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Identification of polysaccharides in pharmaceuticals by capillary gas chromatography*

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Abstract: A sensitive method for the identification of polysaccharides in pharmaceuticals is described. Polysaccharides are isolated by gel filtration and subsequently hydrolysed. The monomeric carbohydrates obtained are transformed into oxime-trimethylsilyl derivatives and analysed by capillary gas chromatography. Profiles of 13 different natural or semi-synthetic polysaccharides are discussed. The profiles of the hydrolysis products can be used to identify the polysaccharides mentioned above. Possible interferences by other polymers are given. The method can be used to identify most polysaccharides used as pharmaceutical adjuvants.

Keywords: *Polysaccharides; pharmaceutical adjuvants; capillary gas chromatography.*

Introduction

Polysaccharides are, among other polymers, frequently used in drug formulations as binding agents, viscosity increasing agents, gelifying agents, coating agents or as active ingredients. Laboratories, involved in the quality control of pharmaceuticals must be able to identify all ingredients in drug formulations, active or not active. However, only limited information is available in the literature on sensitive techniques, which allow a differentiation between individual compounds of this group of the polysaccharides.

Methods, mentioned in the Pharmacopeias are generally designed to identify primary products and they require rather large amounts of sample. These methods most often cannot be applied to finished drug formulations such as tablets, gels, emulsions or suspensions, in which these polysaccharides can be present in microgram quantities. Electrophoresis [1] and high performance liquid chromatography [2] have been used for the quantitative determination of unhydrolysed polysaccharides. These techniques however are not selective enough to differentiate the various polysaccharides.

Several thin layer chromatographic procedures have been described [3–6]. These methods are based on identification of carbohydrate monomers obtained by hydrolytic cleavage of the polymers. The major drawback of these methods is that they are not always applicable to drug formulations, containing only small amounts of polysaccharides. Moreover, due to the strong similarity of the patterns obtained, a discrimination between closely related analogues is frequently not possible. Gas chromatography, most often with packed columns, of hydrolytic or pyrolytic cleavage products offers good

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possibilities [7–12]. However, until now only a restricted number of polysaccharides have been studied. Preuss and Thier [10] performed gas chromatography on trimethylsilylated methylglucosides after hydrolytic cleavage, thus leaving α,β-anomerism intact. Consequently, three to four peaks for each monomeric compound were found in the chromatographic pattern. However of the synthetic analogues of cellulose only the data of carboxymethylcellulose were provided.

In this paper we describe a capillary gas chromatographic procedure based on oxime-trimethylsilylderivative formation of the hydrolysis products of polysaccharides [13]. The results obtained for 13 polysaccharides are discussed.

Experimental

Materials

All reagents used were of analytical grade. Hydroxylamine·HCl was obtained from Janssen Chimica, Beerse, Belgium. Dimethylsulphoxide (DMSO), hexane, sodium acetate, trifluoroacetic acid (TFA), hydrochloric acid (HCl), sodium chloride (NaCl) and sodium azide were obtained from Merck, Darmstadt, FRG.

Sephadex G-50, Sephadex LH and blue dextran were obtained from Pharmacia, Uppsala, Sweden.

The hydrocarbons *n*-decane, *n*-dodecane, *n*-tetradecane, *n*-hexadecane, *n*-octadecane, *n*-eicosane, *n*-docosane and *n*-tetracosane and Power Sil Prep, a mixture of trimethylsilylimidazole (TMSI), bis-trimethylsilylacetamide (BSA) and trimethylchloorsilane (TMCS) (3:3:2), were obtained from Alltech associates, Deerfield, USA. Purified water was obtained from a consecutive Milli RO and Milli Q water purification system.

