

# Agilent G1734AA MassHunter Forensics and Toxicology Dynamic MRM Database Kit

**Quick Start Guide** 

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# 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.



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
chrom	atographic-dependent settings

Compound Name	ISTD?	Precur- sor lon	MS1 Res	Product Ion	MS2 Res	Frag- mentor	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

Compound Name	ISTD?	Precur- sor lon	MS1 Res	Product Ion	MS2 Res	Frag- mentor	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
РСР		244.2	Unit	91	Unit	86	41	2.876	0.4	Positive
РСР		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?	Precur- sor lon	MS1 Res	Product Ion	MS2 Res	Frag- mentor	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	

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

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  $\mu$ g/mL (1 ppm) for all 25 components.

- **a** Dilute 100  $\mu$ L 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 $\mu\text{m},$ 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.

Column Compartment Column - SL, Model G1316B

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

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

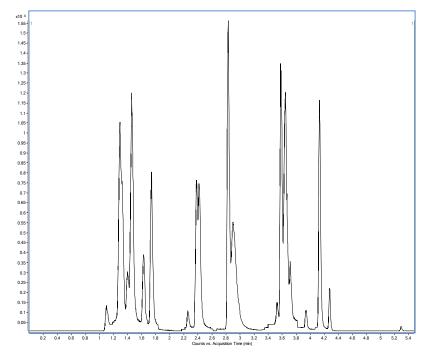
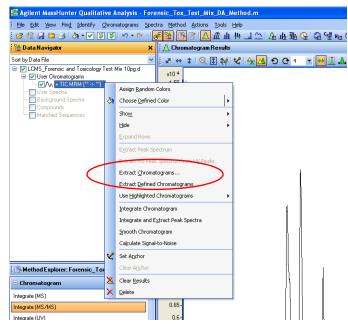


Figure 1 Example LC/MS Forensics and Toxicology Test Mix Total Ion Chromatogram

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





- **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.

Extract Chromatograms		X
List of opened data files  LCMS_Forensis and Toxicology Test	Type: MRM Advanced Excluded Masses MS Groomatogram Advanced Excluded Masses MS levet MS/MS A Polarity: Positive A Scans: Multiple reaction monitor A Transition: All	<b>A</b>
	OK Cance	

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.

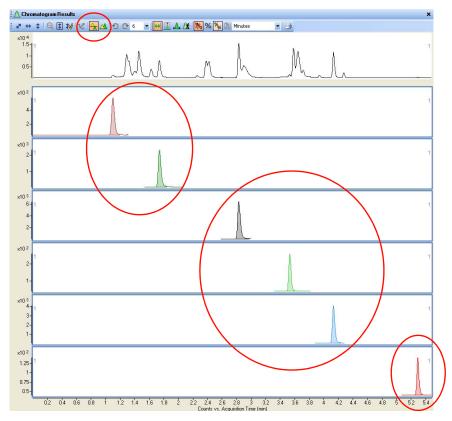


Figure 4 Extracted chromatograms in Qualitative Analysis Chromatogram Results window. The List Mode icon is circled in the toolbar of the Chromatogram Results window shown here.

- **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.

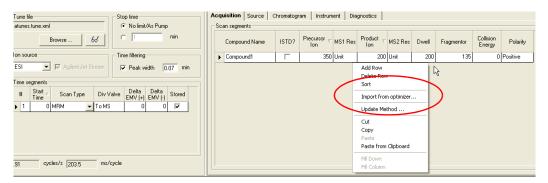


Figure 5 Import from optimizer shown in the Acquisition tab shortcut menu.

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).

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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.

Edit View	R									
ch/Filte	5									
Compound Name	Formula	MW	Polarity	Species	Precursor	Product	Frag	CE	RT	
Strychnine	C21H22N2O2	1	Positive		335.2	184	195	41		1
Strychnine	C21H22N2O2	N	Positive		335.2	156	195	53		Ť
Strychnine	C21H22N2O2	4	Positive	3	335.2	129	195	65	3	T
Strychnine	C21H22N2O2		Positive		335.2	144.1	195	49		T
(1S, 2R)-Ephedrine	C10H15N0		Positive		166.1	148.1	81	9		
(1S, 2R)-Ephedrine	C10H15N0		Positive	2	166.1	115	81	25	2	Ť
(1S, 2R)-Ephedrine	C10H15N0	1	Positive		166.1	117	81	17		Ť
(1S, 2R)-Ephedrine	C10H15N0		Positive	8	166.1	91.1	81	33		Ť
(S,S)-Psuedoephedr	C10H15N0		Positive	8	166.1	148.1	81	5		T
(S,S)-Psuedoephedr	C10H15N0		Positive		166.1	115	81	25		Ť
(S,S)-Psuedoephedr	C10H15N0		Positive	3	166.1	91	81	33	3	T
(S,S)-Psuedoephedr	C10H15N0		Positive		166.1	117	81	17	3	Ť
d-Amphetamine	C9H13N		Positive		136.1	91	66	17		Ť
d-Amphetamine	C9H13N		Positive	8	136.1	119.1	66	5		Ť
d-Amphetamine	C9H13N		Positive		136.1	65.1	66	41		Ť
d-Amphetamine	C9H13N		Positive	3	136.1	51.1	66	61	3	T
MDA	C10H13N02		Positive		180.1	163	61	5		
MDA	C10H13N02		Positive		180.1	105	61	21		T
MDA	C10H13N02	1	Positive		180.1	77	61	41		Ť
MDA	C10H13N02	1	Positive		180.1	135	61	17	1	Ť
MDEA	C12H17N02		Positive		208.1	163	107	9		T
MDEA	C12H17N02		Positive	8	208.1	105	107	25		Ť
MDEA	C12H17N02		Positive		208.1	135	107	21		
MDEA	C12H17N02		Positive		208.1	77	107	49		İ
					( )				10.	>

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.

Save As					? 🗙
Save in:	🗀 Databases		•	⇐ 🗈 💣 📰 •	
My Recent Documents	ContensicTox_Dy Contimizer MH_ForenTox_D	namicMRM_Database			
Desktop My Documents					
My Computer					
My Network	File name:	My Forensic database		•	Save
Places	Save as type:			•	Cancel

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**.

