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Criteria of Identity and Purity in Chromatographic Separations[*,†]

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Summary

One of the fundamental objectives of both experimentalists and theoreticians is to establish chromatography as a primary criterion of identity and purity. In part, meeting this objective consists of the experimental aspect of measuring and cataloging the behavior of all possible compounds and classes, but equally important it consists of describing the theoretical basis for system definitions. These system definitions include the discrimination ability or chromatographic "mesh size," the density or incidence of retention values in the test chromatogram, and the quality of measurements used to compare reference and experimental material. The latter is composed both of the instrument detection capabilities and column efficiencies, and of the experimenter's choice of peak-measurement techniques; the greatest difference in these is between the integral (flow) and incremental (fraction) methods. The uses, advantages, and disadvantages of each depend in a critical manner upon the limiting efficiencies and resolution of which the instrument is capable, but the quality of the measurements can always be improved by the use of the incremental technique. In particular, the incremental method permits the distinction between two nonidentical species to be made without achieving partial peak separation or even in the absence of a perceptible discontinuity in the peak envelope. A theoretical basis for classifications of systems is described. This is based upon the ability to determine the basic parameters characterizing a chromatographic peak and is illustrated by reference to synthetic chromatograms generated by digital computation and by reference to representative column-performance figures in actual separations.

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The inherent simplicity of the chromatographic technique, the variety of compounds for which it may be used, and the readiness with which its accuracy can be enhanced make it a logical choice for many analyses. Its full utility will not be realized, however, until it is unequivocally accepted as a primary standard in the establishment of chemical identity and purity. Partial acceptance as a standard is already evident in the description of radiochemicals as "chromatographically pure in one or more systems," and with the construction of catalogues of homologous compounds in functional classes that permit generalizations about structure and chromatographic behavior, identifications by chromatographic means are gaining acceptance.

Ultimately, the most rigorous test we can conceive is the comparison of the experimental sample with the reference substance it is suspected to be. This reference, by choice of the investigator, is, first, the *arbiter of identity;* the experimental sample and reference may not display any detectable differences, and, second, *the reference is considered to be pure;* that is, purity consists of that level and type of contaminants present in the reference. These definitions are necessary solely because identity and purity are not end points but scale readings. They may be set (in the case of identity) anywhere from class identity to the isotope abundances at various points in the same molecule and (in terms of purity) from being merely the major component to having no other component present at the parts-per-million level of concentration. In this hierarchy, one man's pure reference standard is likely to be the next man's standard mixture.

The first step in establishing categories of systems and associated capabilities for particular discrimination is to define them in such a manner that the ability of particular column or system to recognize identity and purity is expressed in numerical form.* What is

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* Definitions of the ability of chromatographic systems to identify compounds have usually been concerned with the *resolution* of the test compound from a second (known) compound (*1,2*). The analytical capability of the system is then an easily computed relationship between the number of theoretical plates (better, peak dispersion) and the ratio of retention times or elution volumes for the two compounds that would permit the chromatogram to be divided into two fractions, each containing a single compound of specified purity. These computations are largely concerned with the relationship of peak dispersion to cross-contamination between two peaks that are a specified distance apart. To be used in a practical situation, these treatments require a priori stipulation of the identity of possible contaminants, their

required in this situation may be stated as follows: In the chromatographic system used, compound A migrated with a mobility *M*, undergoing a dispersion of the peak σ, and appeared to be a single component. What certainty of identity and purity may the experimenter assume, or rely upon obtaining, from this chromatographic analysis? What are the cardinal properties that determine the certainty or quality of the analysis which has been performed?

To answer these questions, three steps are necessary: first, the parameters which characterize the quality of the analysis must be identified together with the means for their measurement; second, the basis of purity and identity criteria must be demonstrated; and, third, the capabilities and limitations of present systems should be indicated. These steps constitute the task of this paper.

SYSTEM-DESCRIPTION PARAMETERS AND THEIR MEASUREMENT

M. This quantity, which corresponds to the usual mobility or retention volume term in liquid and gas-liquid chromatography (corrected for the dead volume of the column) is more specifically restricted to that distance (volume, time) at which 50% of the peak has been eluted. This requires somewhat more information than is conventionally obtained by triangulation measurements. *M* is also distinguished from the point of maximum peak inflection, i.e., the point of highest concentration in the chromatographic peak. The maximum may or may not correspond to the same quantity as *M*, but it suffers from the handicap of being a subjective determination, the accuracy of which depends upon the peak dispersion and upon the purity of the sample. It is also more severely affected by nonideal conditions than is *M*.

