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Quantitative Determination of Sugars in Gums

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QUANTITATIVE DETERMINATION OF SUGARS IN GUMS BY A MODIFIED GLC METHOD

BY

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THESIS ABSTRACT

Quantitative determination of sugars in gums by a modified GLC method, has generated two manuscripts with the following abstracts:

Abstract I:

A modified GLC method for hexose derivatives.

The procedure involving oxime formation followed by silylation for the analysis of standard hexoses resulted in the formation of unseparated peaks for D-glucose, D-mannose and D-galactose. Therefore, this procedure was modified to obtain clear separation of hexoses. "STOX" was mixed with TMSI or HMDS, and then added to a standard sugar mixture containing D-glucose, D-galactose and D-mannose. The mixture was analyzed by gas chromatography with OV-17/OV-22 as the stationary phase, resulting in three distinct peaks.

Abstract II:

Quantitative determination of sugars in gums by GLC method.

A modified GLC method was utilized for the detection and quantitative determination of sugars derived from commercial gums. The method is suitable for the analysis of Guar gum, Locust bean gum, Tamarind gum, Agar, Carageenan, Xanthan, Gum Ghatti, Gum Arabic, Emulgum, Gum Tragacanth and Gum Karaya.

The polysaccharides were first cleaved by acid hydrolysis followed by derivatization with "STOX" hydroxylamine to form oxime with "TMSI" or "HMDS+TFAA" for silylation. The separation and quantitation was performed by gas liquid chromatography using glass columns. Phenyl-B-D-glucopyranoside was used as internal standard for quantitative analysis. The sugar molar ratios (percent) for all commercial gums tested, were similar to values reported in the literature. Results indicated that this scheme is suitable for gum identification as well as quantification of thickeners isolated from foods.

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PREFACE

This thesis has been prepared according to the manuscript plan. The first manuscript follows the style of the Journal of Chromatography, and the second manuscript follows the style of the Journal of Food Science.

MANUSCRIPT I

A MODIFIED GLC METHOD FOR HEXOSE DERIVATIVES

SUMMARY

The procedure involving oxime formation followed by silylation for the analysis of standard hexoses resulted in the formation of unseparated peaks for D-glucose, D-mannose and D-galactose. Therefore, this procedure was modified to obtain clear separation of hexoses. "STOX" was mixed with TMSI or HMDS, and then added to a standard sugar mixture containing D-glucose, D-galactose and D-mannose. The mixture was analyzed by gas chromatography with OV-17/OV-22 as the stationary phase, resulting in three distinct peaks.

INTRODUCTION

Over the past years a number of procedures (1-3) have been reported for producing volatile derivatives of sugars suitable for use in GLC analysis. The results of earlier investigations (9,10) on the chromatography of carbohydrates in the form of their TMS ethers showed the suitability of these derivatives for analytical applications. Hence, TMS derivatives have found increased use in GLC, largely because they are appropriate for a wide range of functional groups. The major problems inherent to the gas chromatographic separation of mono saccharides is the formation of multiple peaks due to tautomeric forms of reducing sugars. Sweely and co-workers (1,4,5) investigated the possibility of reducing the number of tautomers by converting the sugars into oximes before forming the trimethyl silyl ethers (TMS). Under these conditions however, D-glucose, D-galactose and D-mannose could not be resolved. Separation of these sugars was improved by using high-efficiency capillary columns (6,7). However, these columns often give incomplete separations of closely related compounds and make it difficult to identify each component, even with the use of mass spectrometry (8). Further, one of the disadvantages of glass capillary columns that has limited their wide use is their high cost and relatively short life.

Thus the separation of TMS derivatives of D-glucose, D-galactose and D-mannose appears to be difficult by the

by the derivatization method #18 of Pierce Hand Book (11) on regular columns. Therefore, the purpose of this research was to modify the existing procedure in order to obtain a clear separation of these three hexoses.

EXPERIMENTAL

Materials:

D-glucose, D-galactose and D-mannose standards were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). "STOX" hydroxylamine containing phenyl-B-D-glucopyranoside as internal standard, silylation reagents, Hexamethyldisilazane (HMDS) and Trimethylsilylimidazol (TMSI) and Trifluoroacetic acid (TFAA), silylation grade pyridine as solvent and reaction vials were purchased from Pierce Chemical Co. (Rockford, IL, U.S.A.). Chromatography liquid phase containing 3% OV-17/1.5% OV-22 on 100/120 Supelcoport was obtained from Supelco, Inc. (Bellefonte, PA, U.S.A.).

Apparatus:

A Varian Aerograph Series 1800 gas chromatograph equipped with a flame ionization detector and 1.8mx4mm ID glass column packed with the mixture of OV-17/OV-22 was used. Nitrogen was used as the carrier gas at a flow rate of 35 cm3/min. Hydrogen flow rate was 25 cm3/min. The temperatures of the injector and the detector were 320°C and 350°C, respectively. The analysis was performed using temperature programming from 140°C to 300°C at a heating rate of 10°C/min, with an initial holding for 3 minutes.

Preparation of Derivatives:

The Pierce (1983) procedure #18 for derivatization of sugars was modified as follows: One ml of "STOX" reagent containing the internal standard was mixed with 1 ml of HMDS. To this mixture 0.1 ml trifluoroacetic acid (TFAA) was added, the mixture was shaken for 30 seconds, allowed to stand at room temperature for 15 min, and filtered. The filtrate was added to 10-15 mg of accurately weighed mixture of sugar standards, in a 3.5 ml screw cap septum vial. This mixture was heated for 15 min at 70-75°C and allowed to cool to room temperature for 30 minutes. One microliter of the clear solution was injected into the gas chromatograph. When trimethylsilylimidazole (TMSI) was used for silylation, trifuloroacetic acid (TFAA) was omitted.

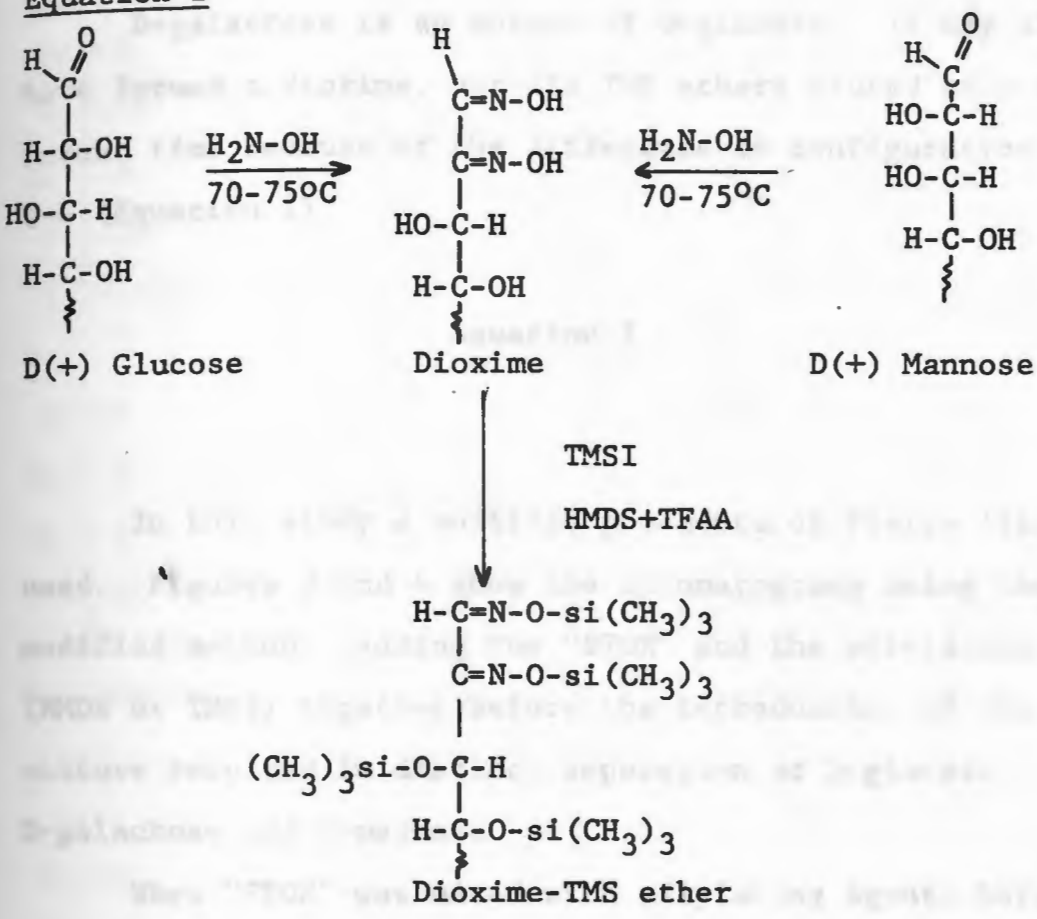
RESULTS AND DISCUSSION

Gas chromatograms obtained by using the Pierce procedures (11) are shown in Figures 1 and 2. The standard mixture of three sugars, D-glucose, D-galactose and D-mannose, gave rise to two peaks. These peaks were undifferentiated under the experimental conditions used. Since hexoses are aldehydes or ketones, they react with hydroxylamine to form oximes. If an excess amount of hydroxylamine is used, the reaction may yield products known as dioximes. The dioxime formation destroys the configuration about C-2 of an aldose, but does not affect the configuration of the rest of the schedule. This is similar to the formation of osazone by phenylhydrazine (12).

