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CHROMATOGRAPHY OF MONOSACCHARIDES AND DISACCHARIDES

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CONTENTS

1 INTRODUCTION

The determination of mono- and disaccharides has been achieved by indirect physical (refractometry, polarimetry, hydrometry) or by semi-empirical chemical methods (volumetric analysis, gravimetry, colorimetry). These chemical methods, which are non-specific in that they detect a class of sugar such as pentoses or reducing sugars rather than individual sugars, are based on either colour reactions effected by the condensation of degradation products of sugars in strong mineral acids with various organic compounds (Bial and Molisch test)[1]; the reducing properties of the carbonyl group (Fehling's test)[1]; or on oxidative cleavage of neighbouring hydroxyl groups[1,2]. However, the determination of individual carbohydrates is of considerable importance and the specificity of such tests has often been enhanced by using fractions separated by paper (PC), thin-layer (TLC) or column chromatography. Today, enzymatic methods are available[3–6] but these are limited to a few sugars. Moreover, because of their high specificity, such methods do not allow the simultaneous determination of several sugars. On specificity requirements and the need for multiple determinations, chromatographic methods offer distinct advantages.

Several reviews[7–15,359–361] attest to the importance of chromatography in carbohydrate analyses. With this consideration in mind, the analytical applications of chromatography to monosaccharides and disaccharides are reviewed. Because of their relevance in gas chromatographic methods, selected derivatives are included. Emphasis is placed on food and clinical applications in the period from 1980 to June 1985. However, earlier work of particular significance is also covered.

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2 PAPER AND THIN-LAYER CHROMATOGRAPHY

PC and TLC have been used extensively in the analysis for sugars. The reasons for this include low cost, simplicity and the ability to simultaneously isolate and identify the sugars. PC was first applied to carbohydrates[16,17] in 1947 and, although partition is the predominant separation mechanism, adsorption phenomena[18] also occur. On paper-partition chromatograms, where compounds partition between aqueous phases held stationary on the paper and mobile organic phases, free sugars migrate as single substances[19–21] indicating that continuous equilibration occurs to prevent the resolution of the anomers present in solution. The relations between mobility of the sugars and their structures were studied primarily by Isherwood and Jermyn[22] and by Levy[23]. Thus, the influence of furan–pyran ring formation and the nature of the ring substituent at C-4 or C-5 (ref 20), *cis–trans* isomerisation[22] at C-2 or C-3, and the type of linkage (-1,4- or -1,6-) in disaccharides[24] have been examined.

For reproducible results in PC the solution to be chromatographed must, as much as possible, be free of non-carbohydrate materials. This means that animal or vegetable extracts should be subjected to preliminary purification[25] to remove impurities such as salts and proteins. Moreover, excessive alkalinity, which may cause epimerization, must be avoided during the preliminary manipulations. Originally, the organic phase of biphasic solvent mixtures[16] such as water-saturated *n*-butanol was proposed for use as mobile phase. However, the temperature dependence of the composition of such systems may result in phase separation during use and, as a result, monophasic solvent systems[26–28] came into use. The latter consist of water, a water-miscible and a water-immiscible organic solvent In such systems the mobility of the sugars, which increases[22] with the water content, is generally the same[29]; namely, pentoses, hexoses, disaccharides and trisaccharides nearest the origin.

Although the PC of carbohydrates is actively pursued[30–34], it has been superceded by TLC for carbohydrate analysis. This view is substantiated by an examination of *Chemical Abstracts* (covering January 1980 to June 1985) where the number of TLC-based methods for carbohydrates outweighs the PC-based methods in the ratio of 7·1. However, such an analysis makes no allowance for the total number of routine analyses performed by PC. This decline in use of PC can be traced to the long development times[35] frequently involved Indeed, development times of up to 16 h are not uncommon.

Although partition TLC of simple sugars on microcrystalline cellulose has had limited use[36–39], it offers the resolution of PC with the advantage that the solvent systems and spray reagents most suitable for PC are directly applicable[36] to cellulose TLC. Carbohydrates, being strongly hydrophilic, require very polar solvents which have relatively slow migration rates[40]. Despite this the separations are considerably faster than those attained by PC[41] although slower than can be achieved on inorganic adsorbents. Moreover, TLC is more sensitive[41] than PC for the determination of sugars. Frequently, TLC separations depend upon preferential adsorption phenomena, and occasionally resolutions must incorporate both partition and adsorption factors as, for example, in separations on silica gel G using butanone–acetic acid–water as eluting solvent[20]. Several inorganic adsorbents have been used for the TLC of sugars including magnesium silicate[42], alumina[43], Kieselguhr[44,45], silica gel[46–55], or mixtures of the last two[56,57], and aminopropyl-bonded silica[58,59]. Caution in the

interpretation of chromatograms is required. For example, aminated sugars are formed[60] when sugars are chromatographed on silica gel G with ammoniacal solvents resulting in hexoses, pentoses and disaccharides being split into at least two different compounds. The silica gel apparently exerts a catalytic effect as the formation of amino sugars is not observed on other adsorbents. The mobility of the sugars on silica gel depends primarily on the molecular weight and the number of hydroxyl groups[49,61] and consequently the diastereoisomers are poorly resolved. Resolution is improved by impregnating silica gel and Kieselguhr with salts of weak acids[44,46,62,63,359].

The effects on chromatographic behaviour of the type and concentration of impregnating salt, which react with the carbohydrates by reasonably well known mechanisms, have been systematically investigated[362] using several solvents. Phosphates were identified as suitable impregnants. Although borates were not examined, excellent separations on thin layers of silica gel impregnated with borate have been reported subsequently[363]. Bisulphite, known for its characteristic addition reactions with aldoses and ketoses, also gave[66] excellent separations of certain sugars. Several other salts including molybdate and tungstate have been investigated[364].

A significant feature of inorganic layers is that more corrosive spray reagents can be employed[64,65] for detection. Thus, sulphuric acid alone or admixed with nitric acid[64] or permanganate[65] has proved suitable for detecting sugars at the microgram level. Various other reagents which have been proposed include naphthol–resorcinol–sulphuric acid[47], aniline–diphenylamine[63] and admixtures of sulphuric acid with anisaldehyde[44], naphthol[62], thymol[66], carbazole[66] and phenol[66]. Reagents suitable for PC are also applicable to TLC and of these iodine vapour warrants mention for despite being less sensitive than sulphuric acid it is non-destructive in the short exposure time required. The adsorbed iodine evaporates when the plate is exposed to the air.

Although PC and TLC are now generally regarded as inferior methods for carbohydrates, it is interesting to note that high-performance TLC has recently been applied[45,58,67] to the separation of sugars.

3 GAS CHROMATOGRAPHY

The application of gas chromatography (GC) to carbohydrates was slower than with other classes of compounds. The major problem was the lack of volatility of the polar compounds and the fact that volatile derivatives could not readily be prepared in quantitative yields. As an alternative to derivative formation, Greenwood *et al.*[68] in 1961 investigated pyrolysis–GC of several sugars. However, this approach does not appear to have been followed by other workers, possibly because of the simultaneous development of suitable derivatising reagents in the early 1960's.

3.1. Derivatives

The first derivatives to be chromatographed[69] were those already used for the chemical analysis of sugars. Thus, the fully methylated methyl glycopyranosides of simple pentoses and hexoses were separated[70] on a column of methylated hydroxyethylcellulose. The anomeric forms of a single monosaccharide were also resolved[70] using this technique and methylated sucrose was successfully chromatographed.

Bishop[71] demonstrated in 1962 that mixtures of methylated disaccharides could be resolved and an extensive literature[9] now covers the methyl derivatives The extent of methylation and the application of the method to different carbohydrates have been examined by several workers[72–75].

The separation of carbohydrates as acetyl derivatives was first described by Gunner *et al.*[76] who found that anomeric glycose acetates gave separate peaks, and derivatives of epimers had different retention times. The technique was further developed by Sawardeker *et al.*[77] and extended to the separation of disaccharides by using lower loadings of more thermostable silicone liquid phases[78,79]. In this procedure the carbonyl group of monosaccharides is reduced with sodium borohydride to the corresponding sugar alcohol which is then acetylated, eliminating the formation of multiple derivatives[77] from a single sugar. Although the preliminary reduction and acetylation steps proceed quantitatively potential difficulties include errors in quantification due to naturally occurring polyols, the possibility of forming a single sugar alcohol from different monosaccharides (*e.g.*, glucose and sorbose), interference by the borate (formed in the reduction step) in the acetylation and tailing peaks caused by the pyridine solvent. Despite this the method has been used widely[80–82].

The occurrence of more than one peak per sugar presents a significant complication in the analysis of carbohydrates. Solutions of carbohydrates undergo mutarotation and an initially pure sugar may result in an equilibrium mixture of the linear form and the α- and β-anomer of both the pyranose and furanose ring forms. Thus, derivatisation of solid α-D-glucose gave[83] a single major peak in the chromatogram Similarly, β-D-glucose showed a single peak. On the other hand, derivatisation of the residue obtained from evaporation of an aqueous equilibrium solution of glucose, showed two peaks on being chromatographed, corresponding to the peaks obtained with the two separate α- and β-anomers. These observations have been extended[83] to many sugars other than glucose. Derivatisation is generally a faster reaction than mutarotation[84] and, hence, compositional changes during derivatisation are assumed minimal. To reduce further any mutarotation of sugars, dimethyl sulphoxide (DMSO) may be used as derivatising solvent whence, for example, the α- to β-D-glucose anomerization rate[85] is essentially zero and remains low in aqueous DMSO. With respect to water, the α-D-glucose anomerization in 50% aqueous pyridine is about 48 times faster than in either 50% aqueous DMSO or 50% aqueous dioxane. Catalysts (for example, 0 2% lithium perchlorate) have been used[84] to effect mutarotation equilibrium prior to derivatisation. In the case of glucose, an aqueous equilibrium mixture contains[83] about 36% α-D-glucopyranose, 64% β-D-glucopyranose and less than 1% of the linear aldose or of either possible furanose form In contrast, Acree *et al.*[86] have determined the composition of an aqueous equilibrium solution of galactose to be 1 0% α-galactofuranose, 3.1% β-galactofuranose, 32.0% α-galactopyranose and 63.9% β-galactopyranose. The importance of solvent is seen by comparing the corresponding compositional data[87], namely, 13.7%, 23.4%, 31.7% and 31.2%, respectively, for pyridine solutions of galactose. Additional factors affecting mutarotation equilibria in aqueous systems include[88] pH and metal ion concentrations.

An alternative approach[89] to acylation is the preliminary conversion of aldoses to the corresponding methyloxime, followed by acetylation of free hydroxyl groups. Since the oximation involves reaction at the C-1 position, the incidence of multiple

derivatives from a single sugar is reduced. Although single peaks are obtained for glucose, fructose, mannose and xylose, some carbohydrates give two peaks[89], presumably due to formation of *syn-* and *anti*-isomers. A similar procedure has been used[81] for disaccharide estimation, one peak being obtained for maltose, but two resulting from lactose.

Bourne *et al.*[90] originally described the derivatisation of sugars with trifluoroacetic anhydride to form the corresponding esters. Several different trifluoroacetylation procedures have been described[91–95] The advantages of trifluoroacetylation are enhanced volatility[91] and sensitivity. Thus, Tamura and Imanari[93] prepared the trifluoroacetate derivatives of glucose, galactose and mannose and exploited the sensitivity obtained with electron-capture detection for their quantification Derivatisation with N-methyl-bis(trifluoroacetamide) is claimed[96] to be more reproducible.

