GAS–LIQUID CHROMATOGRAPHY OF CARBOHYDRATES

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A review is given of materials on the use of the method of gas–liquid chromatography in carbohydrate chemistry.

A quarter of a century has passed since the appearance of the first communication on the gas–liquid chromatography (GLC) of carbohydrates in 1958 [1]. During this period the method has undergone all-sided development, and has become one of the main ones in the analysis of carbohydrate mixtures, and it is playing an enormous role in the structural investigation of carbohydrate-containing compounds. The GLC of carbohydrates is widely used together with other chromatographic and analytical methods and a combination of the advantages of GLC and mass spectrometry (chromato-mass spectrometry) has given a basically new qualitative jump in the elucidation of the structures of the most complex carbohydrate-containing biopolymers, including glycoproteins, peptidoglycans, and lipopolysaccharides. New modifications of the method have also appeared, such as capillary chromatography, and preparative GLC — a promising method for obtaining authentic samples of, in the first place, difficultly accessible methylated sugars — has undergone development. A considerable number of new carbohydrate derivatives have been proposed for more effective separation with the aid of GLC, the possibilities of numerous liquid phases and solid supports have been studied, and the optimum conditions for the performance of the GLC analysis of carbohydrate mixtures have been determined. The investigations of the early period have been considered in detail in numerous monographs and papers with a review nature [2]. In the present review an attempt has been made to analyze the literature on the GLC of carbohydrates beginning from 1974; the citation of earlier papers has been made only in the historical aspect and to confirm individual points.

CONDITIONS FOR SEPARATING CARBOHYDRATES

As is well known, carbohydrates do not possess sufficient volatility for GLC. As volatile carbohydrate derivatives are used the trimethylsilyl ethers of methyl glycosides and of monosaccharides, acetates of polyols and of aldononitriles, trifluoroacetates, and a number of other derivatives.

The trimethylsilyl ethers [3] have come to be used most widely thanks to the ease of their preparation in quantitative yield, their high volatility and stability, and the good separation of the components of a mixture. They are usually prepared by treating the carbohydrates with chlorotrimethylsilane and hexamethyldisilazane in pyridine [3]. The presence of moisture in the samples prevents the reaction from taking place completely, but if the chlorotrimethylsilane is replaced by trifluoroacetic acid, a small amount of water will not affect the occurrence of the reaction [4, 5]. More effective reagents are also used to obtain trimethylsilyl ethers, such as N,O-bis(trimethylsilyl)acetamide and trifluoroacetamide, and also N-trimethylsilylimidazole [6, 7]. When the latter is used, two phases are formed during the reaction, the trimethylsilyl ethers of the sugars passing into the upper phase, which leads to their concentration in a small volume and to an improved separation [8]. Some other reagents have also been proposed for trimethylsilylation [9], but they have not come into wide use.

For sparingly soluble compounds, dimethyl sulfoxide or dimethyl formamide are used as the solvent in the place of pyridine [10-14]. The use of these solvents leads to the formation of an upper phase into which the bulk of the carbohydrate trimethylsilyl ethers passes [10-12]. Sometimes cyclohexane is added to the reaction mixture for the same purpose

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[14], this favoring the concentration of the compounds to be separated in the upper layer to an even greater extent. As is well known, when the trimethylsilyl ethers are used several peaks on the chromatogram correspond to each monosaccharide. To decrease the number of peaks the monosaccharides are sometimes first converted into oximes [15] or methyloximes [16]. Then for each monosaccharide two peaks (syn and anti isomers), one of which usually predominates, can be observed on the chromatograms.

Another type of derivatives used for the separation of carbohydrates with the aid of GLC consists of the acetates, particularly the acetates of the corresponding polyols and aldononitriles, since, in these cases, because of the absence of a tautometric equilibrium, each monosaccharide is represented by a single peak on the chromatograms. Acetylation is achieved by treating samples with acetic anhydride in pyridine or in the presence of sodium acetate. In pyridine, acetylation takes 20 minutes, while when sodium acetate is used complete acetylation is observed only after 2-3 h [18]. On the other hand, acetylation in the presence of N-methylimidazole takes place completely in 5 min at room temperature [19, 20].

The GLC of trifluoroacetates of carbohydrates is used considerably more rarely, in spite of the high volatility of these derivatives and their satisfactory separation. An important factor in the use of the trifluoroacetates is the high sensitivity of an electron capture detector to these derivatives [21, 22].

The type of gas chromatograph is not of fundamental importance for the analysis of carbohydrates, since the chromatographs developed in recent years are, as a rule, fitted with temperature programmers and highly sensitive flame-ionization detectors and are designed for analysis at high temperatures. The chromatographs are frequently equipped with selective detectors (electron-capture and thermionic detectors). Gas chromatographs of the "Tsvet" 100 series are probably the best of the domestic instruments and are completely suitable for carbohydrate analysis. The chromatographs are fitted with a set of metal and, more rarely, glass columns, but chemists prefer to work with the glass columns in view of their inertness and convenience of packing. The use of capillary columns requires modifications to the inlet system and in the connection to the detector in an ordinary gas chromatograph, with the aim in both cases of the maximum decrease in the dead volume. One of the "Biokhrom" domestic instruments is designed directly for the use of capillary columns. The preparation of glass capillary columns several tens of meters long can be carried out under laboratory conditions, but it is not so simple to obtain a column satisfying the necessary demands [23].

Inert sorbents of the "Chromaton" or "Chromosorb" type that are widely used do not possess residual adsorption activity and can be used for separating such polar compounds as carbohydrates. A fairly wide range of phases, depending on the type of volatile derivative, is used for the separation of carbohydrates.

ANALYSIS OF MONO- AND OLIGOSACCHARIDES

The analysis of the monosaccharide compositions of natural or artifical mixtures, especially those obtained in the hydrolytic cleavage of the carbohydrate chains of polysaccharides and of glycoconjugates is used most widely in the practice of the GLC of carbohydrates. The analysis of oligosaccharides with the aid of GLC is encountered comparatively rarely and it is connected with the determination of the composition of the oligosaccharides present in extracts from various natural sources and also with the separation and identification of the oligosaccharides formed as fragments in the cleavage of the carbohydrate chains of polysaccharides and glycoconjugates.

A rapid quantitative analysis of a mixture of mono- and oligosaccharides is achieved when they are in the form of the corresponding trimethylsilyl ethers on a column containing SE-30 [24, 25] with high-rate temperature programming [24]. To improve the separation of the monosaccharides it is desirable to carry out isothermal holding at 135°C for 10 min before switching on the temperature program [7]. And although under these conditions no satisfactory separation of β-mannose and β-galactose, is observed, information on the accurate ratio of the α- and β-anomers of mannose in a mixture permits the determination of the contribution of β-mannose to the combined peak of mannose and galactose [7]. Attempts to use columns with SE-30 for the GLC of mixtures of 6-deoxyhexoses [26], of mono- and oligosaccharides [27], and of methyl glycosides [28-31] have been described, satisfactory separa-

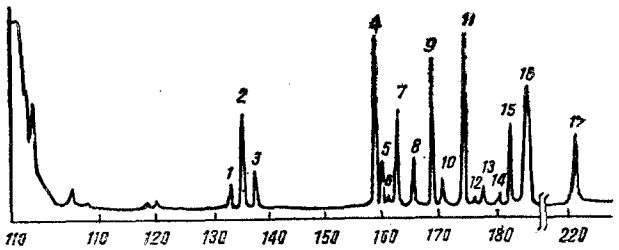

Fig. 1. GLC of a mixture of trimethylsilyl derivatives of methyl glycosides. 1-3) L-Fucose; 4, 6) D-mannose; 5, 7, 8) D-galactose; 9, 10) D-glucose; 11) D-mannitol (internal standard); 12, 14, 16) N-acetyl-D-glucosamine; 13, 15) N-acetyl-D-galactosamine; 17) N-acetylneuraminic acid.

tion being observed in a number of cases. It has been shown that the best results in the separation of the trimethylsilyl ethers of methyl glycosides are achieved on a column containing OV-1 [32] (Fig. 1).

Good results have been obtained in the analysis of a mixture of monosaccharides in the form of trimethylsilyl ethers using as the stationary phase XC-2-1 silicone oil, poly(triethyleneglycol sebacate) [33], and OV-1 [34]. The last-mentioned phase is effective for the GLC of a mixture of aldoses and ketoses in the form of the trimethylsilyl ethers of the corresponding oximes. Although each monosaccharide gives a syn and anti isomer, conditions have been selected under which the isomers issue as a single peak on the chromatogram [15]. On columns containing OV-17 and OV-101 22 monosaccharides have been successfully identified and it has been established that the retention times of the main peaks for each monosaccharide and the ratios of the areas of the peaks of the anomers are characteristic and reproducible. By the GLC of the components of honey using a column containing 3% of OV-101 it has been possible to separate and identify fructose, glucose, sucrose, raffinose, erlose, and melezitose [35].

A column with 1% of OV-101 has been proposed for the quantitative analysis of sugars in blood [36]. The results obtained relating to the concentrations of galactose, fructose, and xylose were close to the results usually obtained by colorimetric and enzymatic methods, but, in contrast to the latter, only a very small amount of blood was necessary for analysis with the aid of GLC.

The use of capillary columns substantially increases the efficiency of separations [37]. With a capillary column (25 m × 0.25 mm) containing OV-101 good results were achieved in the separation of a mixture containing L-fucose, 2,5-anhydromannitol, 2,5-anhydrotalitol, D-mannose, D-glucose, myoinositol, and sialic acid (after previous de-amination) [30]. A similar capillary column (30 × 0.28 mm) has proved effective in the analysis of the neutral monosaccharides in tobacco [38]. A satisfactory separation of monosaccharides in the form of the trimethylsilyl ethers of the corresponding oximes has been observed in capillary columns containing OV-17 [39], SE-30 [40], and SP-2250 [41].

