MassHunter Forensics and Toxicology Triggered MRM Database and Library

Quick Start Guide

Agilent Technologies

What is the MassHunter Forensics and Toxicology Triggered MRM Database and Library?

The MassHunter Forensics and Toxicology Triggered MRM Database and Library contains more than 2800 Forensics and Toxicology analytes with up to 10 transitions. This database, when used with the procedures in this guide, helps you set up your unique Forensics and Toxicology tMRM analysis by simplifying the method development steps.

Triggered MRM occurs when criteria for primary MRMs trigger confirmatory (secondary) MRMs to be acquired for a compound. If the abundances of the Primary MRMs are higher than the set thresholds and other criteria are met, then the confirmatory (or secondary) MRMs are acquired. You can have multiple primary MRMs per compound, and you can specify up to two of these as Trigger MRMs for each compound. You can also have multiple secondary transitions for each compound. All transitions with the same Compound Name belong to the same compound.

The MassHunter Forensics and Toxicology Triggered MRM Database and Library helps minimize method development time for your Forensics and Toxicology analysis, when used with Agilent recommended LC/MS configuration and accessories. It stores up to 10 MRM transitions (a precursor and product ion) per compound of the Forensics and Toxicology analytes included in the database, and their optimized fragmentor and collision energy settings from the Agilent 6400 Series Triple Quadrupole LC/MS instruments. Method development can simply be achieved by importing target compounds from the database to the MassHunter Data Acquisition program. In addition, tMRM reference spectra are provided in the lookup library for compounds present in the comprehensive test mix.

tMRM acquisition is a data-dependent acquisition mode which allows the collection of up to ten MRM transitions per compound, if the signals of the compound's primary MRM transitions exceed user defined thresholds and other criteria are met. See the tMRM technical note (p/n 5990-8461EN), or the Agilent 6400 Series Triple Quadrupole LC/MS *Familiarization Guide* or *Concepts Guide* for more information.

Workflow Overview

This *Quick Start 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.

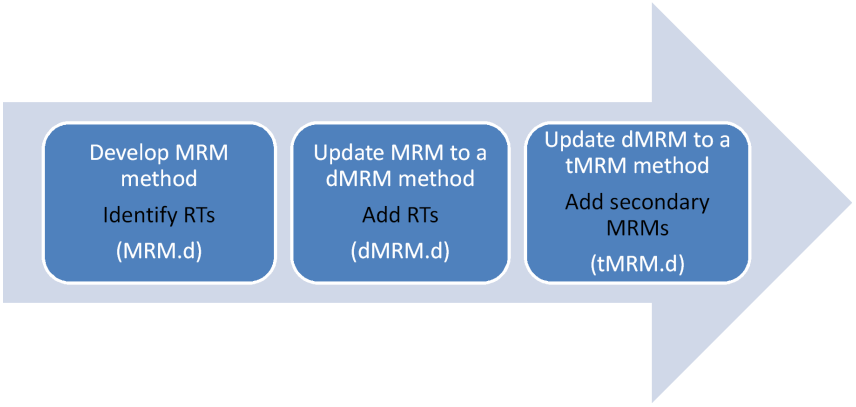


Figure 1 tMRM Method Development Workflow for single standard mix

Single Standard Mix Workflow

The workflow to analyze a single standard mix is:

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

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

Multiple Standard Mix Workflow

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

1. Optimize each tMRM method for each standard mix separately. Use the same LC chromatographic method.
2. Combine these tMRM methods.
3. Re-optimize the parameters for overlapping 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.

Use the *Method Setup Guide* included on the support disc to easily set up methods for multiple standard mixes in the Comprehensive Forensics and Toxicology Test Mix.

The *Method Setup Guide* also contains instructions to optimize LC and MS acquisition parameters for the comprehensive test mix.

Kit Content

Quick Start Guide

MassHunter Forensics and Toxicology Triggered MRM Database and Library Quick Start Guide The Quick Start Guide provides an overview of the MassHunter Forensics and Toxicology Triggered MRM Database and Library, how to use it, and where to find further information. This guide is meant to be used with the Checkout Mix, but you can use the procedures in this guide for your own unique analysis or to analyze the Comprehensive Test Mix.

Installation and Support Discs

MassHunter Forensics and Toxicology Triggered MRM Database and Library Support disc The contents of the disc are:

- Checkout Mix methods:
  - ForTox_TestMix_MRM.m, used to create dMRM method
  - ForTox_TestMix_DMRM.m, used to create tMRM method
  - ForTox_TestMix_TMRM.m

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

- Checkout Mix example Data:
  - ForTox_TestMix_MRM.d
  - ForTox_TestMix_DMRM.d
  - ForTox_TestMix_TMRM.d
- Checkout Mix example report
- *MassHunter Forensics and Toxicology Triggered MRM Database and Library Quick Start Guide* (PDF)
- Technical Notes
- Application Notes
- Database content list
- Comprehensive Test Mix *Method Setup Guide* and method parameters

MassHunter Forensics and Toxicology Triggered MRM Database and Library disc

The contents of this disc are:

- MassHunter Forensics and Toxicology Triggered MRM Database and Library (ForTox_TriggeredMRM_B0601)
- MassHunter Forensics and Toxicology Test Mix Triggered MRM Database (ForTox_TestMix_TriggeredMRM_B0601)

See "Installation" on page 7 for a list of software requirements.

Other Parts

If you purchase the G1734BA MassHunter Forensics and Toxicology Triggered MRM Database and Library Kit, you also receive these parts.

ZORBAX LC Column (p/n 959757-902) Eclipse Plus C18, 2.1 mm x 50 mm, 1.8 µm. Use this column for fast analysis of small sets of targeted analytes.

ZORBAX LC Column (p/n 959758-902) Eclipse Plus C18, 2.1 mm × 100 mm, 1.8 µm. Use this column to analyze the LC/MS Forensics and Toxicology Checkout Mix and the LC/MS Comprehensive Forensics and Toxicology Mix.

Poroshell 120 Column (p/n 693775-902) EC-C18, 2.1 mm x 150 mm, 2.7 µm. Use this column to analyze the LC/MS Comprehensive Forensics and Toxicology Mix and for further method development.

LC/MS Forensic/Toxicology Checkout Test Mix (p/n 5190-0556) One 2-mL ampoule containing 1 mL of Checkout Mix (13 compounds). The contents are listed in "Checkout Mix Content" on page 70.

Where to find more information

Application Notes and Publications You can find information about the MassHunter Forensics and Toxicology Triggered MRM Database and Library for Forensics and Toxicology analysis in the application notes and publications included on the Support Disc.

The Support Disc also includes the acquisition methods used in this kit, in PDF format, so that you can set up nonstandard LC/MS/MS configurations.

The configuration of the example methods is:

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

To run the Checkout Mix, refer to the list of instruments in "Recommended configuration to run the Checkout Mix" on page 8.

See "Forensics and Toxicology LC Parameters" on page 71 for a listing of all of the LC acquisition parameters.

For more information on Agilent products, go to http://www.chem.agilent.com/.

Before You Begin

Installation

To run the Checkout Mix

1 Check that the Agilent 1200 Series LC is properly installed and verified.
2 On the Agilent 1200 Series Binary Pump SL, check that the mixer and damper are bypassed. See "To bypass mixer and damper" on page 12 for details.

   You only need to bypass the mixer and damper if you have a G1312B Agilent 1260 Infinity Binary Pump.
3 Check that the Agilent 6400 Series Triple Quadrupole LC/MS instrument is properly installed and verified.

To search on the Triggered MRM database:

1 Check that the following programs are properly installed:
   - MassHunter Data Acquisition B.06.00 or higher
   - MassHunter Quantitative Analysis B.05.02 or higher.
   - MassHunter Qualitative Analysis B.06.00 or higher
2 Install the MassHunter Forensics and Toxicology Triggered MRM Database and Library. Follow the installation instructions in the Readme file on the database installation disc.
3 Copy the methods from the Support Disc to the **D:\MassHunter\Methods** folder, or to a subfolder in **Methods** folder.

Required reagents and parts (to run the Checkout Mix)

- methanol, highest purity
- 5M ammonium formate (p/n G1946-85021)
- formic acid, highest purity (Agilent p/n G2453-85060 or equivalent)
- ZORBAX LC Column (p/n 959758-902), Eclipse Plus C18, 2.1 mm × 100 mm, 1.8 µm

Recommended configuration to run the Checkout Mix

To run the Checkout Mix, the recommended configuration is:

- Agilent G4226A HiP Sampler
- Agilent G4220A Binary Pump
- Agilent G1316C Column Compartment

Running the Checkout Mix

Do the steps in this section if you purchased the G1734BA MassHunter Forensics and Toxicology Triggered MRM Database and Library Kit, and you want to run the Checkout Mix to collect example data. Otherwise, use the example data that is included with the MassHunter Forensics and Toxicology Triggered MRM Database and Library Support Disc to do the exercises in this guide.

The sample data files provided in the Support Disc were acquired with the Checkout Mix on a system with the LC/MS system configured as described in "Installation" on page 7. Along with the sample data files are the methods with which these data files were acquired. If you review the acquisition method and sample data, you will get an idea of the data acquisition, data processing, and result interpretation when using the MassHunter Forensics and Toxicology Triggered MRM Database and Library.

To review the acquisition method, use the MassHunter Data Acquisition program to open the method file. The following data acquisition settings for the compounds are listed:

- Acquisition method info
- Triple Quadrupole LC/MS settings (see "Forensics and Toxicology LC Parameters" on page 71)
- Sampler settings
- Binary pump settings
- Thermostatted column compartment settings

Refer to "Primary and Secondary Transitions for Triggered MRM" on page 74 for MS/MS transitions and their compound-dependent settings.

To run the Checkout Mix

Run the LC/MS Forensic/Toxicology Checkout Test Mix (p/n 5190-0556) to get a better idea of how the database kit will work for you.

This guide describes the detailed workflow to set up MRM, dMRM and tMRM methods for compounds in the Forensics and Toxicology Checkout Mix. For compounds in Comprehensive Forensics and Toxicology Test Mix, please refer to the *Method Setup Guide* for the general workflow, MS parameters and LC column and mobile phase selections.

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

   Change to the Tune context in the MassHunter Data Acquisition program. Click **Checktune** to verify that 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 µg/mL (100 ppm).

   a Dilute 10 µL of the stock solution to 1.0 mL with 10% MeOH:H_2O to create the final solution concentration of 1 µg/mL (1 ppm).

   For more accurate results, and if conservation of sample is not a concern, dilute 100 µL of the stock solution to 10.0 mL of solvent instead.

   b Transfer 1 mL of the final sample solution to a standard 2-mL sample vial for analysis.

   The final solution is a 1 µg/mL (1 ppm) working solution.

NOTE For some instrument models, this sample concentration is too high. If you consistently see "saturated" warnings listed for some compounds, or if "*" indicators appear routinely above mass peaks in spectra, dilute the sample again by a factor of 10 or more, and inject the diluted sample.

3 Prepare mobile phases A and B.

- A= 5 mM ammonium formate in 0.01% formic acid in water
- B= 0.01% formic acid in methanol

4 Verify the system configuration.

The checkout method uses the system configuration listed in the next table. If your system deviates from this configuration, adjust the method as needed. Refer to the *Method Setup Guide* for the Comprehensive Test Mix that is included on the Support Disc.

Column	ZORBAX LC Column (p/n 959758-902), Eclipse Plus C18, 2.1 mm × 100 mm, 1.8 µm.
Wellplate Sampler	Sampler G1329B
Pump	Binary Pump – SL, Model 1312B If you are using a 1260 Binary Pump, you need to configure with damper and mixer bypassed. See "To bypass mixer and damper" on page 12
Column Compartment	Column – SL, Model G1316C or similar

5 Load the Checkout Mix method ForTox_TestMix_MRM.m.

6 Check that your method is set up to make a 2 µL injection.

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

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

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

   b Run the Checkout 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.

To bypass mixer and damper

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

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

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

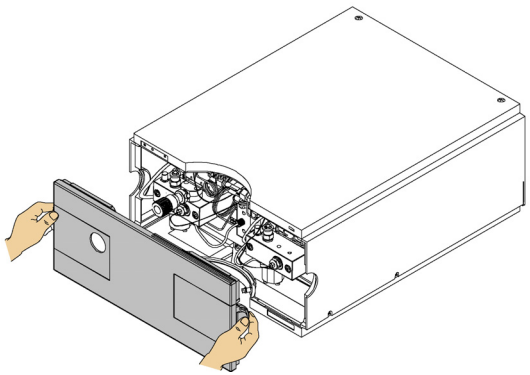
Tools required

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

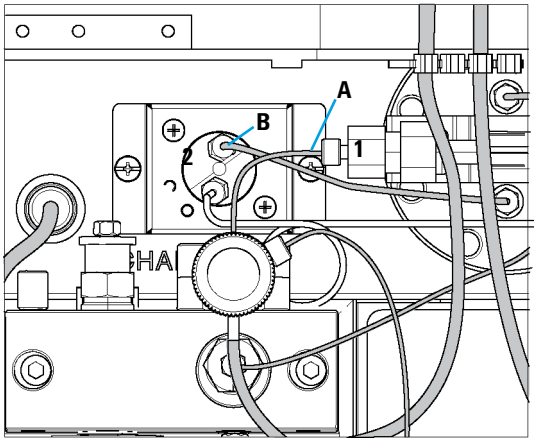
Preparations for this procedure

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

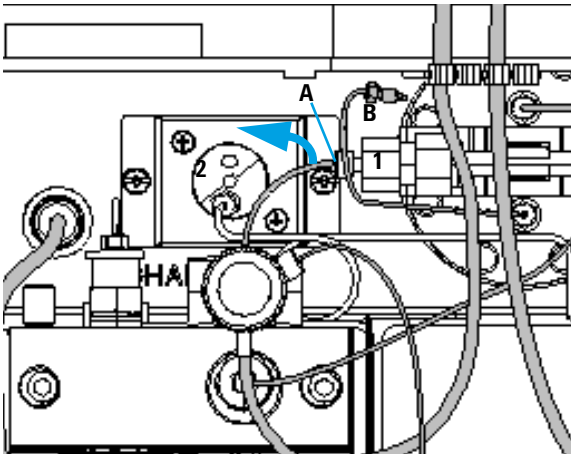
1 Remove the front cover by pressing the clip fastener on both sides of the cover.



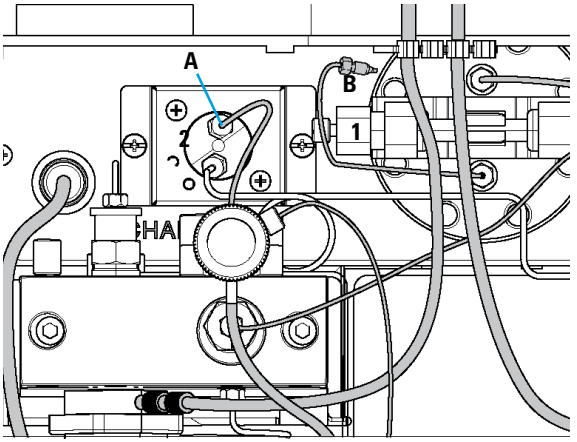
2 Use the 1/4 inch hex driver to remove fitting **B** from port *2* of the pressure sensor.



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



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



Creating an MRM acquisition method from the database

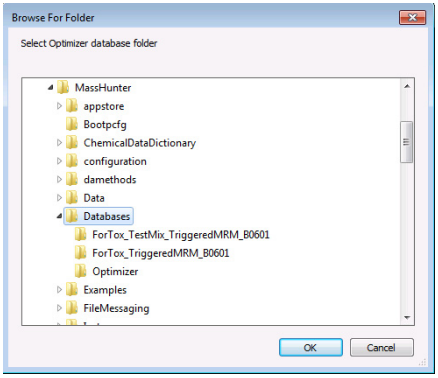
Task 1. Create an MRM method

Before you can create a Dynamic MRM (dMRM) or Triggered MRM (tMRM) Checkout Mix analysis method, you need a standard MRM data acquisition method in which settings such as compound names, ISTD (optional), MRM transitions, fragmentor voltages, and collision energies are defined. With the MassHunter Forensics and Toxicology Triggered MRM Database and Library, you can easily import all of these settings from the database to create an MRM method.

Steps	Detailed Instructions	Comments
1 In the Data Acquisition program, open the default method and save as: ***iii*ForTox_TestMix_MRM.m,** where *iii* are your initials.	**a** Start the Data Acquisition program. **b** Open the **default.m** method. **c** Click **Method > Save As**. **d** Type ***iii*ForTox_TestMix_MRM.m,** where *iii* are your initials.	• You can also load the ForTox_TestMix_MRM.m method from the Support disk.
2 Set the LC parameters according to "Forensics and Toxicology LC Parameters" on page 71. The following configuration was used to collect the included Checkout Mix data: • Agilent G4226A HiP Sampler • Agilent G4220A Binary Pump • Agilent G1316C Column Compartment	**a** In the Method Editor window, click the **HiP Sampler** tab. **b** Enter the parameters from Figure 1 on page 71. **c** Click the **Binary Pump** tab. **d** Enter the parameters from Figure 2 on page 72. **e** Click the **Column Comp.** tab. **f** Enter the parameters from Figure 3 on page 73.	• If you have a different LC model, the LC parameters can be different.

