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THE STANDARDISATION OF THIN-LAYER CHROMATOGRAPHIC SYSTEMS FOR THE IDENTIFICATION OF BASIC DRUGS

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SUMMARY

Three thin-layer chromatographic systems have been selected on the basis of their discriminating power on which to standardise for the identification of basic drugs. They are systems of silica gel sprayed with 0.1 *N* NaOH, dried and run using one of the following solvents: cyclohexane–toluene–diethylamine (75:15:10), chloroform–methanol (90:10) or acetone. They can be used in combination since their correlation coefficients are low. Four reference compounds should be used, equally spaced across the plate. The inter-laboratory variation of measurement of R_F values has been ascertained and the use of a graphical R_F correction method reduced the mean deviation of measurement to 0.02. The measurement of R_F values was found to be less reproducible in the middle of the chromatogram than in regions of very low or very high R_F values.

INTRODUCTION

In recent years much effort has been put into the standardisation of thin-layer chromatographic (TLC) systems for the identification of drugs and other substances. The main advantages gained by such standardisation are (a) analyses would be performed more efficiently since only the more effective systems would be used, (b) chromatographic data built up by one laboratory would be easily transferable to any other laboratory and (c) by using recommended systems disagreements between the findings of two or more laboratories analysing the same sample should be minimised. There are two main criteria to be decided upon before standardisation can occur, *viz.*, to choose the systems and procedures to be used and then to agree to a method of reporting the measured R_F values found for the drugs.

On the choice of the systems to be used the analyst is faced with a bewildering number of systems from which to select. Fortunately the important features which a system should possess have been evaluated and the choice of the systems can now be made. Connors[1] has provided a theoretical relationship between R_F values in two solvent systems which should permit the rational choice of the optimum system for a particular separation. Basically, the author points out that separative power is what is required of a good system, and Massart and Smits[2] state that both resolution and

precision of measurement are very important for efficiency. The work by Moffat *et al.*[3,4] goes even further to allow a quantitative measurement combining both resolution and precision to be used for comparing the efficiencies of different systems. When TLC systems are used in combination it may be that the R_F values in one system are related to those in a second system (Perisho[5], Smalldon[6]) and it is important to recognise that maximum information for identification purposes is only obtained when the R_F value for a compound in one system is independent of its value in another system (Connors[7]).

Recently the measurement of the effectiveness of systems has become possible according to the features mentioned above by calculating their "informing power[2]" or their "discriminating power[3]". A study has also been carried out to include 37 of the paper chromatographic (PC) and TLC systems in common use to compare them for effectiveness and to choose the best four TLC systems for use in identification procedures[4,8]. If these four systems are accepted as the ones on which to standardise then only the method of determining the R_F values needs to be agreed.

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 (R_Fc) is now universally accepted. Galanos and Kapoulas[9] developed a method for use in PC involving the use of two reference compounds and the calculation of the corrected R_F values by a linear regression, *viz.*

$$R_Fc = a\,R_F + b$$

where a and b are constants obtained from the R_F values of the two reference compounds. This concept has been successfully used in TLC by Dhont *et al.*[10–12] who found that an extraordinary gain in reproducibility was obtained in this way. A similar procedure was used utilising three reference compounds and a graphical correction procedure by Phillips and Gardiner[13]. In this latter work the correction graph was non-linear and therefore the method of Galanos and Kapoulas would have been unsuitable. Gasparič[14] uses five reference compounds in his work and demands that they form round, sharp and distinct spots and are also regularly spread over the whole chromatogram.

From the above observations it was decided to examine the best four TLC systems chosen by this laboratory[4,8] to determine the optimum method of reporting R_Fc values and to determine the interlaboratory errors involved in their measurement. This paper presents the results of this investigation.

EXPERIMENTAL

Each of the ten laboratories participating in the trial were asked to use glass-backed plates using the four systems in Table I. The instructions were to dry the plates after spraying, but not to store them in a desiccator.

