# Some Aspects of Carbohydrate Analyses By GLC Techniques

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Since the initial demonstration that mixtures of carbohydrates could be analyzed by gas-liquid chromatography(GLC) of their volatile derivatives, a great deal of work has been applied towards improvement of separations. While no one column type or set of conditions will completely separate all sugars simultaneously, there usually exists an optimum set of conditions for any given mixture. In the case of carbohydrates, trimethylsilyl (TMS) derivatives have been found to be the most generally useful(10)[2]. These derivatives, while somewhat water sensitive, have the advantage of being quickly and easily prepared with a minimum of sample manipulation and a maximum of convenience. Normally, simple dissolution of the sample in derivatizing reagent is sufficient to successfully prepare the desired derivative.

A typical separation of some monosaccharide TMS derivatives is shown in Figure 1. The liquid phase used in this case was SE-52, a relatively nonpolar silicone which is both popular and effective for this type of separation. Note, however, the multiplicity of peaks for each sugar. This is due to anomer formation for each sugar in aqueous solution, the originai source of this sample. Each anomer is derivatized independently, as if it were an individual component. The relative area ratios of anomeric sugars determined from the GLC chart are usually in fairly good agreement with the anomeric ratios obtained by other methods such as polarization or methylation studies. In actual practice, peak multiplicity of TMS derivatives is hardly avoidable since most samples of analytical interest, such as syrups or process streams, contain water. In many cases the relative peak areas of a given sugar will not vary; but often, especially in viscous samples with many components, the area ratios will vary significantly and unpredictably depending on the silylation conditions or the state of the equilibrium(6,7,9). Since the area of each peak is proportional to the amount of material represented by the peak, it is necessary to find the total area of all peaks attributable to the particular sugar of interest if quantitative

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[2] Numbers in parentheses refer to literature cited.

results are desired. If prior aqueous equilibrium has been deliberately established or can be otherwise guaranteed, however, the area of only one clearly separated peak will suffice for quantitation of that component.

Another aspect of sugar analysis is also apparent from Figure 1. In this case, galactose, with three peaks, is not completely separated from the fructose and glucose peaks, making quantitation and identification of these components difficult, if not completely tenuous. Other types of columns can provide better results, depending on the sample, but perfect baseline separation of complex mixtures is seldom achieved.

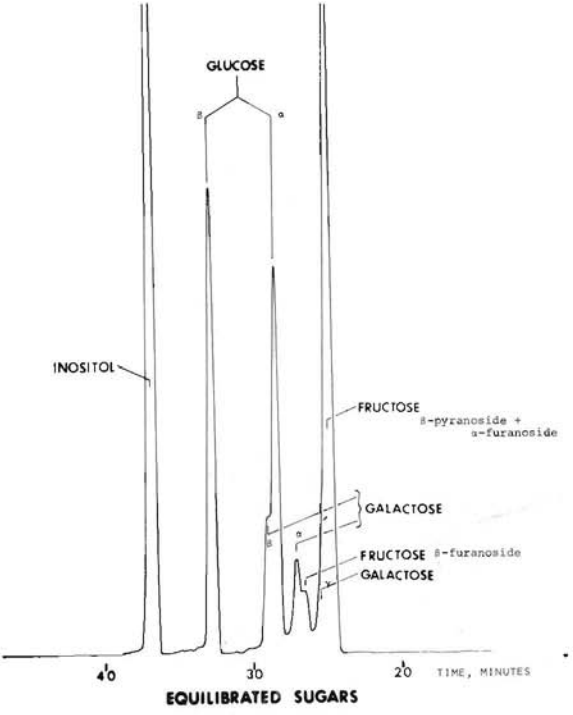
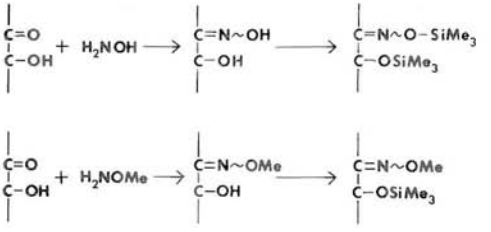



Figure 1.

It would be of considerable interest, then, to examine derivatives other than simple TMS ethers which could tend to reduce the complexity of the chromatogram by either lowering the number of peaks produced for each sugar, or providing a better separation on a stable stationary phase. Possible derivatives briefly examined for this purpose included TMS ethers of some sugar oximes and methoximes. These compounds are prepared by prior reaction of the sugar with either hydroxylamine or methoxylamine hydrochlorides in pyridine, followed by silylation with

a mixture of hexamethyldisilazane and trimethylchlorosilane (Figure 2). In the case of the oxime derivative, the hydroxyl of the oxime function is silylated concurrently with the carbon-bonded hydroxyls, while in the methoxime case, only the C-bonded hydroxyls are reactive. The methoxime derivatives have proven useful as volatile sugar derivatives in mass spectral studies, as well as for GLC(8).