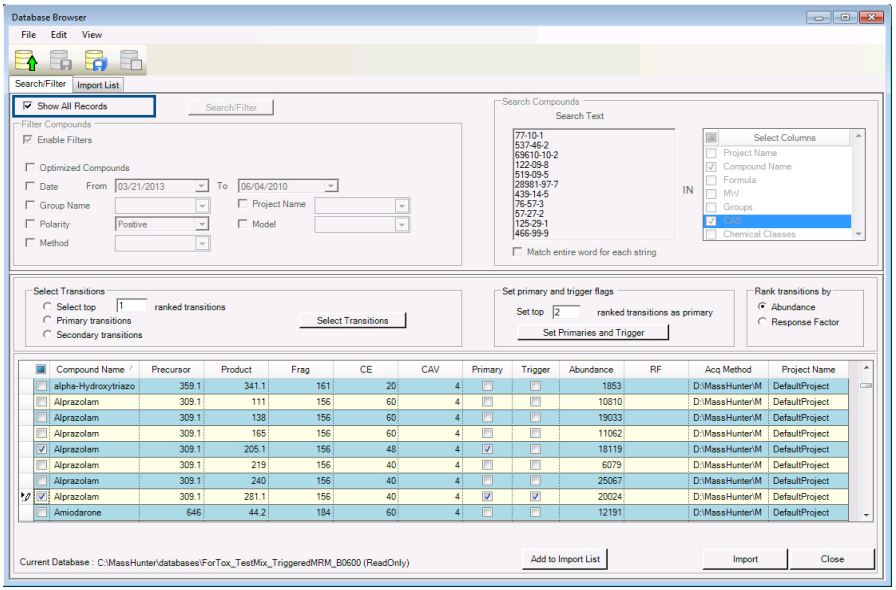
Steps	Detailed Instructions	Comments
3 Set the source parameters and change the method to an MRM method.	The values listed here are for non-iFunnel instruments for use with the Checkout Mix. For iFunnel instruments, refer to the *Method Setup Guide* included on the Support Disc. **a** In the Method Editor window, click the **QQQ** tab. **b** Click the **Source** tab. **c** For **Gas Temp,** type 300. **d** For **Gas Flow,** type 7. **e** For **Nebulizer,** type 40. **f** For **Sheath Gas Temp,** type 375. **g** For **Sheath Gas Flow,** type 11. **h** For **Capillary,** type 3500. **i** For **Nozzle** voltage, type 0. **j** Click the **Acquisition** tab. **k** In the Time segments on the left side of the QQQ tab, select **MRM** as the **Scan Type** for the first Time segment.	• The Scan segments table always has to have at least one row. You manually remove this row after importing transitions from the Database Browser.
4 Open the **ForTox_TestMix_TriggeredMRM_B0601 database** in Database Browser, from the QQQ Acquisition tab.	**a** Right-click the Scan segments table and click **Import from Database Browser**. The Database Browser opens. In the Database Browser, click **File > Open Database**. **b** Select the **ForTox_TestMix_TriggeredMRM_B0601** database in the **\MassHunter\databases** folder. **c** Click **OK**.	

Steps	Detailed Instructions	Comments

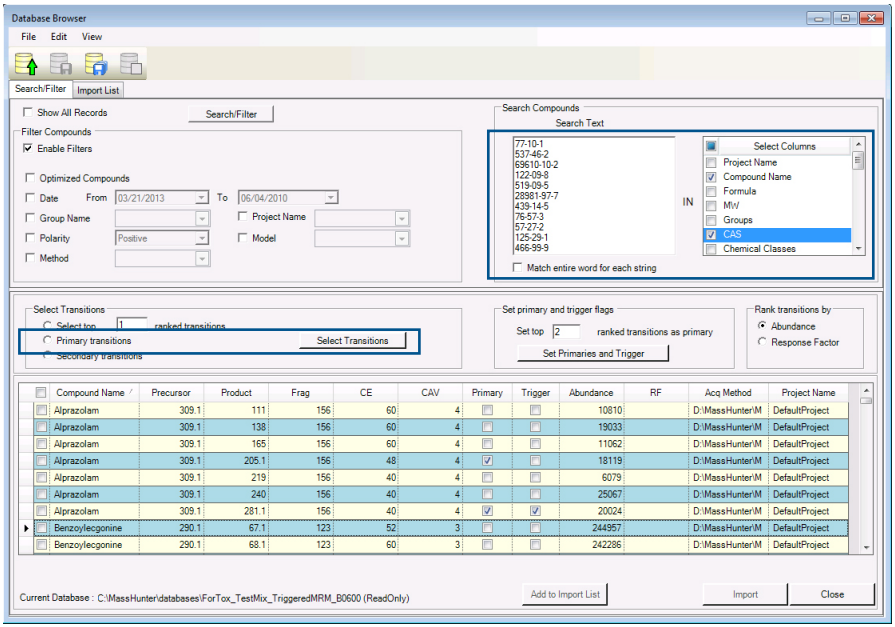


Steps	Detailed Instructions	Comments
5 Select primary transitions. • See "Primary and Secondary Transitions for Triggered MRM" on page 74 for a list of the Primary transitions. • The secondary transition are added when you are creating the triggered MRM method. See "Task 1. Create a tMRM method from a dMRM method" on page 49. Note that in the example data file, when both polarities are available in the **ForTox_TestMix_TriggeredMRM_B0601** database, the analysis was run in positive mode only.	**a** Click the **Compound Name** column header to sort the compounds by Compound Name. **b** Mark the check boxes next to the primary transitions for each of the compounds in the "Primary and Secondary Transitions for Triggered MRM" on page 74. **c** Review the transitions in the table. Clear the check box next to any transitions that you do not want to include.	• Instead of individually marking each check box, you can use the search and filter function with the Select Transitions options to select a number of transitions according to the criteria you have specified. Refer to the help for the Database Browser Search Filter tab in the Optimizer Help. • The CAS number is a reliable item to use to filter the compounds. If you use the Compound Name, you have to spell the name exactly as it is written in the database; otherwise, you get too many random hits which you then have to remove from your import list. Also, you have to write the name or number as a vertical list (a new line for each name or number). • Qualifier and quantifier ions must have the same precursor, so one compound cannot contain both negative and positive polarity transitions. Refer to the *Method Setup Guide* for the more details on choosing the most selective transitions for your analysis.

Steps	Detailed Instructions	Comments

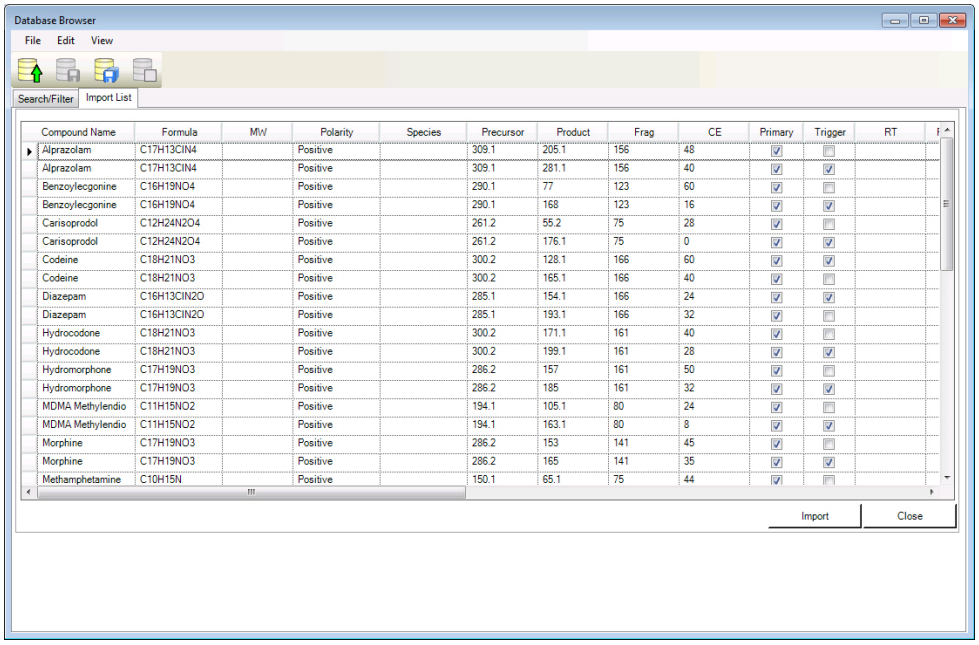


If you mark the Show All Records check box, then all compounds in the database are shown in the table. You scroll through the compounds and mark the Primaries for the compounds you are using.

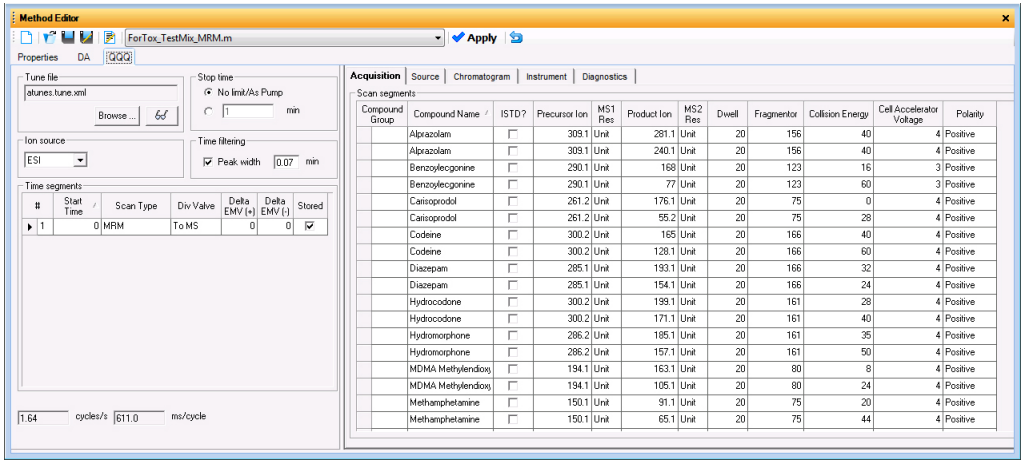


If you clear the Show All Records check box, you can limit the compounds that are shown in the table. In this example, the CAS check box is marked in the Search Compounds group and a list of CAS numbers was typed in the Search Text. Each CAS number was typed on a separate line. Only the compounds with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

Steps	Detailed Instructions	Comments
6 Import transitions to the Data Acquisition program. Remove any negative MRM transition for any compounds with positive MRM transitions.	**a** Click the **Add to Import List** button. **b** Click the **Import List** tab. **c** Review the Import List table. **d** Select all negative MRM transitions for any compounds with positive MRM transition. Right-click the selection, and then click **Remove**. **e** Click the **Import** button.	• Only the transitions that you marked are added to the Import List.



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



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

Task 2. Acquire MRM data and inspect 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.

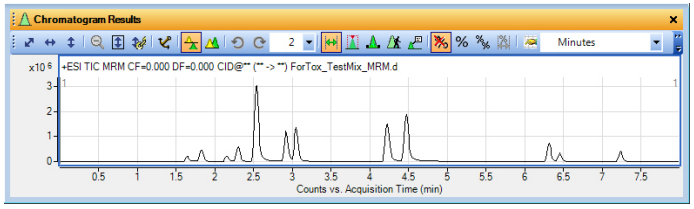
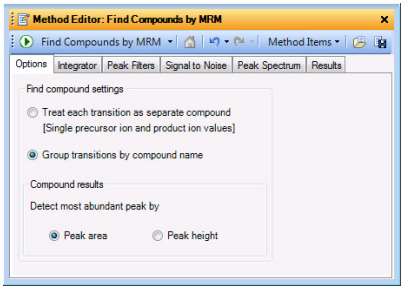
Isomeric compounds are identified during routine LC/MS by injecting an authentic sample of each isomer and determining its retention time under the chromatographic conditions used for the analysis. The retention time is needed for identification in cases where the MS-MS spectra of these isomers are very similar. The LC/MS Forensic/Toxicology Checkout Test Mix (p/n 5190-0556) contains three sets of isomers:

- Morphine and Hydromorphone
- Codeine and Hydrocodone
- Methamphetamine and Phentermine

The retention time order of the compounds in the Checkout Test Mix have been determined using the Eclipse Plus C18 column and mobile phases specified in the "To run the Checkout Mix" on page 10. The expected retention time order is

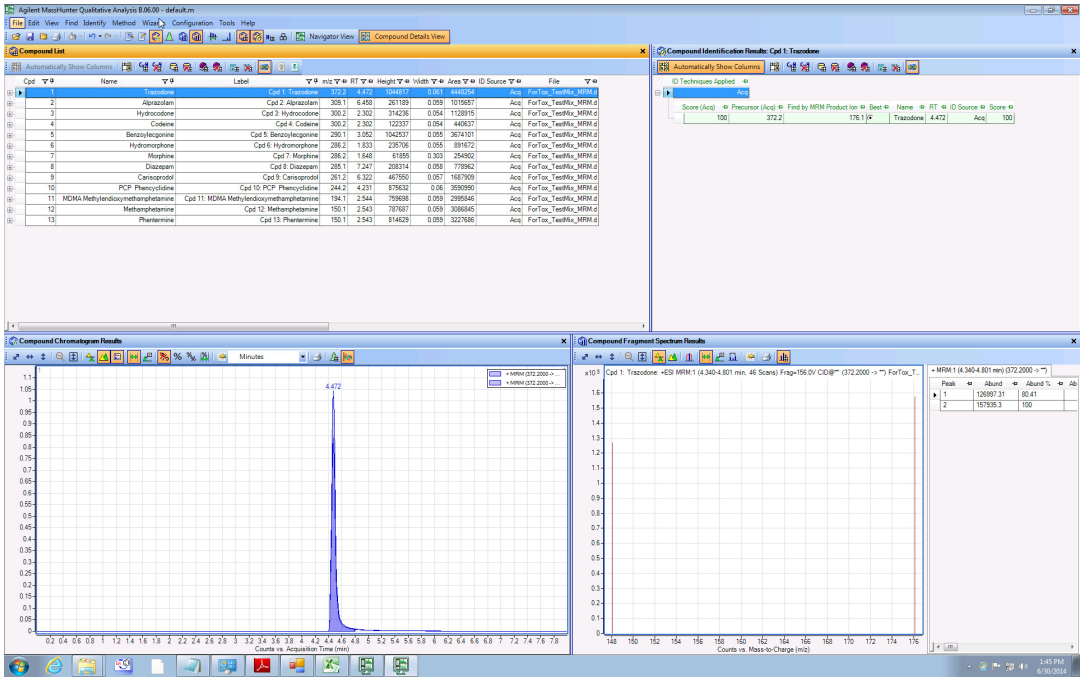
- Morphine
- Hydromorphone
- Codeine
- Hydrocodone
- MDMA/Methylendioxymethamphetamine
- Methamphetamine
- Phentermine
- Benzoylecgonine
- PCP/Phencyclidine
- Trazodone
- Carisoprodol
- Alprazolam
- Diazepam

Steps	Detailed Instructions	Comments
Do this step if you want to acquire data with the Checkout Mix. Otherwise, continue at step 2. **1** Acquire data. • Set up a one-line worklist with the method you just created. • Name the data file ***ForTox_TestMix_MRM*.d**. • Designate a directory path to hold your data files and method.	**a** If necessary, click **View > Worklist** to display the Worklist window. **b** Click **Worklist > Worklist Run Parameters**. Verify that the parameters are set properly. Click **OK**. **c** Click **Worklist > Add Multiple Samples**. **d** Type *ForTox_TestMix_MRM*.d as the data file name **e** Select **ForTox_TestMix_MRM.m** as the method name. **f** Click the **Sample Position** tab. **g** Select the Autosampler, Well-plate or Vial Tray. **h** In the graphic, select a single position. Click **OK.** **i** In the Worklist window, mark the check box to the left of the sample. **j** Click the **Start Worklist Run** icon in the main toolbar, the **Run Worklist** icon in the Worklist toolbar or click the **Worklist > Run** command.	• The Worklist window is tabbed with the Method Editor window by default. Click the **Worklist** tab at the bottom left corner of the program to show the Worklist window. • See also "To run the Checkout Test Mix" on page 11. • Make sure your dwell time for all transitions gives an appropriate cycle time. This criterion determines how many transitions you can put in one time segment. • 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. • Check that all the compounds are at medium level, an adequate analysis concentration so that they are all detected in the sample run and their retention times obtained. For easiest development of a dMRM method, all transitions in these data files must be detected.