Ten aqueous solutions were supplied each containing a different drug at concentrations between 0.5 and 5.0 mg/ml. Four solutions, each containing four drugs, were also supplied to act as reference compounds for each of the four systems (Table I). The above solution (2–5-μl samples) were applied directly to the plates without

TABLE I

TLC SYSTEMS STUDIED

Adsorbent	*Solvent*	*Reference compounds*	
		Compounds	*Amount (mg/ml)*
Silica gel (0.25 mm) sprayed with 0.1 *N* NaOH and dried	Cyclohexane–toluene–diethylamine (75:15:10)	Codeine	2
		Desipramine	2
		Pethidine	2
		Dipipanone	2
Silica gel (0.25 mm) sprayed with 0.1 *N* NaOH and dried	Chloroform–methanol (90:10)	Desipramine	2
		Dipipanone	2
		Caffeine	5
		Meclozine	0.5
Silica gel (0.25 mm) sprayed with 0.1 *N* NaOH and dried	Acetone	Amitriptyline	1
		Procaine	1
		Mepivacaine	1
		Meclozine	0.5
Cellulose (0.1 mm) sprayed with 5% sodium dihydrogen citrate and dried	*n*-Butanol–water–citric acid (87:13:0.48)	Nicotine	2
		Codeine	2
		Caffeine	5
		Phenazone	2

extraction and the systems were run for a distance of 10 cm in fully saturated tanks (paper liners and tanks equilibrated for at least 30 min before use). Visualisation was achieved by means of UV light (254 nm) or acidified iodoplatinate spray.

RESULTS AND DISCUSSION

Table II shows the R_F values for the reference compounds and the ten test drugs found by the laboratories using the chloroform–methanol/silica gel system. Linear correction graphs (*e.g.* Fig. 1) were obtained when the mean R_F values for the reference compounds were taken as their standard values and used on the x-axis and the practically obtained values for those compounds found by each laboratory were used on the y-axis. From their linear nature it is apparent that only two reference compounds are needed and that a first order correction equation such as that proposed by Galanos and Kapoulas is suitable for R_F correction purposes for this system. However, when the data for the other three TLC systems were treated in a similar manner, some non-linear correction graphs were observed, *e.g.* those for the cyclohexane–toluene–diethylamine/silica gel system (Fig. 2). Thus, for a general correction procedure in TLC a linear regression cannot be used and it also follows that more than two reference compounds must be run on the same chromatogram.

From the shapes of some of the curves in Fig. 2, it can be seen that at least four reference compounds are desirable and the reference compound with the highest R_F value should also be chosen to have the highest R_F value of all the drugs which are likely to be analysed. Thus, the reference compounds will give a complete spread of R_F values over the whole chromatogram, and the reference compounds given in Table I are suitable for this purpose for the systems given.

TABLE II

EXPERIMENTAL $R_F \times 100$ VALUES FOR REFERENCE AND TEST DRUGS ON THE CHLOROFORM–METHANOL/SILICA GEL SYSTEM

Drug	*Laboratory*										*Mean*	*Standard deviation*
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*		
Reference compounds												
Desipramine	11	10	11	8	14	15	11	21	7	6	11.4	
Dipipanone	30	33	33	30	31	45	32	53	19	23	32.9	
Caffeine	55	57	56	52	63	63	—	76	54	50	58.4	
Meclozine	74	77	79	70	84	80	79	100	72	73	78.8	
Test compounds												
Amitriptyline	36	38	37	35	44	42	45	57	22	31	38.7	9.3
Amphetamine	13	13	14	13	16	16	17	20	7	8	13.7	4.0
Caffeine	55	57	56	52	64	63	—	71	55	50	58.1	6.6
Codeine	22	21	18	19	23	29	25	28	14	14	21.3	5.2
Dipipanone	30	36	33	32	33	46	40	38	19	22	32.9	8.0
Lignocaine	68	72	73	64	77	73	73	86	63	67	71.6	6.7
Meclozine	74	77	79	71	86	80	81	95	72	73	78.8	7.4
Pethidine	36	39	35	32	42	46	41	46	22	29	36.8	7.6
Quinine	18	16	12	10	23	22	20	22	12	8	16.3	5.5
Strychnine	22	23	20	16	23	32	26	34	12	14	22.2	7.2

