HIGH PERFORMANCE LIQUID CHROMATOGRAPHY OF NATURAL PRODUCTS

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The period 1974–1978 has seen a dramatic increase in the scope and versatility of high-performance liquid chromatography (hplc), due largely to the availability of the necessary instrumentation and to improvements in the efficiency and nature of column packing materials. The technique has been widely adopted in the natural products area, but the results of this work have been scattered throughout a large number of journals and it has been difficult for an investigator to find suitable conditions for a particular separation. This review is thus an attempt to summarize the literature dealing with the applications of hplc to secondary metabolites from 1974 to June 1978.

I have chosen for the purposes of this review to exclude most of the primary metabolites (amino acids, simple carboxylic acids, nucleotides, etc.), since the literature in this area has been reviewed on a number of occasions. These reviews include books devoted to biomedical applications of high pressure liquid chromatography (1–2) and to liquid chromatography in general (3), and to reviews on separation by ion-exchange methods (4), by reverse-phase methods (5), and by combinations of methods (6). In addition, the separations of certain specific classes of primary metabolites have been reviewed, including amino acids and peptides (3, 7), carboxylic acids (3, 8), purine nucleosides (9), and carbohydrates (13–14). Separations of the cytokinins (modified plant nucleosides) are described in two recent papers (15–16), while other representative separations of nucleosides (5, 7–21) and nucleotides (6, 22–26) are given in the references cited.

The material that follows has been organized along biosynthetic lines as far as possible. Separations of acetate-derived materials are discussed first, followed by the mevalonate-derived compounds and, finally, the nitrogen-containing natural products. A final section summarizes the separations of antibiotics and mycotoxins derived from lower plants. No attempt has been made to be comprehensive, since many very similar separations of closely related compounds are reported in the literature, and it is unnecessary to include them all. Enough examples have been given, however, so that each major method reported for a particular class of compounds is included.

There have been no reviews published to date dealing specifically with the hplc of natural plant products, but a number of reviews on pharmaceutical analysis have included discussion of the hplc of various natural products (especially antibiotics, alkaloids, and steroids) (27–31). In addition, a data compilation summarizing separations reported in the period 1969–1972 has appeared (32) and contains references to the hplc of carbohydrates, steroids, and amino acids and peptides.

CARBOXYLIC ACIDS AND LIPIDS.—Hplc of free fatty acids has been handicapped by the fact that most of them are not uv-absorbing at 254 nm, and hence are not detectable by a standard uv-detector. For analytical purposes the problem can be solved by derivatization with a uv-absorbing reagent, and phenacyl groups

(33–35), benzyl groups (36), 2-naphthacyl groups (37), and 4-hydroxymethyl-7-methoxycoumarin groups (38) have been used for this purpose. Conversion to quinoxalones has been used for derivatization of α-ketoacids (39). If derivatization is either not feasible or not desirable, detection may be carried out with a wire transport-flame ionization detector (40) with a differential refractometer (41) or, in certain cases, with an electrochemical detector (42, 42a).

A summary of recent separations of carboxylic acids (including esters and phenolic acids) is given in table 1; a summary of earlier separations by ion-exchange chromatography has appeared elsewhere (43a). A proprietary column for the separation of fatty acids is commercially available and is similar to the octadecylsilyl bonded phase columns described in the table (61).

The separation of lipids by hplc has been reviewed recently (63–65); hence, this section is abbreviated. A comparison of chromatographic methods for glycerol esters has been made (65), and silver-loaded columns have been applied with some success to the separation of isomeric alkene derivatives (67–69), although the reverse-phase technique is also effective at separating geometric isomers (47, 70). Reverse-phase chromatography has been used for the separation of prostaglandins (71), while non-aqueous reverse-phase chromatography is effective at separating glycerides (72). In this latter case, detection can be accomplished with an infrared spectrophotometer. Glycerides may also be separated on silica gel (73) and with a proprietary "triglyceride" column (61).

TABLE 1. *Separation of carboxylic acids and derivatives.*

Compounds separated	Column	Temp.[a]	Mobile phase	Ref.
Short-chain carboxylic acids				
Carboxylic acids	μBondapak C-18	—	1.0 M acetate buffers, various pH	44
Benzoic acids	Lichrosorb SI-60 silanized or Bondapak C-18 Porasil B, coated with 1-pentanol	25°C	Tetrabutylammonium hydrogen sulfate (0.03 M) in pH 7 phosphate buffer, 0.04 M	45
Carboxylic acids from plant tissues	Bio-Sil A, 20–44 μm, with H_2SO_4	—	Cyclohexane-chloroform (1:1), with 4.5–40% t-amyl alcohol	46
Organic acids in foods	Aminex A25	70°	1.0 M sodium formate	41
Long-chain and complex acids				
Long-chain acids	Micropak SH-10	—	Hexane-chloroform (9:1), with 0–70% ethanol	40
Long-chain acids	Vydac RP	—	Methanol-water (60:40)	47
Retinoic acid	Partisil-10	—	Dichloromethane-acetic acid (99:1) Dichloromethane-methanol-acetic acid (97.5:1.5:1.0)	48
Abscisic acid	Biobeads SX-12	—	Tetrahydrofuran	49
Abscisic acid	Zipax SCX	50°	Nitric acid, pH 1.7	50
	Corasil C-18	50°	Phosphoric acid, pH 2.6→ methanol	50
Abscisic acid	Amberlite IRC-50	—	Methanol-water (4:6)	51
	Aminex A-6	—	Methanol-water (4:1)	51
Carboxylic esters				
Long-chain esters	Bondapak C-18 Porasil	—	Acetonitrile-water (85:15)	52
Lauric acid esters	μBondapak C-18	—	Methanol-water (60:40)	53
Long-chain esters	Vydac RP	—	Methanol-water (60:40)	47
Phenacyl esters of long-chain acids	μBondapak C-18	—	Acetonitrile-water (67:33)	33
Phenacyl esters of carboxylic acids	Corasil II, nonyl bonded	40°	Methanol-water (62.5:37.5) Heptane-chloroform (87.5–12.5)	34
Phenacyl esters of carboxylic acids	Corasil II, nonyl bonded	40°	Methanol-water (68:32)	35
Benzyl esters of carboxylic acids	Corasil II	—	Chloroform-heptane (1:1)	36

TABLE 1. *Continued.*

Compounds separated	Column	Temp.[a]	Mobile phase	Ref.
2-Naphthacyl esters of long-chain acids	Corasil C-18			
4-Hydroxymethyl-7-methoxycoumarin esters	Nucleosil 10 C-18	—	Methanol-water (50:50→100:0)	38
Quinoxalones of α-ketoacids	μBondapak C-18	—	Methanol-ammonium acetate (20:80)→methanol	39
Acetates of long-chain unsaturated alcohols	Nucleosil 10 SA, silver loaded	10°	Methanol	39a
Phenolic acids				
Phenolic acids	Beckman PA-28	55°	Citrate buffer pH 3.28 Boric acid-citrate buffer, pH 4.53	54
Phenolic acids	Merckogel SI-150	—	Dichloromethane-ethanol-water (85.4:12.5:2.3)	57
Phenolic acids	μBondapak C-18	—	Water-acetic acid (95:5)	58
Beer flavor compounds	Vydac	—	Hexane, then methanol-chloroform-acetic acid (30:70:1)	76
Phenolic acids	Silica gel saturated with 0.5 M sulfuric acid		Chloroform-cyclohexane (1:1)	60
Homovanillic acid	Vydac SC anion	45°	0.025 M acetate buffer, pH 4.7–0.025 M citrate buffer, pH 5.3 (4:1)	
Vanilmandelic and homovanillic acid	Hitachi gel No. 3010	30°	0.05 M tartrate buffer-methanol (4:1) (pH 2.75–4.8)	55
Sinapic, ferulic, and p-coumaric acids	Zipax SAX	25°	Sodium acetate-citric acid-water (1:1:8)	56
Various phenolic acids	Zipax SAX	25°	0.1 M acetate buffer pH 4.7–0.25 M citric acid, pH 1.8-water (1:1:8)	42a
Chlorogenic acid	Pellidon	—	0.1 M citric acid	59

[a]Column temperature, in degrees Celsius. If no temperature was specified, or if it was specified as ambient, this column is left blank.