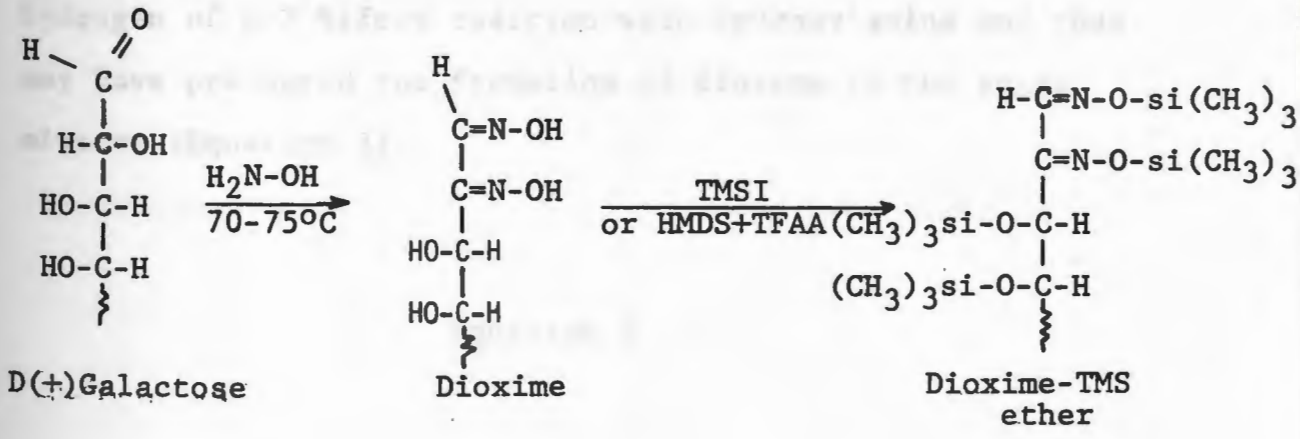
It is known that D-glucose and D-mannose are epimers with respect to C-2 and have the same configuration about C-3, C-4 and C-5. These two hexoses may have formed the same dioxime and therefore, the dioxime-TMS ethers of these two sugars gave rise to a single peak (Equation 1).

Equation 1

Equation 1



Equation 2



D-galactose is an epimer of D-glucose. It may also have formed a dioxime, but its TMS ethers eluted at a different time because of the difference in configuration about C-4 (Equation 2).

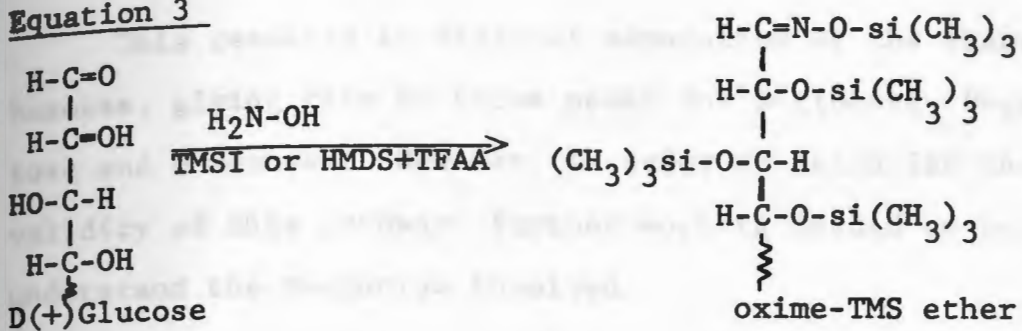
Equation 2

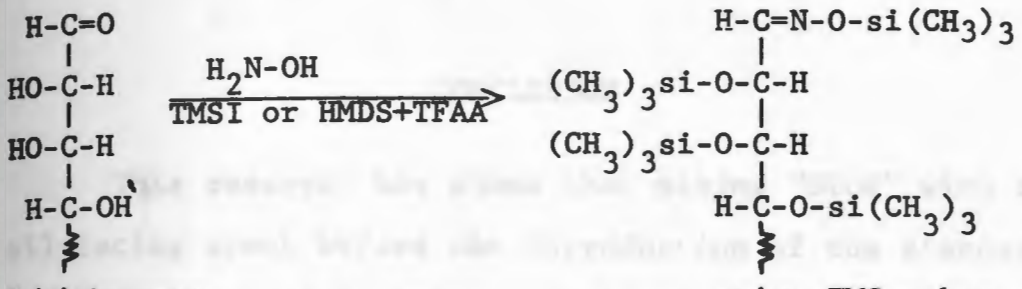
In this study a modified procedure of Pierce (11) was used. Figures 3 and 4 show the chromatograms using the modified method. Adding the "STOX" and the silylating agent (HMDS or TMSI) together before the introduction of the sugar mixture resulted in distinct separation of D-glucose, D-galactose and D-mannose.

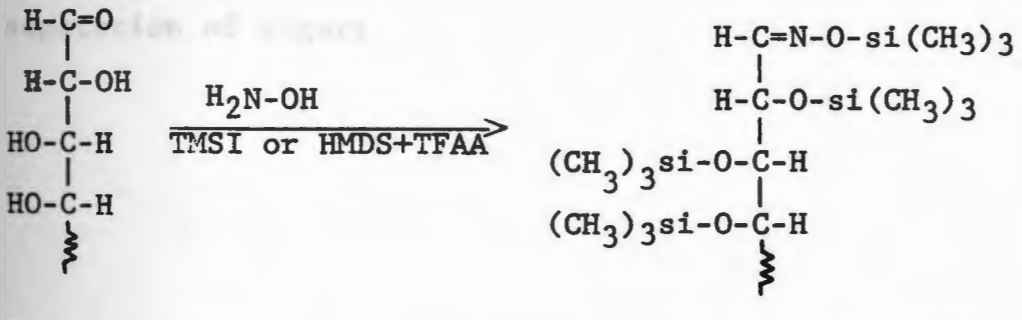
When "STOX" was mixed with silylating agents before sugar addition, a silyl group may have replaced the active hydrogen of C-2 before reaction with hydroxylamine and that may have prevented the formation of dioxime in the sugar mixture (Equation 3).

Equation 3

Equation 3


H-C=O
H-C-OH
HO-C-H
H-C-OH
D(+)Glucose
H_2N-OH
TMSI or HMDS+TFAA
H-C=N-O-si$(CH_3)_3$
H-C-O-si$(CH_3)_3$
$(CH_3)_3$si-O-C-H
H-C-O-si$(CH_3)_3$
oxime-TMS ether


H-C=O
HO-C-H
HO-C-H
H-C-OH
D(+)Mannose
H_2N-OH
TMSI or HMDS+TFAA
H-C=N-O-si$(CH_3)_3$
$(CH_3)_3$si-O-C-H
$(CH_3)_3$si-O-C-H
H-C-O-si$(CH_3)_3$
oxime-TMS ether


H-C=O
H-C-OH
HO-C-H
HO-C-H
D(+)Galactose
H_2N-OH
TMSI or HMDS+TFAA
H-C=N-O-si$(CH_3)_3$
H-C-O-si$(CH_3)_3$
$(CH_3)_3$si-O-C-H
$(CH_3)_3$si-O-C-H
oxime-TMS ether

This resulted in distinct separation of the standard hexoses, giving rise to three peaks for D-glucose, D-galactose and D-mannose. However, in order to establish the validity of this pathway, further work is needed to better understand the mechanism involved.

CONCLUSION

This research has shown that mixing "STOX" with the silylating agent before the introduction of the standard hexoses, resulted in three individual peaks for D-glucose, D-galactose and D-mannose. Therefore, the modified procedure otulined here proves to be an effective tool in clear separation of sugars.

Fig. 1 - Gas chromatogram of three hexoses as their oxime TMS ethers, using "STOX" followed by TMSI as derivatizing agents: (1) and (2) represent D-Mannose, D-gallactose and D-glucose; (3) phenyl-B-D-glucopyranoside as (I-S).

Fig. 2 - Gas chromatogram of three hexoses as their oxime TMS ethers,using "STOX" followed by HMDS + TFAA as derivatizing agents: (1) and (2) represent D-Mannose, D-galactose and D-glucose; (3) phenyl-B-D-glucopyranoside as (I-S).

Fig. 3 - Gas chromatogram of three hexoses, using "STOX + TMSI" as derivatizing agent: (1) D-Mannose; (2) D-galactose; (3) D-glucose; (4) phenyl-B-D-glucopyranoside as (I-S).

Fig. 4 - Gas chromatogram of three hexoses, using "STOX + HMDS + TFAA" as derivatizing agent: (1) D-Mannose; (2) D-galactose; (3) D-glucose; (4) phenyl-B-D-glucopyranoside as (I-S).

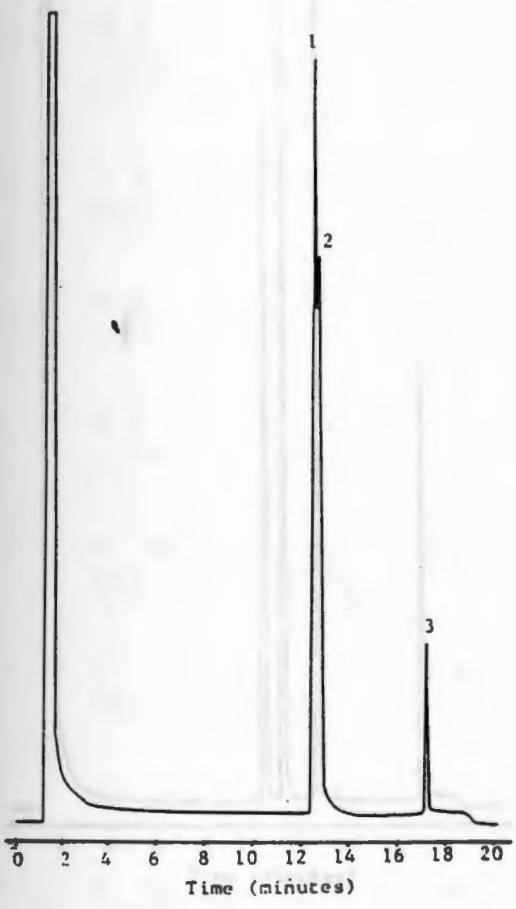

1
2
3
0 2 4 6 8 10 12 14 16 18 20
Time (minutes)

