Decision and Detection Limits for Linear Calibration Curves

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For linear calibration curves, two kinds of lower limits may be connected to the notion of confidence limits—a decision limit, the lowest signal that can be distinguished from the background and a detection limit, the content under which, *a priori*, any sample may erroneously be taken for the blank. From a few algebraical and computational developments, several practical rules are deduced to lower these limits. The influence of the precision of the analytical method, the number of standards, the range of their contents, the various modes of their repartition, and the replication of measurements on the unknown sample are studied from a statistical point of view.

EXTERNAL STANDARDS are of very common use in analytical practice. In many methods, *e.g.*, as in X-ray spectrochemical analysis, the analyst used a linear calibration curve obtained from measurements made on these standards, to estimate the concentration of the unknown. Obviously the sensitivity of his method may be enhanced by a judicious choice of standards, but the quantitative estimate of this enhancement is not straightforward. The various definitions of the detection limits found in the literature, although having different advantages do not explicitly include this influence.

Linning and Mandel (*1*) have presented a very interesting discussion on the determination of the precision of an analytical method involving a calibration curve. They emphasize that, because there is always some scatter in the calibration data, the precision of analysis for an unknown will be poorer than indicated from several repeat determinations on the same sample. Various authors have proposed an objective way to calculate the detection limit of an analytical determination. They suggest that a signal higher than the standard deviation of the background multiplied by a conventionally chosen factor (usually 3), should be considered as characteristic of a detectable amount of the element to be analyzed. Kaiser, in several papers (*2–4*) develops this concept at length and proposes to work at the confidence level of 99.86%, which corresponds to a value of 3 for the factor.

B. Altshuler and B. Pasternak (*5*) have connected the notion of detection limits with the statistical concepts of the errors of the first and second kind; these concepts will be also used in the present text. A review of the published definitions of the limits for qualitative detection and quantitative determination has been done by L. A. Currie (*6*), who proposes to introduce three specific levels: *A decision limit* to which corresponds a *critical level* L_c, the net signal level (instrument response) above which an observed signal may be recognized reliably enough to be detected; at this limit, one may decide whether or not the result of an analysis indicates presence; *A detection limit*, L_D, the "true" net signal level which may be *a priori* expected to lead to detection; this is the limit at which a given analytical procedure may be relied upon to lead to detection; and *A determination limit*, L_Q, the level at which the measurement precision will be satisfactory for quantitative determination.

We will show how estimates of the decision and detection limits may be introduced by considering the confidence limits of the linear calibration curve. The dependence of these limits upon the standards will thereby be made explicit.

DECISION AND DETECTION LIMITS—A NEW APPROACH

In the analytical methods of interest here, the response signals of a certain number of standards are measured and a straight line (the regression line) is passed through the representative points. This line is an estimate of the true calibration line. It may be predicted that any new standard will give a signal falling in the neighborhood of this obtained line. At this point two questions may arise:

Above which level are the signals significantly different from the background?

Above which concentration is a confusion with the nul concentration unlikely?

To seek an answer, let us scrutinize what the expression "in the neighborhood of" really implies. The representative point of a measured signal does not fall exactly on the line for two independent reasons: the drawn calibration line does not exactly coincide with the true calibration line but is only an estimate (this estimate is based on a limited number of standards); and for a given content, the corresponding response signal does not assume a fixed value but is randomly distributed around a mean value, and this distribution is not exactly known. In order to make precise the combined effects of these two uncertainties, one due to the insufficiency of our information, and the other to a lack of perfect reproductivity inherent to the method, four basic hypotheses are necessary.

First, the standards are supposed to be independent. Practically, this means that they should be prepared separately, *i.e.*, in such a way that they will differ in their preparation, as much from each other as from the samples to be analyzed. This condition is not so easily met as seems at first sight.

Second, the variance of the error distribution of the signals around their expectation is supposed to remain constant. Practically, this means that the scatter of the signals does not depend on the contents, in the studied range of these contents.

Third, the contents of the standards are supposed to be accurately known.

Fourth, it is assumed that the observed signals have a gaussian distribution around their expectation. Although this hypothesis is very widely accepted, it is not certain that it is always correct. On the contrary, K. Behrends (*7*) has shown that the error distribution definitely is not gaussian in a number of cases. But, although another type of distribution would yield somewhat different numerical results than those presented here, it would probably not significantly modify the main conclusions.

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(1) F. J. Linning and J. Mandel, ANAL. CHEM., **36** (13), 25A (1964).
(2) H. Kaiser, *Z. Anal. Chem.*, **149**, 46 (1956).
(3) *Ibid.*, **209**, 1 (1965).
(4) *Ibid.*, **216**, 80 (1966).
(5) B. Altshuler and B. Pasternak, *Health Phys.*, **9**, 293 (1963).
(6) L. A. Currie, ANAL. CHEM., **40**, 586 (1968).
(7) K. Behrends, *Z. Anal. Chem.*, **235**, 391 (1967).