By far the most popular of the volatile compounds of sugars used for GC purposes are the trimethylsilyl (TMS) derivatives. Sweeley *et al.*[97] were the first to produce a viable method for carbohydrates based on the formation of these derivatives. Their study included pentoses and hexoses as well as monosaccharides through to tetrasaccharides. A mixture of hexamethyldisilazane (HMDS), trimethylchlorosilane (TMCS) and anhydrous pyridine was added to dried sugars, derivatisation occurring rapidly at room temperature with virtually quantitative yields. Since the extract was injected directly, ammonium chloride (formed as a by-product in the reaction) contamination of the column occurred. Various modifications of the procedure have been described[98,99] including hexane extraction[100] of the derivatised sugars prior to injection. Although ammonium chloride may be removed by centrifugation, solvent extraction[100,101] has the advantage of simultaneously eliminating pyridine which produces a severely tailing peak on some stationary phases.

It was originally believed that the procedure of Sweeley *et al*[97] required rigorous drying for silylation. This is not the case for, although drying prevents anomerisation, water does not prevent silylation as it immediately silylates to hexamethyldisiloxane. Thus, its presence merely requires more silylating reagent and this fact has been used[102] to perform the reaction directly in aqueous solution Brobst and Lott[103] substituted trifluoroacetic acid for TMCS and used a sequential addition of reagents to the sugar. However, the silylation of glucose was claimed[104] to be incomplete by this method.

More powerful silyl donors than HMDS have been proposed For example, N-trimethylsilylimidazole[105–108] and N,O-bis(trimethylsilyl)acetamide (BSA)[109–111], with TMCS as catalyst, have been used to derivatise sugars in a range of biological fluids. However, BSA in pyridine causes the anomerisation of hexoses, and the chromatogram of a single sugar may contain[112] four or five peaks. Other reagents in this category include N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and N-methyl-N-trimethylsilyltrifluoroacetamide[113,114]. Although BSTFA should be a self-catalyzing silyl donor, it has been found necessary[84,111,113] to add TMCS as an additional catalyst for rapid quantitative derivatisation.

Pyridine has been used as solvent in the majority of silylation studies although other solvents, such as DMSO and dimethylformamide[115–118] and including a large excess of silylating reagent itself[15], have also been used. The advantages claimed for these solvents compared to pyridine are lower anomerization rates[85], improved chromatographic behaviour[112] and less noxious odours

TABLE 1

STATIONARY PHASES AND PACKED COLUMNS USED FOR GC OF SUGARS

Derivative	*Column**	*Column packing*	*Ref.*
TMS	12 ft	5% SE-30 on Gas Chrom P	114
	2–6 ft. × 6 mm O D	2–3% SE-30 on silanised Chromosorb W	103
	1.5 m × 3 mm	3% OV-1 on Gas Chrom Q	111
	3 m × 3 mm	13% OV-1 on Gas Chrom Q	137
	6 ft × 3 mm	2% OV-1 on silanized Gas Chrom S AW	116
	2 7 m × 3 mm	10% OV-17 on Gas Chrom Q	109–11
	2–6 ft. × 6 mm O D	2–3% SE-52 on silanised Chromosorb W	103
	6 ft. × 6 mm O.D	3% SE-52 on silanised Chromosorb W AW	97
	2 ft × 6 mm O.D	0 25% SE-52 on glass beads	138
	2 7 m × 3 mm	3% QF-1 on Gas Chrom Q	111
	6 ft × 3 mm	3% SP-2250 on Supelcoport	132
	2 7 m × 3 mm	3% XE-60 (cyanoethyl methyl silicone) on Gas Chrom Q	111
	8 ft × 6 mm O D.	15% EGS on Chromosorb W AW	97
	6 ft × 6 mm	15% EGS on Chromosorb W	98
	6 ft × 2 mm	3% Dexsil 300GC on Chromosorb W AW DMCS	136
Oxime, TMS	15 ft × 3 mm O D	1% SE-30 on Gas Chrom Q	124
	0 5 and 2 0 m × 3 mm	3% SE-30 on Chromosorb W AW DMCS	134
	7 ft. × 6 mm O D	3% SE-30 on Chromosorb W	139
	6 ft × 6 mm O D	3% OV-17 on Chromosorb W AW DMCS	125
	0 5 and 2 0 m × 3 mm	3% SP-2250 on Supelcoport	134
TMS, acetyl	No details	3% SE-52	140
Alditol, TMS	2 ft. × 6 mm O D	3% SE-52 on Diataport S	133
Alditol acetate	8 ft × 6 mm O D	5% XE-60 on Chromosorb W	77
	4 ft × 6 mm O D	10% Carbowax 20M on Chromosorb W	77
	10 ft × 6 mm O D.	3% ECNSS-M (ethylene succinate silicone copolymer) on Chromosorb W	77
	2 m × 3 mm	5% OV-275 on Chromosorb W	122
	2 m × 2 mm	2% Silar-7CP on Chromosorb W AW	141
Alditol trifluoroacetate	1 5 m × 4 mm	2% XF-1105 (cyanoethyl methyl silicone) on Gas Chrom P	142
Trifluoroacetate	6 ft × 4 mm	20% SE-30 on Chromosorb W AW	95
Methyl	1.8 m × 3 mm	1% SE-30 on Gas Chrom Q	75
	1 8 and 3 6 m × 3 mm	1% OV-17 on Gas Chrom Q	75
Oxime, acetyl	6 ft × 2 mm	5% QF-1 on Supelcoport	143
Methoxime, acetyl	9 ft × 4 mm	3% ECNSS-M on Celite	89
Underivatised glucose	2 m × 3 mm	Chromosorb 101 (porous polymer)	135

* Length × internal diameter, except when indicated otherwise

Additional derivatisation procedures have been combined with trimethylsilylation to decrease the incidence of multiple derivatives. Thus, reduction of monosaccharides to their corresponding alditols followed by the formation of (acetate or) TMS ether derivatives avoids the problem of peak multiplicity[119–123] by removing the carbonyl group normally involved in ring formation. However, the method which involves significant chemical manipulation of sugars, may lead to information loss[124] because certain sugars yield the same alcohol. Moreover, ketosugars yield two epimeric alcohols. For example, fructose and glucose yield sorbitol as their reduction product and fructose, in addition, yields mannitol. The alditol-TMS derivatives are claimed[9] to be less satisfactorily resolved than the corresponding acetyl esters and this evidently accounts for the low incidence of their use. Combined oximation–acetylation[89] and oximation–trimethylsilylation[97,124] also reduces the problem of peak multiplicity (two possible derivatives per sugar, with only one found for most sugars[125]) but some problems have been reported[124] due to poor sample stability of the sugar oximes.

Dimethylsilyl (DMS) and halomethyldimethylsilyl derivatives are prepared using similar conditions as those for TMS ethers. As expected the retention times of the halogen compounds are considerably longer than those of TMS ethers and the application of the halogen derivatives is limited[126]. DMS derivatives can be employed to advantage with high-molecular-weight sugars since the DMS ethers have retention times that are half of those of TMS derivatives. Various other derivatives have been studied[127–130] but offer little by way of improved ease of derivatisation, resolution and/or reduction in the number of derivatives.

In summary, the major difficulty in the GC of carbohydrate derivatives relates

TABLE 2

OPEN TUBULAR COLUMNS USED FOR SUGAR ANALYSIS

SCOT = Support-coated open tubular column, WCOT = wall-coated open tubular column

Derivative	*Column*	*Stationary phase*	*Ref*
TMS	30 m × 0 28 mm glass WCOT	SE-52	108
	25 m glass WCOT	SE-54	146
	Short × 0 32 mm glass WCOT	OV-1, SE-30	147
	30 m × 0 25 mm fused-silica WCOT	SE-30	84
Oxime, TMS	15 m × 0 25 mm glass WCOT	SP-2250	145
Alditol, TMS	25 and 50 m × 0 23 mm fused-silica WCOT	OV-101	148
Alditol acetate	25 m × 0 25 mm glass WCOT	OV-275	149
	28 5 m × 0 5 mm glass SCOT	Silar 10C	150
	28 m × 0 5 mm glass SCOT	Silar 10C	151
	6 m × 0 2 mm fused-silica WCOT	BP-75	151
	25 m × 0 2 mm fused-silica WCOT	SE-52	152
	20–25 m × 0 3 mm glass WCOT	Chiral phase	153
Methoxime, TMS	25 m × 0.28 mm glass WCOT	SE-30	129
Oxime, acetyl	60 m × 0 3 mm glass WCOT	SE-30	154–156
N-Ethoxycarbonyl-O-TMS	25 m × 0 28 mm WCOT	OV-101, Chirasil-Val	157

to the production of several products from a single glycose owing to either faulty derivatisation or the formation of anomeric derivatives of possible furanose and pyranose ring forms[131]. With respect to faulty derivatisation, incomplete silylation[112], for instance, may produce several peaks per component[104]. Complications may also arise from the order of addition of reagents and the heating (often necessary for dissolution of the sugars) of the derivatisation mixture[83,97]. Thus, heating and the sequential addition of reagents apparently increase the number of derivatives.

3 2. Columns and packing materials

Columns, constructed of copper[103] and stainless steel[103,124,132–134] have been used successfully for the GC of sugar derivatives. However, as in other applications of GC, the trend has been to the increasing use of more inert glass columns[122,125,135,136]. Several stationary phases are suitable for chromatographing sugar derivatives and, in general, the non-polar TMS derivatives have been chromatographed[9] on non-polar phases while the more polar acetyl esters have been better resolved on more polar phases (Table 1). However, many cases are observed in which incomplete resolution results on non-polar, non-selective phases such as SE-30 and SE-52, while analyses on polar columns, such as polyethylene glycol, polyester and nitrile silicone, are often more selective. On the other hand, polar columns are less useful for chromatographing mixtures containing substances with a wide range of boiling points.

The derivatisation procedure can restrict the choice of a stationary phase. For example, in the typical silylation procedure[84,136] where the reaction mixture is injected directly, the presence of excess unreacted reagent precludes the use of stationary phases with reactive hydrogens. Although loading of the stationary phase on the inert support has varied[9] from 0.25 to 25% (Table 1) low percentages have been more common. Of the various materials used as the inert support, Chromosorb W and Gas Chrom Q have found widest success (Table 1).

Capillary GC is a powerful technique for the analysis of complex samples such as carbohydrate derivatives. Stainless-steel capillaries coated with OV-17 have been utilized[144] for an extensive study of carbohydrate changes during sugar boiling. However, the reduced activity of glass[145] and, more recently, fused-silica capillaries has resulted in the increased use of such columns (Table 2). An added advantage of capillary systems is the ability to employ cold on-column injection[147] thereby reducing the incidence of sample decomposition in the heated injection port. The possibility of catalytic and adsorptive phenomena occurring in the injection port and column has not been studied in relation to carbohydrates

4 COLUMN CHROMATOGRAPHY

Column chromatography of carbohydrates dates back to 1939, when Reich[158] described the separation of azoyl derivatives of sugars. Alumina has found little application in the carbohydrate field since the sugars are too polar and too strongly adsorbed. Moreover, their hydrophilic character makes it impossible to use non-polar solvents, and this limits the choice of suitable solvents. Finally, the basic character of alumina involves the danger of epimerization. Cellulose partition columns[159,160] and ion-exchange columns[161,162] have found limited use. In contrast, low-resolution

charcoal columns dominated[163–165] the column separations of carbohydrates until 1970. Although the development of strong cation exchange columns[166] was a considerable advance in column technology, it is undoubtedly the development of polar bonded-phase materials prepared from silica (5 and 10 μm) which has led to the full advantages of high-performance liquid chromatography (HPLC) becoming applicable to carbohydrate analyses. Indeed, HPLC is now claimed[167] to be superior to GC as a technique for carbohydrate analysis. Thus HPLC often offers direct injection of sample with little or no pretreatment and sugars are not subjected to high temperatures. The interpretation of many HPLC chromatograms is simple because anomeric forms of sugars are normally not resolved[15]. On the other hand, GC detectors are generally the more sensitive and in those cases where only a limited amount of sample is available, GC can be readily employed

Columns and conditions used for the HPLC of carbohydrates are summarised in Table 3. Anion exchange of carbohydrate-borate complexes was first applied[168] in 1952 but was a tedious, time-consuming procedure. By contrast, a current technique[169] can determine all nine naturally occurring aldoses in 65 min. Various other modes of separation have been applied to HPLC of simple sugars and, of these, reversed-phase partition is the most important. Although both alkylated, cyano- and amino-bonded phases have been utilized for carbohydrates, the latter have been most extensively studied. The first evaluation of the preparation and properties of an amino-bonded stationary phase was reported[195] in 1976. Such columns had limited lifetimes because of the hydrolysis of the bonded phase and the reactivity of the amino function. The resulting deterioration required a mobile phase of gradually decreasing water content[196] so that retention times and resolution could be approximately maintained. Moreover, chemically bonded particles are readily aggregated by contact with hydrophilic substances, and the column packing is altered Therefore, guard columns are usually used to extend column life Despite these limitations commercial packings exhibit reasonable stability and provide excellent separations of sugars. *In situ* preparation of amino columns[197] ensures stability of column conditions by constantly regenerating the surface of the stationary phase.

