Triggered MRM: Simultaneous Quantitation and Confirmation Using Agilent Triple Quadrupole LC/MS Systems

Technical Overview

Triggered MRM (tMRM) acquisition is an analytical method which is available for all Agilent Triple Quadrupole LC/MS systems. tMRM acquisition combines MRM with the generation of a product ion spectrum which can then be used for library identification and confirmation. As a result, tMRM analysis decreases analysis time, increases throughput, and allows for fast, sensitive, quantitative and qualitative analysis on a single instrument, in a single analytical run.

How tMRM Acquisition Works

In tMRM analysis, up to 10 MRM transitions (primary and secondary) are defined for each target analyte in the method. The primary transitions are acquired for all analytes, but when the signal of one of the primary transitions exceeds a user-defined threshold, the secondary transitions are triggered and acquired for a specified number of scans (Figure 1).

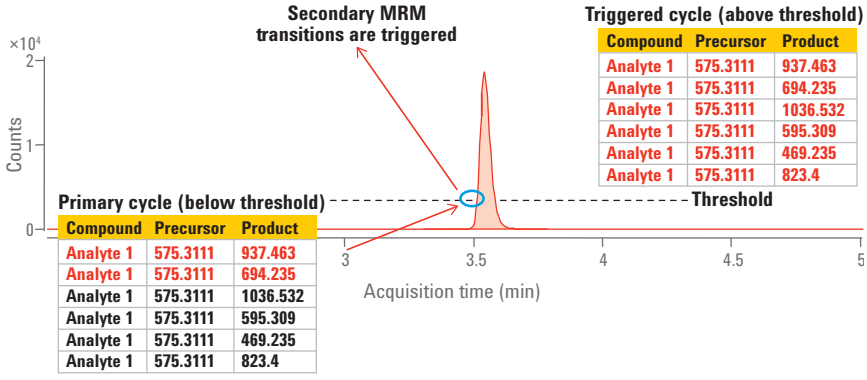

Figure 1. A tMRM experiment with two primary transitions for each analyte. Secondary MRM transitions are triggered when the primary MRM signals cross a user-defined threshold.

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tMRM Library Searching

Using tMRM, the secondary MRM spectra acquired during tMRM experiments are combined with the primary MRM transition spectra for a particular analyte to generate a product ion spectrum. Figure 2 shows nine MRM transitions (two primary and seven secondary transitions) which are combined to make one tMRM product ion spectrum. This product ion spectrum is then used for library search and identification of the compound of interest.

With up to 10 product ions present in each tMRM product ion spectrum for each target analyte, users can confidently confirm target analytes with a library search. The main advantage of using tMRM to generate these product ion spectra, rather than conventional product ion scanning techniques, is that acquiring all 10 MRM transitions takes less than 50 ms, while a typical product ion scan on an ion trap, or triple quadrupole instrument takes approximately 200 ms. This fast cycle time means that more data points are acquired across each peak, and that the quantitative data acquired is more sensitive, robust, and accurate.