All reference products (arabinose, galactose, galacturonic acid, glucose, glucuronic acid, rhamnose, sorbitol, xylose, acacia, tragacanth, methylcellulose, ethylcellulose, sodium carboxymethylcellulose, carboxymethylamylose, hydroxypropylmethylcellulose, hydroxypropylcellulose, pectin, sodium alginate, agar, dextrin, soluble starch, cellulose, starch, guar gum, hydroxyethylcellulose) were of pharmaceutical or analytical grade and were obtained from several pharmaceutical companies. Standard solutions of polysaccharides were prepared at a concentration of 0.01% in water or chloroform (ethylcellulose).

Apparatus

All gas chromatographic analysis were performed on a Varian model 3700 gas chromatograph equipped with a split/splitless capillary injector and a flame ionization detector. The column used was a WCOT 50 m × 0.22 mm CP-Sil-5 CB fused silica column. Temperature settings were as follows: injector 220°C; detector 280°C; oven 150–233°C at 3°C min^{-1}.

Integration and calculation of methylene-units was performed on a Hewlett–Packard model 9816 microcomputer with a modified Nelson model 4416 integration software.

Sample pretreatment

Solid dosage forms: procedure for hydrosoluble polysaccharides. A 100 mg aliquot of carefully ground tablets or an equal amount of the contents of capsules, powders, etc. was weighed into a test tube, 1 ml of purified water was added and the tube shaken for 2 h. Subsequently, the tube was centrifuged at 3000 rpm for 5 min; 800 μl of the supernatant were applied to a Sephadex G-50 gel filtration column.

Solid dosage forms: procedure for ethylcellulose. A 100 mg aliquot of carefully ground tablets or an equal amount of the contents of capsules, powders, etc. was weighed into a test tube, 1 ml of chloroform was added and the tube shaken for 2 h. Subsequently, the tube was centrifuged at 3000 rpm for 5 min. 800 μl of the supernatant were applied to a Sephadex LH gel filtration column.

Liquid dosage forms and hydrous gels. 500 mg of a gel or suspension were diluted with purified water until a suitable solution or dispersion was obtained. After centrifugation at 3000 rpm for 5 min, 800 μl of the supernatant were applied to a Sephadex G-50 gel filtration column.

Liquid emulsions and creams. 500 mg of a liquid emulsion or a cream were vigorously shaken for 2 h with 3 ml hexane and 1 ml purified water in a test tube. Subsequently, the tube was centrifuged until a clear supernatant layer was obtained. 800 μl of the upper aqueous layer were applied to a Sephadex G-50 gel filtration column.

Gel filtration

Gel filtration on a Sephadex G-50 *column.* 11 g of dry Sephadex G-50 were suspended in 100 ml of a 0.1 M NaCl solution. The suspension was allowed to swell for 3 h and subsequently vacuum degassed for 30 min. The swollen gel was poured into a Pharmacia glass column K15/30 (30 × 1.5 cm) and the flow rate through the column was kept at 1.5 ml min^{-1}.

800 μl of a 0.1% solution of Blue Dextran (quality control of the column), a reference solution (determination of the elution volume of the polymeric fraction) or a sample preparation were carefully applied to the top of the column. The solution was allowed to diffuse into the gel. The first 12 ml of the eluate were discarded. The next 5 ml, containing the polymer fraction, were collected and taken to dryness under a stream of nitrogen at 70°C.

*Gel filtration on a Sephadex LH-*20 *column.* 4.5 g of dry Sephadex LH were suspended in 50 ml of dioxan–dichloromethane (2:1). The suspension was allowed to swell for 3 h and subsequently vacuum degassed for 30 min. The swollen gel was poured into a 14 × 1.2 cm glass column and the flow rate through the column was kept at 0.65 ml min^{-1}. 800 μl of a reference solution (determination of the elution volume of the polymeric fraction) or a sample preparation were carefully applied to the top of the column. The solution was allowed to diffuse into the gel. The first 6 ml of the eluate were discarded. The next 2 ml, containing the polymer fraction, were collected and taken to dryness under a stream of nitrogen at 40°C.