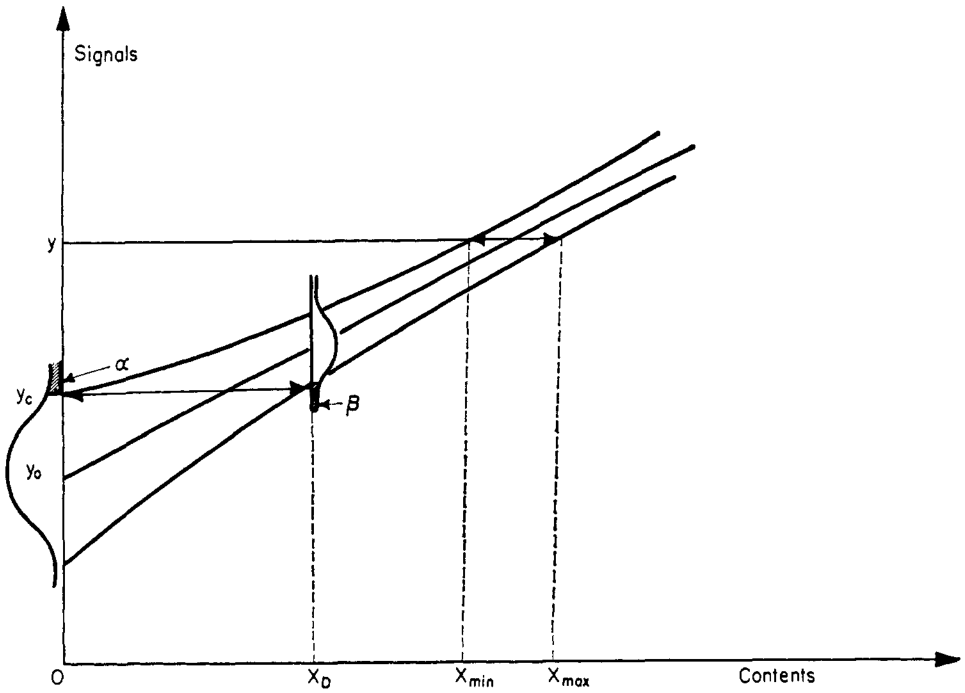

Figure 1. The linear calibration line, with its upper and lower confidence limits. y_c is the decision limit and x_D the detection limit, as explained in text

Starting from these hypotheses, formulas can be established from which several conclusions may be deduced, as will be shown in the next paragraphs. The considerations upon which the reasoning will be based are as follows:

On either side of the regression line, two confidence limits may be drawn, with an *a priori* chosen level of confidence, which we will note as $1-\alpha-\beta$, α and β having prefixed small values, of the order of a few percent (see Figure 1). The regression line and its two confidence limits represent a graphic synthesis of our knowledge about the relationship between content and signal. With it we may predict that an as yet unexplored content will yield a signal falling inside the confidence band. We will do this prediction with $1-\alpha-\beta$ probability; if we did a series of such predictions and then made the measurements, we would observe that, in the long run, we would be right $1-\alpha-\beta$ of the time; α % of the points would fall above the higher limit, and β% under the lower limit. The width of the confidence band depends on: the dispersion of signals for a given content, the knowledge we have of that dispersion and the degree of uncertainty about the true position of the calibration line.

The confidence limits, then, do not represent the dispersion of signals but our capacity to predict likely values for signals, taking into account the actual knowledge we possess of the case.

The confidence band may also be used in reverse; for a measured value y of the signal on a sample of unknown content (see Figure 1) we may predict the range of this content. The intersection of a horizontal line through y with the two confidence limits will define this range $x_{max}-x_{min}$, again with $1-\alpha-\beta$ probability. This, incidentally, is a valid method to estimate confidence limits of contents corresponding to a given signal. In particular, for a measured signal equal to y_C (see Figure 1), the lower limit of content is zero. Signals equal to or lower than y_C have a non-negligible probability to be due to a sample with a nul concentration, and hence we cannot distinguish, with such signals, whether or not the sought element is really present or not.

y_C is then the lowest measurable signal: if we are not ready to take a risk greater than α % to state that the element is present when it is absent (*i.e.*, to take a wrong decision more than α % of the time) we must decide that any signal under y_C must be disregarded. It is then clear that y_C corresponds to L_C as defined by L. A. Currie. More exactly, y_C is the *estimate* of L_C which may be obtained with the knowledge at hand. y_C could also be called the reading threshold, an expression proposed by H. Kaiser in an article published while the present paper was in press [H. Kaiser, ANAL. CHEM., **42** (4), 26A (1970)].

Hence, a measurement being made, we decide whether the sample may be a blank or not. (The blank being a sample which is identical, in principle, to the samples of interest, except that the substance sought is absent, or in such minute quantity that it will give signals not higher than the background). Before making any measurement, on the other hand, we can state that the lowest content we may distinguish from zero is x_D, the abscissa corresponding to y_C on the lower confidence limit. Indeed, with the knowledge in our possession, when we measure an unknown with a content lower than x_D, we run a risk higher than β to obtain a signal lower than y_C, and hence to state that it is a blank. x_D is then our estimate of the "limit of guarantee for purity," as defined by Kaiser (*3*) which in turn is equivalent to the "minimum detectable true activity" of Altshuler and Pasternak (*5*) and to the "detection limit" of Currie (*6*).

