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

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SUMMARY

The efficiencies of fifteen thin-layer silica gel chromatographic systems for separating commonly encountered neutral drugs are compared. The discriminating powers of the systems are measured both individually and in combination. Chloroform–acetone (4:1) is found to be the best system. The combination of the ethyl acetate–methanol–ammonia (85:10:5) system with this gives the best pair of systems. A suitable sequence of spray reagents is also suggested. The chloroform–acetone (4:1) system is recommended as the best system to use when screening for both acidic and neutral drugs.

INTRODUCTION

The measurement of discriminating power[1] has been previously used to select thin-layer chromatographic (TLC) systems which efficiently separate basic[2,3] and acidic drugs[4]. The concepts used in these papers have now been applied to neutral drugs. It would obviously be advantageous if the neutral drugs could be efficiently separated in the systems selected for either basic or acidic drugs. The previously recommended systems[2–4] have therefore been used in this study. In addition, TLC systems used for the benzodiazepines[5,6], a commonly occurring group of neutral drugs, have also been considered.

Four standard reference compounds were chosen for each recommended system. Sequences of spray reagents were also examined.

MATERIALS AND METHODS

The fifteen TLC systems examined (Table I) were the same as those selected for acidic drugs[4]. The silica gel plates and experimental procedure were also as previously reported for the acidic drugs[4].

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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)

The neutral drugs were also run in the three recommended basic drugs TLC systems, *i.e.*, the acetone (13) chloroform–methanol (14) and cyclohexane–toluene–diethylamine (15) systems with KOH treated silica gel plates[3]. The drugs were detected by their UV absorption at 254 and 350 nm and by the following three spray reagents.

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

Furfural reagent: (a) furfuraldehyde (redistilled)–acetone (2:98); (b) conc. sulphuric acid–acetone (4:96). Solution (a) was sprayed first, then solution (b). They were prepared immediately before use.

Acidified potassium iodoplatinate: 5 g potassium iodide in 5 ml platinic chloride solution (5%, w/v) + 5 ml conc. hydrochloric acid. The solution was made up to 100 ml with water.

A total of 34 neutral 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 occurring in poisoning cases in England and Wales[7].

In the preliminary experiments, nine neutral drugs were run in all the systems to exclude the poorer ones. After this preliminary screening the 34 neutral drugs were run in the ten more discriminating systems. Calculations of discriminating power for the TLC systems, both alone and in combination, were made as previously reported[1–4].

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. The three sprays used to detect neutral drugs were acidified potassium permanganate, furfural reagent and acidified iodoplatinate. An attempt was made to overspray one reagent with another. Furfural reagent cannot be sprayed over

TABLE II

UV ABSORPTION AND REACTION TO SPRAY REAGENTS OF THE NEUTRAL DRUGS
+ = positive reaction, Fp = pink fluorescence.

Drug	*UV (nm)*		*Furfural*	*Acidified permanganate*	*Acidified iodoplatinate*
	254	*350*			
Clonazepam	+			+	+
Demoxepam	+			+	+
Lorazepam	+			+	+
Oxazepam	+			+	+
Prazepam	+			+	+
Temazepam	+			+	+
Carisoprodol			+		
Chlorphenesin carbamate	+		+		
Mephenesin carbamate	+		+		
Meprobamate			+		
Methocarbamal	+		+	+	
Methylpentynol carbamate			+	+	
Ethinamate			+	+	
Phenprobamate	+		+		
Styramate	+		+		
Tybamate			+		
Acetanilide	+			+	
Acetylcarbromal	+				
Apronal				+	
Benzocaine	+			+	
Carbromal	+				
Carbimazole	+			+	+
Coumatetralyl	Fp	+		+	
Ethylbiscoumacetate	+	+		+	
Furazolidine	+	+		+	
Diphenadione	+	+		+	
Nicoumalone	+	+		+	+
Phenacemide	+				
Phenacetin	+			+	
Pheneturide	+				
Santonin	+			+	+
Tolazamide	+			+	
Tolbutamide	+				
Tropine				+	+

acidified permanganate and *vice versa*. Acidified iodoplatinate can be sprayed over either of these reagents. The recommended procedure is to spot two samples of drug on the plate, spray the first with furfural reagent, the second with acidified potassium permanganate and then to overspray both with acidified iodoplatinate.

After the preliminary screening the chloroform (5), hexane–ethanol (7) and cyclohexane–toluene–acetic acid (8) systems were discarded as they showed the poorest separation of neutral drugs. All these systems were of low polarity. It appeared that 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. As with the acidic drugs, 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 contained chloroform and acetone (9:1 and 4:1, respectively), were highly correlated ($r = 0.97$). System 1 was discarded as it had a poorer spread of R_F values. Similarily, the chloroform–ethanol (12) and chloroform–methanol (14) systems were highly correlated ($r = 0.98$). System 14 was retained in preference to system 12; the former had previously been selected for basic drugs and it would be convenient if it could also be used for neutral drug separations.

Alkali treated plates were used in all the systems selected for basic drugs[3] *viz.* acetone (13) chloroform–methanol (14) and cyclohexane–toluene–diethylamine (15). However, alkali treatment of plates in these systems showed no marked effect on the separations obtained for the neutral drugs.

Fig. 1 shows the distribution of the R_F values of the 34 neutral drugs in the ten better TLC systems. Since the toluene–acetic acid (9), acetone (13) and cyclohexane–toluene–diethylamine (15) systems showed a poor spread of R_F values they were discarded. The isopropanol–chloroform–ammonia (4) system was also discarded at this stage because it had poor reproducibility and it took 90 min to run compared with 30 min for other systems.