Hydrolysis and derivatization

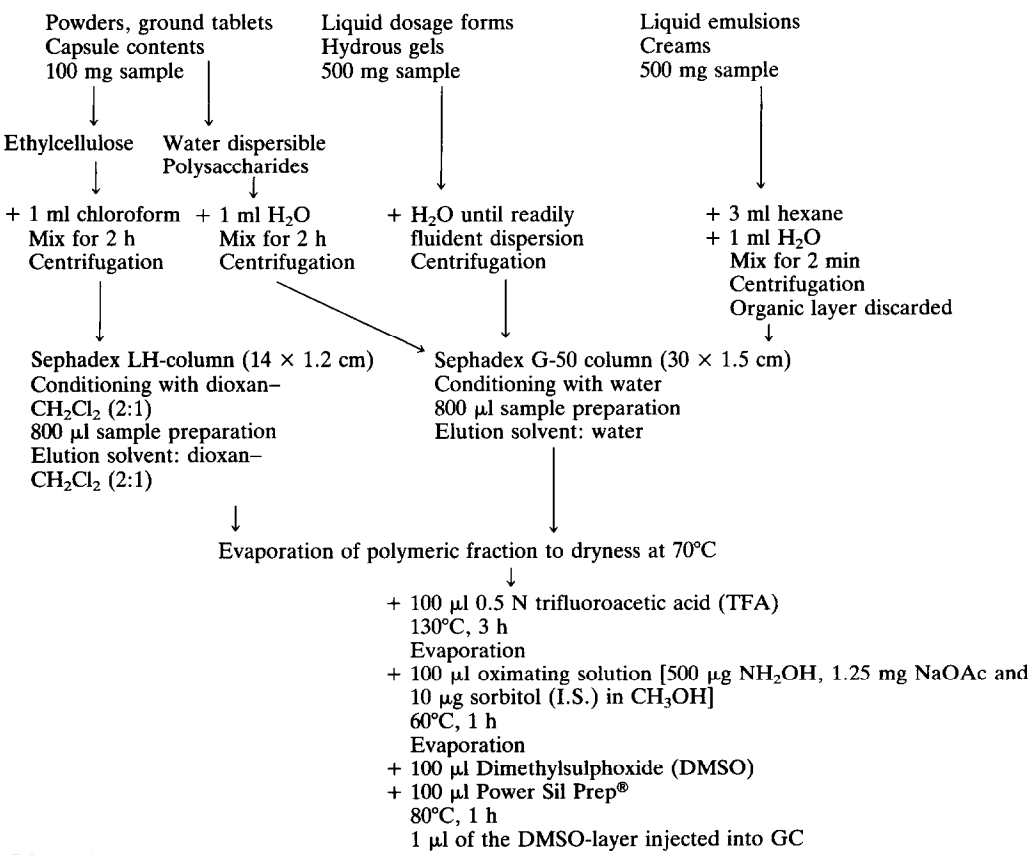
100 μl of a solution of 0.5 N TFA in water were added to the dry polymeric fraction. The vials were placed in an oven at 130°C for 3 h. After cooling, the solution was evaporated under a stream of nitrogen. Subsequently, 100 μl of a methanolic solution containing 500 μg hydroxylamine, 1.25 mg sodium acetate and 10 μg sorbitol (internal standard) were added. The vial was placed in an oven at 60°C for 1 h. After cooling, the reaction mixture was again evaporated under a stream of nitrogen at 60°C. Subsequently, 100 μl of DMSO and 100 μl of Power Sil Prep® were added and the mixture was

vigorously stirred on a vortex mixer. After heating in an oven at 80°C for 1 h, 1 μl of the upper DMSO layer was injected into the gas chromatograph.

Results and Discussion

The overall procedure is summarized in Scheme 1. A gel filtration step on Sephadex G-50 was used to avoid interferences by low molecular weight compounds. Thus, mono- or oligo-saccharides present in pharmaceutical preparations will interfere with the analysis of monomeric carbohydrates, generated during hydrolysis of polysaccharides. Sephadex LH was used for group separation in organic solvents [14].