It is perhaps not useless to remark that y_C and x_D have not a fixed value. For a given method and a given number of standards they will vary because, first α and β may be chosen at will, according to the acceptable levels of risk one is ready to run to derive false conclusions, and second, by making a second series of standards, identical to the actual series, we would obtain signals differing at random from the actual signals. The regression line and the confidence limits we would then draw would not exactly coincide with the actual lines, and y_C and x_D would be somewhat different. In other words, y_C and x_D are random variables and estimates only.

But this is the normal situation whenever randomness is an integral part of the phenomenon.

By way of summary, two sensitivity limits are proposed here: a signal level y_C and a content x_D. These notions are very similar to the lower limits of detection for radioactivity counters proposed by B. Altshuler and B. Pasternak (*5*). The first limit concerns signals and will lead to an *a posteriori* decision, *i.e.*, a decision taken after the signal is measured; the second limit is relative to contents and is inherent to the method; it specifies *a priori* the content which will be safely detected without confusion with blanks. It will be seen that y_C and x_D are directly connected with the statistical notions of the "errors of the first and the second kind," respectively. [A good introduction to these classical notions will be found in (*5*)]. As the direct relationship between these limits and the confidence limits is now established, it is clear that the problem is equivalent to the study of the influence of the standards on the confidence limits: to lower y_C and x_D, the confidence limits must be brought nearer to the regression line.

MATHEMATICAL DEVELOPMENTS

Notations. The following notations have already been introduced: α, β, y_C, x_D. We will also use:

Y_o = estimate of the expectation of the response signal for a blank ($x = 0$) (y_o is the intersection of the calibration line with the axis of ordinates)
y_D = signal corresponding to x_D on the calibration line
x_C = abscissa corresponding to y_C on the calibration line
N = number of standards
x_i = concentration of the element of interest in the *i*th standard ($i = 1,N$)
Σ = the summation sign; stands for $\sum_{i=1}^{N}$
$\bar{x}$ = mean; $\bar{x} = \Sigma x_i/N$
x_1 = lowest concentration within the series of standards
x_N = highest concentration within the series of standards
K = number of standards equal to x_1 in the "three values" repartition of the contents of the standards

$$\lambda_i = \text{dimensionless factor; } \lambda_i = \frac{x_i - x_1}{x_N - x_1} \tag{1}$$

$$\lambda_D = \text{value of } \lambda_i \text{ corresponding to } x_D\text{; } \lambda_D = \frac{x_D - x_1}{x_N - x_1} \tag{2}$$

γ = exponent of the parabolic repartition (Eq 24)
y_i = the observed intensity of a characteristic line of the element of interest measured for the *i*th standard
$\bar{y}$ = mean; $\bar{y} = \Sigma y_i/N$
b = angular coefficient of the regression line, whose equation is:

$$Y = \bar{y} + b(x - \bar{x}) \tag{3}$$

with

$$b = \frac{\Sigma(x_i - \bar{x})(y_i - \bar{y})}{\Sigma(x_i - \bar{x})^2} \tag{4}$$

by the least squares method

Y_i = calculated signal corresponding to x_i

$$Y_i = \bar{y} + b(x_i - \bar{x}) \tag{5}$$

s^2 = the estimate of the residual variance

$$s^2 = \Sigma(y_i - Y_i)^2/(N - 2); \tag{6}$$

s is the estimate of the residual standard deviation

t = Student's t corresponding to $N - 2$ degrees of freedom and $(1 - \alpha)$ or $(1 - \beta)$ confidence level

$$R = \text{range ratio of the standards } R = (x_N - x_1)/x_1 \tag{7}$$

P = factor of s in Equation 16

$$P = t_{1-\alpha}\sqrt{1 + \frac{1}{N} + \frac{\bar{x}^2}{\Sigma(x_i - \bar{x})^2}} \tag{8}$$

P_{III} = third term under the radical in the preceding equation

$$P_{\text{III}} = \frac{\bar{x}^2}{\Sigma(x_i - \bar{x})^2} \tag{9}$$

Q = factor of s in Equation 18

$$Q = t_{1-\beta}\sqrt{1 + \frac{1}{N} + \frac{(x_D - \bar{x})^2}{\Sigma(x_i - \bar{x})^2}} \tag{10}$$

Q_{III} = third term under the radical in the preceding equation

$$Q_{\text{III}} = \frac{(x_D - \bar{x})^2}{\Sigma(x_i - \bar{x})^2} \tag{11}$$

n = number of replicates on each unknown sample.