PHENOLS AND FLAVONES.—Phenolic compounds occur widely in plants, but until recently little attention has been paid to the hplc of simple phenols. The hplc of phenolic acids has been included in the previous section, and the separation of phenolic amines and various phenolic heterocyclic compounds will be discussed later. At this point, it is sufficient to note that phenolic compounds occur in beer, and several papers dealing with the hplc of the components of beer or of hop resins include data on the separation of phenols, phenolic acids, and other compounds such as humulone (74–84). Hplc has also been applied to the separation of the phenolic components of tobacco (85) and cocoa (42a).

Flavones and related compounds have been investigated more extensively, and table 2 summarizes the major publications in this area. Simple flavones have been separated by normal phase (adsorption) chromatography on silica gel and also by reverse-phase chromatography. Although Sephadex LH–20 has been used extensively for the separation of flavones in classical column chromatography (103), no comparable material has been developed for hplc. The situation is a little different with polyamide, which is also used extensively in classical chromatography (103). In this case a commercial polyamide packing is available (62), but it has not yet been extensively used for flavonoid separations. The material of widest application, being suitable both for simple flavonoids and for flavonoid glycosides, is the octadecyl bonded reverse-phase silica packing.

TABLE 2. *Separation of flavonoids and related compounds.*

Compounds separated	Column	Temp.	Mobile phase	Ref.
Simple flavones				
Quercetin, kaempferol, and related compounds	Vydac 101 SC	—	Hexane-chloroform (1:1)→methanol-chloroform-acetic acid (50:50:1)	76
Isoflavonoids	Merckosorb SI 60	—	Hexane-tetrahydrofuran (2:1) diisopropyl ether	86
Flavone ethers	Pellosil HC	—	Diisopropyl ether-methanol (92:8)	86
Flavanone-chalcone	Pellidon	—	Methanol-water (3:1)	86
Hesperitin and hesperidin	μBondapak C-18	—	0.03 M potassium dihydrogen phosphate (pH 4.8), with 10→100% methanol	87
Flavonoids	μBondapak C-18	—	Methanol-water-acetic acid (30:63:5)	37
Isoflavones	Partisil-10 ODS	—	Water-acetonitrile (4:1)	88
Flavanone-chalcone	Pellidon	16°	Water-methanol (40:60)	89
		60°	Water-methanol (60:40)	
Flavonoid glycosides				
Isomeric glycoflavones	LiChrosorb NH$_2$	—	Acetonitrile-water (1:9→9:1)	90
Glycoflavones	Zorbax ODS	50°	Ethanol-0.1 M phosphoric acid (20:80→100:0)	91
Naringin and naringenin rutinoside	μBondapak C-18	—	Acetonitrile-water (20:80)	92
Flavonol glycosides	μBondapak C-18	—	Methanol-water-acetic acid (30:70:5)	93
Hesperidin	μBondapak C-18	—	see above	87
Flavonoid derivatives and related compounds				
Neohesperidin dihydrochalcone	LiChrosorb SI-60 with C-18 Bonded	—	Methanol-water (60:40)	94
Neohesperidin dihydrochalcone	μBondapak C-18	—	Acetonitrile-water (25:75)	95
Catechin and catechin gallates	Vydac	—	Hexane-chloroform (1:1)→methanol-chloroform-acetic acid (50:50:1)	76
	μBondapak C-18	23°	Acetone-water-acetic acid (60:139:1)	96
			0.02 M citrate-phosphate buffer (pH 4.5)	96
			Methanol-0.1 M citrate-phosphate buffer pH 7 (20:80)	96
			Methanol-dimethylformamide-water-acetic acid (2:40:157:1)	
Anthocyanins	Pellidon	—	Chloroform-methane (87:13)	97
Anthocyanidins	μBondapak C-18	—	Water-acetic acid-methanol (71:10:19)	98
	μBondapak C-18	—	Water-acetic acid-methanol (75:5:20)	93
Gallotannin	Corasil II	—	Hexane-methanol-tetrahydrofuran-acetic acid (3000:100:25:1)→methanol-tetrahydrofuran-acetic acid (100:25:1)	99
Flavolignans	μBondapak C-18	—	Methanol-water-acetic acid (40:60:5)	100
				101
			Methanol-water-acetic acid (20:75:5)	93
Podophyllotoxin and related compounds	Lichrosorb RP-8	25°	Acetonitrile-water (4:6)	102

QUINONES.—The hplc of quinones has not been extensively investigated. Complex mixtures of prenylquinones were separated on silica gel with small amounts of dioxane in hexane as eluent (104), while various anthraquinones have been separated on silica gel using a cyclohexane to ethyl acetate gradient (simple anthraquinones) (105), methanol in n-pentane (anthraquinones and their glycosides) (106), and hexane-ethyl acetate-acetic acid, 83:17:1 (*Aspergillus versicolor* quinones) (107). A cyano-type bonded phase was also effective in the separation of the *A. versicolor* quinones with a hexane-chloroform-acetic acid, 65:35:1, solvent system (107), while sennosides A and B have been separated by ion-pair chromatography on a C–18 reverse phase column with an aqueous methanol solvent containing tetrabutyl phosphate (107a).

