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LIQUID CHROMATOGRAPHY OF SUGARS ON SILICA-BASED STATIONARY PHASES

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CONTENTS

1. INTRODUCTION

The use of liquid chromatography for the qualitative and quantitative analysis of organic chemicals has made stormy progress in the last 10 years. Using a very finely particulated solid phase, high eluent flow-rates could be applied without loss of separation efficiency. Because high eluent pressures are necessary under such circumstances, the solid material used must be very pressure resistant.

Nowadays, high-performance liquid chromatography (HPLC) is generally performed with porous silica as the solid phase. This silica can be used either pure or in a modified form. Derivatization of the surface hydroxyl groups with organic silyl compounds results in stationary phases with a wide range of different chemical and physical properties. Because the mobile phase can also have different compositions, a

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TABLE I

SILICA-BASED STATIONARY PHASES USED IN LIQUID CHROMATOGRAPHY OF SUGARS

Trade name	*Supplier*	*Base material*				*Modification*				*Used by (ref.)*
						Chemical				
		Irregular or spherical	*Pore diameter (Å)*	*Surface area (m^2 g)*	*Pore volume (ml g)*	*Alkylamine*	*Alkylnitrile*	*Octadecyl*	*Dynamical by adding amine to the eluent*	
μBondapak carbohydrate	Waters Assoc.	I	125			+				1,2,4,9,10,18,24,29,30,35,36
LiChrosorb NH_2	Merck	I	60	500	0.75	+				26,33,40
Micropak NH_2	Varian	I	60	500	0.75	+				8
Chromosorb NH_2	Johns-Manville					+				33
Partisil-10 PAC	Whatman	I	60	400			+			7
Chromosorb LC 9	Johns-Manville					+				28
No. 71120	Macherey, Nagel & Co.					+				32
LiChrosorb Si 100	Merck	I	100	250	1.0	+				12
Partisil-5	Whatman	I	60	400		+				15
Silasorb	Luchema	I		260		+	*			39
				600		+				39
LiChrosphere Si 100	Merck	S	100	250	1.2	+				3,11,14
LiChrosorb Si 60	Merck	I	60	500	0.75				+	20,31,34,41,42
?	?	I	130	320					+	23
μBondapak C_{18} Corasil	Waters Assoc.	S	(pellicular)					+		25,37,38
LiChrosorb Si 60	Merck	I	60	500	0.75					5,13,14,16,19,22
Partisil	Whatman	I	60	400						17
μPorasil	Waters Assoc.	I		400						21,27
Corasil II	Waters Assoc.	S	(pellicular)							6

* Own modification.

wide variety of phase systems is the result and most separations can be effected in a short time.

The components to be analysed remain in the liquid phase and, therefore, the method is very well suited to the analysis of thermally unstable compounds such as sugars. It is not surprizing that, after the first publications of Linden and Lawhead[1] and Palmer[2] on HPLC for sugar analysis, many others followed[3–42]. In order to obtain rapidly an idea of how to analyse a given sugar mixture, we made a survey of these publications. The different types of silica and the mobile phases used are mentioned, as well as the method of column preparation, possible pre-column derivatization and the detection method applied. Tables are presented that give an overview of the different sugars and sugar-containing samples analysed by the authors with the methods concerned.

2. COLUMN MATERIAL AND PREPARATION

Silica-based HPLC of sugars is carried out with a variety of silicas, as shown in Table 1. However, for both modified and unmodified materials a base quality is used with pore diameter 60–130 Å and surface area 250–600 m^2/g. Chemical modification is generally performed by the manufacturer, introducing alkylamine or octadecyl groups at surface hydroxyl groups through silyl ether bonds. However, the manufacturers do not give exact details.

Schwarzenbach[3,11,14] and in a similar way Hunt *et al.*[12], Jones *et al.*[15] and Kahle and Tesařík[39] carried out their own modification using 3-aminopropyltrieth-oxysilane. Because their chromatographic results are very similar to those obtained using prefabricated amine columns, the manufacturers' procedures will not be very different.

Apart from chemical modification, physical modification is also applied following the publications of Aitzetmüller and co-workers[20,31,42]. A small amount of amine is added to the eluent and the initially pure silica column acquires an amine coating due to simple adsorption.

