TABLE 3 Some Solvent Systems for TLC of Amino Acids on Silica Gel

Solvent System	Ratio	References
Silica gel:		
96% Ethanol-water	7:3	35
n-Propanol-water	7:3	
n-Butanol-acetic acid-water	4:1:1	
n-Propanol-34% NH_4OH	7:3	
n-Propanol water	1:1	58
Phenol-water	3:1	
Isopropanol-water	7:3	59
Butyl acetate-methanol-acetic acid-pyridine	20:20:5:5	25
n-Butanol-formic acid-ethanol	3:1:1	24
n-Butanol-acetic acid-chloroform	3:1:1	22
n-BuOH-HOAc-EtOAc-H_2O	50:20:30:20	60
Cellulose[a]:		
Propan-2-ol-butanone-1 M HCl	60:15:25	61
2-Methylpropan-2-ol-butanone-acetone-methanol-H_2O-conc. NH_3	20:1:14:5	
Butanol-acetic acid-H_2O	4:1:5	63
Methanol-H_2O-pyridine	20:5:1	
Propan-1-ol-8.8% NH_3	4:1	
Chloroform-MeOH-17% NH_3	20:20:9	40
Butanol-acetone-Et_2NH-H_2O	10:10:2:5	
Phenol-water	3:1	
Ethyl acetate-pyridine-HOAc-H_2O	5:5:1:3	64
n-Butanol-acetic acid-H_2O-EtOH	10:1:3:0.3 or 4:1:10:1	65

[a]For good separation, used in pairs for two-dimensional chromatography.

TABLE 4 Some Systems for Two-Dimensional TLC

I direction	II direction	References
Silica gel:		
n-Butanol-HOAc-H_2O (4:1:5, v/v, upper phase)	Phenol-water (15:1, w/w)	66
Chloroform-MeOH-17% NH_3 (2:2:1)	Phenol-H_2O (3:1)	67
n-Butanol-HOAc-H_2O (4:1:5, upper phase)	$CHCl_3$-MeOH-17% NH_3(2:2:1)	
Butanone-pyridine-H_2O-HOAc (70:15:15:2)	$CHCl_3$-MeOH-17% NH_3(2:2:1)	35
Cellulose:		
Propanol-HCOOH-H_2O (40:2:10)	*t*-Butanol-methyl ethyl ketone-0.88 NH_3-H_2O (50:30:10:10, v/v)	68

TABLE 4 (continued)

Propan-2-ol-butan-2-1 M HCl (60:15:25 by vol.)	2-Methyl butanol-butan-2-one acetone-MeOH-H_2O-(0.88) NH_3 (10:4:2:1:3:1) or	
	2-Methylpropanol-butanone-propanone-methanol-H_2O (40:20:2:1:14:5, v/v)	61

TABLE 5 hR_f ($R_f \times 100$) Values for Amino Acids on Different Layers

					E		
	A	B	C	D	FX_A	FX_B	FX_C
Ala	41.9	29.0	32.4	28.8	50.9	51.2	53.6
Ser	26.9	16.1	26.4	24.1	67.1	64.1	67.1
Tyr	50.0	36.1	49.4	45.9	11.9	13.9	15.5
Glu	34.4	22.6	30.0	28.2	34.5	29.4	30.6
Asp	26.3	14.8	25.3	21.8	71.5	68.2	68.6
Arg	25.6	11.0	12.9	10.0	1.8	2.2	2.2
Gly	29.4	14.8	25.9	23.5	55.6	52.4	53.6
Leu	75.0	63.9	51.8	48.8	21.8	17.8	19.4
Ile	73.1	60.0	49.4	47.1	27.8	22.2	23.3
Try	55.6	36.1	54.1	51.8	1.8	2.2	2.2
Met	41.0	22.5	47.3	43.5	28.0	27.2	25.0
Val	63.1	48.4	43.5	41.2	42.5	35.0	34.4
Lys	18.1	7.1	10.0	7.1	7.5	5.0	5.6
His	20.0	7.1	11.7	7.1	10.6	8.9	10.0
Phe	67.5	54.8	52.4	50.0	14.4	11.1	11.7
Thr	32.5	21.3	30.0	27.6	67.1	60.0	57.2
Cys	6.9	3.2	14.1	7.1	55.9	50.0	57.9
Pro	43.8	33.5	24.1	21.2	—	—	—
Time for 17 cm, h	7	11	4.5	7.5	6.5	6	2

A, Baker Flex cellulose sheets; B, Baker Flex microcrystalline cellulose sheets; C, Whatman K6 silica gel plates; D, Whatman high-performance silica gel plates; E, Fixion ion-exchange sheets (Na^+ form). FX_A, no prior treatment; FX_B, layer preequilibrated with equilibration buffer for 16 h; FX_C, layer preequilibrated as for FX_B but at 45°C. Solvent for A, B, C, D, 2-butanol-acetic acid-water (3:1:1); solvent for E and run buffer, 84 g citric acid + 16 g NaOH + 5.8 g NaCl + 54 g ethylene glycol + 4 ml conc. HCl (pH 3.3); solvent equilibration buffer, run buffer diluted 30 times (pH 3.8).

Source: From Ref. 69.

TABLE 2 hR_f Values of Peptides from L-Amino Acids after One-Dimensional TLC on Cellulose

Peptides	Solvent systems				
	A	B	C	D	E
Ala-Ala	65	26	55	68	58
Ala-Asp	56	1	45	44	19
Ala-Glu	64	5	58	56	29
Ala-Gly	50	17	36	46	46
Ala-Phe	94	52	86	84	85
Ala-Ser	52	17	36	41	49
Gly-Ala	52	18	37	43	46
Gly-Asp	43	0	30	29	13
Gly-Gly	34	13	22	29	34
Gly-His	7	16	5	16	32
Gly-Ile	81	49	65	80	75
Gly-Leu	82	51	67	87	80
Gly-Lys	14	11	2	21	27
Gly-Phe	75	50	65	76	67
Gly-Pro	47	17	39	45	44
Gly-Ser	32	13	22	28	26
Gly-Tyr	68	28	45	64	51
Gly-Val	72	36	56	73	62
Leu-Ala	97	56	88	90	89
Leu-Gly	82	52	67	84	83
Leu-Val	100	77	98	96	95
Val-Gly	67	38	56	70	69
Val-Leu	100	80	98	100	95
Ala-Gly-Gly	47	13	34	39	43
Glu-Cys-Gly	16	0	7	7	0
Gly-Gly-Gly	32	8	20	31	30
Val-Gly-Gly	65	28	52	65	61

Solvent systems: A, 2-Propanol-butanone-1N HCl (60:15:25); B, 2-methyl butan-2-ol-butanone-propanone-methanol-water-ammonia (10:4:2:1:3:1); C, 2-propanol-water (3:1); D, 2-propanol-water-acetic acid (15:4:1); E, 2-propanol-water-ammonia (15:4:1).

Source: From Ref. 34.

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Pharmaceuticals and Drugs

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I. INTRODUCTION

Chromatography is utilized extensively in the pharmaceutical industry as a separation tool for qualitative and quantitative analysis. Thin-layer chromatography (TLC), though not as popular as high-performance liquid chromatography (HPLC) or gas chromatography (GC), does claim a share of the market of chromatographic techniques. A literature search of the last 20 years indicates that extensive reviews on TLC systems for pharmaceuticals and drugs have been published (1-5).

Traditional TLC is inexpensive, simple to use, and requires minimal instrumentation, laboratory space, and maintenance. Like most analytical techniques, use of reference standards and controls is essential. Quantitation with current TLC technology is accurate, precise, and reliable (6-9). With the advances made in technology, resolution, sensitivity, reproducibility, and performance are further enhanced with high-performance thin-layer chromatography (HPTLC) (10). Detection is often universal, resulting in mass balance of the analytes. Sensitivity, however, is dependent on the analytes and the detection method. In general, subjective evaluation is needed, and interpretation should be made by trained personnel.

The purpose of this chapter is to provide guidelines for the preparation and analysis of the more popular pharmaceuticals and illicit drugs. Numerous examples are provided for qualitative and quantitative thin-layer chromatographic analysis of pharmaceutical drugs in various dosage forms.

II. APPLICATIONS

Government agencies have stringent requirements for testing of drugs for approval. Hence the monitoring of the stability of drugs upon storage (11-13) and under stress (14-16) is of concern. In addition, determination of the bulk drug purity and impurities profile (9,17-19) is of interest to the pharmaceutical manufacturer as well as to the official drug control laboratories. Maximum limits for undesirable components are set by the authorities of each country.

In toxicology, measurement of drugs in urine, plasma, or gastric fluid for drugs of abuse (20,21) and overdosed medicinal drugs (22) is of great interest to the physician. The misuse of drugs resulting in a patient's confinement to a hospital emergency room requires a systematic approach to identify and confirm the drugs used. Numerous techniques including TLC are available (23,24) to the hospital laboratory. Similarly, for forensic science, illicit drug detection is

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TABLE 1 Approximate Duration of Detectability of Selected Drugs in Urine

Drug	Approximate duration of detectability[a]	Limits of sensitivity of analytic techniques (μmole/liter)
Amphetamine	48 h	0.5 μg/ml (4)
Methamphetamine	48 h	0.5 μg/ml (3)
Barbiturates		
Short-acting	24 h	
Hexobarbital		1.0 μg/ml (4)
Pentobarbital		0.5 μg/ml (2)
Secobarbital		0.5 μg/ml (2)
Thiamylal		1.0 μg/ml (4)
Intermediate-acting	48-72 h	
Amobarbital		1.0 μg/ml (4)
Aprobarbital		1.5 μg/ml (7)
Butabarbital		0.5 μg/ml (2)
Butalbital		1.5 μg/ml (7)
Long-acting	≥7 d	
Barbital		5.0 μg/ml (27)
Phenobarbital		1.0 μg/ml (4)
Benzodiazepines	3 d[b]	1.0 μg/ml (3)
Cocaine metabolites	2-3 d	
Benzoylecgonine		0.5 μg/ml (2)
Ecgonine methyl ester		1.0 μg/ml (3)
Methadone	≈3 d	0.5 μg/ml (1)
1,5-dimethyl-3,3-diphenyl-2-ethylidene pyrrolidine (metabolite of methadone)		0.5 μg/ml (1)
Codeine	48 h	0.5 μg/ml (2)
Morphine		1.0 μg/ml (4)
Propoxyphene	6-48 h	0.5 μg/ml (1)
Norpropoxyphene		1.5 μg/ml (4)
Cannabinoids	3 d,[e] 5 d,[d] 10 d,[e]	
(11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid)	21-27 d[f]	20 ng/ml (65)
Methaqualone	≥7 d	1.0 μg/ml (4)
Phencyclidine	≈8 d	0.5 μg/ml (2)

[a]Interpretation of the duration of detectability must take into account many variables, such as drug metabolism and half-life, subject's physical condition, fluid balance and state of hydration, and route and frequency of ingestion. These are general guidelines only.

