

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)

Supported MassHunter Programs

Make sure these MassHunter programs are installed.

- MassHunter Data Acquisition
 - for Ultivo LC/TQ, 1.2 or later
 - for 6470B, 10.1 or later
 - for 6495C, 10.0 SR1 or later
- MassHunter Quantitative Analysis 10.2 or later.
- MassHunter Qualitative Analysis 10.0 or later.

Familiarization files

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

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

Note that the respective dMRM and tMRM Checkout Mix methods for the 6470B and Ultivo instruments are also included for reference only. These methods work only on an LC/MS system that produces the same retention times as the example data. Any retention time shifts will invalidate the retention time windows in these methods.

- Checkout Mix example Data: This dataset was acquired on a 6470B instrument, and can be used to familiarize yourself with the data analysis workflow regardless of your LC/TQ model.
- Checkout Mix example report

Workflow Overview

This Familiarization Guide uses example data from the Checkout Mix to illustrate the workflow and the familiarization exercises.

Figure 1 summarizes the workflow, which includes incremental method development from MRM, over to dynamic MRM (dMRM) to triggered MRM (tMRM) methods, including identification of retention times (RT), trigger parameters, and secondary transitions.

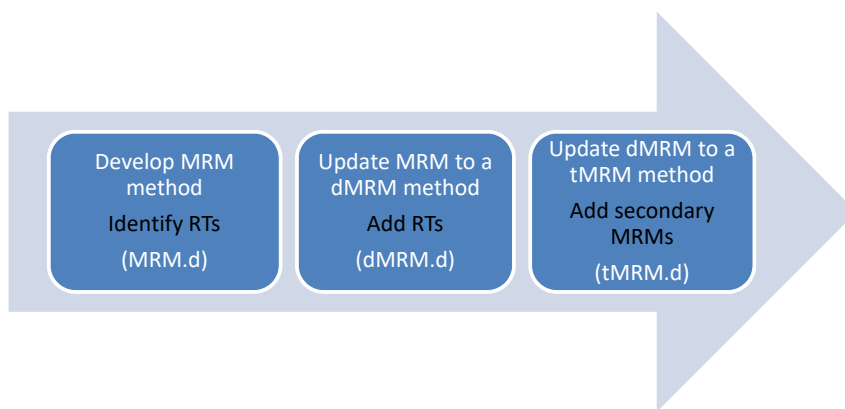


Figure 1. MRM to dMRM to tMRM Method Development Workflow for single standard mix

Single Standard Mix Workflow

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

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

Workflow Overview

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

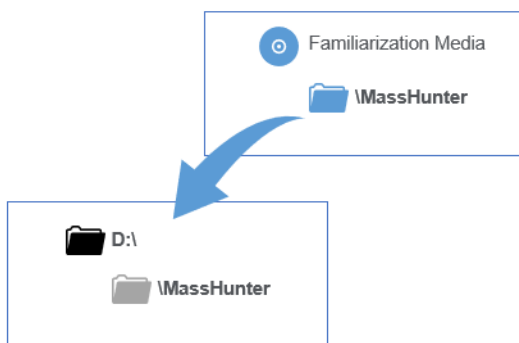
To install the Familiarization files

Before You Begin

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

To install the Familiarization files

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



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

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

Before You Begin

To prepare to run the Checkout Mix

To prepare to run the Checkout Mix

- 1 Make sure that you have these required parts and reagents:
 - Glacial acetic acid
 - ZORBAX LC Column, Eclipse Plus C18, 2.1 mm × 100 mm, 1.8 μm (p/n 959758-902)
- 2 Check that the Agilent 1200 Series, Infinity I, or Infinity II LC system is properly installed and verified.
- 3 If you have an Agilent 1260 Infinity I or Infinity II Binary Pump, bypass the mixer and damper. Refer to your respective user manual for details.
- 4 Check that the Agilent 6400 Series Triple Quad or Ultivo Triple Quad LC/MS System is properly installed and verified.
- 5 Make sure that the supported MassHunter programs are properly installed. See **“Supported MassHunter Programs”** on page 2.
- 6 To use a system configuration that is different from the one described in **“To run the Checkout Mix”** on page 8, create or edit a method for your system configuration and the Checkout Mix method parameters. The Checkout Mix parameters are in the Checkout Mix acquisition method.

Use the MassHunter Data Acquisition program to open and view the method. These data acquisition settings for the compounds are listed:

- QQQ Acquisition method info
- Sampler settings
- Pump settings
- Column compartment settings

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

The three sets of example methods use the following instruments:

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

To run the Checkout Mix

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

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

- 2 Prepare the Checkout Mix.

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

- a Dilute 100 μ L of the stock solution to 10.0 mL with acetonitrile to create Working Solution 1 (1 ppm). Use Working Solution 1 for non-iFunnel systems with an ESI source.

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

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

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

Do this separately for the positive and negative Checkout Mixes.

NOTE

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

- 3 Prepare mobile phases A and B.

- A= 5 mM acetic acid in water (286 μ L 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.

Before You Begin

To run the Checkout Mix

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

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

- 5 Load the method.
 - If you have a non-iFunnel 6400 Series Triple Quad LC/MS system, open **CheckoutMix_MRM_6470B_WithCmpds.m**.
 - If you have an iFunnel 6400 Series Triple Quad LC/MS system, open **CheckoutMix_MRM_6495C_WithCmpds.m**.
 - If you have an Ultivo Triple Quad LC/MS system, open **CheckoutMix_MRM_Ultivo_WithCmpds.m**.
- 6 After loading the method, if your instrument model is different than the 6470A or 6495C, then the tune location may appear in red. Click the **Browse** folder and point to the correct tune folder for your instrument. Then click the most recent tune file, which should be **atunes.TUNE.XML**.
- 7 Check that the method is set up to make a 5 µL injection.
- 8 Click **Sample > Run** to do a single sample run, or create a worklist to make multiple injections.
- 9 If you do not see all the peaks after you process your data:
 - a Extend your **Stop time**.
 - b Run the test mix again.

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

Creating an MRM acquisition method from the database

Task 1. Create an MRM method

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

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

Steps	Detailed Instructions	Comments
1	In the Data Acquisition program, open the appropriate MRM checkout method for your LC/TQ and save as: <i>iii</i> CheckoutMix_MRM.m , where <i>iii</i> are your initials.	<ul style="list-style-type: none"> a Start the Data Acquisition program. b If you have a non-iFunnel 6400 Series Triple Quad LC/MS system, open the CheckoutMix_MRM_6470B.m method. If you have an iFunnel 6400 Series Triple Quad LC/MS system, open the CheckoutMix_MRM_6495C.m method. If you have an Ultivo Triple Quad LC/MS system, open the CheckoutMix_MRM_Ultivo.m method. c Click Method > Save As. d Type <i>iii</i>CheckoutMix_MRM.M, where <i>iii</i> are your initials.
2	Set the LC parameters according to the table on the next page.	<ul style="list-style-type: none"> a In the Method Editor window, click the tab for the configured autosampler. b Enter the parameters. c Click the tab for the configured pump. d Enter the parameters. e Click the Column Comp. tab. f Enter the parameters.

Creating an MRM acquisition method from the database

Task 1. Create an MRM method

Steps	Detailed Instructions	Comments																																														
	<table border="1"> <thead> <tr> <th colspan="4">LC Parameters</th> </tr> </thead> <tbody> <tr> <td>Column</td> <td colspan="3">Agilent ZORBAX LC Column, Eclipse Plus C18, 2.1 mm x 100 mm, 1.8 µm (p/n 959758-902)</td> </tr> <tr> <td>Column temperature</td> <td colspan="3">35 °C</td> </tr> <tr> <td>Injection volume</td> <td colspan="3">5 µL (0.5 µL for Funnel-based instruments)</td> </tr> <tr> <td>Needle Wash</td> <td colspan="3">5 seconds in 50:50 methanol/water</td> </tr> <tr> <td>Mobile phase</td> <td colspan="3">A= 5mM acetic acid in water B= Acetonitrile</td> </tr> <tr> <td>Flow rate</td> <td colspan="3">0.4 mL/min</td> </tr> <tr> <td rowspan="3">Timetable</td> <td>Time (min)</td> <td>A (%)</td> <td>B (%)</td> </tr> <tr> <td>0.00</td> <td>95.00</td> <td>5.00</td> </tr> <tr> <td>12.00</td> <td>5.00</td> <td>95.00</td> </tr> <tr> <td><u>Stoptime</u></td> <td colspan="3">12.00 min</td> </tr> <tr> <td><u>Posttime</u></td> <td colspan="3">3.00 min</td> </tr> </tbody> </table>	LC Parameters				Column	Agilent ZORBAX LC Column, Eclipse Plus C18, 2.1 mm x 100 mm, 1.8 µm (p/n 959758-902)			Column temperature	35 °C			Injection volume	5 µL (0.5 µL for Funnel-based instruments)			Needle Wash	5 seconds in 50:50 methanol/water			Mobile phase	A= 5mM acetic acid in water B= Acetonitrile			Flow rate	0.4 mL/min			Timetable	Time (min)	A (%)	B (%)	0.00	95.00	5.00	12.00	5.00	95.00	<u>Stoptime</u>	12.00 min			<u>Posttime</u>	3.00 min			
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Timetable	Time (min)	A (%)	B (%)																																													
	0.00	95.00	5.00																																													
	12.00	5.00	95.00																																													
<u>Stoptime</u>	12.00 min																																															
<u>Posttime</u>	3.00 min																																															

3 Set the source parameters.

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

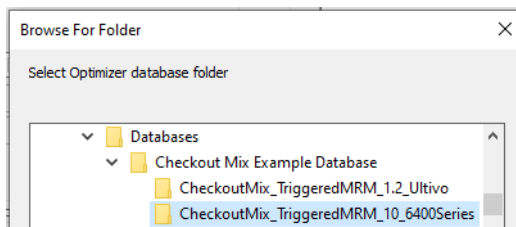
- The Scan segments table always must have at least one row. You manually remove this row after importing transitions from the Database Browser.

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

Creating an MRM acquisition method from the database

Task 1. Create an MRM method

Steps	Detailed Instructions	Comments
4	<p>Open the appropriate database in Database Browser, from the QQQ tab.</p> <ol style="list-style-type: none">For 6400 Series systems, right-click the Scan segments table and click Import from Database Browser. For Ultivo, click Compound Browser. For either system, the Database Browser opens. In the Database Browser, click File > Open Database.For 6400 Series systems, select the CheckoutMix_TriggeredMRM_10_6400 Series database in the \MassHunter\Databases\Checkout Mix Example Database folder. For Ultivo, select the CheckoutMix_TriggeredMRM_1.2_Ultivo database.Click OK.Save the method with a new name. Do not overwrite the provided example method.	



Creating an MRM acquisition method from the database

Task 1. Create an MRM method

Steps	Detailed Instructions	Comments
<p>5 Select primary transitions corresponding to the basic checkout mix compounds.</p> <ul style="list-style-type: none">See “Primary and Secondary Transitions for Triggered MRM” on page 65 for a list of the Primary transitions.The secondary transition are added when you are creating the triggered MRM method. See “Task 1. Create a tMRM method from a dMRM method” on page 44.	<p>a Click the Compound Name column header to sort the compounds by Compound Name.</p> <p>b Ensure the correct check boxes next to the primary transitions are marked according to the “Primary and Secondary Transitions for Triggered MRM” on page 65.</p> <p>c To quickly mark only the primary transitions for the Checkout Mix:</p> <ul style="list-style-type: none">Under Search Compounds, mark the CAS check box. In the Search Text text box, type the CAS numbers for the Checkout Mix. <p>d Under Select Transitions, select Primary transitions and click Select Primary. See next page for filter examples.</p> <p>e Review the transitions in the table. Clear the check box next to any transitions that you do not want to include.</p>	<ul style="list-style-type: none">Instead of individually marking each check box, you can use the search and filter function with the Select Transitions options to select a number of transitions according to the criteria you have specified. Refer to the help for the Database Browser Search Filter tab in the Optimizer Help.The CAS number is a reliable item to use to filter the compounds. If you use the Compound Name, you have to spell the name exactly as it is written in the database; otherwise, you get too many random hits which you then have to remove from your import list. Also, you have to write the name or number as a vertical list (a new line for each name or number).Qualifier and quantifier MRMs can have different precursor ion species but they cannot have different polarities. Compounds that contain halogens often have multiple precursors for the same compound in the database. If a <i>Method Setup Guide</i> is available for your database, refer to the guide for more details on choosing the most selective transitions for your analysis.

Note that in the example data file, when both polarities are available in the **CheckoutMix_TriggeredMRM_1.2_Ultivo** database, the analysis was run in positive mode only.

The CAS numbers for the basic checkout mix (positive ion compounds) are:

2032-59-9
1912-24-9
1563-66-2
333-41-5
60-51-5
35554-44-0
81334-34-1
121-75-5
139528-85-1
19937-59-8
2212-67-1
175013-18-0
148-79-8

Creating an MRM acquisition method from the database

Task 1. Create an MRM method

Steps

Detailed Instructions

Comments

Database Browser

Search/Filter Import List

Show All Records

Filter Compounds

Enable Filters

Optimized Compounds

Date From 12/15/2020 To 06/04/2010

Group Name Project Name Model

Polarity Positive

Method

Search Compounds

Search Text

IN

Match entire word for each string

Select Columns

Project Name

Compound Name

Formula

MW

Groups

CAS

Chemical Classes

Select Transitions

Select top 1 ranked transitions

Primary transitions

Secondary transitions

Set primary and trigger flags

Set top 2 ranked transitions as primary

Set Primaries and Trigger

Rank transitions by

Abundance

Response Factor

<input type="checkbox"/>	Compound Name	Formula	MW	Polarity	Species	Precursor	Product	Frag	CE	CAV	Primary	Trigger
<input checked="" type="checkbox"/>	2.4.5-T	C8H5Cl3O3		Negative		252.9	95	80	60	3	<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	2.4.5-T	C8H5Cl3O3		Negative		252.9	122.9	80	45	3	<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	2.4.5-T	C8H5Cl3O3		Negative		252.9	158.9	80	40	3	<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	2.4.5-T	C8H5Cl3O3		Negative		252.9	194.9	80	10	3	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
<input type="checkbox"/>	2.4.5-TP (Silvex)	C9H7Cl3O3		Negative		266.9	95	80	60	3	<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	2.4.5-TP (Silvex)	C9H7Cl3O3		Negative		266.9	122.9	80	45	3	<input type="checkbox"/>	<input type="checkbox"/>

Current Database : D:\MassHunter\Databases\Checkout Mix Example Database\CheckoutMix_TriggeredMRM_10_6400Series

Add to Import List Import Close

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.

Database Browser

Search/Filter Import List

Show All Records

Filter Compounds

Enable Filters

Optimized Compounds

Date From 12/15/2020 To 06/04/2010

Group Name Project Name Model

Polarity Positive

Method

Search Compounds

Search Text

81334-34-1
6051-5
333-41-5

IN

Match entire word for each string

Select Columns

Project Name

Compound Name

Formula

MW

Groups

CAS

Chemical Classes

Select Transitions

Select top 1 ranked transitions

Primary transitions

Secondary transitions

Set primary and trigger flags

Set top 2 ranked transitions as primary

Set Primaries and Trigger

Rank transitions by

Abundance

Response Factor

<input type="checkbox"/>	Compound Name	Formula	MW	Polarity	Species	Precursor	Product	Frag	CE	CAV	Primary	Trigger
<input type="checkbox"/>	Diazinon (Dimpylate)	C12H21N2O3PS		Positive		305.1	66	105	40	2	<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	Diazinon (Dimpylate)	C12H21N2O3PS		Positive		305.1	84	105	40	2	<input type="checkbox"/>	<input type="checkbox"/>
<input type="checkbox"/>	Diazinon (Dimpylate)	C12H21N2O3PS		Positive		305.1	93	105	40	2	<input type="checkbox"/>	<input type="checkbox"/>
<input checked="" type="checkbox"/>	Diazinon (Dimpylate)	C12H21N2O3PS		Positive		305.1	97	105	40	2	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
<input type="checkbox"/>	Diazinon (Dimpylate)	C12H21N2O3PS		Positive		305.1	100	105	40	2	<input type="checkbox"/>	<input type="checkbox"/>
<input checked="" type="checkbox"/>	Diazinon (Dimpylate)	C12H21N2O3PS		Positive		305.1	169.1	105	32	2	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>

Current Database : D:\MassHunter\Databases\Checkout Mix Example Database\CheckoutMix_TriggeredMRM_10_6400Series

Add to Import List Import Close

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.