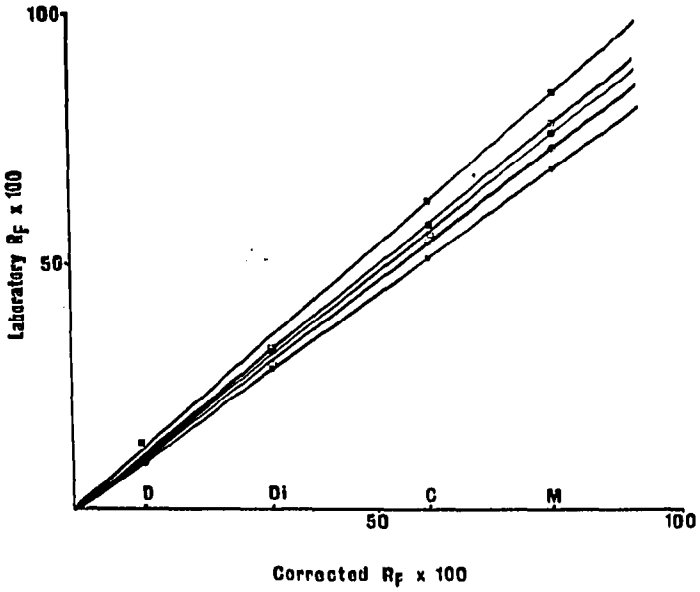

Fig. 1. Correction graphs for five laboratories using the chloroform–methanol/silica gel system. Reference compounds: D, desipramine; Di, dipipanone; C, caffeine; M, meclozine.

Table III gives the graphically corrected R_F values for the ten test drugs as determined using the chloroform–methanol/silica gel system. The excellent gain in reproducibility can be seen by comparing these data with the uncorrected data in Table II. (The mean R_F values for the drugs are practically the same whether corrected

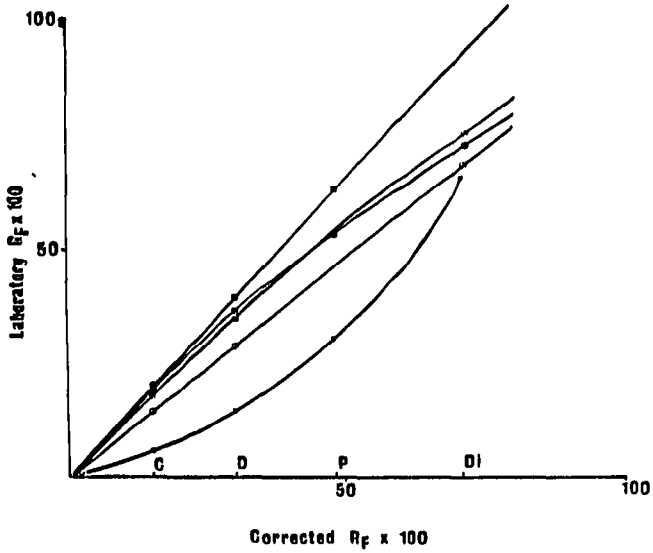

Fig. 2. Correction graphs for five laboratories using the cyclohexane–toluene–diethylamine/silica ge system. Reference compounds: C, codeine; D, desipramine; P, pethidine; Di, dipipanone.

