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National Institute for Bioprocessing Research and Training

Contract Research

CASE STUDY

A Quality by Design (QbD) approach: Development and qualification of a method for critical quality attribute (CQA) monitoring during biotherapeutic product development

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Challenge

A client requested NIBRT Contract Research develop and qualify a robust method to quantify the proportion of sialic acid and mannose-6-phosphate charged *N*-glycans on a glycoprotein biotherapeutic. This developed method would serve towards establishing QbD into the client's product development and manufacturing workflow. The presence of mannose-6-phosphate is crucial to therapeutic targeting, and therefore, makes an ideal quality attribute to monitor as part of the clients control strategy to maintain product consistency.

Solution

A new workflow was developed based on existing NIBRT *N*-glycan testing protocols. Glycans were enzymatically cleaved from the glycoprotein and labelled with a fluorescent 2-AB label. Both sialic acids and mannose-6-phosphate exhibit a negative charge, therefore, weak anion exchange (WAX) HPLC with fluorescent detection was chosen. WAX analysis separates glycans by charge, thereby allowing relative quantitation of charged glycans present. Phosphatase digested glycans were analysed by WAX-HPLC for relative quantitation of sialic acids, and sialidase digested glycans analysed by WAX-HPLC, for relative quantitation of mannose-6-phosphate.

For proof of concept, an in-house *N*-glycan release workflow was utilised and adapted to ensure that the sample preparation procedure was robust and appropriate for use in a QC laboratory. A key feature of this workflow included reducing sample preparation time by 50%. This reduction in procedural time was achieved through incorporating a Rapid™ PNGase F enzymatic digestion and minimising sample drying times. Moreover, digestion volumes were scaled up to avoid pipetting very small volumes, in line with regulatory guidelines. Once the method had been optimised, method verification was performed by assessing the parameters; specificity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ), range and robustness.

Outcome

A comprehensive report was provided to the client detailing the development and qualification of the method. The results provided the client with confidence that the method could be successfully transferred and fully qualified in their own QC lab.

Project Process

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