

UPDATING PESTICIDE RETENTION TIME LIBRARIES FOR THE AGILENT INTUVO 9000 GC

How to Update Retention Time Libraries on Intuvo



Introduction

When applying methods to a new gas chromatographic (GC) system, it is advisable to verify retention times of critical analytes prior to beginning typical workflow production. Often, due to differences in system configuration or column variability, retention times between two similar GC systems may be different. Retention Time Locking (RTL) can be used to ensure repeatable retention times between multiple systems.

If a retention time database is used, in this case pesticide analysis with P&EP MRM database retention times, additional steps can be taken to ensure that retention times match the existing database, or the analytical method must be updated to reflect the new system's retention times.

This technical brief provides instructions on how to retention time lock a pesticide analysis multiple reaction monitoring (MRM) method on an Agilent Intuvo 9000 GC, and subsequently update retention times to take advantage of the P&EP MRM database.

The general workflow for extractor or inert ion sources follows the following steps:

1. Generate a retention time lock chromatogram.
2. Acquire data with the dMRM method and widened left/right RT delta windows.
3. Update retention times using Quantitative Analysis.
4. Import new retention times into the dMRM method.
5. Validate the method.

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Retention Time Locking

When setting up a new system to duplicate a pesticide analysis, use RTL to ensure retention time agreement between the two systems. When acquiring RTL calibration, the system collects five data files with varying flow rates or pressures (Figure 1).

The mass or masses for the RTL compound and the vial number of the standard containing the locking compound was entered before the RTL calibration data were collected. Chlorpyrifos methyl is often used as the locking compound for pesticide analyses. Scanned masses for locking to chlorpyrifos methyl were chosen from the mass spectrum, and is usually locked at 9.143 for a 20-minute method. For a typical 20-minute pesticide analysis, this procedure takes approximately 3 hours. This includes the initial Clean Out run to ensure there are no lingering analytes carried over from previous runs that may interfere with the masses of the RTL compound.

Once the RTL calibration data were collected, the retention times of the locking compound were entered, and the flow rates were adjusted (from the calibration data) to obtain the desired retention time (Figure 2).

Acquire dMRM data

After retention time locking the new system, the retention times of critical compounds throughout the chromatogram were verified to ensure that the MRM windows were still accurate. The MRM method was converted to a dMRM method (Figure 3).

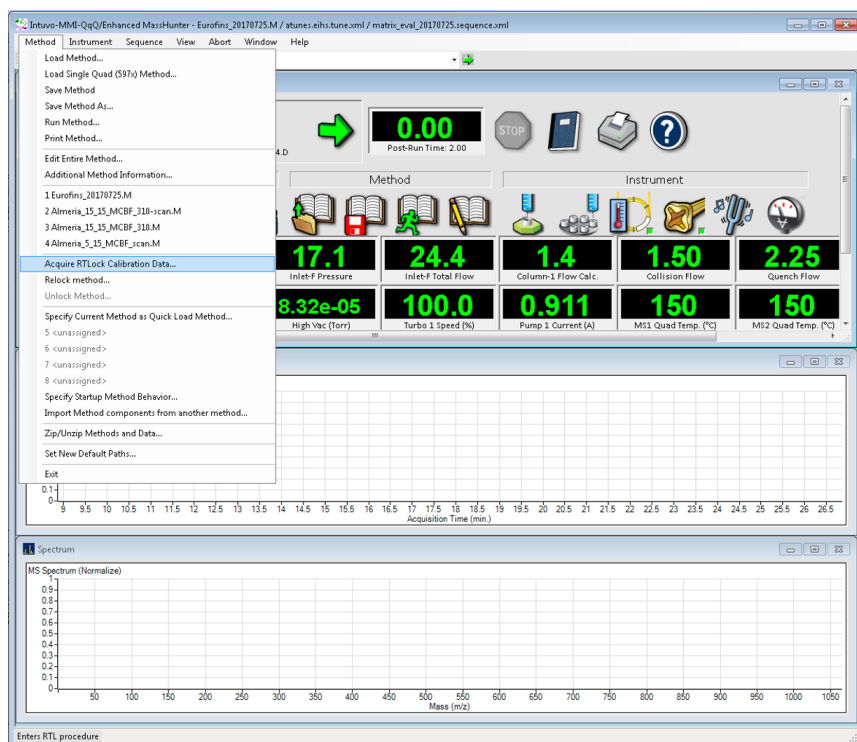


Figure 1. The **Acquire RTL Calibration Data** command can easily be accessed from the Method list. This collects new RTL calibration data. After the initial calibration has been completed, **Relock Method** can be used.

