J. Pharm. Pharmac., 1974, 26, 665–670 *Received February* 13, 1974

The choice of paper and thin-layer chromatographic systems for the analysis of basic drugs

A. C. MOFFAT AND B. CLARE

Home Office Central Research Establishment, Aldermaston, Berks, RG7 4PN, U.K.

Thirty-seven paper and thin-layer chromatographic systems in general use for the analysis of basic drugs have been examined. Their discriminating powers were measured, both individually and when used in combination. The better systems were found to be thin-layer systems of silica gel sprayed with 0·1N NaOH, dried and run using one of the following solvents: (1) chloroform–methanol (90:10), (2) cyclohexane–toluene–diethylamine (75:15:10) and (3) acetone. A thin-layer cellulose system using n-butanol–water–citric acid (87:13:0·48) was suitable if speed was not a requirement and a reversed phase paper system run with an aqueous buffer solution (pH 4·58) at 95° was the fastest system examined. Any of these five systems could be used in combination since their correlation coefficients were never higher than 0·61.

An analyst is often faced with the problem of having to choose a paper or thin-layer chromatographic system, from the hundreds that have been used, for the identification of basic drugs. The collections of R_F data for paper chromatography (Fox, 1969; Sunshine, 1969; Macek, 1972) and thin-layer chromatography (Curry, 1969; Gänshirt, 1969; Šantavý, 1969; Sunshine, 1969; Dumont, Jork & others, 1973; Macek, 1972) are of great help, as are the comprehensive surveys of the literature that are available (Macek, Hais & others, 1968, 1972). However, few comparisons have been made to determine which are the most effective for identification procedures and a need therefore exists for such a comparison to be made. Once the better systems have been chosen, standardization by laboratories on these could occur to enable analyses to be performed more efficiently and chromatographic data would subsequently be easily transferable from laboratory to laboratory.

The most important features of individual systems to be considered are (a) speed, (b) sensitivity, (c) reproducibility, (d) distribution of chromatographic values over the useful range of the system and (e) correlations between systems when more than one is used. A compromise between all the above factors must be obtained to choose the better systems.

In a previous publication (Moffat, Smalldon & Brown, 1974) we presented a method for comparing systems in terms of (c), (d) and (e) by the calculations of the discriminating powers of the individual systems or combination of systems. (The discriminating power is defined as the probability that two drugs selected at random from a large population would be discriminated.) This allows a single value to be assigned to a system, or combination of systems, which is a measure of its effectiveness for the identification of unknown compounds. Eight paper and thin-layer systems

have been examined in this way (Moffat & Smalldon, 1974) and we have now extended the study to include 37 of the paper and thin-layer chromatographic systems in common use.

MATERIALS AND METHODS

The collections of chromatographic data listed above were examined to find those systems in which more than 20 basic drugs had been chromatographed. A total of 11 paper and 26 thin-layer systems were picked and their respective discriminating powers calculated using the original data of each author; of these 37 systems, 14 were chosen as being the better systems and these have been examined further.

One hundred drugs, which are chemically and pharmacologically representative of the basic drugs in common use, were chromatographed using the paper and thin-layer systems listed in Table 1. Systems 1 to 8 in this Table have been reported previously (Moffat & Smalldon, 1974). The other systems used plates which were 20 × 20 cm, had a coating thickness of 0·25 mm, incorporated a fluorescent indicator and were supplied by E. Merck (Darmstadt). All the plates were used as supplied by the manufacturer, except those sprayed with 0·1N sodium hydroxide which were

