satisfactory separation of the derivatives of sulphocarbazole acid is available using a mixed solvent of definite structure, as a mobile phase.

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A quantitative analysis of sorbose by thin-layer chromatography in the presence of some frequently occurring monosaccharides

Thin-layer chromatography is utilized extensively for the analysis of substances including sugars and sugar mixtures. Several authors[1–10] have described solvent systems which separate the various components of monosaccharide mixtures containing, among others, sorbose, by using variously prepared layers. The R_F values given by the authors show that the determination of sorbose is most frequently disturbed by fructose and mannose and more rarely by glycose and xylose. The best separation of sorbose from the other monosaccharides by unidirectional development has been published by WALDI[8] and by LATO and co-workers[9,10]. The two-dimensional chromatograms published by FIGGE[3] also show a good separation; however, it is well known that for quantitative determinations the unidimensional technique is more preferable.

Our present paper describes a method which allows the separation of sorbose from some frequently occurring monosaccharides by unidirectional development and its quantitative analysis.

Separation

30 g of Kieselgel G are mixed with 70 ml of 1/15 M phosphate buffer (pH 7), and a layer 0.25 mm thick is prepared from this mixture. The plates are then dried for 1 h at 100° after spreading. On the plates divided into strips are applied 25–50 μg of the aqueous solution of the following monosaccharides in 10 μl: sorbose, glucose,

galactose, mannose, fructose and xylose. For development the solvent acetone–ethyl acetate–acetic acid–water (50:50:25:5) is used. The solvent front is permitted to reach a height of about 15 cm from the starting point, and then the plate is removed, dried in air, and the development is repeated in the same solvent till the solvent front reaches a height of 15 cm. After drying at 100°, the plate is sprayed with the reagent α-naphthol.

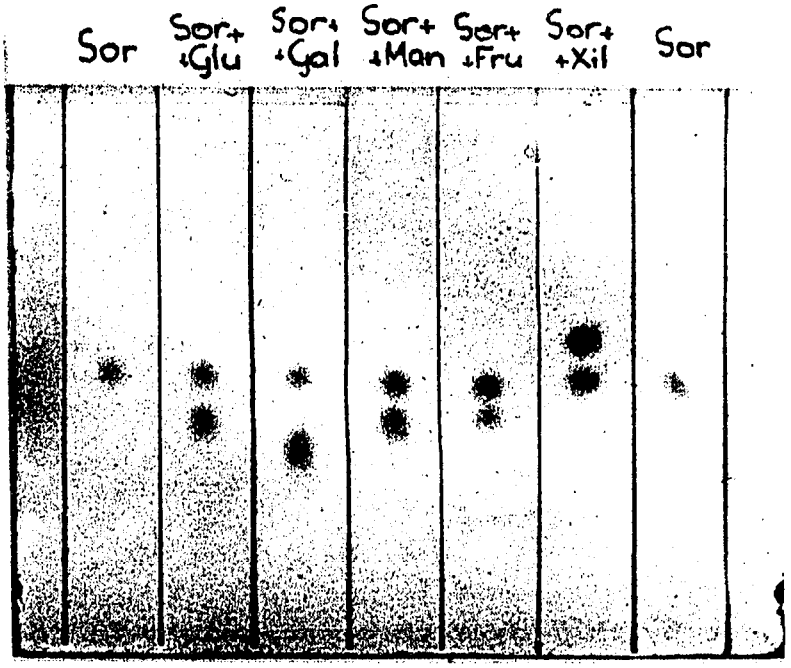

Fig. 1. Sorbose and mixtures of sorbose and various monosaccharides on a Kieselgel G sheet prepared with a phosphate buffer (pH 7) after a single development in the solvent acetone–ethyl acetate–acetic acid–water (50:50:25:5). Spray reagent: α-naphthol.

Fig. 1 shows the chromatogram of pure sorbose and that of a mixture of sorbose and the various monosaccharides after the first development. The spots are already separated after the first development, and, after the second development, shown in Fig. 2, the separation is complete.

