Agilent G1733AA MassHunter Pesticide Dynamic MRM Database Kit

Quick Start Guide

What is the MassHunter Pesticide Dynamic MRM Database Kit?

The MassHunter Pesticide Dynamic MRM Database Kit lets you analyze more than 300 pesticides with 2 transitions each (600 MRM transitions) with enhanced sensitivity, all in a single LC/MS analysis.

The MassHunter Pesticide Dynamic MRM Database Kit helps minimize method development time for your pesticides 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 all pesticides included in the database, and their optimized fragmentor and collision energy settings on

Agilent Technologies

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 G1733AA MassHunter Pesticide Dynamic MRM Database Kit Quick Start Guide The Quick Start Guide provides an overview of the MassHunter Pesticide 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 in this document.

MassHunter Pesticide Dynamic MRM Database Included in the kit is a disk that contains MassHunter Pesticide Dynamic MRM Database B.03.01, along with related software license agreements. See "Installation" on page 4 for a list of software requirements.

MassHunter Pesticide Dynamic MRM Database Kit Support Disk The content of the disk is:

- The Triple Quadrupole LC/MS Dynamic MRM methods to run the test mix (positive and negative)
- A sample chromatogram and Dynamic MRM report obtained with the test mix
- Acquisition methods
- Report templates for creating Dynamic MRM method
- *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
- *Agilent Jet Stream Thermal Gradient Focusing Technology* Technical Note

- *Pesticide Dynamic MRM Compound Database for Screening and Identification Using the Agilent LC/MS Triple Quadrupole Systems* Technical Note
- *Agilent G1733AA MassHunter Pesticide 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 Pesticide Test Mix (p/n 5190-0469) Acidic and basic pesticides sample mixes (3 vials each) for your test runs.

QuEChERS SPE kit (5982-7005) AOAC method sample pack, 3 samples.

QuEChERS SPE kit (5982-7000) EN method sample pack, 3 samples.

Where to find more information

Application Notes and Publications You can find information about the MassHunter Pesticide Dynamic MRM Database for pesticide analysis in the application notes and publications included on the support disk. The support disk also includes the acquisition methods used in this kit, in PDF format, so that you can set up nonstandard LC/MS/MS configurations.

QuEChERS Extraction Procedures and Ready-to-use Kits The QuEChERS (Quick Easy, Cheap, Effective, Rugged and Safe) extraction procedure for pesticide residues in fruits and vegetables is being used by labs around the world. For a training video, references, and ready-to-use kits for doing QuEChERS extractions, go to http://www.chem.agilent.com/en-US/products/consumables/samplepreparation/sampliqspe/sampliqquechers

Alternatively, go to http://www.chem.agilent.com/ and type QuEChERS into the Search text box.

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 47 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 Pesticide Dynamic MRM Database. Follow the installation instruction on the front of the database installation disk.
6. Copy these two folders from the Support Disk to **D:\MassHunter\Methods**:
   - **AJS Methods for Optimizer Pesticide Database**
   - **ESI Methods for Optimizer Pesticide Database**
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
- Glacial acetic acid 99.9% (highest purity)
- Formic acid (highest purity)
- 5M Ammonium formate (highest purity), p/n G1946-85021
- Ammonium acetate (highest purity)
- 5N Ammonium hydroxide (highest purity)

- ZORBAX Rapid Resolution Eclipse Plus C18 HPLC Column, 2.1 x 100 mm, p/n 959764-902, for test mix and for these methods:
  - **Base-Neutral Pesticides Test Mix_DMRM.m**
  - **Acidic Pesticides Test Mix_DMRM.m**
- ZORBAX RRHD Eclipse Plus C18 HPLC Column, 2.1 x 100 mm, p/n 959758-902, for these methods:
  - **300 Pesticides_1200LC_DMRM.m**
  - **75 Pesticides_1200LC_DMRM.m**
- ZORBAX RRHD Eclipse Plus C18 HPLC Column, 2.1 x 150 mm, p/n 959759-902, for this method:
  - **300 Pesticides_1290LC_DMRM.m**
- ZORBAX Rapid Resolution Eclipse XDB-C18 Guard Cartridge, p/n 821125-926, and Guard Hardware Kit, p/n 820888-901, for this method:
  - **Optimizer_Single Pesticide_1200LC_12.5mm-C8.m**

NOTE The retention times contained in the MassHunter Pesticide Dynamic MRM Database were obtained with specific methods and columns. Make sure you use only the column that is specified in the method that you load. The column used for each method can also be found under **Description of Method Selected** within the Open Method dialog box and also in the Properties tab after you load the method.

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 Pesticide Dynamic MRM Database Kit.

To review the Acquisition Method, use the MassHunter Data Acquisition program to load the method file Base-Neutral Pesticides Test Mix_DMRM.m. Note that a copy of the method files in PDF format is included on the support disk. The information can be used to set up non-standard LC/MS/MS configurations.

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 MS/MS transitions for positive ions and their compound-dependent settings

Compound Name	ISTD?	Precursor Ion	MS1 Res	Product Ion	MS2 Res	Fragmentor	Collision Energy	Ret Time (Min)	Delta Ret Time	Polarity
Aminocarb	☐	209.1	Unit	137.1	Unit	120	20	3.125	1	Positive
Imazapyr	☐	262.1	Unit	217.1	Unit	160	15	3.958	1	Positive
Thiabendazole	☐	202	Unit	131.1	Unit	120	30	4.071	1	Positive
Dimethoate	☐	230	Unit	171	Unit	80	10	5.066	1	Positive
Imazalil	☐	297.1	Unit	159	Unit	160	20	5.927	1	Positive
Metoxuron	☐	229.1	Unit	72	Unit	93	14	5.996	1	Positive
Carbofuran	☐	222.1	Unit	123	Unit	120	15	7.025	1	Positive

Table 1 MS/MS transitions for positive ions and their compound-dependent settings (continued)

Compound Name	ISTD?	Precursor Ion	MS1 Res	Product Ion	MS2 Res	Fragmentor	Collision Energy	Ret Time (Min)	Delta Ret Time	Polarity
Atrazine	☐	216.1	Unit	132	Unit	120	20	7.444	1	Positive
Metosulam	☐	418	Unit	175	Unit	144	26	7.481	1	Positive
Metazachlor	☐	278.1	Unit	134.1	Unit	75	18	8.045	1	Positive
Molinate	☐	188.1	Unit	55.1	Unit	78	22	9.138	1	Positive
Malathion	☐	331	Unit	99	Unit	80	10	9.619	1	Positive
Pyraclostrobin	☐	388.1	Unit	163.1	Unit	120	20	10.681	1	Positive
Diazinon	☐	305.1	Unit	153.1	Unit	160	20	10.779	1	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.

The acquisition method parameters for the negative ion test mix are in the test mix method Acid Pesticides Test Mix_DMRM.m.

To run the test mix

Run the test mix (p/n 5190-0469) to get a better idea of how the MassHunter Pesticide 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, then click **Checktune** to verify the instrument is properly tuned. Do an Autotune if Checktune reports any failure.

2 Prepare the test mixes.

The concentration of the test mix stock solution is 100 ppm for both positive and negative mixes.

a Dilute 100 µL of the stock solution to 10.0 mL with acetonitrile to create the interim solution (1 ppm).

b Take 100 µL of the interim solution and dilute it to 10.0 mL with 10:90 acetonitrile:water.

c Transfer an aliquot of the final solution to a standard 2 mL sample vial for analysis.

The final solution is a 10 ppb working solution. Do this separately for the positive and negative test mixes.

3 Prepare mobile phases A and B.

- A= 5 mM acetic acid in water (286 µL glacial acetic acid in 1 L water)
- B= 100% acetonitrile

4 Verify the system configuration.

Load the one-segment test mix method for your instrument that is appropriate for your test mix (positive or negative). These methods use the system configuration as listed below. Systems that deviate from this configuration may not work with this method.