[b]Using therapeutic dosages.

[c]Single use.

[d]Moderate smoker (4 times/wk).

[e]Heavy smoker (smoking daily).

[f]Chronic heavy smoker.

Source: Reprinted from *J. Am. Med. Assoc., 257:* 3110 (1987), by courtesy of the Council on Scientific Affairs, American Medical Association.

helpful in drug traffic control (25) and in sports (26), horse racing (27), and employment screening (28). The rapid and accurate identification of the raw unprocessed material or final product is one facet that contributes to the information data base. Great importance is placed on ensuring the accuracy of the results. Proper documentation of specimens, good control incorporated into the evaluation, and availability of test data and security at every step of the testing contribute to a reliable program. TLC plays a useful role when cost effectiveness is essential. The narcotics laboratory is vital in countries with an illicit drug problem. The actual equipment required by each laboratory depends on the types of samples expected: analyses of drugs in natural products for the drug-producing countries, bulk drug materials or final products for the drug-consuming countries, and drugs in biological fluids for the drug-abusing countries (29,30).

Testing is achieved at two levels: a screening test and a confirmatory test which frequently uses a different technique. Screening or identification tests are designed for maximum sensitivity at the expense of specificity. In other words, screening evaluation should use methods that yield few or no false negative results. Tests for confirmation are, in general, more specific, so as to separate the true positive from the false positive (31).

An important concern in drug testing is the duration of detectability of drugs in urine following the last use. This depends on the sensitivity of the techniques used as well as on the properties of the drugs. Representative drugs are examined in Table 1 for their approximate duration of detectability at the sensitivity limits of the technique employed.

III. GUIDELINES FOR ANALYSIS

A. Sample Preparation

The ideal sample preparation procedure is to dissolve the dosage form with complete recovery of intact compound(s) of interest, and minimum yield of excipients in the solvent(s), with the appropriate concentration that can be directly spotted on the TLC plate. Naturally, not all formulations, even without considering the analytes, fall into such a category.

The general factors to consider during sample preparation are listed in Table 2. Extraneous components in dosage forms can be reduced or removed during sample preparation (32-37), or on the plate as two-phase, two-dimensional chromatography (38), or by use of a preadsorbent strip (39). Besides maximizing the analyte(s) yield in a suitable solvent, the stability of the analyte(s) during the time of analysis should also be considered. Trace amounts of metals that may be present in the silica coating of the thin-layer plate can catalyze autoxidation. In general, prewashing and activating the TLC plate is a good practice. Fabre and co-workers (10) have shown that the use of citric acid solution can chelate any iron present. To extend the storage life of the sample solution during the time of analysis, the mode(s) of potential degradation should be identified. Elimination of the cause, if possible—e.g., aqueous solvent for an active drug that hydrolyzes—should be the first choice. Adjusting the pH of the sample solution (40) and addition of preservative, e.g., butylated hydroxytoluene (BHT), to the solvent system or to the sample solution to reduce oxidation (38) have also been attempted.

Pharmaceutical dosage forms can be classified into various categories (40,41) as described in Table 3. For most formulations on the market, drug amount is not a problem. For the exceptional cases, selection of the solvent for dissolving the analytes as well as detection method become important. Here, one could consider concentration methods, e.g., evaporation, adsorbent column, or enhanced sensitivity of detection.

Though the foregoing discussion has been geared to the analysis of drugs in bulk materials and pharmaceutical dosage forms, similar concepts apply to the analysis of drugs in biological fluids. The objective is to maximize the components

TABLE 2 Factors to Consider in Sample Preparation of Pharmaceutical Dosage Forms

Objective	Factors
1. Dissolution	Solvent selection
	Concentration level
2. Maximize yield of analytes	Filtration
	Heat
	Microwave
	Shaking
	Solvent(s)
	Sonication
	pH
	Enzymes
3. Minimize yield of excipients or extraneous compounds	Extraction
	Filtration
	pH
	Solvent(s)
	Centrifugation
	Adsorption on adsorbent
4. Maximize stability or extend storage life of analytes	pH
	Temperature
	Light
	Moisture
	Solvent compatibility

TABLE 3 Representative Pharmaceutical Dosage Forms

Tablet
Capsule
Liquid
Suspension
Emulsion
Semisolid
Suppository
Aerosol
Parenteral product
Transdermal

of interest and minimize the extraneous components in the test solution. Commercial cleanup columns which are rapid and reliable are available for the analysis of drugs of abuse (22,42).

B. Assay Procedure and Detection

The choice of traditional TLC or HPTLC will depend on the resolution required of the analytes. HPTLC provides improved separation with a shorter assay time but higher equipment cost. A good starting point is to keep the solvent system as simple as the analytes and extraneous components will allow. More than one solvent system will likely be usable, some more rugged than others. To obtain good results, plates should be developed in saturated, preequilibrated tanks. The sample or standard volume spotted on the plate should be kept small to minimize spreading.

Numerous detection sprays and techniques are available. The examples in this chapter should cover the majority of the cases. However, if needed, the reader is directed to Chapter 1, Section VIII on detection.

IV. ANALYSIS OF POPULAR PHARMACEUTICALS AND ILLICIT DRUGS

The most widely prescribed human-formulated drugs from the *American Druggist* (43), animal-formulated drugs from the Animal Health and Nutrition Service (44), as well as the common illicit drugs are complied in Table 4 and Fig. 1. All drugs are listed as the free base, even if a salt form of the drug is used in the formulation. Table 4 provides the thin-layer chromatographic conditions for the prescribed drugs listed by generic name and illicit drugs from Stead et al.'s (1) publication. For pharmaceuticals that are not among the 794 compounds tested by Stead and co-workers, Fig. 1 provides the structures of the commonly used human and animal drugs to assist the reader in deducing suitable TLC systems based on whether the compound is basic, acidic, or neutral.

TABLE 4 $R_f \times 100$ of Some Popular Pharmaceuticals and Illicit Drugs as Classified Under Acid (A), Basic (B), and Neutral (N) in the Eight TLC Solvent Systems Listed in Table 5

		Solvent system							
Compound	Class	1	2	3	4	5	6	7	8
Acetylsalicylic acid	A					18	16	30	31
Amiloride	B	24	0	1	2				
Amitriptyline	B	51	55	32	15				
Atenolol	B	45	0	2	2				
Atropine	B	18	6	3	1				
Caffeine	B	52	3	58	25				
Carbamazepine	B	60	4	56	47				
Carbidopa	N					0	0	2	4
Chlorpheniramine	B	45	33	18	2				
Chlorthalidone	A					4	42	43	23
Cimetidine	B	54	0	9	12				
Clemastine	B	46	48	25	9				
Clonidine	B	62	8	31	53				
Cocaine	B	65	47	47	54				

TABLE 4 (continued)

Compound	Class	Solvent system 1	2	3	4	5	6	7	8
Codeine	B	33	6	18	3				
Desipramine	B	26	20	11	3				
Diazepam	B, N	75	23	73	59	58	77	48	72
Diethylcarbamazine	B	52	17	26	5				
Diflunisal	A					8	16	10	18
Digoxin	N					1	33	5	28
Diphenoxylate	B	74	42	81	70				
Dipyridamole	B	68	0	37	42				
Doxepin	B	51	52	37	13				
Fenoprofen	A	42	9	53	50				
Flurazepam	B, N	62	30	48	40	3	74	3	41
Furazolidone	B					44	0	47	59
Glipizide	B	87	0	41	5				
Haloperidol	B	67	10	27	33				
Hydrochlorothiazide	A					4	34	39	11
Hydrocodone	B	25	4	20	4				
Hydroxyzine	B	68	9	54	19				
Ibuprofen	A					46	7	54	54
Indomethacin	A					16	6	20	38
Ketoprofen	A					27	7	40	41
Levamisole	B	62	18	48	42				
Loperamide	B	70	9	32	22				
Lorazepam	B, N	52	1	36	28	23	45	39	41
Lysergide (LSD)	B	60	3	39	18				
Mebendazole	B	65	0	59	49				
Methaqualone	B	70	37	80	56				
Methyldopa	B	49	1	1	1				
Metoprolol	B	49	8	8	9				
Miconazole	B	73	11	67	3				
Morphine	B	37	0	9	1				
Nadolol	B	42	1	1	1				
Naproxen	A					33	7	45	44
Nicotinic acid	A					1	0	0	4
Nifedipine	B	68	1	65	68				
Nortriptyline	B	34	27	16	4				
Oxazepam	B, N	56	0	40	51	22	44	37	42
Oxycodone	B	50	23	51	39				
Penicillamine	B	36	1	3	3				
Piperazine	B	5	1	1	0				
Prazosin	B	60	1	47	49				

TABLE 4 (continued)

Promethazine	B	50	37	35	17				
Propranolol	B	50	7	10	7				
Sulfadiazine	A					22	4	39	3
Sulfadimethoxine	A					31	10	51	48
Sulfaguanidine	A					1	25	6	7
Sulfamerazine	A					23	8	41	4
Sulfanilamide	A					13	52	46	22
Sulfapyridine	A					16	24	42	34
Sulfathiazole	A					9	9	20	27
Sulindac	A					14	1	10	34
Temazepam	B, N	53	8	59	53	51	63	47	65
Theophylline	B	75	1	30	11				
Thiabendazole	B	67	7	54	53				
Tolmetin	A					13	7	20	30
Trazodone	B	63	9	58	37				
Triamterene	B	51	1	8	4				
Triazolam	B, N	60	1	40	16	5	45	2	41
Verapamil	B	59	23	70	42				
Warfarin	A					64	18	62	64

Source: Reprinted from A. H. Stead, R. Gill, T. Wright, J. P. Gibbs, and A. C. Moffat, *Analyst, 107*: 1106 (1982), by courtesy of the Royal Society of Chemistry, London.