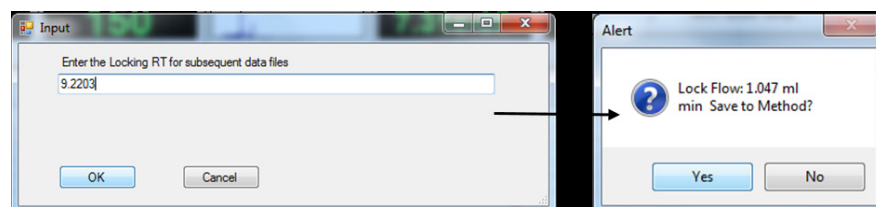


Figure 2. The desired retention time of the locking compound is entered, and the system adjusts the flow rate to achieve the selected retention time.

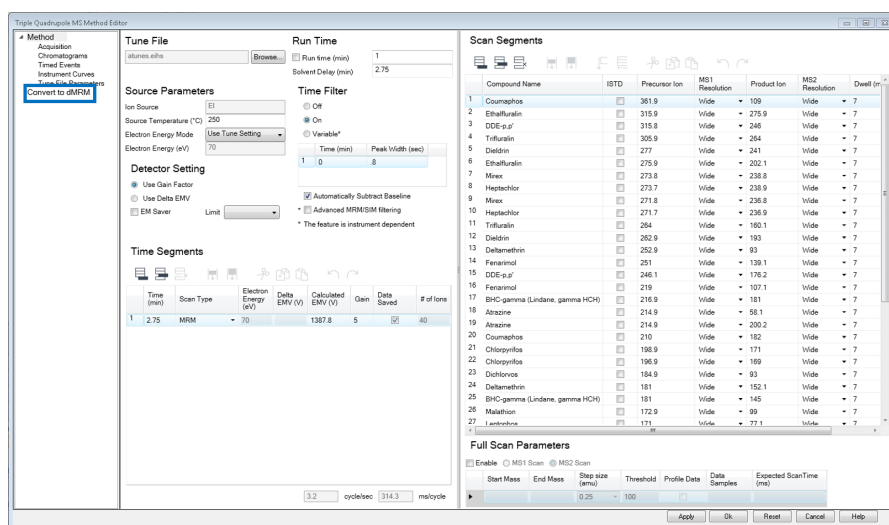


Figure 3. A time-segment-based MRM method was easily converted to a dMRM method with guidance from Agilent MassHunter acquisition software.

By using the existing MRMs, the new dMRM method maintained the existing transitions. The software calculated the analyte retention times by averaging the previously specified start and end time of each segment (Figure 4).

In the dMRM method, the left and right delta windows were widened globally to 2 minutes by using the **Overwrite RT Delta** function (Figure 5).

Depending on the number of compounds and transitions in the dMRM method, the maximum concurrent MRMs (200) may be exceeded (Figure 6).

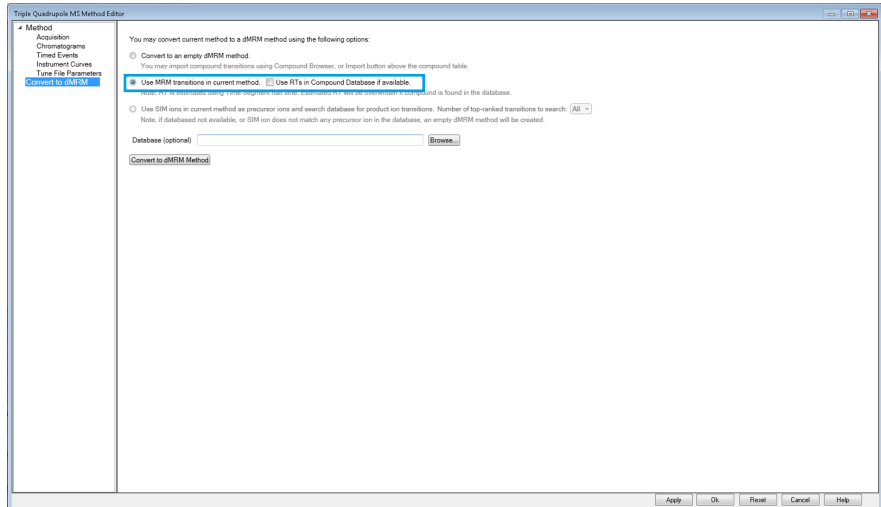


Figure 4. The MRM transitions from the current method were used when converting the method to a dMRM method.

