



# Agilent G1734AA MassHunter Forensic Toxicology Dynamic MRM Database Kit

## Quick Start Guide

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## What is the MassHunter Forensic Toxicology Dynamic MRM Database Kit?

The MassHunter Forensic Toxicology Dynamic MRM Database Kit lets you screen up to 200 Forensic Toxicology analytes with enhanced sensitivity, all in a single LC/MS analysis.

The MassHunter Forensic Toxicology Dynamic MRM Database Kit helps minimize method development time for your Forensic Toxicology analysis, when used with Agilent's recommended LC/MS configuration and accessories. It stores MRM transitions of the Forensic Toxicology analytes included in the database, and their optimized



fragmentor and collision energy settings on an Agilent 6400 Series Triple Quadrupole LC/MS instrument. Method development can simply be done by importing target compounds from the database to the MassHunter Data Acquisition program.

The Dynamic MRM feature of Agilent Triple Quadrupole instruments provides adaptive MRM data collection methodology that requires the instrument collect data only at a predetermined time window (the retention time range for the target compound) for a given MRM transition. More compounds/MRMs can be analyzed in a single run through the Dynamic MRM feature, without losing data quality. See the technical note on Dynamic MRM (p/n 5990-3595EN) for more information.

## Kit Content

**Agilent G1734AA MassHunter Forensic Toxicology Dynamic MRM Database Kit Quick Start Guide (p/n 5990-4265EN)** The Quick Start Guide provides an overview of the MassHunter Forensic Toxicology Dynamic MRM Database Kit, how to use it, and where to find further information. A copy of the Test Mix Report Example is also included on the support disk.

**MassHunter Forensic Toxicology Dynamic MRM Database (p/n G1734-64000)** Included in the kit is a disk that contains MassHunter Forensic Toxicology Dynamic MRM Database B.03.01, along with related software license agreements. See “[Installation](#)” on page 4 for a list of software requirements.

**MassHunter Forensic Toxicology Dynamic MRM Database Kit Support Disk (p/n G1734-90000)** The content of the disk are:

- The Triple Quadrupole LC/MS Dynamic MRM method **LC/MS Forensic Toxicology Test\_Mix\_01.m**
- A sample chromatogram and Dynamic MRM report obtained with the test mix
- Example methods
- Report templates for creating Dynamic MRM method
- Technical note on Dynamic MRM (p/n 5990-3595EN)
- Technical note on the Pesticide Dynamic MRM Database (p/n 5990-4255EN)

- *Agilent G1734AA MassHunter Forensic Toxicology Dynamic MRM Database Kit Quick Start Guide (PDF)*

**ZORBAX LC Column (p/n 959764-902)** Eclipse Plus C18, 2.1mm x 100, 1.8 µm.

**LC/MS Forensic Toxicology Test Mix (p/n 5190-0470)** 4 ampoules containing a Forensic Toxicology LC/MS test mix of 25 components.

## Where to find more information

**Application Notes and Publications** You can find information about the MassHunter Forensic Toxicology Dynamic MRM Database for forensic and toxicology analysis in the application notes and publications included on the support disk.

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

## Before You Begin

### Installation

- 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 32 for details.
- 3 Check that the Agilent 6400 Series Triple Quadrupole LC/MS instrument is properly installed and verified.
- 4 Check that the following programs are properly installed:
  - MassHunter Data Acquisition B.02.01 or higher
  - MassHunter Quantitative Analysis B.03.01 or higher.
  - MassHunter Qualitative Analysis B.03.00 or higher
  - MassHunter Optimizer B.02.01 or higher
- 5 Install the MassHunter Forensic Toxicology Dynamic MRM Database. Follow the installation instruction on the front of the database installation disk.
- 6 If you want to use the example methods that are included with this kit, copy the methods from the support disk to the **D:\MassHunter\Methods** folder, or to a folder under the **Methods** folder.

### Required Reagents and Parts

- LC/MS grade acetonitrile and water
- ZORBAX LC Column, p/n 959764-902
- Formic acid (highest purity)
- Ammonium formate (highest purity)

## Getting Started

The sample data files provided in the support disk were acquired with the test mix on a system with the LC/MS system configured as described in “Installation” on page 4. Along with the sample data files are the Dynamic MRM 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 from using the MassHunter Forensic Toxicology Dynamic MRM Database Kit.

To review the Acquisition Method, use the MassHunter Data Acquisition program to load the method file **LC/MS Forensic Toxicology Test\_Mix\_01.m**. The following data acquisition settings for the positive ion compounds are listed:

- Acquisition method info
- Triple Quadrupole LC/MS settings (see [Table 1](#))
- Wellplate sampler settings
- Binary pump settings
- Thermostatted column compartment settings

**Table 1** Dynamic MS/MS transitions for LC/MS Forensic Toxicology Test Mix analytes and their chromatographic-dependent settings

Compound Name	ISTD ?	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Frag (V)	CE (V)	Ret Time	Ret Window	Polarity
Codeine	<input type="checkbox"/>	300.2	Unit	165.1	Unit	158	45	1.11	0.4	Positive
Codeine	<input type="checkbox"/>	300.2	Unit	58.1	Unit	158	29	1.11	0.4	Positive
Oxycodone	<input type="checkbox"/>	316.2	Unit	298.1	Unit	143	17	1.285	0.4	Positive
Oxycodone	<input type="checkbox"/>	316.2	Unit	256.1	Unit	143	25	1.285	0.4	Positive
d-Amphetamine	<input type="checkbox"/>	136.1	Unit	119.1	Unit	66	5	1.296	0.4	Positive
d-Amphetamine	<input type="checkbox"/>	136.1	Unit	91	Unit	66	17	1.296	0.4	Positive
MDA	<input type="checkbox"/>	180.1	Unit	163	Unit	61	5	1.332	0.4	Positive
MDA	<input type="checkbox"/>	180.1	Unit	105	Unit	61	21	1.332	0.4	Positive
Hydrocodone	<input type="checkbox"/>	300.2	Unit	199	Unit	159	29	1.4	0.4	Positive

**Table 1** Dynamic MS/MS transitions for LC/MS Forensic Toxicology Test Mix analytes and their chromatographic-dependent settings (continued)

