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Monosaccharide Analysis of Serum

INTRODUCTION

The use of high performance anion-exchange chromatography coupled with pulsed amperometric detection (HPAE-PAD) has facilitated the quantification of monosaccharides without prior derivatization.¹ The CarboPac PA1 column is a strong anion-exchange column that separates monosaccharides at high pH while pulsed amperometric detection provides sensitive and specific detection, eliminating interference from most noncarbohydrate matrix components. The analysis of monosaccharides in serum has long been necessary for the assay of certain disease states and the effect of certain pharmaceutical products. During the xylose absorption test, which is administered to evaluate small intestine absorption, the amount of xylose in serum is determined. If there is a bacterial infection of the small intestine, xylose is incompletely absorbed and is present in serum. This test has been reviewed by Craig and Atkinson.² The determination of galactose in serum is necessary when evaluating aldose reductase inhibitors (investigated as antidiabetic drugs) in galactosemic animal models.³ Monitoring blood glucose levels is important in the control and treatment of diabetes. Although simple colorimetric assays are used most often for routine glucose determination, the more specific HPAE-PAD is a useful confirmatory technique.

When determining monosaccharides other than glucose in serum, isocratic NaOH eluent concentrations can be varied over the range of 5 to 20 mM to obtain optimum resolution. A mobile phase of 14 mM NaOH

successfully resolved xylose from glucose (see Figure 1), although Griffin and Williams³ found that 9 mM NaOH was optimum for the separation of galactose and glucose. The separation of glucosamine from a 400-fold excess of glucose required 17 mM NaOH.

To maintain retention and resolution, a 15-min wash with 1.0 M NaOH at a flow rate of 1.0 mL/min is recommended every 5 to 10 injections. After reequilibration, retention and resolution should be verified with standards. Although sample preparation was not necessary for these analyses, if there are interfering peaks, serum protein should be removed. Protein removal can be accomplished with trichloroacetic acid precipitation followed by diethylether extraction, but there are a variety of other methods of protein removal that may better suit the application of interest.⁴

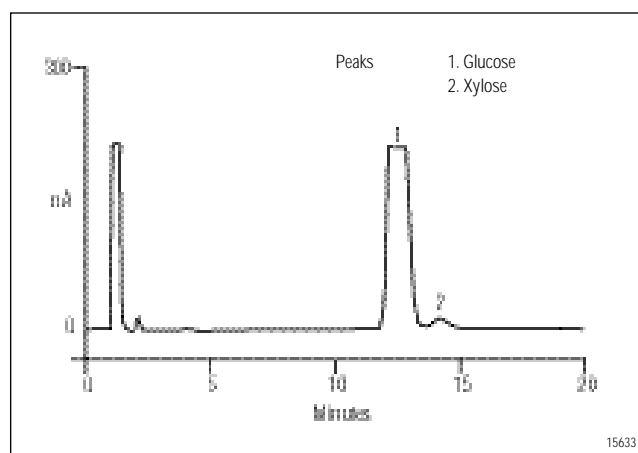


Figure 1. Xylose in serum.

This application employs an electrode working potential of -0.01 V rather than the 0.05 V normally used for carbohydrate detection. A lower working potential minimizes any noncarbohydrate interferents that might be oxidized at the higher potential. For additional information on HPAE-PAD, refer to Dionex Technical Note 20, "Analysis of Carbohydrates by HPAE-PAD."

EQUIPMENT

Any Dionex chromatographic system comprising:
Pulsed Amperometric Detector (PAD-2) or Pulsed Electrochemical Detector (PED) with a gold working electrode
Advanced Gradient Pump (AGP) or Gradient Pump Module (GPM)
Liquid Chromatography Module (LCM-2)
Eluent Degas Module (EDM-2)
Reagent Delivery Module (RDM)
AI-450 Chromatography Workstation or Integrator

REAGENTS AND STANDARDS

Sodium hydroxide solution (50% w/w, low carbonate)
D-Xylose Serum (e.g., Reference Serum, Sigma #R3626)

CONDITIONS

Column:	CarboPac PA1
Eluent:	14 mM NaOH
Sample	
Loop Volume:	25 µL
Flow Rate:	1.0 mL/min
Postcolumn Reagent:	0.3 M NaOH
Postcolumn Flow Rate:	0.5 mL/min
Detector Settings:	t(ms) E(Volt)*
	0–480 -0.01
	481–780 +0.60
	781–1020 0.60
Integration Period:	280–480 ms (PED only)
Sample:	Serum spiked with D-Xylose at 0.22 mM, diluted 10-fold with deionized water

PREPARATION OF SOLUTIONS AND REAGENTS

Eluent: 14 mM NaOH

Dilute 0.73 mL of sodium hydroxide solution (50% w/w, low carbonate) in 1.0 L of helium-sparged 18 M deionized water. Sodium hydroxide pellets will not produce a solution sufficiently low in carbonate unless first diluted to 50% and allowed to settle for several weeks.

Postcolumn Reagent: 0.3 M NaOH

Dilute 16 mL of sodium hydroxide solution (50% w/w, low carbonate) in 1 L of helium-sparged 18 M deionized water.

REFERENCES

1. Rocklin; R.D. and Pohl C.A., *J. Liq. Chromatogr.* **1983**, 6, 1577-1590.
2. Craig R.M. and A.J. Atkinson, Jr., *Gastroenterology* **1988**, 95, 223-231.
3. Griffin B.W. Williams G.W., *Biomed. Chromatogr.* **1990**, 4, 87-88.
4. Dawson; R.M.C., Elliot D.C., Elliot; W.H., Jones K.M. (Eds.), *Data for Biochemical Research*, 3rd ed., Oxford University Press: New York, **1986**; pp. 549-551.

*Potentials are referenced to Ag/AgCl.

