Multiple Reaction Monitoring for Peptide Absolute Quantitation

Multiple Reaction Monitoring Mass Spectrometry (MRM-MS) is a targeted mass spectrometry approach that has been used for decades in clinical reference laboratories for accurate quantification of small molecules in plasma/serum/urine. More recently, this approach has been applied to measure concentrations of candidate biomarker proteins in plasma and cell lysates.[1-2] The MRM approach combined with stable isotope dilution–mass spectrometry (SID-MS) can be used to develop multiplexed, high throughput, accurate and sensitive assays for a moderate number of candidate biomarkers (30-100) and applied to a large number of samples. A Multi-site assessment of precision and reproducibility of MRM-based measurements has been published by the Clinical Proteomics Technology Assessment Consortium (CPTAC) of the National Cancer Institute.[3]

The MRM-MS assay is performed on a triple quadrupole mass spectrometer where an ion of interest (precursor) is preselected with the mass filter Q1, and induced to fragment in the collision cell Q2 by collision-induced dissociation (CID). The resulting "product ions" are mass analyzed using the third quadrupole Q3. The number of target fragment ions is counted over time, resulting in a single reaction monitoring (SRM) trace for each transition (*see* Figure 1). When multiple target fragment ions resulting from multiple precursor ions are monitored, the overall process is termed multiple reaction monitoring. Assay development is an iterative process that involves the selection of proteotypic peptides that will represent the protein of interest, synthesis of heavy isotope labeled analogues of these peptides to be used as internal standards, and the optimization of MRM acquisition parameters so that sensitive and accurate quantitation can be performed without interference from other components in the sample. Finally, MRM-MS assays must be characterized to determine the technical reproducibility and concentration range of the linear response over which quantitation can be performed.

