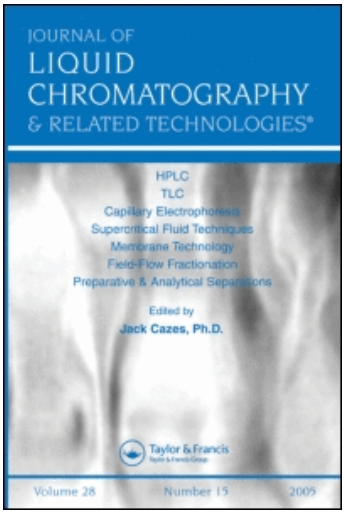

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Review High Performance Liquid Chromatography of Mono-And Oligosaccharides

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Review

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY OF MONO- AND OLIGOSACCHARIDES

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A. SUMMARY

The separation and determination of various monosaccharides and oligosaccharides by different HPLC techniques, published in the period from the second part of 1983 up to December 1987, are reviewed. However, several earlier works of particular significance are also covered. This review includes a systematic description of the important elements in an advanced HPLC system for analysis of saccharides: stationary phases for columns, specific mobile phases, the use of precolumn or postcolumn derivatization and the choise of detector for each specific analysis. The most significant developments of the last decade are the increasing use of bonded-phase

column packings based on silica, as well as postcolumn labeling techniques. The developments leading to advances in the HPLC of mono- and oligosaccharides in recent years are reported and, sometimes, discussed.

This article has been designed to summarize, in a tabular form, the chromatographic conditions of recent HPLC research efforts for analysis of mono- and oligosaccharides. Common and specially developed detection systems for monitoring the HPLC separated saccharides in the column effluent are described and classified.

The applications of HPLC analysis of mono- and oligosaccharides are classified, in a tabular form, in four principal groups.

B. INTRODUCTION

Modern high performance liquid chromatography (HPLC) was introduced as an advanced analytical technique in the late 1960's. The properties which make HPLC such a preferable and widespread technique, namely versatility, simplicity, selectivity, sensitivity, are rooted in the chromatographic theory that evolved from Martin and Synge's [1]. The development of high performance columns for separation of non-volatile substances, as well as of improved and sensitive detectors has accelerated the rapid growth of HPLC.

The parameters of importance in choosing an HPLC system for separation of saccharides are: elution pattern, analysis time, the resolution of complex mixtures and column efficiency, stability and life time.

The mono- and oligosaccharides are an important energy source. They are involved in the structure of polysaccharides, proteins and lipids. Generally the monosaccharides are comprised of the aldoses, ketoses, alditols, aldonic acids, uronic acids. In addition, there are also their modifications, such as deoxy- and deoxyamino derivatives. Oligosaccharides are built up of various combinations of monosaccharides in a diverse position of attachment and of anomeric configuration of glycosidic linkages.

Column chromatography of carbohydrates dates from 1939. when Reich described the separation of azoyl derivatives of sugars [2]. Alumina, as stationary phase, has found minimal application in the saccharides field, since most of these compounds are highly polar and consequently strongly adsorbed. In addition, the basic character of alumina causes sometimes epimerization.

In the 1950's and 1960's cellulose based partition columns [3], and low resolution charcoal columns [4,5] were used. The development of polar bonded-phase materials based on silica gel particles (5-10 μm) has exploited the full advantages of HPLC. Today, HPLC offers an excellent alternative to gas chromatography for the qualitative and quantitative analysis of saccharides with the advantages of high resolution, short analysis time, direct injection of the sample without or with little pretreatment, and easy automation [6]. The HPLC analyses of saccharides available today are sensitive, reliable and reproducible due to the development of new packing materials, detection systems as well as to successive improvements in overall instrumentation. Today, the time unit needed for the analysis is minutes instead of hours. In the last several years HPLC has become a powerful tool for separation of saccharide mixtures.

Several reviews [7,8,9] attest to the importance of chromatography in carbohydrate analyses, including, particularly, HPLC techniques. Precolumn derivatization of saccharides was reviewed by Ross [10]. Partition chromatography of sugars on silica-based stationary phases was reviewed by Verhaar and Kuster [11]. A recent review on HPLC of mono- and oligosaccharides, covering articles up to 1983, was prepared by Sususmu [12]. An extensive review on the application of various types of chromatography for analysis of mono- and disaccharides was published by Robards and Whitelaw [13].

The present review summarizes, in a tabular form, the experimental conditions of HPLC analyses of mono- and oligosaccharides in the period from the second half of 1983 up to the end of 1987. Some earlier literature is also quoted when it is of particular significance. For each case, important chromatographic parameters as such stationary phase, mobile phase, possible pre- or postcolumn derivatization and the type of detection are reported (Tables 1 and 2).

HPLC analysis of saccharides in various natural products, as foods, drinks, juices, dietary fibers, vegetable fibers, oils, beans, roots, biological fluids has been performed by many researchers (Tables 1 and 2). The great amount of works on separation and quantitation of different saccharides by HPLC demonstrates that the recent progress of carbohydrate chemistry has been associated with the development of HPLC.

C. TECHNIQUES AND STATIONARY PHASES FOR HPLC SEPARATION

The analysis of saccharide mixtures by HPLC techniques is more and more widely applied. No general HPLC conditions have been

Table 1. Experimental HPLC conditions used for determination of monosaccharides

No.	Sample	Analyte	Column	Eluent(v/v)	Pre- and post column reaction	Detector	Ref.
1	Human serum	Glucose, mannose	Anion IEX 222, 12μ; three connected columns	0.02M, sodium tetraborate and 0.1M boric acid; pH 8.98		Amperometry	18
2	Hydrolyzates of volcanic ash soil	Aldopentoses and aldohexoses	Anion exchange resin Shimadzu ISA-07/S2504	Gradient: 0.15M boric acid (pH 8.0) to 0.4M boric acid (pH 9.0)	Post-column with monoethanolamine	Fl. λ_{ex} 360 nm λ_{em} 445 nm	19
3	Mixture of reducing mono- and disaccharides	Arabinose, ribose xylose, glucose, mannose, galactose, fructose, rhamnose	1) Shodex SC-1821 2) Hitachi 2633 3) Lichrosorb NH_2	1) H_2O 2) 0.7M borate buffer and 0.01% EDTA acid 3) MeCN-H_2O (3:1)	Post-column with ethylenediamine sulphate	Amperometry	20
4	Mixtures of red. and nonreducing ssaccharides	Reducing pentoses, hexoses. Non reducing saccharides	Aminex A-27 or Shimadzu ISA-07/S2504	Borate buffer (pH 8.7)	Post-column with taurine-2 mol/m^3 sod. metaperiodate at 140°C	Fl.	21
5	Needles of healthy and damaged Picea trees	Ribose, fructose, glucose	Aminex A-25 Borate format	0.4M Boric acid (pH 9.35)	Post-column with ethanolamine-boric acid	Fl. λ_{ex} 342 nm λ_{em} 422 nm	22
6	Market milk; milk products	Glucose, galactose	MCI gel CA0 8S	Stepwise: 0.2M potassium borate (pH 7.8) to 0.5M potassium borate (pH 8.7)	Post-column reaction with 2-cyanoacetamide	UV, 280 nm	23
7	Mixture of mono- and disaccharides	Ribose, fucose, xylose, arabinose, fructose, galactose	1) 4VP (crosslinked) 20% DVB 2) 4VP-Bu as bromide, phosphate, sulphate, nitrate	MeCN-H_2O (4:1)		RI	26
8	Molasses, sugarcane juice syrup, mud	Glucose, fructose	1) Sugar PAK (Ca^{2+}) 2) HPX 87C	Solution of calcium propionate or acetate in water		RI	32
9	Apple juices	Glucose, fructose	Sugar PAK 1 (Ca^{2+})	0.1% calcium acetate in H_2O		RI	33
10	Reducing monosaccharides; sugar beet leaves	Glucose, fructose	Ostion LGKSO 800	H_2O	Post-column with p-aminobenzoic acid hydrazide	UV-Vis	35
11	Human seminal plasma	Ribose, fructose, sorbitol inositol, lactic acid	Aminex HPX-87H	0.01N H_2SO_4		RI	36
12	Cotton leaves	Glucose	Aminex HPX-87H	0.014N H_2SO_4		RI, UV-Vis	37
13	Fermentation mixtures metabolized by intestinal microflora	Glucose, fructose, galactose	Aminex HPX-87H	0.028M H_2SO_4 (pH 1.5) at 40°C		RI	38

Table 1. (Cont.)

No.	Sample	Analyte	Column	Eluent(v/v)	Pre- and post column reaction	Detector	Ref.
14	Hydrolyzates from fibres of barley, sorghum, bran	Glucose, xylose, arabinose, mannose, galactose, rhamnose	1) Alltech-NH_2 2) Aminex HPX-87P	1) MeCN-H_2O 2) H_2O		RI	39
15	Dietary soft drink	Glucose, fructose	HPX-87A	0.01N H_2SO_4		RI	40
16	Mixtures of various mono- and disacchs.	l-arabinose, d-xylose, d-glucose, d-mannose	Aminex HPX-87H	0.01N H_2SO_4		RI	43
17	Cane and beet molasses	Glucose, fructose	1) Resolution carbohydrate in Na^+ or Ca^{2+} form 2) Resolution ODS5 3) Resolution $NH_2$5	1) H_2O 2) H_2O 3) MeCN-0.01M KH_2PO_4 (pH7) (74:26)		RI, UV	45
18	Beer, wine, fruit juice, soft drinks	Ribose, glucose, fructose	1) Aminex HPX-85C 2) Micro Pak-NH_2	1) H_2O 2) 70% aq. MeCN, gradient 90 to 60%	Post-column with 2-cyanoacetamide	FI	46
19	Sucrose after phosphorylysis	Fructose, glucose-1-phosphate	Aminex A-7	0.03M aq. H_2SO_4 and 0.01 to 0.06M aq. CF_3CO_2H		RI	47
20	Food and fecal neutral detergent fiber	Arabinose, xylose, glucose, mannose, galactose, rhamnose	1) Bondapak carbohydrate 2) Aminex HPX-85P	1) MeCN-H_2O (85:15) 2) H_2O		RI	48
21	Saccharide components of proteoglycans	Xylose, fructose, mannose, galactose, fucose	Aminex 87P, with anionic and cationic guard colmns. in series	H_2O	1) Precolumn radioactive labeling 2) Orcinol 3) 2-cyanoacetamide	1) Radioactivity 2) Vis, 420nm 3) UV, 276nm	49
22	Hydrolyzates of bacterial lipopolysaccharides	Glucose, galactose, rhamnose, glyceromannoheptose	1) Nucleosil 5-NH_2 2) HP-87P	1) MeCN-H_2O (75:25) 2) H_2O		RI	50
23	Dry wines	Arabinose, ribose, fructose, rhamnose, xylose	HPX-87P and precolumn - Aminex HPX-87C	H_2O	Post-column with tetrazolium blue	Vis, 520nm	51
24	Enzymatically degrd.cellulose, xylan, arabinan, galactan	Xylose, arabinose, galactose	Aminex HPX-87P (also guard column)	H_2O		RI	52
25	Plant glycosides	Glucose, galactose, mannose, allose	Aminex HPX-87P	H_2O		RI	53

Table 1. (Cont.)