Fig. 1

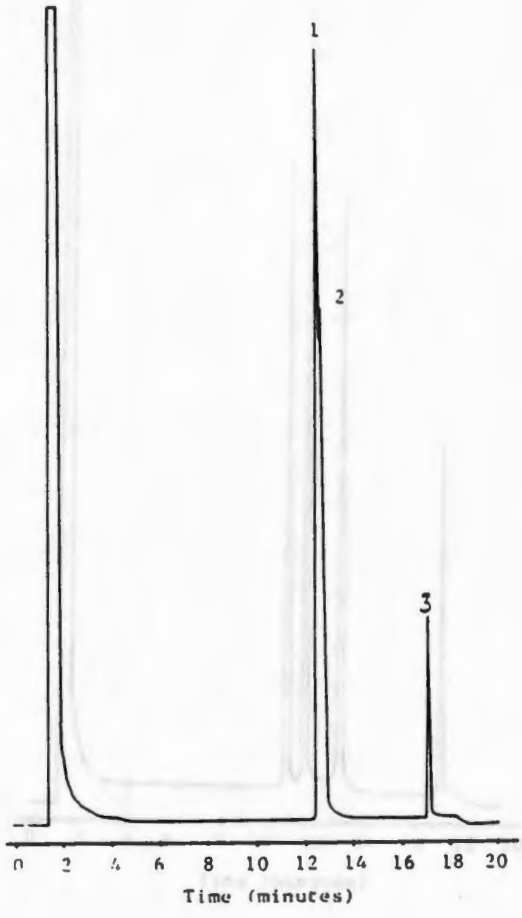

1
2
3
0 2 4 6 8 10 12 14 16 18 20
Time (minutes)

Fig. 2

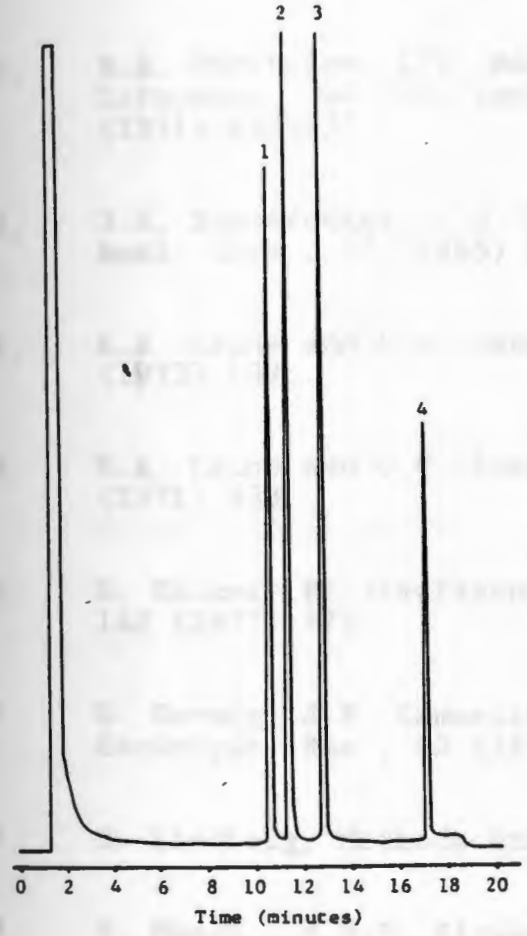

1
2
3
4
0 2 4 6 8 10 12 14 16 18 20
Time (minutes)

Fig. 3

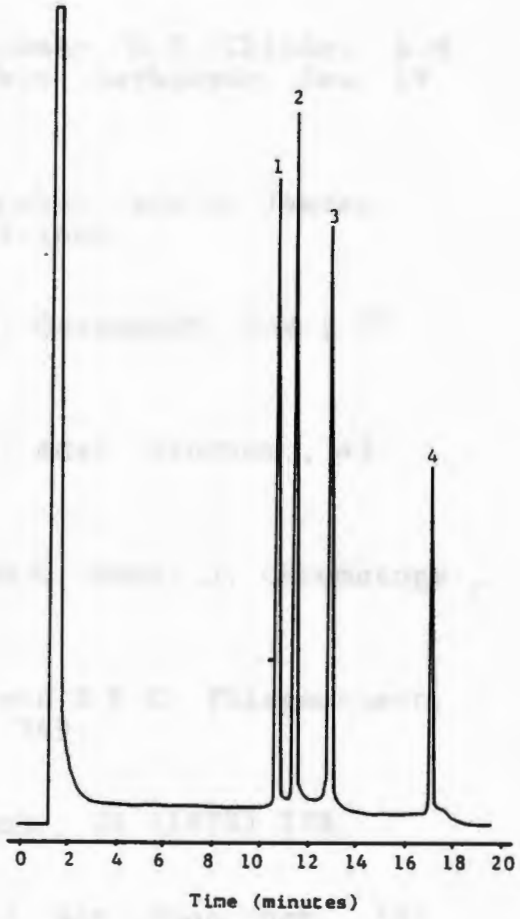

1
2
3
4
0 2 4 6 8 10 12 14 16 18 20
Time (minutes)

Fig. 4

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MANUSCRIPT II

QUANTITATIVE DETERMINATION OF SUGARS IN GUMS BY GAS LIQUID CHROMATOGRAPHY

ABSTRACT

Quantitative Determination of Sugars in Gums by GLC Method

A modified GLC method was utilized for the detection and quantitative determination of sugars derived from commercial gums. The method is suitable for the analysis of Guar gum, Locust bean gum, Tamarind gum, Agar, Carageenan, Xanthan, Gum Ghatti, Gum Arabic, Emulgum, Gum Tragacanth and Gum Karaya. The polysaccharides were first cleaved by acid hydrolysis followed by derivatization with "STOX" hydroxylamine to form oxime with "TMSI" or "HMDS+TFAA" for silylation. The separation and quantitation was performed by gas liquid chromatography using glass columns. Phenyl-B-D-glucopyranoside was used as internal standard for quantitative analysis. The sugar molar ratios (percent) for all commercial gums tested, were similar to values reported in the literature. Results indicated that this scheme is suitable for gum identification as well as quantification of thickeners isolated from foods.

INTRODUCTION

Commercial gums are water soluble or water dispersible hydrocolloids. Their aqueous dispersions usually possess suspending, dispersing, and stabilizing properties. The gums may act as emulsifiers, have gelling characteristics, and be either adhesive or mucilaginous. They may, on occasion, act as coagulants, binders, lubricants, or film formers.

The analysis and identification of commercial gums, sold in the raw or pure state, is comparatively straightforward; various chemical and physical tests have been devised for purposes of identification and confirmation. However, with the extensive use of gums and mixtures of gums in food products, the isolation and identification of these hydrocolloids becomes much more difficult. All the skill, ingenuity, and instrumentation available to the chemist are required for accurate analysis (Glicksman, 1969). Paper chromatography, zone electrophoresis, X-ray crystallography and differential thermal analysis, are several analytical methods available for identification of gums (Smith and Montgomery, 1959). Many identifications for gums by analysis of physical and chemical properties have been reviewed by Glicksman (1969). The current interest in the physiological nature of carbohydrates together with technological developments in food processing and manufacture, has increased the need for methods of analysis to determine in-

dividual sugars in gums used in foodstuffs. Most methods for specific sugars are chromatographic, including column, paper, ion exchange (Whistler and Wolform, 1962), and thin layer (Stahl, 1965) chromatography. For the chromatographic analysis of glucose and maltosaccharides, numerous methods are known (Molnar and Szakacs, 1981). They are paper chromatography, thin- layer chromatography, liquid chromatography, including gel permeation chromatography, as well as gas chromatography.

Conrad and Palmer (1976) gave an account of the current status of the usage of high performance liquid chromatography in the analysis of sugar syrups, but concentrated mainly on the qualitative aspects only. The analysis of carbohydrates and polyols by high-performance liquid chromatography (HPLC) has received considerable attention in recent years, including methods employing different stationary phases (Hendrix et al., 1981).

In recent years gas chromatography (GC) has been adapted to the separation and determination of volatile derivatives of sugars. The first step of the gas chromatographic analysis of saccharides is the preparation of suitable derivatives such as silylation derivatives (Sweely et al., 1963; Kline, D.A. et al., 1970; Prey, V. et al., 1974; Drawert, F., Leupold, G., 1976; Yasui, T. et al., 1979; Zegota, H., 1980; Jaddou, H., Al-Hakin, M., 1980; Iverson, J.L., Bueno, M.P., 1981; and Preuss, A., Thier, H.P., 1982). As a result of their work, gas chromatography

became an efficient tool in the analysis of cartain carbohydrates. The aim of the work proposed here is to devise a rapid quantitative method for the determination of the hydrolyzed sugars of commercial gums. The major benefits of GC as an analytical technique are speed and selectivity.

MATERIALS AND METHODS

Materials:

Commercial Gums: Table 1 lists the various commercial gums used in this study. Standard sugars were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Hydroxylamine containing phenyl-B-D-glucopyranoside as internal standard (STOX), hexamethyldisilazane (HMDS), trimethylsilylimidazol (TMSI), trifluoroacetic acid (TFAA), silylation grade pyridine as solvent and reaction vials were purchased from Pierce Chemical Co. (Rockford, IL, U.S.A.). The GC stationary phase containing 3% OV-17/1.5% OV-22 on 100/200 Supelcoport was obtained from Supelco, Inc. (Bellefonte, PA, U.S.A.).

Apparatus: A Varian Aerograph Series 1800 gas chromatograph equipped with a flame ionization detector and 1.8mm x4mm ID glass column packed with the mixture of OV-17/OV-22 was used. Nitrogen was used as the carrier gas at a flow rate of 35 cm3/min. Hydrogen flow rate was 25 cm/3min. The temperatures of the injector and the detector were 320°C and 350°C, respectively. The analysis was performed using temperature programming from 140°C to 300°C at a heating rate of 10°C/min, with an initial holding for 3 minutes.

Methods:

Hydrolysis: Two hydrolysis procedures were used to assess

the subunits in the gums, according to Adams (1965). The first procedure was used to cleave a-D-hexoglycans. The gum sample (1.5g) was refluxed in 200ml of water with 20ml conc. HCl for 2-1/2 hr. In the second procedure, cleavage of B-D-hexoglycans was accomplished by mixing 1.5g of gum with 5ml of 72% H_2SO_4 and holding the mixture at 25°C for 45 min with stirring at 5 min intervals. Then the mixture was diluted with 140ml of water and autoclaved at 121°C for 1 hr.

