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THIN-LAYER CHROMATOGRAPHIC SEPARATION OF *CINCHONA* ALKALOIDS

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1. INTRODUCTION

Cinchona alkaloids are found in the bark of *Cinchona* and *Remeijia* species of the family *Rubiaceae*. About 35 *Cinchona* alkaloids are known, of which quinine (Q) and quinidine (Qd) are the pharmaceutically most important, quinine because of its antimalarial and quinidine because of its cardiac depressant (antiarrhythmic) properties. These alkaloids have been studied by thin-layer chromatography more extensively than the other *Cinchona* alkaloids such as the stereoisomers cinchonine (C) and cinchonidine (Cd), both of which lack the methoxyl group at C_6, which is present in quinine and quinidine. A number of alkaloids chemically closely related to the four "parent alkaloids" mentioned are known, such as the corresponding dihydro derivatives, in which the vinyl group at C_3 is replaced by an ethyl group.

The dihydro alkaloids are found as common impurities in the vinyl type of alkaloids, and hence the separation of the vinyl and the corresponding dihydro derivatives has been subject of several studies. Because four asymmetric carbons are present in the parent alkaloids (C_3, C_4, C_8 and C_9), theoretically 16 isomers are possible. In nature only four are found, *e.g.*, isomers in which C_8 and C_9 are implicated: the 8*S*, 9*R* series (quinine and cinchonidine) (Fig. 1) and the 8*R*, 9*S* series (quinidine and cinchonine) (Fig. 2). In addition to the parent alkaloids, the epi series also exists, having an 8*S*, 9*S* (epiquinine, epicinchonidine) (Fig. 3) or an 8*R*, 9*R* (epiquinidine, epicinchonine) (Fig. 4) configuration.

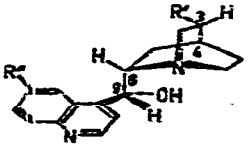

Fig. 1. R′ = vinyl R″ = H cinchonidine (Cd)
R″ = OH cupreine (Cu)
R″ = OCH_3 quinine (Q)
R′ = ethyl R″ = H dihydrocinchonidine (HCd)
R″ = OH dihydrocupreine (HCu)
R″ = OCH_3 dihydroquinine (HQ)

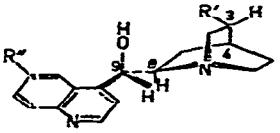

Fig. 2. R′ = vinyl R″ = H cinchonine (C)
R″ = OH cupreidine (Cud)
R″ = OCH_3 quinidine (Qd)
R′ = ethyl R″ = H dihydrocinchonine (HC)
R″ = OH dihydrocupreidine (HCud)
R″ = OCH_3 dihydroquinidine (HQd)

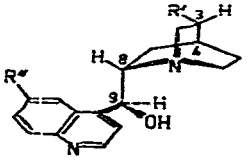

Fig. 3. R′ = vinyl R″ = H epicinchonidine (epiCd)
R″ = OCH_3 epiquinine (epiQ)
R′ = ethyl R″ = H epidihydrocinchonidine (epiHCd)
R″ = OCH_3 epidihydroquinine (epiHQ)

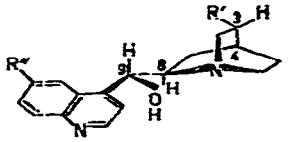

Fig. 4. R′ = vinyl R″ = H epicinchonine (epiC)
R″ = OCH_3 epiquinidine (epiQd)
R′ = ethyl R″ = H epidihydrocinchonine (epiHC)
R″ = OCH_3 epidihydroquinidine (epiHQd)

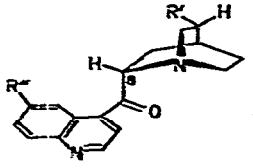

Fig. 5. R′ = vinyl R″ = H cinchoninone
R″ = OCH_3 quinidinone

Other minor alkaloids are cupreine and cupreidine and their dihydro derivatives. They have a hydroxyl group at C_6 and therefore have a more polar character than the other *Cinchona* alkaloids. Oxidation of the alkaloids can lead to the formation of N-oxides (aryl, aliphatic and di-N-oxides) and ketones (C-9, carbonyl) (Fig. 5).

Separation problems in the analysis of *Cinchona* alkaloids can be summarized as follows:

(1) separation of the parent alkaloids, Q, Qd, C and Cd;

(2) separation of the vinyl and dihydro alkaloids;

(3) separation of the epi-alkaloids from the parent alkaloids;

(4) separation of the parent alkaloid and its dihydro, epi-vinyl and epi-dihydro derivative;

(5) separation of the four possible stereoisomers of each group (Q, Qd, epiQ, epiQd and C, Cd, epiC, epiCd, for example);

(6) separation of the four compounds with the same stereochemistry (Q, HQ, Cd, HCd and Qd, HQd, C, HC, for example).

The scope of this study was to survey the literature on the thin-layer chromatographic (TLC) analysis of *Cinchona* alkaloids, to test the TLC systems described in the literature and to choose the systems most suitable for the analytical problems mentioned above.

In Table 1 the literature on the TLC of *Cinchona* alkaloids is summarized. The publications are classified into groups concerning, amongst others, the separation of the alkaloids in plant materials, in drug identification schemes, in drug of abuse screenings and in biological materials. The last two analytical fields are of interest because quinine is often found as an adulterant in drugs of abuse, and the presence of quinine in urine is often used as an indicator of the abuse of heroin.

The solvents described in the literature for the analysis of *Cinchona* alkaloids have been tested in our laboratories for the separation of the alkaloids quinine, quinidine, cinchonine, cinchonidine, dihydroquinine and dihydroquinidine. The solvents giving the best results (Tables 2 and 3) were further tested on the 24 alkaloids summarized in Table 4, leading to the choice of 18 solvents which were found to be most suitable for the different objectives already stated. Table 6 lists solvents which should be used to obtain a specific separation of some *Cinchona* alkaloids. Further, the sensitivity of a number of detection methods was tested and the results are summarized in Table 5. The colours obtained with some of these reagents are summarized in Table 7.

2. EXPERIMENTAL

2.1. Chromatography

Solvents were of "Baker analyzed" quality. The TLC plates were 20 × 20 cm silica gel 60 F_{254} pre-coated aluminium sheets, with a layer thickness of 0.2 mm (E. Merck, Darmstadt, G.F.R.). The plates were stored under normal laboratory conditions and were not activated before use.