The analysis of a mixture of ribose, ribitol, ribonolactone, arabinose, and arabonolactone in the form of their trimethylsilyl ethers on polymethylphenylsiloxane oil showed that each component of the mixture gave a single peak on the chromatogram which permitted quantitative determination to be performed [42]. Complex separation of the trimethylsilyl ethers of arabinose, fucose, mannose, glucose, galactose, glucosamine, and galactosamine was observed when a column containing a mixed phase with 5% of neopentyl glycol succinate and 3% of Apiezon N was used [43]. Good results were obtained in the separation of xylose, mannose, and glucose, in the form of their trimethylsilyl ethers on a column containing 2.5% of SKTFT-50 silicone rubber [44].

The organosilicon polyether ECNSS-M is most frequently used as the liquid phase for the GLC of carbohydrates in the form of polyol acetates [45-48], although a definite disadvantage of this phase is its low maximum working temprature (220°C) and the short life of the column [49]. The use of a column with a phase containing 0.3% of OV-275 and 0.4% of XF-1150 makes it possible to separate many carbohydrates in the form of polyol acetates, including

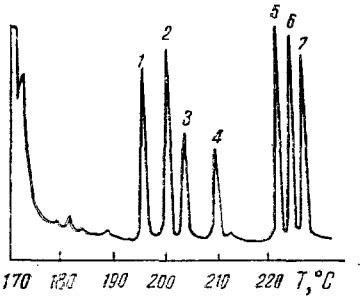

Fig. 2. GLC of a mixture of monosaccharides in the form of polyol acetates on a column containing 3% of OV-225: 1) fucose; 2) arabitol (internal standard); 3) glucosamine (2,5-anhydromannose); 4) galactosamine (2,5-anhydrotalose); 5) mannose; 6) galactose; 7) glucose.

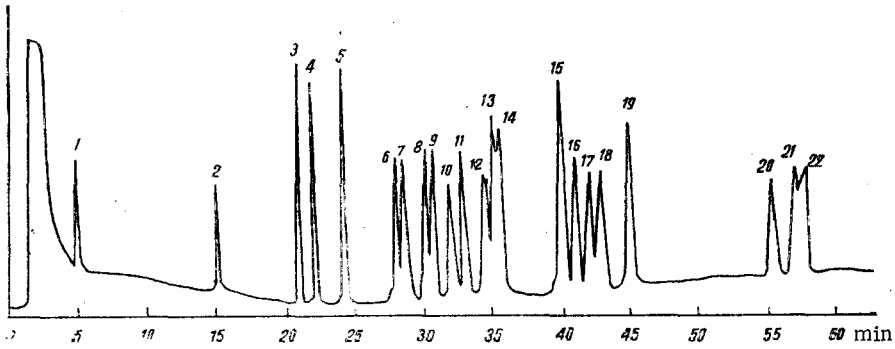

Fig. 3. GLC of 22 monosaccharides in the form of polyol acetates: 1) glycolaldehyde; 2) D-glyceraldehyde; 3) D-erythrose; 4) D-threose; 5) 2-deoxy-D-ribose; 6) L-rhamnose; 7) L-fucose; 8) D-ribose; 9) D-arabinose; 10) 2,5-anhydro-D-mannose; 11) D-xylose; 12) 2,5-anhydrotalose; 13) 2-deoxy-D-galactose; 14) 2-deoxy-D-glucose; 15) D-allose; 16) D-mannose; 17) D-galactose; 18) D-glucose; 19) meso-inositol (standard); 20) D-glucosamine; 21) D-galactosamine; 22) D-mannosamine.

fucose and rhamnose which are not always well separated [50]. Good results are given by the use as liquid phase of Silar-10c [49]. The complete separation of ribose, 2-deoxy-D-glucose, mannose, galactose, glucose, glucosamine, and galactosamine has been achieved on a column containing 5% of Silar-10c [51]. The phase Silar-7CP has been used for preparation of fucose, 2-deoxyribose, 2-deoxyglucose, mannose, glucose, and galactose in 20 min [52]. In the monosaccharide analysis of glycosides the separation of glucose, galactose, glucosamine, galactosamine, and arabinose was observed on a column containing OV-225 [53]. This column was used successfully for the quantitative analysis of a mixture of xylose, galactose, glucose, glucosamine, and galactosamine, which are the main components of glycosphingolipids [54]. A satisfactory separation of the acetates of the polyols of neutral and aminosugars (in the form of the 2,5-anhydrosugars produced by the de-amination of the aminosugars with nitrous acid), except for the incomplete separation of the peaks of glucose and galactose from one another, was observed on a column containing a mixed phase (0.75% of HI EFF-1HP, 0.25% of EGSS-X, and 0.1% of 144-B) [55]. The complete separation of such a mixture was observed on a column containing OV-225 [56] (Fig. 2), which was also used for the complete separation of a mixture of rhamnose arabinose, xylose, 2-deoxyglucose, mannose, galactose, and glucose [18].

A column containing 0.525% of ethylene adipate on Chromosorb W (Tabsorb®) permitted the separation of a large number of monosaccharides, in particular, the GLC of a mixture of 22 monosaccharides has been described [57]. However, some pairs of compounds, such as fucose

and rhamnose, arabinose, and ribose, galactosamine and mannosamine, and some others, are separated only partially, which limits the use of this column for quantitative analysis (Fig. 3).

Good results have been obtained in the separation of neutral monosaccharides and aminosugars in the form of polyol acetates on a column containing Poly A-103, with the complete separation of fucose, arabinose, mannose, and galactose, and also of glucosamine, galactosamine, and mannosamine; only glucose and galactose issue as a single peak [58]. This column was used in a sensitive method for analyzing the monosaccharide composition of a number of glycoconjugates which was based on the introduction of a label into the monosaccharides on the formation of the polyols by reduction with sodium tritioborate [59]. The labeled polyol acetates obtained were separated with the aid of GLC and were analyzed by determining the level of radioactivity of each fraction. A flame-ionization detector was also used for detection. The two methods of detection gave approximatley identical results, but the first method required an amount of the initial substance for analysis two orders of magnitude smaller [59] which is sometimes of no little importance.

D-Glucosamine and L-glycero-D-mannoheptose, which are compponents of bacterial endotoxins, have been successfully separated and identified in the form of polyol acetates with the aid of chromato-mass spectrometry using a column containing SE-30 [60].

Capillary columns are widely used for the separation of carbohydrates in the form of the corresponding polyol acetates. Thus, the separation of monosaccharides on a column (20 m × 0.3 mm) containing a mixture of N-propionyl-L-valine-tert-butylamide polysiloxane with Witoconol LA 23 in a ratio of 9:1 has been described [61]. However, on this column there was an incomplete separation of rhamnose and fucose, of ribose and arabinose, and of some other pairs of monosaccharides. This column proved useful for the separation of 16 monosaccharides, including three aminosugars [62]. However, 4-O-methylglucose and mannose were scarcely separated, and their successful separation was achieved by using a capillary column with the same phase but longer (35 m) [62]. In order to achieve the complete separation of rhamnose from fucose and of ribose from arabinose a capillary column (25 m × 0.25 mm) with OV-275 was used, the successful separation of ten polyol acetates being achieved [63] (Fig. 4). The analysis of hexoses under isothermal conditions at 190°C has been performed successfully on a capillary column (50 m × 0.2 mm) with the nonpolar liquid phase SP-2100, but ribose, fucose, rhamnose, and arabinose had very close retention times [64]. The good separation of a large number of monosaccharides has been observed on capillary columns with the polar phases FAPP and PEG-20 m [64].

The corresponding aldononitrile acetates are widely used for the GLC of monosaccharides. A good chromatogram for nine monosaccharides has been obtained on a column with neopentyl glycol succinate [65]; it is considered that this is one of the best phases for the GLC of carbohydrates in the form of aldononitrile acetates [66-69] (Fig. 5). The separation of 12 monosaccharides on a column containing 10% of LAC-4R-886 has been described [70], but fucose, ribose, xylose, and arabinose were separated only partially. The method has been used for the analysis of the neutral monosaccharide components of glycoproteins [71]. A column containing 5% of XE-60 has been used for the satisfactory separation of eight neutral monosaccharides and the methyl esters of D-galacturonic and D-glucuronic acids; however, fucose, quinovose, and ribose gave a single common peak on the chromatogram [72]. The separation of seven monosaccharides and ten polyols has been performed on a capillary column with SE-30 containing Silanox-101, but it was observed that fucose and xylose were not separated, while ribitol, fucitol, and arabitol were separated only partially [73]. The method has been used for the analysis of carbohydrates in samples of urine [73] and in the tissues of the crystalline lens of the eye [74]. The necessity sometimes arises for analyzing a mixture which, together with monosaccharides contains polyols. In such a case, the monosaccharides have been analyzed in the form of the aldononitrile acetates, and the polyols in the form of their acetates using a column containing 3% of neopentyl glycol succinate [66, 68]. The satisfactory separation of arabinose, ribitol, xylitol, glucose, sorbitol, and dulcitol on a column containing 5% of QF-1 has been reported [75]. The separation of hexosamines in the form of acetates of O-methyloximes and that of neutral sugars in the form of acetates of aldononitriles have been performed on a column containing 1% of diethyleneglycol adipate [76]. Attempts have been made to unify and modernize existing methods for the analysis of neural monosaccharides, aminosugars, and the products of Smith degradation using a single column [77]. However, the proposed column containing 3% of OV-225 was characterized

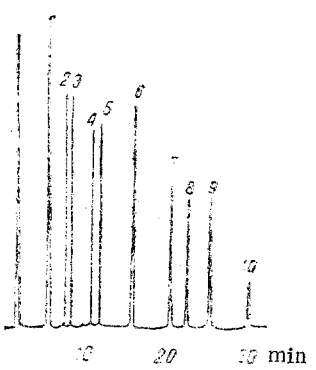

Fig. 4

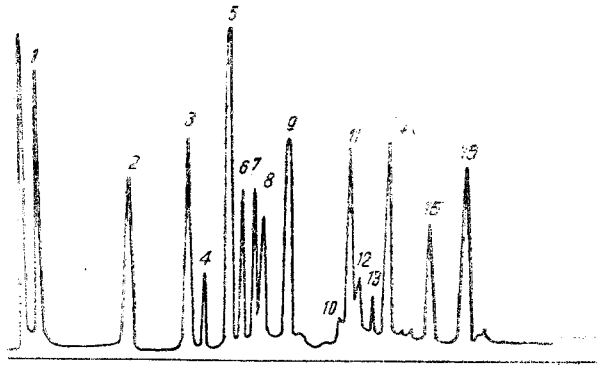

Fig. 5

Fig. 4. GLC of a standard mixture of 10 polyol acetates: 1) erythritol; 2) rhamnitol; 3) fucitol; 4) ribitol; 5) arabitol; 6) xylitol; 7) mannitol; 8) dulcitol; 9) sorbitol; 10) myoinositol.