Creating an MRM acquisition method from the database

Task 1. Create an MRM method

Steps	Detailed Instructions	Comments
6 Import transitions to the Data Acquisition program.	<ol style="list-style-type: none">Click the Add to Import List button.Click the Import List tab.Review the Import List table.Click the Import button.	<ul style="list-style-type: none">Only the transitions that you marked are added to the Import List.Removal of the negative MRM transition for compounds that also have a positive MRM transition ensures that one compound name is associated with only one polarity. One compound cannot have both negative and positive polarity transitions.

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

Creating an MRM acquisition method from the database

Task 1. Create an MRM method

Steps	Detailed Instructions	Comments
7	<p>Review the MRM transitions in the Data Acquisition program.</p> <ol style="list-style-type: none"> Delete the original compound in the Scan segments table. In the provided method, this compound has been named “delete this compound after DB import”. Sort the table by the Compound Name. Review the transitions for each compound. 	<ul style="list-style-type: none"> If a red box appears in the Scan segments table, you click the Apply button in the toolbar. If the red box does not clear, the value is not valid. The example method may not exactly match the transitions in the database. Use the transitions in the database if you find a discrepancy.
8	<p>Change the dwell times.</p> <ol style="list-style-type: none"> In the Scan segments table, type 20 in the column labeled Dwell. Type it in the cell for the first compound (Aminocarb). Right-click the cell and select Fill column so that all compounds have a dwell time of 20 ms. 	<ul style="list-style-type: none"> Make sure your dwell time for all transitions gives an appropriate cycle time. This criterion determines how many transitions you can put within one cycle. For example, for peaks that are 5 seconds wide, use a cycle time of 500 ms to give 10 points across the peak. For 50 compounds with 2 transitions each, use a 2 ms dwell time to give 5.5 ms total per transition.

The screenshot shows the Method Editor software interface. The main window displays the Scan segments table with columns: Compound Group, Compound Name, ISTD?, Precursor Ion, MS1 Res, Product Ion, MS2 Res, Dwell, Fragmentor, Collision Energy, Cell Accelerator Voltage, and Polarity. The Dwell column is highlighted, and a context menu is open over it, showing options like Add Row, Delete Row, Sort, Import from Database Browser..., Update DMRM Method..., Cut, Copy, Paste, Paste from Clipboard, Fill Down, and Fill Column. The Fill Column option is selected. The table lists various compounds such as Aminocarb, Atrazine, Caboluran, Diazinon (Dimethylate), Dimethoate, Imazalil (Enilconazole), Imazapyr, Malathion, and Metazachlor. The Dwell column values are mostly 200, with the first row (Aminocarb) showing 20. The interface also shows a toolbar with 'Apply' and 'Source' buttons, and a sidebar with '6400 Series' and 'cycles/s [5400.9] ms/cycle'.

Creating an MRM acquisition method from the database

Task 1. Create an MRM method

Steps

Detailed Instructions

Comments

The screenshot displays the Method Editor interface for creating an MRM method. The title bar shows the method name: "CheckoutMix_MRM_UltivoB_with compounds.m". The interface is divided into several sections:

- Method Editor:** Includes tabs for Properties, DA, Multisampler, Multisampler Pretreatment, Binary Pump, Column Comp., and QQQ. The "Method" section is active, showing "Acquisition" and "Tune" options.
- Acquisition Parameters:** A table listing various compound groups and their parameters. The "Time filter window (min)" is set to 0.05.
- Time Segments:** A section for defining acquisition time segments, currently showing a segment from 0 to 13 minutes with an MRM scan type.

Compound group	Compound name	ISTD?	Precursor (m/z)	MS1 res	Product (m/z)	MS2 res	Dwell (ms)	Fragmentor (V)	CE (V)	Polarity
Insecticide	Aminocarb	<input type="checkbox"/>	209.1	Unit	137.2	Unit	20	105	24	Positive
Insecticide	Aminocarb	<input type="checkbox"/>	209.1	Unit	152.2	Unit	20	105	12	Positive
Herbicide	Atrazine	<input type="checkbox"/>	216.1	Unit	68	Unit	20	125	40	Positive
Herbicide	Atrazine	<input type="checkbox"/>	216.1	Unit	174.1	Unit	20	125	16	Positive
Acaricide	Carbofuran	<input type="checkbox"/>	222.1	Unit	123.1	Unit	20	80	30	Positive
Acaricide	Carbofuran	<input type="checkbox"/>	222.1	Unit	165.1	Unit	20	80	20	Positive
Acaricide	Diazinon (Dimpylate)	<input type="checkbox"/>	305.1	Unit	97	Unit	20	105	40	Positive
Acaricide	Diazinon (Dimpylate)	<input type="checkbox"/>	305.1	Unit	169.1	Unit	20	105	32	Positive
Acaricide	Dimethoate	<input type="checkbox"/>	230	Unit	125	Unit	20	70	16	Positive
Acaricide	Dimethoate	<input type="checkbox"/>	230	Unit	198.8	Unit	20	70	0	Positive
Fungicide	Imazali (Enilconazole)	<input type="checkbox"/>	297.1	Unit	159	Unit	20	115	20	Positive
Fungicide	Imazali (Enilconazole)	<input type="checkbox"/>	297.1	Unit	201	Unit	20	115	15	Positive
Herbicide	Imazapyr	<input type="checkbox"/>	262.1	Unit	69.1	Unit	20	120	40	Positive

Estimated cycle time (ms/cycle): 587

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

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

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

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

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

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

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

Depending on the delay volume, the compounds Pyraclostrobin and Diazinon can co-elute, separate slightly, or reverse elution order. Imazalil and Metoxuron are also very close in retention time and may reverse elution order.


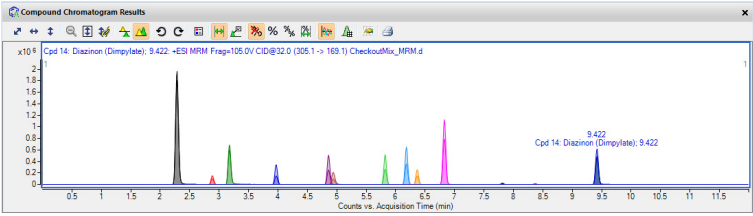
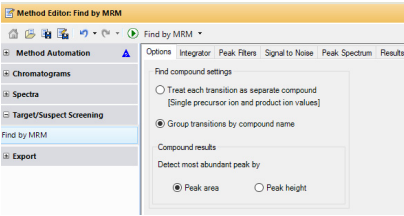
Creating an MRM acquisition method from the database

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

Steps	Detailed Instructions	Comments
<p>Do this step if you want to acquire data with the Checkout Mix. Otherwise, continue at step 2.</p> <p>1 Acquire data.</p> <ul style="list-style-type: none">Set up a one-line worklist with the method you just created.Name the data file CheckoutMix_MRM.d.Designate a directory path to hold your data files and method, different than the path used for the example methods and data.	<p>a If necessary, click View > Worklist to display the Worklist window.</p> <p>b Click Worklist > Worklist Run Parameters. Verify that the parameters are set properly. Click OK.</p> <p>c In the Data File Settings tab, under the File Naming section, type CheckoutMix_MRM.</p> <p>d Click Worklist > Add Multiple Samples.</p> <p>e Select CheckoutMix_MRM.m as the method name.</p> <p>f Click the Sample Position tab.</p> <p>g Select the Autosampler, Well-plate or Vial Tray.</p> <p>h In the graphic, select a single position. Click OK.</p> <p>i In the Worklist window, mark the check box to the left of the sample.</p> <p>j Click the Start Worklist Run icon in the main toolbar, the Run Worklist icon in the Worklist toolbar, or click Worklist > Run.</p>	<ul style="list-style-type: none">The Worklist window is tabbed with the Method Editor window by default. Click the Worklist tab at the bottom left corner of the program to show the Worklist window.See also “To run the Checkout Mix” on page 8.



Creating an MRM acquisition method from the database

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

Steps	Detailed Instructions	Comments
2 Find compounds using the Find Compound by MRM algorithm. <ul style="list-style-type: none">Open the data file CheckoutMix_MRM.d.	<p>Start the Qualitative Analysis program. If it is not running, double-click the Qualitative Analysis 10.0 icon, or that of a later version.</p>  <ol style="list-style-type: none">Click File > Open Data File. The system displays the Open Data File dialog box.From the folder \MassHunter\Data\Checkout Mix Example Data folder, select CheckoutMix_MRM.d, and click Open.Click the Compounds View tab at the top of the Qualitative Analysis screen.If needed, click View > Method Editor. The system displays the Method Editor window.In the Method Automation section, click Workflow, and ensure the Workflow is set to Target/Suspect Screening and that Compound Mining is set to Find by MRM.In the Method Editor Window, in the Target/Suspect Screening section, click Find by MRM. Click the Group transitions by compound name option.Click the Peak area option for Detect most abundant peak by Peak area.	<ul style="list-style-type: none">You can also use the example MRM data file that was installed to the Checkout Mix Example Data folder. If the file is not on your computer, install it from the installation media.
	  <ol style="list-style-type: none">Click Find > Find by MRM.	

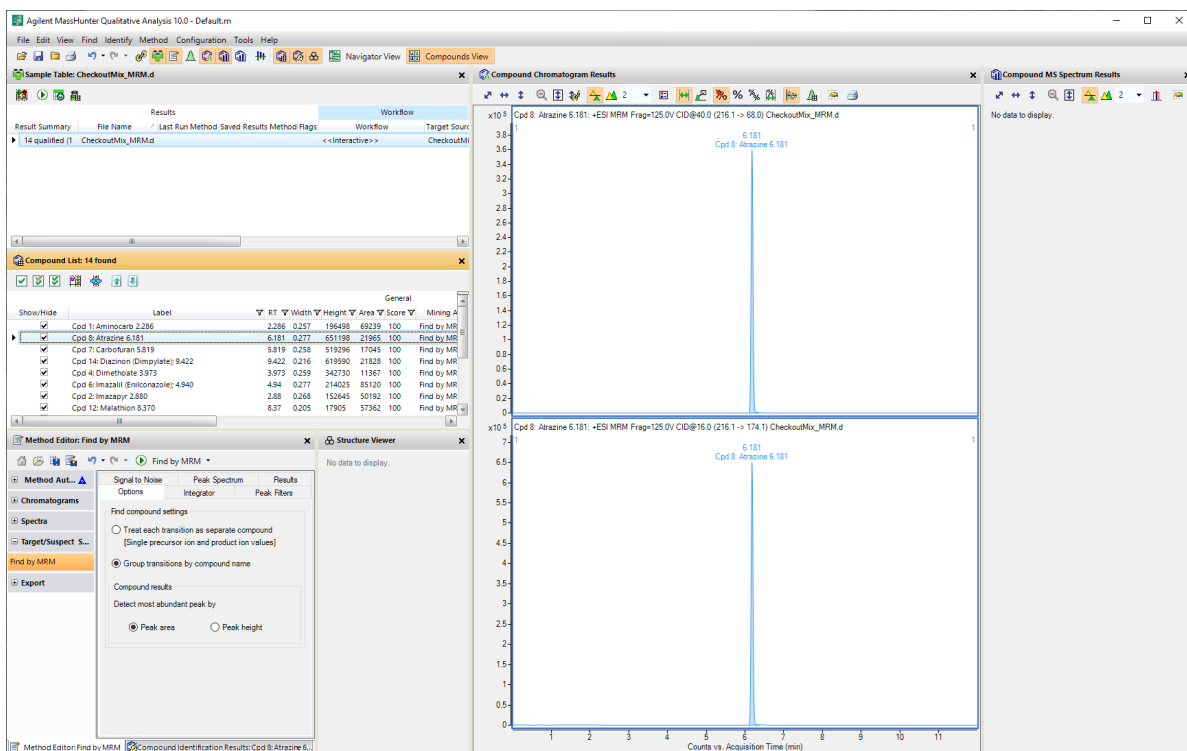
Creating an MRM acquisition method from the database

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

Steps	Detailed Instructions	Comments
<p>3 Review the results of the Find Compounds by MRM algorithm.</p> <ul style="list-style-type: none">Make sure that the primary ions are found for each compound.You cannot edit the retention times of compounds which are identified.	<p>a Click View > Compound List.</p> <p>b Click or use the arrow keys to move through the Compound Table to review one compound at a time. See the figures that follow.</p> <p>c Review each compound. Verify that the primary transitions for each compound were found.</p> <p>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.</p>	<ul style="list-style-type: none">You can also print a Compound Report to review results. You click File > Print > Workflow Report. The Compound Report sorts the compounds by retention time.In the Compound Chromatogram Results window, you can see the abundances for each transition.In Compound List, click each compound, or use the  and  buttons in the compound list window to review the results.

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.

NOTE: You can manually edit these retention times in the Quantitative Analysis program. See “[Task 1. Create a batch file from an existing MRM data file](#)” on page 23.



Creating a Dynamic MRM acquisition method

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

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

For the MassHunter MRM/dMRM/tMRM Database, Agilent recommends that you create a Quantitative Analysis report to specify in **“Task 3. Create a dMRM method using Update dMRM”** on page 32.

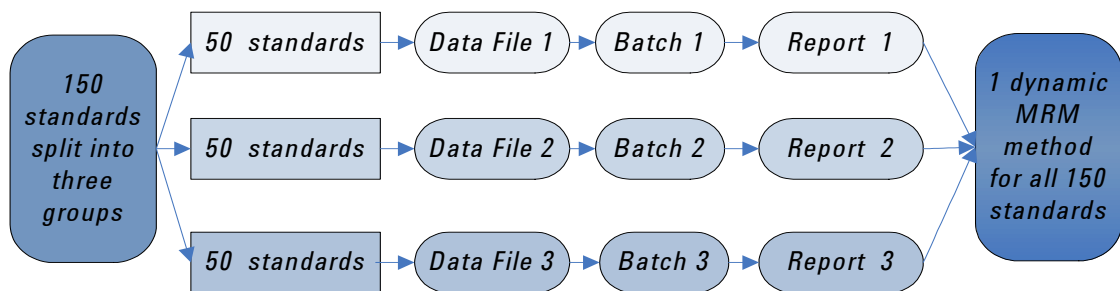



Figure 1. Example process for analyses that have more than 50 compounds

Creating a Dynamic MRM acquisition method

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 .	<p>a Double-click the QQQ Quantitative Analysis (Quant-My-Way) icon.</p>  <p>b Click New Batch.</p> <p>c Navigate to installed data in the folder \MassHunter\Data\Checkout Mix Example Data.</p> <p>d Type CheckoutMix_MRM in the File Name text box.</p> <p>e Click Open.</p> <p>f To add samples, select the file CheckoutMix_MRM.d.</p> <p>g Click OK.</p>	<ul style="list-style-type: none">The file CheckoutMix_MRM.d is installed in the folder \MassHunter\Data\Checkout Mix Example Data folder.You can also use the Checkout Mix data file that you created if you ran the Checkout Mix in the previous exercise. Your results can vary slightly.
2 Create a method for that batch using MRM data.	<p>a Click Method > New > New Method from Acquired MRM data.</p> <p>b Select the CheckoutMix_MRM.d data file, click Open.</p> <p>c Right-click the Method Table and click Collapse All.</p> <p>d Click View > Preset Layouts > Table Top.</p> <p>e Close the Sample Information window.</p>	<ul style="list-style-type: none">You can change which windows are displayed when you use the View menu.You can open or close a single window.You can also load a layout which already has specific windows displayed in specific locations.You can also load or save layouts. See the online Help in the Quantitative Analysis program for more information.

