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Review Article

Applications of Thin-Layer Chromatography in Pharmaceutical Analyses

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THIS REVIEW is a humble effort toward providing a guide to the literature for those pharmaceutical analysts who are not specialists in thin-layer chromatography (TLC), but who are using TLC as one of the many analytical techniques available today. Data on adsorbents, solvent systems, and R_f values are presented so that this review may be used as an intermediate reference between the textbooks and the original journals when they are not conveniently available.

Unfortunately, many good references listed in the abstracting journals were not available to the authors. Because of the repetitive nature of TLC publications, data in the articles omitted will probably appear in future papers. Some classes of compounds, such as amino acids, lipids, and carbohydrates, have been omitted for the sake of brevity. Most of the references reviewed were subsequent to 1959 and for alkaloids, steroids, and vitamins after 1963. Only the first author's name is mentioned in the text but full credit is given to all authors under *References*.

REVIEWS

The popularity of TLC by virtue of its simplicity and economy has produced a large number of general reviews (about 80) since 1960. Most of these were short articles designed for the introduction of the technique. Publication of the texts by Stahl (1), Bobbitt (2), Randerath (3), and Truter (4) consolidated many of the facts and generalizations available from the earlier literature.

Gänshirt's (5) publication summarized some of the earlier work on separation and identification of drug formulations by TLC in his laboratory.

Publications on pharmaceuticals previous to 1960 are included in reviews by Stahl (6,7) in 1961 with sections on pharmaceuticals, steroids, and vitamins. Wollish (8) listed 8 references on pharmaceuticals and showed construction details for an applicator, plate holder, and storage cabinet. Demole (9) gave a general review (French) with 61 references including some alkaloids and vitamins. Teijgeler (10) in 1962 presented a review (Dutch) with 132 references outlining the history, advantages over other forms of chromatography, principles, and reference to six publications on quantitative analyses. The compounds were categorized in terms convenient to the pharmaceutical analysts. Teijgeler (11) brought his review up to date with 64 additional references tabulated according to his original scheme. The book edited by Stahl published in German in 1962 and translated in 1965 (1) is the most convenient starting place for review of the earlier literature, and it is recommended for all analysts active in TLC. It includes chapters by experts in their fields.

A paper by Giacobazzi (12) includes 36 references of pharmaceutical interest. Heft-

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mann's comprehensive bibliography (13) included literature from December 1961 through December 1963 on column, paper, and thin-layer chromatography for many classes of compounds and 30 references specifically for pharmaceuticals.

Russel's (14) review (English) is a very good condensed general outline of TLC particularly in reference to types of development. It could be used as a guide for a short course for the introduction of analysts to the techniques of TLC. No attempts were made by Russel for the classification of compounds.

Zarnack (15) published a similar paper (German) designed for the teaching and practice of drug analyses. Solvent systems and examples are given for analgesics, antipeptics, purines, sulfonamides, and alkaloids with 67 references through 1963.

In a section of a book on biochemical analysis, Mangold (16) presented another condensed summary (90 pages) of the techniques of TLC. No attempt to list classes of compounds was made by the authors. A table of common reagents for detection was shown on page 410. An elutropic series was listed on page 422, and a very good section on means of quantitation was included. A table of increasing affinity of functional groups and adsorption power of adsorbents was shown. In other tables, material and solvents were listed for adsorption, ion exchange, partition, and reversed phase partition TLC. The section was concluded with a very convenient list of manufacturers of TLC apparatus and 87 references.

Coombe (17) gave a brief summary (two pages) on the application of quantitative TLC to pharmaceutical analyses.

Heftmann's (18) bibliography in 1966 with 2,329 references covering TLC, paper, and column chromatography (December 1963–December 1965) gave TLC separate discussion on technique and application to classes of compounds. About 90 references were listed for pharmaceuticals and a separate listing made for steroids, vitamins, and antibiotics. Heftmann's review, even though not specific for TLC nor pharmaceutically oriented, is the best starting point for a literature survey of recent publications. Unfortunately, the only way to find data on specific compounds is to read the whole section on that class and then look up the original references cited. Another survey by Teijgeler (19) added 243 references to his earlier paper.

Special bibliographies furnished by suppliers of apparatus and chemicals are available particularly since the introduction of precoated plates and sheets. One of these by Baitsholt (20) includes sections on pharmaceuticals.

EXPLANATION OF TABLES

There has been much written on the merits of and the factors influencing R_f values. In fact, this itself has been the subject of several publications (21–23). Active workers in the field know that observing a fresh TLC plate or suitable reproduction of such tells much more than observing a table in regard to size of spots, shape, tailing, *etc.* A summary of data using reproduction of plates is not practical in a review article such as this. The original R_f values or averages of ranges of values are listed in the tables. The reference for each publication is listed first. The alphabetical designation for solvent system refers to the list of solvents at the bottom of the table.

An "X" was used to show that the R_f value was not reported or was unavailable to the authors when this review was written.

The compounds are generally listed alphabetically, except when other arrangements make interpretation more convenient. Nomenclature presented the usual problems (24) of various trade names in different languages. Classification of the drugs under the general headings was by convenience and according to type of articles in which the work was reported. These lists certainly should not be used as a therapeutic or pharmacological index.

Some duplication, ambiguity, and inaccuracies are undoubtedly present; but workers active in the specific fields will recognize the various names. The adsorbents are designated by a number which refers to the list in Table I.

TABLE I—ADSORBENTS

1, Silica gel (with or without $CaSO_4$)
2, Silica gel with 0.1 *M* NaOH
3, Silica gel with 0.1 *M* KOH
4, Silica gel with 0.1 *M* $KHSO_4$
5, Silica Gel HF254
6, Silica Gel GF
7, Silica gel with rice starch
8, Silica gel with kieselguhr (1:1)
9, Silica gel with $AgNO_3$
10, Silica gel with EDTA
11, Silica gel with NaEDTA
12, Silica gel with $ZnCO_3$ (1:1)
13, Aluminum oxide (with or without $CaSO_4$)
14, Basic aluminum oxide
15, Silica gel + PEG 200
16, Aluminum oxide with $ZnCO_3$ (1:1)
17, Cellulose
18, Cellulose-MN300
19, Kieselguhr
20, Kieselguhr with formamide
21, Carbon
22, Carbon with acid
23, Polyamide powder

TABLE II—TLC OF ERGOT ALKALOIDS

Ref. →	(28)	(28)	(28)	(28)	(27)	(31)	(32)	(32)	(25)	(25)	(25)	(25)	(25)	(25)	(25)
Solvent[a] →	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
Adsorbent →	1	1	13	13	20	2	1	1	1	1	1	1	1	13	13	2
Compd.	$R_f \times 100$															
Aci-dihydroergotamine						31										
Dihydroergocornine					38		47	48								
Dihydroergocristine					30		47	48	42	30	03	0	07	15	07	69
Dihydroergocryptine					50		47	48								
Dihydroergotamine					09	45	28	29	21	12	0	0	03	07	0	61
Ergocornine	58	59	10	24	50											
Ergocorninine	83	73	31													
Ergocristine	54	56	09		41				51	38	14	05	13	46	15	70
Ergocristinine	80	74	38						61	57	13	0	20	0	27	70
Ergocryptine	60	55	15	30	61											
Ergocryptinine	85	75	46													
Ergonovine	17	12	0			19										
Ergononovinine						36										
Ergometrine							14	17	14	06	0	0	02	03	0	64
Ergometrinine	44	38	01						42	25	03	0	08	12	10	62
Ergosine	35	31	02		17											
Ergosinine	75	68	12													
Ergotamine	31	31	01		11	51	29	32	24	16	0	0	03	10	05	59
Ergotaminine	68	64	07			76			24	51	0	0	14	42	15	68
LSD						61										
Methylergonovine						23										
Methysergide						35										

[a] *A*, ethyl acetate–*N*,*N*-dimethylformamide–ethanol (130:19:1); *B*, benzene-*N*,*N*-dimethylformamide (13:2); *C*, $CHCl_3$–ethyl ether–water (175:25:50); *D*, $CHCl_3$–ethyl ether–water (3:1:1); *E*, ethyl acetate–*n*-heptane–Et_2NH (250:300:1); *F*, $CHCl_3$–MeOH (9:1); *G*, C_6H_6–acetone–ether–10% NH_3 (4:6:1:0.3); *H*, C_6H_6–acetone–ether–25% NH_3 (4:6:1:0.3); *I*, $CHCl_3$–acetone–Et_2NH (50:40:10); *J*, $CHCl_3$–Et_2NH (90:10); *K*, cyclohexane–$CHCl_3$–Et_2NH (50:40:10); *L*, cyclohexane–Et_2NH (90:10); *M*, C_6H_6–ethyl acetate–Et_2NH (70:20:10); *N*, $CHCl_3$; *O*, cyclohexane–$CHCl_3$ (30:70) + 0.05% diethylamine (3 drops); *P*, CH_3OH.

The descriptions of the means of visualization are included in the text. Usually if good separation is obtained, detection is not a problem since there are many means and reagents available, such as I_2 vapor, H_2SO_4, $KMnO_4$, U.V. and fluorescence, and bioautograph. Analysts usually improvise means of detection suitable for their specific problems.

ALKALOIDS

One of the many papers on the separation and identification of alkaloids was an early report by Waldi (25) in 1961. A total of 54 alkaloids were studied using various solvent systems and silica gel or alumina as the adsorbent. The chapter by Waldi in Stahl's book (*Reference 1*, page 279), the chapter on TLC in Stolman's book (26), and the chapters in the book by Marini-Bettolo (*Reference 22*, pages 144, 149, 155) survey the early literature. This review will mention some pertinent studies reported after 1963.

Hydrogenated ergot alkaloids were separated by Hohmann (27) on cellulose powder impregnated with formamide (Table II). The alkaloids were detected by irradiating with a low pressure Hg burner giving a yellow-green fluorescent zone. The method also separated the nonhydrogenated alkaloids which appeared as blue fluorescent zones.

McLaughlin (28) developed a method for the identification and determination of ergot alkaloids. The solvents shown in Table II were chosen from 111 solvent systems studied. Regression analyses for data obtained by a quantitative determination of the eluted alkaloid by reaction with *p*-dimethylaminobenzaldehyde (PDAB) was tabulated.

A TLC procedure using silica gel and C_6H_6–$CHCl_3$–EtOH (2:4:1) was used by Ziner (29) for the quantitative evaluation of the stability of ergot alkaloids in aqueous preparations with the PDAB reaction.

The separation of ergot alkaloids from pharmaceuticals by Sahli (30) was accomplished on silica gel plates using $CHCl_3$–95% EtOH (9:1) as solvent. The plates were examined under U.V. light and the spots were then determined fluorimetrically using blank zones for comparison.

French (31) used TLC for the determination of identity and purity of ergot alkaloids and other active components present in pharmaceutical dosage forms.

Wasicky (33) used microscope slides as a support for silica gel and a small chromatographic chamber with butanol containing 10% acetic acid and saturated with water as the solvent for opium alkaloids. Spots were detected with PDAB reagent.

Brochmann-Hanssen (34) observed the TLC zones (Table III) under U.V. light, then sprayed

TABLE III—TLC OF OPIUM ALKALOIDS

Ref. →	(43)	(36)	(36)	(36)	(36)	(36)	(36)	(36)	(36)	(44)	(34)	(34)	(35)
Solvent[a] →	*A*	*B*	*C*	*D*	*E*	*F*	*G*	*H*	*I*	*J*	*K*	*L*	*M*
Adsorbent →	1	1	1	1	1	1	1	1	1	1	1	1	13
Compd.	$R_f \times 100$												
Piminodine ethanesulfonate[b]		97	98	97	93	78							
Anileridine		95	95	93	77	67							
Cotarnine													
Codeine	26	28	42	40	05	05	11	24	21		35	15	77
Cryptopine											48	40	
Diacetylmorphine													
Hydrocodone bitartrate[c]		36	35	42	05	05							
Dihydrocodeine													
Hydrocodone													
Dihydromorphine		09	16	17	01	02							
Hydromorphone													
Hydromorphone HCl[d]		18	19	21	02	01							
Dionin		40	46	47	05	05							
Heroin		74	67	73	19	11							
Hydromorphone													
10-Hydroxycodeine											17	15	
Laudanidine											47	28	
Laudanine											47	28	
Laudanosine											74	36	
Levorphanol tartrate[e]		57	54	44	20	17							
Meperidine		63	70	70	27	28							
Metapon		28	30	34	03	02							
Methadon		79	81	82	58	60							
Monoacetylmorphine		50	55	56	11	10							
Morphine	12	18	20	23	02	02	05	08	07	05	12	08	03
Nalorphine										60			
Narceine											09	03	
Narcotine	74	80	83	85	72	57	80	89	85		97	87	92
Narcotoline											88	71	
Neopine											38	12	
Alphaprodine HCl[f]		83	79	87	25	22							
Normorphine										42			
Oxymorphone HCl[g]		56	56	58	15	14							
Papaverine	59	73	77	77	53	35	61	81	72		97	77	89
Peronin		53	51	52	09	07							
Phenazocine		96	96	95	80	70							
Protopine											46	42	
Thebaine	45	65	73	74	23	15	34	62	54		65	38	

[a] *A*, xylene–methyl ethyl ketone–MeOH–Et_2NH (20:20:3:1); *B*, EtOH–dioxane–benzene–NH_4OH (5:40:50:5); *C*, $CHCl_3$–dioxane–EtOAc–NH_4OH (25:60:10:5); *D*, EtOH–$CHCl_3$–dioxane–30-60° pet. ether–C_6H_6–NH_4OH–EtOAc (5:10:50:15:10:5:5); *E*, EtOAc–benzene–NH_4OH (60:35:5); *F*, EtOAc–dibutyl ether–NH_4OH (60:35:5); *G*, EtOAc–C_6H_6–acetonitrile–NH_4OH (50:30:15:5); *H*, acetonitrile–$CHCl_3$–EtOAc–NH_4OH (40:30:25:5); *I*, acetonitrile–EtOAc–C_6H_6–NH_4OH (40:25:30:5); *J*, $CHCl_3$–isopropanol (1:3); *K*, CH_3OH–$CHCl_3$ (1:9); *L*, C_2H_5OH–C_6H_6 (1:4); *M*, acetone; *N*, chloroform; *O*, benzene–$CHCl_3$–acetone (70:15:15); *P*, benzene–acetone–ether–10% aq. ammonia (4:6:1:0.3); *Q*, benzene–acetone–ether–25% am-

Continued ⟶

the chromatogram with potassium iodoplatinate. Ikram (35) used modified Dragendorff's spray reagent after TLC (Table III) for the identification of opiates obtained in narcotic seizures. Eight solvent systems were studied by Steele (36) for the same purpose on 26 compounds.