Column	2.1 x 100 mm 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 47
Column Compartment	Column – SL, Model G1316B

5 Check that your method is set up to make a 5 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 15 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 **Test_mix_pos.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 **Test_mix_pos.d** in the **Example Data** folder on the support disk.

See Figure 1.

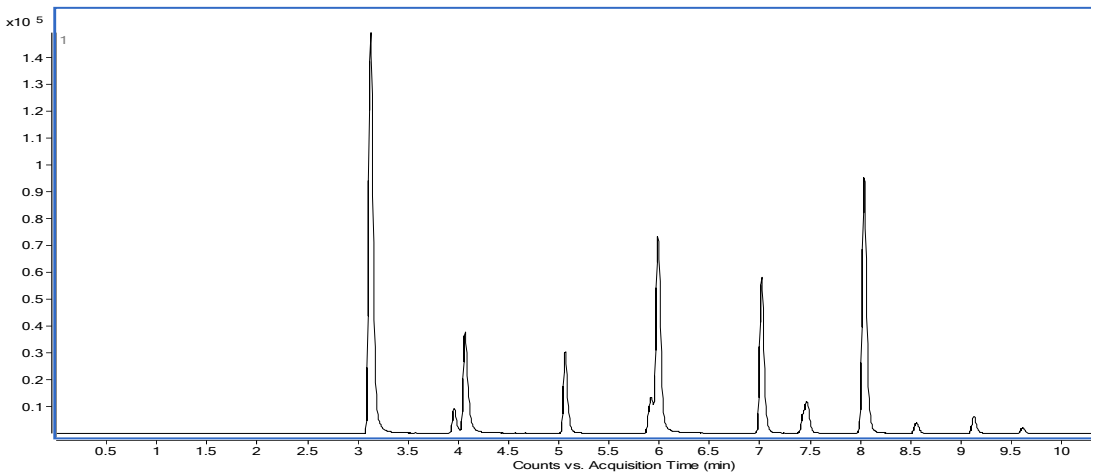

Figure 1 Example test mix total ion chromatogram

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

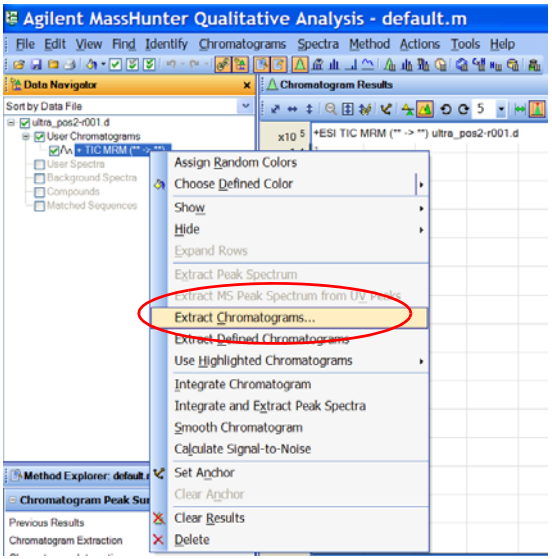

Figure 2 Extract Chromatograms on the TIC MRM shortcut menu.

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**.

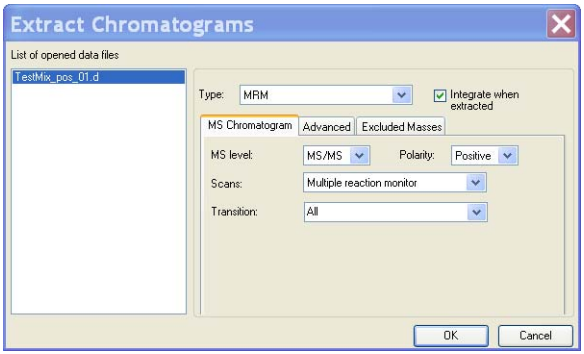

Figure 3 Extract Chromatograms dialog box

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.

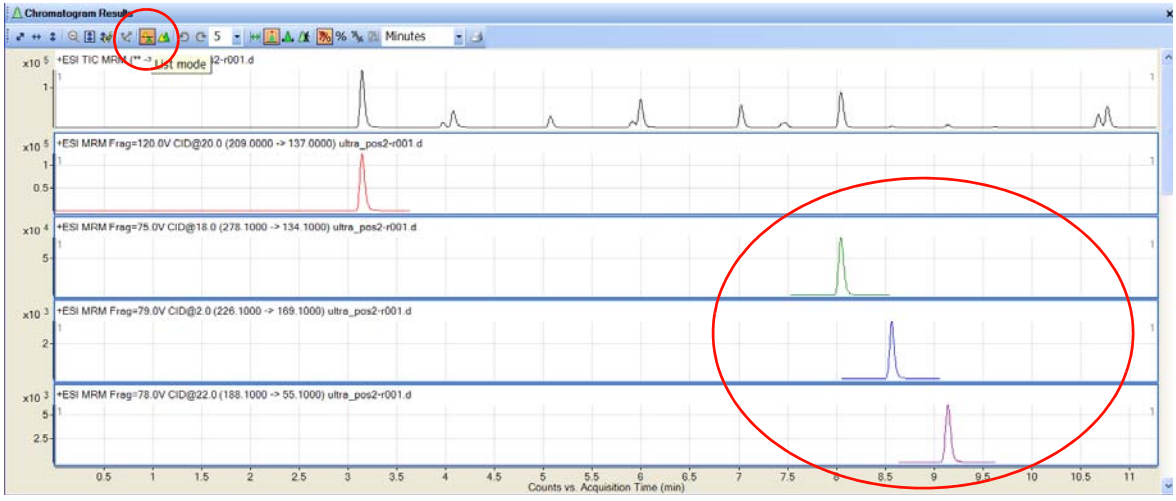

Figure 4 Extracted chromatograms in Qualitative Analysis Chromatogram Results window. The List Mode icon is circled in the toolbar of the Chromatogram Results window shown here.

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 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 14 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 Pesticide 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.

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

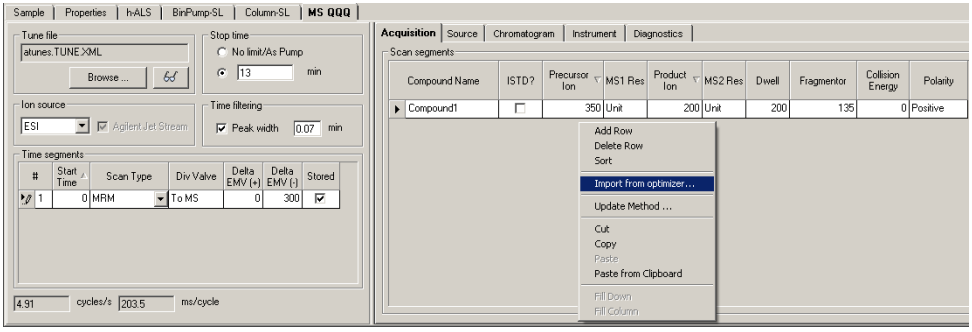

Figure 5 Import from optimizer shown in the Acquisition tab shortcut menu.

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 Pesticide Dynamic MRM Database:

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

b From the **D:\MassHunter\Databases** folder, select the **Pesticide_DynamicMRM_Database_Ver_03.01** 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 Pesticide 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.

Retention times (RT) and RT Window are displayed with all dynamic MRM (DMRM) methods. You can automatically update both parameters in the MassHunter Workstation Data Acquisition program.

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) or display ions (m/z values) in High Resolution, which is very useful for TOF/Q-TOF related pesticide screening.

As an example, if you want to analyze the pesticide compounds in the table below, you can simply copy and paste Compound names, CAS numbers, Formulas etc. into the **Search Text** box (upper right). Then you can mark the appropriate **Select Columns** check boxes, such as

Compound Name, **Trade Names**, and **Other Name**. The latter broadens the search to include alternative names and spellings. To clear the **Search Text** box, press **Ctrl+Z** anywhere within the text box.