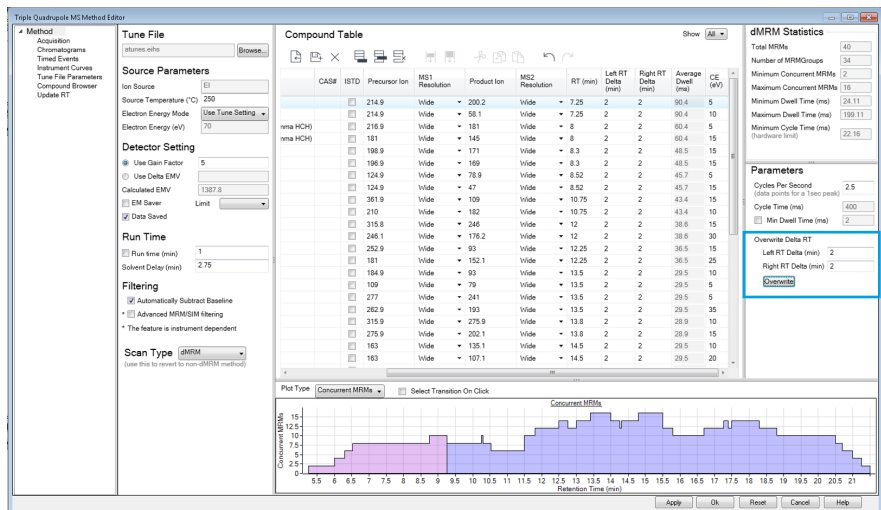


Figure 5. Globally overwriting the left and right retention time delta widens the MRM transition windows to ensure that all the analytes are detected, even if their retention time has shifted slightly.

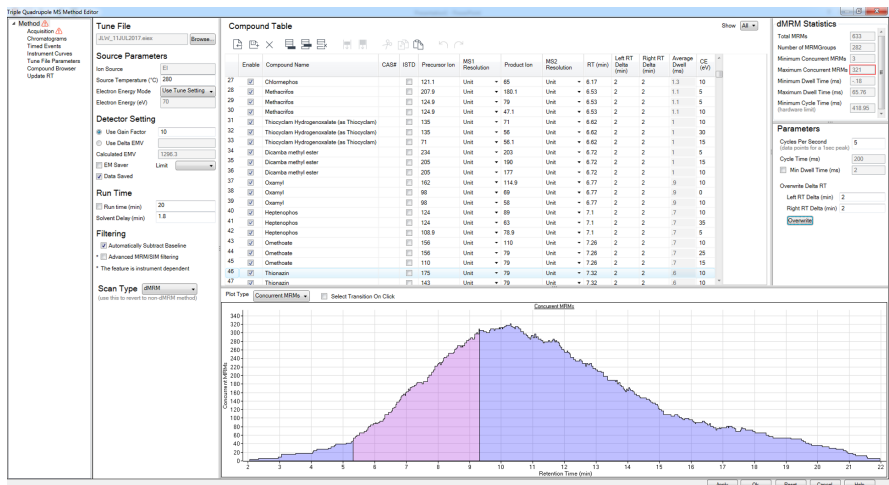


Figure 6. A dMRM method containing a large number of analytes and transitions exceeded the number of concurrent MRMs with the left and right retention time delta set to 2 minutes.

The left and right RT delta windows were narrowed to 1 minute or 0.5 minutes to reduce the number of concurrent MRMs, while maintaining a wide enough window to identify the target analytes. The current retention times were saved as a CSV file (Figure 7).

