Agilent G1734AA MassHunter Forensics and Toxicology Dynamic MRM Database Kit

Quick Start Guide

What is the MassHunter Forensics and Toxicology Dynamic MRM Database Kit?

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

Agilent Technologies

The MassHunter Forensics and Toxicology Dynamic MRM Database Kit helps minimize method development time for your forensics and toxicology analysis, when used with Agilent's recommended LC/MS configuration and accessories. It stores Multiple Reaction Monitoring (MRM) transitions (a pair of precursor and product ions) of the forensics and 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 Forensics and Toxicology Dynamic MRM Database Kit Quick Start Guide The Quick Start Guide provides an overview of the MassHunter Forensics and 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 Forensics and Toxicology Dynamic MRM Database Included in the kit is a disk that contains MassHunter Forensics and Toxicology Dynamic MRM Database B.03.00, along with related software license agreements. See "Installation" on page 4 for a list of software requirements.

MassHunter Forensics and Toxicology Dynamic MRM Database Kit Support Disk

The content of the disk is:

- The Triple Quadrupole LC/MS Dynamic MRM method **LCMS_Forensics_and_ToxicologyTest_Mix_01.m**
- A sample chromatogram and Dynamic MRM report obtained with the test mix
- Acquisition methods
- Report templates for creating Dynamic MRM method

- *An Application Kit for the Screening of Samples for Analytes of Forensic and Toxicological Interest using LC/QQQ MS/MS with a Dynamic MRM Transition Database* Application Note
- *Multi-Residue Pesticide Analysis with Dynamic Multiple Reaction Monitoring and Triple Quadrupole LC/MS/MS* Application Note
- *New Dynamic MRM Mode Improves Data Quality and Triple Quad Quantification in Complex Analyses* Technical Overview
- *Pesticide Dynamic MRM Compound Database for Screening and Identification Using the Agilent LC/MS Triple Quadrupole Systems* Technical Note
- *Agilent G1734AA MassHunter Forensics and Toxicology Dynamic MRM Database Kit Quick Start Guide* (PDF format)

ZORBAX Rapid Resolution Eclipse Plus C18 HPLC Column (p/n 959764-902)

2.1mm x 100, 1.8 µm.

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

Where to find more information

Application Notes and Publications You can find information about the MassHunter Forensics and 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 46 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.03.01 SP1 or higher
   - MassHunter Quantitative Analysis B.03.02 or higher
   - MassHunter Qualitative Analysis B.03.01 or higher
   - MassHunter Optimizer B.03.01 or higher
5. Install the MassHunter Forensics and 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.
7. Copy the content of the **Report Template** folder on the support disk to the **D:\MassHunter\Report Templates\Quant** folder on your system. This report template is used to create Dynamic MRM methods.

Required Reagents and Parts

- LC/MS grade acetonitrile and water
- Formic acid (highest purity)
- Ammonium formate (highest purity)
- ZORBAX Rapid Resolution Eclipse Plus C18 HPLC Column, 2.1 x 100 mm, p/n 959764-902

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 Forensics and Toxicology Dynamic MRM Database Kit.

To review the Acquisition Method, use the MassHunter Data Acquisition program to load the method file **LCMS_Forensics_and_ToxicologyTest_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 Forensics and Toxicology Test Mix analytes and their chromatographic-dependent settings

Compound Name	ISTD?	Precursor Ion	MS1 Res	Product Ion	MS2 Res	Fragmentor	Collision Energy	Ret Time (Min)	Delta Ret Time	Polarity
Codeine	☐	300.2	Unit	165.1	Unit	158	45	1.11	0.4	Positive
Codeine	☐	300.2	Unit	58.1	Unit	158	29	1.11	0.4	Positive
Oxycodone	☐	316.2	Unit	298.1	Unit	143	17	1.285	0.4	Positive
Oxycodone	☐	316.2	Unit	256.1	Unit	143	25	1.285	0.4	Positive
d-Amphetamine	☐	136.1	Unit	119.1	Unit	66	5	1.296	0.4	Positive
d-Amphetamine	☐	136.1	Unit	91	Unit	66	17	1.296	0.4	Positive
MDA	☐	180.1	Unit	163	Unit	61	5	1.332	0.4	Positive
MDA	☐	180.1	Unit	105	Unit	61	21	1.332	0.4	Positive

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

Compound Name	ISTD?	Precursor Ion	MS1 Res	Product Ion	MS2 Res	Fragmentor	Collision Energy	Ret Time (Min)	Delta Ret Time	Polarity
Hydrocodone	☐	300.2	Unit	199	Unit	159	29	1.4	0.4	Positive
Hydrocodone	☐	300.2	Unit	128	Unit	159	65	1.4	0.4	Positive
methamphetamine	☐	150.1	Unit	119	Unit	92	5	1.45	0.4	Positive
methamphetamine	☐	150.1	Unit	91	Unit	92	17	1.45	0.4	Positive
MDMA	☐	194.1	Unit	163	Unit	97	9	1.468	0.4	Positive
MDMA	☐	194.1	Unit	105	Unit	97	25	1.468	0.4	Positive
Strychnine	☐	335.2	Unit	184	Unit	195	41	1.629	0.4	Positive
Strychnine	☐	335.2	Unit	156	Unit	195	53	1.629	0.4	Positive
MDEA	☐	208.1	Unit	163	Unit	107	9	1.735	0.4	Positive
MDEA	☐	208.1	Unit	105	Unit	107	25	1.735	0.4	Positive
Heroin	☐	370.2	Unit	268.1	Unit	149	37	2.256	0.4	Positive
Heroin	☐	370.2	Unit	165	Unit	149	61	2.256	0.4	Positive
Cocaine	☐	304.2	Unit	182.1	Unit	138	17	2.376	0.4	Positive
Cocaine	☐	304.2	Unit	77	Unit	138	61	2.376	0.4	Positive
Meperidine	☐	248.2	Unit	220.1	Unit	128	21	2.419	0.4	Positive
Meperidine	☐	248.2	Unit	174.1	Unit	128	17	2.419	0.4	Positive
Trazodone	☐	372.2	Unit	176	Unit	159	25	2.797	0.4	Positive
Trazodone	☐	372.2	Unit	148	Unit	159	37	2.797	0.4	Positive
PCP	☐	244.2	Unit	91	Unit	86	41	2.876	0.4	Positive
PCP	☐	244.2	Unit	86.1	Unit	86	9	2.876	0.4	Positive
Oxazepam	☐	287	Unit	269	Unit	150	12	3.53	0.4	Positive
Oxazepam	☐	287	Unit	241	Unit	150	20	3.53	0.4	Positive
Nitrazepam	☐	282.1	Unit	236.1	Unit	148	25	3.542	0.4	Positive

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

Compound Name	ISTD?	Precursor Ion	MS1 Res	Product Ion	MS2 Res	Fragmentor	Collision Energy	Ret Time (Min)	Delta Ret Time	Polarity
Nitrazepam	☐	282.1	Unit	180	Unit	148	41	3.542	0.4	Positive
Verapamil	☐	455.3	Unit	165	Unit	158	37	3.554	0.4	Positive
Verapamil	☐	455.3	Unit	150	Unit	158	45	3.554	0.4	Positive
Methadone	☐	310.2	Unit	265.1	Unit	112	9	3.61	0.4	Positive
Methadone	☐	310.2	Unit	105	Unit	112	29	3.61	0.4	Positive
Lorazepam	☐	321	Unit	275	Unit	102	21	3.626	0.4	Positive
Lorazepam	☐	321	Unit	194	Unit	102	49	3.626	0.4	Positive
Alprazolam	☐	309.1	Unit	281	Unit	179	25	3.727	0.4	Positive
Alprazolam	☐	309.1	Unit	205	Unit	179	49	3.727	0.4	Positive
Temazepam	☐	301.1	Unit	255.1	Unit	117	29	3.941	0.4	Positive
Temazepam	☐	301.1	Unit	177	Unit	117	45	3.941	0.4	Positive
Proadifen	☐	354.2	Unit	167	Unit	153	29	4.088	0.4	Positive
Proadifen	☐	354.2	Unit	91.1	Unit	153	45	4.088	0.4	Positive
Diazepam	☐	285.1	Unit	193	Unit	169	45	4.268	0.4	Positive
Diazepam	☐	285.1	Unit	154	Unit	169	25	4.268	0.4	Positive
THC	☐	315.2	Unit	193.2	Unit	150	20	5.277	0.4	Positive
THC	☐	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 times, better signal stability, and higher data quality compared to a traditional MRM method.

To run the test mix

Run the LC/MS Forensics and Toxicology Test Mix to get a better idea of how the MassHunter Forensics and 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 properly tuned. Do an Autotune if Checktune reports any failure.