No.	Sample	Analyte	Column	Eluent(v/v)	Pre- and post column reaction	Detector	Ref.
26	Mixtures of monosaccharides and alditols	d-glucose, d-fructose, d-mannose, d-mannitol, d-glucitol	Aminex HPX-87C (Aminex A7 or Aminex A5)	H_2O		RI	54
27	Low fat plain yogurt, litmus milk	Glucose, galactose	Aminex HPX-87	H_2O		RI	55
28	Gluco-oligomers from hydrothermal degradation of poplar wood	Various pentoses and hexoses	1) μ-Spherogel carbohydrate (Ca^{2+}) 2) Fast carbohydrate (Ca^{2+}) 3) Fast acid (H form) 4) Aminex HPX-42A (Ag^+ form)	H_2O		RI	56
29	1) Degrd. filter paper 2) Degrd. poplar wood	1) Glucose, fructose 2) Xylose, glucose, fructose	1) Aminex HPX-87H 2) μ-Spherogel x 7.5 carbohyd.	1) 0.01N H_2SO_4 2) H_2O		RI	57
30	The sugar chains in glycoproteins	Arabinose, xylose fucose, glucose, galactose, mannose, rhamnose	Shodex DC-613, H^+ form	aq. MeCN 92% or grad. of 80,85,87.5, 90, 92%	Post-column with 2-cyanoacetamide	UV, 280nm	58
31	Mixture of stnd. aldoses	l-arabinose, d-xylose, d-galactose, l-fucose, α/β-d-glucose, d-ribose, d-arabinose d-mannose	Shodex DC-613, Na^+ or Ca^{2+} form	MeCN-H_2O (80:20)	Post-column with 2-cyanoacetamide	UV, 280nm	59
32	Maternal and fetal sheep plasma; amniotic fluid	Mono (hexoses, pentoses); polyols	Sugar-Pak 1, Ca^{2+} form	H_2O		UV, 190nm	60
33	Jejunal aspirates from exper. perfused animals	Glucose, fructose	5μm irregular silica	67% MeCN contg. 0.03% (v/v) 1,4-diaminobutane	Post-column reaction with cuprammonium reagent	UV 280 to 310nm	61
34	Lens, erythrocytes, plasma	Glucose, saccharide alcohols	Zorbax SIL	C_6H_{14}-$CHCl_3$-MeCN (10:3:1.9)	Precolumn: p-nitrobenzoates	UV	65
35	Ripe Kent mangoes	Radiolysis products of D-fructose	Partisil 5	3% MeOH in $CHCl_3$ for 10 min. and 10% MeOH in $CHCl_3$ for add. 10 min.	Precolumn: O-benzyloximes	UV, 254nm	66

Table 1. (Cont.)

No	Sample	Analyte	Column	Eluent(v/v)	Pre- and post column reaction	Detector	Ref.
36	Hen egg albumin, lactose, casein, chondroitin, aggregating proteoglycans	Xylose, fucose, mannose, galactose, glucose	Lichrosorb Si 100	7.6% MeOH - 0.7% H_2O in $CHCl_3$	Precolumn: 2,4-dinitrophenyl-hydrazones	UV, 352nm	68
37	Cardiac glycosides; common food saccharides	Xylose,arabinose, glucose, fructose	Alltech amino	aq. MeCN	Postcolumn photoreduction of anthraquinone derivative by sacchs.	Indirect Fl. λ_{ex} 400 nm λ_{em} 525 nm	77
38	Mixtures of mono, di- and trisaccharides	Xylose,glucose, fructose,mannose, sorbose, galactose	Lichrosorb - NH_2	MeCN-H_2O (80:20)		Electrochemical (Ni electrodes)	78
39	Mixture of commercial monosaccharides	d-lyxose,d-ribose,d-arabinose, d-fructose, d-glucose, d-galactose, d-sorbose, d-mannose	Lichrosorb - NH_2, Nucleosil 5NH_2	$(Me)_2CO$ and H_2O, add small ammounts of AcOH		RI	80
40	Mixture of monosaccharides	Ribose, xylose, glucose, fructose, galactose	Lichrosorb - Si60	THF-H_2O (85-15) or (90:10) and 0.3% diethanolamine	Postcolumn with tetrazolium blue	Vis, 525nm	81
41	Commercial isomerose	Glucose, fructose	Viosfer - NH_2; 5μ	MeCN - H_2O (9:1)		RI	84
42	Mixture of monosaccharides and polyols	Glucose, fructose, galactose, mannitol, xylitol, sorbitol	Sugar Pak I	MeCN-H_2O (25:75) or H_2O		RI	86
43	Fruit juices, human urine	Xylose, ribose, arabinose, glucose, fructose	TSK gel NH_2 - 60	MeCN-H_2O (7:3)	Postcolumn with copper-bis (phenanthroline)	Indirect amperometry	89
44	Sticky cotton	Glucose,fructose	Spherisorb S5 NH_2	MeCN-H_2O (80:20)		RI	90
45	Extract of instant coffee	Arabinose, glucose, fructose, mannose	Spherosorb - NH_2	MeCN-H_2O (4:1)		MS	91
46	Mixture of mono- and disaccharides	Xylose, glucose	Zorbax BP-NH_2	MeCN-H_2O (70:30)		Moving-wire flame ionization	92
47	Various saccharides and their derivatives	1) Monosacchars. 1-O-methyl-glycosides 2) Permethylated monosacchars.	1) LC-18-DB 1) 5μRP-18 gard column 2) Bondapack - NH_2	Ammonium formate buffer (0.1M); pH 5.6		MS	93

Table 1. (Cont.)

No.	Sample	Analyte	Column	Eluent(v/v)	Pre- and post column reaction	Detector	Ref.
48	Musts, wines, champagnes	Glucose, fructose	Lichrosorb - NH_2	MeCN-H_2O (80:20)		RI	95
49	Various beers	Glucose, fructose	Lichrosorb - NH_2	MeCN-H_2O (80:20)		RI	97
50	Glycosides isolated from saponins	Xylose, arabinose, glucose, galactose, rhamnose	Nucleosil - NH_2	MeCN-H_2O (8:2)	Postcolumn with tetrazolium blue	Vis, 546nm	98
51	Cane juice	Glucose, fructose	PN 84730	MeCN-H_2O (4:1) and 0.01% Silica amine modifier No.2		RI	104
52	Saccharide products of formosation reactions	Ribose, arabinose erythrose, glucose, fructose	Separon SIC 18	MeOH-H_2O (6:4) to (7:3)	Precolumn: 2,4-dinitrophenyl-hydrazones	1) UV, 254 nm 2) RI	112
53	Glucoconjugates	Neutral monosaccharides. aminosaccharides	1) TSK-gel G-2000 PW 2) Ultrasphere - ODS 3) Microsorb C_{18} 4) Cosmosil $5C_{18}$ 5) TSK-gel LS-420 6) TSK-gel ODS-120T 7) YMC-gel ODS S-5	Buffer: 0.25M sodium citrate (pH4) containing 1% MeCN	Precolumn with 2-aminopyridine and reduction	Fl λ_{ex} 320 nm λ_{em} 400 nm	113
54	Carbohydrate part of glycoproteins	Fucose, mannose, galactose, glucose, N-acetylhexosamines	1) Zorbax C18 or C-8 2) Spherisorb ODS 11 3) Ultrasphere C-8	MeCN-H_2O (various proportions)	Precolumn: Perbenzoylates of the methyl glycosides	UV, 230nm	115
55	Glycoproteins	Xylose, lyxose, fructose, galactose, mannose	Altech C_{18}	20% (v/v) aq. MeCN contg. 0.01M FOH, 0.04M AcOH and 0.001M $(C_2H_5)_3N$	Precolumn: Dansylhydrazones	UV, 254nm	116
56	Ocean water, sediment hydrolyzates, phytoplankton	Ribose, glucose, fructose, mannose, galactose, rhamnose	Nucleosil-ODS	Citrate buffer (pH 3.78) - MeCN (80:20)	Precolumn: Dansylhydrazones	UV, 230nm	118
57	Tamm-Horsfall human urinary glycoproteins	Fucose, mannose, galactose	TSK-gel LS-410A	8% (v/v) aq. MeCN	Precolumn: Glycamines labelled with fluoresc. reagent NBD-F	Fl	122
58	Fruit juices; human serum; hydrolyzate of serum glycoprotein	Glucose, galactose, mannose	Nova PAK C-18	Gradient: 1) H_2O-MeCN (78:22); 2) MeCN, 10-85% for 45 min.	Precolumn: Dabsylhydrazones	Vis, 425nm	123
59	Mixtures of red. saccharides	Xylose, fucose, glucose, mannose, galactose, rhamnose, deoxyglucose	Hypersil-ODS	1) 25% $(CH_3)_2CO$-0.08M AcOH 2) 25% $(CH_3)_2CO$-10mM Na_3PO_4 (pH 6.5) 3) 10% $(CH_3)_2CO$-0.08M AcOH	Precolumn: Dabsyl or Dansylhydrazones	Fl. λ_{ex} 350 nm λ_{em} 540 nm	124

Table 1. (Cont.)

No.	Sample	Analyte	Column	Eluent(v/v)	Pre- and post column reaction	Detector	Ref.
60	Honey,chocolate; pharmaceutical formulations; mixtures of saccharides	Xylose, arabinose, glucose, galactose, mannose, fructose	1) Hibar-CN 2) Hibar RP-C18 3) Hibar RP-C8 4) Partisphere RP-C18S	MeCN-H_2O (various proportions) + 0.02M FOH and 0.05M boric acid	Precolumn: Dansylhydrazones	Fl. 360 nm ex 440 nm em	126
61	Mixture of saccharides	Glucose, fructose, galactose	HPX-65A (Ag^+ form) HPX-65A in the Pb(II) form	n/a		RI	129
62	Mixtures of monosaccharides	Arabinose, mannose, galactose, rhamnose	Polyol-RSiL; presaturation column	MeCN-H_2O (70:30) with 0.1% TEA		RI	130
63	Coffee drinks; Mixtures of saccharides	Glucose, fructose, xylose, arabinose, ribose, galactose	Lichrospher Diol, 5µm	MeCN-H_2O (80:20)	Post-column with cuprammonium reagent	UV, 315nm	131
64	Products of alcoholic fermentation of wort	Glucose, fructose	Carbohydrate P/N 84038	MeCN-H_2O (75:25)		RI	132
65	Beer worts	Monosaccharides	Carbohydrate P/N 84038	MeCN-H_2O (75:25)		RI	133
66	Cucumber nectar	Glucose,fructose	Carbohydrate analysis	MeCN-H_2O (75:25)		RI	134
67	Soft wheat flours	Glucose,fructose	Carbohydrate analysis	MeCN-H_2O (60:40)		RI	135
68	Mixture of various saccharides	d-glucose, d-xylose; 2-amino-2-deoxy-D-glucopyranose	Copper silica gel	H_2O-MeCN (25:75), $[NH_3]$=0.5M		RI	136
69	Mixture of various red. monosaccharides; hexosamines, dextran.	1) Hexosamines 2) Rhamnose, xylose, fucose, mannose, galactose	1) Hitachi 2617 2) Shodex R Spak DC-613	1) 0.16M borate buffer (pH 7.5) 2) MeCN-H_2O (9:1)	Post-column with 2-cyanoacetamide	Electrochemical	139
70	Pool serum; urine	Glucose, fructose, galactose, arabinose	LS 212	H_2O	Post-column with copper-bis(phenanthroline)	Indirect amperometry	140
71	Hydrolyzed and reduced wood sugar solutions	Glucose, galactose, mannose, xylose	Aminex HPX-85H (micro-guard columns)	H_2O and 0.005M H_2SO_4		RI	141
72	Beer	Various pentoses	Aminex HPX-85H	0.01N H_2SO_4		RI	142

Table 1. (Cont.)