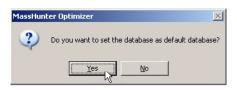


Figure 9

- **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.

e Edi	t View	Import/Export Optin	nization Tools H	elp						
		🖪 🛃 🛸		🔓 🖍 '	🛓 🐔 [	<u>M</u>		9		
ptimizer S	etup Pr	ecursor Ion Selection	Product Ion Selection	Compound Setup						
Show re	esults summ	arv								
		Compound Name	Groups	Formula	Mass	Synonyms	User	ists User N	lotes	References
. · ·		Struchnine	Alkaloid	C21H22N2O2	334.17	oynoriyins	U SU L	lata Gacin	ioles	Hereferees
		(1S, 2R)-Ephedrine	Amphetamine	C10H15N0	165.12					
			Ampricianine							
	-	Acq Method		Polarity	Ion Source	BT	RT Window	Instrument Name		
5	Forensic	Tox_1200LC_DMRM_Po	sitive.m 💙					Morpheous	06/25/2009	
	Prec	ursor Frag	Species	Abundance						
		166.1	81							
		Compound Name	Groups	Formula	Mass	Synonyms	User L	ists User N	lotes	References
		(S,S)-Psuedoephedr	Amphetamine	C10H15NO	165.12	oynonymo	00012	00011	0.00	Hororonooo
		d-Amphetamine	Amphetamine	C9H13N	135.1		N			
÷.		MDA	Amphetamine	C10H13N02	179.09		h\$			
÷.		MDEA	Amphetamine	C12H17N02	207.13					
÷		MDMA	Amphetamine	C11H15N02	193.11					
±		methamphetamine	Amphetamine	C10H15N	149.12					
÷		Methylphenidate	Amphetamine	C14H19N02	233.14		İ			
<b>.</b>		Phenylpropanolami	Amphetamine	C9H13NO	151.1					
÷.		Acetominophen	Analgesic	C8H9ND2	151.06					
		Carbamazepine	anticonvulsant	C15H12N2O	236.1					
÷		Methsuximide	anticonvulsant	C12H13N02	203.1					
		Verapamil	Anti-Hypertensive	C27H38N2O4	454.28					
••••		Clozapine	antipsychotic	C18H19CIN4	326.1					
		Meprobamate	antixiolytic	C9H18N2O4	218.1					
		moprobalitate			285.07					
		7-Aminoclonazepa	Benzo metabolite	C15H12CIN3D						
			Benzo metabolite Benzo metabolite	C15H12CIN30 C16H14N30F	285.07 283.11		ļ	į		

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.

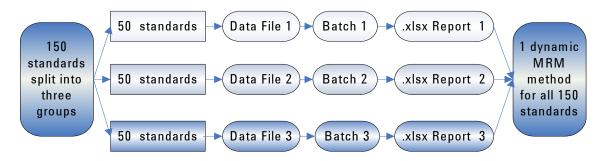


Figure 11 Example process for analyses that have more than 50 compounds

- **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.

Sample Properties h-ALS-SL+ BinPump-SL Column-SL MS QQQ Acquisition Source Chromatogram Instrument Diagnostics Tune file Stop time atunes.tune.xml No limit/As Pump Scan segments Compound Name ISTD? Precursor Ion V MS1 Res Product Ion V MS2 Res Dwell Fragmentor **Collision Energy** min Browse 66 Г 322 Unit 304 Unit 10 134 d6-oxycodone Ion source Time filtering d6-oxycodone Г 322 Unit 247 Unit 134 316.2 Unit 10 134 Oxycodone Г Add Row • Peak width 0.07 min Oxycodone Г 316.2 Unit Delete Row 10 134 25 Time segments Sort 5 Г 306.1 Unit 10 88 Sertraline Start Time Polarity Div Valve Delta EMV Stored # Scan Type Sertraline 306.1 Unit 10 88 25 29 306 Unit 10 166 Positive d6-hvdrococodone ▶ 1 0 MRM To MS 400 **V** Update Method . d6-hydrococodone 306 Unit 166 41 Cut 303 Unit 10 162 41 Codeine-d3 Copy Codeine-d3 303 Unit 10 162 45 Paste from Clipboard Hydrocodone 300.2 Unit 10 158 29 • Hydrocodone 300.2 Unit 10 158 41 Fill Down cycles/s 499.5 ms/cycle Hudrochlorot Fill Column

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.

Update Retention Time?	True
Update Retention Time Window?	True
Add new Compound?	True 💌
If No Peak Was Found for the Compound	Keep old values
Scale Factor of RT Window to Peak Width	3
Cycle Time	500
Threshold	
Peak Abundance Threshold	50
Retention Time Window Threshold	10
Retention Time Window Threshold Unit	Percent
	Jpdate Retention Time Window? Add new Compound? No Peak Was Found for the Compound Scale Factor of RT Window to Peak Width Jpde Time <b>Threshold</b> Peak Abundance Threshold Retention Time Window Threshold

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.

Sample   Properties   h-ALS-SL+   BinPump-SL   Column-SL   MS QQQ Tune file atunes tune.xml	A	cquisition Source	Chroma	ogram In:	strument	Diagnostic	s				
Browse 66		Compound Name	ISTD?	Precursor Ion	MS1 Res	Product Ion	MS2 Res	Fragmentor	Collision Energy	Ret Time (min)	Delta Ret Time
lon source		Gempound1	Г		Unit	200		135	- 0	0	
ESI 🔽 Peak width 0.04 min		Acephate		184	Unit	125	Unit	80	10	0.7735667	0.4048
		Atrazine		216	Unit	132	Unit	120	20	7.59355	0.76
Time segments		Azinphos-methyl		318	Unit	132	Unit	80	10	9.307384	0.931
# Start Scan Type Polarity Div Valve Delta EMV Stored		Carbofuran		222	Unit	123	Unit	120	15	7.083567	0.709
▶ 1 0 Dynamic MRM Pysitive To MS 200 🔽		Diazinon		305	Unit	153	Unit	160	20	11.87535	1.188
		Dimethoate		230	Unit	171	Unit	80	10	4.52365	0.453
		Malathion		331	Unit	99	Unit	80	10	10.50347	1.051
		Metosulam		418	Unit	175	Unit	120	25	7.530317	0.754
cycles/s ms/cycle		Dynamic MRM Paramete Cycle Time 500	ers ms								

