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GENERATION OF UNIQUE PROTEIN SPECIFIC MRM SIGNATURES; USING PEPTIDE INFORMATION FROM ALTERNATE SCANNING LC-MS DATA TO DRIVE MRM DEVELOPMENT

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Proteomics research has resulted in the discovery of a large number of differentially expressed proteins which must be validated to determine their utility as specific markers. The quantitation of these proteins is challenging due to both the inherent complexity associated with the number of tryptic peptides generated and the dynamic range in protein concentration present.

Previously we have described, how, using an alternate scanning LC-MS strategy on a Q-Tof mass spectrometer we can derive a comprehensive inventory of precursor and product ions, peak area intensities and associated physio-chemical properties. Here we show how this experimental data (precursor and fragment m/z values, intensity and retention time) can be utilized to empirically determine those peptides which uniquely identify a protein in a database from a complex sample. In addition the algorithms determine both the 'best' ionising peptide precursor and the most selective fragment ion to determine the most appropriate multiple reaction monitoring (MRM) transition to monitor.

For this study a cytosolic extract from Escherichia coli was digested and analysed by LCMSE on a QTof type instrument. IdentityE processing produced a profile of the proteins present and a comprehensive inventory of the peptide precursor and fragment ions present. This inventory of over 25000 potential MRM transitions was filtered to determine 'proteotypic' peptides each protein in the sample removing any which share a common sequence. Further filtering reduces the candidates to around 5500 by retaining the five most intense precursor ions per protein and their five most intense fragments. These are now automatically assessed for m/z and retention time overlap prior to producing an MRM experimental file which can be transferred to triple quadrupole instrument. Here we show the utility of the automated data sorting tools to build triple quadrupole MRM methods which can be used quantify E.coli proteins.