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OPTIMUM USE OF PAPER, THIN-LAYER AND GAS–LIQUID CHROMATOGRAPHY FOR THE IDENTIFICATION OF BASIC DRUGS

II. PAPER AND THIN-LAYER CHROMATOGRAPHY

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SUMMARY

The concept of discriminating power has been applied to the paper and thin-layer chromatography of 100 basic drugs in eight systems which are representative of the systems in current use. The design of the most effective series of chromatographic systems for the separation and identification of a large drug population is discussed in terms of the individual discriminating powers of the systems and inter-system correlation. It is shown that both the substrate and solvent may have to be changed to obtain significantly different systems.

INTRODUCTION

Paper (PC) and thin-layer (TLC) chromatographic methods have been extensively studied in order to develop screening tests for the detection, identification and quantitation of basic drugs (those drugs extracted from alkaline aqueous solutions by organic solvents) in formulations and biological materials. Many different systems have been advocated for use in general screening methods and for the separation of pharmacologically or chemically similar compounds. If a small group of known compounds is examined it may well prove feasible to separate all the members using one or more chromatographic systems. However, if the whole group of basic drugs is considered, complete separation and identification of each drug may be no longer feasible using a small number of systems.

It is important that the analyst should select the series of systems with the highest discriminating power for the PC and TLC of extracts containing basic drugs. The systems which have been reported for this purpose are too numerous to mention individually, but collections of chromatographic data have been produced. Fox[1] lists data for three PC systems, Curry[2] gives data for eight TLC systems and Sunshine[3] gives eleven PC and thirty-five TLC systems. In many cases the populations of drugs chromatographed in each system are so different that a comparison of the effectiveness of the systems is impossible and the choice of the best series of systems for general screening purposes is very difficult.

A method has been described which measures the effectiveness of chromatographic systems (discriminating power) in terms of the probability of separating

two bases selected at random from a specific drug population[4]. This concept is now applied to the analysis of basic drugs using PC and TLC.

EXPERIMENTAL

Choice of basic drugs

The records, from two forensic science laboratories in Great Britain, involving drugs (6000 items) were searched to obtain those basic drugs which had been received at least twice in any one year during the period 1970–1972. To this list was added a number of other commonly used drugs for which analytical data were available, *e.g.* caffeine and nicotine, to give a total of 100 drugs.

Choice of chromatographic systems

The PC, two reversed-phase PC and five TLC systems were selected as being representative of those which are, or have been, in common use in the field of drug analysis (Table I). Those drugs for which data were unavailable from the literature were chromatographed with the procedures used by the respective authors. Reference compounds were used to ensure compatibility of data. Drug spots were located by means of UV light, spraying with 1% iodine in methanol, acidified potassium iodoplatinate solution, or 1% potassium permanganate solution.

TABLE I

PC AND TLC SYSTEMS STUDIED

Type	*System No.*	*Plate or paper*	*Solvent*	*Reference*
TLC	1	Silica gel dipped or prepared with 0.1 *M* KOH	Cyclohexane–benzene–diethylamine (75:15:10)	5
	2	Silica gel dipped or prepared with 0.1 *M* KOH	Methanol	5
	3	Silica gel dipped or prepared with 0.1 *M* KOH	Acetone	5
	4	Silica gel dipped or prepared with 0.1 *M* $KHSO_4$	Methanol	5
	5	Silica gel dipped or prepared with 0.1 *M* $KHSO_4$	Ethanol (95%)	5
PC	6	Whatman No. 1 paper dipped in 5% sodium dihydrogen citrate	Butanol–water–citric acid (870:130:4.8 g)	6
	7	Whatman No. 1 paper dipped in 10% tributyrin in acetone	Acetate buffer, pH 4.58, run at 95°	7, 8
	8	Whatman No. 1 paper dipped in 10% tributyrin in acetone	Phosphate buffer, pH 7.4, run at 86°	9, 10

Treatment of results

The discriminating power[4] was calculated for each system using error factors of 0.05, 0.10, 0.15, and 0.20 in R_F value and then the discriminating power for each pair was calculated using an error factor of 0.10 for each system. Correlation coefficients were also determined for each pair of systems.