The mixture in either procedure was neutralized with 1N NaOH and filtered through Whatman #4 filter paper. The mixture was dried, 50ml of 80% ethanol was added and the mixture was allowed to stand overnight. After filtering, the filtrates obtained from the two hydrolysis procedures were mixed. The combined filtrates were washed with 30ml of petroleum ether to remove the lipids and the aqueous phase containing the sugars was evaporated to dryness.

Derivatization: Hexamethyldisilazane (HMDS): One ml of "STOX," hydroxylamine containing phenyl-B-D-glucopyranoside as internal standard, was mixed with 1ml of HMDS. To this mixture 0.1ml trifluoroacetic acid (TFAA) was added, the mixture was shaken for 30 seconds, allowed to stand at room temperature for 15 min and filtered. The filtrate was added to 30-40mg of dry hydrolyzed gum, in a 3.5ml screw cap septum vial. This mixture was heated for 15 min at 70-75oC and allowed to cool to room temperature for 30 minutes.

One microliter of the clear solution was injected into the gas chromatograph. When trimethylsilylimidazole (TMSI) was used for silylation, trifluoroacetic acid (TFAA) was omitted.

Peaks were identified by comparing their retention times with those of standard sugars. Internal standard methodology was used to quantitate the known sugars. For the unknown peaks, the response factor used was the average of the highest and the lowest response factors of the eleven individual sugar standards available. The percent of unknown sugars was calculated as follows:

$$\% \text{ Unknown Sugar} = \frac{As \times RRF \times Wi \times 100}{Ai \times Ws}$$

where,

As = area of unknown sugar peak,

RRF = Relative response factor,

Wi = Weight of the internal standard,

Ai = Area of internal standard peak, and

Ws = Weight of the sample.

RESULTS AND DISCUSSION

With a few exceptions, almost all gums are polysaccharides in nature. Usually, one hydrolyzes the gums and identifies the monosaccharides with one of the available analytical methods. Gas chromatography is an ideal quantitative method to analyze these gums. The results of the gas chromatographic analysis of standard sugars are shown in Figures 1 and 2. The retention time (RT) and the relative response factor (RRF) of eleven standard sugars are presented in Table 2. The data were used in identifying and quantifying the monosaccharides present in individual gums, using B-phenyl-D-glucopyranoside as the internal standard.

The results of chromatographic analysis of seed gums, marine gums and microbial gums are presented in Table 3. D-Mannose (17.57%) and D-galactose (5.87%) in a ratio of 2.99:1 were identified as the only sugars in guar gum. The data is also shown in Figure 3. Whistler (1973) reported a 2:1 ratio of D-mannose and D-galactose in guar gum. Similarly locust bean gum contained 26.10% D-mannose and 4.59% D-galactose in 5.70:1 ratio (Figure 4). Several other studies have indicated varying levels of these sugars in locust bean gum giving a ratio of 1.22:1; 4:1; 4.56:1; 5.25:1 and 6.14:1 (Arora, 1983).

In general, until now the ratio for guar gum and locust bean gum was stated as galactose:mannose 1:2 for guar and 1:4 for locust bean, respectively.

Chromatograms of sugars from marine gums are shown in Figures 6 (agar) and 7 (carageenan). Agar contained 15.14% D-galactose and 26.28% 3,6, anhydro L-galactose. However, the unavailability of the latter standard sugar made it difficult to identify, quantitatively. Therefore, the data was calculated by using the average RRF. Carageenan contained 15.68% D-galactose. As seen in agar, 3,6, anhydro L-galactose was also identified in carageenan (34.36%). This gum, when purchased, also contained dextrose used as a preservative (as seen in Figure 7) which was identified as D-glucose (10.68%). The ratio of D-galactose to 3,6, anhydro L-galactose of L-arabinose, D-galactose, D-mannose, D-xylose and D-glucuronic acid in a molar ratio of 10:6:2:1:2, respectively. Figures 10 and 11 show the chromatograms of gum arabic and emul gum, respectively. Both these gums contained the same monosaccharides. L-arabinose, L-rhamnose, D-galactose and D-glucuronic acids were present in gum arabic at 8.94%, 10.21%, 13.90% and 4.81%, and at 19.95%, 18.92%, 14.48% and 1.14% in emul gum, respectively. Whistler (1973) reported concentrations of 34.4% of L-arabinose, 14.2% of L-rhamnose and 42.1% of galactose and 15.5% of D-glucuronic acid. There were two unknown peaks (#2 and 5) in gum arabic (Figure 10). Peak #5 can probably be assumed to be a uronic acid ester. The gas chromatograms of emul gum differed slightly from gum arabic in the overall quantitative distribution of sugars and unknowns. Peak #4 in

Figure 11 is a major unknown peak, which may be a uronic acid ester.

Gum tragacath, another member of the plant exudate gums, contained L-arabinose (9.51%), L-fucose (11.29%), D-xylose (4.76%), D-mannose (4.92%), D-galactose (3.23%), D-glucose (6.04%) and D-galacturonic acid (5.06%). The distinguishing characteristics of this gum, is the presence of L-fucose as a major peak (#2) (Figure 12). This gum also contained two unknown peaks. Peak #6 may probably be a uronic acid ester. The RT of unknown peak #9 was similar to that of glucuronic acid. Therefore, this peak could be glucuronic acid. Preuss and Thier (1982) identified seven monosaccharides fromgum tragacanth with fucose as the principal peak.

Figure 13 shows the quantitative analysis of gum karaya. This gum contained L-rhamnose (11.51%), D-galactose (9.82%) and D-glucuronic acid (0.97%). Preuss and Thier (1982) reported that gum karaya contained 13% L-rhamnose, 18% D-galactose, 21% D-galacturonic acid and 4% glucuronic acid. However, the method used in this study makes it difficult to quantitatively determine the amount of each uronic acid. The unknown peaks #2 and #4 were present in the chromatograms. Peak #4 is probably a uronic acid ester.

The data discussed here was derived by using TMSI silylating agent. Similar results were obtained when HMDS silylating agent was used, which are presented in Tables 3 and 4. From these results it is clear that the quantitative

determination of gums by gas chromatography is generally possible, with the exception of determining uronic acids. By using other available techniques uronic acids and unknown peaks can be further determined quantitatively.

Fig. -1-
Gas chromatogram of Standard Sugars using "STOX + TMSI" as derivatizing agents: A: (1)-L-Arabinose: (2)-L-Fucose; (3)-D-Mannose; (4)-α-L-Galactose; (5)-α-D-Galactose; (6)-D-Glucose; (7)-Internal Standard. B-(8)-L-Rhamnose; (9)-D-Xylose; (10)-D-Galactose; (11)-D-Galacturonic acid; (12)-D-Glucuronic acid; (13)-Internal Standard.

Fig. -2-
Gas chromatogram of Standard Sugars using "STOX + HMDS + TFAA" as derivatizing agents: A - (1)-L-Arabinose; (2)-L-Fucose; (3)-D-Mannose; (4)-α-L-Galactose; (5)-α-L-Galactose; (6)-D-Glucose; (7)-Internal Standard. B: (8)-L-Rhamnose; (9)-D-Xylose; (10)-D-Galactose; (11)-D-Galacturonic acid; (12)-D-Glucuronic acid; (13)-Internal Standard.

Fig. -3A-
*Gas chromatogram of hydrolysate Guar Gum using STOX + TMSI" as derivatizing agents: (1)-D-Mannose; (2)-D-galactose; (3)-Internal Standard.

Fig. -3B-
*Gas chromatogram of hydrolysate Guar Gum using "STOX + HMDS + TFAA" as derivatizing agents: (1)-D-Mannose; (2)-D-galactose; (3)-Internal Standard.

Fig. -4A-
*Gas chromatogram of hydrolysate Locust bean gum using STOX + TMSI as derivatizing agents: (1)-D-Mannose; (2)-D-galactose; (3)-Internal Standard.

Fig. -4B-
*Gas chromatogram of hydrolysate Locust bean gum using "STOX + HMDS + TFAA" as derivatizing agents: (1)-D-Mannose; (2)-D-Galactose; (3)-Internal Standard.

Fig. -5A-
*Gas chromatogram of hydrolysate Tamarind gum using "STOX + TMSI" as derivatizing agents: (1)-D-Xylose; (2)-unknown; (3)-D-galactose; (4)-unknown; (5)-D-glucose; (6)-Internal Standard.

Fig. -5B-
*Gas chromatogram of hydrolysate Tamarind gum using "STOX + HMDS + TFAA" as derivatizing agents: (1)-D-Xylose; (2)-unknown; (3)-D-galactose; (4)-unknown; (5)-D-glucose; (6)-Internal Standard.

Fig. -6A-
*Gas chromatogram of hydrolysate Agar gum using "STOX + TMSI" as derivatizing agents: (1)-unknown; (2)-D-galactose; (3)-3,6 anhydro L-galactose; (4)-Internal Standard.

Fig. -6B-
*Gas chromatogram of hydrolysate Agar gum using "STOX + HMDS + TFAA" as derivatizing agents; (1)-unknown; (2)-D-galactose; (3)-3,6 anhydro L-galactose; (4)-Internal Standard.

Fig. -7A-
*Gas chromatogram of hydrolysate Carageenan gum using "STOX + TMSI" as derivatizing agents: (1)-unknown; (2)-D-galactose; (3)-3,6 anhydro L-galuctose; (4)-D-glucose; (5)-Internal Standard.

Fig. -7B-
*Gas chromatogram of hydrolysate Carageenan gum using "STOX + HMDS + TFAA" as derivatizing agents: (1)-unknown; (2)-D-galactose; (3)-3.6 anhydro L-galactose; (4)-D-glucose; (5)-Internal Standard.

Fig. -8A-
*Gas chromatogram of hydrolysate Xanthan gum using "STOX + TMSI" as derivatizing agents: (1)-D-Mannose; (2)-unknown; (3)-unknown; (4)-glucose; (5)-Internal Standard.