Fig. 5. GLC of monosaccharides in the form of aldononitrile acetates: 1) D,L-glyceraldehyde; 2) D-erythrose; 3) digitoxose; 4) L-rhamnose; 5) 2-deoxy-D-erythro-pentose; 6) D-ribose; 7) D-lyxose; 8) D-arabinose; 9) D-allose; 10) D-mannose; 11) 2-deoxy-D-lyxo-hexose; 12) D-glucose; 13) D-galactose; 14) 5-thio-D-glucose (peracetylated); 15) D-glycerol-D-gluco-heptose.

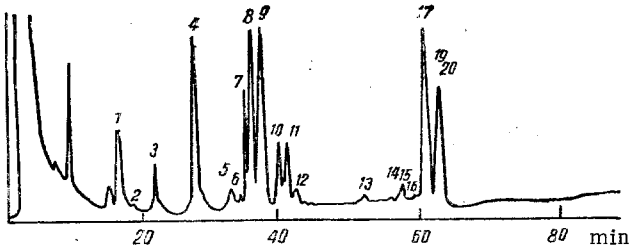

Fig. 6. GLC of methyl glycoside N,O-trifluoroacetates on a packed capillary column (7.0 × 0.5 mm) with 1% of OV-210. 1-3) Fucose; 5-7, 11) galactose; 4) mesoinositol; 8, 12) mannose; 9, 10) glucose; 13, 17, 18, 20) N-acetylglucosamine; 14-16, 19) N-acetylgalactosamine; 21) N-acetylneuraminic acid (peak 21 missing in Russian original — Publisher).

by a low efficiency and did not ensure the complete separation of glucose and galactose, of ribitol and glucosamine (in the form of 2,5-anhydromannose), and of fucose and ribose. The complete separation of ribose, arabinose, xylose, xylitol, mannose, glucose, galactose, and inositol has been achieved on a column containing 3% of HI EFF-3HP using programming of the temperature from 190 to 230° [78].

As already mentioned, the GLC of carbohydrates in the form of the corresponding polyol trifluoroacetates is employed more rarely. These derivatives are used, in particular, for the analysis of the monosaccharide compositions of glycoproteins, the hydrolysis of which is carried out with trifluoroacetic acid [21]. Neutral monosaccharides and aminosugars have been analyzed separately on a column containing 2% of XF-1105. The satisfactory separation of fucose, arabinose (or xylose), and galactose was observed. Aminosugars are separated incompletely. The use of an electron-capture detector made it possible to perform the analysis of 1-10 μg of glycoprotein. A similar result was obtained in the analysis of monosaccharides under the same conditions but in the form of the trifluoroacetates of the corresponding methyl glycosides formed on the methanolysis of the glycoproteins [79]. The use of a capillary column with OV-210 [22], together with an electron-capture detector permitted the sensitivity of the method to be increased even further and the amount of glyco-

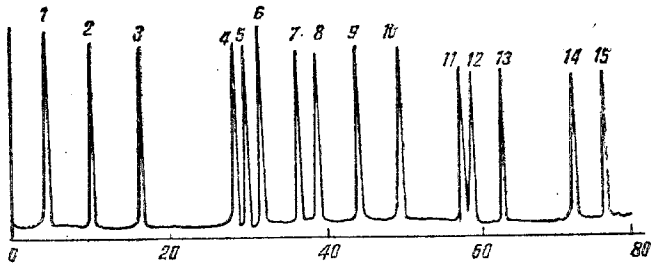

Fig. 7. GLC of diethyl dithioacetals of various aldoses on a SCOT column with SF-96: 1) solvent; 2) glyceraldehyde; 3) erythrose; 4) D-ribose and D-xylose; 5) L-arabinose; 6) inositol (internal standard); 7) L-rhamnose; 8) L-fucose; 9) D-glucuronic acid; 10) D-galacturonic acid; 11) D-glucose; 12) D-mannose; 13) D-galactose; 14) N-acetyl-D-glucosamine; 15) L-acetyl-D-galactosamine.

protein required for analysis to be decreased to 0.1 μg (Fig. 6). The use of a special nitrogen-specific thermionic detector for the analysis of aminosugars in the form of trifluoroacetates has also been described [80].

A comparative study of the GLC of carbohydrates in the form of trifluoroacetates and the trimethylsilyl ethers of the corresponding methyl glycosides has shown that the use of the trimethylsilyl ethers is preferable since the preparation of the trifluoroacetates is characterized by low reproducibility and the possible occurrence of side reactions, while the separation itself requires a special selection of liquid phases and is not always satisfactory [32].

A rapid and convenient method for the analysis of aldoses in the form of the corresponding diethyl dithioacetal trimethylsilyl ethers was proposed in 1979 [81]. In this case, only one peak on the chromatogram corresponds to each monosaccharide. On a SCOT capillary column (50 m × 0.28 mm) with the liquid phase SF-96 at 225°C a good separation of 14 aldoses was achieved (Fig. 7) [81]. A modification of this method has been proposed for the analysis of aminosugars in the presence of neutral monosaccharides and of uronic acids [17]. For this purpose, the aminosugars were first de-aminated with nitrous acid and this was followed by mercaptylation and the preparation of the trimethylsilyl ethers. Under the above conditions a good separation of neutral, basic, and acidic monosaccharides was achieved. The method has been used successfully for the analysis of a number of glycoproteins.

In 1975, it was proposed to use the chromato-mass spectrometry of O-isopropylidene derivatives for the analysis of aldoses [82]. A rapid method that was developed for the quantitative isopropylidenation of monosaccharides consists in the treatment of a mixture of monosaccharides with acetone containing 1% of sulfuric acid, and permits only a single derivative to be obtained from each aldose. On using a column with XE-60 and OV-225, complete separation of mixtures containing fucose, arabinose, xylose, rhamnose, glucose, galactose, and mannose was observed. An analysis took 35-40 min. Subsequently [83], the method was extended to hexuloses and pentuloses with the aim of their identification and quantitative analysis. L-Sorbose, D-psicose, L-erythro-pentulose, L-threo-pentulose, D-fructose, D-galactose, and L-arabinose have been separated successfully on a column containing OV-225. The method has been applied to the analysis of the products of the aldol condensation of trioses [83]. A method based on the use of the trimethylsilyl ethers of butylboronate esters, which are comparatively easy to obtain, give a single peak on chromatograms for each monosaccharide and are well separated on a column containing 3% of OV-225, has been proposed for the quantitative analysis of glucose and fructose in mixtures [84].

For the quantitative analysis of ribose in the presence of ribitol, glucose, and gluconic acid it has been proposed to use as derivatives the trimethylsilyl ethers of oximes: on a column containing 10% of SE-52 the complete separation of ribose from accompanying monosaccharides was achieved [85]. Good results were given by the use of the trimethylsilyl ethers of N-ethoxycarbonyl derivatives of carbohydrates, although their preparation is com-le

plex, including four stages [86]. In this case, to each aldose corresponded a single derivative, while for ketoses the formation of diastereomers was observed. By-products of the synthesis were formed only in insignificant amounts. Unfortunately, the phases OV-101 and Chirasil-Val used do not ensure the satisfactory separation of rhamnose from fucose and of galactose from mannose. The satisfactory separation of all eight aldohexoses in the form of the trifluoroacetates of O-methyloximes and of O-butyloximes has been observed in a capillary column with OV-225 [87].

Although the successful use of methylated polyols as derivatives for the GLC of the monosaccharides on a column with 3% of QF-1 has been described [88], it is doubtful whether these derivatives are finding wide use because of the comparative great complexity of their preparation.

For the performance of the GLC of aminosugars, as a rule, N-acetylation is required, and this is usually carried out before the preparation of the trimethylsilyl ethers [89-92], although good results are not infrequently given by secondary N-acetylation after trimethylsilylation [90, 93], particularly if the reaction is performed on the chromatographic column by the combined introduction of acetic anhydride and the sample under investigation [93].

Recently, stable volatile acyclic derivatives — O-methylaldoximes which are formed by treating hexosamines with O-methylhydroxylamine — have been used for the quantitative analysis of aminosugars with the aid of GLC [76, 94].

In the study of glycoproteins and glycolipids, the necessity frequently arises for the identification of neuraminic acid or its acyl (N-acetyl and N-glycolyl) derivatives. The chromato-mass spectrometry of the trimethylsilyl ethers or acetates of neuraminic acid and its various derivatives — its methyl ester and glycosides, or the corresponding polyol — are usually used for this purpose [95]. Recently, the acyclic O-methylketoximes of neuraminic acid in the form of the trimethylsilyl ethers, which are well separated by GLC on a column containing SE-52 and SP-2250, followed by identification with the aid of mass spectrometry, have been used for this purpose [96].

It appears desirable to consider the GLC of aldonic and uronic acids separately.

The successful separation of aldonic acids is achieved by converting them into permethylated derivatives [88] or trimethylsilyl ethers [15, 42, 97, 98] with the use mainly of the following liquid phases: SE-30, XE-60/EGS, QF-1, OV-1, and OV-17. Good results are given by a capillary column containing SE-30 [97]. For the analysis of glucuronic acids and their lactones with the aid of GLC, the trimethylsilyl ethers are most frequently used as volatile derivatives [12, 17, 90, 100] with columns containing SE-30 [12, 100]. Not bad results are obtained for other liquid phases, as well: XE-60 [12], and SE-52 and DC-200 [99]. The effective separation of neutral monosaccharides and uronic acids in the form of trimethylsilyl ethers of diethyl thioacetals is achieved in a capillary column with the phase SF-97 [17].

