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COMPARISON OF THE RESOLVING PROPERTIES OF A GROUP OF CHROMATOGRAPHY SYSTEMS FOR A COLLECTION OF COMPOUNDS

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SUMMARY

Chromatography systems can be ranked in discriminating power (resolving power) for a specific collection of compounds by determining the standard deviation (S) of the mean R_F of the compounds in each system; the discriminating power decreases with decreasing values for S.

Pairs of chromatography systems (systems 1 and 2, 1 and 3, 2 and 3, etc.) can be ranked in discriminating power by calculating the coefficient of correlation ($r_{1,2}$, $r_{1,3}$, $r_{2,3}$, etc.) between the R_F values of the members of each pair and using the coefficient to "correct" the total standard deviation of the pair for the discriminating power that the two systems have in common. The discriminating power of the various pairs of systems decreases with decreasing values for corrected total standard deviation ($\Sigma S'$). Combinations of three or more systems may be ranked in discriminating power by use of the same general approach.

This method for comparing chromatography systems was applied to the analysis of the R_F values from the chromatography of 163 steroids in seven systems and from the chromatography of 100 basic drugs in seven additional systems. When compared by rank, excellent correlation was found between values for $\Sigma S'$ and discriminating power for both sets of data. A theoretical relationship between $\Sigma S'$ and discriminating power is discussed as well as limitations to use of this method for the evaluation of chromatography systems.

INTRODUCTION

It is commonly known that some chromatography systems provide much better separation of the components of a mixture than others, but it is usually not possible to predict which of several systems is most likely to separate a specific substance from a mixture. We have devised a procedure for comparing the discriminating powers (resolving powers) of a group of chromatography systems and selecting the system that most probably will separate a component from a specific collection of compounds. The procedure is described in this paper, and evaluated by applying it to the chromatography of 163 steroids in each of seven thin-layer chromatography

(TLC) systems, and to the chromatography of 100 commonly used drugs in each of five TLC systems and two paper systems.

CONCEPTS

If a group of three compounds is distributed randomly along the direction of migration on a chromatogram within the range of $0.20 < R_F < 0.80$ and the area occupied by each compound is approximately the same, there is greater probability that the compounds will be separated than if they are distributed randomly within the range of $0.35 < R_F < 0.65$. The greater the range of R_F values over which a group of compounds is distributed, the greater the chance that the individual compounds will occupy separate areas on the chromatogram. This concept implies that the discriminating power of a chromatography system is directly related to the extent of dispersion of the compounds in the system. Since standard deviation of the mean is commonly a good index of dispersion, the discriminating powers of various chromatography systems for a collection of substances should increase in parallel with values for the standard deviation (S) of the mean R_F value.

Combined discriminating power for two or more systems

Unless two chromatography systems have identical discriminating characteristics, their combined discriminating power for a large group of compounds should be greater than their individual discriminating powers. Similarly, unless two chromatography systems have totally different discriminating characteristics, their combined discriminating power for a large group of compounds should be less than the sum of the individual discriminating powers. If the standard deviation of mean R_F is a measure of discriminating power for a single system, an appropriate measure of combined discriminating power of two systems is the standard deviation (S_1) of the mean R_F in the first system plus the portion of the standard deviation (S_2) of the mean R_F in the second system that represents discriminating power not common to both systems. An equation which is used in regression analysis[1] may be employed to calculate the discriminating power (in terms of standard deviation) which is unique to the second system:

$$S_{2.1} = S_2\sqrt{1 - r_{1,2}^2} \tag{1}$$

$S_{2.1}$ is the standard deviation from regression and $r_{1,2}$ is the correlation coefficient of system 2 *vs.* system 1. The expression $\sqrt{1 - r_{1,2}^2}$ represents the fraction of the discriminating power of system 2 (in terms of standard deviation) that should be added to the discriminating power of system 1 to give the total effective standard deviation ($\Sigma S'$)* when the two systems are used in combination. Thus the $\Sigma S'$ of systems 1 and 2 is given by eqn. 2.

$$\Sigma S'_{1,2} = S_1 + S_2\sqrt{1 - r_{1,2}^2} \tag{2}$$

* The total effective standard deviation for a group of systems is equivalent to the sum of the "corrected" individual values for S and may be abbreviated appropriately as $\sum_{n=1}^{N} S'_n$ or, for general use in the text, simply as $\Sigma S'$. When identification of specific systems is required, subscripts will be used.

Since the value of the correlation coefficient of system 1 relative to system 2 is the same as that of system 2 relative to system 1, and since usually S_1 and S_2 will be different in magnitude, values for standard deviation should be ranked and used in a consistent manner to provide congruous data. We will rank the values in the order which provides a maximal value for $\Sigma S'$; hence, in eqn. 2, $S_1 > S_2$.

Values of $\Sigma S'$ for combinations of 3 and 4 systems can be calculated by use of eqns. 3 and 4, respectively:

$$\Sigma S'_{1,2,3} = \Sigma S'_{1,2} + S_3\sqrt{1 - r^2_{1,3}} \cdot \sqrt{1 - r^2_{2,3}} \tag{3}$$

where $\Sigma S'_{1,2} > \Sigma S'_{1,3}$

$$\Sigma S'_{1,2,3,4} = \Sigma S'_{1,2,3} + S_4\sqrt{1 - r^2_{1,4}} \cdot \sqrt{1 - r^2_{2,4}} \cdot \sqrt{1 - r^2_{3,4}} \tag{4}$$

where $\Sigma S'_{1,2,3} > \Sigma S'_{1,2,4}$

The foregoing concepts were developed under the assumption that there is uniform (rectangular) distribution[2] of the compounds being chromatographed over the utilized portion of the chromatogram in each system. However, it is unlikely that a finite group of compounds will be distributed uniformly in any system; therefore, the relationship between $\Sigma S'$ and discriminating power is approximate rather than exact. Before applying the evaluation procedure to a specific group of compounds, an assessment should be made of the frequency distributions of the R_F values in each system. A system in which the distribution of R_F values is grossly non-uniform, *e.g.*, one in which the compounds are distributed over the range $0.05 < R_F < 0.85$ with 50% of the R_F values less than 0.15, should not be included in the scheme of analysis which is being described.

Optimal sequence for use of systems

If numerous chromatography systems are being considered for separation of the components of a mixture, the calculation of $\Sigma S'$ for all possible combinations of systems can be laborious. By use of the following procedure, which is analogous to one described by Dupuis and Dijkstra[3], the "best sequence of systems" can be determined with relatively little effort: (1) for the first chromatogram choose the system with the largest standard deviation, (2) for the second chromatogram choose the system with the largest value for $S_2\sqrt{1 - r^2_{1,2}}$ and (3) for the third chromatogram choose the system with the largest value for $S_3\sqrt{1 - r^2_{1,2}} \cdot \sqrt{1 - r^2_{2,3}}$, etc. With this procedure it is possible to determine the order in which systems should be employed so that maximal probability of resolution is maintained throughout the implementation of the sequence.