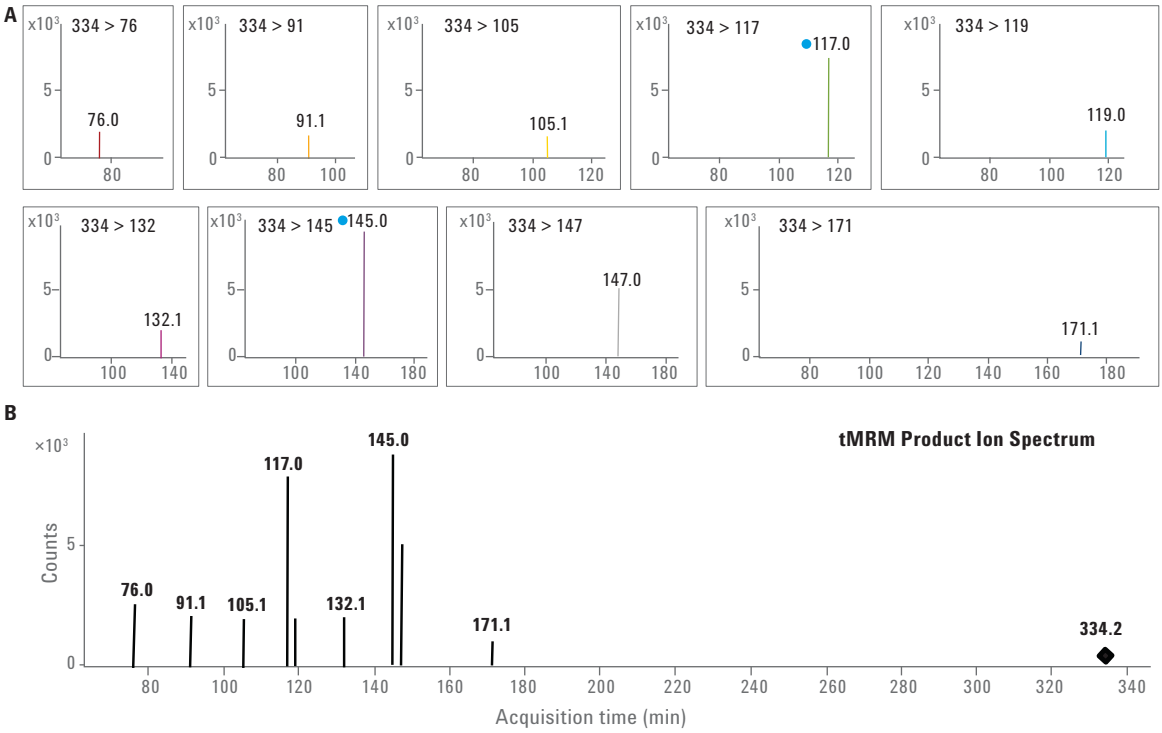


Figure 2. tMRM product ion spectra are generated by combining the primary (●) and secondary MRM spectra for a target analyte **A**. The product ion spectrum **B** may be used for library search and analyte confirmation.

New Enhanced Triggering Functions

The Agilent Mass Hunter B.06 Acquisition for Agilent 6400 Series Triple Quadrupole LC/MS systems adds triggering functions that increase specificity and tMRM data quality:

- **Entrance delay**
  If the signal for the designated primary MRM transitions cross the triggering threshold, the entrance delay postpones triggering for a user-defined number of cycles, moving the acquisition of secondary MRM transitions closer to the apex of the peak.
- **Trigger delay**
  Once the triggering threshold is met, the trigger delay defines the number of cycles to skip between triggers, spreading the acquisition of secondary MRM transitions across a peak. Trigger delay can be combined with the entrance delay function.
- **Trigger window**
  The trigger window confines the activation of all triggering functions to a user-defined window around the expected retention time for a particular peak. This function increases triggering specificity based on the target compounds and known retention times for a particular tMRM method.

Figure 3 shows data acquired using the entrance delay triggering function. Panel **A** shows an entrance delay of 0 scan cycles and Panel **B** shows an entrance delay of three scan cycles. In this example, the entrance delay moves the acquisition of secondary spectra closer to the apex of the peak.