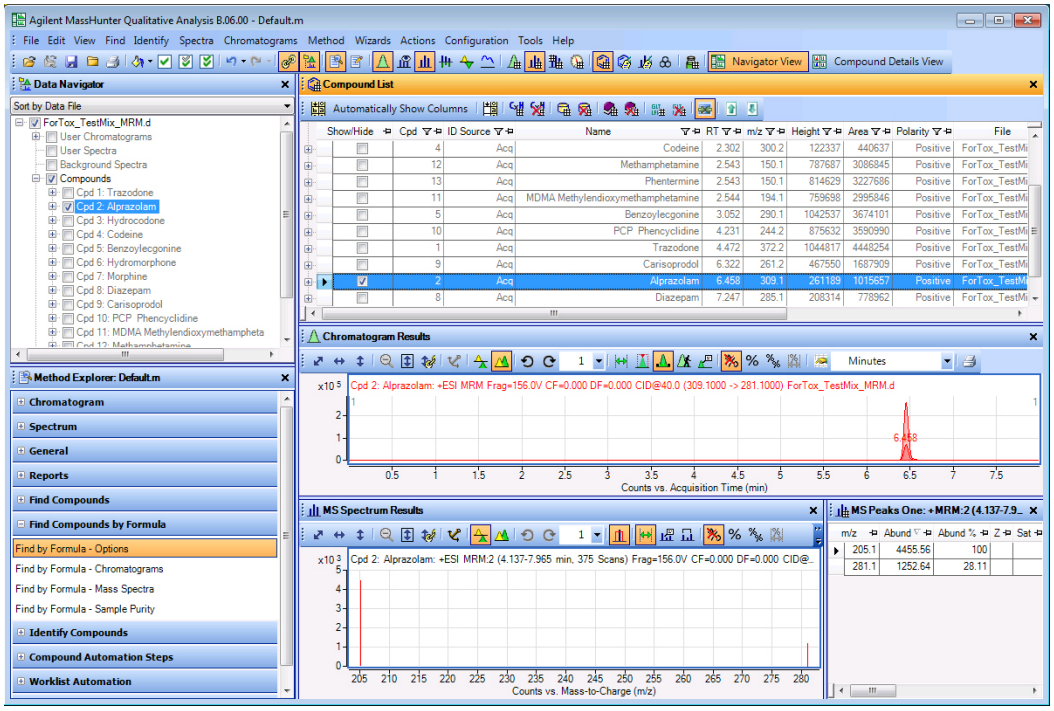
Steps	Detailed Instructions	Comments
2 Find compounds using the Find Compound by MRM algorithm. • Open the data file **ForTox_TestMix_MRM.d**.	**a** Start the Qualitative Analysis program. If it is not running, double-click the **Qualitative Analysis B.06.00** icon ( ). **b** Click **File > Open Data File**. The system displays the "Open Data File" dialog box **c** Select **ForTox_TestMix_MRM.d**, and click **Open**. **d** Click **Method > Method Explorer** or **View > Method Explorer**. The system displays the Method Explorer window. **e** In the Find Compounds section, click **Find by MRM**. **f** In the Method Editor Window, click the **Group transitions by compound name** option. **g** Click the **Peak area** option for **Detect most abundant peak by**.	• If the Find by MRM section is not available, you need to modify the options available in the User Interface Configuration dialog box. You click **Configuration > User Interface Configuration**. Then, you mark the **Unit Mass** check box and the **MS/MS (QQQ, Q-TOF)** check box. Then, click **OK**. • You can also copy the example MRM data file from the Support Disc in the Example Data folder.
		
	h Click **Find > Find Compounds by MRM**.	

Steps	Detailed Instructions	Comments
3 Review the results of the Find Compounds by MRM algorithm. • Make sure that the primary ions are found for each compound. • Switch to the Compound Details view. • It is not possible to edit the retention times of compounds which are identified. • The retention times for pairs of isomers which have identical MRMs are listed under the Retention Time of the compound which is most abundant.	**a** Close the **Method Explorer** and **Method Editor** windows. **b** Click **View > MS Spectrum Peak List 1**. **c** Click **Compound Details View** to change the view. See the figures that follow. **d** Click or use the arrow keys to move through the **Compound Table** to review one compound a a time. **e** Review each compound. Verify that the primary transitions for each compound were found. Qualitative Analysis is the best program to do a quick review of the MRM compound information and to check the chromatography of multiple data files. Manual editing of these retention times can be performed in the Quantitative Analysis program. See "Task 1. Create a batch file from an existing MRM data file" on page 28.	• You can also print a Compound Report to review results. You click **File > Print > Compound Report**. The Compound Report sorts the compounds by retention time. • In the Chromatogram Results window, you can see the abundances for each transition. • In Compound Details View, click each compound, or use the  and  buttons in the compound list window to review the results. • In the Navigator view, you need to select a compound and click **Edit > Show > Only Highlighted** to show only that compound. You can switch compounds in the Data Navigator window, or you can click the  or  buttons in the Compound List window.

Steps	Detailed Instructions	Comments



Steps	Detailed Instructions	Comments



Creating a Dynamic MRM acquisition method

For the MassHunter Forensics and Toxicology Triggered MRM Database and Library, Agilent recommends that you create a Quantitative Analysis report to specify in "Task 3. Create a dMRM method using Update dMRM" on page 38. The check out mix contains many isobaric compounds, and they fragment in similar ways.

The process for 150 standards is described in Figure 1. 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.

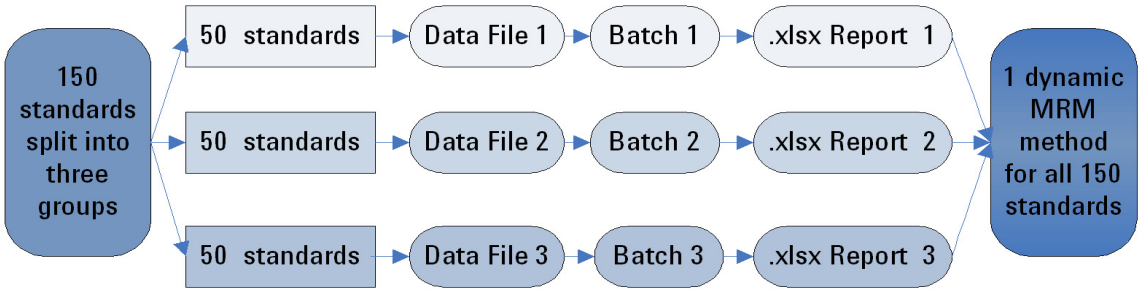
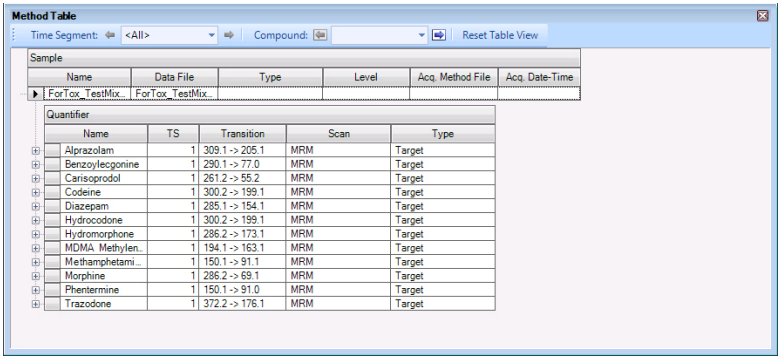


Figure 1 Example process for analyses that have more than 50 compounds

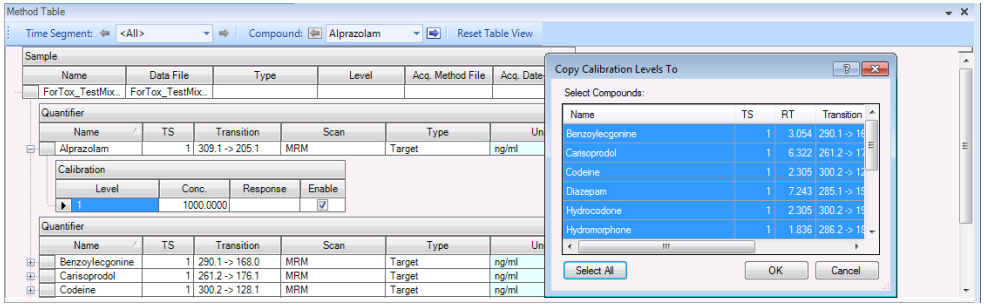
Task 1. Create a batch file from an existing MRM data file

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

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



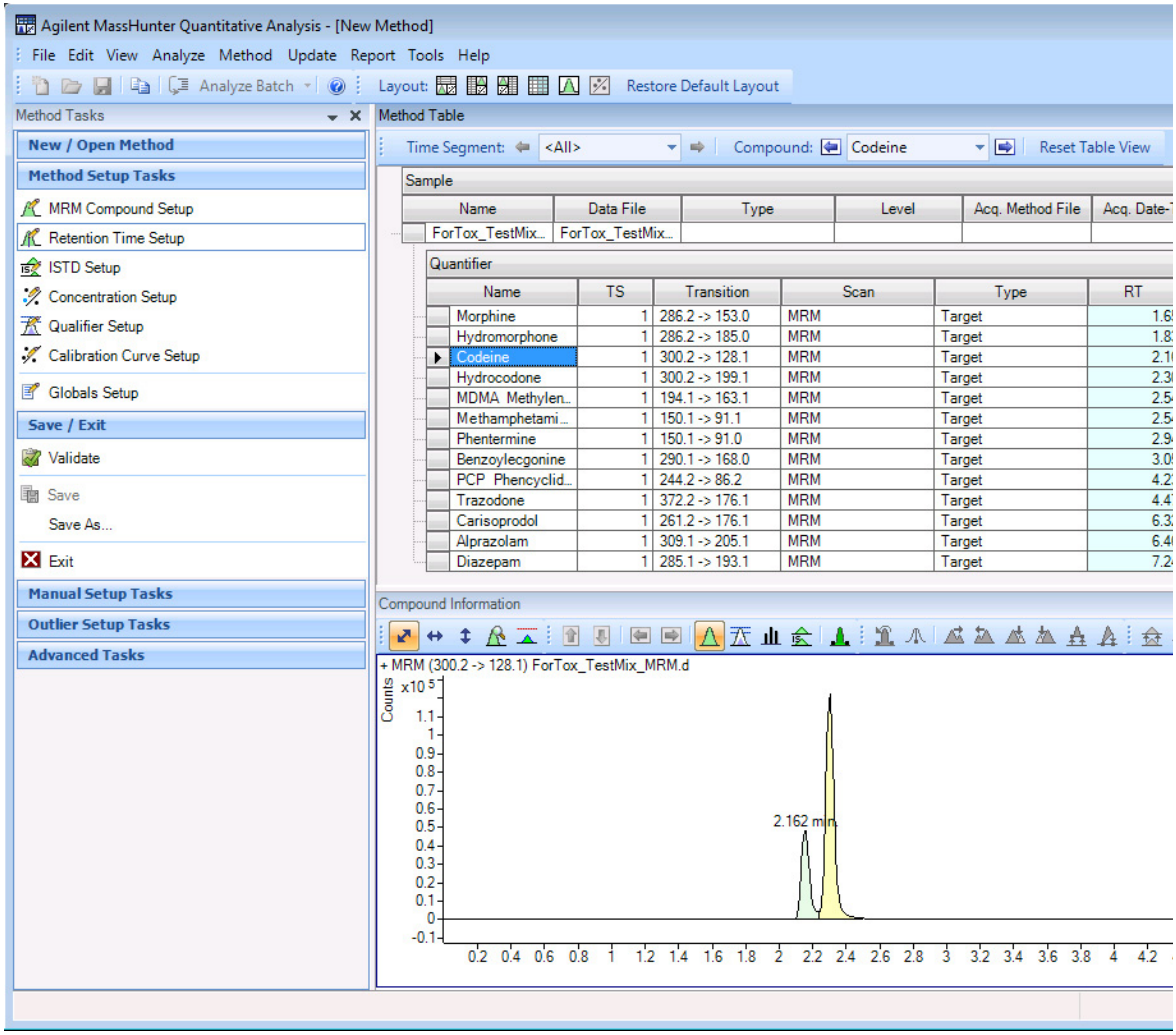
Steps	Detailed Instructions	Comments
3 Set the Concentration Setup, Qualifier Setup, and Calibration Curve Setup. • Add calibration level 1 with a concentration of 1000. • Set the **Uncertainty** to Relative for all qualifiers. • Set the **Curve Fit** to Linear. • Set the **Curve Fit Origin** to **Force**. • Set the **Curve Fit Weight** to None.	**a** Select **Concentration Setup** in the Method Setup Tasks section in the Method Tasks pane. **b** Select the first compound in the table. **c** Right-click the compound row and click **New Calibration Level** from the shortcut menu. **d** In the **Level** column, type `1`. In the **Conc.** column, type `1000`. **e** Right-click in the Level box and click **Copy Calibration Levels To**. **f** Click **Select All**. Click **OK**. **g** Select **Qualifier Setup** in the Method Setup Tasks section. **h** Verify that the **Uncertainty** is Relative. **i** Select **Calibration Curve Setup** in the Method Setup Tasks section. **j** Set **Curve Fit** to **Linear** for all compounds. **k** Set **CF Origin** to **Force** for all compounds. **l** Set **CF Weight** to **None** for all compounds.	• Refer to the online Help in the Quantitative Analysis program for additional help on these tasks. • After you select the option for the first compound in the Method Table, you can right-click the option and click **Fill Down** from the shortcut menu.



Steps	Detailed Instructions	Comments

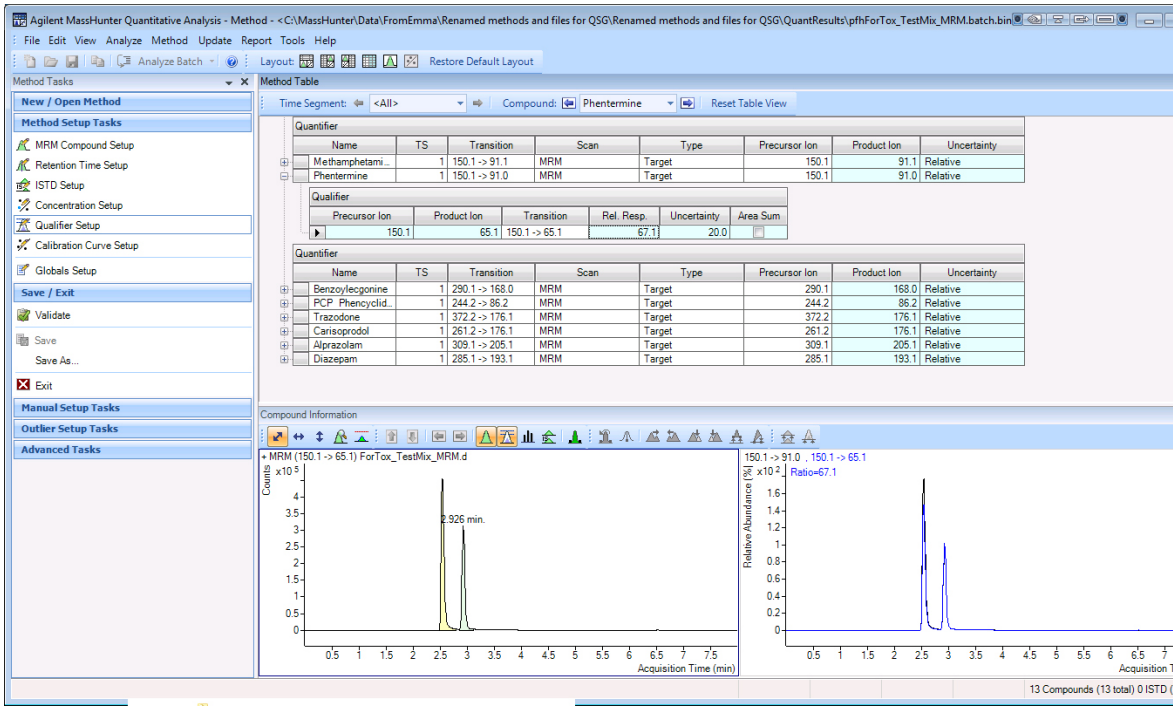
• Resolve the isomers, listed below in retention time elution order:
  • Morphine and Hydromorphone
  • Codeine and Hydrocodone
  • Methamphetamine and Phentermine
• Verify retention time elution order:
  • Morphine
  • Hydromorphone
  • Codeine
  • Hydrocodone
  • MDMA/Methylendioxymethamphetamine
  • Methamphetamine
  • Phentermine
  • Benzoylecgonine
  • PCP/Phencyclidine
  • Trazodone
  • Carisoprodol
  • Alprazolam
  • Diazepam
	m Select **Retention Time Setup** in the Method Setup Tasks section.
	n (optional) Enter `10` for the **Left RT Delta** and **Right RT Delta** for each compound.
	o Verify the retention time order of the isomers is the same as shown in the figure below. 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 isomers that share the same precursor and product ion are seen. In cases where there are multiple isomers, the automatic processing always picks the more abundant peak.
		• When using the example data, the Retention Time for Codeine must be changed to 2.162 minutes.
		• When using the example data, the Retention Time for Phentermine must be changed to 2.946 minutes.
		• When you look at the Chromatogram in the Compound Information window, you can see that multiple peaks exist for each of the isomers. You set the retention time to select the other peak in the chromatogram for Codeine and Phentermine.

Steps	Detailed Instructions	Comments



This image is zoomed in to only show you the

Steps	Detailed Instructions	Comments
• Review qualifier ratios	**p** Select **Qualifier Setup** in the Method Setup Tasks section. **q** Right-click the Method Table and click **Expand All**. **r** Click **View > Window Layout > Restore Default Layout**. **s** Click **View > Sample Information** to close the Sample Information window. **t** Click the Show/Hide Qualifiers button in the toolbar in the Compound Information window. **u** Click on each compound and verify that the Rel. Resp. for each Qualifier matches the value shown in the Compound Information window in the spectrum pane.	• The qualifier ratio shown in the **Rel. Resp.** column was updated for Codeine, Methamphetamine and Phentermine to match the values that are displayed in the spectrum pane in the Compound Information window. • In the example below, the qualifier ratio for Phentermine was changed from 28.5 to 67.1



Steps	Detailed Instructions	Comments
4 Verify method and then save the method and apply the method to the batch.	**a** Click **Method > Validate.** **b** Click **OK** on the message box. Fix any errors, if necessary. **c** Click **Method > Save As**. **d** Type `ForTox_MRM_to_DMRM`. **e** Click the **Save** button. **f** Click **Method > Exit**. **g** Click **Yes** to apply the method to the batch.	
5 Analyze and save the batch.	**a** In the Batch Table window, select **Cal** as the **Type**. **b** Click **Analyze > Analyze Batch.** **c** Click **File > Save Batch**.	
6 Review the batch to resolve errors or messages that are indicated in the Batch Table.	• Resolve isomers. • Check qualifier ratios. • Resolve errors and messages	
7 Save the batch again.	• Click **File > Save Batch**.	