For the combined determination of glucuronic and galacturonic acids in the form of aldonitrile acetates of the corresponding methyl esters, GLC in a column containing 5% of XE-60 is used [72]. In this way a satisfactory separation of glucuronic acids and neutral monosaccharides is achieved.

Good results are also given by the GLC of uronic acids after their reduction to the correspondong polyols followed by their chromatography in the form of n-butylborates on a column with 3% of OV-17 [101]. Definite interest is presented by a new differential approach to the analysis of mixtures of uronic acids and neutral monosaccharides [78]. It consists in analyzing the neutral monosaccharides in the form of the corresponding aldononitrile acetates, and the sum of the neutral monosaccharides and the uronic acids corresponding to them in the form of polyol acetates. The difference between the values corresponds to the amount of uronic acids in the mixture.

In a number of cases, the separation of uronic acids in the form of permethylated derivatives of various glycosides has been described [102, 103].

The analysis of oligosaccharides does not differ fundamentally from the analysis of monosaccharides, but the higher molecular weights and polarities raise the demands on the volatilities of the derivatives of this class of compounds. Oligosaccharides are usually subjected to GLC in the form of corresponding trimethylsilyl ethers [7, 8, 24, 25, 27, 34,

35, 104-112]. Numerous liquid phases are used for this purpose, but most frequently SE-30 [7, 8, 24, 25, 106, 108, 112] and OV-17 [27, 104, 107], which give a good separation of the most diverse oligosaccharides both from one another and from monosaccharides. The efficiency of the separation rises appreciably when capillary columns are used [104, 107].

Attempts have been made to use polyol acetates as derivatives for the GLC of the oligosaccharides [113, 114], but, because of the low volatility of these compounds the method has not come into wide use.

The trimethylsilyl ethers of oximes obtained from oligo- and monosaccharides have been used successfully for quantitative analysis [114-119]. The separation in this way of sucrose, lactose, maltose, raffinose, and melezitose has been described. At the same time, it has been shown [119] that kojibiose, nigerose, cellobiose, neolactose, and melibiose each give two or three peaks on chromatograms obtained on columns with SE-52 and OV-17. This fact cancels the advantages of using oximes in comparison with the trimethylsilyl ethers of oligosaccharides.

Good results have been obtained for the GLC of oligosaccharides in the form of corresponding permethylated polyols on columns with SE-30 and OV-17 [120]. The use in this case of chromato-mass spectrometry for oligosaccharides having a N-acetylhexosamine residue at the reducing end permits the determination of the nature of the glycosidic bond in oligosaccharides directly in a mixture.

GLC AND THE METHOD OF METHYLATION

The method of methylation is one of the main methods in establishing the structures of carbohydrate-containing compounds. GLC has considerably facilitated the separation and analysis of methylated sugars, although this problem is more complicated than the analysis of monosaccharides, which is connected with the large number of methylated derivatives for each initial monosaccharide.

Methylated sugars are most frequently subjected to GLC in the form of polyol acetates, primarily because of the simplicity of their subsequent identification with the aid of mass spectrometry. Good results are obtained on a column with 3% of ECNSS-M [2, 121-130]. And although it gives a satisfactory separation of many partially methylated sugars, nevertheless, because of the low thermal stability of the phase ECNSS-M, columns containing this phase can be used for only a short time. For this reason, the use of the phase OV-225, which has similar characteristics but is distinguished by a higher temperature stability (up to 250°C), is recommended [50, 130-134]. Columns with 3% of OV-225 are usually used for the separation of methylated sugars in the form of the polyol acetates. However, it has been found [134] that columns with 0.3-0.4% of OV-225 on Chromosorb modified with high-molecular-weight polyethyleneglycol ensure a better separation of the methylated derivatives of mannose and galactose than columns with 3% of OV-225 or 3% of ECNSS-M.

The separation of 2,4-di-O-methyl-L-rhamnose, L-rhamnose, and 2,4,6-tri-O-methyl-D-glucose in the form of the corresponding polyol acetates has been performed successfully on a column containing 20% of Apiezon [135]. It has been shown [136] that the Apiezons are frequently superior to ECNSS-M and OV-225. Thus, for example, the tri-O-methyl ethers of galactose are well separated on a column with Apiezon T, while the separation of the methyl ethers of arabinose, rhamnose, and xylose on the same column is comparable with the results obtained on the phases ECNSS-M and OV-225.

A large number of methyl ethers of arabinose, xylose, mannose, galactose, and glucose have been successfully separated on a column with a mixed phase containing 0.3% of OV-275 and 0.4% of XF-1150 [50, 137]. It is particularly important that the 2,3,6-tri- and 3,6-di-O-methyl ethers of glucose and galactose are separated on this column. The use of columns with OV-210 and with Carbowax 20M gives fairly good results [138]. The GLC of the methyl ethers of glucose, galactose, and 3,6-anhydrogalactose on a column with the phase Silar-10C leads to approximately the same pattern as in the case of the use of ECNSS-M, but Silar-10C is characterized by a high thermal stability (up to 275°C) and this is undoubtedly very important in the practical respect [49, 139]. The efficiency of the phase Silar-10C rose appreciably when a capillary column (30 m × 0.28 mm) was used: On this, 32 methyl ethers of rhamnose, arabinose, xylose, glucose, galactose, and mannose, including the 2,3,4,6-tetra-O-methyl derivatives of glucose and mannose were successfully separated [139]. On the other hand, the peaks of 2,4,6- and 3,4,6-tri-O-methylmannoses overlapped completely, which elimi-

nates the possibility of using this column for the structural analysis of mannans by the methylation method [139]. An advantage of a WCOT CP-SIL-5 capillary column (25 m × 0.25 mm) as compared with an ordinary column containing 3% of ECNSS-M has been shown for the case of the separation of a large number of methylated derivatives of L-glycero-D-manno- and D-glycero-D-manno-heptoses [140]. Capillary columns are being used ever more frequently for the analysis of methylated sugars in the form of polyol acetates and give very good results with the use of the most diverse phases: SV-30, OV-101 [141], OV-225 [142], and FAPP [64]. The suscessful separation of the large number of methylated derivatives of monosaccharides and aminosaccharides that are usually formed in the study of glycoproteins and their fragments by the methylation method, has been achieved on capillary columns containing Silar-9CP, Dexsil 410, and OV-101 [143]. In their polarities, the phases Silar-9CP and Dexsil 410 are close to ECNSS-M and OV-17, respectively, but are superior to the latter in thermal stability. The most effective is a column containing the phase Silar-9CP, which is characterized by the highest polarity.

In a number of cases the ethyl ethers of sugars, which are distinguished by a high volatility, permit a better separation to be achieved than the methyl ethers [144]. In this connection, the use of both types of derivatives, supplementing one another, is recommended. This expedient may sometimes be justified, but it will hardly achieve wide use because of the methodological complications.

The GLC of methylated sugars in the form of aldononitrile acetates is used comparatively rarely, but interest in these derivatives is rising because of the possibility of their identification with the aid of chromato-mass spectrometry [65]. Columns with various phases are used for their separation: 5% of butanediyl succinate [145] and its mixture with 5% of Apiezon L [146, 147], QF-1, SP-2340 [148], and 3% of OV-225 and 2.5% of tetramethylcyclobutanediyl succinate [149]. Separation of all possible methylated derivatives of glucose has been observed on columns with QF-1 and SP-2340 [148], while the complete separation of the methyl ethers of L-rhamnose could not be achieved on columns with either QF-1, XE-60, or neopentyl glycol succinate [150].

As is well known, the methanolysis of methylated polysaccharides and glucoconjugates forms methyl glycosides of methylated sugars. They can be separated directly with the aid of GLC, but their acetates or trimethylsilyl ethers are not infrequently used for this purpose [2].

The partial separation of a series of methyl ethers of methyl α-D-galactopyranoside have been observed on a column containing Carbowax 6000 [151]. To improve the results, the mixtures were acetylated and chromatographed on the same column. On a column with diethyleneglycol adipate the α- and β-anomers of methyl 2,3,4,6-tetra- and 2,3,6- and 2,3,4-tri-O-methyl-D-galactosides were successfully separated [152], and a column containing 5% of Versamide 9000 has been used for the analysis of the products of the partial methylation of 2,6-dideoxy-α-D-xylo- and -α-D-lyxo-hexopyranosides [153]. On comparing the possibilities of three phases — ECNSS-M, DEGS, and BDS — it was found that the first of them gave a better separation of seven methylated ethers of α-L-rhamnopyranoside [150].

Methyl glycosides are chromatographed considerably more frequently in the form of acetates and trimethylsilyl ethers. Thus, the methylated derivatives obtained on the partial methylation of α-D-glucopyranoside have been satisfactorily separated in the form of their trimethylsilyl ethers on a capillary column (48 m × 0.5 mm) with OV-101 [154]. But here, the tri-O-methyl ethers and also the 2,3- and 2,4-di-O-methyl ethers, were separated only partially. The retention times on a column containing 3% of neopentyl glycol succinate for the tetra-, tri-, and di-O-methyl ethers of methyl α- and β-D-glucopyranosides and their trimethylsilyl derivatives have been given in the literature [155].

The acetates of methyl glycosides have found wide use in the study of the partial methylation of methyl glycosides. Polyester phases or QF-1 are used for GLC with this aim [156]. Thus, acetates of the methyl glycosides of the methyl ethers of β-D-xylose [157-159], of β-L-arabinose [159, 160], and α-L-rhamnose [161] have been separated on a column with butanediyl succinate. Columns with neopentyl glycol succinate have also been used to separate α-L-rhamnose derivatives [162]. A column with QF-1 has been used for the analysis of acetates of methyl glycosides of methyl ethers of α-L-arabinose, α-D-xylose, α-D-lyxose, and α-L-rhamnose [159], and a column with ECNSS-M for the separation of α-D-mannose derivatives [163]. In the examples given, as a rule, the complete separation of the methylated

sugars was observed, but it was not possible to separate the 16 products of the partial methylation of methyl α-D-glucopyranoside on a single column containing neopentyl glycol succinate [164]: the 2,3-, 3,4- and 3,6-di-O-methyl ethers, and also the 2,6- and 4,6-di-O-methyl ethers, were not separated.

