

MRM³ Quantitation for Highest Selectivity in Complex Matrices

AB SCIEX QTRAP[®] 5500 System Innovations

Mass spectrometry has transformed quantitative analysis to become the method of choice for many assays. More recently, LC-MS/MS has revolutionized quantitative bioanalysis. While single MS filtering offers advantages over non-mass selective techniques, the use of tandem mass spectrometry (MS/MS, or MS²) eliminates interferences and results in a dramatic increase in selectivity which yields a very low baseline, excellent limits of quantification, and very good linearity. As a result, the Multiple Reaction Monitoring (MRM) experiment performed on triple quadrupole mass spectrometers has become the technique of choice for highly sensitive and selective quantification in biological matrices.

In some cases, interferences cannot be eliminated using MRM. More elaborate sample cleanup and chromatography is required to eliminate these interferences. If a high baseline or matrix interference cannot be eliminated, the result is a compromised Lower Limit of Quantification (LOQ) as the detection of compounds in complex matrices is limited by signal-to-noise rather than by raw instrument response. In such cases, the addition of a third MS stage has been shown to greatly increase selectivity and eliminate the high baseline or chromatographic interference. The result is a lower LOQ and better chromatographic peak shape.

Sensitive LC MS/MS/MS analysis requires instrumentation with three key performance features: the ability to perform MS³, ultra high sensitivity to overcome the loss in ion current due to multiple fragmentation steps, and fast scan speeds to keep up with fast LC. These requirements have been difficult to achieve with results comparable to MRM mode until the development of the AB SCIEX QTRAP[®] 5500 LC-MS/MS system.

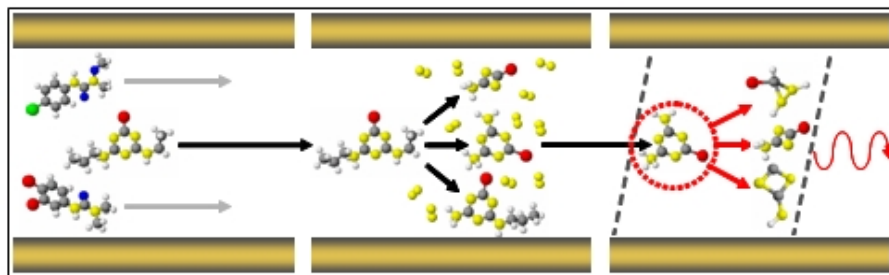


Figure 1. MRM³ for Quantitative Analysis by LC-MS. Analyte ion is first selected in the Q1 quadrupole, then fragmented in Q2 collision cell. Fragment ions are trapped then isolated in the linear ion trap, followed by excitation to perform the second fragmentation step. Second generation product ions are scanned out to the detector.



Key Features of the QTRAP[®] 5500 System for MRM³ Quantification

- MRM³ quantification - On the QTRAP[®] 5500 system, an MS³ scan is performed with a fast cycle time and using a narrow scan range centered around the second generation product ion to be used for quantification. This type of scan is referred to as MRM³ (Figure 1).
- Faster linear ion trap scan speeds – Scan speeds up to 20,000 Da/sec enable MS³ scans with an HPLC compatible cycle time such that extracted ion chromatograms (XICs) of second generation product ions can be extracted and integrated with a sufficient number of data points across the chromatographic peak.
- Better in-trap fragmentation – The new Linear Accelerator™ Trap with pulsed gas valve implemented in the QTRAP[®] 5500 system provides faster, more efficient in-trap fragmentation (Figure 2)
 - Highest available ion trap sensitivity – The QTRAP[®] 5500 system features the highest sensitivity commercial ion trap mass analyzer
 - High selectivity – Unit isolation of precursor ion in Q1 followed by excitation and fragmentation at unit resolution in the ion trap provides the highest available selectivity in MRM³ analysis (Figure 3).

Speed

The speed and efficiency of ion-trap fragmentation has also been greatly improved on the QTRAP[®] 5500 system. Collision gas is now introduced through a high speed pulsed gas valve that enables a rapid increase in pressure in the LIT (Figure 2). Together with an increase in the RF drive frequency, this results in increased fragmentation efficiency and reduced excitation time of 25 ms or less.

