DETERMINATION OF SOME COMPONENTS IN CORN SYRUP BY GAS-LIQUID CHROMATOGRAPHY OF THE TRIMETHYLSILYL DERIVATIVES[1]

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ABSTRACT

Investigation of the separation of the trimethylsilyl derivatives of carbohydrates by gas-liquid chromatography showed that this technique could be used to determine the major mono- through tetrasaccharide components of corn syrup. An improved method of derivatization was developed, applicable in the presence of up to 40 mg. water. Separations were made with SE-52 and SE-30 silicone stationary phases and with both multiple isothermal, and linear temperature-programmed operations. Component data for glucose, maltose, maltotriose, and maltotetraose, in 43 Dextrose Equivalent corn syrup, showed a relative standard deviation of 1.7 to 3.4%. Further application to the separation and determination of sucrose in the presence of corn syrup solids also was described.

The routine analysis of corn syrups for individual sugars is usually restricted to D-glucose, with occasional determination of oligosaccharides by paper-chromatographic methods. While the latter provide extensive information on components, the time required for the separation and determination of the individual sugars may be several days (1). The extension of gas-liquid chromatography (GLC) into the carbohydrate field showed that this powerful separatory technique could rapidly provide component data on complex sugar mixtures, of which corn syrup is an obvious example.

The application of GLC to the separation of carbohydrate derivatives was first reported in 1958 (2). Since then nearly 75 papers have appeared in this field, and references for most of these are given in the 150-page review by C. T. Bishop (3). Later publications include those of Richey *et al.* (4), Sawardeker and Sloneker (5), and Alexander and Garbutt (6), the latter describing a method for the determination of glucose in corn syrups and corn sugars. The work of Sweeley *et al.* (7) with preparation and separation of the trimethylsilyl (TMS) derivatives certainly was a major advance in the application of GLC to carbohydrates. This paper is concerned with the separation of these derivatives on a quantitative basis.

Materials and Methods

Preparation of Derivatives. The usual method for the preparation

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of the TMS derivatives of sugars prescribes solution of the dry sample in pyridine followed by trimethylsilylation with hexamethyldisilazane and trimethylchlorosilane (7). While this method was suitable for the derivatization of mono- and disaccharides, the triose and tetraose components of corn syrup gave yields of derivatives too variable for quantitative work. The improved method presented here avoids tedious sample-drying procedures and is directly applicable to commercial corn syrups. The use of a large excess of hexamethyldisilazane permits as much as 40 mg. water to be present. Specific directions for corn syrup follow:

1. Transfer 60–70 mg. of 80% solids syrup to a stoppered vial.
2. Dissolve in 1.0 ml. pyridine.
3. Add 0.9 ml. hexamethyldisilazane.
4. Add 0.1 ml. trifluoroacetic acid.
5. Shake vigorously for 30 sec. and let stand for 15 min. with occasional shaking. The solution should now be clear; no insoluble precipitates are formed by this method of derivatization. The sample is now ready for GLC separation. A 5-μl. sample, as measured from No. GR 700 Hamilton Constant Rate Syringe, was used throughout this work.

Gas-Chromatographic Apparatus. The gas chromatograph used in this work was an F&M 609, a single-column instrument with flame ionization detector. Columns used were 2-ft. and 6-ft., 0.25 in. o.d., 2–3% silicone (SE-52 and SE-30) on silanized Chromosorb W. Both copper and stainless-steel tubing was used. Column temperatures varied from 140°C. to a maximum of 275°C. Temperature of injection port and block (column outlet to detector) was maintained at 300°C. Gas flows were: carrier, helium at 80 ml./min. at inlet pressure 35 p.s.i., hydrogen 50 ml./min., 8–10 p.s.i., and air at 300–400 ml./min. The instrument was equipped with a Ridgefield Instrument Electronic Integrator Model PX 592. Recorder: span, 1 mv.; chart speed, 15 in./hr.

New columns were conditioned with full carrier gas flow for a least 8 hr. at 250°C.

Calibration Sugars. Reference high-purity sugars were first individually derivatized and separated by GLC, to locate minor impurities. A sugar mixture was then prepared, derivatized, and separated by GLC; the calibration factor for each sugar was obtained from its corresponding peak area. A representative mixture contained 11 mg. dextrose (Bureau of Standards), 11 mg. maltose, and 7–8 mg. each of maltotriose and maltotetraose.