TABLE II

R_F (× 100) VALUES FOR 100 BASIC DRUGS IN EIGHT SYSTEMS*

Drug	*System No.*							
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*
Acetophenazine	3	51	1	15	5	23	58**	0
Ametazole	19	20	47	38	17	8	95	87
Amethocaine	18	50	28	38	14	48	56	0
Amitriptyline	72	50	34	41	28	77	32	0
Amphetamine	34	28	33	59	53	51	85**	88**
Antazoline	8	11	6	61	38	74	85	52
Atropine	9	11	1	31	14	37	94	86**
Benzocaine	6	68	73	63	57	90	17**	16**
Benzphetamine	79	70	85	54	49	75	17	0
Bromodiphenhydramine	50	37	26	48	33	69	31	0
Buphenine	5	68	64	66	68	80	75	5
Butacaine	8	63	71	59	51	78	43	0
Butethamine	5	58	49	58	47	51	82	24
Caffeine	4	55	42	48	34	65	85**	74
Carbetapentane	63	37	30	44	18	76	73	8
Carbinoxamine	29	21	6	5	0	46	78	10
Chlorcyclizine	49	44	30	43	19	74	2	0
Chlordiazepoxide	0§	80§	40§	68**	59**	82	11**	8**
Chlorpheniramine	38	19	6	8	1	45	69**	13**
Chlorpromazine	57	44	37	44	26	68	20	2
Cinchonine	10	37	11	22	9	47	72	10
Clemizole	33	67	61	47	27	77	4	0
Cocaine	58	57	64	26	10	38	71**	12**
Codeine	7	28	6	24	10	16	89	22
Cyclizine	55	46	27	41	16	55	47	0
Cyclopentamine	52	10	2	52	40	64	73**	89**
Desipramine	26	18	3	62	45	65	70	6
Dextropropoxyphene	72	62	60	54	30	75	25	2**
Diamorphine	22	39	20	24	9	33	84	12
Diazepam	33	65	62	65	54	89	26	4**
Diethylpropion	76	69	78	44	27	58	52	0
Dimethoxanate	24	31	13	33	13	54	61	4
Diphenhydramine	52	37	26	45	25	62	70	0
Diphenylpyraline	42	25	11	44	23	46	50	4
Dipipanone	55§	55§	60§	17**	8**	85	16	0
Ephedrine	8	18	2	54	42	45	83**	85**
Ethoheptazine	59	29	11	39	19	52	84	17
Ethopropazine	68	62	82	40	25	72	14	5
Fluphenazine	6	60	25	15	6	36	13	7
Guanethidine	0	3	0	15	5	3	83	82
Hydroxyzine	8	59	39	56	25	67	28	0
Hyoscine	9	54	33	34	13	23	93	57
Imipramine	61	35	18	39	25	63	44	0
Iproniazid	4	64	34	34	22	75	83	79
Isocarboxazid	28	71	70	65	61	94	17	20
Isothipendyl	51	47	32	36	18	58	67**	5**
Levallorphan	31	60	53	63	47	73	74	6
Lignocaine	39	70	69	47	23	62	64	4
Lysergide	0§	70§	35§	49**	30**	47	55**	8**

(*Continued on p. 12*)

TABLE II (*continued*)

