

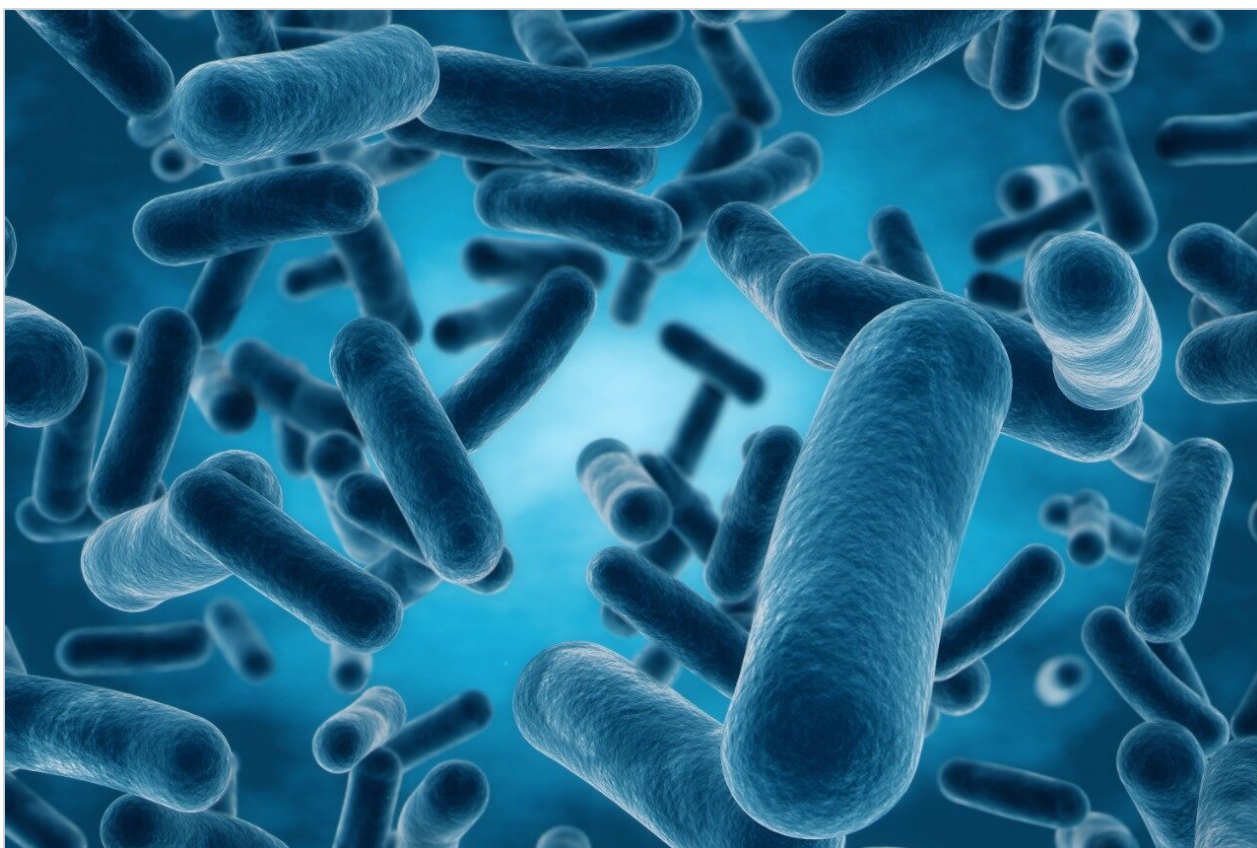


# High Definition Multiple Reaction Monitoring: The Application of Quadrupole Ion Mobility Time-of-Flight Mass Spectrometry for Targeted Proteomics Studies

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## Abstract

This application note demonstrates the benefits SYNAPT G2-Si System for the quantification of proteins and peptides in a new High Definition MRM (HD-MRM) mode.

\*\*\*nanoACQUITY UPLC applications readily transfer to the ACQUITY UPLC M-Class System\*\*\*

## Benefits

- Precise peptide quantification
- Lower limits of detection and quantification
- Complete product ion coverage without duty cycle losses
- Simplified method development

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## Introduction

Recently, targeted LC-MS-based methods have been applied to quantitative proteomics, facilitating highly accurate and multiplexed relative and absolute quantification studies. Both triple quadrupole and high-resolution mass spectrometers have been used. In both instances, the protein is not quantified directly but one or more signature peptides that are released in stoichiometric amounts during enzymatic digestion are monitored as surrogates.