2 Prepare the LC/MS Forensics and 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 Verify the system configuration.

For the analysis of the LC/MS Forensics and Toxicology Test Mix, load the method **LCMS_Forensics_and_ToxicologyTest_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 delay or dead volumes 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 bypassed. See "To bypass mixer and damper" on page 46.

5 Check that your method is set up to make a 1 µL 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 delay volume, dead volumes 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 **LCMS_Forensic and Toxicology Test 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 **LCMS_Forensic and Toxicology Test Mix 10pg.d** in the **Example Data** folder on the support disk.

See Figure 1.

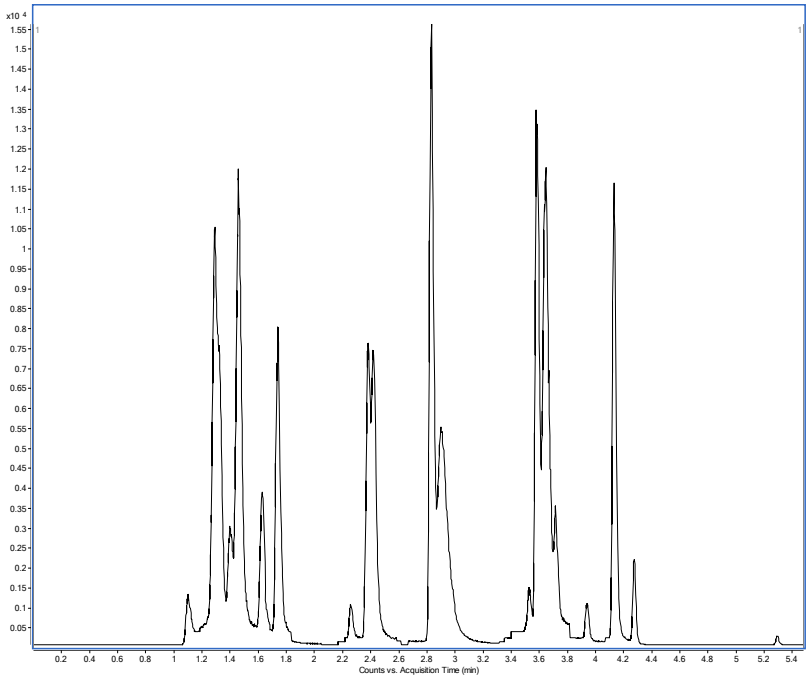


Figure 1 Example LC/MS Forensics and Toxicology Test Mix Total Ion Chromatogram

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

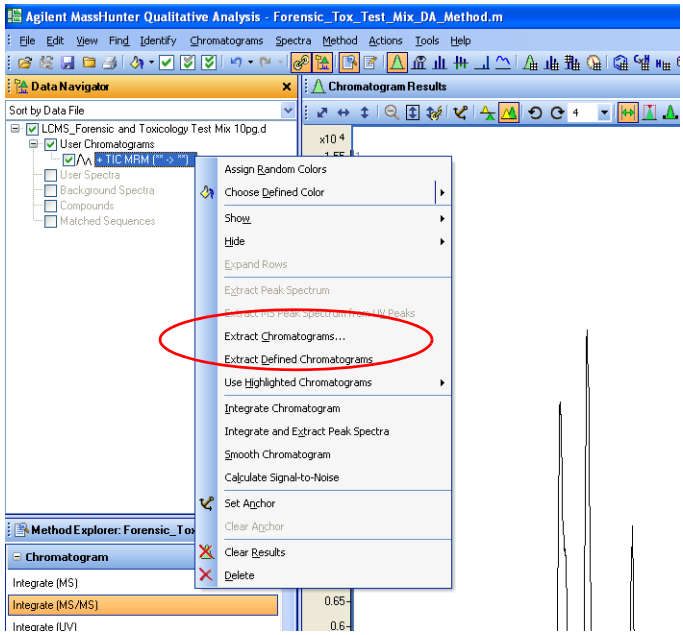


Figure 2 Extract Chromatograms on the TIC MRM shortcut menu.

5 In the Extract Chromatograms dialog box:

- **a** For **Type**, select **MRM**.
- **b** Set **Transition** to **All**. See Figure 3.
- **c** Mark the **Integrate when extracted** check box.

d Click **OK**.

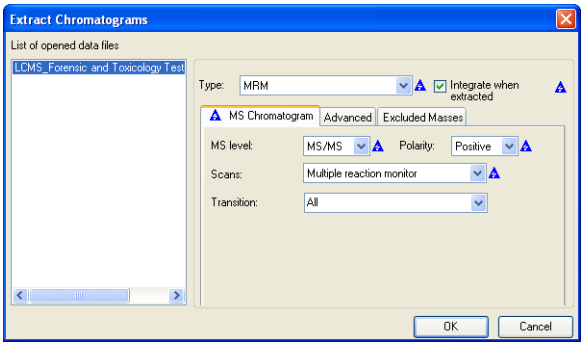


Figure 3 Extract Chromatograms dialog box

After the chromatograms are extracted and integrated, they are displayed on the Chromatogram Results window, as shown 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 the 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 Time 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 Forensics and 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*).

   If you select Dynamic MRM instead, retention times from the database are imported and will overwrite your existing conditions.

   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 Forensics and Toxicology Dynamic MRM Database.

4 Right-click an empty area on the Acquisition tab, then click **Import from optimizer** in the shortcut menu. See Figure 5.

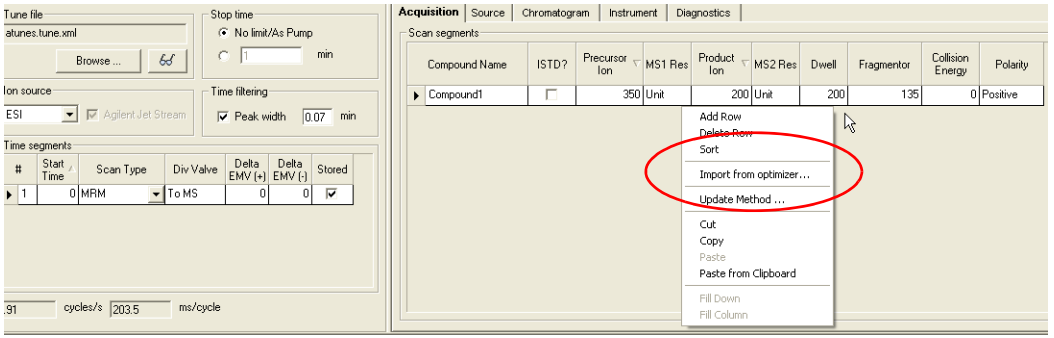


Figure 5 Import from optimizer shown in the Acquisition tab shortcut menu.

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 Forensics and Toxicology Dynamic MRM Database:

a In the Database Browser, click **File > Open Database**.

b From the **D:\MassHunter\Databases** folder, select the **ForensicTox_DynamicMRM_Database** folder. Note that the name of the current database (Read Only) is displayed at the bottom of the Database Browser.

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

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 ion 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. Alternatively, you can select to display or select **Response Factors** for MRM transitions in the Database Browser.

NOTE Note that the only way to add, edit or update database content is to save the information in a MassHunter Optimizer project.

If you want to change the order of the columns, drag a column heading to the desired location.

You can right-click anywhere within the compound table to get a menu of additional options. You can Show/Hide columns (useful for advanced Search/Filter operations).