In addition, the scan speed of the linear ion trap has been increased to a maximum of 20,000 through the use of the faster eQ[™] electronics. This enabling fast MRM3 scan cycles at highest sensitivity.

Selectivity

The combination of features on the QTRAP[®] 5500 system provides the highest level of selectivity. The analyte ion of interest is isolated in the Q1 quadrupole with a user-selected resolution, usually unit resolution (0.7 Th FWHH). It is then fragmented in the Q2 collision cell, providing a broad range of product ions to be selected in the ion trap.

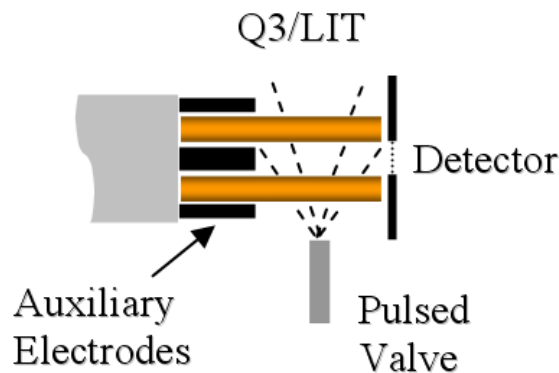


Figure 2. Pulsed Gas Valve. Gas is introduced into the linear ion trap using a high speed pulsed gas valve which rapidly increases LIT pressure and reduces required excitation time for ion trap fragmentation.

The in-trap fragmentation is achieved through the application of a single wavelength / narrow band excitation. As shown in Figure 3, this allows very selective fragmentation. The C12 isotope of a product ion can be specifically excited and fragmented to completion with minimal fragmentation of the C13 isotope. This provides further selectivity advantages in the removal of interfering background. The combination of these features provides unprecedented selectivity and flexibility in the design of the optimal MRM3 quantification experiments.

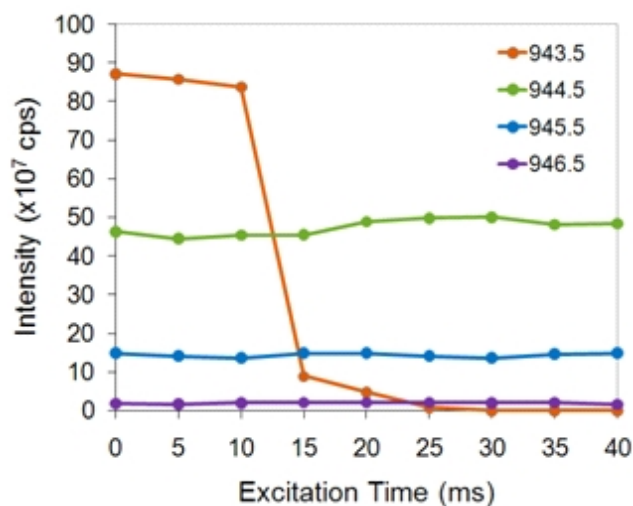
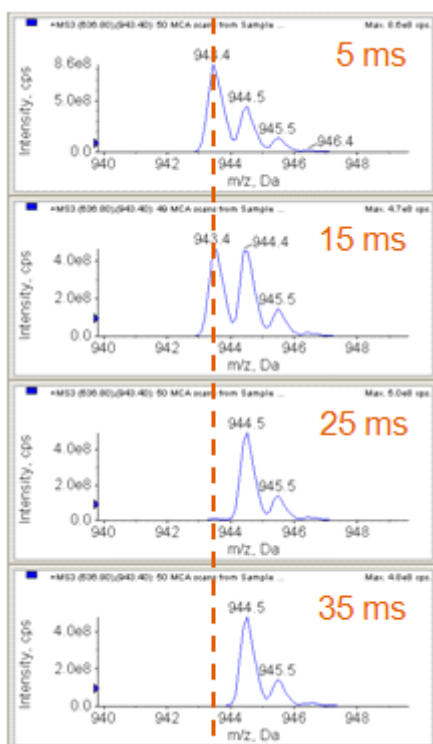


Figure 3. Single Frequency Excitation for Highest Selectivity. Narrow band excitation is used to specifically excite and fragment just the C12 isotope of the ion isolated in the LIT (left). This isotope can be fragmented to completion with no impact on the nearby C13 isotopes (right).