To facilitate use of the data, Table 5 describes the eight standardized solvent systems, four for basic drugs and four for acidic and neutral drugs. When the drug is essentially basic (B) or acidic (A) but can also be extracted into a neutral fraction, the designation B, N or A, N is given by Stead et al. All sample solutions at a concentration of 2 mg/ml are applied to 20 × 20 cm silica gel 60 F(254 nm) TLC plates of 0.25 mm thickness (E. Merck, Darmstadt, FRG). The TLC plates for the four basic systems are pretreated by dipping them into a methanolic solution of potassium hydroxide (0.1 M) and then air-dried. Detection is by absorption or fluorescence, or if that is not possible, by spray reagents.

For the analysis of herbal medicines, Table 6 gives an extensive list of chromatographic systems, and detection methods or sprays compiled from Zhou (45) and from Munshi and Das (46).

TABLE 5 Standardized TLC Systems for Acidic, Basic, and Neutral Compounds

No.	Solvent system	Adsorbent
(1)	Methanol:ammonia (100:1.5)	Silica dipped in 0.1 M KOH and dried
(2)	Cyclohexane:toluene:diethylamine (75:15:10)	Silica dipped in 0.1 M KOH and dried
(3)	Chloroform:methanol (9:1)	Silica dipped in 0.1 M KOH and dried
(4)	Acetone	Silica dipped in 0.1 M KOH and dried
(5)	Chloroform:acetone (4:1)	Silica
(6)	Ethyl acetate:methanol:ammonia (85:10:5)	Silica
(7)	Ethyl acetate	Silica
(8)	Chloroform:methanol (9:1)	Silica

Source: Reprinted from A. H. Stead, R. Gill, T. Wright, J. P. Gibbs, and A. C. Moffat, *Analyst*, *107*: 1106 (1982), by courtesy of the Royal Society of Chemistry, London.

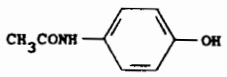
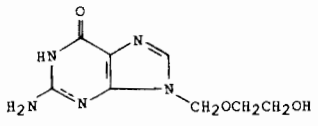
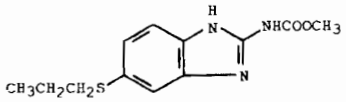
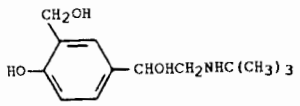
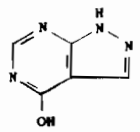
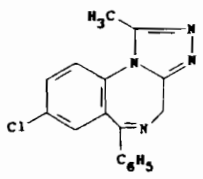
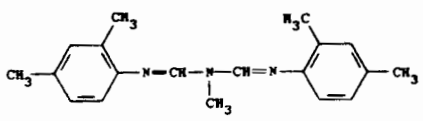
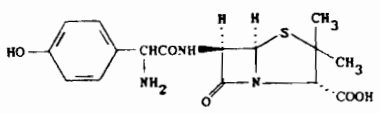
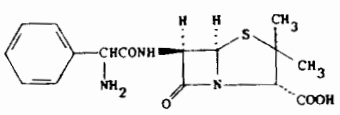
Compounds	Structure
Acetaminophen	
Acyclovir	
Albendazole	
Albuterol	
Allopurinol	
Alprazolam	
Amitraz	
Amoxicillin	
Ampicillin	

FIGURE 1 Structures of some popular human and animal drugs. (The compound listing is reprinted from the *American Druggist*; data collected by Pharmaceutical Data Services, Scottsdale, Ariz., and Animal Health and Nutrition Service, Wood Mackenzie and Co. Structures are reproduced by permission of Merck & Co., Inc., from the *Merck Index*, 11th ed., 1989. Exceptions marked * are copied from USAN and USP *Dictionary of Drug Names*,)

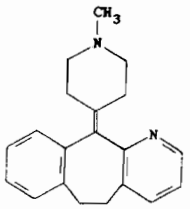
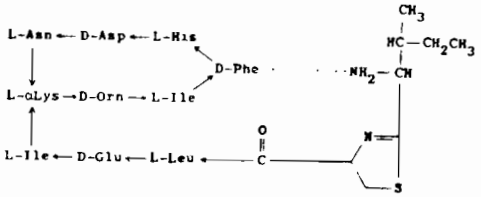
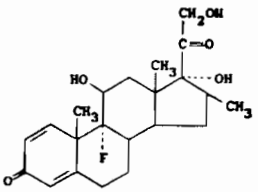
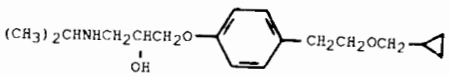
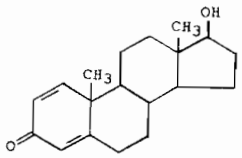
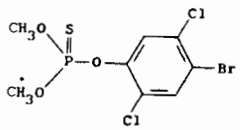
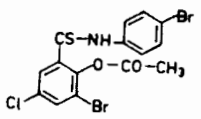
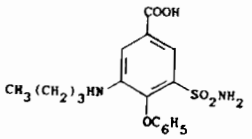
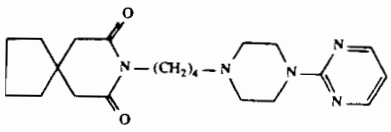
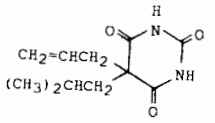
Identification	Structure	R_f 100
Azatadine		
Bacitracin		
Betamethasone		
Betaxolol		
Boldenone		
Bromophos		
Brotianide*		
Bumetanide		

FIGURE 1 (continued)

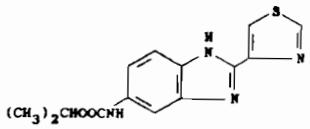
Buspirone



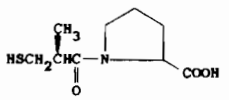
Butalbital



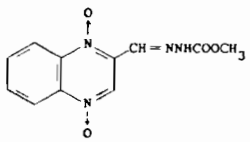
Cambendazole



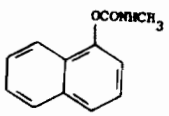
Captopril



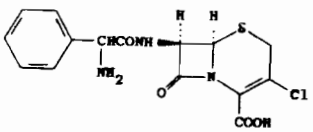
Carbadox



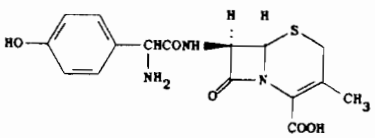
Carbaryl



Cefaclor



Cefadroxil



Cephalexin

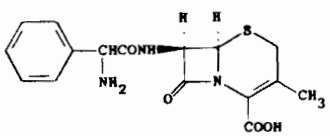
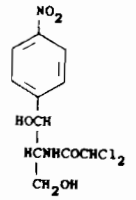
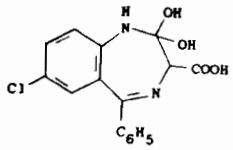


FIGURE 1 (continued)

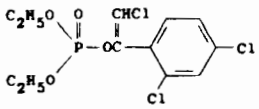
Chloramphenicol



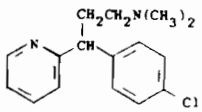
Chlorazepate



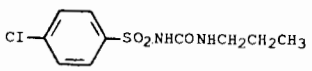
Chlorfenvinphos



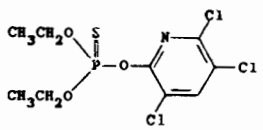
Chlorpheniramine



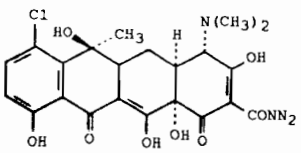
Chlorpropamide



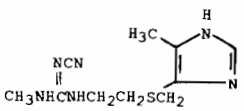
Chlorpyrifos



Chlortetracycline



Cimetidine



Clavulanic acid

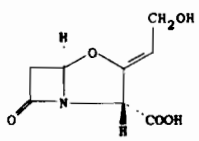
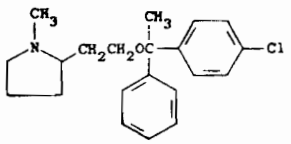
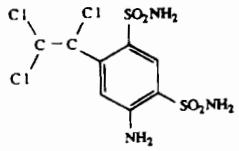


FIGURE 1 (continued)

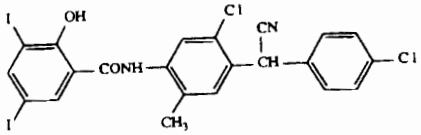
Clemastine



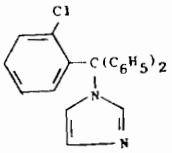
Clorsulon



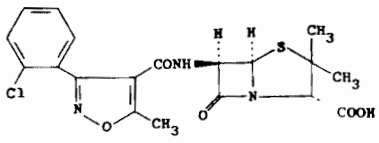
Closantel



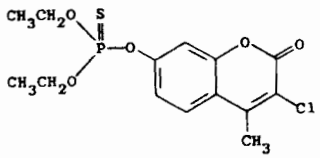
Clotrimazole



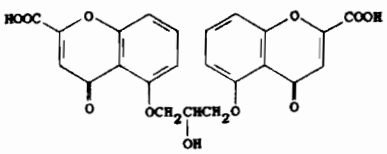
Cloxacillin



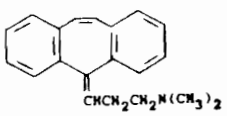
Coumaphos



Cromolyn



Cyclobenzaprine



Cyhalothrin

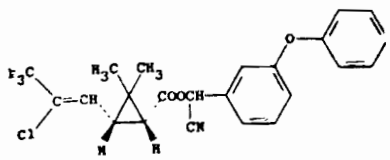


FIGURE 1 (continued)

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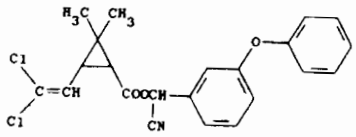
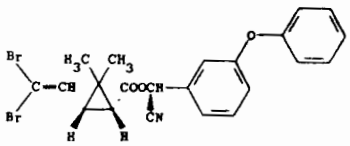
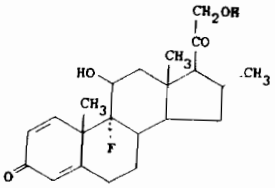