Testing the hypothesis

The experimentally determined discriminating power (DP)[4]* of a chromatography system, or group of systems, for a specific collection of compounds can be quantified by calculating the fraction of the pairs of compounds which is separated by more than a specified amount (*e.g.*, 0.10 R_F unit) in the chromatography system, or group of systems, under consideration. This calculation involves 3 steps: (1) determination of the total number of pairs of compounds in the group which has been chromatographed; number of pairs $= n(n-1)/2$, where $n =$ the number of compounds, (2) calculation of ΔR_F for each pair of compounds and (3) determination of the fraction of ΔR_F values which is greater than the value required for separation of a pair of compounds.

The degree of correlation between total effective standard deviation and discriminating power can be measured by use of Spearman's equation[5] for rank correlation, where

$$r_s = 1 - \frac{6\Sigma d^2}{N(N^2 - 1)} \tag{5}$$

the coefficient of correlation, N is the number of pairs being correlated and d is the difference in the ranking of corresponding values.

A theoretical relationship between standard deviation and discriminating power

Moffat *et al.*[4] showed that with uniform distribution of compounds over an entire chromatogram and no similarity in the discriminating characteristics of the individual systems, the aggregate discriminating power for k systems is given by eqn. 6.

$$\mathrm{DP}_k = 1 - \prod_{i-1}^{k} (2E_i - E_i^2) \tag{6}$$

DP_k represents the combined discriminating power of k systems, $\prod_{i-1}^{k}$ is a product notation which indicates that the number of individual systems (i) may range from 1 to k, and E_i signifies the "error factor" (degree of separation) required to resolve two substances in each respective system. If the error factor is the same for all systems, the product notation can be eliminated and eqn. 7 is obtained.

$$\mathrm{DP}_k = 1 - (2E - E^2)^k \tag{7}$$

* Two compounds may be regarded as having been discriminated[4] in a chromatography system if the difference in their retention values exceeds a certain critical value which is called the error factor. This factor may be the difference in R_F (*e.g.*, 0.10) required for separation of a pair of compounds. The discriminating power of a single system is defined as the probability that two compounds which are randomly selected from the parent population will be discriminated in that system, *i.e.*, will differ in retention value by more than that which is required to separate them. The discriminating power of a series of systems is defined as the probability that two compounds selected at random will be discriminated by at least one of the systems.

Eqn. 7 can be modified to relate DP_k to $\Sigma S'$ for k systems. Let exponent k represent the ratio of $\Sigma S'_{1\ldots k}$ to the standard deviation of one ideal chromatography system as expressed in eqn. 8.

$$DP_k = 1 - (2E - E^2)^{\frac{\Sigma S'_{1\ldots k}}{0.289}} \quad (8)$$

The value 0.289 is the calculated standard deviation from the mean R_F of a chromatography system which distributes a group of compounds uniformly over the range $0 \leqslant R_F \leqslant 1$. Eqn. 8 relates the discriminating power of a chromatography system, or a sequence of systems, to the corresponding $\Sigma S'$ when the error factor is E.

If it is assumed that two substances can be discriminated when they differ in mobility by 0.10 R_F unit, and 0.10 is substituted for E in eqn. 8, eqn. 9 is obtained.

$$DP_k = 1 - (0.19)^{\frac{\Sigma S'_{1\ldots k}}{0.289}} \quad (9)$$

Although eqns. 8 and 9 are exact only if all distributions are rectangular (uniform), they should provide a good estimate of the DP that is associated with a given value for $\Sigma S'$ provided none of the systems has a markedly nonuniform distribution of the R_F values.

RESULTS

Lisboa and co-workers[6–17] have chromatographed a large number of steroids in numerous solvent systems by use of the TLC technique. From their data we have selected all substances which, in each of seven TLC systems, have R_F values in the range of 0.02 to 0.85. In Table I, these steroids are listed with R_F values in each system; the list includes 20 estrogens (numbers 1–20 in the table), 60 androgens (21–80) and 83 pregnanes (81–163) for a total of 163 substances. Since the histograms (Fig. 1) from the R_F values indicate that in none of the seven systems the distributions are markedly nonuniform, values from all of the systems are included in the analysis. The data from Table I will be used to examine the putative relationship between $\Sigma S'$ and discriminating power.

Correlation between DP and $\Sigma S'$ for all 163 steroids

The mean of the R_F values ($\bar{R}_F$) and values of S for 163 steroids in seven TLC systems are given in Table II. Also given in Table II are values for $\sqrt{1 - r^2}$ from all pairs of systems.

Using the data in Table II, values for $\Sigma S'$ were calculated for each of the 21 pairs of systems (eqn. 2), the 35 combinations of three systems (eqn. 3) and the 35 combinations of four systems (eqn. 4). By employing the approach which is outlined under *Testing the hypothesis*, corresponding values for DP were calculated for each of the seven individual systems and for the various combinations of two, three and four systems using an error factor of 0.10 R_F; the total number of pairs of compounds was 13,203. In Fig. 2, the relationship between DP and $\Sigma S'$ is shown for the 163 steroids

TABLE I

R_F VALUES FOR 163 STEROIDS IN SEVEN TLC SYSTEMS

The adsorbant was silica gel G for all systems. The composition of the solvent systems was: A = cyclohexane–ethyl acetate–ethanol (45:45:10); C = cyclohexane–ethyl acetate (50:50); D = chloroform–ethanol (90:10); E = ethyl acetate–*n*-hexane–acetic acid–ethanol (72:13.5:10:4.5); H = benzene–ethanol (80:20); K = benzene–ethanol (90:10); L = chloroform–ethanol (95:5).