Compound Name	ISTD ?	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Frag (V)	CE (V)	Ret Time	Ret Window	Polarity
Hydrocodone	<input type="checkbox"/>	300.2	Unit	128	Unit	159	65	1.4	0.4	Positive
methamphetamine	<input type="checkbox"/>	150.1	Unit	119	Unit	92	5	1.45	0.4	Positive
methamphetamine	<input type="checkbox"/>	150.1	Unit	91	Unit	92	17	1.45	0.4	Positive
MDMA	<input type="checkbox"/>	194.1	Unit	163	Unit	97	9	1.468	0.4	Positive
MDMA	<input type="checkbox"/>	194.1	Unit	105	Unit	97	25	1.468	0.4	Positive
Strychnine	<input type="checkbox"/>	335.2	Unit	184	Unit	195	41	1.629	0.4	Positive
Strychnine	<input type="checkbox"/>	335.2	Unit	156	Unit	195	53	1.629	0.4	Positive
MDEA	<input type="checkbox"/>	208.1	Unit	163	Unit	107	9	1.735	0.4	Positive
MDEA	<input type="checkbox"/>	208.1	Unit	105	Unit	107	25	1.735	0.4	Positive
Heroin	<input type="checkbox"/>	370.2	Unit	268.1	Unit	149	37	2.256	0.4	Positive
Heroin	<input type="checkbox"/>	370.2	Unit	165	Unit	149	61	2.256	0.4	Positive
Cocaine	<input type="checkbox"/>	304.2	Unit	182.1	Unit	138	17	2.376	0.4	Positive
Cocaine	<input type="checkbox"/>	304.2	Unit	77	Unit	138	61	2.376	0.4	Positive
Meperidine	<input type="checkbox"/>	248.2	Unit	220.1	Unit	128	21	2.419	0.4	Positive
Meperidine	<input type="checkbox"/>	248.2	Unit	174.1	Unit	128	17	2.419	0.4	Positive
Trazodone	<input type="checkbox"/>	372.2	Unit	176	Unit	159	25	2.797	0.4	Positive
Trazodone	<input type="checkbox"/>	372.2	Unit	148	Unit	159	37	2.797	0.4	Positive
PCP	<input type="checkbox"/>	244.2	Unit	91	Unit	86	41	2.876	0.4	Positive
PCP	<input type="checkbox"/>	244.2	Unit	86.1	Unit	86	9	2.876	0.4	Positive
Oxazepam	<input type="checkbox"/>	287	Unit	269	Unit	150	12	3.53	0.4	Positive
Oxazepam	<input type="checkbox"/>	287	Unit	241	Unit	150	20	3.53	0.4	Positive
Nitrazepam	<input type="checkbox"/>	282.1	Unit	236.1	Unit	148	25	3.542	0.4	Positive
Nitrazepam	<input type="checkbox"/>	282.1	Unit	180	Unit	148	41	3.542	0.4	Positive
Verapamil	<input type="checkbox"/>	455.3	Unit	165	Unit	158	37	3.554	0.4	Positive

**Table 1** Dynamic MS/MS transitions for LC/MS Forensic Toxicology Test Mix analytes and their chromatographic-dependent settings (continued)

Compound Name	ISTD ?	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Frag (V)	CE (V)	Ret Time	Ret Window	Polarity
Verapamil	<input type="checkbox"/>	455.3	Unit	150	Unit	158	45	3.554	0.4	Positive
Methadone	<input type="checkbox"/>	310.2	Unit	265.1	Unit	112	9	3.61	0.4	Positive
Methadone	<input type="checkbox"/>	310.2	Unit	105	Unit	112	29	3.61	0.4	Positive
Lorazepam	<input type="checkbox"/>	321	Unit	275	Unit	102	21	3.626	0.4	Positive
Lorazepam	<input type="checkbox"/>	321	Unit	194	Unit	102	49	3.626	0.4	Positive
Alprazolam	<input type="checkbox"/>	309.1	Unit	281	Unit	179	25	3.727	0.4	Positive
Alprazolam	<input type="checkbox"/>	309.1	Unit	205	Unit	179	49	3.727	0.4	Positive
Temazepam	<input type="checkbox"/>	301.1	Unit	255.1	Unit	117	29	3.941	0.4	Positive
Temazepam	<input type="checkbox"/>	301.1	Unit	177	Unit	117	45	3.941	0.4	Positive
Proadifen	<input type="checkbox"/>	354.2	Unit	167	Unit	153	29	4.088	0.4	Positive
Proadifen	<input type="checkbox"/>	354.2	Unit	91.1	Unit	153	45	4.088	0.4	Positive
Diazepam	<input type="checkbox"/>	285.1	Unit	193	Unit	169	45	4.268	0.4	Positive
Diazepam	<input type="checkbox"/>	285.1	Unit	154	Unit	169	25	4.268	0.4	Positive
THC	<input type="checkbox"/>	315.2	Unit	193.2	Unit	150	20	5.277	0.4	Positive
THC	<input type="checkbox"/>	315.2	Unit	123.3	Unit	150	30	5.277	0.4	Positive

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.

## To run the test mix

Run the LC/MS Forensic Toxicology Test Mix to get a better idea of how the MassHunter Forensic Toxicology Dynamic MRM Database Kit will work for you.

- 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 the instrument is in tune. Do an Autotune if Checktune reports any failure.

- 2 Prepare the LC/MS Forensic Toxicology Test Mix.

The concentration of the test mix stock solution is 1 µg/mL (1 ppm) for all 25 components.

**a** Dilute 100 µL of the stock solution to 10.0 mL with methanol to create the final solution concentration.

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

The final solution is a 10 ng/mL (10 ppb) working solution.

- 3 Prepare mobile phases A and B.

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

- 4 Run the test mix.

For the analysis of the LC/MS Forensic Toxicology Test Mix, load the method **LC/MS Forensic Toxicology Test\_Mix\_01.m**. This method uses the HPLC system configuration as listed below. Systems that deviate from this configuration may not completely comply with this method due to changes in system dead volume and appropriate adjustments will need to be made to the methodology.

Column	2.1 x 100 ZORBAX Eclipse Plus C18 1.8 µm, p/n 959764-902
Wellplate Sampler	h-ALS-SL+, model# G1367D
Pump	Binary Pump – SL, Model 1312B configured with damper and mixer removed
Column Compartment	Column – SL, Model G1316B



- 5 Check that your method is set up to make a 1  $\mu$ L injection.
- 6 Click **Run > Interactive Sample** to do a single sample run, or create a worklist to make multiple injections.
- 7 If you do not see all the peaks after you process your data:
  - a Extend your Stop time in the method to 10 minutes.
  - b In the MS QQQ > Acquisition tab, set the **Delta Ret Time** to 3 minutes.
  - c Run the test mix again.

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

## To process and interpret test mix data

In this step, you process the data file that you created when you ran the test mix. The figures in this task are based on the example data file **LC/MS Forensic and ToxicologyTest\_mix\_10pg.d** found in the **Example Data** folder on the support disk. Your results may differ slightly.

**1** Open the MassHunter Qualitative Analysis program.

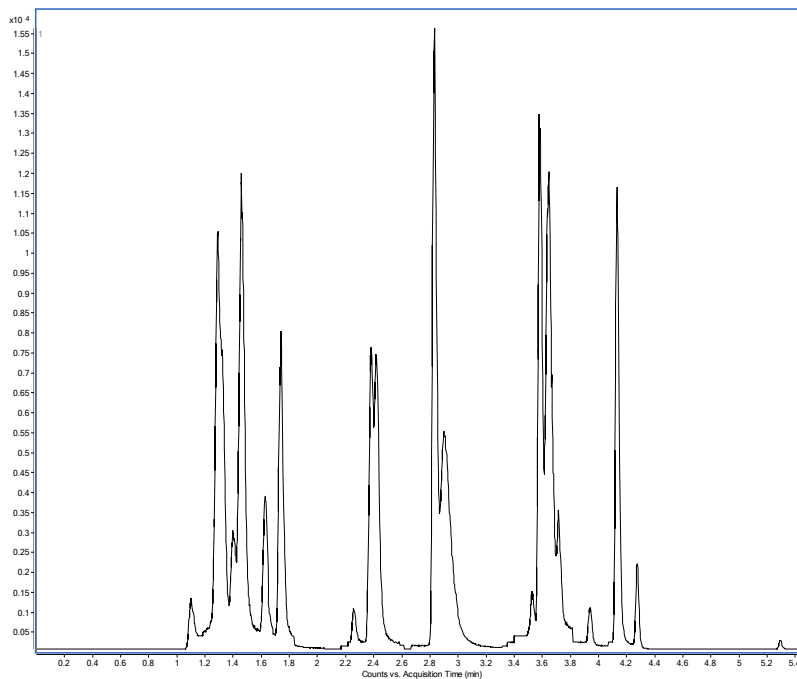
Click **Cancel** if you are asked to open a data file.