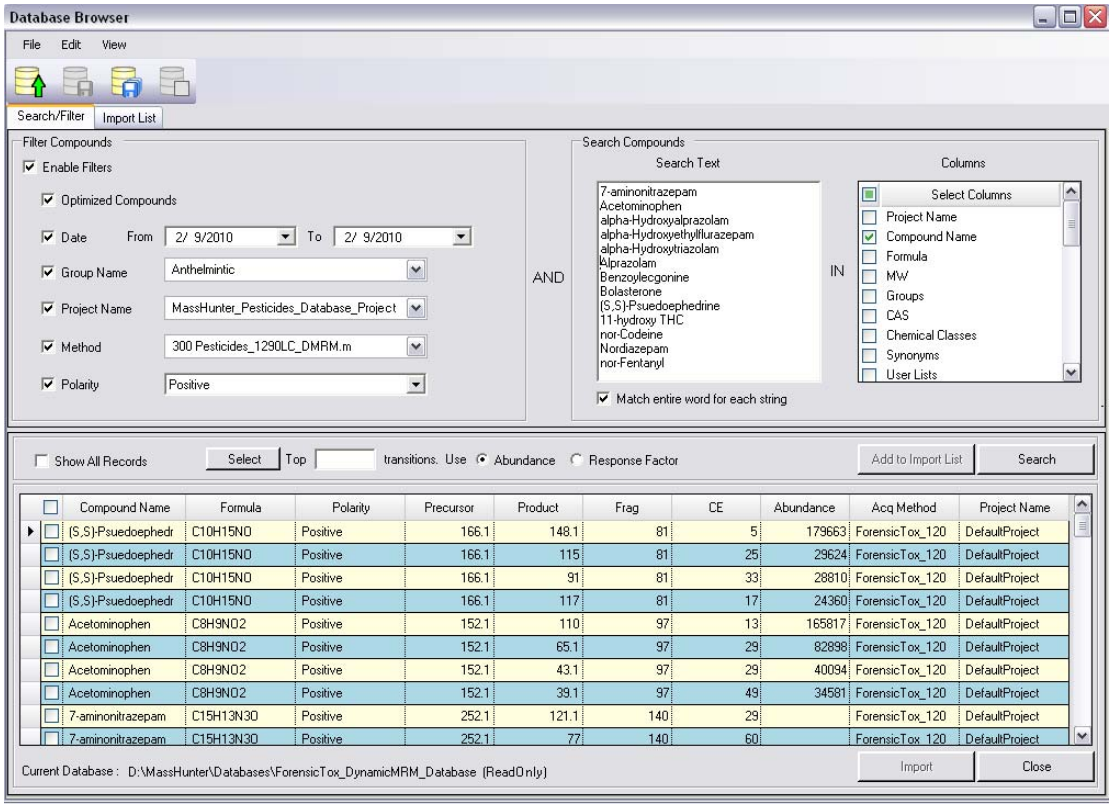


Figure 6 Database Browser with MassHunter Forensics and Toxicology Dynamic MRM Database opened

In Figure 6, several filters were enabled, including **Acquisition Method** and **Polarity**. You can also select **Top 2 transitions** based upon Abundance or response factors to refine the search.

6 Import the required MRM transitions from the database:

a Select the required compound transitions from the MassHunter Forensics and Toxicology Dynamic MRM Database browser.

When MRM transitions are selected, the Import and the Add to Import List buttons in the Database Browser become active.

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

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

Alternatively, you can add the compound transitions (step 6a) to the **Import List**. To do so, click the Add to Import List button, then click the Import List tab to see the compounds that you have added as shown in Figure 7. This lets you continue to search or filter for other compounds, or to select another database for searching. Compounds selected can be sequentially added (or removed) from the Import List. When the list is complete, click **Import** to copy the entries to the Scan Segment table on the MS QQQ > Acquisition tab.

7 Save the method.

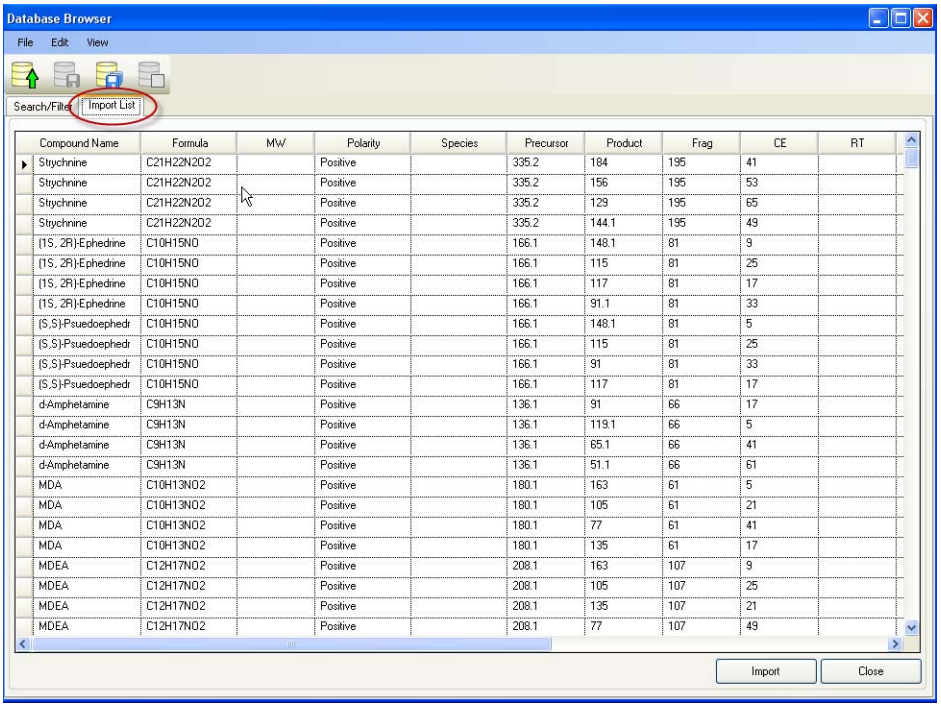


Figure 7 Database Browser window.

To save a database that can be edited

The database files that are installed with this kit are read-only. You can save a database to a new name so that you can edit it.

1 Open the database **ForensicTox_DynamicMRM_Database**.

When the database is initially opened, no filters are enabled, the Search Text box is empty, and no columns are selected. All compound database records are displayed, but none are selected.

2 Click the **Save As Database** icon  .

3 Type a name for the new database in the **File name** text box, then click **Save**. See Figure 8.

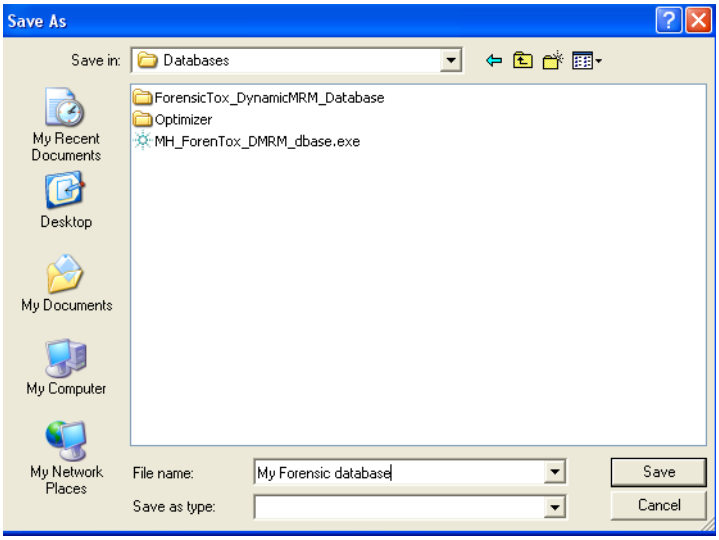


Figure 8 Save As dialog box to save a database that can be edited

To edit a user-created database

1 Open the MassHunter Optimizer program.

2 Click **Import/Export > Import from Database**.

The Database Browser opens.

3 Click the Open Database icon  .

4 Select a user-created database to open from the **D:\MassHunter\Databases** folder.

5 If you are prompted to set the database as the default database, click **Yes**.

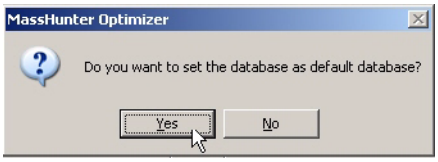


Figure 9

6 In the **Search/Filter** tab, select options in the **Filter Compounds** and **Search Compounds** to select the compounds to import to Optimizer, then **Search**.

7 Click **Import**.

The Optimizer window displays the compounds that you selected.

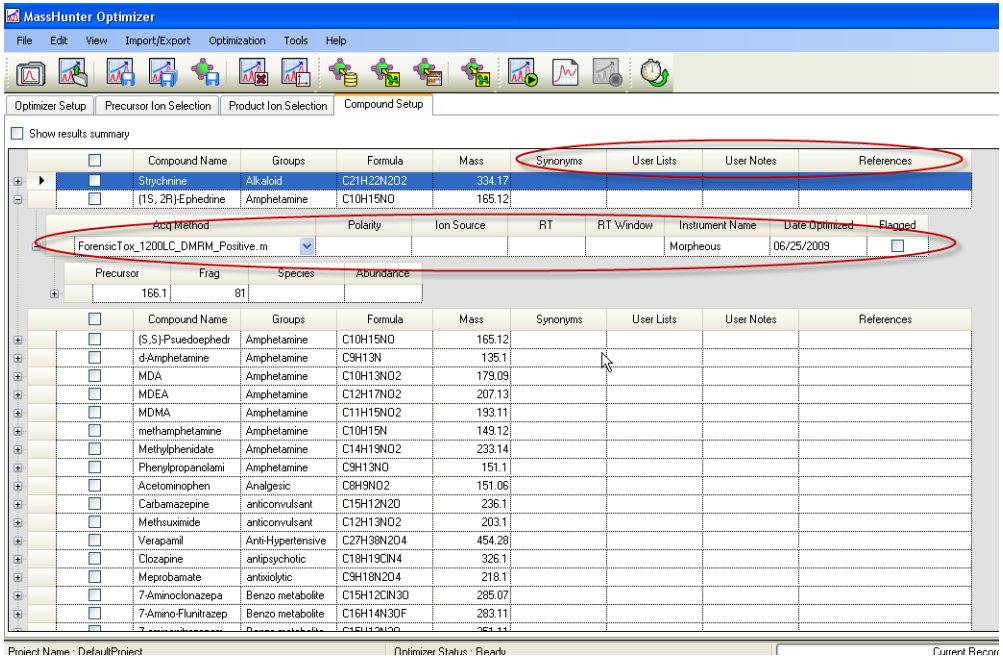


Figure 10 Commonly edited options

8 To select which columns are displayed for editing, right-click in the Compound Setup table and click **Show/Hide Columns**, then mark the check boxes for the columns to display.