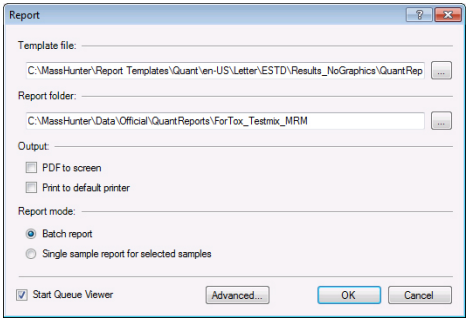
Task 2. Print a report in the Quantitative Analysis program

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

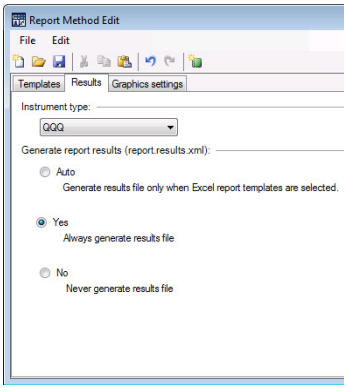
You can use either a Quantitative Analysis report or a data file to create a dMRM method, but the Quantitative Analysis report is recommended. If you use a data file and an error is generated, then none of the compounds in that data file are included in the dMRM method.

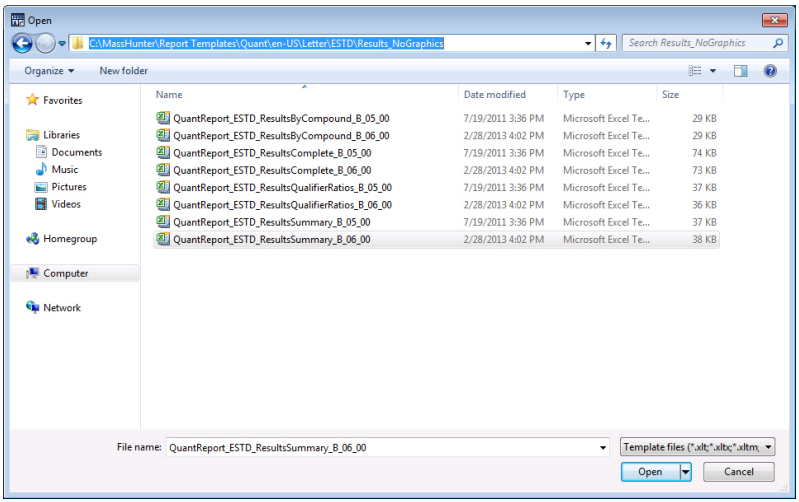
In this task, you:

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

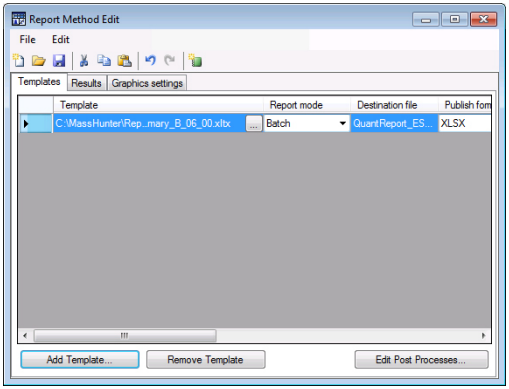
Steps	Detailed Instructions	Comments
1 (*For Quantitative Analysis B.05.02*) Print a report. Use a template that creates a summary report without graphics for fastest report creation.	**a** See "Task 1. Create a batch file from an existing MRM data file" on page 28. **b** Click **File > Save**. **c** Click **Report > Generate**. The system displays the Report dialog box. **d** Select a **Template** file. **e** Select the **Report** folder. This folder name is used in the next task. **f** Click **OK**.	• For this report, you do not need to print the report. You need to click **Advanced** to select a different printer. If you don't want to print this report, click **Advanced** instead.
		

Steps	Detailed Instructions	Comments

1 (*For Quantitative Analysis B.06.00*) Print a report. Use a template that creates a summary report without graphics for fastest report creation.	**a** See "Task 1. Create a batch file from an existing MRM data file" on page 28.
	b Click **File > Save**.
	c Click **Report > Generate**. The Generate Report dialog box opens.
	d Under **Report method**, click **New**. The Report Method Edit program opens.
	e Click **Add Template**. The Open dialog box opens.
	f Navigate to the folder **MassHunter\Report Templates\Quant\en-US\Letter\ESTD\Results_NoGraphics**
	g Select any report in which the filename ends in **B_06_00**. Click **Open**.
		• Reports have one of these file name extensions: *.xlt, *.xltx, *.xltm, *.report.xml.
		• If you select a PDF-document template, you also need to:
		  • In the Report Method Edit program, click the **Results** tab.
		  • Click **Yes**.
		See next figure.
		 

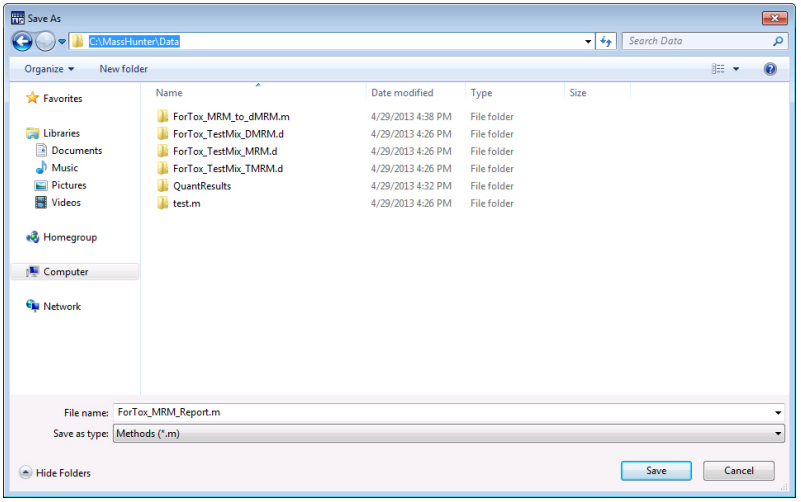
Steps	Detailed Instructions	Comments

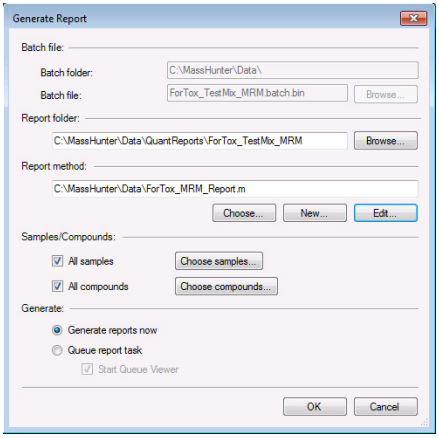


h Click **File > Save Method As**. The Save As dialog box opens.

i Navigate to the **\MassHunter\Data** folder.

j For the **File name**, type `ForTox_MRM_Report`. Click **Save**.



Steps	Detailed Instructions	Comments
	k In the Report Method Edit program, click **File > Exit**. **l** Click **Generate reports now**. **m** Click **OK**.	The report.results.xml file is in the **\MassHunter\Data\QuantReports\ ForTox_TestMix_MRM** folder.
		
2 Check the status of the report using the Queue Viewer program.	**a** Click **Report > Queue Viewer**. **b** Wait for the report to finish printing. **c** Close the **Task Queue Viewer** program.	

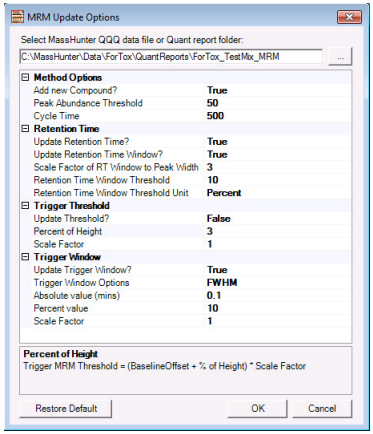
Task 3. Create a dMRM method using Update dMRM

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

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

Steps	Detailed Instructions	Comments
2 Update the method to change from an MRM method to a Dynamic MRM method with the same compounds. **3** If you have isomers in the data file, specify a report instead of a data file for the source of the update, to ensure that you identify the isomers correctly. Do not manually change the Scan Type to Dynamic MRM. If you do, the existing Scan segments table is cleared.	**a** Click the **Acquisition** tab in the QQQ tab in the Method Editor window. **b** Right-click the **Scan segments** table and click **Update DMRM Method**. The MRM Update Options dialog box opens. **c** Select the folder containing the *report.results.xml* file. The name of this folder is shown in the Report dialog box in the Quantitative Analysis program. By default, this file is in a folder in the QuantReports folder. The QuantReports folder is in the same folder as the Batch. By default, the folder has the same name as the Batch. **d** Under **Method Options**, for **Add new compound?**, select **True**. **e** Under **Retention Time**, for **Update Retention Time?**, select **True**. **f** For **Scale Factor of RT Window to Peak Width**, select **2**. **g** For **Retention Time Window Threshold**, select 1. **h** For **Retention Time Window Threshold Unit**, select **Minutes**. **i** Under **Trigger Threshold**, for **Update Threshold?**, select **False**. **j** Under **Trigger Window**, for **Update Trigger Window?**, select **True**. **k** For **Absolute value (mins)**, select **0.5**. **l** Review other parameters. **m** Click **OK**.	• The Update MRM Method tool automatically sets the Scan type to **Dynamic MRM**. • You can select either a data file that was acquired with a **Scan Type** of **MRM** or a Quant Report folder as the input to this dialog box. It is recommended to always specify the Quant Report. The Scan segments are created from one of these two input sources. • 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, particularly when multiple MRMs are being measured at the same time. • The respective dwell times for MRM transitions will also decrease, depending on the number of overlapping peaks and their respective peak widths. • The method is now updated with the transitions, parameters, and retention times found in the Quantitative Analysis report.

Steps	Detailed Instructions	Comments

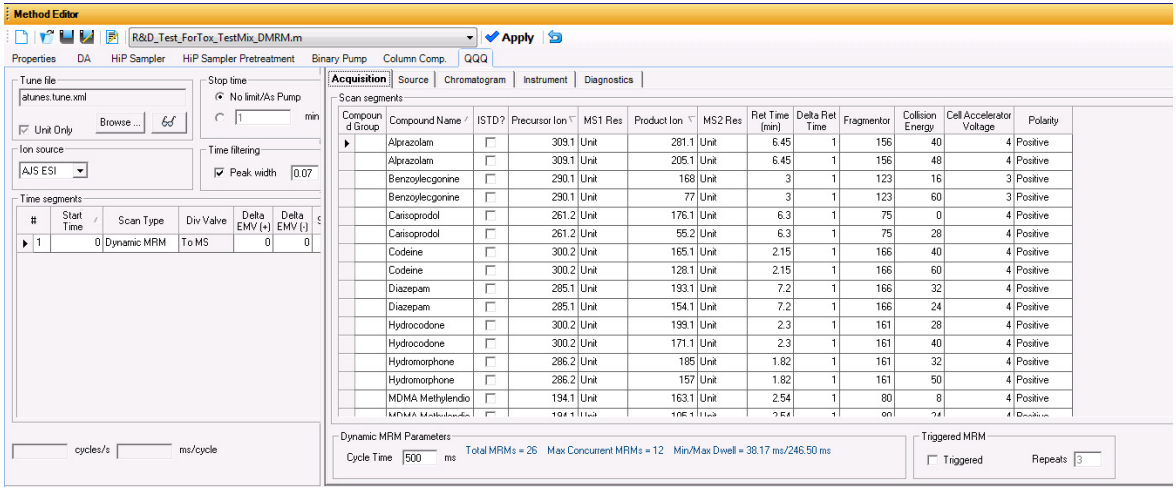


You can update the compounds in the Scan segments table by using a QQQ data file or a Quantitative Analysis report folder. If you select a QQQ data file and an error is generated when you click OK, manually create the report and select the report. See "Task 2. Print a report in the Quantitative Analysis program" **on page 34.**

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

Note that the cycle time in the MRM Update Options dialog box is applied only the first time the method is created using the Update Method function. After that, the cycle time must be manually typed into the QQQ > Acquisition tab.

When you close the method viewer, changes made to the cycle time in the viewer are not entered into the acquisition method.



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

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

p Click **Method > Save**.

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

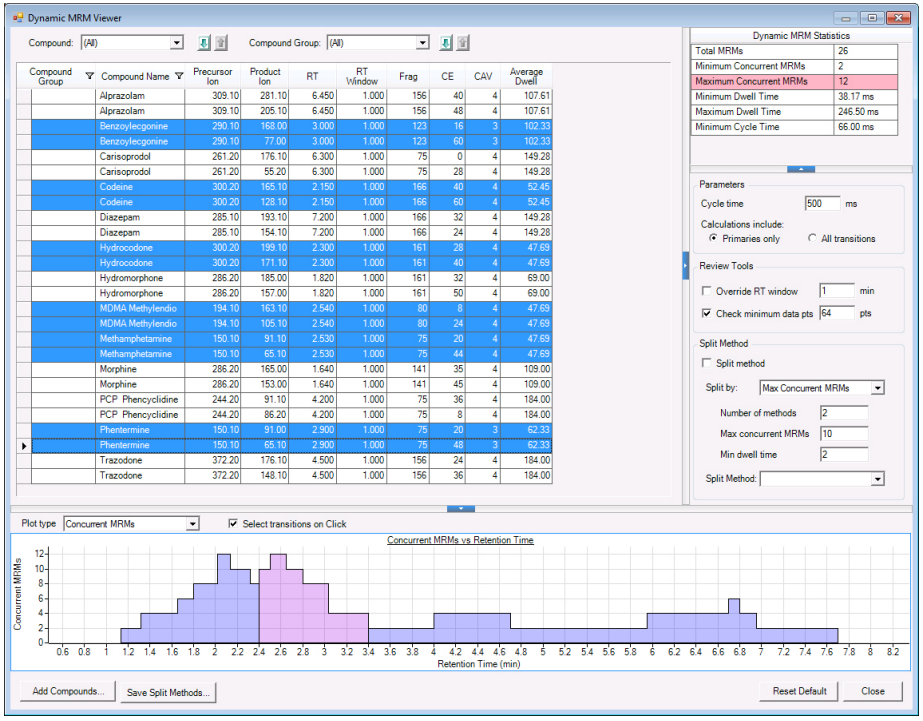
The Dynamic MRM Viewer provides a powerful display to show you important details of your method. The Average Dwell time in milliseconds is shown in the table.

- A dwell time of 3 ms or more is recommended to acquire data for dMRM. If both cycle time and overlapped peaks reduce the dwell time of a transition to below this value, that transition is highlighted and the minimum cycle time and dwell time on the right is also highlighted. Increase the cycle time to increase the minimum dwell time to correct the method problem.
- At a minimum, for good quantitative results, peaks must have at least 10 data points. In an example of a 3 second peak width, a cycle time of 500 ms barely provides this.
- If good quantitative results cannot be obtained because of too many overlapping peaks, select a retention time delta that will give less than 64 points. If you do, select the general integrator in the Quantitative Analysis method used to process standards and samples collected by this method. The default is the Agile integrator.

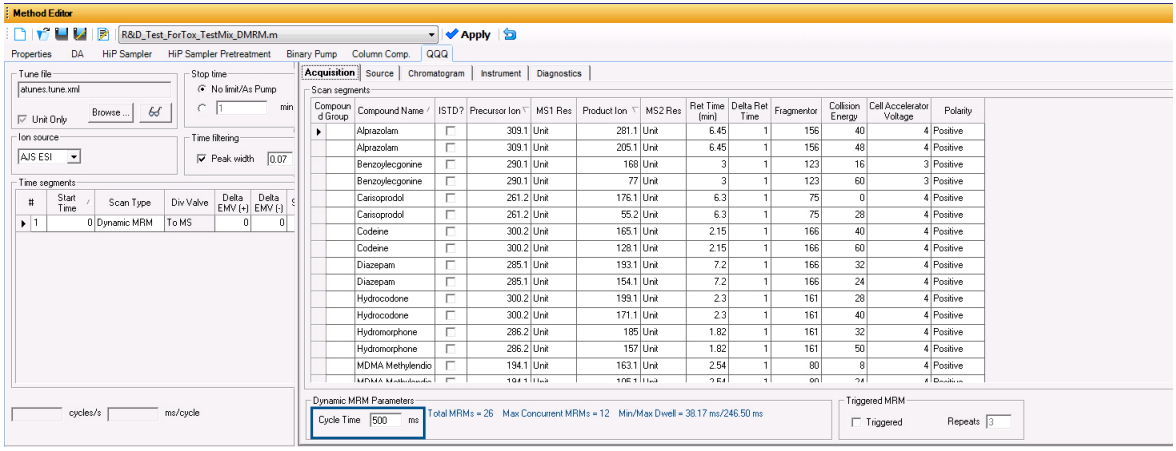
Good, reproducible chromatography will enable a large number of compounds to be analyzed in one method using dMRM.

Steps	Detailed Instructions	Comments
1 Start the Dynamic MRM Viewer dialog box.	• Right-click the Scan segments table and click **Edit DMRM Method**.	
2 Review each compound in the Dynamic MRM Viewer dialog box.	**a** Click each compound in the table. **b** Mark **Check minimum data pts**. **c** Verify in the table that two transitions are shown for each compound. **d** Examine the graphic to review how many concurrent MRMs are being acquired with that compound. **e** Adjust the cycle time so that all criteria for **Minimum Dwell Time**, for the Agile integrator and for good integration are met. If a compound has a red background, then that compound does not meet one of the requirements. **f** Click **Close**.	• To use the Agile integrator, 64 data points are required in the retention time window. Either increase the Delta Ret Time for the transition(s) with less than 64 points, or decrease the cycle time. As a general rule, set the retention time factor based on reproducibility of the chromatography. • The transition table in the Dynamic MRM Viewer shows the average dwell time of each transition based on the number of overlapping transitions and the Cycle Time that appears under Parameters.