Table 2 shows that in half of the references the authors prefer pre-packed columns. For the self-made columns the slurry method and the balanced density method are frequently used.

As shown in Table 3, amine-modified silica columns are almost exclusively used with an acetonitrile (ACN)–water eluent, and the sugars are analysed without derivatization. Pure silica is mainly applied for the separation of sugar derivatives using other eluents; it will be mentioned in section 3.

2.1. Chemically modified amine columns

Jones *et al.*[15] investigated the influence of the silica:silane ratio, the reaction temperature and the reaction time on the amine load using aminopropyl reagent. He found that the amine load was virtually independent of the temperature and time of reaction but was strongly dependent on the amount of reagent added. Too much silane leads to "strand structures" of several silica particles owing to reagent polymerization, especially if the silica has not been sufficiently well dried. Such material cannot be packed properly and columns with low separation efficiency are the result.

TABLE 2

SILICA-BASED HPLC COLUMNS FOR SUGAR ANALYSIS

Column preparation method	*Used by (ref.)*		
	Unmodified silica gel	*Alkylamine modification*	*Octadecyl modification*
Slurry packed	14,20,31, 34,41,42	3,11,12,14, 26,28,39	
Balanced-density packed	13,16,19, 22	7,15,32,33, 40	
Pre-packed	6,17,21,27	1,2,4,8,9,10, 18,24,29,30, 35,36	25,37,38

According to Woidich *et al.*[26], LiChrosorb NH_2 columns are serviceable for approximately 3 months when in permanent use. The amine groups bound by silyl ether are stripped off gradually, and the silica is also slowly dissolved by the eluent; decreased retention and resolution result. Although decreased retention can be compensated for by a lower water content of the ACN–water eluent, the column will finally become of inferior quality. Continuous addition of amine to the eluent was tried in order to overcome this problem.

TABLE 3

PHASE SYSTEMS USED FOR CHROMATOGRAPHIC SEPARATION OF SUGAR COMPOUNDS

Mobile phase		*Stationary phase*		*Separation*		*Ref.*
Acetonitrile–water	*Other composition*	*Silica gel, pure*	*Silica gel, chemical modification*	*Directly*	*After derivatization*	
+			+ Amine	+		1–4,8–12,14,15,18,24, 26,28–30,32,33,35,36, 39,40
+			+ Amine (physically)	+		20,23,31,34,41,42
+			+ Nitrile	+		7
+			+ Octadecyl		+	25
+		+		+		17,22
+		+			+	17
	+		+ Amine	+		32,40
	+		+ Octadecyl	+		37,38
	+	+		+		5
	+	+			+	6,13,14,16,17,19,21,27

2.2. *Physically modified amine columns*

Aitzetmüller[20] used a polyfunctional amine (*A*mine *M*odifier *I*) of concentration 0.01 % together with LiChrosorb Si 60, and obtained results similar to those with chemically modified columns, if slightly higher water contents in the eluent were applied. This higher water content seems to be in contradiction with the experience of Wheals and White[23], who investigated the applicability of several amines and found that retention ability decreased in the order chemically bonded aminopropyl (and no amine added to the eluent), polyamine, diamine, amine. According to White *et al.*[41], 1,4-diaminobutane is better than other amines, including polyamines, if a series of glucose polymers have to be separated. Apparently the proper choice of amine is dependent on the type of sugars to be analysed. Aitzetmüller *et al.*[31] also tested silicas other than LiChrosorb Si 60 and obtained similar column qualities.

Aitzetmüller, Wheals and White and White *et al.* used an amine concentration of 0.01 %. If one starts with an amine-free column it takes a long time to reach an equilibrium state using this concentration. According to Aitzetmüller[42], the procedure can be speeded up by using a 0.1 % concentration overnight, subsequently changing it to 0.01 %.

Aitzetmüller also considers the use of a pre-column to be important. If this column is packed with silica of similar quality, the silica in the analytical column will not hydrolyse owing to the high eluent pH. The pre-column should be mounted before the injection valve in order to cause no increase in peak widths due to the dead volume created on silica dissolution.