9 For each compound record, edit the information in the applicable columns.

10 Save the project.

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 11.

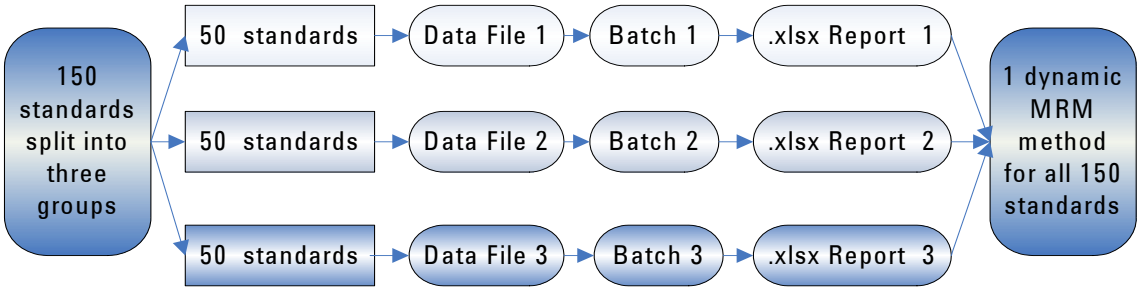


Figure 11 Example process for analyses that have more than 50 compounds

1 Run your standards with the method and chromatography that you created in "To create an MRM method to run your own sample".

- For best results, run standards with subgroups that contain no more than 100 compounds per injection. If you analyze between 50 and 100 compounds, run a medium level calibration with a dwell time of 2 milliseconds.
- 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. Run with one time segment if you have no prior knowledge of retention times.

  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 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 dynamic MRM method, all transitions in these data files must be detected.

2 In the MRM method which you ran in the previous step, click the **MS QQQ > Acquisition** tab.

Note that all transitions are cleared the first time you update the method from MRM to dynamic MRM and are set to what is in the data file you select. When a dynamic MRM method is updated, compounds in the data file that are not in the acquisition method are added. The LC conditions *must* be the same as those used to collect the data files you will use to create the method (so that the retention times will be the same).

3 Right-click the Scan segments table or in the gray area to the right or below it.

The menu shown in Figure 12 appears.

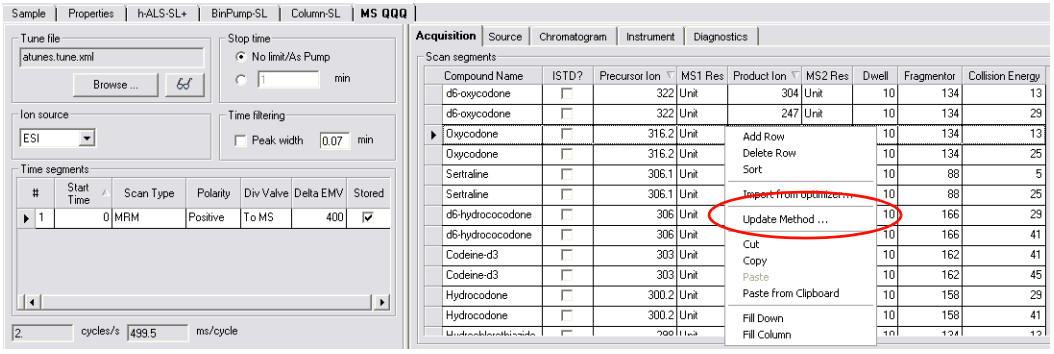


Figure 12 Acquisition tab with Update Method command highlighted

4 Click **Update Method** to open the Dynamic MRM Update Options dialog box shown in Figure 13.

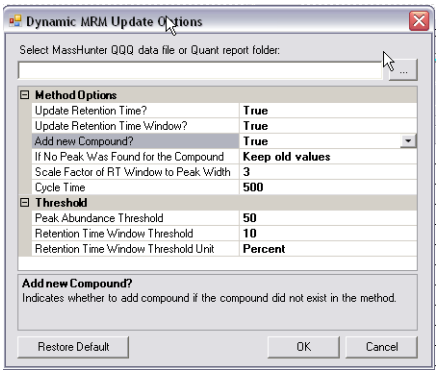


Figure 13 Dynamic MRM Update Options

This dialog box is used to add compounds to the method. Retention times and retention time windows are obtained from the data file that is selected.

5 Select a MassHunter Triple Quad data file or Quantitative report folder.

Click the Browse button to find the file to use.

6 Change the options in the Dynamic MRM Update Options dialog box as needed.

- Set all the **Method Options** parameters to **True**.
- Set **Add new Compound?** to **True**.
- Set **If No Peak Was Found for the Compound** to **Keep old values**.

  For all undetected peaks, the retention time will be set to 0. These undetected MRM transitions will be listed at the top of the Acquisition Scan Segment table when sorted by retention time, which lets you easily find them.

- The retention time window (Delta Ret Time) is scaled to the peak width found for that compound. A scale factor of 2 will create 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. The respective dwell times for MRM transitions will also decrease, depending on the number of overlapping peaks and their respective peak widths. See Figure 14.

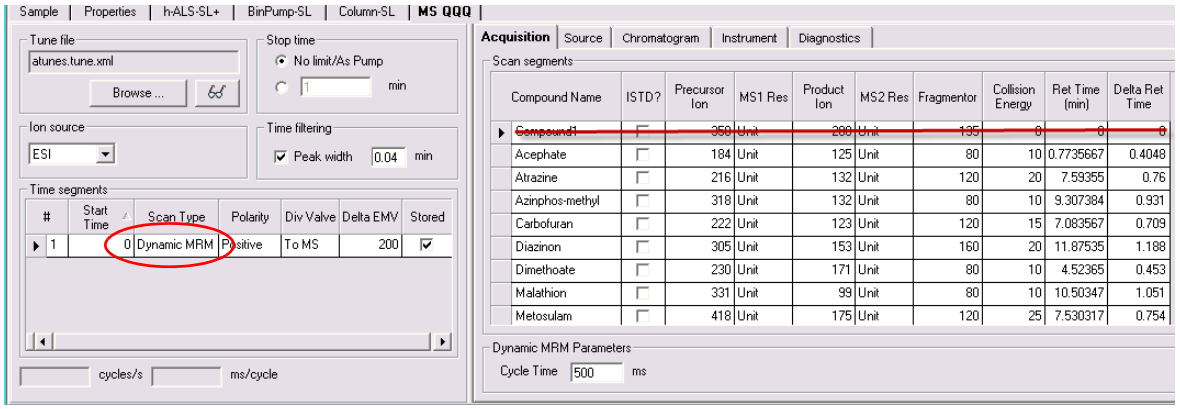


Figure 14

- If you manually select Dynamic MRM in the “Scan Type” under “Time Segments” as shown in Figure 14 before you update the method, the transition table is cleared to contain only “Compound1”. When you update the method, the compounds in the data file are added. As shown in Figure 14 you can delete the extraneous “Compound1”. (To delete the first row, select the row, right-click the table and click **Delete Row**.)

When you choose a data file, whether collected in MRM mode or Dynamic MRM mode, and update the method, the scan type in the method is converted to Dynamic MRM and the compounds in the data file added to the method.

