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Note

Thin-layer chromatographic method for the identification of mono-, di- and trisaccharides

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Many thin-layer chromatographic (TLC) methods for the separation of mono- and disaccharides have appeared since Stahl and Kaltenbach[1] published their method for the separation of sugars in 1961. Some of these methods[2–5] describe the use of impregnated layers where silica gel is first slurried with different reagents before spread-

TABLE I

R_F VALUES AND COLOUR REACTION OF MONO-, DI- AND TRISACCHARIDES ON PRE-COATED SILICA GEL 60 IMPREGNATED WITH NaH_2PO_4

Sugar	*R_F value*	*Colour*
2-Deoxy-ribose	0.83	red
Rhamnose	0.83	green
6-Deoxy-glucose	0.80	green
Xylulose	0.76	green
Fucose	0.65	green
Lyxose	0.65	violet
Xylose	0.63	violet
Ribose	0.60	violet
Tagatose	0.60	red
Sorbose	0.52	red-brown
Mannose	0.50	blue
Sucrose	0.47	violet
Arabinose	0.47	violet
Fructose	0.46	red
Glucose	0.41	blue
Melizitose	0.36	violet
Cellobiose	0.35	blue
Maltose	0.34	blue
Trehalose	0.31	blue
Galactose	0.30	blue
Maltotriose	0.27	blue
Lactose	0.23	blue
Gentiobiose	0.20	blue
Isomaltose	0.17	blue
Raffinose	0.17	blue
Panose	0.14	blue
Melibiose	0.14	blue
Isomaltotriose	0.06	blue

ing on glass plates. The TLC method described here is a new analytical method for the identification of mono-, di- and trisaccharides. It is a development of existing methods but has been greatly improved by using lactic acid in the solvent system and pre-coated TLC plates impregnated with NaH_2PO_4 solution. Pre-coated plates have previously been shown to be well-suited for the separation of mono- and oligosaccharides[6] and very reproducible results can be obtained.

PROCEDURE

Pre-coated 20 × 20 cm silica gel glass plates (Merck art. No. 5715) are impregnated with a 0.5 *M* NaH_2PO_4 solution before use. 69 g $NaH_2PO_4 \cdot H_2O$ are dissolved in 750 ml of water and 250 ml of methanol added. A glass chromatography tank is filled to within 2 cm of the rim with this impregnation solution. The pre-coated silica gel 60 plates are placed in a stainless-steel holder (Shandon No. SAB 2883) and quickly submerged in the tank so that the plates are covered. The plates are allowed to stand overnight in the tank (15–20 h) and then removed and air-dried in the holder