Carbohydrates have been successfully chromatographed[198] on Partisil-10 PAC, a bonded phase containing both cyano and amino groups. The effect of the addition of acids and salts to the aqueous acetonitrile mobile phase has also been examined.

Separations on amino-bonded phase columns have been considered normal-phase partition by others[199] because increasing water content in the mobile phase speeds up the elution. However, it is not clear whether the retention is caused by a competitive interaction of the water and carbohydrate or by adsorption via hydrogen bonding between hydroxyl groups of the carbohydrate molecule and the amino groups of the stationary phase[179]. Indeed, some prefer to call this use reversed-phase adsorption while others feel that there are at least three mechanisms occurring simultaneously: adsorption, partition and surface tension[200].

Ion-moderated partitioning is increasingly being used for the separation of carbohydrates[187]. Porous-polymer-based ion exchangers are used as the stationary phase together with aqueous mobile phase. Both anion and cation exchangers are used, but the latter more frequently. The elution profile is determined by the counterion with separation proceeding in the partition mode[201,202]. However, the change

TABLE 3

HPLC SYSTEMS USED FOR SUGAR ANALYSIS

Mode	*Column*	*Mobile phase*	*Detector*	*Ref.*
Anion exchange: borate complex	15 cm × 4.0 mm, Hitachi 2633	0.50 *M* Borate buffer	Fluorimeter, post-column derivatisation to 2-cyanoacetamide	169
	25 cm × 4.0 mm, Aminex A-25	Borate buffer	UV, 199 nm	170
	12 cm × 8.0 mm, Jeolco LC-R-3	Borate buffer	Visible, 425 nm; post-column derivatisation with orcinol–sulphuric acid	171
Reversed-phase partition	30 cm × 4.0 mm, Bondapak Carbohydrate	Acetonitrile–water (83:17) and (85:15)	Refractometer	172,173
	μBondapak NH_2	Acetonitrile–water (80:20)	Visible, post-column derivatisation with tetrazolium blue	174
	25 cm × 4.6 mm, Amino	70–80% Acetonitrile in water; ethyl acetate–ethanol–water (40:50:10)	Refractometer; UV, 188 nm	175
	25 cm × 4.6 mm, Resolution NH_2 5	Acetonitrile–0.01 *M* KH_2PO_4, pH 7	Refractometer	176
	15 cm × 4.6 mm, Micropak NH_2 (phosphate form)	Acetonitrile–water (70:30)	Refractometer; fluorimeter, post-column derivatisation to 2-cyanoacetamide	177
	15 cm × 4.6 mm, Lichrosorb NH_2 and Nucleosil 5 NH_2	Acetone–water–acetic acid (100:15:1)	Refractometer	178
	25 cm × 4.6 mm, Zorbax NH_2 and Supelcosil LC-NH_2	72–80% Acetonitrile in water	Refractometer	179
	15 cm × 4.6 mm, Resolution ODS 5	Water	Refractometer	176
	25 cm × 4.9 mm, ODS Hypersil	Acetonitrile–water (22:78)	Fluorimeter, pre-column derivatisation to dansyl derivative	180

Ion-moderated partition	100 cm × 4 mm, Aminex A6 (Li^+)	70–85% Ethanol in water	Moving wire	181
	15 and 25 cm × 6.4 mm, Resolution Carbohydrate (Na^+ and Ca^{2+})	Water	Refractometer	176
	30 cm × 7.0 mm, Aminex HPX-85C	Water	Refractometer, fluorimeter	177
	30 cm × 7.8 mm, Aminex HPX-87H	0.01 *N* Sulphuric acid	Refractometer	182
	25 cm × 6.0 mm polystyrene and silica-based cation exchangers (Ca^{2+})	Water	UV, 276 nm, post-column derivatisation to 2-cyanoacetamide	183
	Sugar-Pak 1 (Ca^{2+})	Water	UV, 190 nm	184
	15 cm × 6.0 mm, Shodex RS Pak DC-613 (Na^+ and Ca^{2+})	70–90% Acetonitrile in water	UV, 280 nm, post-column derivatisation to 2-cyanoacetamide	185
	30 cm × 7.8 mm, Aminex HPX-87	Water containing 0.02 g/l calcium propionate	Refractometer	174
	30 cm × 7.8 mm, Aminex HPX-87H and HPX-42A	Water, 0.01 *N* sulphuric acid	Refractometer	186
	20 cm × 9.0 mm, Aminex A7	0.06 *M* Trifluoroacetic acid	Refractometer	187
	25 cm × 4.0 mm, Shodex RS Pak DC-613	Acetonitrile–water (90 10)	Electrochemical	188
Adsorption–normal-phase partition	25 cm × 4.6 mm, Partisil 5	Ethyl acetate hexane (1 49) to (1.199)	UV-260 nm, pre-column derivatisation to dimethylphenylsilyl	189,190
	25 cm × 4.6 mm, Zorbax SIL	Hexane–chloroform–acetonitrile (10·3 1 9) with 0.1% water	UV, 260 nm, pre-column derivatisation to nitrobenzoates	191
	60 cm × 9.0 mm, Porasil A	Methyl ethyl ketone–water–acetone (85 10 5)	Refractometer	192
	25 cm × 2.1 mm, LiChrosorb Si 60	Water–acetonitrile (0 1 99 9)	Refractometer	193
	100 cm × 4.0 mm, Pellosil HC	Dichloromethane	UV, 254 nm, benzoates	194

TABLE 4

TECHNIQUES USED FOR THE DETERMINATION OF MONO- AND DISACCHARIDES

Analyte	*Sample*	*Method*	*Ref*
Sucrose and other sugars	Syrups	TLC on silica gel	220
Glucose, rhamnose, xylose, galactofuranose	Microorganism	GC–MS of TMS ethers	221
Simple sugars	Cereals	HPLC	222
Mono- and disaccharides	Urine	PC after desalting	30
Pentoses, hexoses	Not applied	GC on packed columns	223
Aldoses	Biological	GC of TMS diethyldithioacetals on capillary columns	224
Sugars	Wheat bran hydrolysate	GC of alditol acetates on packed columns	225
Xylose, fructose, glucose, sucrose, maltose, lactose etc	Amylose and inulin hydrolysate	HPLC on amine-bonded phase	226
Mono- and oligosaccharides	Body fluids	Anion-exchange HPLC of borate complexes	227
Mono- and oligosaccharides	Not applied	TLC on phosphate-impregnated silica gel	228
Sorbitol and other sugar alcohols	Bulk sorbitol	HPLC on cation exchanger	229
Mono- and disaccharides	Serum, faeces, urine	TLC on silica gel	230
Mono-, di- and oligosaccharides	–	Reversed-phase HPLC	231
Dissolved carbohydrate hydrolysate	Natural water	GC of alditol acetates	122
Maltose, ribose, xylose, fructose, galactose, glucose	Serum, amylose	HPLC on various columns	232
Mono- and disaccharides	Foods	HPLC on cation exchangers	233
Monosaccharides	[Comparison of UV and refractive index detection]	HPLC on amine-bonded phase	175
Mono-, di- and trisaccharides	Foods	TLC on silica gel	52
Mono- and disaccharides	Body fluids	TLC on silica gel	53
Fucose, mannose, galactose	Serum	GC of alditol acetates on packed columns	141

Mono-, di- and trisaccharides	[Thermal degradation]	GC of underivatised sugars on porous polymer	135
Monosaccharides	Corn bran	GC of TMS alditols on fused-silica WCOT columns	148
Mono- and disaccharides	Not applied	TLC on cellulose	234
Ketoses, aldoses	Not applied	GC–MS of acetyl-oximes	235
Monosaccharides	Plant gums	GC on chiral phase	121
Amino acids, sugars	–	HPTLC on cellulose and silica	236
Alditols	Not applied	HPLC on cation exchanger with several metal ions	237
C_2–C_7 carbohydrates	Reaction products	GC of TMS and TMS-oxime derivatives on packed and capillary columns	238
Glucose, fructose, sucrose, galactose, mannitol, sorbitol	Not applied	HPLC on boronic acid substituted silica	239
Free sugars	Plant materials	GC	240
Sugars	Corn syrup	HPLC on ion exchanger	241
Fructose and other sugars	Urine	HPLC on cation exchanger	242
Sugars	Foods	HPLC on cation exchanger	243
Mono- di- and trisaccharides	[Use of radially compressed columns]	HPLC on modified silica	197
Glucose, mannose, xylose, galactose	Wood pulp	HPLC on various columns	244
Rhamnose, fucose, xylose, arabinose, glucose, galactose, mannose	Rice endosperm, cell wall hydrolysate	GC of methylated alditol acetates on glass SCOT column	245
Glucose, maltose	Foods	HPLC on amine-bonded phase	246
Mono- and disaccharides	Papermill effluent	GC on capillary column	247
Monosaccharides, sugar acids	Microorganisms	GC of alditol acetates and aldonitrile acetates on packed columns	248
Arabinose, xylose, fructose, sorbose glucose	–	GC of TMS ethers	249
Galactose, glucose, mannose	Galactomannans from *Aspergillus* spp.	GC of methylated alditol acetates on WCOT columns	250
Alditols	Diatomaceous ooze sediment	GC of alditol acetates on glass WCOT columns	149

(Continued on p 94)

TABLE 4 *(continued)*

Analyte	*Sample*	*Method*	*Ref.*
Neutral sugars	Hydrolysate from Daemonorops	GC of alditol acetates on glass WCOT columns	153
Monosaccharides	[Comparison of aldose/ketose derivatisation]	GC of N-ethoxycarbonyl-O-TMS ethers on WCOT columns	157
Fructose, glucose, sucrose	Cane molasses	GC of oxime-TMS ethers on glass WCOT columns	145
Arabinose, fructose, glucose, sucrose, trehalose, sugar alcohols	Wine	HPLC	251
Acids, sugars, polyols	Wine	GC of TMS ethers on glass WCOT columns	252
Sugar	Bakery products	Colorimetry, ebulliometry, iodometry	253
Carbohydrates	Meats	Infrared transmission spectrophotometry	254
Aldoses	Glycoconjugates	Anion-exchange HPLC of borate complexes	255
Glucose, fructose, ribose	Plasma lipids	TLC on silica gel 60, alumina, cellulose and polyamide	256
Mono- and oligosaccharides	Cotton	TLC on silica gel	257
Carbohydrates	Wine	GC of TMS-oximes	258
Mono- and disaccharides	—	HPLC on cation exchanger (Li^+)	259
Reducing sugars, uronic acids	Pneumococcal polysaccharide hydrolysate	PC on DEAE-cellulose	31
Reducing sugars	Sugar refinery products	Potentiometry following copper reduction	260
Glucose	[Mutarotation rate and retention mechanism]	HPLC on amine-bonded silica	261
Sorbose, lactose, lycose, sucrose, glucose, fructose, maltose	Candies	TLC on silica gel	262
Twelve sugars	[Several mobile phases examined]	TLC on silica gel, cellulose and Kieselguhr	263