No.	*Compound*[a]	*System*						
		A	*C*	*D*	*E*	*H*	*K*	*L*
1	E	0.72	0.63	0.67	0.84	0.61	0.56	0.53
2	E^{16}	0.65	0.48	0.63	0.78	0.56	0.51	0.44
3	16β,17β-ox-E	0.69	0.56	0.66	0.80	0.59	0.54	0.47
4	16α,17α-ox-E	0.69	0.54	0.66	0.79	0.60	0.51	0.47
5	E-17-one	0.69	0.53	0.65	0.80	0.59	0.51	0.46
6	$E^{9(11)}$-17-one	0.65	0.50	0.64	0.79	0.56	0.52	0.45
7	$E^{6,8}$-17-one	0.63	0.47	0.63	0.77	0.58	0.50	0.42
8	E-7,17-one	0.61	0.36	0.55	0.72	0.55	0.48	0.44
9	7α-ol-E-17-one	0.42	0.12	0.40	0.60	0.47	0.30	0.17
10	11β-ol-E-17-one	0.56	0.27	0.50	0.72	0.51	0.42	0.26
11	15α-ol-E-17-one	0.48	0.18	0.46	0.66	0.45	0.30	0.21
12	4-ol,3-MeO-E-17-one	0.66	0.53	0.76	0.79	0.67	0.63	0.69
13	17β-ol-E	0.61	0.40	0.52	0.74	0.53	0.42	0.30
14	17α-ol-E	0.61	0.43	0.55	0.75	0.52	0.45	0.34
15	17β-ol-E^6	0.57	0.36	0.52	0.73	0.49	0.41	0.29
16	17β-ol-E^7	0.57	0.36	0.53	0.74	0.50	0.41	0.30
17	E-7α,17β-ol	0.40	0.09	0.22	0.59	0.37	0.24	0.08
18	E-11β,17β-ol	0.34	0.08	0.29	0.54	0.39	0.20	0.09
19	E-16α,17β-ol	0.29	0.06	0.21	0.48	0.38	0.17	0.07
20	2-MeO-E-17β-ol	0.58	0.36	0.58	0.72	0.53	0.45	0.41
21	3α,11β-ol-5αA-17-one	0.49	0.17	0.51	0.66	0.57	0.34	0.29
22	3β,11β-ol-5αA-17-one	0.45	0.13	0.43	0.68	0.53	0.28	0.22
23	3α,11β-ol-5βA-17-one	0.44	0.12	0.45	0.64	0.52	0.30	0.24
24	2α,17β-ol-A^4-3-one	0.34	0.11	0.46	0.53	0.44	0.25	0.27
25	4,17β-ol-A^4-3-one	0.52	0.28	0.58	0.70	0.61	0.47	0.46
26	11β,17β-ol-A^4-3-one	0.31	0.05	0.34	0.46	0.45	0.22	0.11
27	11α,17β-ol-A^4-3-one	0.18	0.02	0.26	0.27	0.33	0.13	0.05
28	14α,17β-ol-A^4-3-one	0.27	0.04	0.31	0.39	0.39	0.19	0.11
29	16α,17β-ol-A^4-3-one	0.19	0.02	0.26	0.33	0.38	0.18	0.09
30	17β,19-ol-A^4-3-one	0.28	0.06	0.31	0.39	0.37	0.16	0.12
31	3β,7α-ol-A^5-17-one	0.22	0.02	0.27	0.35	0.36	0.14	0.09
32	3β,11β-ol-A^5-17-one	0.47	0.17	0.48	0.62	0.50	0.29	0.25
33	3β,16α-ol-A^5-17-one	0.43	0.16	0.46	0.67	0.51	0.19	0.30
34	3β,17β-ol-A^5-11-one	0.37	0.10	0.38	0.50	0.40	0.20	0.17
35	3α-ol-5αA-7,17-one	0.40	0.12	0.53	0.54	0.47	0.27	0.32
36	3β-ol-5αA-7,17-one	0.33	0.07	0.50	0.49	0.45	0.23	0.27
37	3α-ol-5αA-11,17-one	0.44	0.14	0.59	0.60	0.59	0.37	0.40
38	3α-ol-5βA-11,17-one	0.38	0.09	0.52	0.61	0.55	0.33	0.31
39	11β-ol-5αA-3,17-one	0.57	0.27	0.63	0.75	0.64	0.44	0.46
40	4-ol-A^4-3,17-one	0.59	0.41	0.72	0.76	0.70	0.62	0.67
41	11β-ol-A^4-3,17-one	0.47	0.17	0.58	0.64	0.55	0.41	0.42
42	11α-ol-A^4-3,17-one	0.37	0.08	0.49	0.49	0.50	0.29	0.26
43	14α-ol-A^4-3,17-one	0.39	0.10	0.54	0.54	0.52	0.33	0.33
44	15α-ol-A^4-3,17-one	0.32	0.06	0.47	0.45	0.46	0.29	0.24
45	16α-ol-A^4-3,17-one	0.38	0.11	0.59	0.58	0.53	0.36	0.42
46	19-ol-A^4-3,17-one	0.31	0.06	0.44	0.43	0.51	0.26	0.23

TABLE I *(continued)*

No.	*Compound*[a]	*System*						
		A	*C*	*D*	*E*	*H*	*K*	*L*
47	17β-ol-19-al-A[4]-3-one	0.39	0.10	0.53	0.56	0.54	0.38	0.35
48	3β-ol-A[5]-7,17-one	0.35	0.08	0.49	0.49	0.45	0.30	0.28
49	3β-ol-A[5]-11,17-one	0.47	0.19	0.59	0.62	0.51	0.37	0.41
50	17β-ol-5αA-3-one	0.56	0.35	0.61	0.73	0.57	0.42	0.49
51	17β-ol-5βA-3-one	0.55	0.30	0.61	0.71	0.59	0.42	0.49
52	17β-ol-A[4]-3-one	0.50	0.22	0.59	0.64	0.52	0.37	0.43
53	17α-ol-A[4]-3-one	0.51	0.23	0.60	0.64	0.52	0.39	0.43
54	17β-ol-A[1,4]-3-one	0.41	0.14	0.55	0.58	0.49	0.31	0.34
55	3α-ol-5αA-17-one	0.56	0.33	0.63	0.74	0.58	0.44	0.50
56	3β-ol-5αA-17-one	0.52	0.30	0.58	0.70	0.56	0.40	0.44
57	3α-ol-5βA-17-one	0.53	0.24	0.58	0.71	0.55	0.39	0.43
58	3β-ol-5βA-17-one	0.57	0.36	0.62	0.75	0.59	0.44	0.50
59	3β-ol-A[5]-17-one	0.53	0.36	0.57	0.73	0.55	0.39	0.43
60	3α-ol-A[5]-17-one	0.54	0.26	0.67	0.68	0.64	0.49	0.52
61	5αA-17β-ol	0.64	0.56	0.64	0.78	0.62	0.57	0.57
62	A[5]-3β-ol	0.65	0.49	0.62	0.75	0.60	0.44	0.52
63	5αA-3α,17β-ol	0.52	0.26	0.50	0.70	0.48	0.33	0.32
64	5αA-3β,17β-ol	0.51	0.25	0.47	0.68	0.49	0.29	0.29
65	5βA-3α,17β-ol	0.46	0.17	0.45	0.63	0.48	0.25	0.23
66	5βA-3β,17β-ol	0.53	0.30	0.50	0.70	0.50	0.34	0.32
67	A[4]-3β,17β-ol	0.50	0.26	0.47	0.70	0.50	0.30	0.32
68	A[5]-3β,17β-ol	0.49	0.26	0.48	0.69	0.50	0.29	0.31
69	A[5]-3β,17α-ol	0.50	0.26	0.49	0.71	0.50	0.30	0.32
70	A[5]-3β,7β,17β-ol	0.28	0.04	0.23	0.43	0.29	0.09	0.07
71	A[5]-3β,11β,17β-ol	0.31	0.06	0.22	0.47	0.33	0.13	0.07
72	5αA-3,17-one	0.66	0.46	0.74	0.78	0.66	0.64	0.69
73	5βA-3,17-one	0.64	0.43	0.74	0.78	0.67	0.63	0.67
74	5αA[1]-3,17-one	0.61	0.38	0.76	0.75	0.66	0.58	0.67
75	5αA[2]-7,17-one	0.67	0.52	0.77	0.81	0.70	0.61	0.70
76	A[4]-3,17-one	0.53	0.30	0.73	0.68	0.64	0.55	0.65
77	A[1,4]-3,17-one	0.49	0.20	0.71	0.62	0.58	0.47	0.58
78	5βA-3,11,17-one	0.52	0.25	0.69	0.70	0.66	0.50	0.58
79	19-al-A[4]-3,17-one	0.48	0.19	0.70	0.63	0.64	0.54	0.61
80	A[4]-3,11,17-one	0.39	0.14	0.67	0.60	0.56	0.48	0.53
81	5αP-3β-ol	0.66	0.48	0.62	0.76	0.64	0.48	0.53
82	5βP-3α-ol	0.67	0.55	0.65	0.77	0.67	0.52	0.59
83	5βP-3α,6α-ol	0.31	0.06	0.29	0.48	0.58	0.23	0.12
84	5αP-3α,20β-ol	0.59	0.34	0.55	0.70	0.56	0.34	0.34
85	5αP-3β,20α-ol	0.52	0.27	0.50	0.65	0.53	0.30	0.33
86	5αP-3β,20β-ol	0.57	0.31	0.52	0.70	0.56	0.33	0.35
87	5βP-3α,20α-ol	0.49	0.22	0.50	0.65	0.52	0.33	0.28
88	5βP-3α,20β-ol	0.54	0.25	0.52	0.68	0.54	0.36	0.31
89	5βP-3β,20β-ol	0.59	0.37	0.57	0.70	0.57	0.41	0.40
90	5βP-3α,17α,20α-ol	0.26	0.05	0.23	0.45	0.39	0.20	0.07
91	5βP-3β,17α,20α-ol	0.34	0.11	0.35	0.55	0.47	0.26	0.15
92	5βP-3α,17α, 20β-ol	0.32	0.08	0.32	0.51	0.44	0.23	0.12
93	5βP-3β,17α,20β-ol	0.39	0.16	0.41	0.61	0.49	0.29	0.19
94	5αP-3β,17α,20α-ol	0.34	0.10	0.33	0.58	0.45	0.25	0.14
95	20β-ol-5αP-3-one	0.64	0.42	0.71	0.76	0.65	0.51	0.60
96	3β-ol-5αP-20-one	0.55	0.33	0.62	0.71	0.58	0.42	0.45