The chromatograms were developed in normal chromatography chambers, the walls of which were lined with filter-paper. The chambers were equilibrated with the solvents for at least 30 min. The temperature was 24 ± 2° and the relative humidity in

TABLE 1

LITERATURE SURVEY OF THE TLC OF *CINCHONA* ALKALOIDS

References dealing with the TLC separation of Cinchona alkaloids:

1	11	23	38	55	73	101	120
2	12	25	39	56	74	112	123
3	14	26	40	62	75	113	124
5	17	30	47	64	76	114	
5	18	33	49	65	79	116	
3	19	34	50	67	93	118	
9	20	36	53	71	99	119	

References dealing with the TLC separation of alkaloids in general, including some Cinchona alkaloids:

4	63	68	72	81	98
43	65	69	77	97	100

References dealing with the TLC separation of various chemical compounds, including some Cinchona alkaloids:

15	35	59	70	82	86	91
21	41	61	73	83	87	92
22	46	63	78	84	88	103
31	54	66	80	85	89	111

References dealing with the TLC identification of Cinchona alkaloids (particularly quinine) in food and beverages:

28	43	44	45

References dealing with the TLC identification of Cinchona alkaloids in biological material (urine, blood, etc.):

10	24	36	58	95	106	109
13	27	42	60	104	107	115
16	29	51	94	105	108	125

References dealing with the analysis of drugs of abuse, including the identification of quinine:

7	24	37	57	82	104	108
10	27	42	58	91	105	109
13	29	51	66	94	106	
16	32	52	78	102	107	

the laboratory was 25 ± 5%. The hR_F values were calculated from at least six chromatograms. Amounts of 5–10 μg of the alkaloids were spotted 1 cm above the bottom of the plates, and the plates were developed over a distance of 10 cm.

TABLE 2

SOLVENT SYSTEMS SUITABLE FOR THE SEPARATION OF VINYL AND DIHYDRO *CINCHONA* ALKALOIDS (SEE ALSO TABLE 6)

Solvent system	*Plates*
Chloroform–methanol–17% ammonia (24:6:0.5)	Silica gel, not activated
Acetone–benzene–diethyl ether–25% ammonia (6:4:1:0.3)	
Chloroform saturated with ammonia	
Methanol	0.1 *M* sodium hydroxide impregnated silica gel
Chloroform–methanol (9:1)	

TABLE 3

SOLVENT SYSTEMS FOR THE TLC SEPARATION OF *CINCHONA* ALKALOIDS

No.	*Solvent system*	*Solvent class (Snyder[127])*	*References*
S1	Chloroform–diethylamine (9:1)	VIII	89, 20, 34, 64, 71, 119
S2	Chloroform–methanol–25% ammonia (85:14:1)	VIII + II	83, 84
S3	Chloroform–acetone–diethylamine (5:4:1)	VIII + VIa	55, 40, 70, 89, 119, 121
S4	Chloroform–acetone–(3 ml 25% ammonia + 17 ml absolute ethanol) (5:4:1)	VIII + VIa + II	117
S5	Chloroform–acetone–methanol–25% ammonia (60:20:20:1)	VIII + VIa + II	40
S6	Chloroform–ethyl acetate–isopropanol–diethylamine (20:70:4:6)	VIII + VIa + II	25, 53
S7	Chloroform–dichloromethane–diethylamine (20:15:5)	VIII + V	11
S8	Dichloromethane–diethyl ether–diethylamine (20:15:5)	V + I	11
S9	Kerosene–acetone–diethylamine (23:9:9)	VIa	2, 18, 39, 74, 116
S10	Acetone–25% ammonia (58:2)	VIa	12
S11	Ethyl acetate–isopropanol–25% ammonia (45:35:5)	VIa + II	
S12	Toluene*–ethyl acetate–diethylamine (7:2:1)	VIb + VIa	17, 89
S13	Toluene*–ethyl acetate–diethylamine (10:10:3)	VIb + VIa	68
S14	Toluene*–diethyl ether–diethylamine (20:12:5)	VIb + I	14, 19, 33, 56, 75, 76, 122
S15	Toluene*–diethyl ether–dichloromethane–diethylamine (20:20:20:8)	VIb + I + V	23
S16	Carbon tetrachloride–*n*-butanol–methanol–10% ammonia (12:9:9:1)	VIb + II + II	
S17	Cyclohexanol–cyclohexane–*n*-hexane (1:1:1) + 5% diethylamine	II	1, 73, 113
S18	Methanol–25% ammonia (100:1)	II	40

* The original solvent described in the literature contains the more toxic benzene. Direct comparison of benzene- and toluene-containing solvents did not show any major difference.

2.2. Detection

The detection limit was determined by spotting 0.01, 0.1, 1, 10 and 100 μg of the parent alkaloids in 10 μl of solution on to a TLC plate. The plates were not developed but were immediately sprayed with the spray reagents. The spray reagents were prepared according to the references mentioned in Table 5 or, if no reference is given there, according to the reagents list in ref. 126.

The alkaloids tested were kindly provided by Drs. H. B. Trijzelaar, ACF Chemiefarma, Maarssen, The Netherlands.

2.3. Literature survey

2.3.1. TLC systems

On surveying the literature, it was found that some solvent systems have been utilized more often than others for the separation of the parent alkaloids. Such

TABLE 4

hR_F VALUES OF *CINCHONA* ALKALOIDS IN SOLVENTS S1–S18, AS DETERMINED IN OUR LABORATORY

The hR_F values found in the literature are also given.
The hR_F values were calculated from at least six chromatograms run under the following conditions: plates, silica gel Si 60 F254 pre-coated aluminium sheets, 20 × 20 cm (Merck); temperature, 24 ± 2°; relative humidity, 25 ± 5%; normal chromatography chamber, saturated for 30 min before use.

Alkaloid	*Solvent*												
	S1	*Ref. 20*	*S2*	*Ref. 83*	*S3*	*Ref. 40*	*S4*	*S5*	*Ref. 40*	*S6*	*S7*	*S8*	*S9*
Quinine (Q)	17	16	44	64	17	24	21	37	42	11	22	23	32
Dihydro-Q	14	16	36		15	23	17	31	32	10	19	21	32
Quinidine (Qd)	28	36	44	66	26	45	26	41	46	21	34	35	41
Dihydro-Qd	24	29	35		24	39	18	31	34	18	31	32	41
Cinchonidine (Cd)	25	29	38		24	40	23	35	40	17	31	31	39
Dihydro-Cd	21	29	30		22	37	16	26	27	15	28	29	40
Cinchonine (C)	32	47	37		32	54	23	34	38	24	40	40	44
Dihydro-C	26	41	28		28	48	15	24	23	20	35	38	44
Epi-Q	52	68	48		42	64	32	37	41	26	56	46	39
Dihydroepi-Q	51		37		41		24	26		40	56	47	42
Epi-Qd	55	73	49		44	68	33	39	43	31	59	50	42
Dihydroepi-Qd	53		38		43		25	24		29	57	49	43
Epi-Cd	54		46		44		34	37		31	57	49	43
Dihydroepi-Cd	52		34		44		25	25		30	56	50	45
Epi-C	55		47		45		33	38		33	58	51	45
Dihydroepi-C	53		35		43		25	25		30	57	51	46
Cupreine (Cu)	1		19		1		8	22		1	1	3	8
Dihydro-Cu	1		15		1		5	15		1	1	2	9
Cupreidine (Cud)	1		20		1		7	21		1	2	3	8
Dihydro-Cud	1		14		1		4	12		1	1	3	8
Quinidinone	60		71		53	76	53	65	74	41	63	57	49
Cinchoninone	60		69		52		53	64		41	61	56	49
HQd ar-N-oxide	12		28		12		9	22		6	16	14	16
HCd ar-N-oxide	9		23		11		6	16		5	13	11	15
Development time (min/8 cm)	15		13		15		13	13		13	12	11	15

solvents, all in combination with silica gel plates, are S1 and S3[89], S9[116] and S14[75] (Table 3). Systems of chloroform–methanol–DEA in different ratios have also been used by several workers[1,3,5,6,8,113]. Solvent S3 was adapted in the USP XVIII for the quality control of *Cinchona* alkaloids and solvent S14 in the British Pharmacopoeia (1973).