Creating a Dynamic MRM acquisition method

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

Steps	Detailed Instructions	Comments
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Method Table

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

Sample					
Name	Data File	Type	Level	Acq. Method File	Acq. Date-Time
▶ CheckoutMix... CheckoutMix...					
Quantifier					
Name	TS	Transition	Scan	Type	
⊞ Aminocarb	1	209.1 -> 137...	MRM	Target	
⊞ Atrazine	1	216.1 -> 174...	MRM	Target	
⊞ Carbofuran	1	222.1 -> 123...	MRM	Target	
⊞ Diazinon (Di...	1	305.1 -> 169...	MRM	Target	
⊞ Dimethoate	1	230.0 -> 125...	MRM	Target	
⊞ Imazalil (Enil...	1	297.1 -> 159...	MRM	Target	
⊞ Imazapyr	1	262.1 -> 217...	MRM	Target	
⊞ Malathion	1	331.0 -> 126...	MRM	Target	
⊞ Metazachlor	1	278.1 -> 134...	MRM	Target	
⊞ Metosulam	1	418.0 -> 175...	MRM	Target	
⊞ Metoxuron	1	229.0 -> 72.1	MRM	Target	
⊞ Molinate	1	188.0 -> 83.2	MRM	Target	
⊞ Pyrethroid	1	388.1 -> 193...	MRM	Target	
⊞ Thibendazo...	1	202.0 -> 175...	MRM	Target	

- | | | |
|--|---|--|
| <p>3 Set the Concentration Setup, Qualifier Setup, and Calibration Curve Setup.</p> <ul style="list-style-type: none"> • Add calibration level 1 with a concentration of 100. • Set the Uncertainty to Relative for all qualifiers. • Set the Curve Fit to Linear. • Set the Curve Fit Origin to Force. • Set the Curve Fit Weight to None. | <p>a Select Concentration Setup in the Method Setup Tasks section in the Method Tasks pane.</p> <p>b Select the first compound in the table.</p> <p>c Right-click the compound row and click New Calibration Level from the shortcut menu.</p> <p>d In the Level column, type 1. In the Conc. column, type 100.</p> <p>e Right-click in the Level box and click Copy Calibration Levels To.</p> <p>f Click Select All. Click OK.</p> <p>g Select Qualifier Setup in the Method Setup Tasks section.</p> <p>h Verify that the Uncertainty is Relative.</p> <p>i Select Calibration Curve Setup in the Method Setup Tasks section.</p> <p>j Set Curve Fit to Linear for all compounds.</p> <p>k Set CF Origin to Force for all compounds.</p> <p>l Set CF Weight to None for all compounds.</p> | <ul style="list-style-type: none"> • Refer to the online Help in the Quantitative Analysis program for additional help on these tasks. • After you select the option for the first compound in the Method Table, you can right-click the option and click Fill Down from the shortcut menu. |
|--|---|--|

Creating a Dynamic MRM acquisition method

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

Steps	Detailed Instructions	Comments
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Method Table

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

Sample					
Name	Data File	Type	Level	Acq. Method File	Acq. Date-Time
CheckoutMix_M...	CheckoutMix_M...				

Quantifier					
Name	TS	Transition	Scan	Type	Units
Aminocarb	1	209.1 -> 137.2	MRM	Target	ng/ml

Calibration			
Level	Conc.	Response	Enable
1	100.0000		<input checked="" type="checkbox"/>

Quantifier				
Name	TS	Transition	Scan	
Atrazine	1	216.1 -> 174.1	MRM	
Carbofuran	1	222.1 -> 123.1	MRM	
Diazinon (Dimpyl...	1	305.1 -> 169.1	MRM	
Dimethoate	1	230.0 -> 125.0	MRM	
Imazalil (Enilcon...	1	297.1 -> 159.0	MRM	
Imazapyr	1	262.1 -> 217.1	MRM	
Malathion	1	331.0 -> 126.9	MRM	
Metazachlor	1	278.1 -> 134.2	MRM	

Copy Calibration Levels To

Select Compounds:

Name	TS	RT	Transition	ISTD Flag
Atrazine	1	6.181	216.1 -> 174.1	<input type="checkbox"/>
Carbofuran	1	5.819	222.1 -> 123.1	<input type="checkbox"/>
Diazinon (Dimpylate)	1	9.422	305.1 -> 169.1	<input type="checkbox"/>
Dimethoate	1	3.973	230.0 -> 125.0	<input type="checkbox"/>
Imazalil (Enilconazole)	1	4.940	297.1 -> 159.0	<input type="checkbox"/>
Imazapyr	1	2.880	262.1 -> 217.1	<input type="checkbox"/>

Select All OK Cancel

- Verify retention time elution order:
 - Aminocarb
 - Imazapyr
 - Thiabendazole
 - Dimethoate
 - Metoxuron
 - Imazalil (Enilconazole)
 - Carbofuran
 - Atrazine
 - Metosulam
 - Metazachlor
 - Molinate
 - Malathion
 - Diazinon (Dimpylate)
 - Pyraclostrobin
- Select **Retention Time Setup** in the Method Setup Tasks section.
- (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.

Creating a Dynamic MRM acquisition method

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

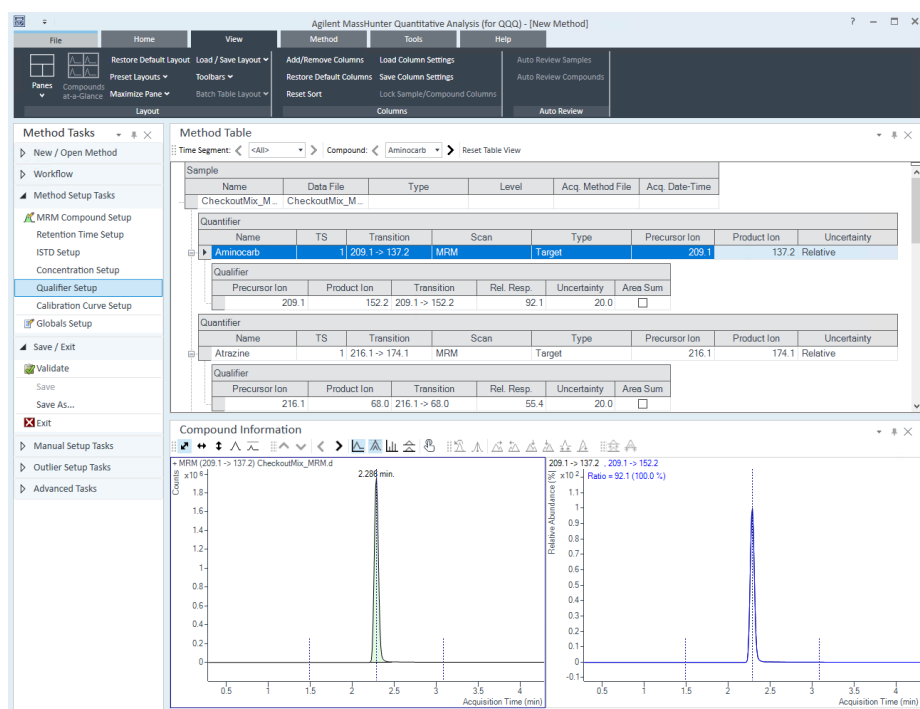
Steps

- Review qualifier ratios

Detailed Instructions

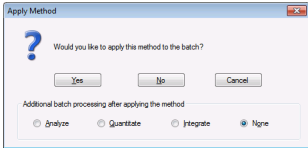
- p Select **Qualifier Setup** in the Method Setup Tasks section.
- q Right-click the Method Table and click **Expand All**.
- r Click **View > Restore Default Layout**.
- s Click **View > Panes > Sample Information** to close the Sample Information window.
- t Click the Show/Hide Qualifiers button in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

Comments



Creating a Dynamic MRM acquisition method

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

Steps	Detailed Instructions	Comments
4	Verify method and then save the method and apply the method to the batch.	
	<ol style="list-style-type: none">Click Method > Validate under the Save/Exit section in Method tasks (left panel).Click OK on the message box. Fix any errors, if necessary.Click Method > Save As.Type <code>Checkout_MRM_to_DMRM</code>.Click the Save button.Click Method > Exit.For the additional batch processing option, select None.Click Yes to apply the method to the batch.	
5	Analyze and save the batch.	
	<ol style="list-style-type: none">In the Batch Table window, select Cal as the Type. Select Level as 1.Click Home > Analyze Batch > Analyze Batch.Click File > Save Batch.	
6	Review the batch to resolve errors or messages that are indicated in the Batch Table.	<ul style="list-style-type: none">Resolve isomers.Check qualifier ratios.Resolve errors and messages
7	Save the batch again.	<ul style="list-style-type: none">Click File > Save Batch.

Task 2. Print a report in the Quantitative Analysis program

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

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

In this task, you:

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

Steps	Detailed Instructions	Comments
1 Print a report. Use a template that creates a summary report for fastest report creation.	<p>a See “Task 1. Create a batch file from an existing MRM data file” on page 23.</p> <p>b Click File > Save.</p> <p>c Click Home > Generate Report. The Generate Report dialog box opens.</p> <p>d Under Report method, click New. The Report Method Edit program opens.</p> <p>e Click Add Template. The Open dialog box opens.</p> <p>f Navigate to the folder MassHunter\Report Templates\Quant\PDF-Reporting.</p> <p>g Select a simple report, such as Gen_ResultsSummary.report.xml. Click Open.</p>	

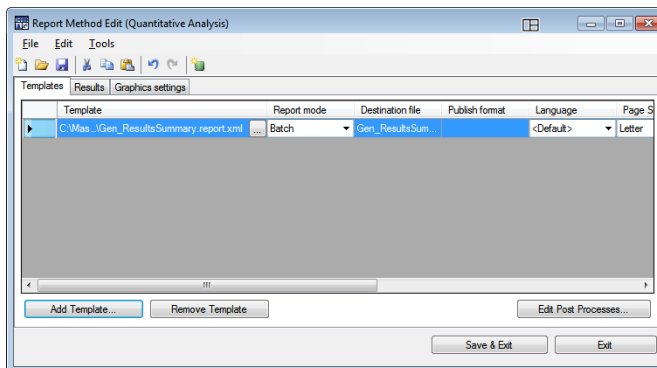
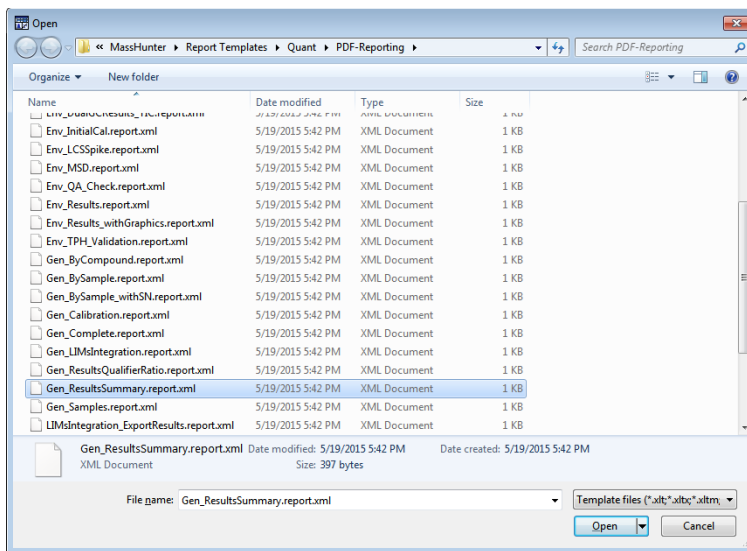
Creating a Dynamic MRM acquisition method

Task 2. Print a report in the Quantitative Analysis program

Steps

Detailed Instructions

Comments

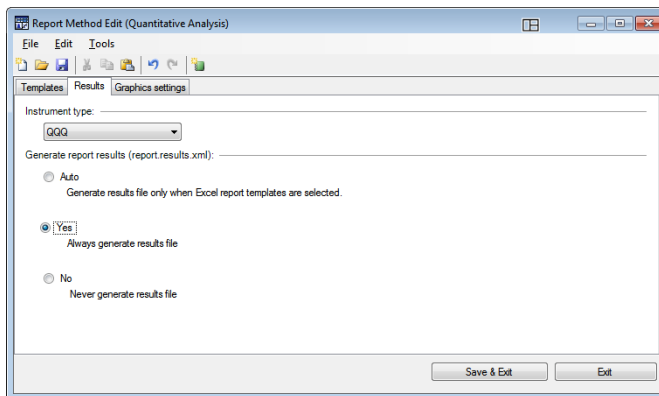


Creating a Dynamic MRM acquisition method

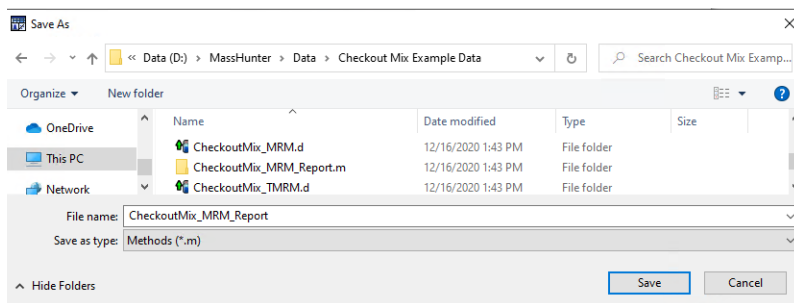
Task 2. Print a report in the Quantitative Analysis program

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



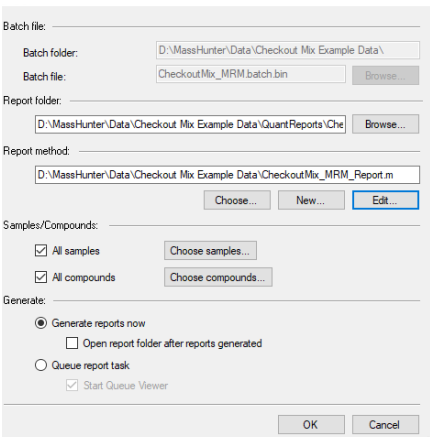
- j Click **File > Save Method As**. The Save As dialog box opens.
- k Navigate to the example data folder.
- l For the **File name**, type CheckoutMix_MRM_Report.
- m Click **Save**.



- n In the Report Method Edit program, click **Save and Exit**.
 - o Click **Generate reports now**.
 - p Click **OK**.
- The report.results.xml file is in the **\\MassHunter\Data\Checkout Mix Example Data\QuantReports\CheckoutMix_MRM** folder.

Creating a Dynamic MRM acquisition method

Task 2. Print a report in the Quantitative Analysis program

Steps	Detailed Instructions	Comments
	 <p>The screenshot shows a dialog box for generating reports. It contains the following fields and options:</p> <ul style="list-style-type: none">Batch file:<ul style="list-style-type: none">Batch folder: D:\MassHunter\Data\Checkout Mix Example Data\Batch file: CheckoutMix_MRM.batch.bin (with a Browse button)Report folder: D:\MassHunter\Data\Checkout Mix Example Data\QuantReports\Che (with a Browse... button)Report method: D:\MassHunter\Data\Checkout Mix Example Data\CheckoutMix_MRM_Report.m (with Choose..., New..., and Edit... buttons)Samples/Compounds:<ul style="list-style-type: none"><input checked="" type="checkbox"/> All samples (with Choose samples... button)<input checked="" type="checkbox"/> All compounds (with Choose compounds... button)Generate:<ul style="list-style-type: none"><input checked="" type="radio"/> Generate reports now<input type="checkbox"/> Open report folder after reports generated<input type="radio"/> Queue report task<ul style="list-style-type: none"><input checked="" type="checkbox"/> Start Queue Viewer <p>Buttons: OK, Cancel</p>	

Creating a Dynamic MRM acquisition method

Task 3. Create a dMRM method using Update dMRM

Task 3. Create a dMRM method using Update dMRM

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

Steps	Detailed Instructions	Comments
1 Open the MRM method CheckoutMix_MRM.m you created on page 10 and save it to a new name with the format ///CheckoutMix_DMRM.m , where /// are your initials.	<p>a In the Data Acquisition program, click Method > Open.</p> <p>b Select the MRM method you created in step 1 on page 10. Click OK.</p> <p>c Click Method > Save As.</p> <p>d Type the new method name with the format ///CheckoutMix_DMRM.m.</p>	<ul style="list-style-type: none">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.