The following advantages and disadvantages of physically modified amine columns were mentioned by Aitzetmüller[42]:

Advantages:

constant retention after prolonged use;

cheap material compared with the expensive modified silica;

easy modification;

the possibility of using eluents with a higher water content, which results in higher sugar solubility and lower peak tailing;

an increased isocratic range, which means that a relatively wide range of sugars with different molecular weights can be separated with an eluent of constant composition;

less sensitive to pollution by samples.

Disadvantages:

preparative chromatography can be difficult owing to the presence of amine in the eluent;

certain detection methods give problems when using amine in the eluent, *e.g.*, UV and moving-wire detection;

acid-containing samples, *e.g.*, from citrus-drinks, cause baseline disturbance owing to the variable amine delivery.

The changed sensitivity to the refractive index (RI) detector as a result of the higher water content of the eluent was considered to be a disadvantage by Aitzetmül-

ler, whereas Woidich *et al.*[26] considered it to be an advantage because the sensitivity for sugars increases.

2.3. *Octadecyl columns*

To separate an acetylated starch hydrolysate, Wells and Lester[25] used a RP-18 column. On changing the ACN content in an ACN–water eluent during the analysis from 10 to 70% a good separation is obtained from DP 1 up to DP 35 (DP = degree of polymerization).

Heyraud and Rinaudo[37] and Fonknechten *et al.*[38] analysed underivatized sugars on an octadecyl column with pure water as the eluent. Both groups applied a low eluent flow-rate (0.1 and 0.33 ml/min, respectively, using 4 mm I.D. columns). Heyraud and Rinaudo used a column temperature of 3.5°C: they stated that the separation efficiency decreases with increasing temperature and with increasing eluent flow-rate.

2.4. *Silica columns*

As mentioned before, pure silica is used almost exclusively for the separation of derivated sugars. Binary or ternary mixtures of organic solvents of different polarity are used as eluents.

Rocca and Rouchouse[5] eluted underivatized sugars on a LiChrosorb Si 60 column with a mixture of ethyl formate, methanol and water. McGinnis and Fang[17] mentioned a separation of underivatized sugars with ACN–water (9:1) as the eluent, whereas Van Olst and Joosten[22] used ACN–water (99.9:0.1).

3. ELUENT SYSTEMS AND ELUTION MECHANISM

3.1. *Chemically and physically modified amine columns*

A mixture of acetonitrile and water is the most commonly applied eluent with this type of column. The water content is usually between 10 and 40% (v/v), depending on the sample composition. An increase in the water content decreases the retention without changing the elution sequence. Fig. 1 shows a plot of elution times of sugars obtained by Meagher and Furst[4] against the number of carbon atoms in the molecule. As can be seen, the elution sequence follows the order of molecular size. Identical results are obtained with similar data from other references. On the one hand it is impossible to change the elution sequence, which is a limitation, but on the other hand the elution time gives qualitative information about the molecular size of the sugar.

The separations are carried out at room temperature. Hunt *et al.*[12] found that elution times decreased with increasing temperature, but at the same time the noise level of the RI detector increased.

Different elution mechanisms are proposed in the literature. Meagher and Furst[4] considered it to be reversed-phase chromatography, probably because of the organic modification of the silica. Because an increased water content in the eluent or an increased polarity speeded up the elution, Rabel *et al.*[7] and Hettinger and Majors[8]