According to Andary[25], solvent S6 gives a complete separation of the parent alkaloids. This solvent was later used by Massa *et al.*[53] in the quantitative analysis of the parent alkaloids.

With chloroform–methanol–DEA (80:20:1) as solvent in combination with base-impregnated silica gel plates, a separation of vinyl and dihydro alkaloids is obtained[3,5]. For this separation various other solvents have also been described: S5[40,123], S10[9], S18[40], acetone–water–25% ammonia (80:20:1)[120] methyl ethyl ketone–

Ref. 74	*S10*	*S11*	*S12*	*Ref. 89*	*S13*	*S14*	*Ref. 76*	*S15*	*Ref. 23*	*S16*	*S17*	*Ref. 1*	*S18*	*Ref. 40*
35	32	49	12	17	18	18	25	20	19	67	41	56	45	50
	24	43	11		17	17		17		60	41		38	39
45	37	55	20	25	28	26	44	29	37	71	60	74	46	52
	27	49	18		26	25	35	27		62	58		37	39
45	33	52	19		27	25	41	27	32	67	57	71	43	48
	25	46	18		25	24	35	25		57	57		35	35
50	34	53	24	27	33	31	54	33	54	65	67	80	39	43
	24	45	22		30	29	49	31		52	65		29	30
	41	48	29		35	30		38		53	37		30	31
	31	40	30		36	31		39		39	38		19	
	39	48	33		41	35		42		52	44		29	32
	30	39	32		40	35		41		39	41		19	
	43	49	34		41	36		42		53	46		30	
	33	40	34		41	37		43		39	45		20	
	41	48	36		43	39		44		51	49		30	
	31	40	36		42	39		44		39	47		19	
	13	29	2	0	3	2		3		51	9		43	
	8	22	2		3	3		3		41	9		34	
	11	29	2		3	2		3		50	9		42	
	7	21	2		3	2		3		36	8		29	
	59	59	44		51	47		51		83	63		54	64
	58	59	44		50	47		51		81	63		54	
	10	25	6		10	6		10		53	41		29	
	8	22	5		8	5		7		41	28		25	
	10	17	14		12	11		12		28	45		13	

ammonia (58:2)[9] and acetone–methanol–DEA (50:50:1)[20,34], all on silica gel plates. Methyl ethyl ketone–methanol–water (6:2:1)[18,19,118,119] was used in combination with 0.1 *M* sodium hydroxide impregnated silica gel plates.

Böhme and Bitsch[18] postulated that polar solvents would be able to separate the vinyl and dihydro alkaloids whereas non-polar solvents would not. Stöver[14] used the reaction of the vinyl alkaloids with mercuriacetate to separate the vinyl alkaloids from the dihydro compounds. The vinyl alkaloids give polar mercuri derivatives, which do not move in the solvent used (S14), whereas the dihydro alkaloids are not affected by mercuriacetate.

For the separation of the epi-alkaloids from the parent alkaloids, solvents S3 and S18 were proposed by Smith *et al.*[40]; Storck and co-workers[20,34] used solvent S1 for this separation.

TABLE 5

TLC DETECTION OF *CINCHONA* ALKALOIDS

Reagent	*Sensitivity**	*Background colour*	*Colour with parent alkaloids*	*Reference*
Quenching, 254 nm	0.1			
Fluorescence, 366 nm (formic acid or sulphuric acid spray)	0.01 Qd, Q, 0.1 C, Cd		Q, Qd light blue; C, Cd dark blue	
Dragendorff's modification:				
Munier–Machebocuf	0.1	Yellow	Orange-red	
Munier	0.01	Light yellow	Orange-red	
Munier, $NaNO_2$	0.01	Light yellow-white	Brown	66
Vágujfalvi	0.1–1	Light yellow	Orange	
Bregoff–Delwiche	0.1	Light yellow	Orange	
Iodine vapour	0.1	Yellow-white	Brown	
Iodine in KI	1	White	Brown	
Iodine in methanol	0.1	Light yellow	Brown	87
Iodine vapour, pyrrole vapour	1	Yellow	Brown	83
Iodine in KI and Ag acetate	1			105
Iron(III) chloride, iodine in KI	0.1	Light green-yellow	Brown	85
Iodoplatinate	0.01–0.1	Violet	Q, Qd violet; C, Cd blue	
Iodoplatinate, acidified	0.1	Dark violet	Q, Qd violet; C, Cd blue	34
Iron(III) hexacyanoferrate(III)	10	Light green-blue	Dark green-blue	100
Iron(III) chloride–perchloric acid	1	Yellow-white	Violet	
Methyl orange	10	Light orange	Orange	104
Tetraphenylborate, quercetin	10		In UV: Q, Qd blue, C, Cd yellow	110
Phenothiazine, iodine vapour	0.1	Violet	Brown	83
Phenothiazine, bromine vapour (ammonia vapour)	1	Violet	Q, light brown; Qd green; C yellow; Cd red-brown	83

* As tested in our laboratories for the parent alkaloids.

TABLE 6

SOLVENT SYSTEMS USED FOR THE TLC SEPARATION OF *CINCHONA* ALKALOIDS

The numbers refer to solvents S1–S18 in Table 3 which can be used to obtain a separation within a group of alkaloids or between two groups of alkaloids or between two alkaloids. Numbers in parentheses indicate that a complete baseline separation was not obtained.

	Parent alkaloids				*Dihydro alkaloids*			
	Q	*Qd*	*Cd*	*C*	*HQ*	*HQd*	*HCd*	*HC*
Parent alkaloids	1, 3, 6–9, 12–15, 17				2, 4, 5, 10, 11, 16, 18			
Q		1, 3, (4), 6–9, (11), 12–15, 17	1–3, 5–10, (11), 12–15, 17	1–3, 5–10, (11), 12–15, 17, (18)	2, 4, 5, (6), 10, 11, 16, 18	1–10, 12–18	1–10, (11), 12–18	1–10, (11), 12–18
Qd			2, (4), 5, 6, (7), (8), 10, (13), (16)	1–3, (4), 5–8, (9), 10, 12–17, (18)	1–18	(1), 2, 4, 5, (6), 10, 11, 16, 18	1, 2, (3), 4–8, 10, 11, 13, (15), 16, 18	2, 4, 5, (8), (9), 10–14, 16–18
Cd				1, 3, 6–9, 12–15, 17	1, 3, 4, 5, 6–15, (16), 17, 18	4, 5, 10, (16), 18	2, 4, 5, 10, 11, 16, 18	(1), 2–14, (15), 16–18
C					1, 3, (5), 6–15, (16), 17, (18)	1, 3, (5), 6, 7, 8, 10, (11), 12–15, (16), 17, (18)	1–3, 5–18	1, 2, 5, 6, (8) 10, 11, (12), (13), (15), 16, 18
Dihydro alkaloids					1, 3, 6–9, 12–15, 17			
HQ						1, 3, 6–9, (11), 12–15, 17	1–3, 5–9, 12–15, (16), 17	1–3, 5–9, (10), 12–18
HQd							2, 5, (8), (10), (11), (15), (16)	(1), 2, (3), 5, (6), (7), 8, (9), 10, (11), 12–14, (15), 16–18
HCd								1, 3, 6–9, 12–15, (16), 17, 18

