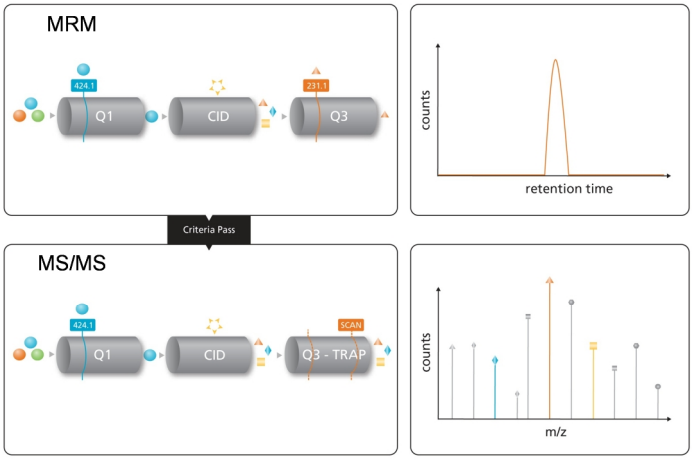
Using Your QTRAP® LC/MS/MS System at Full Potential

A Quick-Start Guide to Upgrade an MRM Acquisition Method to a Scheduled MRM Pro-IDA-MS/MS Acquisition Method in 6 Easy Steps using Analyst® Software

Overview

This document outlines the 6 easy steps to upgrade a basic Multiple Reaction Monitoring (MRM) acquisition method to utilize the *Scheduled* MRM™ Pro algorithm to improve quantitative data quality and to automatically include Enhanced MS/MS scanning to increase confidence in compound identification and confirmation.



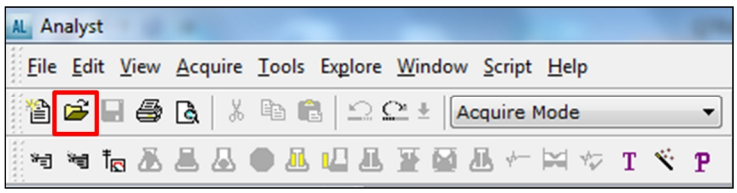
The benefits of this workflow include:

- **Improved data acquisition** by more efficient scheduling of transitions with the advanced algorithm (*Scheduled* MRM™ Pro with Flexible Window Width, Dynamic Window Extension, and MRM-triggered MRM) and automatic MS/MS acquisition using the logic provided by Information Dependent Acquisition (IDA), Dynamic Background Subtraction (DBS), and Dynamic Fill Time (DFT)
- **Improved sensitivity** – Enhanced MS/MS scans are called 'enhanced' because fragment ions are accumulated in Q3 of your QTRAP®, giving you better signal-to-noise for the detected MS/MS spectra
- **Improved selectivity** – multiple fragment ions are detected (beyond just 2 MRM transitions) meaning additional confidence in identification of positive findings
- **Improved data processing** – dual injection approach with automatic quantitation, identification using MRM ratios in MultiQuant™ software and confirmation using MS/MS library searching in MasterView™ software

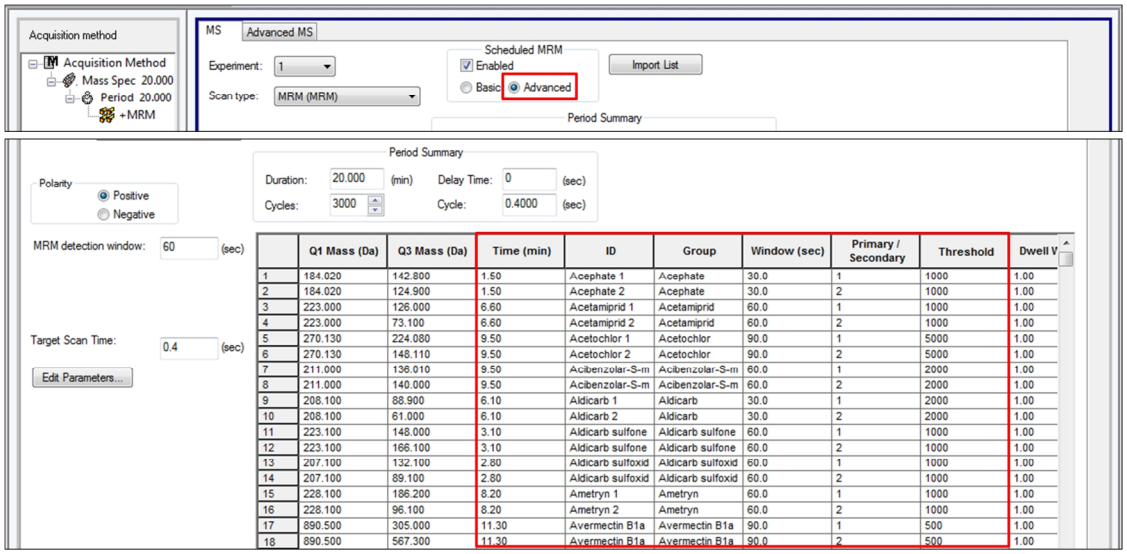
The QTRAP® Software Workflow in Analyst® Software