No.	Sample	Analyte	Column	Eluent(v/v)	Pre- and post column reaction	Detector	Ref.
73	Wine, milk; mixture of mono- and oligosaccharides, organic acids	Glucose, fructose, galactose, ribose, D-xylose	TSK-gel SCX (H^+ form)	0.1M; 0.8M; 0.05M Boric acid solution		Conductivity	143
74	Mixture of various saccharides	Glucose, fructose, sorbose, xylose, arabinose	Sugar-PAK 1	H_2O	Post-column with copper compounds	Indirect potentiometry	144
75	Urine	Rhamnose, mannitol	CHO-620	H_2O		RI	145
76	Strawberry, raspberry purees	Glucose, fructose	Hibarlichrosorb NH_2	MeCN-H_2O (85:15)		RI	146
77	Honey, fruit juices, other food substances	Pentoses	Hipersil 5	MeCN-H_2O (8:2)		RI	149
78	Mandarin juice	Glucose, fructose	μ Bondapak NH_2 Sep-PakC18 cartridge	MeCN (75% in H_2O)		RI	150
79	Lignocellulosic hydrolyzates	Glucose, mannose, xylose, arabinose	Radial PakB	MeCN-H_2O (75:25)+0.1% Sam, amine additive		RI	151
80	Laxative syrup for babies	Glucose, mannitol	1) Hypersil 2) Hypersil ODS	1) isooctane-$(Et)_2O$-MeCN (150:75:15) 2) MeCN-H_2O-tert. butyl ether (80:20:4)	Precolumn benzoylation	UV, 231 nm	154
81	Glycosphingolipid globotetraosyl ceramide; human milk lacto-N-fucopentaose	Twenty-three D-L petnoses; hexoses, heptose, 2 or 6 deoxyhexoses	Supelcosil	Solvent A: $CHCl_3$-MeOH-0.1M sodium tetraborate to pH 3.5 with glac. AcOH, (65:25:4) Solvent B: $CHCl_3$-MeOH-0.1M sodium acetate to pH 3.5 (65:25:4)	Precolumn with 4'-N,N-dimethyl amino-4-amino-azo benzene	Vis, 436nm	155
82	Mixture of saccharides	Various monosaccharides	SCR-101N	n/a	Post-column with arginine and boric acid	Fl. λ_{ex} 320 nm λ_{em} 430 nm	156
83	Honey	Monosaccharides	n/a	MeCN-H_2O	Precolumn with 3-hydroxy-2-naphthoic hydrazide	UV	158
84	Babyfoods; diet or breakfast bars; condiments	Glucose, fructose	μ Bondapak/ carbohydrate	MeCN-H_2O (82:18)		n/a	160
85	Extract of mycelial sample of P.graminis; standard mixture of saccharides	Glucose, fructose, galactose and polyols	Sugar-Pak1	50 mg/l calcium titriplex dihydrate ($CaNa_2$.EDTA)	Post-column with new developed cuprammonium reagent	UV, 280nm	162

Table 2. Experimental HPLC conditions used for determination of oligosaccharides

No.	Sample	Analyte	Column	Eluent(v/v)	Pre- and post column reaction	Detector	Ref.
86	Mixture of reducing mono- and disacchs.	Maltose.	1) Shodex SC-1821 2) Lichrosorb NH_2	1) H_2O 2) MeCN-H_2O (3:1)	Post-column with ethylenediamine sulphate.	Amperometry	20
87	Mixtures of reducing and nonreducing saccharides.	Sucrose, melizitose, raffinose, stachyose.	Hitachi gel 3013N	Borate buffer (pH 7).	Post-column with taurine-2 mol./m^3 sodium metaperiodate, at 130°C.	Fl.	21
88	Needles of healthy and damaged Picea trees.	Saccharose, raffinose.	Aminex A-25, borate form.	0.4M boric acid, (pH 9.35)	Post-column with ethanolamine-boric acid.	Fl. λ_{ex} 342 nm λ_{em} 422 nm	22
89	Market milk; milk products.	Lactose.	MC gel CH08S	Stepwise: from 0.2M potassium borate (pH 7.8) to 0.5M potassium borate (pH = 8.7).	Post-column reaction with 2-cyanoacetamide	UV, 280 nm	23
90	Human breast milk.	Various oligosaccharides.	Hitachi, Custom Resin No. 2630	gradient: 0.1M sodium borate to 0.4M sodium borate buffer, at 55°C.		1) UV, 210 nm for N-acetyl-glucosamine residue. 2) RI for sacchs. without acetamido groups.	24
91	Human meconium; human milk.	Neutral and acetamido oligosaccharides.	1) Shandon ODS 2) APS-Hypersil 3) Micropack AX-5	1) H_2O 2) MeCN-H_2O (gradient). 3) aq. MeCN (different proportions).		UV, 206 nm	25
92	Mixture of mono- and disaccharides.	Sucrose, lactose, maltose.	1) 4VP (cross-linked 20% with DVB 2) 4VP-BU as bromide, phosphate, sulphate, nitrate	MeCN-H_2O (4:1)		RI	26
93	Sugarcane juice, syrup, molasses.	Sucrose (also polysaccharides).	1) Sugar Pak. 2) HPX 87C (Ca^{2+} form).	soln. of calcium propionate or acetate in water.		RI	32
94	Apple juices	Sucrose.	Sugar Pak 1 (Ca^{2+} form).	0.1% calcium acetate		RI	33
95	Brewing intermediates, beer, malt.	Sucrose, maltose, raffinose.	OSTION LGKS 0800 (Ca^{2+} form).	H_2O		RI	34
96	Red. and nonred. oligosacchs.	Sucrose, raffinose.	OSTION LGKS 0800	H_2O	Post-column with p-aminobenzoic acid hydrazide	UV-Vis	35

Table 2. (Cont.)

No.	Sample	Analyte	Column	Eluent(v/v)	Pre- and post column reaction	Detector	Ref.
97	Cotton leaves	Sucrose.	Aminex HPX-87H	0.014N H_2SO_4 (pH 2.1)		1) RI 2) UV-Vis	37
98	Fermentation mixtures metabolized by intestinal microflora.	Sucrose, lactose.	Aminex HPX-87H	0.028M H_2SO_4 (pH 1.5) at 40°C.		RI	38
99	Chondroitin Sulfate, Dermatan Sulfate.	Unsaturated disaccharides. (4 or 6 sulfated and non-sulfated.	Shodex RS (Na^+ form)	MeCN-MeOH-0.5M $HCOONH_4$, pH 4.5 (65:15:20)		UV, 232 nm	41
100	Hyaluronic acid and chondroitin.	Unsaturated, nonsulfated disaccharides.	Shodex RS, Na^+ form.	MeCN-MeOH-0.5M $HCOONH_4$, pH 4.5 (65:15:20)		UV, 232 nm	42
101	Mixture of mono- and disaccharides.	Cellobiose.	Aminex HPX-87-H	0.01N H_2SO_4		RI	43
102	Cane and beet molasses.	Sucrose.	1) Resolution Carbohydrate Na^+ or Ca^{2+} form. 2) Resolution ODS 5 3) Resolution NH_2 5	1) H_2O 2) H_2O 3) MeCN-0.01M KH_2PO_4 pH 7 (74:26)		RI,UV	45
103	Food and fecal neutral detergent fiber.	Cellobiose.	Aminex HPX-85P	H_2O		RI	48
104	Emzymatically degraded cellulose, xylan, arabinan, galactan.	Oligosaccharides up to d.p. 4.	Aminex HPX-87P; also guard column.	H_2O		RI	52
105	Low fat plain yogurt; litmus milk.	Lactose.	Aminex HPX-87	H_2O		RI	55
106	Degraded filter paper. Degraded poplar wood.	Cellobiose; glucooligomers-d.p. 3-8.	1) HPX-42A 2) TSK PW	1) H_2O 2) H_2O		RI	57
107	Jejunal aspirates from perfused animals.	Maltose, lactose, sucrose, maltotriose.	5μm Irregular silica	67% MeCN contg. 0.03% 1.4 diaminobutane.	Post-column with cuprammonium. reagent.	UV, 280 to 310 nm	61

Table 2. (Cont.)

No.	Sample	Analyte	Column	Eluent(v/v)	Pre- and post column reaction	Detector	Ref.
108	Oligosacchs. isomers: synthesized; from human milk.	1) Di- and trisaccharides as acetylated alditols. 2) Trisaccharides as alditols. 3) Trisaccharides as alditols. 4) Pentasaccharides as acetylated alditols.	1) Hypersil, 5μm. 2) Hypersil, 5μm. modified with TEPA 3) APS-Hypersil 4) ODS-Hypersil.	1) CH_2Cl_2-C_6H_{14}-isoPrOH 5%. 2) MeCN H_2O with 0.05% TEPA 3) MeCN-H_2O (pH 2.9) 4) MeCN-H_2O (pH 5.2) MeCN-H_2O (pH 7.0)		1,2,4) Radioactivity monitor. 3) UV, 190-210 nm	62
109	Oligosacchs. from milk and other natural sources.	Reducing oligosacchs. with d.p. 1-6.	Nucleosil Si gel	Ethyl acetate-AcOH-MeOH-H_2O (10:3:3:2)	Precolumn with 4-trifluoroacetamidoaniline under reductive conditions.	RI	67
110	Starch hydrolyzates.	Oligosaccharides of d.p. 1 to 12; 1 to 15.	OSTION KS (Ca^{2+} and Ag^{+} form).	H_2O		RI	69
111	17 different disaccharides.	β-cellobiose, β-gentiobiose, α-lactose, lactulose, maltose, α-trehalose, turanose, etc.	1) Zorbax-NH_2 2) Supelcosil LC-NH_2	MeCN-H_2O (Various compositions).		RI	75
112	Mixtures of different trisaccharides.	Cellotriose; isopanose, isomaltotriose, panose, melezitose, raffinose, xylotriose.	1) Zorbax-NH_2 2) Supelcosil LC-NH_2	1) MeCN-H_2O(72:28); (75:25) 2) MeCN-H_2O(75:25); (80:20)		RI	76
113	Cardiac glycosides; common food saccharides	Lactose, maltose, sucrose; various cardiac glycosides.	1) Merck Lichrosorb RP-8 2) Merck Lichrosorb RP-18 3) Alltech amino	aq. MeCN	Postcolumn photoreduction of anthraquinone derivative by saccharides.	Indirect Fl λ_{ex} 400 nm λ_{em} 525 nm	77
114	Mixtures of mono, di and trisacchs.	Saccharose, maltose, lactose, trehalose, etc.	Lichrosorb-NH_2	MeCN-H_2O (80:20)		Electrochemical (Ni electrodes)	78
115	Powdered milks.	Sucrose, lactose.	Micropack-$NH_2$10	MeCN-H_2O (80:20)		RI	79
116	Mixture of commercial disaccharides.	Anomers of: d-lactose, d-maltose, d-sucrose.	Lichrosorb NH_2; Nucleosil NH_2	$(Me)_2CO$-H_2O and small amounts of AcOH.		RI	80

Table 2. (Cont.)

No.	Sample	Analyte	Column	Eluent(v/v)	Pre- and post column reaction	Detector	Ref.
117	Hydrolyzates from natural starch.	Maltooligo-saccharides.	Separon SIX RPDA	H_2O		RI	82
118	Products of lactose degradation by β-galactosidase.	Oligosaccharides	1) μ-Spherogel 2) Amino Spheri-5	1) H_2O 2) MeCN 75%		RI	83
119	Candy cookies	Fructooligo-saccharides.	Nucleosil 5 NH_2	MeCN-H_2O (65:35)		RI	85
120	Mixtures of di, tri, tetra-saccharides and polyols.	Sucrose, maltose, lactose, raffinose, stachyose and other polyols.	1) μBondapak/Carbo-hydrate 2) Sugar Pak I	1) MeCN-H_2O (75:25) 2) H_2O		RI	86
121	Oligosaccharides (di-to hepta).	Oligosacchs.	Lichrosorb-NH_2	MeCN-H_2O contg. 15 mM K_3PO_4 (pH 5.2)		1) UV; 2) Phenol-H_2SO_4 assays 3) Liquid scintillation counter.	87
122	Partial hydrolyzates or acetolyzates of cyclosophoraose, curdlan, amylose, cellulose, dextran, luteose.	Homogeneous d-gluco-oligosaccharides, (1->3), (1->4), (1->6), and polysaccharides up to d.p.35.	ERC-NH-1171	MeCN-H_2O: for cyclosophoraose (58:42); for curdlan (60:40); for amylose (57:43); for dextran (56:44); for luteose (55:45).		RI	88
123	Human Urine disaccharides.	Lactose.	TSK gel NH_2-60	MeCN-H_2O (7:3)	Post-column with copper-bis (phenanthroline).	Indirect Amperometry	89
124	Sticky cotton.	Sucrose, melezitose.	Spherisorb S5 NH	MeCN-H_2O (80:20)		RI	90
125	Extract of green and roasted coffee.	Sucrose.	Spherosorb-NH_2	MeCN-H_2O (4:1)		MS	91
126	Mixture of mono- and disaccharides.	Sucrose, maltose, lactose.	Zorbax BP-NH_2	MeCN-H_2O (70:30)		Moving-wire flame ionization.	92
127	Mono- and disacchars., 1-O-methylglycosides; O-permethyl mono- to tetrasaccharides	1) Disaccharides. 2) Permethylated di-to tetrasaccharides.	1) LC-18-DB 1) 5μRP-18 guard column. 2) μBondapack-NH_2	Ammonium formate buffer (0.1M); pH 5.6.		MS	93

Table 2. (Cont.)