δM. This quantity has been defined in an earlier paper (3) as

$$\delta M = 4(\text{s.e. } M) \qquad (1)$$

where s.e. *M* is the standard error in the determination of the mobility *M* of a compound in the chromatographic system of choice. It should be noted that δM describes the experimenter's ability

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mobilities, and their approximate concentration. In the present considerations, this amounts to proving that a peak is contaminated *after* an impurity has been demonstrated experimentally, instead of beforehand.

to measure M and is unrelated to conventional expressions of ***resolution*** in which the term 4σ appears. There is, in fact, no formal relation between the efficiency of a column and the precision with which the midpoint of a peak can be determined; the first is concerned with the character of peak dispersion, or the manner in which the area under the peak is distributed; the second is concerned with the techniques for, and reliability of, determinations specifying the x distance to the midpoint of the peak.

σ. The peak dispersion or basis of the column efficiency.

$\delta\sigma$. This quantity expresses the experimenter's ability to detect changes in the peak dispersion of the chromatographic sample and is defined as

$$\delta\sigma = 4(\text{s.e. } \sigma) \tag{2}$$

Unlike δM, whose magnitude relative to M can be reduced by increasing the value of M, $\delta\sigma$ places the more difficult requirement upon the investigator to *reduce* the proportionate error of measurement in a quantity that becomes smaller the more efficient the column becomes.

ρ. The density of retention values or fractional occupancy of sample space is given by

$$\rho = \frac{N}{100\delta M} \tag{3}$$

where N is the total number of compounds whose retention values fall within any given interval of $100\delta M$. This quantity is directly related to the number of unresolved classes still present in the sample prior to analysis and reflects the demand placed upon the system by the experimenter.

The quality of the measurements characterizing the peak depends in a critical fashion upon the techniques used to monitor the chromatogram. A comparison of the three basis procedures: (1) those of *integral measurement* represented by a recorder and integrating device; (2) *flow sampling*, exemplified by the ninhydrin measurements of an automatic amino acid analyzer; and (3) *incremental* or fraction collection is shown in Table 1. In the case of the incremental measurement, the values for each fraction may be used directly in a regression or probit analysis to obtain the first four peak parameters shown. The techniques for this

TABLE 1

The Measurement of Peak Characteristics with Various Monitoring Techniques

Measurement	Integral	Flow sampling	Incremental
M	Graphic estimate	Graphic, can be computed	Computed value
σ	Graphic estimate	Graphic, can be computed	Computed value
δM	Direct determination in single run not possible	Only if computed	Computed value
$\delta\sigma$	Direct determination in single run not possible	Only if computed	Computed value
Statistical quantitation (e.g., cpm)	Rate-dependent	Rate-dependent	Any desired level
Dual properties of single peak	Sequential measurement	Sequential measurement	Coincident measurement
Digitization	Difficult, accuracy range-limited	Moderately difficult	Column-independent

computation have been described in detail elsewhere (*4*). The integral measurement, while providing an estimate of M, depends heavily upon perfect peak symmetry for the point at which 50% has been eluted to coincide with the maximum, and the dispersion is at best obtained from triangulation of the peak upon the base line to yield an approximate 4σ. It follows that measurements made in this manner are *capable* of yielding the desired parameters but are relatively gross and inefficient compared to the other two. Furthermore, those quantities δM and $\delta\sigma$ cannot be obtained within a single run, each value of M and σ being the result of a single measurement; as a consequence, it is necessary to obtain them from multiple runs. This introduces additional variability from differences in column stability and reproducibility. These complications are avoided by the procedures of flow sampling and incremental analysis, in which multiple analyses upon a single peak provide the basis for computing the internal error associated with M and σ.

A further distinction between flow sampling and fraction collection may be drawn where the measurement requires a given statistical reliability or where two or more properties of the same peak must be determined. Neither of these requirements can be met adequately by the flow-sampling technique; the "holding time" for each sample is fixed by the speed at which the column is run and cannot be lengthened without affecting the course of the analysis. This means that when radioactive regions are being monitored, low-counting regions are either counted in a statistically unreliable manner or the column operation time must be lengthened to enable the lowest region to be counted accurately. The problem of conducting multiple measurements on the same peak is also complicated in the flow-sampling system by the requirement of having the measurements made sequentially. Few, if any, detectors can be used to make two different types of measurements at the same time on the same volume of eluate. The sequential or tandem configuration is limited by the accuracy with which the separation or "lapsed time" between detectors can be measured and by the ability to maintain the column flow rate at precisely the setting for which both channels of the recorder display coincidence. In contrast, the fraction collection system, in which segregation of the increments is preserved indefinitely, permits both the desired degree of accuracy in statistical measurements as well as rigorous certainty of analytical coincidence with the fraction interval.