In the analysis of the methylated sugars obtained by the methanolysis of a permethylated sialoglycopeptide, use was made of the chromato-mass spectrometry of the acetates of the partially methylated methyl glycosides of α- and β-D-galactoses, D-mannose, D-glucose, and 2-(N-methylacetamido)-2-deoxy-D-glucose using capillary columns (60 m × 0.35 mm) with Carbowax 20M and OV-101.

Methylated amino sugars are usually subjected to GLC in the form of methyl glycoside acetates or polyol acetates using various liquid phases [166-171]. It has been shown [168] that a column with OV-17 is preferable to a column with ECNSS-M, but satisfactory results on chromatography are by no means always observed. In this connection it must be mentioned that on a column with OV-225 the 3,4,6-tri-, 3,6- and 3,4-di-, and 3-O-methyl ethers of 2-(N-methylacetamido)-2-deoxy-D-glucose, in the form of their trimethylsilyl ethers, were separated completely [134, 172]. In recent years, chromato-mass spectrometry has been widely used for determining the nature of the bond of neuraminic residues in various carbohydrate-containing compounds [173-175]. Thus, the separation of the 4,7,8-, 4,7,9-, and 7,8,9-tri- and the 4,7,8,9-tetra-O-methyl ethers of the methyl glycoside of methyl N-acetyl-N-methyl-β-D-neuraminate and their acetates has been successfully achieved on a column with OV-17 [173]. A column with SE-30 has been used for the separation of the permethyl and the 8-O-acetyl-4,7,9-tri-O-methyl derivatives of neuraminic acid [176].

The analysis of a number of methyl derivatives of neuraminic acid has also been performed on a column with OV-101 [174], while a capillary column with trimethylsilyl ethers as the volatile derivatives has also been used [177]. The separation of seven methyl ethers of the methyl glycoside of methyl N-acetyl-N-methyl-β-D-neuraminate in the form of trimethylsilyl and acetyl derivatives has been described [178, 179].

Definite interest is presented by information [180] on the GLC of methyl ethers of methyl (methyl α-D-glucopyranosid)uronate and their trimethylsilyl derivatives, acetates, and trifluoroacetates on columns with XE-60, ECNSS-M, and butanediyl succinate. The best results were obtained by the use of acetyl derivatives with BDS.

GLC IN THE SOLUTION OF PROBLEMS OF CARBOHYDRATE CHEMISTRY

GLC is not infrequently used for the analysis of products of reactions, for the quantitative analysis of α- and β-anomers, and for determining the D- and L-configurations of monosaccharides. In the study of the periodate oxidation of monosaccharides of methyl glycosides, and of a number of plant glycosides, the aldehydes formed have been analyzed, in the form of the trimethylsilyl ethers of diethyl dithioacetals with the aid of GLC [181-183]. GLC has been used for the separation of oximes of keto sugars obtained by the bromine oxidation of pentoses and hexoses in the form of methyl glycosides and polyols [184, 185]; for the analysis of the products of the oxidation of methyl α-D-glucopyranoside with dimethyl sulfoxide and acetic anhydride [186]; in the study of the oxidation of the methyl ethers of methyl β-D-maltoside [187]; in the analysis of the products of Smith degradation [124]; and in the identification of the mono-O-methyl ethers of aldoses after their oxidation with silver carbonate on Celite [188]. To determine the D- and L-configurations of monosaccharides, the diastereomers of glycosides obtained in reactions with chiral alcohols (butan-2-ol, octan-2-ol), followed by their separation in the form of trimethylsilyl ethers on the capillary column with SE-30, have been used [189-191], and so also have esters of saccharic acids and optically active alcohols [190] in the form of acetates or trimethylsilyl derivatives. It has recently [192] been shown that the per(heptafluorobutanoyl) derivatives are very suitable for determining the configurations of the carbohydrates, permitting an excellent separation of the D- and L- forms of arabinose, fucose, xylose, and mannose, while the D- and L-enantiomers of glucose are best separated in the form of 6-O-trimethylsilyl-α-glucofuranose 1,2:3,5-bis(methylboronate). The separation of the D- and L-forms of galactose and fucose in the form of peracetates of the corresponding bis(ethyl L-lactate) acetals has also been described [193]. The D- and L-forms of arabinose, lyxose, fucose, rhamnose, galactose, glucose, and mannose have been successfully separated in the form of the acyclic diastereoisomeric dithioacetals obtained by the reaction of the monosaccharides with (+)-1-phenyl-

ethanethiol [194]. Here, the acetates or trimethylsilyl ethers were used as the volatile derivatives, being chromatographed on columns with SE-60 or SE-54.

GLC has been used successfully for the quantitative analysis of the α- and β- anomers of alkyl glycosides derived from D-galactose and D-xylose [195], methyl glycosides [196], and methyl rhamnopyranosides [197], and for determining the composition of the products of the isopropylidenation of D-ribose and D-arabionse [198], and of D-mannose [199]. In a study of the relative reactivities of the hydroxy groups of 1,5-anhydro-D-xylitol, the complete separation of all the partially acetylated derivatives, in the form of their trimethylsilyl ethers, was achieved on a capillary column with SE-30 [200]. GLC was performed under analogous conditions in a study of the distribution of hydroxyethyl groups in the glucose residues of starch after its reaction with ethylene oxide [201].

The separation of the benzyl ethers of benzyl α-L-rhamnopyranoside in the form of the corresponding acetates was performed on a column with OV-1 [202], and the benzyl ethers of α-D-glucopyranoside were analyzed in the form of the trimethylsilyl ethers on a column with OV-17 followed by quantitative analysis with the aid of chromato-mass spectrometry [203]. The endo- and exo-isomers of benzylidene derivatives of a number of monosaccharides have been successfully chromatographed on a column with UCW-982, OV-1, and SE-30 [202, 204]. Glucosinolates in the form of their trimethylsilyl ethers have been successfully separated on columns with OV-1 [205] and OV-17 [206] and so have partially acetylated methyl α- and β-glycosides and D-glucose, D-galactose, and D-mannose [207], mono-O-acyl derivatives of glucose [208], plant galactolipids [209], glucocorticoids [210], the methyl- and butylboronates of a number of methyl glycosides [211], the butylboronates of polyhydroxyalkylpyrazines — the products of the condensation of monosaccharides with ammonia — [212], sugar osazones [213], and N-arylglucosamines [214].

Trimethylsilyl ethers have been used as the volatile derivatives in the GLC analysis of the products of the pyrolysis of cellulose [215] and the products of the radiolysis of D-ribose [216] and of D-glucose [217]. To determine the degree of polymerization of oligo- and polysaccharides a method has been proposed which is based on the determination, with the aid of GLC, of the ratio between the polyol acetate formed on the reduction of the terminal monosaccharide residue and the acetates of the aldononitriles obtained from the other links of the carbohydrate chain after the complete hydrolysis of the compound being analyzed [68, 218].

A quantitative analysis of uronic acids in glycuronoglycans has been based on the GLC determination of the carbon dioxide formed on the decarboxylation of the uronic acid residues [219]. The amount of pentosans in a plant raw material has been determined by the GLC estimation of the furfural formed on hydrochloric acid treatment [220]. The quantitative analysis of pentoses, hexoses, and 6-deoxyhexoses has been performed with the aid of the GLC of the furufral, 5-hydroxymethylfurfural, and 5-methylfurfural obtained after the treatment of the sample being analyzed with concentrated hydrochloric acid [221]. The facts given indicate the great possibilities of GLC in the solution of numerous problems of carbohydrate chemistry.

PREPARATIVE GLC OF CARBOHYDRATES

This comparatively new and effective method for the preparative production of various carbohydrate derivatives did not find its merited wide use for a long time because of the general preparative difficulties of separating high-boiling compounds. However, in the last decade interest in this method has risen considerably in view of the promising production of authentic samples of methylated sugars. In 1973, preparative GLC was used for the preparative separation of the methyl ethers of methyl α-D-mannopyranoside [222]. A sample containing 14 mg of a mixture of 11 methyl ethers was deposited on a column (6 m × 0.92 cm) containing 3% of Carbowax 6000, and, as the result of GLC, from 0.6 to 3.2 mg of individual compounds were obtained. The unresolved mixture of 2,3,4- and 3,4,6-tri-O-methyl ethers was chromatographed supplementarily in the form of the trimethylsilyl ethers.

On a similar column containing butandiyl succinate, 9 mg of monomethyl ethers of methyl α-D-mannopyranoside was successfully separated, the yields of the individual compounds amounting to 1-2 mg [163].

In order to obtain authentic samples of methylated derivatives of methyl α-D-galactopyranoside [151], the latter was subjected to partial methylation, and the resulting mixture was first fractionated according to the degree of substitution with the aid of liquid chromatography on silica gel.

The fractions obtained, containing compounds with the same degree of methylation, were deposited on columns containing Carbowax 6000 in an amount of 10 mg per column. The mixtures that were not resolved by GLC were chromatographed additionally on a column containing butanediyl succinate. The yields of individual compounds amounted to 0.1-3 mg.

In 1974, a large-diameter column (2 m × 1.4 cm) containing 10% of butanediyl succinate was used for the separation and preparative production of acetates of the methyl ethers of methyl β-D-xylopyranoside obtained as the result of the partial methylation of the initial glycoside [158]. The use of a such a column with a high concentration of liquid phase permitted the successful separation of about 600 mg of mixture in one GLC cycle. Two columns were used for separating the acetates of the methyl ethers of methyl α-L-rhamnopyranoside: a main column (2 m × 1.4 cm) containing 10% of butandiyl succinate, which ensured the separation of 300 mg of a mixture of almost all the methyl ethers, and an auxiliary column containing 3% of QF-1, on which the separation of a mixture 3- and 4-mono-O-methyl ethers, not separated on the main column, was achieved [161].

In order to fractionate a complex mixture of the products of the partial methylation of methyl α-D-glucopyranoside [164] in the form of their acetates twofold liquid chromatography was first used. This achieved the complete separation of the tri-O-methyl ethers and the partial fractionation of the di- and mono-O-methyl ethers. The unresolved mixtures were deposited in an amount of 100-300 mg on a column (2 m × 1.4 cm) containing 10% of neopentyl glycol succinate, and as a result all the methyl esters were obtained in the individual state.

