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EVALUATION OF WEIGHTED DISCRIMINATING POWER CALCULATIONS AS AN AID TO THE SELECTION OF CHROMATOGRAPHIC SYSTEMS FOR THE ANALYSES OF DRUGS

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SUMMARY

In toxicological analyses some chromatographic separations are more important than others. Two weighting methods for chromatographic data are described which incorporate the importance of particular separations into discriminating power calculations. The data were abstracted from a publication on the separations of acidic drugs on thin-layer chromatographic systems. When compared with non-weighted discriminating power calculations, those obtained with the weighting procedure did not give any advantage.

INTRODUCTION

The use of objective criteria with which to evaluate the separating power of chromatographic systems is now well established. Chromatographic systems have been classified according to their similarities by use of the Poisson distribution[1,2] and techniques such as numerical taxonomy[3]. However, when the selection of the optimal systems for a particular problem is required, the use of informing power[4,5] or discriminating power[6,7] is more useful. Discriminating power is preferable for the comparison and selection of systems, since it enables correlations between chromatographic systems to be made more easily than with informing power.

The concept of discriminating power has been developed for use with a wide variety of physical characteristics, but the major use has been in the choice of chromatographic techniques for the analyses of drugs. Paper and thin-layer chromatographic (TLC) systems have been compared for the analyses of the various chemical classes of drugs, *viz.* bases[8,9], neutrals[10] and acids[11], and gas–liquid chromatographic systems have been compared for the separation of basic drugs[12]. However, one feature of the above work is that all the separations examined were considered to be equally important. This may not always be the case, since some drugs occur far more frequently than others in toxicological analyses. Barbiturates are a good example which,

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with acetylsalicylic acid and paracetamol, are the more commonly occurring acidic drugs found in cases of fatal overdosage. It is obviously advantageous to have a system that separates these drugs. However, a balance must be reached between the separation of the important drugs from each other and the separations of all the other drugs which are likely to be present in a particular analysis.

One way of reaching this compromise is to weight the chromatographic data used to calculate the discriminating power of a particular system. The more important drugs would then make a greater contribution. The separation problem examined in this paper is that of the choice of TLC systems for the routine screening for acidic drugs during toxicological analyses. A non-weighted calculation of discirminating power has previously been reported[11] and we now present an examination of two weighting methods which have been used in order to determine if the weighting of data confers any advantage to the calculation of discriminating power.

METHODS

Chromatographic data

The R_F values for the 51 acidic drugs listed in Table I using the six silica gel TLC systems in Table II were taken from Owen *et al.*[11].

Weighting

The importance of each drug in a separation was described by assigning a weighting to the R_F value (Table I). Two weighting systems were used. The first used the opinions of forensic scientists in Great Britain on the relative importance of acidic drugs in a list of 147. Scientists in each laboratory were requested to give each drug a value of 0, ½ or 1 and the weighting assigned to the drug was the sum of the values obtained from ten laboratories. The second weighting system (Table I) used the number of fatal poisoning cases associated with each drug in England and Wales in one year[13].

Discriminating power measurements

The calculations of discriminating power for the unweighted chromatographic data were made as previously reported[7] using the formula

$$\mathrm{DP} = 1 - \frac{2M}{N(N-1)}$$

Where DP = discriminating power; M = number of pairs of R_F values that matched within a set error factor, *i.e.*, two compounds were regarded as separated or discriminated in a particular chromatographic system if the difference between their $R_F \times 100$ values exceeded the error factor of 10; N = number of R_F values examined.

When the weighted values were used, the computer programme was modified in the following manner. Each of the 51 $R_F \times 100$ values was compared with every other value in turn and if they matched within an error factor of 10, the weightings for the two $R_F \times 100$ values were multiplied together and stored. All these data were then summed (ΣM). The total number of possible matches (ΣT) was calculated in