Fig. -8B-
*Gas chromatogram of hydrolysate Xanthan Gum using "STOX + HMDS + TFAA" as derivatizing agents: (1)-D-Mannose; (2)-unknown; (3)-unknown; (4)-D-glucose; (5)-Internal Standard.

Fig. -9A-
*Gas chromatogram of hydrolysate Gum Ghatti using "STOX + TMSI" as derivatizing agents: (1)-L-Arabinose; (2)-unknown; (3)-D-Xylose; (4)-D-Mannose; (5)-D-galactose; (6)-unknown; (7)-D-glucuronic acid; (8)-Internal Standard.

Fig. -9B-
*Gas chromatogram of hydrolysate Gum Ghatti using "STOX + HMDS + TFAA" as derivatizing agents: (1)-L-Arabinose; (2)-unknown; (3)-D-Xylose; (4)-D-Mannose; (5)-D-galactose; (6)-unknown; (7)-unknown; (8)-D-glucuronic acid; (9)-Internal Standard.

Fig. -10A-
*Gas chromatogram of hydrolysate Gum Arabic using "STOX + TMSI" as derivatizing agents: (1)-L-Arabinose; (2)-unknown; (3)-L-Rhamnose; (4)-D-Galactose; (5)-unknown; (6)-D-Goucuronic acid; (7)-Internal Standard.

Fig. -10B-
*Gas chromatogram of hydrolysate Gum Arabic using "STOX + HMDS + TFAA" as derivatizing agents: (1)-L-Arabinose; (2)-unknown; (3)-L-Rhamnose; (4)-D-galactose; (5)-unknown; (6)-unknown; (7)-unknown; (8)-Internal Standard.

Fig. -11A-
*Gas chromatogram of hydrolysate Emul Gum using "STOX + TMSI" as derivatizing agents; (1)-L-Arabinose; (2)-L-Rhamnose; (3)-D-galactose; (4)-unknown; (5)-D-Glucuronic acid; (6)-Internal Standard.

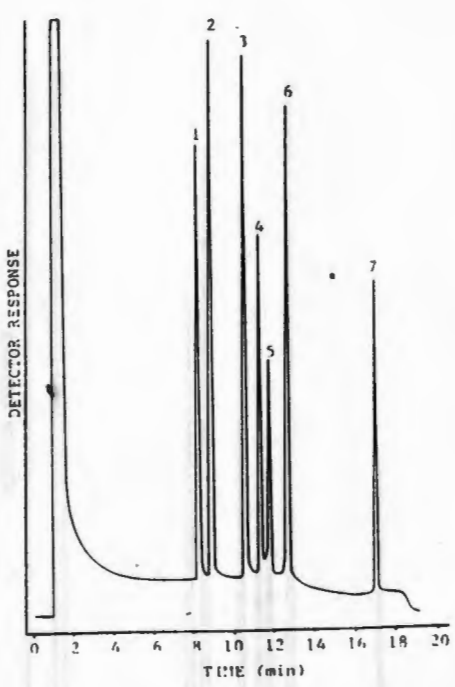
Fig. -11B-
*Gas chromatogram of hydrolysate Emul gum using "STOX + HMDS + TFAA" as derivatizing agents; (1)-L-Arabinose; (2)-L-Rhamnose; (3)-D-galactose; (4)-unknown; (5)-unknown; (6)-D-Glucuronic acid; (7)-Internal Standard.

Fig. -12A-
*Gas chromatogram of hydrolysate Gum Tragacanth using "STOX + TMSI" as derivatizing agents: (1)-L-Arabinose; (2)-L-Fucose; (3)-D-Xylose; (4)-D-Mannose; (5)-D-Galactose; (6)-unknown; (7)-D-glucose; (8)-galacturonic acid; (9)-unknown; (10)-Internal Standard.

Fig. -12B-
*Gas chromatogram of hydrolysate Gum Tragacanth using "STOX + HMDS + TFAA" as derivatizing agents: (1)-L-Arabinose; (2)-L-Fucose; (3)-D-Xylose; (4)-D-Mannose; (5)-D-galactose; (6)-unknown; (7)-D-Glucose; (8)-D-Galacturonic acid; (9)-unknown; (10)-Internal Standard.

Fig. -13A-
*Gas chromatogram of hydrolysate gum Karaya using "STOX + TMSI" as derivatizing agents: (1)-L-Rhamnose; (2)-unknown; (3)-D-galactose; (4)-unknown; (5)-D-Glucuronic acid; (6)-Internal Standard.

Fig. -13B-
*Gas chromatogram of hydrolysate Gum Karaya using "STOX + HMDS + TFAA" as derivatizing agents: (1)-L-Rhamnose; (2)-unknown; (3)-D-galactose; (4)-unknown; (5)-D-galacturonic acid; (6)-D-glucuronic acid; (7)-Internal Standard.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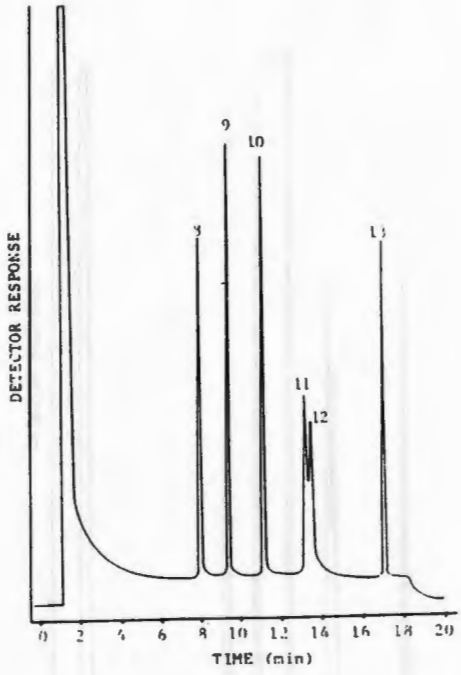
0 2 4 6 8 10 12 14 16 18 20
TIME (min)

A


DETECTOR RESPONSE
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13
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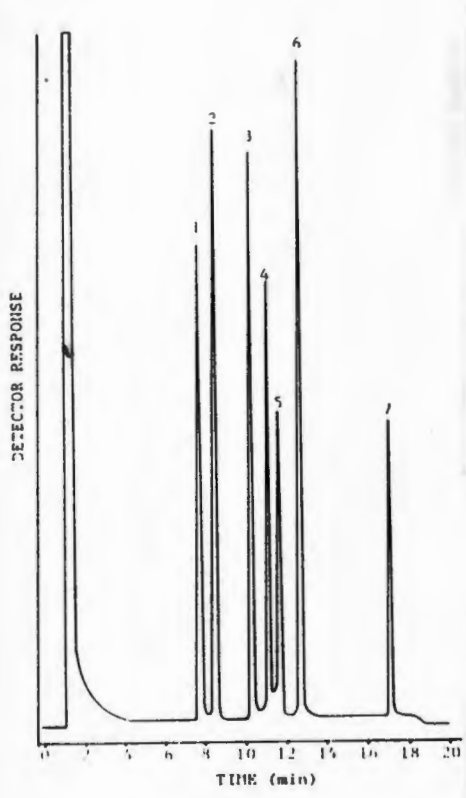
TIME (min)

B

Fig. 1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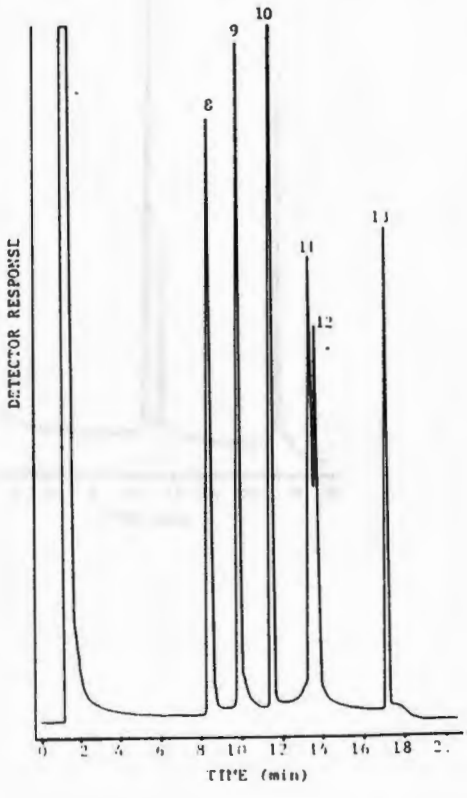
TIME (min)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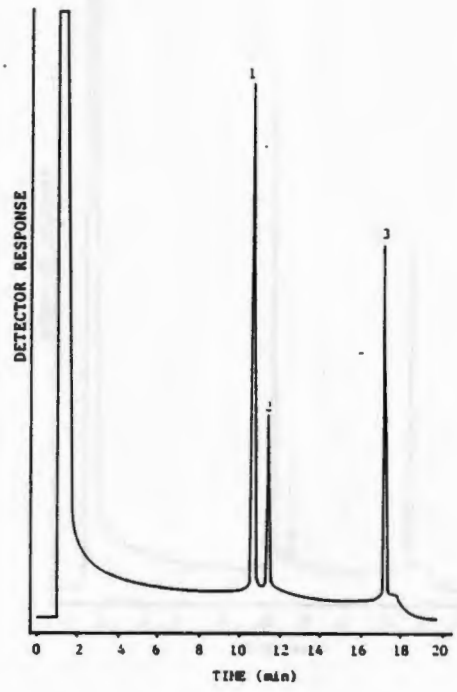
TIME (min)

