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Note

Gas chromatography of alditol acetates on a high-polarity bonded-phase vitreous-silica column

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Monosaccharides are often analysed by gas chromatography of their alditol acetates on polar phases. Recently, glass capillary columns coated with the polar cyanoalkyl silicone phases, OV-275[1] and Silar 10C[2,3] have been used to separate alditol acetates. However, these glass columns are difficult to handle because of their lack of flexibility and poor mechanical strength. This problem can be overcome by using flexible vitreous-silica columns which are easy to install in gas chromatographs and interfaces with mass spectrometers. Vitreous-silica also has lower levels of metal oxides and other impurities that may adsorb reactive compounds or cause catalytic breakdown of the chromatographic phase. Non-polar phases, such as SP-2100, can be coated onto silica columns, but give relatively poor resolution of alditol acetates[4]. Polar phases give good resolution, but are unstable when coated onto the non-polar surfaces of vitreous-silica columns. This instability can be overcome by cross-linking within the phase to give a bonded or immobilized phase[5,6]. In this paper we report the chromatography of alditol acetates on a high-polarity wall-coated open-tubular (WCOT) column, BP-75, produced by bonding the polar phase, OV-275, on vitreous-silica.

EXPERIMENTAL

Materials

Sugars were obtained from the following sources: L-arabinose, BDH, Poole, Great Britain; 2-deoxy-D-ribose, Koch-Light, Colnbrook, Great Britain; D-mannose, Pfanstiel Labs., Waukegan, IL, U.S.A.; D-allose, cellobiose, 2-deoxy-D-glucose, L-fucose, D-galactose, D-glucose, L-rhamnose, D-ribose and D-xylose, Sigma, St. Louis, MO, U.S.A. Myo-inositol and erythritol were from BDH. Laminaritetrose was prepared by the method of Clarke and Stone[7].

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Preparation of alditol acetates

Alditol acetates were prepared from monosaccharides by the method of Blakeney *et al.*[3]. Permethylated, peracetylated alditols were prepared as described by Jansson *et al.*[8].

Gas chromatography

Alditol acetates were separated on a 6 m × 0 2 mm I D , BP-75, vitreous-silica, WCOT column (S G E., Melbourne, Australia) in a Hewlett-Packard 5710A chromatograph equipped with a flame-ionization detector and a S.G E. Unijector capillary injection system, used in the split mode (split ratio 4 1) The end of the column was located at the point within the heated section of the injection splitter at which equivalent splitting of each component was achieved High-purity hydrogen was used as the carrier gas at a flow-rate of 1 3 ml/min. Alditol acetates were separated using a temperature program of 170°C for 4 min followed by a 4°C/min rise to 230°C. Permethylated, peracetylated alditol acetates were separated using a temperature program of 150°C for 4 min followed by a 2°C/min rise to 230°C The injection port and detector were heated to 250°C and 300°C, respectively. Peak areas were recorded using a Hewlett-Packard Model 3380A reporting integrator