To achieve absolute quantification, typically, an accurately determined amount of a standard stable isotope-labeled peptide is added to the sample. The key benefits of triple quadrupole-based SRM/MRM assays are sensitivity, acquisition speed, and linear dynamic range.

However, the specificity of SRM/MRM peptide quantification has been questioned,<sup>1,2</sup> prompting the use of high-resolution accurate mass instruments for peptide quantification. Here, the benefits of the SYNAPT G2-Si System are demonstrated for the quantification of proteins and peptides in a new High Definition MRM (HD-MRM) mode, where T-Wave ion mobility separations are used with MS/MS mode on a quadrupole time-of-flight mass spectrometer, to provide a highly sensitive and selective quantitative assay with maximum response for all product ions of a molecular peptide ion in a single acquisition.

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## Experimental

The cytosolic content of *E.coli*, bovine serum albumin (BSA), and alcohol dehydrogenase (ADH) were digested using trypsin. The digest mixtures/lysates were injected onto a nanoACQUITY UPLC System equipped with an ACQUITY UPLC BEH 1.7  $\mu\text{m}$ , 15 cm x 75  $\mu\text{m}$  column coupled to a SYNAPT G2-Si Mass Spectrometer. The BSA on-column amounts ranged from one attomole to one femtomole on-column. Data were processed with TargetLynx and/or Skyline.<sup>3</sup>

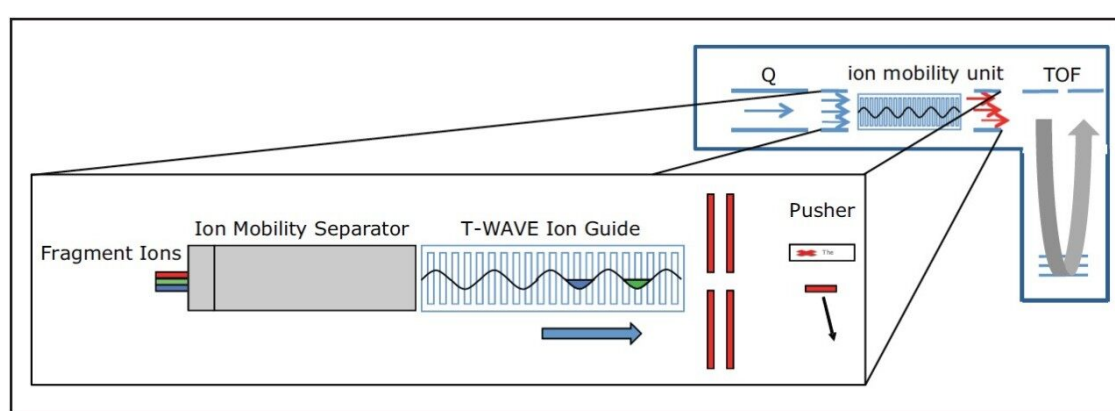
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## Results and Discussion

The High Definition Multiple Reaction Monitoring (HD-MRM) acquisition method of the SYNAPT G2-Si High

Definition MS System provides sensitivity or selectivity benefits for targeted quantification. In this study, we describe the sensitivity and coverage benefits provided by the use of a Wideband Enhancement acquisition mode on SYNAPT G2-Si. Wideband Enhancement uses ion mobility separations to provide maximum duty cycle for all product ions in a single MS/MS acquisition.

Maximum transmission, as shown in Figure 1, is achieved by the synchronization of the pusher region of the time-of-flight analyzer with target  $m/z$  ranges as ion mobility separated ions are released from the Triwave device. The signal intensity of product ions can be increased from five- to ten-fold. Hence, this provides very low limits of detection and quantitation for all potential product ions from a target peptide molecular ion without loss in duty cycle.



*Figure 1. Experiment principle High Definition Multi Reaction Monitoring acquisition. The product ions for a given charge state are separated and focused by ion mobility and the pusher duty cycle synchronized to the mobility (drift) times of the product ions.*

Acquisitions can be conducted in either scheduled<sup>1</sup> or non-scheduled mode, with the former providing the ability to measure the transitions of several hundred components during the course of a single LC-MS experiment. Collision energies can be in silico predicted or derived from previous discovery experiments. Data can be processed and quantified with either TargetLynx or Skyline.