#### 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.

Sample   Properties   h-ALS-SL+   BinPump-SL   Column-SL   MS QQQ										
Tune file Stop time atunes.tune.xml	quisition Source	Chromat	ogram   In:	strument	Diagnostic	s				
Browse 66 0 min	Compound Name	ISTD?	Precursor Ion	MS1 Res	Product Ion	MS2 Res	Fragmentor	Collision Energy	Ret Time (min)	Delta Ret Time
Ion source     Time filtering	Gempound1	Г		Unit	200	Unit	135	- 0	-0	
ESI 🔽 Peak width 0.04 min	Acephate		184	Unit	125	Unit	80	10	0.7735667	0.4048
	Atrazine		216	Unit	132	Unit	120	20	7.59355	0.76
Time segments           ++         Start         Countries         Delayly         Diviviation         Diviviation <thdiviviation< th=""> <thdiviviation< th="">         Divivia</thdiviviation<></thdiviviation<>	Azinphos-methyl		318	Unit	132	Unit	80	10	9.307384	0.931
# Start / Scan Type Polarity Div Valve Delta EMV Stored	Carbofuran		222	Unit	123	Unit	120	15	7.083567	0.709
▶ 1 0 Dynamic MRM Positive To MS 200 🔽	Diazinon		305	Unit	153	Unit	160	20	11.87535	1.188
	Dimethoate		230	Unit	171	Unit	80	10	4.52365	0.453
	Malathion		331	Unit	99	Unit	80	10	10.50347	1.051
	Metosulam		418	Unit	175	Unit	120	25	7.530317	0.754
cycles/s ms/cycle	Dynamic MRM Paramete Cycle Time 500	ers ms								



- 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.

	Compound Name	ISTD?	Precursor Ion	MS1 Res	Product Ion	MS2 Res	Fragmentor	Collision Energy	Ret Time (min)	Delta Ret Time	Re
•	d6-oxycodone	• 🗆		Unit	304	Unit	134	13	5.2531	0.526	Add Row Delete Row
	Oxycodone		316.2	Unit	298.1	Unit	134	13	5.351367	1.21715	Sort
	Sertraline		306.1	Unit	274.8	Unit	88	5	17.25038	1.727	Import from optimizer
	d6-hydrococodone		306	Unit	202	Unit	166	29	6.13785	0.614	
	Codeine-d3		303	Unit	181	Unit	162	41	0	0	Update Method View Method
	Hy socodone		300.2	Unit	199	Unit	158	29	6.377083	1.3783	
	Hydrochlorothiazide		298	Unit	169.8	Unit	134	13	0	0	Copy
	Diclofenac		296	Unit	250	Unit	100	15	22.64233	2.265	Paste
	Diazepam-d5		290.1	Unit	198	Unit	162	33	19.16847	1.917	Paste from Clipboard