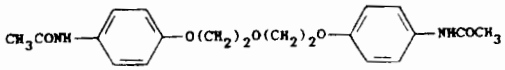
Deltamethrin



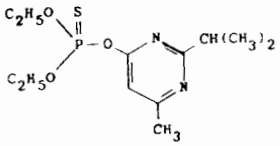
Desoximetasone



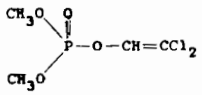
Diamfenetide



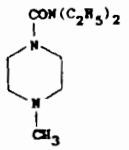
Diazinon



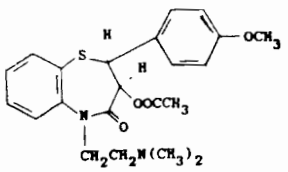
Dichlorvos



Diethylcarbamazine



Diltiazem



Dioxathion

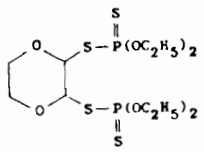
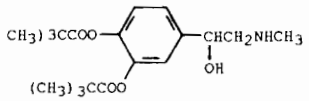
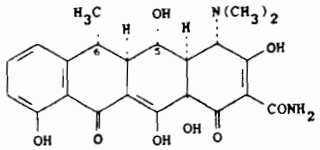


FIGURE 1 (continued)

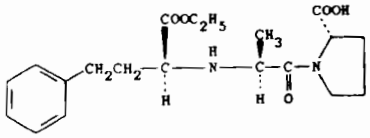
Dipivefrin



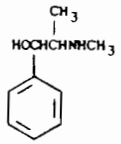
Doxycycline



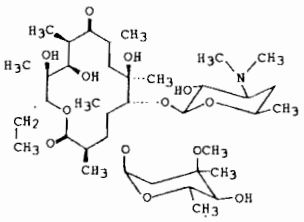
Enalapril



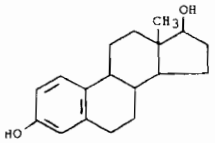
Ephedrine



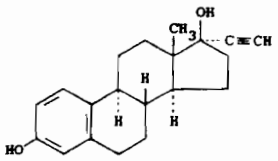
Erythromycin A



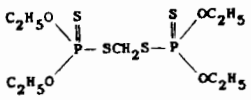
Estradiol



Ethinyl estradiol



Ethion



Ethynodiol

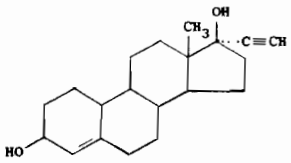


FIGURE 1 (continued)

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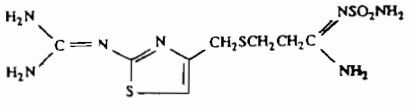
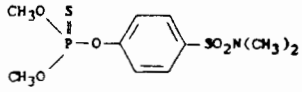
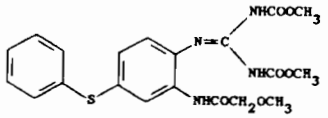


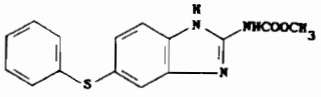
Famphur



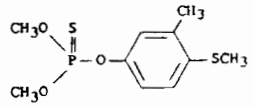
Febantel



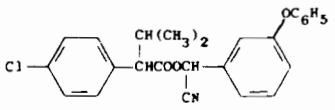
Fenbendazole



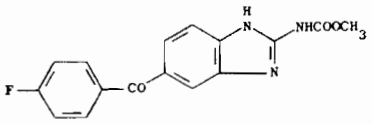
Fenthion



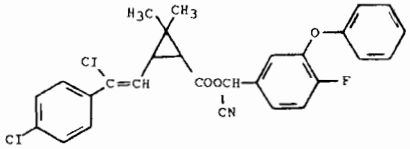
Fenvalerate



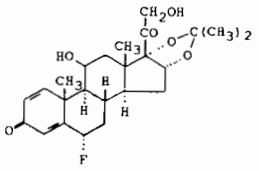
Flubendazole



Flumethrin



Flunisolide



Fluocinonide

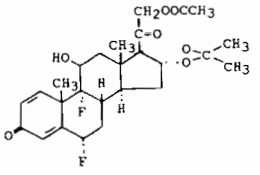
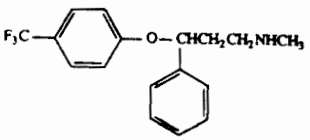
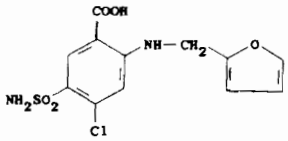


FIGURE 1 (continued)

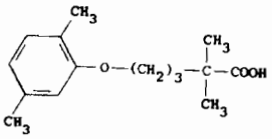
Fluoxetine



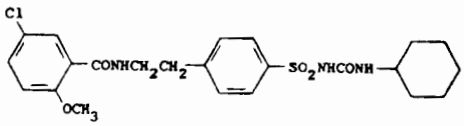
Furosemide



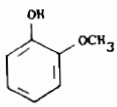
Gemfibrozil



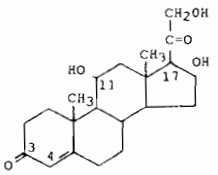
Glyburide



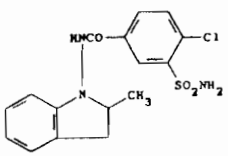
Guaiacol



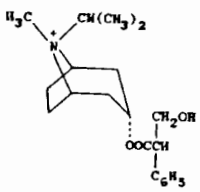
Hydrocortisone



Indapamide



Ipratropium



Isosorbide

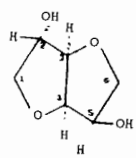
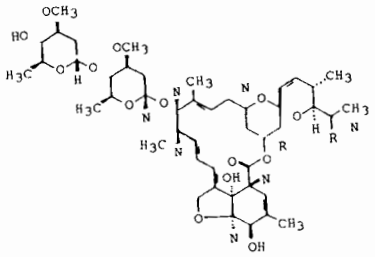
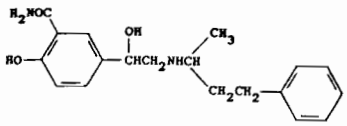


FIGURE 1 (continued)

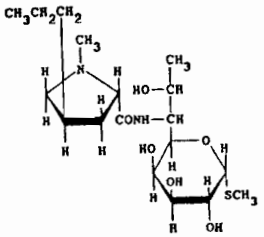
Ivermectin



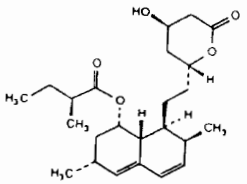
Labetalol



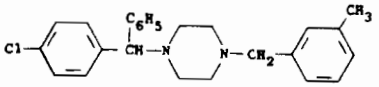
Lincomycin



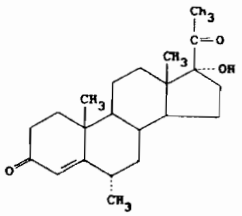
Lovastatin



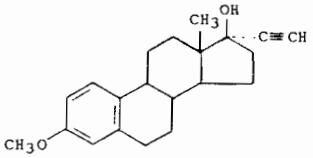
Meclizine



Medroxyprogesterone



Mestranol



Metaproterenol

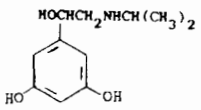
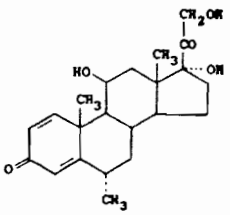
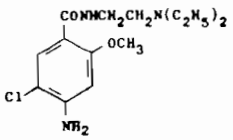


FIGURE 1 (continued)

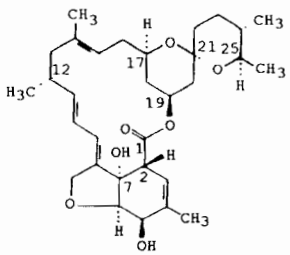
Methylprednisolone



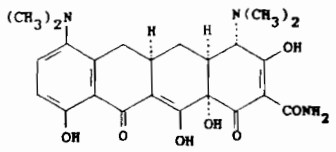
Metoclopramide



Milbemycin α_1



Minocycline



Moenomycin A

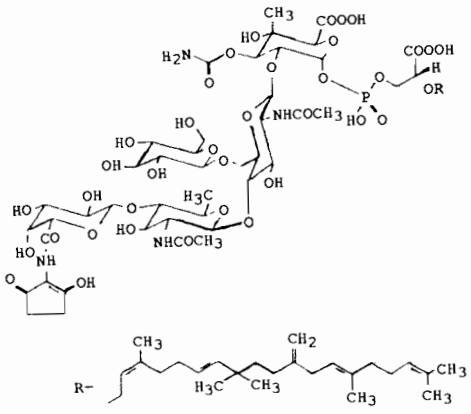
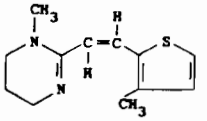
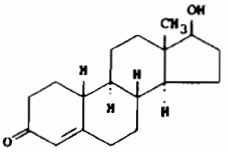


FIGURE 1 (continued)

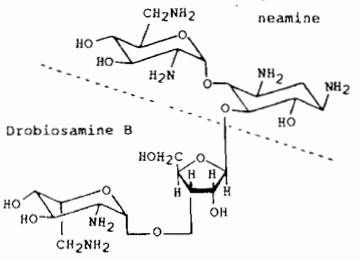
Morantel



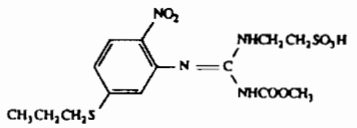
Nandrolone



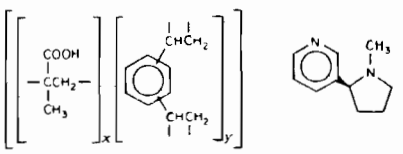
Neomycin B



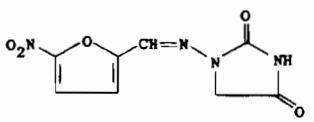
Netobinin



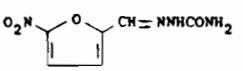
Nicotine polocribe*



Nitrofuratoin



Nitrofurazone



Nitroglycerin

Nitroxynil

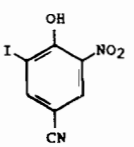
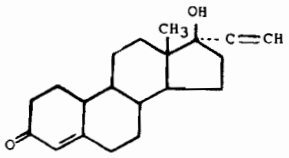
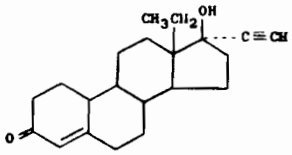