Finally, one may compare the ease with which these three monitoring techniques can be made to yield numerical values, automatically. For the greatest accuracy, it is essential to dissociate the *operation* of the column from the *analysis* of the measurement, both to permit more points to be taken, and also to adjust the sensitivity of the determination according to the requirements of the sample. Usually a sacrifice must be made in choosing between the speed of response necessary to follow the chromatogram in real (i.e., live) time and the range, sensitivity, and accuracy of the numbers that are obtained if the integral or flow-sampling monitoring techniques are employed.

CHROMATOGRAPHIC CRITERIA OF PURITY AND IDENTITY

We have previously reported on the behavior of a chromatographic peak which was contaminated by various percentages of components of differing mobilities (*4*). This study, which was conducted by computer generation of the digital values for each chromatographic component from their algorithm, showed that the peak was displaced according to the relationship

$$M_2 = \frac{n_1 M_1 + n_3 M_3}{n_1 + n_3} \tag{4}$$

where M_2 is the mobility of the mixed peak and n_1 and n_3 are the quantities of the pure component and contaminant with respective mobilities M_1 and M_3. This relationship indicated that a given displacement could be the consequence of a variety of contaminants whose size and mobilities were inversely related. Each contaminant, however, produces a characteristic broadening of the peak, and the purity of the peak could be expressed as

$$\Psi_{(x)} = 2.718 \log \left[\frac{\sigma_1 M_1}{100(M_2 - M_1)}\right] + 1.481 \log \left[\frac{100(\sigma_2 - \sigma_1)}{\sigma_1}\right] - 2.384 \tag{5}$$

where $\Psi_{(x)}$ is the purity function and is the area under the unit normal deviate curve that corresponds to the fractional purity X. X is given by

$$X = \frac{n_1}{n_1 + n_3} \tag{6}$$

and is deduced from the definition

$$X = \frac{1}{(2\pi)^{1/2}} \int_{-\infty}^{\Psi(x)} \exp\left(\frac{-T^2}{2}\right) dT \tag{7}$$

It is apparent that in a displaced peak some of the contaminants would be visible (if present) as a bulge or inflection in the peak, whereas others, larger and closer in mobility, would be detectable only by an increase in peak dispersion. The exact conditions that result in an inflection in the chromatographic peak are determined by the magnitude of the displacement ΔM, or $M_2 - M_1$, the peak dispersion σ, and the per cent impurity responsible for the displacement. One can foresee that as the mobility of the contaminant is increased, even though its magnitude is decreased, the peak corresponding to the impurity will gradually emerge from the envelope of the chromatogram and become visible as a discontinuity. Beyond this point it will appear as a second peak.

This relationship between ΔM, σ, and purity, which does not appear to have been computed, underlies many of our basic assumptions about the term "chromatographically pure." Its solution might indicate the reliance that can be placed upon the belief that a single peak is indicative of purity.

Using a program developed by J. Pagels of this Division, the following analysis was performed. Holding ΔM and σ constant, a series of chromatograms were constructed by the computer in which the proportion of n_1 and n_3 and the value of M_3 were varied in such a manner to move the impurity away from the reference compound while keeping the over-all peak displacement M_2 constant [Eq. (4)]. The downward limb was examined for the appearance of a positive inflection, and by an iterative process the composition of the mixture at which this inflection was first visible could be determined to any desired degree of accuracy. Peak splitting occurred at the compositions shown in Table 2 for values of $\Delta M/\sigma$

TABLE 2

Peak Splitting by Impurities: Maximum Size of Contaminant Which Is Still Visible as Discontinuity in Peak

$\Delta M/\sigma$	Separation[a] $x\sigma M_1 + y\sigma$		$M_3 = M_1 + z\sigma$	$\% n_3$
1.0	1.80	2.00	2.414	41.42
0.9	2.20	2.40	2.610	34.37
0.8	2.40	2.60	2.757	29.00
0.7	2.40	2.60	2.878	24.31
0.6	2.60	2.80	2.989	20.07
0.5	2.80	3.20	3.113	16.06
0.4	2.80	3.20	3.249	12.31
0.3	2.80	3.20	3.378	8.88
0.2	3.20	3.60	3.559	5.62

[a] Point of positive inflection in downward limb of peak occurs between $M_1 + x\sigma$ and $M_1 + y\sigma$.

between 1.0 and 0.2 and were independent of the absolute value of σ. When the displacement exceeds the dispersion ($\Delta M/\sigma > 1.0$), the contamination is so great as to constitute more than 50% of

