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CHOICE OF THIN-LAYER CHROMATOGRAPHIC SYSTEMS FOR THE ROUTINE SCREENING FOR ACIDIC DRUGS DURING TOXICOLOGICAL ANALYSES

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SUMMARY

The efficiencies of fifteen thin-layer silica gel chromatographic systems in separating commonly encountered acidic drugs are compared. The discriminating powers of the systems are measured both individually and in combination. Ethyl acetate and chloroform–methanol (9:1) are found to be the two best systems. The combination of the ethyl acetate–methanol–ammonia (85:10:5) system with either of these gives the best pair of systems. Various sequences of spray reagents are also examined.

INTRODUCTION

Thin-layer chromatography (TLC) is an ideal technique for the screening of drugs in toxicological analyses because of low cost, easy maintenance and selectivity of detection reagents. However, the analyst is often faced with the problem of selecting the most suitable system. Recently much effort has been applied to the production of objective criteria with which to evaluate the separating ability of TLC systems. Numerical taxonomy has been used to classify systems according to their similarities[1], but the measurement of the informing power[2–4] or discriminating power[5,6] is more useful when the selection of optimal systems is required. Discriminating power measurements enable correlations between TLC systems to be made more easily than informing power measurements.

Moffat and Clare[7] have previously used discriminating power to select the more suitable TLC systems for the analyses of basic drugs. The factors they considered to be most important were (1) distribution of chromatographic values over the useful range of the system; (2) correlation between systems when more than one is used; (3) speed; (4) reproducibility and (5) sensitivity. The discriminating power (*i.e.*, the probability that two drugs selected at random will be discriminated) of a

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system or combination of systems is an objective criterion which can be used to assess the first two of these factors.

When the better systems for the analyses of basic drugs were chosen, standardisation of results from different laboratories occurred and chromatographic data became transferable from laboratory to laboratory[8]. In this work a similar selection procedure has been used to choose TLC systems for acidic drugs. We also considered that a reasonable spread of R_F values for important classes of drugs (*e.g.*, barbiturates) was important.

The systems were chosen from; those included in standard texts of drug analysis[9–11]; those used by British forensic science laboratories and literature surveys of TLC systems used for the general screening of drugs[12–14], and also specific drugs *e.g.*, barbiturates[15–19] and thiazide diuretics[20,21]. Only those systems which appeared to show major differences from commonly used systems were abstracted.

The measurement of R_F values without the use of reference compounds, run at the same time, is prone to systematic errors and the use of defined substances as reference compounds with which to convert the practically obtained R_F values to corrected values is now universally accepted. The use of reference compounds has resulted in a significant decrease in the interlaboratory variation in mean R_F values for basic drugs[8]. Since correction graphs, *i.e.*, plots of experimentally obtained R_F values against mean accepted R_F values for the reference compounds, are not always linear[8,22] it is better to have reference compounds that will give a spread of R_F values over the whole system. Therefore four standard reference compounds were chosen for each selected system.

Various sequences of spray reagents were also examined.

MATERIALS AND METHODS

The fifteen systems used are given in Table I. All systems used silica gel plates (20 × 20 cm, 0.25 mm thickness) incorporating a fluorescent indicator (E. Merck,

TABLE I

THIN-LAYER SYSTEMS STUDIED

System No.	*Solvent*
1	Chloroform–acetone (9:1)
2	Chloroform–acetone (4:1)
3	Acetic acid–toluene–ether–methanol (18:120:20:1)
4	Isopropanol–chloroform–ammonia (45:45:10)
5	Chloroform
6	Ethyl acetate–methanol–ammonia (85:10:5)
7	Hexane–ethanol (9:1)
8	Cyclohexane–toluene–acetic acid (75:15:10)
9	Toluene–acetic acid (9:1)
10	Ethyl acetate
11	Dioxane–toluene–ammonia (20:75:5)
12	Chloroform–ethanol (95:5)
13	Acetone
14	Chloroform–methanol (9:1)
15	Cyclohexane–toluene–diethylamine (75:15:10)

Darmstadt, G.F.R.). Paper liners were used in all the tanks and after the addition of the appropriate solvents the systems were allowed to equilibrate for at least 30 min. The drugs were dissolved in either methanol or chloroform; 5-μg quantities were applied to the plates and the systems allowed to run for 10 cm.