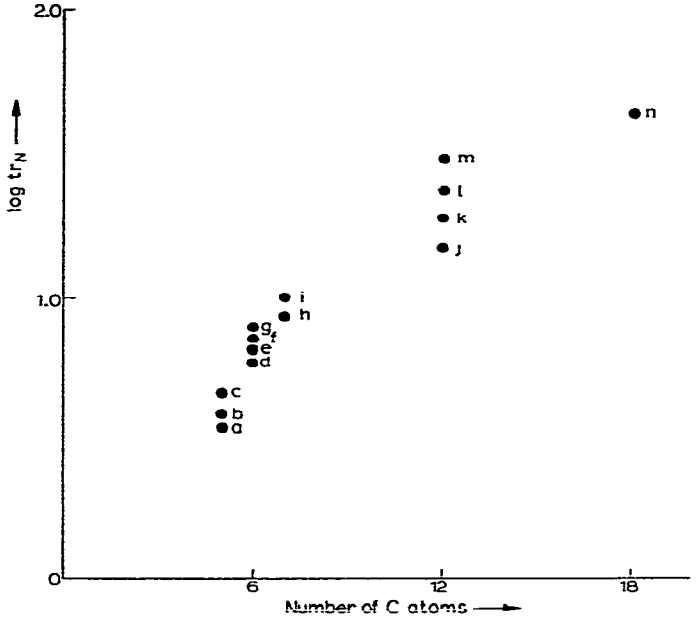


Fig. 1. Plot of elution times (tr_N) of sugars *versus* carbon number (data from Meagher and Furst[4]). Stationary phase, μBondapak carbohydrate; mobile phase, acetonitrile–water. Compounds: (a) ribose; (b) xylose; (c) L-arabinose; (d) fructose; (e) mannose; (f) glucose; (g) galactose; (h) mannoheptulose; (i) glucoheptose; (j) sucrose; (k) maltose; (l) lactose; (m) melibiose; (n) raffinose.

concluded the process to be normal-phase chromatography. Hettinger and Majors assumed that a competitive interaction of water and sugar with the bonded phase caused the retention. Wong-Chong and Martin[29] stated it to be adsorption chromatography, where the eluent polarity is important. Binder[32] explained retention by hydrogen bonding between sugar hydroxyls and amine groups in the solid phase. With more hydroxyl groups, there is more hydrogen bonding and, therefore, increased retention. D'Amboise *et al.*[33] gave the same opinion; further, he stated that retention increases if the hydroxyls are one-sided in the sugar molecule. This explanation seems to be supported by the results of Jones *et al.*[15], who found a linear relation between k' values of several sugars and the amine load. At a high load the k' values become constant, which Jones *et al.* ascribed to phase polymerization after a monolayer of amine has been formed. Verhaar and Kuster[43] measured a higher water content in the stagnant liquid phase in comparison with the eluent using a LiChrosorb NH_2–ACN–water system. This is probably caused by the hydrophilic nature of the amine. Sugars preferred a water-rich phase and so retention occurred.

Attempts have also been made to use alternative eluents with chemically modified silica columns. Rabel *et al.*[7] obtained poor results with methanol–water as the eluent, which they ascribed to the relatively poor solution of the bonded phase in methanol. The results obtained with an ethyl acetate–ethanol–water mixture, as applied by Binder[32], are also less satisfactory than those obtained with ACN–water as the eluent. Similar results to those obtained with the latter eluent can be obtained with a mixture of acetone, ethyl acetate, and water, as reported by Müller and

Siepe[40]. This eluent has the advantage of the absence of poisonous acetonitrile; an increase in the water content has a similar influence on the elution pattern to that in the ACN–water system.

Because elution patterns can hardly be changed, data from different references could be combined, and relative retention times for some common sugars are presented in Table 4.

TABLE 4

RELATIVE RETENTION TIMES OF UNDERIVATIZED SUGARS ON AN ALKYLAMINE-MODIFIED SILICA COLUMN WITH ACETONITRILE–WATER (80:20) AS ELUENT

Sugar	*Relative retention time**	*Sugar*	*Relative retention time**
Ribose	60	Psicose	55
Lyxose	65	Fructose	80
Xylose	70		
Arabinose	80		
Glucose	100	Rhamnose	65
Mannose	90	Fucose	70
Galactose	110		
Maltose	180	Glycerol	40
Cellobiose	180	Xylitol	75
Lactose	200	Arabinitol	80
Saccharose	150	Glucitol	95
Melibiose	240	Mannitol	105
Raffinose	350	Galactitol	105

* Glucose = 100

Normally, amine columns do not separate anomeric forms of reducing sugars. Verhaar and Kusters[43] showed this to be caused by the catalytic activity of the bonded amine for the mutarotation reaction. At high reaction rates the anomeric forms have the same average residence time and a single symmetrical peak is the result, and interpretation of the chromatogram is simplified, as reported by Cerny *et al.*[36]. The addition of acids or salts to the eluent, investigated by Rabel *et al.*[7], influences the peak quality, but no clear mechanism could be given. By converting an amine column into the sulphate form, Kahle and Tesarik[39] succeeded in separating anomeric forms using ACN–water as the eluent and a column temperature of 0°C. For all D-sugars investigated the α-form elutes before the β-form, which should be due to the larger number of equatorial hydroxyl groups for the β-form, and these groups mainly defined the retention.