Equations of y_C and y_D. The equations of the upper and lower curves of Figure 1 (*i.e.*, the confidence limits) are derived [see (*8*) for instance] by considering that any value y corresponding to a given value of x has a gaussian distribution around its calculated value Y. The confidence limits at any point x are then expressed by

$$y = \bar{y} + b(x - \bar{x}) \pm t\sqrt{V[y]} \tag{12}$$

t corresponding to a probability of $1 - \alpha$ for the upper limit and $1 - \beta$ for the lower limit. The variance of y, $V[y]$, is the sum of the variance of Y plus the residual variance

$$V(y) = V[\bar{y} + b(x - \bar{x})] + \sigma^2 \tag{13}$$

with

$$V[\bar{y}] = \frac{\sigma^2}{N},\ V[b] = \frac{\sigma^2}{\Sigma(x_i - \bar{x})^2} \tag{14}$$

The residual variance σ^2 may be replaced by its estimate s^2 and Equation 12 becomes

$$y = \bar{y} + b(x - \bar{x}) \pm st\sqrt{1 + \frac{1}{N} + \frac{(x - \bar{x})^2}{\Sigma(x_i - \bar{x})^2}} \tag{15}$$

In particular, for $x = 0$, the upper limit will be

$$y_C = \bar{y} - b\bar{x} + st_{1-\alpha}\sqrt{1 + \frac{1}{N} + \frac{\bar{x}^2}{\Sigma(x_i - \bar{x})^2}} \tag{16}$$

y_C may be considered as the sum of two terms:

$$y_C = Y_o + P\cdot s \tag{17}$$

with Y_o and P as defined in the list hereabove. We have no possibility to reduce Y_o, the intersection of the calibration line with the axis of ordinates. But we may reduce P and s and hence enhance the decision limit.

For the detection limit, it will be convenient to consider y_D, the ordinate of x_D on the regression line. Developments as in

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(8) K. A. Brownlee, "Statistical Theory and Methodology in Science and Engineering," John Wiley & Sons, Inc., New York, N. Y., 1960.

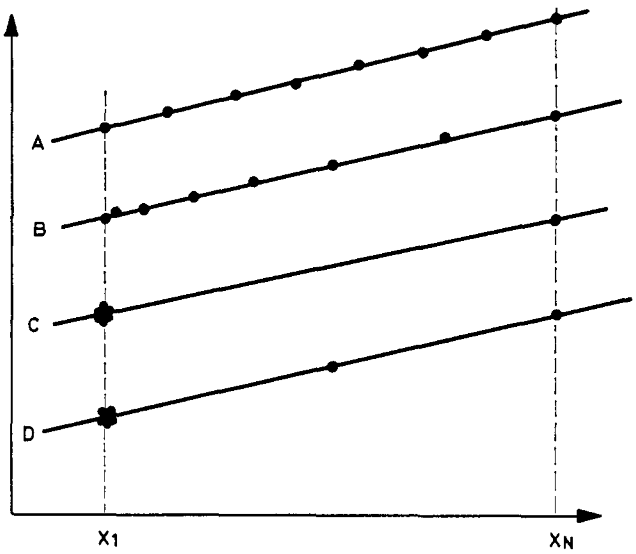

Figure 2. Four types or repartition of the standards

A. **Equidistant or linear**
B. **Parabolic**
C. **In two values**
D. **In three values**

the preceding paragraph will yield

$$y_D = y_C + st_{1-\beta}\sqrt{1 + \frac{1}{N} + \frac{(x_D - \bar{x})^2}{\Sigma(x_i - \bar{x})^2}} \tag{18}$$

and a decrease of y_C will generally bring about a decrease of y_D. y_D may be considered as the sum of three terms

$$y_D = Y_o + Ps + Qs \tag{19}$$

and the problem is thus concerned with the reduction of P and Q.

Computation of P. It will prove useful to express the x's as functions of R, λ_i, and x_1

$$x_i = (1 + \lambda_i R)x_1 \tag{20}$$

where R represents the "range ratio" of the contents of the standards (Equation 7) and where the λ_i's are dimensionless factors which depend on the repartition of the standards. Let us observe that

$$0 \leqslant \lambda_i \leqslant 1, \lambda_1 = 0, \lambda_N = 1 \tag{21}$$

It will readily be seen that the third term of P, P_{III}, does not depend on the scale of the x's, but may be expressed as a function of the range ratio and the λ_i's only:

$$\frac{\bar{x}^2}{\Sigma(x_i - \bar{x})^2} = \frac{\left(\bar{\lambda} + \frac{1}{R}\right)^2}{\Sigma(\lambda_i - \bar{\lambda})^2} \tag{22}$$

hence

$$P = t\sqrt{1 + \frac{1}{N} + \frac{\left(\bar{\lambda} + \frac{1}{R}\right)^2}{\Sigma(\lambda_i - \bar{\lambda})^2}} \tag{23}$$

P may thus be expressed as a function of R, for given values of N and several combinations of the λ_i's. When one may prepare the standards at will, he has, theoretically, an infinity of possible ways of distributing the λ's. In practice, however, these modes of repartition are rather limited. We have here selected four types; the first two are of very common use; the last two have been introduced for reasons which will be clear later on.