The gas chromatographic retention data of reference polysaccharides, as obtained by the present method are summarized in Table 1. Peak characterization is based on methylene-unit values. Representative gas chromatograms are shown in Figs 1–4. The hydrolysis products were identified by comparing the chromatographic profiles of the hydrolysed polysaccharide standards with the monosaccharide standards (Table 2). As the present study was not focussed on the identification of the different hydrolysis products obtained, the hydrolysis products of most cellulose-derivatives were not evaluated in detail.



Scheme 1
Method summary

Table 1
Gas chromatographic retention data (methylene-units) of polysaccharides cleavage products

Acacia	18.03	18.10	18.66	18.82	20.48	20.77
	20.82					
Tragacanth	18.00	18.03	18.10	18.66	18.71	18.76
	18.82	18.90	20.48	20.58	20.77	20.94
	21.29					
Pectin	18.03	18.10	18.66	18.82	20.48	20.66
	20.77	20.83	20.94	21.29		
Alginate	19.95	20.31	20.60	20.78	20.85	20.94
	21.05	21.17				
Agar	19.52	20.48	20.60	20.71	20.77	
Guargum	18.03	18.10	18.52	19.03	19.55	20.48
	20.50	20.77	20.79			
Methylcellulose	16.44	16.58	17.81	18.10	18.18	18.44
	18.74	18.82	18.93	19.06	19.19	19.50
	19.67	19.79	19.89	20.57	20.75	
Ethylcellulose	17.78	18.01	18.75	18.94	19.04	19.13
	19.40	19.49	19.58	19.69	20.14	
Hydroxyethylcellulose	20.58	20.77	22.83	23.28	23.89	24.48
Hydroxypropylcellulose	18.01	20.58	20.77	24.13	24.74	
Hydroxypropylmethylcellulose	16.44	16.58	17.81	18.10	18.18	18.44
	18.74	18.82	18.93	19.06	19.19	19.50
	19.67	19.79	19.89	20.57	20.75	
Carboxymethylcellulose	20.59	20.75	22.00	22.08	22.16	22.28
	22.45					
Carboxymethylamylose	20.59	20.75	22.00	22.08	22.16	22.28
	22.45					

Confirmation of the open chain oxime-trimethylsilyl derivatives generated during the derivatization step was carried out by GC/MS analysis of derivatized reference products. The occurrence of double peaks for aldoses and uronic acids is characteristic and is due to *syn*- and *anti*-isomerism of the oxime-group [13]. The occurrence of double peaks can be avoided when open chain poly-*O*-acetylaldononitriles (Wohl-derivatives) are formed [13]. However, in our hands the latter derivatives yielded lower detector signals, which resulted in reduced sensitivity of the method. However, formation of the 1-methylglycoside-trimethylsilylderivatives according to the procedure of Preuss *et al.* [10] left α,β-anomerism intact. Thus, each monosaccharide gives rise to four peaks, which makes the interpretation of data obtained from complex mixtures rather difficult.

Diluted solutions of hydrochloric or sulphuric acid have been used as an alternative for TFA [9, 11]. However, when using 2 N HCl, we not only observed lower recoveries for several compounds, we also were not able to obtain any chromatographic profile for ethylcellulose, hydroxyethylcellulose, hydroxypropylcellulose and even for cellulose. Furthermore, the profile of the hydrolysis products of agar was difficult to reproduce. This finding can be explained by a decomposition of 3,6-anhydrogalactose, as this compound is not stable in strong acid media [10]. Our results indicate that 0.5 N TFA [8] is an appropriate reagent for all 13 compounds studied. It was also shown that even cellulose was hydrolysed under these conditions.

In comparison with carbohydrates and polyols, uronic acids gave rather poor detector signals. Addition of DMSO during silylation in order to improve solubility [13] did not significantly improve the yield. Nevertheless, this solvent was retained in the procedure to avoid injection of excesses of TMSI into the capillary column.