OXYGEN-CONTAINING HETEROCYCLIC COMPOUNDS.—Relatively few hplc studies have been carried out on this diverse class of natural products, and these have tended to focus on a restricted group of compounds. The summary in table 3

TABLE 3. *Separation of oxygen-containing heterocyclic compounds.*

Compounds separated	Column	Temp.	Mobile phase	Ref.
Furanoterpenoids	Corasil II	—	Ether-pentane (1:1.3:1)	108
Furocoumarins	Corasil I	—	Chloroform-cyclohexane	109
Bergapten	Corasil II	—	Hexane-chloroform (75:25)	110
	μPorasil	—	Isooctane-ethyl acetate-isopropanol (80:1:1)	111
	Zorbax SIL			
	Corasil II	—	Chloroform-hexane-methanol (25:75:0.125)	111
Rotenoids	μPorasil	—	Chloroform-*iso*-octane (35:65)	112
	Zorbax ODS	34°	Methanol-water-phosphoric acid (60:39.9:0.1→85:14.9:0.1)	113
	μBondapak C-18	—	Methanol-water (80:20)	114
Xanthones	Micropak CN	—	Hexane-chloroform (13:7.2:3)	115
	Micropak NH_2	—	Isooctane-chloroform (3:17)	115
			Dioxan-dichloromethane (1:9)	
O-Glycosylxanthones	Micropak CN	—	Hexane-chloroform (1:1)	116
			Isooctane-chloroform (3:7)	
Tocopherols	LiChrosorb SI 60	—	Hexane-dioxan (99:1)	104
	Corasil I	—	Hexane-diisopropyl ether (96:4)	117
	Corasil II	—	Hexane-tetrahydrofuran (199:1)	118
Cannabinoids	μBondapak C-18	—	Methanol-water (75:25)	119
	Phenylcorasil	—	Methanol-water (70:30)	119
	Bondapak C-18 Corasil	—	Acetonitrile-water (45:55)	120
	μPorasil	—	Chloroform-heptane (20:80.80:20)	120
	Partisil-5	—	Methanol-0.2 N sulfuric acid (4:1)	121-123
	Partisil PAC	—	Isooctane-dioxan (3:2)	124
Cannabinoids, dansyl derivatives	Micropak SI-10	—	Hexane→dichloromethane-methanol (98:2)	125

indicates that no one method is suitable for all compounds; the non-polar furocoumarins, for example, separate nicely on a normal-phase column, but polar glycosylxanthones require a polar bonded phase. Considerable attention has been paid to the separation of cannabinoids by hplc, and here a method using the octadecyl reverse-phase column is the most commonly used method, although other column packing materials have also given good results.

TERPENES AND PLANT PIGMENTS.—Separations of various members of these classes are indicated in table 4. The use of a column packed with small-particle

polyethylene powder for the separation of various chlorophylls is noteworthy (150), as is the observation that the addition of small amounts of water to the solvent improves the separation of polar terpenes on normal-phase silica gel packings (134). This result, which has been made use of by other workers also, is probably due to the formation of a partition-type system on the column. Care must be

TABLE 4. *Separation of terpenes and plant pigments.*

Compounds separated	Column	Temp.	Mobile phase	Ref.
Cinnamon oils	Corasil II	—	Cyclohexane→Cyclohexane-ethyl acetate (60:40)	126
Essential oils	μBondapak C-18	—	Methanol-water (1:1)	127
	μStyragel	—	Tetrahydrofuran	127
Sesquiterpene lactones	Porasil A	—	Acetonitrile	126
	μPorasil	—	Hexane-ethyl acetate (85:15)	129
Phorbol and esters	Sil-X	—	Diethyl ether-methanol (9:1)	130
Limonin	μPorasil	35°	Chloroform-acetonitrile (95:5)	131
	μBondapak CN	—	Methanol-water (35:65,40:60)	132
Triterpenes	Silica gel	—		133
	μPorasil	—	Chloroform-methanol-water (190:11:1)	134
	μPorasil	—	Hexane-ether, 50% water saturated (85:15)	134
Juvenile hormones	μPorasil	—	Pentane or hexane with a few %	135
	LiChrosorb SI 60	—	diethyl ether	136
Carotenoids	Woelm B18 alumina deactivated with isopropanol		Benzene-hexane (3:5), with 0.01% BHT	137
	Spherisorb deactivated with 95% ethanol		Tetrahydrofuran-hexane (1:5), water saturated, with 0.1% BHT	137
	Permaphase ODS	—	Methanol-water (95:5)	138
	Alumina	—	Methanol→methanol water (90:10)	139
Retinals	μPorasil	—	Petroleum ether-diethyl ether (98:2)	140
	Zorbax SIL	—	Hexane-diethyl ether	141
Vitamin A	Micropak SI 10	—	Petroleum ether-dichloromethane-*iso*propanol (80:19.30.7)	142
	Vydac ODS	—	Acetonitrile-water (65:35)	143
	Zorbax 5μ	—	Hexane-dichloromethane-*iso*propanol (300:200:15)	143
	LiChrosorb RP	—	Methanol-water (90:10)	144
	Permaphase ODS	—	Methanol-water (63:37)	145
Peiricidins (pyridinoterpenes)	Micropak SI 10	—	Hexane-tetrahydrofuran (95:5)	146
	Micropak CN	—	Hexane-tetrahydrofuran (97:3)	146
	Vydac RP	60°	Methanol-water (70:30,40:60)	146
Carotenoid and porphyrin plant pigments	Bondpak C-18	18°	Methanol-water (80:20)→	147
	Porasil B	28°	methanol-diethyl ether (25:75)	
	Spherisorb 10μ	—	Hexane-acetone (98:2→50:50)	148
	Partisil 5	—	Hexane-acetone (99:1→25:75)	148
Xanthophylls	Sil-S-1 ODS	—	Methanol-water (82:18), with acetone added (1:1) to last 40%	149
Chlorophylls	Microthene FN 500 polyethylene	—	Acetone-water (35:65→25:75)	150
Porphyrins	Partisil 5	—	Cyclohexane-ethyl acetate (40:60)	151
	Micropak CN	—	Heptane-ethyl acetate-isopropanol (60:40:0.5)	152
	Pellionex SAX	—	Methanol→Methanol-acetic acid (85:15)	153
Porphyrin methyl esters	Merckosorb SI-60	—	Heptane-tetrahydrofuran (80:20→50:50)	154
	Corasil II	—	Petroleum ether-ethyl acetate (55:45)	153
	Merckosorb SI-60	—	Hexane→ethyl acetate	153
	Partisil 10	—	Hexane→ethyl acetate	153

taken not to use too much water, otherwise baseline stability for refractive index detectors is destroyed by the sample injection solvent; the recommendation is made that solvents should be 50–75% water-saturated.

STEROIDS.—Hplc has been applied extensively to the separation of steroids, and the analysis of steroid hormones by hplc has been the subject of a recent review (155). Although many of the steroids investigated are not natural products, the separations developed for them can be applied in many cases to related compounds such as the triterpenes which are natural products. Table 5 summarizes some of the recent separations that have been achieved on steroids; because of the similarity of many of these separations, only representative examples have been given.