DERIVATIVE FORMATION

Figure 2.

Although both oxime and methoxime derivatives introduce an extra step in sample preparation, their use reduces the complexity of the resulting chromatogram by lowering the number of significant peaks produced for each sugar (Table 1). The syn- and anti- forms of these derivatives are indicated by the peak multiplicity shown in Table 1. Note that in almost all cases, the multiplicity decreases on going to the oxime or methoxime derivative as compared to the persilyl compounds. Most striking is the coalescence of peaks for arabinose and fructose in both cases when compared to TMS derivatives. The number of peaks observed for fructose depends on the type and efficiency of the column used for the separation. In cases where five peaks are observed, they have been shown to consist of two pyranose, two furanose, and the open chain configurations(5). When fewer peaks are found, some of the individual components coalesce to single peaks. With the two mixed derivatives, fructose appears as two discrete peaks, differing in degree of separation.

**Table 1.—Peak multiplicity of various derivatives.**

| Sugar | TMS | NOH-TMS | NOMe-TMS |
|---|---|---|---|
| Arabinose | 4 | 1 | 1 |
| Fructose | 1-5 | 2 | 2* |
| Galactose | 3 | 2 | 2* |
| Glucose | 2 | 1-2 | 1-2 |
| Mannose | 2 | 2 | 1 |
| Xylose | 2 | 1 | 1 |
| Maltose | 2 | 1 | 1 |

*Nearly coalesced

*Application to Analysis of Corn Syrup Blends.*

The foregoing discussion provides a brief background for the selection of some appropriate derivatives in analyzing particular mixtures of carbohydrates. An approach to one analytical problem will indicate general considerations useful in GLC applications. Of particular interest in this regard was the examination of corn syrups and mixtures of corn syrups with sucrose and with 50% invert syrup for qualitative and semiquantitative characteristics. Such blends are supplied by Spreckels to various customers and are made up according to the desired specifications.

An immediately obvious advantage to analyzing such blends was readily apparent: these would be mixtures of relatively pure components in an aqueous milieu, therefore, no noncarbohydrate interferences would be expected. The proportion of components in a mixture would, of course, be variable.

Corn syrup being a hydrolysis product of starch, contains amounts of glucose, maltose, maltotriose, maltotetraose, and so on, which are dependent on the degree of hydrolysis of the starch. This is indicated by the dextrose equivalent(DE) of the syrup calculated as a percentage equivalent to glucose based on dry solids. In general, the higher the DE of a corn syrup, the greater is the degree of hydrolysis of the starch, and the greater is the proportion of lower DP (DP = degree of polymerization) components. This relationship is indicated in Figure 3. On the other

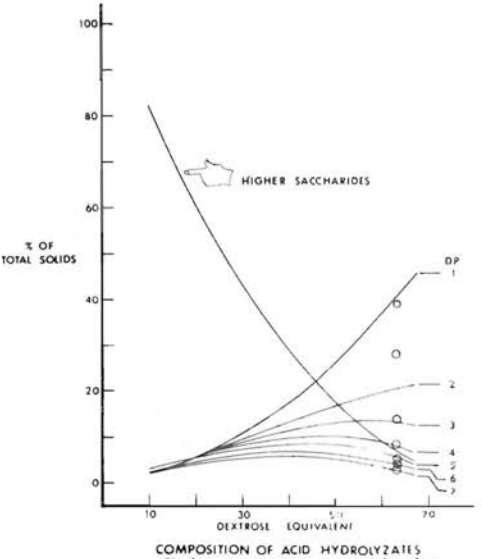



Figure 3.

hand, sucrose is molecularly homogeneous, while 50% invert syrup contains sucrose, fructose, and glucose in equimolar amounts.

Previous reports indicated the feasibility of analyzing TMS derivatives of corn syrup with reasonable quantitative accuracy (1,2). Comparison of the results of GLC analysis with actual weights of components in synthetic mixtures showed good agreement. The analysis by GLC required only minutes, while classical paper chromatography requires up to several days, depending on the separation required. Figure 4 shows a temperature-programmed separation of corn syrup solids as their TMS derivatives. Separate anomers are distinguishable for DP 1 through 3, but differences between anomers are too slight to allow separation of the corresponding higher molecular weight species. By programming to very high temperatures, species up through DP 7 were separated.