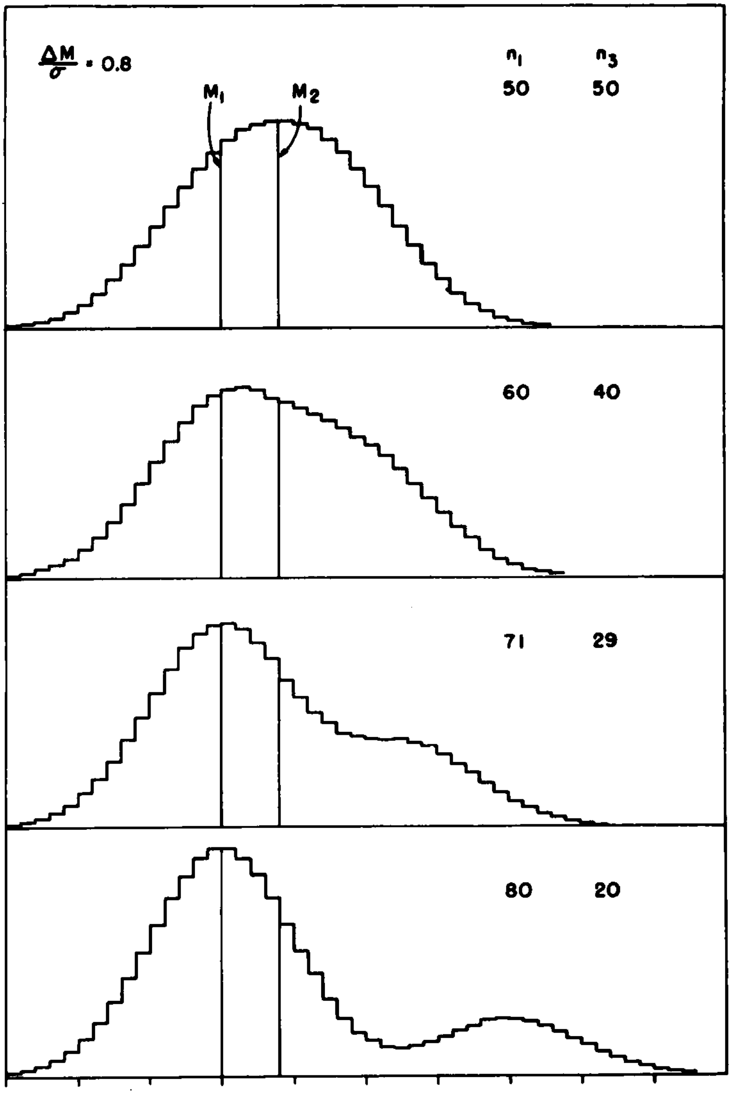

FIG. 1. The visibility of peak discontinuities caused by impurities as a function of the magnitude of the component causing a given displacement. All four peaks are 50% eluted at the point labeled M_2, which is displaced by the distance indicated from the reference value M_1. The ratio of the displacement to the dispersion of the pure peak is 0.8.