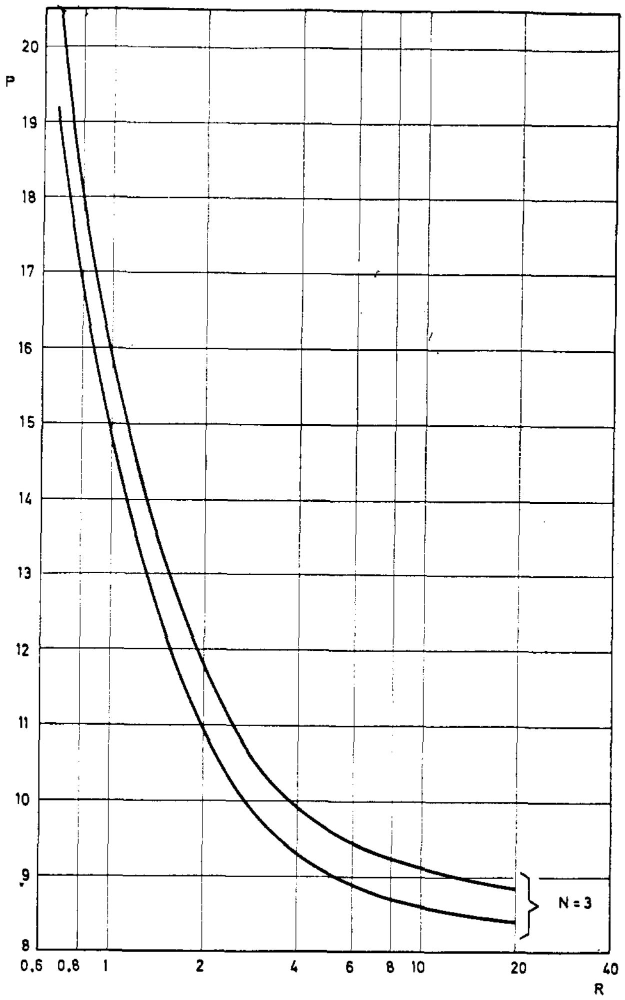

Figure 3. Influence of range (R) on the decision limit, in the case of three standards, with $\alpha = 5\%$; P is in ordinates

Upper curve, linear repartition; lower curve, parabolic repartition with $\gamma = 2$

A) the repartition may be linear—*i.e.*, the x's are equidistant (see Figure 2*A*);

B) the distribution may be parabolic, its general expression being

$$\lambda_i = \left(\frac{i - 1}{N - 1}\right)^\gamma \tag{24}$$

each λ_i corresponding to one x_i by formula 1. In practice, γ is around 2 or 3. An example would be:

10, 12, 18, 29, 43, 61, 85, 110 ppm;

here $\gamma = 2$, $N = 8$, $x_1 = 10$, $x_N = 110$, $R = 10$; this distribution corresponds to line B in Figure 2;

C) a repartition of theoretical importance is one where a certain number of standards all have the smallest permissible content (*i.e.*, equal to x_1) and the others the highest permissible content x_N. This is line C of Figure 2.

D) Finally, we have also studied the disposition

K standards at x_1
1 standard at $(x_1 + x_N)/2$
$N - K - 1$ standards at x_N

This repartition is represented at Figure 2*D*. We will call it the "three values repartition."

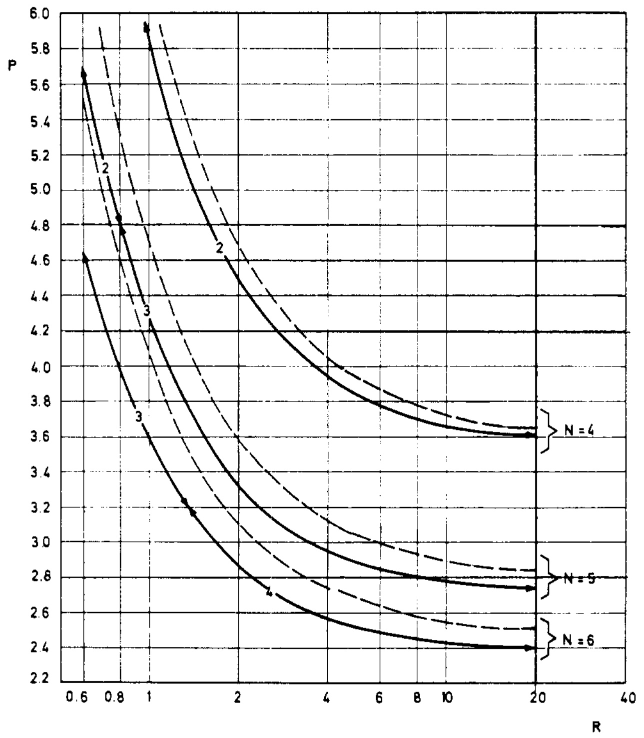

Figure 4. Influence of range R on decision limit, four to six standards, $\alpha = 5\%$. P is in ordinates. For each N, the upper curve corresponds to the parabolic repartition with $\gamma = 2$, and the lower curve to the three values repartition, with the values of K written on the curve