Glucose, mannose, ribose, xylose, arabinose, fucose, galactose, lyxose	[Separation of enantiomers]	GC on chiral stationary phases	264
Carbohydrate	Beer	HPLC on amine-bonded phase	265
Free sugars	Green tea	HPLC	266
Invert sugar	Sugar beet	Colorimetry using autoanalyser	267
Tetroses, aldopentoses	Not applied	GC of methoxime and butoxime pertrifluoroacetates on packed and capillary columns	268
Rhamnose, fucose, arabinose, xylose, mannose, galactose, glucose	Sheep rumen fluid and milled barley straw	GC of methylated alditol acetates on glass WCOT columns	269
Sugars	[Retention mechanism]	HPLC on amine-bonded phase	270
Free sugars	Oilseeds	HPLC on amine-bonded phase	271
Glucose, sucrose, fructose	Cantaloupe melon juice	HPLC on silica	272
Glucose	[Flow injection and electrochemical detection]	HPLC on amine bonded phase	273
Dextrose	Foods	HPLC on cation exchanger (Ca^{2+})	274
Sugars	[Method for determining pore size in exclusion chromatography]	HPLC on cation exchangers	275
Fructose, sucrose, glucose, raffinose	–	GC and HPLC	276
Fructose, sucrose glucose, sorbitol mannoheptulose	Apple and pear juices	HPLC on ODS and amine-bonded phases	277
Fructose, glucose, sucrose, maltose, lactose	Chocolate and cacao	HPLC on amine-bonded phase	278
Monosaccharides	–	GC of alditol acetates on packed columns	279
Fructose, glucose, sucrose, maltose, lactose	Fruit juices	HPLC on amine-bonded phase	280
Mannose, ribose, xylose, glucose, fucose, rhamnose, arabinose, etc	Glycoproteins	GC of TMS ethers on fused-silica WCOT columns	281

(Continued on p 96)

TABLE 4 *(continued)*

Analyte	*Sample*	*Method*	*Ref*
Carbohydrate	Cocoa	Near-infrared reflectance	282
Arabinose, lyxose, fucose, rhamnose, galactose, glucose, mannose	Flax-seed mucilage hydrolysate	Enantiomer separation by GC of dithioacetals	283
Mannose, fructose, fucose, galactose, glucose, rhamnose	Bovine glycoproteins	HPLC on cation exchanger	284
Sugars	Culture media	PC following ion exchange	32
Glucose, fructose	Spruce and pine needles	TLC on silica gel	285
Sugars	Foods	HPLC and GC	286
Reducing sugars	Candy	Fehling's solution	287
Aldoses, ketoses	Serum	GC–MS of methoxylated TMS ethers on glass WCOT columns	288
Ribose, xylose, lyxose, fucose glucose, mannose, sorbose, etc	[Enantiomer separation]	GC of menthyloxime pertrifluoroacetates on WCOT columns	289
Rhamnose, fucose, ribose, xylose, mannose, galactose, glucose, etc	[Column activity tests]	GC of alditol acetates on glass WCOT columns	290
Fructose, glucose, lactose, sucrose	Yogurts	HPLC on amine-bonded phase	291
Glucose, sucrose, fructose, maltose	Pizza extract	HPLC using ion-pairing reagent	292
Fructose, glucose sucrose	Grape musts	GC of TMS ethers (oximes)	293
Glucose, fructose, mannitol, xylose, rhamnose and saccharose	Green olives	PC	294
Fructose, glucose, sucrose, lactose, maltose and sorbitol	Food	GC of TMS-oximes	295
Fructose, glucose, lactose, sucrose, mannitol	Narcotics	GC of TMS ethers on glass WCOT columns	146
Monosaccharides	Plant cell wall hydrolysates	GC of alditol acetates on glass SCOT columns	150

Fructose, glucose, sucrose, maltose, maltotriose	Wort, beer	HPLC using an *in situ*-modified amine column	296
Sugars	[On-column reactions and their influence on elution]	HPLC	297
Glucose, fructose, sucrose	Honey	HPLC	298
Maltose, maltulose, isomaltose	High-fructose syrups	PC	299
Glucitol, galactitol, mannitol, xylitol, arabinitol	[Relation between structure and retention time]	GC of methylated alditol acetates on packed columns	300
Arabinose, mannose, allose, fucose, ribose, xylose, glucose, galactose, etc	Not applied	GC of alditol acetates on fused-silica WCOT columns	301
Sugars	Infant foods	TLC	302
Glucose, galactose	–	HPLC on anion exchanger and amine-bonded phase, TLC	303
Xylose, arabinose	[On-column interaction of aldose and NH_2 groups]	HPLC on *in situ*-modified amine phase	304
Xylose	–	HPLC on anion exchanger	305
Mono-, di- and oligosaccharides	Physiological fluids	HPLC on silica	306
Reducing sugars	Wood hydrolysate, beverages	HPLC of dansyl derivatives on an ODS column	212
Fructose, maltose, glucose, sucrose, raffinose	–	TLC on silica gel	307
Glucose, mannose, galactose, fucose, arabinose, xylose, glucosamine, 2,5-anhydromannose	Chitin, ovalbumin, horseradish peroxidase	GC of alditol acetates on glass WCOT columns	308
Rhamnose, fucose, ribose, xylose, mannose, glucose, galactose, etc	Bacterial cell walls	GC of alditol acetates on packed and WCOT columns	309
Sugar alcohols	Not applied	GC of alditol acetates on fused-silica WCOT columns	310

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TABLE 4 *(continued)*

Analyte	*Sample*	*Method*	*Ref*
Sugars	Foods	Comparison of GC and HPLC	311
Fructose, glucose, sucrose	[Thermodynamic parameters]	HPLC on amine and cobalt(III) complex-bonded phases	199
Aldoses, alditols	–	GC of TMS ethers and alditol acetates on packed columns	312
Mono- and disaccharides	Beverages	HPLC on an amine-bonded phase	313
Fructose, glucose, sucrose	Fruit juices	HPLC on an amine column	314
Sugars	[NaCl interference]	HPLC on aminopropyl columns	315
Galactose, saccharose, maltose, fructose, raffinose, glucose, lactose	–	TLC on phosphate-impregnated Kieselguhr and silica gel	316
Arabinose, lyxose, mannose, ribose, glucose, fucose, galactose, xylose	Gum arabic, gum tragacanth	GC of trifluoroacetylated derivatives	317
Sucrose, glucose, fructose	Molasses	HPLC on several columns	176
Reducing sugars	Beer	HPLC on amine-bonded phase	177
Monosaccharides	[Pre-column derivatisation]	HPLC on amine-bonded phases	189,190
Monosaccharides	[Plasticizers]	GC of alditol acetates on SCOT and WCOT columns	151
Fructose, galactose, glucose, sucrose, lactose, maltose	Yogurt	GC of TMS ethers on packed columns	139
Glucose, fructose, xylitol	Infusion solution	Colorimetry	318
Sugars	Confectionery	Improved HPLC method	319
Fructose, sucrose, glucose, glycerol, ethanol	Wines	HPLC	320
Fructose, glucose, sucrose, maltose, glycerol	Food, beverages	HPLC on amine-modified silica columns	321

Dextrose, fructose, glycerol, ethanol	Wines	HPLC on cation exchanger	322
Sucrose, glucose fructose	Plant tissue	HPLC on cation exchanger	323
Galactose, glucose, lactose, fructose, maltose, sucrose	Infant foods	TLC on silica gel	324
Glucose, sucrose, lactose, opiates	Illicit heroin	HPLC on silica	325
Mono- and disaccharides	[Electrochemical detection]	HPLC	326
Glucose, fructose, sorbitol, lactic acid, citric acid	Infusion solutions	HPLC	327
Glucose, galactose, saccharose, maltose, fructose, raffinose, lactose	–	TLC on Kieselguhr	328
Maltose, glucose	Dough	HPLC	329
Glucose, galactose, rhamnose	–	TLC of dansylated sugars on polyamide	330
Monosaccharides	Plankton, wood sediments	GC of TMS ethers on fused-silica WCOT columns	84
Mono-, di- and trisaccharides	–	TLC on aminopropyl-bonded-phase silica	58,59
Reducing sugars	[Flow injection analysis]	HPLC on amine-bonded phase	331
Glucose	[Electrochemical detection]	HPLC	332
Glucose, fructose, inositol, sorbitol, mannitol	Lens, erythrocytes, plasma	HPLC on silica	191
Sucrose, lactose, glucose, galactose, fructose	Yogurt	HPLC of cation exchanger (Ca^{2+})	333
Lactose, glucose, galactose	Whey, whey permeate	HPLC on exclusion resin	334
Aldoses	[Anomer separation]	HPLC on cation exchanger	185
Glucose, fructose, sugar alcohols	Lens	GC of trifluoroacetates on WCOT columns	335
Sugars	Fruit juices	HPLC	336
Fructose, glucose, sucrose, maltose	Licorice	HPLC on amine-bonded phase	172
Sucrose, raffinose, invert sugar	–	Reversed-phase HPLC	337
Mono- to tetrasaccharides	Foods	Normal- and reversed-phase HPLC	338

(Continued pn p 100)

TABLE 4 *(continued)*

Analyte	*Sample*	*Method*	*Ref*
Reducing sugars	Infusions	TLC on cellulose and HPLC	339
Reducing sugars, sucrose, raffinose, sorbitol	Almonds	GC of TMS ethers on packed columns	340
Fructose, glucose, sucrose maltose, maltotriose	Beer, wort	GC of TMS ethers on packed columns	341
Aldoses	Glycoproteins	HPLC on cation exchanger	342
Sucrose, glucose, fructose	Plant material	TLC on silica gel	54
Glucose, fructose, sucrose, raffinose	Wheat tissue	GC of TMS ethers on packed columns	343
Sucrose	[Adsorption]	GC of TMS ethers on glass WCOT columns	147
Fructose, glucose, sucrose	Nectars	GC of TMS oximes on packed columns	125
Neutral and amino sugars	Microorganisms	GC of alditol acetates on fused-silica WCOT columns	152
Sugar alcohols	Anhydride formation	GC	344
Sucrose, glucose, fructose, raffinose	Sugar beet leaves	HPLC on cation exchanger (Ca^{2+})	345
Reducing sugars	[Pressurized reactor outlet]	HPLC	346
Erythrose, ribose, arabinose, fructose, glucose	Formosation reaction mixtures	HPLC on C_{18} column	347
Sucrose, glucose, galactose	Plants	TLC	348
Sucrose, maltose, raffinose	Beer, malt	HPLC on cation exchanger (Ca^{2+})	349
Sugars	[Retention mechanisms on copper(II)-modified silica gel]	LC	350
Mono- and disaccharides, sugar alcohols	[Details of several systems]	HPLC	351

Sucrose, fructose, glucose	Juices, wines, molasses	HPLC on cation exchanger (Ca^{2+})	352
Raffinose, oligosaccharides	Soy beans	GC of TMS oximes on packed columns	134,353
Neutral and amino sugars	Glycoconjugates	Reversed-phase HPLC	354
Glucose, (xylose)	Body fluids	HPLC on ODS column	180
Disaccharides	[Relation between structure and elution time]	HPLC on amine-bonded phase	179
Glucose-1-phosphate, sucrose, fructose, phosphate	–	HPLC on cation exchanger	187
Glucose, fructose, ribose, mannose, galactose, sugar alcohols	Plasma, amniotic fluid	HPLC on cation exchanger	184
Sugars	Diet composite	GC and HPLC	355
Fructose, glucose, mannose, sugar alcohols	Fructose–mannitol conversion mixture	HPLC on cation exchanger	356
Mono- and disaccharides	[Anomer separation]	HPLC on amine-bonded phase	178
Fructose, glucose, raffinose, sucrose, stachyose, cellobiose	[Detection systems]	HPLC on cation exchangers	183
Monosaccharides	Biomass	HPLC on several columns	186
Reducing sugars	[Optimisation of electrochemical detection]	HPLC on several columns	188
Dextrose, levulose, sucrose	Invert sugar	HPLC on cation exchanger	174
Alcohols, aldehydes, ketones, acids, sugars	[Retention behaviour]	HPLC on cation exchanger	182
Aldoses	Sap of lac trees	GC of alditol acetates on fused-silica WCOT columns	205,357
Glucose, xylose, galactose, arabinose, mannose, erythritol	Wood and pulp hydrolysate	HPLC and PC	358

in elution profile effected by alteration of the metal counterion of cation exchangers suggests that an additional mechanism is involved. Goulding[203] postulated that this was a ligand-exchange interaction between the water molecules in the aquated metal ion and the hydroxyl groups of the aldose molecules.