Steps	Detailed Instructions	Comments



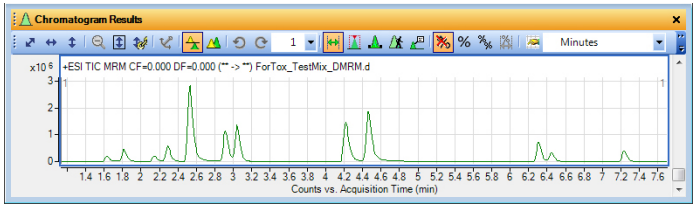
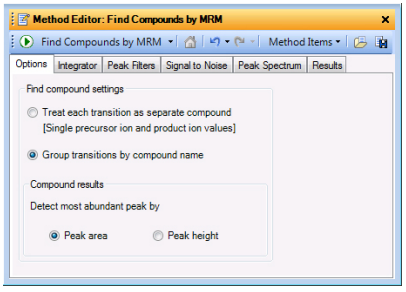
Steps	Detailed Instructions	Comments
		• When you change the cycle time in the Dynamic MRM viewer, you immediately see its effects on the **Minimum Dwell Time** and the **Average Dwell**. • When you decrease the cycle time, you effectively decrease the average dwell time for all transitions. As an alternative, you can increase the retention time window for the compounds that do not meet the 64 point criterion so that the dwell times of only the transitions overlapping with the extended window are decreased. To see the effect of a retention time window increase, you must close the viewer, change the retention time window in the acquisition method, and then open the viewer.
3 Once a cycle time is determined for good integration, set the Cycle Time in the QQQ > Acquisition tab.	**a** Type the **Cycle Time**, if necessary. **b** Save the method. The default setting of 500 ms is recommended for most analysis containing more than 15 compounds.	• When you close the Dynamic MRM Viewer, changes made to the cycle time in the Dynamic MRM Viewer are *not* entered into the acquisition method.



Task 5. Acquire dMRM data and inspect in Qualitative Analysis

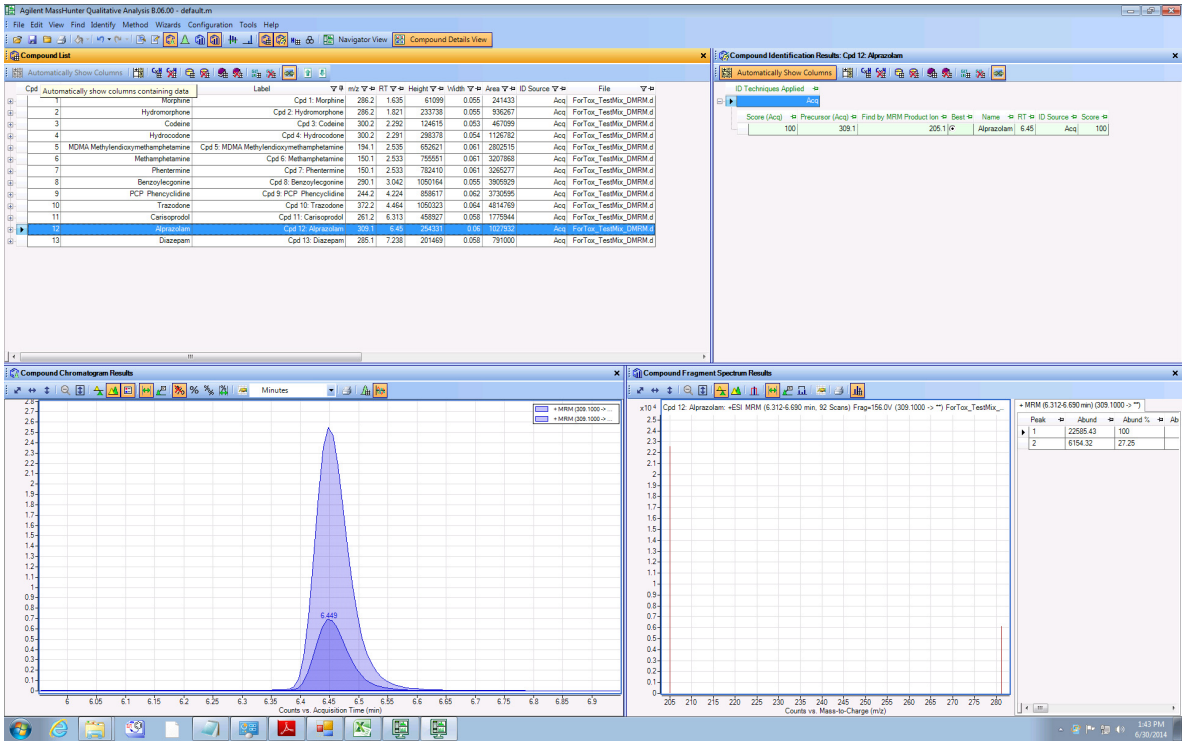
After you acquire the dMRM data file, you examine the data file in the Qualitative Analysis program to verify that the transitions were acquired.

Steps	Detailed Instructions	Comments
Do this step if you want to acquire data with the Checkout Mix. Otherwise, continue at step 2. **1** Acquire data. • Set up a one-line worklist with the method you just created. • Name the data file ***ForTox_TestMix_DMRM*.d**. • Designate a directory path to hold your data files and method.	**a** If necessary, click **View > Worklist** to display the Worklist window. **b** Click **Worklist > Worklist Run Parameters.** Verify that the parameters are set properly. Click **OK**. **c** Click **Worklist > Add Multiple Samples**. **d** Type `ForTox_TestMix_DMRM.d` as the data file name **e** Select **ForTox_TestMix_DMRM.m** as the method name. **f** Click the **Sample Position** tab. **g** Select the Autosampler, Well-plate or Vial Tray. **h** In the graphic, select a single position. Click **OK.** **i** In the Worklist window, mark the check box to the left of the sample. **j** Click the **Start Worklist Run** icon in the main toolbar, the **Run Worklist** icon in the Worklist toolbar or click the **Worklist > Run** command.	• The Worklist window is tabbed with the Method Editor window by default. Click the **Worklist** tab to show the Worklist window. • See also "To run the Checkout Test Mix" on page 11. • Check that all the compounds are at medium level, an adequate analysis concentration so that they are all detected in the sample run and their retention times obtained. For easiest development of a dMRM method, all transitions in these data files must be detected.

Steps	Detailed Instructions	Comments
2 Find compounds using the Find Compound by MRM algorithm in the Qualitative Analysis program. • Open the data file **ForTox_TestMix_DMRM.d**.	**a** Start the Qualitative Analysis program. If it is not running, double-click the **Qualitative Analysis B.06.00** icon,  . **b** Click **File > Open Data File**. The system displays the "Open Data File" dialog box **c** Select **ForTox_TestMix_DMRM.d**, and click **Open**. **d** Click **Method > Method Explorer** or **View > Method Explorer**. The system displays the Method Explorer window. **e** In the Find Compounds section, click **Find by MRM**. **f** In the Method Editor window, click the **Group transitions by compound name** option. **g** Click the **Peak area** option for **Detect most abundant peak by**.	• If the Find by MRM section is not available, you need to modify the options available in the User Interface Configuration dialog box. You click **Configuration > User Interface Configuration**. Then, you mark the **Unit Mass** check box and the **MS/MS (QQQ, Q-TOF)** check box. Then, click **OK**. • You can also copy the example dMRM data file from the Support Disc in the Example Data folder.
		
	h Click **Find > Find Compounds by MRM**.	

Steps	Detailed Instructions	Comments
3 Review the results of the Find Compounds by MRM algorithm. • Make sure that the transitions are found for each compound. • These transitions become the primary transitions when you create a tMRM method. • It is not possible to edit the retention times of compounds which are identified. • The retention times for pairs of isomers which have identical MRMs are listed under the RT of the compound which is most abundant.	**a** Close the **Method Explorer** and **Method Editor** windows. **b** Click **Compound Details View** to change the view. See the figure that follows. **c** Click or use the arrow keys to move through the **Compound Table** to review one compound a a time. **d** Verify that the transitions for each compound were found. Qualitative Analysis is the best program to do a quick review of the MRM compound information and to check the chromatography of multiple data files. Manual editing of these retention times can be performed in the Quantitative Analysis program. See "Task 1. Create a batch file from an existing MRM data file" on page 28.	• You can also print a Compound Report to review results. You click **File > Print > Compound Report**. The Compound Report sorts the compounds by retention time.

Steps	Detailed Instructions	Comments



Creating a Triggered MRM Method

In this section, you create a Triggered MRM (tMRM) method from a Dynamic MRM (dMRM) method.

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 again when standards are diluted in a complex sample matrix due to possible signal/noise differences between compounds in solvent and in matrix.

Triggering parameters and their function

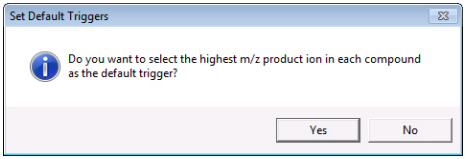
Trigger Entrance Use this parameter to shift the acquisition of secondary ions towards apex of peak. When the signal for the designated primary MRM transitions cross the triggering Threshold, the Trigger Entrance Delay postpones triggering for a user-defined number of cycles, which moves the acquisition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

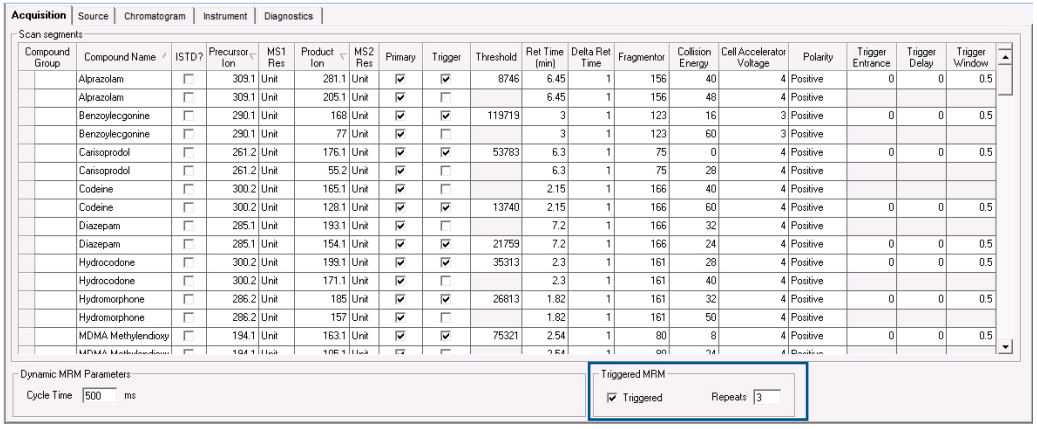
Trigger Window Use this parameter to confine the activation of all triggering functions to a user-defined window around the expected retention time for a particular peak. This function increases triggering specificity based on the target compounds and known retention times for a particular tMRM method.

Task 1. Create a tMRM method from a dMRM method

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

Steps	Detailed Instructions	Comments
1 In the Data Acquisition program, you open the dMRM method: **ForTox_TestMix_DMRM.m** You can open the method that you created or the example method.	**a** Switch to the Data Acquisition program. **b** Open the **ForTox_TestMix_DMRM.m** method.	• An example **ForTox_TestMix_DMRM.m** method is included on the Support disk.
2 Change the method to a tMRM method and start to import the secondary transitions from the Database Browser.	**a** In the Method Editor window, click the **QQQ** > **Acquisition** tab. **b** Mark the **Triggered** check box under Triggered MRM. **c** Click **No** in the Set Default Triggers message box. **d** Manually mark the Triggers shown in the "Primary and Secondary Transitions for Triggered MRM" on page 74. **e** Type 3 for **Repeats**. **f** Right-click the Scan Segments table and click **Import from Database Browser**. The Database Browser opens.	• The Scan segments table always has to have at least one row. • The triggering information is loaded from the Database Browser even if the **Triggered** check box is clear.
		

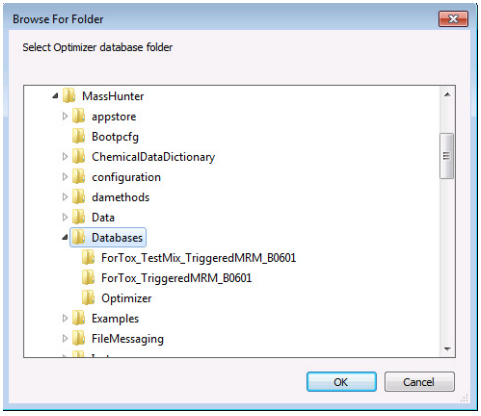
Steps	Detailed Instructions	Comments



3 Open the **ForTox_TestMix_TriggeredMRM_B0601** database in the Database Browser.

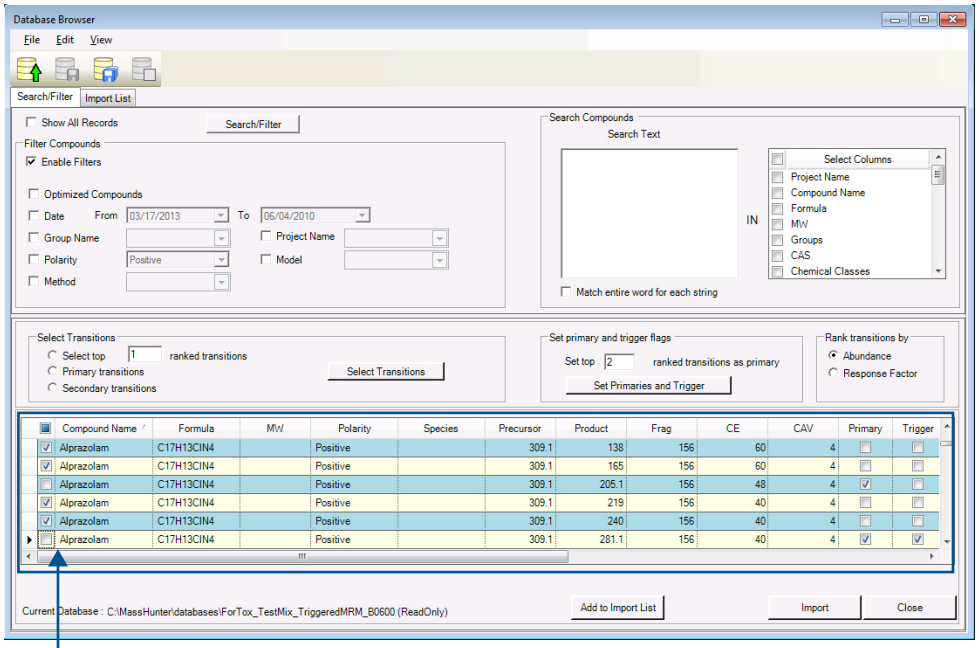
a In the Database Browser, click **File > Open Database**.
b Select the **ForTox_TestMix_TriggeredMRM_B0601** database in the MassHunter\databases folder.
c Click **OK**.

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



Steps	Detailed Instructions	Comments
4 Select secondary transitions.	**a** Click **Abundance** under Rank transitions by. **b** Click the **Secondary transitions** option under Select Transitions. **c** Click the **Compound Name** column header to sort the compounds by Compound Name. **d** Mark the check boxes next to the secondary transitions for each of the compounds in the dMRM method. See "Primary and Secondary Transitions for Triggered MRM" on page 74. **e** Review the transitions in the table. Clear the check box next to any secondary transition that you do not want to include.	• The *Alprazolam* compound has two primary transitions and five secondary transitions. • You can also clear the **Show All Records** check box. Then, you can search for each compound in the database by writing on separate lines the full name or CAS number of each compound in the **Search Text** list, mark the **Compound Name** or **CAS** check box, and then click **Search Filter**. • Once you have the list of desired compounds, click the **Secondary transitions** button and then click **Select Transitions**. All of the secondary transitions for the compounds in the table are marked.

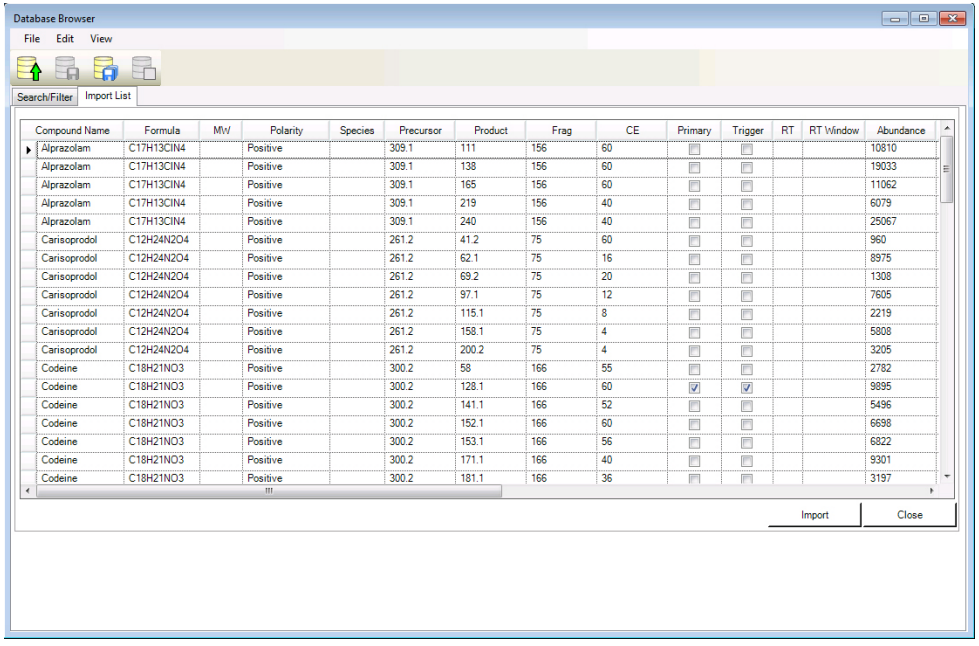
Steps	Detailed Instructions	Comments



Do not mark the two Primary transitions.