**2** Load **default.m** method.

**3** Click **File > Open Data File** and open the data file that you created when you ran the test mix.

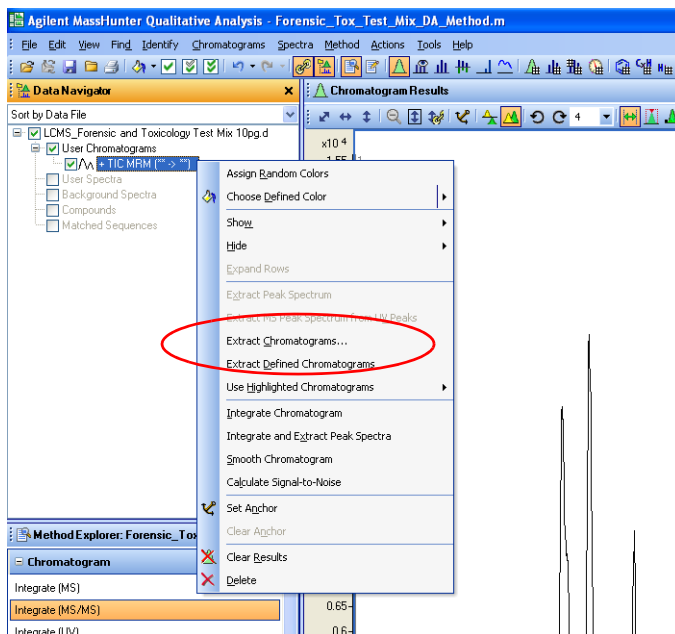
You can also use example data file **LC/MS Forensic and ToxicologyTest\_mix\_10pg.d** in the **Example Data** folder on the support disk.

See [Figure 1](#).



**Figure 1** Example LC/MS Forensic Toxicology Test Mix Total Ion Chromatogram

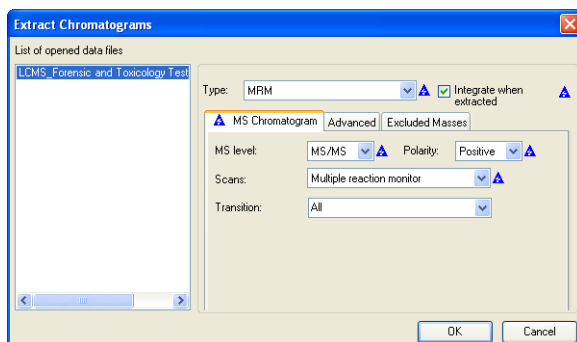
- 4** In the Data Navigator window, right-click **TIC MRM** and then click **Extract Chromatograms** from the shortcut menu.



**Figure 2** Extract Chromatograms on the TIC MRM shortcut menu.

**5** In the Extract Chromatograms dialog box:

- a** Set **Transition** to **All**. See [Figure 3](#).
- b** Mark the **Integrate when extracted** check box.
- c** Click **OK**.



**Figure 3** Extract Chromatograms dialog box

After the chromatograms are extracted and integrated, they are displayed on the Chromatogram Results window, as seen in [Figure 4](#), if the view is in List Mode. Note the segmented chromatograms: the time ranges are the predetermined time windows for the system to collect data for a given MRM transition.



**Figure 4** Extracted chromatograms in Qualitative Analysis Chromatogram Results window. The List Mode icon is circled in the toolbar of the Chromatogram Results window shown here.

- 6 Observe the time retention data for each compound. Look in the Integration Peak List window to see the retention time window.
- 7 If the retention times for either test mix are not close to those given in the method, continue to [“To create an MRM method to run your own sample”](#) on page 15 to update these methods on your system. You can

use the data file created with the expanded retention time window (Delta Ret Window in acquisition), or you can develop a new method as practice. Import these compounds from the database and create a one segment MRM as described in [“To create an MRM method to run your own sample”](#).

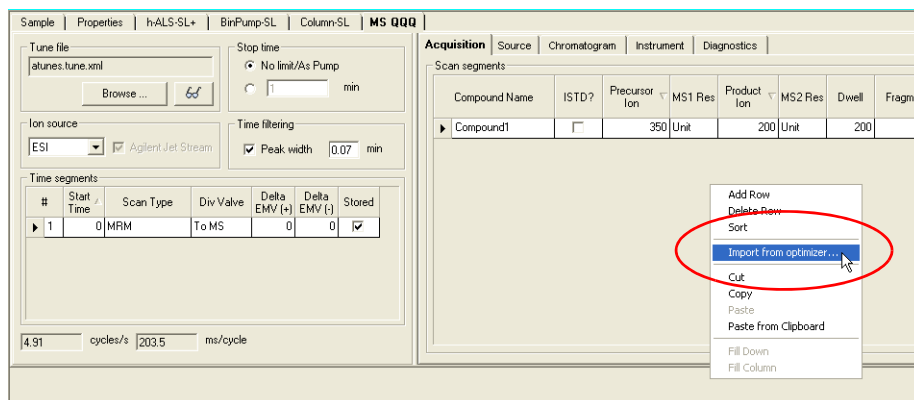
## To create an MRM method to run your own sample

Before you can create a Dynamic MRM analysis method to run your own sample, you need a standard MRM data acquisition method in which settings such as tabular compound names, ISTD (optional), MRM transitions, fragmentor voltages, and collision energies are defined. With the MassHunter Forensic Toxicology Dynamic MRM Database Kit, you can easily import all of these settings from the database to create an MRM method.

- 1 In the MassHunter Data Acquisition program, click the **MS QQQ** tab.
- 2 Click the **Acquisition** tab.
- 3 In the MS QQQ tab, make sure the **Scan Type** is set to **MRM** (not Dynamic MRM).

Refer to the technical note on the Pesticide Dynamic MRM Database (on the support disk) for more information on the benefits of the creation of a single time segment MRM method by use of the MassHunter Pesticide Dynamic MRM Database. The concepts in this technical note also apply to the MassHunter Forensic Toxicology Dynamic MRM Database.

- 4 Right-click an empty area on the Acquisition tab, then click **Import from optimizer** in the shortcut menu.

