

ENHANCED SENSITIVITY OF MRM-BASED PROTEIN QUANTITATION USING A TRIPLE QUADRUPOLE LC/MS SYSTEM WITH DUAL ION FUNNEL

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Assays that are both specific and quantitative for target proteins are critical for preclinical validation of putative biomarkers. Such assays are typically multiplexed, multiple reaction monitoring (MRM) analyses which can provide the high-throughput required. Sensitivity is a key requirement for such assays as protein biomarker concentrations may be quite low in commonly used biofluids such as serum and plasma. Improving the sensitivity of LC/MS can be achieved by enhancing the sampling and transmission of ions in the mass spectrometer. This study investigates the sensitivity gains achieved for peptides using a triple quadrupole mass spectrometer modified with a dual ion funnel. For the initial study, peptides were selected from a standard protein digest mixture and instrument parameters were optimized for optimal sensitivity. Results showed up to a 5-10x increase in sensitivity with the dual ion funnel QQQ for the selected peptides. This improvement lets to achieve low attomol peptide sensitivity using UHPLC systems with 2.1mm columns when high-throughput is required; or use nanoflow HPLC-Chip technology for highest sensitivity. Results are shown for a LC flow-rate study as well as for a robustness study using human plasma.