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An improved method of separating glucosaminitol from galactosaminitol and their amino sugars on an amino acid analyzer

In a recent report on the separation of galactosaminitol from glucosaminitol using the amino acid analyzer, DONALD[1] obtained a good resolution between the two glycitols in about 10 h and with their corresponding amino sugars in 15 h. In the method reported by WEBER AND WINZLER[2] the separation between the two glycitols was not adequate. This latter method therefore is good only if one glycitol was present together with the amino sugars and permits identification but not quantitation of the glycitols.

The method described in the present paper is an improvement of the method of DONALD[1] wherein a complete analysis of hexosamines and their glycitols could be done in the standard 56 cm column of the amino acid analyzer in less than 4 h.

Experimental

Glucosamine·HCl and galactosamine·HCl, A.G., were purchased from Calbiochem. Chromatography on the amino acid analyzer showed that each had only one component. Glucosaminitol and galactosaminitol were prepared by borohydride reduction using the method of CRIMMIN[3].

A Beckman 120C amino acid analyzer equipped with a 0.9 × 56 cm column of Beckman UR-30 resin was used in the separation. The column was equilibrated and eluted with citrate–borate buffer pH 5.06 at a flow rate of 40 ml/h. Ninhydrin flow rate was 20 ml/h. The column temperature was kept at 65°.

The 0.35 *M* sodium citrate buffer pH 5.28 (ref. 4), a standard buffer for basic amino acid analysis, was used for preparing the citrate–borate buffer. To this buffer was added 18.55 g/l boric acid (0.3 *M*) to give a final pH of 5.06.

Results

The separation of galactosaminitol, glucosaminitol, glucosamine and galactosamine is shown in the chromatogram in Fig. 1. The retention times relative to glucosamine are: galactosaminitol 0.72; glucosaminitol 0.76; galactosamine 1.12.

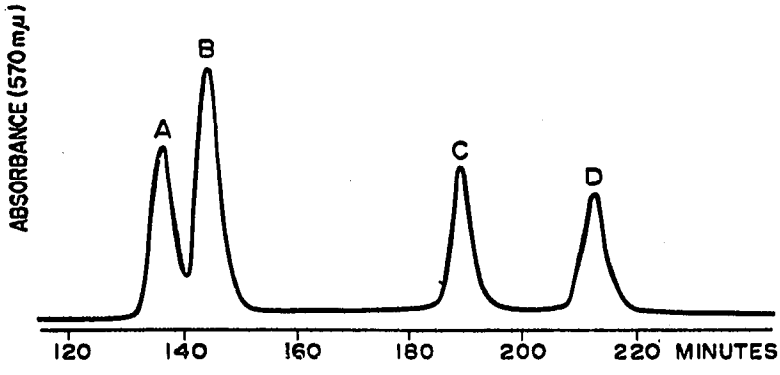

Fig. 1. Chromatographic separation of galactosaminitol (A), glucosaminitol (B), glucosamine (C) and galactosamine (D), on a 0.9 × 56 cm column of Beckman UR-30 resin eluted with 0.35 *M* citrate buffer pH 5.06 containing 0.3 *M* borate.

The Beckman 120C analyzer used in this separation is equipped with a 4–5 mV range recorder bridge[5]. With this expanded scale a normal load of 10–100 nmoles of each sugar base could be determined. The standard 0–5 mV range recorder bridge provided with most instruments allows analysis of 0.14–1.5 μmoles.

An increase in temperature to 65° provides an improved resolution of galactosaminitol from glucosaminitol. To accelerate the analysis by increasing the rate of buffer elution would mean a sacrifice in resolution. The flow rate of 40 ml/h is the ideal condition for the separation of the glycitols with this method. If only one of the sugar alcohols is present the analysis can be accelerated to 68 ml/h.

Chromatograms obtained from Beckman AA-15 resin also produce a similar satisfactory separation of galactosaminitol, glucosaminitol, glucosamine and galactosamine. Resins of similar grade to Beckman UR-30 or AA-15 available from other commercial sources would probably behave in the same manner.

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