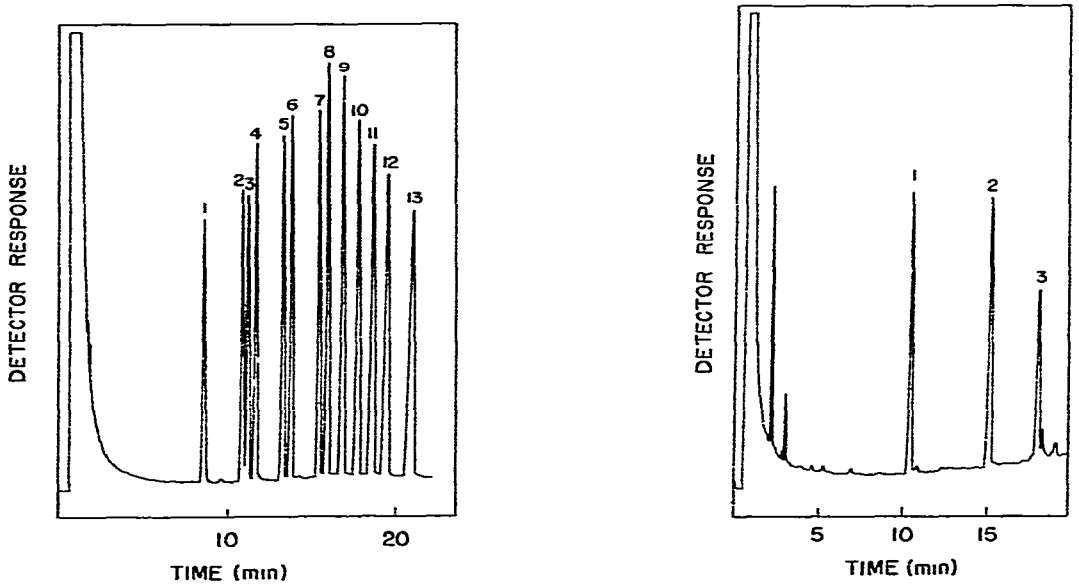

Fig. 1 Separation of 13 alditol acetates on a 6-m B-75 vitreous-silica column Temperature program 170°C for 4 min, then 4°C/min to 230°C Peaks 1 = erythritol, 2 = deoxyribitol, 3 = rhamnitol, 4 = fucitol, 5 = ribitol; 6 = arabinitol, 7 = xylitol, 8 = deoxyglucitol; 9 = allitol, 10 = mannitol, 11 = galactitol; 12 = glucitol, 13 = inositol 2 μl of dichloromethane containing the alditol acetates derived from 3 μg of each monosaccharide was injected

Fig. 2. Separation of permethylated alditol acetates on a 6-m BP-75 vitreous-silica column Temperature program· 150°C for 4 min, then 2°C/min to 230°C Peaks· 1 = 2,3,4,6-tetramethyl-1,5-diacetylglucitol, 2 = 2,4,6-trimethyl-1,3,5-triacetylglucitol, 3 = 2,3,6-trimethyl-1,4,5-triacetylglucitol

RESULTS AND DISCUSSION

The separation of 13 alditol acetates on a BP-75 vitreous-silica WCOT column is shown in Fig. 1. The resolution is comparable with that obtained on a Silar 10C glass support-coated open-tubular (SCOT) column (28 m × 0.5 mm I.D.)[3]. All components were separated in less than 20 min, however, faster separations are possible. For example, by using a temperature program starting at 140°C and rising at 16°C/min to 250°C all 13 alditols, except deoxyribitol and rhamnitol, were separated in less than 9 min. Routine separation of simple mixtures with widely different relative retention times may be completed in even shorter times. Permethylated alditol acetates were also well resolved on this column (Fig. 2).

The flexibility of the vitreous-silica column allowed easy manipulation of the column and the bonded-phase has several advantages[5,6]. Bonded phases have good thermal stability which increases the life of the column and the low phase bleed reduces background in mass spectrometry. Contaminants can be removed by washing with solvents without effecting the chromatographic properties of the column.

The combination of the high resolving power of a polar phase with the practical advantages of a bonded phase on vitreous-silica suggests that this column should find wide application in the analysis of complex mixtures of monosaccharides and in methylation analysis.

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REFERENCES

1 J. Klok, E. H. Nieberg-van Velzen, J. W. de Leeuw and P. A. Schenck, *J. Chromatogr.*, 207 (1981) 273
2 N. Shibuya, *J. Chromatogr.*, 208 (1981) 96
3 A. B. Blakeney, P. J. Harris, R. J. Henry and B. A. Stone, *Carbohydr. Res.*, 1982, in press
4 R. Oshima, A. Yoshikawa and J. Kumanotani, *J. Chromatogr.*, 213 (1981) 142
5 K. Grob, G. Grob and K. Grob, Jr., *J. Chromatogr.*, 211 (1981) 243
6 K. Grob and G. Grob, *J. Chromatogr.*, 213 (1981) 211.
7 A. E. Clarke and B. A. Stone, *Biochem. J.*, 99 (1966) 582.
8 P. Jansson, L. Kenne, H. Liedgren, B. Lindberg and J. Lönngren, *Chem. Commun., Univ. Stockholm*, No. 8 (1976)