A table of reactions of some opium alkaloids and acetylated derivatives with ten reagents and U.V. after separation in MeOH–$CHCl_3$–NH_3 23% (85:15: 0.7) was prepared by Vignoli (37). Continuing a series on TLC without binding agents, Schwarz (38) tabulated R_f values for 19 alkaloids in nine systems including ethylmorphine and codeine. Some of these systems were applied to the separation of mixtures in tablets, extracts, and tinctures.

A method for the separation and the U.V., I.R., and color test identification of acetyl codeine from illicit heroin was described in detail by Nakamura (39). Pfeifer (40) thoroughly studied and tabulated the R_f values of alkaloids of the genus *Papaver* on Silica Gel G with benzene–acetone–methanol (7:2:1) and alumina with heptane–$CHCl_3$–ether (4:5:1). The R_f values of 22 opium alkaloids and 22 rauwolfia alkaloids in $CHCl_3$–acetone–Et_2NH (5:4:1) and chloroform–cyclohexane–Et_2NH (7:2:1) systems along with the fluorescence color and color with iodoplatinate were tested by Kaess (41). Other TLC studies on opium alkaloids are mentioned under *Analgesics and Antipyretics.*

The TLC of cinchona alkaloids (Table IV) was studied intensively by Suszko-Purzycka (45–48) and Steele (36) and also was included in the recent work of Fike (42). The detection of zones was by modified Dragendorff reagent

TABLE III—(*Continued.*)

Ref. → / Solvent[a] → / Adsorbent → / Compd.	(35) N 13	(25) N 13	(35) O 13	(32) P 1	(32) Q 1	(25) R 1	(25) S 1	(25) T 1	(25) U 1	(25) V 1	(25) W 13	(25) X 2	(42) X 2	(42) X 4	(42) Y 2	(42) M 2	(42) Z 4
	$R_f \times 100$																
Piminodine ethanesulfonate[b]																	
Anileridine																	
Cotarnine		0				60	90	43	31	45	25	0					
Codeine	38	12	33	18	24	38	53	16	04	26	27	35	28	24	07	06	10
Cryptopine																	
Diacetylmorphine													39	24	22	20	09
Hydrocodone bitartrate[c]																	
Dihydrocodeine		10				38	54	18	06	28	30	25					
Hydrocodone		48				51	65	21	04	30	43	18	17	11	06	04	03
Dihydromorphine																	
Hydromorphone		05				24	23	08	01	11	08	16	15	13	05	03	05
Hydromorphone HCl[d]																	
Dionin																	
Heroin																	
Hydromorphone				09	13												
10-Hydroxycodeine																	
Laudanidine																	
Laudanine																	
Laudanosine																	
Levorphanol tartrate[e]																	
Meperidine													48	49	55	21	24
Metapon																	
Methadon													37	51	76	43	25
Monoacetylmorphine																	
Morphine	03	03	06	09	09	10	08	0	0	03	0	34	28	23	02	04	10
Nalorphine																	
Narceine		0				03	0	0	0	0	0	0					
Narcotine	77	81	77	78	81	72		51	10	57	79	72					
Narcotoline																	
Neopine																	
Alphaprodine HCl[f]																	
Normorphine																	
Oxymorphone HCl[g]																	
Papaverine	88	85	73	64	69	67		42	03	47	84	70	62	62	11	53	21
Peronin																	
Phenazocine																	
Protopine																	
Thebaine		71		46	51	65		51	16	50	76	40					

monia (4:6:1:0.3); *R*, $CHCl_3$–acetone–Et_2NH (50:40:10); *S*, $CHCl_3$–Et_2NH (90:10); *T*, cyclohexane–$CHCl_3$–Et_2NH (50:40:10); *U*, cyclohexane–Et_2NH (90:10); *V*, benzene–ethyl acetate–Et_2NH (70:20:10); *W*, cyclohexane–$CHCl_3$ (30:70) + 0.05% Et_2NH (3 drops); *X*, CH_3OH; *Y*, cyclohexane–benzene–Et_2NH (75:15:10); *Z*, 95% ethanol. [b] Marketed as Alvodine by Winthrop Laboratories, New York, N. Y. [c] Marketed as Dicodid by Knoll Pharmaceuticals, Orange, N. J. [d] Marketed as Dilaudid by Knoll Pharmaceuticals. [e] Marketed as Levo-Dromoran by Roche Laboratories, Nutley, N. J. [f] Marketed as Nisentil by Roche Laboratories. [g] Marketed as Numorphan by Endo Laboratories, Garden City, N. J.

TABLE IV—TLC OF CINCHONA ALKALOIDS

Ref. → / Solvent[a] → / Adsorbent → / Compd.	(32) A 1	(32) B 1	(47) C 1	(48) C 2	(46) D 2	(45) D 1	(25) E 1	(25) F 1	(25) G 1	(25) H 1	(25) I 1	(25) J 13	(25) K 13	(25) L 2	(42) L 2	(42) L 4	(42) M 2	(42) N 2	(42) O 4
	$R_f \times 100$																		
Cinchonine			30		40	40	38	44	17	07	27	0	22	40	37	22	10	11	09
Cinchonidine			33		40	37													
Dihydrocinchonine			18		30	30													
Dihydrocinchonidine			23		31														
Dihydroquinine			26	37	40	42													
Dihydroquinidine			23	31	38	38													
Quinidine	28	43	37	43	47	46	33	40	15	0	25	12	18	50					
Quinine	23	35	34	47	46	50	19	26	07	0	17	09	18	43	47	37	05	11	14

[a] *A*, benzene–acetone–ether–10% NH_3 (4:6:1:0.3); *B*, benzene–acetone–ether–25% NH_3 (4:6:1:0.3); *C*, $CHCl_3$–MeOH–diethylamine (50:50:1); *D*, $CHCl_3$–MeOH–diethylamine (80:20:1); *E*, $CHCl_3$–acetone–diethylamine (50:40:10); *F*, $CHCl_3$–diethylamine (90:10); *G*, cyclohexane–$CHCl_3$–diethylamine (50:40:10); *H*, cyclohexane–diethylamine (90:10); *I*, benzene–ethyl acetate–diethylamine (70:20:10); *J*, $CHCl_3$; *K*, cyclohexane–$CHCl_3$ (30:70) + 0.05% diethylamine (3 drops); *L*, CH_3OH; *M*, cyclohexane–benzene–diethylamine (75:15:10); *N*, acetone; *O*, 95% EtOH.

and U.V. Rauwolfia alkaloids reported by Ikram (35), Waldi (25), and Zarnack (32) are recorded in Table V. A single tablet assay of reserpine developed by Weaver (49) used Silica Gel G with an acetone–$CHCl_3$ (3:10) solvent. Quantitation was obtained by a comparison of the size

and intensity of the PDAB spots to the standard.

Tropane alkaloids reported by Fike (42), Cuven (50), Zarnack (32), French (31), and Waldi (25) are given in Table VI. Detection was made by modified Dragendorff reagent or iodoplatinate. Kaess (51) reacted a 2% solution of the alkaloid with a few drops of 1 *N* KOH in a sealed capillary tube for 1 hr. before TLC.

Atropine was estimated by Ikram (52) in tropane alkaloids by TLC on alumina with MeOH. The eluted atropine zone in acid was back titrated with base with a relative error of 2%.

Chen (53) separated strychnine and brucine on alumina using as a solvent benzene–ethyl acetate (1:2). The strychnine was eluted from the

TABLE V—TLC OF RAUWOLFIA ALKALOIDS

Ref. →	(35)	(35)	(35)	(32)	(32)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)
Solvent[a] →	*A*	*B*	*C*	*D*	*E*	*F*	*G*	*H*	*I*	*J*	*K*	*L*	*M*
Adsorbent →	13	13	13	1	1	1	1	1	1	1	13	13	2
Compd.	$R_f \times 100$												
Ajmalicine	77												
Ajmaline	24	87	51			47	42	12	03	30	06	13	56
Rauwolscine						55	63	18	04	36	36	15	68
Reserpine	60		89	72	75	72	80	20	0	46	63	35	69
Serpentine	24	75	34			24	15	0	0	04	0	0	0
Serpentinine		86	73			53	56	08	0	10	0	03	12
Yohimbine						63	62	18	03	37	33	15	60

[a] *A*, $CHCl_3$–acetone (85:15); *B*, absolute EtOH; *C*, $CHCl_3$–EtOH–acetone (90:5:5); *D*, benzene–acetone–ether–10% NH_3 (4:6:1:0.3); *E*, benzene–acetone–ether–25% NH_3 (4:6:1:0.3); *F*, $CHCl_3$–acetone–diethylamine (50:40:10); *G*, $CHCl_3$–diethylamine (90:10); *H*, cyclohexane–$CHCl_3$–diethylamine (50:40:10); *I*, cyclohexane–diethylamine (90:10); *J*, benzene–EtOAc–diethylamine (70:20:10); *K*, $CHCl_3$; *L*, cyclohexane–$CHCl_3$ (30:70) + 0.05% diethylamine (3 drops); *M*, CH_3OH.

TABLE VI—TLC OF TROPANE ALKALOIDS

Ref. →	(50)	(31)	(32)	(32)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(42)	(42)	(42)	(42)	(42)
Solvent[a] →	*A*	*B*	*C*	*D*	*E*	*F*	*G*	*H*	*I*	*J*	*K*	*L*	*L*	*L*	*M*	*N*	*O*
Adsorbent →	13	2	1	1	1	1	1	1	1	13	13	2	2	4	2	2	4
Compd.	$R_f \times 100$																
Atropine	89	05	08	20	38	40	16	05	12	0	10	17	11	31	09	01	14
Apoatropine			19	43	54	67	40	20	26	15	40	16					
Homatropine	87	07			37	45	15	05	23	04	24	15					
Scopolamine		39	37	52	56	60	19	03	34	30	0	52	54	34	09	33	13
Tropine	43																
Tropacocaine					65		56	34	45	58	78	35					
Hyoscyamine		05															

[a] *A*, EtOH–pyridine–H_2O (10:60:40); *B*, $CHCl_3$–MeOH (9:1); *C*, benzene–acetone–ether–10% NH_3 (4:6:1:0.3); *D*, benzene–acetone–ether–25% NH_3 (4:6:1:0.3); *E*, $CHCl_3$–acetone–Et_2NH (50:40:10); *F*, $CHCl_3$–Et_2NH (90:10); *G*, cyclohexane–$CHCl_3$–diethylamine (50:40:10); *H*, cyclohexane–Et_2NH (90:10); *I*, benzene–ethyl acetate–Et_2NH (70:20:10); *J*, $CHCl_3$; *K*, cyclohexane–$CHCl_3$ (30:70) + 0.05% diethylamine (3 drops); *L*, CH_3OH; *M*, cyclohexane–benzene–diethylamine (75:15:10); *N*, acetone; *O*, 95% ethanol.