Fig. 2 A B

Fig. 3

A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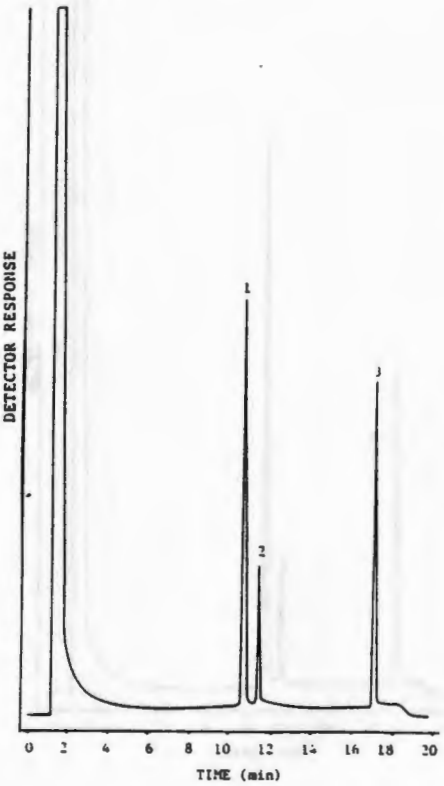
TIME (min)

B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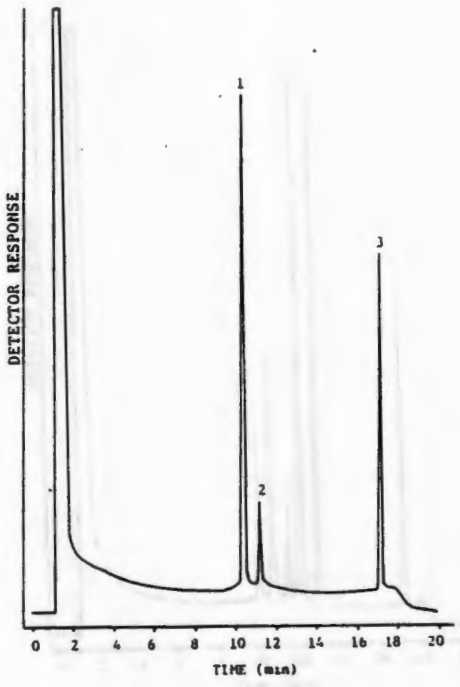
TIME (min)

Fig. 4

A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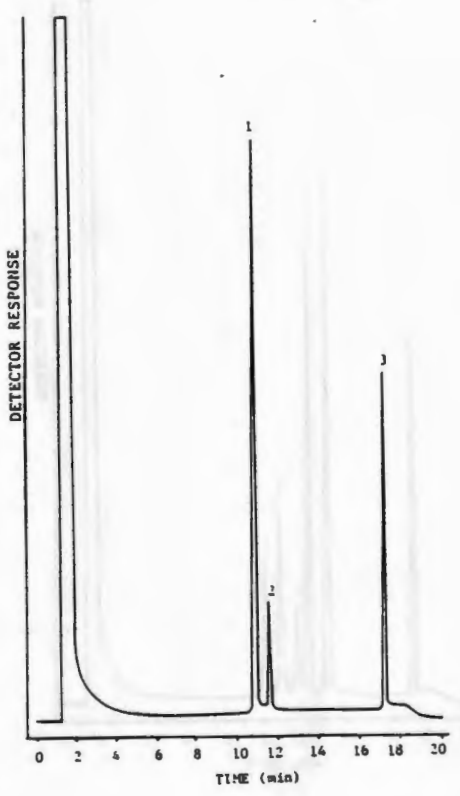
TIME (min)

B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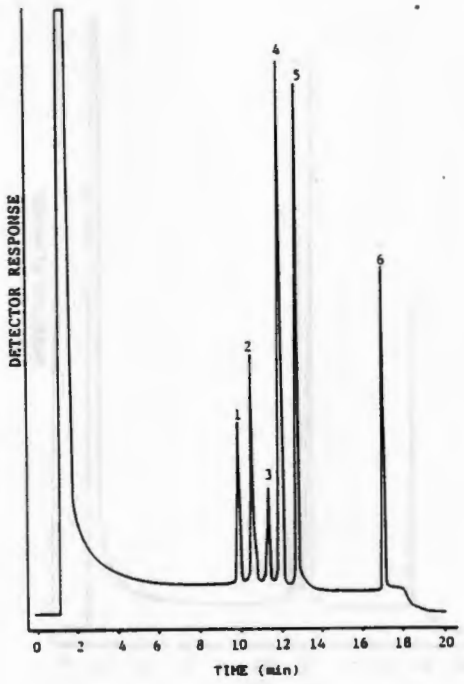
TIME (min)

Fig. 5

A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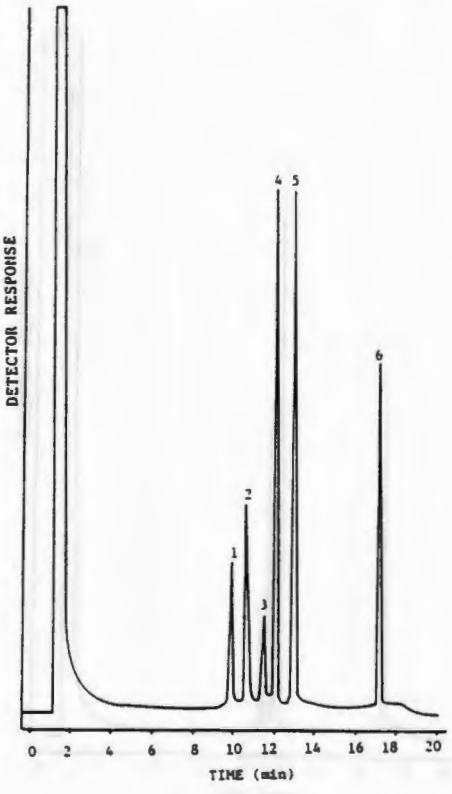
TIME (min)

B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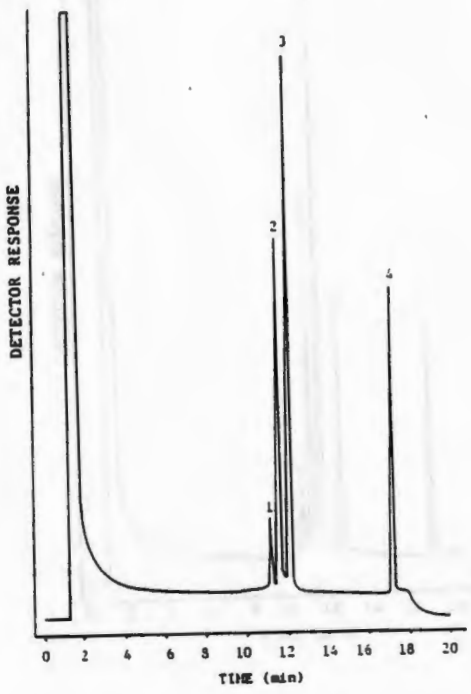
TIME (min)

Fig. 6

A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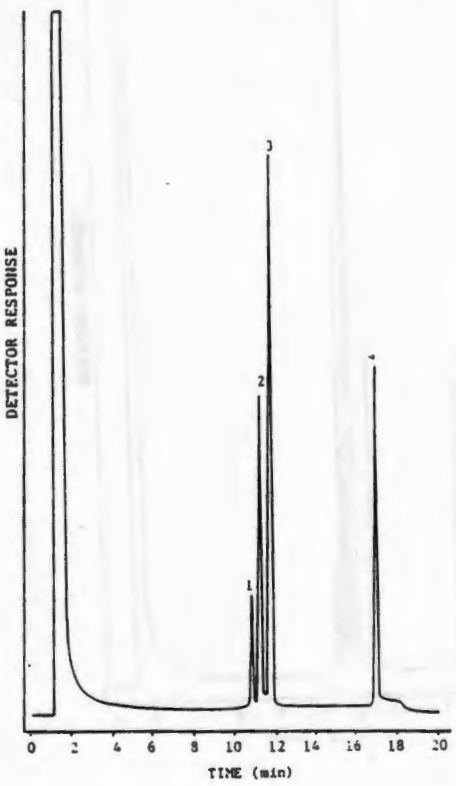
0 2 4 6 8 10 12 14 16 18 20
TIME (min)

B


DETECTOR RESPONSE
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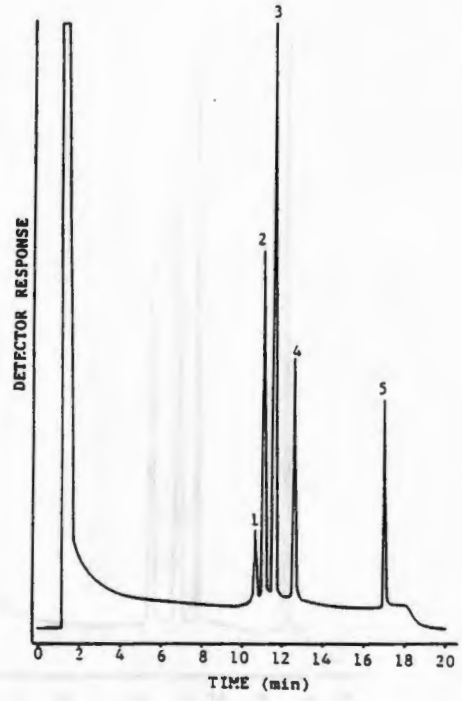
0 2 4 6 8 10 12 14 16 18 20
TIME (min)

Fig. 7

A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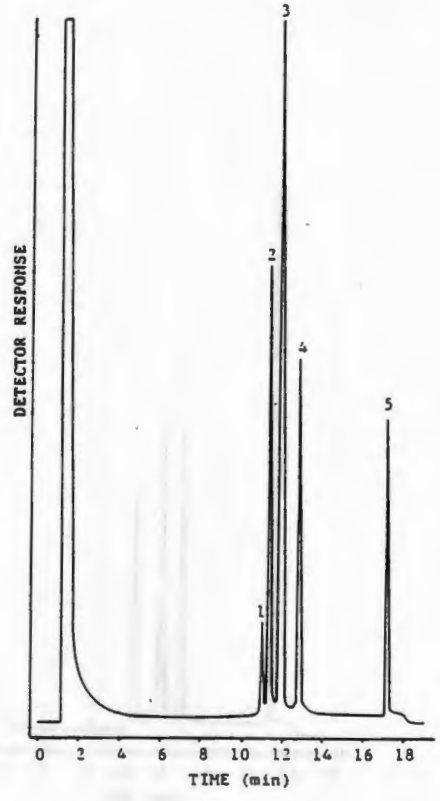
TIME (min)

