Waters™

Note d'application

Targeted High Resolution Quantification with Tof-MRM and HD-MRM

Nick Tomczyk, Alistair Wallace, Keith Richardson, Arkadiusz Grzyb, Jason Wildgoose

Waters Corporation

This is an Application Brief and does not contain a detailed Experimental section.

Abstract

This technology brief enables routine quantification at very low limits while providing the sensitivity and selectivity benefits of high resolution MS and T-Wave ion mobility separations using the SYNAPT G2-S*i* MS acquisition modes, HD-MRM and Tof-MRM.

Benefits

Tof-MRM and HD-MRM workflows enable users to carry out routine targeted quantification with more selectivity, sensitivity, and confidence than ever.

Introduction

High resolution mass spectrometers (HR-MS) combined with liquid chromatography (LC) systems are commonly

used to acquire information rich qualitative data in fields as diverse as metabolite I.D., proteomics, metabonomics, biomarker discovery, biopharmaceutical characterization, polymer analysis, and petroleum analysis. Interest in the quantitative information that can be obtained during these mainly qualitative experiments has increased. Quan/Qual workflows, such as the Waters accurate mass screening platform solution based on MS^E, have been successful in extending the capabilities of HR-MS; however, the majority of dedicated quantification is still performed on tandem quadrupole MS systems. These are typically used as they provide an ideal combination of high sensitivity, selectivity, robustness, reproducibility, and affordability for many routine quantitative assays.

More recently, interest in targeted quantification on HR-MS has increased for a number of practical reasons. The ability to perform both qualitative and quantitative analyses on the same platform extends the versatility of an analytical instrument. Often, complex LC methods and other inlet options can be problematic to migrate due to shifts in RT and performance which then require re-optimization, consequently costing time and effort. In instances like these, the ability to perform the quantification in situ is extremely valuable. In addition to this, HR-MS may provide greater confidence in results by affording the scientist extra selectivity in the form of accurate mass, isotope ratios, and ion mobility separations.

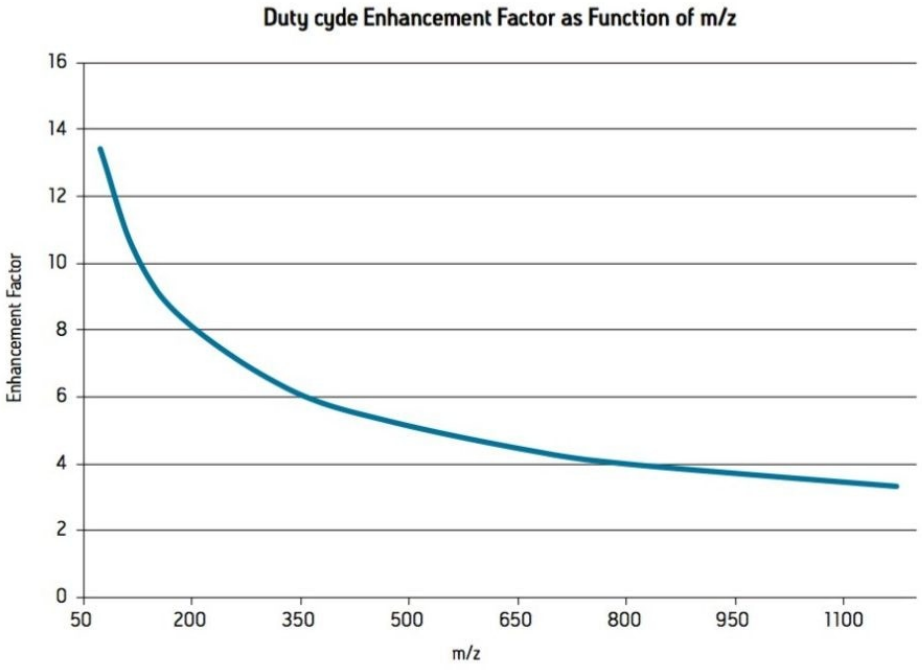

Figure 1. Expected duty cycle enhancement factor (and therefore sensitivity) as a function of m/z.

