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Combination of high resolution HILIC-UPLC-FLD-ESI-QTOF MS/MS with automated data processing for structure determination and quantification of proteins with complex glycan patterns

Optimal functionality and limitation of side effects of recombinant therapeutic proteins require a glycosylation profile that mimics the complexity of the natural human glycosylation as much as possible. As a result, the need for characterization of complex glycosylation patterns in R&D and regulatory environments is evident. The coupling of high resolution chromatography and mass spectrometry is the method of choice for analysis of both simple and complex glycan patterns. However, LC-MS/MS data of glycans are highly complex. Thus, manual data processing and evaluation are time consuming as well as error-prone. We developed an automated method to cope with intense glycoanalysis requirements in biopharmaceutical characterization. We applied MS-sensitive fluorescence labeling of liberated glycans from biopharmaceuticals and high resolution chromatography with fluorescence detection in line coupled to tandem mass spectrometry (HILIC-UPLC-FLD-ESI-QTOF MS/MS). This approach facilitates the unambiguous identification and reasonable quantification of biologically important N-glycan parameters like antennarity, sialic acid, bisecting N-acetylglucosamine, core or outer-arm fucose and glycan sulfation optionally in GxP quality. We developed software that automatically provides such parameters and minimizes user intervention. Automated Bruker QTOF (Compact/Impact II) data evaluation using script based software allowed an evolution of the automated analysis, which facilitated the hybridization of fluorescence and MS data and empirical MS/MS spectra library matching for automatic structure identification and fluorescence based quantification of glycan structures. The feasibility of the method was successfully proven for biopharmaceuticals resulting from Glycotope's human cell lines for production of recombinant proteins with complex glycosylation with improvements in sialylation, galactosylation, fucosylation, antennarity which results in higher bioactivity, stability, serum half-life time and reduced immunogenicity. Even for glycoproteins comprising over 60 individual glycan structures, the complete identification and quantification starting from approximately 10 µg of glycoprotein is available within one day in an automated workflow.

Biography

Sven Bahrke is a leading Scientist at Glycotope GmbH since 2007. As a Specialist in Glycobiology, he was responsible for the establishment of the Glyco-analytic Department, where he implemented all necessary technologies for high standard glycan profiling as well as for MS based protein analysis. Before the appointment at Glycotope, he studied Chemistry at the University of Potsdam and gained experience in the fields of glycobiology and instrumental analytics during his PhD and Postdoctoral Fellowship in the Departments of Chemistry and Biochemistry as well as at the Interdisciplinary Center for Mass Spectrometry, Berlin.

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