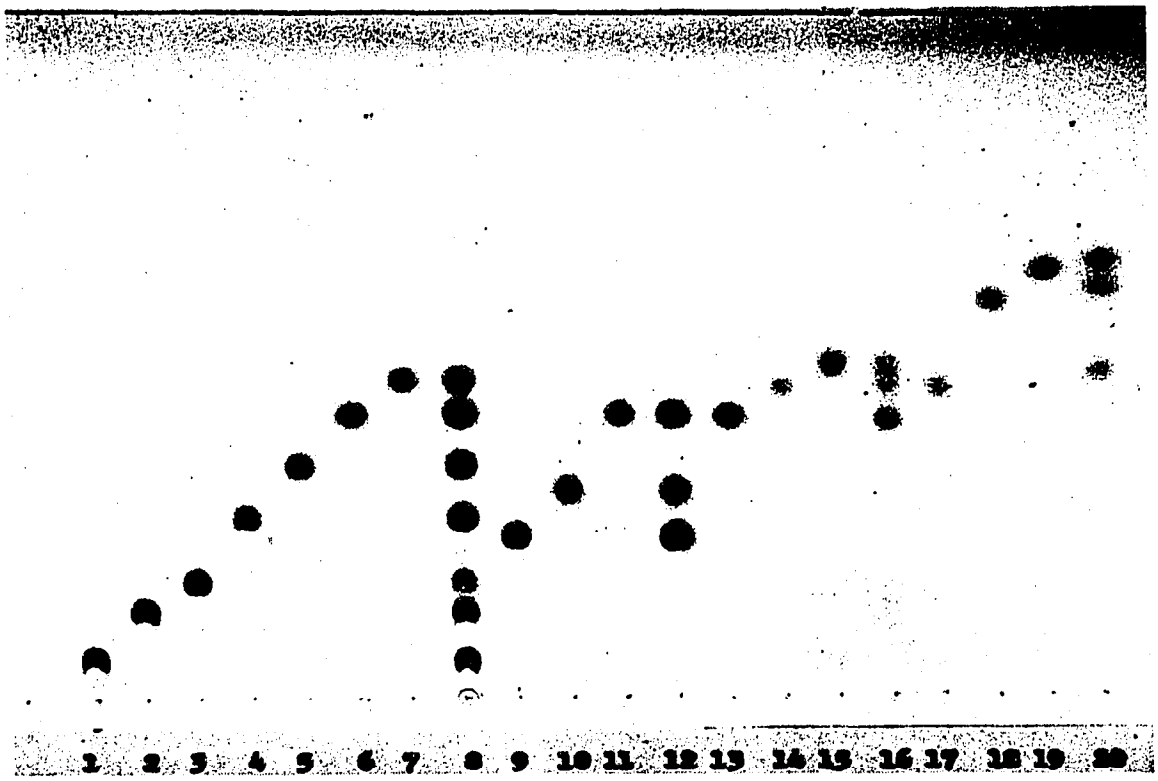

Fig. 1. Chromatogram of mono-, di- and trisaccharides on 20 × 20 cm pre-coated TLC silica gel 60 plates (Merck, Art. No. 5715) impregnated with NaH_2PO_4. Solvent system, acetone–isopropanol–0.1 *M* lactic acid (40:40:20); spraying reagent, aniline–diphenylamine–acetone–80% H_3PO_4 (4 ml:4 g: 200 ml:30 ml). Samples, 1 µl of (1) isomaltotriose, (2) panose, (3) isomaltose, (4) maltotriose, (5) maltose, (6) glucose, (7) sucrose, (8) 1–7, (9) lactose, (10) galactose, (11) glucose, (12) 9–11, (13) glucose, (14) fructose, (15) mannose, (16) 13–15, (17) arabinose, (18) ribose, (19) xylose, (20) 17–19.

in a vertical position. The impregnated plates should be dried at 105° for 60 min and cooled in a desiccator before use. 1-μl samples are applied to the edge of the plate which was facing downwards during impregnation and drying. A concentration of 0.2% is suitable for mono-, di- and tri-saccharide standards. When the samples have dried, the plate is placed in the chromatography tank. The solvent system used is isopropanol–acetone–0.1 *M* lactic acid (4:4:2), and this is prepared about 1 h before use. After 5 h, the solvent front will have migrated about 16 cm. The plate is removed from the tank and dried in a stream of warm air (approx. 60°). The sugar spots are developed on the plates by spraying with aniline–diphenylamine–acetone–H_3PO_4 80% (4 ml: 4 g:200 ml:30 ml) and heating for 30 min in an oven at 105°. The sugars appear as coloured spots on a white background.

DISCUSSION

This TLC method for the separation of mono-, di- and trisaccharides is used routinely for the identification of sugars in fermentation substrates and sugars resulting from enzymatic hydrolysis of starch and other carbohydrates. The method is more suitable for monosaccharide separation than any method previously described because the sugar spots run further without becoming diffuse. This separation has been achieved by using lactic acid in the solvent system and impregnated, pre-coated plates. The lactic acid causes the sugars to run as well defined spots without diffuse edges.

Using impregnated, pre-coated plates it is possible to apply almost twice as many samples on the plates as when laboratory-prepared plates are used. Where several sugars are lying close together, the colour of the spot can be used for identification and compared with known standards. For example, glucose, fructose, mannose, and sorbose are coloured blue, red, blue, and red-brown, respectively, which makes identification possible in spite of the small differences in R_F value. In doubtful cases the sample should be diluted about ten times and re-run on a new TLC plate. This will normally give satisfactory results.

NOTE ADDED IN PROOF

In 1974, E. Merck (Darmstadt, G.F.R.) altered their process for preparing pre-coated silica gel 60 plates. Merck art. No. 5715 no longer gives such well-defined spots as illustrated in Fig. 1. Satisfactory results have been obtained using TLC aluminium sheets silica gel 60, Merck art. No. 5553, 20 × 20 cm.

REFERENCES

1 E. Stahl and U. Kaltenbach, *J. Chromatogr.*, 5 (1961) 351.
2 M. Lato, B. Brunelli, G. Ciuffini and T. Mezzetti, *J. Chromatogr.*, 34 (1968) 26.
3 M. Lato, B. Brunelli, G. Ciuffini and T. Mezzetti, *J. Chromatogr.*, 39 (1969) 407.
4 T. Mezzetti, M. Ghebregzabher, S. Rufini, G. Ciuffini and M. Lato, *J. Chromatogr.*, 74 (1972) 273.
5 M. Ghebregzabher, S. Rufini, G. Ciuffini and M. Lato, *J. Chromatogr.*, 95 (1974) 51.
6 S. A. Hansen, *J. Chromatogr.*, 105 (1975) 388.