

It is a challenge to get high quality MS/MS spectrum of phosphopeptide. First of all, it is harder to ionize phosphopeptides in the source of the mass spectrometer due to the negative charge on the phosphate moiety. Second, the stoichiometry for the phosphorylation is often low. Third, non-phosphopeptide neighbors make it difficult to pick the right precursor ion, especially when the protein and/or tag are very large. Picking more than one ion as precursor means that many peaks in the MS/MS spectrum come from peptides other than the phosphopeptide, thus complicating the data set.

LC/MS/MS has the advantage of separating phosphopeptides from non-phosphopeptides. It also offers the possibility to identify peptides with multiple phosphorylation sites. In this case, both S4 and S6 are phosphorylated as illustrated below. However, no LC/MS/MS will have 100% coverage of the protein. Non-specific cleavage will complicate the data interpretation and sometimes phosphopeptides just get lost during the desalting process especially when they are short in length or very hydrophilic in nature.