3.2. Octadecyl columns

As mentioned before, an excellent separation of acetylated starch hydrolysate was obtained by Wells and Lester[25] by applying a negative water gradient in an ACN–water eluent. Poor results were obtained by Heyraud and Rinaudo[37] using pure water as the eluent for the separation of mono- and disaccharides. The elution

volume of the first and the last sugars in a series of 17 differed by only 1 ml, whereas for the baseline separation of two sugars a difference of 0.4 ml is necessary. According to Heyraud and Rinaudo[37] and Fonknechten *et al.*[38], this system can be better applied to the separation of sugars with different numbers of monomeric units. In about 30 min an almost complete separation can be obtained from DP 1 up to DP 10. Especially at low column temperatures double peaks occur for the anomeric forms because this system lacks a mutarotation catalyst, in contrast to the system mentioned previously.

3.3. Silica columns

Derivatization of sugars with acetyl-, benzoyl- or nitrobenzoate compounds leads to increased retention with increasing molecular weight when using silica columns and a mixture of organic solvents as the eluent. Generally, separate peaks for the anomeric forms are obtained on acetylation and also on benzoylation with benzoyl or nitrobenzoyl chloride. Single peaks are obtained if derivatization is accompanied by ring opening, as carried out by Thompson[21] using as the reagent benzoyl chloride and benzyloxyamine. Because more peaks need more space in the chromatogram, the number of sugars that can be separated is decreased by the occurrence of anomeric forms and, according to Cerny *et al.*[36], quantitative interpretation is also hampered.

Thiem *et al.*[19] analysed acetylated sugars with acetone–hexane as the eluent, and reported a k' value that increased with decreasing eluent pressure or eluent flow. However, in an earlier publication Thiem *et al.*[16] reported the opposite effect.

4. DETECTION METHODS

The different detection methods applied are presented in Table 5. The refractive index detector is most generally used, and almost solely for underivatized sugars, as is clear on comparing the data in Tables 5 and 3. Derivatized sugars are preferably detected with UV absorption. Apart from the methods mentioned in Table 5, the applicability of a micro-polarimeter and mass detector will also be discussed.

4.1. Refractive index detector

This method is universally applicable, only moderately sensitive and very sensi-

TABLE 5

DETECTION SYSTEMS USED IN SILICA-BASED HPLC

Method of detection	*Ref.*
Refraction index	1–12,14,15,17–20,22–24,26, 28–32,34–42
Ultraviolet	6,8,13*,16*,19,21*,27*,32
Visible light	28**, 33**
Moving wire	25

* After pre-column derivatization.

** After post-column derivatization with tetrazolium blue.

tive to temperature fluctuations. The refractive index of the compounds to be detected should differ, of course, from that of the eluent. The sensitivity of the RI detector towards a given compound is dependent on the eluent composition and therefore the method is not suitable for gradient systems. According to Woidich *et al.*[26] and Black and Glover[35], the response for sugars increases with increasing water content of an ACN–water eluent. Johncock and Wagstaffe[34] considered both long-term and short-term instability to be due to temperature fluctuations in the detector cell. Hunt *et al.*[12] mentioned increased noise when using higher column temperatures. Our experience is that at reasonably constant room temperature no special temperature control is necessary.

The detection limit depends on the retention time and the column quality. Minimal detectable amounts of 20 μg of sugars were reported by Palmer[2], whereas Cerny *et al.*[36] mentioned a value of 3 μg at a signal-to-noise ratio of 5. Schwarzenbach[14] reported that the minimal detectable amount can be decreased by a factor of several thousand by using nitrobenzoate derivatization in a pre-column mode.

4.2. Ultraviolet detector

This method is suitable only for UV-absorbing compounds, it is very sensitive and is only moderately influenced by temperature fluctuations. Sugars can be detected directly in the range 185–195 nm. Using an ACN–water eluent, the ACN must be of analytical-reagent grade because impurities generally give strong UV absorption. Derivatized sugars can be detected at higher wavelengths and generally their detectability is improved drastically.