Atrazine	1912-24-9
Atrazine-desethyl	6190-65-4
Atrazine-desethyl-desisopropyl	3397-62-4
Chlorotoluron	15545-48-9
Chloroxuron	1982-47-4
Chloropropham	101-21-3
Crimidine	535-89-7
Cyanazine	21725-46-2
Diuron	330-54-1
Fenuron	101-42-8
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Terbuthylazin	5915-41-3
Terbutyrn	886-50-0

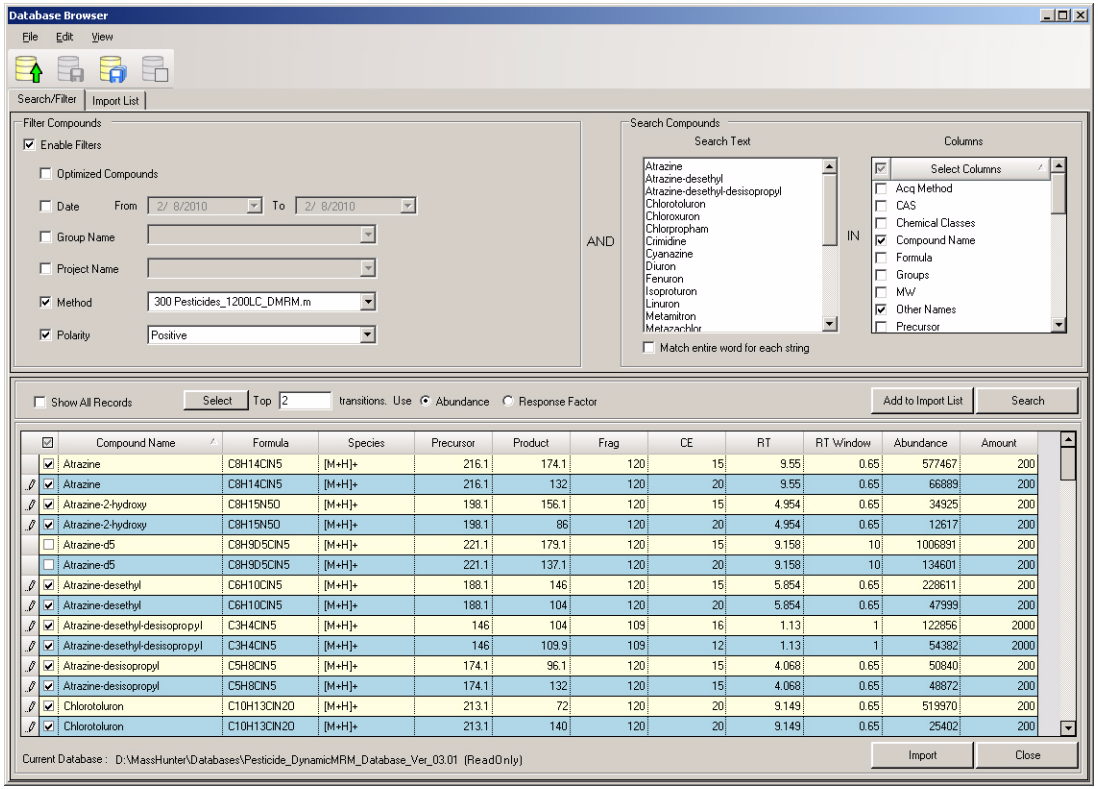


Figure 6 Database Browser with MassHunter Pesticide Dynamic MRM Database opened

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.

Acquisition methods developed for the LC/MS/MS analysis of pesticides are located in two folders under **D:\MassHunter\methods**

- **AJS Methods for Optimizer Pesticide Database** - used for 6460 Triple Quad with either an Agilent 1200 Series LC or Agilent 1290 Infinity LC.
- **ESI Methods for Optimizer Pesticide Database** - used for 6400 Series Triple Quad with a standard ESI source and either an an Agilent 1200 Series LC or Agilent 1290 Infinity LC.

(If you cannot find these folders, copy them from the MassHunter Pesticide Dynamic MRM Database Kit Support Disk.) Each folder contains fourteen acquisition methods developed for both single and multi-analyte LC/MS/MS analysis of various pesticides, or for their optimization using the MassHunter Optimizer program. These acquisition methods are also available as PDF files on the support disk. The PDF format is easily viewable to help you resolve any instrument configuration issues.

In Figure 6, the selected method **300 Pesticides_1200LC_DMRM.m** is a standard analytical LC/MS/MS method developed to analyze 300 pesticides in 20 minutes, with the use of an Agilent 1200 Series LC and 6400 Series LC/MS system. Similar methods for an Agilent 1290 Infinity LC for use with a 6460 (with Agilent Jet Stream Technology) or 6400 Series Triple Quad (with a standard ESI source) are also included.

In summary, methods are included to analyze over 750 pesticides, with the use of various 6400 series instrument configurations, or to optimize single or multiple analytes for addition to a pesticide database. The specific LC column used with the method can be found in the Properties tab, in the method description pane.

6 Import the required MRM transitions from the database:

a Select the required compound transitions from the MassHunter Pesticide Dynamic MRM Database browser.

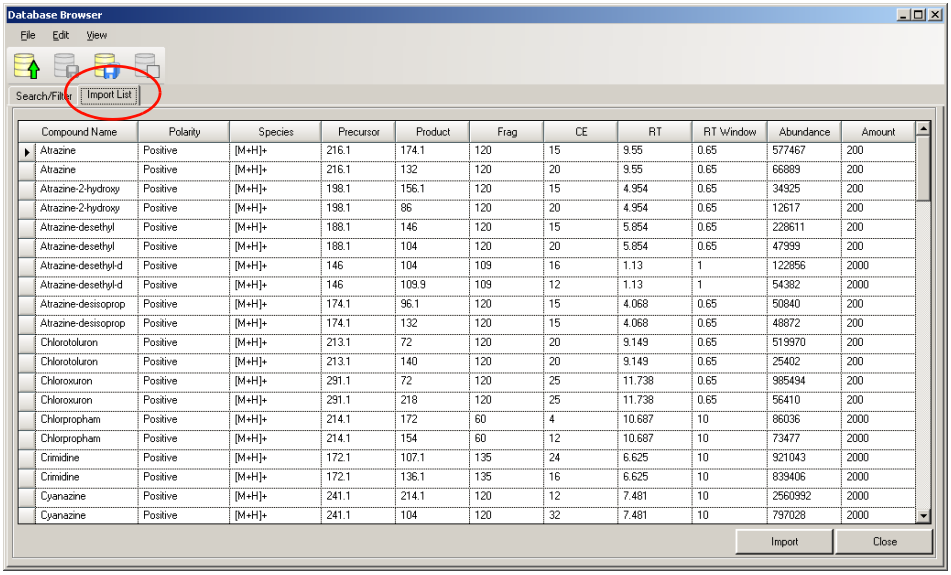
When MRM transitions are selected, the Import and the Add to Import List buttons in the Database Browser become active.

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.