(Continued on p. 250)

TABLE I *(continued)*

No.	*Compound*[a]	*System*						
		A	*C*	*D*	*E*	*H*	*K*	*L*
97	3α-ol-5αP-20-one	0.59	0.36	0.67	0.74	0.59	0.47	0.52
98	3α,6α-ol-5βP-20-one	0.23	0.04	0.29	0.37	0.43	0.15	0.09
99	3α,12α-ol-5βP-20-one	0.31	0.04	0.32	0.44	0.49	0.22	0.10
100	3α,17α-ol-5βP-20-one	0.46	0.15	0.42	0.64	0.57	0.30	0.20
101	3β,17α-ol-5βP-20-one	0.50	0.25	0.52	0.68	0.61	0.36	0.33
102	3β,17α-ol-5αP-20-one	0.47	0.22	0.47	0.65	0.57	0.33	0.30
103	P^4-3,20-one	0.62	0.38	0.75	0.72	0.68	0.56	0.67
104	5αP-3,20-one	0.66	0.53	0.75	0.78	0.70	0.63	0.70
105	5βP-3,20-one	0.64	0.50	0.73	0.76	0.70	0.63	0.69
106	12α-ol-5βP-3,20-one	0.51	0.13	0.56	0.58	0.56	0.31	0.38
107	3α-ol-5βP-11,20-one	0.42	0.10	0.54	0.54	0.54	0.31	0.38
108	3α,17α-ol-5βP-11,20-one	0.49	0.08	0.44	0.62	0.49	0.19	0.19
109	3β,17α-ol-5αP-11,20-one	0.45	0.12	0.37	0.57	0.47	0.24	0.26
110	5αP-3,11,20-one	0.56	0.26	0.76	0.68	0.67	0.53	0.69
111	5βP-3,11,20-one	0.53	0.22	0.73	0.65	0.68	0.49	0.66
112	5βP-3,6,20-one	0.53	0.27	0.74	0.65	0.67	0.51	0.66
113	5βP-3,12,20-one	0.61	0.39	0.77	0.72	0.71	0.57	0.69
114	P^4-3,11,20-one	0.44	0.16	0.70	0.62	0.60	0.44	0.60
115	21-ol-P^4-3,11,20-one	0.21	0.04	0.49	0.37	0.42	0.23	0.27
116	17α,21-ol-P^4-3,11,20-one	0.31	0.05	0.34	0.47	0.43	0.17	0.13
117	20β-ol-P^4-3-one	0.56	0.27	0.63	0.70	0.56	0.38	0.55
118	20α-ol-P^4-3-one	0.50	0.24	0.60	0.67	0.54	0.36	0.52
119	6β,20β-ol-P^4-3-one	0.44	0.16	0.51	0.61	0.55	0.32	0.27
120	6α,20β-ol-P^4-3-one	0.39	0.10	0.48	0.55	0.51	0.27	0.19
121	P^4-3-one	0.70	0.59	0.75	0.83	0.72	0.64	0.72
122	$P^{4,17(20)}$-3-one	0.69	0.56	0.75	0.83	0.70	0.62	0.72
123	$P^{4,6}$-3-20-one	0.57	0.34	0.71	0.73	0.66	0.55	0.67
124	$P^{4,16}$-3,20-one	0.59	0.38	0.72	0.74	0.65	0.55	0.68
125	2β-ol-P^4-3,20-one	0.47	0.18	0.62	0.63	0.56	0.40	0.53
126	2α-ol-P^4-3,20-one	0.47	0.18	0.62	0.62	0.56	0.40	0.53
127	6β-ol-P^4-3,20-one	0.49	0.15	0.55	0.61	0.55	0.30	0.37
128	11β-ol-P^4-3,20-one	0.46	0.14	0.57	0.60	0.54	0.32	0.40
129	11α-ol-P^4-3,20-one	0.31	0.06	0.43	0.43	0.45	0.20	0.24
130	14α-ol-P^4-3,20-one	0.47	0.14	0.55	0.58	0.52	0.30	0.40
131	16α-ol-P^4-3,20-one	0.29	0.06	0.47	0.44	0.52	0.25	0.35
132	17α-ol-P^4-3,20-one	0.56	0.27	0.64	0.72	0.58	0.40	0.52
133	19-ol-P^4-3,20-one	0.39	0.09	0.53	0.51	0.54	0.26	0.27
134	21-ol-P^4-3,20-one	0.40	0.13	0.62	0.55	0.55	0.38	0.51
135	11β,17α-ol-P^4-3,20-one	0.43	0.11	0.40	0.62	0.50	0.22	0.20
136	17α,21-ol-P^4-3,20-one	0.38	0.09	0.41	0.57	0.49	0.23	0.22
137	12β,14α-ol-P^4-3,20-one	0.23	0.02	0.33	0.28	0.35	0.08	0.10
138	5βP-3-one	0.73	0.70	0.79	0.84	0.76	0.75	0.80
139	3β-ol-P^4-20-one	0.54	0.37	0.58	0.72	0.56	0.42	0.48
140	3β-ol-$P^{5,16}$-20-one	0.53	0.37	0.57	0.71	0.55	0.42	0.49
141	3β,15α-ol-P^5-20-one	0.37	0.08	0.38	0.51	0.46	0.23	0.17
142	3β,16α-ol-P^5-20-one	0.35	0.09	0.41	0.52	0.47	0.26	0.21
143	3β,17α-ol-P^5-20-one	0.51	0.28	0.50	0.70	0.53	0.35	0.33
144	3β,21-ol-P^5-20-one	0.48	0.19	0.52	0.63	0.52	0.36	0.36
145	3β,17α,20α-ol-P^5-11-one	0.44	0.13	0.37	0.66	0.46	0.31	0.13
146	3β,11β,17α-ol-P^5-20-one	0.34	0.07	0.31	0.51	0.40	0.23	0.11
147	3β,17α,21-ol-P^5-20-one	0.35	0.14	0.34	0.61	0.43	0.25	0.17
148	3β-ol-P^5-15,20-one	0.44	0.16	0.51	0.60	0.54	0.40	0.37