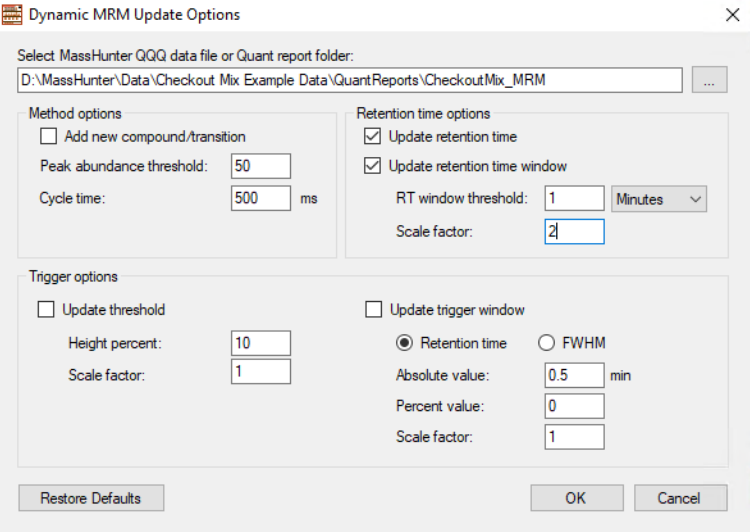
Creating a Dynamic MRM acquisition method

Task 3. Create a dMRM method using Update dMRM

Steps	Detailed Instructions	Comments
<p>2 Update the method to change from an MRM method to a Dynamic MRM method with the same compounds.</p> <p>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.</p> <p>Do not manually change the Scan Type to Dynamic MRM. If you do, the existing Scan segments table is cleared.</p>	<p>a Click the Acquisition tab in the QQQ tab in the Method Editor window.</p> <p>b For 6400 Series, right-click the Scan segments table and click Update DMRM Method. The MRM Update Options dialog box opens. For Ultivo, click Convert to dMRM. Then click Update Method. The MRM Update Options dialog box opens.</p> <p>c Select the folder containing the <i>report.results.xml</i> 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.</p> <p>d Under Method Options, check Add new compound/transition.</p> <p>e For Peak abundance threshold, enter 50.</p> <p>f For Cycle time, enter 500 ms.</p> <p>g Under Retention time options, select Update retention time and Update retention time window.</p> <p>h For RT window threshold, select 1. From the drop-down list, select Minutes.</p> <p>i For Scale factor, select 2.</p> <p>j Under Trigger options, clear the Update threshold check box.</p> <p>k Mark the Update trigger window check box.</p> <p>l For Absolute value (mins), select 0.5.</p> <p>m Review other parameters.</p> <p>n Click OK.</p>	<ul style="list-style-type: none"> • You can select either a data file that was acquired with a Scan Type of MRM or a Quant Report folder as the input to this dialog box. Agilent recommends to use the Quant Report. • The Delta Retention Time is scaled to the peak width found for that compound. A scale factor of 2 creates a retention time window that is 2 times the peak width (baseline to baseline). Choose a larger factor if you want to acquire more data points for the transition. • A Delta Retention Time or Retention Time Window of 1 minute is chosen for this method. A large delta retention time is recommended for early eluting compounds, which tend to have a high background. This ensures sufficient baseline for the peak integration. The automatic calculation which provides a smaller delta retention time is recommend for later eluters. • The dwell times for MRM transitions will depend on the number of overlapping peaks and their respective peak widths. • The method is now updated with the transitions, parameters, and retention times in the Quantitative Analysis report.

Creating a Dynamic MRM acquisition method

Task 3. Create a dMRM method using Update dMRM

Steps	Detailed Instructions	Comments
		<p>You can update the compounds in the Scan segments table by using a QQQ data file or a Quantitative Analysis report folder.</p> <p>If you select a QQQ data file and an error is generated, manually create the report and select the report folder in this location instead of the QQQ data file. See “Task 2. Print a report in the Quantitative Analysis program” on page 28. The folder path of the quant report in the image is for the provided example report. Instead use the location of the report you created in Task 2.</p> <p>Note that all transitions must be detected in each data file, or the program will generate an error when you update the method.</p> <p>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.</p>

Creating a Dynamic MRM acquisition method

Task 3. Create a dMRM method using Update dMRM

Steps

Detailed Instructions

Comments

Method Editor: CheckOutMix_MRM_6470B_dMRM.m

Acquisition Parameters Table:

Compound Group	Compound Name	ISTD?	Precursor Ion	MS1 Res	Product Ion	MS2 Res	Ret Time (min)	Delta Ret Time	Fragmentor	Collision Energy	Cell Accelerator Voltage	Polarity
Insecticide	Azinocarb	<input type="checkbox"/>	209.1	Unit	152.2	Unit	2.28	0.57	105	12		Positive
Insecticide	Azinocarb	<input type="checkbox"/>	209.1	Unit	137.2	Unit	2.28	0.57	105	24		Positive
Herbicide	Altrazine	<input type="checkbox"/>	216.1	Unit	174.1	Unit	6.18	0.56	125	16		Positive
Acaricide	Altrazine	<input type="checkbox"/>	216.1	Unit	68	Unit	6.18	0.56	125	40		Positive
Acaricide	Carbofuran	<input type="checkbox"/>	222.1	Unit	165.1	Unit	5.82	0.5	80	20		Positive
Acaricide	Carbofuran	<input type="checkbox"/>	222.1	Unit	123.1	Unit	5.82	0.5	80	30		Positive
Acaricide	Diazinon (Dimpylate)	<input type="checkbox"/>	305.1	Unit	169.1	Unit	9.41	0.5	105	32		Positive
Acaricide	Diazinon (Dimpylate)	<input type="checkbox"/>	305.1	Unit	97	Unit	9.41	0.5	105	40		Positive
Acaricide	Dimethoate	<input type="checkbox"/>	230	Unit	198.8	Unit	3.97	0.5	70	0		Positive
Acaricide	Dimethoate	<input type="checkbox"/>	230	Unit	125	Unit	3.97	0.5	70	16		Positive
Fungicide	Imazali (Emilconazol)	<input type="checkbox"/>	297.1	Unit	201	Unit	4.94	0.57	115	15		Positive
Fungicide	Imazali (Emilconazol)	<input type="checkbox"/>	297.1	Unit	159	Unit	4.94	0.57	115	20		Positive
Acaricide	Imazapyr	<input type="checkbox"/>	262.1	Unit	217.1	Unit	2.9	0.53	120	20		Positive
Acaricide	Imazapyr	<input type="checkbox"/>	262.1	Unit	69.1	Unit	2.9	0.53	120	40		Positive
Acaricide	Malathion	<input type="checkbox"/>	331	Unit	126.9	Unit	8.37	0.5	80	5		Positive
Acaricide	Malathion	<input type="checkbox"/>	331	Unit	99	Unit	8.37	0.5	80	10		Positive

Dynamic MRM Parameters: Total MRMs = 28, Max Concurrent MRMs = 4, Min/Max Dwell = 122.54 ms/248.02 ms

Triggered MRM: Triggered, Repeats: 3

- o Review the results of updating the MRM method to a dMRM method and then click **Close**.
- p Verify that each row has a **Compound Name**. A blank Compound Name is not allowed.
- q Click **Method > Save**.

Method Editor: CheckOutMix_MRM_Ultivo_from DE.m

Acquisition Parameters Table:

Compound Group	Compound Name	ISTD?	Precursor Ion	MS1 Res	Product Ion	MS2 Res	RT (min)	RT Window (min)	Fragmentor	CE (V)	Average Dwell Time	Polarity
Insecticide	Azinocarb	<input type="checkbox"/>	209.1	Unit	152.2	Unit	2.26	0.77	105	12	113.22	Positive
Insecticide	Azinocarb	<input type="checkbox"/>	209.1	Unit	137.2	Unit	2.26	0.77	105	24	113.22	Positive
Herbicide	Altrazine	<input type="checkbox"/>	216.1	Unit	174.1	Unit	6.15	0.8	125	16	74.91	Positive
Herbicide	Altrazine	<input type="checkbox"/>	216.1	Unit	68	Unit	6.15	0.8	125	40	74.91	Positive
Acaricide	Carbofuran	<input type="checkbox"/>	222.1	Unit	165.1	Unit	5.8	0.71	80	20	94.38	Positive
Acaricide	Carbofuran	<input type="checkbox"/>	222.1	Unit	123.1	Unit	5.8	0.71	80	30	94.38	Positive
Acaricide	Diazinon (Dimpylate)	<input type="checkbox"/>	305.1	Unit	169.1	Unit	9.39	0.62	105	32	82.29	Positive
Acaricide	Diazinon (Dimpylate)	<input type="checkbox"/>	305.1	Unit	97	Unit	9.39	0.62	105	40	82.29	Positive
Acaricide	Dimethoate	<input type="checkbox"/>	230	Unit	198.8	Unit	3.96	0.74	70	0	124.03	Positive
Acaricide	Dimethoate	<input type="checkbox"/>	230	Unit	125	Unit	3.96	0.74	70	16	124.03	Positive
Fungicide	Imazali (Emilconazol)	<input type="checkbox"/>	297.1	Unit	201	Unit	4.98	0.62	115	15	94.95	Positive
Fungicide	Imazali (Emilconazol)	<input type="checkbox"/>	297.1	Unit	159	Unit	4.98	0.62	115	20	94.95	Positive
Herbicide	Imazapyr	<input type="checkbox"/>	262.1	Unit	217.1	Unit	2.84	0.8	120	20	92.79	Positive
Herbicide	Imazapyr	<input type="checkbox"/>	262.1	Unit	69.1	Unit	2.84	0.8	120	40	92.79	Positive
Acaricide	Malathion	<input type="checkbox"/>	331	Unit	126.9	Unit	8.36	0.59	80	5	113.41	Positive
Acaricide	Malathion	<input type="checkbox"/>	331	Unit	99	Unit	8.36	0.59	80	10	113.41	Positive
Metazoanthor	metazoanthor	<input type="checkbox"/>	278.1	Unit	215.1	Unit	6.5	13	70	4	171.31	Positive
Metazoanthor	metazoanthor	<input type="checkbox"/>	278.1	Unit	134.2	Unit	6.5	13	70	15	171.31	Positive
Metazoanthor	Metazoanthor	<input type="checkbox"/>	278.1	Unit	134.2	Unit	6.81	0.71	70	15	102.23	Positive
Metazoanthor	Metazoanthor	<input type="checkbox"/>	278.1	Unit	215.1	Unit	6.81	0.71	70	4	102.23	Positive
Herbicide	Metazachlor	<input type="checkbox"/>	419	Unit	175	Unit	6.39	0.62	140	32	74.71	Positive

Bar Chart: Concurrent MRMs

Statistics:

- Total MRMs: 30
- Minimum Concurrent MRMs: 2
- Maximum Concurrent MRMs: 8
- Minimum Dwell Time (ms): 51.68
- Maximum Dwell Time (ms): 248.02
- Minimum Cycle Time (ms): 16.16
- Cycle time (ms): 500
- Override RT window (ms): 1
- Check retention data (ms): 64

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

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

- A dwell time of 5 ms or more is recommended to acquire dMRM data for this particular analysis. If cycle time and concurrent MRMs reduce the dwell time below this value, the minimum cycle time and minimum dwell time on the right will be highlighted. If the minimum dwell time is below the specification of the instrument, a warning will be provided.
- For some newer Agilent LC/TQ models, the dwell time can be as low as 0.5 ms. Lower dwell times allow for faster cycle times at the cost of data points across a chromatographic peak and chromatographic peak reproducibility.
- For good quantitative results, make sure you have 10 data points across the chromatographic peak.

Steps	Detailed Instructions	Comments
1 Start the Dynamic MRM Viewer dialog box. Note: If you have an Ultivo system, the Dynamic MRM Viewer is displayed automatically, so you can skip this step.	<ul style="list-style-type: none"> • Right-click the Scan segments table and click Edit DMRM Method. 	
2 Review each compound in the Dynamic MRM Viewer dialog box.	<ul style="list-style-type: none"> a Click each compound in the table. b Verify in the table that two transitions are shown for each compound. c Examine the graphic to review how many concurrent MRMs are being acquired with that compound. d Adjust the cycle time so that all criteria for Minimum Dwell Time, and for good integration are met. e Click Close. 	<ul style="list-style-type: none"> • To use the Agile integrator, 64 data points are required in the retention time window. Either increase the Delta Ret Time for the transition(s) with less than 64 points, or decrease the cycle time. As a general rule, set the retention time factor based on reproducibility of the chromatography. • When you change the cycle time in the Dynamic MRM viewer, you immediately see its effects on the Minimum Dwell Time and Maximum Dwell Time.

Creating a Dynamic MRM acquisition method

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

Steps

Detailed Instructions

Comments

Dynamic MRM Viewer

Compound: (All) Compound group: (All)

Compound Group	Compound Name	Precursor Ion	Product Ion	RT	RT Window	Frag	CE	CAV	Average Dwell
	Aminocarb	209.10	152.20	2.010	1.000	380	12	2	151.78
	Aminocarb	209.10	137.20	2.010	1.000	380	24	2	151.78
	Alprazine	216.10	174.10	6.100	1.000	380	16	3	96.06
	Alprazine	216.10	68.00	6.100	1.000	380	40	3	96.06
	Carbifuran	222.10	165.10	5.730	1.000	380	20	2	110.02
	Carbifuran	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
	Diazinon (Dimpylate)	305.10	97.00	9.420	1.000	380	40	2	123.93
	Dimethoate	230.00	198.80	3.850	1.000	380	0	5	110.08
	Dimethoate	230.00	125.00	3.850	1.000	380	16	5	110.08
	Imazalil (Enilconazol)	297.10	201.00	4.640	1.000	380	15	2	110.05
	Imazalil (Enilconazol)	297.10	159.00	4.640	1.000	380	20	2	110.05
	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
	Malathion	331.00	126.90	8.350	1.000	380	5	2	186.47
	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
	Metazachlor	278.10	134.20	6.760	1.000	380	15	5	151.62
	Metosulam	418.02	175.00	6.270	1.000	380	32	3	96.02
	Metosulam	418.02	140.00	6.270	1.000	380	60	3	96.02
	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
	Molinate	188.00	126.10	7.790	1.000	380	25	2	186.45
	Molinate	188.00	83.20	7.790	1.000	380	16	2	186.45
	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
	Thiabendazole	202.00	175.00	2.860	1.000	380	24	2	144.80

Dynamic MRM Statistics

Total MRMs	28
Minimum Concurrent MRMs	2
Maximum Concurrent MRMs	6
Minimum Dwell Time	82.12 ms
Maximum Dwell Time	249.15 ms
Minimum Cycle Time	19.28 ms

Parameters

Cycle time: 500 ms

Calculations include: Pitanes only All transitions

Review tools

Override RT window 1 min

Check minimum data pts 64 pts

Split method

Split method

Split by: Max Concurrent MRMs

Number of methods: 2

Max concurrent MRMs: 10

Min dwell time: 2 ms

Split method: [Dropdown]

Plot type: Concurrent MRMs Select transitions on Click

Concurrent MRMs vs. Retention Time

Add Compounds... Save Split Methods... Reset Default Close

6400 Series

Creating a Dynamic MRM acquisition method

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

Steps

Detailed Instructions

Comments

The screenshot displays the 'Method Editor' interface for a dMRM acquisition method. The 'Acquisition Parameters' table lists various compounds and their associated parameters. The 'Statistics' panel on the right provides summary metrics for the MRM setup.