(Continued on p. 88)

TABLE 6 *(continued)*

	Epi-alkaloids					*Epi-dihydro alkaloids*				
		epiQ	*epiQd*	*epiCd*	*epiC*		*epiHQ*	*epiHQd*	*epiHCd*	*epiHC*
Parent alkaloids	1, 3, 6–8, 12 (2×), 13–15, 18					1, 3, 5, 6, 7, 8, 10, 12 (2×), 13, 14, 15, 16, 18				
Q		1, (2), 3, 6–9, 12–16, 18	1, (2), 3, 6–9, (5), 12–18	1, 3, 6–10, 12–18	1, 3, 6–10, 12–18		1, 2, 3, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15, 16, 18	1–3, 5–10, 12–16, (17), 18	1–3, 5–10, 12–18	1–3, 5–10, 12–18
Qd		1, (2), 3, 5–8, 11–18	1, (2), 3, 6–8, 11–18	1, 3, (4), 5–8, 11–18	1, 3, 5–9, 11–18		1, 2, 3, (4), 5, 6, 7, 8, 10–18	1–3, (4), 5–8, 10–18	1–3, (4), 5–18	1–3, (4), 5–18
Cd		1–4, 6–8, 10, (11), 12–18	1–8, 10, (11), 12–18	1–4, (5), 6–8, (9), 10, (11), 12–18	1–4, 5, 6–10, (11), 12–18		1, 3, 5–8, 10–18	1, 3, 5–8, (9), 10–18	1, 3, 5–18	1, 3, 5–18
C		1–3, (5), 6, 8–12, (13), 15–18	1–3, (5), 6–8, 10–18	1–3, 6–8, 10–18	1–3, 5, 6–8, 10–18		1, 3, 5–8, (9), 10–13, 15–18	1, 3, 5–8, 10–18	1, 3, 5–8, 10–18	1, 2, 5–8, 10–18
Dihydro alkaloids	1, 3, 5–8, 10, 12 (2×), 13–15					1, 3, 6, 7, 8, 12 (2×), 13, 14, 15, 16, 18				
HQ		1–10, 12–16, (18)	1–10, 12–17, (18)	1–10, 12–17, (18)	1–10, 12–17, (18)		1, 3, (4), 5–9, (11), 12–16, 18	1, 3, (4), 5–9, (11), 12–16, 18	1, 3, (4), 5–9, (10), (11), 12–18	1, 3, (4), 5–9, (11), 12–18
HQd		1, 3–8, 10, 12–17, (18)	1, 3–8, 10, 12–17, (18)	1, 3–8, (9), 10, 12–17, (18)	1, 3–10, 12–17, (18)		1, 3, 5–8, 11–18	1, 3, 5–8, (9), 11–18	1, 3, (4), 5–9, 11–18	1, 3, (4), 5–9, 11–18

HCd		1–8, 10, 12–15, (16), 17	1–8, (9), 10, 12–15, (16), 17	1–8, (9), 10, 12–15, (16), 17	1–10, 12–15, (16), 17		1–3, (4), 6–8, (10), 11–18	1–3, (4), 6–8, (9), (10), 11–18	1–4, 6–18	1–4, 6–9, (10), 11–18
HC		1–10, 12, 13, 15, (16), 17	1–8, 10, 12–15, 17	1–8, 10, 12–15, 17, (18)	1–8, 10, 12–15, 17		1–4, 6–8, 10–13, 15–18	1–4, 6–8, 10–18	1–4, 6–8, 10–18	1–4, 6–8, 10–18
Epi-alkaloids	(9), 12 (2×), 13–15, 17					2, (3), 4, 5, 10, 11, 16, 18				
epiQ			(1), 6, 8, (9), 12–15, 17	6, (8), 9, (10), 12–15, 17	1, (3), 6, (7), 8, 9, 12–15, 17		2, 4, 5, 8, (9), 10, 11, 16, 18	(1), 2, 4, 5, 8, 9, 10–18	(1), 2, 4–6, 8–18	(1), 2, 4–6, 8–18
epiQd				(10)	(9), (10), 12, (13), 14, (15)		1–6, 8, 10–16, 18	2, 4, 5, 10, 11, 16, (17), 18	2, 4, 5, (9), 10, 11, 16, 18	2, 4, 5, 9–12, (13), 14, (15), 16, (17), 18
epiCd					(1), 2, (9), (12), (13), (14), (15)		(1), 2–6, 8, 10–14, (15), 16–18	2, 4, 5, 10, 11, 16, (17), 18	2, 4, 5, (9), 10, 11, 16, 18	2, 4, 5, 9–11, (12), (13), 14, (15), 16, 18
epiC							1–6, (7), 8, (9), 10–18	(1), 2, 4–6, (9), 10–14, (15), 16–18	(1), 2, 4, 5, (6), 10, 11, (12), (13), (15), 16, 18	2, 4, 5, (6), 10, 11, 16, 18
Epi-dihydro alkaloids						(9), 12 (2×), 13, 14, 15, 17				
epiHQ								(1), (3), 8, (9), (6), 12–14, (15), 17	(1), (3), 6, 8, (9), (10), 12–14, 15, 17	(1), (3), 6, 8, 9, 12–15, 17
epiHQd									(10), (12), (14), (17)	(9), 12, (13), 14, (15), 17
epiHCd										(12), (13), (14), (15)
epiHC										

TABLE 7

DETECTION OF *CINCHONA* ALKALOIDS: COLOURS OBTAINED AFTER SPRAYING WITH DILUTE SULPHURIC ACID AND IODOPLATINATE SPRAY REAGENT

Alkaloid	*Fluorescence colour (366 nm)*	*Iodoplatinate spray reagent*[20]
Quinine	Light blue	Violet-brown
Quinidine	Light blue	Violet-brown
Dihydroquinine	Light blue	Violet-brown
Dihydroquinidine	Light blue	Violet-brown
Cinchonine	Dark blue	Blue-violet-brown
Cinchonidine	Dark blue	Blue
Dihydrocinchonine	Dark blue	Blue-violet
Dihydrocinchonidine	Dark blue	Blue-violet
Epiquinine	Light blue	Violet-brown
Epiquinidine	Light blue	Violet-brown
Dihydroepiquinine	Light blue	Violet-brown
Dihydroepiquinidine	Light blue	Violet-brown
Epicinchonine	Dark blue	Blue-violet-brown
Dihydroepicinchonine	Dark blue	Blue-violet
Epicinchonidine	Dark blue	Blue-violet-brown
Dihydroepicinchonidine	Dark blue	Blue-violet
Quinidinone	Yellow-green	Yellow-violet
Cinchoninone	Yellow-green	Yellow-violet
Cupreine	Orange-red	Light blue-violet
Dihydrocupreine	Orange-red	Light blue-violet
Cupreidine	Orange-red	Blue-violet-brown
Dihydrocupreidine	Orange-red	Blue-violet-brown