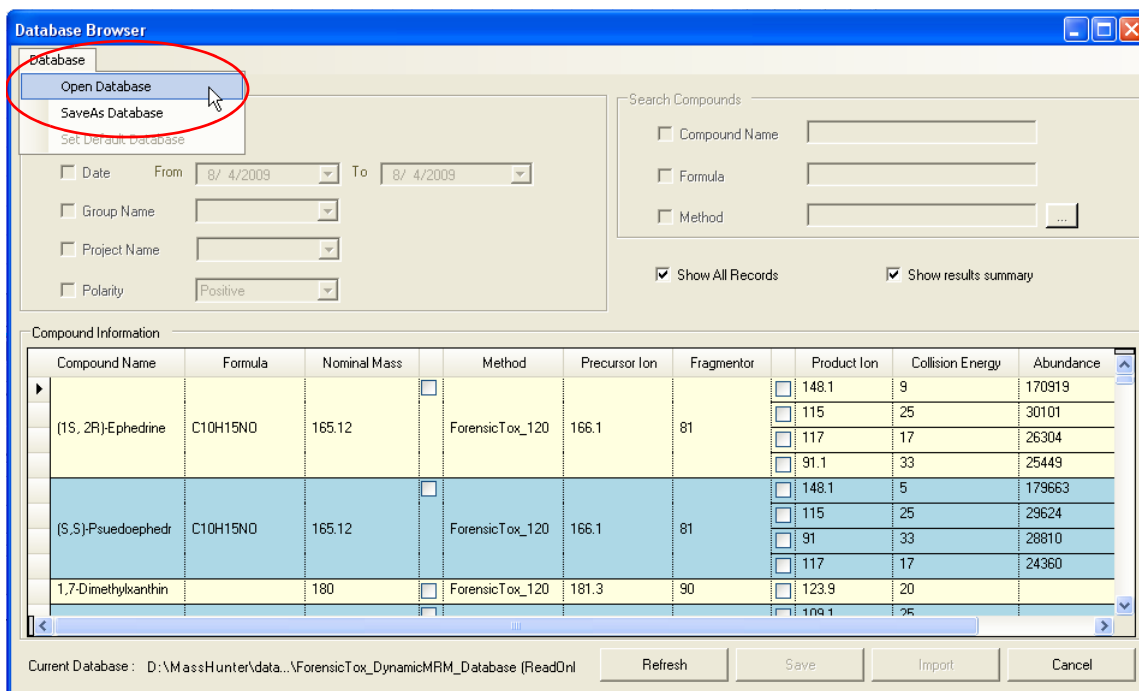


**Figure 5** Import from optimizer shown in the Acquisition tab shortcut menu.

The Database Browser is opened with the default database. You can then select the compounds and product ions needed to import into the acquisition method of your choice.

- 5 Open the MassHunter Forensic Toxicology Dynamic MRM Database:
  - a In the Database Browser, click **Database > Open Database**.
  - b From the **D:\MassHunter\Databases** folder, select the **ForensicTox\_DynamicMRM\_Database** folder.

Figure 6 shows the Database Browser with the MassHunter Forensic Toxicology Dynamic MRM Database loaded.



**Figure 6** Data Browser with MassHunter Forensic Toxicology Dynamic MRM Database opened

The database as it is shipped contains:

- compound name
- formula
- the nominal monoisotopic mass of the compound



- the method(s) that were used for analyses

The parameters for analysis include:

- the precursor that gave the optimal signal and its associated fragmentor voltage
- at least two product ions (if the compound did produce two significant product ions)
- the optimized collision energy for each product ion

In addition, the abundance of each ion is shown so that you can determine the best quantitation and qualifier ions.

**6** Import the required MRM transitions from the database:

- a** Select the required compound transitions from the MassHunter Forensic Toxicology Dynamic MRM Database browser.

When MRM transitions are selected, the Import button in the Database Browser ([Figure 6](#)) becomes active.

- b** To load all selected compound transitions into the MassHunter Data Acquisition method, click **Import**.

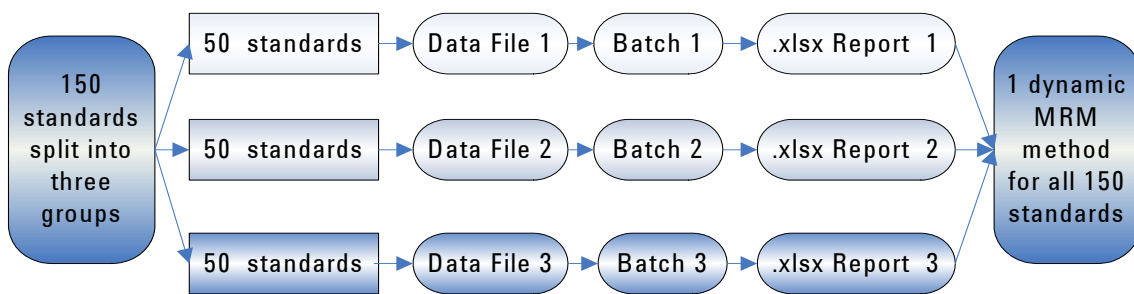
The Database Browser window closes, and the selected transitions appear in the MS QQQ Acquisition tab.

**7** Save the method.

## To create a Dynamic MRM method

To create a Dynamic MRM method, you update your single-time-segment MRM method with additional retention times and retention time windows for every compound in the analysis. To get the retention times for all the compounds, run all the standards with your single time segment MRM method.

The process for 150 standards is described in [Figure 7](#).



**Figure 7** Example process for standards that have more than 50 compounds

### Run the standards with the single time segment MRM method

- 1 Run your standards with the method and chromatography that you created in the previous task.
  - Run standards with subgroups that contain no more than 50 compounds per injection.
  - Make sure your dwell time for all transitions gives an appropriate cycle time.

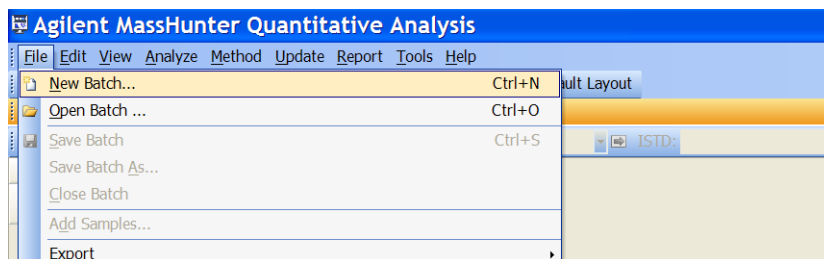
For peaks that are 5 seconds wide, use a cycle time of 500 ms (10 points across the peak). For 50 compounds with 2 transitions each, use a 2 ms dwell time (5.5 ms total per transition).
  - Check that all the compounds are at medium analysis concentration so that they are all captured in the sample run to acquire their retention times.
- 2 After you have run your standards, open the MassHunter Quantitative Analysis program.

## Create batch file for each data file

If you created multiple data files, you create a separate batch file and report for each data file. Then you create a single Dynamic MRM method for all of your standards.

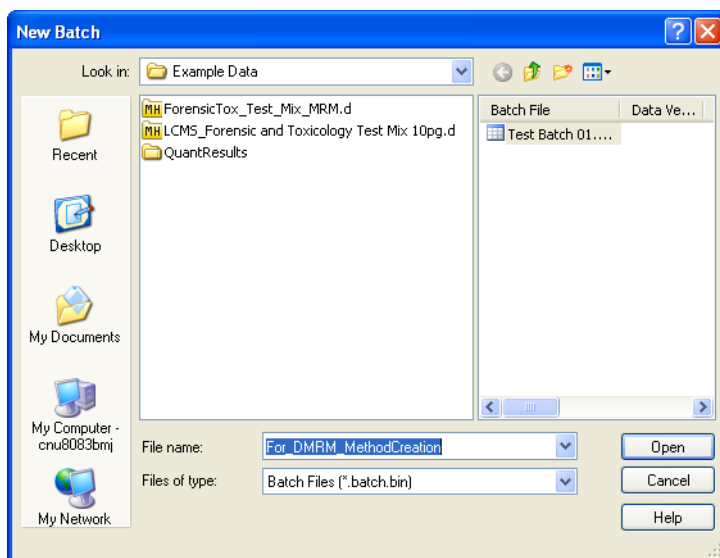
Do the steps in this task for each data file that you created.