Compound Group	Compound name	ISTD?	Precursor (m/z)	MS1 res	Product (m/z)	MS2 res	RT (min)	RT Window (min)	Fragmenter (V)	CE (V)	Average Dwell (ms)	Polarity
Insecticide	Amiocarb	<input type="checkbox"/>	209.1	Unit	152.2	Unit	2.26	0.77	105	12	113.22	Positive
Insecticide	Amiocarb	<input type="checkbox"/>	209.1	Unit	137.2	Unit	2.26	0.77	105	24	113.22	Positive
Herbicide	Alazoxe	<input type="checkbox"/>	216.1	Unit	114.1	Unit	6.15	0.8	125	16	74.61	Positive
Herbicide	Alazoxe	<input type="checkbox"/>	216.1	Unit	68	Unit	6.15	0.8	125	40	74.61	Positive
Acaricide	Carbofuran	<input type="checkbox"/>	222.1	Unit	165.1	Unit	5.8	0.71	80	20	94.38	Positive
Acaricide	Carbofuran	<input type="checkbox"/>	222.1	Unit	123.1	Unit	5.8	0.71	80	30	94.38	Positive
Acaricide	Diazinon (Dimpylate)	<input type="checkbox"/>	305.1	Unit	169.1	Unit	9.39	0.62	105	32	82.29	Positive
Acaricide	Diazinon (Dimpylate)	<input type="checkbox"/>	305.1	Unit	97	Unit	9.39	0.62	105	40	82.29	Positive
Acaricide	Dimethoate	<input type="checkbox"/>	230	Unit	198.8	Unit	3.96	0.74	70	0	124.03	Positive
Acaricide	Dimethoate	<input type="checkbox"/>	230	Unit	125	Unit	3.96	0.74	70	16	124.03	Positive
Fungicide	Imazali (Enilconazole)	<input type="checkbox"/>	297.1	Unit	201	Unit	4.98	0.82	115	15	94.05	Positive
Fungicide	Imazali (Enilconazole)	<input type="checkbox"/>	297.1	Unit	159	Unit	4.98	0.82	115	20	94.05	Positive
Herbicide	Imazapyr	<input type="checkbox"/>	262.1	Unit	217.1	Unit	2.84	0.8	120	20	92.79	Positive
Herbicide	Imazapyr	<input type="checkbox"/>	262.1	Unit	69.1	Unit	2.84	0.8	120	40	92.79	Positive
Acaricide	Malathion	<input type="checkbox"/>	331	Unit	126.9	Unit	8.36	0.59	80	5	113.41	Positive
Acaricide	Malathion	<input type="checkbox"/>	331	Unit	99	Unit	8.36	0.59	80	10	113.41	Positive
	metazachlor	<input type="checkbox"/>	278.1	Unit	210.1	Unit	6.5	13	70	4	171.31	Positive
	metazachlor	<input type="checkbox"/>	278.1	Unit	134.2	Unit	6.5	13	70	15	171.31	Positive
	Metazachlor	<input type="checkbox"/>	278.1	Unit	134.2	Unit	6.81	0.71	70	15	102.23	Positive
	Metazachlor	<input type="checkbox"/>	278.1	Unit	210.1	Unit	6.81	0.71	70	4	102.23	Positive
Herbicide	Metosulam	<input type="checkbox"/>	418	Unit	175	Unit	6.35	0.82	140	32	74.71	Positive

Statistic	Value
Total MRMs	38
Minimum Concurrent MRMs	2
Maximum Concurrent MRMs	8
Minimum Dwell Time (ms)	61.68
Maximum Dwell Time (ms)	243.17
Minimum Cycle Time (ms)	16.16
Cycle time (ms)	500
Override RT window (min)	1
Check minimum data pts (pts)	64

Plot Type: Concurrent MRMs

The plot shows the 'Number of Concurrent MRMs' on the y-axis (ranging from 1 to 9) against time. The bars represent the number of MRMs active at various points in the run. A blue box at the bottom right of the plot contains the text: 'ACTIVATE WINDOWS Go to settings to activate Windows'.

Ultivo after updating to dMRM

Creating a Dynamic MRM acquisition method

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

Steps	Detailed Instructions	Comments
3	<p>Once a cycle time is determined for good integration, set the Cycle Time in the QQQ > Acquisition tab.</p> <p>a Type the Cycle Time, if necessary. b Save the method.</p> <p>The default setting of 500 ms is recommended for most analysis containing more than 15 compounds.</p>	<ul style="list-style-type: none">When you close the Dynamic MRM Viewer, unless you have an Ultivo system, changes made to the cycle time in the Dynamic MRM Viewer are <i>not</i> entered into the acquisition method.

The screenshot shows the 'Method Editor' window with the 'Acquisition' tab selected. The 'Tune file' is 'atunes.tune.xml'. The 'Ion source' is 'AJS ESI'. The 'Time segments' table has one segment: # 1, Start Time 0, Scan Type Dynamic MRM, Div Valve To MS, Delta EMV (+) 0, Delta EMV (-) 0, Stored checked. The '6400 Series' text is prominently displayed. The 'Acquisition' table lists 28 scan segments. The 'Dynamic MRM Parameters' section shows 'Cycle Time' set to 500 ms, 'Total MRMs = 28', 'Max Concurrent MRMs = 6', and 'Min/Max Dwell = 81.56 ms/248.73 ms'. The 'Triggered MRM' section has 'Triggered' checked and 'Repeat' set to 3.

Compound Group	Compound Name	ISTD?	Precursor Ion	MS1 Res	Product Ion	MS2 Res	Ret Time (min)	Delta Ret Time	Fragmentor	Collision Energy	Cell Accelerator Voltage	Polarity
	Aminocarb	<input type="checkbox"/>	209.1	Unit	152.2	Unit	2.01	1	105	12	2	Positive
	Aminocarb	<input type="checkbox"/>	209.1	Unit	137.2	Unit	2.01	1	105	24	2	Positive
	Atrazine	<input type="checkbox"/>	216.1	Unit	174.1	Unit	6.1	1	125	16	3	Positive
	Atrazine	<input type="checkbox"/>	216.1	Unit	68	Unit	6.1	1	125	40	3	Positive
	Carbofuran	<input type="checkbox"/>	222.1	Unit	165.1	Unit	5.73	1	80	20	2	Positive
	Carbofuran	<input type="checkbox"/>	222.1	Unit	123.1	Unit	5.73	1	80	30	2	Positive
	Diazinon (Dimethylate)	<input type="checkbox"/>	305.1	Unit	169.1	Unit	9.42	1	105	32	2	Positive
	Diazinon (Dimethylate)	<input type="checkbox"/>	305.1	Unit	97	Unit	9.42	1	105	40	2	Positive
	Dimethoate	<input type="checkbox"/>	230	Unit	198.8	Unit	3.95	1	70	0	5	Positive
	Dimethoate	<input type="checkbox"/>	230	Unit	1.25	Unit	3.95	1	70	16	5	Positive
	Imazalil (Enilconazole)	<input type="checkbox"/>	297.1	Unit	201	Unit	4.64	1	115	15	2	Positive
	Imazalil (Enilconazole)	<input type="checkbox"/>	297.1	Unit	159	Unit	4.64	1	115	20	2	Positive
	Imazapyr	<input type="checkbox"/>	262.1	Unit	217.1	Unit	2.81	1	120	20	3	Positive
	Imazapyr	<input type="checkbox"/>	262.1	Unit	69.1	Unit	2.81	1	120	40	3	Positive
	Malathion	<input type="checkbox"/>	331	Unit	126.9	Unit	8.35	1	80	5	2	Positive

Dynamic MRM Parameters
Cycle Time: 500 ms
Total MRMs = 28
Max Concurrent MRMs = 6
Min/Max Dwell = 81.56 ms/248.73 ms
Triggered MRM: Triggered
Repeat: 3


Task 5. Acquire dMRM data and inspect in Qualitative Analysis

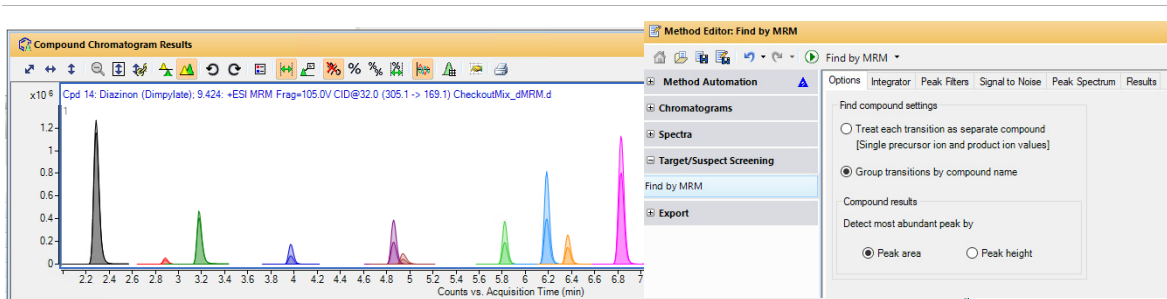
After you acquire the dMRM data file, you examine the data file in the Qualitative Analysis program to verify that the transitions were acquired.

Steps	Detailed Instructions	Comments
<p>Do this step if you want to acquire data with the Checkout Mix. Otherwise, continue at step 2.</p> <p>1 Acquire data.</p> <ul style="list-style-type: none"> • Set up a one-line worklist with the method you just created. • Name the data file CheckoutMix_DMRM.d. • Designate a directory path to hold your data files and method. 	<p>a If necessary, click View > Worklist to display the Worklist window.</p> <p>b Click Worklist > Worklist Run Parameters. Verify that the parameters are set properly. In the Data File Settings tab, under the File Naming section, type <code>CheckoutMix_DMRM</code>. Click OK.</p> <p>c Click Worklist > Add Multiple Samples.</p> <p>d Select the DMRM method you created in step 1 on page 32 as the method name.</p> <p>e Click the Sample Position tab.</p> <p>f Select the Autosampler, Well-plate or Vial Tray.</p> <p>g In the graphic, select a single position. Click OK.</p> <p>h In the Worklist window, mark the check box to the left of the sample.</p> <p>i Click the Start Worklist Run icon in the main toolbar, the Run Worklist icon in the Worklist toolbar or click Worklist > Run.</p>	<ul style="list-style-type: none"> • The Worklist window is tabbed with the Method Editor window by default. Click the Worklist tab to show the Worklist window. • See also “To run the Checkout Mix” on page 8.

Creating a Dynamic MRM acquisition method

Task 5. Acquire dMRM data and inspect in Qualitative Analysis

Steps	Detailed Instructions	Comments
2 Find compounds using the Find Compound by MRM algorithm in the Qualitative Analysis program. <ul style="list-style-type: none">Open the data file CheckoutMix_DMRM.d.	<p>Start the Qualitative Analysis program. If it is not running, double-click the Qualitative Analysis 10.0 icon,</p>  <p>a Click File > Open Data File. The system displays the "Open Data File" dialog box.</p> <p>b Select CheckoutMix_DMRM.d, and click Open.</p> <p>c Click the Compounds View tab at the top of the Qualitative Analysis user interface.</p> <p>d If needed, click View > Method Editor. The system displays the Method Editor window.</p> <p>e In the Method Automation section, click Workflow, and ensure the Workflow is set to Target/Suspect Screening and that Compound Mining is set to Find by MRM.</p> <p>f In the Method Editor window, in the Target/Suspect Screening section, click Find by MRM. Click the Group transitions by compound name option.</p> <p>g Click the Peak area option for Detect most abundant peak by Peak area.</p>	<ul style="list-style-type: none">You can also use the example dMRM data file in the Checkout Mix Example Data folder. If the data file is not on your computer, install it from the installation media.

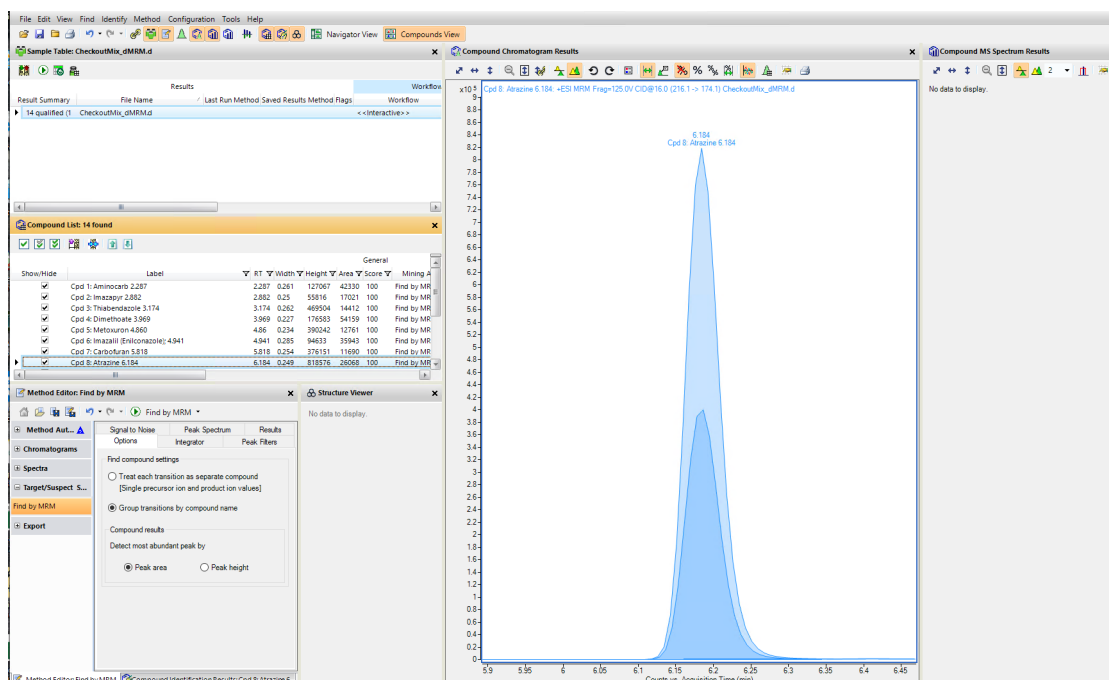


h Click **Find > Find Compounds by MRM**.

Creating a Dynamic MRM acquisition method

Task 5. Acquire dMRM data and inspect in Qualitative Analysis

Steps	Detailed Instructions	Comments
<p>3 Review the results of the Find Compounds by MRM algorithm.</p> <ul style="list-style-type: none"> Make sure that the transitions are found for each compound. These transitions become the primary transitions if you create a tMRM method. You cannot edit the retention times of compounds which are identified. 	<p>a Click View > Compound List.</p> <p>b Click or use the arrow keys to move through the Compound Table to review one compound a time. See the figure that follows.</p> <p>c Verify that the transitions for each compound were found. Qualitative Analysis is the best program to do a quick review of the MRM compound information and to check the chromatography of multiple data files.</p>	<ul style="list-style-type: none"> You can also print a Compound Report to review results. You click File > Print > Workflow Report. The Compound Report sorts the compounds by retention time.



Creating a Triggered MRM Method

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

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

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

Triggering parameters and their function

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

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

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

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

Creating a Triggered MRM Method

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	<p>In the Data Acquisition program, you open the dMRM method. You can open the method that you created in step 1 on page 32 or the example method.</p>	<ul style="list-style-type: none"> Example CheckoutMix_DMRM.m methods for the 6470B and Ultivo instruments can be found in the Checkout Mix Example Methods folder and also on the installation media.
2	<p>Change the method to a tMRM method and start to import the secondary transitions from the Database Browser.</p>	<ul style="list-style-type: none"> For 6400 Series systems, the triggering information is loaded from the Database Browser even if the Triggered check box is clear. This includes the trigger Threshold values if the Trigger MRM Threshold column has a value. Later in this section, we replace the values manually with the values shown “Primary and Secondary Transitions for Triggered MRM” on page 66.
	<p>a Switch to the Data Acquisition program.</p> <p>b Open the DMRM method that you created in step 1 on page 32.</p>	
	<p>a In the Method Editor window, click the QQQ > Acquisition tab.</p> <p>b For 6400 Series, mark the Triggered check box under Triggered MRM. For Ultivo, under Time Segments, change the Scan type to tMRM.</p> <p>c Manually mark the Triggers shown in the “Primary and Secondary Transitions for Triggered MRM” on page 66.</p> <p>d Type 3 for Repeats.</p> <p>e For 6400 Series systems, right-click the Scan Segments table and click Import from Database Browser. The Database Browser opens. For Ultivo, click Compound Browser in the left pane. The Database Browser opens.</p>	

6400 Series

Compound Group	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
	Aninocarb	209.1	Unit	152.2	Unit	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	6937	2.01	1	105	12	2	Positive	0	0	0.5
	Aninocarb	209.1	Unit	137.2	Unit	<input checked="" type="checkbox"/>	<input type="checkbox"/>		2.01	1	105	24	2	Positive			
	Atrazine	216.1	Unit	174.1	Unit	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	2010	6.1	1	125	16	3	Positive	0	0	0.5
	Atrazine	216.1	Unit	68	Unit	<input checked="" type="checkbox"/>	<input type="checkbox"/>		6.1	1	125	40	3	Positive			
	Carbolaran	222.1	Unit	165.1	Unit	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	728	5.73	1	80	20	2	Positive	0	0	0.5
	Carbolaran	222.1	Unit	123.1	Unit	<input checked="" type="checkbox"/>	<input type="checkbox"/>		5.73	1	80	30	2	Positive			
	Diazinon (Dimpylate)	305.1	Unit	169.1	Unit	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	2788	9.42	1	105	32	2	Positive	0	0	0.5
	Diazinon (Dimpylate)	305.1	Unit	97	Unit	<input checked="" type="checkbox"/>	<input type="checkbox"/>		9.42	1	105	40	2	Positive			
	Dimethoate	230	Unit	198.8	Unit	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	1547	3.85	1	70	0	5	Positive	0	0	0.5
	Dimethoate	230	Unit	125	Unit	<input checked="" type="checkbox"/>	<input type="checkbox"/>		3.85	1	70	16	5	Positive			
	Imazali (Enilconazol)	297.1	Unit	201	Unit	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	443	4.64	1	115	15	2	Positive	0	0	0.5
	Imazali (Enilconazol)	297.1	Unit	159	Unit	<input checked="" type="checkbox"/>	<input type="checkbox"/>		4.64	1	115	20	2	Positive			
	Imazapyr	262.1	Unit	217.1	Unit	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	249	2.81	1	120	20	3	Positive	0	0	0.5
	Imazapyr	262.1	Unit	69.1	Unit	<input checked="" type="checkbox"/>	<input type="checkbox"/>		2.81	1	120	40	3	Positive			
	Malathion	331	Unit	126.9	Unit	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	175	8.35	1	80	5	2	Positive	0	0	0.5