Table 1. *Paper and thin-layer systems studied.*

System No.	Adsorbent or paper		Discriminating* power
1	Silica gel dipped or prepared with 0·1N KOH	Cyclohexane–benzene–diethylamine (75:15:10)	0·73
2	Silica gel dipped or prepared with 0·1N KOH	Methanol	0·69
3	Silica gel dipped or prepared with 0·1N KOH	Acetone	0·75
4	Silica gel dipped or prepared with 0·1N $KHSO_4$	Methanol	0·66
5	Silica gel dipped or prepared with 0·1N $KHSO_4$	Ethanol–water (95:5)	0·67
6	Whatman No. 1 paper dipped in 5% sodium dihydrogen citrate	n-Butanol–water–citric acid (87:13:0·48)	0·74
7	Whatman No. 1 paper dipped in 10% tributyrin in acetone	Acetate buffer (pH 4·58), run at 95°	0·75
8	Whatman No. 1 paper dipped in 10% tributyrin in acetone	Phosphate buffer (pH 7·4), run at 86°	0·55
9	Silica gel 60	Ethyl acetate–n-heptane–methanol–ammonia (0·88), (60:30:7·5:2·5)	0·72
10	Aluminium oxide (Type E)	Chloroform	0·71
11	Silica gel 60	Dioxan–chloroform–ethyl acetate–ammonia (0·88), (60:25:10:5)	0·57
12	Silica gel 60	Methanol–ammonia (0·88) (100:1·5)	0·63
13	Silica gel 60 sprayed with 0·1N NaOH	Cyclohexane–toluene–diethylamine (75:15:10)	0·76
14	Silica gel 60 sprayed with 0·1N NaOH	Chloroform–methanol (90:10)	0·78

* Calculated using an error factor of 0·1 in R_F for each system.

dried in an oven at 110° for 20 min and then left at room temperature (20°) for 30 min before use.

Approximately 5 μg quantities of the drugs were applied as aqueous solutions of their salts. The plates were run for a distance of 10 cm in equilibrated tanks. Spots were located by (a) using ultraviolet light (254 nm), (b) spraying with 1% iodine in methanol and (c) spraying with potassium iodoplatinate reagent.

RESULTS AND DISCUSSION

The R_F values of the 100 basic drugs using systems 1 to 8 have been reported (Moffat & Smalldon, 1974) and those using systems 9 to 14 are given in Table 2. The intra-laboratory reproducibilities of all the systems were very similar (Moffat and Hayler, unpublished observations) and therefore the discriminating power for each system was calculated using an error factor of 0·10 in R_F (Table 1).

Thin-layer system 14 had the highest discriminating power ($DP_{14} = 0{\cdot}78$), and this is the chromatographic system of choice if only one system is to be used. System 13 had the next highest discriminating power ($DP_{13} = 0{\cdot}76$) and this would be a good second choice. Both systems have very good distributions of R_F values (Fig. 1)