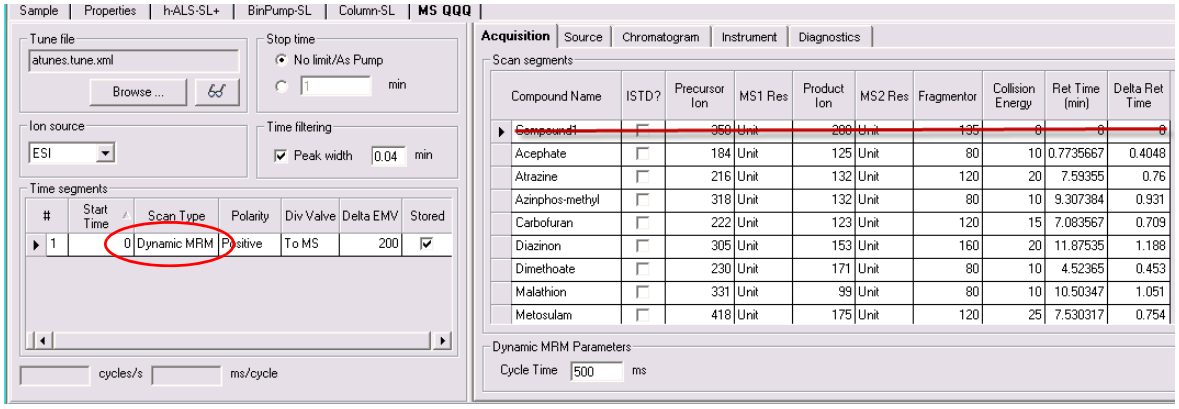


Figure 15

7 Click **OK**.

8 Repeat step 3 through step 6 until all the data files that contain the standards that will be used in the one Dynamic MRM method have been added. Make sure nothing is changed in the method and that all the **Method Options** parameters in the Dynamic MRM Update Options dialog box (Figure 13) are set to **True** and **If No Peak Was Found for the Compound** is set to **Keep old values**.

9 Save the method with an appropriate name.

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.

10 In the Acquisition tab, right-click the Scan segments table or the gray area next to it, and click **View Method**. See Figure 16.

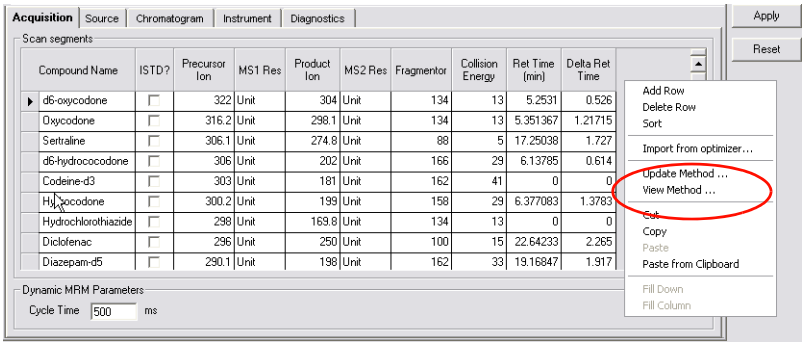


Figure 16 View Method command

The Dynamic MRM Viewer appears. It provides a powerful display to show you important details of your method. See Figure 17.

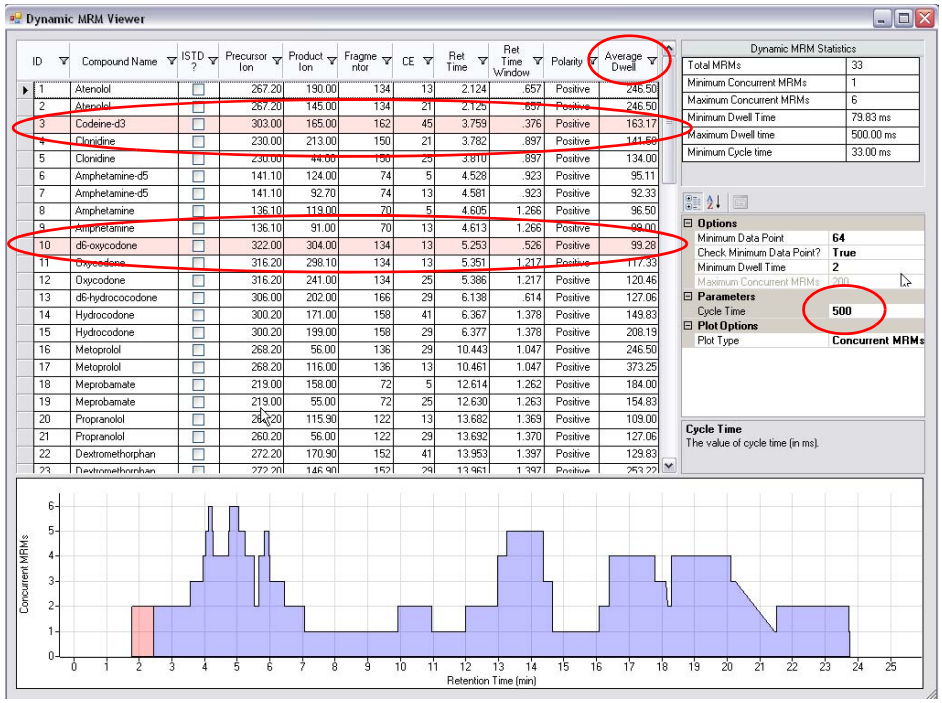


Figure 17 Dynamic MRM Viewer

11 Adjust the cycle time so that all criteria for minimum dwell time, for the MS-MS integrator, and for good integration are met.

- To use the MS-MS 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**. In Figure 17, the two compounds that are highlighted in pink indicate that the MS-MS integrator will not work.

The retention time window *and* the cycle time are set such that fewer than 64 data points will be collected. When the cycle time is decreased to 350 ms as shown in Figure 18, minimum requirements for the MS-MS integrator are met. When you change the cycle time in the viewer, you immediately see its effects on the dwell times.

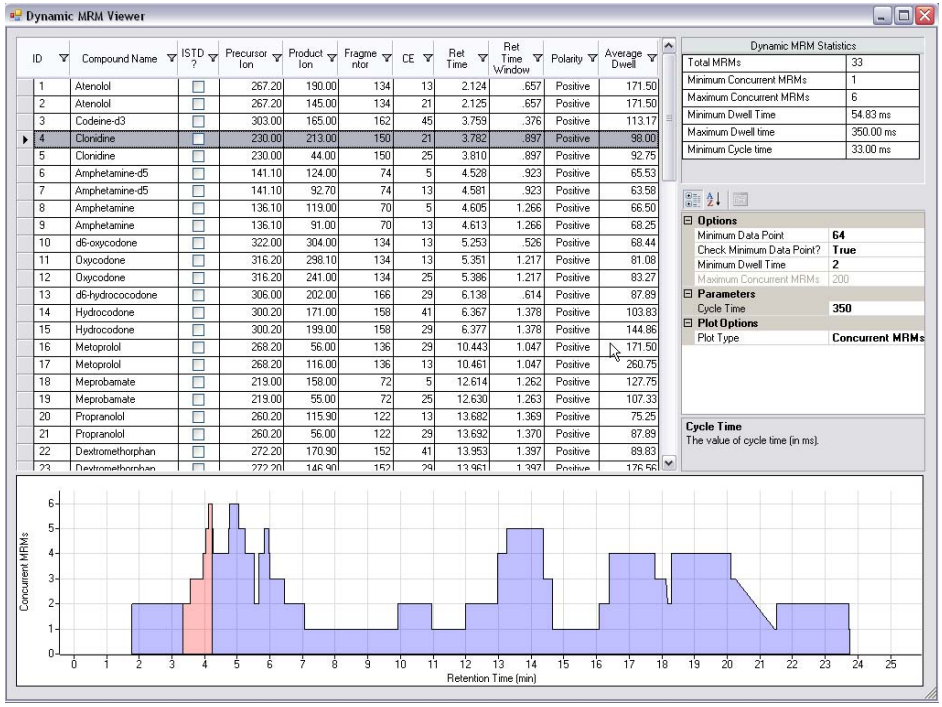


Figure 18 List of corrected transitions

However, 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 again.

- A dwell time of 2 ms or more is required to acquire data for dynamic MRM. 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 300 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 method used to process standards and samples collected by this method. The default is the MS-MS integrator.

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

12 Once a cycle time is determined for good integration (10 or more data points across a peak), type in this value for **Cycle Time** in the MS QQQ > Acquisition tab of the method editor. Figure 19 shows the cycle time setting in the MS QQQ > Acquisition tab.

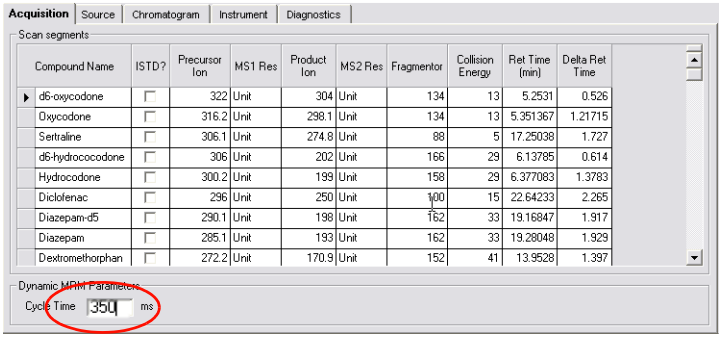


Figure 19 Acquisition tab with the new Cycle Time

Note that the cycle time in the Dynamic MRM Update Method Options dialog box (Figure 13 on page 25) 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 MS 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.