During the course of liquid chromatography mutarotation occurs[19] thereby restricting the separation of sugar anomers. However, successful separations have been achieved[204] on an amino-bonded phase and a phosphoric acid-modified amino packing using low column temperatures to suppress anomerization. In contrast, several anomers have been separated[205] at ambient temperatures using anion-exchange chromatography.

Normal-phase partition and adsorption chromatography have been applied to carbohydrates (Table 3) generally following pre-column derivatisation to perbenzoates[206,207], per-*p*-nitrobenzoates[208], per(dimethylphenylsilyl)ethers[189,190], peracetates[209] and pernaphthoates[210]. However, the need for derivatisation has usually been related to the requirements of detection[180,189–191] and not to altering the chromatographic behaviour of the sugars by endowing them with hydrophobic properties. Dansyl hydrazone derivatives[211,212] have been chromatographed on reversed-phase[213] and normal-phase[214] systems whereas adsorption chromatography of the N-acetyl-α-methoxybenzyl derivatives of glycamines has enabled separation of D- and L-enantiomers of aldoses[215].

Glycamine derivatives[216] can be separated by cation exchange and finally, underivatised sugars have been chromatographed on the bonded-phase anion-exchange resin, Bondapak AX/Corasil, using mixtures of acetone, ethanol and water[217] and ethyl acetate, propan-2-ol and water[218] as mobile phases.

4.1. Detectors

Detection systems for carbohydrates have recently been reviewed by Honda[10] and for this reason only a brief overview is presented here. However, choice of detection system is an important consideration in HPLC of sugars because, unlike GC, there is no universal LC detector and carbohydrates lack chromophoric and fluorophoric groups necessary for UV and fluorescence detection. Indeed, problems with detection have limited[219] the application of HPLC to carbohydrate analysis. Refractivity measurement is suitable but is highly susceptible to changes of column temperature and solvent composition. Moreover, the low sensitivity of refractive index detectors restricts application to relatively concentrated samples[15], such as soft drinks, confectionery and syrups.

Detection based on absorbance in the near-ultraviolet (180–210 nm)[170,184] is non-selective and involves high capital and running costs due to the requirement for expensive instrumentation and mobile phases. Pre-column[180] and post-column[177] derivatisation to provide colorimetric[174], UV-absorbing[191] or fluorescent[169] species provides the best means of detection with currently available instrumentation.

5. APPLICATIONS

Table 4 summarises the chromatographic analyses of mono- and disaccharides cited by *Chemical Abstracts* during the period January 1980 to June 1985. An attempt has been made also to indicate the range of non-chromatographic techniques which

have been applied during this same period. The distribution of citations between the various chromatographic techniques for the period was 52% HPLC, 31% GC, 14% TLC and 3% PC. The greater use of HPLC relative to GC is even more pronounced when considering foods and clinical–biochemical applications. For foodstuffs, practical considerations strongly favour the use of HPLC over GC since component sugars of foods are generally present in adequate concentrations to be detected by the less sensitive detectors of HPLC. On the other hand, biochemical applications are more likely to require the greater sensitivity of GC detectors. The increasing use of pre- and post-column derivatisation techniques in HPLC coupled with UV and fluorescence detectors has had an obvious impact. However, the main advantage of HPLC, namely, the ability to inject directly with little or no sample preparation, is partially eliminated by pre- and post-column derivatisation.

6 SUMMARY

Chromatographic procedures for measuring monosaccharides and disaccharides are reviewed. Although gas and high-performance liquid chromatographic methods predominate, interest continues in the older techniques using paper and thin layers However, the most significant developments of the last decade are the increasing use of open tubular columns and bonded-packings based on silica.

REFERENCES

1 D. T Plummer, *An Introduction to Biochemistry*, McGraw-Hill, Maidenhead, 1978, pp 173–192.
2 L. F Fieser and M Fieser, *Reagents for Organic Synthesis*, Wiley, New York, 1967, p 815
3 H U. Bergmeyer and E Bernt, *Methods of Enzymatic Analysis*, Academic Press. New York, 1963, p 127
4 J. M. Sempere, C Gancedo and C A Senio, *Anal Biochem* , 12 (1965) 509
5 W Postel, F. Drawert and W. Hagen, *Dtsch Lebensm.-Rundsch* , 67 (1971) 107
6 K Zurcher and A Hadorn, *Mitt Geb Lebensmittelunters Hyg.*, 68 (1977) 200
7 E Heftmann, *Chromatography*, Van Nostrand-Reinhold, New York, 1967, Ch 20
8 G J Dickes and P V Nicholas, *Gas Chromatography in Food Analysis*, Butterworth, London, 1976, Ch 15
9 M F Laker, *J Chromatogr* , 184 (1980) 457
10 S Honda, *Anal Biochem* , 140 (1984) 1
11 E V Evtushenko and Yu S Ovodov, *Khim Prir Soedin.*, (1984) 411
12 E H Reimerdes and K D Rothkitt, *Fresenius' Z Anal Chem.*, 318 (1984) 220
13 D Patoraux-Prome and J C. Prome, *Gas Chromatography–Mass Spectroscopy Applications in Microbiology*, Plenum, New York, 1984, pp 105–156
14 A Soerensen and M. Guldborg, *Publ — Statens Levnedsmiddelinst (Den)*, 104 (1984) 54 pp
15 K J Schaffler and P G Morel du Boil, *Sugar Technol Rev* , 11 (1984) 95
16 S M Partridge, *Nature (London)*, 158 (1947) 270
17 S M Partridge, *Biochem J* , 42 (1948) 238
18 C. K. Hordis and G. N. Kowkabany, *Anal Chem* , 30 (1958) 1210.
19 H. Jaeger, A Ramel and O Schindler, *Helv. Chim Acta*, 40 (1957) 1310
20 R J Ferrier and P M Collins, *Monosaccharide Chemistry*, Penguin, Harmondsworth, London, 1972, pp. 293–297
21 A. E Flood, E L Hirst and J K N Jones, *J Chem Soc* , (1948) 1679
22 F A Isherwood and M A. Jermyn, *Biochem J* , 48 (1951) 515
23 M F Levy, *Anal. Chem* , 26 (1954) 1849
24 A Jeanes, C S Wise and R J Dimler, *Anal Chem* , 23 (1951) 415
25 J E. Courtois, *Mises au Point de Chimie Analytique*, Vol IV, Masson, Paris, 1956, p 1.
26 M A Jermyn and F A. Isherwood, *Biochem. J* , 44 (1949) 402

27 L Hough, J K N Jones and W H Wadman, *J. Chem. Soc.*, (1950) 1702
28 J E Courtois and U Ariyoshi, *C.R Hebd. Seances Acad Sci*, 250 (1960) 1369
29 I Smith, *Chromatographic and Electrophoretic Techniques, Vol 1, Chromatography*, William Heinemann, Bath, 1969, p 311
30 M Piccardo, L Russo and M Rosa, *Boll -Soc Ital Biol Sper*, 55 (1979) 1039
31 G Caldes, B Prescott and P. J Baker, *J. Chromatogr*, 234 (1982) 264
32 L I Trusova and L Ya Telishevskaya, *Tr., Vses Gos Nauchno-Kontrol'n Inst Vet. Prep*, (1980) 29, 114
33 D M Alperin, V P Idoyaga-Vargas and H Carminatti, *J Chromatogr*, 242 (1982) 299.
34 P W Simon and R E Freeman, *Hort. Science*, 20 (1985) 133
35 J C Touchstone, *Quantitative Thin Layer Chromatography*, Wiley, New York, 1973, p 81
36 A Schweiger, *J Chromatogr*, 9 (1962) 374.
37 M. L Wolfrom, D L Patin and R M de Lederkremer, *J. Chromatogr*, 17 (1965) 488
38 J W Walkley and J. Tillman, *J Chromatogr.*, 132 (1977) 172.
39 M Hoton-Dorge, *J Chromatogr*, 116 (1976) 417
40 B A Lewis and F Smith, in E. Stahl (Editor), *Thin Layer Chromatography*, George Allen and Unwin, London, 1969, p 807
41 I Smith, *Chromatographic and Electrophoretic Techniques, Vol 1, Chromatography*, William Heinemann, Bath, 1969, p 318
42 H Grasshof, *Dtsch Apotheker-Z.*, 103 (1963) 1396
43 P Barbetti and S. C Leopizzi, *Ann Chim (Rome)*, 65 (1975) 403
44 E Stahl and U Kaltenbach, *J Chromatogr*, 5 (1961) 351
45 K. C Guven, *Acta Pharm Turc.*, 27 (1985) 1
46 V. Prey, H Berbalk and M Kausz, *Mikrochim Acta*, (1961) 968.
47 G Pastuska, *Fresenius' Z Anal Chem*, 179 (1961) 427
48 F Micheel and O. Berendes, *Mikrochim Acta*, (1963) 519
49 G W Hay, B A Lewis and F Smith, *J Chromatogr*, 11 (1963) 479
50 S A. Hansen, *J. Chromatogr.*, 107 (1975) 224
51 B B Pruden, G Pineault and H Loutfi, *J. Chromatogr*, 115 (1975) 477
52 R Gauch, U Leuenberger and E Baumgartner, *J. Chromatogr*, 174 (1979) 195
53 Ž Žilić, N. Blau and M Knob, *J Chromatogr*, 164 (1979) 91
54 P. Macuha and A Chudziak, *Pol'nohospodarstvo*, 30 (1984) 774
55 A. I Makarov, *Fiziol. Biokhim Kul't. Rast*, 16 (1984) 292.
56 L. Wassermann and H Hanus, *Naturwissenschaften*, 50 (1963) 351
57 V Prey, H Scherz and E Bancher, *Mikrochim Acta*, (1963) 567
58 L W Doner and L. M Biller, *J Chromatogr*, 287 (1984) 391
59 L W Doner, C. L Fogel and L M. Biller, *Carbohydr Res*, 125 (1984) 1
60 H Weicker and R. Brossmer, *Klin Wochenschr*, 39 (1961) 1265
61 E Guilloux and S Beaugiraud, *Bull Soc Chim Fr*, (1965) 261
62 H Jacin and A R Mishkin, *J. Chromatogr*, 18 (1965) 170
63 P G Pifferi, *Anal Chem*, 37 (1965) 925.
64 G G Dutton, K B Gibney, P E Reid and K N Slessor, *J Chromatogr*, 20 (1965) 163
65 H Ertel and L Horner, *J Chromatogr*, 7 (1962) 268
66 S Adachi, *J Chromatogr*, 17 (1965) 295
67 K Y Lee, D Nurok and A Zlatkis, *J Chromatogr*, 174 (1979) 187
68 C T Greenwood, J H Knox and E Milne, *Chem Ind (London)*, (1961) 1878
69 A G McInnes, D H Ball, F P Cooper and C T Bishop, *J Chromatogr*, 1 (1958) 556
70 H. W. Kircher, *Anal. Chem.*, 32 (1960) 1103
71 C T Bishop, *Methods Biochem Anal*, 10 (1962) 1
72 D Anderle, P Kováč and H Anderlová, *J Chromatogr*, 64 (1972) 368
73 B. Fournet and J Montreuil, *J Chromatogr*, 75 (1973) 29
74 W J Lewicki and J R Edwards, *Anal. Lett*, 3 (1970) 151
75 R M Thompson, R D. Coffin, M R. Glick and J F Fitzgerald, *Clin Chim Acta*, 84 (1978) 185
76 S W. Gunner, J K W Jones and M B Perry, *Can. J Chem*, 39 (1961) 1892
77 J. S. Sawardeker, J H. Sloneker and A. Jeanes, *Anal Chem.*, 37 (1965) 1602
78 W J A Vanden Heuvel and E C Horning, *Biochem. Biophys Res Commun*, 4 (1961) 399
79 H. G Jones and M B. Perry, *Can. J Chem*, 40 (1962) 1339
80 E Pitkanen, *Clin Chim Acta*, 38 (1972) 221