Table 2. *R_F (X100) for 100 basic drugs in 6 t.l.c. systems.*

Drug	System						Drug	System					
	9	10	11	12	13	14		9	10	11	12	13	14
Acetophenazine	41	17	56	67	04	47	Mephentermine	39	30	59	29	55	15
Ametazole	10	87	10	29	75	01	Mepivacaine	72	83	94	82	48	87
Amethocaine	71	87	88	69	27	60	Mepyramine	76	86	93	64	58	52
Amitriptyline	89	92	95	61	84	59	Methadone	91	91	96	58	89	40,
Amphetamine	42	13	58	44	35	19	Methapyrilene	80	92	95	67	65	55
Antazoline	40	68	70	29	11	13	Methaqualone	84	96	95	85	65	97
Atropine	22	17	37	24	11	06	Methotrimeprazine	91	97	96	73	75	65
Benzocaine	84	83	93	78	12	87	Methyl phenidate	79	88	90	77	57	64
Benzphetamine	98	97	97	78	92	93	Methylamphetamine	40	54	60	43	50	28
Bromodiphenhydramine	79	94	95	63	69	67	Morphine	16	01	20	51	02	24
Buphenine	65	05	76	74	08	29	Naphazoline	19	20	37	20	07	10
Butacaine	86	69	95	72	14	62	Nialamide	48	05	75	85	10	36
Butethamine	72	19	86	77	08	51	Nicotine	67	59	79	71	62	62
Caffeine	46	62	77	66	05	81	Nicotinyl alcohol	34	79	55	78	08	40
Carbetapentane	77	77	94	54	74	40	Nikethamide	60	89	75	71	26	85
Carbinoxamine	48	63	82	51	42	34	Nitrazepam	61	97	87	81	01	74
Chlorcyclizine	81	94	94	67	64	74	Nortriptyline	53	53	82	36	46	23
Chlordiazepoxide	97	21	70	76	04	74	Orphenadrine	82	94	87	65	75	63
Chlorpheniramine	59	71	90	45	54	37	Papaverine	66	93	94	80	16	91
Chlorpromazine	92	97	97	57	75	63	Perphenazine	38	33	69	69	12	01
Cinchonine	79	18	74	59	10	24	Pethidine	69	76	90	62	60	63
Clemizole	89	95	94	80	51	89	Phenelzine	87	06	98	82	58	20
Cocaine	88	94	94	76	71	73	Phenindamine	82	88	89	72	67	82
Codeine	35	24	62	47	11	39	Pheniramine	48	51	77	49	52	30
Cyclizine	83	91	93	72	77	67	Phenmetrazine	47	86	64	64	25	42
Cyclopentamine	46	32	63	23	52	33	Phenylpropanolamine	22	05	33	52	13	08
Desipramine	31	26	65	32	35	21	Phenyramidol	83	46	92	85	16	77
Dextropropoxyphene	94	95	96	80	89	80	Pipamazine	48	12	61	81	01	32
Diamorphine	51	69	80	58	26	64	Piperidolate	01	00	00	02	00	03
Diazepam	88	89	93	79	36	94	Piperocaine	93	86	97	68	78	62
Diethylpropion	94	94	95	80	90	88	Pramoxine	90	86	98	81	62	94
Dimethoxanate	58	83	82	45	32	43	Procaine	79	48	93	71	12	64
Diphenhydramine	86	92	91	63	69	58	Procyclidine	93	96	98	56	90	64
Diphenylpyraline	68	77	86	52	69	58	Promazine	82	94	95	52	66	65
Dipipanone	96	91	96	56	95	61	Promethazine	86	95	96	61	62	78
Ephedrine	33	18	45	31	12	08	Propiomazine	83	86	92	71	55	84
Ethoheptazine	63	81	89	41	70	43	Prothipendyl	64	79	89	50	69	52
Ethopropazine	93	92	96	68	93	69	Pyrrobutamine	79	92	95	62	83	78
Fluphenazine	44	37	73	75	13	50	Quinine	36	13	51	64	04	50
Guanethidine	01	00	00	03	01	05	Strychnine	24	74	55	26	09	29
Hydroxyzine	55	47	79	80	17	77	Thenyldiamine	81	92	88	62	65	61
Hyoscine	40	20	69	71	13	67	Thiopropazate	84	91	94	79	51	91
Imipramine	87	98	90	51	79	57	Thioridazine	86	91	92	58	66	73
Iproniazid	80	18	49	79	01	49	Thonzylamine	78	76	88	66	56	68
Isocarboxazid	74	80	95	74	08	95	Tranylcypromine	68	49	79	72	51	68
Isothipendyl	83	89	94	59	67	57	Trifluoperazine	72	87	83	65	54	75
Levallorphan	84	29	93	70	40	46	Trimeprazine	92	91	94	66	84	80
Lignocaine	83	85	95	86	65	94	Tripelennamine	83	83	92	64	70	63
Lysergide	53	45	82	74	08	68	Triprolidine	70	58	84	61	73	18
Meclozine	97	99	98	77	87	98	Yohimbine	64	51	87	81	10	76

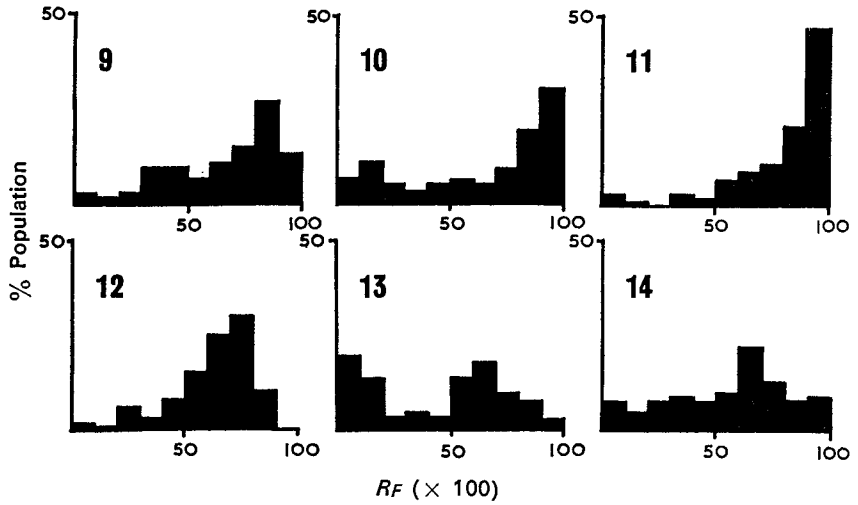

FIG. 1. Frequency distributions of R_F values for 100 basic drugs in six thin-layer systems: 9, silica gel–ethyl acetate–n-heptane–methanol–ammonia (0·88) (60:30:7·5:2·5); 10, aluminium oxide-chloroform; 11, silica gel–dioxan–chloroform–ethyl acetate–ammonia (0·88) (60:25:10:5); 12, silica gel–methanol–ammonia (0·88) (100, 1·5); 13, silica gel sprayed with 0·1N NaOH–cyclohexane–toluene–diethylamine (75:15:10); 14, silica gel sprayed with 0·1N NaOH–chloroform–methanol (90:10).

