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Note

High-performance liquid chromatography of maltosaccharides

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We have previously reported several improvements for fractionation and quantitative or qualitative determination of maltooligosaccharides using carbon column chromatography[1], paper densitometry[2], gas–liquid chromatography[3], gel filtration chromatography[4] and multiple descending paper chromatography[5]. These studies were closely related to our work on the fine structure of the starch molecule and on the mechanism of action of amylases. Recently, high-performance liquid chromatography (HPLC) has been recognized as a quick, convenient and accurate method of quantitative analysis of carbohydrates [6–10].

In this paper, we summarize our results on the quantitative analysis of maltosaccharides by HPLC. We also try to determine the range of error in the determination of higher maltooligosaccharides when maltose is used as a standard compound.

EXPERIMENTAL

Preparation of maltooligosaccharides

A 200-ml volume of 2% short-chain amylose (mean degree of polymerization, $\overline{D.P.}$ = 18) was incubated with 5.5 I.U.* of bacterial liquefying α-amylase (Crystalline; Seikagu Kogyo, Tokyo, Japan) at 40°C for 30 min, then deionized on Amberlite MB-3 resin. The deionized hydrolyzate was lyophilized. Each pure maltooligosaccharide was prepared by macro paper chromatography. The concentrations of oligosaccharides were determined by the phenol–sulphuric acid method[11].

HPLC apparatus, column and operating conditions

A Trirotor HPLC apparatus (Nihon Bunko, Tokyo, Japan) was used with a Jascopak SN-01 column (25 cm × 4.6 mm, Nihon Bunko) and a 5-cm SN-01 guard column or a μBondapak carbohydrate column (30 cm × 4.0 mm, Waters Assoc.).

* One International Unit (I.U.) according to the International Commission on Enzymes is the amount of enzyme that will hydrolyze 1 μmol of glycosidic bonds in 1 min under optimal conditions.

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The elution of carbohydrate was detected with a differential refractometer (Shodex SE-11). The column was eluted with acetonitrile–deionized water (65:35 v/v) at room temperature. Samples of 10–30 μl were injected into the HPLC and eluted at a velocity of 2–3 ml/min. All the solvents and samples were degassed ultrasonically and filtered with a membrane filter (0.45 μm pore size).

RESULTS

Fractionation of maltooligosaccharides with Jascopak SN-01

The partial hydrolyzate of short-chain amylose and fractionated G_1, G_2, G_3 ··· G_7* were analyzed by HPLC with the Jascopak SN-01 column under the conditions described in Experimental.

Fig. 1 shows the elution profile of the mixture of maltooligosaccharides. The quantitative analysis of G_1 to G_8 is easily done within 20 min. Various amounts of pure G_1, G_2, G_4 and G_6 were injected into the HPLC in order to construct a calibration curve between detector response and the amount of maltooligosaccharides. The detector response, K, is defined as the slope of the calibration curve for each saccharide.

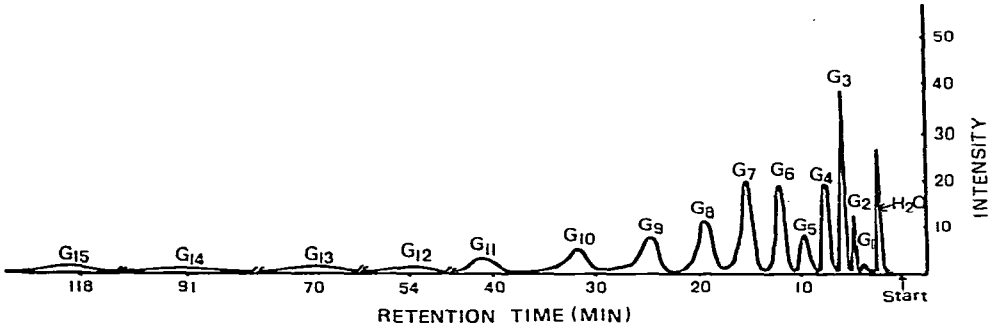

Fig. 1. Chromatogram of maltooligosaccharides mixture on Jascopak SN-01.

Fig. 2 shows the relation between peak height and the amount of saccharides. The slope of the curves gradually decreases with increasing molecular weight. The K value obtained from the G_1 curve is more than three times that from the G_6 curve. The values for G_1, G_2, G_4 and G_6 are 1.63, 1.35, 0.85 and 0.55 respectively.

Fig. 3 shows the relation between peak area, calculated as the area under a triangle, and the amount of saccharides. It is seen that the four curves are close to each other. The K values for G_1, G_2, G_4 and G_6 are 1.16, 1.23, 1.19 and 1.24.

Fig. 4 was obtained by plotting the peak weight (mg) against the amount of saccharides. Peak weight was measured by cutting and weighing the peak of each saccharide from a Xerox copy of the chromatogram. We found that the closest calibration curve was obtained when the peak weights were employed. The K values are 1.00 for G_1 and G_6, 0.99 for G_2 and G_4.

