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Note

The simultaneous separation and analysis of aminodeoxyhexitols and alditols by automated ion-exchange chromatography

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The separation and determination of alditols is important, as they occur widely in Nature[1], and are encountered in the course of structural investigation of polysaccharides and glycoproteins. The cleavage, using alkaline borohydride, of oligosaccharide side-chains from the protein core of some glycoproteins results in the formation of the alditol corresponding to the sugar originally linked to the amino acid. The hydrolysis of the resulting, reduced oligosaccharides may give both alditols and aminodeoxyalditols. Although gas–liquid chromatography has been used[2] for the analysis of such mixtures, ion-exchange methods[9] would eliminate the need to convert the alcohols into volatile derivatives. Separation of neutral alditols on ion-exchange resins in the borate form, based on the original experiments of Zill and co-workers[3], has been achieved, although rather broad elution peaks were obtained. A much improved method, which required about 24 h for one run, was described by Spencer[4]. However, these methods are only for the separation of neutral alditols. Aminodeoxyalditols are usually determined separately on a cation-exchange resin[5]. This paper describes a rapid, automated method for the analysis of mixtures of neutral and aminodeoxyalditols, using an ion-exchange resin in the borate form.

EXPERIMENTAL

Materials. — 2-Amino-2-deoxy-D-galactitol and 2-amino-2-deoxy-D-glucitol were prepared by the reduction of the corresponding sugars with sodium borohydride. Other alditols were obtained commercially.

Chromatographic system. — Type-S Chromobeads anion-exchange resin (Technicon Instruments Co. Ltd.; an 8% cross-linked, high-capacity polystyrene) was washed successively with 2M hydrochloric acid, water, 2M sodium hydroxide, and water, and then converted into the borate form by washing with 0.5M boric acid. The column (6 × 750 mm) was packed in short sections at a pressure of 200 p.s.i. and at a column operating-temperature of 75°, giving a resin bed of 700 mm. More resin was added as the bed shrank during the first few cycles until the bed stabilized (7–8 runs).

The column was eluted with a borate–chloride gradient supplied from the

Autograd, a nine-chamber gradient device, at 70 ml/h. The Autograd composition is shown in Table I. After each chromatographic run, the borate form of the resin was regenerated by pumping aqueous potassium tetraborate (10% w/v) through the column until the effluent was chloride-free, and the column was then equilibrated with the starting buffer (0.1M boric acid, pH 7). The regeneration may be performed overnight or in 2 h at a flow rate of about 100 ml/h. A sample containing 0.1–1μmole of each component in ~0.5 ml of borate buffer (0.1M, pH 7) was applied to the column. Ethylene glycol was added as an internal standard.

TABLE I

AUTOGRAD COMPOSITION[a] FOR SEPARATION OF POLYOLS

Chamber	*0.1M H_3BO_3 (ml)*	*0.4M H_3BO_3 (ml)*	*0.4M H_3BO_3/0.2M NaCl (ml)*
1	29	5	1
2	29	5	1
3	17	17	1
4		34	1
5		34	1
6		34	1
7			35
8			35
9			35

[a]All buffers were titrated to pH 7 with 2M NaOH.

Analytical system. — The column eluate was analysed by using the automated pentane-2,4-dione procedure[6] to determine the formaldehyde produced on periodate oxidation of the alcohols.

Analysis of neutral saccharides. — The chromatographic system was similar to that described above. The column was eluted with a borate–chloride gradient. The Autograd composition is shown in Table II. The eluate was analysed by the automated cysteine–sulphuric acid assay, as described before[7].

TABLE II

AUTOGRAD COMPOSITION[a] FOR SEPARATION OF SACCHARIDES

Chamber	*0.1M H_3BO_3 (ml)*	*0.4M H_3BO_3 (ml)*	*0.4M H_3BO_3/0.2M NaCl (ml)*
1	32		3
2	32		3
3	32		3
4		32	3
5		32	3
6		32	3
7		25	10
8			35
9			35

[a]All buffers were titrated to pH 7 with 2M NaOH.

RESULTS AND DISCUSSION

The present system is based on one developed in this laboratory for the analysis of neutral monosaccharides[7]. The separation of a mixture of aminodeoxyhexitols and alditols is shown in Fig. 1. A high operating-temperature of 75° and a high flow-rate were employed so as to obtain sharp peaks. In order to minimize the damage to the resin and other adverse effects at this elevated temperature, the use of alkaline buffers was avoided, and a chloride–borate buffer system of pH 7 was used. The elution times of the alditols were critically dependent on the rate of increase of concentration of both borate ion and chloride ion in the eluant. The use of both borate and chloride ion coupled with a high elution-rate considerably shortened the elution time of the alditols, while maintaining good resolution, and a run could be completed in ~4 h.

