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National Institute for Bioprocessing Research and Training

Contract Research

CASE STUDY

N-glycan characterization of a complex biotherapeutic before and after process change to comply with ICH Q5E guidelines

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Challenge

A client requested NIBRT Contract Research to perform *N*-glycan characterisation of pre- and post-change batches following a change in their working cell bank (WCB). ICH Q5E guidelines describe the principles for assessing the comparability of biotechnological/biological products before and after process change. For glycoproteins, these requirements state that *'... the carbohydrate content is determined. In addition, the structure of the carbohydrate chains, the oligosaccharide pattern is analysed, to the extent possible.'* Glycosylation is considered a critical quality attribute (CQA) and therefore must be assessed to ensure the required product quality, safety, and efficacy is achieved. Glycans exhibit multiple levels of heterogeneity, this coupled with the isobaric nature of glycans pose a significant analytical challenge.

Solution

A released glycan approach was employed to determine glycan structure and abundance. *N*-glycans were enzymatically cleaved and fluorescently labelled to increase detection sensitivity. Following sample preparation, a combination of technologies were used in order to obtain the highest possible level of structural information. Hydrophilic interaction liquid chromatography (HILIC) separation allowed for the high-resolution separation of the complex glycan profile separating isomeric, charged, and branching variations of glycans. Glucose units (GU) were generated using a dextran ladder standard to normalise retention time and to facilitate data analysis. Exoglycosidase sequencing elucidated the linkage of monosaccharides. Exoglycosidase enzymes cleave a terminal monosaccharide with a specific glycosidic linkage. The characteristic GU shift in the HILIC profile was used to interpret the data and elucidate the glycan sequence. Weak anion exchange (WAX) chromatography, which separates glycans on the basis of charge, allowed for the relative quantitation of charged glycans. 2D-LC WAX-HILIC reduced complexity of the highly complex glycosylation profile. Liquid chromatography–mass spectrometry (LC-MS) with fluorescence detection confirmed the elucidated glycan compositions. Data from all sources was interpreted to assign glycan structures leveraging on the experience of the subject matter expert (SME) analyst.

Outcome

A comprehensive report was provided to the client detailing the *N*-glycan characterisation in the pre- and post change samples. Confident assignments of glycan structures including linkage information were reported for each peak alongside peak abundance. Critical features such as abundance of charged species and abundance of immunogenic alpha-gal epitope were also reported. Data from the analysis was included in the client dossier of data which demonstrated that product quality had not been impacted by the change in WCB.

Project Process

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