Hettinger and Majors[8] and Binder[32] used 192 and 188 nm, respectively, for direct detection. According to Hettinger and Majors, for glucose and fructose the sensitivity is approximately 10 times higher than that in RI detection. For sugars of higher molecular weight the sensitivity decreased to the values for RI detection. Binder analysed a number of monosaccharides and found almost equal sensitivity for the two methods.

Thiem and co-workers[16,19] detected acetylated sugars at 220 nm. In the eluent used, acetone–*n*-hexane, only a small amount of acetone can be used because of its own absorption. Lehrfeld[6] and White *et al.*[27] detected benzoyl esters of sugars at 254 nm, a commonly available fixed wavelength in UV detectors, and obtained a 1000-fold increase in sensitivity. According to Nachtmann and Budna[13], an even higher sensitivity can be achieved by esterification with nitrobenzoate, so this could be a good method in trace analysis.

A disadvantage of derivatization, especially with large ester groups, could be the reduction in the differences in molecular structure, through which the separation of hexoses, for example, could become more difficult. However, for sugars with a different number of hydroxyl groups such as starch hydrolysate products, separation could be more efficient. Using an eluent composition that does not absorb UV light at the choosen wavelength, a gradient system can be used, as shown by Lehrfeld[6].

4.3. Visible light–fluorescence detection

In comparison with RI detection, an increased sensitivity for sugars can be

TABLE 6

APPLICATION OF SILICA-BASED CHROMATOGRAPHIC MATERIALS TO SUGAR ANALYSIS IN DRINKS, FOODS, PROCESS AND PLANT MATERIALS AND OTHER SUGAR-CONTAINING SAMPLES

Material	*Ref.*	*Material*	*Ref.*
Drinks:		*Proces materials:*	
Apple cider	2,9	Cellulose hydrolysate	2,9,37,43
Beverage	7	Dextran	43
Breakfast drinks	26	Fructose isomerization	24
Fruit drinks	9,11,14, 36,40,42	Glucose isomerization	22
		High fructose syrup	9,22
Lemonades	11,42	Inulin hydrolysate	33
Milk	7,9,30	Polyglycerol	9
		Starch syrup	3,7,9,25,28,37,42
Foods:		Xylan	28,43
Breakfast cereals	15		
Carrot purée	40	*Plant materials:*	
Cocoa	9	Cotton seed	10
Confectionery	34	Maple sugar	7
Corn products	40	Soybeans	7,9,10,15,31,35
Fruit purée	40	Sugar cane	29
Honey	9,11,42	Sun flowers	10
Ice cream	15,36	Tomato cell wall	9
Jam	42	Wheat straw	18
Kidney beans	9	Wood	28
Peanuts	10		
Sauces	40	*Others:*	
Toffees	12	Intravenous high calorie electrolyte solution	7
		Narcotics	23
		Pharmaceutical syrup	13
		Rat urine	4
		Tobacco humectant	14
		Toothpaste	14

TABLE 7

METHODS OF SUGAR DERIVATIZATION FOR HPLC ANALYSIS

Derivatization method	*Ref.*
Methylation	17
Acetylation	16,17,19,25
Benzoylation:	
With benzoyl chloride	6,27
With 4-nitrobenzoyl chloride	13,14,27
With benzyloxyamine hydrochloride	21

obtained by post-column derivatization, already used in sugar analysis with ion exchangers and borate buffers[44]. In HPLC narrow peaks are obtained, so the continuous flow reaction system in wide-bore glass tubes is replaced by a reaction system in capillary tubes having a much lower peak broadening. Because these tubes have a