Results

The GLC separation of the calibration mixture is shown in Fig. 1.

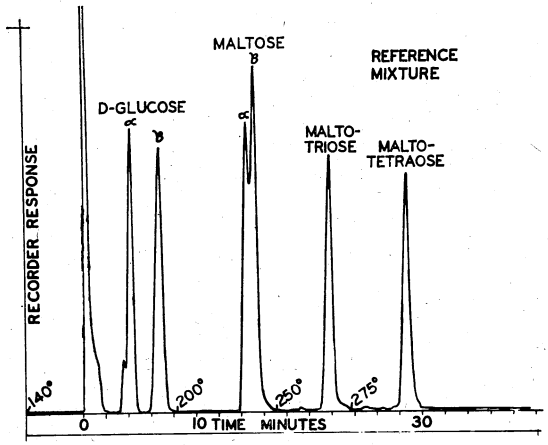

Fig. 1. Chromatogram of a TMS-derivatized calibration mixture on a 2-ft. (copper), 2% silicone (SE-52) column, using a multiple isothermal temperature program.

This separation was made with a 2-ft. SE-52 column and with a multiple isothermal operation. Alpha and beta D-glucose were separated at 140°, maltose at 200°, maltotriose at 250°, and maltotetraose at 275°C. Column temperature changes were made, as shown, immediately following peak elution, and this permitted manual correction for baseline drift and the establishment of a quiet base line prior to elution of the next peak. The area of each peak was measured by the integrator, and from this the calibration factor for each sugar was calculated. In practice it was convenient to express calibration factors as integrator counts per unit weight based on the starting sample weight only; and without bringing into the calculation the sample volume, as it and also the sample size injected were the same for all samples. To illustrate, 10 mg. maltose was derivatized, 5 μl. was injected, and an area of 250 integrator counts was obtained. The maltose calibration factor was then 250 divided by 10 (mg.), or 25. Similarly, for D-glucose (alpha plus beta) the factor was 36, and for maltotriose and maltotetraose, 24. The factors to be used for any one day were determined at the beginning of a day's work, and this daily calibration continued for 2 weeks with new columns. Thereafter, column bleed loss was less than 1 mg. per day and calibration on alternate days was satisfactory.

The maltose used in the calibration mixture was separately derivatized at various sample weights, and at constant sample weight with

various amounts of added water. Results showed detector linearity from 5 to 40 mg. of sample. Water in increasing increments of 10 mg. and from 10 to 50 mg. was added to 10 mg. maltose; the mixture was derivatized and GLC-separated. Erratic results were obtained at the 50-mg. water level, but at 40 mg. results were satisfactorily reproducible, and the same calibration factors were obtained as without added water.

This same multiple isothermal GLC separation, applied to TMS-derivatized, 43 Dextrose Equivalent (D.E), corn syrup unmixed (CSU), is shown in Fig. 2. The selection of a sample weight as de-

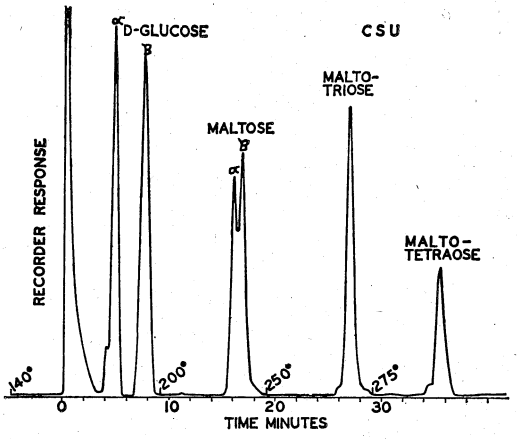

Fig. 2. Chromatogram of TMS-derivatized 43 D.E. corn syrup on a 2-ft. (copper), 2% silicone (SE-52) column, using a multiple isothermal temperature program.

