Rapid Glycoprotein Sialic Acid Determination by High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection

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Overview

Purpose: To develop a high-speed, direct chromatographic method for the rapid detection and quantitation of sialic acids in glycoprotein samples.

Methods: Anion-exchange chromatography with pulsed amperometric detection (PAD) was used to quantify sialic acids in glycoprotein samples. PAD analysis was performed on a Dionex CarboPac PA20 ion-exchange column with a Dionex IONPAC AG120-AE anion-exchange guard column. Samples were hydrolyzed using a method developed by the Varki laboratory. The method is designed to quickly and accurately determine the amounts of Neu5Ac and Neu5Gc in glycoprotein samples.

Results: The method provided rapid analysis of sialic acids, allowing for the determination of sialic acids in as little as 1 minute per sample. The method was highly precise and accurate, with repeatability of ±0.12 pmol and recovery ranging from 94% to 106% for Neu5Ac and Neu5Gc, respectively.

Introduction

Due to the biological role of glycoproteins in disease processes, the analysis of sialic acids in biological fluids is important. This is especially true for glycoproteins found in therapeutic protein products. Although over 50 natural sialic acids have been identified, two forms, Neu5Ac and Neu5Gc, are commonly determined in therapeutic glycoproteins. This is due to their potential to generate large numbers of samples. Supporting this process requires high-throughput analysis to allow quick transition from early research studies to the clinical stage of development. Therefore, the use of selected methods, such as high-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS), have been identified to have time, limits, and needs for optimization.

Methods

Sample Preparation

Human transferrin (a. transferrin), bovine apo-transferrin (b. apo-transferrin), human transferrin (h. transferrin), sheep fetuin (f. fetuin), and rat prothrombin (r. prothrombin) were used as the glycoproteins for sample preparation. The proteins were hydrolyzed by the method of Varki et al. and diluted in DI water 1/100. Samples were injected within 24 hours of hydrolysis.

Results

Figure 1. Sialic acids (neuraminacids)

Quantitative analysis of Neu5Ac and Neu5Gc was performed using a Dionex CarboPac PA20 ion-exchange column with a Dionex IONPAC AG120-AE anion-exchange guard column. The method was validated for linearity, precision, and accuracy. The linear range for Neu5Ac was 1–100 pmol, with a coefficient of determination of 0.999. The linear range for Neu5Gc was 1–100 pmol, with a coefficient of determination of 0.998.

Conclusion

The developed method is rapid, accurate, and highly precise for the determination of sialic acids in glycoprotein samples. This method is suitable for therapeutic protein product characterization and early research studies.

References