Figure 16 View Method command

Atendol       267.20       190.00       134       13       2124       557       Positive       245.50       Maximum Concurrent MPMs       1         Aurold       2.87.20       145.00       134       21       21.25       597       Positive       245.50       Maximum Concurrent MPMs       6         Codeined3       0.303.00       155.00       152       21       3.782       897       Positive       111.60       Maximum Could Time       500.00 ms         Chrinian       2.3000       4100       100       2.3       3101       897       Positive       111.60       Maximum Owell Time       500.00 ms         Amphetamine       141.10       92.00       74       15       4.581       528       Positive       92.33         Amphetamine       138.10       91.00       70       13       4.581       1.286       Positive       92.33         Amphetamine       138.10       91.00       70       13       4.591       1.286       Positive       92.33         Amphetamine       138.10       91.00       70       13       4.591       1.286       Positive       92.46         Geocycodone       30.00       20.00       136       136	ID 🛛	Compound Name		Precursor v	Product V	Fragme 🗸	CE 🛛	Ret <del>∨</del> Time ⊽	Ret Time ❤ Window	Polarity	Average 🗸	Dyn Total MRMs	amic MRM St	atistics 33
Atrodal         287.20         145.00         134         21         21.25         607         Penitive         246.50         Maimum Concurrent MRMs         6           Condine         303.00         165.00         162         45         3.76         907         Positive         11.817           Condine         230.00         121.00         150         21         3.78         997         Positive         14.10         230.00         165         452         3.278         Positive         134.00         134         0           Ampletamine         141.10         124.00         74         5         4.528         3.22         Positive         9.233         Ampletamine         3.81.0         1136.00         70         13         4.613         1.266         Positive         9.630         9.97         9.	1	Atenolol		267.20	190.00	134	13			Positive	246 501	Minimum Concurre	nt MRMs	1
Codeine-d3         933.00         185.00         182         45         3.759         .376         Positive         163.17         Minimum Ovel Time         78.33 ns           Concidine         230.00         121.00         150         21         3.769         Positive         14.160         500.00 ns           Concidine         230.00         44.00         150         20         3.781         .897         Positive         13.4         500.00 ns         Minimum Ovel Time         500.00 ns           Amphetamine         1         141.10         124.00         74         5         4.528         922         Positive         93.11           Amphetamine         1         135.10         91.00         70         13         4.613         1.266         Positive         93.28           Amphetamine         1         316.20         294.10         134         13         5.257         Positive         94.20         Minimum Date Point?         True           Opycodone         316.20         294.10         134         25         536         1.217         Positive         127.66         Hydiocodone         20.02.00         168         1.217         Positive         127.66         Hydiocodone         500.72 <td>2</td> <td></td> <td>Maximum Concurre</td> <td>ent MRMs</td> <td>6</td>	2											Maximum Concurre	ent MRMs	6
Daradine         23000         21300         150         21         3.782         .897         Positive         11.160         Marinam Duell time         \$00.00 ms           Dioridine         2.0000         4400         100         23         3100         1397         Positive         134.00           Amphetamine-65         141.10         92.00         74         5         4.528         323         Positive         92.33           Amphetamine         136.10         19.00         70         13         4.581         1.266         Positive         93.28           Amphetamine         136.10         91.00         70         13         4.513         1.266         Positive         93.28           Synochone         322.00         304.00         134         13         5.537         5.567         Positive         127.66           Osynochone         300.20         166         28         6.138         6.14         Positive         127.66           Hydiococodone         300.20         198.00         128         6.377         1.378         Positive         127.66           Hydiococodone         300.20         136         128         1.377         Positive         28.375	3											Minimum Dwell Tim	ne	79.83 ms
Doridine         23000         4400         100         20         3101         897         Politive         134.00           Amphetamine-d5         1         141.10         22.00         74         5         4.528         323         Positive         95.11           Amphetamine-d5         1         141.10         27.00         74         5         4.605         1.268         Positive         92.33           Amphetamine         1         32.00         30.00         70         5         4.605         1.268         Positive         92.83           Amphetamine         1         36.10         91.00         70         5         4.605         1.268         Positive         92.83           Opcodene         32.00         30.00         134         13         5.251         526         Positive         120.46           Ghydrocodone         30.00         134         13         5.261         1.272         Positive         120.46           Ghydrocodone         300.20         170.10         158         41         6.367         1.378         Positive         123.60           Hydrocodone         300.20         170.00         158         21         1.378	3											Maximum Dwell tim	e	500.00 ms
Anghetamine-d5       141.10       124.00       74       5       4.528       .923       Positive       95.11         Anghetamine-d5       141.10       92.70       74       13       4.581       .923       Positive       92.33         Anghetamine       135.10       91.00       70       13       4.613       1.226       Positive       94.00         d6:ogcodone       2220       304.00       134       13       5.253       1.226       Positive       93.28         Oxycodone       316.20       298.10       134       13       5.253       1.217       Positive       120.46         d6:rydioccodone       306.20       171.00       158       41       6.367       1.378       Positive       144.88         Hydioccodone       300.20       171.00       158       23       6.377       1.378       Positive       143.68         Hydioccodone       300.20       175.00       156       13       10.443       10.47       Positive       23.25         Metoprol0       288.20       116.00       136       13       10.443       10.47       Positive       134.03         Metoprol0       288.20       156.00       72 <t< td=""><td>5</td><td></td><td></td><td></td><td></td><td></td><td></td><td>102.22.31.51</td><td></td><td></td><td>134.00</td><td>Minimum Cycle time</td><td>•</td><td>33.00 ms</td></t<>	5							102.22.31.51			134.00	Minimum Cycle time	•	33.00 ms
Anghetamine-d5       141.10       92.70       74       13       4.581       .923       Positive       92.33         Anghetamine       135.10       113.00       70       5       4.605       1.266       Positive       96.50         Anghetamine       135.10       91.00       70       13       4.513       1.206       Positive       99.28         Anghetamine       135.10       134       13       5.253       .526       Positive       99.28         Drysodone       316.20       298.10       134       13       5.253       .526       Positive       99.28         Orysodone       316.20       298.10       134       13       5.253       .526       Positive       17.33         Operatione       316.20       298.10       134       13       5.263       Positive       120.4         Operatione       300.20       177.100       158       41       6.367       1.378       Positive       120.6         Hydococonce       300.20       179.00       158       23       1.379       Positive       28.50       10.443       1.047       Positive       28.50         Metopolal       288.20       56.00       136	6	Contract of the second s			1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1						1.2.2.2.2.2			
Anghetamine         136.10         119.00         70         5         4.605         1.266         Positive         96.50           Anghetamine         136.10         51.00         70         13         4.613         1.266         Positive         96.50           Goycodone         322.00         304.00         134         13         5.253         526         Positive         93.83           Goycodone         316.20         228.10         134         13         5.251         1.217         Dauber         117.73           Goycodone         316.20         228.10         134         13         5.268         1.217         Dauber         117.73           Goycodone         300.20         170.00         158         24         6.367         1.378         Positive         148.93           Hydocodone         300.20         170.00         158         23         6.377         1.378         Positive         24.600           Metopolol         288.20         150.00         72         5         12.614         1.262         Positive         24.600           Metopolol         286.20         150.00         72         5         12.630         1.263         Positive         <	7			2										
Ampresidinine         136.10         91.00         70         13         4.613         1.268         Positive         49.00           d6-soycodone         322.00         304.00         134         13         5.253         5.268         Positive         93.28           Osycodone         316.20         29.310         134         13         5.253         1.212         Positive         192.88           Osycodone         306.20         22.000         134         25         5.386         1.217         Positive         120.46           d6'rydococodone         300.20         170.00         158         41         6.387         1.378         Positive         120.66           Hydiococodone         300.20         193.00         158         29         6.377         1.378         Positive         127.66           Hydiococodone         28.20         156.00         72         5         12.614         1.047         Positive         373.25           Metopolol         28.820         1159.00         72         13         136.82         1.369         Positive         194.01           Metopolol         28.200         56.00         122         23         1.369         Positive	8											824		