FIGURE 1 (continued)

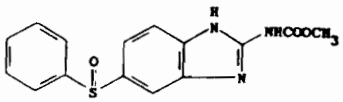
Norethindrone



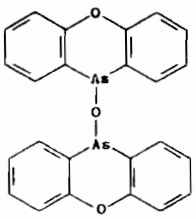
Norgestrel



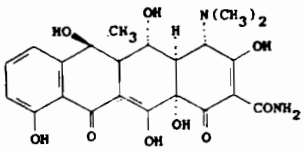
Oxfendazole



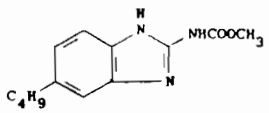
Oxyclozanide



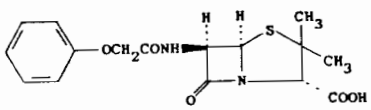
Oxytetracycline



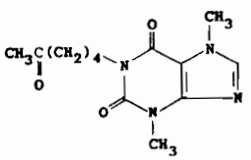
Parbendazole



Penicillin V



Pentoxifylline



Permethrin

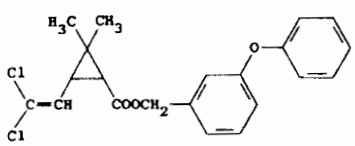
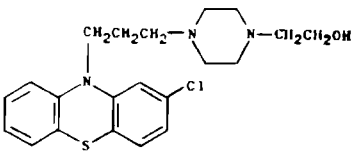
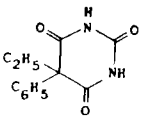


FIGURE 1 (continued)

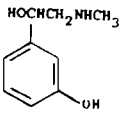
Perphenazine



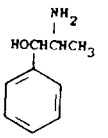
Phenobarbital



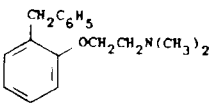
Phenylephrine



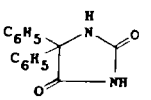
Phenylpropanolamine



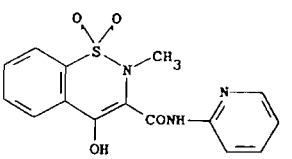
Phenyltoloxamine



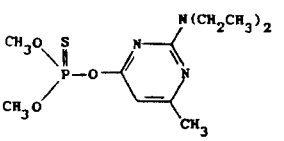
Phenytoin



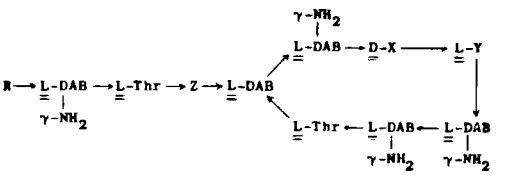
Piroxicam



Pirimiphos-methyl



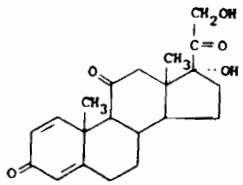
Polymyxin



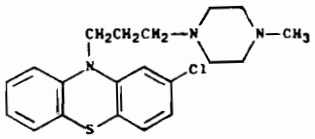
DAB=α γ-diaminobutyric acid

FIGURE 1 (continued)

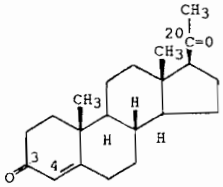
Prednisone



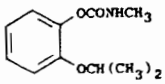
Prochlorperazine



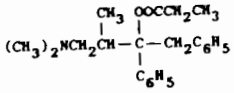
Progesterone



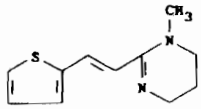
Propoxur



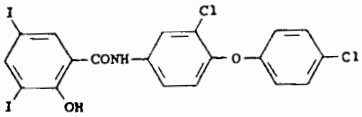
Propoxyphene



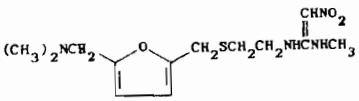
Pyrantel



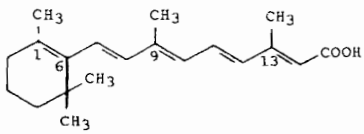
Rafoxanide



Ranitidine



Retinoic acid



Ronnel

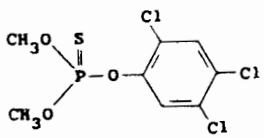
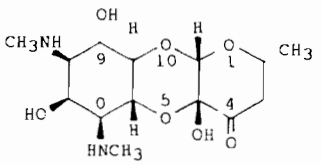
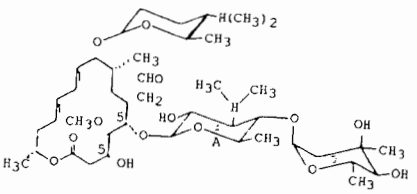


FIGURE 1 (continued)

Spectinomycin

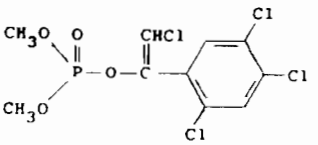


Spiramycin

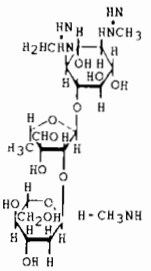


Spiramycin I, R=H.

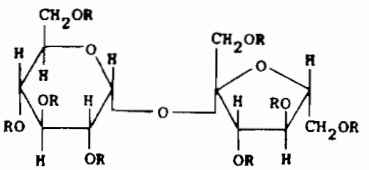
Stirofos



Streptomycin

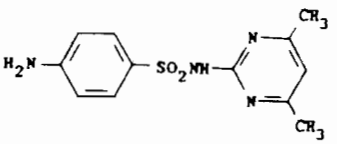


Sucrafate

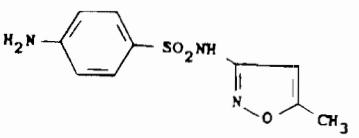


R = $SO_3[Al_2(OH)_5]$

Sulfamethazine



Sulfamethoxazole



Sulfaquinoxaline

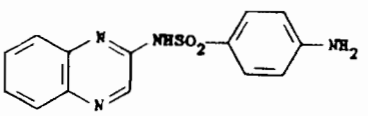
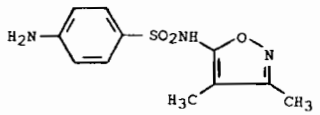
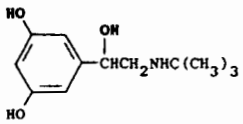


FIGURE 1 (continued)

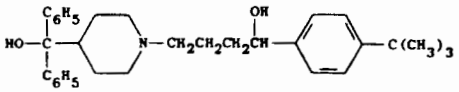
Sulfisoxazole



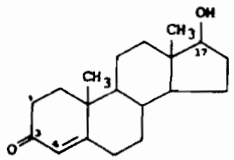
Terbutaline



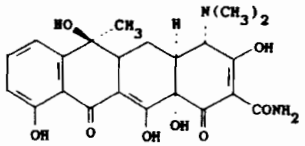
Terfenadine



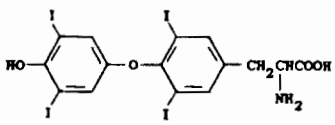
Testosterone



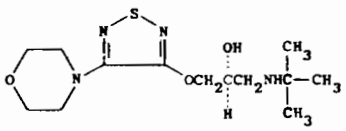
Tetracycline



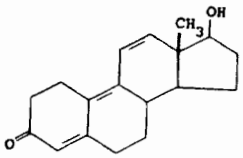
Thyroxine



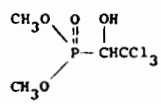
Timolol



Trenbolone



Trichlorfon



Triclabendazole

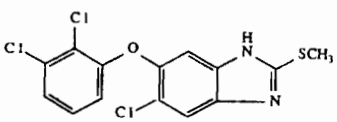
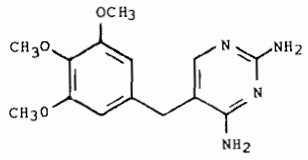
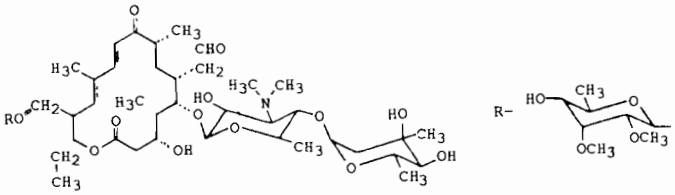


FIGURE 1 (continued)

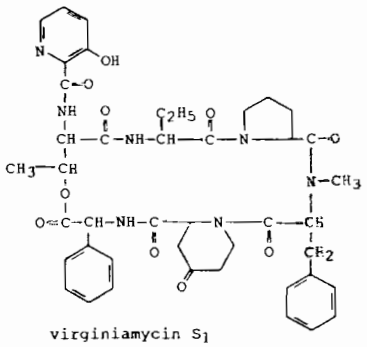
Trimethoprim



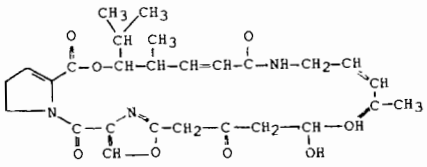
Tylosin



Virginiamycin



virginiamycin S_1



virginiamycin M_1

Zeranol

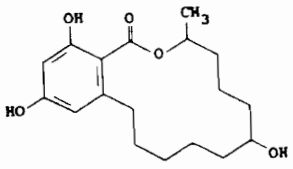


FIGURE 1 (continued)