Dynamic MRM Parameters

Cycle Time: 500 ms Total MRMs = 28 Max Concurrent MRMs = 6 Min/Max Dwell = 81.56 ms/248.73 ms
 Primary Only Total MRMs = 28 Max Concurrent MRMs = 6 Min/Max Dwell = 81.56

Triggered MRM

Triggered Repeats:

Creating a Triggered MRM Method

Task 1. Create a tMRM method from a dMRM method

Steps

Detailed Instructions

Comments

Ion source: AJS ESI

Stop time:
 As pump/No limit
 Limit (min) 13

Time filter window (min) 0.05

Time Segments

Start time (min)	Scan type
0	dMRM

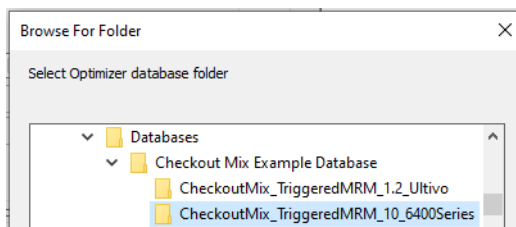
Statistics

Total MRMs	30
Minimum Concurrent MRMs	2
Maximum Concurrent MRMs	8
Minimum Dwell Time (ms)	61.48
Maximum Dwell Time (ms)	249.17
Minimum Cycle Time (ms)	16.16
Cycle time (ms):	500
Number of Repeats:	3
<input type="checkbox"/> Override RT window (min)	1
<input type="checkbox"/> Check minimum data pts (pts)	64

Calculations include:
 Primaries
 All transitions

3 For 6400 Series, open the **Checkout_Mix_TriggeredMRM_10_6400Series** database. For Ultivo, open the **CheckoutMix_TriggeredMRM_1.2_Ultivo** database in the Database Browser.

- In the Database Browser, click **File > Open Database**.
- Select the appropriate database in the folder **\\MassHunter\Databases\Checkout Mix Example Database**.
- Click **OK**.



Creating a Triggered MRM Method

Task 1. Create a tMRM method from a dMRM method

Steps	Detailed Instructions	Comments
4 Select secondary transitions. The CAS numbers are: 2032-59-9 1912-24-9 1563-66-2 333-41-5 60-51-5 35554-44-0 81334-34-1 121-75-5 139528-85-1 19937-59-8 2212-67-1 175013-18-0 148-79-8	<ol style="list-style-type: none">Click the Secondary transitions option under Select Transitions.Click the Compound Name column header to sort the compounds by Compound Name.Mark the check boxes next to the secondary transitions for each of the compounds in the dMRM method. See “Primary and Secondary Transitions for Triggered MRM” on page 66.Review the transitions in the table. Clear the check box next to any secondary transition that you do not want to include.	<ul style="list-style-type: none">The <i>Aminocarb</i> compound has two primary transitions and four secondary transitions.You can also clear the Show All Records check box. Then, you can search for each compound in the database by writing on separate lines the full name or CAS number of each compound in the Search Text list, mark the Compound Name or CAS check box, and then click Search Filter.To speed this step, you can copy in the entire list of CAS numbers shown in the first column of this table.Once you have the list of desired compounds, click the Secondary transitions button and then click Select Transitions. All of the secondary transitions for the compounds in the table are marked.

Database Browser

Search/Filter Import List

Show All Records Search/Filter

Filter Compounds

Enable Filters

Optimized Compounds

Date From 03/02/2016 To 06/04/2010

Group Name Project Name

Polarity Positive Model

Method

Select Compounds

Search Text

81334-34-1
67129-08-2
60-51-5
35554-44-0
333-41-5
2212-67-1
2032-59-9
19937-59-8
1912-24-9
175013-18-0
1563-66-2

Select Columns

Project Name
Compound Name
Formula
MW
Groups
 CAS
Chemical Classes

Match entire word for each string

Select Transitions

Select top 1 ranked transitions

Primary transitions

Secondary transitions

Select Transitions

Set primary and trigger flags

Set top 2 ranked transitions as primary

Set Primaries and Trigger

Rank transitions by

Abundance

Response Factor

Compound Name	Formula	MW	Polarity	Species	Precursor	Product	Frag	CE	CAV	Primary	Trigger
Aminocarb	C11H16N2O2		Positive		209.1	67.2	105	60	2	<input checked="" type="checkbox"/>	<input type="checkbox"/>
Aminocarb	C11H16N2O2		Positive		209.1	77.2	105	60	2	<input type="checkbox"/>	<input type="checkbox"/>
Aminocarb	C11H16N2O2		Positive		209.1	94.2	105	56	2	<input type="checkbox"/>	<input type="checkbox"/>
Aminocarb	C11H16N2O2		Positive		209.1	122.1	105	44	2	<input type="checkbox"/>	<input checked="" type="checkbox"/>
Aminocarb	C11H16N2O2		Positive		209.1	137.2	105	24	2	<input checked="" type="checkbox"/>	<input type="checkbox"/>
Aminocarb	C11H16N2O2		Positive		209.1	152.2	105	12	2	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>

Current Database: D:\MassHunter\Databases\Pesticides tMRM Database B.06.00.Exa...\CheckoutMix_TriggeredMRM_B0600

Add to Import List Import Close

Do not mark the two Primary transitions.

Creating a Triggered MRM Method

Task 1. Create a tMRM method from a dMRM method

Steps	Detailed Instructions	Comments
5 Import secondary transitions to the Data Acquisition program. (If you are using these steps to customize your own method, remove negative MRM transitions from any compound with positive MRM transitions.)	<ul style="list-style-type: none">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.	<ul style="list-style-type: none">• The compound Aminocarb has four secondary transitions.• Only the transitions that you marked are added to the Import List.• All transitions that have the same Compound Name are part of the same compound.
NOTE	<ul style="list-style-type: none">• Qualifier and quantifier ions must have the same polarity, so one compound cannot contain both negative and positive polarity transitions. If you want to include both polarities for one compound while you develop a method, you must rename the compounds in the method to "<i>compoundname_pos</i>" and "<i>compoundname_neg</i>". When the best polarity and transitions are found for a compound, remove from the method all other transitions for the compound. Then remove "_pos" or "_neg" from the remaining compound name.• To ensure good signal/noise ratios for the secondary transitions, the superfluous secondary transitions which are not required for confirmation must be removed. Secondary transitions required for confirmation should be as unique as possible to a particular analysis or have intense ion peaks.	

Creating a Triggered MRM Method

Task 1. Create a tMRM method from a dMRM method

Steps

Detailed Instructions

Comments

Compound Name	Formula	MW	Polarity	Species	Precursor	Product	Frag	CE	Primay	Trigger	RT
Aminocarb	C11H16N2O2				209.1	67.2	105	60	<input type="checkbox"/>	<input type="checkbox"/>	
Aminocarb	C11H16N2O2				209.1	77.2	105	60	<input type="checkbox"/>	<input type="checkbox"/>	
Aminocarb	C11H16N2O2				209.1	94.2	105	56	<input type="checkbox"/>	<input type="checkbox"/>	
Aminocarb	C11H16N2O2				209.1	122.1	105	44	<input type="checkbox"/>	<input type="checkbox"/>	
Atrazine	C8H14ClN5				216.1	43.1	125	48	<input type="checkbox"/>	<input type="checkbox"/>	
Atrazine	C8H14ClN5				216.1	62.1	125	56	<input type="checkbox"/>	<input type="checkbox"/>	
Atrazine	C8H14ClN5				216.1	79	125	24	<input type="checkbox"/>	<input type="checkbox"/>	
Atrazine	C8H14ClN5				216.1	104	125	28	<input type="checkbox"/>	<input type="checkbox"/>	
Atrazine	C8H14ClN5				216.1	132	125	20	<input type="checkbox"/>	<input type="checkbox"/>	
Atrazine	C8H14ClN5				216.1	145.9	125	20	<input type="checkbox"/>	<input type="checkbox"/>	
Carbofuran	C12H15NO3				222.1	55.2	80	24	<input type="checkbox"/>	<input type="checkbox"/>	
Carbofuran	C12H15NO3				222.1	78	80	50	<input type="checkbox"/>	<input type="checkbox"/>	
Carbofuran	C12H15NO3				222.1	124	80	20	<input type="checkbox"/>	<input type="checkbox"/>	
Carbofuran	C12H15NO3				222.1	137	80	16	<input type="checkbox"/>	<input type="checkbox"/>	
Carbofuran	C12H15NO3				222.1	166	80	4	<input type="checkbox"/>	<input type="checkbox"/>	
Carbofuran	C12H15NO3				222.1	207	80	12	<input type="checkbox"/>	<input type="checkbox"/>	
Diazinon (Dimpylate)	C12H21N2O3PS				305.1	66	105	40	<input type="checkbox"/>	<input type="checkbox"/>	
Diazinon (Dimpylate)	C12H21N2O3PS				305.1	84	105	40	<input type="checkbox"/>	<input type="checkbox"/>	
Diazinon (Dimpylate)	C12H21N2O3PS				305.1	93	105	40	<input type="checkbox"/>	<input type="checkbox"/>	

6 Review the secondary transitions in the Data Acquisition program.

- In the Acquisition tab, sort the table by the **Compound Name**.
- Review the primary and secondary transitions for each compound.

- If a red box appears in the Scan segments table, you click the **Apply** button in the toolbar. If the red box does not clear, the value is not valid.

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

- Sort the table by the Trigger column.
- For each Trigger transition, type 1 for the **Trigger Entrance**. You can type 1 in the first row, right-click and select **Fill down**.
- For each Trigger transition, type 2 for the **Trigger Delay**.
- For each Trigger transition, type 0.5 for the **Trigger Window**.

- See the QQQ Concepts guide or the online Help for more information on these values.

8 Update the **Trigger Threshold**.

- In the MRM Update Options dialog box, select to update the Trigger Threshold from the MassHunter QQQ data file or Quant report folder.

- See **“Task 3. Create a dMRM method using Update dMRM”** on page 33.
- You can also enter the Threshold directly.

Creating a Triggered MRM Method

Task 1. Create a tMRM method from a dMRM method

Steps

Detailed Instructions

Comments

Acquisition	Source	Chromatogram	Instrument	Diagnostics																			
Scan segments														Threshold	Ret Time (min)	Delta Ret Time	Fragmentor	Collision Energy	Cell Accelerator Voltage	Polarity	Trigger Entrance	Trigger Delay	Trigger Window
Aminocarb	<input type="checkbox"/>	209.1	Unit	152.2	Unit	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	6937	2.01	1	105	12	2	Positive	1	2	0.5						
Altrazine	<input type="checkbox"/>	216.1	Unit	174.1	Unit	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	2010	6.1	1	125	16	3	Positive	1	2	0.5						
Carbofuran	<input type="checkbox"/>	222.1	Unit	165.1	Unit	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	728	5.73	1	80	20	2	Positive	1	2	0.5						
Diazinon (Dimpylate)	<input type="checkbox"/>	305.1	Unit	169.1	Unit	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	2788	9.42	1	105	32	2	Positive	1	2	0.5						
Dimethoate	<input type="checkbox"/>	230	Unit	198.8	Unit	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	1547	3.85	1	70	0	5	Positive	1	2	0.5						
Imazalil (Enilconazol)	<input type="checkbox"/>	297.1	Unit	201	Unit	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	443	4.64	1	115	15	2	Positive	1	2	0.5						
Imazapyr	<input type="checkbox"/>	262.1	Unit	217.1	Unit	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	249	2.81	1	120	20	3	Positive	1	2	0.5						
Malathion	<input type="checkbox"/>	331	Unit	126.9	Unit	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	175	8.35	1	80	5	2	Positive	1	2	0.5						
Melazachlor	<input type="checkbox"/>	278.1	Unit	210.1	Unit	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	2855	6.76	1	70	4	5	Positive	1	2	0.5						
Metosulam	<input type="checkbox"/>	418.02	Unit	175	Unit	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	404	6.27	1	140	32	3	Positive	1	2	0.5						
Metoxuron	<input type="checkbox"/>	229	Unit	72.1	Unit	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	2155	4.74	1	95	16	3	Positive	1	2	0.5						
Molinate	<input type="checkbox"/>	188	Unit	126.1	Unit	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	69	7.79	1	90	25	2	Positive	1	2	0.5						
Pyraclostrobin	<input type="checkbox"/>	388.11	Unit	193.8	Unit	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	9558	9.42	1	95	8	2	Positive	1	2	0.5						
Thiabendazole	<input type="checkbox"/>	202	Unit	175	Unit	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	2263	2.86	1	130	24	2	Positive	1	2	0.5						

Dynamic MRM Parameters: Cycle Time ms Total MRMs = 93 Max Concurrent MRMs = 22 Min/Max Dwell = 21.36 ms/248.71 ms Primary Only Total MRMs = 28 Max Concurrent MRMs = 6 Min/Max Dwell = 81.56

Triggered MRM: Triggered Repeats

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

- Click the **Method > Save Method As** command.
- Type **iiiCheckoutMix_TMRM.m**.
- Click the **Save** button.

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

- Right-click the Scan segments table and click **Edit DMRM Method**. The Dynamic MRM Viewer dialog box is opened. If you have an Ultivo system, you can skip this step because the Dynamic MRM Viewer is displayed automatically.
- Switch between the **Primaries only** button and the **All transitions** button if the **Dynamic MRM Statistics** information is not updating.
 - Inspect the Dynamic MRM Statistics in the upper right corner. You can modify the **Cycle time** and see how the minimum and maximum **Dwell Times** are changed.
 - While newer Agilent instruments enable a **Dwell Time** of 0.5 ms, a **Dwell Time** of 5 ms per transition is recommended for this particular analysis. When you click **All transitions**, **Maximum Concurrent MRMs** value can change.
 - If the minimum **Dwell Time** was lower than 5 ms, then you can change the **Cycle time** to a larger value to increase the **Dwell** time.

