

## Notes

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CHROM. 5166

### Gas chromatographic determination of fructose and glucose in syrups

Since MCINNES *et al.*<sup>1</sup> first reported the gas chromatography of carbohydrate derivatives, a vast number of publications have appeared in the literature on the subject. BROBST AND LOTT<sup>2</sup> demonstrated that it is possible to trimethylsilylate syrups containing glucose, maltose, maltotriose, and maltotetraose. Their method involved the use of hexamethyldisilazane and trifluoroacetic acid, which could not be premixed, and which generated considerable heat during reaction.

Since then, N-(trimethylsilyl)imidazole (TSIM)<sup>3</sup> has been shown to be an excellent silylating reagent for several wet sugars. It reacts quickly and smoothly, does not liberate great amounts of heat when reacting, involves the use of only one reagent, and minimizes anomerization.

When a 21 % w/v solution of TSIM was used in this laboratory as the silylating agent for the sugars in a high-fructose corn syrup, entirely unsatisfactory results were obtained. While glucose and maltose were rapidly and completely silylated, TMS-fructose appeared as three peaks on an OV-17 and as two peaks on an OV-101 column. Addition of more TSIM and/or heating did not remedy the situation.

MASADA *et al.*<sup>4</sup> described a method for determining fructose in honey by converting the carbohydrates to their TMS derivatives with hexamethyldisilazane and trimethylchlorosilane (TMSCl). The two major drawbacks of this method were the extremely long (2 h) reaction time for derivatization of fructose, and the appearance of large unidentified peaks in the chromatograms of TMS-fructose.

BETHGE *et al.*<sup>5</sup>, SEMENZA *et al.*<sup>6</sup> and CURTIUS *et al.*<sup>7</sup> have also demonstrated the appearance of multiple peaks in gas chromatograms of TMS-fructose, and presented evidence which indicated the multiplicity to be the result of the presence of the open chain, as well as mutarotation of the pyranose and furanose forms of the sugar.

Work done in this laboratory indicates that by changing the silylation reagent to a combination of TSIM and TMSCl in pyridine, fructose can be readily silylated and chromatographed as a single derivative. Other reagent combinations such as TSIM and N,O-bis(trimethylsilyl)acetamide (BSA) were found to be unsatisfactory.

### Experimental

The instrument used for this work was a Varian Aerograph Model 1800, equipped with hydrogen flame detectors. The column used for quantitative analyses was a stainless steel coil, 5 ft. long and 1/8 in. O.D., packed with 5 % OV-101 on 80/100 mesh Gas-Chrom Q. The temperatures were: column, 165°; injection port, 200° with a Pyrex insert; detector, 200°. The flow rates were: carrier gas — nitrogen, 20 ml/min; detector gas — hydrogen, 30 ml/min; and air, 300 ml/min. All injections were made with a micro syringe, using an injection volume of approximately 1  $\mu$ l. The instrument was operated at a sensitivity of  $64 \times 10^{-11}$  A/mV.

The silanating reagent was prepared by mixing four volumes of anhydrous pyridine, one volume of TSIM, and one volume of TMSCl in an air-tight injection vial. This mixture is stable for extended periods if stored in a cool dry place.