TABLE 6 TLC Determination of Active Components in Herbal Medicinal Plants

Latin name	Components	Plate	Development solvent	Visualization	R_f (a)	R_f (b)
Tevetia peruviana	Peruvoside	Alumina	$CHCl_3$:MeOH (10:1)	Iodine	0.37	
	Nerrifolin				0.52	
	Cerberin				0.72	
Digitalis lanata	Lanatoside A	Celite	$CHCl_3$:acetone:EtOAc:EtOH	Iodine	0.75	
D. purpurea	Lanatoside B	Formamide	(8:2:0.5:0.5)		0.45	
	Lanatoside C				0.27	
	Desacetyl lanatoside A		$CHCl_3$:*n*-BuOH (9:1)		0.3	
	Desacetyl lanatoside B				0.85	
	Digitoxin		$CHCl_3$:EtOAc:benzene (1:3:2)		0.73	
	Gitoxin				0.38	
	Digoxin				0.22	
Securinega suffruticosa	Securinine	Alumina	$CHCl_3$	UV	0.5	
Rauwolfia serpentina	Reserpine	Cellulose	Pet. ether (90–120°C):CCl_4		0.4	
R. verticillata	Rescinamine		(12:8)		0.3	
R. vomitoria	11-Demethylreserpine	Formamide			0.5	
Crotalaria sessiliflora	Monocrotaline	Silica gel G	Benzene:anh. EtOH (96:4)	Dragendorff	0.1	
			$CHCl_3$:MeOH (8:2)	Iodine	0.26	
Solanaceous plants	Hyoscyamine	Alumina (a)	Xylene:acetone:anh. EtOH:	Dragendorff/	0.50	0.34
Datura stramonium	Scopolamine		diethylamine (50:40:10:0.6)	Wagner	0.72	0.92
D. innoxia	Anisodamine				0.22	0.23
D. tatula	Anisodine				0.07	0.85
Anisodus tanguticus	Cuscohygrine				0.6	0.12
Przewalskia shebbeari	Tropane	Silica gel G (b)	$CHCl_3$:MeOH:NH_4OH			0.04
P. tangutica	Apotropine		(8.5:1.5:0.07)			0.77

TABLE 6 (continued)

Latin name	Components	Plate	Development solvent	Visualization	R_f (a)	R_f (b)
Sophora flavescens	Matrine	Silica gel G	Benzene:acetone:EtOAc-NH_4OH (2:3:4:0.2) (c)	Dragendorff	0.37	0.84
	Sophocarpine				0.43	0.84
	Sophoridine				0.21	0.71
	Oxymatrine		$CHCl_3$:MeOH:NH_4OH (5:0.6: 0.3) (d)	Morin (UV)	0	0.43
	Oxysophocarpine				0	0.33
Corydalia ambigua	Tetrahydropalmatine	Silica gel G	Hexane:$CHCl_3$:MeOH (5:3:0.5)		0.45	
	Corydaline				0.71	
	Protopine				0	
	Tetrahydroberberine				0.61	
Vinca minor	Vincamine	Silica gel G	*i*-Propyl ether:xylene:acetone: MeOH (2:2:1:0.5)	Dragendorff	0.5	
Ergota	Ergometrine	Silica gel G	*i*-Propyl ether:benzene:ace- tone:MeOH:diethylamine (2:2:1:0.5:0.1)	UV	0.27	
	Ergotamine				0.42	
	Ergocristine				0.68	
	Lysergicamide				0.33	
Cephalotaxus hainanensis	Cephalotaxine	Silica gel G/1 N NaOH	Ether:acetone (2:1)	Iodine	0.8	
	Harringtonine				0.40	
	Homoharringtonine				0.46	
	Isoharringtonine				0.58	
	Desoxyharringtonine				0.87	
Partially synthesized harringtonine	2'R	Silica gel G/1% HCl	$CHCl_3$:MeOH (7:3)	Iodine	0.5	
	2'S				0.56	
Cissampelos pareira	Hayatine	Silica gel G	$CHCl_3$:MeOH (8:0.5)	Iodine	0.6	
Polygonum aviculare	Avicularin	Silica gel G	$CHCl_3$:MeOH:HCOOH (8:2:0.1)	$AlCl_3$	0.5	
		Polyamide	36% HOAc:EtOH (9:1)	UV	0.3	
Pycnostelma paniculatum	Paeonol	Silica gel G	Hexane:$CHCl_3$:anh. EtOH (7:3:1)	UV	0.5	
Paeonia suffriticosa	Paeonol	Silica gel G	Hexane:$CHCl_3$:EtOAc (7:3:1)	Iodine	0.55	
Acacia catechu	Catechin	Silica gel G	$CHCl_3$:MeOH (8:2)	Iodine	0.5	
Rhododendron dahuricum	Rhodotoxin	Silica gel G	$CHCl_3$:MeOH (9:1) Toluene:MeOAc:HCOOH (5:4:1)	$SbCl_3$	0.5	
	Farrerol	Silica gel G/10% $NaHSO_3$	$CHCl_3$:MeOH (7:0.5)	UV	0.4	

	Farrerol	Silica gel G/10% $NaHSO_3$	Toluene:MeOAc:HCOOH (5:4:1) $CHCl_3$:MeOH (7:0.5)	UV	0.4

Panax sanchi	Saponin C_1	Silica gel G	1,2-Dichloroethane:*n*-BuOH: MeOH:H_2O (3:4:1.5:2)	NH_4HSO_4	0.68
	Saponin D_1				0.50
	Saponin D_2				0.42
	Saponin E_1				0.18
Arisia alyxiaofolia	Saponin	Silica gel G	$CHCl_3$:MeOH-*n*-BuOH:HOAc: H_2O (2.5:1:1.5:1.5:0.2)	Vanillin	0.46
Panax ginseng	Saponins R_o, R_a, R_{b1}, R_{b2}, R_c, R_e, R_d, R_f, R_{g1}, R_{g2}	Silica gel G	$CHCl_3$:MeOH:H_2O (70:55:10) (60:42:11)	UV Iodine 50% H_2SO_4	0.2-0.8
	Oleanolic acid		$CHCl_3$:ether (1:1)		0.72
	Panaxdiol				0.47
	Panaxtriol				0.17
Agave americana	Hecogenin	Silica gel G	$CHCl_3$:MeOH (95:5)	Vanillin	0.6-0.7
Paeonia lactiflora	Paeoniflorin	Silica gel G	$CHCl_3$:MeOH:EtOAc (4:2:0.5)	Iodine	0.42
P. veitchii	Paeonol				0.96
	Oxypaeoniflorin				0.10

Rheum palmatum		Silica gel G	Pet. ether:hexane:HCOOEt:	Self-colored	(e)	(f)
R. emodii	Chrysophanol		HCOOH (1:3:1.5:0.1) (e)		0.67	0.89
R. officinale	Physcion				0.58	0.89
R. tanguticum	Emodin		Benzene:HCOOEt:MeOH:HCOOH		0.24	0.70
	Aloe-emodin		(3:1:0.2:0.05) (f)		0.17	0.60
	Rhein				0.17	0.52
	Rhaponticin		*i*-Propyl ether:MeOH:*n*-BuOH (3:0.5:0.5)	UV	0.60	
Curcuma aromatica	Curcumol	Silica gel G	Pet. ether (30-60°C):acetone: EtOAc (94:5:1)	Iodine	0.30	
			Pet. ether:acetone (96:4)		0.11	
Agrimonia pilosa	Agrimophol	Silica gel G/0.1% $FeCl_3$	Pet. ether (60-90°C):EtOAc: HOAc (10:25:0.5)	Colored	0.74	
Ardisia japonica	Bergerin	Silica gel G	$CHCl_3$:EtOAc:HCOOH (5:4:2)	$FeCl_3/Fe(CN)_6^{-3}$	0.4	

TABLE 6 (continued)

Latin name	Components	Plate	Development solvent	Visualization	R_f
Schizandra chinensis	Schizandrol B	Silica gel G	Toluene-EtOAc (6:4)	P-Mo acid	0.52
	Schizandrol ester B				0.65
	Schizandrin B		Toluene-EtOAc (9:1)		0.59
	Schizandrin C				0.75
Silybum marianum	Silybin	Silica gel G	Toluene:EtOAc:HOAc (27:13:4)	UV	0.26
Ilex chinensis	Protocatechualdehyde	Silica gel G	$CHCl_3$:MeOH:HCOOH (90:8:2)	Self-colored	0.6
Salvia miltiorrhiza	Protocatechualdehyde	Silica gel G	$CHCl_3$:MeOH:HCOOH (90:8:2)	Self-colored	0.6
	Cryptotanshinone		Benzene:EtOAc (9:1)	Self-colored	0.6
Angelica sinensis	Ferulic acid	Silica gel G	Benzene: $CHCl_3$:MeOH (2:2:0.6)	UV	0.55
Livisticum officinale	Ligustilide		CCl_4:xylene: $CHCl_3$:hexane		0.37
	n-Butylenephthalide		(4:3:2:0.5)		0.46
Artemisia cina	α-Santonin	Silica gel G/alumina	EtOH: NH_4OH (8:0.3)	UV	0.45
	β-Santonin				0.55
Apium graveolens	3-*n*-Butylphthalide	Silica gel G	$CHCl_3$:benzene (2:1)	Iodine	0.61
	3-*n*-Butyl-4,5-dihydrophthalide				0.51
Berberidaceae aristata	Berberine	Silica gel G	Methanol: NH_4OH (24:1)	UV	0.28
B. asistata	Berberine				
B. insignia	Berberine				
B. lycium	Berberine				
B. umbellata	Berberine				
B. wallichiana	Berberine				
B. vulgaris	Berberine				

Source: Reprinted from T. H. Zhou, *Proc. Sino-West Ger. Symp. Chromatogr.* (1983), p. 549, by courtesy of the author except for the Berberidaceae, which are from G. K. Munshi and S. K. Das, *Indian Drugs Pharm. Ind.*, *14*: 17 (1979), by courtesy of the authors.

V. EXAMPLES

Representative examples of TLC analysis of active compounds in pharmaceutical dosage forms are given in this section.

A. Flurazepam Hydrochloride and Related Compounds in Capsules*

1. Materials and Reagents

Standards: Flurazepam is USP Reference Standard, U.S. Pharmacopeial Convention, Rockville, Md. Related compounds are from Hoffman-La Roche, Manati, P.R.
Sample: Flurazepam capsule.
Diluent solvent: Methanol.
Applicator: 10-µl disposable micropipet (Microcaps, Drummond Scientific, Broomall, Pa.
TLC plate: 5 × 20 cm or 20 × 20 cm precoated silica gel GF plates (0.25 mm thick, Analtech, Newark, Del.).
Solvent system: Diethyl ether:methylene chloride:diethylamine:triethylamine (90:10:2:1).