Results and Discussion

The new MRM and HD-MRM methods make use of the unique SYNAPT G2-S*i* ion optics to deliver very low limits of detection and quantification (with 100% detection duty cycle for transitions of interest). The new methods incorporate RADAR (*m/z*, rt, int) and HD RADAR (*m/z*,rt, dt, int), respectively, providing full scan accurate mass information at regular time points during a chromatographic run to assist in method development and the evaluation of matrix effects. The MRM method editor ensures simplified method set up and TargetLynx Software provides automated data processing and review.

Due to the nature of targeted quantification, it is essential to maximize analytical duty cycle to achieve the best possible sensitivity for specific ions. Maximum transmission can be achieved by the synchronization of the pusher region (on the time-of-flight analyzer) with target *m/z* ranges as ions are released from the Triwave device. There are two such modes that can provide in excess of 10x sensitivity improvement. 'Target Enhancement' provides duty cycle enhancement for narrow *m/z* ranges (optimized collision energy per single transition), and Wideband Enhancement uses ion mobility separations to provide full duty cycle for a full *m/z* range (using common collision energy conditions for all transitions). The theoretical enhancement is m/z-dependent, as shown in Figure 1.

MRM acquisitions on tandem quadrupole instruments work by mass selecting a precursor ion in Q1 fragmenting to a known target ion in a collision cell, mass selecting for this ion in Q2 and recording the ion current on a detector. The Tof-MRM and HD-MRM acquisition modes on the SYNAPT G2-S*i* work in a similar fashion. The chosen parent ion is selected by the quadrupole, and fragmented in the collision cell to a known target ion. The enhanced duty cycle mode is then used to preferentially monitor the target ion, based on an EIC for this fragment. Like tandem quadrupole MRM, this method greatly increases selectivity by pushing LODs lower than ever. Table 1 gives an overview of commonly used quantification in addition to Tof-MRM and HD-MRM.

MS type	Experiment	MS1	Collision cell	MS2	Quantitation trace	Selectivity points
Tandem Quad	MRM	Precursor selection	CID	Target selection	TIC	RT, Transition
SYNAPT G2-S*i*	TOF MS	→	→	AccMass	EIC	RT, AccMass
SYNAPT G2-S*i*	TOF MRM	Precursor selection	CID	Target Enhanced AccMass	EIC	RT, Transition, AccMass
SYNAPT G2-S*i*	HD-MRM 1	Precursor selection	CID^{trap} → IMS	All frag Enhanced AccMass	EIC	RT, Transition, AccMass
SYNAPT G2-S*i*	HD-MRM 2	Precursor selection	IMS → CID^{trans}	Target Enhanced AccMass	EIC	RT, DT, Transition, AccMass

Table 1. Overview of quantitation strategies available, adding Tof-MRM and HD-MRM.

A comparison of two injections of 1.25 pg of testosterone in solvent on an ACQUITY UPLC 2.1 x 100 mm Column is shown in Figure 2. The traces are extracted ion chromatogram (EIC - 289.217 *m/z*) from a full scan MS (upper trace) and EIC of the transition 289.217 > 97.065 from an MRM experiment (lower trace). In the MRM experiment, the testosterone derived peak at 1.75 minutes is significantly cleaner with signal-to-noise increased more than four times.