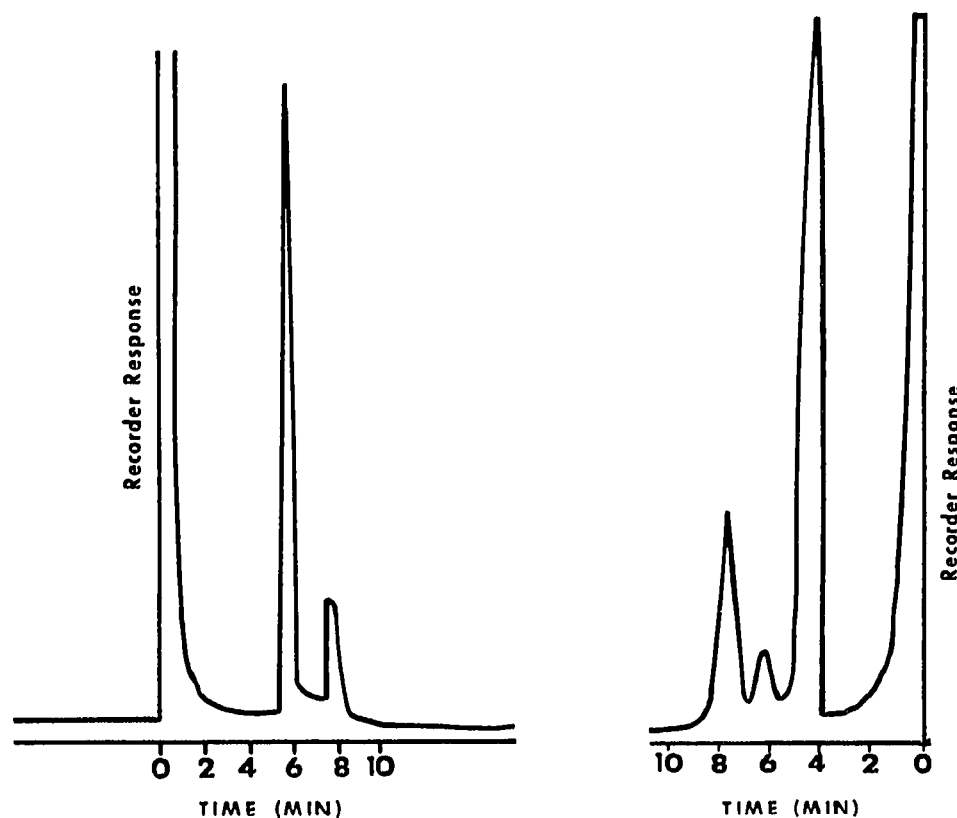


Fig. 1. Elution pattern on an OV-101 column of a sample of fructose syrup derivatized with TSIM in pyridine.

Fig. 2. Elution pattern on an OV-17 column of a sample of fructose syrup derivatized with TSIM in pyridine.

Samples were prepared by accurately weighing about 50 mg of 80 % solid high-fructose corn syrup and about 20 mg of *n*-eicosane into a small injection vial. The vial was then sealed with an appropriate crimping tool, and 5 ml of the silanating mixture were injected. The mixture was shaken vigorously for about 5 min, at which time it was ready for injection into the chromatograph.

Standards were prepared by weighing appropriate amounts of  $\alpha$ - and  $\beta$ -glucose, fructose, and internal standard into an injection vial and treating as described in the above paragraph.

Calculations were made on the basis of relative response factors as follows:

$$K = \frac{(\text{mg of } n\text{-eicosane in standard}) (\text{area of sugar peak for standard})}{(\text{mg of sugar in standard}) (\text{area of } n\text{-eicosane peak for standard})}$$

Weight percentage of sugar in sample =

$$\frac{(\text{Mg of } n\text{-eicosane in sample}) (\text{area of sugar peak for sample}) (100)}{(K) (\text{area of } n\text{-eicosane peak for sample}) (\text{mg of sample taken})}$$