Drugs were detected by their UV absorption at 254 and 350 nm and by the following four spray reagents.

Mercuric chloride–diphenylcarbazone: new solution prepared daily containing (a) diphenylcarbazone 0.1 g in 50 ml ethanol; (b) 1 g mercuric chloride in 50 ml ethanol. Solutions (a) and (b) were mixed just before spraying.

Acidified potassium permanganate: 1 g potassium permanganate in 100 ml 0.25 *M* sulphuric acid.

Van Urk: 1 g *p*-dimethylaminobenzaldehyde in 90 ml ethanol + 10 ml conc. hydrochloric acid. The plates were heated at 100° for 5 min after spraying.

Ferric chloride: Ferric chloride (5%, w/v) aqueous solution.

A total of 51 acidic drugs were selected as representative of those that occurred during toxicological examinations. They were chosen from those submitted to British forensic science laboratories and from those commonly occuring in poisoning cases in England and Wales[23]. In the preliminary experiments 23 acidic drugs (Table II) were run in all the systems to exclude the poorer ones. After this preliminary screening, the 51 acidic drugs were run in the six more discriminating systems. Calculations of discriminating power for the TLC systems, both alone and in combination, were made as previously reported[5–7].

RESULTS AND DISCUSSION

Table II lists those drugs which absorbed UV light at 254 and 350 nm and also their response to spray reagents. Fluorescence after irradiation at 254 nm is also included. Barbiturate absorption at 254 nm was often poor unless the plate was run under alkaline conditions. The developed thin-layer chromatograms were also sprayed with 1% KOH in ethanol to increase the UV absorption of barbiturates in neutral systems. The mercuric chloride–diphenylcarbazone spray is a common detection reagent for barbiturates; however, other drugs also respond to this spray. Normally, white spots are obtained on a purple background. In contrast, lilac-purple spots were obtained on a pink background after the barbiturates had been run in alkaline systems (*e.g.*, system 6). If plates run in alkaline systems were heated before spraying (100° for 5 min) barbiturates again showed as white spots on a purple background.

The four sprays used to detect acidic drugs were diphenylcarbazone–mercuric chloride, acidified permanganate, Van Urk reagent and ferric chloride. An attempt was made to overspray one reagent with another. However, the incompatibility of several of these sprays and the confusing results obtained by overspraying indicated that it would be better to spot two aliquots of drug on the plate. The first should be sprayed with diphenylcarbazone–mercuric chloride reagent followed by acidified permanganate and the second should be sprayed with Van Urk reagent and oversprayed with ferric chloride. It was better to fade the background on the diphenylcarbazone–mercuric chloride sprayed plate by heating in an oven at 100° before spraying with acidified permanganate.

Fig. 1 shows the distribution of R_F values of the 23 acidic drugs in the 15

TABLE II

UV ABSORPTION AND REACTION TO SPRAY REAGENTS OF THE ACIDIC DRUGS
+ = Positive reaction; F = fluorescence; p = purple; w = white; y = yellow; b = brown; bl = blue; ft = faint; pk = pink; r-b = reddish brown; w-bl = whitish blue; pk-b = pinkish brown.