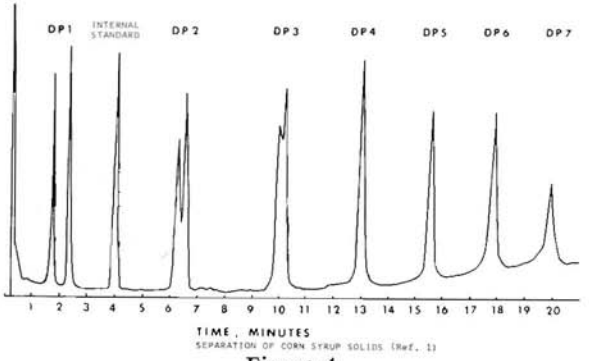



Figure 4.

With the blends of sucrose and invert with corn syrup, a major question was whether sucrose would be separable from the corn syrup components. For unmodified TMS derivatives, it was known from prior results that glucose and fructose were separable, so no problem would arise in that regard. Later, it was found that sucrose could be separated easily from maltose by utilizing TMS derivatives. Figure 5 shows a sucrose-corn syrup blend with fairly good separation obtained between sucrose and the two maltose peaks. A somewhat less complicated chromatogram could be obtained by using oxime derivatives in which maltose would appear as a single peak with better separation from sucrose, but the separation is adequate with TMS derivatives for most purposes.

Table 2 shows the relationships between the different possible blends of corn syrup with sucrose and with 50% invert

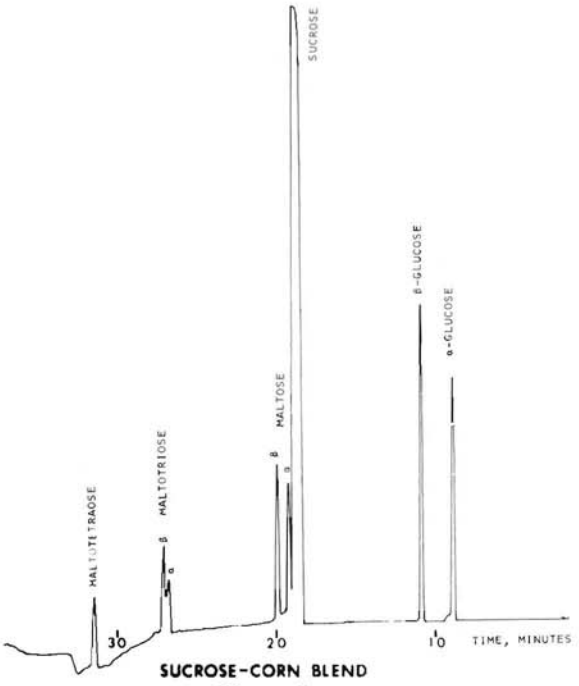



Figure 5.

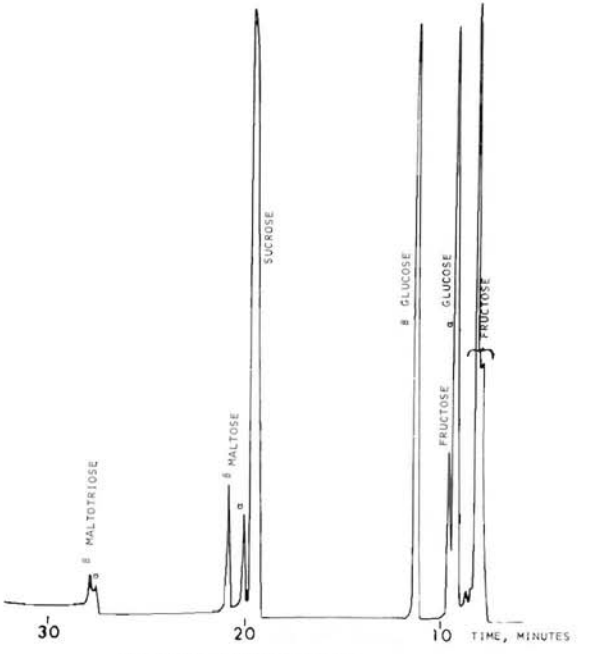
syrup. Brief inspection of the table reveals that each blend yields a unique combination of components which, in turn, provides a qualitative identification of the blend. Examination of peak areas or heights serves to quantify the proportion of each component in the blend. Figures 5 and 6 are examples of each blend type.

Table 2.—Composition of corn syrup blends.

| | Corn | Corn-Sucrose | Corn-Invert |
|---|---|---|---|
| Fructose | | | X |
| Sucrose | | X | X |
| Glucose | X | X | X |
| Maltooligosaccharides | X | X | X |

An additional piece of information is also potentially available from the chromatogram. Since the proportion of each component in a corn syrup is related to the degree of conversion of the parent starch, and hence, to the dextrose equivalent of the syrup (Figure 3), the DE may be estimated by determining the ratio of one or two maltooligosaccharides to total corn syrup solids or to one another. Alternatively, a total of all individual components allows one to estimate an approximate DE. The

available data (4) indicate little compositional difference in corn syrups derived from simple acid hydrolysis as compared to syrups obtained by a combination of acid and enzyme processing.