TABLE 5. *Separation of steroids.*

Compounds separated	Column	Temp.	Mobile phase	Ref.
Steranes	Woelm alumina	—	Pentane	156
Sterols	Bondapak C-18 Porasil B	—	Hexane-isopropanol (99.5:0.5)	157
	μBondapak C-18	—	Methanol-chloroform-water (71:12:17,71:16:13)	158
	μPorasil	—	Hexane-ethyl acetate (97.5:2.5)	159
	Spherisorb ODS	—	Methanol-water (115:95)	
	Hypersil ODS	60°	Methanol-pH 5 buffer (45:55)	160
Sterol acetates	Bondapak C-18 Corasil	—	Methanol-chloroform-water (85:6:9)	158
Sapogenin benzoates	LiChrosorb RP8	—	Acetonitrile-water (8:2)	161
Withaferin A and metabolites	μPorasil	25°	Ethyl acetate-hexane (5:1) Ethyl acetate	162
Ecdysones	Bondapak phenyl Corasil	20°	Water→water-ethanol (80:20)	163
	Corasil II	20°	Chloroform-ethanol (8:1)	163
	Corasil II	—	Chloroform-ethanol (4:1,9:1,14:1)	164
Bufadienolide aglycones	μBondapak C-18	—	Methanol-water (2:1) Acetonitrile-water (1:1) Tetrahydrofuran-water (2:3)	165
	μPorasil	—	Hexane-tetrahydrofuran (1:1)	
Cardenolide aglycones	μBondapak C-18	—	Methanol-water (2:1) Acetonitrile-water (2:3) Tetrahydrofuran-water (1:2)	165
	μPorasil	—	Hexane-tetrahydrofuran (1:2)	
17-Ketosteroids	Micropak CH	45°	Methanol-water (43:57)	166
Estrogens	μBondapak C-18	30°	Acetonitrile-water (24:76) Methanol-water (41:54)	167
Vitamin D and related compounds				
Cholecalciferol, ergocalciferol	μPorasil	—	Hexane-chloroform-tetrahydrofuran (30:70:1)	168
Vitamin D_2	Zorbax Sil	—	Heptane (50% water saturated)-ethyl acetate-dichloromethane (84:4:12)	169
Vitamin D_2 isomers	Zorbax Sil	—	Pentane-ethyl ether-methanol (2000:60:3); pentane-chloroform (45:55)	170
Vitamin D_3	Partisil-10	—	*Iso*octane-ethanol (984:16)	171
Vitamin D_3 metabolites	Zorbax Sil	—	Dichloromethane-methanol (98:2)	172
Vitamins D_2 and D_3	μPorasil	—	Light petrol-1,2-dichloroethane-tetrahydrofuran (85:8:7)	173
Vitamin D_2 and D_3 metabolites	Zorbax Sil	—	Skellysolve B-isopropanol (90:10)	174
Hydroxylated Vitamin D_3	Zipax ODS Permaphase	—	Methanol-water (3:7→8:2)	175
25-Hydroxycholecalciferol	Zorbax ODS	—	Acetonitrile-methanol-water (18:1:1)	177
Ergosterol, sitosterol, and related steroids	Sil-X-1	—	*Iso*octane-ethyl acetate (37:3)	178
Steroid hormones				
Estradiol, estrone, etc.	μBondpak C-18	—	Acetonitrile-water (55:45)	156

TABLE 5. *Continued.*

Compounds separated	Column	Temp.	Mobile phase	Ref.
Aldosterone, cortisone	Amberlite LA-1 on Plaskon polymer	—	Water	156
Canrenone	Sil-X	—	Heptane-*iso*propanol (96.4)	179
Methandrostenolone	LiChrosorb SI 60	—	Hexane-*iso*propanol-1,2-dichloroethane (82:15:3)	180
Progesterone	Zipax ODS Permaphase	43°	*Iso*propanol-water (18:82)	181
Estrogens	μBondapak-NH_2	—	Heptane-*iso*propanol (4:1)	182
	Partisil-10 ODS	—	Methanol-0.1% ammonium carbonate (55:45)	183
	Partisil-5	—	Hexane-ethanol (95:5)	183
Equine estrogens	Zipax ETH Permaphase	40°	Hexane-tetrahydrofuran (98:2)	184
Progesterone, etc.	μBondapak C-18	—	Acetonitrile-water (45:55)	185
Ethynodiol diacetate	μBondapak C-18	—	Methanol-water (4:1)	186
Corticosteroids, estrogens, etc.	LiChrosorb SI 100 with formamide	25°	Dichloromethane (saturated with formamide)	187
Corticosteroids	Styrene-divinyl benzene copolymer	—	Methanol-water (9:1)	188
	Silica gel	—	Chloroform-methanol (97:3)	189
	Silica gel	—	Dichloromethane-ethanol (95:5)	190
	Zorbax-SIL	20°	Dichloromethane-ethanol-water (968:20:12)	191
	μBondapak C-18	—	Methanol-water (45:55)	192
			Acetonitrile-water (60:40)	192
Hydrocortisone and hydrocortisone phosphate	μBondapak C-18	—	10^{-2} M Tetrapentylammonium hydroxide in methanol-water (420:500) at pH 7.5	193
Bile acids				
Isomeric bile acids	Zorbax ODS	25°	Methanol-water (8:2) pH2	194
Bile acids	μBondapak C-18	—	Acetonitrile-0.3% ammonium carbonate (4:9.4:11)	195
Esterified bile acids	Micropak-NH_2	—	2,2,4-trimethylpentane-dichloromethane (1:1→1:9)	196
Conjugated steroids				
Conjugated 17-keto steroids	Micropak-CH	45°	Methanol-water (20:80,30:70)	166
Conjugated estrogens	μBondapak C-18	30°	Methanol-water (41:59), Acetonitrile-water (24:76)	167
Conjugated bile acids	Corasil II	—	*Iso*propanol-ethyl acetate-water-ammonia (130:300:25:1.5)	197
	Corasil II	—	Acetonitrile-acetic acid-formic acid-water (500:10:5.10)	197
Steroid glucuronides	LiChrosorb RP-18 with *n*-pentanol as stationary phase	25°	Phosphate buffer (pH 7) or 0.041 M tetrapropyl ammonium bromide in phosphate buffer, pH 6.4	198
Cardiotonic steriod conjugates (bufotoxins, bufogenins)	μBondapak C-18	—	Methanol-water (2:1)	199
			Methanol-0.2 M $(NH)_4H_2PO_4$ (2:1)	
			Tetrahydrofuran-water (1:2,1:3)	
Conjugated estrogens	Zipax-ETH Permaphase	60°	Heptane-*iso*propanol (98.9:1.1)	200
	LiChrosorb RP18	—	Acetonitrile-0.05 M phosphate buffer, pH 8, with 0.1% CTMABr	201
	LiChrosorb RP8	—	Methanol-0.1 M sodium perchlorate, 0.05 M phosphate, pH 8 (40:60)	201
	LiChrosorb RP2	—	Butanol-0.05 M phosphate, pH 8 (7:93)	201
			Acetonitrile-0.05 M phosphate, pH 8 (15:85)	201

STEROID AND OTHER PLANT GLYCOSIDES.—The separation of polar plant glycosides by classical techniques has generally proved more difficult than the separation of less polar secondary metabolites. The great advantage of hplc in this area is that it allows the use of a variety of column packings, particularly including the octadecylsilyl (ods) reverse-phase packing. The two most popular methods for separation of the plant glycosides seem to be the use of the ods packing or the use of a normal-phase silica gel packing with a mobile phase containing a small amount of water.

