ABSTRACT

The analysis of glycoproteins represents a significant challenge in glycoproteomics, primarily due to the macro- and microheterogeneity of protein glycosylation. Hydrophilic interaction liquid chromatography (HILIC) is a convenient alternative to reversed-phase chromatography, commonly used in glycoproteomic analysis. This dissertation thesis discusses the potential of HILIC in glycoproteomic analysis, ranging from the separation of glycopeptides on polar stationary phases to the use of HILIC in sample preparation processes.

First, the effect of acetonitrile concentration on glycopeptide precipitation was investigated, depending on the type of glycan attached. Subsequently, three commercially available stationary phases were tested: a column containing a silica gel modified with five hydroxyl groups, an amide stationary phase, and a zwitterionic stationary phase. Their efficiency in separating glycopeptide isomers, differing only in branching and/or linkage position, was compared. Further research was devoted to the separation of human immunoglobulin G glycopeptides using relatively new columns that have not yet been characterized in glycoproteomic analysis. These columns, provided by Advanced Chromatography Technologies, included unmodified silica gel (HILIC-A), aminopropyl-modified sorbent (HILIC-B) and polyhydroxyl-functionalized stationary phase (HILIC-N). All HILIC columns demonstrated better separation of glycoforms compared to reverse-phase separation, but exhibited different separation efficiencies. Moreover, a mixed-mode retention mechanism of glycopeptides was observed in the case of HILIC-A and HILIC-B columns.

In this dissertation thesis, a model was developed to predict the retention windows of glycopeptides in HILIC mode. This model can serve as a complementary tool to glycopeptide identification by mass spectrometry, thereby reducing their false discovery rate.

The final section of this thesis focused on the study of the experimental conditions for glycopeptide enrichment utilizing solid-phase extraction (SPE) on polar stationary phases operating in HILIC mode. Two stationary phases, a commercially available aminopropyl, and an in-house-synthetized polyaniline-coated silica gel, were tested. The impact of different experimental conditions on the enrichment efficiency was investigated, with each tested parameter playing a crucial role on the enrichment.