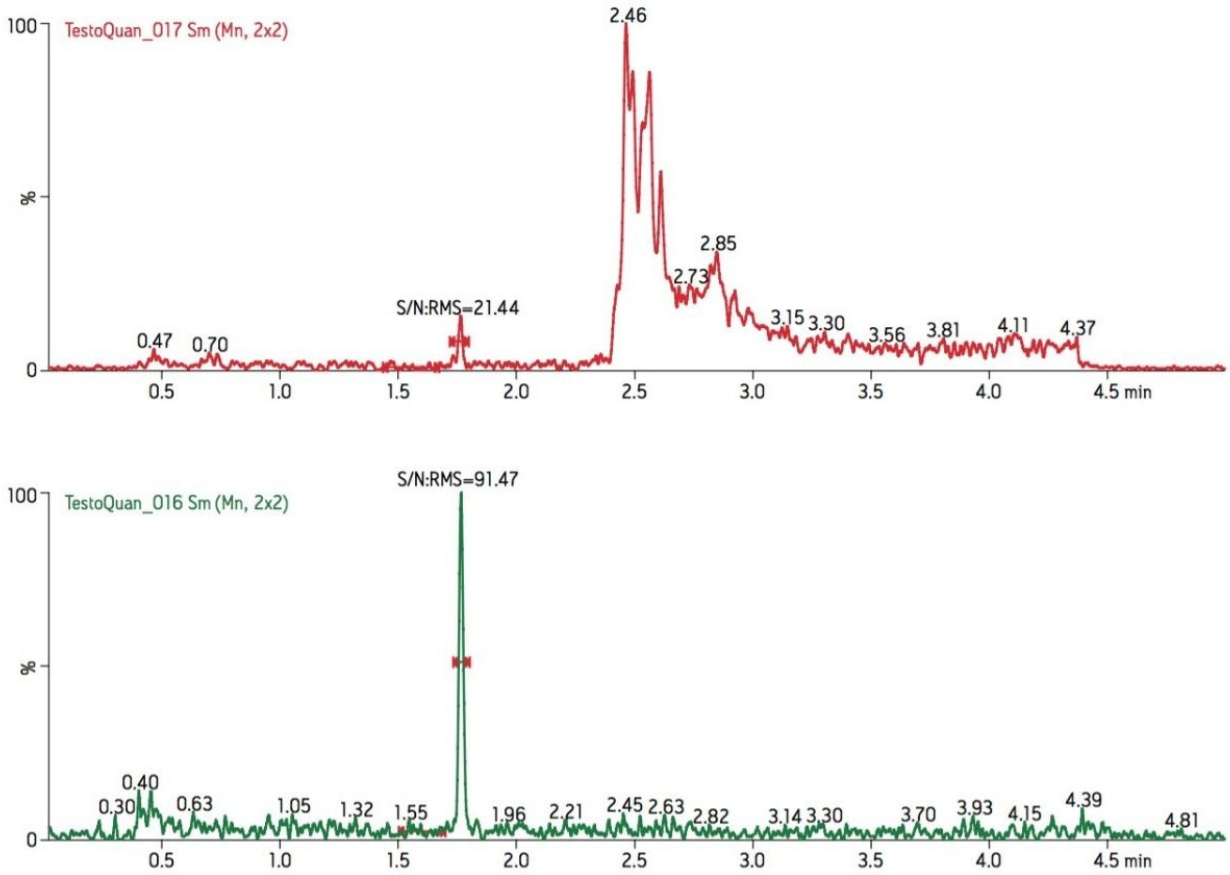

Figure 2. 1.25 pg of testosterone in solvent on column (ACQUITY UPLC 2.1 x 100 mm Column). Lower trace shows EIC of the transition 289.217 > 97.065 from an MRM experiment. Upper trace shows EIC of parent mass 289.217 from an MS experiment.

Figure 3 shows a similar comparison, however only 250 fg of testosterone in solvent on column. In this specific experiment, this was found to be the limit of detection for the MRM experiment. The peak, however, is totally absent in the MS experiment.