No.	Sample	Analyte	Column	Eluent(v/v)	Pre- and post column reaction	Detector	Ref.
128	Glycosaminoglycans, enzymatically treated.	Unsaturated disaccharides.	TSK-gel NH_2-60	MeCN-0.1M acetate buffer (pH 5.6) (90:20)	Precolumn: Dansylhydrazones.	Fl.	94
129	Wines, champagnes.	Sucrose.	Lichrosorb NH_2	MeCN-H_2O (80:20)		RI	95
130	Enzyme digestion products of hyaluronic acid and of chondroitin sulphate isomers.	Unsaturated tetra- and hexasaccharide.	Zorbax NH_2	4% MeOH and diff. % of 0.5M ammonium formate, pH 5.5		UV, 232 nm	96
131	Various beers.	Saccharose, maltose, maltotriose.	Lichrosorb-NH_2	MeCN-H_2O (80:20)		RI	97
132	Oligosaccharides	Eight disaccharides; laminarioligosacchs.; isomalto-oligosacchs.	Lichrosorb Si60	MeCN-H_2O contg. 0.05% 1.4-diaminobutane.	Precolumn coupling with different UV absorbing reagents.	UV, 229 nm 254 nm	103
133	Glycopeptides, glycoproteins.	Various oligosaccharides (di- to dodeca)	Hypersil modified by 50% aq. MeCN contg. 0.1% of 1,6-diaminohexane.	1) gradient 80->60% aq. MeCN and 0.01% 1,6 DAH 2) isocratic-80% aq. MeCN and 0.01% 1,6 DAH	Precolumn reductive amination with 2-amino pyridine.	Fl. λ_{ex} 230 nm λ_{em} 340 nm	105
134	Digested amylose by α-β amylase and diastase.	Maltose, maltotriose, maltopentaose	Radial-PaK	45-55% MeCN contg. 0.01% 1.4-diaminobutane.		RI	106
135	Starch and cellulose hydrolyzates.	Oligomers of dextrose units.	1) Polygosil RP-18 2) Nucleosil RP-8 3) Synchropak R 101 4) Synchropak R 103 5) Synchropak R 110	1) H_2O at different pH (2.0, 6.5, 10) addition of NaCl or alcohols. 2) H_2O 3,4,5) H_2O or H_2O and pentanol-1.		RI	108
136	Starch (corn) hydrolyzates.	Maltose, sucrose, stachyose, raffinose, difructose anhydrides, etc.	1) Spherisorb S5 ODS 2 2) Nucleosil 5C 18 3) Vydac 201 HSB 5 RF 4) Spherisorb S5 C8 5) Spherisorb S5 C6 6) Vydac 201 TPB 5 RF 7) Spherisorb 5 C18 8) Shandon PB178, WP 300, C18	H_2O		RI, MS	109

Table 2. (Cont.)

No.	Sample	Analyte	Column	Eluent(v/v)	Pre- and post column reaction	Detector	Ref.
137	Corn syrup; β-cyclodextrin, maltotriose.	Derivatized products of reductive cleavage of high oligomers.	Spherisorb C-6; Spherisorb C-18.	MeCN-H_2O (0.15M ammonium hydroxide)	Precolumn with naphthoyl chloride or 2-aminopyridine.	Fl. λ_{ex} 232 nm λ_{em} >320 nm	110
138	Two series of oligosaccharides.	Malto-oligosaccharides; isomalto-oligosaccharides	1) Dextropak Cartridge ODS 2) LiChrosorb RP-8 3) LiChrosorb SI-60 RP8	1,2,3) H_2O with addition of cationic, anionic or nonionic surfactants, TEU or inorganic salts.		RI	111
139	Soybean seed meal.	Sucrose, raffinose, stachyose.	Dextropak C18	H_2O		RI	114
140	Regenarating rat liver.	Asparagine-linked oligosaccharides.	TSK-gel-LS 410-C18	0.1M phosphate buffer (pH3.8) contg. 0.1-1.0% 1-butanol.	Precolumn with 2-amino pyridine	Fl. λ_{ex} 320 nm λ_{em} 400 nm	117
141	Ocean water, sediment hydrolyzates, phytoplankton.	Cellobiose, maltose, gentiobiose, lactose.	Nucleosil-ODS	Citrate buffer (pH 3.78) -MeCN (80:20)	Precolumn: Dansylhydrazones	UV, 230 nm	118
142	Reduced cellodextrins, maltodextrins, isomaltodextrins.	Various oligosaccharides.	1) Lichrosorb RP-18 2) Lichrosorb RP-8 3) ODS-1 (C-18). 4) ODS-2 (C-18).	H_2O. for cellodextrins: MeOH-H_2O (10%-90%)		RI	119
143	Legume seeds; lupine seeds, soybeans, fermented soybean products.	Sucrose, stachyose, verbascose, raffinose.	Lichrosorb RP-18	H_2O; for efficient sepn. of verbascose: 0.3M $(NH_4)_2SO_4$		n/a	120
144	Partial acid hydrolyzate of cyclodextrin.	Maltose-to maltohexaose.	Irica RP-18	1) MeOH-H_2O-MeCN (30:65:5). 2) MeOH-H_2O-MeCN (30:50:20).	Precolumn: 1) Quinoxalines; 2) Acetylated quinoxalines.	UV, 320 nm	121
145	Mixtures of reducing saccharides.	Lactose, cellobiose, gentiobiose.	Hypersil-ODS, 3μm	1) 25%$(CH_3)_2CO$-0.08M AcOH. 2) 25%$(CH_3)_2CO$-10mM Na_3PO_4 (pH 6.5) 3) 10%$(CH_3)_2CO$-0.08M AcOH	Precolumn: 1,2) Dabsyl hydrazones 3) Dansyl hydrazones	Fl. λ_{ex} 350 nm λ_{em} 540 nm	124
146	Biological samples.	Malto- and isomalto oligosaccharides.	1) Ultrasphere, 5-μm 2) Microsorb, C-8, 3μm.	Linear gradient: from MeCN-H_2O (4:1) to pure MeCN.	Precolumn benzoylation.	UV, 230 nm.	125

Table 2. (Cont.)

No.	Sample	Analyte	Column	Eluent(v/v)	Pre- and post column reaction	Detector	Ref.
147	Honey, chocolate; pharmaceutical formulations.	Lactose, maltose.	1) Hibar CN 2) Hibar RPC-18 3) Hibar RP C-8 4) Partisphere RPC -18S	MeCN-H_2O (various proportions) -0.02M FOH and 0.05M boric acid.	Precolumn: Dansylhydrazones.	Fl. 360 nm ex. >440 nm em.	126
148	Corn syrup solids; maltodextrins.	Oligosacchs: d.p. 1-10 (1) d.p. overall (2)	1) Resolve C_{18} 2) E-High A and E-500 μBondagel.	1) H_2O 2) H_2O contg. 0.15M NaCl.		RI	127
149	Sugar-beets, fodder sugar-beets.	Sucrose.	μBondapak/carbohydrate.	MeCN-H_2O (3:1).		RI	128
150	Mixture of saccharides.	Maltose, lactose, sucrose, maltotriose, lactulose.	HPX-65A (Ag^+_{2+} form). HPX-65A (Pb^{2+} form)	n/a		RI	129
151	Mixture of various oligosaccharides.	Maltose, lactose, maltotriose, raffinose.	Polyol-RSiL	MeCN-H_2O (68.6:31.4) and add. of 0.1% TEA.		RI	130
152	Starch, enzymatically degraded.	Malto-oligosacchs. d.p. 1-5.	Lichrospher Diol, 5μm.	MeCN-H_2O (80:20)	Post-column with cuprammonium reagent.	UV, 315nm.	131
153	Products of alcoholic fermentation of wort.	Maltose, maltotriose, maltotetraose. oligosacchs., d.p.>4.	SugarPak I T 30731-230	H_2O		RI	132
154	Beer worts.	1) Di- and trisaccharides; (various). 2) Oligosaccharides with up to 10 glucose units.	Carbohydrate, P/N 84038	1) MeCN-H_2O (75:25) 2) MeCN-H_2O (60:40)		RI	133
155	Cucumber nectar.	Sucrose.	Carbohydrate.	MeCN-H_2O (75:25)		RI	134
156	Soft wheat flours.	Saccharose, maltose.	Carbohydrate-Analysis.	MeCN-H_2O (60:40)		RI	135
157	Mixture of various saccharides.	Maltose, sucrose, lactose.	Copper silica gel.	H_2O: MeCN (25:75), $[NH_3]$ = 0.5M		RI	136
158	Heparin.	Di, tetra, hexa, octa and decasaccharide.	1) SAX analytica column. 2) GPC-HPLC G3000SW and G2000SW.	1) NaCl, pH 3.5 2) 0.5M NaCl		UV, 232 nm	138

Table 2. (Cont.)

No.	Sample	Analyte	Column	Eluent(v/v)	Pre- and post column reaction	Detector	Ref.
159	Dextran.	Malto- and iso-maltodextrins.	Gelko C-610	H_2O	Post-column with 2-cyanoacetamide.	Electro-chemical	139
160	Pool serum; urine.	Maltose, lactose.	LS 212	H_2O	Post-column with copper-bis (phenanthroline).	Indirect Amperometry	140
161	Wine, milk, mixture of mono- and oligo-saccharides; organic acids.	Lactose, sucrose, maltose, raffinose.	TSK-gel SCX (H^+ form).	0.1M; 0.8M; 0.05M boric acid solution.		Conductometry	143
162	Mixture of various saccharids.	Sucrose, lactose, maltose.	Sugar-PAK 1	H_2O	Post-column with copper compounds	Indirect Potentiometry	144
163	Urine.	Lactulose.	CHO-620 cation-exchange.			RI	145
164	Strawberry and raspberry purees.	Saccharose.	Hibarlichrosorb NH_2	MeCN-H_2O (85:15)		RI	146
165	Hyaluronate	Even and odd numbered oligo-saccharides.	1) Lichrosorb-NH_2 2) Ultrasil NH_2	0.1M KH_2PO_4 (pH 4.75)		UV, 206 nm.	147
166	Milk treated with β-galacto-sidase	Lactose; three disaccharides and one trisaccharide.	Amino Spheri-5	MeCN-H_2O (75:25)		RI	148
167	Mandarin juice.	Sucrose.	μBondapak NH_2, Sep-Pak C-18.	MeCN (75% in H_2O)		RI	150
168	Lignocellulosic hydrolyzates	1) Cellobiose, 2) oligomers (d.p. 2 to 5)	1) Radial Pak B 2) Dextropak	1) MeCN-H_2O (75:25) +0.1% Sam, an amine additive. 2) H_2O.		RI	151
169	Glycoproteins. (the sugar chains).	Various oligo-sacchs.	TSK-gel LS410, ODS	0.1M CH_3COONH_4 buffer, pH 4 contg. 0.25% 1-butanol.	Post-column with 2-amino pyridine.	Fl.	152
170	Amylose EX-1	Maltooligo-sacchs.	Cosmosil 5 C-18	0.1M CH_3COONH_4 buffer, pH 4 contg. 0.05% 1-butanol.	Post-column with 2-amino pyridine.	Fl. λ_{ex} 320 nm λ_{em} 400 nm	153
171	Laxative syrup for babies	Sucrose, raffinose.	1) Hypersil 2) Hypersil ODS	1) Isooctane-$(Et)_2O$-MeCN (150:75:15) 2) MeCN-H_2O-tert. butyl methyl ether (80:20:4).	Precolumn benzoylation.	UV, 231 nm	154

Table 2. (Cont.)