Thus, it appears desirable for the preparative GLC of methylated saccharides to use columns of large diameter containing high concentrations of liquid phase (up to 10%). In spite of the fact that the efficiency of such columns is comparatively low [158], they permit the deposition of up to 1 g of mixture and the production directly or with the aid of additional fractionation of authentic specimens in the individual state with yields of up to several hundreds of milligrams, which is sufficient for performing a large number of structural and analytical investigations.

CONCLUSIONS

The period of establishing GLC in carbohydrate chemistry is long past, and in recent years the process of improving and modernizing the procedure and of expanding the sphere of application of the method has been proceeding. There is no doubt that GLC has become a universal method for solving numerous analytical and structural problems of carbohydrate chemistry and for obtaining a number of difficultly accessible authentic specimens. Chromatography in capillary columns, which, as a rule, is distinguished by a very high efficiency of separation and which requires only small amounts of the initial samples for analysis, is being used ever more widely.

It may be assumed with confidence that in the near future GLC will receive new development and will even further expand its sphere of application in carbohydrate chemistry.

LITERATURE CITED

1. A. G. McInnes, D. H. Ball, F. R. Cooper, and T. Bishop, J. Chromatogr., 1, 556 (1958).
2. C. T. Bishop, Adv. Carbohydr. Chem., 19, 95 (1964); Yu. S. Ovolov, The Gas-Liquid Chromatography of the Carbohydrates [in Russian], Vladivostok (1970); G. G. S. Dutton, Adv. Carbohydr. Chem. Biochem., 28, 11 (1973); G. G. S. Dutton, Adv. Carbohyd. Chem. Biochem., 30, 9 (1974).
3. C. C. Sweeley, R. Bentley, M. Makita, and W. W. Wells, J. Am. Chem. Soc., 85, 2497 (1963).
4. W. E. Sable and G. W. Cathey, Agron. J., 62, 36 (1970).
5. C. Miyazaki, Kanzei, Chuo Bunsekishoho, 15, 93 (1975); Chem. Abstr., 87, 290065 (1977).
6. A. E. Pierce, Silylation of Organic Compounds, Pierce Chemical Co. Rockford, Illinois (1968).
7. C. W. Ford, Anal. Biochem., 57, 413 (1974).

8. M. Martinez, D. Nurok, and A. Zlatkis, Anal. Biochem., 86, 552 (1978).
9. M. Donike, J. Chromatogr., 42, 103 (1969).
10. W. C. Ellis, J. Chromatogr., 41, 325 (1969).
11. A. Gindzienski and D. Kilczewska, Chem. Anal. (Warsaw), 24, 167 (1979).
12. J. F. Kennedy, S. M. Robertson, and M. Stacey, Carbohydr. Res., 49, 243 (1976).
13. R. Malinen, E. Sjöström, Carbohydr. Res., 39, 335 (1975).
14. D. J. Leblanc and A. J. S. Ball, Anal. Biochem., 84, 574 (1978).
15. G. Petersson, Svensk. Papperstidn., 78, 27 (1975).
16. R. A. Laine and C. C. Sweeley, Carbohydr. Res., 27, 199 (1973).
17. S. Honda, K. Kakehi, and K. Okada, J. Chromatogr., 176, 367 (1979).
18. R. S. Selvendran, J. F. March, and S. Ring, Anal. Biochem., 96, 282 (1979).
19. R. Wachowaik, K. A. Connors, Anal. Chem., 51, 27 (1979).
20. G. D. McInnes, Carbohydr., Res., 108, 284 (1982).
21. Y. Arakawa, T. Imanari, and Z. Tamura, Chem. Pharm. Bull., 24, 2032 (1976).
22. M. M. Wrann and C. W. Todd, J. Chromatogr., 147, 309 (1978).
23. B. A. Rudenko, Capillary Chromatography [in Russian], Moscow (1978).
24. D. V. Phillips and A. E. Smith, Anal. Biochem., 54, 95 (1973).
25. V. L. Yarovenko, B. M. Nakhmanovich, D. M. Makeev, and E. M. Belov, Ferment. Spirt. Promst., 6 (1977).
26. B. Koop, J. Jurenitsch, and W. Kubelka, J. Chromatogr., 210, 291 (1981).
27. H. Jaddou and M. Al-Hakim, J. Agric. Food Chem., 28, 1208 (1980).
28. T. T. Gorovich and N. K. Abubakirov, Khim. Prir. Soedin., 241 (1975).
29. M. Yokota and T. Mori, Carbohydr. Res., 59, 289 (1977).
30. J. Mononen, Carbohydr. Res., 88, 39 (1981).
31. J. P. Kamerling, J. Gerwig, J. F. G. Vliegenthart, and J. R. Clamp, Biochem. J., 151, 491 (1975).
32. A. A. Akhrem, G. V. Avvakumov, O. V. Sviridov, O. A. Strel'chonok, Vestsi Akad. Navuk BSSR, Ser. Khim. Navuk, No. 5, 95 (1978).
33. L. P. Stepovaya and Yu. I. Khol'kin, in: Chromatographic Analysis in Wood Chemistry [in Russian], Riga (1975), p. 18.
34. A. Ericsson, J. Hansen, and L. Dalgard, Anal. Biochem., 86, 552 (1978).
35. A. Patetta and A. Manino, Cron. Chim., 57, 9 (1978); Chem. Abstr., 91, 138942 (1979).
36. A. Doussin, S. Charonnat, and M. Guernet, Ann. Biol. Chem. (Paris), 35, No. 1, 19 (1977); Chem. Abstr., 87, 64830 (1977).
37. J. Jurenitsch, B. Kopp, J. Gabber-Kolacsek, and W. Kubelka, J. Chromatogr., 210, 337 (1981).
38. T. Sakaki, S. Munakata, H. Sakuma, and S. Sugawara, Agric. Biol. Chem., 46, 827 (1982).
39. D. Anderle, J. Königstein, and V. Kováčik, Anal. Chem., 49, 137 (1977).
40. E. Jellum, P. Storseth, J. Alexander, P. Heiland, O. Stokke, and E. Teig, J. Chromatogr., 126, 487 (1976).
41. K. J. Schäffler, P. G. Morel du Boil, J. Chromatogr., 207, 251 (1981).
42. V. M. Svetlaeva, L. M. Kirshchenya, M. Ts. Yanotovskii, Khim.-Farm. Zh., 132 (1977).
43. D. Podhradsky, M. Podhradska, and J. Andrašina, Chem. Zvesti, 31, 87 (1977).
44. Z. E. Bryantseva, V. R. Bretosh, and V. E. Moskaleva, in: Chromatographic Analysis in Wood Chemistry [in Russian], Riga (1975), p. 31.
45. R. A. Laine, W. J. Esselman, and C. C. Sweeley, Methods Enzymol., 28, 159 (1972); H. Sloneker, in: Methods in Carbohydrate Chemistry, Vol. VI, R. L. Whistler and J. N. BeMiller, eds., Academic Press, New York (1972), pp. 20-24.
46. A. Misaki, Y. Tsumuraya, M. Kakuta, H. Takemoto, and T. Igarashi, Carbohyd. Res., 75, C8 (1979).
47. N. E. Anderson and E. M. Clydesdale, J. Food, Sci., 45, No. 2, 336 (1980).
48. L. H. Krull and G. E. Inglett, J. Agric. Food Chem., 28, 917 (1980).
49. S. Hirase, K. Watanabe, and R. Takano, Agric. Biol. Chem., 42, 1065 (1978).
50. A. G. Darvill, D. P. Roberts, and M. A. Hall, J. Chromatogr., 115, 319 (1975).
51. J. Stadler, Anal. Biochem., 74, 62 (1976).
52. C. W. Gehrke, T. P. Waalkes, E. Borek, W. F. Swartz, T. F. Cole, K. C. Kuo, M. Aberloff, D. S. Ettinger, N. Rosenshen, and R. C. Yound, J. Chromatogr. 162, 507 (1979).
53. R. Kanna, P. N. Seng, and H. Debuch, J. Chromatogr., 92, 95 (1974).
54. L. A. Torello, A. J. Yates, and D. K. Thomson, J. Chromatogr., 202, 195 (1980).
55. W. H. Porter, Anal. Biochem., 63, 27 (1975).
56. A. A. Akhrem, G. V. Avvakumov, O. V. Sviridov, and O. A. Strel'chonok, Vestsi Akad. Navuk BSSR, Ser. Khim. Navuk, No. 3, 98 (1978).