13 Save the method.

To update a Dynamic MRM method to include data files with errors

Do these steps only if you are unable to successfully create a Dynamic MRM method with the use of the Update Method function directly from a data file.

For example, if you get an error message such as that shown in Figure 20 when you update the method with a data file, none of the compounds in that data file are included in the dynamic MRM method that you are creating. You can use the steps in this topic to add the valid compounds from that data file.

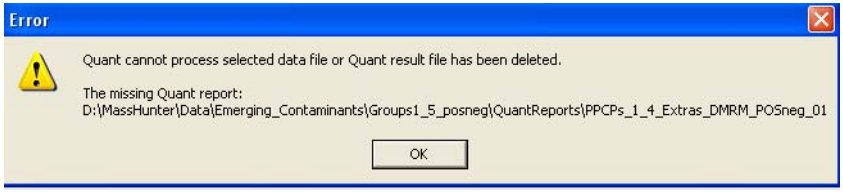


Figure 20

In this topic, you:

- Manually generate a report for each data file.
- Remove all errors in the manually generated quantitation method.
- Update the dynamic MRM method with a Quant Report, using the Update Method tool.

Do these steps after you run your standards, for only the data files that cannot be used to automatically create the dynamic MRM method.

Create a batch file for each data file

To process multiple data files, you create a separate batch file and report for each one. You will use the report file instead of the data file to update your dynamic MRM method.

Do the steps in this task for each data file that you need to process.

1 Open the MassHunter Quantitative Analysis program.

2 Create a new batch in the folder that contains the MRM data you collected.

   a Click **File > New Batch**. See Figure 21.

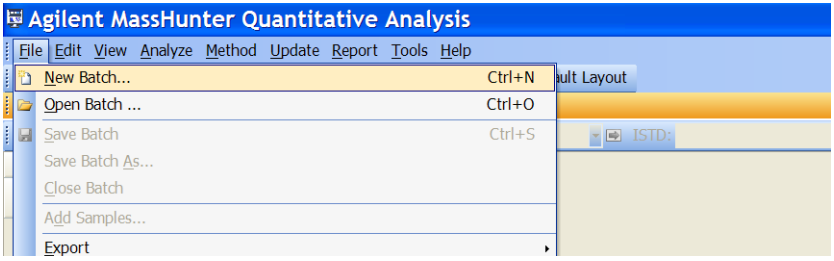


Figure 21 New Batch from the File menu

b Type a **File name** for the batch, then click **Open**. See Figure 22.

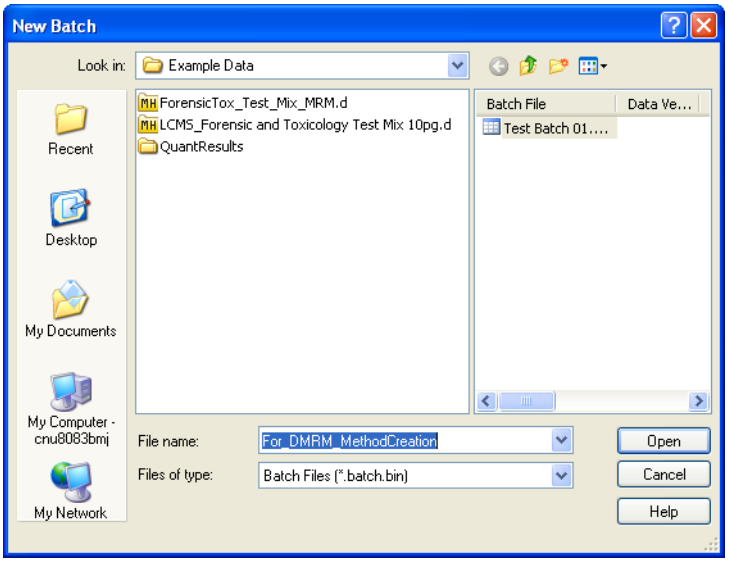


Figure 22 New Batch dialog box

3 Load the single time segment MRM data of your first standard that failed with the Update Method function.

a Click **File > Add Samples**. See Figure 23.

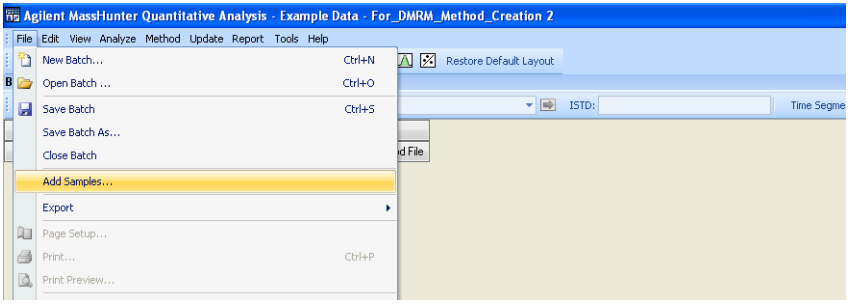


Figure 23 Add Samples from the File menu

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 24.

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

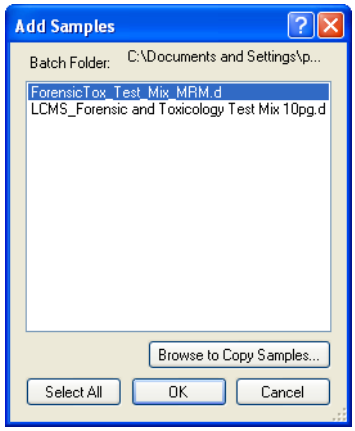


Figure 24 Add Samples dialog box

4 Create a new method.

a Click **Method > New > New Method from Acquired MRM Data**. See Figure 25.

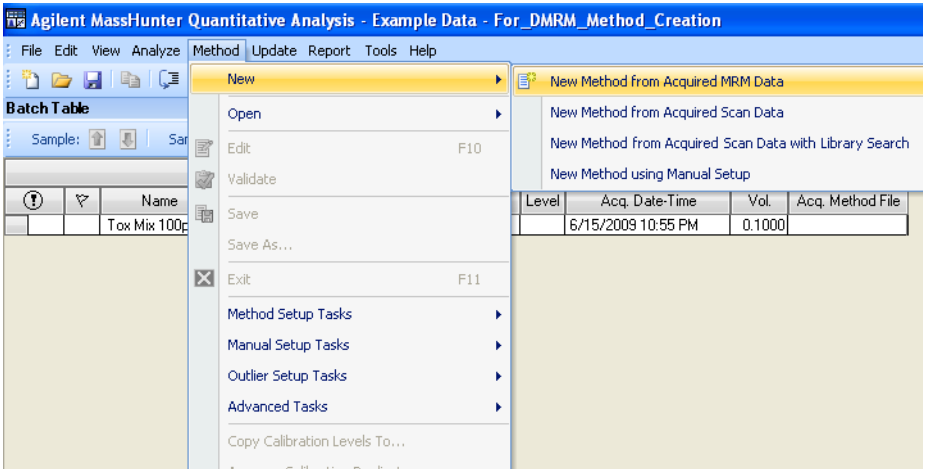


Figure 25 New Method from Acquired MRM Data selected

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

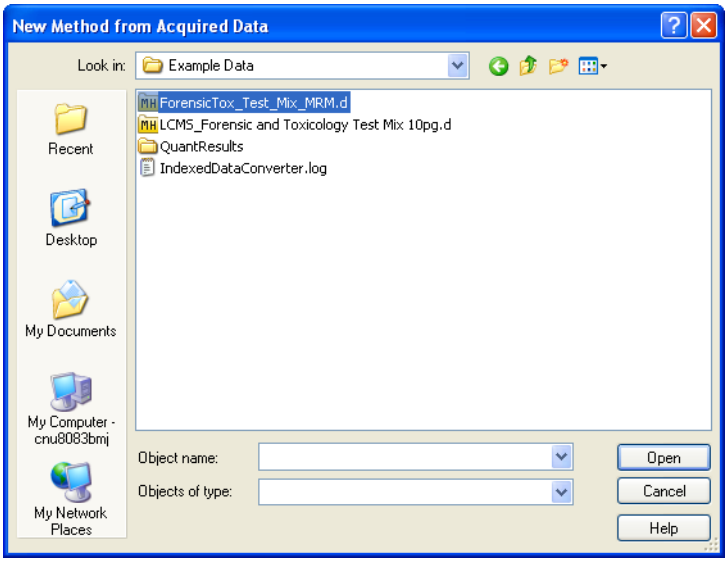


Figure 26 New Method from Acquired Data dialog box

c In the Quantitative Analysis program, from the **Method Setup Tasks** list, click **Concentration Setup**. See Figure 27.