The benefit of Wideband Enhancement is demonstrated in Figure 2. Here, [Glu<sup>1</sup>]-Fibrinopeptide B solution was infused at a constant flow rate. Data were acquired by normal CID MS/MS and targeted HD-MRM. In both cases, the same number of ToF integrations were combined. For this particular experiment, >10 fold signal increase was observed. As shown in Figure 3, for example BSA and ADH peptides, summation of several product ions gives rise to increased sensitivity.

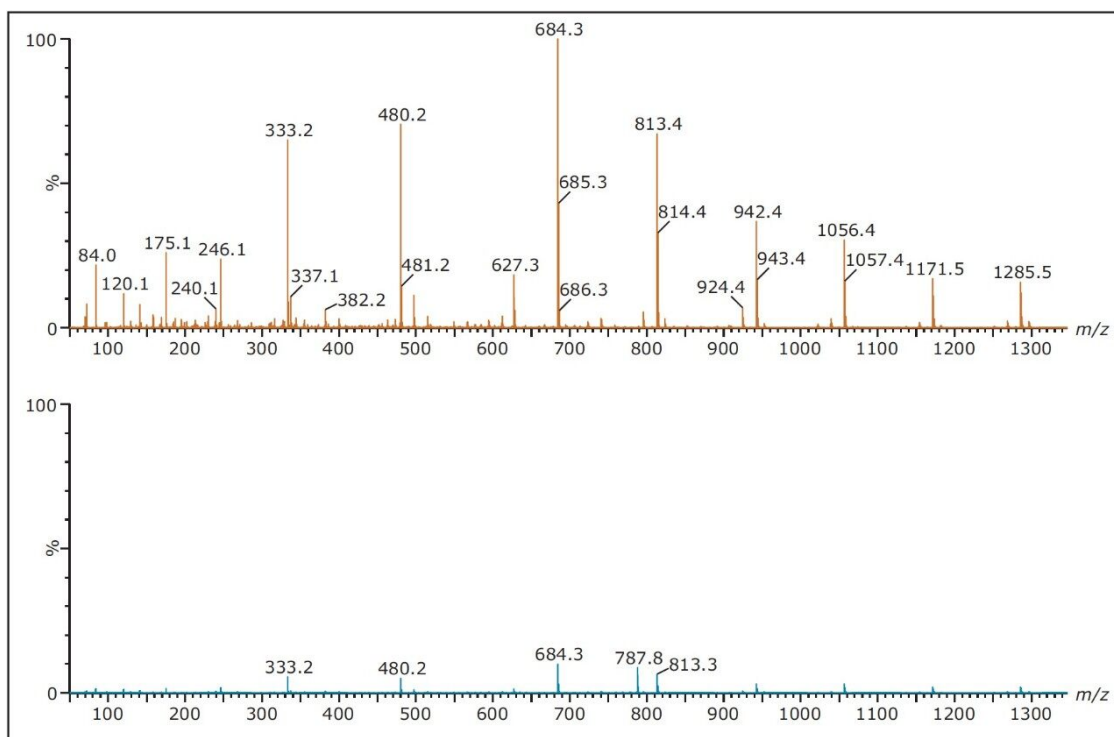
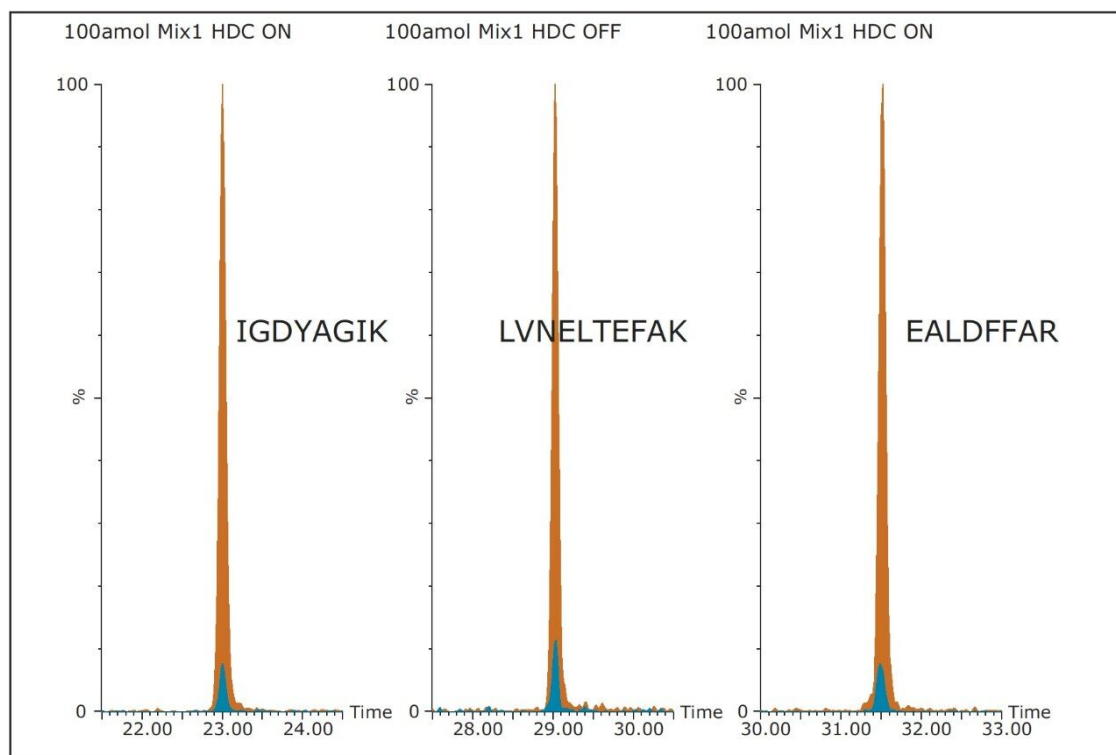