* Symbols G_1, G_2, G_3, ...etc. represent glucose, maltose, maltotriose, etc.

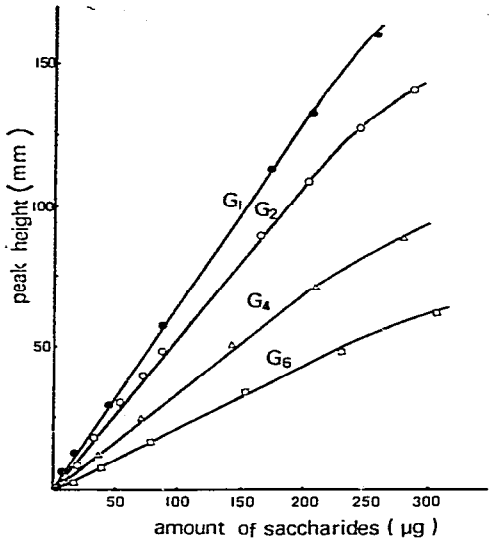

Fig. 2. Calibration curves obtained by plotting peak height against the amounts of each saccharide. Column: Jascopak SN-01.

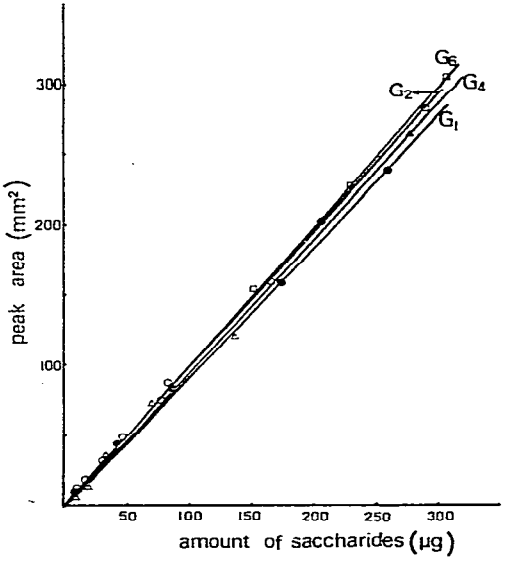

Fig. 3. Calibration curves obtained by plotting peak area against the amount of each saccharide. Column: Jascopak SN-01.

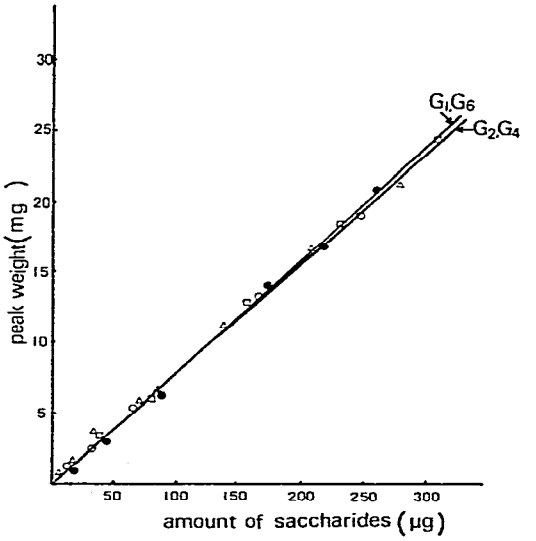

Fig. 4. Calibration curves obtained by plotting peak weight against the amount of each saccharide. Column: Jascopak SN-01.

Fractionation of maltooligosaccharides on μBondapak carbohydrate

The results of the fractionation of maltooligosaccharides on a μBondapak carbohydrate column are shown in Fig. 5. Glycerol was added as an internal standard. The column satisfactorily fractionated oligosaccharides up to G_6 or G_7, even

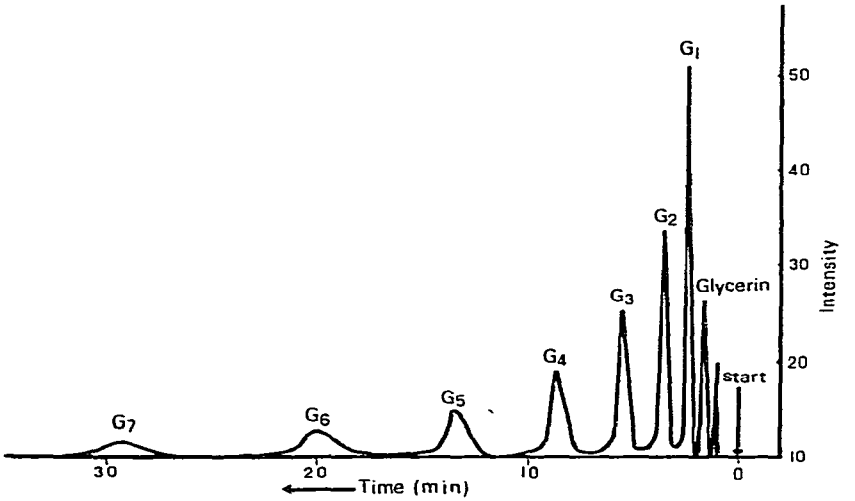

Fig. 5. Chromatogram of maltooligosaccharides mixture on μBondapak carbohydrate.

