How to Verify and Update Retention Times in *Scheduled* MRM™ Acquisition Methods using Analyst® and MultiQuant™ Software

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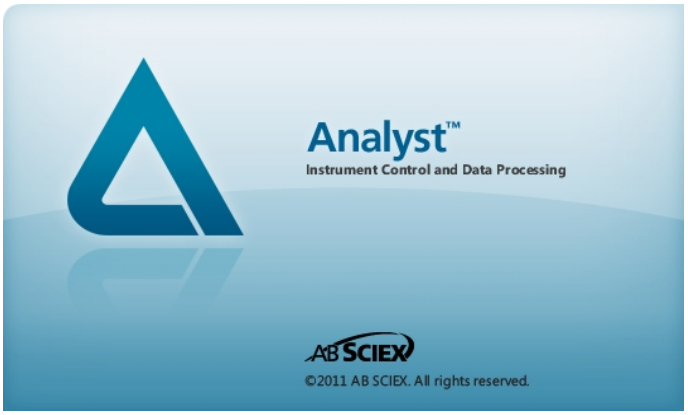
Overview

The following document describes a procedure to update retention times and other parameters when transferring acquisition methods using the *Scheduled* MRM™ algorithm from one laboratory to another.

The following steps need to be completed to update the acquisition method:

1. Open original acquisition method in Analyst® software and export MRM method table into Excel worksheet.
2. Run standard mix using the original acquisition method.
3. Process and review in Analyst® or MultiQuant™ software to verify and update retention times. Copy updated retention times from result table into Excel worksheet.
4. Copy MRM method table from updated Excel worksheet into Analyst® software.
5. Adjust *Scheduled* MRM™ parameters and save updated acquisition method.



1. Export MRM Method

Open the original acquisition method in Analyst® software (Figure 1).

Two MRM transitions are monitored per compound. Target analytes are in alphabetical order. A large ‘MRM detection window’ of 180 seconds is recommended for verification to ensure that all compounds will be detected after transferring the method to another LC system.

Highlight the MRM table to export all parameters. This can be done by simply clicking the top-left corner of the table. Copy the complete table using ‘Ctrl+C’ (Figure 2).

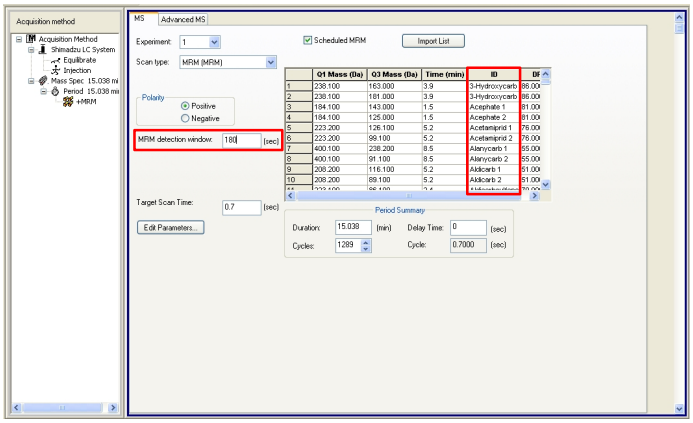


Figure 1. Original *Scheduled* MRM™ method

Paste the table into an empty worksheet in Excel using ‘Ctrl+V’. Add a header row and delete all empty and duplicate columns (Figure 3).