- 1 Create a new batch in the folder that contains the MRM data you just collected.
  - a Click **File > New Batch**. See [Figure 8](#).



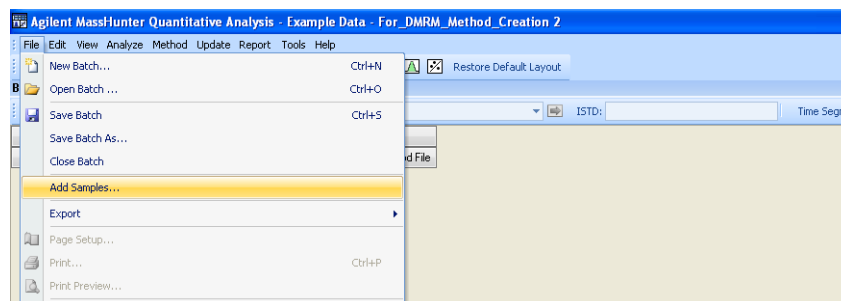
**Figure 8** New Batch from the File menu

- b Type a **File name** for the batch, then click **OK**. See [Figure 9](#).



**Figure 9** New Batch dialog box

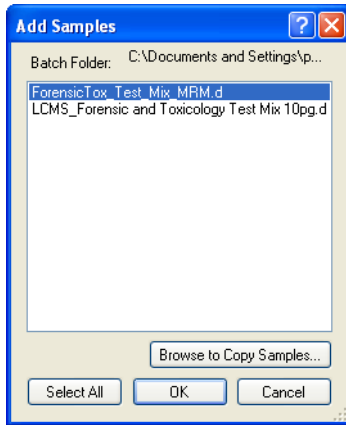
- 2 Load the single time segment MRM data of your standards.
  - a Click **File > Add Samples**. See [Figure 10](#).



**Figure 10** Add Samples from the File menu

- b Select the acquired MRM data file from the **Add Samples** list (or use the model data file **ForensicTox\_Test\_Mix\_MRM.d**, which is located in the **Example Data** folder on the support disk), then click **OK**. See [Figure 11](#).

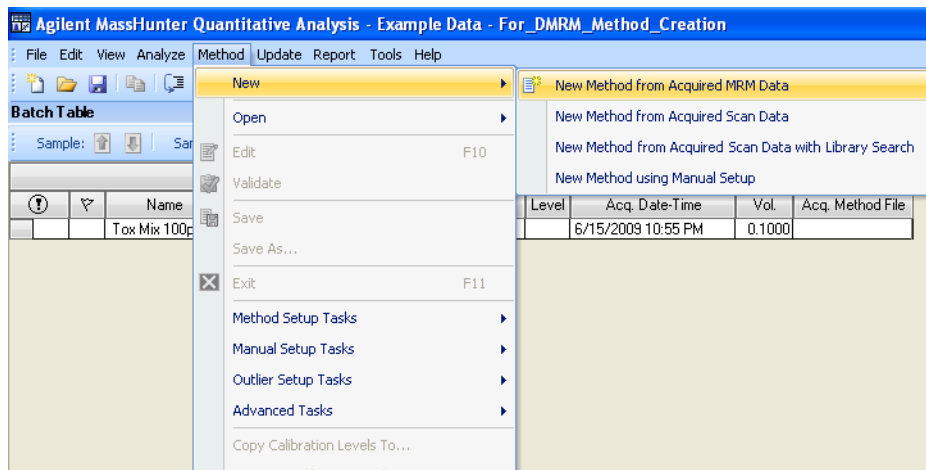
Select only one file. Do not click **Select All**.



**Figure 11** Add Samples dialog box

**3** Create a new method.

- a Click **Method > New > New Method from Acquired MRM Data**. See [Figure 12](#).



**Figure 12** New Method from Acquired MRM Data selected

b Click the data file that you just added to the batch, then click **Open**.

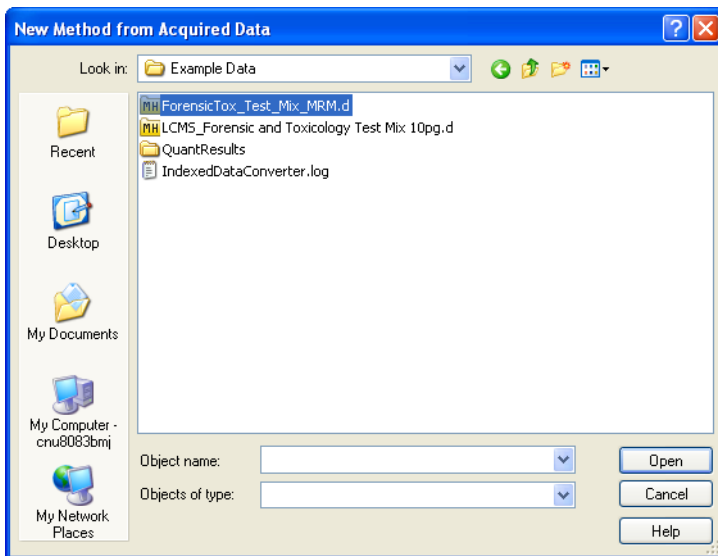


Figure 13 New Method from Acquired Data dialog box

c In the Quantitative Analysis program, from the **Method Setup Tasks** list, click **Concentration Setup**. See Figure 14.

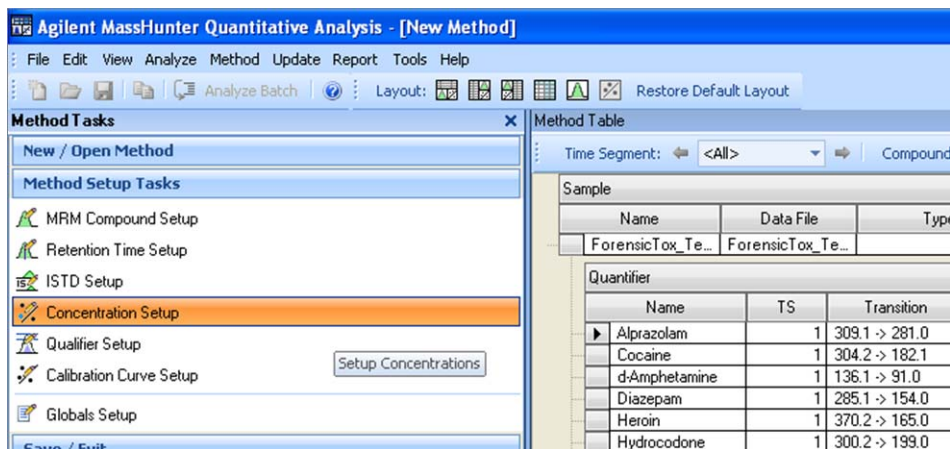


Figure 14 Concentration Setup under Method Setup Tasks

- d Click the name of the first compound in the **Quantifier** table.
- e Change **Dil. High Conc.** for the first compound to 1.
- f Change **Dil. Pattern** to 1:2.
- g In the Method Table header, change **# of Levels** to 1.
- h Click **Create Levels**.