Figure 2. Product ion spectrum of [Glu<sup>1</sup>]-Fibrinopeptide B infused at 0.5  $\mu$ L/min and integrated over 30 seconds with and without ion mobility synchronization of an ion push for the singly charged product ions across the complete MS/MS acquisition range, improving signal-to-noise at an approximate minimum of ten-fold.



*Figure 3. Summed peptide product ion mass extracted chromatogram acquired without Wideband Enhancement (blue) and with Wideband Enhancement (brown).*

Further benefits are demonstrated by the on-line analysis of selected peptides from a tryptic digest of bovine serum albumin. The peptides were separated by nanoscale LC-MS and quantified by targeted HD-MRM. The results, shown in Figure 4, illustrate the quantification of one of the peptides over at least four orders of dynamic range, and the integration of the signal response by Skyline of three product ions/pseudo transitions.

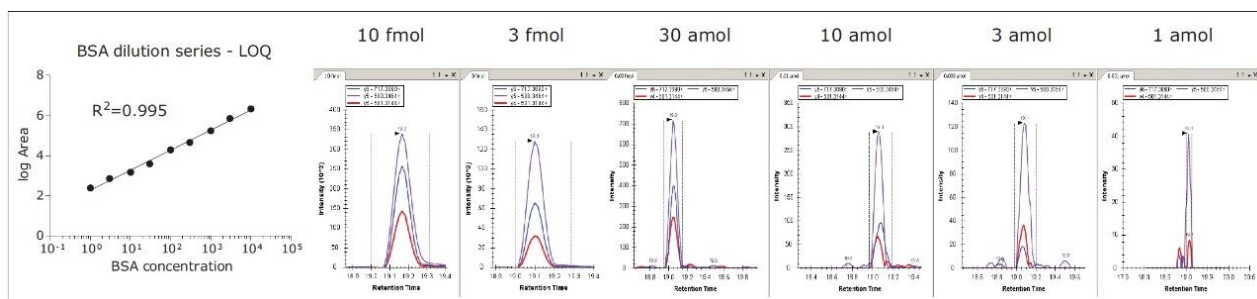


Figure 4. Integration of the area under the curve of a single transition from 10 fmol to 1 amol for CCTESLVNR from a bovine serum albumin digest on column (left), and MRM chromatograms of three high resolution product ions of the specific peptide (right).

## Conclusion

- The HD-MRM workflow provides a unique method, whereby low limits of detection and quantification can be realized for all products of a target compound/peptide from a single MS/MS acquisition.
- HD-MRM acquisitions also enable quantification of specific product ions following acquisition rather than defining the specific target ions prior to acquisition.
- The workflow can also utilize HD RADAR Technology, which provides the ability to simultaneously acquire full scan ( $m/z$  versus dt) data across the chromatographic separation, and assists method development through better understanding of potential matrix effects.

## References

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