2.3.2. Two-dimensional chromatography

Several workers have proposed two-dimensional chromatography for the complete separation of the four parent alkaloids. Van Severen[1] applied solvents S17 and chloroform–methanol–DEA (80:2:0.2). Kamp *et al.*[73] modified this method by first running the chromatograms in chloroform–methanol–DEA (80:20:1) followed by S17, because of difficulties in drying the plates after using S17 for the first development. Kamp *et al.* described two other combinations for two-dimensional TLC on silica gel plates: chloroform–*n*-butanol (1 + 1) saturated with 10% ammonia followed by S9 or S17. Böhme and Bitsch[18] used methyl ethyl ketone–methanol–water (6:2:1) followed by benzene–isopropanol–DEA (4:2:1) on 0.1 *M* sodium hydroxide impregnated silica gel plates. Instead of the latter solvent, Vermes[33] used benzene–diethyl ether–DEA (20:12:5) for the second run. Wyesekera *et al.*[62] described a combination of the solvents chloroform–methanol–17% ammonia (24:6:0.05) and diethyl ether–DEA (17:1) on 0.1 *M* sodium hydroxide impregnated silica gel plates for the separation of the alkaloids present in *Cinchona* bark. Pound and Sears[120] proposed the solvents acetone–water–ammonia (25%) (80:20:1) and benzene–DEA (1:1) for the separation of the parent alkaloids and the dihydro bases of quinine and quinidine.

2.3.3. Reaction chromatography and TAS technique

Reaction chromatography of *Cinchona* alkaloids has been described by Wilk and Brill[90]. Before developing the plates with the solvents they were exposed to iodine vapour for 18 h. After development with benzene–methanol–acetone–acetic acid

(70:20:5:5) characteristic patterns of spots were observed for the alkaloids. Kaess and Mathis[93] used several reagents in connection with the *Cinchona* alkaloids: treatment with chromic acid leads to the formation of less polar ketones, but in small yields, and treatment of quinine with potassium ethanolate (0.1 *M*) leads to the formation of a number of unidentified compounds. With acetyl chloride or acetic anhydride *Cinchona* alkaloids give less polar acetyl compounds. According to the authors, the reactions can be helpful in the identification of the alkaloids.

Jolliffe and Shellard[101] found that the results obtained with thermofractography (TAS) of *Cinchona* alkaloids from the bark were inconsistent. Investigations by Stahl and Schmitt[99,114] showed that the pure *Cinchona* alkaloids could be volatilized at temperatures above 180° without decomposition. However, from *Cinchona* bark only decomposition products, simple quinoline derivatives, were obtained. They could be used to characterize *Cinchona* bark with the TAS technique. Chmel and Chmelová-Hlavatá[118] succeeded in the thermofractography of the alkaloids present in *Cinchona* bark. They mixed 20–40 mg of bark with 20 mg of lithium hydroxide and applied a temperature of 300°. As the propellant 100 mg of $Ni(NH_3)Cl_2$ were used. As solvent for TLC they used methyl ethyl ketone–methanol–water (6:2:1) on 0.05 *M* potassium hydroxide impregnated plates.

2.3.4. Quantitative analysis

Oswald and Flück[75] analysed *Cinchona* alkaloids quantitatively by measuring the spot areas. Later, several workers described methods for quantitative analysis by extraction of the spots from the TLC plates followed by fluorimetric[40,69] or spectrophotometric determination[17,19,20,34,43,44]. The alkaloids were extracted with different solvents: chloroform[43,44], ethanol–acetone (1:1)[95,115], absolute ethanol[20], methanol–25% ammonia (9:1)[18,19], 0.1 *M* hydrochloric acid[17] or 0.05 *M* sulphuric acid[40,123]. Dilute hydrochloric acid or 2 *M* sulphuric acid were used to dissolve alkaloids and the stationary phase (magnesium oxide)[69,79].

For fluorimetric analysis after extraction an excitation wavelength of 350 nm has been used[40,69,115,123] and the emission measured at 450 nm[69] and 455 nm[40,123] for quinidine and quinine, respectively, in dilute acid. For indirect spectrophotometric determination wavelengths of 332 nm[18,19], 324 nm[20,34] and 366 nm[17] have been used for quinine and 316 nm for cinchonine[20,34]. Direct fluorimetric analysis of the TLC plates has been performed after acidification, with excitation wavelengths of 361 nm[45] or 345 nm[40] for quinine and 335 nm[60] or 365 nm[36] for quinidine, and emission wavelengths of 438 nm[45] or 430 nm[40] for quinine and 455 nm[36] or 430 nm[60] for quinidine. Röder *et al.*[23] described the direct fluorimetric analysis of the four parent alkaloids on TLC plates after immersion in diethyl ether–concentrated sulphuric acid (95:5). Quinine and quinidine have a fluorescence maximum at 460 nm, excitation at 365 nm. Under these conditions cinchonine and cinchonidine do not interfere. Cinchonine and cinchonidine can be determined without interference from quinine and quinidine by excitation at 313 nm and measuring the emission at 390 nm. In this way the four main alkaloids can be determined quantitatively without being separated completely. Ebel and Herold[124] used this method for the determination of quinine.

To obtain a complete separation of quinine from the other *Cinchona* alkaloids before quantitative analysis, Böhme and Bitsch[18,19] improved the separation by

first developing the plates with a polar solvent, in which quinine has a high R_F value, followed by a reversed development with a less polar solvent, in which quinine has a low R_F value. In this way quinine could be separated from quinidine, cinchonine, cinchonidine, dihydroquinidine and dihydroquinine.

Massa and co-workers[53,96] studied the optimal conditions for direct measurement on the plates. The four main alkaloids were separated with the solvent ethyl acetate–chloroform–isopropanol–DEA (70:20:4:6) on silica gel plates. For direct photodensitometric determination on the plate after spraying with ethanol–concentrated sulphuric acid (10%), the absorption maxima for quinine and quinidine were found to be 330 nm and for cinchonine and cinchonidine 288 nm. The detection limit was found to be 100 ng. Using fluorescence after spraying with 1% sulphuric acid in ethanol, quinine and quinidine could be determined by using an excitation wavelength of 365 nm and measuring at 450 nm. In this way the authors found the detection limit to be 1 ng. The use of an excitation wavelength of 313 nm permitted the determination of the emission of cinchonine and cinchonidine at a wavelength of 410 nm, with a detection limit of 5 ng (cinchonidine has its fluorescence maximum at 420 nm). Quinine and quinidine are also excited at 313 nm, but have their fluorescence maximum at 454 nm.

Okumura *et al.*[97] used a modified flame-ionization detection method for the quantitative analysis of a number of alkaloids, *e.g.*, quinine, on sintered silica gel or aluminium oxide on glass rods.

3. RESULTS AND DISCUSSION

None of the TLC systems described in the literature is able to separate all known *Cinchona* alkaloids in one run, but using solvents S6, S12, S13, S14 and S15 an optimal separation is obtained. From all the solvents and sorbents tested in our study some general conclusions can be drawn.