scribed under *Preparation of Derivatives* (above) gives a chromatogram with peak areas for all sugars similar to that of the calibration mixture; hence, extrapolation is avoided in calculation of the various sugar percentages. The manual correction for base-line drift following a step-up in column temperature (necessary to return the recorder pen to the integrator threshold) resulted in exclusion of the small isomaltose peak between the maltose and maltotriose. This peak will be shown later in the chromatogram from a linear temperature-programmed operation. Again, as in Fig. 1, the alpha and beta anomers of D-glucose are completely separated, a partial separation of the maltose anomers is obtained, and maltotriose and maltotetraose are eluted as single peaks. The broad base of the maltotriose peak was consistently observed, and a separate investigation of this peak with a one-half sample size and a fourfold increase in instrument sensitivity showed a pair of peaks in the leading edge and a peak and shoulder in the following edge, all near the base line. The fact that the broad

base was always observed suggests that as yet unknown TMS derivatives of trisaccharides are present rather than a partial derivative of a lower-molecular-weight oligosaccharide. Tests with isomaltotriose showed that its TMS derivative was eluted midway between maltotriose and maltotetraose and not in the following edge of the maltotriose peak.

The separation of the TMS derivative of maltotetraose in a practical operating time is certainly an analytical spectacular. This derivative has a molecular weight near 1,700 and is perhaps the largest molecule ever to be eluted in a GLC separation.

The column temperatures employed in this multiple isothermal operation may be varied, depending on the sugar of interest. For example, in this laboratory, where most glucose determinations are made in an automated system using glucose oxidase-peroxidase, the column temperature is often started at 200°C. and the glucose quickly eluted without measurement. The maltose is then eluted and the appropriate temperature changes made to separate the maltotriose and maltotetraose. This program reduces the over-all separation time by 6 to 8 min.

Composition data for several samples from the same lot of 43 D.E. CSU are shown in Table I. These data were obtained from five repli-

TABLE I
COMPOSITION OF 43 D.E. CSU

SUGAR	RANGE	MEAN	S.D.	RELATIVE S.D.
	%	%		%
D-glucose (alpha and beta)	20.2–21.0	20.5	0.35	1.7
Maltose	14.4–16.3	15.3	0.55	3.6
Maltotriose	15.5–16.8	16.0	0.44	2.8
Maltotetraose	9.8–10.9	10.3	0.35	3.4

cate samples each run on two different days, and represent sample weights of 46–51 mg. dry substance. Replicates of the same sample run on the same day showed better precision, e.g. a relative standard deviation of 2.0 to 2.3% for maltose. In general, the precision of the method compares favorably with some paper-chromatographic methods (8).

With the exception of the maltotriose content, results by other methods of analysis are in good agreement with those by the GLC technique. Published data obtained by paper chromatography show, for 40–45 D.E. corn syrups: disaccharides 13–15%, trisaccharides 11–12%, and tetrasaccharides 10% (9). The mean value of 16.0% maltotriose as obtained by GLC shows the effect of the large base of this

peak on peak area. This high value could not be due to a faulty calibration factor, because similar results were obtained with three calibration mixtures. By using the integrator to center-cut this peak, values of 13–14% maltotriose were obtained. However, such a method is obviously quite empirical and subject to individual interpretation. This sample had a glucose content of 20.7% as determined by the glucose oxidase-peroxidase method.

Linear Temperature Programming. The inconvenience of manually correcting for base-line drift so as to maintain the recorder pen just below the integrator threshold suggested the use of a linear temperature-programmed operation. Such a separation of 43 D.E. CSU is shown by Fig. 3. This was made with a 2-ft. SE-30 column from 140° to 275°C. at 4.6° per min. and with holding at the 275°C. maximum until the maltotetraose was eluted. The expected base-line drift is readily apparent but does not present a serious problem until a temperature of 250°C. is reached, or near the point of elution of maltotriose. Holding at 275°C. established a new base line before the elution of maltotetraose, thus permitting elution of the latter without severe peak distortion. As indicated earlier, this chromatogram shows the small isomaltose peak between maltose and maltotriose. Area measurements of the major peaks were made with the integrator for the glucose and maltose peaks and peak height times width at half-peak height for the maltotriose and maltotetraose peaks. Calibration factors were obtained in like manner.

Comparative data for the linear temperature program and the

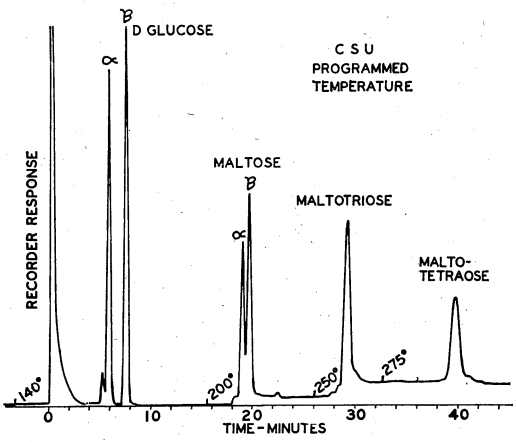

Fig. 3. Chromatogram of TMS-derivatized 43 D.E. corn syrup on a 2-ft. (stainless-steel), 2% silicone (SE-30) column, using linear temperature programming from 140° to 275°C. at 4.6°C. per min. Temperature held at 275°C. until the maltotetraose derivative was eluted.