2. Detection

Short- and long-wavelength ultraviolet (UV) light.

3. Experimental

Standard preparation: Standard solutions of flurazepam hydrochloride (100,000 µg/ml), related compounds A-D, F (100 µg/ml), and E (200 µg/ml) are prepared in methanol. The solutions are stable for several weeks if kept away from light.
Sample preparation: The contents of one capsule is dissolved in methanol to give a solution containing about 0.1% flurazepam hydrochloride.
Assay procedure: Solutions of standard and samples are applied to a TLC plate at 1.5- to 2.0-cm intervals and 2.5 cm away from one of the edges of the plate. The plate is allowed to air-dry at ambient temperature and then develop to about 12 cm from the origin. After air-drying inside a hood, examine under short- and long-wavelength UV light.

Figure 2 shows the structures of flurazepam and related structures with their respective R_f values.

B. Preservatives and Excipients in Creams†

1. Materials and Reagents

Diluent solvent: Ethanol, chloroform, petroleum ether, and water.
Solvent system: A: *n*-pentane and diethyl ether; B: *n*-butanol:water: glacial acetic acid (20:5:2).

2. Detection

a. Short- and long-wavelength UV light (254 and 365 nm).
b. ANS spray reagent: 0.1% 8-anilino-naphthalene-I-sulfonic acid ammonium salt (Merck) in water.

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*Source: C. M. Klein and C. A. Lau-Cam, *J. Chromatogr.*, *350*: 273 (1985).

†Source: F. J. van de Vaart, A. Hulshoff, and A. W. M. Indemans, *Pharm. Weekbl. Sci. Ed.*, *5*: 109 (1983).

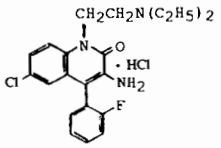
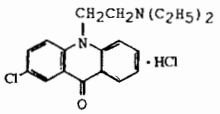
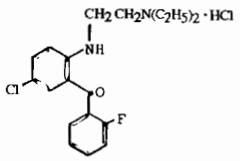
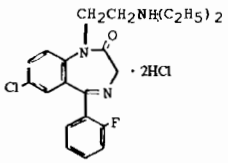
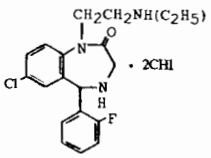
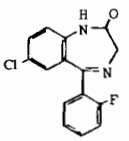
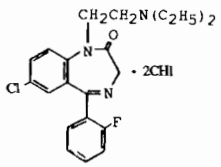
Identification	Structure	$R_f \times 100$
A		71.0
B		64.5
C		83.5
D		26.0
E		30.5
F		43.5
Flurazepam hydrochloride		60.5

FIGURE 2 Structures of Flurazepam and related compounds and their R_f values. Reprinted from C. M. Klein and C. A. Lau-Cam, *J. Chromatogr.*, *350*: 273 (1985), by courtesy of Elsevier Science Publishers.

c. Permanganate (MnO_4) spray reagent: 3% potassium permanganate in 0.5 M sulfuric acid.
d. BCC spray reagents for chlorocresol: (1) 0.4% 2,6-dibromo-quinone-4-chlorimide (Merck) in methanol; (2) 10% sodium bicarbonate in water; after spraying with (1) and (2), a blue color slowly develops.
e. Ninhydrin (Ninh) spray reagent: 0.3% ninhydrin (Merck) in *n*-butanol. After spraying, the plate is heated at 110°C until purple spots appear.

3. *Experimental*

Standard preparation: Reference solutions contain 1% of the reference compound in a suitable solvent. Solutions of sorbic acid, methyl hydroxybenzoate, propyl hydroxybenzoate, chlorocresol, and cetrimide contain 0.1% of these compounds.

Sample preparation:

a. Standard cream sample. 500 mg of the cream are vigorously shaken with 3 ml of chloroform until the cream is completely dispersed. Ethanol is then added until a clear or almost clear solution is obtained (typically 2-4 ml). For creams containing a considerable amount of soft paraffin, the addition of more chloroform (up to 85% of the total volume) is favorable; when complete dissolution of the cream cannot be obtained, the sample solution is gently warmed immediately before application onto the plate. Clear or almost clear sample solutions containing 5-10% of the cream can be prepared in this way.
b. Concentrated cream sample. 500 mg of the cream are transferred to a separation funnel and shaken with 10 ml of ethanol and 10 ml of light petroleum ether (b.p. 40-60°C). Water is added until separation of the phases occurs. The ethanolic layer is collected and evaporated by gentle heating on a water bath. The residue is redissolved in 1 ml of a mixture of equal parts of ethanol and chloroform.

Assay procedure:

System A. The chamber is saturated (10-15 min) with *n*-pentane by adding 15 ml of this solvent to one trough of the chamber. In order to promote and maintain saturation, the chamber is lined with filter paper at that side. 0.5 µl of both the sample and the reference solutions are applied to the plate. The eluent, 6 ml of diethyl ether, is then pipetted into the other trough. Immediately afterward the plate is placed in the chamber and developed over a distance of 7 cm. Run times are approximately 10 min.

System B. 0.5 µl of both the sample and reference solutions are applied to the plate, which is developed over a distance of 7 cm under saturated conditions (ca. 90 min). The eluent can be evaporated by heating the plate at 100°C for about 15 min.

Table 7 discusses the R_f values and detection of some common cream preservatives and excipients.

C. All-*trans* and 13-*cis*-Retinoic Acids in Gels*

1. *Materials and Reagents*

Standards: Tretinoin is USP Reference Standard, 13-*cis*-retinoic acid can be purchased from BASF Wyandotte (Parsippany, N.J. As an alternative, the cis isomer can be prepared by exposing 3-5 ml of the tretinoin standard stock solution to 365-nm UV light for 1 h at room temperature (ca. 30% isomerization).

Sample: 0.025% and 0.01% Retin-A-Gel R (Ortho Pharmaceutical Corp., Raritan, N.J.

*A. M. DePaolis, *J. Chromatogr.*, *258*: 314 (1983).

TABLE 7 R_f Values and Detection of Some Common Cream Preservatives and Excipients

Substance	$R_f \times 100$ System A	$R_f \times 100$ System B	Detection[a]
Arachis oil[b]	37	>80	ANS/MnO_4
Benzoic acid	24	81	UV 254 nm
Benzyl alcohol	25	78	UV 254 nm
Butylhydroxyanisole	35	80	BCC
Cetiol v	60	>80	ANS/MnO_4
Cetomacrogol 1000[c]	0	0-28	ANS
Cetostearyl alcohol	24	>80	ANS
Cetrimide	0	23	ANS
Chlorocresol	27	80	BCC
Cholesterol	21	>80	ANS/MnO_4
Dioctyl sulfosuccinate	0	64	ANS
Glycerol	3	36	MnO_4
Glyceryl monostearate[c]	10	>80	ANS
Isopropyl myristate	60	>80	ANS
Lauryl sulfate	0	57	ANS
Macrogol ethers/esters of fatty acids[c]	0	0-30	ANS
Methyl hydroxybenzoate	22	83	UV 254 nm
Mygliol	31	>80	ANS
Paraffins	76	>80	ANS
Phenoxyethanol	19	70	UV 254 nm
Phenylethanol	25	81	UV 254 nm
Polysorbates (Tweens)[c]	0	0-40	ANS
Propyl gallate	15	74	UV 254 nm
Propyl hydroxybenzoate	22	83	UV 254 nm
Propylene glycol	8	53	MnO_4
Sorbic acid	18	75	UV 254 nm
Sorbitan esters of fatty acids (Spans)[c]	0-40	68->80	ANS
Sorbitol	0	14	MnO_4
Spermaceti[b]	68	>80	ANS/MnO_4
Stearic acid	27	>80	ANS
Triethanolamine	0	6	MnO_4/Ninh
White beeswax[b]	68	>80	ANS

[a]ANS = 0.1% 8-anilino-naphthalene-1-sulfonic acid ammonium salt in water; MnO_4 = 3% potassium permanganate in 0.5 M sulfuric acid; Ninh = 0.3% ninhydrin in *n*-butanol; BCC = 0.4% 2,6-dibromo-quinone-4-chlorimide in methanol and 10% sodium carbonate in water.

[b]More than one spot is detected; the R_f value of the main component is given.

[c]Spot pattern between the given R_f value.

Source: Reprinted from F. J. van de Vaart, A. Hulshoff, and A. W. M. Indemans, *Pharm. Weekbl. Sci. Ed.*, 5: 109 (1983).

Diluent solvent: BHT-methanol solution was prepared by dissolving 5 g of butylated hydroxytoluene (Tenox-BHT, food grade, Eastman Kodak, Rochester, N.Y. in 1 liter of methanol.
Applicator: 50-µl Hamilton microsyringe, Reno. Nev.
HPTLC plate: LHP-KF silica gel, 10 cm × 10 cm, 200-µm thickness with yellow-green fluorescent indicator under 254-nm UV light, and preadsorbent strip.
Solvent system: Diethyl ether:cyclohexane:acetone:glacial acetic acid (40:60:2:1).

2. *Detection*

a. Long-wavelength UV light source (365 nm)
b. Visualization spray consists of concentrated sulfuric acid:ethanol (8:92).

3. *Experimental*

Standard preparation: Tretinoin and 13-*cis*-retinoic acid are dissolved in BHT-methanol at 0.2 mg/ml. The cis isomer is further diluted (1:25) to about 8 µg/ml in BHT-methanol.
Sample preparation: After discarding the first 1-2 g of the gel from the tube, about 3 g of both gels are accurately weighed into separate 25-ml low-actinic volumetric flasks. The matrix is dispersed with about 15 ml of BHT-methanol in a Vortex Genie mixer (Scientific Industries) for 2 min and diluted to volume with BHT-methanol.
Assay procedure: Prewash the plate with methanol:acetone (1:1). A 50-µl aliquot for the 0.025% solution and 100 µl for the 0.01% solution is spotted onto the preadsorbent layer of a HPTLC plate adjacent to standard test solutions. After drying under nitrogen, the plate is developed for a distance of 9 cm (about 10 min). Then the plate is dried using warm air (2 min, hair dryer). The spots are visualized under UV light both before and after the detection spray. The plate is heated at 110°C for 5 min, which produces pink fluorescent spots for all-*trans* and 13-*cis*-retinoic acids against a dark background.