(1) Silica gel plates in combination with basic solvents or base-impregnated silica gel plates together with neutral solvents give the best results without tailing, except for the epi-alkaloids, which in all solvents tested show some tailing.

(2) The use of ammoniacal solvents or of base-impregnated plates usually leads to the separation of the vinyl alkaloids from the dihydro alkaloids. An increase in the pH of the mobile or stationary phase leads to an improvement in the vinyl–dihydro alkaloid separation. In addition to solvents S2, S4, S5, S10, S11, S16 and S18 found in Table 3 the solvents listed in Table 2 can be used successfully for the vinyl–dihydro alkaloid separation. On comparing ammonia-containing solvents with those containing diethylamine, it is observed that in the former both Cd and epiCd have R_F values equal or higher than those of C and epiC, whereas the opposite is true in the latter. The same observation is made for Q, Cd and Qd, C. Although the differences in the R_F values are smaller, a similar behaviour is observed for the epi-alkaloids.

(3) Chromatography with ammonia-containing systems leads to deterioration of the separation of the four stereoisomers of each group, such as Q, Qd, epiQ and epiQd.

(4) If diethylamine in solvent S3 is replaced with ammonia, as proposed by Puech *et al.*[117] for the analysis of tropane alkaloids (S4), an improved separation of the vinyl and dihydro alkaloids is obtained, but the separation of the parent

alkaloids and the epi-alkaloids deteriorates in comparison with the original diethylamine-containing solvent. Solvents containing diethylamine are in general suitable for the separation of the various compounds within each group of stereoisomers.

Summarizing, and considering the aims mentioned in the Introduction, the following conclusions can be drawn about the analysis of *Cinchona* alkaloids:

(1) An almost complete separation of the parent alkaloids can be achieved with solvents S6, S7, S8 and S13. Solvents S1, S3, S9, S12, S14, S15 and S17 give a nearly complete separation of three of the alkaloids and a partial separation of the fourth. None of these systems give a complete "baseline" resolution of Qd and Cd. This is achieved only in solvent S2. The solvents methanol and chloroform–methanol (9:1) on base-impregnated plates are able to separate the pair cinchonine–cinchonidine from the pair quinine–quinidine.

(2) The vinyl alkaloids can be separated from the dihydro alkaloids in solvents S2, S4, S5, S10, S11, S16 and S18 and in the solvents given in Table 2.

(3) The epi compounds usually have R_F values higher than those of the parent alkaloids. The best separation from the parent alkaloids is obtained with solvents S1, S3, S6, S7, S8, S12, S13, S14, S15 and S18. The best separation of the epi-alkaloids is obtained with solvents S6, S12, S13 and S17. In solvent S17 the epi compounds have R_F values between those of Q and Qd and it is therefore less suitable for the separation of the epi-alkaloids from the parent alkaloids.

(4) Separation of the parent alkaloid from its dihydro, epi-vinyl and epi-dihydro derivative is achieved with solvents S16 and S18 (not for HCd–epiCd) and partly by solvents S2 and S6. Solvents S16 and S18 give poor resolutions of Q and Qd and of C and Cd, which makes it unsuitable for the separation of the stereoisomers of each group.

(5) Separation of the four stereoisomers of each group can be obtained with solvents S1, S6, S12, S13, S14 and S15.

(6) Separation of the four derivatives of the quinine group can best be accomplished with solvents S2, S5 and S15 and partly with solvents S7, S8, S13 and S16. The quinidine group can be separated best with solvents S10 and S16 and partly with solvents S2, S5, S6, S7, S8, S13 and S18. The group of epi-quinine can be only partly resolved with solvents S9, S12, S14 and S18. The epi-quinidine group can be partly resolved with solvents S10 and S17.

The phenolic alkaloids cupreine and cupreidine were not separated in any of the solvents, nor were their dihydro derivatives separated from each other. The same was the case with quinidone and cinchoninone. The separation of the cupreine series of alkaloids, quinidone and cinchoninone from the other alkaloids does not give any problem, as can be seen from Table 4.

The keto compounds can be separated from the other alkaloids in solvents S4, S8, S11, S12, S14, S15 and S16. For the cupreine series low R_F values were found in all of the systems tested, except for solvents S11 and S16. In the latter solvent the alkaloids of the cupreine series coincide with several of the other alkaloids, which makes this solvent less useful for the separation of all alkaloids.

3.1. Detection

For the TLC detection of *Cinchona* alkaloids the fluorescence of these com-

pounds in acidic conditions has been widely utilized. The developed plate is sprayed with dilute sulphuric acid or 25% formic acid in water, immersed in a mixture of diethyl ether and concentrated sulphuric acid (95 + 5)[23] or exposed to formic acid vapour. The aryl-methoxyl-containing alkaloids show a strong blue fluorescence, stronger at 366 nm than 254 nm, whereas the methoxyl-free alkaloids have a weak, dark blue fluorescence (Table 7). As can be seen from Table 5, the modification of Dragendorff's reagent according to Munier and Munier and Macheboeuf is the most sensitive of the spray reagents. The iodoplatinate reagent, which gives different colours with some of the alkaloids (Table 7), is also sensitive.

4. SUMMARY

The TLC analysis of *Cinchona* alkaloids is reviewed. From the TLC systems described in the literature, 18 solvents were found to be most suitable for the separation of the 24 *Cinchona* alkaloids tested. The sensitivity of a number of detection methods described for *Cinchona* alkaloids is reported. Some general conclusions concerning the optimal conditions for specific separations are drawn.

