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Measurement of uncertainty and discrimination limit in purity tests of drug quality

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Abstract

Because of baseline fluctuation in an instrumental analysis, a purity test can overlook an illegitimate drug which contains an undesirable substance in more amount than a prescribed reference value. This paper proposes a probability theory to predict the lowest (average) signal, $E[Y_2]$, of the substance which can be discriminated from the (average) reference signal, $E[Y_1]$, with a high probability (here, 95%) in liquid chromatography (LC). The difference between the lowest signal and reference signal, $E[Y_2] - E[Y_1]$ (> 0), is referred to here as a discrimination limit. The repetition of experiments to estimate the standard deviation of measurements is unnecessary for the probability theory, but a mathematical treatment of instrumental baselines (Fourier transform, etc.) is essential. The Monte Carlo simulation is carried out in which the reference signal and predicted signal for the discrimination limit are overlaid randomly 5000 times on real LC baselines. The result is satisfactory: the observed probability for the right answer is 94.3 or 94.8%; the theoretical one is 95%. The normality of the measurement distribution is examined for LC and capillary electrophoresis to verify the fundamental assumption of the proposed theory. © 1997 Elsevier Science B.V.

Keywords: Uncertainty; Discrimination limit; Purity test; Pharmacopoeia; Chromatography; Capillary electrophoresis; Limit of detection; Probability theory; Statistics

1. Introduction

As stated by Thompson [1], the uncertainty of measurement is of great importance in an analytical community and should be made explicit as a universal practice. This paper focuses on the uncertainty and restricted precision, called the discrimination limit, of purity tests in pharmacopoeias [2,3]. The indeterminate error of instrumental analyses is the only concern of this paper and the other errors such as those associated with sampling and calibration are not considered.

As an example, hydrocortisone sodium phosphate decomposes into hydrocortisone and sodium phosphate and the Pharmacopoeia of Japan allows the decomposition product to be present up to one hundredth of the original drug content in weight. The purity test for the drug in liquid chromatography is stipulated as follows: the hydrocortisone peak, Y_2, for the sample solution of the drug is not larger than the peak, Y_1 for the standard solution of hydrocortisone [2].

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We consider the performance of the purity test to signal rejection for an illegitimate drug such as hydrocortisone in hydrocortisone sodium phosphate, from the statistical viewpoint. The instrumental measurement (e.g. peak area) is inevitably disturbed by the baseline noise, which blurs the true value. Nevertheless, if the decomposition product in the sample is present in much larger amount than in the reference standard, the peak area, Y_2, for the sample solution will be observed to be larger than the area, Y_1, for the reference standard. In this situation, the right answer ($Y_2 > Y_1$) will result with almost 100% probability. When the reference and sample contain equal amounts of the decomposition product, then the opposite conclusions ($Y_2 > Y_1$ and $Y_2 < Y_1$) will be drawn with the same probability ($= 50\%$). Therefore, there must exist a limiting amount (e.g. concentration), C_2, for the sample which is slightly larger than the reference amount, C_1, and which leads to a sufficiently high probability (say 95%) for the right answer.

If the contaminant concentration falls above the limiting concentration, C_2, the purity test can find the irregularity with the probability of more than 95%. In other words, the risk for the purity test to overlook the irregularity is at most only 5%. However, the loophole of the test exists between the standard and limiting concentrations.

The discrimination limit is defined as the minimum increase in analyte amount which can be discriminated, with a high possibility, from the original amount [4,5]. The discrimination limit of the purity test can be regarded as the difference between the limiting concentration and reference concentration: $C_2 - C_1$ or similarly $E[Y_2] - E[Y_1]$ where $E[Y_i]$ denotes the mean of the observed value, Y_i. The discrimination limit is subject to many factors such as the concentration of the standard and the stochastic properties of an analytical apparatus used. In general, the smaller the discrimination limit, the more precise the test, as long as the same probability for the right judgment is referred to (here, 95%).

The aim of this paper is to provide a probability theory for determining the discrimination limit of the purity tests in separation science. A probability theory of quantitative analyses recently proposed by Hayashi and Matsuda is employed for predicting the uncertainty of measurement, i.e. standard deviation (S.D.) or relative standard deviation (R.S.D.) of Y_1 and Y_2 [6–10]. The rest of this section describes the reasons why this study does not rely on the widespread statistical method and why the above probability theory is selected from many candidates.