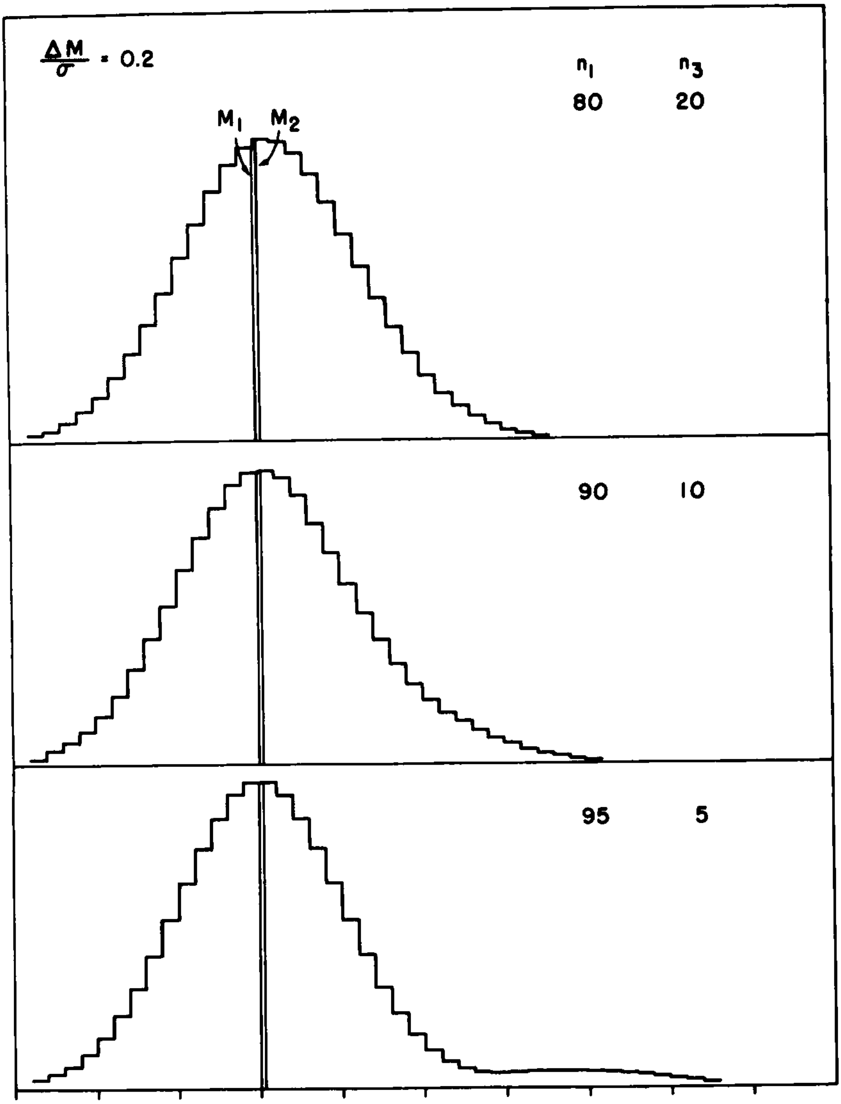

FIG. 2. Peak splitting by impurities causing a displacement-to-dispersion ratio of 0.2 for three exemplary compositions, of which the last produces a perceptible discontinuity.

the sample, whereas below values of 0.2 for $\Delta M/\sigma$ impurities do not produce visible peak splitting at any concentration. The emergence of an impurity and the occurrence of peak splitting at values of $\Delta M/\sigma$ of 0.8 and 0.2 are illustrated in Figs. 1 and 2. Inflection occurs at 29 and 5% impurity, respectively; *above* this concentration (i.e., large contaminants producing the same displacement) the presence of impurities in the peak is only suggested by distortion. The contaminated peak can only be differentiated from the reference by virtue of its displacement and broadening. The ability to carry out this differentiation clearly depends upon the values of δM and $\delta\sigma$ attainable in the system.

We have already said that by definition the reference is pure. This means that if the unknown is impure, its mobility will be displaced and it will be distinguished from the reference and rejected as nonidentical. Therefore, the ultimate criterion of purity can only be met by complete identity in the mobilities of both reference and unknown material and in the coincident values for the peak dispersion. As long as there is a measureable displacement between the two peaks, they are either not identical (in which case one cannot, except under limited circumstances, infer anything about the purity of the second peak) or, if the experimenter already *knows* them to be identical substances, the purity can be computed by means of Eq. (5).

If structural and chemical species are being separated, the establishment of identity will hinge directly upon the value of ρ, in the following manner. When successive compounds in an elution series are separated by intervals of random length, the series density at which two retention values are likely to coincide can be formulated as an occupancy problem. Knowing ρ, one may specify the required δM to provide any level of confidence that two compounds will *not* be regarded as identical when they are indeed different. In the instance where all members of a class have not been mapped in the chromatographic retention system, the experimenter is faced with the necessity of specifying a δM adequate to distinguish a known member from all other possibilities for that class, both known and unmapped. He can provide an estimate for ρ from the interval separating the lowest from the highest member of the series and the combinatorial ensemble of functional groups that are possible and whose retention values fall between these two extremes. The ratio of this ensemble to the total multiples of δM in the interval is the fundamental density required and is reduced by division of the unit interval into smaller increments, as dictated by the analytical requirements and the system capability.

As additional members of a class are mapped, an examination of the distribution of intervals between retention values will indicate whether the distribution is of the first, second, or third type, shown in Fig. 3. Type I may be regarded as consisting of clusters of retention values in which the separation factors for various functional groups are small relative to the homologous-series factor. Type II represents the Poisson-type distribution of intervals, in which the separation factors interact in a random fashion to produce a variety of intervals centered about an average value. Type III collections are those in which the separation factors are regular, equal, and additive, resulting in uniform spacing between successive compounds.

I CLUSTER

II POISSON

III UNIFORM

FIG. 3. Varieties of distributions of retention values in a polyfunctional class.

Note that at low-to-intermediate densities ($\rho = 0.05$ to 0.30) the Poisson model of Type II is the most pessimistic expectation of coincidence; uniformity, even in close-packed retention values,

TABLE 3

Relative Retention Values of Some Sterol Acetates on Code 12 Silica Gel Eluted with 16% Benzene/Pentane (Cholesterol Acetate = 1.000)