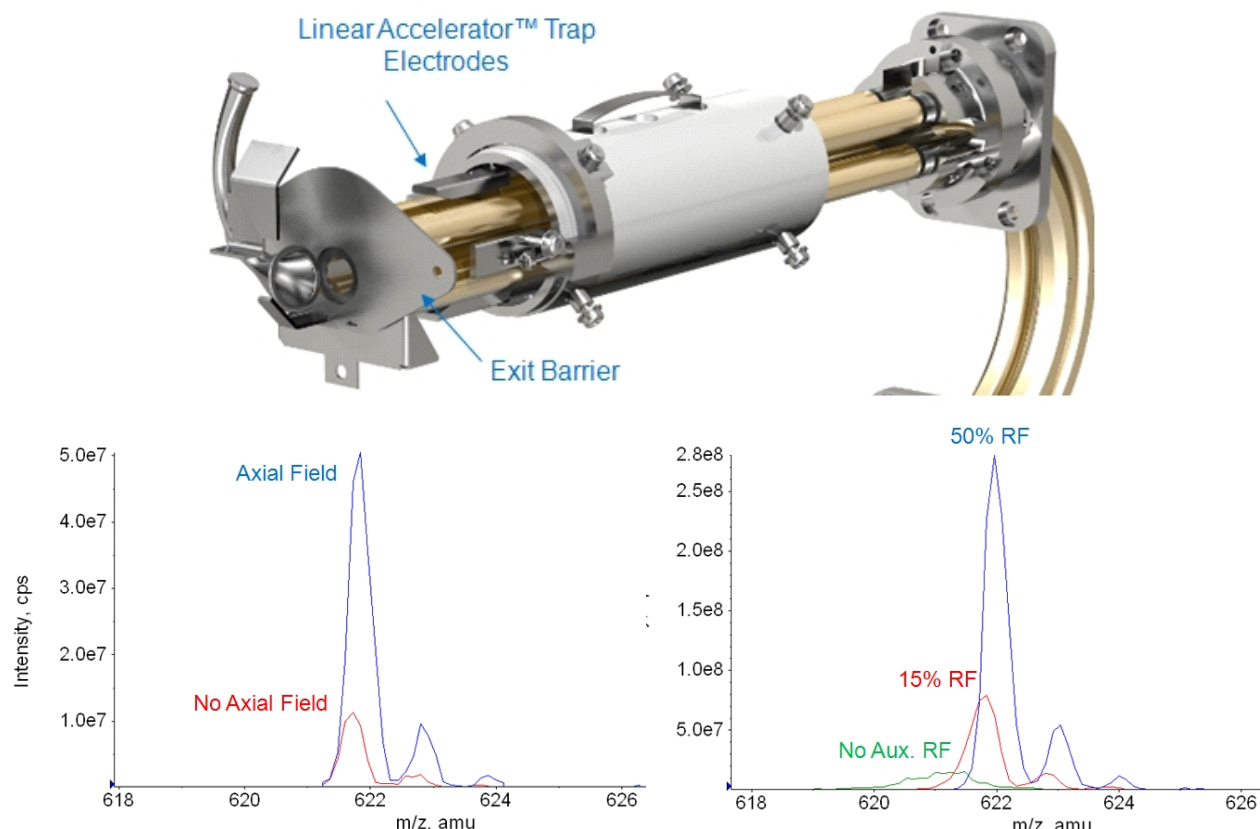


Figure 4. Linear Accelerator™ Trap Innovations for Sensitivity. Addition of electrodes in this new trap design (top) significantly improves the sensitivity of trap scanning by moving the trapped ions into the extraction region before axial ejection from the trap (bottom left). Further sensitivity gains are achieved by the addition of RF to the exit barrier of the trap (bottom right).

Sensitivity

Linear Accelerator™ Trap technology has resulted in ground breaking improvement in the handling of ions inside the linear ion trap of the QTRAP® 5500 system, resulting in up to 100x more sensitivity. Trapped ions are manipulated within the linear ion trap through the use of auxiliary DC fields provided by the addition of small electrodes (Figure 4, top). Ions are gently moved toward the extraction region of the linear ion trap during the cooling period by a voltage applied to the trap collar. A potential barrier is created by increasing the potential on the auxiliary electrodes just before the mass scan to complete the ion concentration process. The application of this axial field has a significant effect on sensitivity (Figure 4, bottom left).