Open your MRM acquisition method in Analyst® software

Open any MRM method that contains your list of targeted MRM transitions.



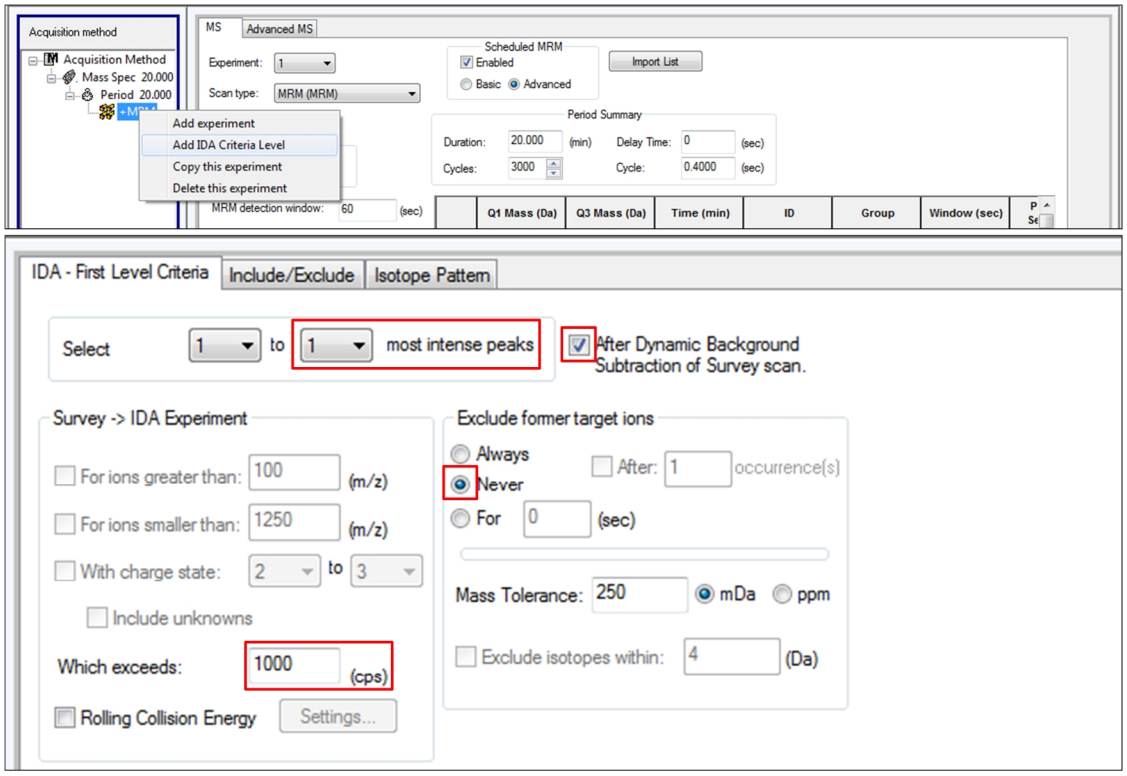
Enable *Scheduled* MRM™ Pro



- Check the ‘Scheduled MRM Advanced’ button
- Specify compound specific scheduling parameter:

1. Retention time (min)
2. Name of transition (ID) and Group
3. Detection window (sec)
4. Primary and secondary transition to define quantifier and qualifier ion in each group
5. Intensity threshold to define MRM-triggered MRM and dynamic window exclusion

