

High sensitivity phosphoprotein analysis using a combination of variable flow chromatography and precursor ion discovery on a Q-ToF mass spectrometer.

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Mass spectrometry has firmly established itself as the primary technique for identifying proteins. Currently, the mass spectrometry technique providing the highest degree of specificity and sensitivity is electrospray LC-MS/MS. However in the case of post translationally modified peptides only a very limited sub set of the peptides present are required to be fragmented and often these low intensity peaks can be missed. A solution to this problem is a method that allows specific post translationally modified peptides to be identified during the course of an LC-MS experiment. In the case of phosphotyrosine, a low mass immonium ion at m/z 216 can be detected. This characteristic ion is used to direct the mass spectrometer to fragment potential phosphopeptide precursor ions which are present at that time point in the low energy data. In this case several precursor ions may require MS/MS interrogation at one decision making time-point and implementation of a chromatographic technique known as variable flow chromatography allows greater time to interrogate these peaks. This approach will be discussed with examples of where this methodology has been used for the targeted analysis of phosphorylated peptides.