TABLE I

WEIGHTING ASSIGNED TO DRUGS

Drug	*Weighting*	
	Scale of 1 to 10	*Number of deaths**
Warfarin	10	1
Quinalbarbitone	9	616
Amylobarbitone	9	599
Pentobarbitone	9	250
Butobarbitone	9	206
Acetylsalicylic acid	9	167
Paracetamol	9	111
Phenobarbitone	9	102
Glutethimide	9	30
Salicylic acid	9	8
Cyclobarbitode	9	4
Barbitone	9	5
Primidone	9	1
Saccharin	9	0
Lysergic acid	9	0
Phenytoin	8	7
Salicylamide	8	7
Frusemide	8	2
p-Aminophenol	8	0
Thiopentone	7	2
Phenazone	7	0
Indomethacin	7	0
p-Aminosalicylic acid	7	0
Methohexitone	6	1
Hydrochlorothiazide	6	1
Phenolphthalein	6	1
Hexobarbitone	6	0
Bemegride	6	0
Bendrofluazide	6	0
p-Aminobenzoic acid	6	0
Benzoic acid	6	0
Nicotinic acid	6	0
Methyldopa	5	1
Ethosuximide	5	0
Chlorothiazide	5	0
Gentisic acid	5	0
Sulthiame	5	0
Chlorpropamide	5	0
Dicoumarol	5	0
Phensuximide	4	0
Hydroflumethiazide	4	0
Sulphamethizole	4	0
Sulphathiazole	3	1
Sulphacetamide	3	0
Sulphadimidine	3	0
Sulphafurazole	3	0
Sulphamethoxazole	3	0
Sulphanilamide	3	0
Nalidixic acid	3	0
Benzthiazide	3	0
Carbenoxolone	2	0

* From Office of Population Census[13].

a similar manner by using all combinations of $R_F \times 100$ values. The discriminating power was then calculated using the formula

$$DP = 1 - \frac{\Sigma M}{\Sigma T}$$

The computer search programme is given as in the Appendix.

RESULTS AND DISCUSSION

Table II shows the DP values for the six TLC systems for the separation of the 51 acidic drugs. Using unweighted data the best system is either the chloroform–methanol (9:1) or ethyl acetate system, both of which have DP values of 0.74. The other systems all exhibit DP values between 0.71 and 0.60 and provide a reasonable separation of the drugs.

TABLE II

DISCRIMINATING POWERS OF SILICA GEL TLC SYSTEMS FOR ACIDIC DRUGS CALCULATED WITH AND WITHOUT WEIGHTINGS FOR THE DRUGS

Calculated using an error factor of 10 in $R_F \times 100$.

Solvent system	*Discriminating power*		
	No weighting	*Weighting*	
		Scale of 1 to 10	*Number of deaths*
Chloroform–methanol (9:1)	0.74	0.76	0.36
Ethyl acetate	0.74	0.73	0.33
Chloroform–acetone (4:1)	0.71	0.73	0.39
Ethyl acetate–methanol–ammonia (85:10:5)	0.69	0.71	0.54
Acetic acid–toluene–ether–methanol (18:120:20:1)	0.62	0.59	0.15
Acetone	0.60	0.62	0.36

When the DP values are calculated using the weighting system on a scale of 1 to 10, the best system is chloroform–methanol (9:1) (DP value 0.76) followed closely by the ethyl acetate and chloroform–acetone systems (DP values 0.73). The worst system (acetic acid–toluene–ether–methanol, DP value 0.59) is also the second least discriminating when unweighted DP values are used. Thus, whether unweighted or weighted systems on a scale of 1 to 10 are used, the results of DP measurements and ranking systems in order of effectiveness are very similar.

In contrast to these results, a very different picture is seen when unweighted DP values are compared with those weighted according to the number of deaths (Table II). In the latter situation, the maximum DP value (0.54) is for the ethyl acetate–methanol–ammonia (85:10:5) system. The two best systems from unweighted values, *i.e.*, the chloroform–methanol (9:1) and ethyl acetate systems, have DP values of only 0.36 and 0.33, respectively, using the weighted DP values, and the acetic acid–toluene–ether–methanol (18:120:20:1) system has a DP value of only 0.15. These

low DP values are caused by the very high incidence of poisonings (and thus weightings) from the five barbiturates, acetylsalicylic acid and paracetamol all of which have weightings of over 100 (Table I). These drugs, because of their high weightings, control the number of matched pairs and therefore it appears that the DP values are based almost exclusively on the power of the system to separate these seven drugs. Table III gives the R_F values for these seven drugs in the six TLC systems examined. In the acetic acid–toluene–ether–methanol (18:120:20:1) system, six of the seven drugs have $R_F \times 100$ values in the range 34–42 and all match with an error factor of 10. A low DP value of 0.15 is therefore not surprising. As the ethyl acetate–methanol–ammonia (85:10:5) system has the greatest spread of R_F values for the barbiturates, this leads to the highest observed DP value (0.54).