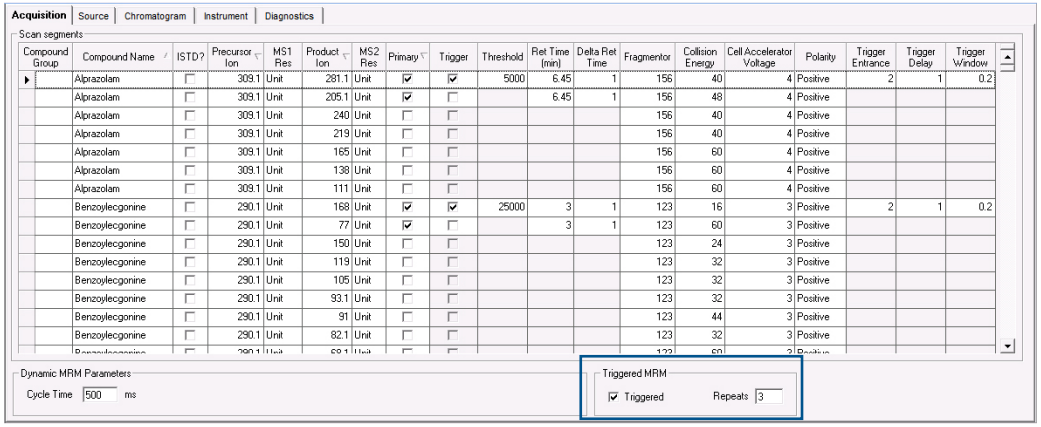
5 Import secondary transitions to the Data Acquisition program. Remove any negative MRM transition for any compounds with positive MRM transitions.	**a** Click the **Add to Import List** button. **b** Click the **Import List** tab. **c** Review the Import List table. **d** Select all negative MRM transitions for any compounds with positive MRM transition. Right-click the selection, and then click **Remove**. **e** Click the **Import** button.	• The compound Alprazolam has five secondary transitions. • Only the transitions that you marked are added to the Import List. • All transitions that have the same **Compound Name** are part of the same compound.

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



Compound Name	Formula	MW	Polarity	Species	Precursor	Product	Frag	CE	Primary	Trigger	RT	RT Window	Abundance
Alprazolam	C17H13ClN4		Positive		309.1	111	156	60					10810
Alprazolam	C17H13ClN4		Positive		309.1	138	156	60					19033
Alprazolam	C17H13ClN4		Positive		309.1	165	156	60					11062
Alprazolam	C17H13ClN4		Positive		309.1	219	156	40					6079
Alprazolam	C17H13ClN4		Positive		309.1	240	156	40					25067
Carisoprodol	C12H24N2O4		Positive		261.2	41.2	75	60					960
Carisoprodol	C12H24N2O4		Positive		261.2	62.1	75	16					8975
Carisoprodol	C12H24N2O4		Positive		261.2	69.2	75	20					1308
Carisoprodol	C12H24N2O4		Positive		261.2	97.1	75	12					7605
Carisoprodol	C12H24N2O4		Positive		261.2	115.1	75	8					2219
Carisoprodol	C12H24N2O4		Positive		261.2	158.1	75	4					5808
Carisoprodol	C12H24N2O4		Positive		261.2	200.2	75	4					3205
Codeine	C18H21NO3		Positive		300.2	58	166	55					2782
Codeine	C18H21NO3		Positive		300.2	128.1	166	60	☑	☑			9895
Codeine	C18H21NO3		Positive		300.2	141.1	166	52					5496
Codeine	C18H21NO3		Positive		300.2	152.1	166	60					6698
Codeine	C18H21NO3		Positive		300.2	153.1	166	56					6822
Codeine	C18H21NO3		Positive		300.2	171.1	166	40					9301
Codeine	C18H21NO3		Positive		300.2	181.1	166	36					3197

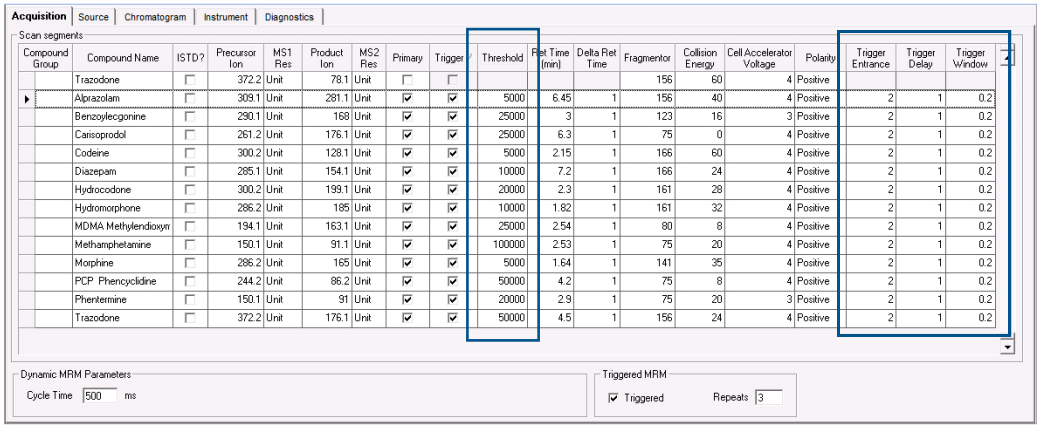
Steps	Detailed Instructions	Comments
6 Review the secondary transitions in the Data Acquisition program.	**a** In the Acquisition tab, sort the table by the **Compound Name**. **b** Review the primary and secondary transitions for each compound.	• If a red box appears in the Scan segments table, you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



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
	Alprazolam		309.1	Unit	281.1	Unit	☑	☑	5000	6.45	1	156	40	4	Positive	2	1	0.2
	Alprazolam		309.1	Unit	205.1	Unit	☑			6.45	1	156	48	4	Positive			
	Alprazolam		309.1	Unit	240	Unit						156	40	4	Positive			
	Alprazolam		309.1	Unit	219	Unit						156	40	4	Positive			
	Alprazolam		309.1	Unit	165	Unit						156	60	4	Positive			
	Alprazolam		309.1	Unit	138	Unit						156	60	4	Positive			
	Alprazolam		309.1	Unit	111	Unit						156	60	4	Positive			
	Benzoylecgonine		290.1	Unit	168	Unit	☑	☑	25000	3	1	123	16	3	Positive	2	1	0.2
	Benzoylecgonine		290.1	Unit	77	Unit	☑			3	1	123	60	3	Positive			
	Benzoylecgonine		290.1	Unit	150	Unit						123	24	3	Positive			
	Benzoylecgonine		290.1	Unit	119	Unit						123	32	3	Positive			
	Benzoylecgonine		290.1	Unit	105	Unit						123	32	3	Positive			
	Benzoylecgonine		290.1	Unit	93.1	Unit						123	32	3	Positive			
	Benzoylecgonine		290.1	Unit	91	Unit						123	44	3	Positive			
	Benzoylecgonine		290.1	Unit	82.1	Unit						123	32	3	Positive			

7 Enter the **Trigger Entrance Delay**, **Trigger Delay** and **Trigger Window** values.	**a** Sort the table by the Trigger column. **b** For each Trigger transition, type 2 for the **Trigger Entrance**. **c** For each Trigger transition, type 1 for the **Trigger Delay**. **d** For each Trigger transition, type 0.2 for the **Trigger Window**. **e** For each Trigger transition, type the threshold from "Primary and Secondary Transitions for Triggered MRM" on page 74.	• See the QQQ Concepts guide or the online Help for more information on these values. • The trigger **Threshold** values are brought over automatically from the Database Browser if the Trigger MRM Threshold column has a value. This column is not visible by default in the Database Browser. • In the MRM Update Options dialog box, you can also select to update the Trigger Threshold from the MassHunter QQQ data file or Quant report folder. See "Task 3. Create a dMRM method using Update dMRM" on page 38. • You can also enter the Threshold directly.

Steps	Detailed Instructions	Comments

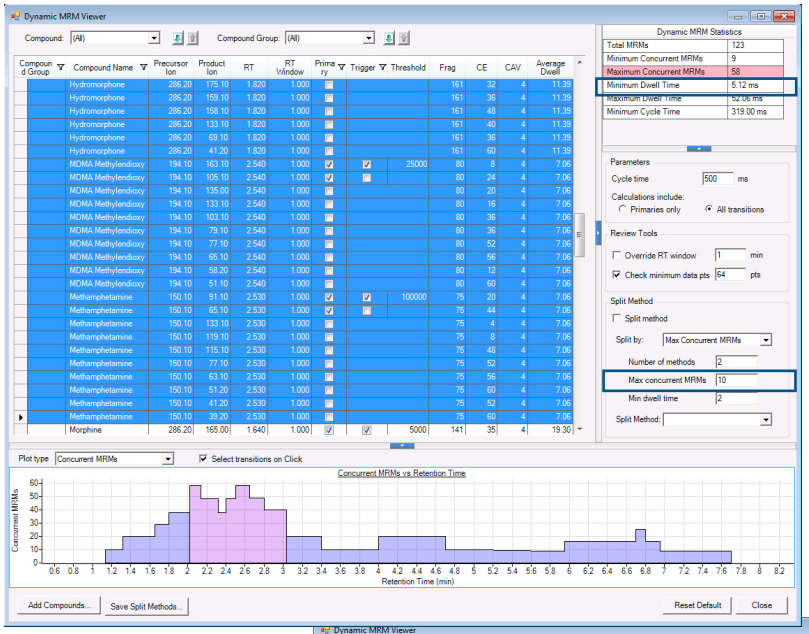


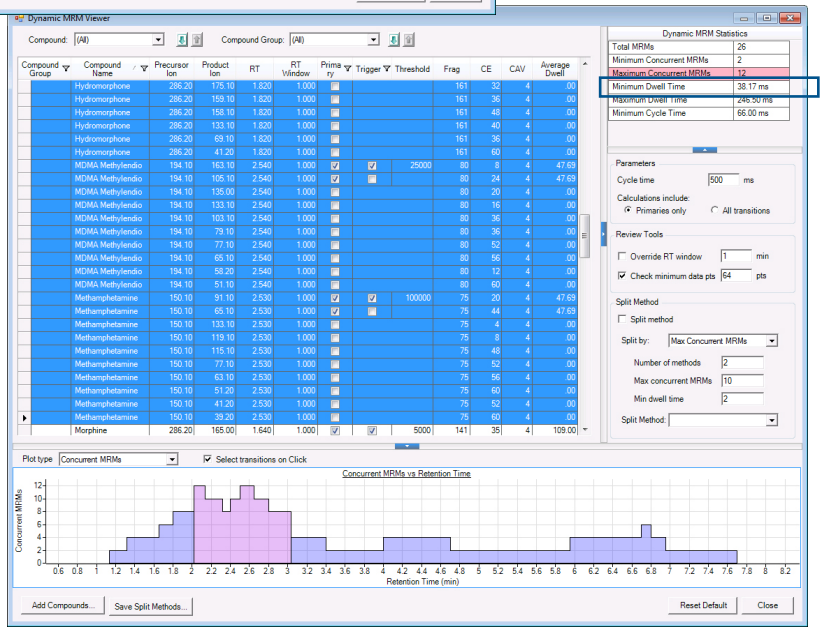
8 In the Data Acquisition program, Save the method to a new method name, ***iii*ForTox_TestMix_TMRM.m**, where ***iii*** are your initials.

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

Steps	Detailed Instructions	Comments
9 Review the method in the Dynamic MRM Viewer dialog box.	**a** Right-click the Scan segments table and click **Edit DMRM Method**. The Dynamic MRM Viewer dialog box is opened. **b** Switch between the **Primaries only** button and the **All transitions** button if the **Dynamic MRM Statistics** information is not updating.	• Inspect the Dynamic MRM Statistics in the upper right corner. You can modify the **Cycle time** and see how the **Average Dwell Time** is changed. If the **Average Dwell Time** is less than 5 ms, and especially if it is less than 3 ms, then signal-to-noise is poor. • A **Dwell Time** of 8 ms or higher per transition is fine. • When you click **All transitions**, the **Maximum Concurrent MRMs** value indicates a warning. The **Maximum Concurrent MRMs** value is set to a value which is higher than the default **Max concurrent MRMs** shown under Split Method. However, if you look at the **Dwell Time** when you look at **Primaries only**, the times are all much higher than 8 ms, so this method does not need to be changed. • If the **Dwell** was lower than 5 ms, then you can change the **Cycle time** to a larger value to increase the **Dwell** time. • If you click **Primaries only**, the dwell times are all much higher than 8 ms.

Steps	Detailed Instructions	Comments





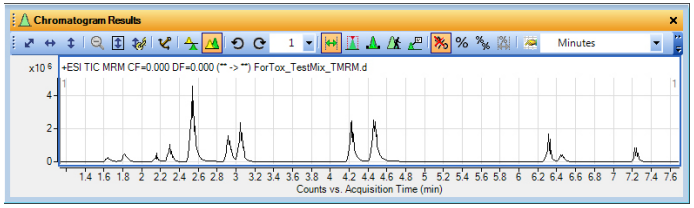
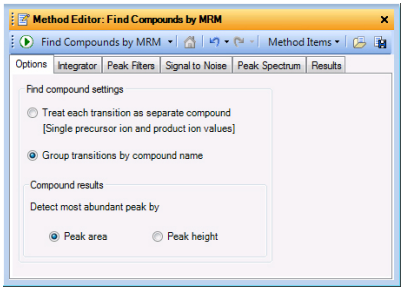
The image to the left shows All transitions. If all of the secondary ions are acquired at the same time, then the Minimum Dwell time is 5.12 ms. However, in the image below, the Primaries only option is clicked. The Minimum Dwell Time is 38.17 ms.

Steps	Detailed Instructions	Comments
10 Adjust the cycle time.	**a** See step 9 on page 56 for details.	• The cycle time can be optimized for each analysis. The default cycle time is 500 ms. For methods that contain more than 15 compounds, the cycle time usually needs to be at least 500 ms. Use the Dynamic MRM Viewer to see what the Minimum Dwell Time is and increase the cycle time so that the Minimum Dwell Time is at least 8.

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

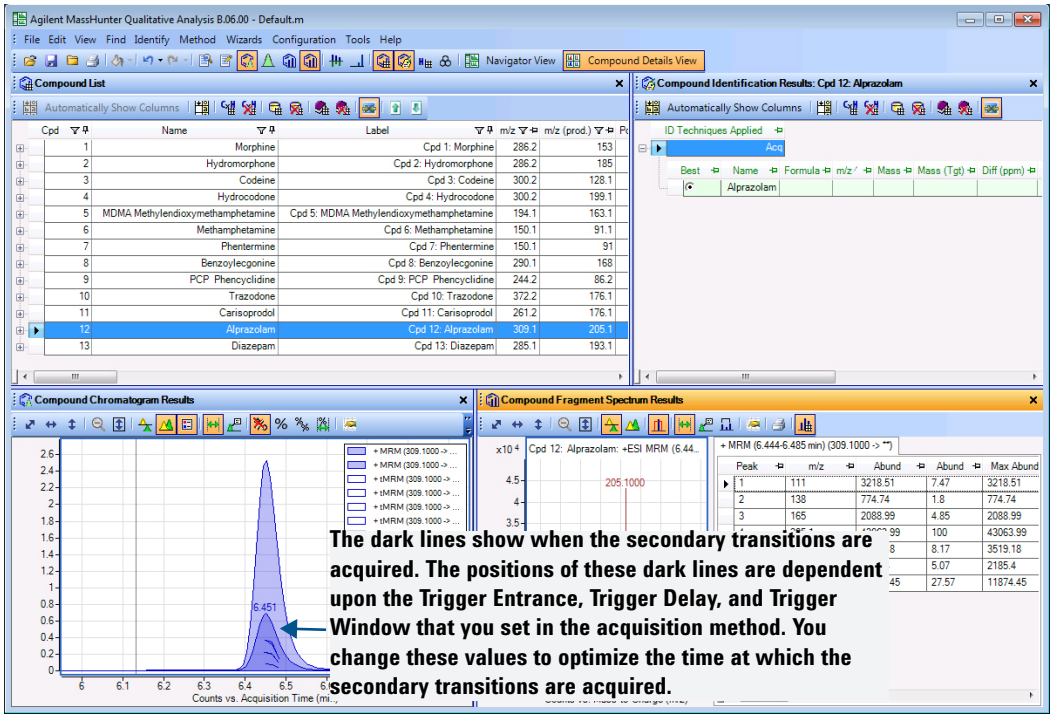
After you acquire the tMRM data file, you examine the data file in the Qualitative Analysis program to verify that all of the Primary and Secondary transitions were acquired.

Steps	Detailed Instructions	Comments
Do this step if you want to acquire data with the Checkout Mix. Otherwise, continue at step 2. **1** Acquire data. • Set up a one-line worklist with the method you just created. • Name the data file ***ForTox_TestMix_TMRM*.d**. • Designate a directory path to hold your data files and method.	**a** If necessary, click **View > Worklist** to display the Worklist window. **b** Click **Worklist > Worklist Run Parameters.** Verify that the parameters are set properly. Click **OK**. **c** Click **Worklist > Add Multiple Samples**. **d** Type `ForTox_TestMix_TMRM.d` as the data file name **e** Select **ForTox_TestMix_TMRM.m** as the method name. **f** Click the **Sample Position** tab. **g** Select the Autosampler, Well-plate or Vial Tray. **h** In the graphic, select a single position. Click **OK.** **i** In the Worklist window, mark the check box to the left of the sample. **j** Click the **Start Worklist Run** icon in the main toolbar, the **Run Worklist** icon in the Worklist toolbar or click the **Worklist > Run** command.	• The Worklist window is tabbed with the Method Editor window by default. Click the **Worklist** tab to show the Worklist window. • This step is optional because you can perform the next step with an example data file that comes with the program. If you prefer, you can create your own data file as described in this step. • See also "To run the Checkout Test Mix" on page 11.