Drug	*System No.*							
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*
Meclozine	69	71	84	74	60	92	0	0
Mephentermine	50	15	3	52	39	62	82**	92**
Mepivacaine	37	62	57	43	30	62	79	12
Mepyramine	42	33	24	12	1	32	71	5
Methadone	76	37	43	51	25	74	59	0
Methapyrilene	47	36	27	14	2	32	71	5
Methaqualone	35§	75§	60§	69**	62**	94	6	4
Methotrimeprazine	56	56	65	46	23	65	13	4
Methylamphetamine	46	18	4	50	39	56	87**	89**
Methyl phenidate	55	56	35	54	45	63	76**	30**
Morphine	2	28	4	23	10	14	88	81
Naphazoline	5	8	1	43	30	51	81	76
Nialamide	0	55	9	35	23	78	78	77
Nicotine	53	52	29	8	2	7	99	39
Nicotinyl alcohol	3	60	40	30	5	16	89	81
Nikethamide	24	64	44	38	25	86	65	68
Nitrazepam	0§	75§	60§	64**	60**	92	63**	5**
Nortriptyline	35§	20§	10§	6**	6**	74	55	4
Orphenadrine	60	48	32	45	22	67	71	0
Papaverine	11	62	53	62	21	49	8	0
Perphenazine	6	57	20	12	4	23	19	0
Pethidine	55	48	21	49	24	0	83	3
Phenelzine	51	72	75	62	49	38	84**	95**
Phenindamine	55	53	35	41	25	63	37	0
Pheniramine	40	18	5	6	1	27	92	20
Phenmetrazine	24	44	11	49	37	49	83**	88**
Phenylpropanolamine	9	35	50	58	56	44	90**	94**
Phenyramidol	14	68	66	57	40	52	86	29
Pipamazine	0	60	32	48	27	50	36	3
Piperidolate	70	65	65	42	25	76	14	0
Piperocaine	63	45	42	47	29	68	79	5
Pramoxine	52	61	55	48	28	61	6	0
Procaine	5	52	47	39	18	31	89	27
Procyclidine	78	36	39	52	41	84	53	4
Promazine	50	36	25	39	20	58	33	2
Promethazine	46	47	37	45	23	65	23	2
Propiomazine	42	59	53	40	26	77	7	4
Prothipendyl	53	37	20	24	15	55	50	4
Pyrrobutamine	62	39	34	59	40	84	11	4
Quinine	5	47	11	37	14	46	71	11
Strychnine	13	17	3	17	7	30	81**	55**
Thenyldiamine	47	32	25	12	1	27	65	7
Thiopropazate	44	66	67	30	11	53	67	7
Thioridazine	52	45	31	41	27	76	67	5
Thonzylamine	41	38	27	29	12	52	73	5
Tranylcypromine	55	57	58	58	56	45	79	0
Trifluoperazine	45	49	19	10	2	34	6	3
Trimeprazine	64	55	62	44	22	70	29	94
Tripelennamine	50	35	27	12	3	35	74	57
Triprolidine	41	45	13	18	2	59	74	28
Yohimbine	9	68	61	55	11	54	64	7

* Data taken from original papers except where stated.
** This study.
§ Data recorded by Smalldon[11].

RESULTS AND DISCUSSION

The R_F values for the 100 basic drugs in the eight systems are given in Table II. Although there are many thousands of synthetic and natural basic drugs, the sample of 100 used in this study is representative of the population of interest since 98% of the items submitted to laboratories, in which a basic drug was found, contained one of those listed in Table II.

Table III gives the discriminating power *DP* for each system at a range of error factors. The inter-laboratory reproducibility, and hence the error factor *E*, should ideally be determined for each system. This was prohibited by the amount of work needed and therefore an estimate of the likely error factors was made from experience.

TABLE III

DISCRIMINATING POWERS FOR SINGLE CHROMATOGRAPHIC SYSTEMS AT VARIOUS ERROR FACTORS

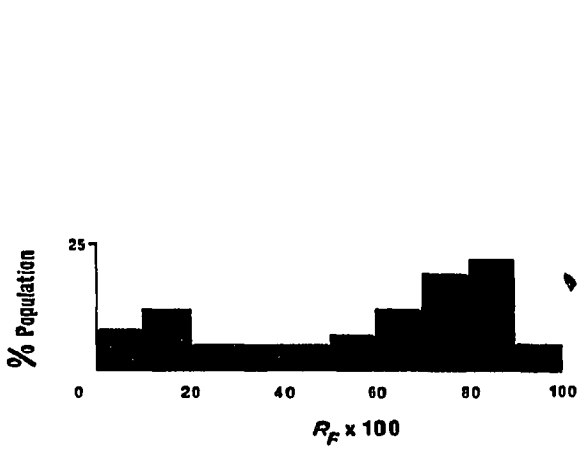
Total number of possible pairs = 4950.