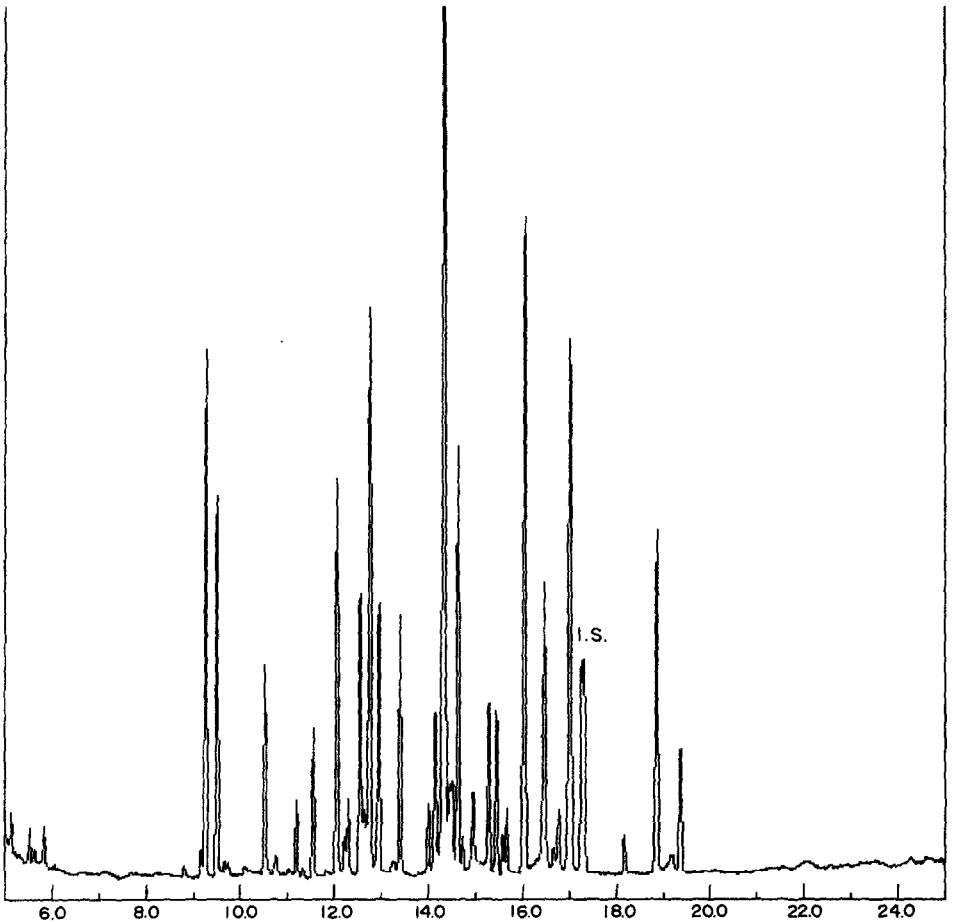


Figure 1
Typical profile obtained from methylcellulose (composition of monomers: 2-, 3- or 6-monomethylglucose, 2,3-, 2,6- or 3,6-dimethylglucose, 3,4,6-trimethylglucose). Key: I.S., internal standard (sorbitol).

Table 2
Gas chromatographic retention data (methylene-units) of carbohydrate-monomers and some interfering compounds

Xylose-*O*-TMS-5	18.00–18.04
Arabinose-*O*-TMS-5	18.03–18.10
Lauric acid-TMS-1	18.41
Rhamnose-*O*-TMS-5	18.66–18.82
Sorbitol-TMS-6 (I.S.)	19.99
Galactose-*O*-TMS-6	20.48–20.77
Glucose-*O*-TMS-6	20.58–20.77
Glucuronic acid-*O*-TMS-6	20.82–21.05
Galacturonic acid-*O*-TMS-6	20.94–21.29
Stearic acid-TMS-1	22.40
Palmitic acid-TMS-1	20.40
Oleic acid-TMS-1	22.08

