nibrt
National Institute for Bioprocessing Research and Training

Contract Research

CASE STUDY

Development and qualification of an improved method to quantify NANA and NGNA sialic acids present on a therapeutic glycoprotein

CASE STUDY

Development and qualification of an improved method to quantify NANA and NGNA sialic acids present on a therapeutic glycoprotein

Challenge

A client requested NIBRT Contract Research develop a new method for the quantification of *N*-acetylneuraminic acid (NANA) and *N*-glycolylneuraminic acid (NGNA) sialic acids on a therapeutic glycoprotein product. The client believed that their original method, which used a sialidase enzyme to cleave sialic acids from the glycoprotein, was inefficient, and the cause of a high degree of variability in their results. As such, the existing client method did not meet the performance criteria for regulated work due to low reproducibility.

Solution

NIBRT Contract Research developed a new method, based on a commercially available kit, which uses mild acid hydrolysis to release the sialic acids from the glycoprotein, which are subsequently labelled with a fluorescent dye and analysed by UPLC-FLR. NIBRT Contract Research worked closely with the client to develop a bespoke strategy for optimising each aspect of the method. Ongoing updates were provided, and method optimisation discussed as relevant, to ensure the maximum amount of information could be gathered for each experiment. During the acid hydrolysis step, the team carried out a panel of experiments to achieve optimum experimental conditions, including, varying the type and concentration of acid used.

An orthogonal method was also used to verify the level of sialic acid release, where portions of the hydrolysed samples were subjected to *N*-glycan release, followed by 2-AB labelling and Weak Anion Exchange (WAX) HPLC analysis. Comparison of hydrolysed 2-AB labelled samples to a non-hydrolysed 2-AB sample allowed determination of the level of sialic acids present. 2-AB labelling efficiency and selection of final conditions to use for method verification were determined by analysing data for different label volumes and incubation times. Once optimised conditions were set, method verification was performed by assessing the parameters; specificity, accuracy, precision, linearity, limit of detection (LOD), limit of quantification (LOQ), range and robustness. The robustness experiments also included assessment of different lot numbers of kit components.

Outcome

A comprehensive report was provided to the client detailing the successful development and qualification of a more robust sialic acid quantification method. The newly developed method showed greatly improved performance characteristics compared to the client's pre-existing method. These results provided the client with confidence that the new method could be successfully transferred and fully qualified in their own QC lab.

Project Process

nibrt
National Institute for Bioprocessing Research and Training

Contact Us

Tel: +353 1 215 8100 Email: contract.research@nibrt.ie
Web: www.nibrt.ie/contract-research /
Foster Avenue, Mount Merrion, Blackrock, Co. Dublin, A94 X099, Ireland