TABLE II

COMPOSITION OF 43 D.E. CSU: COMPARATIVE DATA FOR LINEAR AND MULTIPLE ISOTHERMAL TEMPERATURE PROGRAMMING

SUGAR	SAMPLE 1 [a]		SAMPLE 2		SAMPLE 3	
	A	B	A	B	A	B
	%	%	%	%	%	%
D-glucose (alpha and beta)	21.1	20.8	21.0	20.4	20.4	20.6
Maltose	15.6	14.9	14.6	15.8	15.4	15.8
Maltotriose	16.1	15.8	15.3	15.8	16.0	16.2
Maltotetraose	11.1	10.4	9.4	10.4	10.1	9.8

[a] Column A was a linear temperature-programmed separation and column B a multiple isothermal separation.

multiple isothermal operation are shown in Table II. These data show good agreement between the two methods of changing column temperatures and with all percentages within the range shown in Table I. Data of run B were obtained with a 2-ft. SE-52 column; hence, satisfactory agreement also was obtained between separations provided by two different silicone stationary phases.

Although the linear temperature-programmed operation is not altogether satisfactory for peak area measurement, it still provides useful data. Improvement can be expected by the use of a dual-column instrument or base-line drift corrector, or a combination of both.

Sucrose-Corn Syrup Separation. The lack of direct methods for the analysis of corn syrup-sucrose mixtures suggested a further extension of this technique to the separation and determination of sucrose in the presence of corn syrup. Therefore, a 16-mg. sample of hard candy was TMS-derivatized and separated as shown in Fig. 4. This separation

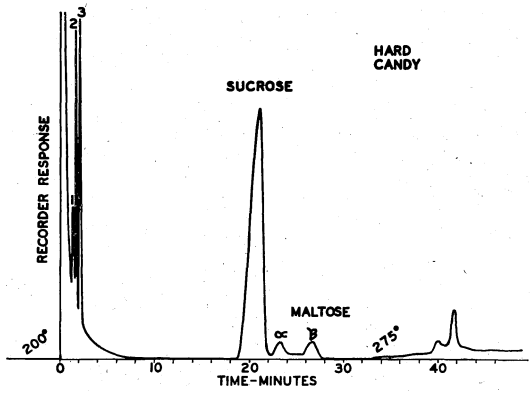

Fig. 4. Chromatogram of TMS-derivatized hard candy on a 6-ft. (stainless-steel), 3% silicone (SE-52) column, run isothermally at 200°C. Peak 1, fructose; 2, alpha-D-glucose; 3, beta D-glucose.

was made with a 6-ft. SE-52 column and at a constant temperature of 200°C. The longer column was required to make the difficult separation of sucrose from alpha maltose. Fructose present from the inversion of sucrose was not well separated from alpha D-glucose at 200°C. Lowering the column temperature to 150°C. does permit this separation, but nearly doubles the time, even with the elution of the sucrose at 200°C. In practice, following maltose elution, the column temperature was raised immediately to 275°C. to clear the column of the other derivatized oligosaccharides.

Four separate hard candy samples were analyzed for sucrose in this manner, and the results were compared with a standard chemical method based on the determination of reducing sugars before and after invertase inversion. Data were: for the GLC method, 56.1, 48.9, 54.8, and 53.5% sucrose; and for the chemical method, 56.0, 47.7, 55.8, and 55.6% sucrose.

Discussion

The methods and data presented here predict an interesting future for GLC in the carbohydrate field. Preparation of the TMS derivative in the presence of water simplifies sample preparation and reduces analysis time. Further improvement in speed and separation will come with new stationary phases and nonporous supports. Preliminary work here with glass beads coated with SE-52 shows rapid separation of TMS derivatives at lower temperature; e.g., the maltotetraose peak from CSU can be readily eluted at 235°C. Further work is in progress. The number of applications is legion.

Acknowledgments

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