Drug	*UV (nm)*		*DPC–$HgCl_2$*	*Acidified $KMnO_4$*	*Van Urk*	*$FeCl_3$*
	254	*350*				
Amylobarbitone*	+		+			
Barbitone*	+		+			
Butobarbitone	+		+			
Cyclobarbitone	+		+	+		
Hexobarbitone	+		+	+		
Methohexitone	+	+	+	+		
Pentobarbitone	+		+			
Phenobarbitone*	+		+			
Quinalbarbitone*	+		+	+		
Thiopentone*	+		+	+		
Bemegride*	+		+			
Glutethimide*	+		+			
Ethosuximide	+					
Phensuximide	+			+		
Primidone*	+					
Phenytoin*	+		+			
Bendrofluazide	Fp	+				
Benzthiazide	+					
Chlorothiazide	+		+			
Hydrochlorothiazide*	+		+			
Hydroflumethiazide	Fp	+		+ft		
Frusemide*	+	+		+	+pk-b	
Sulphacetamide*	+		+bl	+	+y	
Sulphadimidine	+		+bl	+	+y	
Sulphafurazole	+		+bl	+	+y	
Sulphamethizole	+		+bl	+	+y	
Sulphamethoxazole	+		+bl	+	+y	
Sulphanilamide*	+		+bl	+	+y	
Sulphathiazole	+		+bl	+	+y	
Phenazone	+		+	+	+pk	+r-b
Acetylsalicylic acid*	+					
p-Aminophenol*	+			+	+y	+p**
Indomethacin	+			+		
Saccharin*	+					
Salicylic acid*	Fp	+		+		+p
p-Aminobenzoic acid	+			+	+y	
p-Aminosalicylic acid	Fbl			+	+y	+p**
Benzoic acid	+					
Gentisic acid	Fp	+		+		+y
Carbenoxolone	+			+ft		
Sulthiame*	+					
Chlorpropamide*	+					
Lysergic acid	Fp	+	+bl	+	+p	
Methyldopa	+		+w-bl	+	+p	
Nalidixic acid	+					
Nicotinic acid	+					
Dicoumarol*	+	+		+		
Paracetamol*	+			+		ft-bl
Warfarin*	+			+		
Salicylamide*	Fp	+		+		+p
Phenolphthalein	+			+		

* Drugs used in preliminary screen.

** Overspraying with $FeCl_3$ resulted in a spot which was a mixture of yellow and purple (reddish brown).

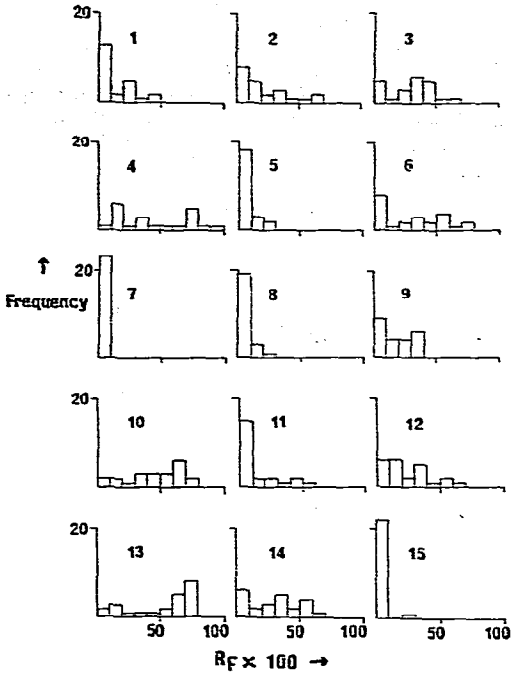

Fig. 1. Frequency distribution of $R_F \times 100$ values of 23 acidic drugs in 15 TLC systems (for identification of systems see Table I).

TLC systems. The systems with the poorest separation of drugs were those of low polarity *viz.* chloroform (5), hexane–ethanol (7), cyclohexane–toluene–acetic acid (8) and cyclohexane–toluene–diethylamine (15). In each of these four systems the large hydrocarbon content of the solvent did not overcome the adsorptive power of the silica which led to very low R_F values for the drugs. Toluene–acetic acid (9) gave a better spread of R_F values, as did dioxane–toluene–ammonia (11), but in both cases the distribution of R_F values was not sufficiently large to be useful. The more discriminating systems were the more polar ones, *i.e.*, alcohols or ketones in combination with chlorinated hydrocarbons, or solvents such as acetone or ethyl acetate.

As expected, systems 1 and 2, which both contain chloroform and acetone, (9:1 and 4:1, respectively) were highly correlated ($r = 0.97$), with system 2 having a better spread of R_F values. Similarly, the chloroform–ethanol (12) and chloroform–methanol (14) systems were highly correlated ($r = 0.96$).

All the systems, with the exception of the isopropanol–chloroform–ammonia (4) system, gave reproducible results. As system 4 also took 1½ h to run, compared with 30 min for the other systems, it was discarded. After consideration of the features mentioned above, systems 2, 3, 6, 10, 13 and 14 were chosen for further examination and all 51 drugs were run on these six systems. The results are given in Table III. Drugs were grouped, where possible, according to their chemical or pharmacological nature.