d6-ouycodone       322.00       304.00       134       13       5.253       .526       Positive       99.28         Drysodace       316.20       238.10       134       13       5.253       .526       Positive       99.28         Drysodace       316.20       238.10       134       13       5.251       .121       Peaker       17.33         Dysodace       316.20       241.00       134       25       5.386       1217       Positive       120.46         d6+ydococodone       300.20       177.100       158       41       6.367       1.378       Positive       120.66         Hydococodone       300.20       177.00       158       41       6.367       1.378       Positive       120.86         Metoprolol       288.20       56.00       136       23       10.443       1.047       Positive       28.19         Metoprolol       288.20       56.00       72       25       12.630       1.263       Positive       194.00         Meprobanale       219.00       55.00       72       25       12.630       1.268       Positive       194.00         Proparadol       262.00       150.00       72       25	9				91.00	70						🗉 Options		
Orgensea         Image: Constraint of the second secon	10													
Opcodore         316.20         241.00         134         25         5.386         1.217         Positive         120.46           d8+ydacocodore         306.00         202.00         166         23         6.138         .514         Positive         127.66           Hydacocodore         300.20         171.00         158         41         6.377         1.378         Positive         14.83           Hydacocodore         300.20         195.00         158         23         6.377         1.378         Positive         246.50           Metopolol         288.20         56.00         136         23         10.443         1.047         Positive         246.50           Metopolol         288.20         115.00         138         13         10.461         1.047         Positive         373.25           Metopolol         280.20         55.00         72         5         12.614         1.282         Positive         198.00           Poptanolol         280.20         55.00         72         12.38         Positive         109.00         109.00           Poptanolol         280.20         55.00         72         13.582         1.389         Positive         109.00	11	2011 - 100 -				12.872	727	20100.000	1.		100000			
d6-hydiocaccdone       306.00       202.00       166       23       6.138       .614       Positive       127.06         Hydiocacdone       300.20       177.00       158       41       6.367       1.378       Positive       148.83         Hydiocacdone       300.20       177.00       158       41       6.367       1.378       Positive       148.83         Metoprolal       288.20       56.00       136       23       10.443       1.047       Positive       208.19         Metoprolal       288.20       156.00       136       23       10.443       1.047       Positive       246.50         Meprobamate       219.00       155.00       72       25       12.630       1.263       Positive       194.00         Poparadol       286.20       115.90       72       25       12.630       1.263       Positive       194.00         Poparadol       280.20       155.00       72       25       12.630       1.263       Positive       194.00         Poparadol       280.20       150.00       72       28       1.379       Positive       127.06         Dextomethorphan       272.20       170.90       152       41	12													
Hydrocodone       300.20       171.00       158       41       6.367       1.378       Positive       143.63         Hydrocodone       300.20       193.00       158       24       6.377       1.378       Positive       248.63         Metopolol       268.20       55.00       136       23       10.443       1.047       Positive       248.63         Metopolol       268.20       116.00       136       13       10.441       1.047       Positive       237.25         Metopolone       219.00       158.00       72       5       12.614       1.262       Positive       184.03         Proparolol       288.20       115.00       72       5       12.614       1.262       Positive       194.00         Poparolol       288.20       156.00       72       25       1.263       Positive       104.00         Poparolol       288.20       115.00       122       13       13.682       1.370       Positive       127.06         DevidoneHorphan       272.20       170.90       152       41       13.570       Positive       127.06         DevidoneHorphan       727.271       145.491       147.92       13.961       1.92 <td>13</td> <td>12 7 2 5 1 2 4 1 9 0 1 2 5 1 5</td> <td></td> <td>011100.000</td> <td></td>	13	12 7 2 5 1 2 4 1 9 0 1 2 5 1 5		011100.000										
Hydrocodone       300.20       193.00       158       23       6.377       1.378       Positive       208.19         Metopold       268.20       55.00       136       23       10.443       1.047       Positive       246.50         Metopold       288.20       116.00       136       13       10.461       10.47       Positive       246.50         Metopolamate       219.00       55.00       72       5       12.614       1.262       Positive       194.00         Mepolamate       219.00       55.00       72       25       12.630       1.263       Positive       194.00         Propanold       286.20       115.90       122       13       13.682       1.369       Positive       194.00         Propanold       286.20       170.90       152       41       13.997       Positive       127.06         Dextromethorphan       272.20       170.90       152       41       13.977       Positive       128.93         Dextromethorphan       272.721       146.91       157.2       74       13.907       Positive       78.32       The value of cycle time [m ms].	14					107.01		100000	1. 10. 10. 10. 10.			Cycle Time	(	500
Metopolol         288.20         56.00         136         23         10.443         1.047         Politive         246.50           Metopolol         288.20         116.00         136         13         10.451         1.047         Positive         246.50           Metopolol         288.20         116.00         136         13         10.461         1.047         Positive         373.25           Meprobamate         21.900         55.00         72         25         12.630         1.263         Positive         154.83           Properavolol         286.20         115.90         72         25         12.630         1.263         Positive         109.00           Properavolol         280.20         55.00         72         22         1.368         Positive         127.06           Destromethorphan         272.20         170.90         152         41         13.953         1.397         Positive         127.06           Destromethorphan         272.70         170.80         152         41         13.961         1.927         29.37	15		1	2	199.00	158	29	6.377						
Metopolal         288.20         116.00         136         13         10.461         1.047         Positive         373.25           Meprobanale         219.00         158.00         72         5         12.614         1.262         Positive         194.00           Meprobanale         219.00         55.00         72         25         12.63         Positive         194.00           Proparalol         24.200         115.90         122         13         13.882         1.370         Positive         109.00           Proparalol         286.20         170.90         152         41         13.953         1.370         Positive         127.60           Destromethorphan         272.20         170.90         152         41         13.953         1.370         Positive         127.60           Destromethorphan         272.20         170.90         152         41         1.397         Positive         293.29         ✓	16				10.312.52							Plot Type		Concurrent MR
Meprobamate         □         219.00         158.00         72         5         12.614         1.262         Positive         184.00           Meprobamate         □         219.00         55.00         72         25         12.630         1.263         Positive         154.83           Progranold         □         28.20         155.90         122         13         13.892         1.370         Positive         105.00           Destromethorphan         □         272.20         170.90         152         41         13.953         1.397         Positive         128.83         The value of cycle time [mms].           Destromethorphan         □         272.721         146.90         157.2         24         13.971         Positive         128.83         The value of cycle time [mms].	17	and a second produced												
Meprobanate         219.00         55.00         72         25         12.630         1.263         Population         154.83           Propravolul         286,200         115.90         122         13         13.682         1.369         Positive         190.00           Propravolul         260.20         55.00         122         23         13.362         1.369         Positive         127.06           Dextromethorphan         272.20         170.90         152         41         13.953         1.337         Positive         122.63           Dextromethorphan         272.20         170.90         152         41         13.953         1.337         Positive         122.63           Dextromethorphan         272.20         170.90         152         41         13.953         1.337         Positive         123.63           Dextromethorphan         272.20         170.90         152         41         13.961         1.337         Positive         128.63           Dextromethorphan         272.20         126.64         145.94         13.961         1.397         Positive         128.76	18													
Propriancial         Image: Second seco	19													
Propranolol         280.20         56.00         122         23         13.692         1.370         Positive         127.06         The Yalue of cycle time (n ms).           Destrometrophan         272.20         170.90         152         41         13.953         1.397         Positive         128.93           Destrometrophan         272.20         170.90         152         41         13.953         1.397         Positive         128.93           Destrometrophan         272.20         146.90         152         24         13.961         1.937         Positive         293.93         ✓	20													
Dextomethorphan         Image: Constraint of the second secon	21	Propranolol		0	56.00	122	29	13.692	1.370	Positive	127.06			
Insutromethornhom         Im         277 201         146 901         152         241         13 961         1 3977         Proteinue         263 251         Im		1			170.90			13.953	1.397	Positive		I he value of cycle	time (in ms).	
	22 23 6- 5- 4- 3- 2-													
	1-												N	

