

Simplifying the Hunt for Optimal SRM Transitions: Utilizing Discovery Data to Expedite Targeted Peptide Quantitation

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Greater emphasis has been placed on advancing proteomics studies from discovery and/or relative quantitation to validated quantitative methods in an effort to establish clinical assays. The typical workflow involves first performing discovery based experiments to identify protein expression levels that are confidently changing between a control and treated samples and generate product ion information used to sequence the precursor peptide. The difficulty arises in transferring discovery based methods directly over to validated quantitation methods since each is generally performed on separate mass spectral platforms. Low confidence has been placed on relating relative product ion abundance obtained from ion trap CID to that observed using a triple quadrupole mass spectrometer due to the difference in ion activation mechanisms and the timescale of the excitation. Thus, the only information transferred from one method to the other is protein id, peptide sequence, and the most abundant charge state resulting in further method development to complete the SRM assay. Common approaches to determine SRM transitions are based on a set of accepted rules to determine the best possible ion pair(s), which are then searched against the matrix database to determine the uniqueness of each mass pair. We contend that the relative abundance of product ions originating from ion trap CID can be used to directly assign the most sensitive ion pairs for the targeted SRM methods.

We will present direct comparison of relative product ion abundance measurements for 100 plasma peptides between an ion trap and a triple quadrupole mass spectrometer. The selected peptides are broken down into sequence length ranging from 7 to 15 residues to determine consistency across the typical biomarker properties. Success rates for matching the most abundant

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product ions from each method to those predicted will be consolidated and reported.

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