CAPSULES
preliminary notes and applications from Bioanalytical Systems, Inc.

Determination of Reducing Sugars in Serum, Urine, and Edibles

Purpose

Determination of reducing sugars (carbohydrates) in serum, urine, and edible products.

Serum and urine generally contain an overwhelming amount of glucose relative to other sugars. The amounts of these minor carbohydrates are not usually determined, due to a lack in sensitivity and selectivity of existing methods. The instability and corrosive nature of the reagents used in the exisiting techniques is also a problem. Determination of the distribution profile of minor sugars may provide useful information as regards the diagnosis and treatment of various disease states.

Existing Methods

Following LC separation, sugars can be detected directly using a refractive index detector or reacted with appropriate reagents to form colored or fluorescent products. Measurement of sugars by changes in the refractive index and by colorimetric methods lacks sensitivity and specificity. The fluorimetric method has a reported detection limit of 2 ng.

Reference

Amperometric Detection of Reducing Carbohydrates in High-Performance Liquid Chromatography Using an Amino-Bonded Column and Acetonitrile-Water as the Eluent, N. Watanabe, J. Chromatogr. 330(1985) 333-338.

Conditions

Detector: BAS LC-4B/17A (P/N, MF-9094)
Electrode: Glassy Carbon
Potential: +0.04 V vs. Ag/AgCl
Column: 5 µm amino-bonded silica, 250 x 4.6 mm TSK gel NH_2 - 60, Toyo Soda, Tokyo, Japan)
Mobile Phase: 70% acetonitrile, 30% water. Flow rate was 0.35 mL/min. Oxygen was removed by outgassing with added nitrogen gas.

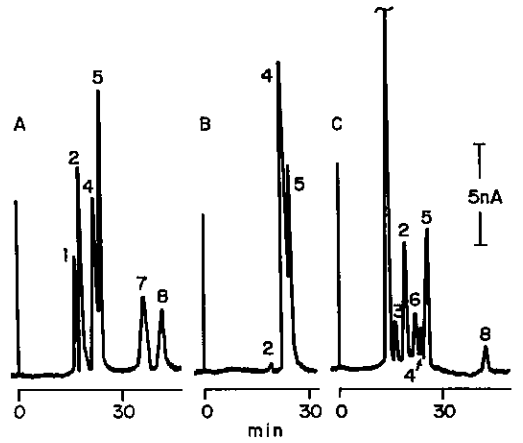


Figure 1. Chromatograms of A) mixture of standard carbohydrates, B) apple juice diluted 1:20,000, and C) human urine diluted 1:50. Peaks: 1 = rhamnose, 2 = xylose, 3 = ribose, 4 = fructose, 5 = glucose, 6 = arabinose, 7 = maltose, 8 = lactose. Redrawn from above-cited reference.

Post Column: Reagent solution 0.1 M Na_2HPO_4, pH 10.9, containing 1 mM copper bis(phenanthroline) (CBP), infused at 0.8 mL/min. The mixture of column effluent and reagent solution reacts during passage through a Teflon coil maintained at 98.5°C. Oxygen was removed from the reagent solution by passage through a Teflon tube kept under vaccum.
Detection Limit: 5 pmol for glucose, 0.05 ppm. concentration, 0.9 ng injected in 20 µL.
Linear Range: 5 pmol to 2 nmol for all sugars tested.

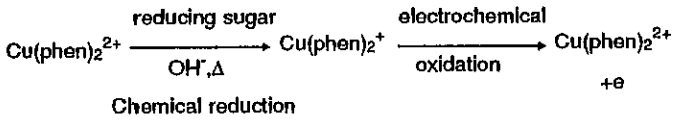
Sample Preparation

Deproteinization by mixing with an equal volume of acetonitrile followed by centrifugation.

Comments

The reducing ability of carbohydrates is coupled with the redox reaction of CBP as mediator. CBP,

reduced by carbohydrates in alkaline solution at elevated temperature in a post-column reaction, is oxidized at the surface of a glassy carbon electrode. Detection scheme:



Several advantages are realized with this detection scheme. The method exhibits greater sensitivity for reducing sugars than other published procedures. Selectivity is excellent, resulting from an optimized post-column reaction coupled to the added selectivity of the electrochemical reaction. Sample preparation is simple and the reagent solution is stable for long-term operation. The method is universally applicable to all of the separation modes for carbohydrates.

Related References

1. N. Watanabe, and M. Inoue, Anal. Chem. 55(1983) 1016-1019. Reported detection limit for glucose was 1 pmol (0.2 ng).

2. N. Watanabe, G. Toda, and Y. Ikeda, Bunseki Kagaku (Jap. Anal.) 33(1984) E241-E248.

3. The results presented in this report can be duplicated utilizing a BAS 200 Problem Solver, or a BAS LC-400 Analyzer.

The information in this publication is supplied as a service to our customers. Performance of the methodology has not necessarily been verified by the BAS technical staff. However, Lee Elrod of BAS Analytics, has experimented with Dr. Watanabe's method and finds that it works well. Dr. Watanabe is a good friend of BAS in Japan. We are pleased to acknowledge this excellent contribution.