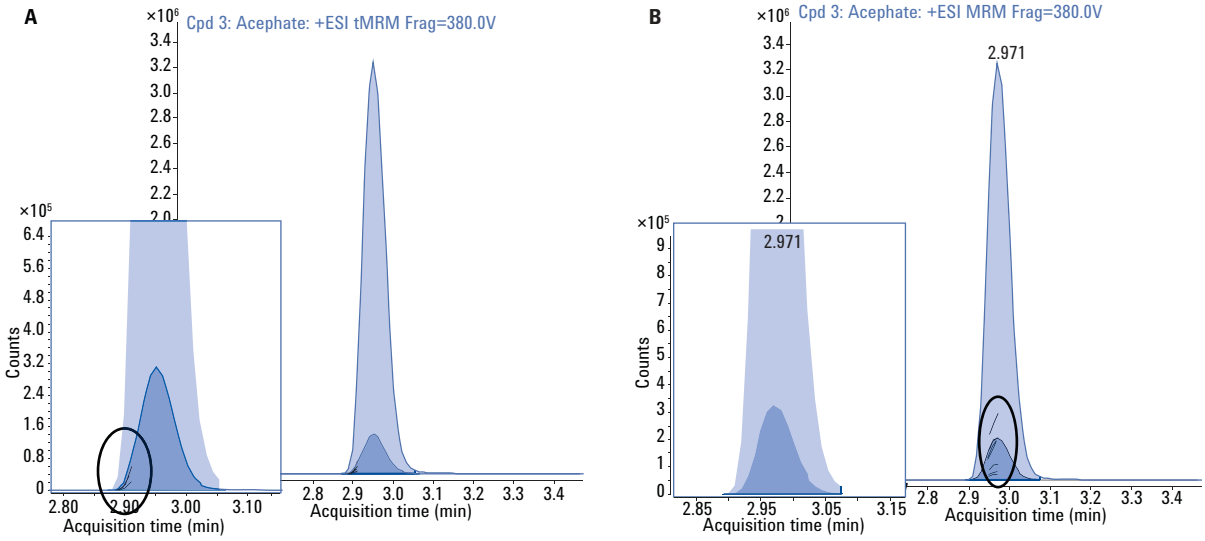


Figure 3. Data acquired for acephate using the entrance delay triggering function.

Avoiding False Positives with tMRM Acquisition

Tebufenpyrad is a toxic insecticide which can be present in ginger. However, there is an endogenous compound found in ginger which co-elutes with tebufenpyrad and shares the same two primary MRM transitions with a similar qualifier ion ratio. In a typical triple quadrupole analysis, without tMRM analysis, a ginger extract could easily give a false positive result for tebufenpyrad. However, the product ion spectra generated by tMRM analysis allow differentiation of the endogenous ginger compound from the tebufenpyrad contaminant. In order to acquire the same quantitative and qualitative data without tMRM acquisition, one would have to first analyze the extract using a fast scanning triple quadrupole, and then reanalyze the sample using an ion trap or a time-of-flight (TOF) instrument for qualitative analysis.

Figure 4 shows a comparison of the tebufenpyrad tMRM library spectrum and the endogenous compound spectrum found in the ginger extract. The peaks circled in the tebufenpyrad spectrum differ significantly or are absent from the endogenous ginger compound. In this case, a false positive result was avoided due to the qualitative data generated by tMRM acquisition.

Rapid Quantitative Analysis with tMRM Acquisition

The tMRM product ion spectrum for tebufenpyrad was generated while quantitation was performed, without sacrificing the quality of either result. Triggering additional MRM transitions with a primary MRM threshold means that the tMRM cycle time is always as short as possible, maximizing the number of data points acquired across each peak. When the number of data points acquired across an analytical peak is maximized, the sensitivity and accuracy of the quantitative analysis is optimal. Quantitation using tMRM is also compatible with dynamic MRM[1] (Stone, 2009). This is ideal for confirmation and identification of large numbers of analytes, such as those encountered in pesticide residue screening. In addition, the tMRM method allows each MRM to be acquired at optimal collision energy and hence maximizes sensitivity.