**INVERT-CORN BLEND**
Figure 6.

From a practical standpoint, the analysis of corn syrups or blends containing corn syrup presents some problems. One is in column selection for high temperature programmed operation which is required to provide adequate separation. In the examples shown here, a Dexsil 300 column was selected for all separations even though peak multiplicity, especially for fructose, is greater with this phase than with others. Dexsil provides a better separation than is actually called for, but it is much better than any other available phase in terms of excellent high temperature stability. The better separation permitted examination of corn syrup components only through DP 4, however. There is no other phase which exhibits long term stability at temperatures of 350-400°C. Degradation of the solid support has been noted to occur before volatility of Dexsil becomes significant. Under the severe conditions imposed by high temperature operation of chromatographic columns, one alternative is, therefore, to operate a column at temperatures which exceed the thermal stability of the phase, causing continuous degradation and ulti-

mate destruction of the phase which, in turn, requires periodic column replacement, with concurrently irreproducible separations. Another alternative is to use a high temperature phase with tolerable separation characteristics, but which is stable and reproducible over the long run. This alternative was chosen for this work.

Other phenomena which appear under conditions of high temperature operation are associated with leakage at couplings and fittings, as well as bleed of more or less volatile components from injector septa and column end plugs. Bleeding of ordinary septa causes grave instabilities in the baseline of the programmed runs at high temperatures, as well as ghosting phenomena associated with residues of previous runs. These problems can be largely alleviated by substituting teflon coated silicone septa for the ordinary silicone variety which are normally used at lower temperatures. The teflon coating, being inert, acts as a vapor barrier, preventing volatile septum impurities from entering the column. They are, however, less rugged than ordinary septa and need to be replaced after every few injections. Foil coated septa can also be used, but suffer from similar disadvantages. In the case of column connections with injector port and detector, silicone O-rings are used to seal the fittings of the glass columns to the chromatograph. They tend to become hard and brittle after a few days of high temperature operation and must be replaced when leaks develop. Asbestos cord, impregnated with Dexsil, has, however, been mentioned (3) as an effective column seal at high temperatures. This technique was used successfully in the work reported here to eliminate leaks caused by high temperature operation at the column fittings. Leakage at the septum still remains a problem, however.

## Methods

*Preparation of Derivatives.* The sugar oxime and methoxime derivatives (Figure 2) were prepared by dissolving a previously dried equilibrated sugar mixture in a pyridine solution containing a slight excess of either hydroxylamine hydrochloride or methoxylamine hydrochloride. The resulting solution was warmed at 80°C for an hour and the solvent was then removed by a gentle stream of nitrogen in a sand bath at 60°C. The dried sample was derivatized with Sweeley's reagent (10). Excess solvent and reagents were removed by nitrogen as before, and the sample was taken up in carbon tetrachloride for injection into the chromatograph. Corn syrup and corn blends were derivatized directly with Sweeley's reagent after prior drying *in vacuo* over phosphorus pentoxide.

*Chromatography.* Gas chromatography was carried out on a Varian-Aerograph Model 2100 chromatograph equipped with columns of either 5% SE-52 on 80/100 mesh Chromosorb WHP (Figure 1) or 5% Dexsil 300 on 80/100 mesh Gas-Chrom Q (Figures 5 and 6). When chromatographing monosaccharide derivatives on either phase, the temperature was programmed from 130 to 250°C at 2°/minute with injectors and detectors maintained at 275°C. When the corn syrup or corn blend samples were examined, only the Dexsil columns were employed and were programmed from 100 to 350°C at 10°/minute with injectors and detectors at 390°C.

## Conclusion

Since the advent of trimethylsilyl derivatives of carbohydrates, a multitude of techniques and procedures has been developed for their qualitative and quantitative analysis by GLC. In many cases, such as the analysis of corn syrup blends, these derivatives provide satisfactory separations in a relatively short period of time. More extensive and less complicated separations and analyses may be had by using different derivatives, such as oxime or methoxime trimethylsilyl ethers. These require somewhat more sample preparation than do simple TMS ethers, but result in less complicated chromatograms due to a reduction in peak multiplicity. Once an appropriate derivative is selected for a particular mixture, limitations on carbohydrates analysis of higher saccharides in particular can become a matter of the ultimate volatility of the derivative as well as the chromatographic system. This is evident in the analysis of corn syrup blends for higher molecular weight components. Stringent requirements are placed on the ultimate stability of the column and its associated hardware, both in terms of high temperature stability and freedom from leakage of carrier gas and sample. Newly developed phases and mechanical conveniences are beginning to show promise in alleviating these limitations, but much room for future improvement is apparent.

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