From the data in Table III the discriminating powers of the individual systems and the paired systems were calculated (Tables IV and V, respectively). It was con-

TABLE III

$R_F \times 100$ VALUES OF THE ACIDIC DRUGS IN SIX SELECTED TLC SYSTEMS

Drug	*Solvent system*					
	2 Chloroform–acetone (4:1)	*3 Acetic acid–toluene–ether–methanol (18:120:20:1)*	*6 Ethyl acetate–methanol–ammonia (85:10:5)*	*10 Ethyl acetate*	*13 Acetone*	*14 Chloroform–methanol (9:1)*
Amylobarbitone	48	37	49	66	78	57
Barbitone	38	30	36	61	75	51
Butobarbitone	48	42	45	66	79	54
Cyclobarbitone	48	38	36	63	77	53
Hexobarbitone	63	43	59	66	79	68
Methohexitone	76	46	70	72	82	74
Pentobarbitone	47	39	45	68	79	55
Phenobarbitone	38	34	27	63	74	52
Quinalbarbitone	52	42	56	69	77	51
Thiopentone	71	48	62	73	79	72
Bemegride	50	34	70	55	76	62
Glutethimide	60	40	77	63	76	71
Ethosuximide	50	35	65	57	75	56
Phensuximide	67	43	75	61	77	76
Primidone	9	26	33	22	60	31
Phenytoin	30	38	42	66	72	51
Bendrofluazide	23	10	70	73	81	36
Benzthiazide	13	6	10	48	81	30
Chlorothiazide	3	1	4	21	66	10
Hydrochlorothiazide	4	2	31	40	71	10
Hydroflumethiazide	7	4	41	48	77	12
Frusemide	2	21	4	10	19	7
Sulphacetamide	17	6	1	44	72	30
Sulphadimidine	22	13	11	46	75	43
Sulphafurazole	22	13	4	52	76	33
Sulphamethizole	8	9	6	28	63	30
Sulphamethoxazole	25	20	6	57	77	40
Sulphanilamide	14	6	53	56	74	24
Sulphathiazole	7	6	6	22	64	28
Phenazone	14	8	41	7	24	46
Acetylsalicylic acid	8	38	7	16	21	18
p-Aminophenol	18	0	55	44	63	30
Indomethacin	10	42	4	25	37	35
Saccharin	0	17	4	1	12	4
Salicylic acid	5	43	7	7	8	9
p-Aminobenzoic acid	16	36	3	42	60	28
p-Aminosalicylic acid	1	34	3	11	5	7
Benzoic acid	20	43	5	31	44	32
Gentisic acid	2	26	5	10	12	5
Carbenoxolone	6	37	1	20	33	39
Sulthiame	23	10	52	42	76	42
Chlorpropamide	35	43	8	55	71	41
Lysergic acid	0	0	1	0	0	0
Methyldopa	0	0	0	0	0	0
Nalidixic acid	27	24	3	23	48	53
Nicotinic acid	3	9	2	2	4	6
Dicoumarol	13	57	26	32	72	31
Paracetamol	14	7	42	35	64	28
Warfarin	61	36	11	73	80	60
Salicylamide	34	35	49	56	74	41
Phenolphthalein	36	25	54	59	76	40

TABLE IV

DISCRIMINATING POWERS FOR SIX TLC SYSTEMS

	System No.					
	2	*3*	*6*	*10*	*13*	*14*
Discriminating power*	0.71	0.62	0.69	0.74	0.60	0.74
Spread of $R_F \times 100$ values** of						
Barbiturates	38	18	43	12	8	23
Thiazide diuretics	20	9	66	62	15	26
Sulphonamides	18	14	52	35	14	19

* D.P. values were calculated using an error factor of 10 in $R_F \times 100$.
** The differences between the largest and smallest $R_F \times 100$ value for drugs in that group.

TABLE V

DISCRIMINATING POWERS FOR PAIRS OF CHROMATOGRAPHIC SYSTEMS

Values were calculated using an error factor of 10 in $R_F \times 100$ for each system.