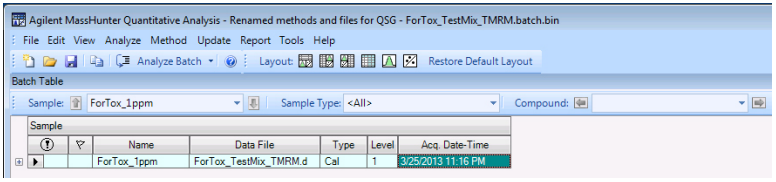
Steps	Detailed Instructions	Comments
2 Find compounds using the Find Compound by MRM algorithm. • Open the data file **ForTox_TestMix_TMRM.d**.	**a** Start the Qualitative Analysis program. **b** Click **File > Open Data File**. The system displays the "Open Data File" dialog box **c** Select **ForTox_TestMix_TMRM.d**,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

Steps	Detailed Instructions	Comments
3 Review the results of the Find Compounds by MRM algorithm. • Make sure that the primary ions are found for each compound.	**a** Close the **Method Explorer** and **Method Editor** windows. **b** Click the **Compound Details View** button. **c** Close the Compound MS Spectrum Results window. **d** In the Compound Chromatogram Results window, click the **Overlaid mode** button and the **Show Legend in Overlaid** mode button. **e** In the Compound Fragment Spectrum Results window, click the **Spectrum Peak List** button. **f** Review each compound. Verify that the primaries and secondaries for each compound were found.	• You can also print a Compound Report to review results. You click **File > Print > Compound Report**. The Compound Report sorts the compounds by retention time. • If you are using the Navigator View, then in the Data Navigator window, the primary transitions are labeled MRM and the secondary transitions are labeled tMRM. • If you are using the Compound Details View, then in the legend in the Compound Chromatogram Results window, the primary transitions are labeled MRM, and the secondary transitions are labeled tMRM. • The Retention Times of the isomers will not be resolved until "Task 3. Create a Reference Library in the Quantitative Analysis program" on page 63.
	g Select **Cpd 12**. You click this compound in the Compound List. **h** In the Compound Fragment Spectrum Results window, verify that these transition are all found: • 309.1 -> 111 (Secondary) • 309.1 -> 138 (Secondary) • 309.1 -> 165 (Secondary) • 309.1 -> 205.1 (Primary) • 309.1 -> 219 (Secondary) • 309.1 -> 240 (Secondary) • 309.1 -> 281.1 (Primary)	• In the MS Peaks One table, you check the abundance for each Primary and Secondary transition. • In the Chromatogram Results window, you can click the Walk Chromatogram tool to review each of the spectra across a peak. You can determine when the Secondaries are acquired. • In the Compound Chromatogram Results window, you can see lines which indicate the abundances for each Secondary transition.

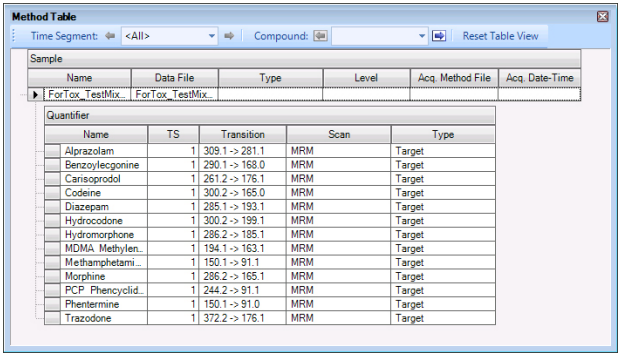
Steps	Detailed Instructions	Comments
	i Continue checking each compound to verify that the Primary and Secondary transitions were acquired. See "Primary and Secondary Transitions for Triggered MRM" on page 74 for a list of the transitions to verify.	• In the Compound Fragment Spectrum Results table, you can check the abundance for each Primary and Secondary. • If you are using the Navigator View, then in the Chromatogram Results window, you can click the **Walk Chromatogram** tool to review each of the spectra across a peak. You can determine when the Secondaries are acquired.



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

Steps	Detailed Instructions	Comments
1 Start the Quantitative Analysis program.	• Click the QQQ Quantitative Analysis icon.	
2 Set up a batch and add the TMRM data file. • Add the data file **ForTox_TestMix_TMRM.d**.	**a** Click **File > New Batch**. **b** Navigate to the location of the TMRM data file. **c** Type `ForTox_TestMix_TMRM` for the **Batch file** name. **d** Click **Open**. **e** Click **File > Add Samples**. **f** Select the **ForTox_TestMix_TMRM.d** data file. **g** Select **Cal** as the **Type**. **h** Type `1` for the **Level**.	
		
3 Set up the TMRM method.	**a** Click **Method > New > New Method from Acquired MRM Data**. **b** Select the **ForTox_TestMix_TMRM.d** data file. **c** Click **Open**. **d** Right-click the Method Table window and click **Collapse All**.	• If you added more than one sample, then you select one of the calibration data files to create the method.

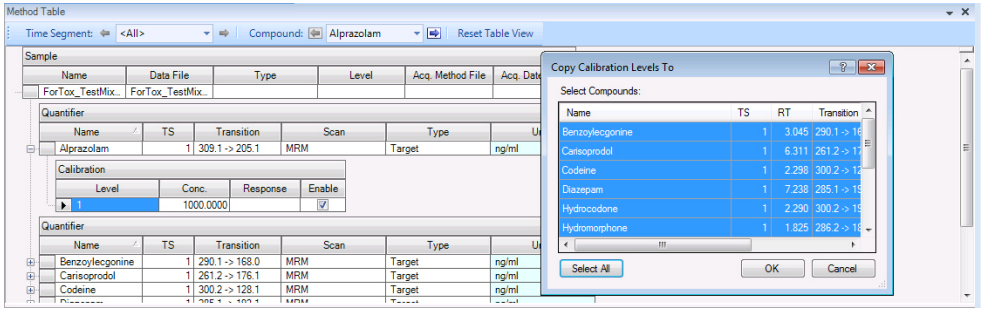
Steps	Detailed Instructions	Comments



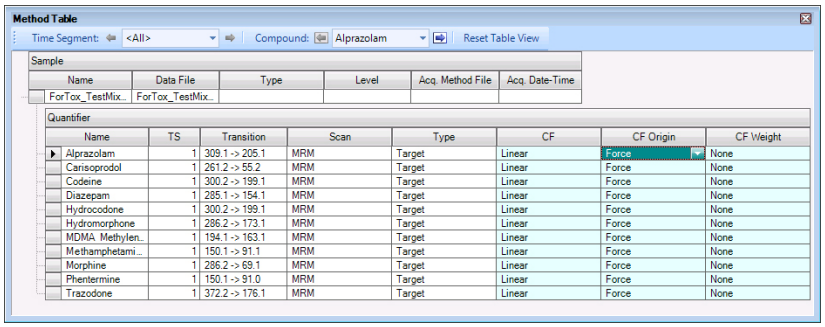
4 Set the Concentration Setup.
- Add calibration level 1 with a concentration of 1000.

a Select **Concentration Setup** in the Method Setup Tasks section in the Method Tasks pane.
b Select the first compound in the table.
c Right-click the compound row and click **New Calibration Level** from the shortcut menu.
d In the **Level** column, type `1`. In the **Conc.** column, type `1000`.
e Right-click in the Level box and click **Copy Calibration Levels To**.
f Click **Select All**. Click **OK**.

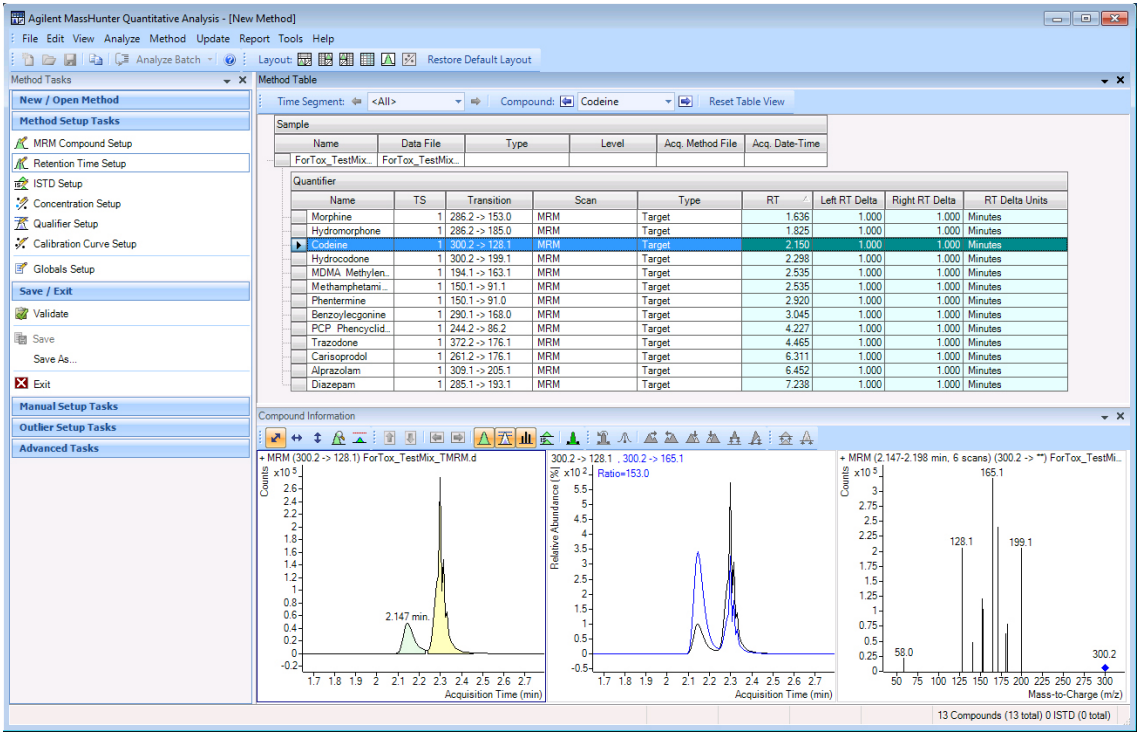
- Refer to the online Help in the Quantitative Analysis program for additional help on these tasks.



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

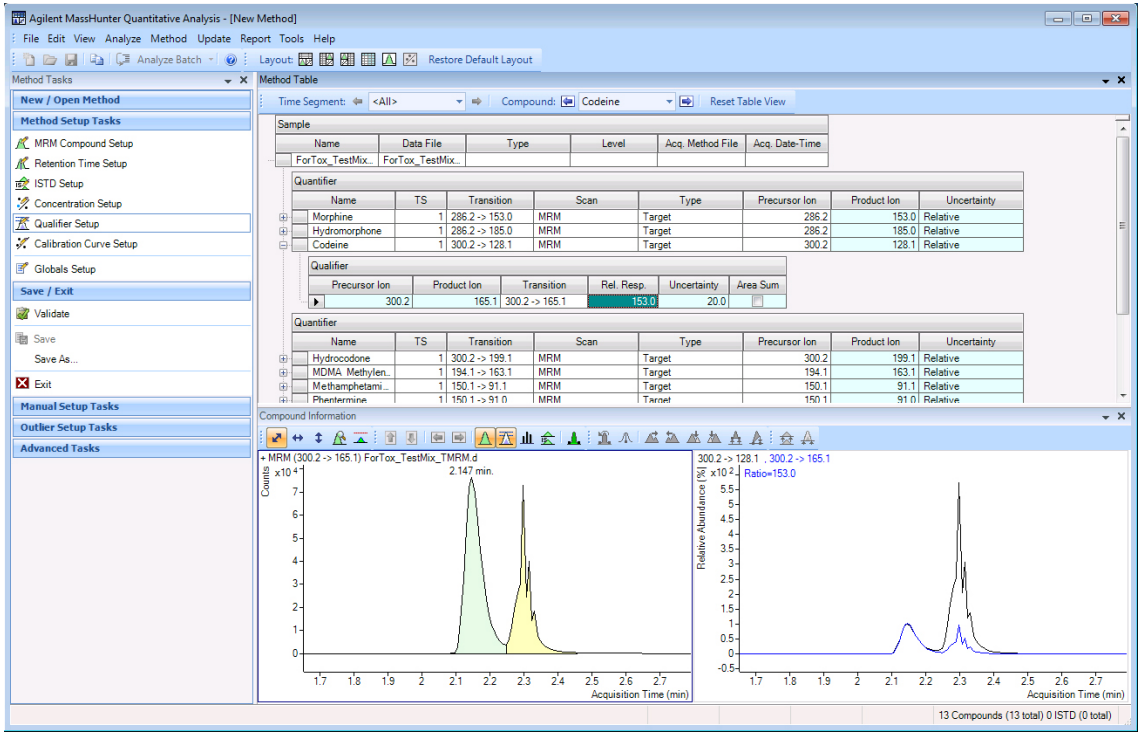


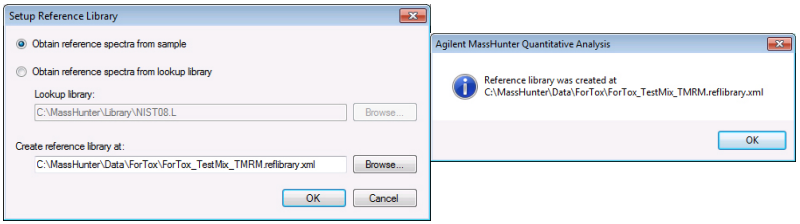
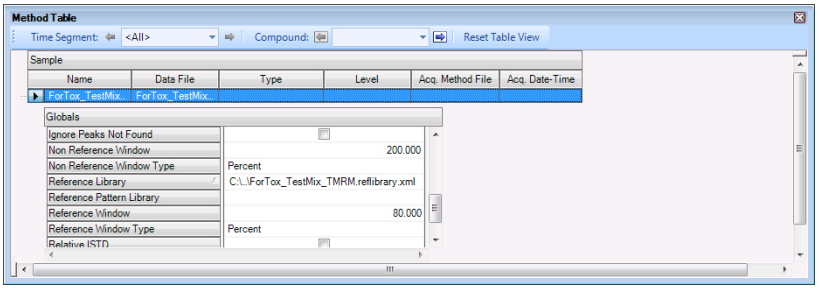
Steps	Detailed Instructions	Comments
6 Resolve the isomers. The RT elution order is: • Morphine • Hydromorphone • Codeine • Hydrocodone • MDMA/Methylendioxymetham-phetamine • Methamphetamine • Phentermine • Benzoylecgonine • PCP/Phencyclidine • Trazodone • Carisoprodol • Alprazolam • Diazepam	**a** Select **Retention Time Setup** in the Method Setup Tasks section. **b** Verify the retention time order of the isomers is the same as shown in the figure below. Resolve any retention time issues for the isomeric compounds.	• The Retention Time for Codeine, Hydrocodone, and Phentermine must be changed when using the example data. • When using the example data, the Retention Time for Codeine must be changed to 2.150 minutes. • When using the example data, the Retention Time for Hydrocodone must be changed to 2.298 minutes. • When using the example data, the Retention Time for Phentermine must be changed to 2.920 minutes.

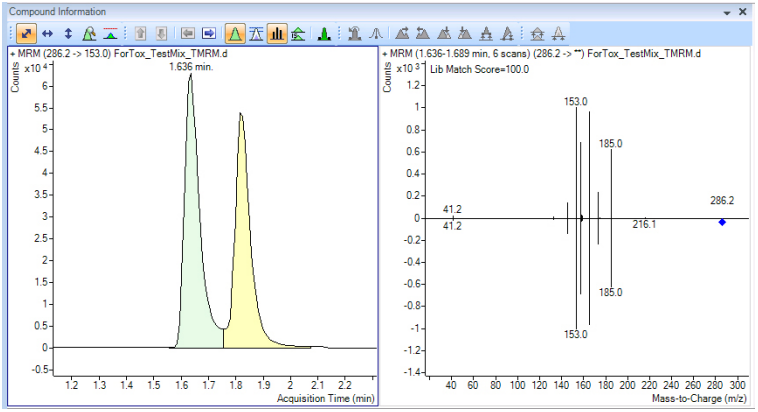


Name	TS	Transition	Scan	Type	RT	Left RT Delta	Right RT Delta	RT Delta Units
Morphine	1	286.2 -> 153.0	MRM	Target	1.636	1.000	1.000	Minutes
Hydromorphone	1	286.2 -> 185.0	MRM	Target	1.825	1.000	1.000	Minutes
Codeine	1	300.2 -> 128.1	MRM	Target	2.150	1.000	1.000	Minutes
Hydrocodone	1	300.2 -> 199.1	MRM	Target	2.298	1.000	1.000	Minutes
MDMA_Methylen...	1	194.1 -> 163.1	MRM	Target	2.535	1.000	1.000	Minutes
Methamphetami...	1	150.1 -> 91.1	MRM	Target	2.535	1.000	1.000	Minutes
Phentermine	1	150.1 -> 91.0	MRM	Target	2.920	1.000	1.000	Minutes
Benzoylecgonine	1	290.1 -> 168.0	MRM	Target	3.045	1.000	1.000	Minutes
PCP_Phencyclid...	1	244.2 -> 86.2	MRM	Target	4.227	1.000	1.000	Minutes
Trazodone	1	372.2 -> 176.1	MRM	Target	4.465	1.000	1.000	Minutes
Carisoprodol	1	261.2 -> 176.1	MRM	Target	6.311	1.000	1.000	Minutes
Alprazolam	1	309.1 -> 205.1	MRM	Target	6.452	1.000	1.000	Minutes
Diazepam	1	285.1 -> 193.1	MRM	Target	7.238	1.000	1.000	Minutes

Steps	Detailed Instructions	Comments
7 Review qualifier ratios	**c** Select **Qualifier Setup** in the Method Setup Tasks section. **d** Right-click the Method Table and click **Expand All**. **e** Click the Show/Hide Qualifiers button in the toolbar in the Compound Information window. **f** Click on each compound and verify that the Rel. Resp. for each Qualifier matches the value shown in the Compound Information window in the spectrum pane. **g** Click Method > Validate and fix any errors.	• The qualifier ratio shown in the Rel. Resp. column was updated for Codeine, Methamphetamine, Phentermine. • In the example below, the qualifier ratio for Codeine was changed from 46.0 to 153.0.