Update Retention Times

A standard was run with the wider windows using Agilent MassHunter Quantitative Analysis, and the retention times were updated in an existing quantitative analysis method. With the batch containing the new standard open and selected, a previously saved Quant method was loaded. In the method editor view, **Update Retention Times** was selected from the Update menu (Figure 8). **Select All** was used to update all retention times in the existing method, at which point the method was saved, validated, and applied to the batch (Figure 9).

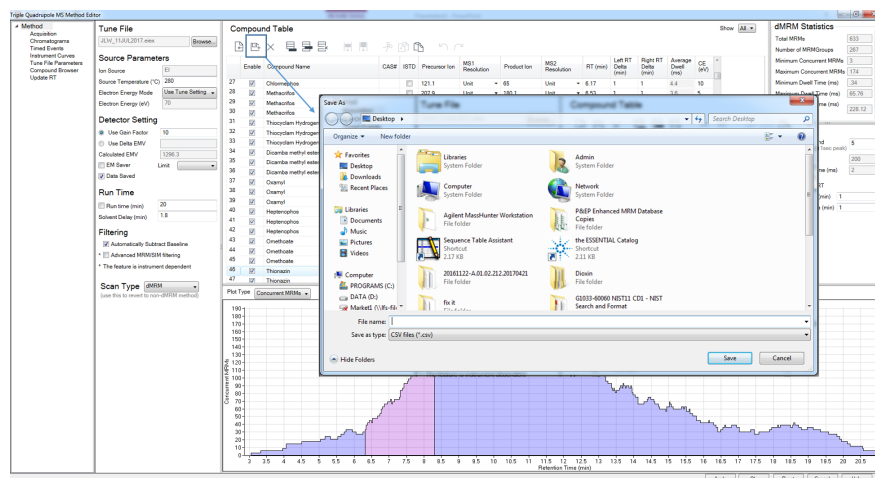


Figure 7. The dMRM compound table was exported by clicking the **Save** icon, and saving the table as a CSV file locally.

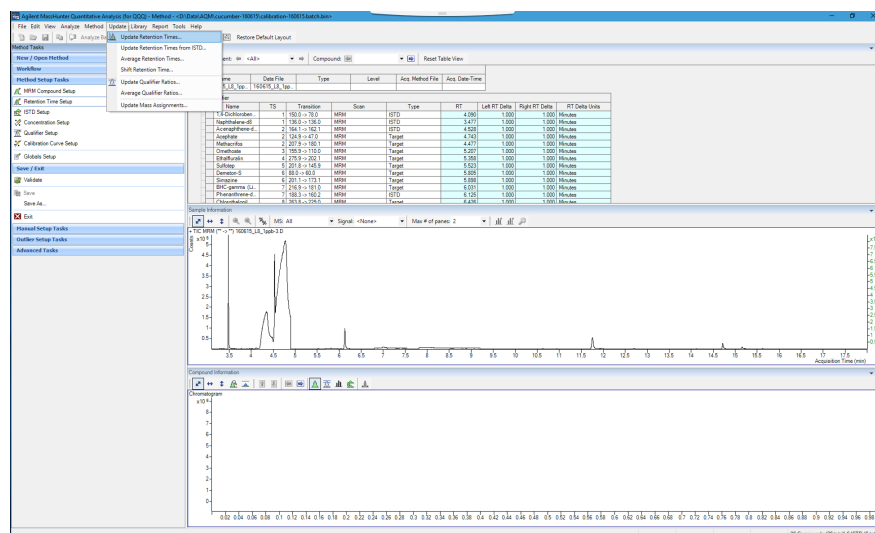


Figure 8. **Update Retention Times** was accessed in the Method Editor view from the **Update** menu in Agilent Mass Hunter Quantitative Analysis.