TABLE VII—TLC OF OTHER ALKALOIDS

Ref. →	(32)	(32)	(55)	(54)	(54)	(56)	(56)	(56)	(25)	(25)	(25)	(25)	(25)	(25)	(25)	(25)
Solvent[a] →	*A*	*B*	*C*	*D*[b]	*E*[b]	*F*	*G*	*H*	*H*	*I*	*J*	*K*	*L*	*M*	*N*	*O*
Adsorbent →	1	1	1			13	1	13	13	1	1	1	1	1	13	2
Compd.	$R_f \times 100$															
Aconitine									36	68		35	03	49	60	65
Brucine	10	25							50	42	63	18	0	19	54	12
Cephaline									25	56	63	19	02	23	17	37
Colchicine	15	20							11	47	41	04	0	04	0	57
Emetine									38	67		40	06	45	58	50
Hydrocodon	18	25														
Leurocristine			16	51												
Leurosidine			06	23												
Leurosine			45		27	27	35	20								
Oxycodon	64	69														
Pethidine	48	60														
Physostigmine	47	60							59	65		32	04	44	50	46
Pilocarpine	25	37							32	41	52	09	0	13	25	55
Sparteine	11	32							0	70		68	68	55	55	05
Strychnine	20	40							57	53	76	28	05	38	60	22
Vincaleucoblastine				24		36										

[a] *A*, benzene–acetone–ether–10% aq. ammonia (4:6:1:0.3); *B*, benzene–acetone–ether–25% ammonia (4:6:1:0.3); *C*, $CHCl_3$–MeOH (95:5); *D*, 5% EtOH in acetonitrile; *E*, 30% acetonitrile in benzene; *F*, $CHCl_3$–ethyl acetate (1:1); *G*, ethyl acetate–EtOH (3:1); *H*, $CHCl_3$; *I*, $CHCl_3$–acetone–Et_2NH (50:40:10); *J*, $CHCl_3$–Et_2NH (90:10); *K*, cyclohexane–$CHCl_3$–Et_2NH (50:40:10); *L*, cyclohexane–Et_2NH (90:10); *M*, benzene–ethyl acetate–Et_2NH (70:20:10); *N*, cyclohexane–$CHCl_3$ (30:70) + 0.05% Et_2NH (3 drops); *O*, CH_3OH. [b] Silica gel with 0.1 *N* LiOH.

chromatogram and determined by polarography. The differentiation of *Vinca rosea* alkaloids by TLC (Table VII) has been reported by Jakovljevic (54) with ceric ammonium sulfate in phosphoric acid as the reagent. The same reagent was used by Farnsworth (55) for a study on *Catharanthus* alkaloids shown in Table VII. In a study by Cone (56), the R_f values of 26 vinca alkaloids were obtained. Some of the veratrum alkaloids were resolved by cyclohexane–EtOH (17:3) on Silica Gel HF254 by Zeitler (57) and observed under U.V. after treatment with trichloroacetic acid and heat.

ANALGESICS-ANTIPYRETICS

The early work of Gänshirt (5) was the beginning of many articles on the separation of acetylsalicylic acid, phenacetin, and caffeine. These components from extracts of tablets and suppositories were identified by U.V., potassium ferricyanide, and with chlorine, HCl, and then NH_3. His system was later used for a laboratory experiment in teaching pharmaceutical analysis by Conners (58). Simple equipment for coating onto microslides and spray applicators were described. The authors stated: "Despite this simplification the procedures described are entirely practical for serious analytical and research use." This was a very conservative statement, for these "simple" techniques have been used to solve many age-old problems such as the measurement of *p*-chloroacetanilide and acetanilide in phenacetin (59–61) by U.V. and permanganate and for the determination of

TABLE VIII—TLC OF ANALGESICS–ANTIPYRETICS

Ref. →	(5)	(58)	(64)	(65)	(65)	(15)	(15)	(69)	(69)	(70)	(66)	(62)	(71)
Solvents[a] →	*A*	*A*	*B*	*C*	*D*	*E*	*F*	*G*	*H*	*I*	*J*	*K*	*L*
Adsorbent →	1	1	1	1	1	1	1	1	1	5	13	1	1
Compd.	$R_f \times 100$												
Acetylsalicylic acid	X	97		0	0	83	19	0	26		20		
Acetylmethadol													
Acetophenetidin	X	45	X	36	70	57	81	25	57	30			
Amidopyrine			X	42	65	04	75						
Aminopyrine								16	21				
Anileridine													08
Antipyrine								03	09				
Cocaine													39
Caffeine	X	10	X	47	61	25	53	04	13	10			
p-Chloroacetanilide													
Dimethylaminoantipyrine													
Ethoheptazine													12

Ref. →	(71)	(72)	(73)	(67)	(68)	(68)	(68)	(68)	(68)	(68)	(68)	(60)	(31)
Solvents[a] →	*M*	*N*	*O*	*P*	*Q*	*R*	*S*	*T*	*U*	*V*	*W*	*X*	*Y*
Adsorbent →	1	1	1	1	1	1	1	1	1	1	1	1	2
Compd.	$R_f \times 100$												
Acetylsalicylic acid													
Acetylmethadol					64	60	99	40	38	52	62		
Acetophenetidin			70									X	67
Amidopyrine													
Aminopyrine													
Anileridine	37												
Antipyrine			50										
Cocaine	75												
Caffeine			05										70
p-Chloroacetanilide												X	
Dimethylaminoantipyrine			64										
Ethoheptazine	24												

Ref. →	(5)	(58)	(64)	(65)	(65)	(15)	(15)	(69)	(69)	(70)	(66)	(62)	(71)
Solvents[a] →	*A*	*A*	*B*	*C*	*D*	*E*	*F*	*G*	*H*	*I*	*J*	*K*	*L*
Adsorbent →	1	1	1	1	1	1	1	1	1	5	13	1	1
Compd.	$R_f \times 100$												
Isopropylantipyrine								59	72	50			
dl-Methadone													42
Merperidine													14
Dextromethorphan													11
Morphine													0
Normorphine													0
Codeine				24	43	03	38						
Norcodeine													
Heroin													
Nalorphine													
Methyldihydromorphine													
Hydromorphone													

(*Continued on next page.*)

TABLE VIII—(*Continued.*)

Ref. →	(71)	(72)	(73)	(67)	(68)	(68)	(68)	(68)	(68)	(68)	(68)	(60)	(31)
Solvents[a] →	*M*	*N*	*O*	*P*	*Q*	*R*	*S*	*T*	*U*	*V*	*W*	*X*	*Y*
Adsorbent →	1	1	1	1	1	1	1	1	1	1	1	1	2
Compd.	$R_f \times 100$												
Isopropylantipyrine													
dl-Methadone	58				34	59	99	17	17	55	62		
Merperidine	32												
Dextromethorphan	20												
Morphine	0	33			29	27	11	21	07	54	34		
Normorphine	0	15			08	48	04	07		66	62		
Codeine	05				30	29	39	25	08	53	30		
Norcodeine					12	50	13	09	06	63	49		
Heroin					37	35	76	35	15	61	32		
Nalorphine					71	55	35	67	25	59	41		
Methyldihydromorphine					16	24	25	15		45	26		
Hydromorphone					11	21	17	13		41	25		

Ref. →	(5)	(58)	(64)	(65)	(65)	(15)	(15)	(69)	(69)	(70)	(66)	(62)	(71)
Solvents[a] →	*A*	*A*	*B*	*C*	*D*	*E*	*F*	*G*	*H*	*I*	*J*	*K*	*L*
Adsorbent →	1	1	1	1	1	1	1	1	1	5	13	1	1
Compd.	$R_f \times 100$												
Ethylmorphine													
Dihydrohydroxymorphinone													
Dihydromorphine													
Hydrocodone													
Dihydrohydroxycodeinone													
6-Acetylmorphine													
n-Allylmorphine													
Paracetamol						46	67	16	48				
Papaverine				57	64	11	80						
Pethidine													
Phenazone				24	53	21	60						

Ref. →	(71)	(72)	(73)	(67)	(68)	(68)	(68)	(68)	(68)	(68)	(68)	(60)	(31)
Solvents[a] →	*M*	*N*	*O*	*P*	*Q*	*R*	*S*	*T*	*U*	*V*	*W*	*X*	*Y*
Adsorbent →	1	1	1	1	1	1	1	1	1	1	1	1	2
Compd.	$R_f \times 100$												
Ethylmorphine					33	25	46	27	08	53	33		
Dihydrohydroxymorphinone					46	29	34	24	10	45	28		
Dihydromorphine					15	21	10	10		43	29		
Hydrocodone					17	25	41	19		42	23		
Dihydrohydroxycodeinone					46	24	87	29	16	32	34		
6-Acetylmorphine		55			38	40	64	29	19	37	37		
n-Allylmorphine		85											
Paracetamol													
Papaverine													
Pethidine					42	41	97	36	20	46	44		
Phenazone													

Ref. →	(5)	(58)	(64)	(65)	(65)	(15)	(15)	(69)	(69)	(70)	(66)	(62)	(71)
Solvents[a] →	*A*	*A*	*B*	*C*	*D*	*E*	*F*	*G*	*H*	*I*	*J*	*K*	*L*
Adsorbent →	1	1	1	1	1	1	1	1	1	5	13	1	1
Compd.	$R_f \times 100$												
Phenylbutazone						90	40						
Piminodine													
d-Propoxyphene													48
Quinine				28	17	02	55						
Salicylic acid											28	35	
Salicylsalicylic acid											72		
p-Hydroxyisophthalic acid													
Salicylamide						61	59						

Ref. →	(71)	(72)	(73)	(67)	(68)	(68)	(68)	(68)	(68)	(68)	(68)	(60)	(31)
Solvents[a] →	*M*	*N*	*O*	*P*	*Q*	*R*	*S*	*T*	*U*	*V*	*W*	*X*	*Y*
Adsorbent →	1	1	1	1	1	1	1	1	1	1	1	1	2
Compd.	$R_f \times 100$												
Phenylbutazone													
Piminodine					88	73	99	85	76	69	50		
d-Propoxyphene	71				73	68	97	54	56	53	61		
Quinine													
Salicylic acid													
Salicylsalicylic acid													
p-Hydroxyisophthalic acid				31									
Salicylamide													

[a] *A*, MeOH–AcOH–ether–benzene (1:18:60:120); *B*, cyclohexane–acetone (4:5); *C*, benzene + 5% EtOH; *D*, chloroform + 2% *n*-BuOH; *E*, butyl acetate–$CHCl_3$–85% formic acid (6:4:2); *F*, butyl acetate–acetone–*n*-BuOH–10% NH_3 (5:4:3:1); *G*, ether; *H*, ether–ethyl acetate (1:4); *I*, $CHCl_3$–ethyl acetate (1:1); *J*, pet. ether–ethyl acetate–AcOH (85:10:5); *K*, hexane–AcOH–$CHCl_3$ (85:15:10); *L*, benzene–NH_3 atmosphere; *M*, $CHCl_3$–NH_3 atmosphere; *N*, MeOH–*n*-BuOH–benzene–water (60:15:10:15); *O*, $CHCl_3$–acetone–water (2:9:0.5); *P*, benzene–dioxane–AcOH (90:25:4); *Q*, EtOH–pyridine–dioxane–water (50:20:25:5); *R*, EtOH–AcOH–water (60:30:10); *S*, EtOH–dioxane–benzene–NH_4OH (5:40:50:5); *T*, MeOH–*n*-BuOH–benzene–water (60:15:10:15); *U*, *tert*-amyl alcohol–*n*-butyl ether–water (80:7:13); *V*, *n*-BuOH–AcOH–water (4:1:2); *W*, *n*-BuOH–concentrated HCl saturated with water (90:10); *X*, cyclohexane–acetone–isobutylketone–MeOH–H_2O (100:80:30:5:1); *Y*, $CHCl_3$–MeOH (9:1).

salicylic acid in acetylsalicylic acid by reflectance spectroscopy following TLC by Frodyma (62).

Gänshirt (63) also published a table showing reaction of seven common analgesics with reagents already mentioned plus $AgNO_3$–NH_3, Cl_2 + benzidine, I_2, and rhodamine B. Gänshirt (64) reported the accuracy and relative standard deviation results from quantitative TLC for caffeine was 113% ± 12%, amidopyrine as 98.6% ± 5%, and phenacetin as 100% ± 27% from ten replicates. Sarsunova (65) gave solvent systems of choice for the separation of various combinations of acetylsalicylic acid, phenacetin, amidopyrine, caffeine, and quinine. Bailey (66) was able to measure 0.05% or less of salicylsalicylic acid (SSA) in salicylic acid (SA) by U.V. at 310 mμ following TLC. Verification was obtained by the shifting of maxima of SSA to 345 mμ in acid alcohol while SA remained at 310 mμ. Shelly (67) extracted TLC to detect *p*-hydroxyisophthalic acid in SA. Mule (68) applied TLC along with gas chromatography and U.V. spectrophotometry for the identification of analgesics in human urine, plasma, and tissue homogenates. The data on R_f values were organized by Mule according to chemical families and offer a good reference for toxicological studies. Some of these data from the latter authors are presented in Table VIII. Fike (42) gave a comprehensive list of 140 drugs including some analgesics with a separate table for 112 basic drugs and 28 phenothiazines. Five solvent systems were used and a correlation of structure and R_f values discussed. (See Table III.)

ANTIBIOTICS

Nicolaus (74) published one of the early papers on antibiotics using agar containing triphenyltetrazolium for microbiological detection of zones.

