub>13</sub> H <sub>15</sub> N <sub>3</sub> O <sub>3</sub>	261.1113413676
8	Malathion/121-75-5	100.4 µg/mL	0.5 µg/mL	C <sub>10</sub> H <sub>19</sub> O <sub>6</sub> PS <sub>2</sub>	330.0360662899
9	Metazachlor/67129-08-2	100.2 µg/mL	0.5 µg/mL	C <sub>14</sub> H <sub>16</sub> CIN <sub>3</sub> O	277.0981898649
10	Metosulam/139528-85-1	100.4 µg/mL	0.5 µg/mL	$C_{14}H_{13}CI_2N_5O_4S$	417.0065300909
11	Metoxuron/19937-59-8	100.2 µg/mL	0.5 µg/mL	C <sub>10</sub> H <sub>13</sub> CIN <sub>2</sub> O <sub>2</sub>	228.0665553841
12	Molinate/2212-67-1	100.4 µg/mL	0.5 µg/mL	C <sub>9</sub> H <sub>17</sub> NOS	187.103084902
13	Pyraclostrobin/175013-18-0	100.2 µg/mL	0.5 µg/mL	C <sub>19</sub> H <sub>18</sub> CIN <sub>3</sub> O <sub>4</sub>	387.0985837956
14	Thiabendazole/148-79-8	100.4 µg/mL	0.5 µg/mL	C <sub>10</sub> H <sub>7</sub> N <sub>3</sub> S	201.0360679755
	Acetonitrile	Solvent		C <sub>2</sub> H <sub>3</sub> N	41.0265

#### **Reference** Checkout Mix Content

#	Chemical Name/CAS #	Concentration / Units	Tolerance (+/-)	Formula	Mass
1	Acifluorfen/50594-66-6	100.2 µg/mL	0.5 µg/mL	$C_{14}H_7CIF_3NO_5$	360.9964846522
2	2,4,5-T/93-76-5	100.4 µg/mL	0.5 µg/mL	C <sub>8</sub> H <sub>5</sub> Cl <sub>3</sub> O <sub>3</sub>	253.9304271564
3	Bentazone/25057-89-0	100.2 µg/mL	0.5 µg/mL	C <sub>10</sub> H <sub>12</sub> N <sub>2</sub> O <sub>3</sub> S	240.0568629945
4	Dinoseb (Subitex)/88-85-7	100.4 µg/mL	0.5 µg/mL	C <sub>10</sub> H <sub>12</sub> N <sub>2</sub> O <sub>5</sub>	240.0746215091
5	2,4,5-TP (Silvex) (Fenoprop)/93-72-1	100.2 µg/mL	0.5 µg/mL	C <sub>9</sub> H <sub>7</sub> Cl <sub>3</sub> O <sub>3</sub>	267.9460772202
6	Hexaflumuron/86479-06-3	100.4 µg/mL	0.5 µg/mL	C <sub>16</sub> H <sub>8</sub> Cl <sub>2</sub> F <sub>6</sub> N <sub>2</sub> O <sub>3</sub>	459.9816167569
	Acetonitrile	Solvent		C <sub>2</sub> H <sub>3</sub> N	41.0265

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

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 Spe	ed	100.0 μL/i	min	
Eject Spee	ed	100.0 μL/i	min	
Draw Posi	ition Offset	0.0 mm		
Wait Time	e After Drawing	2.0 s		
Sample Fl	ush Out Factor	5.0		
Vial/Well	bottom sensing	No		
Injection				
Injection I	Mode	Standard i	njection	
Injection	Volume	5.00 μL		
High throug	;hput			
Automati	c Delay Volume Reduction	No		
Overlapped Injection				
Enable	e Overlapped Injection	No		
Valve Switching				
Valve Mo	vements	0		
Valve Sw	vitch Time 1			
Switch	n Time 1 Enabled	No		
Valve Sw	vitch Time 2			
Switch	n Time 2 Enabled	No		
Valve Sw	vitch Time 3			
Switch	n Time 3 Enabled	No		
Valve Sw	vitch Time 4			
Switch	n Time 4 Enabled	No		
Stop Time				
Stoptime	Mode	As pump/	No limit	
Post Time				
Posttime	Mode	Off		

Figure 1 HiP Sampler parameters

**LC 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/min <sup>2</sup>
Max. Flow Ramp Down	100.000 mL/min <sup>2</sup>
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
Timotable	
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	А	100.0 % H20 (migrated)	H20 w/ 5mM acetic acid	100.0 % H20 (migrated)		Ch. 1	Yes	95.00 %
2	В	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 w	hen front door open	Yes	
Left Tempe	erature Control		
Tempera	ature Control Mode	Tempera	ture Set
Tempera	ature	35.00 °C	
Enable	Analysis Left Temperature		
Enab	le Analysis Left Temperature On	Yes	
Enab	le Analysis Left Temperature Value	0.80 °C	
Right Tem	perature Control		
Right ter	mperature Control Mode	Tempera	ture Set
Right ter	mperature	35.00 °C	
Enable	Analysis Right Temperature		
Enab	le Analysis Right Temperature On	Yes	
Enab	le Analysis Right Temperature Value	0.80 °C	
Stop Time			
Stoptime	e Mode	As pump	/injector
Post Time			
Posttime	e 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**.

Compound Name	Prim ary?	Trig- ger	Thresh- old	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	Prim ary?	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

Primary and Secondary Transitions for Triggered MRM

Compound Name	Prim ary?	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
lmazapyr	Yes	Yes	249	262.1	Unit	217.1	Unit	3.83	0.63	120	20	3
lmazapyr	Yes			262.1	Unit	69.1	Unit	3.83	0.63	120	40	3
lmazapyr				262.1	Unit	220.1	Unit			120	20	3
lmazapyr				262.1	Unit	202.1	Unit			120	20	3
lmazapyr				262.1	Unit	149	Unit			120	20	3
lmazapyr				262.1	Unit	131	Unit			120	40	3
lmazapyr				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

Primary and Secondary Transitions for Triggered MRM

Compound Name	Prim ary?	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

Primary and Secondary Transitions for Triggered MRM

Compound Name	Prim ary?	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

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



## Reference

To bypass mixer and damper



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## In This Guide

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

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