TABLE I *(continued)*

No.	*Compound**	*System*						
		A	*C*	*D*	*E*	*H*	*K*	*L*
149	3β-ol-P^5	0.64	0.51	0.61	0.79	0.65	0.50	0.50
150	3β,20α-ol-P^5	0.50	0.27	0.48	0.66	0.49	0.33	0.33
151	3β,20β-ol-P^5	0.53	0.31	0.50	0.69	0.50	0.34	0.36
152	3β,15α,20β-ol-P^5	0.30	0.06	0.28	0.48	0.38	0.17	0.12
153	3β,15α,20α-ol-P^5	0.19	0.02	0.15	0.35	0.32	0.10	0.06
154	3α,16α,20α-ol-P^5	0.30	0.02	0.32	0.42	0.42	0.19	0.11
155	3α,16α,20β-ol-P^5	0.27	0.02	0.22	0.41	0.38	0.16	0.08
156	3β,16α,20α-ol-P^5	0.30	0.14	0.38	0.46	0.43	0.22	0.15
157	3β,16α,20β-ol-P^5	0.25	0.08	0.26	0.44	0.36	0.14	0.10
158	3β,17α,20α-ol-P^5	0.38	0.11	0.36	0.57	0.44	0.23	0.14
159	3β,17α,20β-ol-P^5	0.41	0.16	0.40	0.61	0.47	0.30	0.22
160	3β,18,20β-ol-P^5	0.41	0.11	0.36	0.58	0.44	0.22	0.18
161	3β,20α,21-ol-P^5	0.26	0.05	0.24	0.46	0.36	0.15	0.10
162	3β,20β,21-ol-P^5	0.29	0.07	0.26	0.48	0.40	0.18	0.10
163	3β,11β,17α,20α-P^5	0.29	0.05	0.21	0.51	0.36	0.19	0.06

* The structure of the steroid nucleus is designated as follows: E = estra-1,3,5(10)-trien-3-ol; 5αA = 5α-androstane; 5αP = 5α-pregnan; etc. Double bonds are indicated by superscripts. Ketone, hydroxyl, aldehyde, methoxyl and epoxy functions are indicated by -one, -ol, -al, -MeO and -ox, respectively.

through combinations of 4 systems. It is apparent that DP increases as $\Sigma S'$ increases. The points tend to fall along, though slightly above, the solid line which represents the theoretical relationship between DP and $\Sigma S'$ as given by eqn. 9. By substituting each pair of the experimental values for $\Sigma S'$ and DP into eqn. 9 and solving for the average

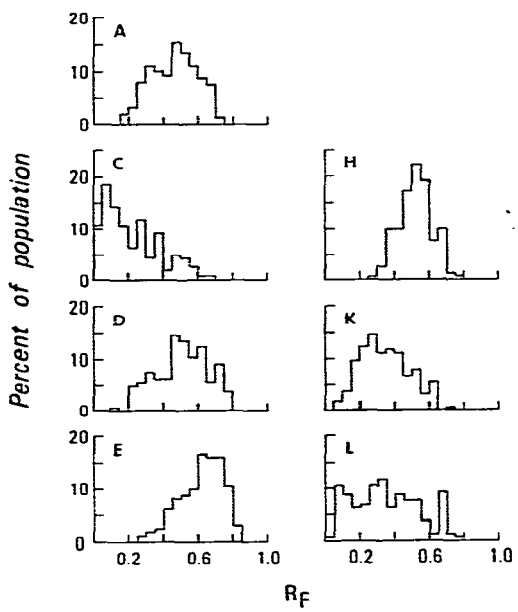

Fig. 1. Frequency distributions of R_F values from the chromatography of 163 steroids in seven TLC systems. See Table I for a list of the compounds and the composition of the solvent systems.

TABLE II

MEAN AND STANDARD DEVIATION OF THE R_F VALUES FOR 163 STEROIDS IN SEVEN TLC SYSTEMS AND VALUES OF $\sqrt{1 - r^2}$ FOR 21 PAIRS OF SYSTEMS

System	$\overline{R_F}$	*S*	*Value of* $\sqrt{1 - r^2}$* *for pairs of systems*						
			L	*C*	*D*	*K*	*A*	*E*	*H*
L	0.354	0.190	0.000	0.633	0.251	0.359	0.586	0.652	0.391
C	0.227	0.160		0.000	0.648	0.484	0.350	0.447	0.642
D	0.514	0.152			0.000	0.382	0.553	0.628	0.381
K	0.355	0.139				0.000	0.468	0.533	0.396
A	0.468	0.131					0.000	0.264	0.552
E	0.623	0.125						0.000	0.605
H	0.527	0.095							0.000

* The fractional amount by which two systems differ in discriminating characteristics is given by the corresponding value for $\sqrt{1 - r^2}$. For systems L and C the value is 0.633. $\Sigma S'$ for use of systems L and C in sequence is $0.190 + (0.160)(0.633) = 0.291$.

