# Isomeric separation of glycopeptides by HILIC LC coupled to MS/MS

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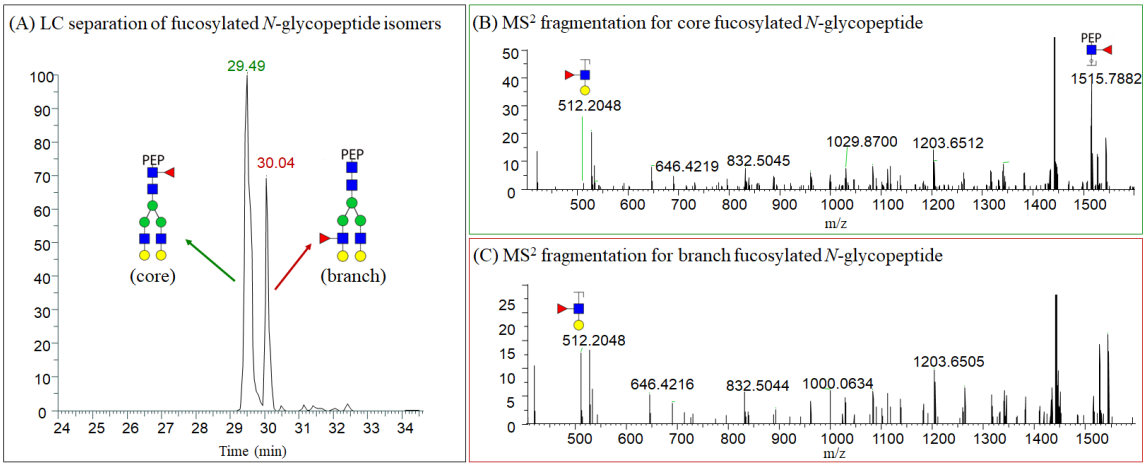
Glycosylation is a critical biological process that can profoundly influence the function of proteins. The structurally different glycans attached on specific site of proteins have been shown to be involved in a variety of disease progressions. Despite the recent advances in LC-MS/MS methodologies, the profiling of site-specific glycosylation is still a challenge due to the glycan isomeric structures attached on the same peptide backbones. Hydrophilic interaction liquid chromatography (HILIC) is a powerful technique for the separation of glycopeptides, as it has the ability to retain highly hydrophilic analytes [1][2]. Additionally, long column length can increase the absolute column efficiency and separation ability.

In this study, we focused on the isomeric separation of N-glycopeptides using a 75 µm × 350 mm HILIC column with 5-hydroxyl groups under a nanoflow rate. To achieve better resolutions on glycopeptide isomers, different LC conditions were optimized using standard glycoprotein digests, including hemopexin, immunoglobulin G (trastuzumab) and haptoglobin.

Many glycopeptide isomers were successfully separated, including core and branch-fucosylated N-glycopeptide isomers (Figure 1) and sialylated N-glycopeptide isomers. In addition, the comparison of C18 and HILIC trap columns coupled with HILIC analytical columns was evaluated in detail. Finally, we applied this approach to separate glycopeptide isomers derived from complex biological samples, including plasma and cell lysates. HILIC not only proved to be a useful tool for the comprehensive characterization of glycoproteins and their isomers, but also a good potential alternative to C18-based glycoproteomics workflows.




PEP, MVSHHN[184]LTTGATLINEQWLLTTAK.

Figure 1. LC-MS and MS2 of core- and branch- fucosylated N-glycopeptide isomers from haptoglobin digests.

Bibliographic references:

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