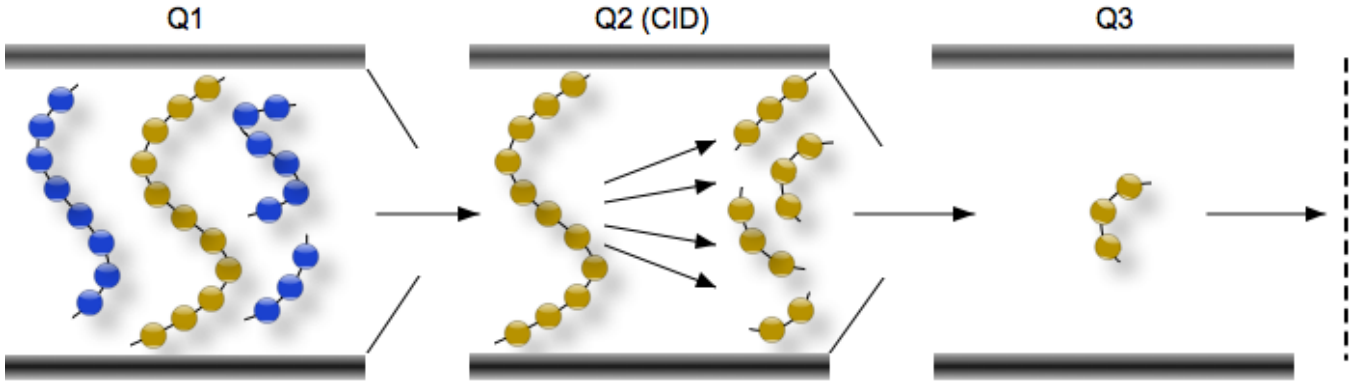


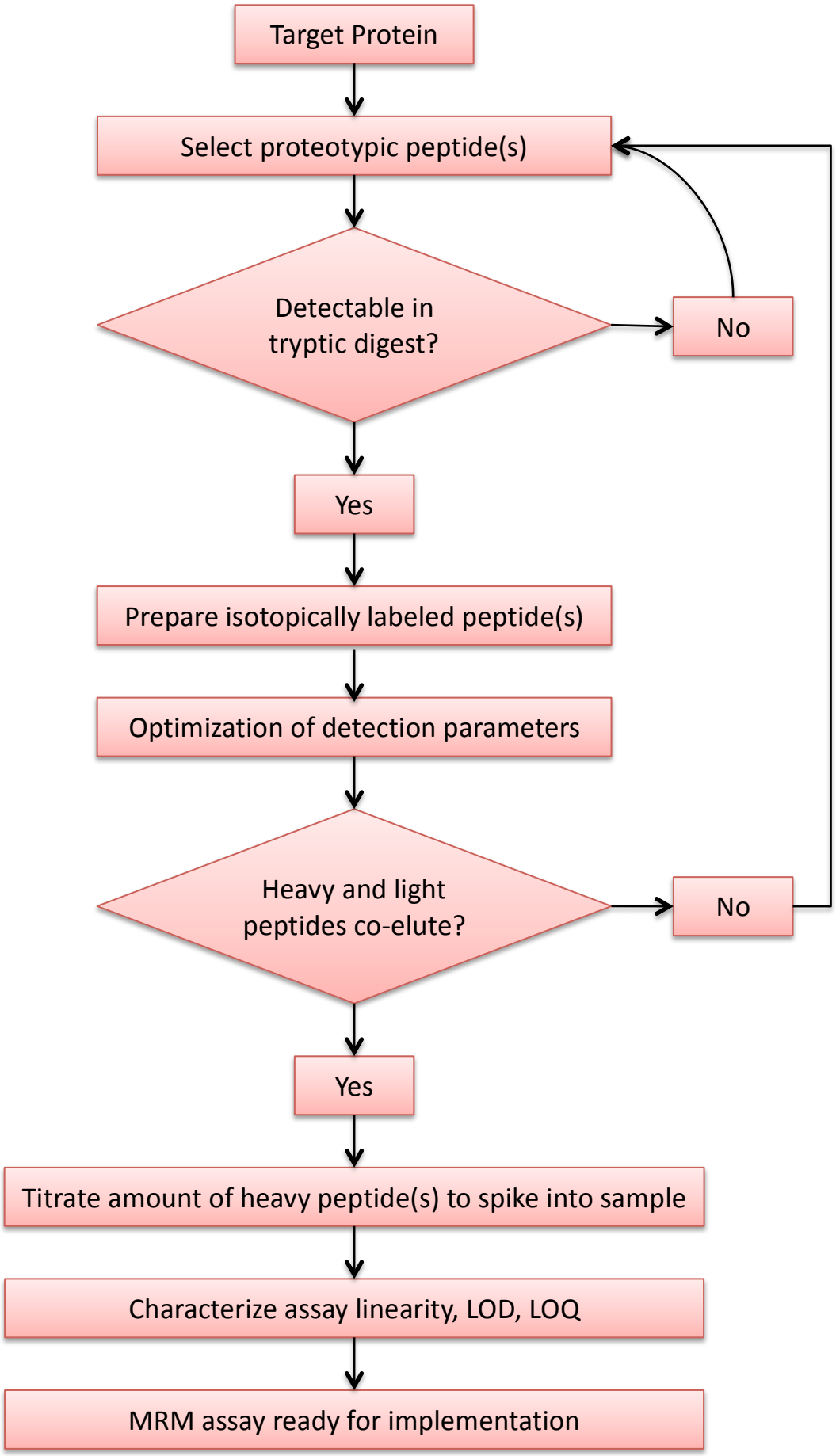
Figure 1. Schematic of the multiple reaction monitoring (MRM) scanning technique on a triple quadrupole mass spectrometer. The targeted parent ion (depicted in yellow as an uncharged species for clarity) is selected in the first quadrupole (Q1) and enters the second quadrupole (Q2) where it undergoes collision-induced dissociation (CID). One or more fragment ions are then selected according to predefined transitions and the ensuing signal provides the spectral counts for quantification. Where more than one transition is selected for a given precursor ion, the accumulative counts are used for quantification.

In the proteomics core facility, MRM-MS assays are developed on the Agilent 6490 iFunnel triple quadrupole LC/MS system, a high throughput platform capable of determining 400 transitions per method. This QQQ can be used with either the Agilent 1290 Infinity UHPLC or the Agilent 1260 HPLC-Chip Cube MS interface. Both systems employ Agilent's iFunnel technology that incorporates a thermal gradient "Jet Stream", a hexabore capillary at the vacuum interface for increased ion sampling and dual stage ion funnel. It is the most sensitive QQQ currently

available with attogram/zeptomole level sensitivity and six orders of magnitude of linearity. These performance characteristics make it particularly well suited for MRM-MS analysis. Furthermore, the dynamic MRM (DMRM) algorithm on the Agilent 6490 QQQ automatically constructs DMRM timetables for multiple analytes throughout the LC/MS analysis based on retention time window for each analyte. This allows the instrument to acquire data only during the stated retention time windows thereby reducing the number of concurrent ion transitions, maximizing dwell time and therefore improved sensitivity, better peak symmetry and 10-fold increase in signal-to-noise ratio. The workflow required to develop and optimize an MRM-MS assay for multiple analytes is illustrated below.

References

1. Keshishian, H. et al. (2007) Quantitative, multiplexed assays for low abundance proteins in plasma by targeted mass spectrometry and stable isotope dilution*. Mol. Cell. Proteomics* 6, 2212-2229
2. Kitteringham, N.R. et al. (2009) Multiple reaction monitoring for quantitative biomarker analysis in proteomics and metabolomics. *J. Chrom. B* 877, 1229-1239
3. Addona, T. et al. (2009) A Multi-site Assessment of Precision and Reproducibility of Multiple Reaction Monitoring-based Measurements by the NCI-CPTAC Network: Toward Quantitative Protein Biomarker Verification in Human Plasma. *Nature Biotechnology* 27, 633-641


Target Protein
Select proteotypic peptide(s)
Detectable in tryptic digest?
No
Yes
Prepare isotopically labeled peptide(s)
Optimization of detection parameters
Heavy and light peptides co-elute?
No
Yes
Titrate amount of heavy peptide(s) to spike into sample
Characterize assay linearity, LOD, LOQ
MRM assay ready for implementation