The R_F values of the 34 neutral drugs in the six remaining TLC systems are given in Table III.

From the data in Table III the discriminating powers of the individual and the paired systems were calculated (Tables IV and V). It was also considered important that the benzodiazepines should be separated if possible. The difference

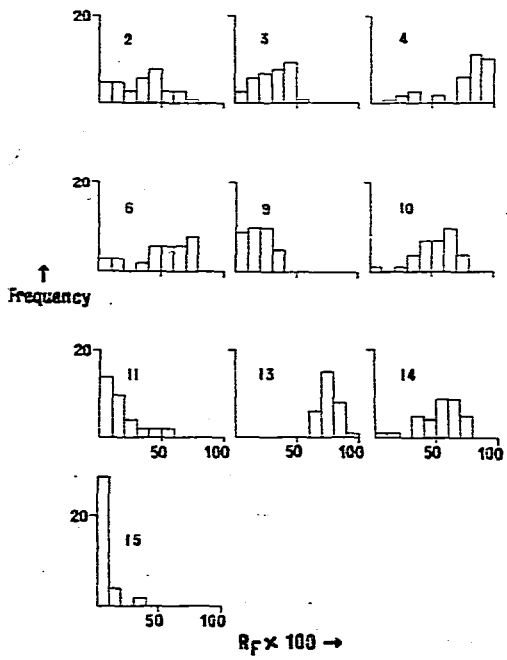

Fig. 1. Frequency distribution of $R_F \times 100$ values of 34 neutral drugs in the 10 more discriminating TLC systems (for identification of systems see Table I).

TABLE III

$R_F \times 100$ VALUES OF NEUTRAL DRUGS IN SIX SELECTED TLC SYSTEMS
For solvent systems, see Table I.

	Solvent system					
Drug	*2*	*3*	*6*	*10*	*11*	*14*
Clonazepam	34	26	64	53	13	56
Demoxepam	16	15	38	30	2	41
Lorazepam	20	16	40	48	5	44
Oxazepam	18	17	38	44	5	43
Prazepam	60	36	77	65	53	75
Temazepam	48	27	58	56	21	69
Carisoprodol	34	35	71	63	18	57
Chlorphensin carbamate	12	15	47	44	57	31
Mephenesin carbamate	13	15	52	45	5	39
Meprobamate	9	17	57	41	3	34
Methocarbamol	9	9	40	33	2	36
Methylpentynol carbamate	47	41	70	69	28	56
Ethinamate	49	42	70	70	27	60
Phenprobamate	49	42	72	68	30	62
Styramate	12	21	54	49	3	30
Tybamate	35	38	68	65	18	54
Acetanilide	42	23	66	52	19	50
Acetylcarbromal	48	26	50	58	10	62
Apronal	33	44	67	64	17	58
Benzocaine	59	40	74	75	36	69
Carbromal	52	42	71	65	28	67
Carbimazole	64	28	41	55	7	77
Coumatetralyl	73	49	13	74	40	78
Ethylbiscoumacetate	3	31	19	33	8	19
Furazolidine	23	7	42	26	10	53
Diphenadione	8	53	40	32	6	55
Nicoumalone	53	36	13	63	3	61
Phenacemide	20	38	58	51	10	47
Phenacetin	37	21	63	43	16	53
Pheneturide	30	43	66	63	14	61
Santonin	65	33	71	60	42	78
Tolazamide	42	39	6	52	0	71
Tolbutamide	49	44	8	72	0	66
Tropine	0	0	7	0	6	0

between the largest and smallest R_F (Table IV) for these drugs in each system was considered as an indication of the separation.

The chloroform–acetone (2) system was the most discriminating and would be the best system to use for the separation of neutral drugs (Table IV). System 2 also produced a good separation of benzodiazepines. The highest combined discriminating power (DP) was obtained by combining the ethyl acetate–methanol–ammonia (6) system with system 2 ($DP_{2,6} = 0.88$). Neutral drugs should not be run in the recommended basic drug TLC systems[3] as poor spreads of R_F values were obtained in two of these systems.

The first choice of a TLC system for the routine screening of neutral drugs is therefore the chloroform–acetone (2) system and if more discrimination is required

TABLE IV

DISCRIMINATING POWERS FOR SIX TLC SYSTEMS

	System No.					
	2	*3*	*6*	*10*	*11*	*14*
Discriminating power*	0.75	0.60	0.70	0.64	0.57	0.66
Spread of $R_F \times 100$ values of benzodiazepines**	44	21	39	35	51	34

* D.P. values were calculated using an error factor of 10 in $R_F \times 100$ values.
** The difference 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*	*11*	*14*
2	0.85	0.88	0.86	0.83	0.83
3		0.81	0.73	0.81	0.80
6			0.83	0.82	0.85
10				0.82	0.82
11					0.82

TABLE VI

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

Solvent	*Compounds*	$R_F \times 100$
Chloroform (4)	Methohexitone	73
Acetone (1)	Quinalbarbitone	55
	Clonazepam	35
	Paracetamol	15
Ethyl acetate (85)	Prazepam	81
Methanol (10)	Temazepam	63
Ammonia (5)	Hydrochlorothiazide	34
	Sulphadimidine	13

this should be combined with the ethyl acetate–methanol–ammonia (6) system. Suitable reference compounds for use with these two systems are given in Table VI.

If one were to select a TLC system for both acidic and neutral drugs the chloroform–acetone (2) system would be most appropriate because it showed a good separation of acidic drugs and it was one of the better systems for separating neutral drugs. Jackson and Clatworthy[8] have previously recommended this system.

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