Comprehensive LC-PAD/MS Analysis of N-glycans using SweetSep™ HPAEC Column

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Abstract

In the field of glycomics, understanding the functional aspects and biological implications of glycoproteins requires a comprehensive understanding of their structure, for example, the glycosylation sites or the type of glycan moieties. Despite the significance of these insights, the analysis of glycans in glycoproteins presents a major challenge due to the vast number of different glycan isomers present in glycoproteins, each with varying abundances. Therefore, to improve the structure elucidation workflow in glycomics, there is a need for the development of analytical methods that enable the separation, detection, and quantification of glycans from glycoproteins.

The typical approach for glycan analysis of glycoproteins involves enzymatic release of the glycans from the glycoproteins, followed by chemical derivatization of the glycans. Subsequently, the mixture of glycans can be separated and identified using liquid chromatography such as HILIC with fluorescence and MS detection. High-performance anion exchange chromatography combined with pulsed amperometric detection (HPAEC-PAD) is an alternative analytical technique that allows for the separation and detection of glycans without the need for derivatization. When coupled with MS, HPAEC-PAD becomes a powerful analytical workflow for the high-resolution separation, sensitive detection, quantification, and accurate identification of glycans from glycoproteins.

In our study, HPAEC-PAD/MS was conducted using a SweetSep™ AEX200 anion-exchange column. The AEX200 stationary phase is based on a highly monodisperse 5 µm resin poly(divinylbenzene-co-ethylvinylbenzene) coated with quaternary amine functionalized latex nanoparticles (crosslinking degree of 80%). This stationary phase has a high ion-exchange capacity of 86 µeq per 200×4 mm ID column.

Several glycan standards were successfully separated using HPAEC at high pH using NaOH/NaOAc, followed by parallel PAD/MS detection using post-column flow splitting. Due to the non-volatile nature of NaOH/NaOAc, a desalter was employed for post-column removal of the sodium ion before MS detection. However, the use of a desalter may contribute to peak dispersion and thus loss in resolution. Therefore, an alternative anion-exchange separation approach was evaluated using a volatile buffer system that is typically used for MS, i.e. NH_4OH/NH_4OAc. The use of the volatile buffer eliminates the necessity of using a desalter, thus simplifying HPAEC-PAD/MS. The results highlight the potential of the SweetSep™ column for glycan separation using HPAEC-PAD/MS, enabling both quantification and identification of individual glycans.