TABLE 6. *Separation of steroid and other plant glycosides.*

Compounds separated	Column	Temp.	Mobile phase	Ref.
Digitalis glycosides and aglycones	Zipax SCX	45°	Amyl alcohol-water (4:96)	202
	μBondapak C-18	25°	Acetonitrile-water (25:75,25:75→40:60, 0:100→30:70)	203
Digitalis glycosides of the cardenolide group	LiChrosorb SI-60	—	*n*-Pentanol-acetonitrile-isooctane-water (175:60:620:10)	204
			t-Butanol-acetonitrile-heptane-water (204:93:712:10.4)	204
Digoxin and other cardenolides	Silica gel	—	Cyclohexane-ethanol-acetic acid (60:9:1)	205
Digitalis glycosides	LiChrosorb SI 60	—	Dichloromethane-methanol (95:5, saturated with water)	206
	Nucleosil C-18	—	Acetonitrile-water (37:63)	206
			Dioxan-water (45:55)	
	Nucleosil C-18	—	Acetonitrile-dioxan-water (20:20:60)	207
Cardiac glycosides in milkweed plants	μBondapak C-18	—	Acetonitrile-water (25:75,30:70)	208
Senna glycosides	Nucleosil C-18	—	Acetonitrile-0.01 M Sodium bicarbonate (1.5:98.5→50:50)	209
				210
	Corning CPG	—	pH 6 buffer	210
Ginsenosides	Porasil A	—	Chloroform-methanol-ethyl acetate-water (2:2:4:1), lower phase)	211
Glycyrrhizin	Permaphase AAX	50°	*Iso*propanol-pH 5.2 phosphate buffer (30:70)	212
	Permaphase AAX	45°	Ethanol-pH 5.2 phosphate buffer with 0.005 M sodium perchlorate 1:1	212
Nitrobenzoyl esters of digitalis glycosides	Mercksorb SI 60	—	Hexane-chloroform-methanol (10:1:0.5) Hexane-dichloromethane-acetonitrile (10:3:3)	213
Iridoid and secoiridoid glucosides	μBondapak C-18	—	Methanol-water (40:60, 20:80, etc)	
Benzaldehyde cyanohydrin glycosides	μBondapak C-18	—	Acetonitrile-water-acetic acid (5:94:1)	215
	μPorasil	—	Ethyl acetate-methanol (97:3)	

CARBOHYDRATES.—The liquid chromatographic analysis of carbohydrates has been extensively studied, and several reviews of this area have appeared (10–14). This section will therefore only summarize the major results of recent research.

Ion-exchange chromatography continues to be widely used in carbohydrate separations, particularly for mono-, di-, and tri-saccharides, with some workers using anion exchange (216–218), some using cation exchange (217–220), and

others using combinations of both methods (221–226). The use of borate complexes to separate sugars on ion-exchange columns has also been reported (227–229), while the incorporation of boronic acid residues into the column has also yielded good separations of vicinal diols (230). Molecular exclusion chromatography has been applied to the separation of polysaccharides (231), oligosaccharides (232) and substituted carbohydrates (233).

Separations have, surprisingly, also been achieved on silica gel (234, 235). In this case, however, the polar solvent systems used presumably give rise to a polar adsorbed layer on the column packing and, thus, bring about separation by a liquid-liquid partition mechanism. Most recent developments have centered around the use of bonded-phase packings, however, and both amino bonded phases (236–240) and cyano bonded phases (241) have been found to be very effective at separating various carbohydrates.

Carbohydrate derivatives have also proved amenable to separation by hplc, and results have been reported for separations of benzoylated carbohydrates (242), 4-nitrobenzoylated carbohydrates (243), methylated carbohydrates (244), and polyhydric alcohols resulting from periodate cleavage of carbohydrates (245). Finally, a ligand exchange separation of amino sugars has been reported (246).

SIMPLE AMINES.—Studies on the separation of simple amines have been dominated by studies of clinically important biogenic amines and drugs. Little work has been done on simple basic plant natural products. The studies that have been done are summarized in table 7.

Most of the early studies on the separation of biogenic amines and related

TABLE 7. *Separation of amines.*

Compounds separated	Column	Temp.	Mobile phase	Ref.
Putrescine, spermine, etc., (as tosyl derivatives)	Permaphase ETH	30°	Acetonitrile-water (4:6)	249
	Zipax ETH	35°	Acetonitrile-water (4:6)	250
Polyamines	Partisil SCX	—	Pyridine acetate buffers, pH 3.5	251
Catecholamines	Zipax SCX	40°	Sodium dihydrogen phosphate, 0.035–0.35 M	252
	Zipax SCX	20°	Sodium dihydrogen phosphate, 0.15 M	253
	Zipax SCX	—	0.1 M perchloric acid	254
Biogenic amines, general	Zipax SCX	—	0.2 M ammonium phosphate, pH 7.0	255
Biogenic amines, acetyl derivatives	Spherosil XCA 400	25°	Ethanol-water-dichloromethane (15:17:968, lower layer)	256
Catecholamines, o-methyl metabolites	Zipax SCX	—	0.1 M perchloric acid	257
Catecholamines and metabolites (including acidic metabolites)	Spherisorb A20 alumina	—	Butanol-acetic acid-diethyl ether-water (4:1:0:1,4:1:1:1)	258
	Sperisorb S5 with 0.1 M $HClO_4$, 0.9 M $NaClO_4$	—	Butanol-dichloromethane (40:60)	258
	ODS/TMS silica	—	Water-methanol-sodium 1-dodecane sulfonate (72.5:27.5:0.02)	258
	Silica gel coated with 0.1 M $HClO_4$, 0.9 M $NaClO_4$	—	Ethyl acetate-hexane (9:1)	259
			Butanol-dichloromethane (2:3)	260
	LiChrosorb RP 18	70°	0.1 M phosphate buffer, pH 2.1	260
	Partisil 10 ODS	25°	0.2 M phosphate buffer, pH 1.9	261
			0.1 M phosphate buffer, pH 9.4	262
				263

compounds used the ion exchange method, and two reviews deal with the applications of this method to amines (247) and with general chromatographic methods for biogenic amines (298). More recent studies have utilized other methods, and the technique of soap chromatography has been shown to give excellent separations of the biogenic amines and their acidic metabolites (258). Electrochemical detection has also been used to good effect in this area (257).

Several publications dealing with the chromatography of drugs of abuse have appeared; these include many examples of the separation of simple amines such as amphetamines, nicotine, methadone, etc. (264–267). The reviews on pharmaceutical analysis mentioned earlier also contain examples of the separation of amines (27–31).

AMINO ACIDS, PEPTIDES AND PROTEINS.—A full discussion of the chromatographic separation of amino acids, peptides, and proteins is beyond the scope of this review, but some of the more recent developments in the area are discussed below.

The separation of amino acids is a common and important application of liquid chromatography. After the introduction of ion exchange methods (268), these techniques were widely adapted and served as the basis of commercially available amino acid analyzers (269, 270). Recent developments have included the use of a single column for analysis (271, 272), improved instrumentation (273), and separations of methylated amino acids (274, 275). It has also been shown that separations of amino acids may be obtained on non-polar bonded-phase columns, with (276) or without (277) the addition of "ion-pairing" reagents. The resolution of enantiomeric amino acids by liquid chromatography is a goal of much current research, and the D- and L- isomers of proline have been partially separated by ligand-exchange chromatography (278). The most dramatic achievement in this field to date, however, has been the resolution of several amino acid methyl esters through chiral complexation to a host covalently bound to silica gel (279). The diastereomeric N-camphor-10-sulfonamides of the 4-nitrobenzyl esters of some racemic amino acids have also been separated by hplc (280).

Amino acid derivatives are more amenable to separation by non-ionic chromatography, and major emphasis has been given to the phenylthiohydantoin derivatives, which have been separated both by normal and reverse-phase methods (281–286). Separation of dansyl amino acids has also been reported (187).