System No.	*Error factor*			
	0.05	*0.10*	*0.15*	*0.20*
1	0.839	0.730	0.645	0.562
2	0.837	0.688	0.564	0.439
3	0.863	0.749	0.659	0.569
4	0.813	0.657	0.525	0.410
5	0.808	0.672	0.535	0.412
6	0.862	0.742	0.620	0.508
7	0.867	0.753	0.650	0.567
8	0.661	0.549	0.492	0.442

Dhont *et al.*[12,13] found that the standard deviations (σ) for the determination of inter-laboratory TLC R_F values for single and multi-component systems are 0.12 and 0.06, respectively. Only three determinations carried out under essentially different conditions (tank saturation, activation, etc.) were used for the calculation of σ's and therefore they may be taken as upper limits of irreproducibility. When using two reference standards and the calculation of corrected R_F^c values[14] with the same systems, they found that the values of σ were reduced to 0.02 and 0.03, respectively. The inter-laboratory reproducibility of R_F values can therefore be dramatically improved by using R_F^c values and for the purposes of this paper we have estimated the error factors of all the systems to be 0.10.

It can be seen from Table III that if the value of *E* can be reduced for a given system, the discriminating power is greatly increased, *e.g.* System 4 has nearly twice the *DP* at an *E* of 0.05 than it has at an *E* of 0.20. It is therefore apparent that R_F^c rather than R_F values should be used in future identification procedures. However, assuming that all the systems have similar error factors, the order of systems in terms of discriminating power is virtually the same regardless of the actual value of *E*.

At our estimated error factor of 0.10 the maximum possible *DP* is 0.81 and the system with the highest discriminating power is System 7 ($DP_7 = 0.753$) followed by Systems 3, 6, and 1 (Table III). System 7 has a high *DP* because it has nearly a rectangular distribution of R_F values over the whole chromatogram, as shown in Fig. 1. This may be compared with Fig. 2, which shows that no less than 61% of all the R_F values in System 8 are 0.10 or lower, and this explains why it has the smallest discriminating power ($DP_8 = 0.549$). It is interesting to note that the best and the worst systems studied are both reversed-phase systems and only the mobile phase is different. The pH of the aqueous buffer is obviously critical and reproducible results are only obtained if the buffer solutions are made exactly to the correct pH.



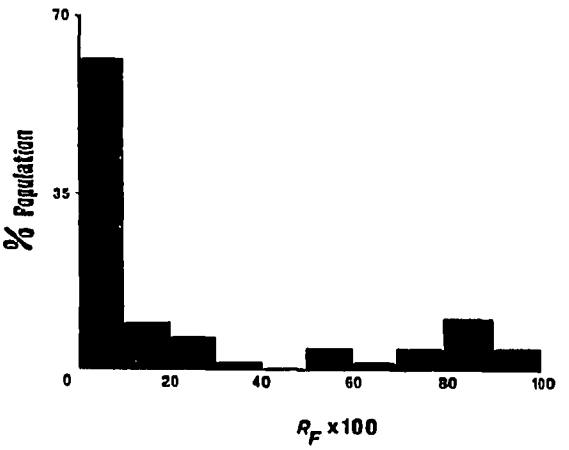


Fig. 1. Frequency distribution of R_F values of 100 basic drugs using System 7 (Whatman No. 1 paper dipped in 10% tributyrin in acetone/acetate buffer, pH 4.58, run at 95°).

Fig. 2. Frequency distribution of R_F values of 100 basic drugs using System 8 (Whatman No. 1 paper dipped in 10% tributyrin in acetone/phosphate buffer, pH 7.4, run at 86°)..