81 H. Schwind, F Scharbert, R Schmidt and R Katterman, *J Clin Chem Clin Biochem*, 16 (1978) 145
82 L. J Griggs, A. Post, E R White, J A. Finkelstein, W. E. Moeckel, K. G Holden, J. E Zarembo and J A Weisbach, *Anal Biochem*, 43 (1971) 369
83 I M Campbell and R Bentley, in R F Gould (Editor), *Advances in Chemistry Series, Carbohydrates in Solution, ACS Symposium*, American Chemical Society, Washington, DC, 1971, pp 1–19.
84 G L Cowie and J I Hedges, *Anal Chem.*, 56 (1984) 497
85 H Jacin, J M Slanski and R J Moshy, *J Chromatogr*, 37 (1968) 103
86 T E Acree, R S Shallenberger, C Y Lee and J W Einset, *Carbohydr. Res*. 10 (1969) 355.
87 T E Acree, R S Shallenberger and L R Mattick, *Carbohydr Res*, 6 (1968) 498
88 G. G. S Dutton, *Adv Carbohydr. Chem Biochem*, 30 (1973) 38
89 D. Murphy and C A Pennock, *Clin Chim Acta*, 42 (1972) 67
90 E J Bourne, C. E M Tatlow and J C Tatlow, *J. Chem Soc*, (1950) 1367
91 M Vilkas, H.-I. Jan, G Boussac and M -C Bonnard, *Tetrahedron Lett*, (1966) 1441
92 J Shapira, T Putkey, A Furst and G A McCasland, *Carbohydr. Res.*, 25 (1972) 535
93 Z. Tamura and T Imanari, *Chem Pharm Bull*, 15 (1967) 246
94 J P Zanetta, W. C Breckenridge and G Vincendon, *J Chromatogr.*, 69 (1972) 291
95 M A. Luke, *J. Assoc Off Anal Chem*, 54 (1971) 937.
96 J. E Sullivan and L R Schewe, *J Chromatogr Sci*, 15 (1977) 196
97 C C Sweeley, R. Bentley, M Makita and W W Wells, *J Am Chem Soc.*, 85 (1963) 2497
98 W W Wells, T Chin and B. Weber, *Clin. Chim Acta*, 10 (1964) 352
99 T. Bhatti and J R. Clamp, *Clin Chim. Acta*, 22 (1968) 563
100 J de Neef, *Clin. Chim Acta*, 26 (1969) 485
101 J Lehrfeld, *J Chromatogr Sci.*, 9 (1971) 757.
102 A H Weiss and H Tambawala, *J Chromatogr. Sci*, 10 (1972) 120.
103 K M Brobst and C E Lott, *Cereal Chem.*, 43 (1966) 35
104 R Bentley and N Botlock, *Anal Biochem*, 20 (1967) 312
105 W C Butts and R L Jolley, *Clin Chem*, 16 (1970) 722
106 T Vander Meulen and R A Hudson, *Anal Biochem*, 53 (1973) 639
107 K Osaka, M Kuboyama, I. Kiyosawa, T Suzaki and M Itoh, *J Nutr. Sci Vitaminol*, 21 (1975) 129
108 S L Smith, M. Novotny and E L. Weber, *Clin. Chem.*, 24 (1978) 545.
109 M F Laker and J N Mount, *Clin Chem*, 26 (1980) 441
110 M. F Laker and W G Gunn, *Clin Chim. Acta*, 96 (1979) 265
111 M. F Laker, *J. Chromatogr*, 163 (1979) 9
112 A E Pierce, *Silylation of Organic Compounds*, Pierce Chemical Co, Rockford, IL, 1968
113 C Petersson, *Carbohydr Res.*, 33 (1974) 47.
114 E. C. Horning and M G Horning, *Methods Med Res*, 12 (1970) 396
115 P E Reid, B Donaldson, D. W Secret and B Bradford, *J. Chromatogr*, 47 (1970) 199
116 W W Wells, *Methods Med Res*, 12 (1970) 115.
117 D V Phillips and A E Smith, *Anal. Biochem*, 54 (1973) 95.
118 A. Ericsson, J Hansen and L. Dalgaard, *Anal Biochem*, 86 (1978) 552.
119 E P Crowell and B. B Burnett, *Anal. Chem.*, 39 (1967) 121
120 L G Borchardt and C V Piper, *Tappi*, 53 (1970) 257
121 G Holzer, J Oró, S J Smith and V M. Doctor, *J Chromatogr*, 194 (1980) 410
122 M Ochiai, *J Chromatogr*, 194 (1980) 224
123 M S Sweet and E. M Perdue, *Environ Sci Technol.*, 16 (1982) 692
124 B S Mason and H T. Slover, *J Agric Food Chem*, 19 (1971) 551
125 B D Tanowitz and D M Smith, *Ann Bot*, 53 (1984) 453.
126 W R Supina, R F Kruppa and R Henley, *J Am Oil Chem Soc*, 44 (1967) 965
127 J R. Clamp, *Biochem Soc Trans*, 5 (1977) 1693.
128 J R. Clamp, *Biochem Soc Symp.*, 40 (1974) 3
129 P. Størset, O Stokke and E Jellum, *J Chromatogr*, 145 (1978) 351
130 F Eisenberg, *Carbohydr Res*, 19 (1971) 135
131 I M Morrison and M B Perry, *Can J Biochem*, 44 (1966) 1115
132 B W. Li, P J Schuhmann and J. M Holden, *J. Agric Food Chem*, 31 (1983) 985
133 T. Cayle, F Viebrock and J Schiaffino, *Cereal Chem.*, 45 (1968) 154
134 I Molnar-Perl, M Pinter-Szakacs, A Kövágó and J Petróczy, *J Chromatogr*, 295 (1984) 433

135 K Uobe, K Nishida, H Inoue and M Tsutsui, *J Chromatogr.*, 193 (1980) 83
136 G A Janauer and P Englmaier, *J Chromatogr* , 153 (1978) 539
137 B Mueller and G Goeke, *Dtsch Lebensmitt -Rundsch* , 68 (1972) 222
138 K. M Brobst and C E. Lott, *Proc Am. Soc Brew Chem.*, (1966) 71.
139 D. J Heaf and D J Galton, *Clin Chim Acta*, 63 (1975) 41
140 J F. Aloia, *J Lab Clin Med* , 82 (1973) 809
141 C W Gehrke, T. P Waalkes, E Borek, W F Swartz, T F Cole, K C. Kuo, M Abeloff, D. S Ettinger, N Rosenshein and R C Young, *J Chromatogr* , 162 (1979) 507
142 H Nakamura and Z Tamura, *Clin Chim Acta*, 39 (1972) 367
143 G I. Manius, T M Y Liu and L -F L Wen, *Anal Biochem* , 99 (1979) 365
144 P G Morel du Boil and K J Schaffler, *Proc S African Sug Technol Assoc* , (1978) 96
145 K J Schaffler and P G Morel du Boil, *J Chromatogr* , 207 (1981) 221
146 I Barni Comparini, F Centini and A Pariali, *J Chromatogr* , 279 (1983) 609
147 H Traitler, S. Del Vedovo and T F Schweizer, *J High Resolut Chromatogr Chromatogr Commun* , 7 (1984) 558
148 A. G Bradbury, D J Halliday and D G. Medcalf, *J Chromatogr* , 213 (1981) 146
149 J Klok, E H Nieberg-van Velzen, J W de Leeuw and P A Schenck, *J Chromatogr* , 207 (1981) 273
150 A B Blakeney, P. J Harris, R J Henry and B A. Stone, *Carbohydr Res* , 113 (1983) 291
151 R J. Henry, P J Harris, A B Blakeney and B A Stone, *J. Chromatogr* , 262 (1983) 249.
152 M D Walla, P Y Lau, S L Morgan, A Fox and A. Brown, *J Chromatogr* , 288 (1984) 399.
153 C. Green, V M. Doctor, G Holzer and J Oró, *J Chromatogr.*, 207 (1981) 268.
154 J Szafranek, C D Pfaffenberger and E C Horning, *Anal Lett.*, 6 (1973) 479
155 C D. Pfaffenberger, J Szafranek, M G Horning and E C Horning, *Anal Biochem* , 63 (1975) 501
156 C D Pfaffenberger, J Szafranek and E C Horning, *J Chromatogr* , 126 (1976) 535.
157 H Frank, H J Chaves das Neves and E. Bayer, *J Chromatogr* , 207 (1981) 213
158 W S Reich, *C R Hebd Seances Acad Sci* , 208 (1939) 589
159 L Hough, J K N Jones and W. H. Wadman, *J Chem Soc* , (1949) 2511
160 R. Dedonder, *Bull Soc Chim Biol* , 34 (1952) 144
161 J X Khym and L. P Zill, *J Am Chem Soc.*, 73 (1951) 2399.
162 J K N Jones and R. A Wall, *Can J Chem* , 38 (1960) 2290
163 A Tiselius, *Ark. Kemi Mineral. Geol* , 14B (1940) 4
164 R L Whistler and D F Durso, *J Am Chem Soc* , 72 (1950) 677
165 R S Alm, *Acta Chem Scand* , 6 (1952) 1186
166 J S Hobbs and J G Lawrence, *J Sci Food Agric* , 23 (1972) 45
167 D J Folkes and P W. Taylor, in R. Macrae (Editor), *HPLC in Food Analysis*, Academic Press, London, 1982, p 150
168 J. X Khym and L P. Zill, *J Am Chem Soc* , 74 (1952) 2090
169 S Honda, M Takahashi, K Kakehi and S Ganno, *Anal Biochem* , 113 (1981) 130
170 L A T Verhaar and J M H Kirkx, *Carbohydr Res* , 62 (1978) 197
171 J F Kennedy and J E Fox, *Carbohydr Res* , 54 (1977) 13
172 P S Vora and R M Tuorto, *J Assoc Off Anal Chem* , 67 (1984) 764 and 529
173 R B Meagher and A Furst, *J Chromatogr* , 117 (1976) 211
174 M Wnukowski, *Int Sugar J* , 86 (1984) 170
175 H Binder, *J Chromatogr* , 189 (1980) 414
176 E Rajakylä and M Paloposki, *J. Chromatogr* , 282 (1983) 595
177 T D Schlabach and J Robinson, *J Chromatogr* , 282 (1983) 169
178 M. Moriyasu, A. Kato, M Okada and Y Hashimoto, *Anal Lett.*, 17 (1984) 689
179 Z L Nikolov, M. M. Meagher and P J Reilly, *J Chromatogr* , 319 (1985) 51
180 D A Finden, R R Fysh and P C White, *J Chromatogr* , 342 (1985) 179
181 T G Lawrence, *Chimia*, 29 (1975) 367
182 R. Pecina, G Bonn, E Burtscher and O. Bobleter, *J. Chromatogr* , 287 (1984) 245
183 P Vrátný, R W Frei, U A. Th Brinkman and M W Nielen, *J Chromatogr.*, 295 (1984) 355
184 J A Owens and J S Robinson, *J Chromatogr* , 338 (1985) 303
185 S Honda, S. Suzuki and K Kakehi, *J Chromatogr* , 291 (1984) 317
186 G. Bonn, R Pecina, E Burtscher and O Bobleter, *J Chromatogr* , 287 (1984) 215
187 R M Stikkelman, T. T Tjioe, J P van der Wiel and F van Rantwijk, *J Chromatogr* , 322 (1985) 220