The separation of peptides and small proteins has also been achieved. Protected peptides can be separated on silica gel (287). Unprotected peptides do not separate well on silica, but they can be separated by ion exchange chromatography (251, 288–291). The claim has been made that hplc with non-polar stationary phases is superior to chromatography or ion-exchange columns for peptide separations (277), although it is not clear that the microparticle ion-exchange columns will prove inferior to the reverse-phase columns.

Proteins may also be separated by hplc, either by steric exclusion chromatography (299) or by reverse-phase chromatography (295–297).

ALKALOIDS.—The separation of alkaloids by hplc has been reviewed (300, 301), and numerous examples of separations of alkaloids are also included in reviews on pharmaceutical analysis by hplc (27–31). The separations of alkaloids are summarized in table 8.

One requirement for the separation of basic compounds, including alkaloids, amines, and some antibiotics, is that the pH of the mobile phase be above 7 and,

TABLE 8. *Separation of alkaloids.*

Compounds separated	Column	Temp.	Mobile phase	Ref.
Miscellaneous simple alkaloids				
Piperine	Porasil A	—	Chloroform	304
Nicotine and cotinine	Micropak SI 10	—	Ethyl acetate-isopropanol-ammonia (80:3:0.4)	305
Tetrahydroisoquinoline alkaloids	Vydac SCX	—	0.1 M Citric acid-0.2 M Na_2HPO_4 (48:32:20)	306
	LiChrosorb SI 60	—	Acetonitrile-ammonia (96.4)	
	μPorasil	—	Chloroform-1% concentrated ammonia in methanol	
Pyrrolizidine alkaloids	μBondapak CN	—	Tetrahydrofuran-0.1 M ammonium carbonate, pH 7.8 (16.84)	308
Pilocarpine	LiChrosorb RP-18	22°	5% Aqueous KH_2PO_4, pH 2.5-methanol (97:3)	309
Tropane alkaloids				
Atropine, apoatropine, scopolamine	Partisil 10	—	Diethyl ether-methanol-diethylamine (70:30:1, 90:10:1)	310
Atropine, scopolamine, cocaine	Merckosorb SI 60	20°	Diethyl ether-methanol (60:40) chloroform-methanol (80:20)	301
Tropane alkaloids	Sil-X	—	Tetrahydrofuran-ammonia (100:1)	311
Cocaine, benzoylecogonine	Partisil-10 ODS	40°	Acetonitrile-0.25 M phosphate buffer, pH 2.7 (83:17)	312
Hyoscyamine, scopolamine	Silica gel coated with picric acid	—	Chloroform, saturated with 0.06 M picric acid	313
Miscellaneous complex alkaloids				
Steroidal alkaloids	Porasil A	—	Hexane-acetone (1:2)→97% aqueous acetone	314
Colchicine and colchicoside	Silica gel, silanised	—	Acetonitrile-water (10:90)→acetonitrile	315
d-tubocurarine chloride	μBondapak C-18	20°	A 0.25 M Tetramethyl ammonium hydroxide in water-methanol (75:25)	316
			B Buffer-methanol (55:45) 90%A 10%B→15%A 85%B	
Purine alkaloids				
Theophylline and metabolites	Aminex A-5	55°	0.5 M $NH_4H_2PO_4$, pH 3.65	317
Theophylline	Durapak OPN	37°	Hexane-isopropanol (83:17)	318
Theophylline	Partisil 10	—	Heptane-chloroform-methanol (56:39:5)	319
	Vydac TP	—		
Caffeine, theophylline, and theobromine	LiChrosorb SI-60	—	Dichloromethane-ethanol-water (1872:94:34)	320
Cinchona alkaloids				
Cinchona alkaloids	Corasil II coated with 10–60% Poly G-300	—	Heptane-ethanol (10:1), 40% saturated with Poly G-300	321
Quinine, cinchonine	Poragel PT Cu	60°	Ethanol-0.2 M ammonia (1:2)	322
Quinine, quinidine, etc.	Silica gel	25°	Tetrahydrofuran-ammonia (99.8:0.2)	323
Quinone, cinchonine, etc.	Merckosorb SI-60	20°	Chloroform-methanol (8:2) Diethyl ether-methanol (7:3)	301
Morphine alkaloids				
Morphine, codeine	Poragel PT Cu	50°	Ethanol-0.2 M ammonia (1:2)	324
Morphine	μBondapak C-18	—	Methanol-0.1 M KH_2PO_4 (7:93)	325
Morphine, hereoin, thebaine, etc.	Merckosorb SI-60	—	Chloroform-methanol (9:1)	301
			Diethyl ether-methanol (8:2)	301
Morphine, heroin, etc.	Corasil II, coated with 60% Poly G-300	—	Heptane-ethanol (10:1), 40% saturated with Poly G-300	321
Thebaine	Corasil II	—	Hexane-chloroform-methanol-diethylamine (900:75:25:0.1)	326
Thebaine	μBondapak C-18	—	Methanol-0.3% ammonium carbonate (4:1)	327
Morphine, codeine, thebaine	μBondapak C-18	—	Acetonitrile-0.1 M NaH_2PO_4, pH 4.8 (1:3,1:19)	328

