

Temperature Effects on Invert Sugar Analysis

Application Note

Food and Pharmaceutical

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Introduction

Invert sugar is produced by the action of the glycoside hydrolase enzyme invertase or an acid on sucrose, which splits each sucrose disaccharide molecule into its component glucose and fructose monomer molecules, one of each. In practical terms, measured on equivalent dissolved weights, invert syrups are sweeter than sucrose solutions.

The separation of invert sugar on an Agilent Hi-Plex Ca column is affected by changes in operating temperature. According to the USP, this separation should be run at 40 °C. However, at this temperature, the peak shape of glucose is particularly poor.



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Experimental

A standard solution containing the main components of invert sugar was made to contain 20 mg/mL of glucose and fructose and 10 mg/mL of sucrose. Ten μL of the solution were injected at temperatures ranging from 40 °C to 85 °C.

Conditions

Column Agilent Hi-Plex Ca, 7.7 x 300 mm, 8 μm (p/n PL1170-6810)
Mobile phase 100% DI H_2O
Flow rate 0.4 mL/min
Temperature Various
Detector RI

Results and Discussion

At 40 °C, the peak for glucose was split and maintained this shape all the way up to 60 °C. This could be a result of continuous interconversion of the glucose molecules between open-chain and cyclic form, in addition to α - and β -ring structures. At these temperatures, the rate of interconversion is slow enough to be detected on the RI. At higher temperatures ≥ 65 °C, where the speed of interconversion is much greater, a single glucose peak is eluted. For all of the sugars, peak width decreases and efficiency increases continuously to 85 °C. At 85 °C, the resolution between the sugar molecules would allow the flow rate to be increased to give a faster separation.

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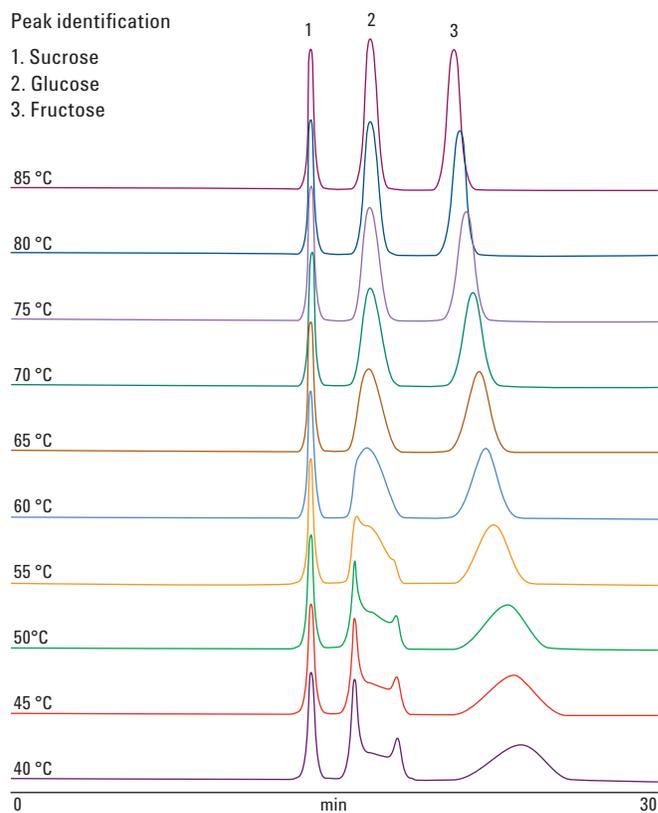


Figure 1. Raw data chromatograms showing the main components of invert sugar on an Agilent Hi-Plex Ca column at different operating temperatures.

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