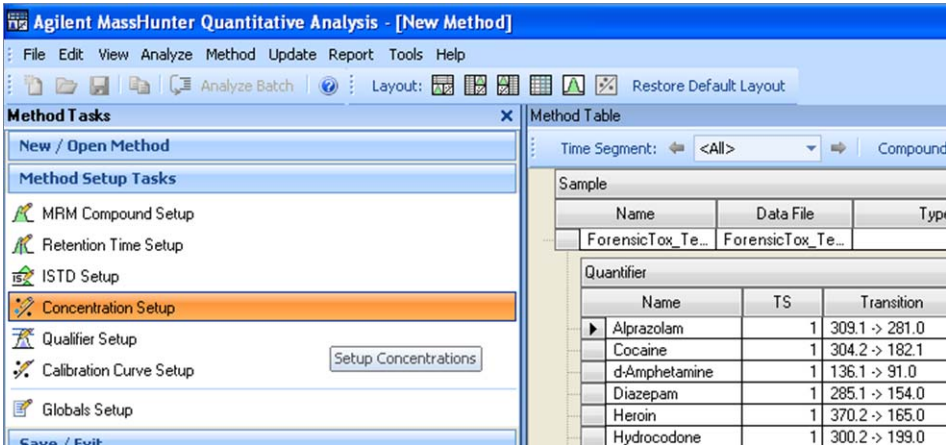


Figure 27 Concentration Setup under Method Setup Tasks

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.

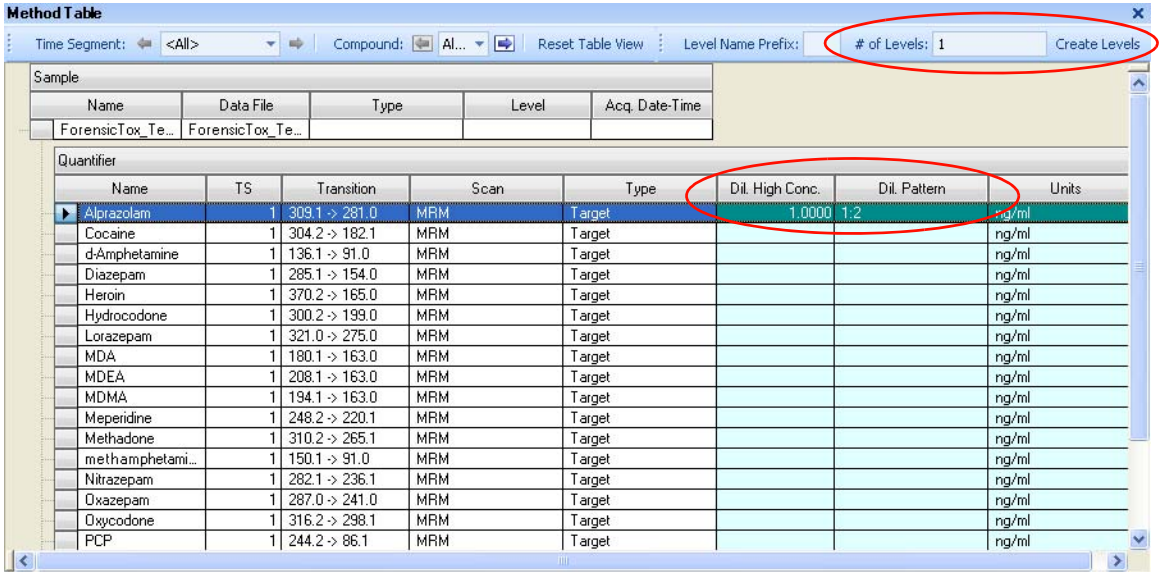


Figure 28 Quantifier table with first compound selected

i After the level is created, right-click the name of the first compound and click **Copy Calibration Levels To**. See Figure 29.

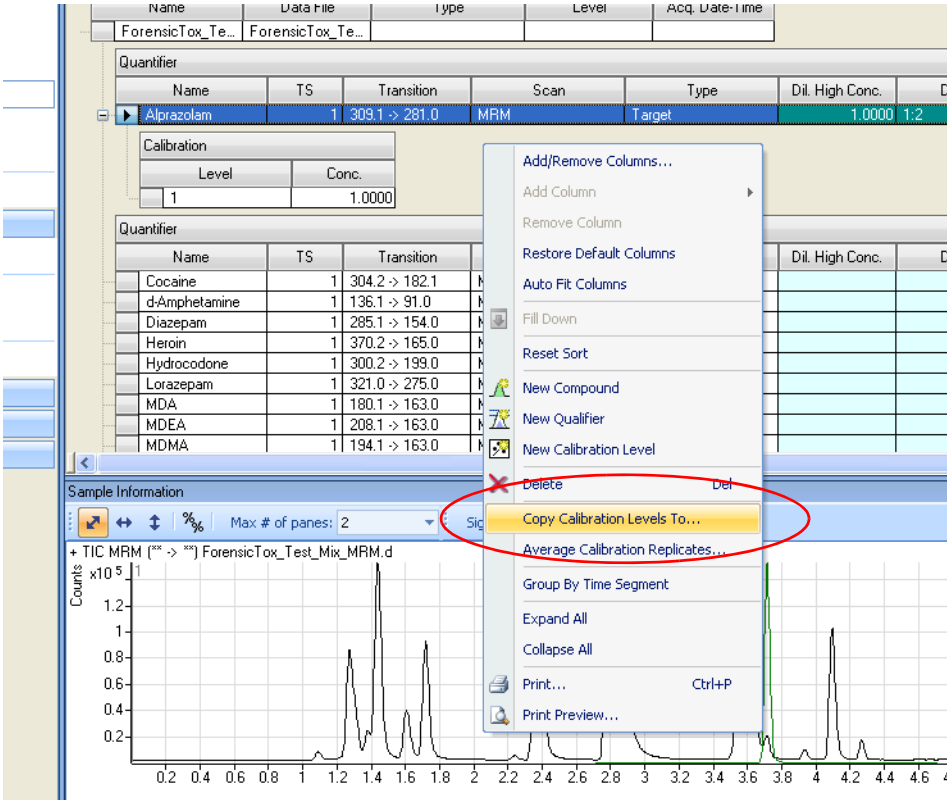


Figure 29 Copy Calibration Levels To selected

5 Click **Select All** to select all compounds in the data file. See Figure 30.

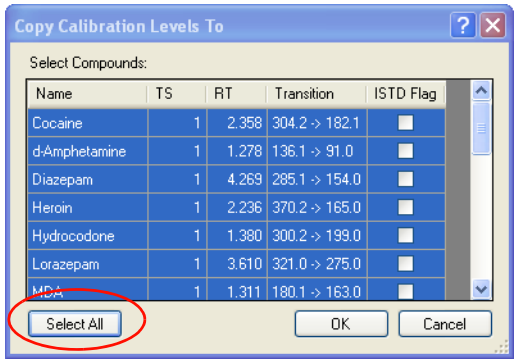


Figure 30 Copy Calibration Levels To dialog box

j Click **OK**.

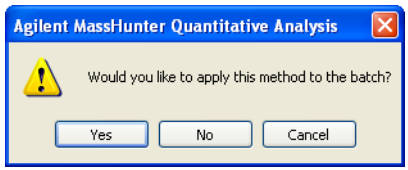
k Click **Validate,** then click on each error and correct it. To correct an error, type a value for the parameter that is missing, or delete that transition from the method. Typical errors are “retention time cannot be zero” or “missing qualifier ratio.”

Make sure the method is validated with no errors before you continue.

l Save the method. As a way to keep track of the method, use the same name as the data file, such as **ForensicTox_Test_Mix_MRM.quantmethod.xml**.

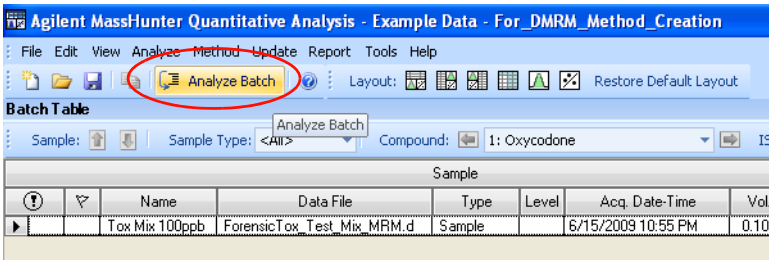
m Click **Method > Exit** to close the Method Table.

6 Click **Yes** to apply the method to the batch.



7 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.



8 Save the batch again, now that the results are processed.

9 Generate a report for the data file:

a Click **Report > Generate**.