Sterol acetate	Retention value
4,4-Dimethylcholest-5-ene-3β-ol	0.640
4,4-Dimethylcholestane-3β-ol	0.667
4,4,14α-Trimethylcholestane-3β-ol	0.709
4,4,14α-Trimethylcholest-8-ene-3β-ol	0.73
4β-Methylcholestane-3β-ol	0.750
Cholest-3,5-diene-3-β-ol	0.786
4β-Methylcholest-5-ene-3β-ol	0.794
4,4,14α-Trimethylcholest-7,9-diene-3β-ol	0.804
4α-Methylcholestane-3β-ol	0.804
4,4,14α-Trimethylcholest-7-ene-3β-ol	0.83
Cholestane-3β-ol	0.860
4,4-Dimethylcholest-8(14)-ene-3β-ol	0.870
4α-Methylcholest-5-ene-3β-ol	0.901
14α-Methylcholestane-3β-ol	0.918
14α-Methylcholest-8-ene-3β-ol	0.944
4,4-Dimethylcholest-7-ene-3β-ol	0.956
Cholest-5-ene-3β-ol	1.000
Sitosterol	1.04
14α-Methylcholest-7,9-diene-3β-ol	1.077
Stigmasterol	1.09
14α-Methylcholest-7-ene-3β-ol	1.105
Cholest-8(14)-ene-3β-ol	1.206
4α-Methylcholest-7-ene-3β-ol	1.22
4,4,14α-Trimethylcholest-8,24-diene-3β-ol	1.34
Cholest-7-ene-3β-ol	1.405
4α-Methylcholest-7,9-diene-3β-ol	1.435
4,4,14α-Trimethylcholest-7,24-diene-3β-ol	1.48
Cholest-7,9-diene-3β-ol	1.640
4,4,14α-Trimethylcholest-7,9,24-triene-3β-ol	1.66
Cholest-5,7-diene-3β-ol	1.77
Cholest-7,14-diene-3β-ol	1.955
Cholest-5,24-diene-3β-ol	1.98
Ergosterol	2.04
Cholest-8,24-diene-3β-ol	2.42

carries with it a premium in the form of the certainty that a given δM, however small, will always achieve a separation.

The spacing of an actual chromatographic series is shown in Table 3, which lists an ensemble of 34 sterol acetates the retention volumes of which on a liquid-solid silica gel column have been mapped in our laboratory (5). Figure 4 shows the distribution of *intervals* separating successive members, in increments that are 1% of the retention volume of the reference sterol, cholesterol. This increment anticipates that it would be possible to measure M with a standard error of 0.25%, and hence δM would be 1%. Two intervals were found that were less than the δM selected, indicating that a mistake in the identity of two pairs of sterols was possible if the single criterion of $M \pm \delta M$ was used, whereas the remainder could be readily distinguished from one another with this "mesh size." A test of the distribution of intervals showed, however, that they were more uniform than would be expected on the basis of the Poisson model (illustrated in Fig. 4 by construction from a table of random numbers), indicating that the structural features (double-bond position, methyl-group substitution, etc.) had predictable, regular effects upon the sterol retention volume.

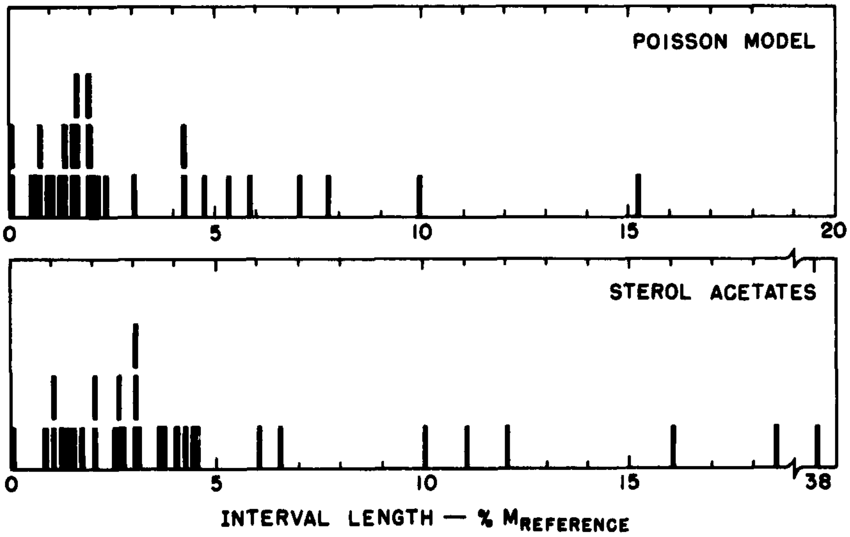

FIG. 4. Lower figure: the intervals between successive sterol acetate retention values as a percentage of the reference sterol, ranked according to size of the interval. Upper figure: intervals between an equal number of retention values created from a table of random numbers, ranked according to the size of the interval.

Identity can therefore be established (at least with probabilistic certainty) *when the value for* δM *is either less than the minimal separation* between any two pairs of compounds the migration of which is known, *or when the density of retention values,* although incompletely mapped, *is low relative to the value of* δM.