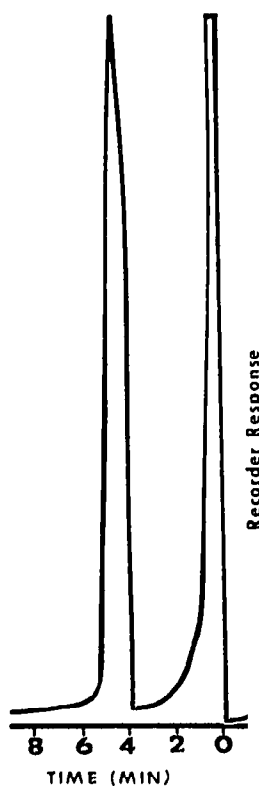
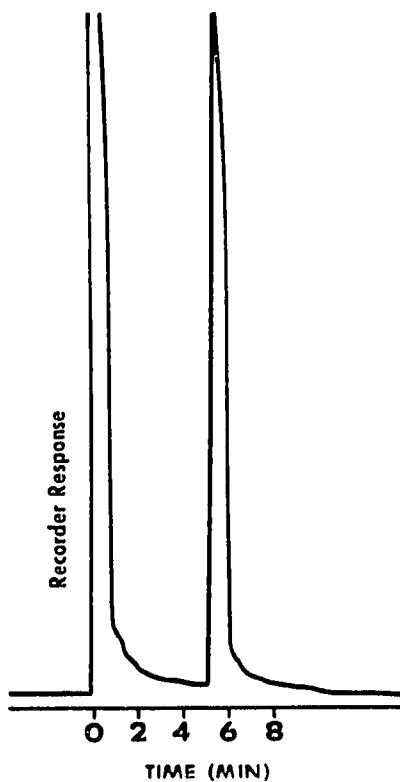
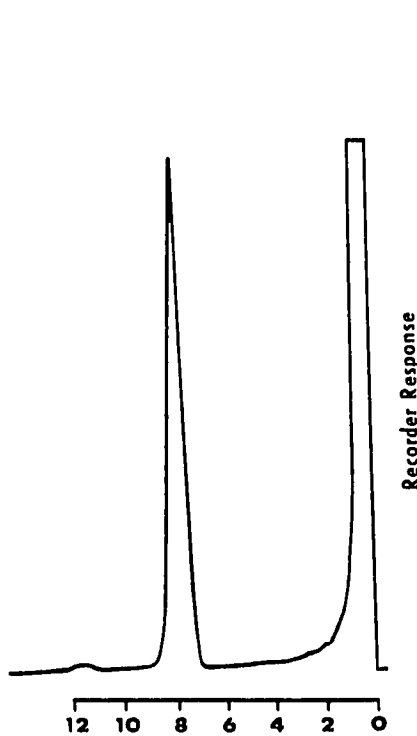
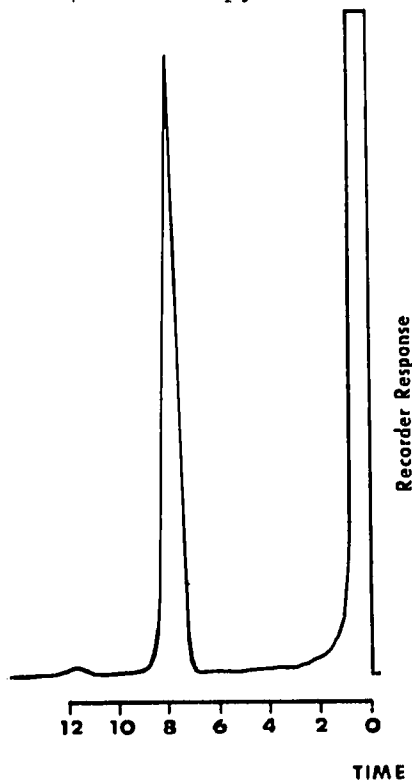


Fig. 3. Elution pattern on an OV-101 column of a sample of fructose syrup derivatized with TSIM/TMScI in pyridine.

Fig. 4. Elution pattern on an OV-17 column of a sample of fructose syrup derivatized with TSIM/TMScI in pyridine.



TIME (MIN)

Fig. 5. Elution patterns on an OV-17 column of TMS- $\beta$ -glucose derivatized with 21% w/v TSIM in pyridine (left), and TSIM/TMScI in pyridine (1:1:4) (right).

### Results and discussion

Fig. 1 shows the chromatographic results one might expect on a column of 5% OV-101 on 80/100 mesh Gas-Chrom Q when trying to silanate a syrup of pure fructose with TSIM in pyridine. Fig. 2 shows the same sample chromatographed on a column of 5% OV-17 on 80/100 mesh Gas-Chrom Q. Conditions for the two chromatograms were identical except for the column temperature, which was 165° for the OV-101, and 170° for the OV-17. It can be seen that the OV-17 stationary phase resolves one additional component. When the silanating reagent was changed to TSIM/TMSCl in pyridine (1:1:4), chromatograms such as those of Figs. 3 and 4 were obtained.

To check whether the use of the proposed reagent mixture resulted in anomerization of sugars, a sample of  $\alpha$ -glucose was derivatized with TSIM/TMSCl in pyridine. For comparison, a similar sample was derivatized with a 21% w/v solution of TSIM in pyridine. This latter combination has been reported<sup>3</sup> to result in little or no anomerization. As can be seen from Fig. 5, the results were virtually identical.