No.	Sample	Analyte	Column	Eluent(v/v)	Pre- and post column reaction	Detector	Ref.
172	Glycosaminoglycans after enzymatic digestion.	Non-sulphated disaccharides.	Hyperchrome packed with Nucleosil 5SB	Saline solutions.		UV, 230 nm	157
173	Human gastric juice.	Varian reduced oligosaccharides.	Chemopak Hypersil	H_2O		UV, 210 nm.	159
174	Baby foods; diet or breakfast bars; condiments.	Sucrose, maltose, lactose.	μBondapak/carbohydrate.	MeCN-H_2O (82:18)		n/a	160
175	Mixture of oligosaccharides.	Lactose, raffinose, malto-oligosaccharides. (1->6).	Column packed with SZ-5532 (Showadenko).	Glycerol-MeCN-H_2O-MeOH (10:63:18:9)		MS	161
176	Extract of mycelial sample of P. graminis.	Trehalose, sucrose, maltose.	Sugar-Pak 1	50 mg/l calcium titriplexdihydrate ($CaNa_2EDTA$)	Post-column with new developed Cuprammonium reagent.	UV, 280 nm.	162
177	Milk, milk products.	Lactose.	Partisil 10 carbohydrate.	MeCN-H_2O (4:1)		RI	163

established that allow analysis of all the different mono- and oligosaccharides due to the great multiplicity of structures. Tables 1 and 2 summarize the specific chromatographic conditions used according to the nature of the saccharide samples and, sometimes, to the requirements of the detector. Comparison of the various separation systems is very difficult because many parameters affect the choice of an HPLC separation system for saccharides: elution pattern, total time of analysis, toxicity of mobile phase, column efficiency, stability and life time of the column packing material.

Many types of HPLC saccharide separation techniques have been developed, according to the nature of the column packing material. The following HPLC variants are presented in this article:

1. Anion exchange.
2. Cation exchange.
3. Silica gel (naked).
4. Amino columns: chemically bonded or dynamically modified silica gel.
5. Chemically bonded alkyl silica gel.
6. Diol and "carbohydrate" columns.
7. Miscellaneous.

The applications of silica gel based cation exchanger and of polyol derivatized silica gel are relatively new to saccharide HPLC separations. Each of the HPLC techniques produces a typical elution profile, enabling to solve a specific analysis problem. The HPLC separation of saccharides can be carried out according to one of the following principal interactions:

a. Formation of an anionic borate complex of the saccharide and separation of this complex on anion-exchange resins.
b. Ligand exchange based on the interactions of the saccharide with cations in the resin.
c. Separation by cation exchange. The chromatographic mode is simple and enables possibility of using different cations in the resins: sodium, silver, lithium, calcium. In this case, the ionized forms of the analyzed solutes are preferentially sorbed onto ionic sites having opposite charges fixed on a solid matrix. The exchange process involves competition between solute ions present in the mobile phase and the counter ions paired with the oppositely charged functional groups fixed on the stationary phase. In order to maintain the electroneutrality of both phases, the separation process involves a displacement of the counter ions, originally

associated with these ionic sites, by the ion to be analyzed. In saccharide analysis the character of the cation of the exchange resin is an important factor which determines the quality of the separation.

d. Partition chromatography of the analyzed saccharides on cation exchangers. This chromatographic technique is based on the partition of ions (saccharides) between a polar mobile phase and the non-polar resin backbone. The ion-exchange sites are typically immobilized in small beads of resin that are formed by a cross-linked polymer.

e. Saccharides separation on silica gel via hydrogen bonding with the silanol groups of the silica.

f. Separation of saccharides on silica gel with chemically bonded aminoalkyl group. This separation technique is simple and effficient [14].

g. Separation of saccharides can be accomplished on silica gel modified with mono or polyfunctional amine. The saccharides in the mobile phase form complexes with the amine, also present in the mobile phase.

h. Separation on cupric silicate according to the hydrophilicity of the saccharides [15].

1. Anion exchange

The anion-exchange of saccharide-borate complexes was first applied in 1952 [16]. This HPLC technique includes formation of negatively charged complexes of certain saccharides (polyols) with boric acid or its salts followed by ion-exchange of these complexes on strong basic anion-exchange resins. The elution of the saccharides is

controlled by using a solution of borate buffer as mobile phase [16]. Anion-exchange of saccharide-borate complexes is a frequently used HPLC technique. It requires gradient elution and column regeneration for optimal separations. Sometimes widening of peaks due to continuous dissociation of the borate complex during the elution was observed. The stability of the complexes depends on the pH [17].

Monosaccharides in human serum have been separated on strong anion-exchange columns using as eluent a mixture of tetraborate-boric acid (pH 8.98) [18]. HPLC analysis of the monosaccharides in hydrolyzates of volcanic ash soil, using an anion-exchange column and gradient elution with boric acid (0.15M, pH 8.0 to 0.4M, pH 9.0), has been reported. A postcolumn labeling with monoethanolamine, enabling fluorescence detection, have been carried out [19]. A recently described HPLC method for determination of mono - and reducing disaccharides involves, in addition to the use of other columns, separation on anion-exchange column [20]. Fluorescence detection has been used for HPLC determination of reducing and nonreducing saccharides, which have been separated as borate complexes on anion-exchange resin. The postcolumn labeling has been performed with taurine in sodium metaperiodate solution at 140°C [21]. Anion-exchange column has been used for HPLC separation of reducing saccharides present in the needles of air-polluted healthy and damaged Picea trees. The mobile phase was 0.4M boric acid (pH 9.35) [22]. HPLC anion-exchange separation of mono - and disaccharides, present in milk and milk products, has been achieved using 0.2M potassium borate buffer (pH 7.8) and 0.5M potassium borate buffer (pH 8.7) as the mobile phase for disaccharides and monosaccharides respectively [23]. The HPLC separation of several human milk oligosaccharides, using an

anion-exchange borate form column and a linear gradient of sodium borate (0.1M-0.4M) buffer, pH 8.0, has been reported recently [24]. The application of an anion- exchange column for HPLC separation of gluco-oligomers, N-acetylglucosamine and of high mannose-type oligosaccharides has been reported. The mobile phase was aqueous acetonitrile. UV absorbance (208 nm) detection has been used [25].

The application of vinylpyridinium (VP) polymers for the anion-exchange HPLC separation of mono- and oligosaccharides has been recently described [26]. The effects of counter ions (bromide, phosphate, sulphate, nitrate) and of the length of the alkyl chain in the pyridinium polymer have been examined. Among the 4VP polymers the order of retention of saccharides is phosphate > sulphate > bromide > nitrate. In all cases the eluent was acetonitrile-water (4:1) [26]. Additional details on the chromatographic conditions are presented in Tables 1 and 2.

2. Cation exchange

The application of ion-exchange materials as chromatographic supports was reported already in 1948 by Applezweig [27]. Ion-exchange chromatography has been used since the 1950's for separation of both organic and inorganic substances. Today this chromatographic method is applied with high degree of sensitivity for HPLC separation of monosaccharides as well as oligosaccharides mixtures. This advance has been made possible by the preparation of very small rigid beads (5-15 μ diameter) of ion-exchange resin. The most popular packings are crosslinked polystyrene-based and silica-based ion exchangers. On these cation-exchange resins, oligosaccharides are separated on the basis of size-exclusion and ligand-exchange mechanisms. Therefore,

both the internal pore size and the presence of the resin with bound metal ligands affect the quality of the separation [28]. The majority of the columns are packed with either 4% or 8% divinylbenzene crosslinked resins. Packing of 4% crosslinked resin in the H^+ form enables separation of oligosaccharides with d.p. 1 to 5. 4% crosslinked resin in the Ag^+ form separates higher oligosaccharides; at least d.p.6 [29]. The elution order is with increasing molecular weight [29]. In the case of polystyrene-based cation exchangers, the influence of column dimensions, as well as of the eluent flow rate, on the analysis time has been extensively investigated [30].

Silica-based cation exchange packing is more pressure stable, but it has the disadvantage of matrix dissolution in alkaline eluent [31].

Columns containing cation-exchange resin in the calcium form have been used for HPLC separation of mono- and oligosaccharides present in sugarcane juice, syrups, molasses, mud [32], in apple juices, foods [33] and for analysis of complex mixtures of different saccharides [86]. The mobile phase was a low concentration solution of calcium propionate or acetate in water [33]. Successful HPLC separation of di- and trisaccharides, found in brewing intermediates and in beer [34], as well as of reducing and nonreducing mono- and oligosaccharides [35] has been achieved by using cation-exchange column. In both cases, the elution has been carried out with water.

Methods which involve the use of crosslinked polystyrene based strong cation-exchange resin for HPLC separation of different saccharides from various sources, have been recently reported. The mobile phase was aqueous solution of sulphuric acid [36,37,38]. Saccharide components (pentoses, hexoses) of cereal fibers poly-

saccharides have been determined by HPLC separation on a cation-exchange resin loaded with lead and using water as the mobile phase [39]. HPLC analysis of monosaccharides in nonalcoholic beverages has been carried out by using a cation-exchange column and 0.01N H_2SO_4 as the eluent [40]. Cation-exchange resin in the sodium form has been employed for HPLC separation of unsaturated disaccharides prepared from chondroitin sulphate and dermatan sulfate isomers [41] as well as from hyaluronic acid and chondroitin [42]. In the both cases the eluent was a mixture of acetonitrile, methanol and 0.5M ammonium formate (pH 4.5).

Investigation on the elution behaviour of different saccharides, using a cation-exchange technique, showed that hexoses and pentoses have shorter retention times than the corresponding deoxysaccharides [43]. The application of strongly acidic cation exchangers, in the silver or calcium form, for separation of oligosaccharides (d.p. 1-15) in starch hydrolysates has been reported. The effect of the resin form, particle size, temperature and mobile phase (water) flow rate on column performance has been tested [69]. Recently, it has been found that a cation-exchange resin in the silver form with a crosslinking of 6% is suitable for the analysis of mono- and disaccharides. Acid-catalyzed hydrolysis of sugars at elevated column temperatures could be suppressed by using column in the mixed silver-lead(II) form. This mixed column connected in series with a 8% crosslinked cation-exchange column resin in the lead(II) form enabled the separation of sucrose, maltose, lactose, glucose, galactose and fructose [129].

Additional examples of the application of cation-exchange column packings for separation of various saccharides by HPLC are as follows: analysis of mono- and disaccharides in pool serum and urine using

water as mobile phase [140]; quantitation of xylose, glucose, galactose and mannose in hydrolysed and reduced wood sugar solutions, using water and 0.005M sulfuric acid as the mobile phase [141]; method for rapid and accurate quantitative separation of various pentoses (d-xylose, arabinose) in beer, for elution, 0.01N H_2SO_4 was used [142]; separation of aldomonosaccharides (pentoses, hexoses), di- and trisaccharides as well as alditols found in wine and milk samples [143]; separation of mixtures of different mono- and disaccharides on sugar - PAK1 column, using water as the eluent [144]; separation at 85°C of rhamnose, mannitol and lactulose, excreted in urine, water was used as the mobile phase [145].

The introduction of fixed-ion resin columns has led to the development of ion-moderated partition chromatography and to the design of specific resin columns for saccharide analysis. The resin is used in a non-ion exchange mode and the counter ion remains on the column and effects the desired separation [44]. In this case the partition of the solute proceeds between a resin swelled by water and a mobile phase of water alone or mixture water/organic solvents. Water is most often used as the mobile phase in the ion-moderated mode of HPLC separation of saccharides, particularly mono- and disaccharides. The resins used for this mode of HPLC separations are cation exchangers (sodium, calcium, lead forms). An ion-moderated technique has been used for HPLC separation of mono - and disaccharides present in cane and beet molasses. Columns containing cation-exchange resins in the sodium or calcium form have been used. It has been found that the cation- exchange resin in the sodium form was the most suitable for the separation of saccharides in both types of molasses [45].

