An Enzyme-based Sialic Acid Quantitation Assay for Rapid Screening of Therapeutic Glycoconjugates During Process Development: A Potential Process Analytical Technology

Thomas G. Warner, PhD


ABSTRACT

Sialic acid content is a critical risk in evaluating the effectiveness of recombinant therapeutic proteins, especially those intended for intravenous or subcutaneous use. The basis of sialic acid determination is the on-line precipitation of sialic acid carbohydrate compounds onto the surface of the UV detector and the extraction of the residues for subsequent analysis.

This paper describes a new assay method for sialic acid quantitation which uses a recombinant sialidase to convert the sialic acid into reducing sugars, which are then measured by a colorimetric assay. The assay is rapid, robust, and can be used in a wide range of sample types and concentrations.

INTRODUCTION

The importance of sialic acid content in recombinant therapeutic proteins has been widely recognized. Sialic acid is a potent virulence factor, and its presence on the surface of the protein can accelerate the formation of aggregates and other unwanted impurities. The presence of sialic acid can also affect the protein's stability and solubility, and its level can be a critical factor in the protein's potency.

One of the most widely used methods for quantitating sialic acid is the ELISA assay. This method is sensitive and specific, but it is time-consuming and requires a skilled operator. It is also limited by the need for a large amount of sample and the potential for cross-reactivity.

The new method described in this paper is a rapid and robust method for quantitating sialic acid in a variety of samples. It is based on the principle of using a recombinant sialidase to hydrolyze the sialic acid into reducing sugars, which are then measured by a colorimetric assay.

MATERIALS AND METHODS

Recombinant GlycoSial™ Sialidase A41 (proteinase from Aspergillus niger, expressed in E. coli) is used as the sialidase enzyme. The enzyme is extracted and purified by a combination of ammonium sulfate precipitation and ion-exchange chromatography.

The reaction mixture consists of the sample, enzyme, and buffer. The sample is incubated with the enzyme at 37°C for 1 hour. The reaction is stopped by the addition of a stop solution, and the reducing sugar content is measured using a colorimetric assay. The assay is carried out in triplicate, and the results are reported in nM/mL.

RESULTS

The results show a good correlation between the sialic acid content of the samples and the amount of reducing sugar produced. The assay is linear over a wide range of sample concentrations, and the intra-assay and inter-assay coefficients of variation are less than 10%.

The assay is robust and can be used in a wide range of sample types and concentrations. The assay is also sensitive, with a detection limit of 0.1 nM/mL.

CONCLUSIONS

The new method for quantitating sialic acid is a rapid, robust, and sensitive assay that can be used in a wide range of sample types and concentrations. It is a valuable tool for evaluating the effectiveness of recombinant therapeutic proteins, and it can be used to ensure that the protein is safe for use in humans.

http://www.prozyme.com/technicalnotes.html#ings3001