Fig. 1 illustrates a model for the discrimination limit. The S.D., σ_2, of the sample measurements is larger than that of the reference, because experiments have proved that the S.D. of measurements increases with increasing analyte amount in many

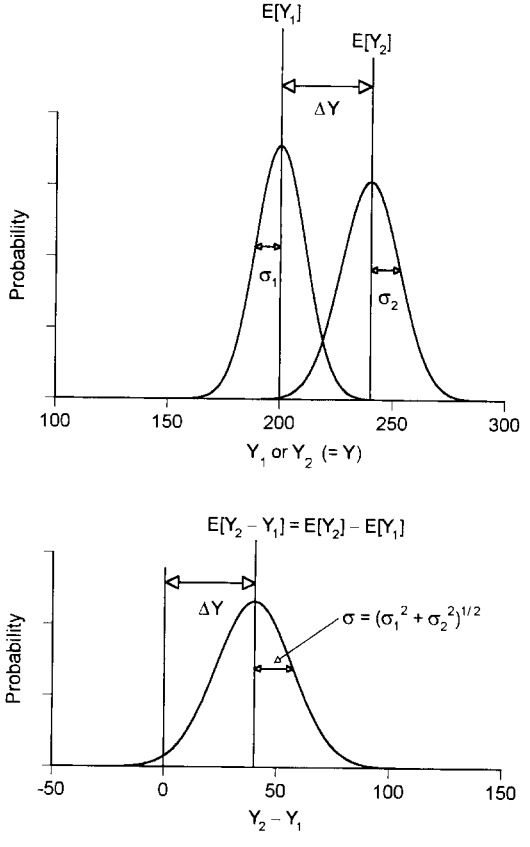

Fig. 1. Distributions of responses (upper) and distribution of their difference (lower). For clear presentation, the risk of the wrong answer ($Y_2 - Y_1 < 0$) is set at ca. 1% in the figure. The observed quantities, Y_1 and Y_2, for the reference and sample in an instrumental analysis are described as the independent Gaussian random variables characterized by the means, $E[Y_1]$ and $E[Y_2]$, and the standard deviations (S.D.), σ_1 and σ_2, respectively. $\sigma_1 = 11$ and $\sigma_2 = 13$.

instrumental analyses [1,11]. The lower figure of Fig. 1 also shows the distribution of the observed difference, $Y_2 - Y_1$, so that the right answer ($Y_2 - Y_1 > 0$) is obtained with a probability.

Given two sample solutions yielding the average measurements, $E[Y_1]$ and $E[Y_2]$ (Problem A), we can easily calculate the probability of the right answer ($Y_2 - Y_1 > 0$) by acquiring the measurement statistics for the solutions, σ_1 and σ_2: the S.D., σ, of the difference, $Y_2 - Y_1$, is known to be $(\sigma_1^2 + \sigma_2^2)^{1/2}$. A more attractive and practical problem is to assess the discrimination limit from the reference standard only with a given probability of the right answer (Problem B). For this purpose, it is imperative to express the uncertainty of measurement, σ_2 (and σ_1) as a function of concentration. This paper highlights Problem B. We assume sufficient accuracy with which the 'true' signals can be estimated experimentally as $E[Y_1]$ and $E[Y_2]$, because statistics tells that the averages converge even more rapidly to the true values than S.D.

If the measurement uncertainty, σ_1 or σ_2, can be known over a wide concentration range, it is easy to solve the above problems, A and B (see below). In practice, however, the most serious difficulty is to estimate the uncertainty, σ_1 or σ_2, with accuracy but as little effort as possible. Referring to the χ^2 distribution with $n-1$ degrees of freedom, we can know that 95% of the root-mean-square estimates of S.D. from 50 measurements scatter between 80 to 120% of the true value. This degree of variability between the individual S.D. values will be tolerable in the above problems, but the 50 experiments are unfavorable for slow instrumental analyses. For five measurements, 95% of the statistical S.D. values spread widely between 35 to 167% of the true value. It is almost impossible to obtain an exact answer to the above problems from the S.D. values based on a small number of measurements. The probability theory used in this paper can provide reliable S.D. values: the corresponding scattering is 78–122% from a single baseline of 2048 data points [12,13]. This prediction ambiguity can be reduced by analyzing more baselines, and the repetition of measurement can be dispensed with [12,13].

The theoretical or empirical prediction of the measurement R.S.D. has been studied extensively, for LC, [6–8,14–19], capillary electrophoresis (CE), [20] and spectroscopy [10,21–24]. Any prediction method for the response uncertainty cannot be perfect and contains some error in it, but there have been few publications specifying not only a theoretical framework but also the prediction error except for the probability–theoretic studies [12,13]. It is important to compare the statistical and probability–theoretic approaches according to the uncertainty accompanied with the same purpose.