TABLE III

$R_F \times 100$ VALUES* OF THE SEVEN DRUGS ASSOCIATED WITH MORE THAN 100 FATAL POISONINGS

Drug	*Number of deaths***	*Silica gel solvent system*					
		Chloroform–acetone (4:1)	*Acetic acid–toluene–ether–methanol–(18:120:20:1)*	*Ethyl acetate–methanol–ammonia–(85:10:5)*	*Ethyl acetate*	*Acetone*	*Chloroform–methanol (9:1)*
Quinalbarbitone	616	52	42	56	69	77	51
Amylobarbitone	599	48	37	49	66	78	57
Pentobarbitone	250	47	39	45	68	79	55
Butobarbitone	206	48	42	45	66	79	54
Phenobarbitone	102	38	34	27	63	74	52
Acetylsalicylic acid	167	8	38	7	16	21	18
Paracetamol	111	14	7	42	35	64	28

* From Owen *et al.*[11].
** From Office of Population Census[13].

In principle, the calculation of DP values after weighting the chromatographic values, appears to be a reasonable approach to the selection of TLC systems for various groups of drugs. However, when the weightings are high for one group of compounds, the resultant DP is biased so heavily in that direction that it would be just as effective to use only the data for that group of compounds. Weightings on a scale of 1 to 10 do not appear to give very different DP values from those made without weighting and therefore do not seem to give any advantage.

In conclusion, the weighted DP calculations made in this work are of limited value. If a separation of a small group of compounds is important within a screening procedure for a large number of compounds it is probably better to measure the individual DP values for those particular separations that are required and then choose the appropriate chromatographic system.

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APPENDIX

Written in Fortran IV for a Hewlett-Packard Model 2100 computer.

```
FTN
      PROGRAM WDP
      COMMON IPACK (640)
      DIMENSION W (400)
      DIMENSION IN (400, 15), IERR (15), IPONT (15)
      DATA W/400*1./
      WRITE (1,2)
2     FORMAT ("INSERT NO OF DATA ITEMS AND SYSTEMS")
      READ (1,*) N, ISYST
      DO 10 I=1, ISYST
      DO 20 J=1, N
      READ (5,*) IN (J,I)
20    CONTINUE
      PAUSE
10    CONTINUE
12    WRITE (1,11)
11    FORMAT ("INSERT ITEM NUMBER AND WEIGHT (0  TO STOP)?-")
      READ (1,*)I,AW
      IF (I.EQ.0) GO TO 6
      W(I)=AW
      GO TO 12
6     WRITE (1,3)
      B=0
3     FORMAT ("INSERT ORDER OF SEARCH AND SYSTEMS REQUIRED")
      READ (1,*) IORD, IPONT
      WRITE (1,4)
4     FORMAT ("INSERT ERROR FACTORS TO BE USED IN THE SAME ORDER")
      READ (1,*) IERR
      A=0.0
      DO 50 I=1, (N-1)
      DO 40 J=(I+1),N
      B=B+W(I)*W(J)
      DO 30 K=1,IORD
      IF (IABS(IN(I, IPONT(K))-IN(J,IPONT(K))).LE.IERR(K))30,40
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30    CONTINUE
      A=A+W(I)*W(J)
40    CONTINUE
50    CONTINUE
      IF(ISSW(15).LT.0)WRITE(1,61)B
61    FORMAT("TOTAL NUMBER PAIRS:",F10.0)
      DP=1.0-A/B
      WRITE (1,5)DP
5     FORMAT("DISCRIMINATING POWER=",F5.3//)
      GO TO 6
      END
      END $
```

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