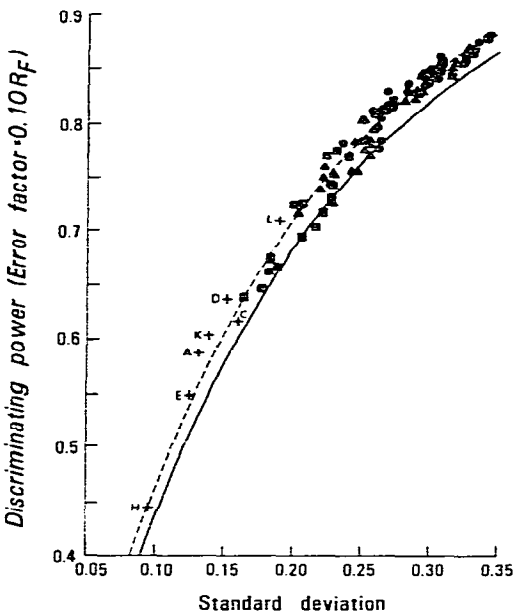

Fig. 2. Relationship of discriminating power to standard deviation for the chromatography of 163 steroids. + = Values from individual systems; ■ = values from systems used in pairs; ▲ = values from systems used in triplets; ● = values from systems used in quadruplets. For individual systems, values for standard deviations (S) were taken from Table II; for combinations of two, three and four systems, values for standard deviation ($\Sigma S'$) were calculated by use of eqns. 2, 3 and 4, respectively. Values for discriminating power (DP) were calculated by use of the approach outlined under *Testing the hypothesis* and a computer search program which is analogous to one given by Moffat *et al.*[4]. The solid line represents eqn. 9; the dashed line represents eqn. 10.

value of the term enclosed in parentheses, eqn. 10 was obtained. This equation is represented by the dashed line in Fig. 2.

$$DP_k = 1 - (0.167)^{\frac{\Sigma S'_{1\ldots k}}{0.289}} \quad (10)$$

The coefficient of correlation, r_s, between the 98 points for DP *vs.* $\Sigma S'$ in Fig. 2 is 0.986. When values for the 98 points are calculated by use of an error factor of 0.15 R_F, r_s is 0.983. These values for r_s indicate that the association between DP and $\Sigma S'$ for the collection of 163 steroids in the seven TLC systems is very good.

When evaluated on the basis of $\Sigma S'$, the order in which to use the seven TLC systems for maximal probability of resolution throughout the sequence was determined to be L, C, E, D, H, A and K. According to DP, using an error factor of 0.10 R_F, the optimal sequence is L, C, E, D, K, H and A. The discrepancy in the order for K, H and A is not very significant since use of the first four systems provides a DP of 0.884 and the use of the last three provides an additional DP of only 0.015.

Estimation of $\Sigma S'$ from a small number of compounds

It was of interest to determine the goodness of the association between $\Sigma S'$ that was calculated for a small, representative group of steroids and the DP that was calculated for the entire collection of 163 compounds. Thus, groups of 10, 20 and 50 steroids were drawn randomly from Table I and the corresponding values for mean R_F and for standard deviation of the mean were calculated for the compounds in each group of steroids in each of the seven systems. These calculated results are given in Table III along with values for $\sqrt{1 - r^2}$ for each pair of systems.

Findings for $\Sigma S'$, calculated for each random sample through combinations of four systems, compare favorably by rank with the corresponding values for DP which were calculated for the parent group of 163 compounds when an error factor of 0.10 R_F is used. For the samples of 10, 20 and 50 compounds, values for r_s are 0.980, 0.980 and 0.954, respectively.

The best orders in which to use the seven TLC systems as determined from values for $\Sigma S'$ for the different samples are: from the sample of 10 compounds, L, C, E, D, H, K, A; from the sample of 20 compounds, L, C, E, D, K, A, H; from the sample of 50 compounds, L, C, E, H, D, K, A. These sequences are very similar to the sequence determined for the entire collection of 163 compounds. Differences in the order in the latter part of the sequence are not very significant since about 97% of the total DP of the seven systems is achieved by use of the first three systems.

Selection of systems for estrogens, androgens and pregnanes

Values for mean R_F and standard deviation of the mean were determined for the 20 estrogens, 60 androgens and 83 pregnanes included in the collection of steroids. These data are presented in Table IV along with values for $\sqrt{1 - r^2}$ that were determined for each pair of systems using only the R_F values from each subclass of compounds.

$\Sigma S'$ correlates well with DP for selecting chromatography systems for the subclasses of steroids. Values for r_s, comparing $\Sigma S'$ and DP (error factor = 0.10 R_F)

TABLE III

MEAN AND STANDARD DEVIATION OF THE R_F VALUES FOR RANDOM SAMPLES OF STEROIDS IN SEVEN TLC SYSTEMS AND VALUES OF $\sqrt{1-r^2}$ FOR PAIRS OF SYSTEMS

See footnote to Table II for an explanation of the term $\sqrt{1-r^2}$.

System	$\overline{R_F}$	*S*	*Value of* $\sqrt{1-r^2}$ *for pairs of systems*						
			L	*C*	*D*	*K*	*A*	*E*	*H*
			*(A) For a random sample of 10 steroids**						
L	0.419	0.203	0.000	0.656	0.193	0.291	0.582	0.623	0.372
C	0.240	0.168		0.000	0.687	0.471	0.277	0.362	0.557
D	0.555	0.163			0.000	0.384	0.578	0.606	0.365
K	0.371	0.148				0.000	0.443	0.495	0.284
A	0.487	0.132					0.000	0.209	0.477
E	0.630	0.134						0.000	0.134
H	0.559	0.102							0.000
			*(B) For a random sample of 20 steroids***						
L	0.392	0.212	0.000	0.558	0.182	0.245	0.480	0.550	0.304
C	0.229	0.157		0.000	0.619	0.428	0.341	0.421	0.561
D	0.529	0.172			0.000	0.337	0.481	0.533	0.267
K	0.364	0.162				0.000	0.381	0.452	0.296
A	0.477	0.127					0.000	0.219	0.442
E	0.628	0.119						0.000	0.486
H	0.541	0.113							0.000
			*(C) For a random sample of 50 steroids****						
L	0.372	0.209	0.000	0.596	0.226	0.329	0.516	0.587	0.397
C	0.232	0.158		0.000	0.626	0.444	0.346	0.416	0.629
D	0.522	0.165			0.000	0.392	0.502	0.573	0.402
K	0.363	0.146				0.000	0.436	0.497	0.396
A	0.479	0.124					0.000	0.239	0.564
E	0.633	0.114						0.000	0.632
H	0.540	0.103							0.000

* The sample consisted of compounds 107, 85, 121, 66, 155, 114, 129, 132, 103 and 101.

** The sample consisted of compounds 107, 85, 121, 66, 155, 114, 129, 132, 103, 101, 128, 23, 105, 144, 157, 78, 71, 73, 160 and 150.

*** The sample consisted of compounds 107, 85, 121, 66, 155, 114, 129, 132, 103, 101, 128, 23, 105, 144, 157, 78, 71, 73, 160, 150, 45, 67, 127, 58, 99, 111, 110, 156, 81, 84, 18, 50, 51, 14, 119, 21, 40, 7, 136, 11, 52, 17, 138, 83, 26, 49, 95, 163, 117 and 154.

through combinations of four systems, are 0.976, 0.980 and 0.970 for the estrogens, androgens and pregnanes, respectively.

Heterogeneous collection of drugs

Since the foregoing correlations between DP and $\Sigma S'$ were based on 163 steroids which are rather similar in structure, it seemed desirable to make correlations on data from the chromatography of a more heterogeneous group of compounds. Accordingly, the R_F values of a group of 100 commonly used basic drugs in seven chromatography systems[18]* have been examined to find the association between $\Sigma S'$

* Since the R_F values for the drugs are listed elsewhere in a single table[18], the data will not be reproduced in this paper.

