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National Institute for Bioprocessing Research and Training

Contract Research

CASE STUDY

Development of a cIEF method to determine the charge isoform distribution of a complex biotherapeutic which exhibits a low pI

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Challenge

A client requested that NIBRT Contract Research develop a method to determine the charge isoform distribution of their complex biotherapeutic which exhibited a low isoelectric point (pI) and several glycosylation sites. The analysis of charged isoform pattern is a regulatory requirement in accordance with the ICH guidelines (ICH Q6B). The associated pI of the biotherapeutic can be altered by post translational modifications (PTMs) such as deamidation, oxidation and glycosylation. The pI of the biotherapeutic can also become chemically modified during the purification process and sample storage. Charge variants can have an effect on safety, quality and efficacy of a biotherapeutic therefore it is critical that comprehensive characterization is performed.

Solution

NIBRT Contract Research developed a capillary isoelectric focusing (cIEF) method to assess and quantify the charge isoform distribution in the biotherapeutic. Initially appropriate pI markers were selected and evaluated to assess their suitability as bracketing markers to the pIs exhibited by the charge variants in the sample. During method development, a panel of experiments was performed to achieve optimal separation conditions, including varying the concentration of urea, IDA and L argenine and altering the ratio of pharmalytes (ampholytes). A calibration curve was constructed using the known pI of the markers and the migration time in order to extrapolate the pIs exhibited by the biotherapeutic. Once optimal conditions had been determined, method qualification was performed by assessing method specificity, accuracy, precision, linearity, limit of detection (LOD), limit of quantification (LOQ) and range. Following successful method qualification, drug product samples from different batches were analysed to compare the charge isoform distribution.

Outcome

A comprehensive report was provided to the client detailing the cIEF method details, qualification data, and results from the sample analysis. The pI and abundance of each charge variant was reported. The data from this analysis enabled the client to assess any batch-to-batch variation in charge isoform distribution during development.

Project Process

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