Systematic LC-MS analysis of labile post-translational modifications in complex mixtures

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Most proteins are post-translationally modified and the characterization of modified peptides in complex mixtures generated by enzymatic digestion of multiple proteins remains a major analytical challenge. We describe an integrated LC-MS workflow implemented on a hybrid quadrupole time-of-flight (Q-ToF) instrument to detect modified peptides in a complex peptide sample and establish the nature of the modification. The method is based on the alternating acquisition of full mass spectra under different collision conditions inducing the cleavage of the substituents. Modified peptides are detected based on their specific fragmentation generating the nonmodified peptide backbone and reporter ions in the low mass region. The two mass analyzer stages of a Q-ToF instrument are used to eliminate the low mass chemical background in the quadrupole and thus facilitate the detection of low mass reporter ions in the ToF. Off-line data processing enables detection of one (or even multiple) modifications and the modified candidates are subsequently sequenced in a directed MS/MS mode. The technique was applied to the analysis of O-GlcNAc peptides, a very complex mixture of N-linked glycopeptides, and a phosphotyrosine peptide.