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

Steps	Detailed Instructions	Comments
10 Analyze the batch, review the results, and save the batch.	**a** In the Batch Table window, select **Cal** as the **Type**. **b** Click **Analyze > Analyze Batch.** **c** If the Compound Information window is not open, click **View > Compound Information**. **d** Click the Show/Hide Spectrum button. **e** Click **File > Save Batch**.	
11 Review the batch to resolve errors or messages that are indicated in the Batch Table.	• Resolve isomers. • Check qualifier ratios. • Resolve errors and messages	If an error is reported for a compound qualifier ratio: **a** Click **Method > Edit**. **b** Click **Update > Update Qualifier Ratios**. **c** Select the compounds for update and click **OK**. **d** Click **Method > Exit**. **e** Click **Yes** to apply the method to the batch. **f** Check qualifier ratios. **g** Resolve errors and messages. **h** Click **File > Save Batch**.
	**The LibMatchScore is 100 because you are comparing against the same spectrum.** **The matching algorithm applies penalties when less than five peaks are present in the spectrum:** **75% (1 peak)** **88% (2 peaks)** **94% (3 peaks)** **97% (4 peaks)**	

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 LC/MS Forensic/Toxicology Checkout Test Mix (p/n 5190-0556)

#	Chemical Name/CAS #	Concentration / Units	Tolerance (+/-)	Formula	Mass
1	PCP/Phencyclidine/77-10-1	100.0 µg/mL	0.5%	$C_{17}H_{25}N$	243.1987
2	Methamphetamine/537-46-2	100.0 µg/mL	0.5%	$C_{10}H_{15}N$	149.12045
3	MDMA/Methylendioxymethampheta mine/69610-10-2	100.0 µg/mL	0.5%	$C_{11}H_{15}NO_2$	193.11028
4	Phentermine/122-09-8	100.0 µg/mL	0.5%	$C_{10}H_{15}N$	149.12045
5	Benzoylecgonine/519-09-5	100.0 µg/mL	0.5%	$C_{16}H_{19}NO_4$	289.32636
6	Alprazolam/28981-97-7	100.0 µg/mL	0.5%	$C_{17}H_{12}ClN_4$	308.08287
7	Diazepam/439-14-5	100.0 µg/mL	0.5%	$C_{16}H_{12}ClN_2O$	284.07164
8	Codeine/76-57-3	100.0 µg/mL	0.5%	$C_{17}H_{21}NO_2$	299.15214
9	Morphine/57-27-2	100.0 µg/mL	0.5%	$C_{17}H_{19}NO_3$	285.33766
10	Hydrocodone/125-29-1	100.0 µg/mL	0.5%	$C_{17}H_{21}NO_2$	299.15214
11	Hydromorphone/466-99-9	100.0 µg/mL	0.5%	$C_{17}H_{19}NO_3$	285.33766
12	Carisoprodol/78-44-4	100.0 µg/mL	0.5%	$C_{12}H_{24}N_2O_4$	260.32996
13	Trazodone/19794-93-5	100.0 µg/mL	0.5%	$C_{19}H_{22}ClN_5O$	371.15129
	Acetonitrile	Solvent		C_2H_3N	41.05192

Forensics and Toxicology LC Parameters

Name:	HiP Sampler	Model:	G4226A
Auxiliary			
Draw Speed		100.0 µL/min	
Eject Speed		100.0 µL/min	
Draw Position Offset		0.0 mm	
Wait Time After Drawing		2.0 s	
Sample Flush Out Factor		5.0	
Vial/Well bottom sensing		Yes	
Injection			
Injection Mode		Injection with needle wash	
Injection Volume		2.00 µL	
Needle Wash			
Needle Wash Location		Flush Port	
Wash Time		10.0 s	
High throughput			
Automatic Delay Volume Reduction		No	
Overlapped Injection			
Enable Overlapped Injection		No	
Valve Switching			
Valve Movements		0	
Valve Switch Time 1			
Switch Time 1 Enabled		No	
Valve Switch Time 2			
Switch Time 2 Enabled		No	
Valve Switch Time 3			
Switch Time 3 Enabled		No	
Valve Switch Time 4			
Switch Time 4 Enabled		No	
Stop Time			
Stoptime Mode		As pump/No limit	
Post Time			
Posttime Mode		Off	

Figure 1 Parameters for the G4226A HiP Sampler (1290 LC)

Name: Binary Pump **Model:** G4220A

Flow	0.400 mL/min
Use Solvent Types	Yes
Stroke Mode	Synchronized
Low Pressure Limit	0.00 bar
High Pressure Limit	1200.00 bar
Max. Flow Ramp Up	100.000 mL/min²
Max. Flow Ramp Down	100.000 mL/min²
Expected Mixer	No check
Stroke A	
Automatic Stroke Calculation A	Yes
Compress A	
Compressibility Mode A	Compressibility Value Set
Compressibility A	45 10e-6/bar
Compress B	
Compressibility Mode B	Compressibility Value Set
Compressibility B	75 10e-6/bar
Stop Time	
Stoptime Mode	Time set
Stoptime	9.00 min
Post Time	
Posttime Mode	Time set
Posttime	3.50 min

Timetable

Timetable

	Time	Function	Parameter
1	0.50 min	Change Solvent Composition	Solvent composition A: 95.00 % B:5.00 %
2	1.50 min	Change Solvent Composition	Solvent composition A: 70.00 % B:30.00 %
3	5.50 min	Change Solvent Composition	Solvent composition A: 40.00 % B:60.00 %
4	9.00 min	Change Solvent Composition	Solvent composition A: 5.00 % B:95.00 %

Solvent Composition

	Channel	Ch. 1 Solv.	Name 1	Ch2 Solv.	Name 2	Selected	Used	Percent
1	A	100.0 % Water V.03	5mM AF 0.01% FA	100.0 % Water V.03		Ch. 1	Yes	95.00 %
2	B	100.0 % Methanol V.03	0.01% FA	100.0 % Acetonitrile V.03		Ch. 1	Yes	5.00 %

Figure 2 Parameters for the G4220A Binary Pump (1290 LC)

Name: Column Comp.	Model: G1316C
Ready when front door open	Yes
Left Temperature Control	
Temperature Control Mode	Temperature Set
Temperature	45.00 °C
Enable Analysis Left Temperature	
Enable Analysis Left Temperature On	Yes
Enable Analysis Left Temperature Value	0.80 °C
Right Temperature Control	
Right temperature Control Mode	Temperature Set
Right temperature	45.00 °C
Enable Analysis Right Temperature	
Enable Analysis Right Temperature On	Yes
Enable Analysis Right Temperature Value	0.80 °C
Stop Time	
Stoptime Mode	As pump/injector
Post Time	
Posttime Mode	Off

Figure 3 Parameters for the G1316C Column Compartment

Primary and Secondary Transitions for Triggered MRM

The Primary and Secondary transitions for the Forensics and Toxicology Checkout Mix analytes 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 2 Primary and secondary positive transitions for Forensics and Toxicology Checkout Mix analytes

Compound Name	Primary?	Trig-ger	Thresh-old	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Ret Time (min)	Delta RT (min)	Frag	CE	CAV
Alprazolam	Y	Y	5000	309.1	Unit	281.1	Unit			156	40	4
Alprazolam	Y			309.1	Unit	205.1	Unit			156	48	4
Alprazolam				309.1	Unit	240	Unit			156	40	4
Alprazolam				309.1	Unit	219	Unit			156	40	4
Alprazolam				309.1	Unit	165	Unit			156	60	4
Alprazolam				309.1	Unit	138	Unit			156	60	4
Alprazolam				309.1	Unit	111	Unit			156	60	4
Benzoylecgonine	Y	Y	25000	290.1	Unit	168	Unit			123	16	3
Benzoylecgonine	Y			290.1	Unit	77	Unit			123	60	3
Benzoylecgonine				290.1	Unit	150	Unit			123	24	3
Benzoylecgonine				290.1	Unit	119	Unit			123	32	3
Benzoylecgonine				290.1	Unit	105	Unit			123	32	3
Benzoylecgonine				290.1	Unit	93.1	Unit			123	32	3
Benzoylecgonine				290.1	Unit	91	Unit			123	44	3
Benzoylecgonine				290.1	Unit	82.1	Unit			123	32	3
Benzoylecgonine				290.1	Unit	68.1	Unit			123	60	3
Benzoylecgonine				290.1	Unit	67.1	Unit			123	52	3

Table 2 Primary and secondary positive transitions for Forensics and Toxicology Checkout Mix analytes

Compound Name	Primary?	Trig-ger	Thresh-old	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Ret Time (min)	Delta RT (min)	Frag	CE	CAV
Carisoprodol	Y	Y	25000	261.2	Unit	176.1	Unit			75	0	4
Carisoprodol	Y			261.2	Unit	55.2	Unit			75	28	4
Carisoprodol				261.2	Unit	200.2	Unit			75	4	4
Carisoprodol				261.2	Unit	158.1	Unit			75	4	4
Carisoprodol				261.2	Unit	115.1	Unit			75	8	4
Carisoprodol				261.2	Unit	97.1	Unit			75	12	4
Carisoprodol				261.2	Unit	69.2	Unit			75	20	4
Carisoprodol				261.2	Unit	62.1	Unit			75	16	4
Carisoprodol				261.2	Unit	41.2	Unit			75	60	4
Codeine	Y	Y	5000	300.2	Unit	128.1	Unit			166	60	4
Codeine	Y			300.2	Unit	165.1	Unit			166	40	4
Codeine				300.2	Unit	183.1	Unit			166	28	4
Codeine				300.2	Unit	181.1	Unit			166	36	4
Codeine				300.2	Unit	171.1	Unit			166	40	4
Codeine				300.2	Unit	153.1	Unit			166	56	4
Codeine				300.2	Unit	152.1	Unit			166	60	4
Codeine				300.2	Unit	141.1	Unit			166	52	4
Codeine				300.2	Unit	58	Unit			166	55	4
Diazepam	Y	Y	10000	285.1	Unit	154.1	Unit			166	24	4
Diazepam	Y			285.1	Unit	193.1	Unit			166	32	4
Diazepam				285.1	Unit	257.1	Unit			166	20	4
Diazepam				285.1	Unit	241.1	Unit			166	36	4
Diazepam				285.1	Unit	228.1	Unit			166	20	4
Diazepam				285.1	Unit	222.1	Unit			166	24	4

Table 2 Primary and secondary positive transitions for Forensics and Toxicology Checkout Mix analytes

Compound Name	Primary?	Trigger	Threshold	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Ret Time (min)	Delta RT (min)	Frag	CE	CAV
Diazepam				285.1	Unit	165.1	Unit			166	60	4
Diazepam				285.1	Unit	135.1	Unit			166	24	4
Diazepam				285.1	Unit	91.1	Unit			166	44	4
Hydrocodone	Y	Y	20000	300.2	Unit	199.1	Unit			161	28	4
Hydrocodone	Y			300.2	Unit	171.1	Unit			161	40	4
Hydrocodone				300.2	Unit	183.1	Unit			161	28	4
Hydrocodone				300.2	Unit	181.1	Unit			161	36	4
Hydrocodone				300.2	Unit	165.1	Unit			161	48	4
Hydrocodone				300.2	Unit	153.1	Unit			161	52	4
Hydrocodone				300.2	Unit	141.1	Unit			161	52	4
Hydrocodone				300.2	Unit	128.1	Unit			161	60	4
Hydrocodone				300.2	Unit	55	Unit			161	56	4
Hydromorphone	Y	Y	10000	286.2	Unit	185	Unit			161	32	4
Hydromorphone	Y			286.2	Unit	157	Unit			161	50	4
Hydromorphone				286.2	Unit	159.1	Unit			161	36	4
Hydromorphone				286.2	Unit	41.2	Unit			161	60	4
MDMA Methylendioxy-methamphetamine	Y	Y	25000	194.1	Unit	163.1	Unit			80	8	4
MDMA Methylendioxy-methamphetamine	Y			194.1	Unit	105.1	Unit			80	24	4
MDMA Methylendioxy-methamphetamine				194.1	Unit	135	Unit			80	20	4
MDMA Methylendioxy-methamphetamine				194.1	Unit	133.1	Unit			80	16	4
MDMA Methylendioxy-methamphetamine				194.1	Unit	103.1	Unit			80	36	4

Table 2 Primary and secondary positive transitions for Forensics and Toxicology Checkout Mix analytes

Compound Name	Primary?	Trigger	Threshold	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Ret Time (min)	Delta RT (min)	Frag	CE	CAV
MDMA Methylendioxymethamphetamine				194.1	Unit	79.1	Unit			80	36	4
MDMA Methylendioxymethamphetamine				194.1	Unit	77.1	Unit			80	52	4
MDMA Methylendioxymethamphetamine				194.1	Unit	65.1	Unit			80	56	4
MDMA Methylendioxymethamphetamine				194.1	Unit	58.2	Unit			80	12	4
MDMA Methylendioxymethamphetamine				194.1	Unit	51.1	Unit			80	60	4
Methamphetamine	Y	Y	100000	150.1	Unit	91.1	Unit			75	20	4
Methamphetamine	Y			150.1	Unit	65.1	Unit			75	44	4
Methamphetamine				150.1	Unit	119.1	Unit			75	8	4
Methamphetamine				150.1	Unit	77.1	Unit			75	52	4
Methamphetamine				150.1	Unit	63.1	Unit			75	56	4
Methamphetamine				150.1	Unit	51.2	Unit			75	60	4
Methamphetamine				150.1	Unit	41.2	Unit			75	52	4
Methamphetamine				150.1	Unit	39.2	Unit			75	60	4
Morphine	Y	Y	5000	286.2	Unit	165	Unit			141	35	4
Morphine	Y			286.2	Unit	153	Unit			141	45	4
Morphine				286.2	Unit	173.1	Unit			141	28	4
Morphine				286.2	Unit	159.1	Unit			141	28	4
Morphine				286.2	Unit	158.1	Unit			141	48	4
Morphine				286.2	Unit	145.1	Unit			141	36	4
Morphine				286.2	Unit	133.1	Unit			141	40	4
Morphine				286.2	Unit	41.2	Unit			141	52	4

Table 2 Primary and secondary positive transitions for Forensics and Toxicology Checkout Mix analytes

Compound Name	Primary?	Trig-ger	Thresh-old	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Ret Time (min)	Delta RT (min)	Frag	CE	CAV
PCP Phencyclidine	Y	Y	50000	244.2	Unit	86.2	Unit			75	8	4
PCP Phencyclidine	Y			244.2	Unit	91.1	Unit			75	36	4
PCP Phencyclidine				244.2	Unit	159.1	Unit			75	8	4
PCP Phencyclidine				244.2	Unit	117.1	Unit			75	28	4
PCP Phencyclidine				244.2	Unit	115.1	Unit			75	60	4
PCP Phencyclidine				244.2	Unit	81.1	Unit			75	16	4
PCP Phencyclidine				244.2	Unit	69.2	Unit			75	40	4
PCP Phencyclidine				244.2	Unit	67.2	Unit			75	20	4
PCP Phencyclidine				244.2	Unit	65.1	Unit			75	60	4
PCP Phencyclidine				244.2	Unit	41.2	Unit			75	60	4
Phentermine	Y	Y	20000	150.1	Unit	91	Unit			75	20	3
Phentermine	Y			150.1	Unit	65.1	Unit			75	48	3
Phentermine				150.1	Unit	133	Unit			75	8	3
Phentermine				150.1	Unit	105	Unit			75	16	3
Phentermine				150.1	Unit	77	Unit			75	48	3
Phentermine				150.1	Unit	63	Unit			75	60	3
Phentermine				150.1	Unit	51.1	Unit			75	60	3
Phentermine				150.1	Unit	41.1	Unit			75	52	3
Phentermine				150.1	Unit	39.1	Unit			75	60	3
Trazodone	Y	Y	50000	372.2	Unit	176.1	Unit			156	24	4
Trazodone	Y			372.2	Unit	148.1	Unit			156	36	4
Trazodone				372.2	Unit	135.1	Unit			156	44	4
Trazodone				372.2	Unit	133.1	Unit			156	44	4
Trazodone				372.2	Unit	121.1	Unit			156	56	4

Table 2 Primary and secondary positive transitions for Forensics and Toxicology Checkout Mix analytes

Compound Name	Primary?	Trigger	Threshold	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Ret Time (min)	Delta RT (min)	Frag	CE	CAV
Trazodone				372.2	Unit	120.2	Unit			156	60	4
Trazodone				372.2	Unit	105.1	Unit			156	56	4
Trazodone				372.2	Unit	92.8	Unit			156	56	4
Trazodone				372.2	Unit	80.1	Unit			156	60	4
Trazodone				372.2	Unit	78.1	Unit			156	60	4

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In This Guide

This Quick Start Guide describes how to use the MassHunter Forensics and Toxicology Triggered MRM Database and Library.

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