HPLC Analysis of Sugars and Glycoproteins in Biological Systems

Application Note

Bioanalysis

Author

Linda Lloyd

Agilent Technologies, Inc.

Introduction

Free sugars, other than glucose and fructose, rarely occur in nature. However, other sugars such as the pentoses (arabinose, xylose, and ribose) and the hexoses (mannose and galactose) are found as integral parts of biological macromolecules. The function of the carbohydrate components of these macromolecules is diverse; they can be important either biochemically, for example targeting sequences in glycoproteins, or structurally, as in DNA and RNA. The sequences of monosaccharides are only obtained as breakdown products during fermentation.

Levels of lactate and pyruvate found in blood serum are important indicators of health. Elevated blood lactate levels are indicative of diabetes, and pyruvate of possible heavy metal poisoning. It is important to reduce sample handling to a minimum during analysis of complex biological fluids. Because samples can often be analyzed directly using Agilent Hi-Plex H columns, the potential for errors is reduced. Hi-Plex columns are well suited to the fast analysis of sugars and glycoproteins in plant and animal tissues.

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Experimental

Instrumentation

Column	Agilent Hi-Plex H, 7.7 × 300 mm, 8 µm (p/n PL1170-6830)
Detector	RI

Materials and Reagents

Mobile phase	0.005 M H_2SO_4 (sugars), 0.0005 M H_2SO_4 (compounds of physiological significance)

Conditions

Flow rate	0.6 mL/min
Temperatures	60 °C (sugars), 55 °C (compounds of physiological significance)

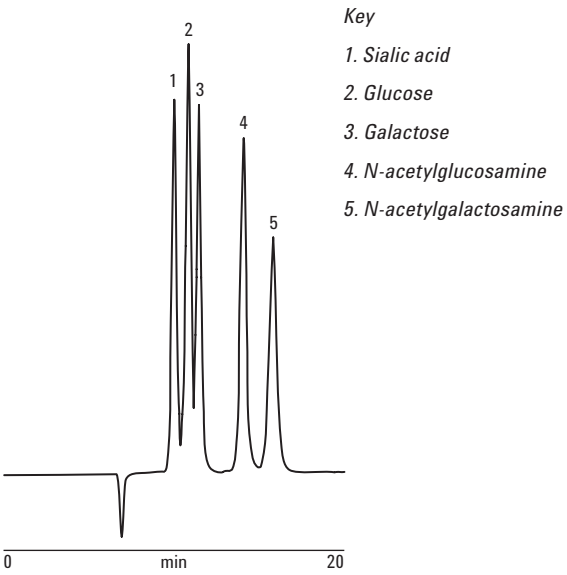

Figure 1. Good separation of glucose and galactose from their derivatives achieved by HPLC with Agilent Hi-Plex H columns.

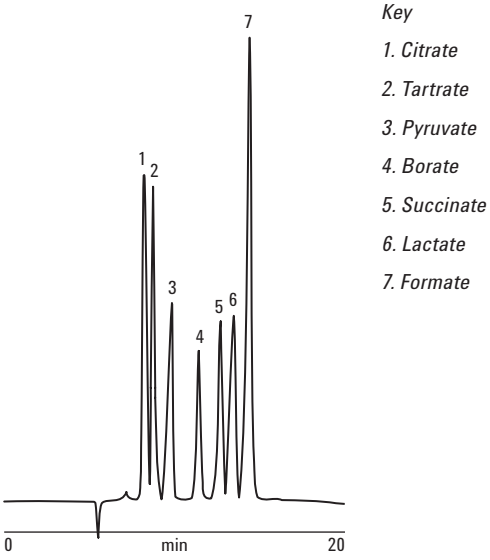

Figure 2. The presence of lactate and pyruvate revealed by HPLC with Agilent Hi-Plex H columns.

Results and Discussion

Figure 1 shows how Agilent Hi-Plex H columns separate glucose- and galactose-free sugars from their derivatives N-acetylglucosamine and N-acetylgalactosamine. The latter are found in the cell membranes of higher organisms as carbohydrate residues, linking the amino acid chain to the carbohydrate component of membrane glycoproteins. Figure 2 shows good resolution of lactate and pyruvate from other salts found in animal tissues.

Conclusion

Agilent Hi-Plex columns are packed with sulfonated resin, giving a fundamental improvement in performance to overcome the problems of low efficiencies and high back-pressures encountered with soft gels. The columns are available in hydrogen form for fast analysis of glycoproteins and sugars in biological systems. Accurate determination of composition and content is ensured.

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