In addition, a radio frequency is applied to the exit lens of the Linear Accelerator Trap resulting in further sensitivity gains (Figure 4, bottom right). These two innovations enable better than unit resolution to be obtained in the trap scan modes at these very high scan speeds.

Removal of Tough Interferences

Innovations in scanning speed, selectivity and sensitivity on the QTRAP® 5500 system enable successful implementation of the MRM³ workflow for a wide range of analytes^{3,4}. Sometimes, background noise or interferences can limit the detection of an analyte. Shown in Figure 5 is an example of an interference that has the same MRM transition as Clenbuterol and elutes at the same retention time. Use of MRM³ can completely remove this interference and enable a much lower detection of this analyte.

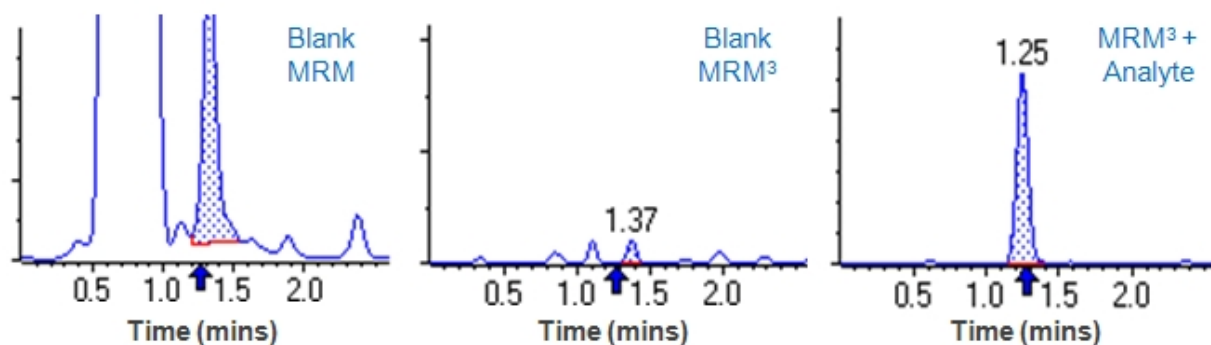


Figure 5. Analysis of Clenbuterol in Urine. Analysis of Clenbuterol in urine by MRM is plagued by the presence of a large co-eluting interference. Left – MRM for Clenbuterol used to analyze the urine blank. Middle – MRM³ analysis of the urine blank shows the interference is completely gone. Right – MRM³ analysis of 0.5 ng/mL clenbuterol spiked into urine. 10x better LOQ obtained with MRM³ than MRM due to substantial reduction in interference (data not shown).

Conclusions

- MRM³ is an effective strategy for quantitation of analytes when high background or interferences make standard MRM quantitation difficult (Figure 5).
- MRM³ can be used to achieve similar LOQ's with less sample preparation and simplified or faster chromatography.
- MRM³ has been successfully applied to the detection and quantitation of small molecules, peptides, and protein biomarkers.
- MRM³ is significantly improved on the QTRAP[®] 5500 system technology making it a useful tool for quantitation in tough matrices.
- The QTRAP[®] 5500 LC-MS/MS system is the highest performance triple quadrupole and linear ion trap system available on the market today providing users with many powerful quantitative and qualitative tools.

References

1. Collings BA, (2007) Fragmentation of ions in a low pressure linear ion trap. *J. Am. Soc. Mass Spectrom.* 18, 1459-1466.
2. Collings BA, and Romaschin AR, (2009) MS/MS of ions in a low pressure linear ion trap using a pulsed gas. *J. Am. Soc. Mass Spectrom.* 20, 1714-1717.
3. Fortin T. et al, (2009) Multiple Reaction Monitoring Cubed for Protein Quantification at the Low Nanogram/Milliliter Level in Nondepleted Human Serum. *Anal. Chem.* ePub. Oct 19, 2009.
4. Niessen J. et al, (2009) Human platelets express OATP2B1, an uptake transporter for atorvastatin. *Drug. Metab. Dispos. Fast Forward.* Feb 23, 2009.

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Publication number: 0920210-02