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LC-ESI-MS OF CARBOHYDRATES USING GRAPHITISED CARBON

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The term glycosylation describes the enzymatic attachment of carbohydrates to biological macromolecules, in particular proteins, to form glycoproteins. Glycoslation, along with phosphorylation, are very commonly observed post-translational modifications of proteins – approximately one-third of all proteins produced by eukaryotes (animal and plant cells) are believed to be glycoproteins. Glycosylation fulfills a vast array of diverse biological roles, however with the exception of a handful of essential biological roles, the role of glycosylation is generally appreciated to be the “fine-tuning” of a glycoprotein’s structure and function. Although carbohydrates have been studied for many years by traditional chemistry, robust and sensitive methods for the analysis of carbohydrates in biological systems have only begun to emerge in the past two decades.

One of the major impediments for carbohydrate analysis is the presence of contaminants such as ionic salts, protein, and detergents. In the case of proteins and peptides, these problems have been largely overcome through the widespread use of hydrocarbon-based reversed-phase columns and cartridges. Here we describe the use of a graphitised carbon guard column for gradient liquid chromatography separations of carbohydrates in an analogous fashion to traditional reversed-phased LC-ESI-MS for peptides and proteins. This system allows for online desalting of carbohydrate mixtures using volatile, non-ionic solvents, as well as for the separation of different classes of oligosaccharides, including the separation of isobaric and linkage-isomeric carbohydrates, which are then analysed by ESI-TOF-MS. We have successfully used carbon-column LC-MS to facilitate the separation and analysis of released N-linked carbohydrates, -eliminated O-linked carbohydrates, as well as sulfated and phosphorylated O-linked carbohydrates from various glycoprotein sources. Data will also be presented from carbohydrates released and analysed from glycoproteins separated by 2-dimensional electrophoresis.