Monosaccharides in beer, white wine and soft drinks have been analyzed using metal cations mode of separation on cation exchange resin [46].

The application of ion-moderated HPLC for separation of fructose, sucrose and glucose-1-phosphate, using divinylbenzene (8%) crosslinked sulphonated polystyrene, has been reported. For elution, 0.03M sulphuric acid and 0.01-0.06M aqueous trifluoroacetic acid have been used [47].

The ability of heavy metal (lead) cation-exchange columns for efficient HPLC separation of different mono- and oligosaccharides has been investigated. In the case of HPLC separation of neutral monosaccharides and cellobiose, commonly present in food and fecal neutral detergent fiber, it has been found that the column packing used, Aminex HPX-85P, was 2-3 times more sensitive than a normal-phase column, enabling the determination of cellobiose [48]. In another case, optimal conditions have been developed for the ion-moderated partition HPLC separation of radioactively labelled monosaccharide components of proteoglycans [49]. In a recently reported study on the HPLC separation of mono- and oligosaccharide components in underivatized hydrolysates of various bacterial lipopolysaccharides, satisfactory resolution has been obtained when a cation-exchange column in the lead form has been used [50]. A series of studies on the HPLC analysis of mono- and oligosaccharides based on a cation loaded column, were recently carried out: determination of pentoses and hexoses in dry wines [51]; separation the monomeric and oligomeric (d.p. up to 4) saccharide products of enzymatic degradation of cellulose, xylan, arabinan and galactan. The resin in the lead form enables the separation of oligosaccharides of low d.p. [52], and of glucose, mannose, allose and galactose, found in plant glycosides

[53]. Cation-exchange mode of HPLC has also been used to analyse mixture of monosaccharides and sugar alcohols [54], to separate glucose, galactose and lactose in low-fat plain yogurt and in lactose containing microbiological media [55]. A cation-exchange resin in the silver form has been used for HPLC separation of monosaccharides in gluco-oligomers [56] and of saccharides obtained upon hydrothermal hydrolysis of filter paper and of poplar wood [57]. The mobile phase in the above cases was water. Aldopentoses and aldohexoses, known to be present in the sugar chains of animal and plant glycoproteins, have been separated in partition mode on a highly crosslinked cation-exchange column packing [58]. A combined partition and ligand-exchange mechanism has been proposed in the case of HPLC analysis of aldose anomers [59]. Finally, the HPLC separation of monosaccharides and their derivatives, contained in plasma from fetal and maternal sheep, has been reported [60].

In conclusion, cation-exchange chromatography is still a powerful method for HPLC separations of mono- and oligosaccharides. Further chromatographic details for the application of the cation-exchange mode of separation by HPLC are compiled in Tables 1 and 2.

3. Silica (naked)

Adsorption chromatography is useful mode of HPLC and the widely used adsorbent is silica. This stationary phase can be used either pure or in a modified form. A wide range of mobile phases, from non-polar hydrocarbons to very polar systems, have been used with silica adsorbents. Since the mobile phase can have different compositions, the result is various separation systems. Most of the separations can be obtained in a short time.

3.1 Silica gel, without precolumn derivatization of the analyte

This is the simplest method for HPLC separation of monosaccharides. In this case the retention of the saccharides depends of the hydrogen bond between the silanol groups of the stationary phase and the hydroxyl groups in the saccharide structure. In the most cases, a moderate resolution has been obtained.

HPLC separation of mono- and oligosaccharides found in jejunal aspirates from experimentally perfused phase, has been carried out on a silica packing phase followed by a postcolumn reaction with cuprammonium reagent and UV monitoring at 285 to 310 nm [61]. In another case, oligosaccharides isomers, released from certain glycoproteins present in human milk, have been separated on silica gel. The separation of the disaccharides and the first trisaccharide from the other trisaccharides has been achieved with a mobile phase of dichloromethane, hexane and 5% isopropanol [62].

3.2 Silica gel and precolumn derivatization of the analyte.

Precolumn derivatization has been performed for three main reasons: (a) to protect the configuration of the separated saccharides, since during the chromatographic procedure epimerization as well as isomerization of the saccharide molecule can occur; (b) to enhance detection and (c) to change the chromatographic behavior. Usually the esters (perbenzoates, acetates) of the saccharides are prepared and a mixture of light hydrocarbons (pentane, hexane) is used as mobile phase. Historically, the precolumn derivatives as dansylhydrazones [63] and O-benzyl-oximes [64] are used most frequently for the separation of saccharides on silica. Usually, comparing to the original saccharides, the precolumn derivatives are more hydrophobic.

Glucose and saccharide alcohols in human and rat lens, erythrocytes and plasma have been analyzed by making a precolumn derivatization with p-nitrobenzoyl chloride followed by separation on silica packing phase and UV monitoring [65]. HPLC quantitation of the radiolysis products of d-fructose has been achieved by a precolumn derivatization to the corresponding O-benzyloximes and their separation on a silica gel column with gradient elution. The good separation obtained using these derivatives as well as the possibility of removing most of the unchanged d-fructose make this method preferable for the analysis of irradiated saccharide solutions [66]. Application of silica gel column for the separation of reducing oligosaccharides (d.p. 1 to 6) present in human milk, hydrolysate of chitin and faeces of breast-fed children has been reported. The method involves precolumn derivatization, under reductive conditions (sodium cyanoborohydride), to the corresponding aminoalditol derivatives [67]. Silica columns were used for the separation of neutral saccharides, present in treated lactose, hen egg albumin, casein and chondroitin. The procedure involves a precolumn derivatization to the corresponding 2,4-dinitrophenylhydrazones and elution with 7.6% methanol and 0.7% water in chloroform [68]. Silica, as well as chemically bonded octadecylated-silica columns have been compared for their separation efficiency in analysis of mono, di- and trisaccharide present in laxative syrup for babies. Here, precolumn benzoylation has been performed for preliminary cleaning [154]. The saccharide components (pentoses, hexoses, heptose and 2 or 6- deoxy-hexoses) of glycosphingolipid globotetraosyl ceramide and the human milk oligosaccharide lacto-N-fucopentaose have been determined by precolumn derivatization with 4′-N,N-dimethylamine-4-aminoazo benzene

following by HPLC separation of the corresponding derivatives on silica column phase [155].

4. Amino columns

4a. Chemically bonded

Silica gel can be used either in the native form or in a modified form. The term "chemically bonded phase" applies to a covalent bonding, via siloxane bridges, of organic moiety (ligate) to the surface of the silica support.

A wide variety of physical and chemical properties of this stationary phase is obtained by derivatizing the surface silanol groups with organic silyl compounds.

The chemical nature of the ligate dictates whether the column material is polar or nonpolar sorbent or ion-exchanger. The amino-bonded silica column packings were first introduced in 1975 [70]. Chemically bonded phases containing aminoalkyl function such as -aminopropyl group at the silica surface are widely employed in HPLC analysis of saccharides, requiring a highly polar surface. Such packings are most frequently used with nonpolar organic solvents or aqueous-organic mixtures (aqueous methanol or aqueous acetonitrile) as the mobile phase.

The separation of saccharides on chemically bonded aminoalkyl-silica is not always quantitative. This is due to the possible interaction between reducing saccharide and the amino group of the ligate (formation of Schiff's base) and to a self hydrolysis of the basic material [71, 72]. It has been shown that aldopentoses and galactose react with the amino function of the stationary phase when aqueous acetonitrile is used as the eluent [14].

The possible interaction between the analyzed reducing saccharides and the amino group influences the elution profile. The aminoalkyl-silica packing phases have relatively short lifetime because of the hydrolysis of the bond during the elutions [71, 72]. Stabilization of aminopropyl-silica phases is achieved by crosslinking, but a decrease of the retention times can be obtained. In order to restore the retention times, the acetonitrile content of the eluent must be increased. Stability of aminoalkyl-silica phase during the elution is attained "in situ" by constantly regenarating the surface of the phase [71]. Despite these possible limitations, the new commercial packings are stable and provide satisfactory separation of saccharides. Table 3 compiles certain aminoalkyl groups bonded to silica used in HPLC.

The HPLC selectivity of different chemically bonded amines for the separation of saccharides has been discussed [73]. The influence of various parameters on the quantitation of the HPLC analysis of mixture of mono- and disaccharides has been investigated [14]. Studies

Table 3

Chemically bonded aminoalkyl groups

-aminopropyl	R ≡ $Si\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}NH_2$
diamino	R ≡ $Si\text{-}(CH_2)_3\text{-}NH\text{-}CH_2\text{-}CH_2\text{-}NH_2$
dimethylamino	R ≡ $Si\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}N(CH_3)_2$

on the influence of the water content in the mobile phase on the retention time as well as the mechanism of the partition of saccharides on amino-bonded silica columns, have been reported [74, 75, 76]. The composition of the eluent determines the elution profile. In practice, the desired HPLC analysis of saccharides is obtained by varying the polarity of the mobile phase, causing a change of the retention times of the individual components [14].

A great number of studies on the HPLC separation of mono- and oligosaccharides, found in different natural products and systems, on chemically bonded aminoalkyl-silica packing material have been reported in the last several years. The experimental details are compiled in Tables 1 and 2. It has been found that when the trisaccharides are composed solely of glycosyl units, retention times increased in the order of bonding (1 -> 3), (1 -> 4), (1 -> 6) following the pattern of the disaccharides [76]. The application of an aminoalkyl-silica column for separation of common food saccharides and cardiac glycosides by HPLC has been studied [77]. A satisfactory separation of saccharides component in certain fibre polysaccharides from cereals [39] and of glucose, fructose, sucrose in cane and beet molasses [45] has been achieved. HPLC separation of mixtures of mono, di- and trisaccharides on aminoalkyl-silica gel column has been combined with an electrochemical detection [78]. An aminoalkyl-silica column has been used for HPLC separation of monosaccharides in beer, white wine, soft drinks [46] as well as of disaccharides present in powdered milk [79]. A complete separation of the anomers of mono- and disaccharides has been reported [80]. A newly developed bonded propylaminoethanol column provided a more stable alternative to commercial aminopropyl-silica or to silica gel treated with amine

modifier [81]. The application of an octadecyl-silica phase doped with primary amino group (-aminopropyl) for HPLC separation of different malto- oligosaccharides, found in hydrolysates from starch, has been reported [82]. An aminoalkyl column has been used for the HPLC follow-up of the formation of oligosaccharides during β-galactosidase action on lactose [83]. Monosaccharides in a commercial isomerose (fructose - high glucose syrup) have been separated on amino-bonded column packing using a mobile phase of acetonitrile-water (9:1) [84].

Finally, the widespread application of chemically bonded aminoalkyl-silica column materials for HPLC separation of different mono- and oligosaccharide mixtures is shown by the following serie of recently performed analyses: [20, 50, 62, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 146, 147, 148, 149, 150].

Tables 1 and 2 present the experimental HPLC conditions for the determination of mono- and oligosaccharides respectively, including the use of chemically bonded amino columns.

4b. Dynamically modified silica gel

Dynamically modified silica gel with amino group is created "in situ", using a modifier containing an amino moiety [99]. Various techniques have been developed which use silica columns and reversed-phase columns for separation of saccharides by adding small amounts of amines (modifiers) to the eluent [100, 73]. In the case that the eluent contains n-alkylamine, octadecyl-silica gel can be used as packing support instead of silica gel [100]. During the separation procedure, the "in situ" amino-modified columns are relatively stable since they are continuously generated [101]. The efficiency of amino-modified silica columns is of the same order as the efficiency

of chemically bonded amino-silica columns and sometimes slightly better [102, 73].

Silica gel modified by certain di- or polyamines gives a relatively effective and stable column packing for HPLC of mono- and oligosaccharides. HPLC separation of trisaccharides as alditols on silica modified "in situ" with tetraethylene pentamine has been achieved in a study on the analysis of oligosaccharide isomers released from certain glycoproteins [62]. Dynamically amino-modified silica with 0.05% (w/v) 1,4-diaminobutane has been used for HPLC separation of eight disaccharides and of laminari - and isomaltooligosaccharides. In this application, precolumn reductive amination followed by coupling with UV-absorbing reagents, of the analyzed oligosaccharides, have been performed [103].