TABLE 8. *Continued.*

Compounds separated	Column	Temp.	Mobile phase	Ref.
Morphine, cryptopine, papaverine, narcotine, etc.	Corasil II	—	Hexane→chloroform-methanol-diethylamine (100:300:1)	329
Opium alkaloids (21)	μBondapak C-18	—	Methanol-water (40:60) with 0.005 M *n*-heptane sulfonic acid	330
Heroin, morphine	Bondapak C-18 Corasil	—	Acetonitrile-0.1% ammonium carbonate	266
Indole alkaloids				
Oxindole alkaloids	Bondapak C-18 Corasil	60°	Methanol-water (80:20)	331
Strychnine, brucine	Merckosorb SI-60	20°	Diethyl ether-methanol-diethylamine (90:10:1, 70:30:1)	332 301
Strychnos alkaloids	Merckosorb SI-60	20°	Diethyl ether-methanol-diethylamine (50:50:1)	333
Strychnine	μPorasil	—	Chloroform-methanol (90:10)	334
Rauwolfia alkaloids	ION-X-SC	—	Methanol-0.2 M $(NH_4)_2HPO_4$, pH 7 (7:3)	335
Reserpine	Bondapak C-18 Corasil	—	Methanol-0.5% ammonium chloride, pH 5.6 (55:45)	336
Alstonine, serpentine	Merckosorb SI-60	30°	Diethyl ether-methanol-diethylamine (70:30:1)	332
Catharanthus alkaloids	Woelm alumina W-200	—	Ethyl acetate-dichloromethane (25:75)	337
	Woelm alumina W-200	—	Dichloromethane-ethanol (100:0→100:5)	337
Catharanthus alkaloids	LiChrosorb RP-8	—	Acetonitrile-0.01 M $(NH_4)_2CO_3$ (47:53)	338
Tabernaemontana alkaloids	Bondapak C-18 Porasil	—	Methanol-water-ammonia (90:5:5)	339
	Porasil B	—	Chloroform-methanol-ammonia (99:0.8:0.2)	
	Porasil B	—	Hexane-chloroform (1:9)	
Ergot alkaloids				
Ergot alkaloids	Corasil	—	Chloroform-methanol-ethyl acetate-acetic acid (60:20:50:3) Chloroform-methanol (100:5)	340
Ergotamine	μBondapak C-18	—	Acetonitrile-0.01 M $(NH_4)_2CO_3$ (1:2)	341
	Nucleosil C-18	—		
Ergot alkaloids	Nucleosil C-18	—	Acetonitrile-0.01 M $(NH_4)_2CO_3$ (8:94→60:40)	209
Ergot alkaloids	LiChrosorb RP-2, RP-8, RP-18	—	Acetonitrile-0.02% $(NH_4)_2CO_3$ (42:58) etc.	342
Ergot alkaloids	LiChrosorb SI-60	—	Hexane-chloroform-ethanol (40:40:10)	343
	LiChrosorb RP-18	—	Acetonitrile-0.01 M $(NH_4)_2CO_3$ (40:60)	343
Clavines and lysergic acid derivatives	Micropak NH_2	25°	Diethyl ether-ethanol (80:20) Chloroform-isopropanol (90:10, 80:20)	344
Lysergic acid diethylamide	Bondapak C-18 Corasil	—	Methanol-0.1% $(N_4)_2CO_3$ (6:4)	345
	Sil-X	—	Acetonitrile-diisopropylether	346
	Corasil II	—	(40:60, 25:75)	
	Partisil-S	—	Methanol-0.2% NH_4NO_3 (11:9)	347
	Spherisorb 500S	—	Methanol-0.25 M Na_2HPO_4 pH8 (65:35) (10% orthophosphoric acid)	348
	Spherisorb S5W		Methanol-0.2 M NH_4NO_3 (60:40)	348
Alkaloid derivatives				
Dansyl derivatives of alkaloids (cephaeline, emetine, ephedrine, etc.)	LiChrosorb SI-100	—	*Di* isopropyl ether-isopropanol-ammonia (48:2:0.3)	349
		—	*Di* isopropyl ether (saturated with ammonia-isopropanol, 99:1)	349

typically, in the range 8–10. This relatively high pH is necessary to suppress ionization of the basic compounds and to give narrow peaks, but it has the disadvantage that silica gel begins to dissolve at pH 8 and above. A recent study of the stability of silica gel and bonded phase silica gel under basic conditions has been reported (302), and the conclusion is reached that at least some bonded phase silica gels are stable for several weeks at pH values up to 11. The situation is less encouraging with silica gel itself, which dissolves considerably faster than the bonded phase packings. Even bonded phase packings are not stable indefinitely: it has been reported that several commercially available bonded phase packings were prone to lose their bonded phases, even at pH values of 8 or less (303). Nevertheless, it does appear that a bonded phase packing with a slightly basic mobile phase will be the method of choice for many alkaloid separations in the future, although not all basic compounds separate well on this type of column (264).

ANTIBIOTICS AND MYCOTOXINS.—The separation of antibiotics by hplc has been reviewed (350, 350a); a summary of antibitotic separations is given in table 9.

TABLE 9. *Separation of antibiotics.*

Compounds separated	Column	Temp.	Mobile phase	Ref.
Polyene macrolides				
Candicidin, levorin, trichomycin	Permaphase ODS	55°	Methanol-0.5 m phosphate buffer, pH 7 (2:8)→methanol	351
Nystatin, eurocidin, etc.	Vydac RP	30°	Methanol-tetrahydrofuran-water (90:50:420)	352
	Bondapak C-18 Corasil		Methanol-acetonitrile-water (200:100:200)	352
Miscellaneous aromatic antibiotics				
Novobiocin	Zipax HCP	—	Methanol-0.01 M phosphate buffer pH 7 (15:85)	353
	Micropak CH	—	Methanol-1% acetic acid (60:40)	350a
Rifampin	Permaphase ODS	50°	Water→methanol	354
Rifamycin	Micropak NH_2	—	Chloroform-methanol (97:3)	355
Virginiamycin	Bondapak C-18 Corasil	—	Acetonitrile-water (1:1)	350
	LiChrosorb RP8	25°	Acetonitrile-water (4:6)	355a
Griseofulvin	Permaphase ETH	—	Hexane-chloroform (95:5)	356
6-Demethylgriseofulvin	Pellasil HS	—	Heptane→heptane-methnol-isopropanol (94:3:3)	357
Kanamycin	Zipax SCX	—	0.01N ammonium phosphate, pH 9.1 0.01 M potassium EDTA, pH 9.5	358
Macrolide antibiotics				
Erythromycin	Spherisorb S5 ODS	—	Methanol-water-ammonia (70:30:1), pH 4.8	359
	μBondapak C-18	—	Acetonitrile-methanol-0.2 M ammonium acetate-water (45:10:10:35)	360
	Corasil II	—	Chloroform	350
Leucomycin	JASCOPACK SV-02-500	—	Methanol-0.067 M acetate buffer, pH 4.9-acetonitrile (35:60:5)	361
β-lactam antibiotics				
Ampicillin	Vydac P150 AX	—	0.2 M Sodium nitrate in 0.01 M Sodium borate, pH 9.15	362
Amoxycillin and ampicillin	LiChrosorb RP8	—	0.067 M Potassium dihydrogen phosphate, pH 4.6	363
Penicillins and cephalosporins	LiChrosorb RP8	—	0.05 M Phosphate buffer, pH 7-methanol (5:1)	364
Penicillin G	Bondapak AX/Corasil	—	0.0045 M Citric acid-disodium phosphate, pH 3.8	354
Pencillin G	Vydac P150 AX	—	0.03 M Sodium nitrate in 0.01 M sodium borate, pH 9.1	362