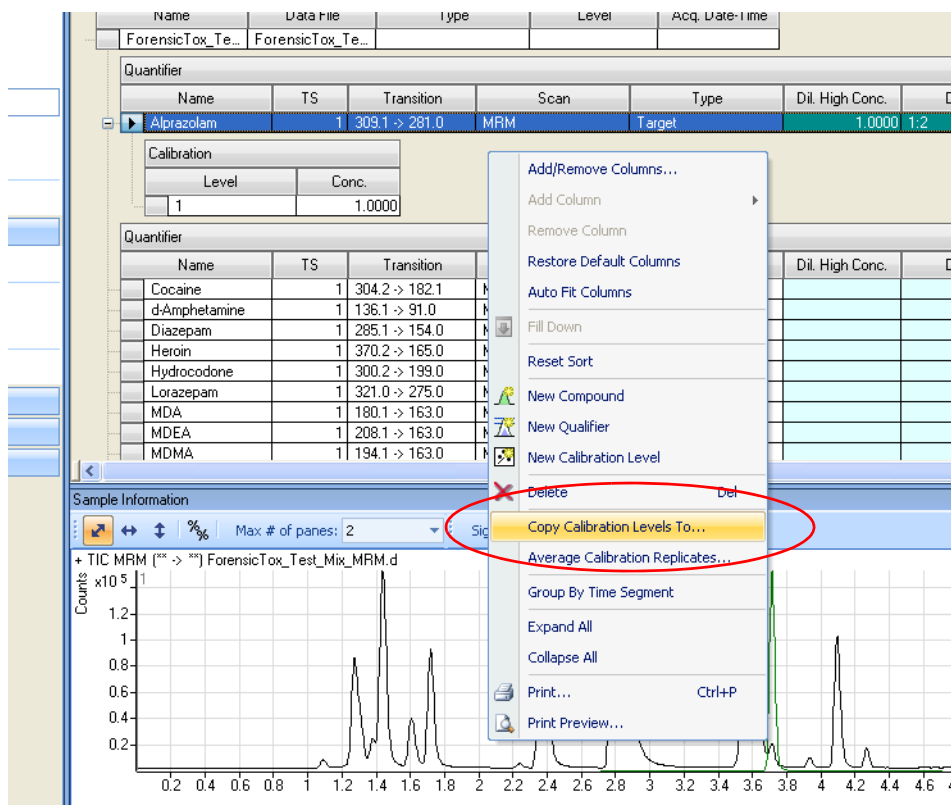
You do not need to change the **Units** settings.

The screenshot shows the 'Method Table' window. At the top, there are controls for 'Time Segment', 'Compound', 'Reset Table View', 'Level Name Prefix', and '# of Levels: 1'. The 'Create Levels' button is to the right. Below this is a 'Sample' table with one row: 'ForensicTox\_Te...'. The main 'Quantifier' table has the following data:

Name	TS	Transition	Scan	Type	Dil. High Conc.	Dil. Pattern	Units
Alprazolam	1	309.1 -> 281.0	MRM	Target	1.0000	1:2	ng/ml
Cocaine	1	304.2 -> 182.1	MRM	Target			ng/ml
d-Amphetamine	1	136.1 -> 91.0	MRM	Target			ng/ml
Diazepam	1	285.1 -> 154.0	MRM	Target			ng/ml
Heroin	1	370.2 -> 165.0	MRM	Target			ng/ml
Hydrocodone	1	300.2 -> 199.0	MRM	Target			ng/ml
Lorazepam	1	321.0 -> 275.0	MRM	Target			ng/ml
MDA	1	180.1 -> 163.0	MRM	Target			ng/ml
MDEA	1	208.1 -> 163.0	MRM	Target			ng/ml
MDMA	1	194.1 -> 163.0	MRM	Target			ng/ml
Meperidine	1	248.2 -> 220.1	MRM	Target			ng/ml
Methadone	1	310.2 -> 265.1	MRM	Target			ng/ml
methamphetamine	1	150.1 -> 91.0	MRM	Target			ng/ml
Nitrazepam	1	282.1 -> 236.1	MRM	Target			ng/ml
Oxazepam	1	287.0 -> 241.0	MRM	Target			ng/ml
Oxycodone	1	316.2 -> 298.1	MRM	Target			ng/ml
PCP	1	244.2 -> 86.1	MRM	Target			ng/ml

**Figure 15** Quantifier table with first compound selected

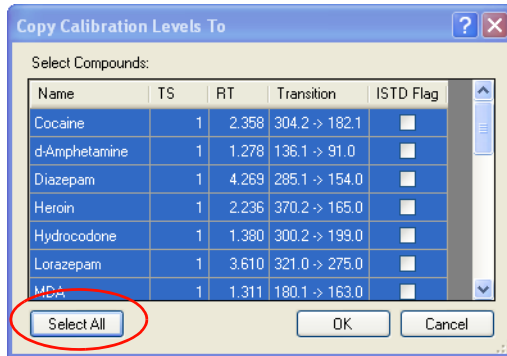
- i After the level is created, right-click the name of the first compound and click **Copy Calibration Levels To**. See [Figure 16](#).



**Figure 16** Copy Calibration Levels To selected

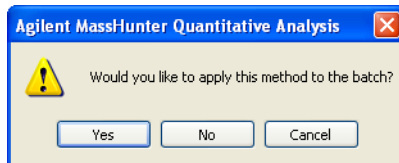


- j Click **Select All** to select all compounds in the data file. See Figure 17.



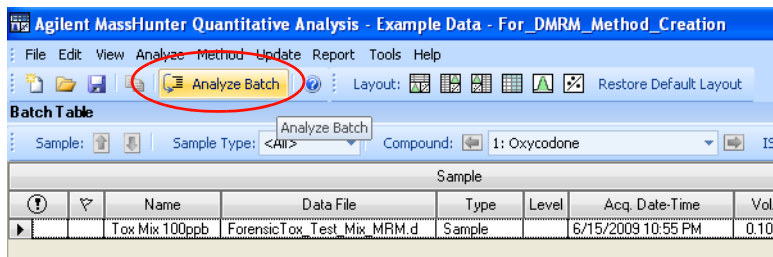
**Figure 17** Copy Calibration Levels To dialog box

- k Click **OK**.
- l Click **Method > Exit** to close the Method Table.
- 4 Click **Yes** to apply the method to the batch.



5 Save the batch and click **Analyze Batch** to run the batch analysis.

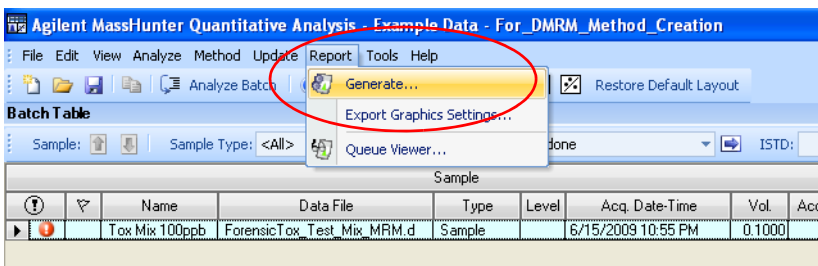
At this point, you are only interested in getting the retention times out of the analysis.