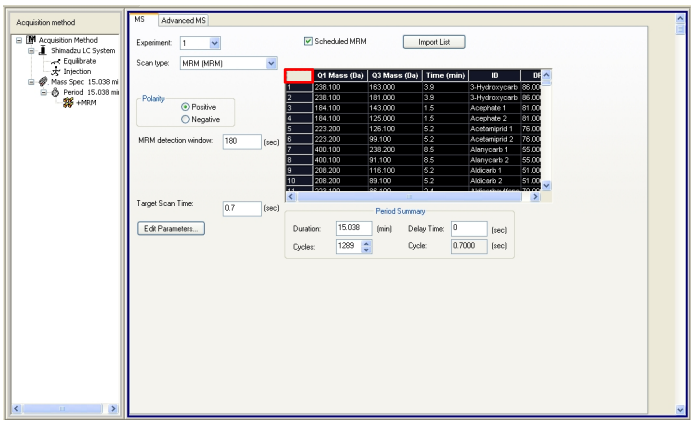


Figure 2. Highlight the MRM table and copy parameters

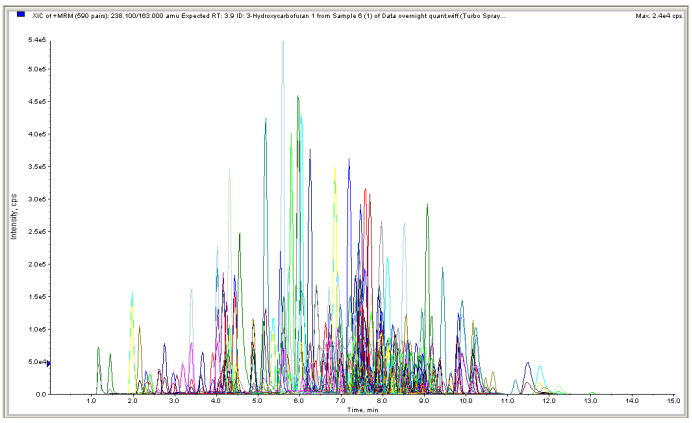


Figure 4. Multi-pesticide standard at 1 ng/mL analyzed using an AB SCIEX QTRAP® 5500 system

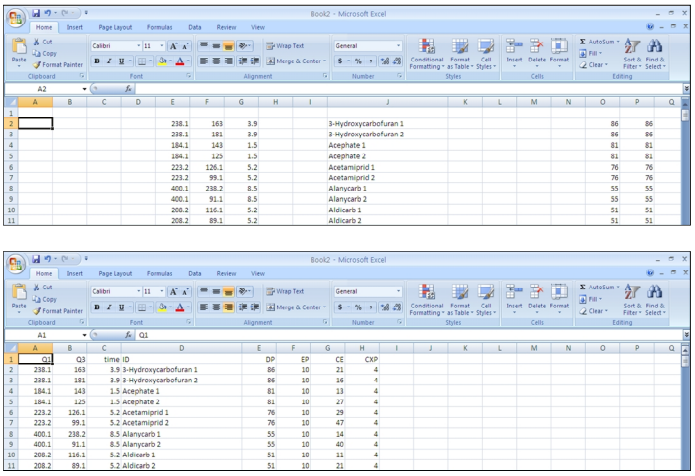


Figure 3. Paste parameters into Excel (top), add a header row and remove empty and duplicate columns

2. Run Standard Mix

Run a mix of all standards using the original acquisition method (Figure 4). The concentration must be high enough to provide sufficient signal. The mix should be dissolved in aqueous mobile phase to avoid distorted peak shape of early eluting compounds.

Note: when the target list is very extensive it might be helpful to also run sub-mixes separately to reduce isobaric interferences.

3. Review Data to Update Retention Times

3.1. Using Analyst® Software

Build a new quantitation method in Analyst® software. Analyte names, MRM transitions, and retention times will be populated automatically (Figure 5).

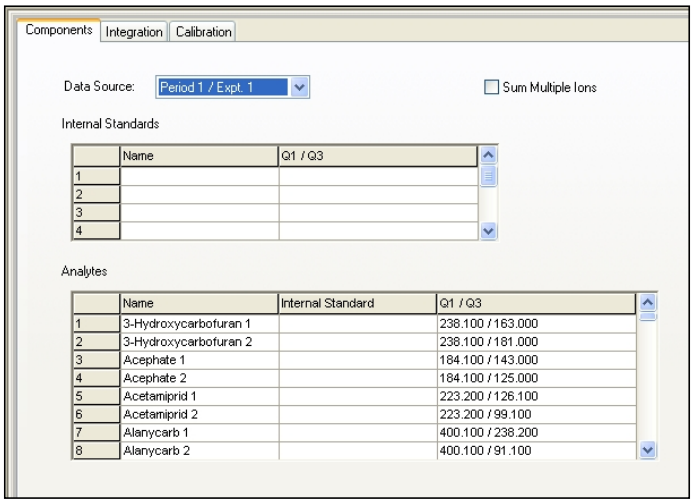


Figure 5. Build a new quantitation method

For reliable integration the following default settings are recommended: MQ3 algorithm with ‘Noise Percentage’ 80%, ‘Peak-Splitting Factor’ 1, ‘Report Largest Peak’ off, ‘Minimum Peak Height’ 100 cps, ‘Minimum Peak Width’ 3 sec, and ‘Smoothing Width’ 3 points.