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.

ID 7	Compound Name		Precursor v	Product v	Fragme 😽	CE V	Ret 🗸	Ret Time ❤	Polarity 🗸	Average 😽	56	Dynamic MRM St Total MRMs	atistics
-		1	lon *	lon •	ntor		Time *	Window		Dwei		Minimum Concurrent MRMs	1
1	Atenolol		267.20	190.00	134	13	2.124	.657	Positive	171.50		Maximum Concurrent MBMs	6
2	Atenolol		267.20	145.00	134	21	2.125	.657	Positive	171.50		Minimum Dwell Time	54.83 ms
3	Codeine-d3		303.00	165.00	162	45	3.759	.376	Positive	113.17	718		
4	Clonidine		230.00	213.00	150	21	3.782	.897	Positive	98.00		Maximum Dwell time	350.00 ms
5	Clonidine		230.00	44.00	150	25	3.810	.897	Positive	92.75	L	Minimum Cycle time	33.00 ms
6	Amphetamine-d5		141.10	124.00	74	5	4.528	.923	Positive	65.53			
7	Amphetamine-d5		141.10	92.70	74	13	4.581	.923	Positive	63.58		HE AL COM	
8	Amphetamine		136.10	119.00	70	5	4.605	1.266	Positive	66.50	100		
9	Amphetamine		136.10	91.00	70	13	4.613	1.266	Positive	68.25	E	Options	
10	d6-oxycodone		322.00	304.00	134	13	5.253	.526	Positive	68.44		Minimum Data Point	64
11	Oxycodone		316.20	298.10	134	13	5.351	1.217	Positive	81.08		Check Minimum Data Point? Minimum Dwell Time	True 2
12	Oxycodone		316.20	241.00	134	25	5.386	1.217	Positive	83.27		Maximum Concurrent MRMs	200
13	d6-hvdrococodone		306.00	202.00	166	29	6.138	.614	Positive	87.89	E	B Parameters	
14	Hydrocodone		300.20	171.00	158	41	6.367	1.378	Positive	103.83		Cycle Time	350
15	Hydrocodone		300.20	199.00	158	29	6.377	1.378	Positive	144.86	E	E Plot Options	1
16	Metoprolol		268.20	56.00	136	29	10.443	1.047	Positive	N 171.50		Plot Type	Concurrent MR
17	Metaprolol		268.20	116.00	136	13	10.461	1.047	Positive	260.75			
18	Meprobamate		219.00	158.00	72	5	12.614	1.262	Positive	127.75			
19	Meprobamate		219.00	55.00	72	25	12.630	1.263	Positive	107.33			
20	Propranolol		260.20	115.90	122	13	13.682	1.369	Positive	75.25			
21	Propranolol		260.20	56.00	122	29	13.692	1.370	Positive	87.89		Cycle Time	
1.1.1.1												I he value of cycle time (in ms).	
23					1.00	29			12011 22 20 12				
22	Dextromethorphan Devtromethorphan		272.20 977 90	170.90 146 90	152	41	13.953	1.397	Positive	89.83		The value of cycle time (in ms).	
1-	0 1 2	3 4	5 6	7 8	ė	J L	1 12	13 14	15 16	5 17 18		19 20 21 22 2	3 24 25

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.

	Compound Name	ISTD?	Precursor Ion	MS1 Res	Product Ion	MS2 Res	Fragmentor	Collision Energy	Ret Time (min)	Delta Ret Time	
	d6-oxycodone		322	Unit	304	Unit	134	13	5.2531	0.526	
I	Oxycodone		316.2	Unit	298.1	Unit	134	13	5.351367	1.21715	
I	Sertraline		306.1	Unit	274.8	Unit	88	5	17.25038	1.727	
1	d6-hydrococodone		306	Unit	202	Unit	166	29	6.13785	0.614	
I	Hydrocodone		300.2	Unit	199	Unit	158	29	6.377083	1.3783	
	Diclofenac		296	Unit	250	Unit	1,00	15	22.64233	2.265	
I	Diazepam-d5		290.1	Unit	198	Unit	162	33	19.16847	1.917	
1	Diazepam		285.1	Unit	193	Unit	162	33	19.28048	1.929	
	Dextromethorphan		272.2	Unit	170.9	Unit	152	41	13.9528	1.397	

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.