Compound Name	Polarity	Species	Precursor	Product	Frag	CE	RT	RT Window	Abundance	Amount
Atrazine	Positive	[M+H]+	216.1	174.1	120	15	9.55	0.65	577467	200
Atrazine	Positive	[M+H]+	216.1	132	120	20	9.55	0.65	66889	200
Atrazine-2-hydroxy	Positive	[M+H]+	198.1	156.1	120	15	4.954	0.65	34925	200
Atrazine-2-hydroxy	Positive	[M+H]+	198.1	86	120	20	4.954	0.65	12617	200
Atrazine-desethyl	Positive	[M+H]+	188.1	146	120	15	5.854	0.65	228611	200
Atrazine-desethyl	Positive	[M+H]+	188.1	104	120	20	5.854	0.65	47999	200
Atrazine-desethyl-d	Positive	[M+H]+	146	104	109	16	1.13	1	122856	2000
Atrazine-desethyl-d	Positive	[M+H]+	146	109.9	109	12	1.13	1	54382	2000
Atrazine-desisoprop	Positive	[M+H]+	174.1	96.1	120	15	4.068	0.65	50840	200
Atrazine-desisoprop	Positive	[M+H]+	174.1	132	120	15	4.068	0.65	48872	200
Chlorotoluron	Positive	[M+H]+	213.1	72	120	20	9.149	0.65	519970	200
Chlorotoluron	Positive	[M+H]+	213.1	140	120	20	9.149	0.65	25402	200
Chloroxuron	Positive	[M+H]+	291.1	72	120	25	11.738	0.65	985494	200
Chloroxuron	Positive	[M+H]+	291.1	218	120	25	11.738	0.65	56410	200
Chlorpropham	Positive	[M+H]+	214.1	172	60	4	10.687	10	86036	2000
Chlorpropham	Positive	[M+H]+	214.1	154	60	12	10.687	10	73477	2000
Cirimidine	Positive	[M+H]+	172.1	107.1	135	24	6.625	10	921043	2000
Cirimidine	Positive	[M+H]+	172.1	136.1	135	16	6.625	10	839406	2000
Cyanazine	Positive	[M+H]+	241.1	214.1	120	12	7.481	10	2560992	2000
Cyanazine	Positive	[M+H]+	241.1	104	120	32	7.481	10	797028	2000

Figure 7 Database Browser with Import List tab circled

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 **Pesticide_DynamicMRM_Database_Ver_03.01**.

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.

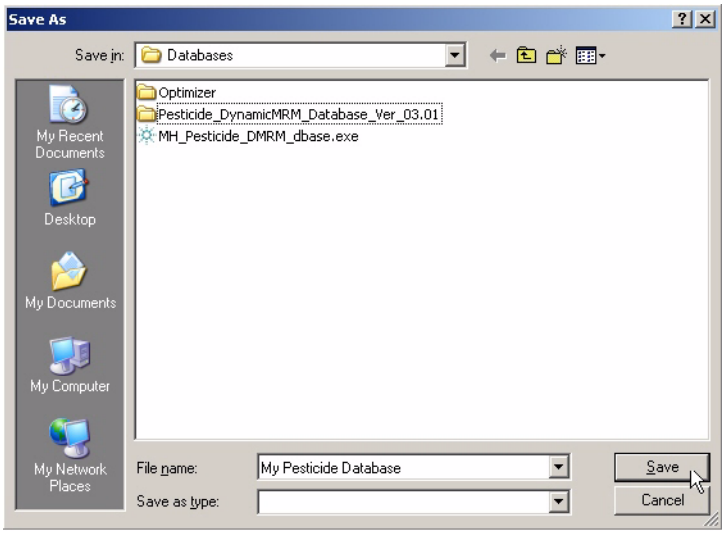


Figure 8 Save As dialog box to save a database that can be edited

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**.

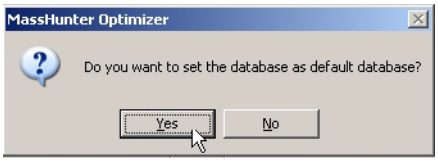


Figure 9

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.

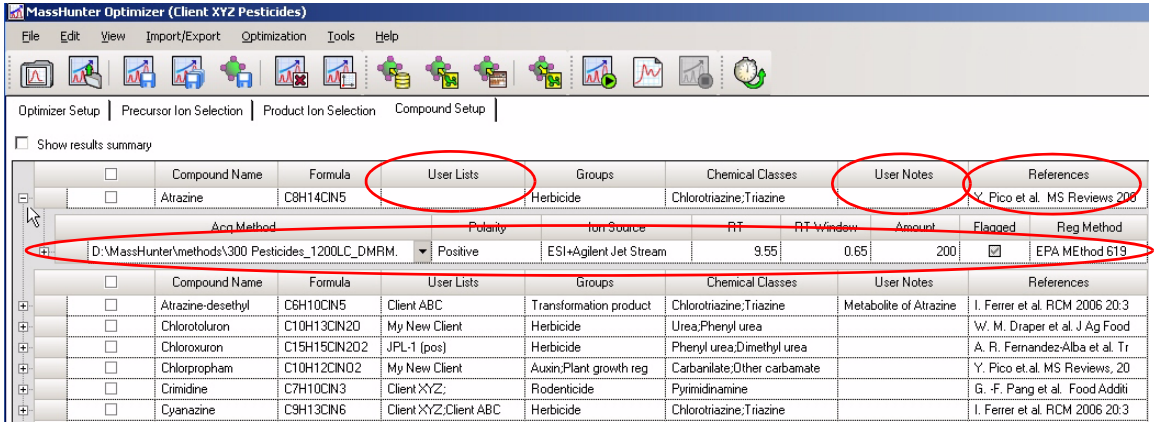


Figure 10 Commonly edited options

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.

NOTE The compound records are saved as a Project. The default project is MassHunter_Pesticides_Database_Project. A compound record can be associated with multiple projects if desired (semicolon delimiter).

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.

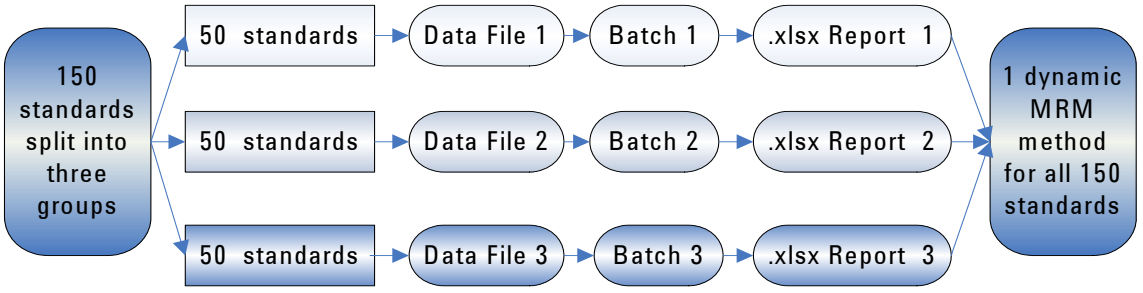


Figure 11 Example process for analyses that have more than 50 compounds

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.

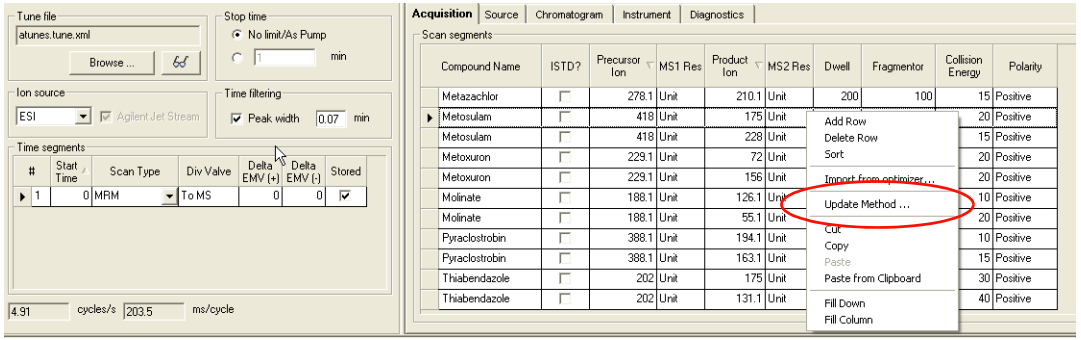


Figure 12 Acquisition tab with Update Method command highlighted

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

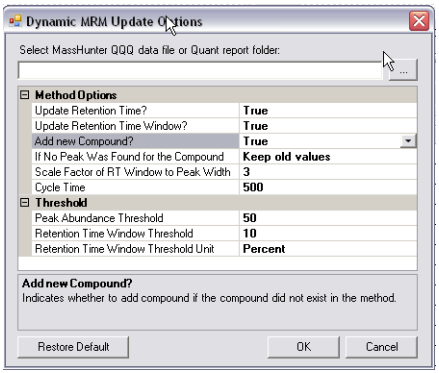


Figure 13 Dynamic MRM Update Options

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.