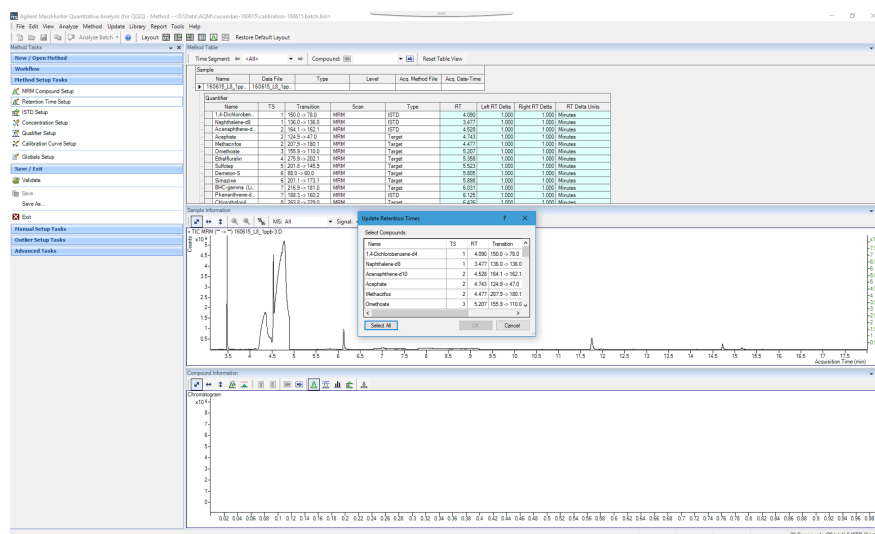


Figure 9. Every analyte in the given method was updated in one action from one standard run.

Import New Retention Times in the dMRM Method

With updated retention times in the Quant method, the compound table was exported from the batch to a CSV file (Figure 10).

The updated retention times from the standard run and the new Quant method were updated in the previously exported and saved CSV file from the dMRM method, and saved with a new name (Figure 11). The critical pieces of information in the CSV file are the compound column and RT column.

The retention times in the dMRM method were updated from the new CSV file (Figure 12), in MassHunter acquisition Method Editor.

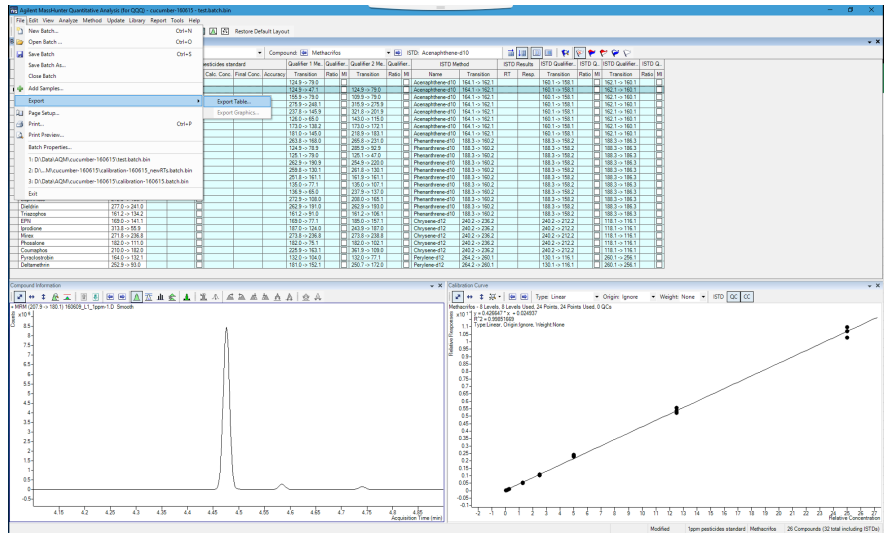


Figure 10. Exporting the Compound Table resulted in a CSV file that was saved locally.

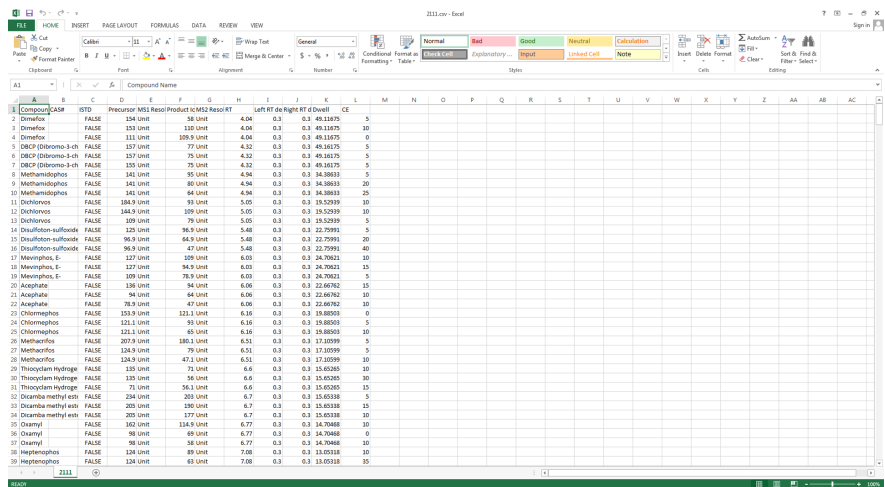


Figure 11. The exported CSV file from the dMRM method contained the compound name in one column and the retention time in another column. In this example, each MRM transition is matched with its respective compound's retention time.