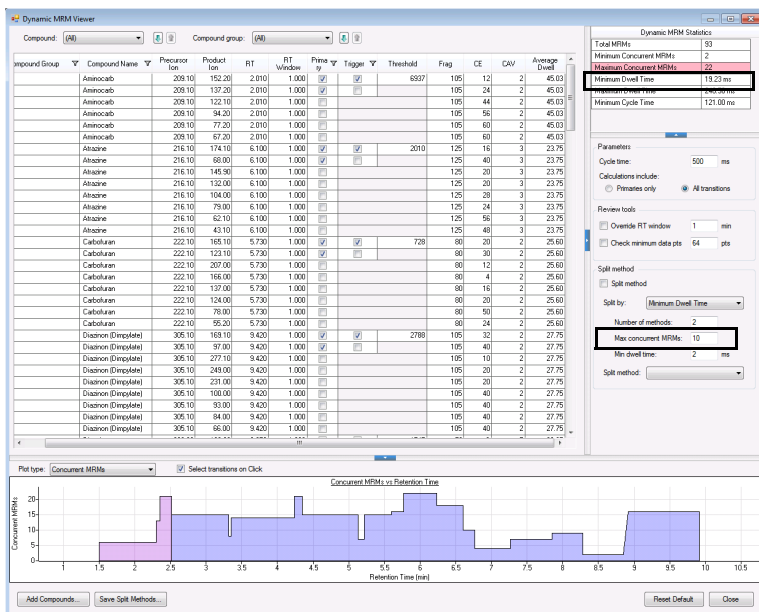
Creating a Triggered MRM Method

Task 1. Create a tMRM method from a dMRM method

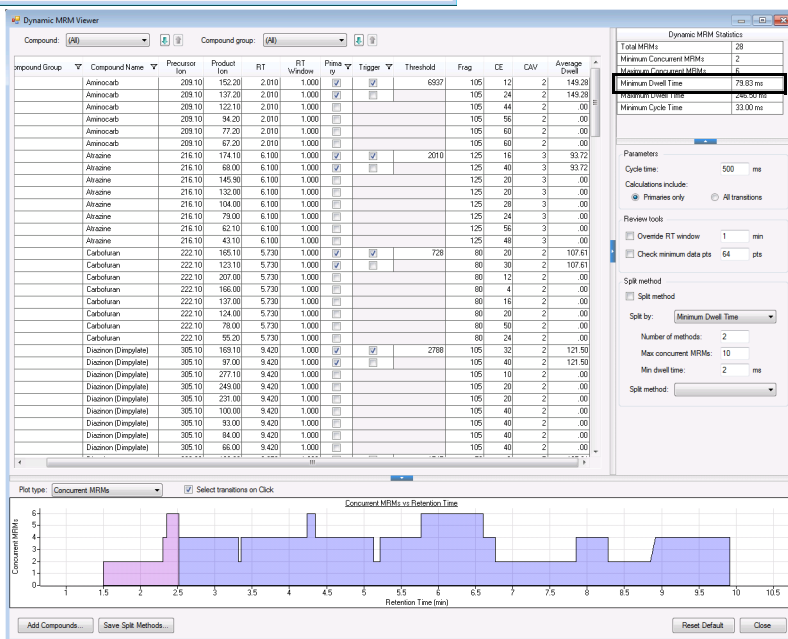
Steps

Detailed Instructions

Comments



The image to the left for the 6400 Series shows All transitions. If all of the secondary ions are acquired at the same time, then the Minimum Dwell time is 19.23 ms. However, in the image below for the 6400 Series, the Primaries only option is clicked. The Minimum Dwell Time is 79.83 ms.



Creating a Triggered MRM Method

Task 1. Create a tMRM method from a dMRM method

Steps	Detailed Instructions	Comments
11 Adjust the cycle time.	a See step 10 on page 50 for details.	<ul style="list-style-type: none">The cycle time can be optimized for each analysis. The default cycle time is 500 ms. For methods that contain more than 15 compounds, the cycle time usually needs to be at least 500 ms. Use the Dynamic MRM Viewer to see what the Minimum Dwell Time is and increase the cycle time so that the Minimum Dwell Time is at least 5.

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

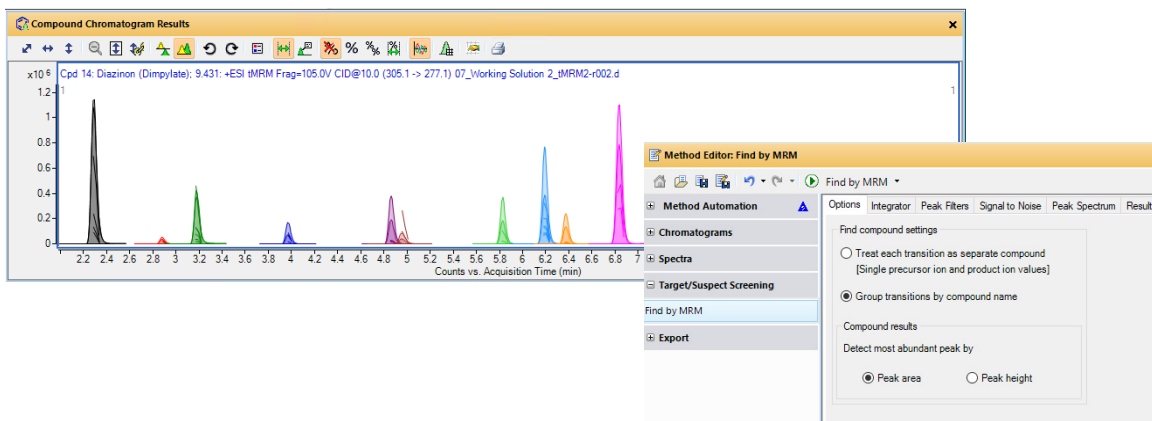
After you acquire the tMRM data file, you examine the data file in the Qualitative Analysis program to verify that all of the Primary and Secondary transitions were acquired.

Steps	Detailed Instructions	Comments
<p>Do this step if you want to acquire data with the Checkout Mix. Otherwise, continue at step 2.</p> <p>1 Acquire data.</p> <ul style="list-style-type: none"> Set up a one-line worklist with the method you just created. Name the data file CheckoutMix_TMRM.d. Designate a directory path to hold your data files and method. 	<p>a If necessary, click View > Worklist to display the Worklist window.</p> <p>b Click Worklist > Worklist Run Parameters. Verify that the parameters are set properly. In the Data File Settings tab, under the File Naming section, type <code>CheckoutMix_tMRM</code>. Click OK.</p> <p>c Click Worklist > Add Multiple Samples.</p> <p>d Select the tMRM method you just created in the previous task as the method name.</p> <p>e Click the Sample Position tab.</p> <p>f Select the Autosampler, Well-plate or Vial Tray.</p> <p>g In the graphic, select a single position. Click OK.</p> <p>h In the Worklist window, mark the check box to the left of the sample.</p> <p>i Click the Start Worklist Run icon in the main toolbar, the Run Worklist icon in the Worklist toolbar or click Worklist > Run.</p>	<ul style="list-style-type: none"> The Worklist window is tabbed with the Method Editor window by default. Click the Worklist tab to show the Worklist window. This step is optional because you can perform the next step with an example data file that comes with the program. If you prefer, you can create your own data file as described in this step. See also "To run the Checkout Mix" on page 8.

Creating a Triggered MRM Method

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

Steps	Detailed Instructions	Comments
2 Find compounds using the Find Compound by MRM algorithm. <ul style="list-style-type: none">Open the data file CheckoutMix_TMRM.d.	<ol style="list-style-type: none">Start the Qualitative Analysis 10.0 program.Click File > Open Data File. The system displays the "Open Data File" dialog box.Click the Compounds View tab at the top of the Qualitative Analysis screen.Select the tMRM method you created in step 9 on page 49, and click Open.Click the Compounds View tab at the top of the Qualitative Analysis screen.If needed, click View > Method Editor. The system displays the Method Editor window.In the Method Automation section, click Workflow, and ensure the Workflow is set to Target/Suspect Screening and that Compound Mining is set to Find by MRM.In the Method Editor window, in the Target/Suspect Screening section, click Find by MRM. Click the Group transitions by compound name option.Click the Peak area option for Detect most abundant peak by.	<ul style="list-style-type: none">The peaks in the TIC have a jagged appearance due to the triggering. This is the expected appearance. When the secondary transitions are acquired, the abundance in the TIC is increased immediately.You can also use the example tMRM data file in the Checkout Mix Example Data folder. If this file is not on your computer, install it from the installation media.



j Click Find > Find Compounds by MRM.

Creating a Triggered MRM Method

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

Steps	Detailed Instructions	Comments
<p>3 Review the results of the Find Compounds by MRM algorithm.</p> <ul style="list-style-type: none">Make sure that the primary ions are found for each compound. <p>In the example data and example database, the compound Malathion does not have any secondary transitions.</p>	<p>a Close the Compound MS Spectrum Results window.</p> <p>b In the Compound Chromatogram Results window, click the Overlaid mode button and the Show Legend in Overlaid mode button.</p> <p>c Click View > Compound Fragment Spectrum Results.</p> <p>d In the Compound Fragment Spectrum Results window, click the Spectrum Peak List button.</p> <p>e Review each compound. Verify that the primaries and secondaries for each compound were found.</p>	<ul style="list-style-type: none">You can also print a Compound Report to review results. You click File > Print > Workflow Report. The Compound Report sorts the compounds by retention time.If you are using the Navigator View, then in the Data Navigator window, the primary transitions are labeled MRM and the secondary transitions are labeled tMRM.If you are using the Compound Details View, then in the legend in the Compound Chromatogram Results window, the primary transitions are labeled MRM, and the secondary transitions are labeled tMRM.The Retention Times of the isomers will not be resolved if they have unique transitions until Task 3. Create a Reference Library in the Quantitative Analysis program on page 57.
	<p>f Select Cpd1:Aminocarb. You click this compound in the Compound List.</p> <p>g In the Compound Fragment Spectrum Results window, verify that these transition are all found:</p> <ul style="list-style-type: none">209.1 -> 67.2 (Secondary)209.1 -> 77.2 (Secondary)209.1 -> 94.2 (Secondary)209.1 -> 122.1 (Secondary)209.1 -> 137.2 (Primary)209.1 -> 155.2 (Primary)	<ul style="list-style-type: none">In the Compound Chromatogram Results window, you can see lines which indicate the abundances for each Secondary transition.
	<p>h Continue checking each compound to verify that the Primary and Secondary transitions were acquired. See Primary and Secondary Transitions for Triggered MRM on page 66 for a list of the transitions to verify.</p>	<ul style="list-style-type: none">In the Compound Fragment Spectrum Results table, you can check the abundance for each Primary and Secondary.If you are using the Navigator View, then in the Chromatogram Results window, you can click the Walk Chromatogram tool to review each of the spectra across a peak. You can determine when the Secondaries are acquired.

Creating a Triggered MRM Method

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

Steps

Detailed Instructions

Comments

The dark lines show when the secondary transitions are acquired. The positions of these dark lines are dependent upon the Trigger Entrance, Trigger Delay, Trigger Repeat number, and Trigger Window that you set in the acquisition method. You change these values to optimize the time at which the secondary transitions are acquired.

Compound Chromatogram Results

Label	RT	Width	Height	Area	Score	Mining A
Cpd 7: Carbendazim 5.830	5.83	0.232	371549	11402	100	Find by MRM
Cpd 8: Atrazine 6.188	6.188	0.239	768465	24519	100	Find by MRM
Cpd 9: Metolachlor 6.376	6.376	0.225	239815	77345	100	Find by MRM
Cpd 10: Metazachlor 6.839	6.839	0.233	110138	38474	100	Find by MRM
Cpd 11: Molinate 7.822	7.822	0.232	20151	60601	100	Find by MRM
Cpd 12: Malathion 8.377	8.377	0.227	16078	47564	100	Find by MRM
Cpd 13: Pradofastin 9.426	9.426	0.283	443563	15055	100	Find by MRM
Cpd 14: Diazinon (Dimpylate) 9.431	9.431	0.214	666816	21910	100	Find by MRM


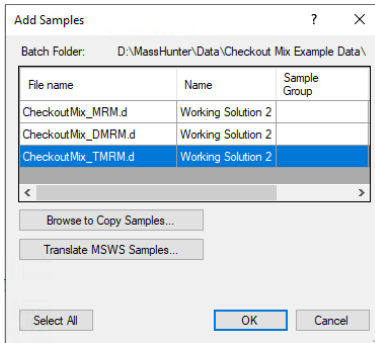
Compound Fragment Spectrum Results

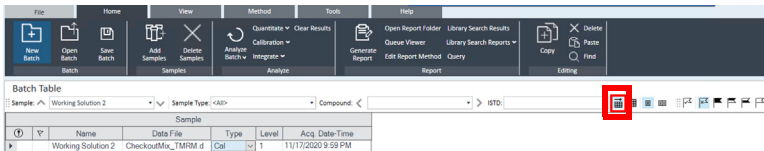
Peak	Abund	Abund %	Abund % (Norm)	Max Abund	m/z	m/z (CAG)	Label
1	19915.96	17.06		19915.96	43.1		
2	8230.29	7.05		8230.29	62.1		
3	58171.37	49.83		58171.37	68		
4	9225.44	7.9		9225.44	79		
5	13553.01	11.61		13553.01	104		
6	5863.58	5.02		5863.58	132		
7	4984.04	4.27		4984.04	145.9		
8	116747.91	100		116747.91	174.1		

Creating a Triggered MRM Method

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

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

Steps	Detailed Instructions	Comments
1 Start the Quantitative Analysis program.	<p>a Click the QQQ Quantitative Analysis (Quant My Way) icon.</p> 	
2 Set up a batch and add the TMRM data file. <ul style="list-style-type: none">• Add the data file CheckoutMix_TMRM.d.	<p>a Click New Batch.</p> <p>b Navigate to the location of the TMRM data file.</p> <p>c Type CheckoutMix_TMRM for the Batch file name.</p> <p>d Click Create Batch. The Add Sample window opens.</p> <p>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.</p> <p>f Select the CheckoutMix_TMRM.d data file and click OK.</p> <p>g Check that Flat Table (shown in red in the next figure) is selected. Then select Cal as the Type.</p> <p>h Type 1 for the Level.</p>	
3 Set up the TMRM method.	<p>a Click Method > New > New Method from Acquired MRM Data.</p> <p>b Select the CheckoutMix_TMRM.d data file.</p> <p>c Click Open.</p> <p>d Right-click the Method Table window and click Collapse All.</p>	<p>• If you added more than one sample, then you select one of the calibration data files to create the method.</p>



Creating a Triggered MRM Method

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

Steps

Detailed Instructions

Comments

Method Table

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

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

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

- Select **Concentration Setup** in the Method Setup Tasks section in the Method Tasks pane.
 - Select the first compound in the table.
 - Right-click the compound row and click **New Calibration Level** from the shortcut menu.
 - In the **Level** column, type 1. In the **Conc.** column, type 100.
 - Right-click in the Level box and click **Copy Calibration Levels To**.
 - Click **Select All**. Click **OK**.
- Refer to the online Help in the Quantitative Analysis program for additional help on these tasks.

Method Table

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

Sample	Name	Data File	Type	Level	Acq. Method File	Ac
CheckoutMix_TMRM.d	CheckoutMix_TMRM.d					
Quantifier						
Name	TS	Transition	Scan	Type	Units	
Aminocarb	1	209.1 -> 137.2	MRM	Target	ng/ml	
Calibration						
Level	Conc.	Response	Enable			
1	100.0000		<input checked="" type="checkbox"/>			
Quantifier						
Name	TS	Transition	Scan			
Atrazine	1	216.1 -> 174.1	MRM			
Carbofuran	1	222.1 -> 123.1	MRM			
Diazinon (Dimpylate)	1	9.431 305.1 -> 169.1	MRM			
Dimethoate	1	3.968 230.0 -> 125.0	MRM			
Imazalil (Eniconazole)	1	4.951 297.1 -> 159.0	MRM			
Imazapyr	1	2.683 262.1 -> 217.1	MRM			
Malathion	1	331.0 -> 126.9	MRM			
Metazachlor	1	278.1 -> 134.2	MRM			

Copy Calibration Levels To

Select Compounds:

Name	TS	RT	Transition	ISTD Flag
Atrazine	1	6.188	216.1 -> 174.1	<input type="checkbox"/>
Carbofuran	1	5.830	222.1 -> 123.1	<input type="checkbox"/>
Diazinon (Dimpylate)	1	9.431	305.1 -> 169.1	<input type="checkbox"/>
Dimethoate	1	3.968	230.0 -> 125.0	<input type="checkbox"/>
Imazalil (Eniconazole)	1	4.951	297.1 -> 159.0	<input type="checkbox"/>
Imazapyr	1	2.683	262.1 -> 217.1	<input type="checkbox"/>

< Select All OK Cancel >

Creating a Triggered MRM Method

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

Steps	Detailed Instructions	Comments
5 Change the calibration curve. <ul style="list-style-type: none">Force the origin to be included.	<ol style="list-style-type: none">Select Calibration Curve Setup in the Method Setup Tasks section in the Method Tasks pane.Set the CF Origin to Force for the first compound.Right-click this value and click Fill Down.	<ul style="list-style-type: none">We only have one data file so we need to include the origin.The Fill Down command copies the value of the current cell to all of the other rows in the table.