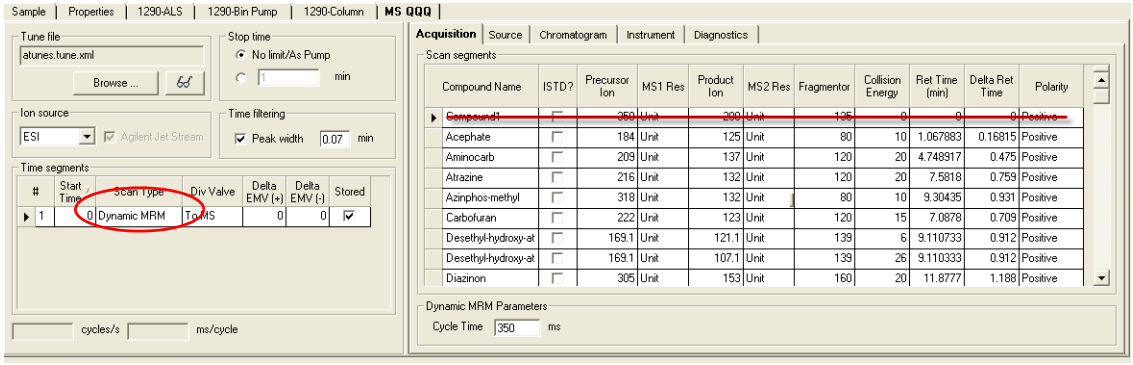


Figure 14

- If you manually select Dynamic MRM in the “Scan Type” under “Time Segments” as shown in Figure 15 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 15 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.

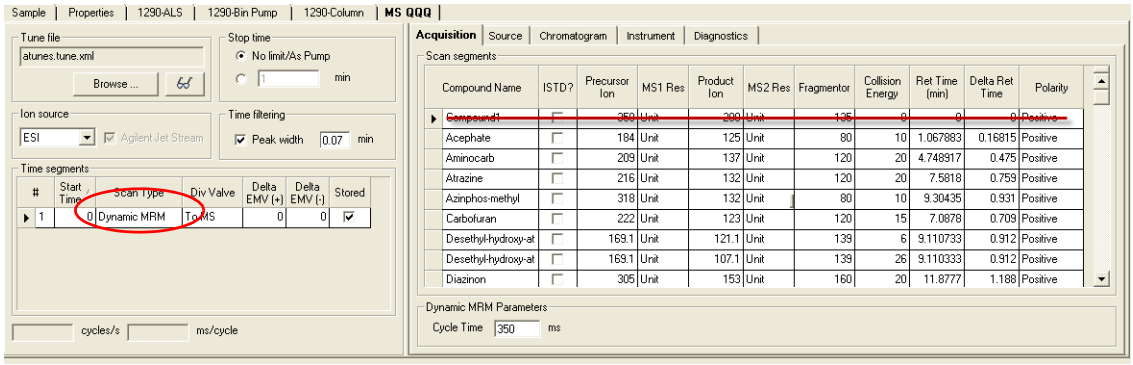


Figure 15

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.

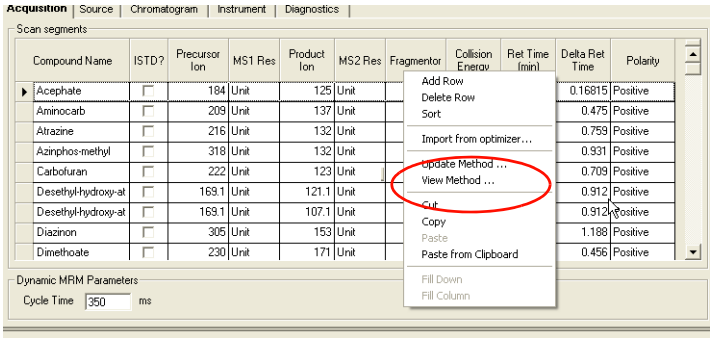


Figure 16 View Method command

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

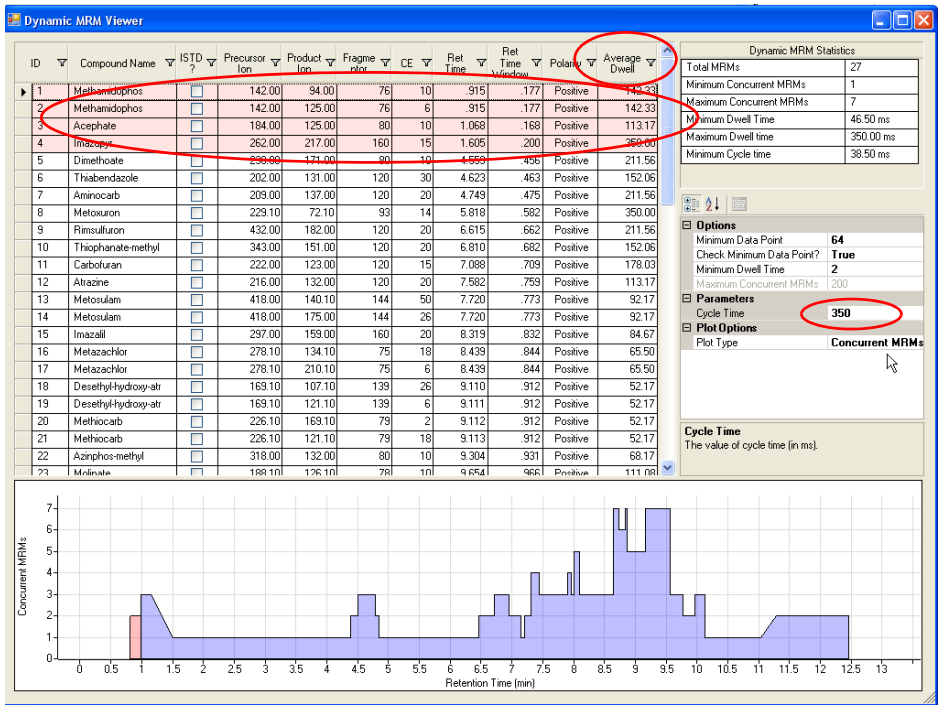


Figure 17 Dynamic MRM Viewer

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 three compounds (4 DMRM transitions) 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 100 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.

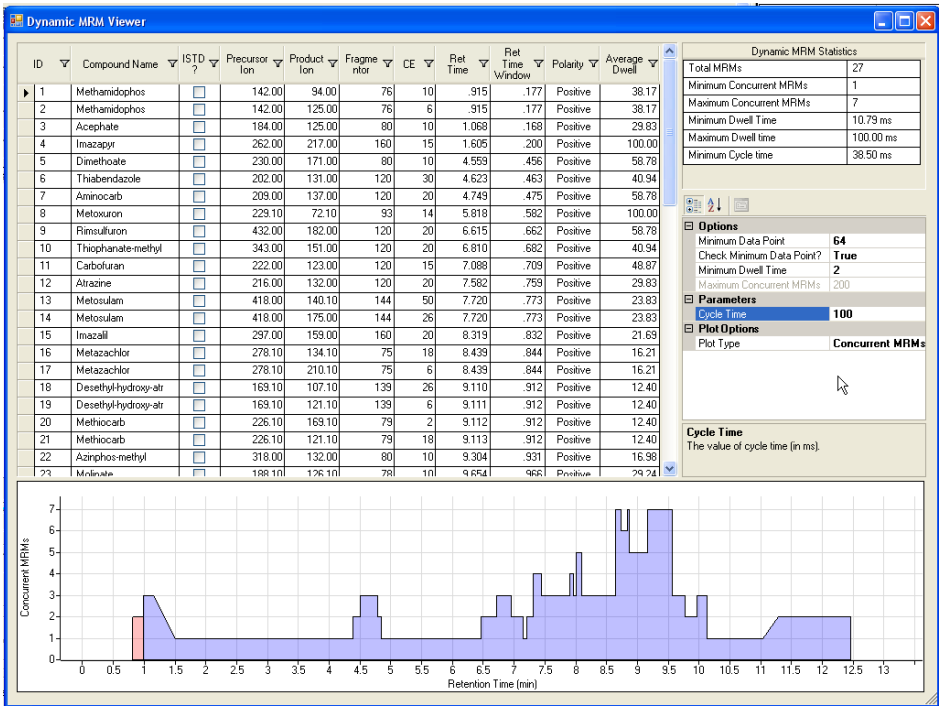


Figure 18 List of corrected transitions

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.