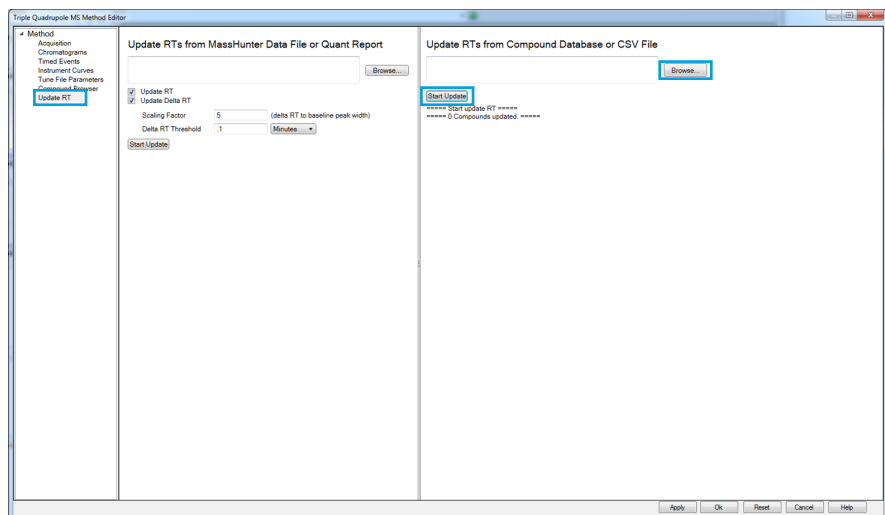


Figure 12. The saved CSV file containing the updated retention times was selected as the file to use for Update RT.

Using the **Update RT** feature, the saved CSV file was selected before starting the update. After updating the retention times, the compound list was shown, indicating the changed retention times (Figure 13). The method's previous retention times are listed in parentheses.

Alternatively, the retention times can be updated from a quant report using a similar procedure. A report was generated from Quantitative Analysis (Figure 14), and the resulting report was used to update retention times in the acquisition method editor.

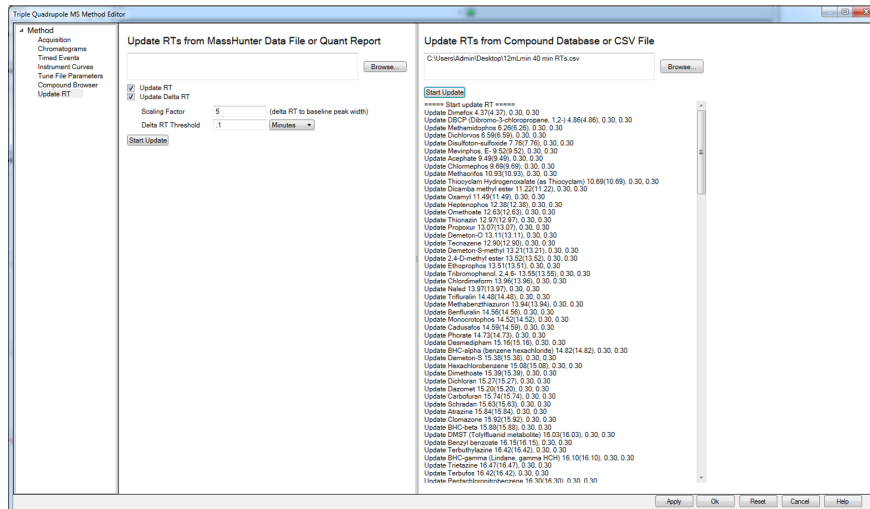


Figure 13. After updating the retention times, the compound list was shown, indicating the changes made.