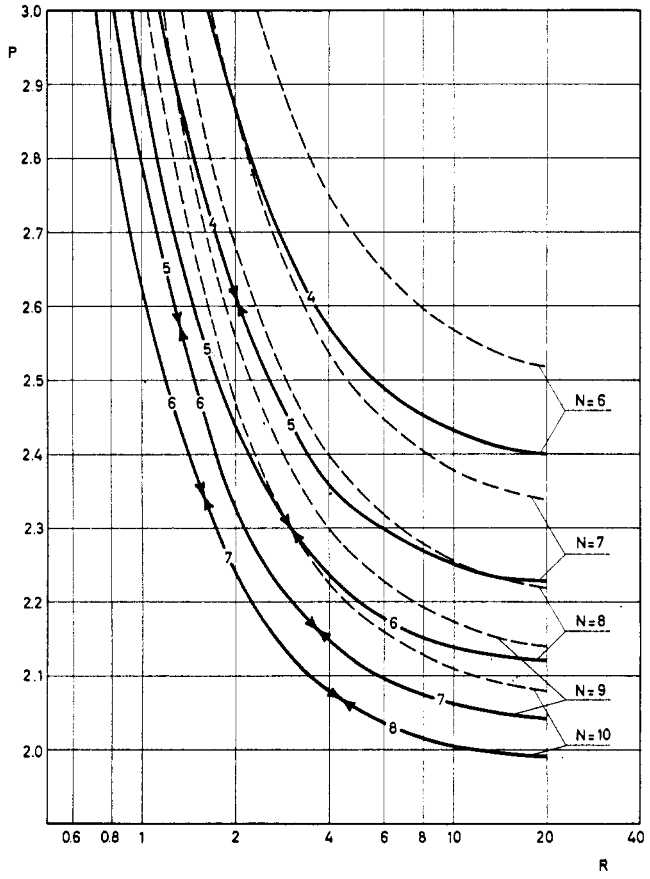

Figure 5. Same as Figure 4, for six to ten standards

Values of P as a function of R have been computed for these four types of repartition and for different values of N. The computations have been done with the program TABFUN (9) on the IBM 360/65 of the CETIS at Ispra (Italy). The principal results are presented in Figure 3 for $N = 3$ and in Figures 4 and 5 for $N = 4$ to 10. For the three graphs, R is in abscissae and P in ordinates. In Figure 3, the upper curve corresponds to the linear repartition, and the lower curve to the parabolic repartition with $\gamma = 2$. In Figures 4 and 5, the three values repartition is represented by plain curves. The values of K which give the smallest P are written on these curves, the field of validity being limited by arrows. Thus, for $N = 10$, $R = 3$, it is seen on Figure 5 that K must be equal to 7. Except for R inferior to 4, an unusual occurrence, K is equal to $N - 2$. The parabolic repartition with $\gamma = 2$ is represented on the same graphs as dotted lines. For the sake of clarity, the curves corresponding to the linear repartition have not been included in graphs 4 and 5. Had they been represented, the parabolic curves would have been roughly at mid-distance between them and the three values curves.

Estimation of Q. Q_{III} contains x_D and hence, unlike P, Q may not be expressed as a function of t, R, N, and the λ's. But, on the other hand, if we could make $\bar{x}$ equal to x_D, Q_{III} would vanish. In practice, as x_D is known only after the standards are measured, it is not possible to realize a complete equality, but a fair approximation will be sufficient. It is thus to be recommended that the contents of the standards be chosen in such a way that $\bar{x}$ will fall in the neighborhood of the region where x_D will most probably be.

Contrary to x_C, which is obtained after few computations, the algebraic expression for x_D is really cumbersome [see (8), § 11.5]. A graphical solution will be quicker: compute L_C by Equation 16, possibly using the graphs of Figures 3 to 5, compute three or four points of the lower confidence limit by Equation 15 using the minus sign, and draw the line through the points with a French curve. The intersection of this line with $y = L_C$ has x_D as its abscissa. Let us observe that

$$x_C > x_D - x_C \geqslant \left(st_{1-\beta}\sqrt{1 + \frac{1}{N}}\right)\Big/ b \qquad (25)$$

the equality on the right being carried out when $x_D = \bar{x}$. When that condition is fulfilled,

$$Q = t_{1-\beta}\sqrt{1 + \frac{1}{N}} \qquad (26)$$

This expression may be used as an estimate of Q in analytical practice.

WAYS TO IMPROVE DECISION AND DETECTION LIMITS

Enhancing the sensitivity of an analytical method can be obtained by improving the precision of the method (s); increasing the number of standards (N); increasing the range of the contents of these standards (R); optimizing the repartition of these standards within this range (the λ_i's and $\bar{x}$); and performing replicate measurements on the unknown sample (n).

Precision. The residual standard deviation s is a good measure of the goodness of fit of the observed signals of the standards, or, in other words, of the precision of the method. It is clear from Equations 17 and 19 that there is a direct relationship between precision and sensitivity: improving the precision will lower the limits y_C and x_D.

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(9) A. Hubaux and M. Lecloux, Tabulation de Fonctions, CEEA Report EUR 2987.f (1966).

Number of Standards. The influence of N is, of course, important, see Figures 3 to 5, especially between 3 and 6. This is due to the fact that t, $1/N$, and $\Sigma(x_i - \bar{x})^2$ all depend on N. Obviously, as results from Equation 18, the influence of N on y_D will be important also. Although the equation cannot be computed in a general way, it may be said, in first approximation, that Q will diminish in the same proportion as P, when N increases.