Create a result table using the built quantitation method using the ‘Quantitation Wizard’. Review all peaks. It is recommended to review MRM transitions in pairs. Update integration if needed by (1) highlighting the peak, (2) clicking the ‘Select Peak’ icon, and (3) clicking ‘Accept’ (Figure 6).

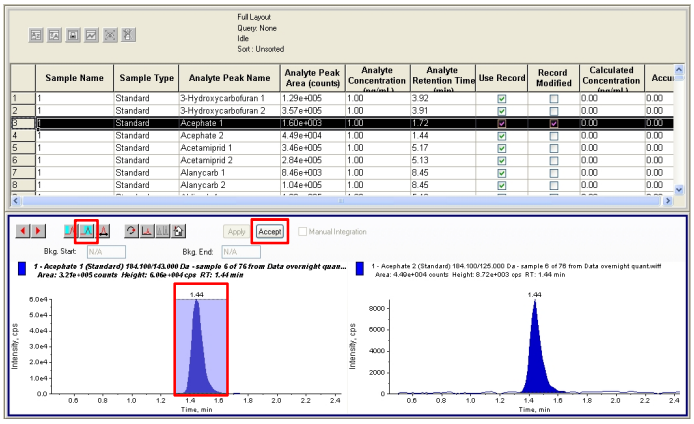


Figure 6. Review all peaks and update retention times if needed

Copy and paste the retention time column into the Excel worksheet after completing the peak review (Figure 7).

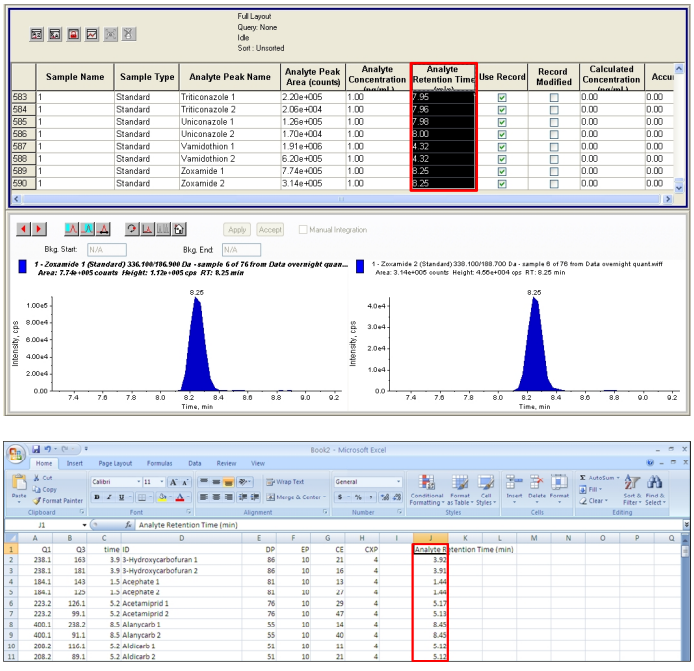


Figure 7. Copy and paste updated retention times into Excel

Plotting original and updated retention times is useful to identify possible integration errors (Figure 8).

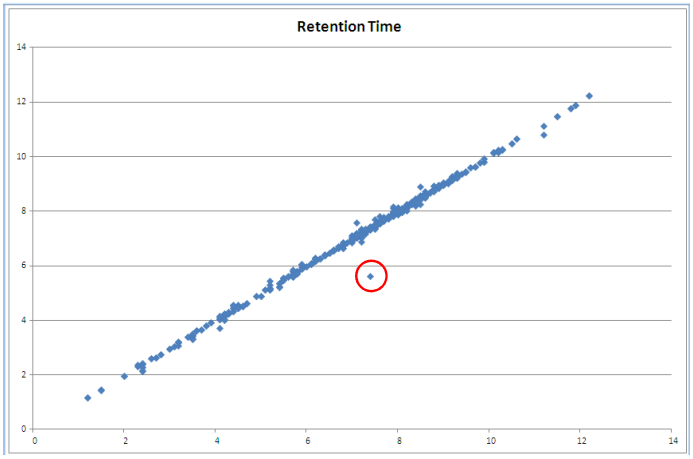


Figure 8. Chart comparing original and updated retention times to identify possible integration errors

Replace the original retention times with updated retention times in the Excel worksheet once all review is completed (Figure 9).