CAPABILITIES AND LIMITATIONS OF PRESENT SYSTEMS

The crucial question is: How do the values of δM and $\delta\sigma$ characterize a given chromatographic system in terms of its ability to identify a solute and thereby determine its purity? These values must be compared to the hierarchy of differences in identity (and purity) in which the analyst may be interested, and this comparison is shown in Table 4. Although exact values for a given class of separation factors cannot be given, they can be classified in order of magnitude and the requirement for δM associated with such separation factors can be readily judged. Thus most homologous members of a series will be separated by factors of 1.01 to 1.10, and, within the same variety of molecule, most structural differences will give rise to differences in mobility of at least 0.3% if the system displays any selectivity toward the class of compounds. Thus, if in the use of a system, one can reduce the uncertainty associated with the determination of a mobility to less than 0.1%, or a δM of approximately 0.3%, it should in general be adequate to distinguish the identities of two chemical varieties. Recent evidence appearing in the literature has, in fact, suggested that the identification ability of chromatographic system extends to isotopic

TABLE 4

Separation Factors and δM Requirements for Distinctions between Two Nonidentical Chromatographic Solutes

Separation factor	δM, %M	Classification of distinction
1.10	10	
1.01	1	Homologous members
1.003	0.3	Most structural differences
1.0010	0.1	
1.0003	0.03	Isotopic differences between same species
1.00010	0.01	
1.00003	0.003	Intramolecular isotope distribution differences

species—often under conditions where no such capability was desired. A recent tabulation (*6*) has shown that more than 50 compounds exhibit perceptible separation of one isotopic species from a second labeled species of the same molecule or from the unlabeled material itself during chromatography, and in some of these studies the *position* of the isotope in the molecule determined the degree of separation observed. It may be expected, therefore, that (as indicated in Table 4) chromatographic assay of isotope location in some molecules is a genuine possibility that depends solely on the attainment of the appropriate values of δM.

An estimate of the present capabilities of chromatographic systems employing fraction-collection techniques is shown in Table 5.

TABLE 5

Performance Characteristics of Some Analytical Fractionations[a]

System	M/σ	σ	δM, %M	$\delta\sigma$, %σ	Comments	Ref.
Partition chromatography	9.6	6.01	0.454	6.16	Celite–aldosterone diacetate	(*7*)
Adsorption chromatography	19.3	5.70	0.180	5.61	Silica gel–cholesterol acetate	(*8*)
Gas-liquid chromatography	28.2	2.33	0.243	10.3	2% SE-30–testosterone acetate	(*9*)
Paper chromatography	81.6	0.838	0.058	3.34	Aldosterone	(*7*)
	143.3	0.914	0.12	18.8	Estradiol	(*10*)
Ion-exchange chromatography	94.2	2.30	0.064	8.86	Amino acids	(*11*)
	135.3	4.56	0.028	5.79	Amino acids	(*11*)
	139.9	1.39	0.010	1.35	Amino acids	(*12*)
Countercurrent distribution	99.0	5.77	0.035	3.47	Pentoses	(*13*)
Thin-layer chromatography[b]	18.7	1.36	0.516	9.89	Triglycerides	(*14*)

[a] All systems employed fraction-collection techniques to monitor the separation process with one exception.

[b] Thin-layer-chromatography data provided by Dr. F. Snyder.

These data were collected during a review of isotopic fractionation during analytical separations observed by a variety of investigators (*6*), and, through the courtesy of these workers, the primary values were made available for computation of the column efficiencies,

δM and $\delta\sigma$. In all likelihood these are not the limiting values attainable, but they represent an across-the-board selection available for comparison. The most striking feature of this comparison is that even with columns of low efficiency (under 1000 plates), the values of δM are less than 0.5% of the retention volume; there is also a tendency for the values of δM to decrease with increasing column efficiency. This table also illustrates that a small value of $\delta\sigma$ is more difficult to attain than for δM. In part this is because any departure from ideality increases the value of $\delta\sigma$, and in part it is because the same fraction size used to determine δM represents a larger error in the determination of $\delta\sigma$ by a factor equal to M/σ. Both δM and $\delta\sigma$ can be reduced, however, by a decrease in fraction size to the point where the fraction represents an interval equivalent to 0.20 to 0.25σ (7). Evidence for this is provided in the last two examples of ion-exchange chromatography, where fraction sizes of 0.6σ and 0.3σ were used. The latter enabled measurement of σ to within 0.35% and of the mobility to within 0.0025%, or 25 parts per million. This corresponds to the ability to measure a separation factor of 1.0001 or less.

