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Note

Separation of some polyhydric alcohols by high-performance liquid chromatography

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The separation of polyhydric alcohols by various types of chromatography has been reported by several authors. Thin-layer chromatography on various adsorbents, with use of such complexing agents as boric acid[1], ammonium borate[2] and lead(II) in alkaline medium[3], has been applied to separations of mannitol and sorbitol in the presence of other polyhydric alcohols and sugars[4–8]. Ion-exchange resins can be used for the partition chromatography of polyhydric alcohols, retention of a polar non-electrolytic compound occurring by partition between adsorbed water at the surface of the resin and the mobile phase; separation have been described by Samuelson[9] and others[10–13].

Recently, I have shown the feasibility of separating some polyhydric alcohols on silica gel to which aminoalkyl groups had been chemically bonded, by using water–acetonitrile as mobile phase[14]. This normal-phase partition system gave good separations of many polyhydric alcohols, but could not be used to separate xylitol from arabitol (which is necessary in the analysis of cellulose hydrolysates).

The detection of small amounts of compounds with a differential refractometer poses serious problems. By forming the nitrobenzoate derivative, as described by Nachtmann *et al.*[15,16], I was able to improve the sensitivity by a factor of several thousand.

EXPERIMENTAL

The apparatus and column systems were as described previously[14,17,18]; separation parameters are given in the legends to the chromatograms.

Materials

The columns were packed with Aminex Q-15-S (Bio-Rad Labs., Richmond, Calif., U.S.A.), LiChrosorb SI-60, 5 μm (Merck, Darmstadt, G.F.R.) or an aminopropylsilica gel[14].

Reagents

4-Nitrobenzoyl chloride puriss.p.a. (Fluka, Buchs, Switzerland) was twice recrystallized from *n*-pentane puriss.p.a. (Merck); pyridine puriss.p.a. (Fluka) was refluxed for 2 h over potassium hydroxide puriss.p.a. (Fluka), distilled at 115° and

stored over potassium hydroxide tablets. It is essential for trouble-free derivatization that these reagents be as pure as possible. 4-Dimethylaminopyridine purum (Fluka), sodium hydrogen carborate Ph.H.VI (Siegfried, Zofingen, Switzerland), sodium carbonate puriss.p.a. (Fluka), chloroform for UV spectroscopy (Fluka), *n*-hexane purum (Merck), dichloromethane puriss.p.a. (Merck) and Uvasol acetonitrile (Merck) were used without further purification.

Standards

The polyhydric alcohol standards were obtained in highly pure grades from Fluka and from Sigma (St. Louis, Mo., U.S.A.).

Derivatization

The polyhydric alcohols were dissolved in pyridine (the concentration of this sample solution should not exceed 5 mg/ml), 50 μl of this solution were placed in a 10-ml round-bottomed flask, and 150 μl of a fresh solution prepared by dissolving 100 mg of 4-nitrobenzoyl chloride in 1 ml of pyridine were added. The mixture was well shaken and allowed to react for 10 min at room temperature, then pyridine was removed by heating at 80° *in vacuo* (water pump), and the residue was dried under high vacuum; special attention must be paid to this step, as any trace of pyridine will give rise to a group of peaks that can interfere with the separation of the derivatives. The pyridine-free residue was dissolved in 2 ml of a solution of 250 mg of 4-dimethylaminopyridine in 100 ml of 5% aqueous sodium carbonate in order to hydrolyze the excess of reagent. The solution, which was usually turbid, was well shaken and kept at room temperature for 10 min, then extracted with 2 ml of chloroform, and the extract was washed once with 2 ml of 5% aqueous sodium hydrogen carbonate and twice with 3 ml of 0.05 *N* hydrochloric acid containing 5% of sodium chloride (*cf.* Nachtmann *et al.*[16]). A portion of the chloroform extract was directly injected into the liquid chromatograph.

RESULTS AND DISCUSSION

Separation systems

The separation of a few glycols and sugar alcohols on an aminoalkylsilica gel column is shown in Fig. 1; this system provides rapid separation at room temperature, and both aqueous and alcoholic samples can be analyzed directly. However, sorbitol and mannitol are eluted at the same k' value, and xylitol and arabitol are not completely separated from each other. By using a column of Aminex Q-15-S (Fig. 2), sorbitol and mannitol can easily be separated, as can xylitol and arabitol, but sorbitol and xylitol are eluted together. Disaccharide alcohols, *e.g.*, maltitol, are eluted at lower k' values than monosaccharide alcohols in this system.