Caution: To reduce isomerization and oxidation, the following precautions are taken:

1. Work is done with minimum light exposure and/or fluorescent or yellow light.
2. Standards and samples are prepared in low-actinic volumetric flasks or translucent volumetric flasks wrapped in aluminum foil.
3. BHT is added to diluent solvent.

D. Aminacrine Hydrochloride in Suppositories*

1. *Materials and Reagents*

Standard: Aminacrine hydrochloride (Sigma Chemical Co. or equivalent)
Sample: 6-, 12-, and 14-mg suppositories.
Diluent solvent: Acidic ethanol is concentrated HCl:ethanol (1:99).
TLC plate: 20 × 20 cm glass, precoated with 250-µm layer of silica gel with or without fluorescent indicator.
Solvent system: Ethyl acetate:methanol:concentrated ammonium hydroxide (17:3:2).

*Source: E. A. Bunch, *J. Assoc. Offic. Anal. Chem.*, *66*: 140 (1983).

2. *Detection*

Long-wavelength UV light and TLC scanner.

3. *Experimental*

Standard preparation: Dissolve accurately ca. 25 mg of aminacrine hydrochloride in 100.0 ml of acidic ethanol and dilute with acidic ethanol to 0.0015 mg/ml for the 6-mg suppository and to 0.00175 mg/ml for the 12- and 14-mg suppositories.

Sample preparation:

Molded suppository. Determine average individual weight and composite 5 suppositories by heating in a 70°C oven. Mix well and cool until solidified. Accurately weigh equivalent to 6 mg of drug into a 150-ml beaker. Add ca. 40 ml of acidic ethanol and heat on a steam bath for 10 min with occasional stirring. Cool and quantitatively transfer to a 100-ml volumetric flask and dilute to volume with acidic ethanol. Dilute 5.0 ml of filtrate to 200.0 ml with acidic ethanol.

Gelatin-encapsulated suppository. Slit 5 capsules along the seams with a scalpel and extrude the contents as completely as possible into a 250-ml beaker. Place capsules in a beaker and add ca. 100 ml of acidic ethanol. Heat on a steam bath for 10 min with occasional stirring and probing of the capsules with a glass rod. Quantitatively transfer to a 200-ml volumetric flask with acidic ethanol. Repeat the extraction with 50 ml and then with 35 ml of additional acidic ethanol. Examine the capsules for completeness of content extraction. Discard sample if appreciable residue remains in any capsule. Cool and dilute to volume with acidic ethanol. Filter the solution, discarding the first 10 ml of filtrate. Dilute 5.0 ml of filtrate to 200.0 ml with acidic ethanol.

Assay procedure:

Qualitative: Line a chromatographic tank with filter paper and add developing solvent. Spot 3 µl of sample and standard solutions on the TLC plate. Develop to 10 cm above the starting line and air-dry. Locate and mark the spots under long-wavelength UV light. Compare by R_f.

Quantitative: The compound can be quantitated on a TLC scanner by scanning the sample solution and equivalent standard solution at 400 nm. Calculate the contents for sample from the formula:

$$\frac{A}{A'} \times C \times \frac{S}{W} \times 4{,}000 = \text{mg aminacrine hydrochloride per molded suppository}$$

$$\frac{A}{A'} \times C \times \frac{C}{T} \times 40{,}000 = \text{mg aminacrine hydrochloride per gelatin suppository composite}$$

where A and A' = net absorbance of sample and standard solutions, respectively; C = mg aminacrine hydrochloride per milliliter; W = sample weight; S = average suppository weight; and T = number of suppositories in composite.

E. Codeine Phosphate and Acetaminophen in Tablets*

1. *Materials and Reagents*

Standard: Codeine phosphate and acetaminophen are pharmacopeial standards.

Sample: 10 mg of codeine phosphate and 500 mg of acetaminophen in the tablet.

Diluent solvent: Ethanol:water (1:1).

Applicator: 5-µl micropipet.

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*Source: H. N. Al-Kaysi, *Anal. Lett.*, *19*: 915 (1986).

TLC plate: Precoated silica gel G-60, F (254 nm) on aluminum plate (Merck, Darmstadt, GFR), 0.25 mm thick, used as received.
Solvent system: *n*-Butanol:methanol:toluene:water:acetic acid (3:4:1:2:0.1).

2. *Detection*

Long-wavelength (366 nm) UV light; Shimadzu CS-920 TLC scanner.

	λ_{max} (nm)	$R_f \times 100$
Codeine phosphate	285	28
Acetaminophen	260	91

3. *Experimental*

Standard preparation: Four dilutions of a standard stock that bracket the concentration of interest.
Sample preparation: Ten tablets are powdered and the weight equivalent to the average weight of one tablet is dissolved in 50 ml of diluent solvent.
Assay procedure: Develop in a saturated, filter-paper-lined, glass chamber at room temperature. After development, the plate is air-dried. The compounds are identified by comparison of R_f values to standards. The spots can also be quantitated on the Shimadzu CS-920 TLC scanner (Japan) or equivalent. Detection is by reflection measurement, absorbance mode, with working curve linearizer set at 1, slit width 1.2 mm. Calibration curves are constructed for each component by plotting peak areas versus concentrations.

Note: Sample solution is stable for at least 36 h at room temperature.

VI. CONCLUSIONS

Thin-layer chromatography has and will probably always have a role in the analysis of pharmaceuticals and illicit drugs. It has been upgraded to a high-performance quantitative technique in recent years with improvements in technology. However, it is unlikely to displace the well-established gas and high-performance liquid chromatographic techniques.

DISCLAIMER

All methods are reproduced without modification. For health reasons, it is recommended that solvents with questionable safety according to OHSA should be substituted.

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TABLE 2 TLC of Pyrrolizidine Alkaloids

Alkaloid	Limit of detection (μg)	R_f	
		Solvent 1[a]	Solvent 2[b]
Anacrotine	0.5	0.40	0.61
Integerrimine	2	0.62	0.82
Jacobine	1	0.37	0.79
Jacoline	0.5	0.29	0.52
Jaconine	2	0.61	0.79
Monocrotaline	1	0.39	0.63
Monocrotaline N-oxide	—	0.17	0.05
Platyphylline	25	0.46	0.78
Retronecine	<0.5	0.05	0.21
Retorsine	2	0.35	0.54
Riddelliine	2	0.34	0.54
Riddelliine N-oxide	—	0.19	0.04
Senecione	1	0.62	0.82
Senecione N-oxide	—	0.38	0.15
Seneciphylline	2	0.61	0.82
Spectabiline	2	0.37	0.68

[a]Solvent 1 = chloroform-methanol-17% ammonium hydroxide (165:31:4).

[b]Solvent 2 = chloroform-acetone-ethanol-ammonium hydroxide (5:3:1:1).

Source: Adapted from Ref. 73.

TABLE 3 TLC of Alkaloids

Alkaloid	R_f			Color
	Solvent 1[a]	Solvent 2[b]	Solvent 3[c]	
Aconitine	0.68	0.35	0.49	Red-brown
Ajmaline	0.47	0.12	0.03	Beige
Apoatropine	0.54	0.40	0.26	Violet-blue
Arecoline	0.66	0.56	0.48	White
Aspidospermine	0.65	0.54	0.49	White
Atropine	0.38	0.16	0.12	Violet-blue
Boldine	0.16	0.03	0.05	Beige
Brucine	0.42	0.18	0.19	Violet-brown
Bulbocapnine	0.65	0.35	0.54	White
Cephaeline	0.56	0.19	0.23	White
Chinchonine	0.38	0.17	0.27	Beige
Cocaine	0.73	0.65	0.58	Violet
Codeine	0.38	0.16	0.26	Magenta
Colchicine	0.47	0.04	0.04	Pale gray
Cotarnine	0.60	0.43	0.45	Violet

TABLE 3 (continued)

Alkaloid	R_f Solvent 1[a]	Solvent 2[b]	Solvent 3[c]	Color
Cupreine	0.03	0.00	0.00	Red-brown
Dihydrocodeine	0.38	0.18	0.28	Violet-blue
Dihydrocodeinone	0.51	0.21	0.30	Violet
Dihydroergocristine	0.42	0.03	0.07	Brown
Dihydroergotamine	0.21	0.00	0.03	Brown
Dihydromorphinone	0.24	0.08	0.11	Brown-yellow
Emetine	0.67	0.40	0.45	Red-brown
Ergocristine	0.51	0.14	0.13	Beige
Ergocristanine	0.61	0.13	0.20	Pale brown
Ergometrine	0.14	0.00	0.02	White
Ergometrinine	0.42	0.03	0.08	Violet-blue
Ergotamine	0.24	0.00	0.03	Rose
Ergotaminine	0.24	0.00	0.14	Rose
Homatropine	0.37	0.15	0.23	Violet-blue
Hordenine	0.33	0.14	0.28	White
Hydrastinine	0.66	0.58	0.50	Violet-blue
Lobeline	0.68	0.48	0.48	Red-brown
Morphine	0.10	0.00	0.03	Deep blue
Narceine	0.03	0.00	0.00	Deep blue
Narcotine	0.72	0.51	0.57	Pale yellow
Papaverine	0.67	0.42	0.47	Yellow
Physostigmine	0.65	0.32	0.44	Rose
Pilocarpine	0.41	0.09	0.13	Pale brown
Psicaine	0.66	0.60	0.53	Yellow
Quinidine	0.33	0.15	0.25	Pale yellow
Quinine	0.19	0.07	0.17	Yellowish
Rauwolscine	0.55	0.18	0.36	Pale yellow
Reserpine	0.72	0.20	0.46	White
Sarpagine	0.12	0.00	0.00	Beige
Scopolamine	0.56	0.19	0.34	Violet
Scopoline	0.60	0.44	0.44	White
Serpentine	0.24	0.00	0.04	Red-brown
Sparteine	0.70	0.68	0.55	Violet
Strychnine	0.53	0.28	0.38	Yellow
Thebaine	0.65	0.51	0.50	Red-brown
Tropacocaine	0.65	0.56	0.45	Violet
Yohimbine	0.63	0.18	0.37	Violet-brown

[a]Solvent 1 = chloroform-acetone-diethylamine (5:4:1).

[b]Solvent 2 = cyclohexane-chloroform-diethylamine (5:4:1).

[c]Solvent 3 = benzene-ethyl acetate-diethylamine (7:2:1).