The quantitation of glucose and fructose, generated by the inversion of sucrose during the sugarcane processing, has been achieved by modifying 10 μm silica particles with amines [104]. Oligosaccharides (di-to dodeca saccharide), precolumn derivatized by reductive 2-aminopyridyl amination, have been separated on silica modified with 0.1% 1,6-diaminohexane (DAH). Gradient or isocratic elution with aqueous acetonitrile containing 0.01% - DAH has been carried out [105]. An analytical method for quantitative determination of linear malto-oligosaccharides with various d.p. using HPLC separation on a silica column amino-modified with 1,4-diaminobutane in 50% acetonitrile, has been developed. The relationship between peak area and amount of oligosaccharides was linear [106]. Monosaccharides (pentoses, hexoses) and oligomers (d.p. 2 to 5), found in lignocellulosic hydrolysates, have been separated on dynamically modified silica by using acetonitrile-water (75:25) mobile

phase with the addition of an amine modifier [151]. Additional details concerning the application of the dynamically prepared amino columns are presented in Tables 1 and 2.

5. Chemically bonded alkyl columns

Chemically bonded nonpolar phases containing a hydrocarbonaceous moiety bound via silyl ether bond to the silica surface are widely used. The most frequently used phases for HPLC analysis of saccharides are bonded octyl and octadecyl silica gel but shorter chains also find applications [107]. Aqueous or aqueous-organic eluents are generally used with such columns.

The first reports on the apllication of RP-HPLC to saccharides, using water as the eluent, were published during 1979-1980 [8]. The retention behaviour of oligomers in starch and cellulose hydrolyzates on alkylbonded silica gel columns with varying chain length (between octyl and octadecyl) and varying support pore diameter has been investigated. The influence of eluent pH and addition of sodium chloride or alcohols to the eluent was studied. It was concluded that a neutral aqueous eluent is better than one of low or high pH. The presence of sodium chloride resulted in an increased retention time and better resolution [108]. Octadecyl-bonded silica gel can be used for the HPLC of underivatized and derivatized saccharides that include oligosaccharides. Generally the retention of saccharides on octadecylated-silica is relatively short and specific. This fact is of importance in the case of analytical HPLC separations of mixtures of mono, di- and permethylated di- to tetrasaccharides [93]. It has been shown that various chemically bonded alkyl-silica packings have the capacity of separating oligosaccharides when the eluent is pure

water. The separation can be performed at room temperature but by lowering the temperature an increase in the resolution and retention times is obtain. Shortening the bonded alkyl chain, or using silica with larger pore diameter, can change significantly the retention behaviour of oligosaccharides. For identification of saccharides in complex mixtures reversed phase HPLC is a good alternative, to either ion-exchange or amino-bonded silica packing materials [109]. A comparison on the use of different chemically bonded phases versus dynamically modified silica packings for separation of oligosaccharides, containing neutral and acetamido saccharides, has been reported [25].

Precolumn derivatizations have been used with alkyl-bonded silica gels, for the HPLC separation of very polar saccharides, which alone are not sensitive to common optical detectors. Most of these derivatives contain aromatic or heterocyclic substituents which either absorb in the UV region, fluoresce intensely or require specific detection. For example, derivatization with naphthoyl chloride [110]; 2-aminopyridyl derivatives (amination) [110, 113, 117]; 2,4-dinitrophenyl hydrazones [112]; methanolysis and perbenzoylation of the methyl glycosides [115]; the corresponding benzoates [125], dansyl hydrazones [116, 118, 124, 126], dabsyl hydrazones [123, 124]; preparation of the quinoxalines and acetylated quinoxalines [121] and preparation of the corresponding glycamines which have been labeled with the fluorescent reagent NBD-F [122].

The popularity of the various alkyl-bonded column materials for HPLC separation of mono- and oligosaccharides can be demonstrated by the great number of publications which appeared during the last years: Separation and quantitation of monosaccharides [93, 112, 113, 115,

116, 118, 122, 123, 124, 126, 150, 154] as well as of oligosaccharides with different degree of polymerization [25, 62, 93, 108, 109, 110, 111, 114, 117, 118, 119, 120, 121, 124, 125, 126, 127, 128, 150, 152, 153, 154].

Tables 1 and 2 present the experimental conditions when alkyl-bonded column packings are used.

6. Diol and "carbohydrate" columns

The application of diol-bonded silica gel as stationary phase for HPLC separation of saccharides has been reported [130, 131]. The polarity of this stationary phase is similar to, or higher than, that of silica gel itself, but the selectivity is different. In most cases of using diol-bonded silica, the elution pattern is similar to that obtained with aminopropylated silica gel. It has been found that diol derivatized silica gel separates mono, di- and trisaccharides, each group from another and also the individual saccharides within each class. The mobile phase used for separation of the monosaccharides and oligosaccharides was acetonitrile-water in proportion (70:30) or (68.6:31.4) respectively. The retention time is a function of the acetonitrile content of the eluent [130]. In this study it was necessary to use triethylamine (0.1%) in the mobile phase to prevent separation of the two anomeric forms of the analyzed saccharide. However, the addition of triethylamine can reduce the life span of the column packing. To alleviate this problem,a precolumn is used. Usually the polyol-bonded silica phase is more stable for saccharide analysis than the popular aminopropyl-bonded silica [130]. Another application of diol-bonded silica is the separation of monosaccharides and maltooligosaccharides in coffee drinks, biological fluid and

enzymatic degraded starch. The mobile phase was a mixture of acetonitrile-water (80:20). In this case the column effluent reacts with cuprammonium reagent and the product is monitored by absorbance detection at 315 nm [131].

Carbohydrate columns have been applied for HPLC analysis of saccharides: glucose and fructose in products of alcoholic fermentation of wort have been separated on a Carbohydrate column, using acetonitrile-water mixture (75:25) as the mobile phase [132]; the same column has been used for the separation of monosaccharides as well as of oligosaccharides (containing glucose units) found in beer worts [133]; separation by HPLC of monosaccharides and sucrose, present in cucumber nectar using acetonitrile-water (75:25) mobile phase [134]; separation of glucose, fructose, saccharose and maltose, found in soft wheat flours with acetonitrile-water (60:40) eluent [135]; determination of the lactose content of milk and milk products by using Partisil 10 Carbohydrate as the stationary phase and mixture of acetonitrile-water (4:1) as the mobile phase [163].

7. Miscellaneous

The application of HPLC for quantitative separation of saccharides includes additional specific stationary phases.

a) Copper silicate column packing

Several transition metals, including copper, form complexes with silica gel. The complexes are used as a stationary phase in the liquid chromatography. When an ammoniacal solution of copper sulfate is percolated through a silica gel phase, the following reaction takes place:

$$2\ (\equiv Si\text{-}OH) + Cu(NH_3)_4SO_4 \rightarrow (\equiv Si\text{-}O)_2Cu(NH_3)_x,(H_2O)_y + (NH_4)_2SO_4$$
$$1 \leq X \leq 2$$

The result is that almost 40% of the original silanol groups are substituted. This stationary phase contains the silanol groups coated with water and cupric silicate molecules. Usually the mobile phase used is a mixture of acetonitrile-water-ammonia. Certain mono- and disaccharides and also 2-amino-2-deoxy-D-glucopyranose have been separated satisfactorily on a copper silica gel phase with a mobile phase of water-acetonitrile-ammonia [136]. The retention of the analyte components is explained as follows: The retention depends on a ligand-exchange mechanism involving complexes between the solutes and copper (II); the retention of the neutral saccharides is based on partition phenomena between the water of solvation of the ammonia molecules contained on the stationary phase and water in the mobile phase [136].

b. Cyanoethyl-bonded silica

Bonded phase of intermediate polarity usually has cyanoethyl groups covalently bonded to silica support.

The separation efficiency of cyano-bonded silica has been compared to that of various alkyl-bonded column materials (octyl- and octadecyl-silica) for the HPLC analysis of mono- and disaccharides, as mixtures, in honey, chocolate and pharmaceutical formulations. The analyzed saccharides have been (precolumn) derivatized to the corresponding dansyl hydrazones. The derivatives were eluted, according to the separation conditions, with a mixture of acetonitrile-water (different proportions), 0.02M formic acid and sometimes addition of 0.05M boric acid [126].

c. Gel permeation, size exclusion

Gel permeation chromatography is one of the techniques for HPLC separation of oligosaccharides. Stationary phases such as Sephadex gels, Polyacrylamide gels and sulfonated styrene - divinylbenzene copolymer are used. Gel permeation chromatography separates mainly on the basis of molecular size and sometimes on the basis of chemical structure [137]. Oligosaccharides, produced by enzymatic depolymerization of heparin, have been determined by GPC techniques. The eluent used was 0.5M NaCl [138]. Malto- and isomaltodextrines, obtained from dextran, have been separated by GPC using acetonitrile-water (9:1) as the mobile phase. The column eluite was post-column derivatized with 2-cyanoacetamide followed by oxidation [139]. A comparative study has been carried out on the separation efficiency of various column packings, including gel permeation, for the 2-amino-pyridyl derivatives of neutral and amino saccharides, found in glyco-conjugates. The method is highly sensitive and picomole amounts of the saccharides can be detected [113]. The overall molecular weight distribution of oligosaccharides, present in starch hydrolysis products, was determined using gel permeation (size exclusion). Aqueous solution of 0.15M NaCl was used as the mobile phase [127].

D. HPLC SEPARATION OF SACCHARIDE ANOMERS

For complete HPLC analysis of saccharides, the separation of optical isomers is often necessary. During the course of HPLC analysis, the separation of saccharide anomers can be restricted since mutarotation occurs. Recent development of new column packings,

having high efficiency, and of specific derivatization reagents enables in the most cases the separation of such isomers.

An improved method for analysis of aldose anomers by HPLC on a highly crosslinked cation-exchange resin of sodium and of calcium forms has been described. A combined partition and ligand- exchange mode was proposed. In this case, it has been found that increasing the acetonitrile content in the eluent, gave a better separation of aldose anomers; the peak splitting due to anomers can be eliminated by the addition of 0.001M triethylamine, which catalyses the mutarotation process [59].

The separation of mono- and disaccharides anomers (pyranose, furanose) on amino-silica columns, using acetone-water-acetic acid as the eluent at a low temperature, has been reported. Resolution patterns showed remarkable changes with decreasing column temperature. It was found that at very low temperatures the mutarotation of the saccharide anomers has been suppressed and the pyranose anomers of the examined mono- and disaccharides were completely separated. The furanose anomers were also separated from an equilibrium mixture under controlled conditions. The column effluent has been monitored with a refractive index detector [80]. In the case of the separation of mono, di- and trisaccharides on a Polyol phase, triethylamine (0.1%) was added to the mobile phase to prevent separation of the anomeric forms [130].

E. DETECTION

The liquid chromatographic detector is a device which indicates the presence of, and measures the amount of the separated components

in the column effluent. Proper choice of detection system is important since, unlike gas chromatography, there is no universal LC detector. The principal detection modes used for HPLC analysis of saccharides can be classified as follows: 1) For underivatized sugars: differential refractometry, ultra-violet absorption, mass spectrometry; 2) pre- or postcolumn reactions followed by absorption or fluorescence monitoring of the derivatized saccharides in the column effluent. Detectors such as refractive index, ultra-violet and visible spectrophotometer, fluorometer, mass-spectrometer, amperometer and potentiometer have been used to varying degrees of success.

Generally, mono- and oligosaccharides cannot be detected directly by absorption in the ultra-violet and visible regions, or by fluorescence since they lack chromophores and fluorophores respectively. Direct measurement of the absorption in the near-ultraviolet region provides a more sensitive method, but it is non-selective. Considerable efforts have been made in improving the detection sensitivity and efficiency by the use of pre- and postcolumn reactions.