Complete separation of all the above-mentioned polyhydric alcohols was possible by adsorption chromatography on silica gel after derivatization (Fig. 3). The main advantage of derivatization, however, is the gain in sensitivity; a 10,000-fold improvement for monosaccharide alcohols is typical. The derivatization step was found to be linear in the range 2 to 750 μg for monosaccharide alcohols, and the NMR

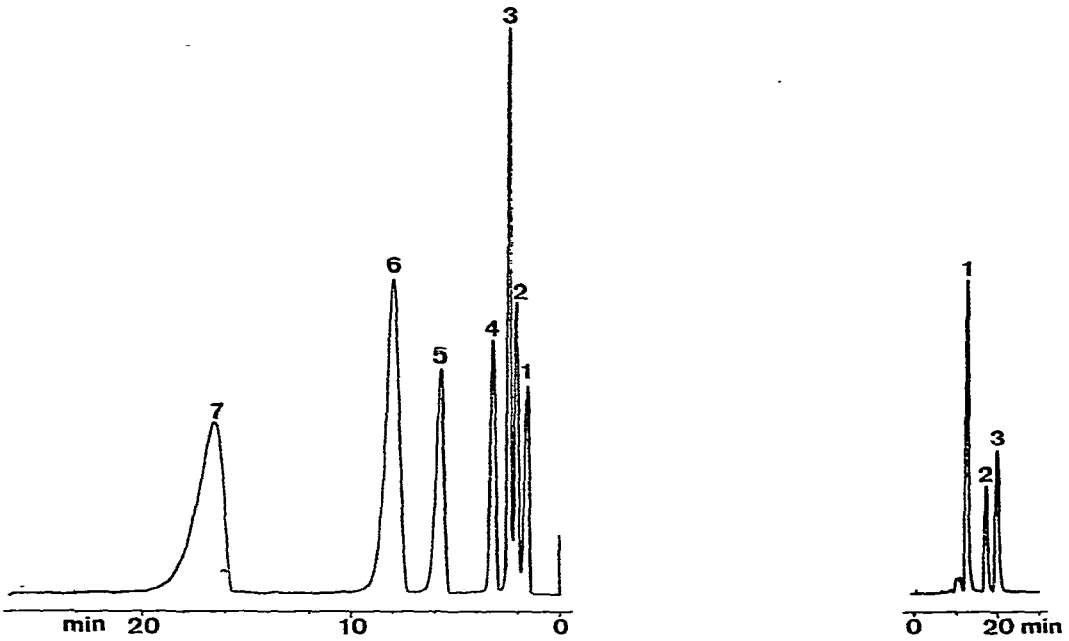

Fig. 1. Chromatogram of polyhydric compounds on a column (250 × 3 mm) of aminoalkylsilica gel (5 μm) at room temperature with a mobile phase of water–acetonitrile (1:3) at 1.0 ml/min. Peaks: 1 = water; 2 = propylene glycol; 3 = ethylene glycol; 4 = glycerol; 5 = xylitol; 6 = sorbitol; 7 = maltitol.

Fig. 2. Chromatogram of polyhydric compounds on a column (1 m × 4 mm of Aminex Q-15-S (22 μm) at 85° with a mobile phase of water at 0.6 ml/min. Peaks: 1 = maltitol; 2 = xylitol; 3 = arabitol.

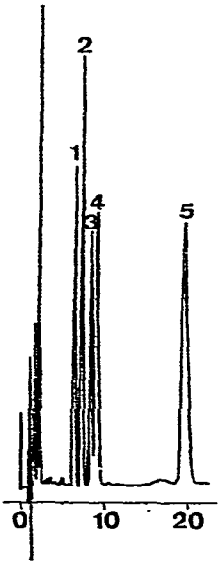

Fig. 3. Chromatogram of polyhydric alcohol derivatives on a column (250 × 3 mm) of LiChrosorb SI-60 (5 μm) at room temperature with a mobile phase of hexane–chloroform–acetonitrile (5:2:1) at 0.8 ml/min. Peaks: 1 = arabitol; 2 = xylitol; 3 = mannitol; 4 = sorbitol; 5 = maltitol.

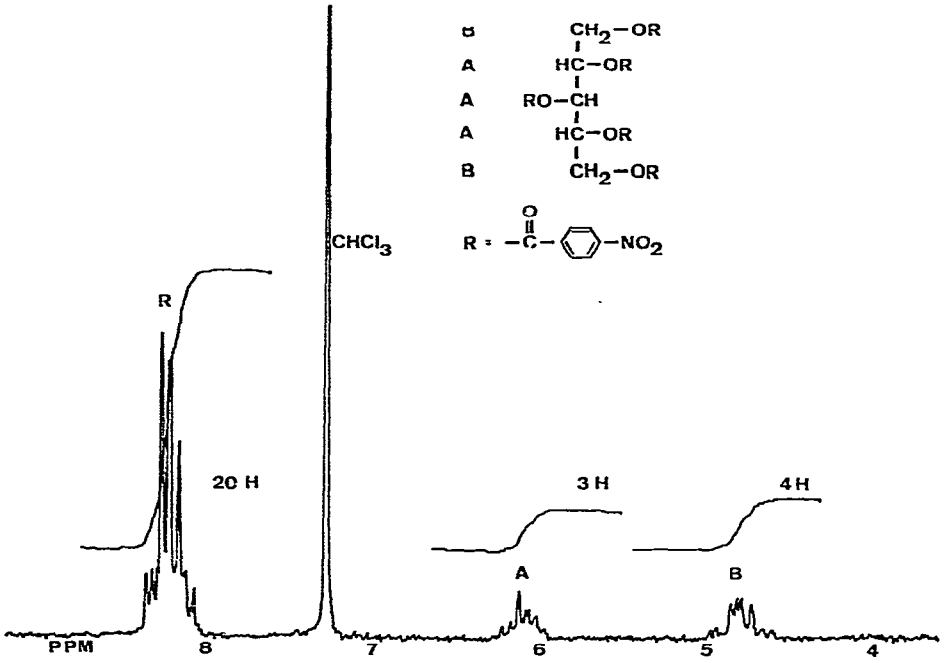

Fig. 4. Section of the 100-MHz nuclear magnetic resonance spectrum of the 4-nitrobenzoyl derivative of xylitol.