Quantitative determination

Quantitative determination is carried out after the above-mentioned second development. The calibrating curve is prepared as follows: 100, 75, 50 and 25 μg of sorbose are applied to the parallel strips of the plate and developed as described above. The two strips at the edges of the sheet are sprayed with the reagent α-naphthol with the rest of the plate covered by a glass plate. The spot with the R_F value corresponding to sorbose is scratched off or sucked off from the undeveloped strips of the plate with a suitable device and is transferred to a small G4 glass filter. The glass filter is fixed in a 2-ml test tube with a glass stopper, and the spot is eluted in several portions with a total of 2 ml of distilled water, the liquid being sucked off after the addition of each portion. Elution is carried out at room temperature with several minutes between additions of the eluting portions. Using the 2-ml eluate, an anthrone color reaction is carried out using 0.2 % anthrone reagent with sulfuric acid (95 wt. %) as described

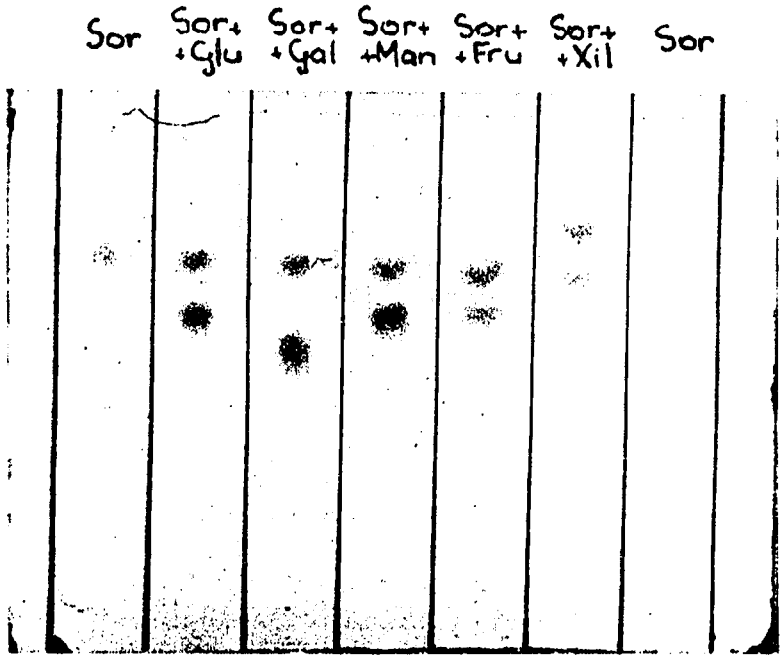

Fig. 2. Sorbose and mixtures of sorbose and various monosaccharides on a Kieselgel G sheet prepared with a phosphate buffer (pH 7) after a double development with the solvent acetone–ethyl acetate–acetic acid–water (50:50:25:5). Spray reagent: α-naphthol.

in the literature[11]. To the 2-ml eluate cooled in ice 4 ml of the anthrone reagent are added in small portions and then heated for 10 min on a boiling water bath. After cooling on an iced water bath, the extinction is measured photometrically at 620 nm. The blank test is prepared by dropping a solution containing no sorbose on one of the strips, by eluting the spot having the R_F value equal to that of sorbose and by further treatment used for the spots containing sorbose. By this method we obtained the following extinction values corresponding to the different quantities of sorbose (Table I).

The averages of these extinction values were presented graphically as a function of

TABLE I

A COMPARISON OF EXTINCTION VALUES OBTAINED APPLYING VARIOUS QUANTITIES OF SORBOSE

Quantity of sorbose applied to plate (μg)	*Extinction values*	*Average*
100	0.330, 0.335, 0.302, 0.310, 0.334	0.322
75	0.254, 0.246, 0.220, 0.218, 0.262	0.240
50	0.124, 0.148, 0.174, 0.170, —	0.154
25	0.095, 0.092, 0.080, 0.073, 0.085	0.085

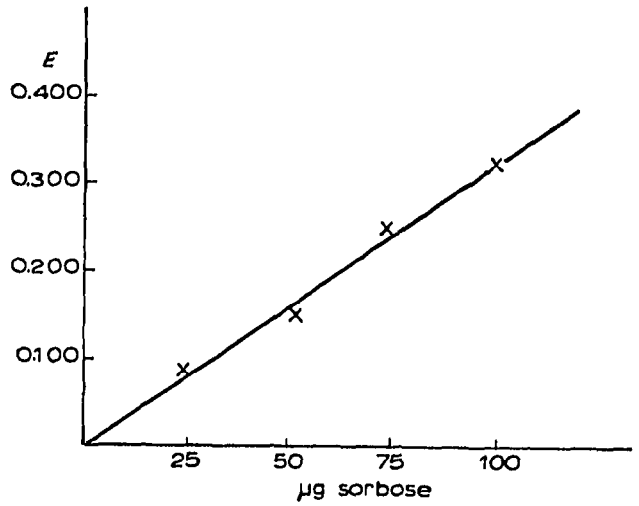

Fig. 3. Calibration curve obtained by plotting the average of the extinction values *versus* quantity of sorbose applied to plate.

concentration. The calibration curve obtained in this way is shown in Fig. 3. The unknown sorbose concentration is found with the aid of this calibration curve.

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