TABLE 8

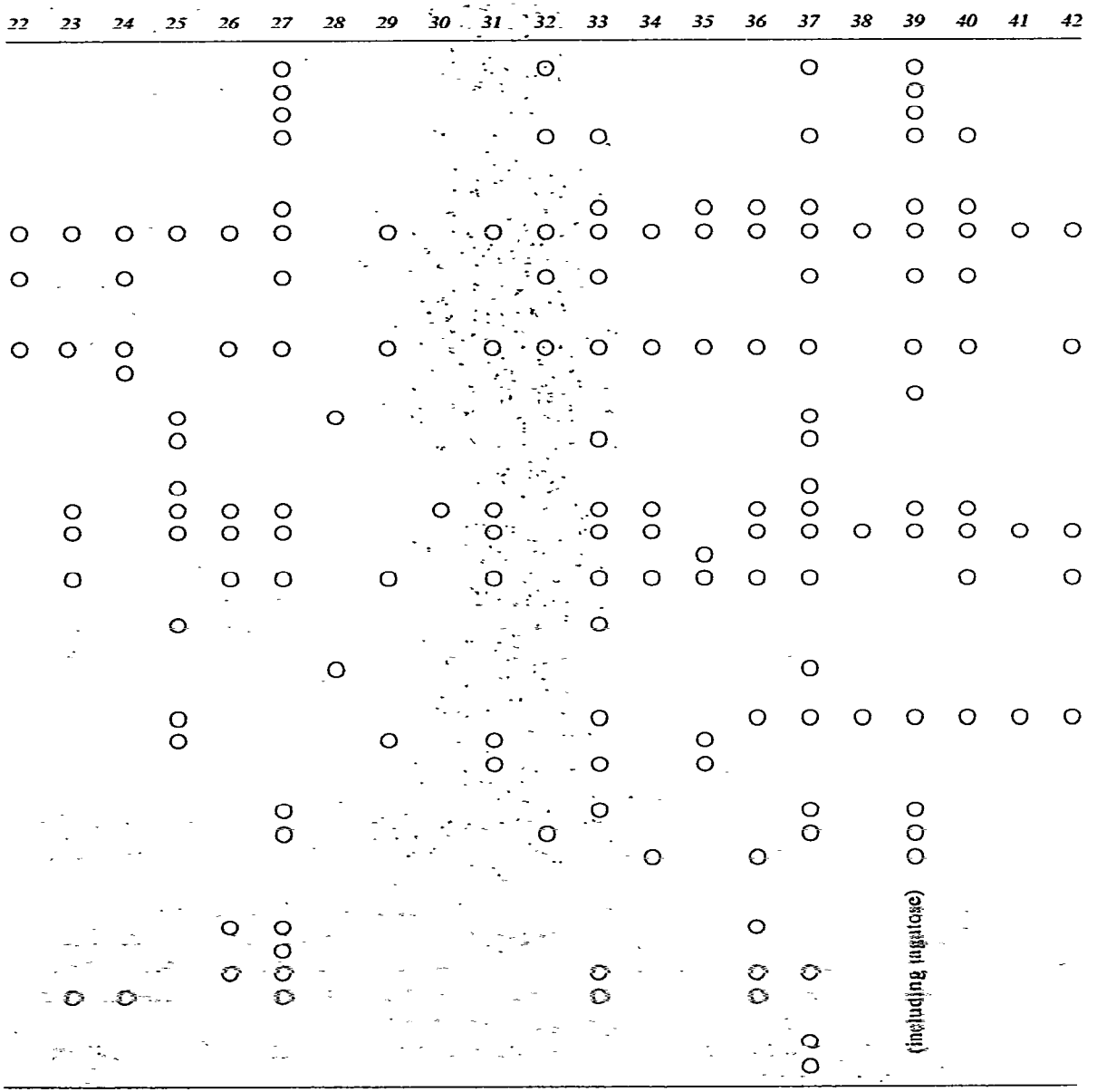
SUGAR COMPOUNDS CHROMATOGRAPHED

Sugar compounds	*Ref.*																			
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*	*19*	*20*	*21*
Arabinose		○	○	○	○		○	○	○								○	○	○		○
Lyxose							○												○		
Ribose		○	○	○	○		○	○				○					○		○		○
Xylose		○	○	○	○	○	○	○	○								○	○	○		○
Allose																○			○		
Galactose	○	○		○	○	○	○	○	○							○	○		○		○
Glucose	○	○	○	○	○	○	○	○	○	○	○	○	○		○	○	○	○	○	○	○
Idose																○			○		
Mannose		○		○		○	○		○							○	○	○	○		
Tallose																○			○		
Fructose	○	○	○	○	○		○	○	○		○	○	○		○		○	○		○	○
Psicose									○												
Sorbose					○																
Cellobiose		○			○		○		○								○	○	○		
Gentiobiose									○										○		
Isomaltose																					
Lactose		○	○	○	○	○	○	○	○			○	○		○				○	○	○
Maltose	○	○	○	○	○	○	○		○		○				○				○	○	○
Melibiose	○			○			○														
Sucrose	○	○	○	○	○	○	○	○	○	○	○	○	○		○				○	○	○
Trehalose					○			○		○											○
Cellotriose (and higher)		○																○			
Maltotriose (and higher)	○		○			○	○		○												
Raffinose	○			○	○		○	○	○	○										○	
Stachyose									○	○											
Fucose		○		○			○														
Rhamnose		○	○	○					○										○		
Glycerol			○	○		○			○		○		○							○	
Erythritol																	○				
Arabinitol									○					○			○				
Xylitol			○											○							○
Galactitol					○				○												
Glucitol			○		○				○		○		○	○						○	
Mannitol					○				○		○			○							○
Cellobitol																					
Maltitol																					

(including rare sugars)

(including altrose)

high flow resistance, an additional pump is necessary which can deliver a liquid flow at the proper pressure. Post-column derivatization with tetrazolium blue and absorption measurement at 530 nm was used by Noël *et al.*[28] and D'Amboise *et al.*[33]. Another type of post-column derivatization is the reaction of sugars with cerium(IV),



as mentioned by Katz and Pitt[45]. This permits fluorescence measurement, as reported by Mrochek *et al.*[46].

4.4. Moving-wire detection

In this method, underneath the column exit a clean platinum wire is moved continuously and becomes moistened with the pure eluent or with eluent containing the component to be detected. The eluent is evaporated and the residual material is combusted to carbon dioxide in a pyrolysis oven. This carbon dioxide is reduced to methane, and the methane is detected with a flame-ionization detector (FID). The method can be combined very well with a gradient system, as reported by Wells and Lester[25]. However, the manufacturer, Philips–Pye Unicam, took this detector out of production in 1978.

4.5. Polarimetric detection