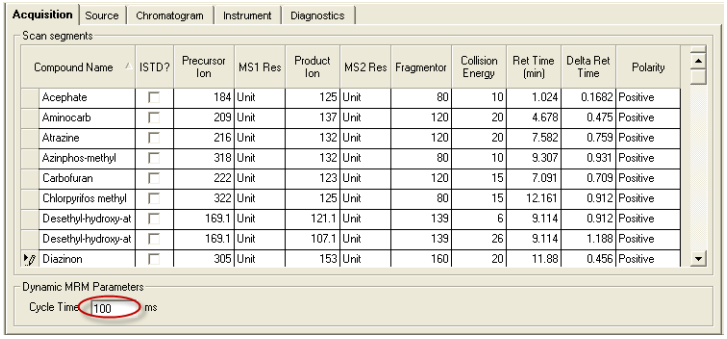


Figure 19 Acquisition tab with the new Cycle Time

Note that the cycle time in the Dynamic MRM Update Method Options dialog box (Figure 13 on page 26) 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.

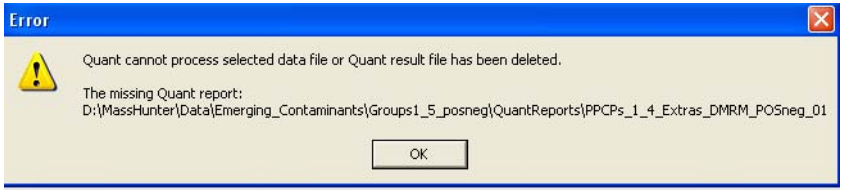


Figure 20

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.

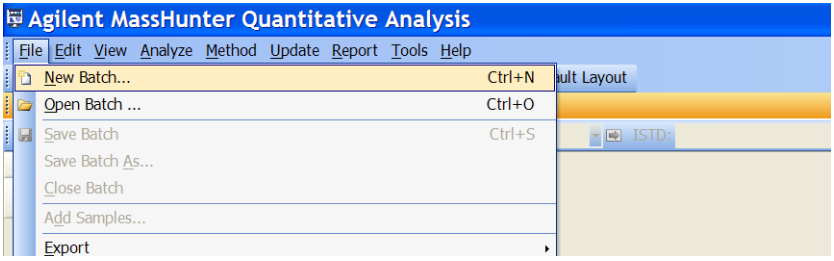


Figure 21 New Batch from the File menu

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

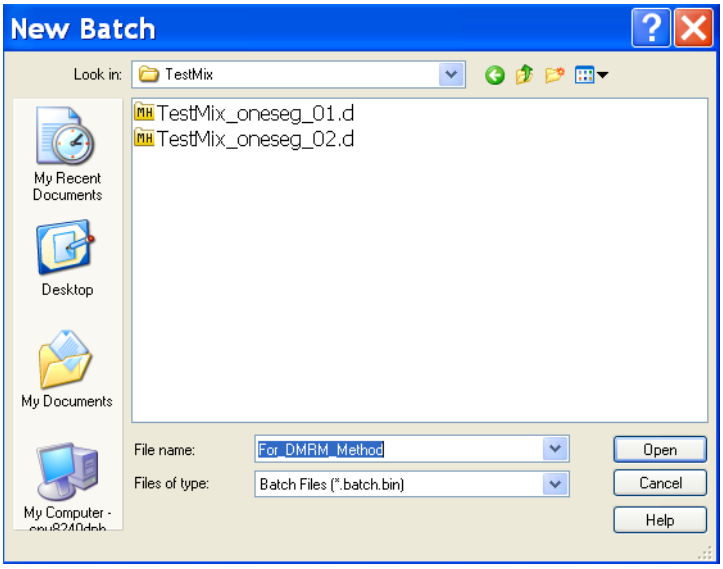


Figure 22 New Batch dialog box

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.

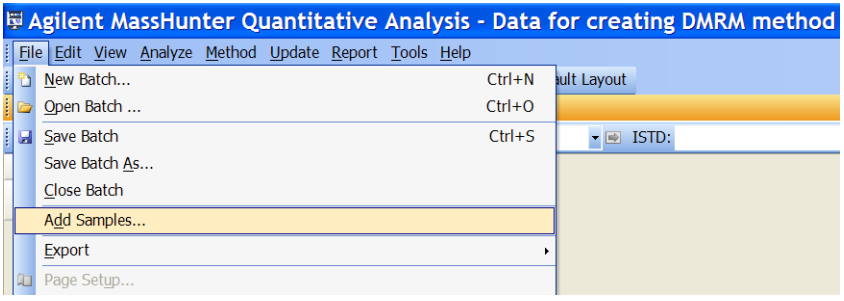


Figure 23 Add Samples from the File menu

b Select the acquired MRM data file from the **Add Samples** list, then click **OK**. See Figure 24.