188 S. Honda, T Konishi and S Suzuki, *J Chromatogr*, 299 (1984) 245
189 C A White, S W Vass, J. F Kennedy and D G Large, *J Chromatogr*, 264 (1983) 99
190 C A White, S. W Vass, J F Kennedy and D G Large, *Carbohydr Res*, 119 (1983) 241
191 M Petchey and M J Crabbe, *J Chromatogr.*, 307 (1984) 180.
192 G P Belue, *J. Chromatogr*, 100 (1974) 233
193 H van Olst and G E Joosten, *J Liq Chromatogr*, 2 (1979) 111
194 R. S. Ward and A Pelter, *J Chromatogr. Sci*, 12 (1974) 570.
195 R. Schwarzenbach, *J Chromatogr.*, 117 (1976) 206
196 J C Linden and C L Lawhead, *J Chromatogr*, 105 (1975) 125
197 D L Hendrix, R. E. Lee, Jr, J G Baust and H James, *J Chromatogr.*, 210 (1981) 45
198 F M Rabel, A G Caputo and E T Butts, *J Chromatogr*, 126 (1976) 731
199 C A Chang, *Anal Chem*, 55 (1983) 971
200 R W Yost, L S Ettre and R D Conlon, *Practical Liquid Chromatography — An Introduction*, Perkin-Elmer, Norwalk, CT, 1980, p. 79.
201 P. Jonsson and O Samuelson, *Anal Chem*, 39 (1967) 1156
202 E Martinsson and O Samuelson, *J Chromatogr*, 50 (1970) 429
203 R W Goulding, *J Chromatogr.*, 103 (1975) 229
204 V. Kahle and K Tesařík, *J. Chromatogr*, 191 (1980) 121
205 R Oshima, N Takai and J Kumanotani, *J Chromatogr.*, 192 (1980) 452.
206 C. A White, J F Kennedy and B T. Golding, *Carbohydr Res.*, 76 (1979) 1
207 R Oshima and J Kumanotani, *J. Chromatogr*, 265 (1983) 335.
208 F. Nachtmann and K W Budna, *J Chromatogr*, 136 (1977) 279
209 J Thiem, J Schwentner, H. Karl, A Sievers and J. Reimer, *J Chromatogr*, 155 (1978) 107.
210 J Golik, H W Liu, M Dinovi, J Furukawa and K. Nakanishi, *Carbohydr. Res*, 118 (1983) 135
211 H. Iwase, S.-C. Li and Y -T Li, *J Chromatogr*, 267 (1983) 238.
212 K. Mopper and L. Johnson, *J Chromatogr*, 256 (1983) 27
213 W F. Alpenfels, *Anal Biochem*, 144 (1981) 153
214 M Takeda, M Maeda and A Tsuji, *J Chromatogr*, 244 (1982) 347.
215 R Oshima, Y Yamauchi and J Kumanotani, *Carbohydr Res*, 107 (1982) 169
216 F. Perini and B P. Peters, *Anal. Biochem*, 123 (1982) 357
217 H Hyakutake and T. Hanai, *J Chromatogr*, 108 (1975) 385
218 J N Little, D F Horgan, R L. Cotter and R V Vivilecchia, in E Grushka (Editor), *Bonded Stationary Phases in Chromatography*, Ann Arbor Sci Publ., Ann Arbor, MI, 1974, p 54
219 A Pryde and M T Gilbert, *Applications of High Performance Liquid Chromatography*, Cambridge University Press, Cambridge, 1979, p. 136
220 S Farag, *J Am Soc Sugar Beet Technol*, 20 (1979) 251
221 H A Aluyi and D B Drucker, *J. Chromatogr*, 178 (1979) 209
222 J W. De Vries, J C Heroff and D C Egberg, *J Assoc Off. Anal Chem*, 62 (1979) 1292
223 Y N Elkin, *J Chromatogr*, 180 (1979) 163
224 S Honda, N Yamauchi, S. Suzuki and K Kakehi, *Koen Yoshishu-Seitai Seibun no Bunseki Kagaku Shimpojumu*, 4th (1979) 65; *C A*, 92 (1980) 176702u
225 N E. Anderson and F. M. Clydesdale, *J Food Sci*, 45 (1980) 336
226 M D'Amboise, D Noel and T Hanai, *Carbohydr Res.*, 79 (1980) 1.
227 S Honda, Y Matsuda, M. Takahashi, K Kakehi and S Ganno, *Anal Chem.*, 52 (1980) 1079
228 N Iizima, M Fujihara and T Nagumo, *J Chromatogr*, 193 (1980) 464.
229 J Dokladalova, A Y Barton and E A Mackenzie, *J Assoc. Off. Anal Chem*, 63 (1980) 664.
230 Y. Vaysse, P Pilardeau and M. Garnier, *Pathol Biol*, 28 (1980) 206
231 A Heyraud and M. Rinaudo, *J Liq. Chromatogr*, 3 (1980) 721
232 T. Kato and T Kinoshita, *Anal Biochem*, 106 (1980) 238
233 J L Di Cesare, *Chromatogr. Newsl*, 8 (1980) 52
234 N I Kovalevskaya, *Lab Delo*, (1980) 565
235 F. R Seymour, E C Chen and J E. Stouffer, *Carbohydr Res.*, 83 (1980) 201
236 H E Hauck and H. Halpaap, *Chromatographia*, 13 (1980) 538
237 L. Petrus, V. Bilik, L. Kuniak and L Stankovic, *Chem Zvesti*, 34 (1980) 530
238 D E Willis, *J. Chromatogr Sci.*, 21 (1983) 132
239 M Glad, S Ohlson, L Hansson, M -O Månsson and K Mosbach, *J Chromatogr*, 200 (1980) 254
240 H Englyst, *Basic Clin Nutr*, 3 (1981) 71

241 D. L. Kiser, R. L. Hagy and R. L. Olson, in G. Charalambous (Editor), *Anal. Control Less Desirable Flavors Foods Beverages, [Proc. Symp.] 1980, 71*, Academic Press, New York, *C.A.*, 94 (1981) 137883a
242 J. C. Kuo and E. S. Yeung, *J. Chromatogr.*, 223 (1981) 321
243 P. Hou, B. G. Archer and R. Won, *Altex Chromatogram*, 4 No. 2 (1981) 7
244 F. E. Wentz, A. Dale Marcy and M. J. Gray, *J. Chromatogr. Sci.*, 20 (1982) 349
245 N. Shibuya, *J. Chromatogr.*, 208 (1981) 96
246 R. Macrae and J. Dick, *J. Chromatogr.*, 210 (1981) 138
247 G. A. Reid, A. W. Harper, P. F. Sanders and K. B. Pugh, *Chem. Ind. (London)*, No. 2, (1981) 64
248 J. Lehrfeld, *Anal. Biochem.*, 115 (1981) 410
249 T. Li, C. Wu, T. Jiang, Y. Zhang and X. Fan, *Fen Hsi Hua Hsueh*, 9 (1981) 295, *C.A.*, 95 (1981) 187542e
250 E. Barreto-Bergter, L. Hogge and P. A. Gorin, *Carbohydr. Res.*, 97 (1981) 147
251 W. Flak, *Mitt. Klosterneuburg*, 31 (1981) 204
252 P. De Smedt, P. A. Liddle, B. Cresto and A. Bossard, *J. Inst. Brew.*, 87 (1981) 349
253 S. Sidorenko and A. Vangelov, *C.A.*, 96 (1982) 5011w.
254 O. C. Bjarnoe, *J. Assoc. Off. Anal. Chem.*, 64 (1981) 1392.
255 S. Honda, M. Takahashi, Y. Nishimura, K. Kakehi and S. Ganno, *Anal. Biochem.*, 118 (1981) 162
256 R. Segura and X. Navarro, *J. Chromatogr.*, 217 (1981) 329
257 J. Bourely, *Coton Fibres Trop. (Fr. Ed.)*, 35 (1980) 189
258 K. Iwano, S. Sato, A. Mizuno, Y. Takahara, E. Sano, Y. Kizaki, K. Tsuji, N. Umeda, A. Totsuka and H. Kawashima, *Nippon Jozo Kyokai Zasshi*, 76 (1981) 778, *C.A.*, 96 (1982) 67195t.
259 R. A. Boykins and T. Y. Liu, *Chromatogr. Sci.*, 18 (1981) 347
260 J. Dobrzycki and M. Ludwicki, *Gaz. Cukrow.*, 89, No. 3 (1981) 65
261 L. A. T. Verhaar and B. F. M. Kuster, *J. Chromatogr.*, 234 (1982) 57
262 Y. Ou, *Shipin Kexue (Beijing)*, 13 (1981) 35
263 J. I. Vucetic, M. M. Vrvic and B. M. Vujovic, *Med. Podmladak*, 32 (1980) 151, *C.A.*, 96 (1982) 123113d
264 W. A. Koenig, I. Benecke and S. Sievers, *J. Chromatogr.*, 217 (1981) 71
265 M. Toth, E. Laszlo and J. Morvai, *Sorbar*, 28 (1981) 127, *C.A.*, 96 (1982) 141082x.
266 T. Anan, H. Takayanagi, K. Ikegaya and M. Nakagawa, *Nippon Shokuhin Kogyo Gakkaishi*, 28 (1981) 632; *C.A.*, 96 (1982) 141283p
267 B. Ilcha, Z. Folbergerova and M. Friml, *LC, Listy Cukrov.*, 97 (1981) 271
268 P. Decker and H. Schweer, *J. Chromatogr.*, 236 (1982) 369
269 J. A. Lomax and J. Conchie, *J. Chromatogr.*, 236 (1982) 385
270 P. Orth and H. Engelhardt, *Chromatographia*, 15 (1982) 91
271 R. Yamauchi, K. Kato and Y. Ueno, *Nippon Nogei Kagaku Kaishi*, 56 (1982) 123, *C.A.*, 96 (1982) 216127b.
272 N. L. Wade and S. C. Morris, *J. Chromatogr.*, 240 (1982) 257
273 W. Buchberger, K. Winsauer and C. Breitwieser, *Fresenius' Z. Anal. Chem.*, 311 (1982) 517
274 S. Hughes and D. C. Johnson, *J. Agric. Food Chem.*, 30 (1982) 712.
275 T. Crispin and I. Halász, *J. Chromatogr.*, 239 (1982) 351
276 H. M. Rodriguez and A. M. Escudey, *Bol. Soc. Chil. Quim.*, 27 (1982) 319
277 P. E. Shaw and C. W. Wilson, *J. Chromatogr. Sci.*, 20 (1982) 209
278 C. Corradi, D. Carreri and G. Micheli, *Boll. Chim. Unione Ital. Lab. Prov., Parte Sci.*, 33 (1982) 33
279 A. Okahira, H. Kobatake and H. Kushida, *Bunseki Kagaku*, 31 (1982) 163
280 T. T. Fang and K. J. Chang, *Kuo Li T'ai-wan Ta Hsueh Nung Hsueh Yuan Yen Chiu Pao Kao*, 21 (1981) 62, *C.A.*, 97 (1982) 108606u
281 M. F. Chaplin, *Anal. Biochem.*, 123 (1982) 336
282 K. J. Kaffka, K. H. Norris, F. Kulcsar and I. Draskovits, *Acta Aliment.*, 11 (1982) 271
283 M. R. Little, *Carbohydr. Res.*, 105 (1982) 1.
284 A. K. Shukla, N. Scholz, E. H. Reimerdes and R. Schauer, *Anal. Biochem.*, 123 (1982) 78.
285 I. Aguinagalde and R. Hueser, *An. Edafol. Agrobiol.*, 40 (1981) 1485
286 C. Date, H. Tanaka, K. Yoshikawa, T. Baba, M. Hayashi, K. Owada, K. Okazaki, Y. Nakayama and R. Ishii, *Osaka City Med. J.*, 28 (2) (1982) 67
287 K. Ma, *Shipin Kexue (Beijing)*, 27 (1982) 57, *C.A.*, 97 (1982) 143191b
288 O. Pelletier and S. Cadieux, *J. Chromatogr.*, 231 (1982) 225
289 H. Schweer, *J. Chromatogr.*, 243 (1982) 149