TABLE IX—TLC OF ANTIBIOTICS

Ref. →	(84)	(83)	(83)	(85)	(89)	(92)
Solvent[a] →	*Q*	*V*	*W*	*Z*	*AB*	*AF*
Adsorbent →	1	18	18	1	1	1
Compd.	$R_f \times 100$					
Actinomycin C	70					
Actinomycin F	50					
Amidinomycin		53				
Bacitracin A					35	
Bacitracin F					22	
Catenulin		15				
Erythromycin A				X		20
4-Acetylerythromycin				X		
2-4-Diacetylerythromycin				X		
Erythromycin hemiacetal				X		
Fradiomycin		10				
Gentamycin		35				

Ref. →	(74)	(78)	(78)	(80)	(83)	(83)	(92)	(90)
Solvent[a] →	*A*	*P*	*P*	*Q*	*V*	*W*	*AE*	*AC*
Adsorbent →	1	22	21	1	18	18	1	1
Compd.	$R_f \times 100$							
Gramicidin				94				
Kanamycin					17			
Neomycin A		61	54		10			
Neomycin B		21	24					33
Neomycin C		43	45					33
Netropsin						39		

Ref. →	(74)	(74)	(74)	(74)	(74)	(74)	(74)	(74)	(74)	(74)	(74)	(80)	(81)	(82)	(82)	(82)	(83)	(83)	(94)	(91)
Solvent[a] →	*B*	*C*	*D*	*E*	*F*	*G*	*H*	*I*	*J*	*K*	*L*	*Q*	*R*	*S*	*T*	*U*	*V*	*AF*	*AG*	*S*
Adsorbent →	1	1	1	1	1	1	1	1	1	1	1	1	19[b]	10	10	10	18	18	17	10
Compd.	$R_f \times 100$																			
Tetracycline	36	38	60	61	50	50	23	50	67	68	64		X	23	26	38				36
Epitetracycline																				36
Anhydrotetracycline	10	45	64	68	50	50	23	53	69	68	64								100	50
4 Epianhydrotet.																			60	40
Chlortetracycline	30	43	64	72	60	58	24	50	69	67	65		X	30	35	49				
Anhydrochlorotet.	10	46	65	75	52	52	19	55	70	70	65									
Oxytetracycline	58	41	62	70	55	60	23	52	68	69	65		X	27	31	46				
Tylosin																		83		
Tyrocidin												35								
Viomycin																	21			
Zyomycin																	15			

Ref. →	(74)	(78)	(78)	(80)	(83)	(83)	(92)	(90)
Solvent[a] →	*A*	*P*	*P*	*Q*	*V*	*W*	*AE*	*AC*
Adsorbent →	1	22	21	1	18	18	1	1
Compd.	$R_f \times 100$							
Acetyloleandomycin							20	
Diacetyloleandomycin							50	
Triacetyloleandomycin							70	
Paromomycin					15			
6-Aminopenicillinic acid	09							

Ref. →	(74)	(75)	(75)	(77)	(83)	(83)	(87)	(92)	(92)
Solvent[a] →	*A*	*M*	*N*	*O*	*V*	W	*AA*	*AE*	*AF*
Adsorbent →	1	1	1	7	18	18	1	1	1
Compd.	$R_f \times 100$								
Pen. G K		16		67					
Pen. G NA									75
Pen. V acid	23			71					
Pen. V K		68	64						
Pen. P K		62	58						
Procaine pen.				66					
DBED pen.				68					
Me pen. V K		72	68						
Streptomycin					44	31			
Dihydrostreptomycin					44	31			
Hydroxystreptomycin					32	23			
Dihydrost. phenyl hyd.							92		

[a] *A*, BuOH buffered to 4.6; *B*, citric acid 10%; *C*, BuOH–MeOH–citric acid, 10% (40:10:2); *D*, BuOH–MeOH–citric acid 10% (40:15:20); *E*, BuOH–MeOH–citric acid 10% (40:20:20); *F*, BuOH–MeOH–citric acid 10% (40:30:20); *G*, BuOH–MeOH–citric acid 10% (40:40:20); *H*, citric acid 10% saturated with BuOH + 5% MeOH; *I*, citric acid 10% saturated with BuOH + 10% MeOH; *J*, citric acid 10% saturated with BuOH + 15% MeOH; *K*, citric acid 10% saturated with BuOH + 20% MeOH; *L*, citric acid 10% saturated with BuOH + 25% MeOH; *M*, acetone–MeOH (50:80); *N*, isopropanol–MeOH (30:70); *O*, butyl acetate–*n*-BuOH–AcOH–PO_4 buffer–MeOH (80:5:40:24:5) penicillin hydrol. 0.5 hr. in MeOH–HCl at 50°; *P*, 0.5 *N* H_2SO_4; *Q*, BuOH–AcOH–H_2O (10:1:3); *R*, $CHCl_3$–Me_2CO (1:1); *S*, *n*-BuOH saturated with H_2O circular TLC; *T*, *n*-BuOH–tartaric acid–water (100:6:100) circular TLC; *U*, *n*-BuOH–oxalic acid–water (100:5 Gm.:100) circular TLC; *V*, *n*-PrOH–pyridine–AcOH–H_2O (15:10:3:12); *W*, BuOH + 2% *p*-toluene sulfonic acid; *Z*, methylene chloride–MeOH–benzeneformamide (80:20:20:3); *AA*, BuOH–H_2O–MeOH (40:20:10) + 1 Gm. *p*-toluene sulfonic acid; *AB*, BuOH–AcOH–H_2O (4:1:5); *AE*, $CHCl_3$–MeOH–toluene (80:17:23); *AF*, MeOH–acetone (60:40); *AG*, EDTA 0.1 *M* – 0.1% NH_4Cl pH 4.5, then two-dimensional in $CHCl_3$ saturated with above solution. [b] Adjusted to pH 3.7 with PO_4 buffer.

Experimental details were given for preparation of the TLC plates of Silica Gel G with buffers pH 6.85, 4.5, and 2.2, and the agar inoculation with *Sarcina lutea* for rifomycin, *Bacillus subtilis* for tetracycline, and *Staphylococcus aureus* for penicillin. After cooling, another layer of agar was applied to protect the tetrazolium from the air. Preliminary chilling before incubation allowed the antibiotics to diffuse through the agar. They found that sensitivity of TLC was 10 times greater than paper chromatography, with detection limits of 0.1 mcg. chemically and 0.001 mcg. microbiologically. Fischer (75) used iodine–azide solution for detecting 1–2 mcg. including a white and a yellow spot for some penicillins. Only the single component R_f values are reported in Table IX.

Nussbaumer (76) studied the effect of 40 tablet excipients on the identification of penicillins and found most of them were removed by TLC. A penicillin V spot (77) was removed for measurement at 276 mμ after separation, on silica gel plus rice starch, from phenoxyacetic acid and 20 possible tablet excipients.

Brodasky (78) used acid and neutral carbon black for TLC of neomycin A, B, and C followed by agar diffusion inoculated with *Bacillus pumilus*. Bromophenol blue spray 15 min. after 5% $KMnO_4$ was found by Akita (79) to lower the detection limit for antibiotics over $KMnO_4$ alone. The acid oxidation products produce blue or greenish colors which lasted for 1 month. Tyrocidin and gramicidin (80) were observed on Silica Gel G by U.V. and reaction with *o*-tolidine in AcOH after treatment with Cl_2. Sprays of $SbCl_3$ 50% in AcOH and H_2SO_4, and fluorescence under 350 mμ intensified by NH_4OH or NaOH identified three tetracyclines for Sonanini (81) on regular and circular TLC. Kapadia (82) also used circular TLC for the same compounds. EDTA was incorporated into Silica Gel G to prevent iron from forming complexes with the tetracyclines. A regular TLC plate was inverted over a Petri dish containing the development solvent. A roll of filter paper served as the wick. Ferric chloride and fluorescence were the means of visualization.

Nine basic water-soluble antibiotics were separated on MN cellulose by Ito (83) with oxidized nitroprusside and ninhydrin used for visualization. Actinomycins were separated by Cassani (84) into C and F groups (Table IX). Other systems isolated C_1, C_2, C_3, F, and F_3 portions. Erythromycins were detected on Silica Gel G with phosphomolybdic acid by Anderson (85) and 0.05 mcg. of erythromycin by *Streptococcus lactis* on agar using a reprint from 0.5 mm. Al_2O_3 by Meyers (86). Preliminary treatment of streptomycin and dihydrostreptomycin with phenylhydrazine allowed the separation of these two antibiotics (87). Streptomycin yields 2 spots and dihydrostreptomycin one spot following the reaction and subsequent TLC. The spots of these two antibiotics were removed (88), centrifuged with PO_4 buffer pH 7.9, and the supernatant used for microbiological assay with *B. subtilis*. The procedure was recommended for the control of injectables of streptomycin. The degradation of bacitracin was followed by TLC by Nussbaumer (89) with the spots detected by U.V. and ninhydrin. The microbiological inactivity of the new spot confirmed the technique. Other quantitative TLC work by Foppiano (90) on neomycin was based on its ribose moiety determined with orcinol–ferric chloride–AcOH reagent. Assays after TLC invariably were lower (0.5 to 13%) than before chromatography. The variation of the TLC method was no greater than an official method. Circular TLC with silica gel and disodium EDTA of degradation products in tetracycline using NH_3 and U.V. for detection was reported by Rustici (91).

Dragendorff's reagent detected 2 mcg. spots after TLC of mono-, di-, and triacetyl derivatives of oleandomycin by Gantes (92). Kline (93) used a paint sprayer to coat silica gel and kieselguhr TLC plates with agar inoculated with *B. subtilis* or *S. lutea*. Sensitivities were 0.1 mcg. for streptomycin, 0.25 mcg. for tylosin and erythromycin, and 0.005 units for penicillin.

ANTIHISTAMINICS

Antihistamine compounds have been considered along with tranquilizers by Cochin (95). The phenothiazine derivatives respond to reagents described later under *Psychotropic Drugs*. In a quantititative study, Morrison (96) used ceric sulfate (5%) as a reagent and measured the area of the spot by comparison to a plastic sheet imprinted with circles and ellipses of known area. A formula was used for calculating the concentration of unknown drugs with accuracies for concentrations of 25–50 mcg. of about 3%. Thirty replicates on six different capsule preparations gave recoveries from 94.3–104.5% of label with relative standard deviations 5.8 to 11.4%. Fike (97) used three solvent systems and two spray reagents (Dragendorff and 1% solution ammonium vanadate in H_2SO_4) to identify all but six of 30 antihistamines.

TABLE X—TLC OF ANTIHISTAMINIC AND RELATED COMPOUNDS

Ref. →	(95)	(95)	(95)	(95)	(96)	(67)	(97)	(97)	(42)	(42)	(42)	(42)	(42)
Solvent[a] →	A	B	C	D	E	F	G	G	H	G	I	G	J
Adsorbent →	1	1	1	13	1	1	2	4	2	2	2	4	4
Compd.	$R_f \times 100$												
Antazoline	31	72	40	38		08	11	61					
Bromodiphenylhydramine	86	80	33	63		50	37	48	08	11	06	61	38
Brompheniramine	42	47	16	48									
Buclizine									73	72	84	75	64
Carbinoxamine	33	40	17	63		29	21	05					
Chlorcyclizine	90	68	40	49		49	44	43	49	44	30	43	19
Chlorothen	70	56	29	66		43	37	15	43	37	28	15	02
Chlorpheniramine	40	41	20	52	27	38	19	08	38	19	06	08	01
Clemizole						33	67	47	33	67	61	47	27
Covatin						58	54	52					
Cyclizine						55	46	41	55	46	27	41	16
Diphenhydramine	91	61	25	53	55	52	37	45	52	37	26	45	25
Diphenylpyraline						42	25	44	42	25	11	44	23
Doxylamine	52	38	17	60									
Hydroxyzine	27	70	62	84		08	59	56	08	59	39	56	25
Meclizine						69	71	74	69	71	84	74	60
Methaphenilene						55	46	44	55	46	40	44	24
Methapyrilene	83	52	30	56	38	47	36	14					
Phenindamine	90	67	48	65	35	55	53	41	55	53	35	41	25
Pheniramine	68	38	19	63		40	08	06	40	18	05	06	01
Phenyltoloxamine						46	51	42					
Pyrilamine	82	43	27	56	35	42	33	12	42	33	24	12	01
Pyrrobutamine	84	82	32	78		62	39	59	62	39	34	59	40
Thenyldiamine						47	32	12	47	32	25	12	01
Thenylpyramine									47	36	27	14	02
Thonzylamine	65	57	32	59		41	38	29	41	38	27	29	12
Tripelennamine	92	48	23	62		50	35	12	50	35	27	12	03
Triprolidine	40	48	26	81		41	40	17	41	45	13	18	02

[a] *A*, benzene–dioxane–aq. NH_3 (60:35:5); *B*, EtOH–AcOH–water (50:30:20); *C*, MeOH–BuOH (60:40); *D*, BuOH–butyl ether–AcOH (40:80:10); *E*, AcOH–water (20:80); *F*, cyclohexane–benzene–diethylamine (75:15:10); *G*, MeOH; *H*, cyclohexane–benzene–Et_2NH (75:15:10); *I*, acetone; *J*, 95% ethanol.