B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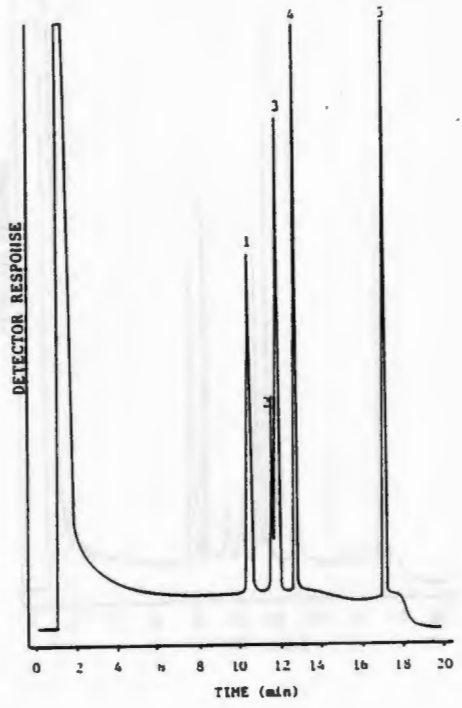
TIME (min)

Fig. 8

A


DETECTOR RESPONSE
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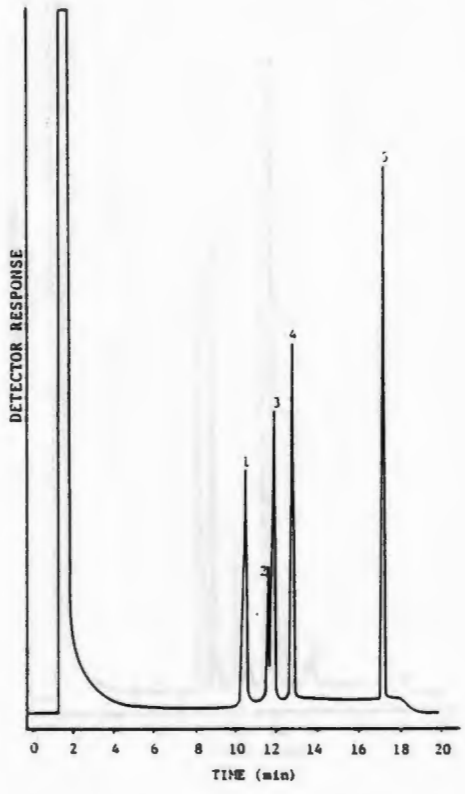
TIME (min)

B


DETECTOR RESPONSE
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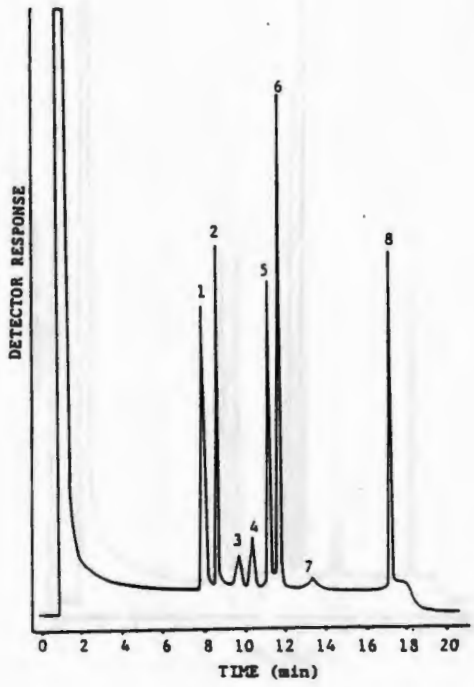
TIME (min)

Fig. 9

A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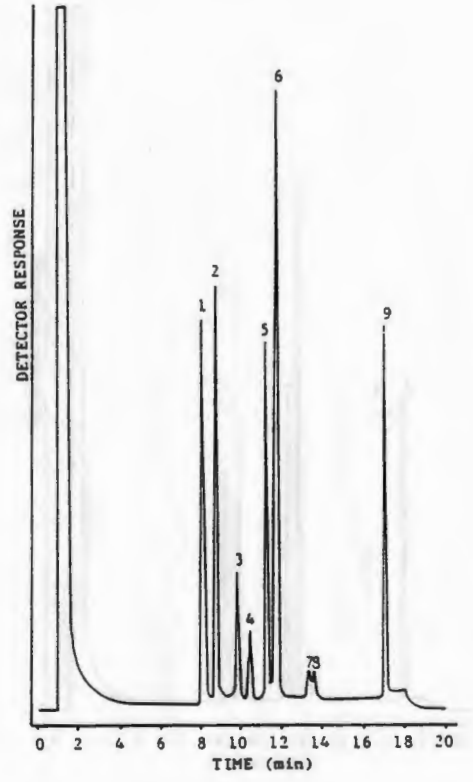
TIME (min)

B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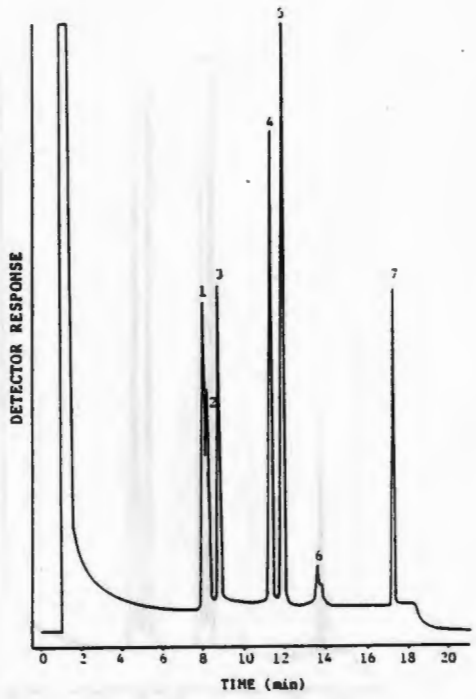
TIME (min)

Fig. 10

A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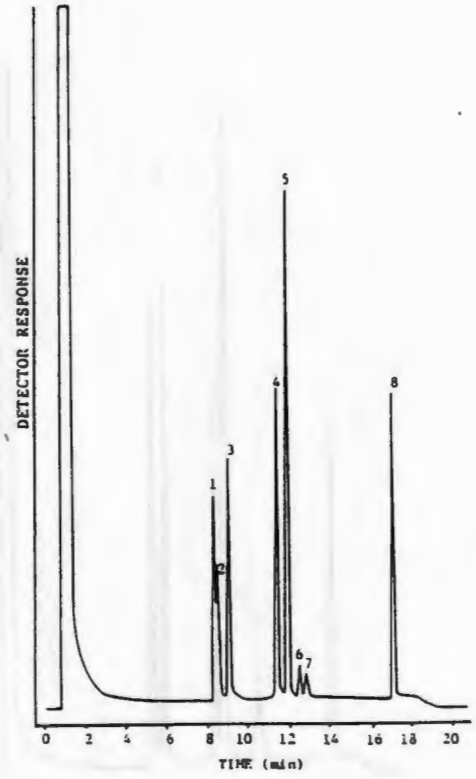
TIME (min)

B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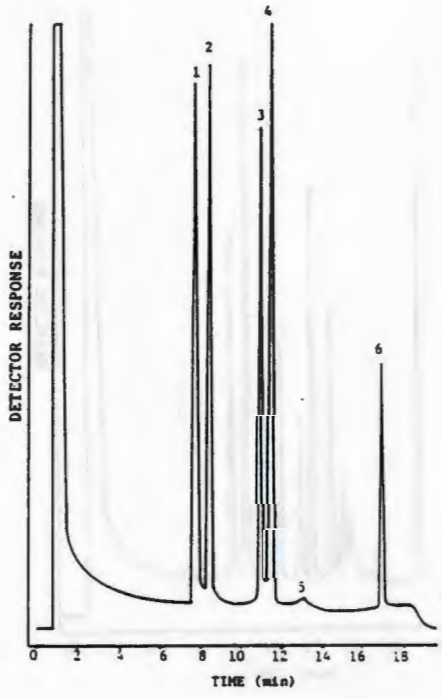
TIME (min)

Fig. 11

A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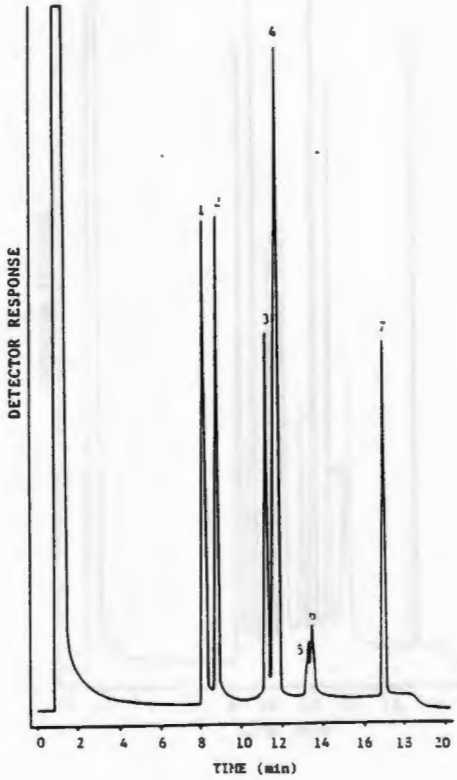
TIME (min)