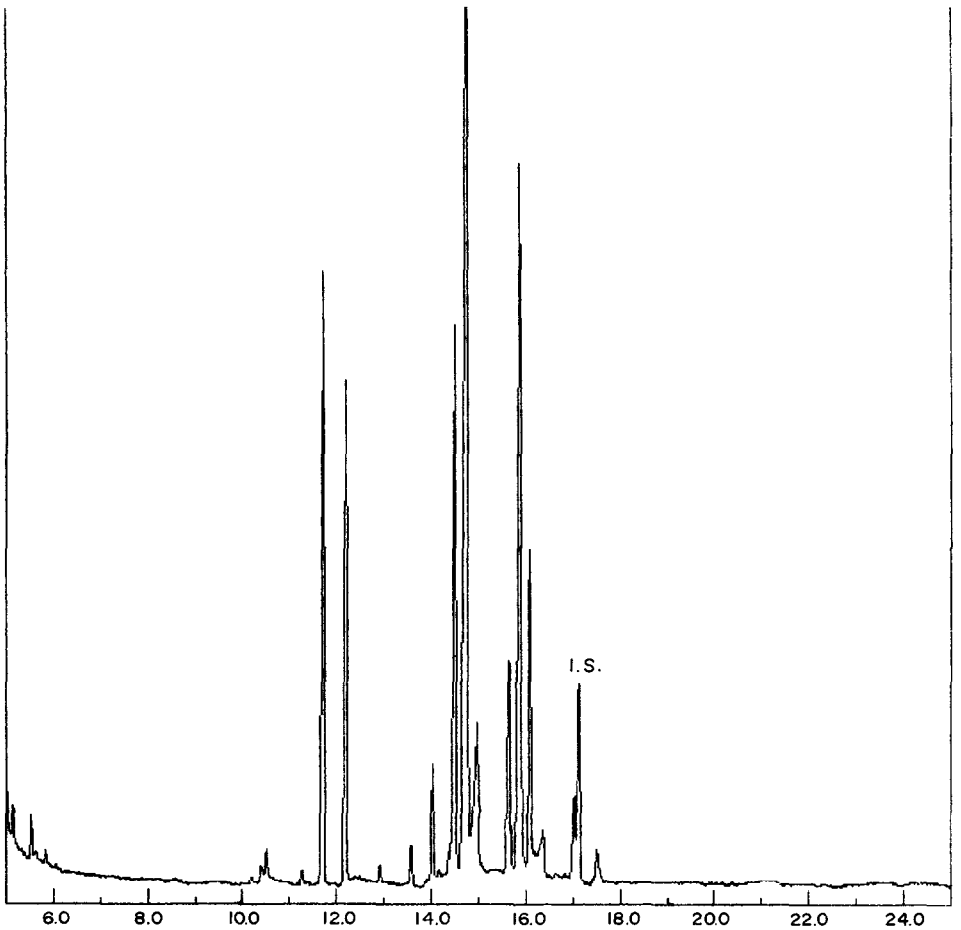


Figure 2
Typical profile obtained from ethylcellulose (composition of monomers: 2-, 3- or 6-monoethylglucose, 2,3-, 2,6- or 3,6-diethylglucose, 3,4,6-triethylglucose). Key: I.S., internal standard (sorbitol).

When applying the overall procedure to 400 mg of starch, cellulose, dextrin or soluble starch, no glucose peaks occurred in the chromatogram. It was therefore concluded that these compounds are not sufficiently hydrosoluble to interfere with the proposed method. Starch and cellulose can be identified easily by a microscopic technique using iodine and iodinated zinc chloride as colouring reagents.