Range of Contents of Standards. The influence on P of the range ratio R is expressed in Equation 23 and illustrated by Figures 3 to 5. P is very sensitive to small values of the range ratio, say under 10. From 10 to 20, on the other hand, P goes down only a few percent. Above 20, the gain becomes completely negligible. This consideration may be of use in a number of cases: if the lowest possible standard is at 10 ppm, there is no gain in sensitivity by preparing standards with more than 200 ppm. On the other hand, if we have at our disposal only standards between 80 and 120 ppm (R = 0.5) we should expect a really poor sensitivity, unless s is very small, that is unless all the observed signals fall neatly on the regression line. Let us note that, when a "blank" is available, x_1 will be very small (it will never be exactly equal to zero) and the range ratio will take a very high value. For all practical purposes, the right extremity of the graphs (R = 20) may be used in this case.

The influence of R on Q is not so readily computed. It may be said however, that this influence will be small as long as $\bar{x}$ remains in the neighborhood of x_D, and nil when the two coincide.

Modes of Repartition of Standards. Very often, N is fixed by economical considerations, various conditions determine the value of x_1 and x_N, and s is made as small as practically possible by a careful preparation of the standards and a good checking of the measurements. When N, R, and s are fixed, however, one still has the liberty to distribute the λ_i's in the manner best suited for his purposes. Generally, two aims are pursued: first, to check the linearity of the content/signal relation, and second, to lower the sensitivity limit as much as possible. There is no strategy which optimizes both aims: to obtain a maximum of information on the linearity, the x's should be as far as possible from each other, or, in other words, they should be equidistant (see Figure 2*A*). On the other hand, the best disposition which we have found after some computations to enhance the sensitivity is to have a certain number of standards with the smallest admissible content (*i.e.*, equal to x_1) and the other standards with the maximum permissible content x_N (see Figure 2*C*). When the range ratio is greater than 4 or 5, there should be $N - 1$ standards equal to x_1, and only one equal to x_N. The diminution of P, by adopting the second scheme instead of the first, may be as high as 30% when the range ratio is small and remains of the order of 10 to 15% when this ratio tends to infinity. But unfortunately, this disposition is impossible to adopt in practice because there would not be any control on linearity and because any error on the standard at x_N would be impossible to assess.

As an alternative, we have studied what appears to be the best substitute, the three values distribution, as illustrated at Figure 2*D*, where there is a check on linearity and on the absence of gross errors. The values of P obtained from Equation 23 with this disposition are plotted as plain curves on Figures 4 and 5. As a comparison, the values of P corresponding to the parabolic distribution with $\gamma = 2$ are given as dotted curves. Computations have shown that this parabolic distribution yields a lower P than the equidistant distribution (2.25 instead of 2.42 with $N = 8$, $R = 10$). But on the other hand, this P is still notably higher than the corresponding P of the three values disposition: 2.13. With the parabolic repartition, this value of 2.13 is not even reached by the use of 9 standards ($P = 2.17$). Thus, with the three values disposition, we may gain the effect on sensitivity of more than one standard. When the range ratio is smaller and N bigger, the gain is still more important: 8 standards with the three values disposition will give a sensitivity as good (for $R = 3$ or less) as 10 standards distributed parabolically: a gain of two standards!

With the three values repartition, $\bar{x}$ is low and, hence, more likely to fall near x_D, thus contributing also to reduce Q. More specifically, it may be shown that, if we take the three values mode with $K = N-2$, the third term of Q is equal to

$$Q_{\text{III}} = \frac{\left(\lambda_D - \dfrac{1.5}{N}\right)^2}{1.25 - \dfrac{2.25}{N}} \tag{27}$$

(the developments are straightforward and too long to be given here). From this equation it will be clear that if λ_D is not too far from $1.5/N$, Q_{III} will be conspicuously smaller than unity; hence, the exact coincidence of $\bar{x}$ and x_D is not required.

Replication on the Unknown. By making n replicates on the unknown sample, the residual variance is divided by n and P must be replaced by P_n, with

$$P_n = t\sqrt{\frac{1}{n} + \frac{1}{N} + \frac{\bar{x}^2}{\Sigma(x_i - \bar{x})^2}} \tag{28}$$

It should be emphasized that this equation applies to replicates which may really be considered as "independent" from each other. It is readily seen that

$$P_n{}^2 = P^2 - \left(1 - \frac{1}{n}\right)t^2 \tag{29}$$

and hence that replication may conspicuously improve the sensitivity. Let us also remark that the influence of replications will have somewhat more effect when P diminishes. For instance, with $N = 4$ and $\alpha = 5\%$, t is 2.92; if P is equal to 3.7, four replications ($n = 4$) will yield $P_4 = 2.7$, a gain of 27%. With $N = 10$, $t_{95\%} = 1.86$; if $P = 2.0$, $n = 4$ will yield $P_{10} = 1.19$, a gain of 40%.

Likewise, Q must be replaced by Q_n, and in symmetry with Equation 29, we have

$$Q_n{}^2 = Q^2 - \left(1 - \frac{1}{n}\right)t^2 \tag{30}$$

Hence, the effect of replication on y_D will be about the same as the effect on y_C.