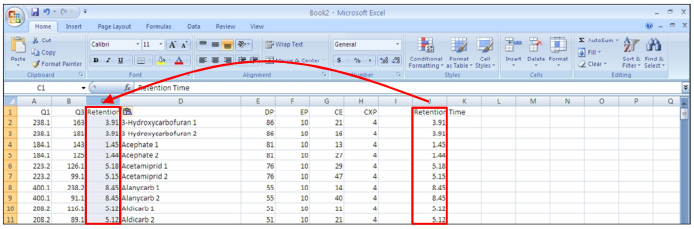


Figure 9. Replace original retention times with updated retention times

3.2. Using MultiQuant™ Software

Create a new quantitation method and result table in MultiQuant™ software. Analyte names, MRM transitions, and retention times will be populated automatically. Group names should be added for easier data review (Figure 10).

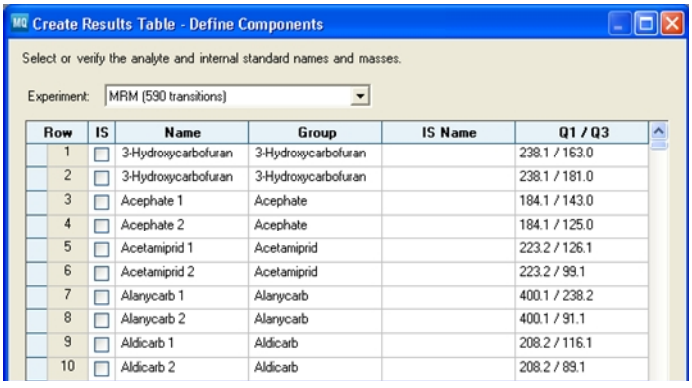


Figure 10. Build a new quantitation method

For reliable integration the following default settings are recommended: MQ4 algorithm with ‘Gaussian Smooth Width’ 1.0 points, ‘Report Largest Peak’ off, ‘Min. Peak Width’ 3 sec, ‘Min. Peak Height’ 100 cps, ‘Noise Percentage’ 80%, and ‘Peak Splitting’ 1.

Review all peaks. It is recommended to review MRM transitions in pairs. Defining group names allows to overlay MRM pairs. Update integration if needed by simply highlighting the peak (Figure 11).