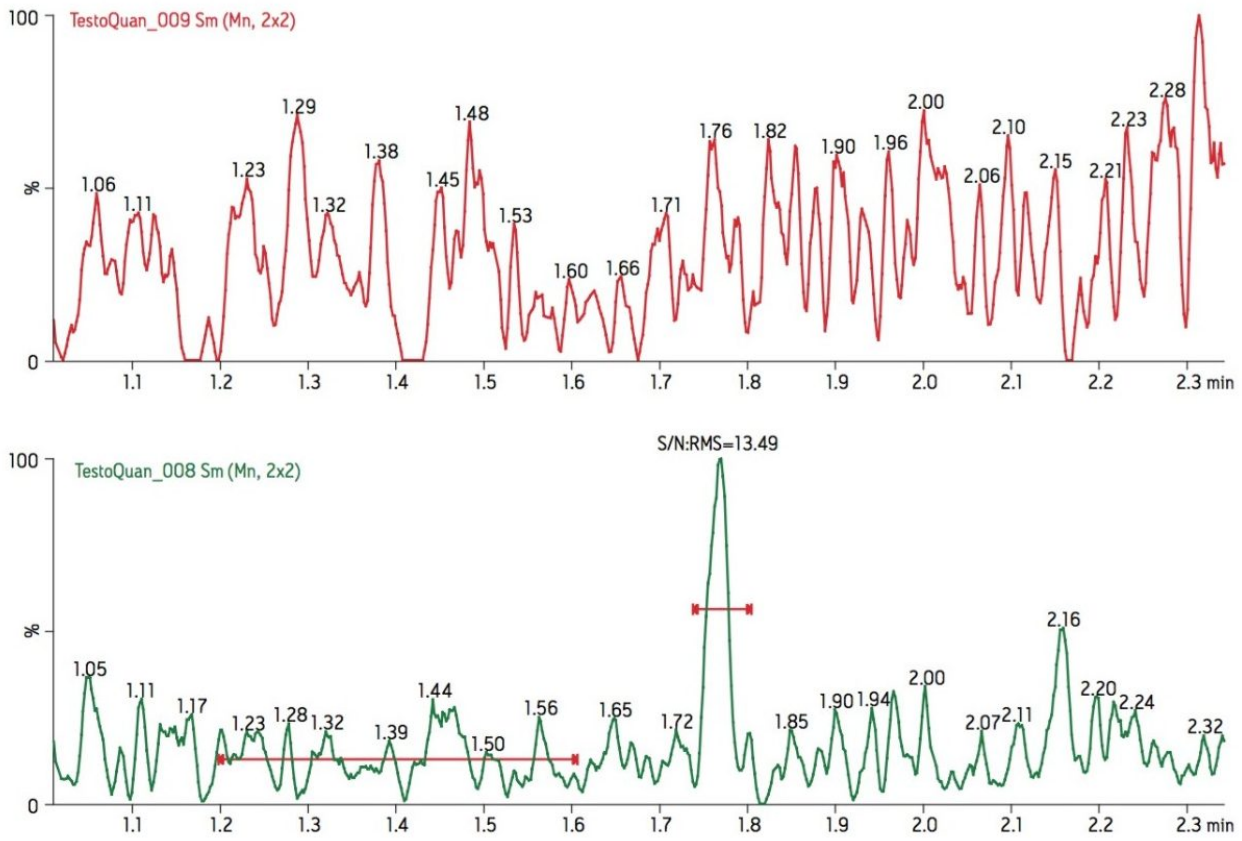

Figure 3. 250 fg of testosterone in solvent on column (ACQUITY UPLC 2.1 x 100 mm Column). Lower trace shows EIC of the transition 289.217 > 97.065 from an MRM experiment. Upper trace shows EIC of parent mass 289.217 from an MS experiment.

In addition to the MRM mode, the SYNAPT G2-S*i* offers HD-MRM. This mode offers two distinct analytical advantages over MRM alone with the unique advantages of T-wave ion mobility.

One of the advantages it offers is the ability to enhance all fragments of a target precursor by making use of Wideband Enhancement. The analyst is, therefore, free to decide which transition to use for quantification post-acquisition without loss of sensitivity. This could be particularly useful when an analytical method is being used across many matrices where potential interferences are unknown. Figure 4 shows combined spectra of the 527.75^{2+} peptide ion from consecutive HD-MRM experiments of 500 amol on column loads of digested Phosphorylase B. on an ACQUITY UPLC 2.1 x 100 mm Column. The upper panel shows the resultant spectrum when Wideband Enhancement is used and lower spectrum from normal duty cycle. An increase in sensitivity is

observed for all fragment ions, and all possible y-ion fragments are clearly observed when Wideband Enhancement is used.