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.

	gilent MassHunter Quantitative Analysis Edit View Analyze Method Update Report Tools Help		
2	New Batch	Ctrl+N	ult Layout
<b>&gt;</b>	Open Batch	Ctrl+O	
	Save Batch	Ctrl+S	👻 🖬 IST
	Save Batch As		
	Close Batch		
	A <u>d</u> d Samples		
	Export	•	

Figure 21 New Batch from the File menu

New Batch					? 🛛
Look in:	🚞 Example Data		*	I 🕫 🕫 🔃	
📁 Recent	MH ForensicTox_Te MH LCMS_Forensic OuantResults	st_Mix_MRM.d and Toxicology Test Mix 10pg.	.d	Batch File	Data Ve
Desktop					
My Documents					
				<	>
My Computer - cnu8083bmj	File name:	For_DMRM_MethodCreation		~	Open
	Files of type:	Batch Files (*.batch.bin)		~	Cancel
My Network					Help

**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.

111 A	gilent	MassHu	nter	Quanti	tative	Analys	is - Ex	ample	Data - Fo	_DMRM_	Method	I_Creation	1 <b>2</b>	
File	Edit	View An	alyze	Method	Update	e Repo	't Tool	ls Help	I.	_				
1	New E	Batch							Ctrl+N	Δ 🗵	Restore	Default Lay	out	
B 📂	Open	Batch							Ctrl+O					
	Save	Batch							Ctrl+S			*	-	ISTD
	Save	Batch As												
	Close	Batch								d File				
	Add S	amples												
	Expor	t								•				
ĺ0	Page	Setup												
8	Print.								Ctrl+P					
D,	Print P	Preview												

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.

Add Samples 🔹 💽 🔀
Batch Folder: C:\Documents and Settings\p
ForensicTox, Test, Mix, MRM, d LCMS_Forensic and Toxicology Test Mix 10pg.d
Browse to Copy Samples Select All OK Cancel

Figure 24 Add Samples dialog box

- 4 Create a new method.
  - **a** Click **Method > New > New Method from Acquired MRM Data**. See Figure 25.

ns Agilent MassHunter Q	uantitative Analysis - E	xample Data - Fo	_DMI	RM_Method_Creation		
; File Edit View Analyze M	1ethod Update Report To	ools Help				
i 🗅 🗁 🔒 i 🖬 i 📮 🗧	New	•	B <sup>O</sup> N	lew Method from Acquired M	IRM Data	
Batch Table	Open	•	N	lew Method from Acquired S	can Data	
Sample: 👔 👢 🛛 Sar	Edit	F10	N	lew Method from Acquired S	ican Data	with Library Search
	Validate		N	lew Method using Manual Se	tup	
() 🕅 Name	Save		Leve		Vol.	Acq. Method File
Tox Mix 100p				6/15/2009 10:55 PM	0.1000	
	Save As					
E	X Exit	F11				
	Method Setup Tasks	۱.				
	Manual Setup Tasks	+				
	Outlier Setup Tasks	•				
	Advanced Tasks	•				
	Copy Calibration Levels	То				
		р 1				

Figure 25 New Method from Acquired MRM Data selected

New Method fro	om Acquired Dat	a					? 🗙
Look in:	🚞 Example Data		*	6	1 🖻	•	
D Recent	MH ForensicTox_Te MH LCMS_Forensic CuantResults IndexedDataCo	and Toxicology Test M	ix 10pg.d				
Desktop							
My Documents							
My Computer - cnu8083bmj							
	Object name:				~	]	Open
	Objects of type:				*	]	Cancel
My Network Places							Help

**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.

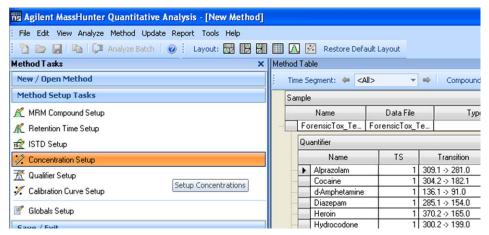


Figure 27 Concentration Setup under Method Setup Tasks

- 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.

mple									
Name	Data File	Тур	•	Level	Acq. Date-	Time			
ForensicTox_Te	ForensicTox_T	e							
Quantifier									
Name	TS	Transition		Gcan	Туре	6	Dil. High Conc.	Dil. Pattern	Units
Alprazolam	1	309.1 -> 281.0	MBM	Tai	get		1.0000	1:2	g/ml
Cocaine	1	304.2 -> 182.1	MRM	Tai	get				ng/ml
d-Amphetamine	1	136.1 -> 91.0	MRM	Tai	get				ng/ml
Diazepam	1	285.1 -> 154.0	MRM	Tai	get				ng/ml
Heroin	1	370.2 -> 165.0	MRM	Ta	get				ng/ml
Hydrocodone	1	300.2 -> 199.0	MRM	Ta	get				ng/ml
Lorazepam	1	321.0 -> 275.0	MRM	Ta	get				ng/ml
MDA	1	180.1 -> 163.0	MRM	Tai	get				ng/ml
MDEA	1	208.1 -> 163.0	MRM	Tai	get				ng/ml
MDMA	1	194.1 -> 163.0	MRM	Tai	get				ng/ml
Meperidine	1	248.2 -> 220.1	MRM	Tai	get				ng/ml
Methadone	1	310.2 -> 265.1	MRM	Tai	get				ng/ml
methamphetami		150.1 -> 91.0	MRM	Tai	get				ng/ml
Nitrazepam	1	282.1 -> 236.1	MRM	Tai	get				ng/ml
Oxazepam	1	287.0 -> 241.0	MRM	Tai	get				ng/ml
Oxycodone	1	316.2 -> 298.1	MRM	Tai	get				ng/ml

Figure 28Quantifier 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.

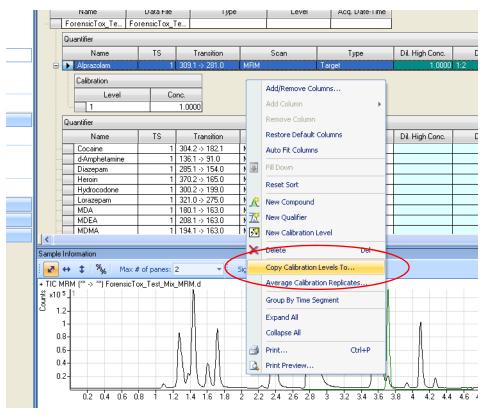


Figure 29 Copy Calibration Levels To selected

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

Copy Calibration	Levels	То			?>
Select Compounds					
Name	TS	RT	Transition	ISTD Flag	^
Cocaine		2.358	304.2 -> 182.1		
d-Amphetamine		1.278	136.1 -> 91.0		
Diazepam		4.269	285.1 -> 154.0		
Heroin		2.236	370.2 -> 165.0		
Hydrocodone		1.380	300.2 -> 199.0		
Lorazepam		3.610	321.0 -> 275.0		
MDA	1	1.311	180.1 -> 163.0		~
Select All			OK	Car	ncel

**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.

- I 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.