6 Save the batch.

7 Generate a report for the data file:

a Click **Report -> Generate**.



**Figure 18** Generate on the Report menu

b Under **Report Folder**, select the folder where you want to save this report. See [Figure 19](#).

c Click **Add** to add a new template.

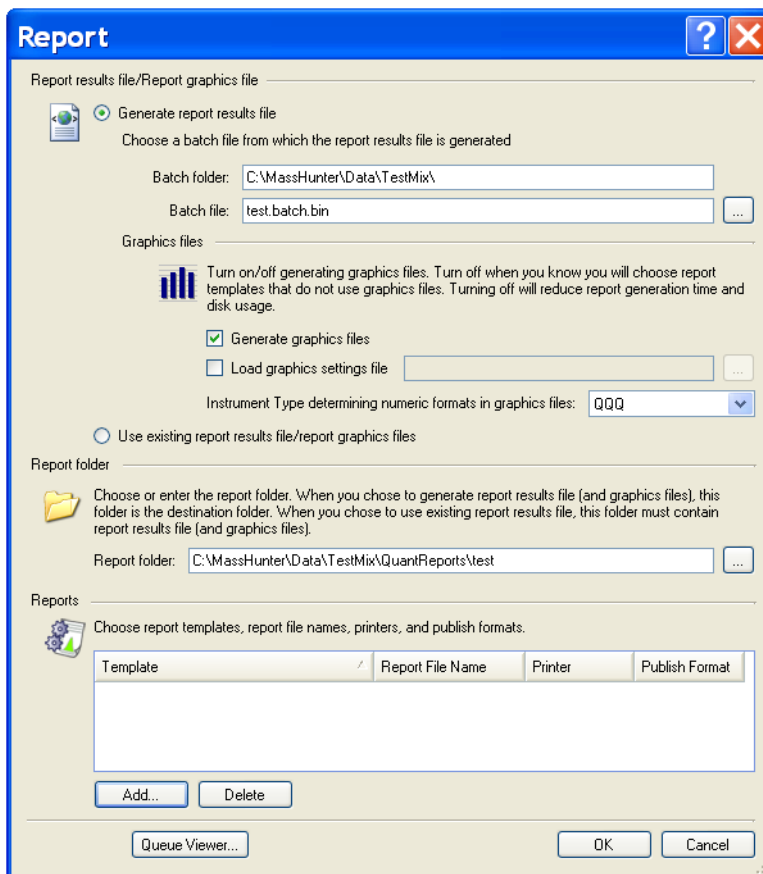
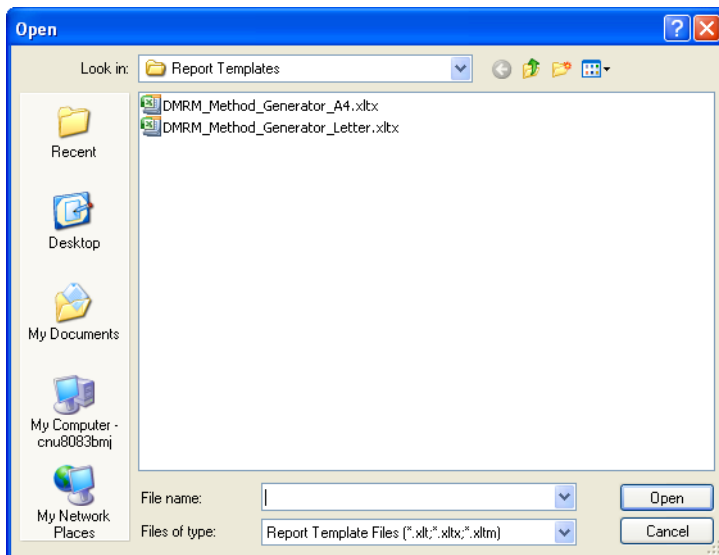


Figure 19 Report dialog box

- d In the **Report Templates** folder on the MassHunter Forensic Toxicology Dynamic MRM Database Kit support disk, click **DMRM\_Method\_Generator\_Letter.xlsx** or **DMRM\_Method\_Generator\_A4.xlsx**, then click **Open**. See [Figure 20](#).



**Figure 20** Open dialog box, with choice of A4 or Letter report templates.

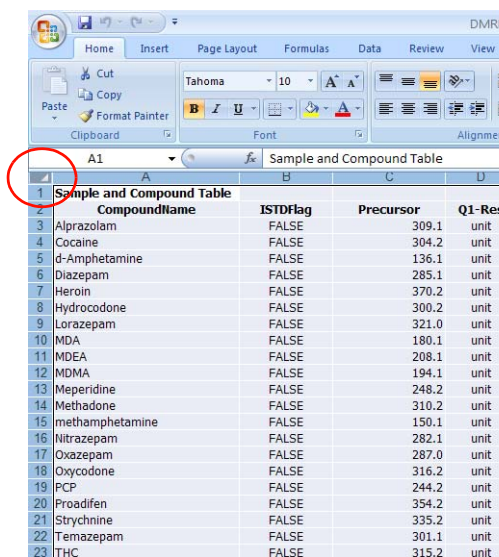
- e In the Report dialog box, specify the **Printer** to use, the **Publish Format** and the **Report File Name**, then click **OK**.

MassHunter can take one to two minutes to generate the report. If you want to check on the progress, click **Report > Queue Viewer**.

- f After the report is generated, go to the folder where you saved the report and open the report file. The report is in .xlsx format.
- 8 Repeat the steps in this topic for every mix of standards you want to go into the one Dynamic MRM method you are creating.

## Create final Dynamic MRM method

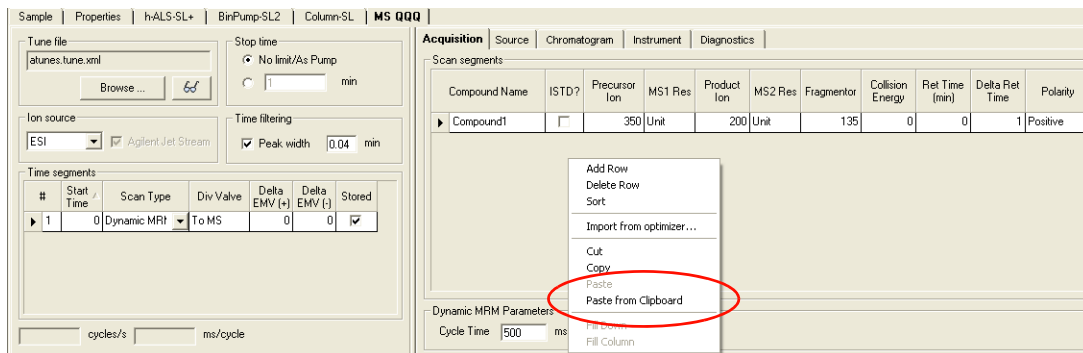
- 1 For the first data file:
  - a Open the Excel report for that data file.
  - b Click the **Report 2** tab to open that worksheet.
  - c Select all content in the spreadsheet. To do so, you can press **Ctrl + A** or click the upper left cell as indicated in the [Figure 21](#) on page 29.
  - d Copy the selected cells to the Clipboard. To do so, press **Ctrl + C**.