Add IDA criteria



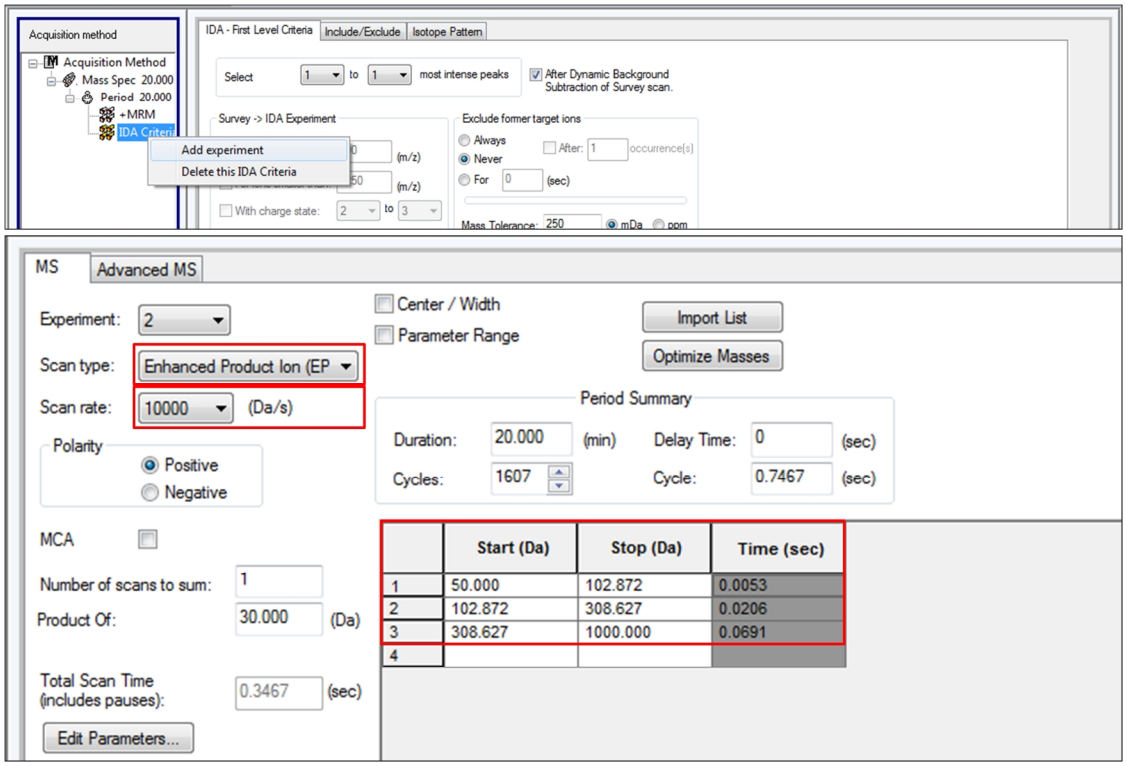
- Right click on your MRM experiment and select ‘Add IDA Criteria Level’
- Set-up the criteria by using these recommended settings:

6. Monitor most intense peak
7. Activate Dynamic Background Subtraction (DBS)
8. Set the automatic exclusion to ‘Never’
9. Set the IDA threshold to 1000 cps (depending on MRM background)

Add MS/MS experiment

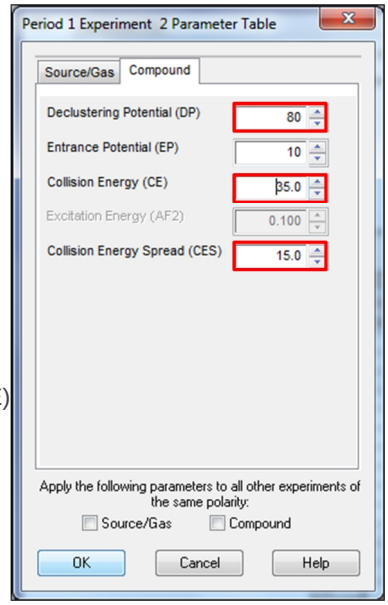
- Right click on your IDA criteria and ‘Add Experiment’
- Set-up your MS/MS scan:

1. Change scan type to ‘Enhanced Product Ion’
2. Select a scan rate of 10000 Da/s
3. Specify a mass range to cover all target compounds of your MRM list, like 50 to 1000

