

A new strategy for glycopeptide enrichment using combining ZIC-HILIC and molecular weight cut-off filter

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Glycosylation, the most common protein post-translational modifications (PTMs), plays a key role in biological and disease processes. However, glycosylation takes place with low abundance and exhibits a broad dynamic range across different glycoproteins. Additionally, large amounts of non-glycosylated peptides in complex mixtures would hinder the detection of low-abundant glycopeptide signals during LC-MS/MS analysis. These characteristics present significant difficulties in N-glycosylation analysis and require an efficient approach, such as a specific glycopeptide enrichment method. For glycopeptide isolation, zwitterionic hydrophilic interaction liquid chromatography (ZIC-HILIC) has been widely used. In this study, we show that the glycopeptide enrichment step was improved by a combination of ZIC-HILIC and molecular weight cut-off (MWCO) filter. MWCO filter-aided method increased the number of identified glycopeptides compared to method and decreased the percentage of non-glycopeptide-matched spectra by 27.8 % compared to ZIC-HILIC method without MWCO filter step. These results indicate that removal of non-glycopeptides was effectively achieved using MWCO. These findings indicate that the site-specific characterization of glycoproteins from complex sample can be improved using the MWCO-aided intact glycopeptide enrichment method.