REFERENCES

1 R. van Severen, *J. Pharm. Belg.*, 17 (1962) 40.
2 H. Feltkamp, *Deut. Apoth.-Ztg.*, 102 (1962) 1269.
3 A. Suszko-Purzycka and W. Trzebny, *J. Chromatogr.*, 16 (1964) 239.
4 M. van Schantz, *Thin-Layer Chromatogr. Proc. Symp. Rome*, (1963) 122; *C.A.*, 62 (1965) 70849.
5 A. Suszko-Purzycka and W. Trzebny, *J. Chromatogr.*, 17 (1965) 114.
6 A. Suszko-Purzycka and W. Trzebny, *Chem. Anal. (Warsaw)*, 9 (1964) 1103; *C.A.*, 63 (1965) 431c.
7 J. A. Steele, *J. Chromatogr.*, 19 (1965) 300.
8 A. Suszko-Purzycka and W. Trzebny, *Poznan. Tow. Przyj. Nauk, Pr. Kom. Farm.*, 4 (1966) 43; *C.A.*, 65 (1966) 8668e.
9 M. Petkovic, *Arh. Farm.*, 15 (1965) 437; *C.A.*, 66 (1967) 3180m.
10 V. P. Dole, W. K. Kim and I. Englitis, *J. Amer. Med. Ass.*, 198 (1966) 115.
11 R. Adamski and J. Bitner, *Farm. Pol.*, 24 (1968) 17; *C.A.*, 69 (1968) 46085j.
12 M. Petkovic, *Arh. Farm.*, 17 (1967) 193; *C.A.*, 69 (1968) 54299x.
13 M. Ono, B. F. Engelke and C. Fulton, *Bull. Narcot.*, 21 (1969) 31.
14 D. J. Stöver, *Pharm. Weekbl.*, 104 (1969) 738.
15 Hung-Cheh Chiang and Tzong-Min Chiang, *J. Chromatogr.*, 47 (1970) 128.
16 J. G. Montalvo, E. Klein, D. Eyer and B. Harper, *J. Chromatogr.*, 47 (1970) 542.
17 L. Hörhammer, H. Wagner and J. Hölzl, *Deut. Apoth.-Ztg.*, 110 (1970) 227.
18 H. Böhme and R. Bitsch, *Arch. Pharm. (Weinheim)*, 303 (1970) 456.
19 H. Böhme and R. Bitsch, *Arch. Pharm. (Weinheim)*, 303 (1970) 418.
20 J. Storck, J. P. Papin and D. Plas, *Ann. Pharm. Fr.*, 28 (1970) 25.
21 Hung-Cheh Chiang and Chu-Chi Liu, *J. Chin. Chem. Soc.*, 17 (1970) 101.
22 V. Vukecovic, *Bull. Sci. Cons. Acad. Sci. Arts RSF Yougosl. Sect. A*, 15 (1970) 238; *C.A.*, 73 (1970) 123554y.
23 K. Röder, E. Eich and E. Mutschler, *Pharm. Ztg.*, 115 (1970) 1430.
24 S. J. Mulé, *J. Chromatogr.*, 55 (1971) 255.
25 C. Andary, *Trav. Soc. Pharm. Montpellier*, 30 (1970) 307.
26 S. Gill, *Gdansk. Tow. Nauk. Rozpr. Wydz*, 37 (1970) 175; *C.A.*, 75 (1971) 64040u.
27 G. A. Jansen and I. Bickers, *S. Med. J.*, 64 (1971) 1072.
28 H. Hey, *Z. Lebensm.-Unters.-Forsch.*, 148 (1972) 1.
29 M. M. Baden, N. N. Valanju, S. K. Verma and S. N. Valanju, *Amer. J. Clin. Pathol.*, 57 (1972) 291.

30 M. Sarsunová, B. Kakác and L. Krasnec, *Z. Anal. Chem.*, 260 (1972) 291.
31 F. Pellerin and D. Mancheron, *Int. Symp. Chromatogr. Electrophor. Lect. Pap. 6th. 1970*, Ann Arbor Sci. Publ., Ann Arbor, Mich., 1971, p. 536.
32 C. W. Gorodetzky, *Toxicol. Appl. Pharmacol.*, 23 (1972) 511.
33 M. V. Vermes, *Acta Pharm. Hung.*, 43 (1973) 25; *C.A.*, 78 (1973) 133371d.
34 J. Storck and J. P. Papin, *Bull. Soc. Chim. Fr.*, (1973) 105.
35 F. Pellerin, D. Dumitrescu-Mancheron and C. Chabrelie, *Bull. Soc. Chim. Fr.*, (1973) 123.
36 C. Mulder and D. B. Faber, *Pharm. Weekbl.*, 108 (1973) 289.
37 J. Paul and F. Conine, *Microchem. J.*, 18 (1973) 42.
38 M. Vermes-Vincze and Z. Vincze, *Acta Pharm. Hung.*, 43 (1973) 49; *C.A.*, 79 (1973) 5481y.
39 H. Thieleman, *Sci. Pharm.*, 41 (1973) 47.
40 E. Smith, S. Barkan, B. Ross, M. Maienthal and J. Levine, *J. Pharm. Sci.*, 62 (1973) 1151.
41 G. Cesaire, F. Fauran, C. Pellissier, J. Goudote and J. Mondain, *Bull. Mem. Fac. Mixte Med. Pharm. Dakar*, 17 (1969) 245; *C.A.*, 79 (1973) 97027f.
42 W. T. Fischer, A. D. Baitsholts and G. S. Grau, *J. Chromatogr. Sci.*, 10 (1972) 303.
43 G. Bärwald and J. Prucha, *Monatsschr. Brau.*, 26 (1973) 190.
44 G. Bärwald and J. Prucha, *Brauwissenschaften*, 26 (1973) 299.
45 P. J. Beljaars and P. J. Koken, *J. Ass. Offic. Anal. Chem.*, 56 (1973) 1284.
46 A. M. Guyot-Hermann and H. Robert, *J. Pharm. Belg.*, 28 (1973) 557.
47 K. C. Guven and N. Guven, *Eczacilik Bull.*, 15 (1973) 77; *C.A.*, 80 (1974) 52412g.
48 D. W. Chasar and G. B. Toth, *J. Chem. Educ.*, 51 (1974) 22.
49 M. Petkovic, *Arh. Farm.*, 23 (1973) 1; *C.A.*, 80 (1974) 63802k.
50 M. Petkovic, *Acta Farm. Jugosl.*, 24 (1974) 23; *C.A.*, 81 (1974) 25833j.
51 J. M. Meola and M. Vanko, *Clin. Chem.*, 20 (1974) 184.
52 F. Conine and J. Paul, *Mikrochim. Acta*, 3 (1974) 443.
53 V. Massa, P. Susplugas and R. Taillade, *Trav. Soc. Pharm. Montpellier*, 32 (1974) 141.
54 H. Sybirska and H. Gajkzinska, *Bromatol. Chem. Toksykol.*, 7 (1974) 189; *C.A.*, 81 (1974) 164121p.
55 M. Šaršūnová and J. Hrivnāk, *Pharmazie*, 29 (1974) 608.
56 D. D. Datta and C. Ghosh, *East. Pharm.*, 17 (1974) 113.
57 P. A. F. Pranitis and A. Stolman, *J. Chromatogr.*, 106 (1975) 485.
58 R. J. Kokoski and M. Jain, *Clin. Chem.*, 21 (1975) 417.
59 D. Zivanov-Stakic, D. Radulovic and V. Brzulja, *Arh. Farm.*, 25 (1975) 29; *C.A.*, 83 (1975) 152434w.
60 J. M. Steyn and H. K. L. Hundt, *J. Chromatogr.*, 111 (1975) 463.
61 T. Inoue, M. Tatsuzawa, T. Ishii and Y. Inoue, *Eisei Shikenjo Hokoku*, 93 (1975) 31; *C.A.*, 85 (1976) 10490d.
62 R. O. B. Wijesekera, L. S. Rajapakse and D. W. Chelvarajan, *J. Chromatogr.*, 121 (1976) 388.
63 D. Giacopello, *J. Chromatogr.*, 19 (1965) 172.
64 R. R. Paris, R. Rousselet, M. Paris and J. Fries, *Ann. Pharm. Fr.*, 23 (1965) 473.
65 V. Schwarz and M. Sarsunová, *Pharmazie*, 19 (1964) 267.
66 I. Sunshine, W. W. Fike and H. Landesman, *J. Forensic Sci.*, 11 (1966) 428.
67 A. Eichhorn and L. Kny, *Zentralbl. Pharm.*, 112 (1973) 567.
68 P. D. Swaim, V. M. Loyola, H. D. Harlan and M. J. Carlo, *J. Chem. Educ.*, 51 (1974) 331.
69 E Ragazzi and G Veronese, *Mikrochim. Acta*, (1965) 966.
70 G. S. Tadjer and A. Lustig, *J. Chromatogr.*, 56 (1971) D44-D47.
71 R. R. Paris and M. Paris, *Bull. Soc. Chim. Fr.*, (1963) 1597.
72 J. Zarnack and S. Pfeifer, *Pharmazie*, 19 (1964) 216.
73 W. Kamp, W. J. M. Onderberg and W. A. Seters, *Pharm. Weekbl.*, 98 (1963) 993.
74 J. M. G. J. Frijns, *Pharm. Weekbl.*, 103 (1968) 929.
75 N. Oswald and H. Flück, *Pharm. Acta Helv.*, 39 (1964) 293.
76 F. Wartmann-Hafner, *Pharm. Acta Helv.*, 41 (1966) 406.
77 E. Röder, E. Mutschler and H. Rochelmeyer, *Arch. Pharm. (Weinheim)*, 301 (1968) 624.
78 W. W. Fike, *Anal. Chem.*, 38 (1966) 1697.
79 E. Ragazzi, G. Veronese and C. Giatobazzi, in G. B. Marini-Bettólo (Editor), *Thin-layer Chromatography*, Elsevier, Amsterdam, 1964, p. 149.
80 E. Nováková and J. Večerková, *Cesk. Farm.*, 22 (1973) 347.