TABLE 6

Purity Function of Some Analytical Systems

	$\Psi(x)$	$\%n_1$	α
Thin-layer chromatography	0.233	58.0	1.00517
Partition chromatography	1.835	96.6	1.00454
Adsorption chromatography	2.804	99.7	1.00180
Gas-liquid chromatography	1.946	97.4	1.00213
Paper chromatography	1.544	93.6	1.00058
	1.900	97.1	1.00120
Ion-exchange chromatography	3.236	99.94	1.00064
	4.730	> 99.99	1.00028
	3.636	99.99	1.00010
Countercurrent distribution	4.442	> 99.99	1.00035

The values shown in Table 5 for δM are several orders of magnitude smaller than the dispersions of the peaks, indicating that the ability to *identify* compounds far exceeds the system capability for *resolving* them or determining their purity by graphical means. Instead, the predictions of Eq. (5), governing the band-broadening by impurities, can be used to express the abilities of these systems to establish purity. If we replace $100(M_2 - M_1)/M_1$ by $\delta M\%$ and $100(\sigma_2 - \sigma_1)/\sigma_1$ by $\delta\sigma\%$, Eq. (5) becomes

$$\Psi_{(x)} = 2.718 \log \left(\frac{\sigma}{\delta M\%}\right) + 1.481 \log (\delta\sigma\%) - 2.384 \qquad (8)$$

Solution of Eq. (8) provides a value for the maximum purity of the chromatographic peak that can be inferred from the quality of the measurement, provided that both reference and unknown coincide within the limits of δM and $\delta\sigma$. Values for these systems are shown in Table 6 and indicate that unequivocal demonstration of purities as high as 99.99% can be obtained under appropriate conditions in existing chromatographic systems.

DISCUSSION

Using the numerical parameters which have been described here, one can define the ability of any chromatographic system to establish the identity and purity of the members of any class of chemical compounds. The central values limiting this ability are δM, the uncertainty associated with the position of the midpoint; $\delta\sigma$, the uncertainty of the peak dispersion; and ρ, the sample density. For most identifications, the value of ρ will have been reduced by prior class fractionation to the point where identity is a matter of choosing from a very limited number of possibilities. It is then a matter of measuring δM and $\delta\sigma$ to establish that they are sufficiently small to distinguish between these possibilities.

The experimenter has the ability to increase the capability of any given system by manipulating either the efficiency of the column or the techniques for peak measurement. It is evident in the comparisons that have been made that the substitution of incremental measurements for integral measurements confers a considerable advantage in identity and purity measurements. Because these are more readily installed and improved than is the column efficiency, the best analytical strategy is to begin with the most effective column available and then devote subsequent effort to making fraction collection of 8 to 20 divisions in the peak compatible with the speed, volume, and sensitivity requirements of the column.

The present state of chromatography is such that identifications or distinctions as subtle as those at the isotopic level are both possible and practical. Ranked on the basis of their ability to determine identity, ion-exchange and paper chromatography (shown in Table 5), appear to be the systems of choice when coupled with the incremental techniques, whereas ion-exchange and adsorption chromatography offer, according to Table 6, the greatest certainty of chromatographic purity. It is obviously of interest to obtain further estimates of column capabilities from others who may be concerned with these attributes of their analytical system. To this end, the computational procedures, either as programs, or as a service for those without access to machines, are offered to interested individuals without charge, in the hope that it will facilitate their efforts and encourage others to publish comparable numbers for their systems.

REFERENCES

1. E. Glueckauf, *Trans. Faraday Soc.*, **51**, 34 (1955).
2. J. C. Giddings, *Dynamics of Chromatography* Vol. 1, Pt. I, Dekker, New York, 1965, p. 272.
3. P. D. Klein and S. Tyler, *Anal. Chem.*, **37**, 1280 (1965).
4. P. D. Klein and Kunze-Faulkner, *Anal. Chem.*, **37**, 1245 (1965).
5. P. D. Klein, J. C. Knight, and P. A. Szczepanik, *J. Am. Oil Chemists Soc.*, **43**, 275 (1966).
6. P. D. Klein, in *Advances in Chromatography* (J. C. Giddings and R. A. Keller, eds.), Vol. 3, Dekker, New York, 1966, p. 3.
7. J. H. Laragh, J. E. Sealey, and P. D. Klein, *Proc. Symposium on Radiochemical Methods of Analysis*, Vol. 2, I.A.E.A., Vienna, 1965, p. 353.
8. P. D. Klein, D. W. Simborg, and P. A. Szczepanik, *Pure Appl. Chem.*, **8**, 357 (1964).
9. M. A. Kirshner and M. B. Lipsett, *J. Lipid Res.*, **6**, (1965).
10. E. V. Jensen and H. I. Jacobsen, *Recent Progr. Hormone Res.*, **18**, 387 (1962).
11. K. A. Piez and H. Eagle, *J. Am. Chem. Soc.*, **78**, 5285 (1956).
12. P. D. Klein and P. A. Szczepanik, unpublished work, 1966.
13. L. M. Marshall and D. E. Cook, *J. Chromatog.*, **15**, 97 (1964).
14. F. Snyder and H. Kimble, *Anal. Biochem.*, **2**, 510 (1965).

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