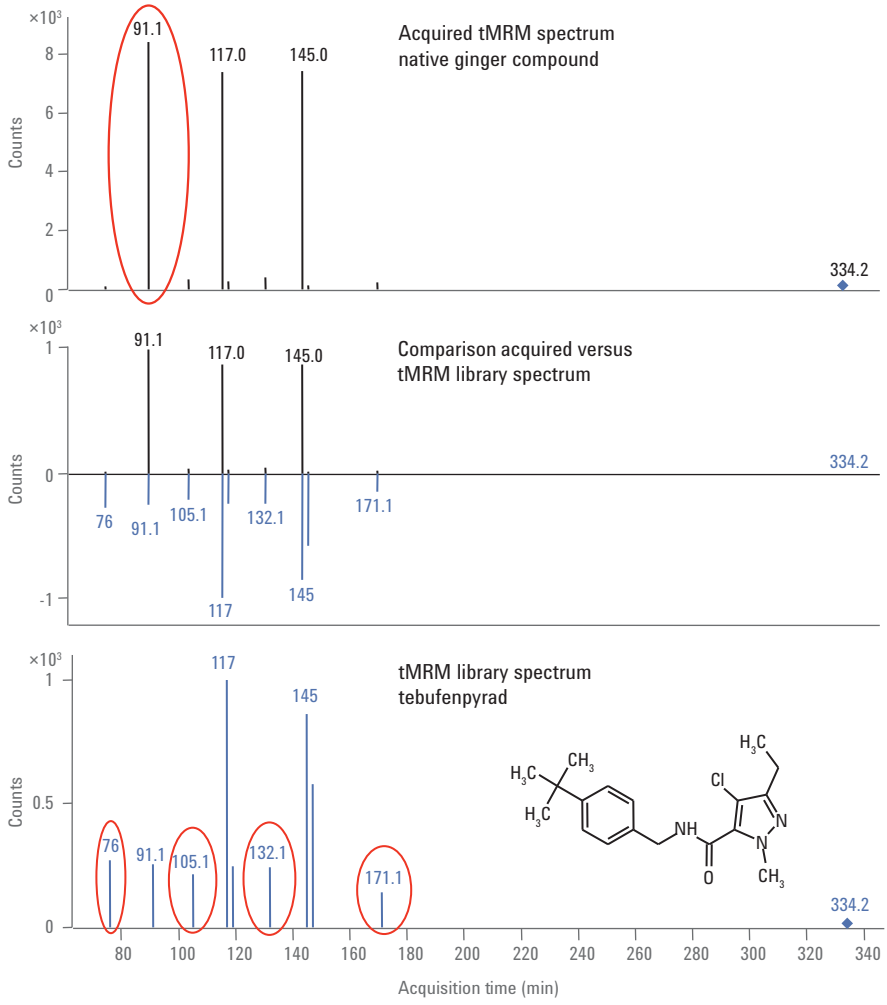
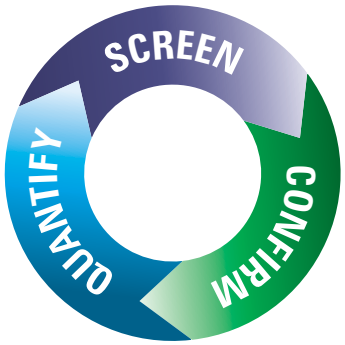


Figure 4. The comparison of an acquired tMRM spectrum for an endogenous ginger compound compared with the authentic library tMRM spectrum for tebufenpyrad.

Conclusions

tMRM is a data dependent scan function which increases throughput, provides both quantitative and qualitative information, and minimizes the cost of analyses. Standard product ion scanning techniques require approximately 200 ms per transition, at least four times longer than a tMRM cycle. The longer cycle time for a typical product ion scan means that fewer data points are acquired across each analytical peak, compromising sensitivity and robustness for qualitative and quantitative results. In addition, the LC methods for instruments with longer product ion scan cycle times are often lengthened to minimize analyte co-elution. With tMRM, high throughput LC methods with narrow peak widths can be easily adopted on a triple quadrupole LC/MS instrument, enabling quantitative analysis and qualitative confirmation on a single instrument, in a single injection.



Reference

1. New Dynamic MRM Mode
Improves Data Quality and Triple Quad
Quantification in Complex Analyses.
Agilent publication 5990-3595EN.

www.agilent.com/chem/QQQ

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