81 H. C. Hsiu, J. T. Huang, T. B. Shih, K. L. Yang, K. T. Wang and A. L. Lin, *J. Chin. Chem. Soc.*, 14 (1967) 161.
82 A. Noirfalise and G. Mees, *J. Chromatogr.*, 31 (1967) 594.
83 R. A. Egli, *Z. Anal. Chem.*, 259 (1972) 277.
84 R. A. Egli, *Deut. Apoth.-Ztg.*, 110 (1970) 987.
85 F. Schmidt, *Deut. Apoth.-Ztg.*, 114 (1974) 1593.
86 A. C. Moffat and B. Clare, *J. Pharm. Pharmacol.*, 26 (1974) 665.
87 A. C. Moffat, K. W. Smalldon and C. Brown, *J. Chromatogr.*, 90 (1974) 1; A. C. Moffat and K. W. Smalldon, *J. Chromatogr.*, 90 (1974) 9.
88 A. C. Moffat, *J. Chromatogr.*, 110 (1975) 341.
89 D. Waldi, K. Schnackerz and F. Munter, *J. Chromatogr.*, 6 (1961) 61.
90 M. Wilk and U. Brill, *Arch. Pharm. (Weinheim)*, 301 (1968) 282.
91 E. Vidic and J. Schütte, *Arch. Pharm. (Weinheim)*, 295 (1962) 342.
92 K. F. Ahrend and D. Tiess, *Wiss. Z. Univ. Rostock, Math.-Naturwiss. Reihe*, 22 (1973) 951.
93 A. Kaess and C. Mathis, *Int. Symp. Chromatogr. Electrophor. Lect. Pap. 4th, 1966*, Ann Arbor Sci. Publ., Ann Arbor, Mich., 1968, p. 525.
94 R. E. Stoner and C. Parker, *Clin. Chem.*, 20 (1974) 309.
95 G. Härtel and A. Harjanne, *Clin. Chim. Acta*, 23 (1969) 289.
96 V. Massa, *Int. Symp. Chromatogr. Electrophor. Lect. Pap. 6th, 1970*, Ann Arbor Sci. Publ., Ann Arbor, Mich., 1971, p. 470.
97 T. Okumura, T. Kadono and A. Iso'o, *J. Chromatogr.*, 108 (1975) 329.
98 J. A. Vinson and J. E. Hooyman, *J. Chromatogr.*, 105 (1975) 415.
99 E. Stahl and W. Schmitt, *Arch. Pharm. (Weinheim)*, 308 (1975) 570.
100 M. H. Hashmi, S. Parveen and N. A. Chughtai, *Mikrochim. Acta*, (1969) 449.
101 G. H. Jolliffe and E. J. Shellard, *J. Chromatogr.*, 48 (1970) 125.
102 T. M. Holdstock and H. M. Stevens, *Forensic Sci.*, 6 (1975) 187.
103 J. Bäumler and S. Rippstein, *Pharm. Acta Helv.*, 36 (1961) 382.
104 S. Thunell, *J. Chromatogr.*, 130 (1977) 209.
105 K. K. Kaista and J. H. Jaffe, *J. Pharm. Sci.*, 61 (1972) 679.
106 R. C. Baselt and L. J. Casarett, *J. Chromatogr.*, 57 (1971) 139.
107 K. G. Blass, R. J. Thibert and T. F. Draisey, *J. Chromatogr.*, 95 (1974) 75.
108 M. Debackere and L. Laruelle, *J. Chromatogr.*, 35 (1968) 234.
109 R. L. Nemen, *J. Chem. Educ.*, 49 (1972) 834.
110 R. Neu, *J. Chromatogr.*, 11 (1963) 364.
111 E. Marozzi and G. Falzi, *Farmaco, Ed. Prat.*, 20 (1965) 302.
112 P. Vácha, P. Čuba, V. Preininger, L. Hruban and F. Šantavý, *Planta Med.*, 12 (1964) 406.
113 P. Braeckman, R. Van Severen and L. de Jaeger-van Moeseke, *Deut. Apoth.-Ztg.*, 104 (1964) 1211.
114 E. Stahl and W. Schmitt, *Arch. Pharm. (Weinheim)*, 307 (1974) 925.
115 E. Härtel and A. Korhonen, *J. Chromatogr.*, 37 (1968) 70.
116 K. H. Müller and H. Honerlagen, *Mitt. Deut. Pharm. Ges.*, 30 (1960) 202.
117 A. Puech, M. Jacob and D. Gaudy, *J. Chromatogr.*, 68 (1972) 161.
118 K. Chmel and V. Chmelová-Hlavatá, *J. Chromatogr.*, 118 (1976) 276.
119 K. Chmel and V. Chmelová-Hlavatá, *Cesk. Farm.*, (1975) 433.
120 N. J. Pound and R. W. Sears, *Can. J. Pharm. Sci.*, 10 (1975) 122.
121 *United States Pharmacopoeia XVIII (1970)*, United States Pharmacopeial Convention, Bethesda, pp. 581 and 582.
122 *British Pharmacopoeia (1973)*, Her Majesty's Stationery Office, London, pp. 407 and 409.
123 *United States Pharmacopoeia XIX (1975)*, United States Pharmacopeial Convention, Rockville, pp. 434 and 436.
124 S. Ebel and G. Herold, *Z. Anal. Chem.*, 266 (1973) 281.
125 J. Christiansen, *J. Chromatogr.*, 123 (1975) 57.
126 E. Stahl, *Dünnschichtchromatographie*, Springer, Berlin, 2nd ed., 1967, p. 815.
127 L. C. Snyder, *J. Chromatogr.*, 92 (1974) 223.