The profiles obtained from carboxymethylcellulose (CMC), and carboxymethylamylose (CMA) were identical. As a consequence, a simple gas chromatographic differentiation between these compounds is not possible. As ethylcellulose is not soluble in water, an alternative isolation method had to be used. Often, ethylcellulose is present as a film coating which can easily be removed by simple physical separation from the rest of the tablet. When used for micro-encapsulation this procedure is not feasible. In this case extraction of ethylcellulose with an organic solvent followed by gel filtration with a hydrophobic gel has proven to be a valuable alternative.

Several polymers, not belonging to the group of the polysaccharides, can be present in the polymer fraction. Although most polymers do not decompose under the hydrolytic

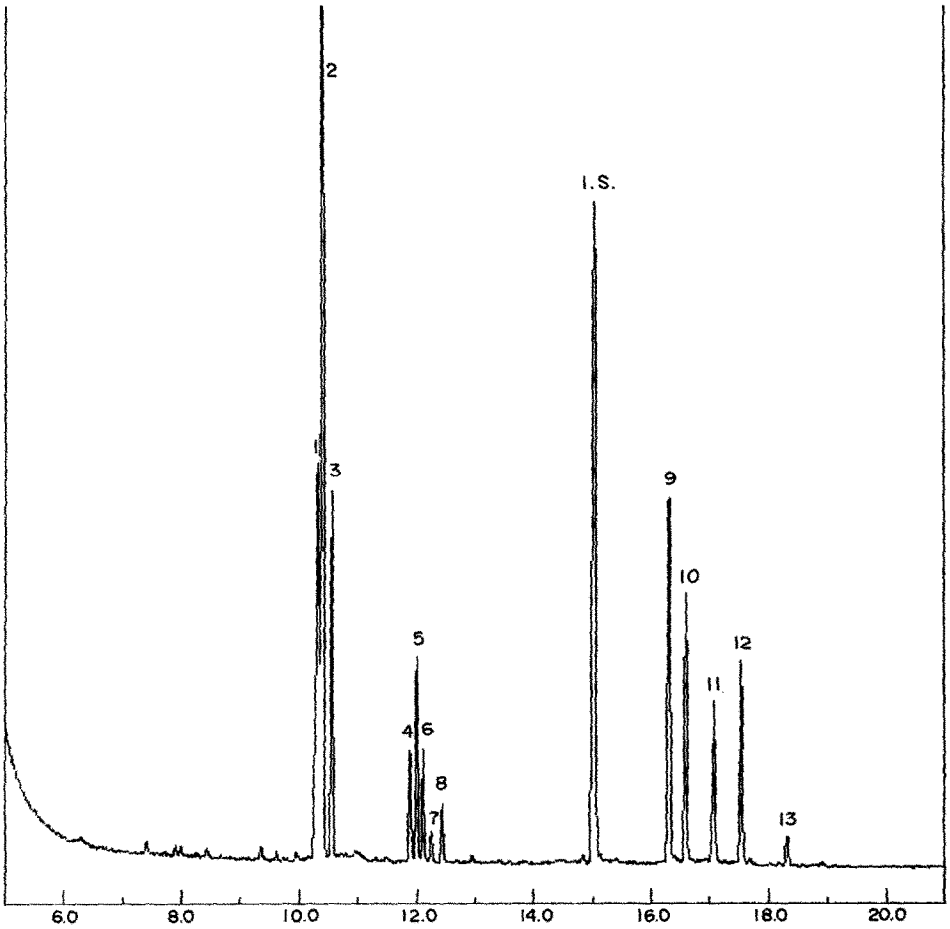


Figure 3
Typical profile obtained from tragacanth. Key: (1) xylose pk.I; (2) xylose pk.II + arabinose pk.I; (3) arabinose pk.II; (4) rhamnose pk.I; (5) fucose pk.I; (6) unknown; (7) rhamnose pk.II; (8) fucose pk.II; I.S., internal standard (sorbitol); (9) galactose pk.I; (10) glucose pk.I; (11) galactose pk.II + glucose pk.II; (12) galacturonic acid pk.I; (13) galacturonic acid pk.II.

reaction conditions, a particular group, i.e. those carrying an ester group, generated free fatty acids after hydrolysis. Thus, esters of polyethylene glycol (polysorbates, PEG-stearate . .) were cleaved into corresponding PEG- and fatty acid-fragments. These decomposition products gave rise to supplementary peaks, easily recognized and well separated from the sugar moieties (Table 2). Moreover, the finding of these peaks is useful in confirming the presence of fatty acid containing polymers.