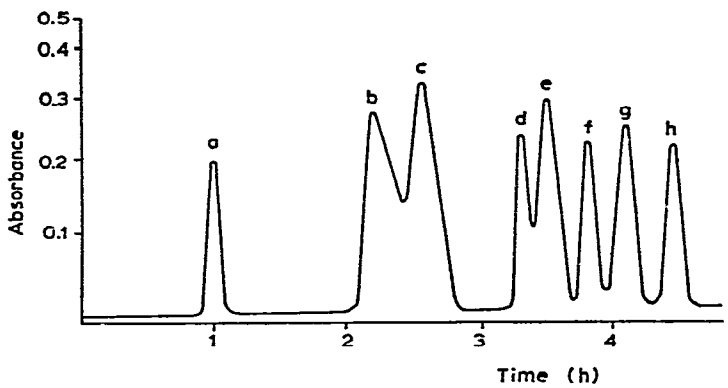

Fig. 1. Separation of polyols: (*a*) ethylene glycol, (*b*) 2-amino-2-deoxy-D-galactitol, (*c*) 2-amino-2-deoxy-D-glucitol, (*d*) xylitol, (*e*) D-arabinitol, (*f*) D-mannitol, (*g*) D-glucitol, and (*h*) galactitol.

Better resolution between 2-amino-2-deoxy-D-galactitol and 2-amino-2-deoxy-D-glucitol (Fig. 1) could be achieved by decreasing the borate concentration in both chambers 3 and 4. However, this led to rather broad elution peaks. Decreased borate concentration in chambers 1 and 2 gave sharp but less well-resolved peaks. The present conditions are optimal for sharpness and resolution of the elution peaks.

The colour development in the pentane-2,4-dione assay depends on the amount of salt present. Provided that the level of salt is constant, each peak area was found to be proportional to the amount of alcohol present in a range from 0.1–1.0 μmole of alcohol.

The foregoing method has been used in this laboratory for the analysis of aminodeoxyhexitols and alditols encountered in the course of structural investigation of glycoproteins from human gastric mucosa[8], in particular 2-amino-2-deoxy-galactitol, 2-amino-2-deoxyglucitol, and galactitol.

The response of the eluate to automated analysis is diminished as the flow rate through the column is increased. For a given amount of sample, the components are

present in larger volumes of buffer, and their concentration in the eluate, and consequently the peak heights are decreased. By decreasing the column flow-rate to 37 ml/h, a run takes ~8 h, and the response is nearly doubled. This is desirable when sensitivity rather than speed is required.

By using the column conditions and flow rates described here (*i.e.*, 70 ml/h, 75°), neutral saccharide determination can be performed in ~4 h, rather than 7.5 h as described by us previously[7]. However, the sensitivity of the method is decreased for the reasons given above. A separation of neutral saccharides, using the present conditions, is shown in Fig. 2.

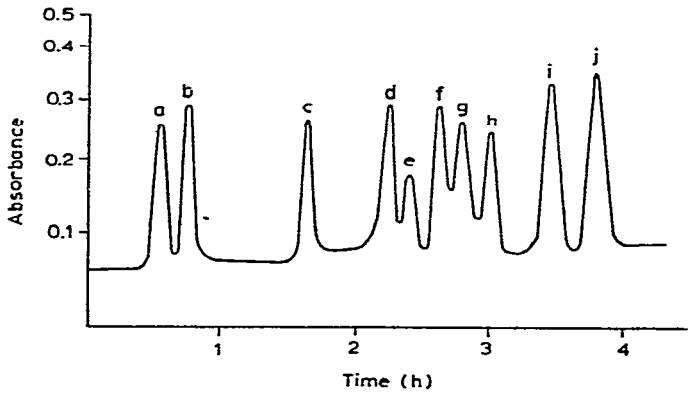

Fig. 2. Separation of neutral saccharides: (*a*) trehalose, (*b*) cellobiose, (*c*) L-rhamnose, (*d*) D-ribose, (*e*) D-mannose, (*f*) L-fucose, (*g*) L-arabinose, (*h*) D-galactose, (*i*) D-xylose, and (*j*) D-glucose.

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