

Simultaneous Quantitation of Mannose, Glucose, Fructose, and Galactose in Human Plasma and Serum by LC-MS/MS

Ryan Schmich¹; Sharon DeChenne¹; Chad Christianson¹; Jennifer Zimmer¹

¹Alturas Analytics Inc., Moscow, Idaho

Introduction

Monosaccharides such as mannose, glucose, fructose, and galactose in human plasma and serum can be biomarkers for diseases such as diabetes, cancer, and various metabolic disorders. LC-MS/MS analysis offers a sensitive and robust method for simultaneous quantitation of mannose, glucose, fructose, and galactose. These small, hydrophilic sugars are well suited to HILIC chromatography. Since the molecular weights are identical and there are no unique MS fragments for these sugars, chromatographic resolution is the only method of differentiating between them for LC-MS/MS analysis.

Methods

Plasma and serum samples were extracted with a simple protein precipitation method, with the final extract consisting of at least 75% water due to the hydrophilic nature of these compounds. Since mannose, glucose, fructose, and galactose are endogenous in human plasma and serum, surrogate matrix was evaluated for preparation of the calibrators and QCs for accurate detection of endogenous sugar levels.

The extract was injected onto a HILIC chromatographic column for sugar separation prior to analysis on an AB Sciex 6500+. The AB Sciex 6500+ showed improved sensitivity over previous methods utilizing less sensitive platforms.

Preliminary Data

Mannose, glucose, fructose, and galactose are known to be endogenous in human plasma and serum in varying levels. This presents challenges for finding a suitable control matrix with a true zero concentration, in order to accurately assess baseline levels of the sugars in plasma or serum. To overcome these challenges, a surrogate matrix was investigated as the control matrix. Due to the hydrophilic nature of these compounds, solubility issues or non-specific binding of these sugars was expected if the injection solvent was <50% water in the final 96-well plate prior to injection on the LC-MS/MS system. Therefore it was determined that the final injection solution should consist of at least 75% water.

Sufficient resolution of the four underivatized sugars presented a significant challenge due to the physical similarities. Several chromatographic columns were evaluated for sufficient resolution of the sugars.

Calibrator and QC results showed good quantitation for typical regulated LC-MS/MS methods per FDA guidance. The back-calculated accuracy of the calibrators and QCs was within 15% of the nominal concentration (20% at the LLOQ level), and the %CV was within 15% (20% at the LLOQ level).

Novel Aspect

Simultaneous quantitation of mannose, glucose, fructose, and galactose by LC-MS/MS without derivatization.