Applications of the various detection modes for HPLC analysis of mono- and oligosaccharides in real samples are presented in Tables 1 and 2 respectively. In addition, Tables 4, 5 and 6 summarize the various classes of the detection systems.

a. Underivatized sugars

a.1 Refractive index (RI)

Among the methods for the detection of saccharides in HPLC, refractive index measurement is the most popular. This detector is stable, simple to operate and the sample is not destroyed. The RI detector has relatively good sensitivity. However, it is highly

Table 4. Detection (Underivatized)

Detector	Item number in Tables 1 and 2
Refractive index	7, 8, 9, 11, 12, 13, 14, 15, 16, 17, 19, 20, 22, 24, 25, 26, 27, 28, 29, 39, 41, 42, 44, 48, 49, 51, 61, 62, 64, 65, 66, 67, 68, 71, 72, 75, 76, 77, 78, 79, 90, 92, 93, 94, 95, 97, 98, 101, 102, 103, 104, 105, 106, 110, 111, 112, 115, 116, 117, 118, 119, 120, 122, 124, 129, 131, 134, 135, 136, 138, 139, 142, 148, 149, 150, 151, 153, 154, 155, 156, 157, 163, 164, 166, 167, 168, 177.
UV absorption	12, 17, 32, 90, 91, 97, 99, 100, 102, 108, 121, 130, 158, 165, 172, 173.
Mass spectrometry	45, 47, 125, 127, 136, 175.
Miscellaneous	1, 38, 46, 73, 108, 114, 126, 161.

Table 5. Detection (Precolumn reactions)

Detector	Item number in Tables 1 and 2
UV-Vis absorption	34, 35, 36, 52, 54, 55, 56, 58, 80, 81, 83, 132, 141, 144, 146, 171.
Fluorescence	53, 57, 59, 60, 128, 133, 137, 140, 145, 147.
Radioactive labeling	21.

Table 6. Detection (Postcolumn reactions)

Detector	Item number in Tables 1 and 2
UV-Vis absorption	6, 10, 21, 23, 30, 31, 33, 40, 50, 63, 85, 89, 96, 107, 152, 176.
Fluorescence	2, 4, 5, 18, 37, 82, 87, 88, 113, 169, 170
Miscellaneous	3, 43, 69, 70, 74, 86, 123, 159, 160, 162.

sensitive to column effluent temperature changes and to mobile phase composition. For the former, a thermostatic control is essential for operation at the most sensitive range of the detector. In the case of RI detection of various saccharides, gradient elution is impossible [102]. Particular applications of refractive index monitoring of mono- and oligosaccharides in various samples are presented in Tables 1 and 2 and summarized in Table 4.

a.2 Ultra-violet absorption (UV)

Historically, UV absorption detectors played an important role in the development of HPLC. Although the UV detector is not universal, it is used in HPLC systems because it is relatively insensitive to changes in the eluting solvent when this solvent is a non-ultraviolet absorber. This detector does not need strict temperature control. Direct ultra-violet monitoring is not widely applicable for saccharides due to their low absorptivity in this wavelengths region.

In recent years important developments of the ultra-violet detector enabled improved detection of saccharides at low wavelengths (near 190-200 nm) since most saccharides have their ultra-violet

absorption maxima below 200 nm. Tables 1, 2 and 4 show the application of the ultra-violet absorption for monitoring, without derivatization, of various saccharides separated by HPLC methods.

a.3 Mass spectrometry

The main advantage of using a mass spectrometer as an HPLC detector is that it is possible to obtain a spectral identification of a specific compound. The coupling of HPLC and MS is difficult. One of the interfaces between HPLC and MS is the thermospray. The principles of thermospray ionization and its use in LC/MS have been described by Blakley and Vestal [164]. The application of fast-atom-bombardment mass spectrometry for detection of malto-oligosaccharides (d.p. 2 to 6), separated by HPLC, has been reported [161]. Recently developed mass spectrometry detectors seem to be insensitive to changes of column temperature and solvent composition. However, this detector is not in wide use. Applications of mass-spectrometry for detection of saccharides are presented in Tables 1 and 2 and summarized in Table 4.

a.4 Miscellaneous

1. Electrochemical detection: This is another mode of monitoring saccharides separated by HPLC [78]. This form of detection is based on the electrochemical reaction of a solute in a cell designed for minimal peak dispersion and maximum electrochemical response. The electrochemical monitoring is possible when the compound in the column effluent flow is electrochemically active under the experimental conditions used and the mobile phase is conducting.
2. Specific detection systems have been developed for determination of saccharides in the HPLC column effluent: Conductivity, Amperometry, Moving-wire flame ionization, Radioactivity monitor.

Tables 1 and 2 present the application of the above mentioned detection systems, which are compiled in Table 4.

Pre- and postcolumn reactions

Saccharides have no chromophores to permit their direct photometric monitoring in ordinary ultraviolet and visible regions. The lack of chromophores and fluorophores in saccharides has been overcome by chemical derivatization (labeling) with strong ultraviolet absorbing or fluorescing functional groups. Precolumn derivatization reagents, on one hand, insert in the sugar to be analyzed an absorbing (UV, visible) or fluorescing label and, on the other hand, reduce the sugar polarity, thus allowing the use of the more general reversed-phase columns. A great number of derivatization methods have been developed [165, 166]. Tables 5 and 6 give a list of pre- and postcolumn reactions used in the HPLC analysis of mono- and oligosaccharides.

b. Precolumn reactions

Precolumn reactions have been carried out in order to improve the HPLC separation and detectability of saccharides on certain column packings (naked silica, chemically bonded alkyl-silica gels). Most of the derivatives contain aromatic or heterocyclic substituents which either absorb in the ultraviolet-visible region, fluoresce intensely or require a different but specific detector (Tables 1, 2 and 5).

b.1 Ultraviolet and visible absorption

Various saccharide derivatives were prepared by using UV-visible absorbing reagents: p-nitrobenzoates [65]; O-benzyloximes [66]; 2,4-dinitrophenylhydrazones [68,112]; coupling with specific UV

absorbing reagents [103]; perbenzoylates of the prepared methyl glycosides [115]; dansylhydrazones [116,118]; quinoxalines and acetylated quinoxalines [121]; dabsylhydrazones [123]; benzoylation [125]; substituted azobenzene [155]; 3-hydroxy-2-naphthoic hydrazide [158].

b.2 Fluorescence

The following precolumn reactions of saccharides to form fluorescing derivatives have been reported: with 2-aminopyridine [105, 110, 113, 117]; with naphthoyl chloride [110]; preparation of glycamines labelled with NBD-F [122]; dansylhydrazones [124, 126]; dabsylhydrazones [124].

c. Postcolumn reactions

Historic methods of postcolumn treatment of saccharides involved color reactions with some chromogenic reagents in strong mineral acids. In these cases the proposed mechanism is dehydration of the saccharide molecules to formation of furfural derivatives which condense with chromogenic reagents (orcinol, anthrone) to give colored compounds having absorption maxima at 420 nm [167] and 640 nm [168] respectively.

At present, automated instrumentation has been introduced for performing postcolumn reactions: automatic injectors, double-plunger reagent pumps, photometric or fluorometric detector and temperature-regulated columns [169]. Postcolumn reaction detectors have contributed successfully to the HPLC analysis of saccharides since these detectors have, in many cases, demonstrated high selectivity and sensitivity. The applications of postcolumn reactions with HPLC separated mono- and oligosaccharides to give products which absorb in

the UV and visible regions or which fluoresce, are presented in Tables 1 and 2 and compiled in Table 6.

c.1 Ultraviolet and Visible absorption

Reagents for postcolumn reaction with saccharides which absorb in the UV-VIS range are as follows:

Ultraviolet region: 2-cyanoacetamide [23, 49, 58, 59]; cuprammonium reagent [61, 131, 162].

Visible region: p-aminobenzoic acid hydrazide [35]; orcinol [49]; tetrazolium blue [51, 81, 98].

c.2 Fluorescence

Compounds containing aromatic ring or multiple conjugated double bonds have fluorescence spectra in the visible or ultraviolet regions. Electron donating groups as -OH, $-OCH_3$ and $-NH_2$ enhance fluorescence. Compounds which are nonfluorescent can be converted into fluorescent derivatives by postcolumn coupling with fluorogenic reagents. The main advantages of fluorescence monitoring are marked specificity, high sensitivity and stability to thermal change. Fluorescence labeling is possible with gradient elution. However, the solvents used must be transparent, both to the exciting ultraviolet and the fluorescence wavelength.

The fluorogenic reagents used for postcolumn reactions with saccharides are as follows: monoethanolamine-boric acid [19, 22]; taurine-sodium methaperiodate [21]; 2-aminopyridine under reductive conditions [113, 152, 153]; arginine-boric acid [156]; photoreduction of anthraquinone derivative by the saccharide and measurement the change in the fluorescence [77]; 2-cyanoacetamide [46].

c.3 Miscellaneous

The requirements for more specific and sensitive monitoring of HPLC separated saccharides brought the development of new detection

systems: Amperometry [20]; Indirect amperometry [89, 140]; Electrochemical [139] and Indirect potentiometry [144].

F. APPLICATIONS

The greater use of HPLC relative to gas chromatography is more pronounced in the case of food, clinical - biochemical and biological applications. In the case of biochemical applications, which require greater sensitivity of the detectors, the increasing use of pre- and postcolumn derivatization techniques coupled with ultraviolet or fluorescence detection enables us to obtain satisfactory results. Recent advances in the HPLC analysis of oligosaccharides have made it possible to study the structure of complex biological compounds, such as glycoproteins, glycosaminoglycans, cyclodextrins and lipopolysaccharides. The oligosaccharide chains of these biological systems have been analyzed by HPLC following enzymatic or chemical treatment.

For foodstuff, the use of HPLC is favoured over gas chromatography since saccharides in foods are generally present in adequate concentrations to be monitored by the less sensitive detectors of HPLC. The application of HPLC techniques, including the detection systems, to the analysis of mono- and oligosaccharides is very common in the food and beverage industry.

A great number of articles on the HPLC analysis of the saccharides in hydrolyzates of pulp (paper), woods as well as in different types of plant-tissue, seeds, worts and beets have been recently published.

Table 7. Applications (Biological, Biochemical, Biomedical, Pharmaceutical)

Item number in Tables 1 and 2
1, 11, 13, 20, 21, 22, 24, 30, 32, 33, 34, 36, 37, 43, 50, 53, 54, 55, 57, 58, 60, 69, 70, 75, 80, 81, 85, 90, 91, 98, 100, 103, 104, 107, 108, 113, 118, 123, 128, 130, 133, 134, 140, 144, 146, 147, 152, 158, 159, 160, 163, 165, 166, 169, 171, 172, 173, 176.

Table 8. Applications (Food, Beverages)

Item number in Tables 1 and 2
6, 9, 15, 18, 23, 27, 35, 37, 43, 45, 48, 49, 58, 60, 63, 66, 67, 69, 72, 73, 76, 77, 78, 83, 84, 89, 93, 95, 105, 109, 110, 113, 115, 117, 119, 125, 129, 131, 147, 155, 156, 159, 161, 164, 167, 174, 177.

Table 9. Applications (Wood, Pulp, Plants, Seeds, Worts, Beets)

Item number in Tables 1 and 2
5, 8, 10, 12, 14, 17, 25, 27, 28, 29, 44, 51, 64, 65, 71, 79, 88, 93, 97, 102, 105, 106, 124, 135, 136, 137, 139, 143, 148, 149, 153, 154, 168.

Table 10. Applications (Miscellaneous)

Item number in Tables 1 and 2
2, 3, 4, 7, 16, 19, 26, 31, 38, 39, 40, 42, 46, 47, 52, 56, 59, 61, 62, 68, 74, 82, 86, 87, 91, 101, 111, 112, 114, 116, 120, 121, 122, 126, 127, 132, 138, 141, 145, 150, 151, 157, 162, 175.

The HPLC separation efforts cited in the present review are classified, according to the following application fields: biological, biochemical, biomedical, pharmaceutical (Table 7), food, beverages (Table 8), pulp, cellulosic, lignocellulosic materials, plant-tissue, seeds, worts, beets (Table 9) and other applications (Table 10).

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