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SITE-SPECIFIC GLYCAN ANALYSIS OF PROTEINS IN CELL CULTURE CONDITIONED MEDIA AND SUB-CELLULAR FRACTIONS BY LC-MS/MS FOR UNDERSTANDING THE IMPACT OF PROCESS CONDITIONS ON *N*-GLYCOSYLATION

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Protein glycosylation, which involves the attachment of sugar residues to proteins, is an important post-translational modification that can influence the structure, pharmacological activity and stability of therapeutic proteins. The mechanisms by which cells modify and process these sugars therefore needs to be well understood and tightly controlled during protein production to ensure consistent product quality. Typically, glycosylation patterns are determined for the secreted therapeutic protein found in the conditioned media (CM). However, since glycosylation occurs through enzymatic reactions within the cellular endomembrane system, the glycosylation profile of the therapeutic protein in different intracellular compartments can also provide valuable insight into the static and dynamic properties of the cell culture system that impact protein glycosylation. This study focuses on determination of glycosylation patterns in CM and different sub-cellular fractions for three distinct *N*-glycosylation sites of a therapeutic Fc-fusion protein, by applying the Selected Reaction Monitoring (SRM) approach coupled with liquid chromatography-tandem mass spectrometry (LC-MS/MS). SRM is a rapidly evolving technique for the reliable quantification of low abundance glycopeptides, since it reduces noise and enhances signal intensity by monitoring a select number of predetermined transitions. This mode of analysis was successfully applied for comprehensive site-specific glycan profiling of the therapeutic protein from CM and sub-cellular fractions during CHO cell culture, providing an overview of intermediate and final glycan species formed during the glycosylation process. The distinct glycan distribution patterns for the Fc-fusion protein in CM and different sub-cellular fractions, revealed through this effort, provide a window for understanding the impact of cell culture conditions on the glycosylation pathway, and possibly identify the bottlenecks in generation of glycan species of interest.