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

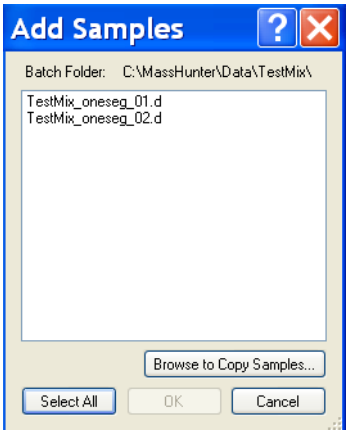


Figure 24 Add Samples dialog box

4 Create a new method.

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

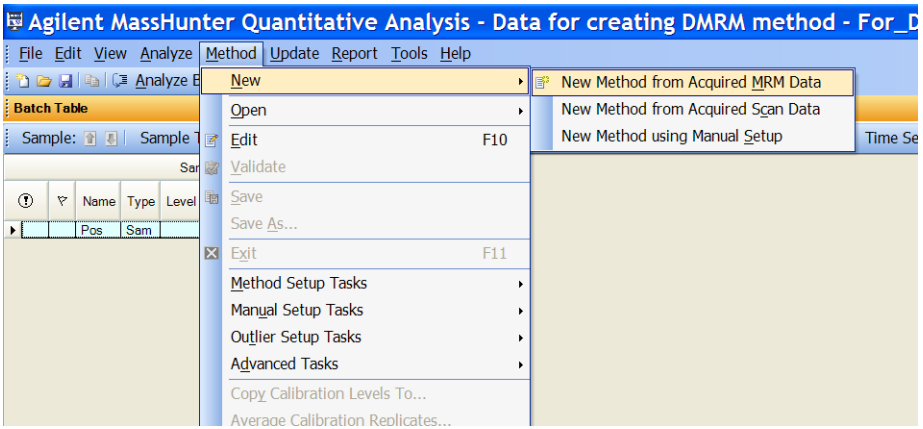


Figure 25 New Method from Acquired MRM Data selected

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

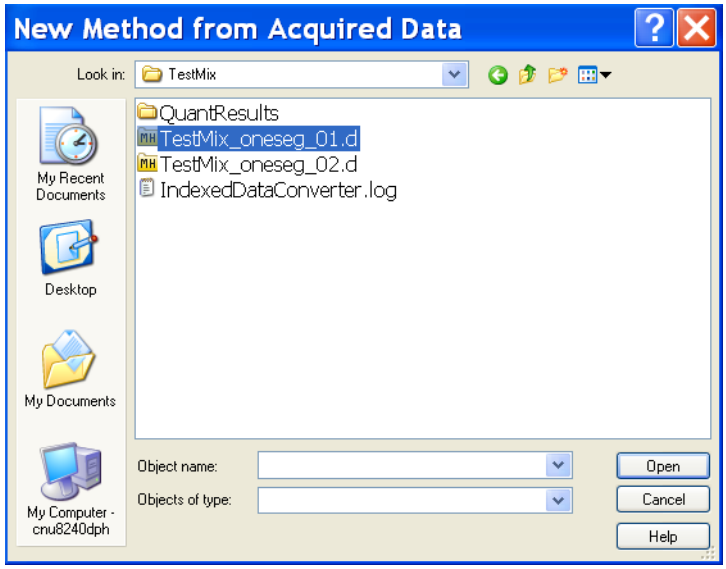


Figure 26 New Method from Acquired Data dialog box

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

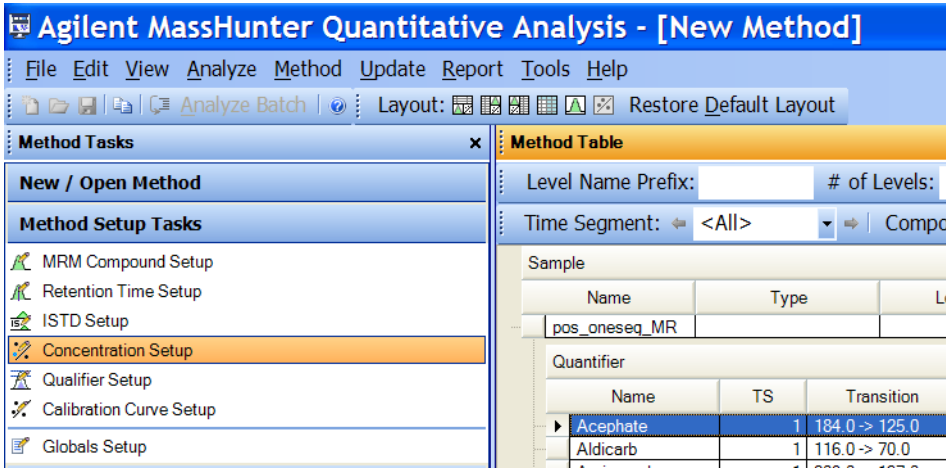


Figure 27 Concentration Setup under Method Setup Tasks

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.

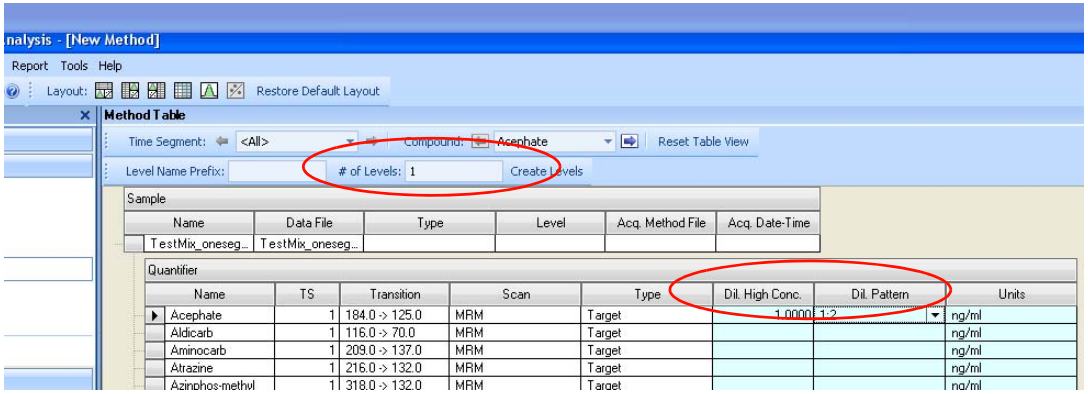


Figure 28 Quantifier table with first compound selected

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

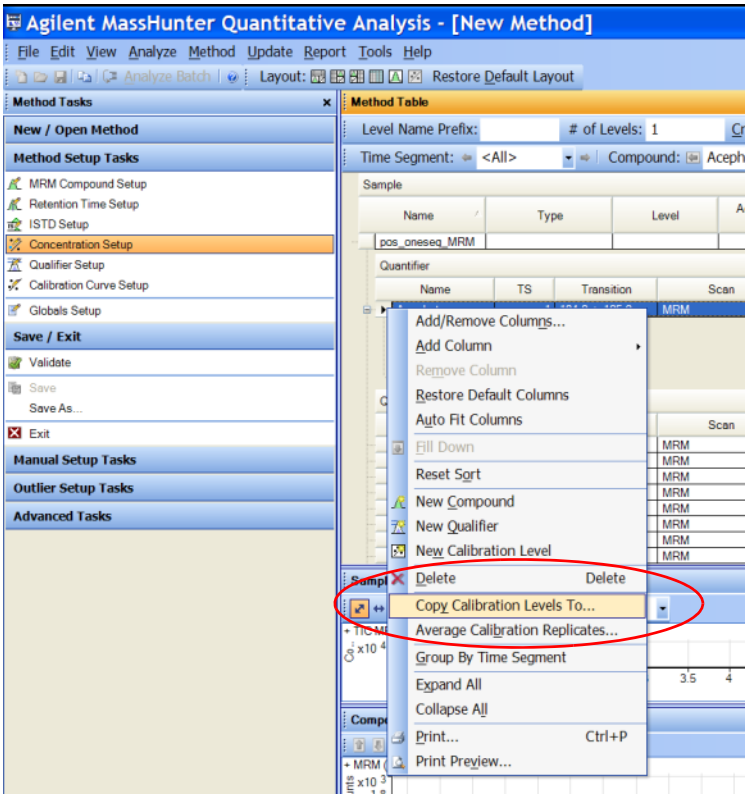


Figure 29 Copy Calibration Levels To selected

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

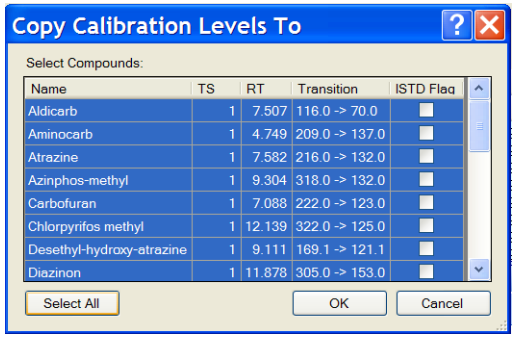


Figure 30 Copy Calibration Levels To dialog box

j Click **OK**.

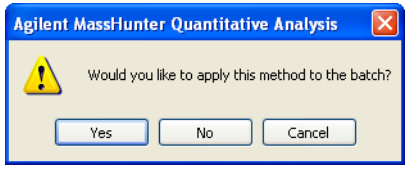
k Click **Validate,** then click on each error and correct it. To correct an error, type a value for the parameter that is missing, or delete that transition from the method. Typical errors are “retention time cannot be zero” or “missing qualifier ratio.”

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 **TestMix_oneseg_01.quantmethod.xml**.

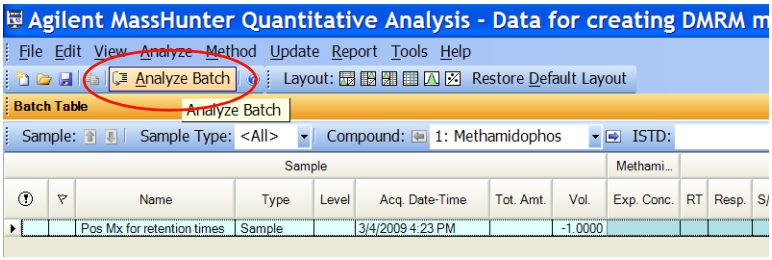
m Click **Method > Exit** to close the Method Table.

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**.