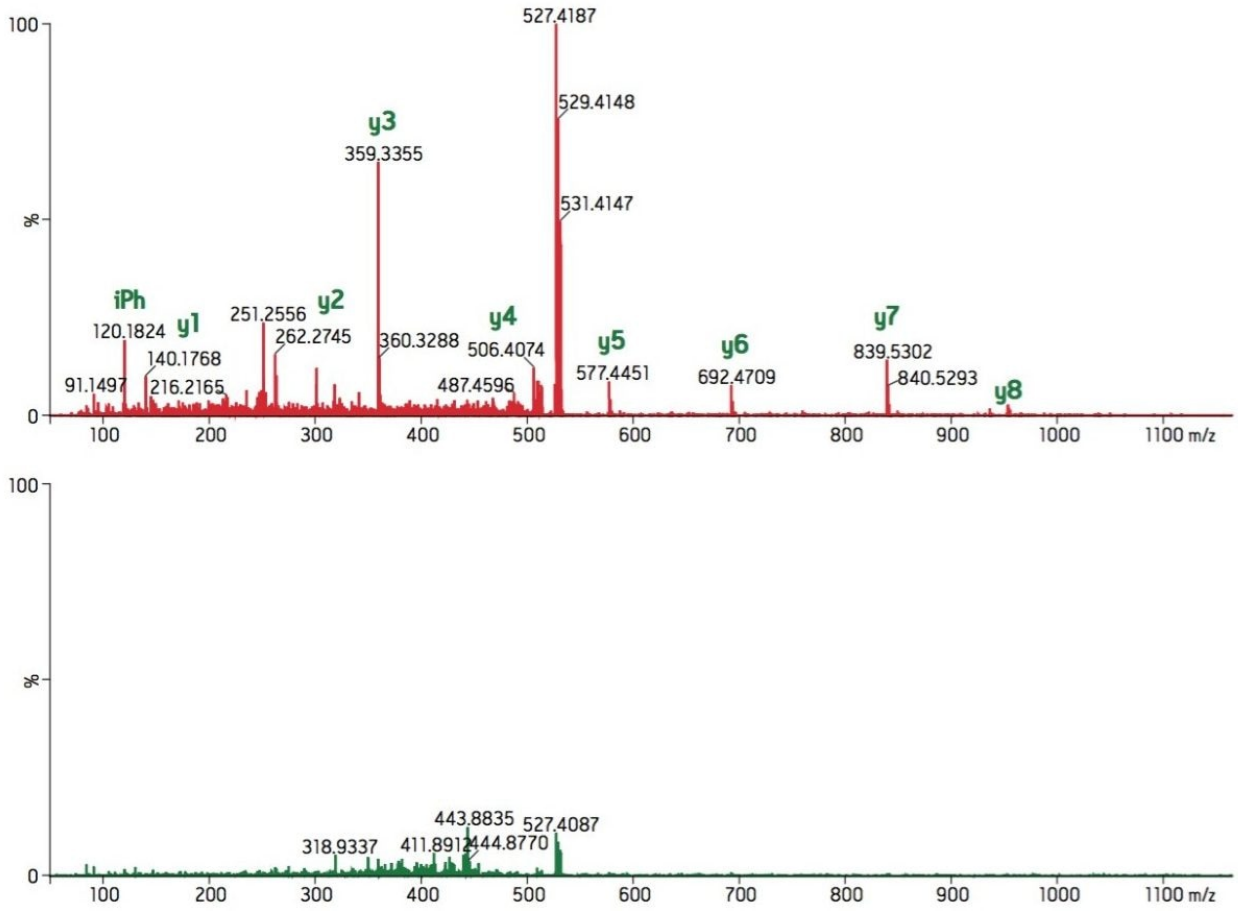

Figure 4. Combined spectra of the 527.752+ peptide ion from consecutive HD-MRM experiments of 500 amol on column loads of Phosphorylase B. tryptic digest using an ACQUITY UPLC 2.1 x 100 mm Column. The upper panel shows resultant spectrum when Wideband Enhancement is used and lower spectrum from normal duty cycle.

Figure 5 shows a comparison of EICs for three transitions including 527.7 > 839.4, 527.7 > 359.3, and 527.7 > 120.1 from the two HD-MRM experiments. In all cases, the top trace is obtained using Wideband Enhancement and bottom trace with normal duty cycle.

