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Note

Qualitative thin-layer chromatographic separation of 1,5-anhydroglucitol in the presence of other carbohydrates on silica gel impregnated with borate buffer

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Considerable attention has recently been focused on carbohydrates in body fluids[1,2]. Changes in the monosaccharide content are frequently associated with pathological conditions. The presence of 1,5-anhydroglucitol in cerebrospinal fluid (CSF) was established by Pitkänen[3] and confirmed by Smith *et al.*[4]. The separation of 1,5-anhydroglucitol from other polyols and sugars is desirable in an investigation of its role in diseases. Most common carbohydrates can be separated easily using thin-layer chromatography (TLC). We found that the separation of 1,5-anhydroglucitol can be performed on plates impregnated with borate buffer. We used nine specially selected reference polyols and sugars, and the separation of 1,5-anhydroglucitol from them was satisfactory under the conditions suggested.

EXPERIMENTAL

Materials and methods

All solvents were analytical-reagent grade reagents and were used without further purification. Borate buffer (0.1 *M*) was prepared from boric acid and sodium tetraborate.

Spray reagents

The stock solution contained 4.2 g of sodium metaperiodate in 75 ml of water. The spray reagent was prepared from the stock solution by dilution with acetone (1:100) just before use. Five minutes after the application of metaperiodate reagent the chromatograms were sprayed with *o*-tolidine solution containing 184 mg of *o*-tolidine, 95 ml of acetone and 0.6 ml of glacial acetic acid.

Chromatographic plates

The commercial plates used were DC-Karten SI, 10 × 20 cm (Riedel-De Häen, Hannover, G.F.R.), and high-performance thin-layer chromatographic (HPTLC) plates (silica gel 60 RP-2; E. Merck, Darmstadt, G.F.R.). Our coated plates were prepared from Kieselgel G nach Stahl (E. Merck), making the slurry in a borate-buffered solution (methanol–water, 1:1). Glass plates (10 × 20 cm) were

then coated with this slurry to a thickness of 0.25 mm. The plates were allowed to dry at room temperature for 24 h and stored at the humidity and temperature of the laboratory air.

The commercial plates were impregnated by soaking them in buffered methanol solution and allowing them to dry in air.

Application of sugar solutions

A micropipette (Drummond, Broomall, Pa., U.S.A.) was used to spot the samples. The sugar solutions were prepared at a concentration of 0.5% in water and spots of 1–2 μl were applied.

Development

The development solvent was 1-butanol–acetone–water (5:4:1). The plates were allowed to develop by ascending chromatography to a height of about 10 cm in closed glass tanks at room temperature. The average development time was 30–45 min. The plates were dried in air.

RESULTS AND DISCUSSION

The identification of 1,5-anhydroglucitol in the presence of other polyols and sugars is not possible on TLC plates using normal methods[5]. It has been reported[6] that 1,5-anhydroglucitol does not form a chelate with boric acid. We therefore decided to compare the influence of borate impregnation on the R_F values of 1,5-anhydroglucitol and other sugars and to find the optimal concentration of boric acid and pH of the impregnating solution. Boric acid solution (0.1 *M*) was chosen for the impregnation of the plates as it gave the best separation and did not produce diffused or tailed spots, and a good separation of 1,5-anhydroglucitol from other sugars was obtained. The optimal pH of the impregnating solution was *ca.* 8.4. If the pH was greater than 9 the migration of 1,5-anhydroglucitol was too slow, and if it was less than 8 the separation of 1,5-anhydroglucitol from other carbohydrates was poor. We tested several mixtures of the following eluates on borate-impregnated plates: methanol, ethanol, 1-propanol, 2-propanol, 1-butanol, ethyl acetate, acetone, acetic acid, methyl ethyl ketone and water.

A good separation was obtained with the solvent system 1-butanol–acetone–water (5:4:1), which favoured the migration of carbohydrates with a wide range of R_F values.

Symmetrical spots were obtained both on HPTLC plates and on the home-made plates. The good separation of 1,5-anhydroglucitol from the other compounds is evidently due to the poor chelate-forming capacity of 1,5-anhydroglucitol with boric acid. The other sugars and polyols seem to be easily chelated on plates. The chelation is clearly dependent on the boric acid concentration and on the pH of the impregnating solution (Fig. 1). In order to achieve a good separation we used impregnation with 0.1 *M* borate buffer. This buffer was more concentrated than those commonly used for carbohydrate separations[7].

Table I and Fig. 1 summarize the data on the migration and the R_F values of the ten carbohydrates run on TLC plates.

The method permits a rapid, one-dimensional and reproducible separation of 1,5-anhydroglucitol from other closely migrating reference carbohydrates.

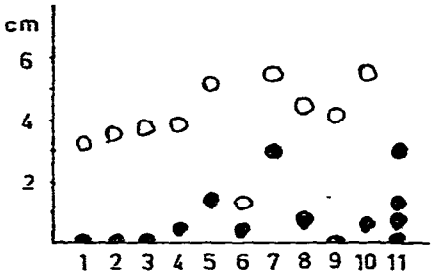

Fig. 1. Influence of borate impregnation on the TLC migration of the following carbohydrates: 1 = sorbitol; 2 = mannitol; 3 = xylitol; 4 = arabinitol; 5 = erythritol; 6 = inositol; 7 = 1,5-anhydroglucitol; 8 = glucose; 9 = fructose; 10 = xylose; 11 = mixture of 1, 5, 7 and 8. Open spots, methanol-impregnated plates; closed spots, borate buffer-impregnated plate, pH 8.5.

TABLE I

R_F VALUES OF CARBOHYDRATES RUN ON HPTLC PLATES AND ON HOME-MADE PLATES

The plates were impregnated either non-buffered or with borate buffer. 1-Butanol–acetone–water (5:4:1) was used as the eluent.

Carbohydrate	*HPTLC plate*			*Home-made plate*		
	Non-buffered	*Buffered*		*Non-buffered*	*Buffered*	
		pH 8.25	*pH 8.5*		*pH 8.25*	*pH 8.5*
Sorbitol	0.20	0.05	0	0.41	0.01	0.01
Mannitol	0.26	0.09	~0	0.49	0.04	0.02
Xylitol	0.32	0.10	0.02	0.45	0.02	0.01
Arabinitol	0.40	0.16	0.05	0.55	0.07	0.03
Erythritol	0.57	0.36	0.17	0.64	0.17	0.12
Inositol	0.03	0.03	0.02	0.34	0.16	0.08
1,5-Anhydro-glucitol	0.59	0.53	0.37	0.70	0.44	0.36
Glucose	0.35	0.25	0.13	0.65	0.20	0.13
Fructose	0.35	0.14	0.04	0.58	0.05	0.03
Xylose	0.59	0.35	0.12	0.72	0.16	0.11

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