

Frequently asked questions (FAQs) MRM peptide quantitation

Q. What is MRM quantitation?

A. Multiple reaction monitoring (MRM) is a highly specific and sensitive mass spectrometry technique useful for quantitating pre-defined peptides of interest. The method is best practised utilising a triple quadrupole mass spectrometer where the precursor peptide mass is selected in Q1 and a diagnostic CID fragment ion is selected in Q3. A signal is registered only when a pre-defined fragment ion arises from the pre-defined precursor.

Q. Is MRM compatible with absolute or relative quantitation?

A. The technique is compatible with both modes of quantitation. For absolute quantitation a peptide standard is required to generate a standard curve.

Q. What are the characteristics of peptides that are compatible with MRM using a 4000 QTRAP mass spectrometer?

A. In principle, the majority of peptides produced by enzymatic digestion are amenable to MRM analysis. However, in practise peptides between 7-17 amino acids in length are the best candidates for MRM analysis. Peptides generated using a tryptic digest are ideal since they often produce predictable fragment ions. Peptides produced from other proteases may be compatible with MRM provided a clear CID spectrum can be generated.

Q. How many MRM transitions can be followed in a single LC-MS run?

A. Approximately 80 different MRM transitions.

Q. How sensitive is an MRM assay?

A. Limit of detection differs for each peptide and sample matrix. Purified peptides can be accurately quantitated at low femto-molar concentration. In complex samples such as plasma there are reports of detecting peptides by MRM from proteins (L-selectin) present at 1 nano-molar (low μg) concentration.

Q. What is the workflow required for absolute quantitation and is it more expensive than relative quantitation?

A. Designing and running MRM assays for absolute quantitation requires considerable operator time and requires sample replicates. Therefore the costs are considerably more than relative quantitation. For absolute quantitation, synthetic, isotopically labelled peptide standards are needed to optimize MS parameters and to build a standard curve. A quantitation processing method is required to measure ion area and deduce quantity from the standard curve.