TABLE XI—TLC OF BARBITURATES

Ref. →	(98)	(26)	(105)	(98)	(98)	(102)	(103)	(105)	(105)	(31)	(31)	(107)	(107)	(107)
Solvents[a] →	A	A	A	B	C	D	E	F	G	H	I	J	K	L
Adsorbent →	1	1	1	1	1	1	1	1	1	2	2	1	20	20
Compd.	$R_f \times 100$													
Allylbarbituric acid		48								54	71			
Amobarbital	60	48	38	42	52	61	74	40	55			58		
Barbital	50	33	24	38	40	48		34	45	40	67	31	13	
Butabarbital	62	42	32	49	52	58		37	50			53		
Cyclobarbital	68		32	55	38	51		36	50			30	46	
Diallylbarbituric acid	55			43	48	62	0					34	24	
Hexobarbital	77	68	46	49	58	61		41	54			77		
Itobarbital	67			48	50									
Mephobarbital	98	79		85	60							64		90
Pentobarbital	57	47	36	40	49	64	40	40	55	61	73	66		50
Phenobarbital	50	33	25	36	26	46	51	27	49	29	64	20		
Secobarbital	64	55	41	46	54	75	29	39	59			63		43

[a] *A*, $CHCl_3$–acetone (9:1); *B*, benzene–AcOH (9:1); *C*, dioxane–benzene–aq. NH_3 (20:75:5); *D*, diisopropyl ether–$CHCl_3$ (1:1); *E*, acetone–*n*-butyl alcohol–NH_3 (9:9:2), preliminary treatment of sample with H_2SO_4; *F*, benzin–dioxane (5:2) DMF stationary phase; *G*, benzol–ether (1:1); *H*, isopropanol–NH_3 25%–$CHCl_3$ (45:10:45); *I*, $CHCl_3$–MeOH (9:1); *J*, ethyl acetate–hexane–NH_4OH (20:9:10); *K*, $CHCl_3$–CCl_4 (2:1); *L*, $CHCl_3$–CCl_4 (1:1).

BARBITURATES

The analyses of the barbiturates and related compounds has received exhaustive and often repetitive attention from many authors. Most of the work was designed for the identification of barbiturates in blood, urine, and tissue samples [Cochin (98), Walker (99), Bogan (100), Eberhardt (101), Shellard (102), Petzold (103)]. Stolman's book (26) is an excellent starting reference for separation on Silica Gel G using the most popular system of $CHCl_3$–acetone (9:1). (Designated *A* in Table XI.) Pretreatment of the barbiturates by H_2SO_4 on the plate by Petzold (103) allowed separation of five common (U.S.) drugs. The same technique was suggested later by Walker (99) following earlier use of this on paper chromatography. Qualitative analyses in pharmaceuticals have received less attention. Phenobarbital was identified in a suppository

containing amidopyrine and quinine by Kraus (104) using benzene–96% ethanol (37:3) on silica gel–$CaSO_4$ (4:1) and other barbiturates by Shellard (102) in galenicals and tablets using diisopropyl ether–$CHCl_3$ (1:1) on Silica Gel G. Sahli (105) was able to separate 15 compounds using three systems, *A*, *F*, *G*, in Table XI. Using a system similar to *H*, Table XI, Kiger (106) differentiated 23 barbiturates on silica gel.

Many detection reagents are available. Mercurous salts gave grey, black, and white colors [Sahli (105), Kraus (104), Stolman (26)]. Cobalt nitrate, then NH_3 vapor, formed a deep blue color (102). Different colors of fluorescence were recorded (101) at 254 and 366 mμ with and without $HgNO_3$. Silver acetate–diphenylcarbazone gave purple colors (103). Permanganate was used to differentiate unsaturated substituents (98) by the yellow colors produced.

CARDIAC GLYCOSIDES AND CARDENOLIDES

The section on cardiac glycosides by Waldi in Stahl's book (1) described the evolution of the work on TLC of the cardiac glycosides. Stahl (108) was able to separate most of these compounds on silica gel. Chloramine–trichloroacetic acid reagent and U.V. were used for visualization.

Mixtures of scillarin and digitoxin were resolved on silica gel and identified by Steinegger and van der Walt (109) with $SbCl_3$. Reichelt (110), using silica gel without binder inactivated with water or acetic acid, observed separation of many of the cardenolides in water saturated by the reagent benzene–alcohol (3:1) reported by Stahl (108).

Duncan (111) was able to postulate possible success of scale up from TLC to column chromatography by the R_f found on material from *Pachycarpus concolor*. Bobbitt (2) discussed in detail Duncan's formula of

$$r = \frac{a}{b + 0.1a}$$

where a and b are the R_f values of the fast and slow moving substance, and pointed out that even though r is greater than 1, good column separation may not be obtained.

Faucennet (112) reported a greater sensitivity on TLC than paper chromatography for cardenolides of digitalis with H_3PO_4–Br_2 reagent. Sonanini (113) chose previously mentioned reagents for observing several compounds separated on kieselguhr impregnated with formamide. Momose (114) introduced 1,3,5-trinitrobenzene 0.1% in DMF–5% aqueous Na_2CO_3 as a reagent to produce orange-red spots with oleandrin, digitoxin, digoxin, and ouabain.

Commercial K-strophanthin analyses by Lukas (115) revealed four components on silica gel with a BuOH–MeOH–formamide (17:2:1) system.

Corona (116) identified five components of K-strophanthin on Silica Gel G and observed another unidentified spot using $CHCl_3$–AcOH–MeOH (85:2:13) solvent.

CENTRAL STIMULANTS

Many reports of other central stimulants such as the sympathomimetic amines are included in review articles previously discussed. Stolman's book (26) shows tables taken from literature for several of these compounds.

Noirfalise's (117) comprehensive paper on central stimulants tabulated responses of many of these compounds to U.V., $KMnO_4$, Dragendorff, iodoplatinate, PDAB, $HClO_4$–KNO_3–$FeCl_3$, and furfural–H_2SO_4. Beckett (118) found multiple spots formed when the amines were treated with trichloroacetic acid in the solvent system used but not with the same system without the acid on cellulose powder (Table XII). Some of the primary amines and their *N*-methyl derivatives are included in Fike's (42) discussion on correlation of structure to R_f. The identification by Troup (119) of the degradation products of phenylephrine in tablet formulations containing aspirin were accomplished by TLC and detection with diazotized *p*-nitroaniline.

Catecholamines and their metabolites were assayed quantitatively following TLC by de Potter (120). Fluorimetric measurement of the eluted spots indicated recoveries of 97–100% for norepinephrine, epinephrine, and metanephrine.

CRUDE DRUGS AND EXTRACTS

The use of TLC in pharmacognosy is a field of its own. Analysts in this work have taken advantage of the simplicity and utility of TLC for the identification of tinctures, fluid extracts, and powdered extracts. It is used routinely in the authors' laboratory for the identification of about 40 such preparations (121). A partial summary of the literature is shown in Table XIII.

DIURETICS

Margasinski (131) applied TLC before and after hydrolysis of benzothiadiazine derivatives to obtain separation on Al_2O_3 with visualization by PDAB. Without the buffer in the ethyl acetate solvent, Adam (132) separated most of these and other compounds of the same series and observed them under U.V. at 250 mμ. (Table XIV.)

TABLE XII—TLC OF CENTRAL STIMULANTS

Ref. →	(118)	(119)	(120)	(117)	(117)	(117)	(42)	(42)	(42)	(42)	(42)
Solvent[a] →	A	B	C	D	E	F	G	H	I	H	J
Adsorbent →	17	1	18	1	1	1	2	2	2	4	4
Compd.	$R_f \times 100$										
Amphetamine	75			38	30	11	34	28	33	59	53
Adrenaline											
Benzphetamine							79	70	85	54	49
Cyclopentamine							52	10	02	52	40
Dextroamphetamine				39	30	11					
Ephedrine	74						08	18	02	54	42
Epinephrine	55						01	07	11	62	40
Hydroxyamphetamine							03	29	36	59	21
Histamine	27										
Iproniazid				46	62	69	04	64	34	34	22
Isocarbazid				74	69	81	28	71	70	65	61
Isophenaline	60										
Methamphetamine							46	18	04	50	39
Metanephrine			X								
Nialamide				21	54	62	0	55	09	35	23
Norepinephrine	47		X				02	21	15	66	50
Normetanephrine			X								
Phenylephrine		X					05	21	33	60	45
Phenylethylamine	72										
Phenylpropanolamine							09	35	50	58	56
Propylhexedrine							54	15	03	54	46

[a] *A*, *n*-BuOH–AcOH–H_2O (4:1:5); *B*, $CHCl_3$–AcOH–H_2O (5:1:1); *C*, *n*-BuOH saturated with 3 *N* HCl; *D*, acetone–NH_3 25% (99:1); *E*, MeOH–NH_3 25% (99:1); *F*, $CHCl_3$–MeOH (50:50); *G*, cyclohexane–benzene–Et_2NH (75:15:10); *H*, MeOH; *I*, acetone; *J*, 95% EtOH.

TABLE XIII—IDENTIFICATION OF CRUDE DRUGS OR ADULTERANTS BY TLC

Recent Lit. Drug	Ref.
Review	(122)
Aloe	(123, 124)
Althaea	(125)
Datura stramonium	(125)
Hyoscyamus niger	(125)
Digitalis lanta	(125)
Cassia angustifolia	(125)
Umbelliferen roots	(126)
Pimpinella soxifraga	(127)
Coptis japonica	(128)
Senna	(129)
Volatile oils	(130)

TABLE XIV—TLC OF DIURETICS

Ref. →	(131)	(131)	(132)	(132)
Solvent[a] →	A	A	B	C
Adsorbent →	13	13[b]	13	13
Compd.	$R_f \times 100$			
Chlorothiazide	10	87	X	
Hydrochlorothiazide	94	90	X	X
Trichloromethiazide			X	X
Benzothiazide	62	54		
Thiabutazide			X	
Polythiazide	100	98	X	
Methylclothiazide			X	
Hydroflumethiazide			X	X
Bendroflumethiazide			X	
Cyclopentathiazide	100	86		

[a] *A*, ethyl acetate, pH 6.7–7.2, with NH_4OH; *B*, ethyl acetate; *C*, ethyl acetate–C_6H_6 (80:20). [b] After hydrolyses.

DYES, EXCIPIENTS, FLAVORS, AND PRESERVATIVES

Although TLC offers a very fast method for separation of dyes, the comprehensive summary of paper chromatography of dyes (133) should be consulted before attacking a complicated dye mixture problem with TLC.

Gasparic (134) listed 87 dyes separated on Al_2O_3 with benzene, and Synodinos (135) separated 7 FD&C dyes on $CaCO_3$ with BuOH–EtOH–water (2:1:1) + 10% NH_3. Stahl's book (1) offers a guide to other systems.

The excipients in pharmaceuticals pose the greatest problems and are the least mentioned in the literature. Those working in stability assays of creams, ointments, and suppositories have experienced many frustrations by the obscuration of good TLC by the smearing action of PEG's, surfactants, flavors, and preservatives, particularly when attempting to observe minor amounts of degradation products. There are a few publications which may offer guidance in these types of problems. Mannitol and sorbitol were separated using isopropanol–0.1 *N* boric acid (85:15) (136). Breithurd (137) dissolved PEG's in methylene chloride. The 2% solutions were applied to silica gel plates and developed with acetone–NH_4OH 20% (85:15).

Vakhtina (138) used alumina with $CHCl_3$–EtOH (98:2) for glycols with molecular weights up to 2000. Thoma (139) found that $CHCl_3$–MeOH–H_2O (3:25:12) would separate the stearates of PEG. Hartsaw (140) studied several excipients with some common systems and general means of visualization (Table XV).

TABLE XV—TLC OF EXCIPIENTS

Ref. →	(140)										Visualization			
Solvent[a] →	*A*	*B*	*C*	*D*	*E*	*F*	*G*	*H*	*I*	*J*	*d*	*e*	*f*	*g*
Adsorbent →	1	1	1	1	1	1	1	1	1	1				
Compd.	$R_f \times 100$													
Polyethylene glycol										49	−	+	−	+
Cetyl alcohol	71	70							02		+	+	−	+
Citric acid				04		04					−	+	+	+
Cocoa butter	77	81	82	81	91	93		73	73	80	−	+	+	+
Cream of tartar				04		04				02	−	−	+	+
Ethylcellulose	80	71									−	+	−	+
Ethyl *p*-hydroxybenzoate	61	65	68	25	54	74	76	07	06	66	+	+	+	+
Glucose										14	−	+	+	+
Glycerin	0		07	02	02	25	79	0		33	−	−	+	+
Heavy mineral oil										78	−	−	+	+
Lactose				04		04				05	−	+	+	+
Lt. paraffin oil	77	77									−	+	−	+
Mannitol										18	−	+	+	+
Paraffin hard		79									−	+	−	−
Polyethylene glycol 200	01	07	07	0	14	20		07	14[c]		−	−	−	+
Polyoxyl 40 stearate										54	−	+	+	−
Sorbitan sesquioleate	79	66	75	32	61	89		0	0	77	−	+	+	+
	64[b]	83	82	60	88									
Spermaceti	79	81	82	81	88				73	77	−	+	−	−
Stearic acid		93			63						−	+	−	−
Sucrose				04		04				09	−	−	+	+
White petrolatum	80	80	82	81	88				73	77	−	+	−	−
White wax	80	82	82		88				73	77	−	+	−	−
Magnesium stearate										50	−	−	+	+
Agar[b]											−	+	−	+
$CaSO_4$[b]											−	+	−	−
Cellulose[b]											−	−	−	−
Citrus pectin[b]											−	+	+	+
Gelatin soft[b]											−	+	+	+
Kaolin[b]											−	+	−	−
Acacia powder[b]											−	+	−	+
Methylcellulose[b]											−	+	+	+
Silicon dioxide[b]											−	+	−	−
Polyvinyl pyrrolidine[b]											−	+	+	+
Starch powder[b]											−	+	−	+
Talc											−	−	−	−

[a] *A*, cyclohexane–acetone (40:50); *B*, $CHCl_3$–EtOH (9:1); *C*, $CHCl_3$–EtOH–heptane (1:1:1); *D*, cyclohexane–$CHCl_2$–AcOH (40:50:10); *E*, MeOH–benzene–AcOH (8:45:4); *F*, ethyl acetate–MeOH–AcOH (80:10:10); *G*, MeOH–acetone–triethanolamine (50:50:1.5); *H*, cyclohexane–acetone–Et_2NH (70:20:10); *I*, $CHCl_3$–Et_2NH (9:1); *J*, BuOH–5 *N* NH_4OH–MeOH (60:20:20). [b] Where no R_f value listed, the compound remained at point of application or it gave no reaction to reagents. [c] Two zones formed. [d] U.V. [e] I_2. [f] $KMnO_4$. [g] H_2SO_4 sprayed over $KMnO_4$.