B


DETECTOR RESPONSE
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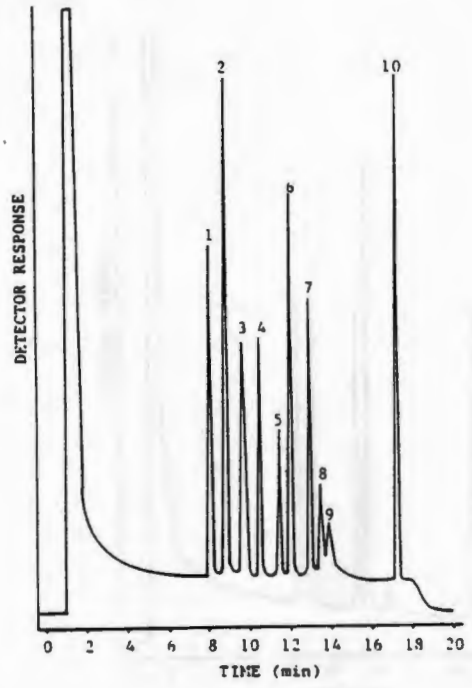
TIME (min)

Fig. 12

A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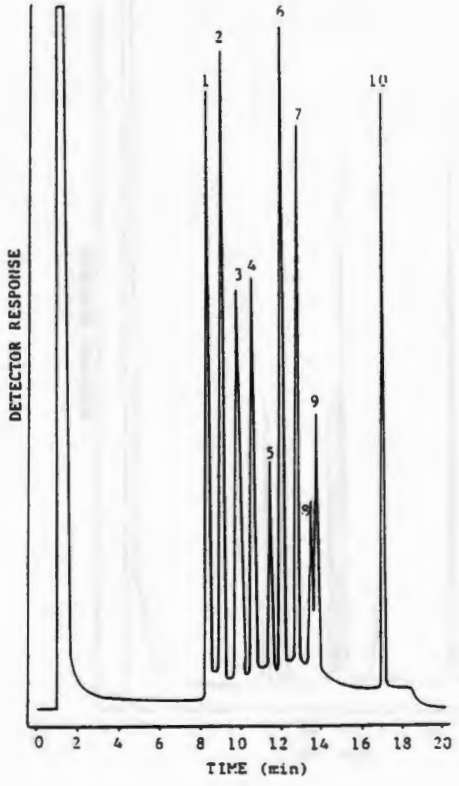
TIME (min)

B


DETECTOR RESPONSE
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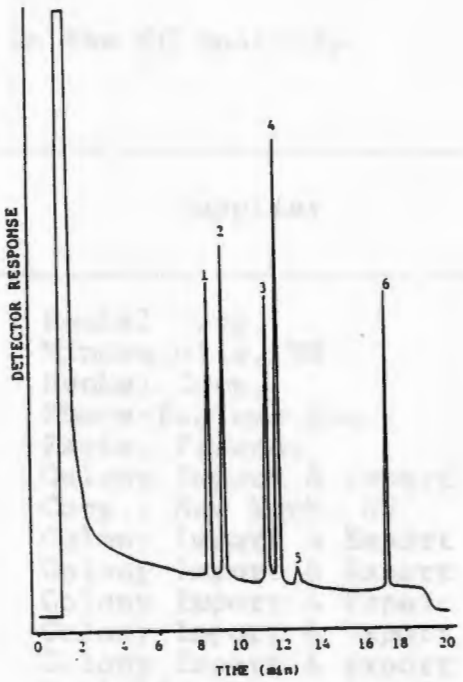
0 2 4 6 8 10 12 14 16 18 20
TIME (min)

Fig. 13

A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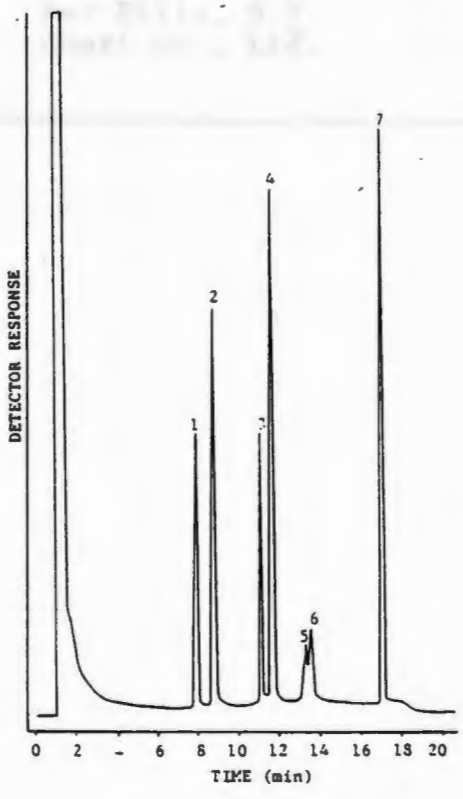
TIME (min)

B


DETECTOR RESPONSE
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TIME (min)

TABLE 1

Commercial Gums Used in the GC Analysis

Gum	Grade	Supplier
Guar Gum	Supercol UH	Henkel Corp. Minneapolis, MN
Locust Bean	Supercol 911	Henkel Corp.
Xanthan Gum	Rhodigel 23	Rhone-Poulenc Inc., Paris, France.
Sod. Carageenan	Satigum CD	Colony Import & Export Corp., New York, NY
Gum Tragacanth	USP Type A	Colony Import & Export Corp.
Gum Ghatti	#1	Colony Import & Export Corp.
Gum Arabic	Mechanical	Colony Import & Export Corp.
Gum Karaya	4 Star	Colony Import & Export Corp.
Agar Agar	USP	Colony Import & Export Corp.
Emul Gum	IRX 25631	Colloides Naturels, Inc. Far Hills, N.Y.
Tamarind	Glyloid 3S	Chori Co., Ltd.

TABLE 2

GC Analysis of Standard Sugars

Standard Sugars	Peak No.	RT		RRF	
		TMSI	HMDS	TMSI	HMDS
L-Arabinose	1	7.98	7.49	0.7347	0.8417
L-Fucose	2	8.88	8.33	0.7490	0.6754
D-Mannose	3	10.56	10.25	0.7289	0.7273
a-L-Galactose	4	11.18	11.05	1.0330	1.0724
B-L-Galactose	5	11.79	11.61	1.4302	1.4669
D-Glucose	6	12.81	12.65	0.8650	0.7013
Phenyl-B-D-Gluco-pyranoside (I.S.)	7	17.15	17.25	1.0000	1.0000
L-Rhamnose	8	8.42	8.08	0.8192	0.9632
D-Xylose	9	9.46	9.56	0.7928	0.9639
D-Galactose	10	11.34	11.26	0.7683	0.8296
D-Galacturonic Acid	11	13.13	13.33	1.3002	1.3491
D-Glucuronic Acid	12	13.45	13.64	1.6580	1.6102
Phenyl-B-D-Gluco pyranoside (I.S.)	13	17.18	17.22	1.0000	1.0000

*(I.S.) = Internal Standard.
*RT = Retention Time.
*RRF = Relative Response Factor.

TABLE 3

Sugar Contents of Seed Gums, Marine Gums and Microbial Gums

Gum	Sugar Monomers	(%) Known Sugars		(%) Unknown Sugars	
		TMSI	HMDS	TMSI	HMDS
Guar Gum	D-Mannose	17.57	12.44	-	-
	D-Galactose	5.87	4.67	-	-
Locust Bean Gum	D-Mannose	26.10	16.65	-	-
	D-Galactose	4.59	3.55	-	-
Tamarind	D-Xylose	6.49	7.05	-	-
	Unknown (1)	-	-	9.72	9.14
	D-Galactose	3.47	4.13	-	-
	Unknown (2)	-	-	18.69	18.59
	D-Glucose	17.10	12.24	-	-
Agar	Unknown (1)	-	-	3.70	5.34
	D-Galactose	15.14	14.75	-	-
	3,6 anhydro. L-Galactose	26.28	28.58	-	-
	D-Glucose	10.68	10.58	-	-
Xanthan Gum	D-Mannose	6.06	6.54	-	-
	Unknown (1)	-	-	3.42	3.94
	Unknown (2)	-	-	8.52	8.40
	D-Glucose	9.63	7.79	-	-

TABLE 4

Sugar Content of Plant Exudate Gums

Gum	Sugar Monomers	(%) Known Sugars		(%) Unknown Sugars	
		TMSI	HMDS	TMSI	HMDS
Gum Ghatti	L-Arabinose	8.97	13.44	-	-
	Unknown (1)	-	-	11.55	13.44
	D-Xylose	2.42	4.96	-	-
	D-Mannose	2.27	2.45	-	-
	Unknown (2)	-	-	16.33	18.17
	Unknown (3)	-	-	-	-
	D-Glucuronic Acid	1.84	1.76	-	-
Gum Arabic	L-Arabinoside	8.94	9.56	-	-
	Unknown (1)	-	-	7.64	6.77
	L-Rhamnose	10.21	13.18	-	-
	D-Galactose	13.90	14.17	-	-
	Unknown (2)	-	-	23.86	25.34
	Unknown (3)	-	-	-	1.60
	Unknown (4)	-	-	-	1.59
	D-Glucuronic Acid	4.81	-	-	-
Emul Gum	L-Arabinose	19.95	24.96	-	-
	L-Rhamnose	18.92	23.61	-	-
	D-Galactose	14.48	14.50	-	-
	Unknown (1)	-	-	24.48	31.45
	Unknown (2)	-	-	-	5.07
	D-Glucuronic Acid	1.14	6.74	-	-

TABLE 4
(cont)

Gum	Sugar Monomers	(%) Known Sugars		(%) Unknown Sugars	
		TMSI	HMDS	TMSI	HMDS
Gum Tragacanth	L-Arabinose	9.51	17.51	-	-
	L-Fucose	11.29	10.25	-	-
	D-Xylose	4.76	14.06	-	-
	D-Mannose	4.92	6.65	-	-
	D-Galactose	3.23	4.79	-	-
	Unknown (1)	-	-	10.11	16.70
	D-Glucose	6.04	9.07	-	-
	D-Galacturonic Acid	5.06	6.09	-	-
	Unknown (2)	-	-	2.13	6.13
Gum Karaya	L-Rhamnose	11.51	9.88	-	-
	Unknown (1)	-	-	13.03	12.07
	D-Galactose	9.82	7.25	-	-
	D-Galacturonic	-	3.42	-	-
	D-Glucuronic	0.97	5.20	-	-

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