Conclusion

A highly sensitive and selective procedure is presented for the identification of polysaccharides in pharmaceutical products. Thirteen polysaccharides have been evaluated by this procedure. Discrimination between various polysaccharides is possible but some polysaccharides cannot be unambiguously differentiated. Gas chromatographic profiles can provide supplementary information on the fatty acid content of hydrolysable polymers different from polysaccharides.

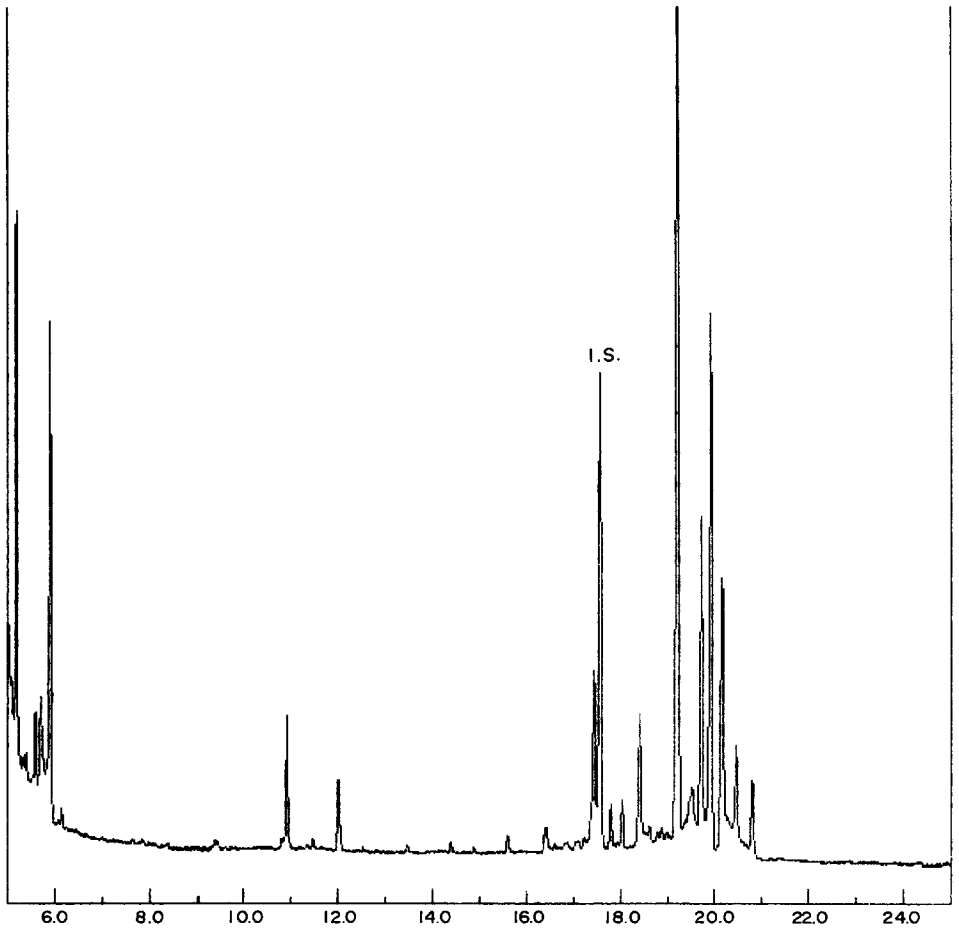


Figure 4
Typical profile obtained from alginate (composition of monomers: D-mannuronic acid and L-guluronic acid). Key: I.S., internal standard (sorbitol).

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