For the partial resolution of nine preservatives Copius-Peereboom (141) used cellulose plates with a 20 cm. solvent travel. (Table XVI.) By using two developments on silica gel, Gossele (142) was able to separate these preservatives on a 20 × 20-cm. plate with temperature control below 22°. All of the compounds were observed under 366 mμ with a fluorescent indicator present, but other color reagents were used for additional differentiation.

Pinson (143) identified and determined four of the same compounds in codeine syrups and other syrups containing carboxymethylcellulose.

Antioxidants in vitamin A were detected after TLC by phosphomolybdic acid and ammonia vapor (144). Attaway (145) studied 60 flavor esters on silica gel using benzene or trifluorotrichloroethane–methylene chloride solvents and 5% vanillin in 96% H_2SO_4 reagent.

TABLE XVI—TLC OF PRESERVATIVES

Ref. →	(141)	(142)	(144)	(144)	(143)
Solvent[a] →	*A*	*B*	*C*	*D*	*E*
Adsorbent →	17	8	1	23	8
Compd.	$R_f \times 100$				
Benzoic acid	50	82			X
Sorbic acid	58	76			X
Salicylic acid	56	69			
Dehydroacetic acid	09	62			
Bromoacetic acid		43			
Propyl-*p*-HO-benzoate	90	30			X
Ethyl-*p*-HO-benzoate	86	25			
Methyl *p*-HO-benzoate	75	20			
p-HO-Benzoic acid	09	11			
o-Phenylphenol	95				
Hydroxyanisole			31		
Butyl hydroxytoluene			79		
Isoamyl gallate				61	
Nordihydroguaiaretic acid				45	

[a] *A*, BuOH–NH_3 35%–water (70:20:10); *B*, pet. ether–$CHCl_3$–formic acid (10:4:1); *C*, $CHCl_3$; *D*, $CHCl_3$ one dimension, then MeOH second dimension; *E*, *n*-pentane–AcOH (88:12).

TABLE XVII—TLC OF LOCAL ANESTHETICS

Ref. →	(42)	(42)	(42)	(42)	(42)	(146)	(146)	(146)	(146)	(146)	(146)	(146)	(146)	(146)	(146)	(147)
Solvent[a] →	A	B	C	B	D	E	F	G	H	I	J	K	L	M	N	O	P
Adsorbent →	2	2	2	4	4	13	13	13	13	13	13	13	13	13	13	13	1
Compd.	Rf × 100																
Benzocaine	06	68	73	63	57	18	25	48			53	52	62	70	75	31	
Butacaine	08	63	71	59	51												
2-Chloroprocaine	03	53	52	46	26												
Cinchocaine						10	19	46	65	75	42	51	47	75	61	20	
Cocaine	58	57	64	26	10	17	34	65	75	82	57	67	60	80	70	30	X
Cyclomethycaine	66	45	42	46	29												
Dibucaine																	X
Diocaine						10	17	55	67	80	35	51	52	81	80	24	
Ethylaminobenzoate																	X
β-Eucaine						14	16	28	53	72	41	44	45	51	30	21	
Hexylcaine																	X
Hostocaine						10	17	26	42	68	27					05	X
Lidocaine	39	70	69	47	23	10	17	50	56	78	52	62	56	81	65	22	X
Larocaine						12	18	37	54	70	40	50	45	79	68	24	
Mesocaine						05	16	35	57	74	50	64	55	80	62	23	
Meprylcaine																	X
Onocaine																	X
Phenacaine	12	63	76	61	55	06	24	52	60	77	37	37	55	77	70	27	
Piperocaine	63	45	42	47	29												
Procaine	05	52	47	39	18	0	14	31	52	65	27	34	43	60	54	11	X
Proparacaine																	X
Psicaine						08	18	46	65	78	55	64	57	78	63	25	
Tetracaine	18	50	28	38	14	08	15	40	63	72	40	43	56	59	58	20	X
Tropacocaine																	X
Tutocaine						10	16	43	62	71	45	41	57	60	55	22	

[a] *A*, cyclohexane–benzene–Et_2NH (75:15:10); *B*, MeOH; *C*, acetone; *D*, 95% EtOH; *E*, benzene; *F*, benzene–EtOH (98:2); *G*, benzene–EtOH (95:5); *H*, benzene–EtOH (90:10); *I*, benzene–EtOH (80:20); *J*, $CHCl_3$; *K*, $CHCl_3$–EtOH (99:1); *L*, $CHCl_3$–BuOH (98:2); *M*, $CHCl_3$–acetone (1:1); *N*, ether; *O*, ether–pet. ether (1:1); *P*, benzene–acetone–NH_4OH (80:20:1).

LOCAL ANESTHETICS

A major contribution in the TLC of local anesthetics was made by Sarsunova (146). He recommended specific systems for pairs of compounds, including local anesthetics in a large group of drugs. Iodine and Dragendorff were effective reagents for these compounds (Table XVII). Fuwa (147), in a series of drug analyses by TLC, included 14 local anesthetics along with a study of hypnotics and anthelmintics. Other compounds were separated in BuOH–AcOH–H_2O (5:1:4) on silica gel (148).

ORAL HYPOGLYCEMIC DRUGS

In a series of papers on drug analyses Reisch (149) reported on the resolution of carbutamide, chlorpropamide, and tolbutamide with visualization by ninhydrin spray. Neidlein's (150) work included hypoglycemic compounds with other sulfonamides.

Strickland (151) studied the above compounds plus acetohexamide and phenformin HCl in six solvent systems. The R_f values cited in Table XVIII for his work (151) are the average of 50 measurements.

PSYCHOTROPIC DRUGS

In an early report on chromatography by paper, electrophoresis, and thin layer of phenothiazine drugs, Mellinger (152) found silica gel thin layer "by far, the best procedure for any phenothiazine drug." A comprehensive study of solvent systems was reported on 23 compounds (Table XIX). Fluorescence under 263 mμ gave a sensitive means of detection, but in the presence of acids, decomposition was evident. Light protected tanks were used for chromatography in acid systems. Various colors were obtained with 40% H_2SO_4. Addition of ferric salts or PDAB to the H_2SO_4 or a palladium chloride reagent gave no additional differentiation. Paulus (153) also observed a better color differentiation with H_2SO_4–EtOH 10% than with Dragendorff, $KMnO_4$, H_2O_2, and KNO_3 solutions. Margasinski (154) added iodoplatinate, H_2SO_4 16% + 1 ml. formalin to the list of color reagents. Adank (155) pointed out the utility of analyses of trace contaminants by TLC.

TABLE XVIII—TLC OF ORAL HYPOGLYCEMIC AGENTS

Ref. →	(149)	(150)	(151)	(151)	(151)	(151)	(151)
Solvent[a] →	A	B	C	D	E	F	G
Adsorbent →	1	6	1	1	1	1	1
Compd.	Rf × 100						
Acetohexamide			52	67	49	50	42
Carbutamide	X	45					
Chlorpropamide	X		59	70	53	52	35
Phenformin HCl			03	52	24	32	03
Tolbutamide	X	54	75	76	65	66	33

[a] *A*, BuOH–$CHCl_3$–Et_2NH (7:7:1); *B*, BuOH–$CHCl_3$–acetone–Et_2NH (9:1:1:1); *C*, acetone–benzene–water (65:30:5); *D*, acetone–BuOH–water (20:50:30); *E*, BuOH saturated with water; *F*, BuOH–formamide–water (50:10:50) upper phase; *G*, dioxane–NH_3 0.88 sp. gr.–water (100:3:10).

TABLE XIX—TLC OF PSYCHOTROPIC DRUGS

Ref. →	(152)	(152)	(152)	(152)	(152)	(152)	(153)	(153)	(164)	(164)	(154)	(154)	(154)
Solvent[a] →	*A*	*B*	*C*	*D*	*E*	*F*	*G*	*H*	*I*	*J*	*K*	*L*	*M*
Adsorbent →	1	1	1	1	1	1	1	1	1	1	1	1	1
Compd.	$R_f \times 100$												
Acetophenazine	12	36	04		18	38							
Acetylpromazine													
Alimemazine													
Amitriptyline									X	X			
Aminopromazine													
Butyrylperazine	08	31	06	28	10	45							
Chlordiazepoxide								64					
Chlorpromazine	37	44	28	14	23	66	05	100	X	X	21	21	12
Chlorpromazine sulfoxide	05	10	01	09	05	27							
Chlorprothixine	34	78		20	36	88	69	100	X	X			
Dimethoxinate													
Dixyrazine													
Ethopropazine													
Fluphenazine	37	56	09	68	27	57							
Imipramine	45	55	24	13	16	66	47	100	X	X			
Isothypendyl	26	39	24	15	11	61							
Levomepromazine													
Mepazine	29	46	13	13	13	62							
Mepazine sulfoxide													
Meprobamate													
Methdilazine													
Methoxypromazine	15	26	12	12	09	45							
Methotrimeprazine													
Methylpromazine													
Nortriptyline													
Perphenazine	28	57	07	48	24	53					14	14	19
Pipamazine	38	71		41	37	79							
Prochlorpemazine													
Prochlorperazine	10	31	06	24	08	55							
Prochlorperazine sulfoxide													
Proketazine	19	45	06	53	25	44							
Promazine	16	31	12	11	11	50	37	92	X	X	21	21	12
Promazine sulfoxide													
Promethazine											12	23	18
Promethazine sulfoxide													
Prothipendyl	16	21	11	11	08	44							
Thiazinamine													
Thioridazine	24	39	14	24	15	64	45	100					
Thioridazine sulfoxide	03	13	01	09	03	33							
Thiopropazate													
Thiopropazine	64	79	49	65	53	81							
Thiethylperazine	14	50		23	13	61							
Triflupromazine	36	50	48	22	22	72					14	30	17
Triflupromazine sulfoxide													
Trimeprazine													
Trifluoperazine	18	33	09	34	12	51							
Trifluoperazine sulfoxide	03	11	02	17	03	31							
Trimeprazine													

[a] *A*, *tert*–BuOH–1 *N* NH_3 (90:10); *B*, *n*-PrOH–1 *N* NH_3 (88:12); *C*, ether saturated with water; *D*, 70% MeOH; *E*, 85% *n*-PrOH; *F*, *n*-BuOH saturated with 1 *N* NH_3; *G*, benzene–acetone–NH_3 25% (50:10:5); *H*, benzene–EtOH–NH_3 25% (50:10:5); *I*, BuOH–AcOH–H_2O (88:5:7); *J*, BuOH–AcOH–H_2O (65:15:20); *K*, acetone–ethyl acetate–EtOH 95% (5:4:1) saturated with NH_4 lactate pH 3; *L*, acetone–ethyl acetate–EtOH 95% (5:4:1) saturated with NH_4 lactate pH 7; *M*, acetone–

Continued ⟶

Imipramine HCl[1] was found to contain eight impurities with a composite total of less than 0.2%. Pharmacological evidence was obtained to show that the impurities would not cause unfavorable side effects at the low concentration present.

Cochin (95) in the development of methods for phenothiazine in body fluids preferred H_2SO_4 for color differentiation on 26 compounds including the sulfoxide metabolic products. Noirfalise (156) tried cellulose as an adsorbent and used I_2 vapor visualization in addition to others listed.

[1]Marketed as Tofranil by Geigy Pharmaceuticals, Ardsley, N. Y.

Rusiecki (157) found the same problems with decomposition during exposure to light. With proper protection TLC was recommended for quality control of ampuls and suppositories for possible degradation products.