TABLE 9. *Continued.*

Compounds separated	Column	Temp.	Mobile phase	Ref.
Penicillin V	Vydac P150 AX	—	10% Methanol in 0.03 M sodium nitrate and 0.01 M Sodium borate, pH 9.1	362
Benzylpenicillins	ODA SIL-X-II Vydac RP Bondapak C-18/Porasil B	—	Methanol-0.05 M ammonium carbonate (3:7)	366
Cephalosporins	Zipax SAX	—	0.02 M Sodium dihydrogen phosphate pH 85	367
	Zorbax ODS	50°	Methanol-0.05 M Ammonium carbonate (30:70)	
Cephalosporin C	μBondapak NH_2	20°	Acetic acid-methanol-acetonitrile-water (1.4:2.8:10:85:8)	368
	Zipax SAX	—	0.035 M boric acid, pH 9.6	369
Cephalosporins	ODS SIL-X-II Bondapak C-18/Porasil B	—	Methanol-0.05 M ammonium carbonate (5:95)	365
Cephradine, cephalexin	μBondapak C-18 ODS SIL-X-II	—	Methanol-0.03% ammonium carbonate (5:05)	370
Cephalexin	Micropak CH	50°	Methanol-0.01 M phosphoric acid (10:90)	350a
Cephaloridine	Bondapak phenyl/Corasil	—	Methanol-0.2 M ammonium acetate (1:4)	371
Cefoxitin, cephalothin	Zipax SAX	—	0.025 M acetic acid, pH 5.0	372
Polypeptide antibiotics				
Gramicidin	Zorbax ODS	60°	Methanol-0.005 M ammonium sulfate (74:26)	373
Bacitracin	Bondapak C-18/Corasil	—	Methanol-0.02 M phosphate buffer, pH 4.5 (5:95)→methanol-acetonitrile-buffer (40:20:40)	374
Circulin, colistin, polymyxin	μBondapak C-18	—	Acetonitrile-water-0.2 M phosphate buffer, pH 2.0 (20:70:10)→acetonitrile-methanol-water-buffer (50:20:29:1)	375
Actinomycin	Bondapak C-18/Corasil	22°	Acetonitrile-water (1:1)	376
Oligosaccharide antibiotics				
Gentamycin	μPorasil	—	Water-methanol-diethylamine (60:40:0.5)	377
	LiChrosorb RP 8	—	0.2 M sodium sulfate, 0.015 M sodium pentane sulfonate-acetic acid (99.1:0.1)	377
Bleomycin	μBondapak C-18	20°	Methanol-water (85:15) with 0.005 M ammonium formate	378
	Porasil A	20°	Methanol-0.3% ammonium	378
	μPorasil	—	formate, pH 6.4 (1:1)	379
Tetracyclines				
Tetracyclines	Vydac TP RP	60°	0.1 M tetraammonium EDTA-diethanolamine, pH 7.3-isopropanol-water (1:8:5:86)	380
Tetracycline	μBondapak C-18	—	0.01 M sodium dihydrogen phosphate, pH 2.4-acetonitrile (7:3,6:4)	381
Tetracycline	μBondapak C-18	—	0.02 M phosphate buffer, pH 2.5-acetonitrile (9:1→4:6)	382
Tetracycline and rolitetracycline	Pellionex CP-128	—	0.1 M sodium ions, 0.003 M EDTA, pH 4.35-ethanol (60:40)	383
Tetracyclines	Zipax HCP	—	0.02 M sodium dihydrogen phosphate, 0.01 M phosphoric acid pH acid pH 2.5-methanol (87:13)	384
Tetracyclines	Vydac 401TP	—	0.010 M EDTA, 0.2% sodium citrate, triethylamine (pH 8.0)-acetonitrile (9:1)	385
Tetracyclines	SC-TAS silica	—	0.1 M perchloric acid-acetonitrile (3:1)	386

TABLE 9. *Continued.*

Compounds separated	Column	Temp.	Mobile phase	Ref.
Doxycycline	Vydac 201TP	20°	0.14 M ammonium carbonate, pH 8.4-acetonitrile (96:4)	387
	LiChrosorb RP8	20°	Citrate-phosphate buffer, pH 2.2-acetonitrile (65:35)	387
	LiChrosorb RP8	20°	Glycine, pH 2.1-acetonitrile (72:28)	387
Daunorubicin and daunorubicinol	Pellionex CP-128	25°	Acetonitrile-water-0.1 M phosphoric acid (25:65:10)	388
	Zorbax SIL	—	Dichloromethane-methanol-25% ammonia-water (90:9:0.1:0.8)	389
Doxorubicin hydrochloride	Zorbax SIL	—	Isopropanol-0.5 M sodium acetate (96.2:3.8)	390
Doxorubicin and metabolites	Bondapak Phenyl/Corasil	—	Acetonitrile-1% ammonium formate (16:84→50:50)	391
Doxorubicin and daunorubicin	LiChrosorb RP2	25°	0.01 M phosphoric acid-ethanol (27.8:8.6)	392
	LiChrosorb RP8			
	LiChrosorb RP18		0.01 M phosphoric acid-acetonitrile (33.3:7.6)	392

In the mycotoxin area, most of the work on hplc has been carried out on the aflatoxins and their metabolites, but several other common mycotoxins have also been investigated. This work is summarized in table 10.

CONCLUSION.—This review has attempted to summarize in convenient form the literature on the hplc of natural products that has been published in approxi-

TABLE 10. *Separation of mycotoxins.*

Compounds separated	Column	Temp.	Mobile phase	Ref.
Patulin	Zorbax-SIL	—	Isooctane-diethyl ether-acetic acid (750:250:0.5)	393
Patulin	Zorbax-SIL	—	Isooctane-dichloromethane-methanol (84:15:1)	394
Altertoxin and related *Alternaria* metabolites	Zorbax-SIL	—	Petroleum ether-tetrahydrofuran (90:10→70:30)	395
Rubratoxins	μBondapak C18	—	Acetonitrile-water-ethyl acetate (11:9.9:3)	396
	μPorasil	—	Acetonitrile-chloroform	396
	μPorasil	—	Acetonitrile-chloroform-ethyl acetate	396
Rubratoxins, patulin, aflatoxins, zearalenone, penicillic acid, ochratoxins, sterigmatocystin	μBondapak C18	—	Acetonitrile-water-acetic acid (55:45:2.45:55:2)	
Sterigmatocystin	μBondapak C18	—	0.1 M potassium dihydrogen phosphate-acetonitrile (7:5)	398
Sterigmatocystins and *versicolorins*	μPorasil	—	Hexane-propanol-acetic acid (99.3:0.7:0.1)	107
	μPorasil	—	Hexane-ethyl acetate-acetic acid (88:17:1)	107
	Partisil 10 PAC	—	Hexane-chloroform-acetic acid (65:35:1)	107
	Zorbax SIL	—	Chloroform-dichloromethane (25% water saturated)-hexane-acetic acid (5:20:75:0.1)	399

TABLE 10. *Continued.*

Compounds separated	Column	Temp.	Mobile phase	Ref.
Aflatoxins	μPorasil	—	Chloroform-dichloromethane methanol (75:25:0.5)	400
Aflatoxins	Partisil 5	—	Dichloromethane (water saturated)-methanol (99.7:0.3)	401
Aflatoxins	Vydac 101 SC	—	Chloroform-isooctane (2:1)	401
Aflatoxins	μPorasil	—	Chloroform (water saturated)-cyclohexane-acetonitrile (25:7.5:1.0)	402
Aflatoxins	Corasil II	—	Hexane-chloroform-ethanol (500:497:3)	403
Aflatoxins	Spherisorb ODS	25°	Water-acetonitrile-methanol (15:3:2)	404
Aflatoxins	μBondapak C18	—	Acetonitrile-water (35:65)	405
Aflatoxin metabolites	Zorbax SIL	—	Dichloromethane-chloroform (50% water saturated)-methanol (60:40:05)	406
Aflatoxin degradation products	μPorasil	—	Chloroform-ethanol (99.25:0.75)	407

mately the last four years. Insofar as the technique is rapidily developing, many of the separations included in this review have been rendered obsolete by improvements in column technology and packing materials. In particular, the current dominance of the octadecylsilyl bonded phase packing is not clearly reflected in the tables; it has been estimated that approximately 80% of current separations are done on bonded phase columns of this general type (302, 409). For this reason, and also because many researchers have carried out separations that have not yet been reported in the open literature, it is tentatively proposed to prepare an updated version of this review in approximately two years' time. Opinions on the value of this review and suggestions for improvement would be welcomed; in addition, copies of reprints or of unpublished data relating to the hplc of natural products would be gladly received for inclusion in the next review.

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