which account for their very high discriminating powers. In contrast, these distributions may be compared with that of system 11 in which 64% of R_F values were above 0·80 and for which a low discriminating power was obtained ($DP_{11} = 0·57$).

The requirements for systems to be used in combination are that they should have good individual discriminating powers and show low correlations between them. Table 3 lists the combined discriminating powers of all the pairs of systems made

Table 3. *Discriminating powers for pairs of chromatographic systems with an error factor of 0·10 for each system.*

	3	6	7	9	10	13	14
1	0·92	0·92	0·93	0·90	0·90	0·82	0·92
3		0·93	0·93	0·90	0·91	0·92	0·90
6			0·93	0·91	0·91	0·93	0·94
7				0·92	0·92	0·93	0·94
9					0·86	0·90	0·91
10						0·90	0·91
13							0·94

up of the 8 individually most discriminating systems. The most discriminating pairs were those of combinations between systems 3,6,7,13 and 14 when the combined discriminating powers were between 0·93 and 0·94. A good two-dimensional chromatogram can be obtained by using the two thin-layer systems 13 and 14 (Fig. 2), and this is the combination of choice when two systems are required.

When systems are highly correlated, e.g. thin-layer systems 1 and 13 which differ only in the substitution of toluene for benzene and have a correlation coefficient of

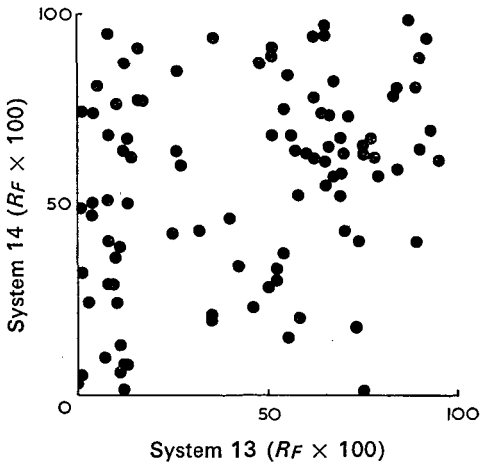

FIG. 2. The correlation of R_F values for 100 basic drugs using system 13 (silica gel sprayed with 0·1N NaOH–cyclohexane–toluene–diethylamine 75:15:10) and system 14 (silica gel sprayed with 0·1N NaOH–chloroform–methanol 90:10). Correlation coefficient 0·33.

0·91 (Table 4), their combined discriminating power is not much higher than the individual discriminating powers of the systems ($DP_1 = 0{\cdot}73$; $DP_{13} = 0{\cdot}76$; $DP_{1,13} = 0{\cdot}82$). This is due to the order of running of spots in one system being very similar to the order of running in the second system. Combinations of systems of low discriminating powers also produce a combination of relatively poor performance ($DP_9 = 0{\cdot}72$; $DP_{10} = 0{\cdot}71$; $DP_{9,10} = 0{\cdot}86$) even though they have low correlation (correlation coefficient = 0·64, Table 4).

Table 4. *Correlation coefficients between chromatographic systems.*

	3	6	7	9	10	13	14
1	0·26	0·28	—0·29	0·56	0·60	0·91	0·30
3		0·45	—0·43	0·55	0·33	0·22	0·61
6			—0·53	0·50	0·31	0·22	0·41
7				—0·46	—0·41	—0·27	—0·52
9					0·64	0·63	0·72
10						0·70	0·65
13							0·33

Further combinations of systems enable even larger discriminating powers to be obtained. The best combination of any three systems was 7, 13 and 14 ($DP_{7,13,14} = 0{\cdot}982$) with any combination of three from systems 3,6,7,13 and 14 giving a combined discriminating power of 0·98. If four systems are to be used the discriminating power increases to 0·994 using systems 6,7,13 and 14.