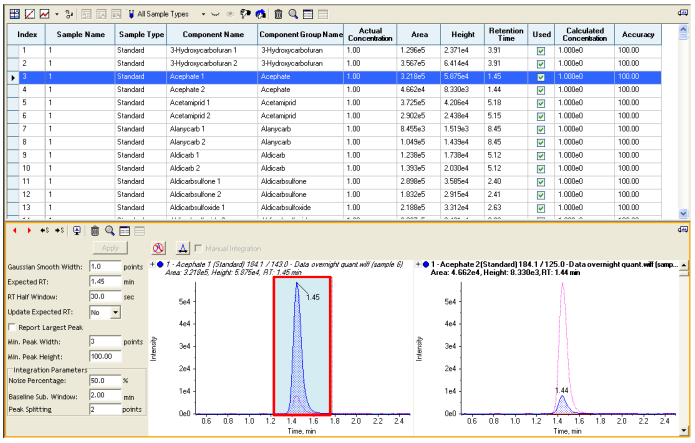


Figure 11. Review all peaks and update retention times if needed

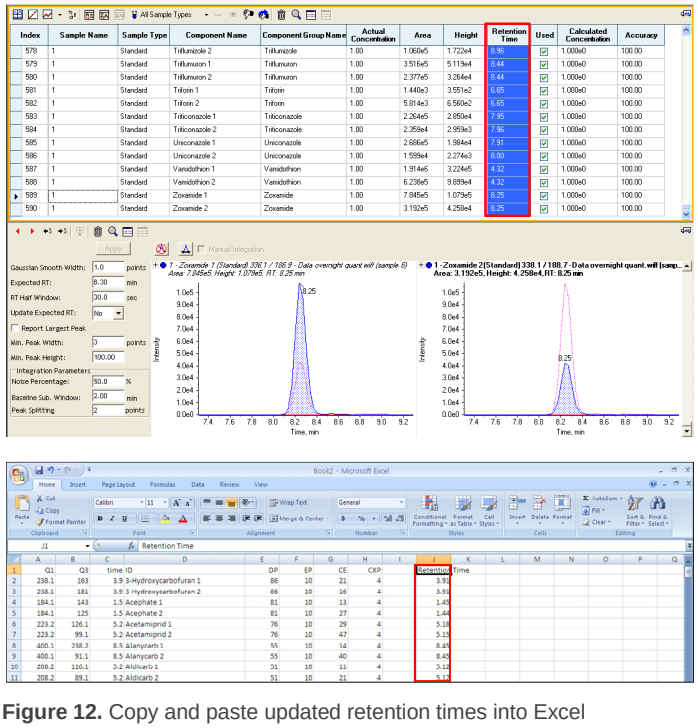


Figure 12. Copy and paste updated retention times into Excel

Copy and paste the retention time column into the Excel worksheet after completing the peak review (Figure 12).

Plotting original and updated retention times is useful to identify possible integration errors (Figure 8).

Replace the original retention times with updated retention times in the Excel worksheet once all review is completed (Figure 9).

4. Update Acquisition Method

Activate all compound dependent parameters in the acquisition method before pasting by right-click on the method table and checking all 4 parameters (Figure 12).

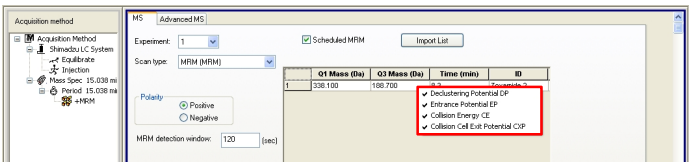


Figure 12. Activate all compound dependent parameters in the acquisition method by right-click on the table

Copy and paste the complete MRM table with updated retention times from Excel into Analyst® software (Figures 13 and 14).

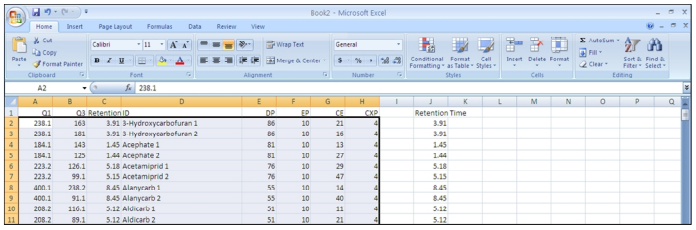


Figure 13. Copy and paste updated method table into Analyst® software

5. Adjust *Scheduled* MRM™ Parameters and Save Updated Acquisition Method

Adjust the ‘MRM detection window’ and ‘Target scan time’ depending on chromatographic peak shape (Figure 14).

The ‘MRM detection window’ is an estimate of the LC peak width and chromatographic reproducibility. It should be large enough to contain the entire LC peak plus any shifts of retention time.

The ‘Target scan time’ defines how often the chromatographic peak should be sampled. This is determined from the peak width at the base. The best accuracy and reproducibility is between 10-15 points across the LC peak.

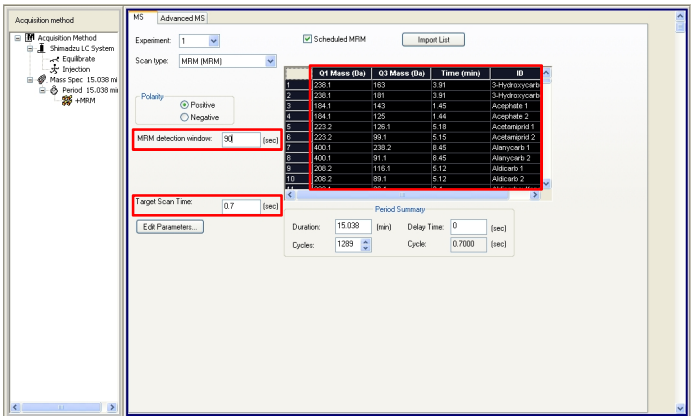


Figure 14. Adjust Scheduled MRM™ parameters in the updated acquisition method

After updating the acquisition method save the .dam file using a new name.

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