Method Table

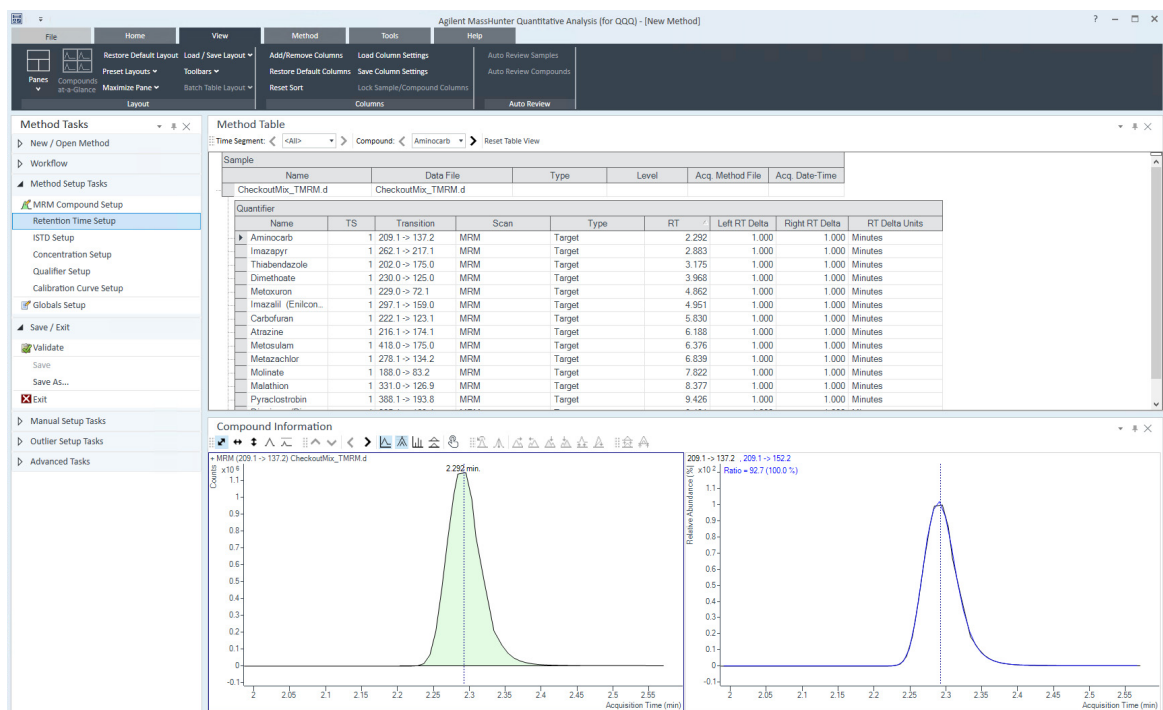
Time Segment: <All> Compound: Aminocarb Reset Table View

Sample	Name	Data File	Type	Level	Acq. Method File	Acq. Date-Time	
CheckoutMix_TMRM.d	CheckoutMix_TMRM.d						
Quantifier							
Name	TS	Transition	Scan	Type	CF	CF Origin	CF V
Aminocarb	1	209.1 -> 137.2	MRM	Target	Linear	Force	None
Atrazine	1	216.1 -> 174.1	MRM	Target	Linear	Force	None
Carbofuran	1	222.1 -> 123.1	MRM	Target	Linear	Force	None
Diazinon (Dimpy...	1	305.1 -> 169.1	MRM	Target	Linear	Force	None
Dimethoate	1	230.0 -> 198.8	MRM	Target	Linear	Force	None
Imazalil (Enicon...	1	297.1 -> 159.0	MRM	Target	Linear	Force	None
Imazapyr	1	262.1 -> 217.1	MRM	Target	Linear	Force	None
Malathion	1	331.0 -> 126.9	MRM	Target	Linear	Force	None
Metazachlor	1	278.1 -> 134.2	MRM	Target	Linear	Force	None
Metosulam	1	418.0 -> 175.0	MRM	Target	Linear	Force	None
Metoxuron	1	229.0 -> 72.1	MRM	Target	Linear	Force	None
Molinate	1	188.0 -> 83.2	MRM	Target	Linear	Force	None
Pyraclostrobin	1	388.1 -> 193.8	MRM	Target	Linear	Force	None
Thiabendazole	1	202.0 -> 131.0	MRM	Target	Linear	Force	None

Creating a Triggered MRM Method

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

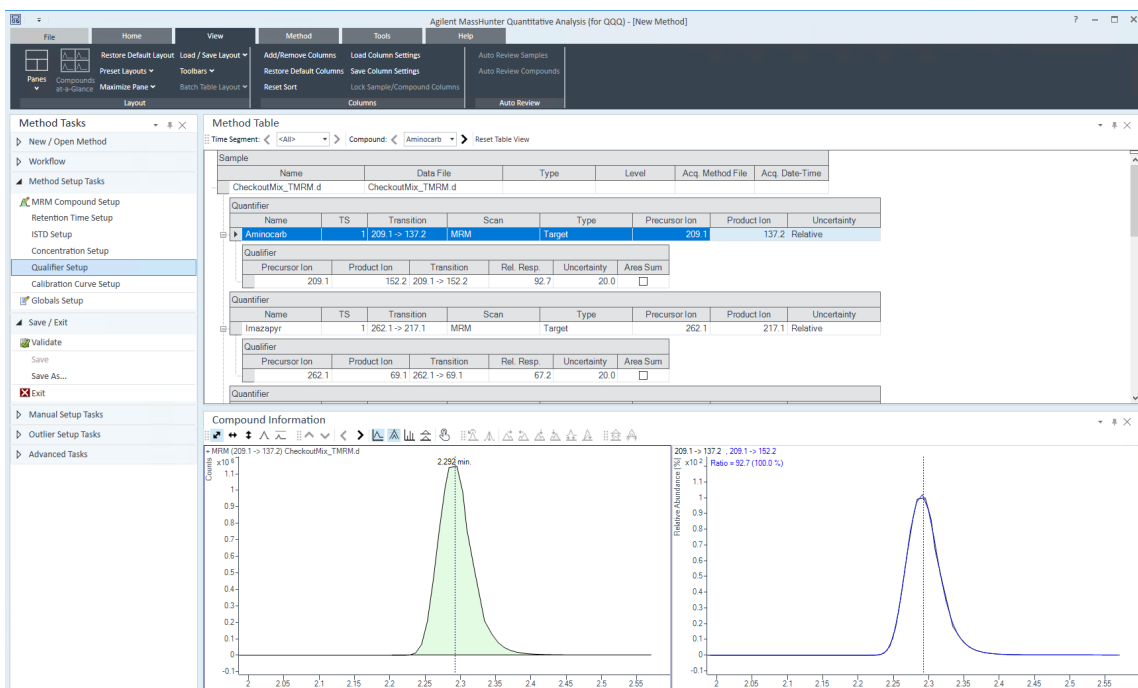
Steps	Detailed Instructions	Comments	
6	<p>Resolve the RTs. The RT elution order is:</p> <ul style="list-style-type: none">AminocarbImazapyrThiabendazoleDimethoateMetoxuronImazalil (Enilconazole)CarbofuranAtrazineMetosulamMetazachlorMolinateMalathionDiazinon (Dimpylate)Pyraclostrobin	<p>a Select Retention Time Setup in the Method Setup Tasks section.</p> <p>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.</p>	<p>Depending on the delay volume, the compounds Pyraclostrobin and Diazinon can co-elute, separate slightly, or reverse elution order.</p>



Creating a Triggered MRM Method

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

Steps	Detailed Instructions	Comments
7	Review qualifier ratios.	<ul style="list-style-type: none">c Select Qualifier Setup in the Method Setup Tasks section.d Right-click the Method Table and click Expand All.e Click the Show/Hide Qualifiers button in the toolbar in the Compound Information window.f Click on each compound and verify that the Rel. Resp. for each Qualifier matches the value shown in the Compound Information window in the spectrum pane.g Click Method > Validate and fix any errors.



8	Set up the Reference Library.	<ul style="list-style-type: none">a Click Method > Library > Setup Reference Library.b Click Obtain reference spectra from sample.c Verify that Create reference library at is set to the folder you wish to use.d Click OK.e Click OK in the "Reference library was created" message. <ul style="list-style-type: none">• 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".
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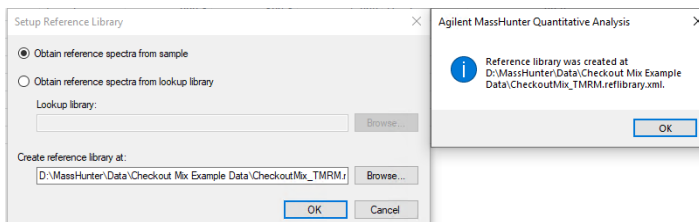
Creating a Triggered MRM Method

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

Steps

Detailed Instructions

Comments



- f Select **Globals Setup** in the Method Setup Tasks section in the Method Tasks pane.
- g Verify that the Reference Library is set to the Reference Library you just created.

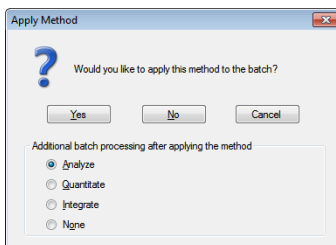
Method Table

Time Segment: < -AB> > Compound: < Aminocarb > Reset Table View

Sample	Name	Data File	Type
CheckoutMix_TMRM.d	CheckoutMix_TMRM.d		
Globals			
Apply Multiplier to ISTD		<input type="checkbox"/>	
Apply Multiplier to Matrix Spike		<input checked="" type="checkbox"/>	
Apply Multiplier to Surrogate		<input checked="" type="checkbox"/>	
Apply Multiplier to Target		<input checked="" type="checkbox"/>	
Bracketing Type	None		
Correlation Window			0.100
Dynamic Background Subtraction		<input type="checkbox"/>	
Ignore Peaks Not Found		<input type="checkbox"/>	
Library Method			
Non Reference Window			70.000
Non Reference Window Type	Percent		
Reference Library	E:\MassHunter\Data\CheckoutMix_TMRM.reflibrary.xml		
Reference Pattern Library			
Reference Window			80.000
Reference Window Type	Percent		
Relative ISTD		<input type="checkbox"/>	
Standard Addition		<input type="checkbox"/>	

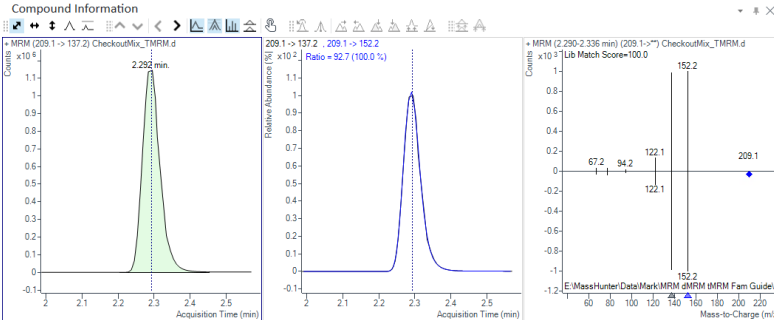
- 9 Save the method and set additional batch processing to analyze.

- a Click **Method > Save As**.
- b Type **Quant_Checkout_TMRM** in the **File name**.
- c Click **Save**.
- d Click **Method > Exit**.
- e Verify that **Additional batch processing after applying the method** is set to **Analyze**.
- f Click **Yes** to apply the method to the batch.



Creating a Triggered MRM Method

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

Steps	Detailed Instructions	Comments
10 Review the results, and save the batch.	<ol style="list-style-type: none">Inspect the results.If the Compound Information window is not open, click View > Panes > Compound Information.Click the Show/Hide Spectrum button.Click File > Save Batch.	
		
<p>The LibMatchScore is 100 because you are comparing against the same spectrum.</p> <p>The matching algorithm applies penalties when less than five peaks are present in the spectrum:</p> <ul style="list-style-type: none">75% (1 peak)88% (2 peaks)94% (3 peaks)97% (4 peaks)		
11 Inspect the Library Match Score. Review the batch to resolve errors or messages that are indicated in the Batch Table.	<ul style="list-style-type: none">Check library match scoresCheck qualifier ratios.Resolve errors and messages <p>NOTE: The sample data included for this exercise does not contain isomers. But if your sample does, you would resolve isomers at this time.</p>	<p>If an error is reported for a compound qualifier ratio:</p> <ol style="list-style-type: none">Click Method > Update > Update Qualifier Ratios.Select the compounds for update and click OK.Click Method > Exit.Click Yes to apply the method to the batch.Check qualifier ratios.Resolve errors and messages.Click File > Save Batch.

Reference

Checkout Mix Content

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

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

#	Chemical Name/CAS #	Concentration / Units	Tolerance (+/-)	Formula	Mass
1	Aminocarb/2032-59-9	100.2 µg/mL	0.5 µg/mL	C ₁₁ H ₁₆ N ₂ O ₂	208.1211777698
2	Atrazine/1912-24-9	100.4 µg/mL	0.5 µg/mL	C ₈ H ₁₄ ClN ₅	215.0937731936
3	Carbofuran/1563-66-2	100.2 µg/mL	0.5 µg/mL	C ₁₂ H ₁₅ NO ₃	221.1051933528
4	Diazinon (Dimpylate)/333-41-5	100.4 µg/mL	0.5 µg/mL	C ₁₂ H ₂₁ N ₂ O ₃ PS	304.1010497716
5	Dimethoate/60-51-5	100.2 µg/mL	0.5 µg/mL	C ₅ H ₁₂ NO ₃ PS ₂	228.9996212071
6	Imazalil (Enilconazole)/35554-44-0	100.4 µg/mL	0.5 µg/mL	C ₁₄ H ₁₄ Cl ₂ N ₂ O	296.0483185037
7	Imazapyr/81334-34-1	100.2 µg/mL	0.5 µg/mL	C ₁₃ H ₁₅ N ₃ O ₃	261.1113413676
8	Malathion/121-75-5	100.4 µg/mL	0.5 µg/mL	C ₁₀ H ₁₉ O ₆ PS ₂	330.0360662899
9	Metazachlor/67129-08-2	100.2 µg/mL	0.5 µg/mL	C ₁₄ H ₁₆ ClN ₃ O	277.0981898649
10	Metosulam/139528-85-1	100.4 µg/mL	0.5 µg/mL	C ₁₄ H ₁₃ Cl ₂ N ₅ O ₄ S	417.0065300909
11	Metoxuron/19937-59-8	100.2 µg/mL	0.5 µg/mL	C ₁₀ H ₁₃ ClN ₂ O ₂	228.0665553841
12	Molinate/2212-67-1	100.4 µg/mL	0.5 µg/mL	C ₉ H ₁₇ NOS	187.103084902
13	Pyraclostrobin/175013-18-0	100.2 µg/mL	0.5 µg/mL	C ₁₉ H ₁₈ ClN ₃ O ₄	387.0985837956
14	Thiabendazole/148-79-8	100.4 µg/mL	0.5 µg/mL	C ₁₀ H ₇ N ₃ S	201.0360679755
	Acetonitrile	Solvent		C ₂ H ₃ N	41.0265

Reference
Checkout Mix Content

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

#	Chemical Name/CAS #	Concentration / Units	Tolerance (+/-)	Formula	Mass
1	Acifluorfen/50594-66-6	100.2 µg/mL	0.5 µg/mL	C ₁₄ H ₇ ClF ₃ NO ₅	360.9964846522
2	2,4,5-T/93-76-5	100.4 µg/mL	0.5 µg/mL	C ₈ H ₅ Cl ₃ O ₃	253.9304271564
3	Bentazone/25057-89-0	100.2 µg/mL	0.5 µg/mL	C ₁₀ H ₁₂ N ₂ O ₃ S	240.0568629945
4	Dinoseb (Subitex)/88-85-7	100.4 µg/mL	0.5 µg/mL	C ₁₀ H ₁₂ N ₂ O ₅	240.0746215091
5	2,4,5-TP (Silvex) (Fenoprop)/93-72-1	100.2 µg/mL	0.5 µg/mL	C ₉ H ₇ Cl ₃ O ₃	267.9460772202
6	Hexaflumuron/86479-06-3	100.4 µg/mL	0.5 µg/mL	C ₁₆ H ₈ Cl ₂ F ₆ N ₂ O ₃	459.9816167569
	Acetonitrile	Solvent		C ₂ H ₃ N	41.0265

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

Primary and Secondary Transitions for Triggered MRM

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

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

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

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

Reference

Primary and Secondary Transitions for Triggered MRM

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

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

Reference

Primary and Secondary Transitions for Triggered MRM

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

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

Reference

Primary and Secondary Transitions for Triggered MRM

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

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

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In This Guide

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

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