TABLE IV

MEAN AND STANDARD DEVIATION OF THE R_F VALUES FOR DIFFERENT CLASSES OF STEROIDS IN SEVEN TLC SYSTEMS AND VALUES OF $\sqrt{1-r^2}$ FOR PAIRS OF SYSTEMS

See footnote to Table II for an explanation of the term $\sqrt{1-r^2}$.

System	$\overline{R_F}$	*S*	*Value of* $\sqrt{1-r^2}$ *for pairs of systems*						
			L	*C*	*D*	*K*	*A*	*E*	*H*
			(A) For 20 estrogens						
L	0.355	0.164	0.000	0.390	0.282	0.228	0.427	0.467	0.222
C	0.366	0.177		0.000	0.334	0.303	0.234	0.280	0.360
D	0.532	0.151			0.000	0.221	0.310	0.319	0.225
K	0.427	0.125				0.000	0.274	0.303	0.217
A	0.571	0.123					0.000	0.133	0.373
E	0.718	0.096						0.000	0.420
H	0.523	0.080							0.000
			(B) For 60 androstanes						
L	0.369	0.174	0.000	0.585	0.212	0.234	0.532	0.600	0.368
C	0.206	0.134		0.000	0.674	0.591	0.347	0.475	0.646
D	0.532	0.137			0.000	0.316	0.567	0.618	0.348
K	0.356	0.135				0.000	0.550	0.629	0.333
A	0.455	0.118					0.000	0.248	0.531
E	0.616	0.127						0.000	0.559
H	0.526	0.094							0.000
			(C) For 83 pregnanes						
L	0.345	0.207	0.000	0.566	0.236	0.328	0.521	0.616	0.405
C	0.207	0.157		0.000	0.610	0.417	0.390	0.442	0.550
D	0.497	0.161			0.000	0.388	0.498	0.607	0.373
K	0.337	0.141				0.000	0.440	0.490	0.343
A	0.452	0.131					0.000	0.277	0.453
E	0.605	0.121						0.000	0.551
H	0.529	0.109							0.000

TABLE V

MEAN AND STANDARD DEVIATION OF THE R_F VALUES FOR 100 BASIC DRUGS IN SEVEN CHROMATOGRAPHY SYSTEMS AND VALUES OF $\sqrt{1-r^2}$ FOR PAIRS OF SYSTEMS

Values were calculated from data given in ref. 18. For an explanation of the term $\sqrt{1-r^2}$, see the footnote to Table II.

System	$\overline{R_F}$	*S*	*Value of* $\sqrt{1-r^2}$ *for pairs of systems*						
			7	*1*	*3*	*6*	*2*	*5*	*4*
7	0.569	0.288	0.000	0.958	0.901	0.851	0.887	0.984	0.974
1	0.349	0.238		0.000	0.967	0.961	1.000	1.000	1.000
3	0.354	0.233			0.000	0.893	0.595	0.885	0.865
6	0.560	0.219				0.000	0.922	0.803	0.812
2	0.465	0.184					0.000	0.936	0.918
5	0.256	0.174						0.000	0.432
4	0.408	0.172							0.000

and DP. Table V gives values for mean R_F and S for each of these drugs in each of the seven systems and includes calculated values for $\sqrt{1 - r^2}$ from each of the theoretically possible 21 pairs of systems. From these values, $\Sigma S'$ has been determined for the seven individual systems, the 21 combinations of pairs, the 35 combinations of triplets and the 35 combinations of quadruplets. Calculations of the corresponding values for DP using an error factor of 0.10 have been performed and a plot of the values of $\Sigma S'$ *vs.* DP is displayed in Fig. 3 along a line which was calculated from eqn. 9 using an error factor of 0.10. The correlation coefficient, r_s, between $\Sigma S'$ and DP is 0.985; this correlation is essentially the same as that found between $\Sigma S'$ and DP for the 163 steroids at an error factor of 0.10.

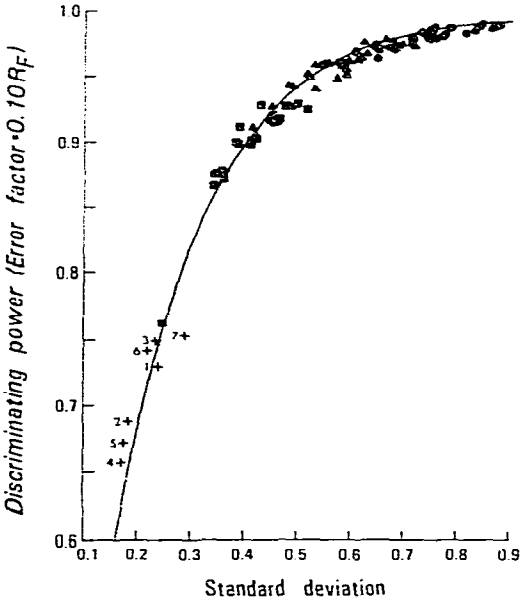

Fig. 3. Relationship of discriminating power to standard deviation for the chromatography of 100 basic drugs. + = Values from individual systems; ■ = values from systems used in pairs; ▲ = values from systems used in triplets; ● = values from systems used in quadruplets. For individual systems, values for standard deviation (S) were taken from Table V; for combinations of two, three and four systems, values for standard deviation ($\Sigma S'$) were calculated by use of eqns. 2, 3 and 4, respectively. Values for discriminating power (DP) were calculated by use of the approach outlined under *Testing the hypothesis* and a computer search program which is analogous to one given by Moffat *et al.*[4]. The solid line represents eqn. 9.

The optimal sequences for use of the seven systems as predicted by values for $\Sigma S'$ and DP (error factor = 0.10) are similar; the sequence determined from values for $\Sigma S'$ is 7, 1, 3, 6, 5, 2 and 4 and that determined by DP is 7, 3, 6, 1, 5, 2 and 4.

DISCUSSION

The accuracy with which the discriminating power of chromatography systems can be ranked by use of values for S and $\Sigma S'$ is determined largely by the degree of similarity in the shapes of their histograms (R_F *vs.* frequency). Minor skewing in the

shape of a histogram may cause the corresponding chromatography system, and combinations which involve the system, to be slightly out of order relative to DP predicted by S and $\Sigma S'$, but generally these differences do not invalidate the conclusions drawn from an analysis based on values for S. Dissimilarity in the shapes of the histograms of systems C and D (Fig. 1) probably accounts for the discrepancy in the predicted and found discriminating power of these two systems (Fig. 2), yet overall correlation between DP and $\Sigma S'$ for all seven systems and combinations involving pairs, triplets and quadruplets thereof is good ($r_s = 0.986$).

If a particular system has a histogram which is markedly different from those of the other systems in the comparison, the results of an analysis based on values for S can be very misleading. Thus, prior to evaluating a group of systems using values for S, an assessment of the frequency distributions of the R_F values in each system should be made. A visual scan of the table of R_F values is usually sufficient to recognize a system which has a highly unusual distribution.