290 J R Hudson, S L Morgan and A Fox, *J High Resolut Chromatogr Chromatogr Commun.*, 5 (1982) 285
291 Y K Lee, M H Song and S B Chun, *Hanguk Ch'uksan Hakhoe Chi*, 24 (1982) 326
292 R B Wills, R A Francke and B P Walker, *J Agric Food Chem*, 30 (1982) 1242
293 J E. Marcy and D E Carroll, *Am. J Enol Vitic*, 33 (1982) 176
294 J Fernandez-Bolanos, M. J Fernandez Diez, M Rivas Moreno and A. Gil Serrano, *Grasas Aceites (Seville)*, 33 (1982) 208
295 C Date, H Tanaka, K. Yoshikawa, Y Ueda, T Baba, M. Hayashi, Y. Tanaka, T. Shimada and H Yamamoto, *Nippon Eiseigaku Zasshi*, 37 (1982) 516, *C A.*, 97 (1982) 214356x
296 G K Buckee and D E. Long, *J Am Soc Brew Chem*, 40 (1982) 137
297 L A Verhaar, *Starch/Staerke*, 34 (1982) 351
298 J. Jary, M. Marek and J Bacilek, in W Baltes, P B Czedik-Eysenberg and W Pfannhauser (Editors), *Recent Developments in Food Analysis, Proceedings of the 1st European Conference on Food Chemistry, 1981*, Verlag Chemie, Weinheim, 1982, p 453
299 S Osaki and Z. Yoshino, *Denpun Kagaku*, 29 (1982) 210
300 A Okahira and H Kobatake, *Bunseki Kagaku*, 31 (1982) 304
301 A B. Blakeney, P J Harris, R. J Henry, B A. Stone and T Norris, *J Chromatogr*, 249 (1982) 180
302 M. Fayez, M El-Tarras, A S Ahmed and F A Emam, *Egypt J. Pharm Sci.*, 21 (1982) 215
303 T Kato, F Inuma and T. Kinoshita, *Nippon Kagaku Kaishi*, (1982) 1603
304 B Porsch, *J Chromatogr*, 253 (1982) 49
305 T Kato and T Kinoshita, *Bunseki Kagaku*, 31 (1982) 615
306 G K Grimble, H M Barker and R. H Taylor, *Anal Biochem*, 128 (1983) 422
307 E Elmer and J Kwasnik, *Pr Nauk Akad. Ekon. im Oskara Langego Wroclawiu*, 199 (1982) 49, *C.A*, 98 (1983) 122225w
308 R J Henry, A B Blakeney, P. J Harris and B A Stone, *J Chromatogr*, 256 (1983) 419
309 A. Fox, S L Morgan, J R Hudson, Z. T Zhu and P Y Lau, *J Chromatogr*, 256 (1983) 429
310 R Oshima, J Kumanotani and C Watanabe, *J Chromatogr*, 250 (1982) 90
311 J L Iverson and M P Bueno, *J. Assoc Off Anal Chem*, 64 (1981) 139.
312 V G Kostenko and G G Senchenko, *Zh Anal. Khim*, 38 (1983) 300
313 R. Gaub, *Monatsschr Brau*, 36 (1983) 125
314 P E Shaw and C W Wilson, *J Sci. Food Agric*, 34 (1983) 109
315 J W De Vries, H L Chang, J C Heroff and K D Johnson, *J Assoc Off Anal Chem.*, 66 (1983) 197
316 T Hodisan, E Moraru and G. Murgu, *Stud Univ Babes-Bolyai, [Ser.] Chem*, 27 (1982) 34.
317 H Schweer, *J Chromatogr*, 259 (1983) 164
318 A. Baer and H Kraemer, *Krankenhauspharmazie*, 4 (1983) 115, *C A*, 99 (1983) 10918p
319 S Johncock and P Wagstaffe, *Analyst (London)*, 105 (1980) 581
320 C Otoguro, S Ogino and M Watanabe, *Nippon Jozo Kyokai Zasshi*, 78 (1983) 220
321 R. E Lee, D Friday, R Rojas, H James and J. G Baust, *J Liq Chromatogr*, 6 (1983) 1139
322 S Hughes and D C Johnson, *Anal. Chim Acta*, 149 (1983) 1
323 G. G McBee and N. O Maness, *J Chromatogr*, 264 (1983) 474
324 M T. Valdehita, A Carballido and M D. Tenorio, *An Bromatol*, 33 (1983) 283
325 P C White, I Jane, A Scott and B E Connett, *J Chromatogr*, 265 (1983) 293.
326 W Buchberger. K Winsauer and C Breitwieser, *Fresenius' Z Anal Chem*, 315 (1983) 518
327 M Ishikawa, M Yamamoto, T Masui and M Nara, *Shizuoka-ken Eisei Kankyo Senta Hokoku*, 25 (1982) 131, *C A*, 99 (1983) 146179f.
328 E Moraru, T Hodisan and C. Sarbu, *Rev Chim (Bucharest)*, 34 (1983) 144
329 L Bianco and M Marucchi, *Boll Chim Unione Ital Lab Prov, Parte Sci*. 34 (1983) 209
330 A Murakami, E Otani, F. Yamamura, K Hirota, K Maeda and H Nishizawa, *Kyoritsu Yakka Daigaku Kenkyu Nenpo*, (27) (1982) 27, *C A*, 99 (1983) 186768d
331 D Betteridge, N G Courtney, T J. Sly and D G Porter, *Analyst (London)*, 109 (1984) 91
332 W Buchberger, K Winsauer and C Breitwieser, in E. Pungor (Editor), *Modern Trends in Analytical Chemistry, Part A, Electrochemical Detection in Flow Analysis, Proceedings of Scientific Symposium, Matrafured, October 17–20, 1982 (Analytical Chemistry Symposia Series*, Vol. 18, Part A), Elsevier, Amsterdam, 1984, p 267
333 C. Vidal-Valverde, C Martin-Villa and J Herranz, *J Dairy Sci*, 67 (1984) 759
334 I J Jeon, S J Galitzer and K J Hennessy, *J Dairy Sci*, 67 (1984) 884.
335 M Tomana, J T Prchal, L C Garner, H W Skalka and S A Barker, *J Lab Clin Med*, 103 (1984) 137.

336 E D Coppola, *Food Technol (Chicago)*, 38 (4) (1984) 88
337 G Palla, *Anal. Chem*, 53 (1981) 1966
338 R Galensa, *Z Lebensm -Unters -Forsch*, 178 (1984) 199
339 J Guardiola and K W Schultze, *Fresenius' Z Anal Chem*, 318 (1984) 237
340 F Saura-Calixto, J Canellas and A Garcia-Raso, *J Agric Food Chem*, 32 (1984) 1018
341 I Kabzev, S. Manchev and N Genov, *Nauchni Tr.-Vissh Inst Khranit. Vkusova Prom-st, Plovdiv*, 30 (1983) 27.
342 S Honda and S Suzuki, *Anal. Biochem*, 142 (1984) 167
343 A V Maidanyuk, Yu P. Shalin, Z A Dobrovolskaya and A S Shcherbatenko, *Fiziol Biokhim Kul't. Rast*, 16 (1984) 500
344 G J Gerwig, J P Kamerling and J F Vliegenthart, *Carbohydr Res*, 129 (1984) 149
345 P Vratny, U. A Brinkman and R. W Frei, *Anal Chem*, 57 (1985) 224.
346 J H Van den Berg, H W Horsels and R. S. Deelder, *J Liq Chromatogr*, 7 (1984) 2351.
347 M Matisova, E Gasparovicova, L. Koudelka and M Singliar, *Chem Prum*, 34 (1984) 543
348 I I Gavrilyuk, *Prikl Khromatogr*, (1984) 294, *C A*, 102 (1985) 109171g
349 S Ondrousek, G Basarova and H Loukova, *Kvasny Prum*, 30 (1984) 98
350 F Guyon, M Caude and R Rosset, *Analusis*, 12 (1984) 321
351 C Vidal-Valverde, C Martin-Villa, B Olmedilla and I Blanco, *J Liq Chromatogr.*, 8 (1985) 75
352 A C Duarte-Coelho, E D Dumoulin and I T Guetaun, *J Liq Chromatogr*, 8 (1985) 59
353 I Molnar-Perl, M Szakacs-Pinter, A Kovago and J Petroczy, *Carbohydr. Res*, 138 (1985) 83
354 H Takemoto, S Hase and T Ikenaka, *Anal. Biochem*, 145 (1985) 245.
355 B W Li, P. J. Schuhmann and W. R Wolf, *J. Agric Food Chem*, 33 (1985) 531
356 M Makkee, A P Kieboom and H Van Bekkum, *Int Sugar J.*, 87 (1985) 55
357 R Oshima, A Yoshikawa and J. Kumanotani, *J Chromatogr*, 213 (1981) 142
358 R C. Pettersen, V. H. Schwandt and M J Effland, *J Chromatogr Sci*, 22 (1984) 478.
359 M Ghebregzabher, S Rufini, B Monaldi and M. Lato, *J Chromatogr.*, 127 (1976) 133
360 L. A Th Verhaar and B F. M. Kuster, *J. Chromatogr*, 220 (1981) 313
361 H Scherz, G. Stehlik, E Bancher and K Kaindl, *J Chromatogr*, 10 (1968) 1
362 Yu S. Ovodov, E V Evtushenko, V E Vaskovsky, R G Ovodova and T F Solo'eva, *J Chromatogr*, 26 (1967) 111
363 M Ghebregzabher, S Rufini, G. Ciuffini and M Lato, *J Chromatogr*, 95 (1974) 51
364 T Mezzetti, M Lato, S Rufini and G Ciuffini, *J Chromatogr*, 63 (1971) 329