TABLE III

CORRECTED R_F × 100 VALUES DERIVED FROM TABLE II FOR THE CHLOROFORM–METHANOL/SILICA GEL SYSTEM

Drug	*Laboratory*										*Mean*	*Standard deviation*
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*		
Amitriptyline	39	39	37	40	42	35	45	37	35	41	39.0	3.2
Amphetamine	14	14	14	15	15	14	17	11	13	13	14.0	1.6
Caffeine	58	58	57	59	60	58	—	50	60	59	57.7	3.0
Codeine	24	22	18	22	22	24	25	16	25	23	22.1	3.0
Dipipanone	32	37	33	38	31	39	40	24	32	32	33.8	4.8
Lignocaine	73	74	74	72	72	70	73	65	69	76	71.8	3.1
Meclozine	79	79	79	80	81	78	81	75	79	80	79.1	1.7
Pethidine	39	39	35	38	39	39	41	28	35	37	37.0	3.7
Quinine	19	17	12	12	22	18	20	13	22	14	16.9	3.9
Strychnine	24	24	20	19	22	26	26	19	22	22	22.4	2.6

or uncorrected.) All the silica gel systems showed an increase in reproducibility by using the graphical correction procedure (Table IV) although the cellulose system did not show an improvement in reproducibility and had the largest standard deviation of corrected R_F values. Any of the silica gel systems would therefore be an excellent choice as a TLC system for the identification of basic drugs and they can be used in combination since their correlation coefficients are less than 0.62 (ref. 8).

The mean values for the standard deviations in Table IV can only be used for comparison purposes since the measurement of R_F values is less reproducible in the middle of the chromatogram than it is in regions of very low or very high R_F values

TABLE IV

MEAN STANDARD DEVIATIONS OBTAINED FOR THE TEN TEST DRUGS USING THE FOUR TLC SYSTEMS

System	*Standard deviation (× 100)*	
	Calculated from experimental values in Table II	*Calculated from corrected values in Table III*
Cyclohexane–toluene–diethylamine/silica gel	2.8	2.2
Chloroform–methanol/silica gel	6.8	3.1
Acetone/silica gel	6.2	3.7
n-Butanol–water–citric acid/cellulose	5.7	5.7

(Fig. 3). Thus, when an unknown drug is to be identified using one of the three silica gel systems, the R_F value obtained should be corrected graphically from the R_F values of the four reference compounds and an appropriate error factor applied when searching through literature R_F values for a possible identity according to the appropriate standard deviation of measurement.

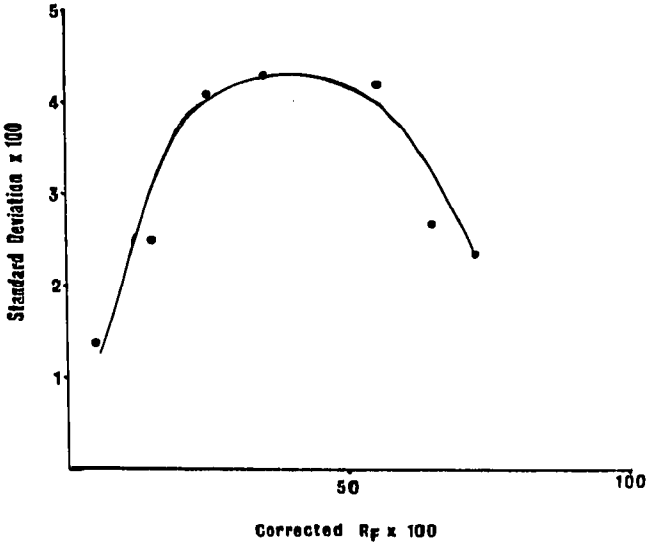

Fig. 3. Relationship between the standard deviation of measurement and magnitude of the corrected R_F value for drugs using the silica gel systems.

Now that the most efficient TLC systems have been chosen, the method of correcting R_F values by using four reference compounds and the measurement of interlaboratory variations in measuring corrected R_F values have been accomplished it is the author's hope that this information may aid laboratories to standardise on the TLC systems for the identification of basic drugs.

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