A further advantage of replication is that it will yield estimates of the residual variance. It will then be possible to test whether this variance remains constant, as supposed in the present developments; if it does, a better estimate of this variance may be obtained and thus a t with more degrees of freedom may be used, and this smaller t will also contribute to diminish y_C and x_D.

CONCLUSIONS

The definition of the decision and detection limits is here attached to the concept of confidence limits. This presents the advantage that the influence of the standards on the sensitivity may be quantitatively estimated. The most important conclusions are:

A direct relationship exists between the precision of the method and its sensitivity.

There is, as expected, an important gain in sensitivity when increasing the number of standards from 3 to 6. Above 10 standards, the gain is of the order of 2 to 1% on P and Q for one additional standard.

The range ratio should be higher than 10, a condition which is easily met in most cases. But there is no need that it be higher than 20.

When blanks may be added to the series of standards, Figures 3 to 5 may also be used, the results for $R = \infty$ being practically equal to those of $R = 20$.

Where it is important to have a limit of detection as low as possible, it may be of advantage to distribute the standards into three groups of contents only: K standards with the lowest possible content, $N - K - 1$ standards with the highest possible content and one at midway between. The value of K may be read on Figures 4 and 5, where it is seen that when R is greater than 4, $K = N - 2$. This distribution in three values will allow a gain which may be of one or even two standards, when used instead of the more common equidistant or parabolic distributions.

The mean content of the standards, $\bar{x}$, should fall in the neighborhood of the presupposed value of x_D, a requirement which will be easier to meet with the three values repartition.

Replicate measurements on the unknown samples conspicuously improve the decision and detection limits; this improvement may be computed by Equations 29 and 30.

ILLUSTRATIONS

Case 1. In order to be accepted, an organic material should have a chlorine content inferior to 3.5 ppm. The material is to be analyzed by X-ray fluorescence and the lowest possible content for reliable standards (x_1) is 1 ppm Cl. As has been shown, the range of the standards, R, should be around 20, hence $x_N = 21$ ppm. α and β are both chosen as 5%. Six standards are prepared and measured, with contents distributed in the three-values mode, yielding as equation of the regression line $y = 2286 + 54.4\,x$ (x in ppm, y in counts, for a counting time of 100 seconds) with a standard deviation of 40.0 counts. From Figure 5, $P = 2.39$, hence $y_C = 2382$ and $x_C = 1.77$ ppm. Graphical estimate of x_D yields 3.2 ppm. This value is too high, but duplicates on the unknown will give $P_2 = 1.89$, $Q_2 = 2.08$, and hence $x_D = 2.51$ ppm.

Case 2. Only three standards of a particular impurity in an alloy are available. The contents are 89, 91, and 144 ppm. Hence $\bar{x} = 108$ and $R = 0.62$, a low value indeed! Careful analysis of the three standards gives a regression line with equation: $y = 64690 + 45.2\,x$ counts (100 sec counting time) and residual standard deviation of 400 counts. $t_{95\%}$ for 1 degree of freedom $= 6.314$. Hence $P = 17.1$ by Equation 8, from which $x_C = 151$ ppm! It may only be concluded that this poor series of standards is really inappropriate. The addition of one standard at 400 ppm (supposing linearity remains) would yield $R = 3.5$, hence $P = 4.0$ and $x_C = 35$ ppm. The decision limit may be lowered by adding to the series a standard with a higher content.

ACKNOWLEDGMENT

We express thanks to our colleagues, L. Farese, F. Girardi, and J. Larisse, for fruitful discussions on various aspects of the concepts exposed here.

RECEIVED for review May 21, 1969. Accepted February 24, 1970.

Computer Evaluation of Continuously Scanned Mass Spectra of Gas Chromatographic Effluents

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Efficient utilization of the vast amount of data produced by a continuously scanning mass spectrometer coupled to a gas chromatograph required the development of novel data processing techniques. One of the most useful is the display of the change in abundance of certain ions during the gas chromatogram (called "mass chromatogram"). This technique permits detection of the presence or absence of homologous series of compounds as well as specific substances of known or predictable mass spectra. The selection of the *m/e* values to be plotted can be based on a knowledge of the chemical system under investigation or can be supported by an evaluation of the data itself. Applications of these approaches to geochemical and biomedical problems are discussed.

THE DESIRABILITY of obtaining mass spectral information on practically all components of a complex mixture led to the design of a gas chromatograph–mass spectrometer system which uses a computer to continuously and automatically record mass spectra of the gas chromatographic effluent (*1*). The need to efficiently utilize the resulting data at a speed comparable to that at which they are acquired made it necessary to develop entirely new approaches to this problem. One approach was the computerized searching of reference mass spectra files (*2–4*). These techniques relieve the chemist from a great deal of routine work but, because of the limited number of spectra in the reference file (*ca.* 7500 are now available), search results sometimes do not indicate a definite compound. Frequently, the suggestions of such a library search, even though not conclusive, aid in the manual identification of the spectra (*2–4*). In the course of using these library search techniques for an extended time, several other approaches were developed for certain problems presented by

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