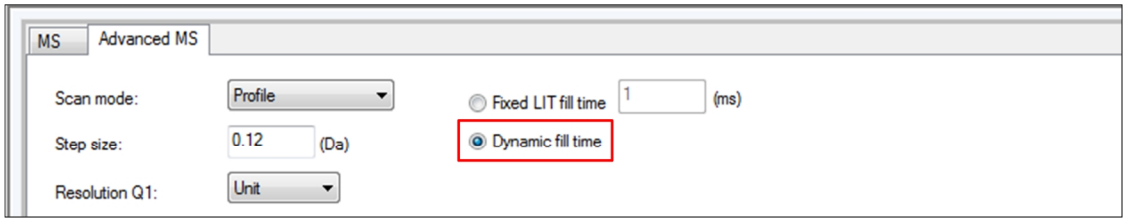


Edit MS and MS/MS parameters

- Set Declustering Potential (DP) to a value near the average DP of all compounds in your MRM list
- Set Collision Energy (CE) to 35 V and Collision Energy Spread (CES) to 15 V for best library search results



Verify that Dynamic Fill Time (DFT) is active



- Go to the 'Advanced MS' tab and verify that DFT is activated

Data Example

MS/MS library search confirms questionable MRM ratio

A grapes sample was extracted using a QuEChERS procedure, diluted 10x to minimize possible ion suppression and analyzed by LC-MS/MS using the AB SCIEX QTRAP® 6500 system. The *Scheduled* MRM™ Pro chromatogram is shown in Figure 1. A number of pesticides were identified and quantified at a concentration above 5 µg/kg.

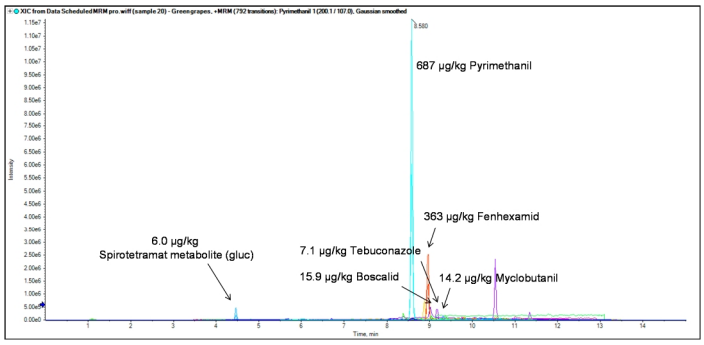


Figure 1. *Scheduled* MRM™ Pro chromatogram of a grapes sample

Identification was based on the MRM ratio calculated in MultiQuant™ software. The example presented in Figure 2 shows the results for Pyrimethanil. It can be seen in the Peak Review window that the MRM ratio is outside the 20% tolerance.

We performed confirmatory analysis of a second sample extract using the MRM-triggered MS/MS method. Data were processed in MasterView™ software. Figure 3 shows the excellent MS/MS library match with a FIT 99.5% confirming the presence of Pyrimethanil.

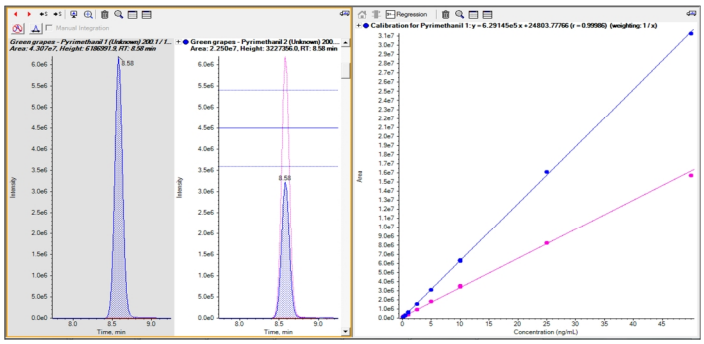


Figure 2. Data review for Pyrimethanil in grapes, quantifier and qualifier ion (left) with the MRM ratio outside the tolerance levels, and calibration line (right)

Figure. MS/MS library searching results for Pyrimethanil in grapes, the FIT of 99.5% confirms the presence of the detected pesticide

For additional support on implementing this workflow in your own lab, or for support on other AB SCIEX products, visit our website or email us at support@absciex.com.

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