La utilización de volúmenes reducidos de suero o plasma (100-200 µl) no es un impedimento para un análisis diferencial de expresión por técnicas 2-D DIGE, SELDI-TOF o LC-MS.

## Referencias

[1] Pavon EJ, Munoz P, Lario A, Longobardo V, Carrascal M, Abián J, et al. Proteomic analysis of plasma from patients with systemic lupus erythematosus: increased presence of haptoglobin alpha2 polypeptide chains over the alpha1 isoforms. Proteomics 2006; 6: 282-92.

# Developing MRM Assays for Peptide Quantitation: *The MIDAS™ workflow and Qtrap™ technology*

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In the field of proteomics, most of the information needed on biological samples of interest extends beyond just the identity of the protein. Once the protein identification is made from a variety of MS and orthogonal experiments, a more in-depth study is necessary to characterize isoforms, sites of post-translational modification, or even sites of cleavage after activation or secretion. Additionally, constructing methods to determine the presence of a specific protein or peptide in a complex mixture is of greater importance as the field of biomarker discovery and validation grows. Robust and sensitive techniques for this targeted discovery and characterization of peptides and proteins are necessary.

The information known about the sample, such as the protein sequence or a hypothesized post-translational modification, allows more specific questions to be addressed. Normal information dependent acquisition techniques will not always detect the components of interest if they are of low abundance, or are poorly amenable to MS analysis. A more hypothesis-driven acquisition approach is often more effective such as the MIDAS™ workflow (Figure 1 and 2). The utility and power of this approach is explored here.

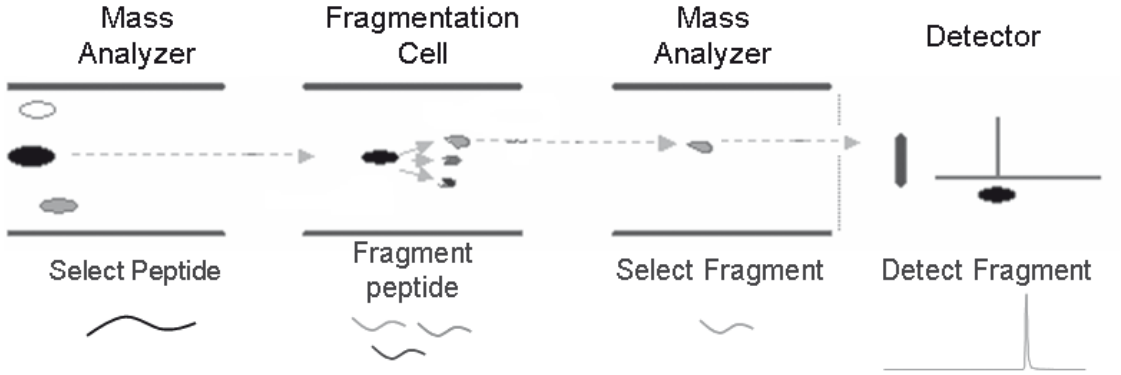


**Figure 1.** *Schematic of the Multiple Reaction Monitoring (MRM) scan for high selectivity and sensitivity. Q1 is set to transmit only the parent m/z of the peptide , the collision energy is optimized to produce a diagnostic charged fragment of this peptide in Q2, and Q3 is set to transmit this diagnostic fragment only. Because of the short dwell times required (10-50 ms) and the ability to change rapidly between MRM transitions, many components (transitions) in a mixture can be monitored simultaneously in a single LC/MS/MS run.*

## 1. Key Features of the MIDAS™ Workflow on the 4000 Q TRAP™ and 5500 Q TRAP™ Systems

• Extremely high selectivity and sensitivity for detecting peptides in complex mixtures.

• High sensitivity and high quality MS/MS for confirmation of peptide sequence.

• Multiple Reaction Monitoring (MRM) can provide quantitative information between samples.

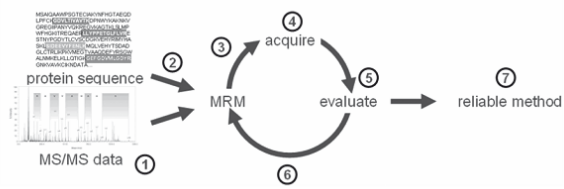


**Figure 2:** *Creating a reliable MRM-based method for peptide quantitation is an iterative procedure that takes input from various sources. Choosing which peptides, and then which fragment ions, can be based on either 1 previously acquired MS/MS data or from 2 non-MS based techniques and peptide fragmentation prediction. The next step is to 3 create an instrument specific method (MRM-triggered MS/MS method otherwise known as a MIDAS Workflow method) and then use this method to 4 acquire data. The selected MRM transitions are then 5 evaluated for their suitability for quantitative measurements (i.e.signal/noise, intensity, etc.) and the MS/MS is evaluated for how well it confirms the peptide ID. This process is 6 repeated until 7 a set of MRM transitions are generated that reliably identify the peptides and can be used for quantitation.*

## 2. The MIDAS™ Workflow

Typically in an information dependent acquisition (IDA), peaks are selected from full scan MS data, and are then used for dependent MS/MS experiments. The MRM scan can also be used as survey scan which enables the user to influence the choice of precursor ion selection for MS/MS. In combination with ab initio prediction of theoretical fragments and modifications of interest, MRM driven IDA provides all of the advantages of MRM sensitivity, S/N gains, and high selectivity, with full scan MS/MS data for confirmation, and even database searching. Figure 3 highlights the general schema utilized in these experiments. Since each MRM transition is rapid, many of these can be combined in a single survey scan spanning hundreds of potential peptides and modifications. After a peak is detected by MRM, an Enhanced Resolution (ER) scan can be performed to obtain accurate charge and m/z information, and then MS/MS is performed. In many cases the detection of an MRM transition can be sufficient for confirmation, but the MS/MS data can provide additional validation of identity.

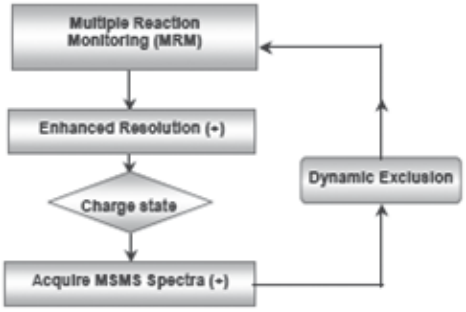


**Figure 3**. *MRM Initiated Detection and Sequencing using the MIDAS™ Workflow. The sensitivity and specificity of an MRM scan can be used as a survey scan for Information Dependent Acquisition (IDA) to 'dig deeper' into a biological sample. The MRM scan detects the peptide at high sensitivity and triggers a full scan MS/MS for confirmation of peptide identity.*

## 3. Applications of the MIDAS™ Workflow

• Targeting low level phosphorylation sites on a protein of known sequence to identify or confirm modification location

• Validating weak protein identifications by specifically obtaining more MSMS on additional peptides for that protein

• Detection of low level proteins in complex mixtures

• Quantitation of proteins/peptides with accompanying MS/MS for identity confirmation

## Conclusions

The unique hybrid nature of the triple quadrupoles linear ion traps 4000 Q TRAP® and 5500 Q TRAP® systems allow for targeted powerful workflows. Combining the specificity and sensitivity of Multiple Reaction Monitoring (MRM) with the high quality MS/MS allows for the targeted discovery of peptides and post-translational modifications. Good MS/MS can be obtained on peptides at extremely low amounts on column (1 amol), easily track.