Agilent	MassHunter Quantitative Analysis	×
⚠	Would you like to apply this method to the b	atch?
	Yes No Cancel	

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.

ii.	Agil	ent M	assHunter Qua	intitative Analysis - Examp	le Data - Fo	r_DMR	M_Method_Creation		
£	File Edit View Anal <del>yze Method Upd</del> ate Report Tools Help								
🗄 🎦 🗁 🛃 🌘 📜 Analyze Batch 🕜 🗄 Layout: 🔜 🔛 🔛 🛄 \Lambda 📝 Restore Default Layout									
Ba	Batch Table								
Sample: 👔 🎚 Sample Type: Analyze Batch Compound: 🗰 1: Oxycodone 🔹 🖬 IS									
Sample									
	•	8	Name	Data File	Туре	Level	Acq. Date-Time	Vo	
_	T	T T	Tox Mix 100ppb	ForensicTox Test Mix MRM.d	Sample	1	6/15/2009 10:55 PM	0.10	

- 8 Save the batch again, now that the results are processed.
- **9** Generate a report for the data file:
  - a Click **Report > Generate**.

📅 Agilent MassHunter Quantitative Analysis - Example Data - For _DMRM _Method _Creation								
File Edit View Analyze Method Update Report Tools Help								
🗄 🎦 🗁 🛃 📄 🖓 Analyze Batc	Generate		) 🛛	Restore Default Layo	out			
Batch Table Export Graphics Settings								
Sample: 🝸 🎩 Sample Type: <all> 資 Queue Viewer done 🔻 🖃 ISTD:</all>								
Sample								
⑦ ♥ Name	Data File	Туре	Level	Acq. Date-Time	Vol.	Acc		
🕨 🔮 🛛 Tox Mix 100ppb 🛛 Forensi	cTox_Test_Mix_MRM.d	Sample	I	6/15/2009 10:55 PM	0.1000	I		

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**.

Report				? 🗙						
Report results file/Report graphics	file									
🔊 💿 Generate report resi	ults file									
Choose a batch file	Choose a batch file from which the report results file is generated									
Batch folder:	Batch folder: C:\MassHunter\Data\TestMix\									
Batch file:	Batch file: test.batch.bin									
Graphics files										
Turn on/off generating graphics files. Turn off when you know you will choose report templates that do not use graphics files. Turning off will reduce report generation time and disk. usage.										
<b>—</b> L	Load graphics settings file									
Instru	ument Type determinin;	g numeric formats in grap	hics files: QQQ	*						
🔘 Use existing report r	esults file/report graph	ics files								
Report folder										
	folder. When you chos	hose to generate report r e to use existing report n								
Report folder: C:\Mas	sHunter\Data\TestMix	<\QuantReports\test								
Reports										
Choose report templates	s, report file names, prir	nters, and publish formats	а.							
Template		Report File Name	Printer	Publish Format						
Add D	elete									
Queue Viewer			OK	Cancel						

**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.

	28					
ease select MassHunter QQQ data file or Quant report folder:						
🗉 🍕 co_pos_oneseg_MRM_01.d						
🖽 婿 co_pos_oneseg_MRM_02.d						
🖃 🚞 QuantReports						
🖃 🗁 co_pos_oneseg_MRM_01						
🚞 PeakChromatogram						
🚞 PeakQualifierChromatogram						
🚞 PeakQualifiers	20					
🛅 PeakSpectrum						
🗀 SampleChromatogram						
🚞 TargetCompoundCalibration						
OK Cancel						
•	· ·					
Peak Abundance Threshold 50						
Retention Time Window Threshold 10						
Retention Time Window Threshold Unit Percent						
Update Retention Time?						
Update Retention Time? Indicates whether to update the retention time of a new method.						
	Cancel					
Retention Time Window Threshold 10						

**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.

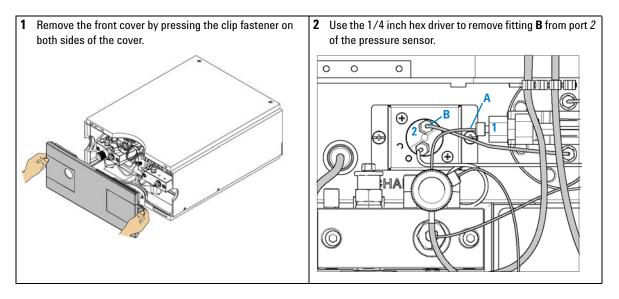
**Tools required** • Wrench, 1/4-inch x 5/16-inch (p/n 8710-0510)

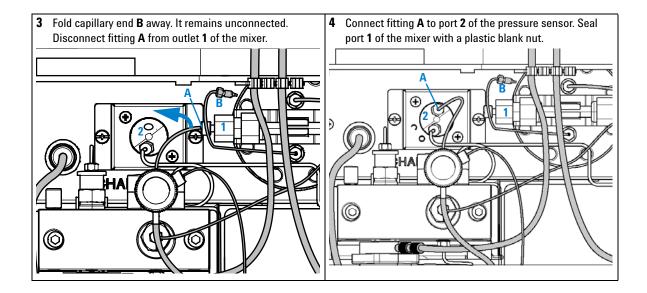
- Wrench, open end, 14-mm (p/n 8710-1924)
- Hex Driver, 1/4-inch, slitted (p/n 5023-0240)

#### **Preparations for** • Flush the system (water if buffers were used, otherwise IPA).

this procedure

• Turn the flow off.





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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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