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Note

High-pressure liquid chromatography of polyhydric alcohols on silica gel columns

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Sequential periodate oxidation, borohydride reduction and acid hydrolysis of a polysaccharide leads to products commonly referred to as polyhydric alcohols. Previous work by Smith and co-workers[1,2] relates how these polyhydric alcohols can be assayed and the results used to complement methylation analyses in structural studies.

In this paper, a high-pressure liquid chromatographic (HPLC) method is described for the separation and identification of some polyhydric alcohols and related compounds. This method is also suitable for separating a large number of hydroxylated compounds not mentioned here.

EXPERIMENTAL

Apparatus and materials

A Waters Ass. (Framingham, Mass., U.S.A.) liquid chromatograph (Model 202) equipped with a 1000-p.s.i. pumping system and both ultraviolet and differential refractometer detectors was employed. All chromatograms were obtained at room temperature.

Samples of glycoaldehyde, ethylene glycol, glycerol and isoerythritol were obtained in a highly pure grade from both Sigma, St. Louis, Mo., U.S.A. and Matheson, Coleman and Bell, Baton Rouge, La., U.S.A. All carbohydrates and derivatives of carbohydrates assayed were purchased from Sigma.

Porasil A (75–125 μm silica gel) was obtained from Waters Ass.

Column preparation

A stainless-steel column, 2 ft. × 3/8 in. O.D., was clamped in a vertical position, and an external vibrator was attached. The column was vibrated continuously for several hours while silica gel was slowly introduced at the top. When completely packed, the column was attached to a solvent output line on the liquid chromatograph. The column was then allowed to purge overnight with an appropriate solvent in order to remove small foreign matter which would damage the ultraviolet and refractometer cells.

RESULTS AND DISCUSSION

A number of solvent systems were tried with the previously described silica gel column: the best resolution was obtained with a mixture of methyl ethyl ketone, water and acetone in the proportions 85:10:5. Fig. 1 shows the separation of glycoaldehyde from related polyhydric alcohols using this solvent system. The identity of the peaks was confirmed by collecting samples directly from the liquid chromatograph and analyzing them by gas–liquid chromatography (GLC). This GLC analysis followed a procedure previously described by Laver *et al.*[3].

The elution times of the polyhydric alcohols decreased as the number of hydroxyl groups decreased. It was also noted that by altering the proportion of water, the elution times of the compounds could be controlled (the greater the water content, the shorter was the elution time for all of the compounds).

For comparison, several other compounds were assayed on the silica gel column under identical conditions. Fig. 2 shows the chromatogram of these compounds.

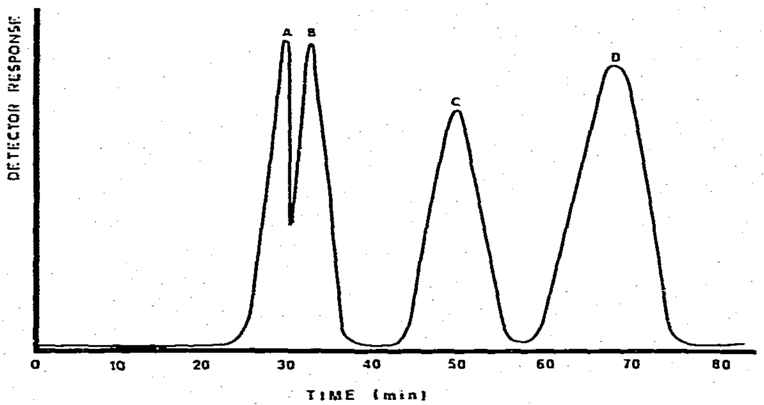

Fig. 1. HPLC refractive index chromatogram of related polyhydric alcohols. Column Porasil A (silica gel), 2 ft. × $^3/_8$ in. O.D.: solvent, methyl ethyl ketone–water–acetone (85:10:5): flow-rate, 2.1 ml/min. (A) glycoaldehyde: (B) ethylene glycol: (C) glycerol: (D) isoerythritol.

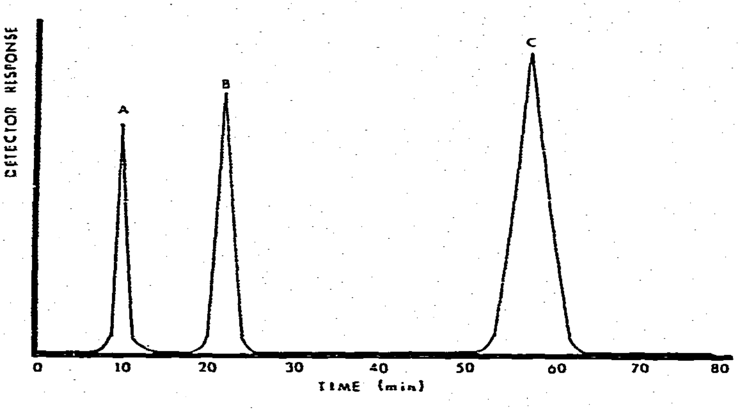

Fig. 2. HPLC refractive index chromatogram of (A) β-D-galactose pentaacetate, (B) phenyl-β-D-glucopyranoside, (C) methyl-α-D-glucopyranoside. Column Porasil A, 2 ft. × $^3/_8$ in. O.D.: solvent, methyl ethyl ketone–water–acetone (85:10:5); flow-rate, 2.1 ml/min.

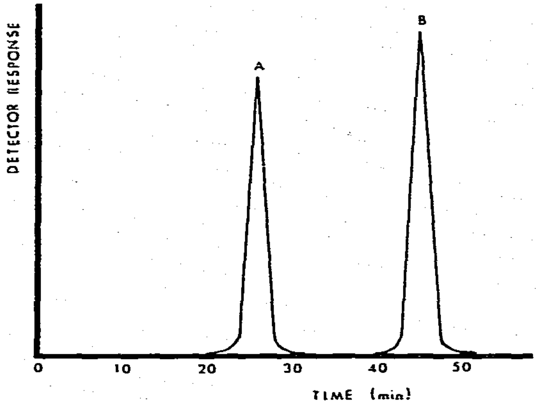

Fig. 3. HPLC refractive index chromatogram of (A) phenyl-β-D-glucopyranoside, (B) α-D-glucose. Column: Porasil A, 2 ft. × $^3/_8$ in. O.D.; solvent, ethyl acetate–2-propanol–water (50:35:25); flow-rate: 1.2 ml/min.

One other solvent system, ethyl acetate–2-propanol–water (50:35:25), appeared suitable for use with silica gel; however, the 2-propanol in this system increased the overall viscosity of the solvent mixture. This viscosity factor caused an increase in line pressure that was sufficient to reduce the flow-rates by nearly half. Fig. 3 shows the results of some initial work with this solvent system.

The technique discussed here has advantages over both thin-layer and GLC. Analyses are rapid with only negligible losses in sensitivity, and compounds can be assayed directly without any initial derivative formation (trimethylsilyl, acetyl or trifluoroacetyl).

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