57. K. Schmid, and L.-C. H. Chen, Prep. Biochem., 6, 27 (1976).
58. W. Niedermeier and M. Tomana, Anal. Biochem., 57, 363 (1974).
59. P. Prehm and A. Scheid, J. Chromatogr., 166, 461 (1978).
60. R. Chaby and L. Szabo. Eur. J. Biochem., 70, 115 (1976).
61. G. Holzer, J. Oró, S. J. Smith, and V. M. Doctor, J. Chromatogr., 194, 410 (1980).
62. C. Green, V. M. Doctor, G. Holzer, and J. Oró, J. Chromatogr., 207, 268 (1981).
63. J. Klok, E. H. Niebérg-van Velzen, J. W. de Leeuw, and P. A. Schenck, J. Chromatogr., 207, 273 (1981).
64. R. Oshima, A. Yoshikawa, J. Kumanotani, J. Chromatogr., 213, 132 (1981).
65. B. A. Dmitriev, L. V. Backinowsky, O. S. Chizhov, B. M. Zolotarev, and N. K. Kochetkov, Carbohydr. Res., 19, 432 (1971).
66. J. K. Baird, M. J. Holroyde, and D. C. Ellwood, Carbohydr. Res., 27, 464 (1973).
67. R. Varma and R. S. Varma, J. Chromatogr., 128, 45 (1976).
68. R. Varma and R. S. Varma, J. Chromatogr., 139, 303 (1977).
69. F. R. Seymour, E. C. M. Chen, and S. H. Bishop, Carbohydr., Res., 73, 19 (1979).
70. R. Varma, R. S. Varma, and A. H. Wardi, J. Chromatogr., 77, 222 (1973).
71. R. Varma, R. S. Varma, W. S. Allen, and A. H. Wardi, J. Chromatogr., 86, 205 (1973).
72. T. T. Gorovich and N. K. Abubakirov, Khim. Prir. Soedin., 523, (1975).
73. C. D. Pfaffenberger, J. Szafrenek, M. G. Horning, and E. C. Horning, Anal. Biochem., 63, 501 (1975).
74. C. D. Pfaffenberger, J. Szafranek, and E. C. Horning, J. Chromatogr., 126, 535 (1976).
75. G. J. Mawnius, T. M. Li, and L.-F. L. Wen, Anal. Biochem., 99, 365 (1979).
76. T. P. Mawhinney, M. S. Feather, G. J. Barrero, and J. R. Martinez, Anal. Biochem., 101, 112 (1980).
77. S. H. Turner, and R. Cherniak, Carbohydr. Res., 95, 137 (1981).
78. J. Lehrfeld, Anal. Biochem., 115, 410 (1981).
79. M. Tomana, W. Niedermeier, and C. Spivey, Anal. Biochem., 89, 110 (1978).
80. T. Shinohara, J. Chromatogr., 207, 262 (1981).
81. S. Honda, N. Yamauchi, and K. Kakeni, J. Chromatogr., 169, 287 (1979).
82. S. Morgenlie, Carbohydr. Res., 41, 285 (1975).
83. S. Morgenlie, Carbohydr. Res., 80, 215 (1980).
84. P. J. Wood, J. R. Siddiqui, and J. Weisz, Carbohydr., Res., 42, 1 (1975).
85. R. J. Stroz, Carbohydr. Res., 72, 207 (1979).
86. H. Frank, H. J. C. Das Neves, and E. Bayer, J. Chromatogr., 207, 213 (1981).
87. P. Decker and H. Schweer, J. Chromatogr., 243, 372 (1982).
88. J. N. C. Whyte, J. Chromatogr., 87, 163 (1973).
89. J. R. Etchison and J. J. Holland, Anal. Biochem., 66, 87 (1975).
90. D. G. Pritchard and C. W. Todd, J. Chromatogr., 133, 133 (1977).
91. B. Kozulic, B. Ries, and P. Mildner, Anal. Biochem., 94, 36 (1979).
92. S. J. Rickart, C. C. Sweeley, J. Chromatogr., 147, 317 (1977).
93. A. A. Akhrem, G. V. A. Avvakumov, O. V. Sviridov, and O. A. Strel'chyonok, J. Chromatogr., 166, 123 (1978).
94. T. P. Mawhinney, M. S. Feather, J. R. Martinez, and G. J. Barbero, Carbohydr. Res., 75, C21 (1979).
95. J. Mononen and J. Karkkainen, FEBS Lett., 59, 190 (1975).
96. T. P. Mawhinney, M. A. Madson, R. H. Rice, M. S. Featherm and G. J. Barbero, Carbohydr. Res., 104, 169 (1982).
97. J. Szafranek, C. D. Pfaffenberger, and E. C. Horning, J. Chromatogr., 88, 149 (1974).
98. G. Petersson, J. Chromatogr. Sci., 15, 245 (1977).
99. M. Wurst, V. Vancura, and L. Kalachova, J. Chromatogr., 91, 469 (1974).
100. S. Inoue, M. Miyawaki, Anal. Biochem., 65, 164 (1975).
101. L. Vadas, H. S. Prihar, B. K. Pugasetti, and D. S. Feingold, Anal. Biochem., 114, 294 (1981).
102. R. M. Thompson and N. Gerber, J. Chromatogr., 124, 321 (1976).
103. H. Ehrsson, T. Walle, and S. Wikström, J. Chromatogr., 101, 206 (1974).
104. D. Nurok and T. J. Reardon, Carbohydr. Res., 56, 165 (1977).
105. G. A. Janauer, and P. Englmaier, J. Chromatogr., 153, 539 (1978).
106. K. A. Killick, Carbohydr. Res., 82, 1 (1980).
107. D. Nurok, J. Chromatogr. Sci., 14, 305 ((1976).
108. P. L. Coduti and C. A. Buch, Anal. Biochem., 78, 21 (1977).
109. T. Pietrzykowski, H. Gruszecka, A. Borys, and S. Kubacki, Zes. Probl. Postepow Nauk Pol., 187, 145 (1977); Chem. Abstr., 87, 186359 (1977).

110. J. Copikova and F. Kvasnieka, Listy Cukrow, 95, No. 4, 93 (1979).
111. A. Ostaniewicz, K. Wesolowska, S. Kubacki, and A. Borys, Gaz. Cukrow, 85 (5), 110 (1977).
112. C. Womersley, Anal. Biochem., 112, 182 (1981).
113. M. Deki, and Y. Hakamada, Zanzei Chuo Bunsekishoho, 15, 115 (1975); Chem. Abstr., 87, 95036 (1975).
114. H. Schwind, F. Scharbet, R. Schmidt, and R. Kattermann, J. Clin. Chem. Biochem., 16, 145 (1978).
115. M. J. Prager, M. A. Miskiewicz, J. Assoc. Off. Anal. Chem., 69, 262 (1979).
116. A. M. Jamiesson, J. Am. Soc. Brew. Chem., 34, 44 (1976); Chem. Abstr., 84, 178149 (1976).
117. K. Kuercher, H. Hadorn, C. Strack. Mitt. Geb. Lebensmittelunters. Hyg. 66, 92 (1975); Chem. Abstr., 84, 313262 (1975).
118. S. Adam and W. G. Jennings, J. Chromatogr., 115, 218 (1975).
119. T. Toba and S. Adachi, J. Chromatogr., 135, 411 (1977).
120. S. E. Zurabyan, T. N. Mirzayanova, B. V. Rozynov, V. L. Sadovskaya, and A. Ya. Khorlin, Bioorg. Khim., 4, 1402 (1978).
121. J. Jones, in: Methods in Carbohydrate Chemistry, Academic Press, New York (1962ff) [Russian translation, Moscow (1975), p. 26].
122. B. Lindberg, Methods Enzymol., 28, 178 (1972).
123. K. Kato, K. Mutohi, T. Egashira, M. Kiura, and Y. Ueno, Agric. Biol. Chem., 42, 1073 (1978).
124. T. Mukumoto and H. Yamaguchi, Carbohydr. Res., 59, 614 (1977).
125. G. R. Woolard, E. B. Rathbone, and L. Novellie, Carbohydr. Res., 51, 239 (1976).
126. J.-P. Benedetto, M. Bruneteau, and G. Michel, Eur. J. Biochem., 63, 313 (1976).
127. A. K. Chakraborty, H. Friebolin, H. Niemann, and S. Stirm., Carbohydr. Res., 59, 525 (1977).
128. L. D. Kennedy and R. W. Bailey, Carbohydr. Res., 49, 451 (1976).
129. A. S. Rao and N. Roy, Carbohydr. Res., 74, 349 (1979).
130. J. F. Kennedy, J. S. Sandhy, and D. A. T. Southgate, Carbohydr. Res., 75, 265 (1979).
131. J. H. Pazur and L. S. Forsberg, Carbohydr. Res., 58, 222 (1977).
132. L. P. T. M. Zavenhuizen, Carbohydr. Res., 26, 409 (1973).
133. H. Rauvala, Carbohydr. Res., 26, 409 (1973).
134. A. A. Akhrem, G. V. Avvakumov, and O. A. Strelchonok, J. Chromotgr., 176, 207 (1979).
135. Y. M. Choy and G. G. S. Dutton, Can. J. Chem., 52, 684 (1974).
136. H. Parolis and D. McGarvie, Carbohydr. Res., 62, 363 (1978).
137. A. Amemura, M. Hisamatsum, S. K. Ghai, and T. Haraba, Carbohydr. Res., 91, 59 (1981).
138. P. N. Lipke, W. C. Raschke, and C. E. Ballou, Carbohydr. Res., 37, 23 (1974).
139. N. Shibuya, J. Chromatogr., 208, 96 (1981).
140. J. Radziejewska-Lebrecht, D. H. Shaw, D. Borowiak, I. Fromm, and H. Mayer, J. Chromatogr., 179, 113 (1979).
141. L.-E. Franzen and S. Svensson, Carbohydr. Res., 73, 309 (1979).
142. Y. V. Anjaneyalu and D. C. Gowda, Carbohydr. Res., 75, 251 (1979).
143. R. Geyer, H. Geyer, S. Kühnhardt, W. Mink, and S. Strim. Anal. Biochem., 121, 263 (1982).
144. D. P. Sweet, P. Albersheim, and R. H. Shapiro, Carbohydr. Res., 40, 199 (1975).
145. F. R. Seymour, R. D. Plattner, and M. E. Slodki, Carbohydr. Res., 44, 181 (1975).
146. F. R. Seymour, E. C. M. Chen, and S. H. Bishop, Carbohydr. Res., 68, 113 (1979).
147. F. R. Seymour, M. E. Slodki, R. D. Plattner, and A. Jeines, Carbohydr. Res., 53, 153 (1977).
148. D. Anderle and P. Kovac, Chem. Zvesti, 30, 355 (1976).
149. B. W. Li, T. W. Cochran, and J. R. Vercellotti, Carbohydr. Res., 59, 567 (1977).
150. F. Janecek, R. Toman, S. Karácsonyi, and D. Anderle, J. Chromatogr., 173, 408 (1979).
151. B. Fournet, J.-M. Dhalluin, Y. Leroy, J. Montreuil, and H. Mayer, J. Chromatogr., 153, 91 (1978).
152. S. Ito and Y. Fujino, Agric. Biol. Chem., 44, 1181 (1980).
153. J. Jarý and M. Marek, Collect. Czech. Chem. Commun., 46, 2410 (1981).
154. S. Pekkala, B. Holmbom, and E. Avela, Kemia-kemi, 1, 25 (1974).
155. T. Matsubura and A. Hayashi, Biomed. Mass Spectrom., 62 (1974).
156. Yu. N. El'kin, J. Chromatogr., 180, 163 (1979).
157. Yu. S. Ovodov and E. V. Evtushenko, Carbohydr. Res., 27, 169 (1973).