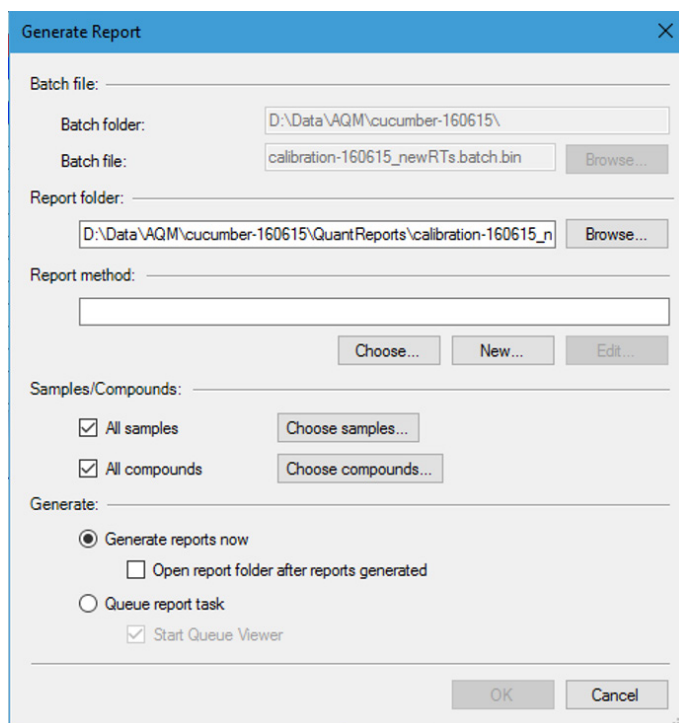


Figure 14. Generating a report in Quantitative Analysis gave an alternative means of updating the retention times in the acquisition method.

If a time-segment-based MRM method is preferred for the final, updated method, a transitions file can be created from the updated Quant method (Figure 15). By specifying a Time Segment schema (Figure 16), the resulting file can be imported into the compound table (Figure 17).

Validate the Method

After applying the changes to the method, the standard was verified before continuing with calibration and sample analysis.

Conclusions

A step-by-step procedure was shown for retention time locking, and updating retention times in a library or database method is demonstrated. The process was relatively simple and straightforward. An additional analytical run beyond the retention time locking calibration was required. For this process to be optimal, a standard or sample containing known target analytes is required.

These results may vary depending on the ion sources installed in the mass spectrometer. If the method is optimized for sensitivity, the higher flow rates likely obtained by this procedure may result in nonoptimal performance.

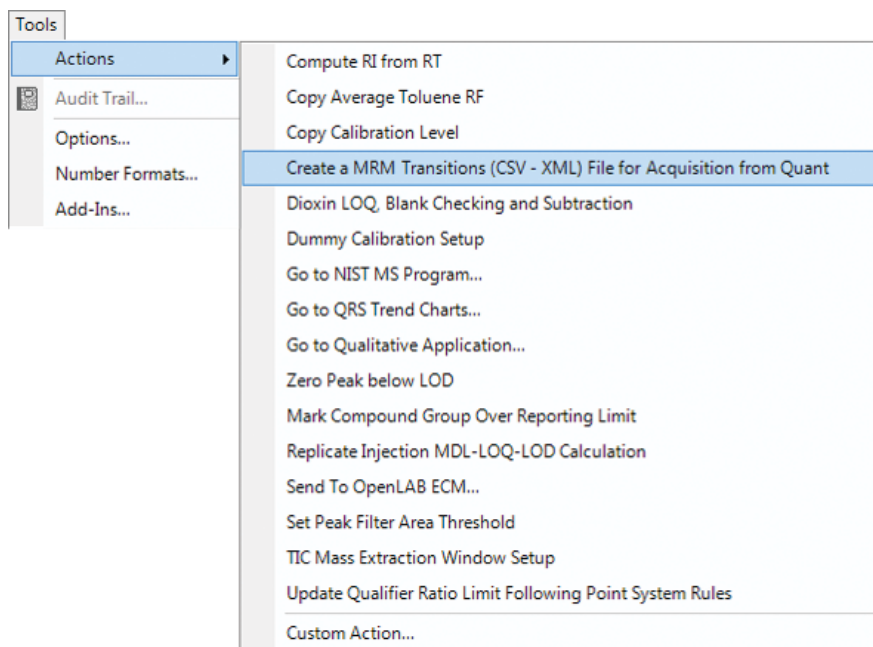


Figure 15. A CSV or XML file containing updated retention times and the existing MRM transitions in the new Quant method.