though the peaks were broad. It was difficult to analyze maltooligosaccharides higher than 10, which was easily done on the Jascopak SN-01 column. Fig. 6 shows the calibration curves for each oligosaccharide plotted as peak area against concentration. The *K* values calculated by various methods with and without internal standard are shown in Table I.

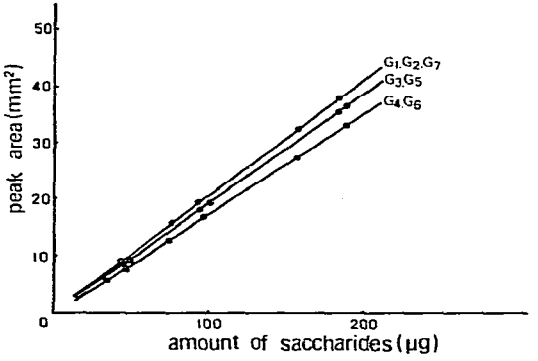

Fig. 6. Calibration curves obtained by plotting peak area against the amount of each saccharide. Column: μBondapak carbohydrate.

DISCUSSION

Because of the difficulty in obtaining commercially pure maltooligosaccharides as standard compounds for chromatography, one of the purposes of this study was to determine the range of error which might arise by using pure maltose, which is fairly readily obtainable, as a standard compound for the determination of higher malto-

TABLE I

DETECTOR RESPONSE VALUES (K) OF MALTOSACCHARIDES

Response values: H, based on peak height of maltooligosaccharide; A, based on peak area of maltooligosaccharide; H_I, based on peak height of internal standard; A_I, based on peak area of internal standard.

	H	A	H/H_I	A/A_I
G_1	0.18	0.21	0.91	1.36
G_2	0.14	0.21	0.59	1.11
G_3	0.11	0.20	0.52	1.17
G_4	0.06	0.18	0.27	1.05
G_5	0.05	0.20	0.23	1.13
G_6	0.03	0.17	0.14	1.01
G_7	0.03	0.21	0.15	1.37

oligosaccharides. Tables II and III show the correction factors for each maltooligosaccharide when G_2 is used as a standard. The correction factor (C.F.) was defined as:

$$\frac{K \text{ value of } G_2}{K \text{ value of maltooligosaccharide}}$$

As seen in Table II, the C.F. values of G_1, G_4 and G_6 are very close to 1 when the peak areas or peak weights are measured. This indicates that G_2 could be used as the standard compound for maltooligosaccharide determination with high accuracy because all the standard curves shown in Figs. 4 and 5 are very close to each other.

TABLE II

CORRECTION FACTORS OF EACH MALTOOLIGOSACCHARIDE WHEN G_2 IS USED AS STANDARD COMPOUND (JASCOPAK SN-01)

Correction factors were calculated as described under Discussion.

	Based on peak height	*Based on peak area*	*Based on peak weight*
G_1	0.83	1.06	0.99
G_4	1.59	1.03	1.00
G_6	2.45	0.99	0.99

TABLE III

CORRECTION FACTORS OF EACH MALTOOLIGOSACCHARIDE WHEN K VALUE OF G_2 IS USED (μBONDAPAK CARBOHYDRATE)

	H	A	H/H_I	A/A_I
G_3	1.27	1.05	1.13	0.94
G_4	2.33	1.17	2.19	1.06
G_5	2.80	1.05	2.57	0.98
G_6	4.67	1.24	4.21	1.10
G_7	4.67	1.00	3.93	0.81

Using the correction factors, the amount of each maltooligosaccharide is easily determined accurately from;

$$\mu g \text{ of maltooligosaccharide} = (\mu g \text{ as } G_2) \times \text{C.F.}$$

Correction factors obtained from the results on the μBondapak column were widely scattered when compared with the data obtained on Jascopak SN-01 (Fig. 3).

The experimental results described show that maltose can be employed as a standard saccharide for the determination of maltooligosaccharides with high accuracy, if the peak area or peak weight is measured. We have obtained satisfactory results with the Jascopak SN-01 column in terms of peak resolution and reproducibility. We also tested the SN-01 column packed in our laboratory and obtained exactly the same resolution of oligosaccharides. The use of the latter column reduced the cost of the column in HPLC to 5–10% of the commercially packed column.

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