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The Analytical Challenges of Glycomics

Comprehensive analysis of protein glycosylation represents one of the most challenging bioanalytical tasks as glycans are very complex groups of molecules. The lack of chromophore / fluorophore moieties and, in many instances, easily ionizable groups usually require derivatization of carbohydrates before their analysis by high performance bioanalytical techniques, such as liquid chromatography, electrophoresis and mass spectrometry. Full structural elucidation of glycans also utilizes consecutive enzymatic digestion by an array of exoglycosidases, followed by LC or capillary electrophoresis separation of the digests. Hyphenation with offline weak anion exchange chromatography (WAX) fractionation is an additional powerful tool, especially to decipher highly sialylated complex sugars. This presentation will cover the state of the art of liquid phase separation methods for structural elucidation of protein glycosylation mostly focusing on capillary electrophoresis (CZE and CGE) but also including the main approaches of liquid chromatography and microfluidics methods, as well as their combination with mass spectrometry. Particular attention will be paid to the analysis of recombinant glycoprotein therapeutics (both innovative and biosimilars), a recently emerging class of very successful new generation drugs with the main goal to demonstrate structural and functional equivalence of the products. Comprehensive characterization of their glycosylation is crucial in the biotechnology and biopharmaceutical industry, especially in clone selection, process development and lot release.