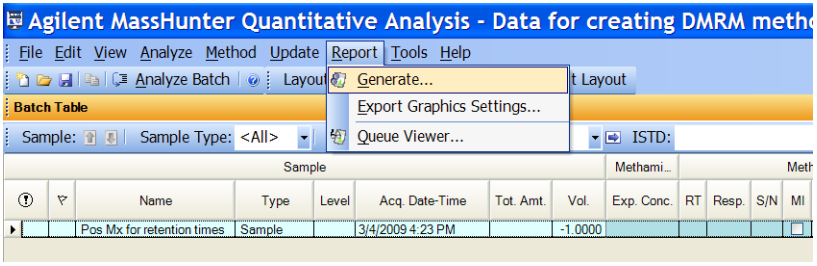


Figure 31 Generate on the Report menu

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**.

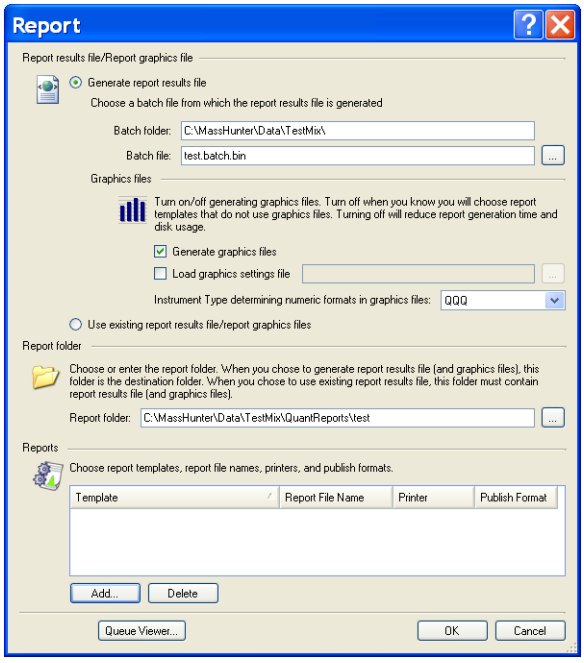


Figure 32 Report dialog box

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

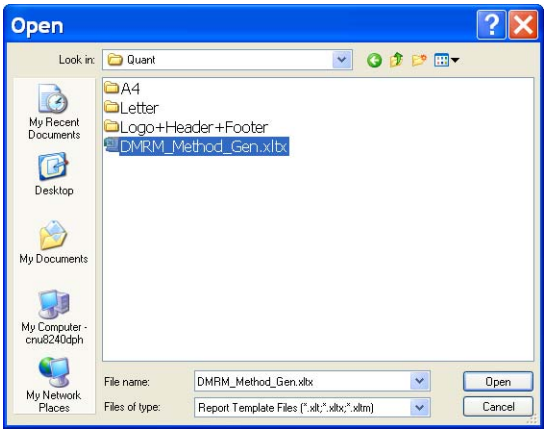


Figure 33 Open dialog box, with DMRM_Method_Gen.xltx selected

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 35) 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 25) and click **Update Method**.

   b In the Dynamic MRM Update Options dialog box (Figure 13 on page 26), 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.

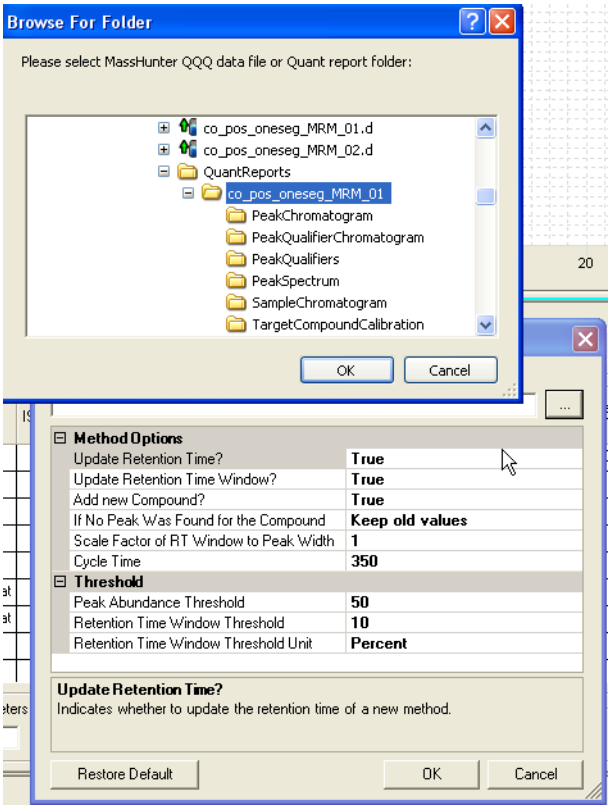


Figure 34 Navigation to Quant Report folder. Select the Quant report folder of the data file to be used to update the method.

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.

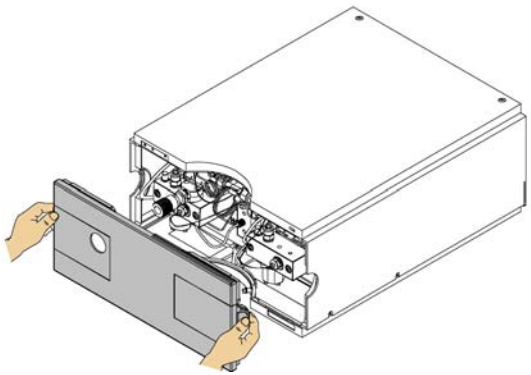
Tools required

- 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)

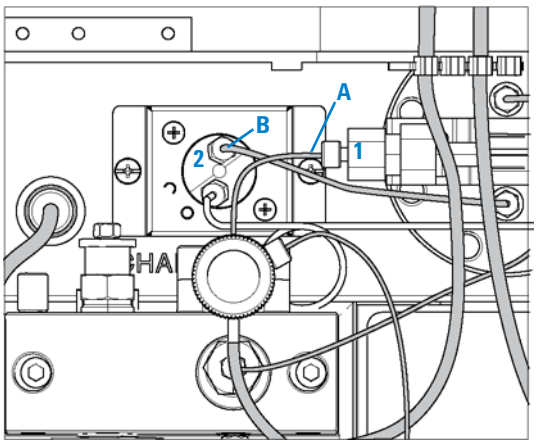
Preparations for this procedure

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

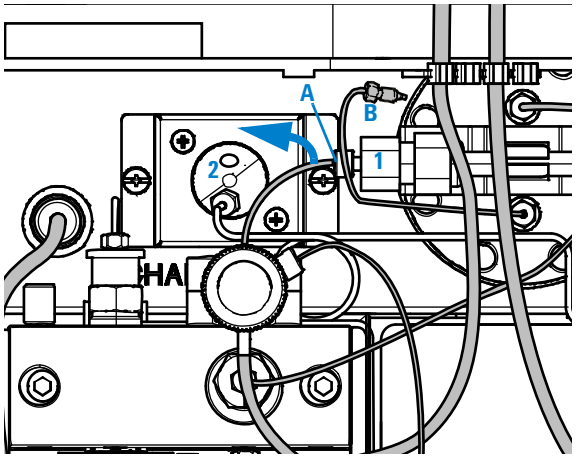
1 Remove the front cover by pressing the clip fastener on both sides of the cover.



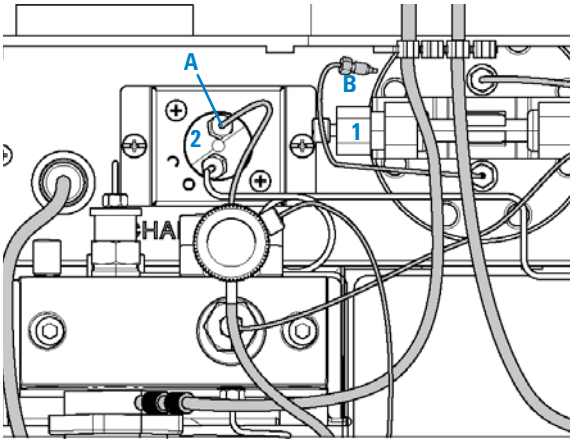
2 Use the 1/4 inch hex driver to remove fitting **B** from port *2* of the pressure sensor.



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 Pesticide Dynamic MRM Database Kit.

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Printed in USA
Second Edition, May 2010

5990-5742EN

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