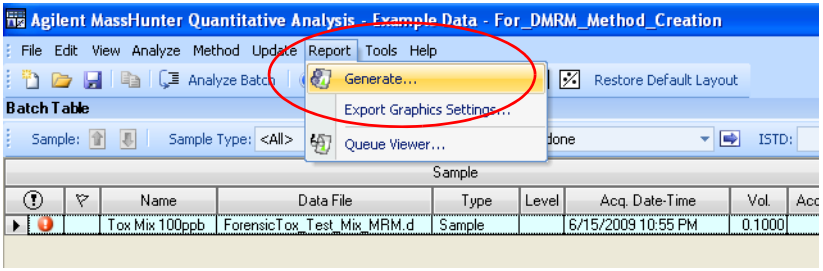


Figure 31 Generate on the Report menu

b Under **Report Folder**, select the folder where you want to save this report. Use the default data folder. See Figure 32.

c Depending on your version of the MassHunter Quantitative Analysis program, either click **Add** under **Reports**, or click the Browse button (...) next to **Template file**.

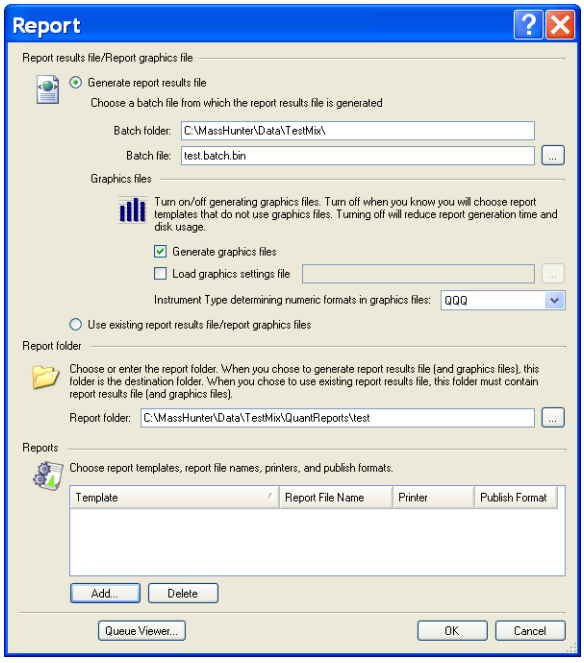


Figure 32 Report dialog box

d In the **D:\MassHunter\Report Templates\Quant** folder, click **DMRM_Method_Gen.xltx**, then click **Open**. See Figure 33.

Figure 33 Open dialog box, with DMRM_Method_Gen.xltx selected

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

f Depending on your version of the MassHunter Quantitative Analysis program, either click **Queue Viewer** to open the Queue Viewer and then minimize the viewer, or mark the **Queue Viewer** check box.

g Click **OK**.

MassHunter can take one to two minutes to generate the report. Use the Queue Viewer to check on the progress.

10 Repeat this entire topic (starting from the top of "Create a batch file for each data file" on page 34) for every group of standards that you need to process manually.

Create final Dynamic MRM method

1 For the first data file for which Quant reports were manually generated:

   a In the MassHunter Acquisition software, right-click within the Acquisition tab (Figure 12 on page 24) and click **Update Method**.

   b In the Dynamic MRM Update Options dialog box (Figure 13 on page 25), click the Browse button (...) and select the Quant folder (inside of the data folder) of the manual report that was generated as shown in Figure 34.

      The method is now updated with the transitions, parameters, and retention times found in the Quant report.

2 Repeat step 1 for each data file for which a quant report was manually generated.

You can always use that Quant Report folder to update methods, which is a faster process than using the data file again.

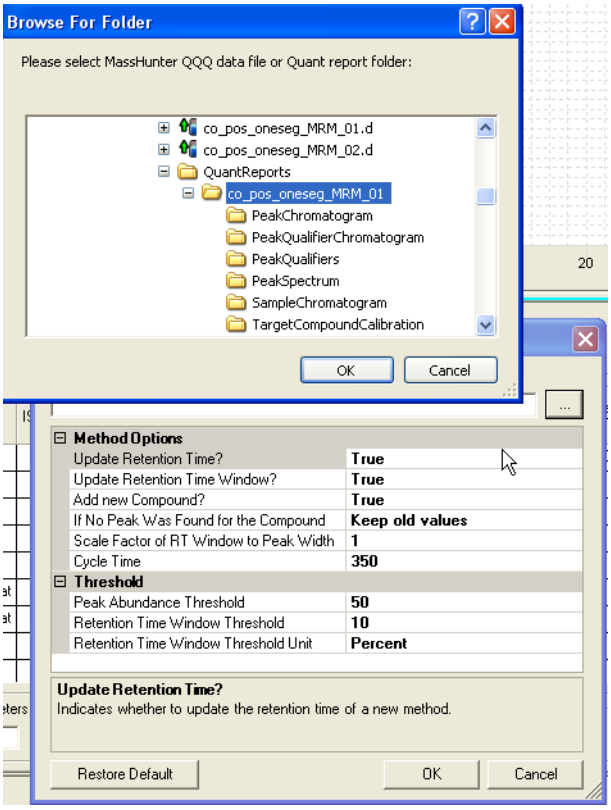


Figure 34 Navigation to Quant Report folder. Select the Quant report folder of the data file to be used to update the method.

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.

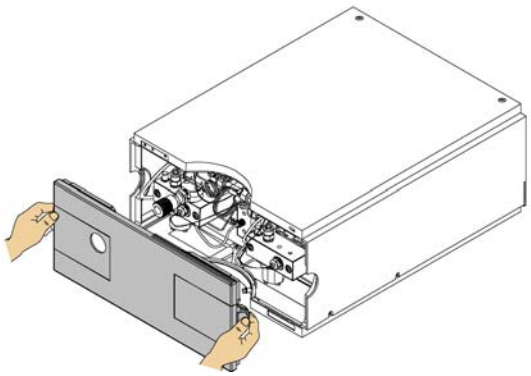
Tools required

- 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)

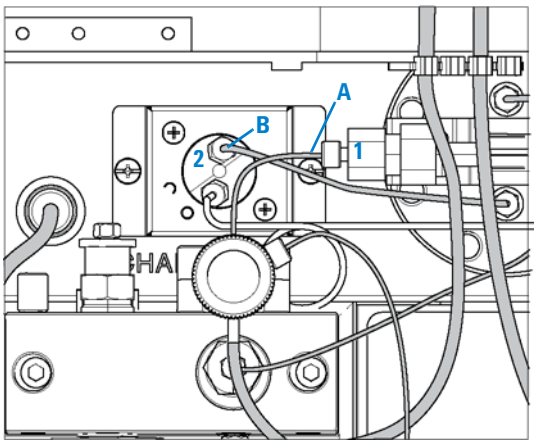
Preparations for this procedure

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

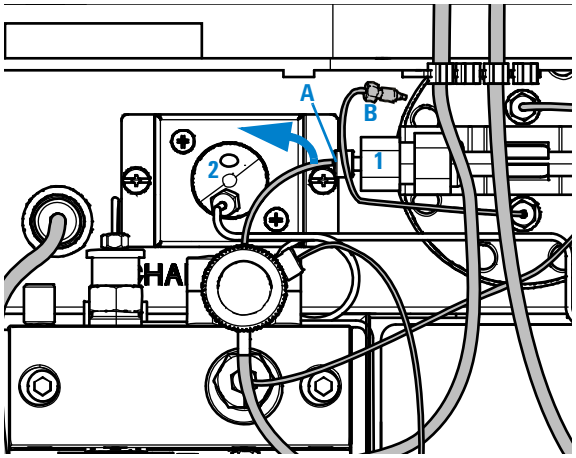
1 Remove the front cover by pressing the clip fastener on both sides of the cover.



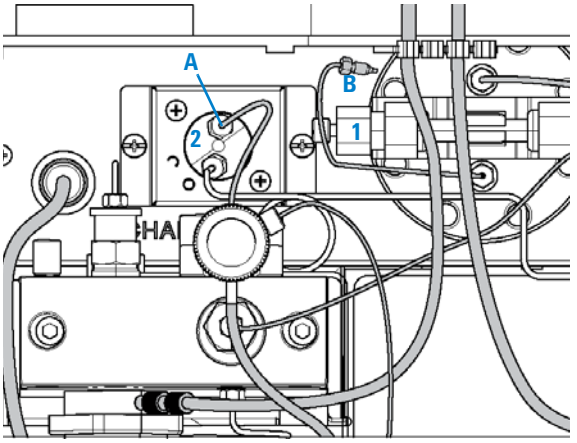
2 Use the 1/4 inch hex driver to remove fitting **B** from port *2* of the pressure sensor.



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 Forensics and Toxicology Dynamic MRM Database Kit.

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