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Note

A simple thin-layer chromatographic technique for the separation of mono- and oligosaccharides

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Thin-layer chromatography (TLC) is a relatively simple technique and has been extensively utilised in the separation of sugars. Many variations of the process have been recommended since Stahl and Kaltenbach[1] published their paper in 1961, indicating the degree of difficulty in effecting the separation of these similar compounds. Impregnated Kieselguhr layers[2], impregnated silica layers[3], coupled silica layers[4], combined with multiple and two-dimensional development have all been recommended.

Analysis times using silica are generally lengthy, although the introduction of a vapour programming technique[5] has been reported as giving separation of some mono- and disaccharides within 3 h. The inclusion of lactic acid in the solvent system[6] has been recommended for the separation of 28 sugars in 5 h, and triple development on cellulose[7] has also been reported as giving effective separations.

This paper describes the chromatographic separation of 18 sugars with a simple system that offers the advantage of speed and minimum manipulation by the use of commercially available plates requiring no pre-treatment.

EXPERIMENTAL

Materials

Glass plates (20 × 20 cm) pre-coated with Cellulose F (0.10 mm) were obtained from E. Merck, Darmstadt, G.F.R. (Art. 5718/0025). As developing solvent ethyl acetate–pyridine–water–glacial acetic acid–propionic acid (50:50:10:5:5) was used. The last three volumes should be measured accurately to maintain reproducibility. The visualising reagent was prepared as follows: 0.5 g diphenylamine, 1 ml aniline, 5 ml orthophosphoric acid (85%) were mixed and diluted to 50 ml with acetone. This reagent may be stored in a refrigerator for several weeks. Sugar solutions were 1% w/v in water.

Procedure

Samples (1 μl) are applied onto the baseline of the plate which is then placed in a paper-lined pre-saturated chromatography tank. Two solvent runs of approx. $1\frac{3}{4}$ h each are performed, air drying the plate between each run. The plates are then sprayed with visualising reagent and heated at 105° until the spots are of sufficient

TABLE I

AVERAGE R_G VALUES FOR VARIOUS SUGARS

Sugar	*R_G value (glucose = 100)*	*Sugar*	*R_G value (glucose = 100)*
Rhamnose	192	Glucose	100
Ribose	176	Galactose	82
Lyxose	162	Sucrose	60
Fucose	162	Maltose	48
Xylose	154	Cellobiose	39
Arabinose	134	Lactose	29
Sorbose	129	Iso-maltose	26
Fructose	127	Maltotriose	25
Mannose	115	Raffinose	13

intensity. Any dark background may be alleviated by placing the plate in a source of steam (*e.g.* over a boiling water bath) for a few minutes.

RESULTS AND DISCUSSION

The R_G values of 18 sugars are reported in this study (Table I), with good separation of 11 of them from a mixture. A typical chromatogram is illustrated in Fig. 1.

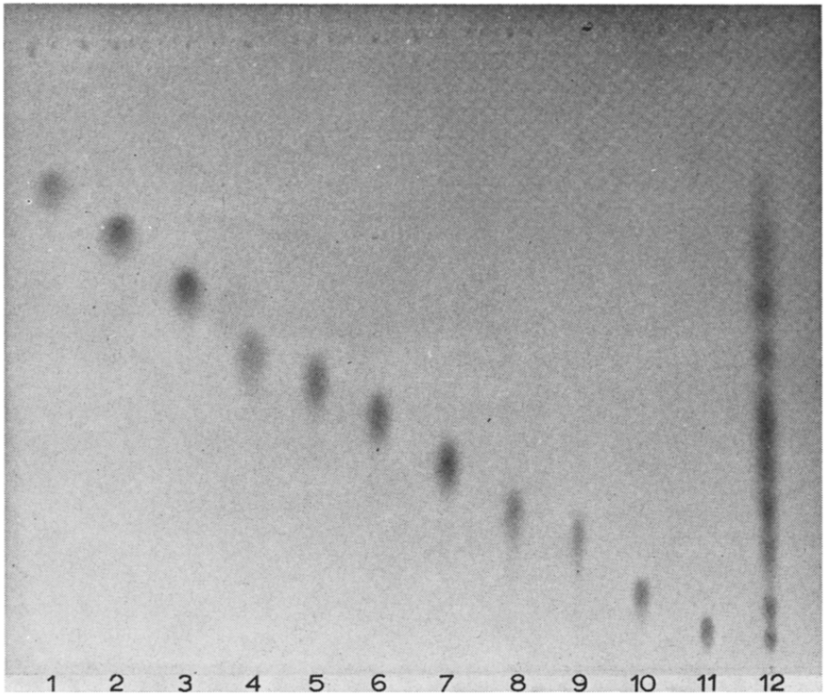

Fig. 1. TLC of 11 sugars on 20 × 20 cm pre-coated cellulose plates. Solvent system, ethyl acetate–pyridine–water–acetic acid–propionic acid (50:50:10:5:5). Two consecutive runs. 1 = Rhamnose, 2 = ribose, 3 = xylose, 4 = fructose, 5 = mannose, 6 = glucose, 7 = galactose, 8 = sucrose, 9 = maltose, 10 = lactose, 11 = raffinose, 12 = mixture of 1–11.

The colours of the spots include shades of yellow, gold, olive, blue and violet, but depend to some extent on the degree of spraying and heating. Of particular interest is the ability of the system to partially separate fructose and mannose, not achieved satisfactorily with other simple TLC systems. The colours of the spots due to fructose, mannose and glucose (gold, brown and blue, respectively) further help to confirm their identities.

The advantage of this particular system is its simplicity. It does not require the use of specially prepared plates or the use of buffer-impregnated or coupled-layer sorbents; commercially available pre-coated plates are sufficient. Furthermore the method is both readily reproducible and more rapid than most. Development time is under $3\frac{1}{2}$ h with a total analysis time of 5 h. Up to twenty samples may be examined simultaneously on the same plate, as the procedure involves only two runs of a one-dimensional development.

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