The development of a micro-design of the polarimeter made this instrument applicable in HPLC, as shown by Yeung *et al.*[47] and Böhme[48]; the latter applied it to the detection of sugars. Because the anomeric forms give a different response, an additional problem occurs in quantitative analysis. If anomers elute in the same time, for accurate quantitation the anomer ratio should be constant or known. However, the polarity of the response can give additional qualitative information. The applicability to gradient elution using optically inactive eluents is another advantage. The sensitivity for sugars is lower than that in direct RI or UV detection.

4.6. Mass detection[49]

In the mass detector, designed by Applied Chromatography Systems Ltd., the eluent stream containing the solute is nebulized and carried by an air stream through a heated column. The eluent evaporates, leaving a fine mist of solute particles which pass through a light beam. Light scattering occurs and is detected by a photomultiplier. Except with eluents to which salts have been added, the method can also be used for sugar detection and the minimal detectable amount is about 500 ng.

5. MISCELLANEOUS

5.1. Sample pre-treatment

Before injection on to the chromatographic column can be carried out, the sample generally needs pre-treatment. Sugars often occur in natural substances together with proteins and fats, which interfere in the analysis. According to Meagher and Furst[4], proteins can cause considerable pressure resistance in the column if not removed, which can be effected by ultrafiltration or dialysis. Because the pre-treatment is closely related to the origin of the sample, a survey of sugar-containing products analysed by different authors is given in Table 6. In the publications concerned, good descriptions of the pre-treatments are generally given.

Another pre-treatment is derivatization, as mentioned before. Table 7 lists the different methods used.

5.2. *Choice of chromatographic system*

The choice of the chromatographic system depends on a number of factors, such as available apparatus and sugar concentration in the sample. Because it is impossible to mention all factors, Table 8 lists many sugars that have been analysed. This table makes it easy to find information about the analysis of a particular sugar.

6. SUMMARY

A review is given of sugar analysis by liquid chromatography using silica columns. Aspects covered are column materials and preparation, chemically and physically modified amine columns, octadecyl- and unmodified silica columns; eluent composition and elution mechanisms for the different types of columns used; detection methods, RI and UV detectors, visible light, fluorescence, moving-wire, polarimetric and mass detection; and sample preparation and origin of samples.

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