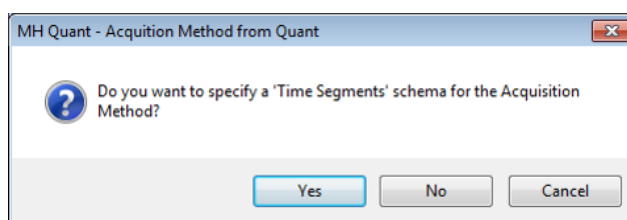


Figure 16. Selecting **Yes** to **specify a 'Time Segments' schema** allows the CSV file to be imported into the compound table of a time-segment-based MRM method.

Compound Table								Show	All
	Import Compound List	Compound Name	CAS#	ISTD	Precursor Ion	MS1 Resolution	Product Ion	MS2 Resolution	RT (min)
1	<input checked="" type="checkbox"/>	Dimefox		<input type="checkbox"/>	154	Unit	58	Unit	4.06
2	<input checked="" type="checkbox"/>	Dimefox		<input type="checkbox"/>	153	Unit	110	Unit	4.06
3	<input checked="" type="checkbox"/>	Dimefox		<input type="checkbox"/>	111	Unit	109.9	Unit	4.06
4	<input checked="" type="checkbox"/>	DBCP (Dibromo-3-chloropropane, 1,2-)		<input type="checkbox"/>	157	Unit	77	Unit	4.33
5	<input checked="" type="checkbox"/>	DBCP (Dibromo-3-chloropropane, 1,2-)		<input type="checkbox"/>	157	Unit	75	Unit	4.33
6	<input checked="" type="checkbox"/>	DBCP (Dibromo-3-chloropropane, 1,2-)		<input type="checkbox"/>	155	Unit	75	Unit	4.33
7	<input checked="" type="checkbox"/>	Methamidophos		<input type="checkbox"/>	141	Unit	95	Unit	4.95
8	<input checked="" type="checkbox"/>	Methamidophos		<input type="checkbox"/>	141	Unit	80	Unit	4.95
9	<input checked="" type="checkbox"/>	Methamidophos		<input type="checkbox"/>	141	Unit	64	Unit	4.95
10	<input checked="" type="checkbox"/>	Dichlorvos		<input type="checkbox"/>	184.9	Unit	93	Unit	5.06
11	<input checked="" type="checkbox"/>	Dichlorvos		<input type="checkbox"/>	144.9	Unit	109	Unit	5.06
12	<input checked="" type="checkbox"/>	Dichlorvos		<input type="checkbox"/>	109	Unit	79	Unit	5.06
13	<input checked="" type="checkbox"/>	Disulfoton-sulfoxide		<input type="checkbox"/>	125	Unit	96.9	Unit	5.5
14	<input checked="" type="checkbox"/>	Disulfoton-sulfoxide		<input type="checkbox"/>	96.9	Unit	64.9	Unit	5.5
15	<input checked="" type="checkbox"/>	Disulfoton-sulfoxide		<input type="checkbox"/>	96.9	Unit	47	Unit	5.5
16	<input checked="" type="checkbox"/>	Mevinphos, E-		<input type="checkbox"/>	127	Unit	109	Unit	6.05
17	<input checked="" type="checkbox"/>	Mevinphos, E-		<input type="checkbox"/>	127	Unit	94.9	Unit	6.05
18	<input checked="" type="checkbox"/>	Mevinphos, E-		<input type="checkbox"/>	109	Unit	78.9	Unit	6.05
19	<input checked="" type="checkbox"/>	Acephate		<input type="checkbox"/>	136	Unit	94	Unit	6.08
20	<input checked="" type="checkbox"/>	Acephate		<input type="checkbox"/>	94	Unit	64	Unit	6.08
21	<input checked="" type="checkbox"/>	Acephate		<input type="checkbox"/>	78.9	Unit	47	Unit	6.08
22	<input checked="" type="checkbox"/>	Chlormephos		<input type="checkbox"/>	153.9	Unit	121.1	Unit	6.18

Figure 17. The CSV file containing updated retention times from Quantitative Analysis can be imported into the acquisition method with time-segment-based MRMs.

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