

# Simultaneous Quantitation and Confirmation of Peptides with Triggered MRM Acquisition

## Application Note

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### Introduction

Assays that are both specific and quantitative for target proteins are critical in many application areas, such as preclinical validation of putative biomarkers. These assays are typically multiplexed, multiple reaction monitoring (MRM) analyses where high-throughput is required. Since many biological samples are highly complex, it is important to also confirm identification of the peak and monitor for any underlying impurities which might impact quantitative results.

Triggered Multiple Reaction Monitoring (tMRM) acquisition, which is available on all Agilent Triple Quadrupole LC/MS systems, combines dynamic MRM for quantitative analysis with the generation of MRM-based product ion spectra for library identification and confirmation. The tMRM method decreases analysis time, increases throughput, and allows for quantitative and qualitative analysis on a single instrument, in a single analytical run.



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## Using the tMRM Method

For LC/MS triple quadrupole analysis, identification of target peptides typically includes retention time,  $m/z$  value, and abundance data for two or more transitions. Expanding the number of transitions monitored per peptide will improve the confidence of the identification, especially in a complex matrix. However, increasing the number of MRM transitions for each peptide results in shorter dwell times or an increased cycle time for each MRM scan, making it difficult to achieve the desired sensitivity and peak sampling.

tMRM offers an alternative mode, where the triple quadrupole only monitors the primary MRM transitions used for quantification in the specified retention time window. Detection of the primary transition for the targeted peptide triggers several cycles that include the additional secondary MRM transitions. The combination of all the transitions for a given peptide can be used to create an MRM-based product ion spectrum to compare against a user-created reference spectrum for additional confirmation of peptide identification. Each target

peptide is allowed a total of 10 MRM transitions in tMRM mode and these 10 transitions can be any combination of primary and secondary types. This tMRM acquisition mode maximizes the dwell time for all possible target peptides in the primary MRM quantitation phase, and also acquires sufficient MRM data to create a product ion spectrum. By allowing optimized collision energy for each product ion and maximizing dwell times, tMRM is significantly more sensitive than conventional product ion scanning.

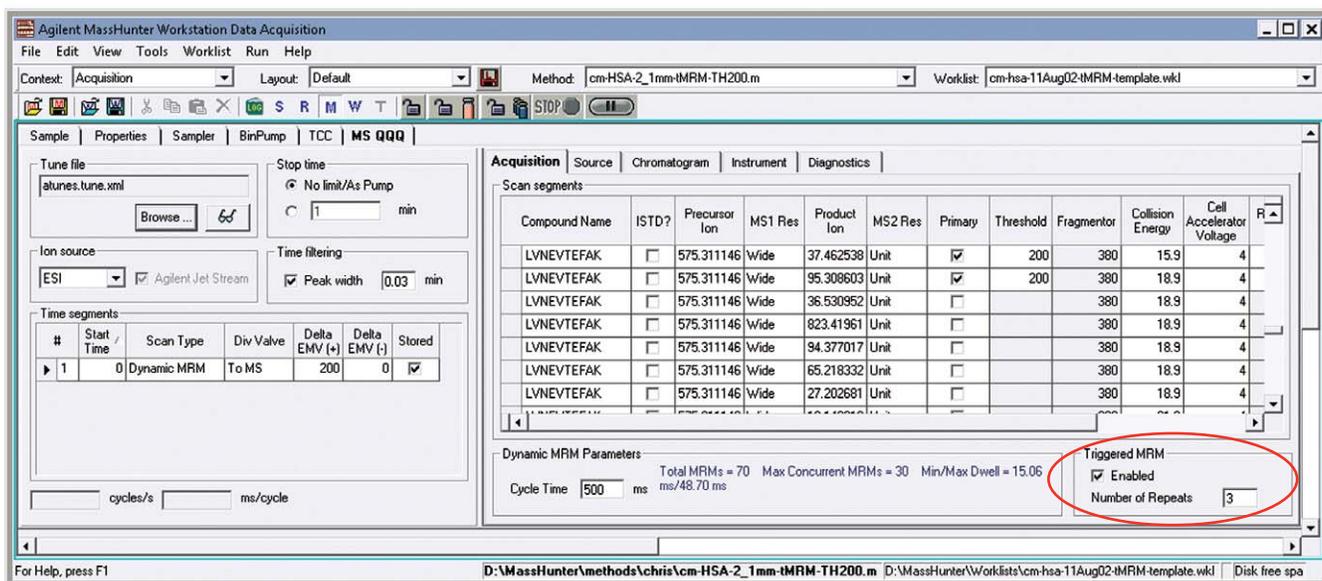


Figure 1. Agilent MassHunter acquisition software allows a dynamic MRM method to be easily changed to a tMRM method by selecting the **Triggered MRM Enabled** box and specifying the number of repeats. In this example, two primary transitions were selected and eight additional transitions are used as triggered MRM's for confirmation.