Not included in the analysis of the chromatographic data on the 100 drugs were the R_F values of the compounds in system 8 (ref. 18); the values range from 0.00 to 0.95 but 61% of them are less than 0.10. Thus, the histogram is grossly skewed ("L-shaped") and is markedly different in form from those of the other seven systems. The value for S (0.322) implies that system 8 is the best system for separating drugs (see Table V); however, DP for system 8 (error factor = 0.10 R_F) is only 0.549, which indicates that system 8 is the poorest system for resolving drugs (see Fig. 3).

If all of the systems being compared have relatively rectangular distributions, the points from a plot of DP *vs.* $\Sigma S'$ will fall on or near the theoretical curve as represented by eqn. 8, provided that the value for error factor is less than the values of S for most of the systems. If the value for error factor approaches or exceeds the values for S, values found for DP generally will be less than those calculated by use of eqn. 8, regardless of the shapes of the histograms. Since the histograms of the seven systems used to chromatograph the basic drugs are relatively rectangular, the points shown in Fig. 3 fall near the theoretical line. In contrast, the histograms of the seven TLC systems used to chromatograph the steroids (Fig. 1) tend to be somewhat "bell-shaped." Consequently, most of the points in Fig. 2 fall above the theoretical curve (error factor is less than most values for S), and when an error factor of 0.15 R_F is employed, most of the points from a plot (not shown) fall below the theoretical line (error factor approaches or exceeds most of the values for S).

As indicated by the value for S, and verified by the value for DP, system L is the best single system of those examined for resolving the mixture of steroids. In fact, the DP provided by system L (using an error factor of 0.10) is greater than that provided by 7 of the 21 combinations of two systems, and nearly equivalent to that provided by one combination of three systems (Fig. 2). This observation highlights the importance of using a sequence that is composed of systems which have both high individual discriminating powers, and little similarity with the other systems in the sequence relative to resolving characteristics[18]. Because systems A and H are of appropriate polarity and are quite different in composition, one might predict intuitively that use of these systems in combination would provide more discriminating power for a mixture of steroids than use of system L alone. However, neither system A nor system H has particularly high individual discriminating power and the resolving properties of the two systems are not greatly different. Thus, comparison of the values for $\Sigma S'_{A,H}$

(0.183) and S_L (0.190) or of values for the corresponding discriminating powers ($DP_{A,H} = 0.674$ and $DP_L = 0.710$) should convince one that his initial prediction is suspect.

The seven TLC systems used for the steroids have rather similar resolving characteristics; in fact, only 6 of the 21 values for $\sqrt{1 - r^2}$ (Table II) are greater than 0.6 whereas 8 of the values are less than 0.4. The $\Sigma S'$ of all seven systems is 0.353, which is only 35% of the amount that would have been available if the systems had had no similarities in discriminating properties. The first three systems in the optimal sequence, L, C and E, provide 93% of the $\Sigma S'$ produced by all seven systems. It is apparent that each of the remaining systems, D, H, A and K, has resolving characteristics which are very similar to those of one or more of the systems which precede it in the sequence, and that little discriminating power is gained by using more than the first three systems.

In contrast to the foregoing observations, the seven systems used for drugs generally have distinctly different resolving properties. Of the 21 values for $\sqrt{1 - r^2}$ (Table V), 12 are greater than 0.9 and only one is less than 0.5. The $\Sigma S'$ for all seven systems used for drugs is 1.131, which corresponds to 75% of the amount that would have been available if the systems had had no similarities in discriminating properties. Only 78% of the $\Sigma S'$ for all seven systems is provided by the first four systems in the optimal sequence, *viz.*, 7, 1, 3 and 6.

The theoretical relationship between DP and $\Sigma S'$ as expressed by eqn. 9 indicates that if $\Sigma S'$ of 0.121 discriminates half of the pairs of compounds (DP = 0.50), an additional $\Sigma S'$ of 0.121 will discriminate half of the residual pairs so that $\Sigma S'$ of 0.242 corresponds to DP of 0.75; similarly, $\Sigma S'$ of 0.363 corresponds to DP of 0.875, $\Sigma S'$ of 0.484 corresponds to DP of 0.918, etc. Application of this concept in a general way to a practical problem may help one evaluate the real usefulness of a particular system. Suppose that a mixture of basic drugs, taken from the population of 100, has been chromatographed successively in systems 7, 1, 3 and 6. Since these four systems provide a DP of 0.991, it may seem, from a conventional point of view, that the use of the next system in the optimal sequence, *i.e.*, 5, could provide little additional discrimination. However, examination of the situation with the foregoing concept in mind indicates that use of system 5, which will contribute additional $\Sigma S'$ of 0.122, will provide a 50% chance of resolving a pair of drugs that was not resolved in any of the first four systems.

A procedure for evaluating chromatography systems which is analogous in some aspects to the one described in this paper has been published by Massart and co-workers[19–21]. Their procedure involves quantifying the resolving power of individual systems by determining information content and classifying systems according to their resemblance in chromatographic behavior by means of numerical taxonomy. This latter technique requires determination of either correlation coefficients or taxonomic distances, reduction of the resulting resemblance matrix, and construction of a dendrogram. Additional analyses of the R_F values of the 100 basic drugs[20] and of the R_F values of the 163 steroids[22] have been conducted using the method of Massart and co-workers to select the sequence of systems which is most likely to separate a mixture of drugs and the sequence most likely to separate a mixture of steroids. In general, optimal sequences determined on the basis of values for S and $\sqrt{1 - r^2}$, as outlined

elsewhere in this paper, correspond well[22] with analogous sequences constructed on the basis of information content from dendrograms which were based on correlation coefficients.

The foregoing statistical treatment of the R_F values of the 163 steroids and of the 100 basic drugs has shown that, if none of the systems being compared has a markedly skewed frequency distribution, there is good rank correlation between $\Sigma S'$ and DP. In addition, optimal sequences for the separation of drugs and steroids which were determined on the basis of values for S and $\sqrt{1 - r^2}$ generally correlate well with those determined on the basis of DP or by the method of Massart and co-workers. Having established these correlations with two rather large collections of data, we suggest that our procedure for evaluating chromatography systems is applicable to practical problems which may involve only a few compounds and several systems.

In principle, the procedure of evaluation is adaptable to any mode of chromatography and any class of compounds. The user may apply only that portion of the procedure which is applicable to his problem. If he is interested primarily in a qualitative evaluation of resolving power and the similarity of the systems to one another, he may obtain this information from values of S and $\sqrt{1 - r^2}$. If he wants a semi-quantitative evaluation of one or more specific systems, or sequences of systems, he can obtain this information by determining values for $\Sigma S'$ and calculating values for DP.

Frequently, an investigator publishes chromatographic data in the form of tables which consist of the R_F values for 10–20 structurally-similar compounds in 3–4 different systems. If he appended these tables with values for mean R_F, S and $\sqrt{1 - r^2}$, he would give the reader a much fuller description of the properties of the systems. These additional items of description should be of value to the reader in selecting the system(s) most suitable for his purpose.

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