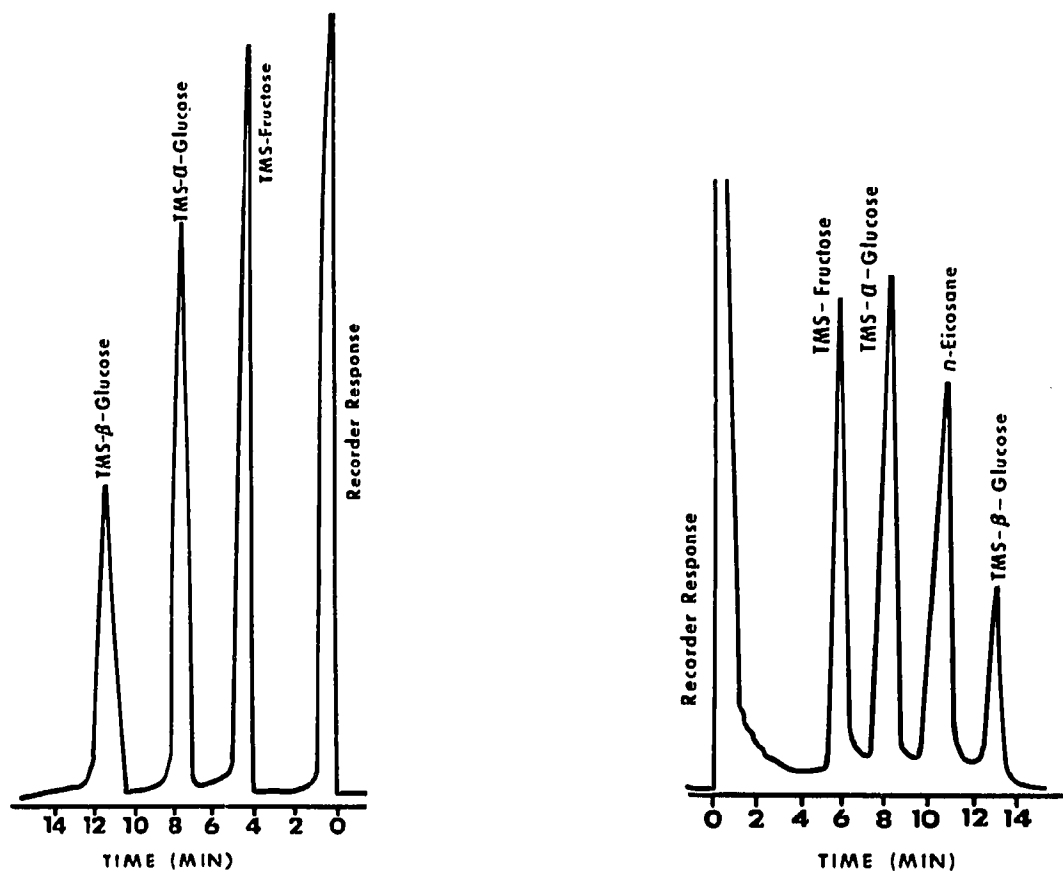


Fig. 6. Elution pattern on an OV-17 column of TMS-fructose, TMS- $\alpha$ -glucose, and TMS- $\beta$ -glucose.

Fig. 7. Elution pattern on an OV-101 column of TMS-fructose, TMS- $\alpha$ -glucose, *n*-eicosane, and TMS- $\beta$ -glucose.

*n*-Eicosane proved to be an excellent internal standard for glucose-fructose mixtures when using OV-101 columns. The retention time of the hydrocarbon is about midway between those of TMS- $\alpha$ -glucose and TMS- $\beta$ -glucose. For these reasons,

silanation conditions were established using an OV-17 column, and analytical work was done using an OV-101 one. Fig. 6 shows the chromatographic separation of TMS-fructose, TMS- $\alpha$ -glucose, and TMS- $\beta$ -glucose on an OV-17 column. Fig. 7 shows the separation of TMS-fructose, TMS- $\alpha$ -glucose, *n*-eicosane, and TMS- $\beta$ -glucose on an OV-101 liquid phase. HALPERN *et al.*<sup>8</sup> discussed in detail the advantages of using inert hydrocarbons as internal standards with samples such as these. A typical analysis of a sample of high-fructose liquid glucose was  $27.7 \pm 2.4$  % fructose and  $43.5 \pm 1.1$  % glucose. Thus precision is quite adequate.

The proposed reagent mixture provides the advantages of speed, convenience, and completeness of derivatization over other systems reported in the literature for quantitative gas chromatography of syrups containing fructose. Reagents can be premixed, no special precautions need be taken because of heat generation, and derivatization is extremely fast.

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