When more than one chromatographic systems are used, the combined discriminating power obviously increases and the discriminating powers for the 28 possible pairs of systems (at an error factor of 0.10 in R_F value for each system) are given in Table IV. The pair with the largest discriminating power is System 3 and System 7 ($DP_{3,7} = 0.929$), followed by Systems 3 and 6, 6 and 7, and then 1 and 7. The reasons why these are the best combinations of systems may be seen by examining the factors necessary to produce a large *DP* of combined systems.

Firstly the two systems must themselves have large *DP*'s. System 8 has the lowest individual *DP* and consequently, in combination with any other system, although the *DP* increases from 0.549 to between 0.834 and 0.875, it does not approach the $DP_{3,7}$ of 0.929.

The second factor governing the size of the second-order *DP* is the correlation between systems. The correlation coefficients of each pair of systems are given in Table V. Systems 4 and 5 are both silica gel, with methanol and ethanol (95%), respectively, used as the mobile phase. The order of elution of the drugs on each system is virtually the same, as can be seen from the high correlation coefficient of 0.902 and the graphical plot shown in Fig. 3. Because the systems are highly cor-

TABLE IV

DISCRIMINATING POWERS FOR PAIRS OF CHROMATOGRAPHIC SYSTEMS WITH AN ERROR FACTOR OF 0.10 IN R_F FOR EACH SYSTEM

Total number of possible pairs = 4950.

System No.	*System No.*						
	2	*3*	*4*	*5*	*6*	*7*	*8*
1	0.903	0.917	0.898	0.902	0.917	0.925	0.875
2		0.866	0.875	0.876	0.912	0.921	0.844
3			0.900	0.899	0.928	0.929	0.871
4				0.763	0.871	0.915	0.839
5					0.878	0.916	0.834
6						0.927	0.865
7							0.873

TABLE V

CORRELATION COEFFICIENTS BETWEEN CHROMATOGRAPHIC SYSTEMS FOR 100 BASIC DRUGS

System No.	*System No.*						
	2	*3*	*4*	*5*	*6*	*7*	*8*
1	0.007	0.256	0.027	0.000	0.277	−0.288	−0.324
2		0.804	0.396	0.352	0.387	−0.462	−0.368
3			0.502	0.465	0.449	−0.434	−0.279
4				0.902	0.583	−0.226	0.000
5					0.596	−0.177	−0.068
6						−0.525	−0.285
7							0.544

related, the value of $DP_{4,5}$ is only 0.763 (Table IV) and even the combination of one of these systems with System 8 provides a higher *DP* ($DP_{4,8}=0.839$; $DP_{5,8}=0.834$).

The attainment of a large *DP* for systems used in combination is therefore achieved by large individual discriminating powers and low correlation between systems, although the best result usually involves a compromise between these two factors, *viz.* Systems 3 and 7, as shown in Fig. 4. The usefulness of the concept Discriminating Power is now apparent, since a single numerical value can be obtained which expresses the effectiveness of chromatographic systems whether used singly or in combination.

The series of three systems with the largest discriminating power is 3, 6, and 7 ($DP_{3,6,7}=0.979$) followed by Systems 1, 6, and 7 and then Systems 1, 3 and 7. The series of four systems with the largest discriminating power is 1, 3, 6, and 7 ($DP_{1,3,6,7}=0.993$). Thus, if a compound in Table II is to be identified using these four systems then, on average, 1.7 compounds will be found to be chromatographically similar[4].

Locating sprays can be used for the characterisation of drugs in chromatographic systems, but because the hue and colour intensity depend upon the freshness

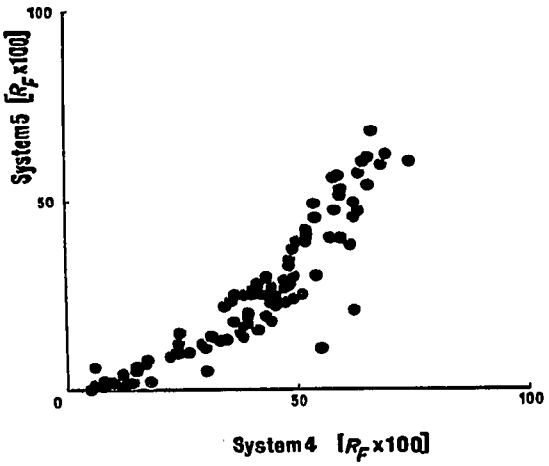


Fig. 3. Correlation of R_F values of 100 basic drugs using System 4 (silica gel with 0.1 *M* $KHSO_4$/methanol) and System 5 (silica gel with 0.1 *M* $KHSO_4$/ethanol, 95%). Correlation coefficient = 0.902.