From the above data the best single system or combination of systems can be chosen depending upon the number required. If sensitivity is of paramount importance, and therefore a thin-layer system is to be preferred, the choice should be between systems 14, 13 or 3. Although system 6 is a paper system, a cellulose plate (0·25 mm or 0·10 mm thickness) with the same solvent system will give equivalent

R_F values, with equivalent reproducibility, but with greater sensitivity (Haywood & Moss, 1968; Smalldon, 1971) and therefore may be included with systems 14,13 and 3.

The other major feature that has to be considered in the choice of a system is the time required for a chromatographic run. This is especially important in such applications as clinical toxicology. System 6, even as a t.l.c. system, takes 3–4 h for a 10 cm run (0·10 mm plate) and is therefore not suitable for such purposes. Both systems 13 and 14 take between 1 and 1½ h for a 10 cm run, but system 3 takes only 50 min since acetone has a low viscosity. The fastest running system of those studied was the reversed phase system number 7 which took only 15–20 min for a 10 cm run and this may be the best choice when time is a limiting factor. Unfortunately it must be run at elevated temperatures and hydrolysis of the tributyrin causes an unpleasant smell. It may therefore be unpopular for these reasons.

In conclusion, if paper and thin-layer chromatographic systems are to be chosen which are quick to run, sensitive and pleasant to work with, the choice is between thin-layer systems 14,13 and 3 (in decreasing order of discriminating power), viz silica gel sprayed with 0·1N sodium hydroxide solution and run using (14) chloroform–methanol (90:10), (13) cyclohexane–toluene–diethylamine, (75:15:10) and (3) acetone. The reversed phase paper system (7) run with an aqueous buffer solution (pH 4·58) at 95° can be used when sensitivity is not too important and the thin-layer cellulose system using n-butanol–water–citric acid (87:13:0·48) can be used when speed is not a requirement. Combinations of any of these five systems may be made since their correlation coefficients are all lower than 0·61.

Acknowledgements

We thank Miss M. Dutton for technical assistance, Mr. C. Brown for help in the computer evaluations and Mr. K. W. Smalldon for useful discussions.

REFERENCES

CURRY, A. S. (1969). In *Isolation and Identification of Drugs*, p. 43. Editor: Clarke, E. G. C. London: Pharmaceutical Press.

DUMONT, E., JORK, H., KRAUS, Lj., ROZUMEK, K.-E., SCHORN, P. J. & STAHL, E. (1973). *Drug Analysis by Chromatography and Microscopy*, Ann Arbor: Ann Arbor Science.

FOX, R. H. (1969). In *Isolation and Identification of Drugs*, p. 31. Editor: Clarke, E. G. C. London: Pharmaceutical Press.

GÄNSHIRT, H. (1969). In *Thin-layer Chromatography*, 2nd edn, p. 506. Editor: Stahl, E. Berlin: Springer.

HAYWOOD, P. E. & MOSS, M. S. (1968). *Analyst, Lond.*, **93**, 737–739.

MACEK, K. (1972). *Pharmaceutical Applications of Thin-layer and Paper Chromatography*, Amsterdam: Elsevier.

MACEK, K., HAIS, I. M., KOPECKÝ, J. & GASPARIĆ, J. (1968). *Bibliography of Paper and Thin-layer Chromatography* 1961–1965 Amsterdam: Elsevier.

MACEK, K., HAIS, I. M., KOPECKÝ, J., GASPARIĆ, J., RÁBEK, V. & CHURÁČEK, J. (1972). *Bibliography of Paper and Thin-layer Chromatography* 1966–1969. Amsterdam: Elsevier.

MOFFAT, A. C. & SMALLDON, K. W. (1974). *J. Chromat.*, **90**, 9–17.

MOFFAT, A. C., SMALLDON, K. W. & BROWN, C. (1974). *Ibid.*, **90**, 1–7.

ŠANTAVÝ, F. (1969). In *Thin-layer Chromatography*, 2nd edn, p. 421. Editor: Stahl, E. Berlin: Springer.

SMALLDON, K. W. (1971). *J. forens. Sci. Soc.*, **11**, 171–176.

SUNSHINE, I. (1969). *Handbook of Analytical Toxicology*, Cleveland: Chemical Rubber Co.