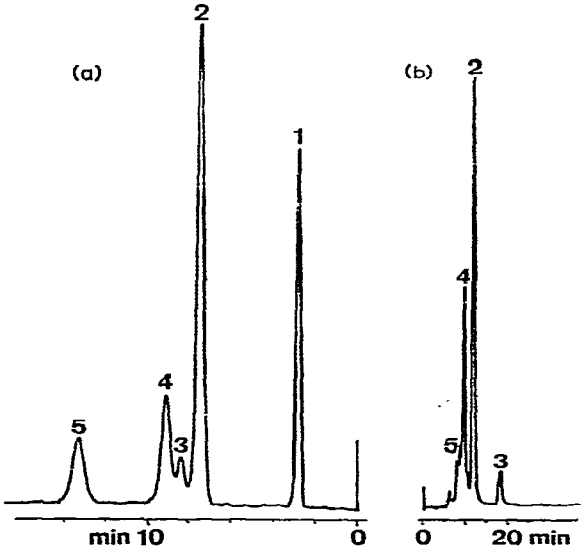

Fig. 5. Chromatograms of apple juice. Conditions: (a) as in Fig. 1, but with water–acetonitrile (1:4) as mobile phase; (b) as in Fig. 2. Peaks: 1 = water; 2 = fructose; 3 = sorbitol; 4 = glucose; 5 = saccharose.

spectra (Fig. 4) of the derivatives showed that all hydroxyl groups had been converted[19].

Applications

Determination of sorbitol in apple juice. The apple juice can be injected directly. Fig. 5a shows the separation on an aminoalkylsilica gel column, and Fig. 5b is the chromatogram of the same sample on Aminex Q-15-S. The Aminex system has the advantage of a baseline-separated sorbitol peak, which facilitates determination of this alcohol. The retention time is short, and, since no sample preparation or clean-up is necessary, the entire analysis is simple.

Determination of humectants in tobacco. The humectants most commonly in tobacco (propylene glycol, ethylene glycol, glycerol and sorbitol) can be identified and determined in a single operation. The powdered tobacco is extracted with water, the extract is cleaned-up on a short column (a Pasteur pipette) filled with an aminoalkylsilica gel (200 mesh) prepared in the same manner as the packing material for the aminoalkylsilica gel column[14], and then injected. The chromatogram of such an extract is shown in Fig. 6.

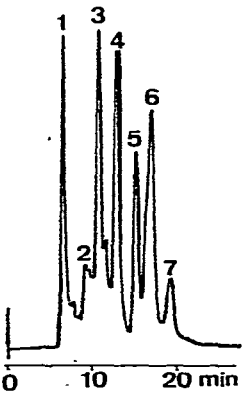

Fig. 6. Chromatogram of tobacco extract (conditions as in Fig. 2). Peaks: 1 = higher polysaccharides; 2 = disaccharides; 3 = glucose; 4 = fructose; 5 = glycerol; 6 = ethylene glycol and propylene glycol; 7 = sorbitol.

Determination of xylitol in tooth-paste. Since tests at the University of Turku, Finland[20], have shown that xylitol has an anti-caries effect, this sweetener has become increasingly used in, *e.g.*, dietary food products, soft drinks, chewing-gum and tooth-paste. Its determination by liquid chromatography is simple, as the product itself, a solution or an extract of it can usually be directly analyzed. Tooth-paste was mixed with water to form a thin slurry, which was then centrifuged, the supernatant liquid was filtered over Celite, and the filtrate was injected into the liquid chromatograph. The chromatogram (Fig. 7) of this filtrate shows all the polyhydric alcohols present in the paste.

The high-performance liquid chromatographic systems discussed here have the following advantages over other techniques: analyses are rapid; the compounds can often be assayed directly, without prior separation from common monosaccharides;

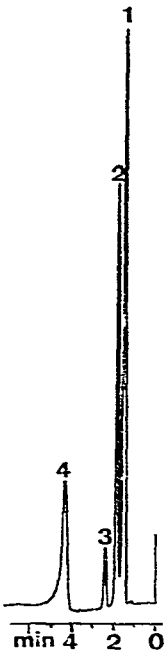

Fig. 7. Chromatogram of tooth-paste extract (conditions as in Fig. 1). Peaks: 1 = water; 2 = ethylene glycol; 3 = glycerol; 4 = xylitol.

and with very small amounts of sample, the sensitivity can be improved by derivatization with 4-nitrobenzoyl chloride.

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