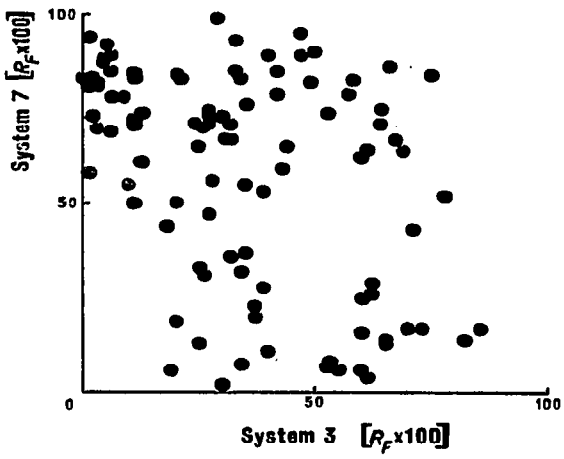


Fig. 4. Correlation of R_F values of 100 basic drugs using System 3 (silica gel with 0.1 *M* KOH/acetone) and System 7 (Whatman No. 1 paper dipped in 10% tributyrin in acetone/acetate buffer, pH 4.58, run at 95°). Correlation coefficient = −0.434.

of reagent, amount of drug in the spot, and presence of interfering substances, the results must be used with caution. The reactions of drugs with different spray reagents have been used as part of a systematic identification procedure[15]. However, most authors prefer to use spray reagents merely as locating agents when screening samples for the presence of basic drugs[3,16,17]. Therefore, although many spray reagents can be used to detect and locate any spots on the chromatogram, the searching system for identification should in future be based on the $R_F{}^c$ values and the colour reactions used only as a means of confirmation.

The eight systems used in this study are representative of those in common use and the correlation coefficients in Table V indicate how additional systems may be designed. Systems 2 to 5 use single solvents and, in general, are highly correlated showing that, although numerically different R_F values may be obtained by using a more polar solvent and the same substrate, the final order of elution of the compounds is similar. This is not surprising since there is a nearly rectilinear relationship between partition coefficients for a series of congeners in different solvent systems[18] and this has been shown to apply to chromatographic solvents[19]. Attempts to overcome this involve the use of a single mobile phase and a range of substrates, *viz.* cellulose, acetylated cellulose, aluminium oxide, and silica gel[20]. However, excluding the acetylated cellulose system, which showed poor separation, the remaining three pairs had correlation coefficients of between 0.79 and 0.81, which is not better than the results obtained in this study using the same substrate and different mobile phases (Table V).

The only system that shows a negligible correlation with all the other systems is System 1 (the mixed solvent system). The three components of the mobile phase obviously allow a completely different kind of separation to be obtained compared

to that achieved using a single solvent on the same substrate. Therefore, when creating a new system to be used in conjunction with existing systems, a combination of change of substrate and the change from single to mixed solvent or *vice versa* may be necessary to obtain a truly different system. When the system is designed, its *DP* can then be compared to those of the systems already in use to decide if it merits use in drug identification procedures.

The practical limitations of the systems may influence the choice of a system even if it has a high *DP*. For example, TLC is more sensitive and rapid than PC, and TLC may therefore be favoured by analysts to save time and sample. Also the best system was System 7, which must be run at elevated temperatures and has an objectionable smell because of the tributyrin and is therefore unpleasant to use. An analyst should therefore consider the practical constraints in conjunction with the relevant discriminating powers when choosing the most effective systems for the identification of basic drugs.

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