System	*3*	*6*	*10*	*13*	*14*
2	0.86	0.86	0.88	0.87	0.83
3		0.89	0.86	0.84	0.88
6			0.90	0.89	0.90
10				0.86	0.89
13					0.87

sidered important that various groups of acidic drugs, *viz.* barbiturates, thiazide diuretics and sulphonamides, should be separated within their respective groups, hence the difference between the largest and smallest R_F values for drugs in each group were calculated (Table IV). From Table IV it can be seen that the ethyl acetate (10) and chloroform–methanol (14) systems each had the highest discriminating power (DP = 0.74) and would be the best screening systems to use for the separation of acidic drugs. However these systems showed a poor separation of barbiturates and a reasonable separation of barbiturates was regarded as an important consideration for the TLC system, as these were one of the commonest type of drugs encountered in fatal poisonings. It would therefore be important to pair system 10 or 14 with either the ethyl acetate–methanol–ammonia (6) or chloroform–acetone (2) systems which both showed a good separation of barbiturates. The highest combined discriminating power was obtained by combining system 6 with system 10 or 14 (DP = 0.90, Table V). Strongly acidic drugs containing carboxyl groups were observed to streak badly in systems 2, 10 and 14. The effect was not particularly noticeable in system 6 as it was an alkaline system and strongly acidic drugs were retained near the baseline. More important was the observation that the R_F values of strongly acidic drugs containing carboxylic groups increased as the amount applied to the TLC plate was increased. Applications of 20 μg of these drugs showed consistent small increases in R_F values compared with 5-μg applications (Table VI). Larger applications showed even higher R_F values. Other carboxylic acids in this study which behaved similarly were frusemide, *p*-aminosalicylic acid, carbenoxolone, nalidixic acid and indomethacin. However, if these acids were run in the acetic acid–toluene–ether–methanol (3)

TABLE VI

THE EFFECT OF INCREASING THE CONCENTRATION OF THE STRONGLY ACIDIC DRUGS ON $R_F \times 100$ VALUES

For solvent systems, see Table I.

Drug	$R_F \times 100$									
	System									
	10		*6*		*2*		*3*		*14*	
	5 μg	*20 μg*	*5 μg*	*20 μg*	*5 μg*	*20 μg*	*5 μg*	*20 μg*	*5 μg*	*20 μg*
Bendrofluazide*	65	65	68	68	29	29	14	14	33	34
Benzoic acid**	26	34	2	2	19	24	48	49	29	35
Gentisic acid**	4	8	3	3	1	5	29	30	2	5
Nicotinic acid**	0	2	1	1	2	3	10	10	2	5
Aspirin**	13	19	6	6	8	14	43	43	20	28
Chlorpropamide*	37	41	8	8	38	41	45	45	50	51
Salicylic acid**	6	12	8	9	5	11	46	46	8	12
p-Aminobenzoic***	39	43	1	1	18	22	37	38	25	26

* No streaking of spots observed.

** Drugs streak badly in all systems except 3 in which distinct round spots were observed (all contain carboxylic groups).

*** Slight streaking of spots observed.

system no altered R_F values were observed as different quantities of acid were spotted onto the plate. Distinct round spots were also obtained with this system. The acetic acid decreases the ionisation of these acids and presumably also inactivates many of the acidic adsorbing sites on the silica plate. A better spread of R_F values for the strongly acidic drugs was obtained with this system than the cyclohexane–toluene–acetic acid (8) or toluene–acetic acid (9) systems.

The choice of a TLC system for the routine screening of acidic drugs during toxicological analyses is therefore somewhat of a compromise. The first choice would be either the ethyl acetate or chloroform–methanol system. If more discrimination

TABLE VII

REFERENCE COMPOUNDS FOR USE WITH THE RECOMMENDED TLC SYSTEMS FOR ACIDIC DRUGS

Solvent	*Compounds*	$R_F \times 100$
Ethyl acetate (85)	Prazepam	81
Methanol (10)	Temazepam	63
Ammonia (5)	Hydrochlorothiazide	34
	Sulphadimidine	13
Ethyl acetate	Quinalbarbitone	68
	Salicylamide	55
	Phenacetin	38
	Sulphathiazole	20
Chloroform(9)	Prazepam	72
Methanol (1)	Phenacetin	52
	Sulphafurazole	33
	Hydroflumethiazide	13

is required either of these should be combined with the ethyl acetate–methanol–ammonia system. Suitable reference compounds for use with these systems are given in Table VII. If it is only necessary to chromatograph strongly acidic drugs the acetic acid–toluene–ether–methanol system should be used to prevent changes in R_F values caused by carboxylic groupings. In the authors' experience methanol had very little effect in this system and could be removed.

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