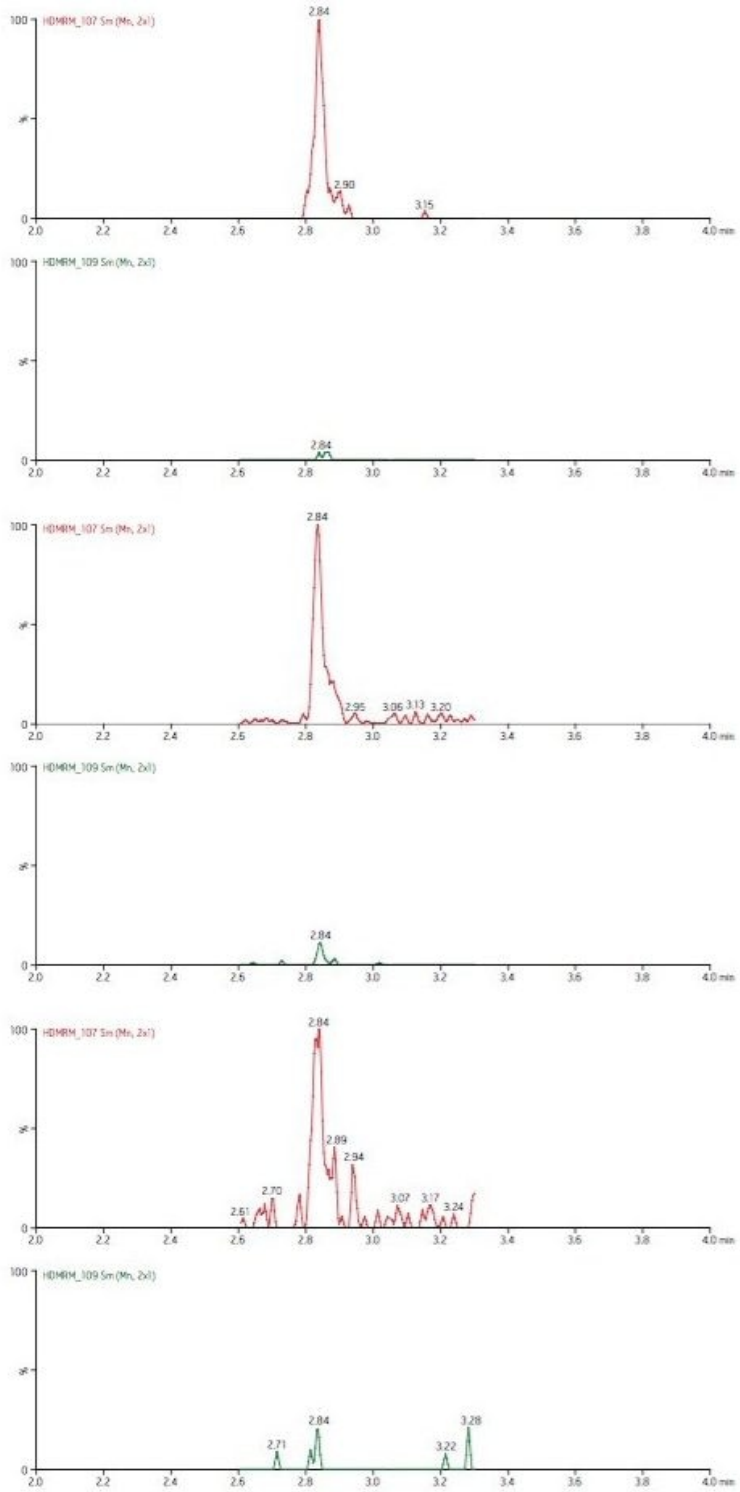

Figure 5. Comparison of EICs for three transitions including 527.7 > 839.4, 527.7 > 359.3, and 527.7 > 120.1 from

the two HD-MRM experiments. In all cases, the top trace is with Wideband Enhancement and bottom trace normal duty cycle.

In addition, HD-MRM mode offers the possibility to separate isobaric species by T-Wave ion mobility and induce fragmentation post-IMS separation while enhancing selectivity on transitions. This can be useful when quantifying specific isomers and protomers.

Conclusion

- Tof-MRM and HD-MRM increase sensitivity by up to 10 times by making the use of enhanced duty cycle routine to maximize instrument sensitivity in targeted quantification.
- Tof-MRM and HD-MRM take selectivity to another dimension by combining traditional MRM levels of selectivity with accurate mass, isotope ratios, and ion mobility separations.
- Intuitive compound driven method editor
- Optional RADAR and HD-RADAR scanning to enhance method development

Featured Products

SYNAPT G2-Si High Definition Mass Spectrometer <https://www.waters.com/134740622>

RADAR <https://www.waters.com/134798882>

TargetLynx Application Manager <https://www.waters.com/513791>

Available for purchase online:

ACQUITY UPLC BEH C18, 1.7 µm, 2.1 mm X 100 mm Column <
https://www.waters.com/waters/partDetail.htm?partNumber=186002352>

720004728, June 2013