Heyndrickx (158) separated meprobamate from sulfathiazole for subsequent colorimetric assay. Hynie (159) used benzidine–KI reagent for meprobamate and metabolites in urine. Rusiecki (160) extracted chlorpromazine from decomposition products (157) and eluted TLC plates with MeOH for assay at 268 mμ. Noirfalise (161) continued

TABLE XIX—(*Continued.*)

Ref. →	(156)	(95)	(95)	(95)	(165)	(165)	(165)	(158)	(166)	(161)	(161)	(161)	(161)	(42)	(42)	(42)	(42)	(42)
Solvent[a] →	*N*	*O*	*P*	*Q*	*R*	*R*	*R*	*S*	*T*	*U*	*V*	*W*	*X*	*Y*	*Z*	*AA*	*Z*	*AB*
Adsorbent →	17	1	1	1	1[b]	1[c]	1	1	1	1	1	1	1	2	2	2	4	4
Compd.	$R_f \times 100$																	
Acetophenazine														03	51	01	15	05
Acetylpromazine						65	54											
Alimemazine										39	64	64	39					
Amitriptyline		74	65	37										72	50	34	41	28
Aminopromazine	41									14	41	41	14					
Butyrylperazine																		
Chlordiazepoxide																		
Chlorpromazine	23	94	70	30		83	75			37	49	49	37	57	44	37	44	26
Chlorpromazine sulfoxide		26	47	19	86													
Chlorprothixine		80	73	46														
Dimethoxinate														24	31	13	33	13
Dixyrazine										61	39	39	61					
Ethopropazine														68	62	82	40	25
Fluphenazine	03	34	58	68		43	27			59	33	33	59	06	60	25	15	06
Imipramine		86	77	29										61	35	18	39	25
Isothypendyl														51	47	32	36	18
Levomepromazine						90	86			42	64	64	42					
Mepazine		88	57	29										55	49	37	43	21
Mepazine sulfoxide		16	59	24	74													
Meprobamate								75										
Methdilazine														44	29	14	39	18
Methoxypromazine														46	37	26	40	19
Methotrimeprazine														56	56	65	46	23
Methylpromazine														53	39	28	42	23
Nortriptyline										15								
Perphenazine														06	57	20	12	04
Pipamazine		12	72	56								43	34	0	60	32	48	27
Prochlorpemazine										44	23							
Prochlorperazine	13	70	27	32		43	27			59	33	33	59	46	41	14	07	02
Prochlorperazine sulfoxide		13	28	13	88													
Proketazine														04	57	17	12	04
Promazine		62	38	37								18	33	50	36	25	39	20
Promazine sulfoxide		17	46	15	70													
Promethazine	54	70	59	22		77	67			44	44	44	44	46	47	37	45	23
Promethazine sulfoxide		22	57	30	78													
Prothipendyl																		
Thiazinamine	64									03	03	03	03					
Thioridazine		97	65	20						32	43	43	32	52	45	31	41	27
Thioridazine sulfoxide																		
Thiopropazate		97	67	70														
Thiopropazine		69	33	40										44	66	67	30	11
Thiethylperazine														44	47	14	08	02
Triflupromazine		95	79	40			85	77						57	52	48	48	31
Triflupromazine sulfoxide		26	48	24	93													
Trimeprazine		96	64	30														
Trifluoperazine														45	49	19	10	02
Trifluoperazine sulfoxide																		
Trimeprazine														64	55	62	44	22

ethyl acetate–EtOH 95% (5:4:1) saturated with NH_4 lactate pH 9; *N*, 5% $(NH_4)_2SO_4$ saturated with iso-BuOH; *O*, benzene–dioxane–NH_3 (60:35:5); *P*, ethanol–AcOH–H_2O (50:30:20); *Q*, MeOH–BuOH (60:40); *R*, benzene–dioxane–NH_3 (10:80:10); *S*, $CHCl_3$–acetone (4:1); *T*, MeOH–$CHCl_3$ (1:2); *U*, acetone–MeOH (1:1); *V*, acetone–NH_3 (1:1); *W*, acetone–NH_3 (99:1); *X*, $CHCl_3$–MeOH (1:1); *Y*, cyclohexane–benzene–Et_2NH (75:15:10); *Z*, MeOH; *AA*, acetone; *AB*, EtOH 95%. [b] 30°. [c] 10°.

the series of papers on phenothiazine compounds and showed tables for color reaction with various compounds.

Reaction of psychotropic drugs on TLC with bromine and bromine + aniline were recorded by Clarke (162). Additional R_f values for phenothiazines on silica gel with a $CHCl_3$–EtOH (70:30) system were given by Moza (163).

STEROIDS

The section on steroids by Waldi in Stahl's book (1) followed by a series (13 pages) by Neher in Marini-Bettolo's book (22) on TLC of steroids and by Cavina and Vicari in Marini-Bettolo's book (22) on the qualitative and quantitative analyses of natural and synthetic corticosteroids by TLC (14 pages) covered the literature through 1962–1963. These authors all gave excellent details on techniques, reagents, and listings for specific separation. Heftmann's (167) article on TLC of steroids in chromatographic reviews (13 pages, 180 references) again surveyed earlier work and extended the review

through mid 1964. Stevens (168) covered a similar period (4 pages) with a more selective bibliography. Tschesche wrote a chapter (13 pages) in another book (169) on the same subject.

To bring the literature up to date from 1964 through 1965 is beyond the scope of this review. For example, there were at least 20 publications on the TLC of estrogens and androgens in urine.

Some publications (1964–1966) have been selected for the TLC of pure steroid compounds. They are listed in Table XX. A mass of R_f values and correlations with structure are available in these publications. Many of the means of visualization and solvent systems are listed in these references. Some articles on assay of steroids in pharmaceuticals during the same period are mentioned in the subsequent paragraphs.

Bican-Fister (180) applied a chloroform dilution of oil solutions of progesterone and testosterone to Silica Gel HF254. Development in cyclohexane–ether (8:2) and removal of U.V. absorbing spots permitted the quantitative assay of these compounds by the INH reaction with 98% recovery and relative standard deviation of about 3.6%. Cavina (181), with added sophistication, used continuous elution with petroleum ether–ethyl ether–AcOH (70:30:1 or 50:50:1) to separate testosterone propionate, progesterone, 19-nortestosterone propionate, and estradiol cyclopentylpropionate from oily solutions. The steroid spots were eluted with $CHCl_3$, evaporated, and dissolved in EtOH for measurement in U.V. from 225–250 mμ or by INH reaction. Recoveries of 95–99.4% were obtained. The U.V. method was found to be more precise than the INH reagent. Bennett (182) successfully separated some closely related steroids by an improved continuous development technique on 50 × 200 × 0.25-mm. Silica Gel G plates.

TABLE XX—TLC OF STEROIDS

Subject	Recent Lit. Ref.
Δ^4-3-Oxo-C_{21}-steroids (37 compd.) (means of visualization with 28 reagents)	(170)
Δ^4-3-Oxo steroids of androstane series (36 compd.)	(171)
Δ^5-3-Hydroxy-C_{19}-steroids (16 compd.)	(172)
Δ^4-3-Ketosteroids of pregnane series (37 compd.)	(173)
Saturated 21-deoxypregnane steroids (55 compd.)	(174)
Corticosteroids (11 compd.)	(175)
Corticosteroids (28 compd.)	(176)
Adrenal corticosteroids (26 compd.)	(177)
3-β-Hydroxy-Δ^5-steroids (13 compd. and 6 estrogens)	(178)
Δ^5-3-β-Hydroxysteroids (8 compd.)	(179)

Methyltestosterone and three related compounds were extracted from tablets and capsules with acetone and from liquids with $CHCl_3$ for subsequent TLC by Castren (183). After development in benzene–ethyl acetate (7:3), the methyltestosterone 2,4-dinitrophenylhydrazone was determined colorimetrically with accuracy of ± 5%.

Jacobsohn (184) photographed the H_2SO_4 color reaction zones of TLC plates of estrone, estradiol, and estriol, irradiated by U.V. The densities of spots were measured in a Photovolt densitometer and quantitative relationships of densities and concentrations were established.

Jensen (185) used TLC to study the stability of steroid ophthalmic solutions. $CHCl_3$ extracts of the solutions were evaporated and applied to Silica Gel G plates. Fluprednisolone (R_f 0.12) and the acetate (R_f 0.50) zones were removed and determined by blue tetrazolium to within ±2%. Comer (186) compared the results of the tetrazolium determination and a TLC scintillation method for measurement of the stability of ^{14}C-labeled fluorandrenolone cream. Jakovljevic (187) used TLC to verify a column chromatographic method for the same steroid. Tishler (188) separated the reaction products of the determination of methandrostenolone with MeOH–HCl solution and was able to postulate reaction mechanisms.

Schultz (189) applied TLC on silica gel, using (90:10) benzene–ether for development solvent, for the determination of 17α-ethynylestradiol-3-methyl ether (R_f 0.32) in the presence of 19-nortestosterone and 6-chloro-6-dehydro-17α-acetoxyprogesterone in tablets. The steroid zone was removed and measured at 278 mμ with accuracy of 95.5–98.5%.

Various colors formed initially, finally, and the colors in U.V. (366 mμ) with 50% H_2SO_4 spray and then heat at 78° were recorded (190) for 141 compounds.

SULFONAMIDES

Wollish (8) used the separation of five sulfonamides as examples in a review of advances in TLC in 1961. Klein (191) and Kho (192) used two-step systems for separation with diazotization for detection of N^1-substituted and bromocresol purple for the N^4-substituted sulfonamides. Bican-Fister (193) used alkaline coupling with betanaphthol after diazotization to detect 0.25 mcg. amounts after separation and the Bratton-Marshall reagent for quantitation after elution. In another study by Bican-Fister (194) the quantitative data showed a relative standard devia-

TABLE XXI—TLC OF SULFONAMIDES

Ref. →	(191)	(192)	(8)	(198)	(198)	(198)	(198)	(198)	(198)	(198)	(198)	(198)	(198)	(198)	(198)	(198)
Solvent[a] →	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
Adsorbent →	1	1	1	13	13	13	13	13	13	13	13	13	13	13	13	13
Compd.	$R_f \times 100$															
Sulfacarbamide				16	33	66	71	41	34	32	29	19	13	37	45	63
Sulfacetamide	42	X		05	20	26	20	24	17	09	07	05	03	17	40	56
Sulfadiazine	47		40	09	18	08	03	35	50	38	33	31	23	25		
Sulfadimerazine																
Sulfadimethoxine																
Sulfadimidine				34	58	77	52	73	60	60	58	55	36	35	50	78
Sulfaethidol																
Sulfamerazine	57															
Sulfamethazine	64															
Sulfamethizole																
Sulfamethoxin				20	31	37	33	57	56	48	42	50	38	40		
Sulfamethoxydiazine																
Sulfamethoxypyrazine																
Sulfamethoxypyridazine				22		54	55	76	69	53	48	40	28	21	50	71
Sulfamethylpyrimidine																
Sulfanilamide	53	X		16	38	66	71	41	34	30	27	19	15	40	42	63
Sulfaguanidine					01	05	05	18	12	08	08	08	05	05	12	
Sulfaquinoxaline	66															
Sulfapyridine																
Sulfathiazole	50	X		08	22	03	05	34	25	12	10	06	05	06	38	55
Succinyl sulfacetamide		37														
Succinyl sulfathiazole		43		08	21	02	03	34	34	12	10	06	04	06	38	55
Succinyl sulfanilamide		49														
Phthalylsulfacetamide		51														
Phthalylsulfathiazole		61		08	08	04	07	34	34	12	10	34	04	05	38	55
Phthalylsulfanilamide		66														
Sulfisoxazole[b]			70													
Acetyl sulfisoxazole[c]			90													
Sulfadimethoxine[d]			80													
Sulfanilic acid			02													

Ref. →	(193)	(201)	(199)	(199)	(199)	(199)	(197)	(193)	(195)	(196)	(202)
Solvent[a] →	Q	R	S	T	U	V	W	X	Y	Z	AA
Adsorbent →	1	13	1	1	1	1	1	1	1	1	1
Compd.	$R_f \times 100$										
Sulfacarbamide											
Sulfacetamide	38	X					31		27		47
Sulfadiazine	50		48	42	87	35	39	53		61	65
Sulfadimerazine										72	
Sulfadimethoxine							67		47		72
Sulfadimidine			52	47	82	35					76
Sulfaethidol		X									
Sulfamerazine	59		45	45	87	45	44	60	35	67	
Sulfamethazine	64						52	72			
Sulfamethizole							25				41
Sulfamethoxin											
Sulfamethoxydiazine											
Sulfamethoxypyrazine							70				
Sulfamethoxypyridazine	68						61	38	40		
Sulfamethylpyrimidine							46				
Sulfanilamide	36		67	65		60	43	61	29	37	52
Sulfaguanidine	16	X	65	64	82	30	15	04	06		
Sulfaquinoxaline											
Sulfapyridine							37				69
Sulfathiazole	38	X	59	55	84	42	41	14	17	43	60
Succinyl sulfacetamide											
Succinyl sulfathiazole			61	58		41					
Succinyl sulfanilamide											
Phthalylsulfacetamide											
Phthalylsulfathiazole			63	61		45					
Phthalylsulfanilamide											
Sulfisoxazole[b]							51	51			
Acetyl sulfisoxazole[c]								81			
Sulfadimethoxine[d]											
Sulfanilic acid											

[a] *A*, $CHCl_3$–EtOH–heptane (1:1:1) plus 1.2% H_2O; *B*, MeOH–EtOH for 5 cm. (1:1) then *n*-propanol–.05 *N* HCl (4:1) for 5 cm.; *C*, $CHCl_3$–EtOH–heptane (1:1:1); *D*, benzene–EtOH (9:1); *E*, benzene–EtOH (8:2); *F*, ether–MeOH (9:1); *G*, ether–EtOH (9:1); *H*, $CHCl_3$–MeOH (80:15); *I*, $CHCl_3$–EtOH (80:15); *J*, $CHCl_3$–*n*-propanol (80:15); *K*, $CHCl_3$–isopropanol (80:15); *L*, $CHCl_3$–*n*-BuOH (80:15); *M*, $CHCl_3$–amyl aclohol (80:15); *N*, $CHCl_3$–acetone (1:1); *O*, $CHCl_3$–AcOH (95:5); *P*, $CHCl_3$–AcOH (90:10); *Q*, $CHCl_3$–MeOH (100:10); *R*, benzene–AcOH–MeOH (8.5:1:0.5); *S*, NH_3 30%–*sec*-BuOH–isopropanol–water (1.5:3.5:4.1); *T*, NH_3 30%–*sec*-BuOH–isopropanol–water (1.5:4:4:0.5); *U*, diethylamine (abs.)–isopropanol–water (1:5:4); *V*, diethylamine (abs.)–*sec*-BuOH–isopropanol–water (1.5:4:4:0.5); *W*, $CHCl_3$–BuOH–pet. ether (1:1:1); *X*, ether; *Y*, benzene–BuOH–pyridine (30:5:5); *Z*, $CHCl_3$–MeOH–$HCONMe_2$ (100:10:5); *AA*, $CHCl_3$–MeOH–water (160:40:2.5). [b] Marketed as Gantrisin by Roche Laboratories, Nutley, N. J. [c] Marketed as Acetyl Gantrisin by Roche Laboratories. [d] Marketed as Madribon by Roche Laboratories.

tion of about 4%. Tablets were extracted with 70% EtOH–NH_3 and the extract applied in three 3-μl. portions to Silica Gel G. Suppositories were extracted with petroleum ether–water–NH_3 and the aqueous layer applied to Silica Gel G. Suspensions were shaken with EtOH–NH_3 and centrifuged. The solution was applied to the TLC plates as in tablets. Pastor (195,196) separated and identified 11 sulfonamides.