158. E. V. Evtushenko, and Yu. S. Ovodov, J. Chromatogr., 97, 99 (1974).
159. E. V. Evtushenko and Yu. S. Ovodov, Khim. Prir. Soedin., 677 (1975).
160. E. V. Evtushenko and Yu. S. Ovodov, Khim. Prir. Soedin., 753 (1977).
161. E. V. Evtushenko, N. M. Vakhrusheva, and Yu. S. Ovodov, Khim. Prir. Soedin., 142 (1979).
162. E. V. Evtushenko, N. M. Vakhrusheva, and Yu. S. Ovodov, J. Chromatogr., 196, 331 (1980).
163. B. Fournet, Y. Leroy, and J. Montreuil, J. Chromatogr., 92, 185 (1974).
164. E. V. Evtushenko and Yu. S. Ovodov, Khim. Prir. Soedin., 18 (1982); E. V. Evtushenko and Yu. S. Ovodov, Khim. Prir. Soedin., 21 (1982).
165. B. Fournet, G. Strecker, Y. Leroy, and J. Montreuil, Anal. Biochem., 116, 489 (1981).
166. S. K. Kundu, R. W. Ledeen, and P. A. J. Gorin, Carbohydr. Res., 39, 329 (1975).
167. S. K. Kundu, R. W. Ledeen, and P. A. J. Gorin, Carbohydr. Res., 39, 179 (1975).
168. T. Tai, K. Yamashita, and A. Kobata, J. Biochem. (Tokyo), 78, 679 (1975).
169. J. Finne and H. Rauvala, Carbohydr. Res., 58, 57 (1977).
170. G. O. H. Schwarzmann and R. W. Jeanloz, Carbohydr. Res., 34, 161 (1974).
171. K. Stellner, H. Saito, and S.-I. Hakomori, Arch. Biochem. Biophys., 155, 464 (1973).
172. A. A. Akhrem, G. V. Avvakumov, I. V. Sidorova, and O. A. Strel'chonok, Vestsi Akad. Navuk BSSR. Ser. Khim. Navuk, No. 4, 111 (1979).
173. A. K. Bhattacharjee and H. J. Jennings, Carbohydr. Res., 51, 253 (1976).
174. S. Inoue and G. Matsumura, Carbohydr. Res., 74, 361 (1979).
175. I. Mononen, Carbohydr. Res., 104, 1 (1982).
176. H. Rauvala and J. Kärkkainen, Carbohydr. Res., 56, 1 (1977).
177. C. Bruvier, Y. Leroy, J. Montreuil, B. Fournet, and J. P. Kamerling, J. Chromatogr., 210, 487 (1981).
178. H. van Halbeek, J. Haverkamp, J. P. Kamerling, J. F. G. Vliegenthart, C. Verslius, and R. Schauer, Carbohydr. Res., 60, 51 (1978).
179. J. Haverkamp, J. P. Kamerling, J. F. G. Vliegenthart, and R. W. Ven, and R. Schauer, FEBS Lett., 73, 215 (1977).
180. D. Anderle and P. Kováč, J. Chromatogr., 91, 463 (1974).
181. S. Honda, K. Takeda and K. Kakehi, Carbohydr. Res., 73, 135 (1979).
182. S. Honda, N. Hamajima, and K. Kakehi, Carbohydr. Res., 68, 77 (1979).
183. S. Honda, S. Okuyama, Y. Kishi, S. Suzuki, and K. Kakehi, Carbohydr. Res., 164, 9 (1979).
184. O. Larm, E. Scholander, and O. Theander, Carbohydr. Res., 49, 69 (1976).
185. P. Decker and H. Schweer, Carbohydr. Res., 49, 201 (1976).
186. E. Wiren, L. Ahrgren, and N. de Belder, Carbohydr. Res., 49, 201 (1976).
187. P.-E. Jansson, L. Kenne, and S. Svensson, Acta Chem. Scand., B, 30, 61 (1976).
188. S. Morgenlie, Carbohydr. Res., 73, 315 (1979).
189. G. J. Gerwig, J. R. Kamerling, and J. F. G. Vleigenthart, Carbohydr. Res., 62, 349 (1978).
190. G. J. Gerwig, J. P. Kamerling, and J. F. G. Vliegenthart, Carbohydr. Res., 77, 1 (1979).
191. K. Leontein, B. Lindberg, and J. Lönngren, Carbohydr. Res., 62, 359 (1978).
192. A. L. Leavitt, and W. R. Sherman, Carbohydr. Res., 103, 203 (1982).
193. W. Zablocki, E. J. Behrman, and G. A. Barber, J. Biochem. Biophys. Methods, 1, 253 (1979).
194. M. R. Little, Carbohydr. Res., 105, 1 (1982).
195. L. B. Schroeder, K. M. Counts, and F. C. Haigh, Carbohydr. Res., 37, 368 (1974).
196. G. Wulff, U. Schroeder, and J. Wichelhaus, Carbohydr. Res., 72, 280 (1979).
197. E. V. Evtushenko and Yu. S. Ovodov, Khim. Prir. Soedin., 87 (1976).
198. J. Gelas and D. Horton, Carbohydr. Res., 45, 181 (1975).
199. J. Gelas and D. Horton, Carbohydr. Res., 67, 371 (1978).
200. J. Szafranek, A. Wisniewski, J. Gajdus, and J. Kusmiers, J. Chromatogr., 168, 445 (1979).
201. J. W. Mourits, H. G. Merkus, and L. de Gatan, Anal. Chem., 48, 1557 (1976).
202. A. Lipták, P. Fügedi, and P. Nánaśi, Carbohydr. Res., 65, 209 (1978).
203. J. M. Küster, H. Luftmann, and I. Dyong, Chem. Ber., 109, 2223 (1976).
204. J. Harangi and A. Liptak, J. Chromatogr., 147, 401 (1978).
205. K. Olsson, O. Theander, and P. Aman, Carbohydr. Res., 58, 1 (1977).
206. J. Eagles, G. R. Fenwick, and R. K. Heaney, Biomed. Mass Spectrom., 8, 278 (1981).
207. H. B. Boren, P. J. Garegg, L. Kenne, A. Pilotti, A. Svensson, and C.-G. Swahn, Acta Chem. Scand., 27, 3557 (1973).
208. K. Yoshimoto, K. Tahara, S. Suzuki, K. Sasaki, Y. Nishikawa, and Y. Tsuda, Chem. Pharm. Bull., 27, 2661 (1979).

209. J. P. Williams, G. R. Watson, M. Khan, S. Leung, A. Kuksas, O. Stachnyk, and J. J. Myher, Anal. Biochem., 66, 110 (1975).
210. H. Tokunaga, T. Kimura, and J. Kawamura, Yakugagku Zasshi, 99, 800 (1979); Chem. Abstr., 91, 206577 (1979).
211. V. N. Reinhold, F. Wirtz-Peitz, and K. Biemann, Carbohydr. Res., 37, 203 (1974).
212. H. Tsuchida, K. Kitamura, H. Komoto, and N. Akimori, Carbohydr. Res., 67, 549 (1978).
213. A. Soler and R. P. A. Garcia, Ann. Univ. Murcia Cien., 32, 21 (1975) (Publ. 1978); Chem. Abstr., 90, 168843 (1979).
214. N. I. Kaletina, S. A. Pronkina, A. A. Bezzubov, and B. N. Stepanenko, Zh. Obshch. Khim., 50, 1195 (1980).
215. F. Shafizadeh, R. H. Furneaux, T. T. Stevenson, and T. G. Cochran, Carbohydr. Res., 67, 433 (1978).
216. C. von Sonntag and M. Dizdaroglu, Carbohydr. Res., 58, 21 (1977).
217. S. Kawakishi, Y. Kito, and M. Namiki, Carbohydr. Res., 39, 263 (1975).
218. J. M. Morrison, J. Chromatogr., 108, 361 (1975).
219. L. P. Kosheleva, G. Y. Il'chenko, and L. I. Glebko, Anal. Biochem., 73, 115 (1976).
220. O. D. Livshits, M. I. Mazela, L. G. Sibiryakova, in: Chromatographic Analysis in Wood Chemistry [in Russian], Riga (1975), p. 39.
221. G. F. Aleksandrova and Yu. N. Khol'kin, in: All-Union Scientific Conference on the Chemistry and Technology of Furan Compounds. Abstracts of Lectures [in Russian], (Ya. P. Stradin', ed., Riga (1979), p. 75.
222. B. Fournet and J. Mountreuil, J. Chromatogr., 75, 29 (1973).

MODIFICATION OF THE TITRIMETRIC METHOD OF ANALYZING PECTIN SUBSTANCES

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A potentiometric variant of the titrimetric method of analyzing pectin substances has been developed which is distinguished by adequate accuracy and good reproducibility. The modified method has been tested on methyl (methyl 2,3-di-O-methyl-α,β-galactopyranosid)uronate and has been used for determining the free (K_f) and esterified (K_e) carboxy groups of the pectin substances of mint. The methods of isolating, purifying, and fractionating the pectin substances and determining their K_f and K_e values are given. Formulas are presented for calculating the percentage values of K_f and K_e.

The development of methods for the complex utilization of the wastes of a number of processing sectors of the national economy is widely based on biotechnological processes at the present time [1-3]. Acidic polysaccharides that are valuable for the food industry and for medicine can be obtained both from wastes of a food nature (apple pomace, sugar-beet pulp, citrus rind) and also from nonfood wastes [4-6].

To evaluate the practical suitability of the acidic polysaccharides obtained, rapid methods of determining the quality and, in particular, the amount of free carboxy groups, and also the degree of their esterification, are important. The methods described in the literature [7-10], and also the widely used titrimetric method [11], have a number of disadvantages and require certain corrections. The disadvantages include, in the first place, the large size of the sample of substance to be determined (of the order of 1 g) and, in the second place, the fact that in the determination of the amount of esterified carboxy groups the excess of HCl (0.5 N) is back-titrated with dilute NaOH solution (0.1 N) in the presence of Hinton's mixed indicator, the color of which changes slowly; this interferes with the strict fixation of the equivalence point.

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