**Figure 21** Click the gray triangle in the upper left corner of the worksheet to select all contents.

- e Open the MassHunter Data Acquisition program and load the single time segment MRM method from which you want to create a Dynamic MRM method.
- f In the Method Editor pane, click **MS QQQ > Acquisition**.
- g From the **Scan Type** list, select **Dynamic MRM**.

- h Right-click an empty area on the Acquisition tab, then click **Paste from Clipboard**. See [Figure 22](#).



**Figure 22** Paste from Clipboard on the shortcut menu

Now the scan segments table contains your standards with all proper MRM settings, including retention times for Dynamic MRM runs.

- i Delete the first dummy compound (compound1) and if the **Delta Ret Time** setting of 1 minute is not appropriate for your chromatographic conditions, change to an appropriate retention time window.

The screenshot shows the 'Acquisition' tab with a table of scan segments. The table lists various compounds with their MRM parameters. The 'Delta Ret Time' column is highlighted in yellow.

Compound Name	ISTD?	Precursor Ion	MS1 Res	Product Ion	MS2 Res	Fragmentor	Collision Energy	Ret Time (min)	Delta Ret Time	Polarity
Alprazolam	<input type="checkbox"/>	309.1	Unit	281	Unit	179	25	3.715	1	Positive
Cocaine	<input type="checkbox"/>	304.2	Unit	182.1	Unit	138	17	2.358	1	Positive
d-Amphetamine	<input type="checkbox"/>	136.1	Unit	91	Unit	66	17	1.278	1	Positive
Diazepam	<input type="checkbox"/>	285.1	Unit	154	Unit	169	25	4.269	1	Positive
Heroin	<input type="checkbox"/>	370.2	Unit	165	Unit	149	61	2.236	1	Positive
Hydrocodone	<input type="checkbox"/>	300.2	Unit	199	Unit	159	29	1.38	1	Positive
Lorazepam	<input type="checkbox"/>	321	Unit	275	Unit	102	21	3.61	1	Positive
MDA	<input type="checkbox"/>	180.1	Unit	163	Unit	61	5	1.311	1	Positive
MDEA	<input type="checkbox"/>	208.1	Unit	163	Unit	107	9	1.72	1	Positive

**Figure 23** Scan segments table with scans from first set of standards imported.

- 2 For each additional set of standards that was run:
  - a Open the Excel report with the **MRM\_Method\_Generator\_Letter.xlsx** or **DMMR\_Method\_Generator\_A4.xlsx** template that you opened earlier, then click **Open**. See [Figure 20](#) on page 28.
  - b Copy and paste the **Report 2** worksheet to the Dynamic MRM method Scan segments table in the MS QQQ > Acquisition tab.
  - c Set the cycle time appropriate to the peak width of your chromatography.

Dynamic MRM will maximize the dwell time for each transition based on the cycle time you have selected.

- d Save the method in MassHunter Data Acquisition program with a different name from the original single segment MRM method.

## To bypass mixer and damper

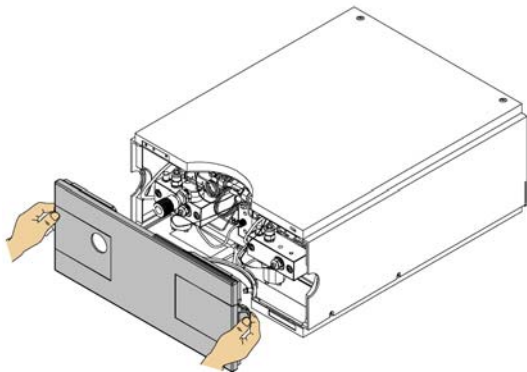
The Binary Pump SL is delivered in standard configuration (damper and mixer connected). This step shows how to bypass the damper and mixer and convert the pump to low delay volume mode.

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

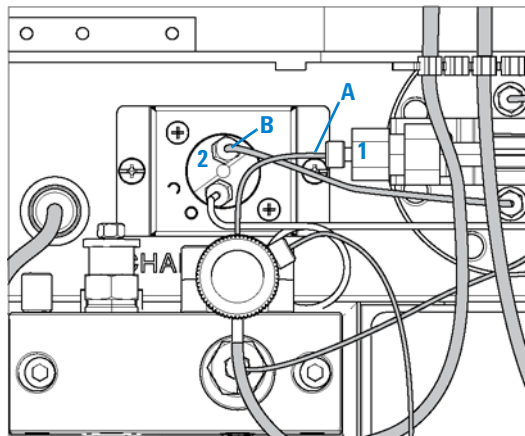
- Tools required**
- Wrench, 1/4-inch x 5/16-inch (p/n 8710-0510)
  - Wrench, open end, 14-mm (p/n 8710-1924)
  - Hex Driver, 1/4-inch, slitted (p/n 5023-0240)

- Preparations for this procedure**
- Flush the system (water if buffers were used, otherwise IPA).
  - Turn the flow off.

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

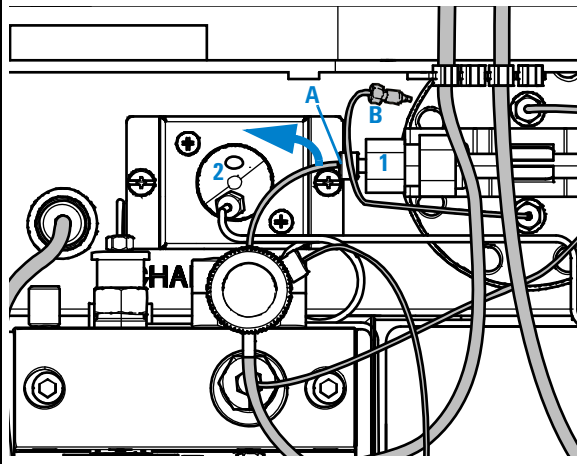


- 2** Use the 1/4 inch hex driver to remove fitting **B** from port 2 of the pressure sensor.

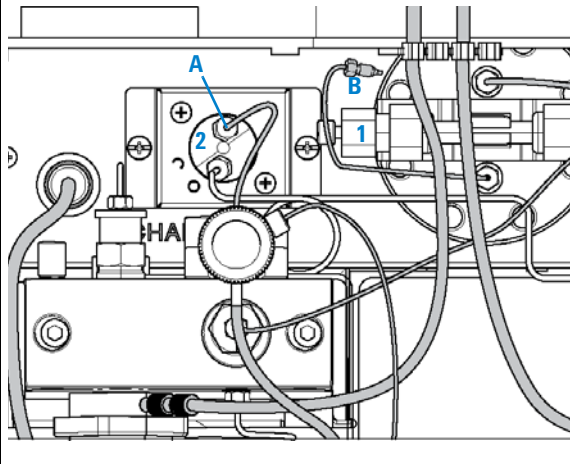




**3** Fold capillary end **B** away. It remains unconnected. Disconnect fitting **A** from outlet **1** of the mixer.



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



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## **In This Guide**

This Quick Start Guide describes how to use the MassHunter Forensic Toxicology Dynamic MRM Database Kit.

For Forensic Use. This information is subject to change without notice.

If you have comments about this guide, please send an e-mail to [feedback\\_lcms@agilent.com](mailto:feedback_lcms@agilent.com).

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