Karpitschka (197) found better separation when EtOH in the Wollish system was replaced by butanol and a wedged-shaped adsorbent used.

Sarsunova (198) screened 13 systems for several components and made a table for the most useful systems for separation of specific pairs of sulfonamides. They used $CHCl_3$–EtOH (100:8) for separation of sulfathiazole, sulfadimidine, and sulfacetamide. For quantitative assay the tablets were extracted with acetone–ethanol and applied to Al_2O_3. After separation, the spots were located with I_2 vapor and removed with a vacuum filter, and the sulfonamides measured at 270 and 290 mμ. The relative standard deviations of 2.5%, 4.1%, and 3.2% were obtained for the three components with six replicates.

There have been so many solvent systems tried (see Table XXI) that it is difficult to find a new one. Even so, publications are still appearing with new combinations and color reagents. Other means of detection are 2% vanillin in acetic acid (199), coupling with dimethyl α-naphthylamine after nitrous acid (196) and 3% ninhydrin in BuOH (200). Other studies on TLC of sulfonamides include those of Poethke (201), Wehrli (202), Lin (203), and Fogg (204).

VITAMINS

A convenient starting point for a survey on TLC of vitamins is the chapter on vitamins in Stahl's book (1). Recent reviews by Katsui (205–207) on techniques, plates, adsorbents, solvent systems, and spray reagents, followed by reviews on fat-soluble and water-soluble vitamins, brought the literature up to date in 1964. This review will attempt to cover some of the work since that time.

Castren (208) was able to separate vitamins A, D, and E in multivitamin preparations after alkaline hydrolysis on silica gel. Oxidation products of vitamin A appeared on TLC of old preparations. Quantitative analyses of vitamins A and E by the linear relationship of area of spots, observed by U.V. or phosphomolybdic acid, to concentration were possible. Various components of vitamin A were observed on alumina (209) by fluorescence in U.V. Varma (210) studied the separation of vitamin A and related compounds in six systems and used one of these systems (*F* in Table XXII) for nonsaponifiable fractions of a liquid multivitamin sample to identify anhydrovitamin A, retrovitamin A alcohol, and vitamin A alcohol. John (211) observed the same compounds after TLC under U.V. and listed blue, green, or yellow colors formed with $SbCl_3$. Silica Gel G treated with PEG 200 was used (212) for the separation of vitamin A alcohol, D_2,D_3, and β-tocopherol.

David (213) used cellulose and other adsorbents and several solvent systems other than *O* in Table XXIII for TLC of thiamine and nitrates. A $Fe(CN)_3$ reagent was used for visualization.

Vorobéva (214) was able to separate vitamin B_{12}, factor B, and factor III on silica gel contain-

TABLE XXII—TLC OF A VITAMINS

Ref. →	(208)	(226)	(210)	(210)	(210)	(210)	(210)	(210)	(210)	(226)	(211)	(211)	(211)	(212)
Solvents[a] →	*A*	*B*	*C*	*D*	*E*	*F*	*G*	*H*	*I*	*B*	*K*	*U*	*M*	*N*
Adsorbent →	1	1	13	13	13	13	13	13	13	13	1	1	1	15
Compd.	$R_f \times 100$													
Vit. A	X	X								X				
Anhydr. vit. A			63	90	97						93	82	82	
β-Carotene			06	80							100	100	100	
Retro-vit. A_1 acetate			0	36	90						70	47	49	
Vit. A_1 acetate				19	88									
Retro-vit. A_1 alcohol					12	36	42				20	08	08	32
Vit. A_1 alcohol					06	16	28	48						
Vit. A_1 epoxide					03	12		32						
Vit. A_2 alcohol					0	08	26	28	58					
Vit. A_1 acid						0		0	05		0	0	0	
Vit. A_1 palmitate											91	76	79	
Anhydro vit. A_2											87	69	61	

[a] *A*, benzene–ethyl acetate (7:3); *B*, $CHCl_3$; *C*, cyclohexane; *D*, cyclohexane–benzene (95:5); *E*, cyclohexane–MeOH (.25%); *F*, cyclohexane–MeOH (99:1); *G*, cyclohexane–MeOH (97:3); *H*, cyclohexane–EtOH (97:3); *I*, cyclohexane–EtOH (92:8); *K*, pet. ether–acetone (94:6); *M*, isooctane–acetone (97:3); *N*, pet. ether–benzene (1:1); *U*, hexane–acetone–MeOH (135:15:3).

ing gypsum and NaCN. Combustion of the organic material in a furnace allowed the detection of cobalt with β-nitroso-α-naphthol. The amount of cobalt could be determined from the eluted spots at 367 and 420 mμ.

Using basic alumina, Popova (215) separated additional analogs with a detection limit of 0.5 mcg. apparently based on the color of the compounds. The procedure was applicable to the control of the purity of concentrates of commercial preparations. The same authors (216) obtained improved separations on neutral alumina using NH_4OH to form cobalichromes.

Sasaki (217) found that cellulose MN300 gave good resolution of cyanocobalamin, hydroxycobalamin, and three coenzymes.

Chen (218) showed that fluorescent dyes sprayed on TLC plates of tuna oil gave less sensitivity to vitamin D_2, D_3, ergosterol, and 7-dehydrocholesterol than heating with H_2SO_4, but permitted a degree of qualitative differentiation. Pasalis (219) incorporated $AgNO_3$ and rhodamine into silica gel to separate some saturated and unsaturated esters of D_2 and D_3. Parekh (220) used TLC for the purification of ^{3}H-vitamin D_3 until all the U.V. fluorescent by-products were removed (Table XXIV). A quantitative assay of vitamin D in a pharmaceutical preparation was made possible by Heaysman (221) with wedges removed from the side of silica gel plates to form an elongated area for sample application and formation of concentric arcs following solvent development. Removal of the area containing vitamin D and subsequent rechromatography produced zones measured by the area of color found with H_2SO_4. The range found from eight tablet (800 units) assays was 700–920 units. The system of cyclohexane–ether (4:1) did not differentiate vitamins D_2 and D_3.

The TLC of tocopherols has been studied extensively and reported in recent publications. Skinner (222, 223) tabulated melting points, R_f, and spray reagents for many phenolic compounds related to tocopherols and their oxidation products and Sturm (224) published a quantitative determination of individual tocopherols. Peanut oils from various sources were assayed spectrophotometrically with bathophenanthroline–$FeCl_3$ after saponification and TLC. The average recovery of α-tocopherol was 93.2% ± standard deviation of 6.85%. Schmandke (225) obtained separation of α, γ, and δ-tocopherol on Al_2O_3–$ZnCO_3$ (3:1) plate with $CHCl_3$ as the solvent.

TABLE XXIII—TLC OF B VITAMINS

Ref. →	(208)	(226)	(213)	(214)	(215)	(216)	(217)
Solvents[a] →	*A*	*J*	*O*	*P*	*Q*	*R*	*S*
Adsorbent →	1	13	1	1	14	15	18
Compd.	$R_f \times 100$						
Vit. B_1		X	80				
Vit. B_1 PO_4			47				
Vit. B_1 $DiPO_4$			30				
Vit. B_1 Tri-PO_4			01				
Vit. B_6		X					
Nicotinamide		X					
Cyanocobalamin				X	62	46	X
Factor B				X	74		
Pseudo vit. B_{12}					25		
Factor B_{12}					46		
Factor V (nB)					12		
Factor A					37		
Hydroxycobalamin						30	X

[a] *A*, benzene–ethyl acetate (7:3); *J*, acetic acid–acetone–MeOH–benzene (5:5:20:70); *O*, *n*-propanol–PO_4 buffer pH 4.9–H_2O (60:20:20); *P*, water saturated with *sec*-BuOH; *Q*, BuOH–isopropanol–water (1:1:1); *R*, BuOH–isopropanol–water (1.5:1:1.25) NH_4OH to pH 8.5; *S*, *sec*-BuOH–0.1 *M* acetate buffer pH 3.5–MeOH (4:12:1).

QUANTITATIVE TLC

Many references were made to quantitative TLC methods under the various drug classes. Some other articles of special significance to pharmaceutical analysts are listed in Table XXV. The lack of precision and accuracy of the di-

TABLE XXIV—TLC OF D VITAMINS AND TOCOPHEROLS

Ref. →	(208)	(226)	(212)	(218)	(220)	(219)	(217)	(217)	(217)	(217)
Solvents[a] →	*A*	*B*	*N*	*T*	*U*	*V*	*B*	*X*	*B*	*B*
Adsorbent →	1	13	15	1	1	9	1	1	16	12
Compd.	$R_f \times 100$									
Vit. D	X	X								
Vit. D_2			47	32						
Vit. D_3			47	32	58					
7-Dehydrocholesterol				18	46					
Ergosterol				18						
D_2 stearate						79				
D_2 oleate						57				
D_2 linolenate						30				
α-Tocopherol	X	X	58				29	49	72	83
β-Tocopherol							20	33		
γ-Tocopherol							20	33	42	80
δ-Tocopherol							12	22	31	67

[a] *A*, benzene–ethyl acetate (7:3); *B*, $CHCl_3$; *N*, pet. ether–benzene (1:1); *T*, dichloromethane; *U* hexane–acetone–MeOH (135:15:3); *V*, hexane–benzene (1:2); *X*, pet. ether–ether (5:1).

TABLE XXV—QUANTITATIVE TLC

Subject	Ref.	Direct Measurement	Elution Measurement
Bile acids	(227)		U.V.
Cinchona alkaloids	(228)	Area by planimeter	
Cholesterol	(229)		Colorimetric
Coal tar	(230)	Spectrofluorometric	
Dyes	(231)	Spectral reflectance	
Furoic acid	(232)		U.V., I.R., GLPC
2-Nitro-4-acetamidophenetol	(233)		Polarographic
Noradrenaline	(234)	Area	Colorimetric
Opium alkaloids	(235)		U.V.
Phospholipids	(236)	Cellophane tape, then densitometer	
Review	(237)	Review	Review
Review	(238)	Wt. area relationship	
Selected pharmaceuticals	(239)	Area by Densicord model 542	
Senna glycoside	(240)		U.V.
Sorbic acid	(241)		Colorimetric
Steroids	(242)	Fluorescence recording photometer	
Technique	(243)	Reflectance spectroscopy	
Technique	(244)	Reflectance spectroscopy	
Technique	(245)	Fluorescent scanning photometer	
Technique	(246)	Scanning photometer	
Technique	(247)	Photometric color and fluorescence	
Technique	(248)		I.R.
Technique	(249)	Area	
Technique	(250)	Plate made transparent, then microphotometer	
Tropane alkaloids	(228)	Area by planimeter	

TABLE XXVI—RADIO-TRACER WITH TLC METHODS

Subject	Ref.	Direct Measurement	Elution Measurement
Benzoic acid-^{14}C	(251)	Methane flow counter	
Lipids-^{14}C,-^{3}H	(252)		Scintillation counter
Prochlorperazine	(253)		Scintillation counter
Sodium iodide-^{131}I	(254)	Radiochromatogram scanner	
Tritium-labeled compd.	(255)	Autoradiography	
Steroid cream-^{14}C	(186)		Scintillation counter

rect measurement instrumentation or the tedious nature of removal of adsorbent containing the desired compounds are the major disadvantages of quantitative TLC. This is probably the technology in TLC that will receive the most advances in the next few years. Table XXVI lists references on the application of radioactive tracers and TLC for analyses. This combination offers some exciting possibilities in the development of pharmaceutical assays, especially in the evaluation of stability.

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