## Results and Discussion

In the experiment shown here, a mixture of seven synthetic peptides (p/n: G2455-85001) was analyzed in a simple matrix composed of a trypsinized bovine serum albumin (BSA) standard (p/n: G1900-85000) at 10 fmol/ $\mu$ L on a 6490 triple quadrupole LC/MS system. The tMRM method used two primary transitions and eight secondary transitions per peptide. The reference spectra were generated from a high-level standard during method creation in Agilent

MassHunter Quantitative Analysis software. The most abundant (or user specified) primary transition is used for quantitation and the other primary transition is used as a qualifier, where changes in the ratio of response between quantifier and qualifier transitions can be used to flag potential interferences in samples (Figure 2A). The combined transitions are used to create the product ion spectrum, which is then automatically compared against the reference spectrum to generate a product ion spectrum match score (Figure 2B).

## Conclusions

tMRM acquisition is a data-dependent mode capable of providing quantitative and qualitative data on a single instrument, in a single injection, while achieving outstanding quantitative sensitivity and accuracy. It can be used to distinguish target peptides from nearby or co-eluting peptides, and to avert false positives with the inclusion of qualitative analysis with library matching.

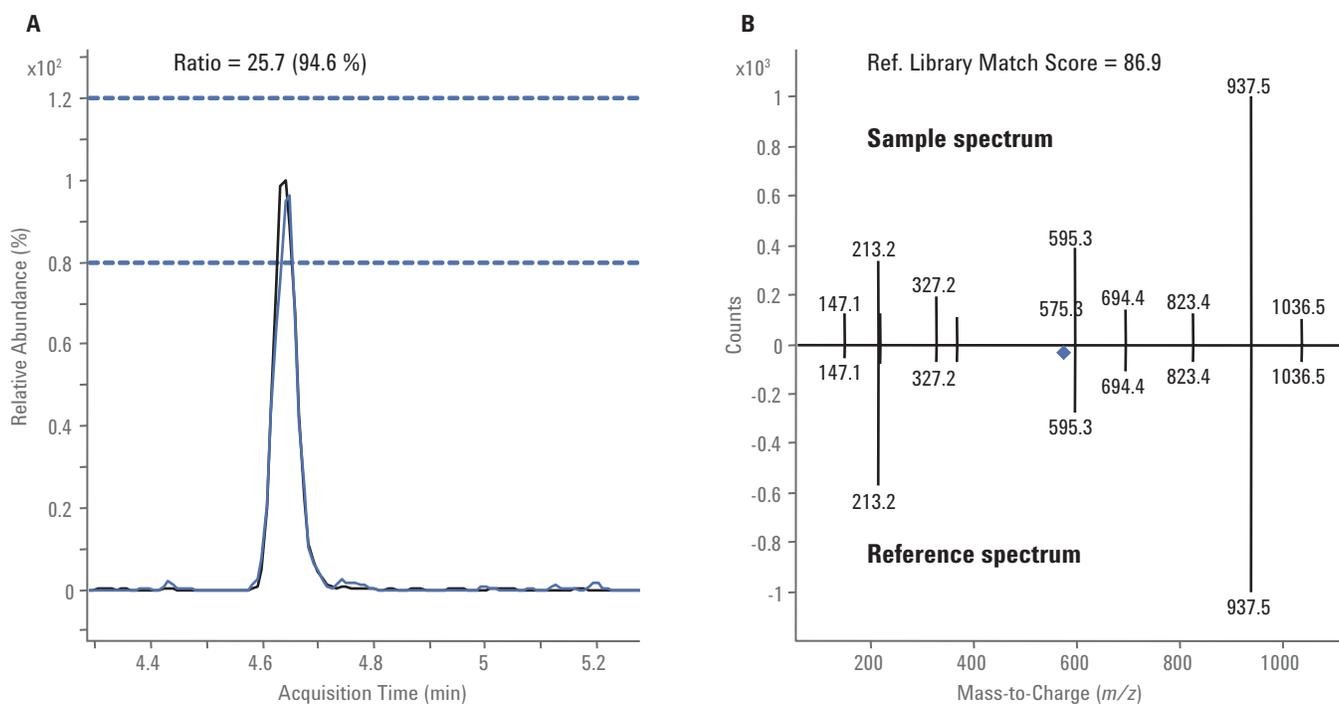


Figure 2. The results for one peptide, LVNEVTEFAK, showing the qualifier to quantifier ratio for the primary transitions (A) and a comparison of the reference spectrum to the product ion spectrum (B).

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