2. Theory

2.1. General consideration

This subsection derives the equation to give an answer to Problem B. Throughout this paper, the discrimination limit, ΔY, is assumed to be positive. The negative discrimination limit represents the risk of the test to label an allowable drug as illicit.

If the difference between the average measurements, $\Delta Y (= E[Y_2] - E[Y_1])$, is much larger than the standard deviation ($\Delta Y \gg \sqrt{\sigma_1^2 + \sigma_2^2}$), the discrimination between Y_1 and Y_2 is considered perfect. That is, the observed difference, $Y_2 - Y_1$, would almost always be positive. If $k\sqrt{\sigma_1^2 + \sigma_2^2} = \Delta Y$ ($k = 1.6$), Y_1 and Y_2 can satisfactorily be discriminated with 5% risk for the wrong answer ($Y_2 - Y_1 < 0$).

If the response S.D. varies only slightly with the response intensity, then σ_2 can be approximated as $\sigma_2 = \sigma + (\mathrm{d}\sigma/\mathrm{d}Y)\,\Delta Y$ where σ_1 and Y_1 are described as σ and Y for the general purpose. The condition of the discrimination limit that $k\sqrt{\sigma_1^2 + \sigma_2^2} = \Delta Y$ can be described:

$$k\sqrt{\sigma^2 + \left(\sigma + \frac{\mathrm{d}\sigma}{\mathrm{d}Y}\Delta Y\right)^2} = \Delta Y \qquad (1)$$

Squaring the above equation and solving the quadratic equation for ΔY, we can obtain (see Appendix):

$$\Delta Y = \frac{k^2\sigma\frac{d\sigma}{dY} + \sqrt{2}k\sigma\sqrt{1-\frac{1}{2}k^2\left(\frac{d\sigma}{dY}\right)^2}}{1-k^2\left(\frac{d\sigma}{dY}\right)^2} \quad (2)$$

If $k(d\sigma/dY) \ll 1$, Eq. (2) can be simplified:

$$\Delta Y = \sqrt{2}k\sigma + k^2\sigma\frac{d\sigma}{dY} \quad (3)$$

This is the general equation of the discrimination limit of the purity test. Throughout this paper, $k = 1.6$ and the probability for the right answer is 95%. The above assumption that $k(d\sigma/dY) \ll 1$ is discussed below.

If the measurement error is homoscedastic ($d\sigma/dY = 0$), Eq. (3) takes the simplest form:

$$\Delta Y = \sqrt{2}k\sigma \quad (4)$$

This equation is quite similar to ones that appear in the literature concerning limit of detection [7,21,25,26]. It can be directly derived from the assumption that $k\sqrt{\sigma_1^2+\sigma_2^2} = \Delta Y$ where $\sigma_1 = \sigma_2 = \sigma$.

If $\Delta Y > 0$, the denominator in the right side of Eq. (2) should be positive and we can get the limit, called discriminability limit here, above which two samples cannot be discriminated:

$$\frac{d\sigma}{dY} < \frac{1}{k} \quad (5)$$

This inequality demonstrates that two samples cannot be distinguished experimentally, if the increase ratio of the instrumental error, $d\sigma$, to the analyte signal intensity, dY, exceeds the discriminability limit, $1/k$. That is, too much increase in the measurement uncertainty disturbs the discriminability.

2.2. Examples of response uncertainty (scedasticity)

This subsection gives a brief review of the probability theory to describe the response R.S.D., as a function of the mean signal intensity, $E[Y]$ ($= E[Y_1]$), in separation science. The detailed explanations and experimental verification of the theory were given elsewhere [6–8,10,13,20].

The 'false area' created by the baseline drifts is the major cause of the measurement uncertainty in instrumental analysis, if the sample concentration is sufficiently low. The R.S.D. of measurements can be described as [8]:

$$\begin{aligned}
\sigma_M^2/E[Y]^2 = {} & \frac{(k_f-k_c)\tilde{w}^2}{E[Y]^2} \text{ (first term)} \\
& + \frac{1}{(1-\rho)} \\
& \left(k_f - k_c - 2\rho\frac{1-\rho^{k_f-k_c}}{1-\rho} + \rho^2\frac{1-\rho^{2(k_f-k_c)}}{1-\rho^2}\right) \\
& \frac{\tilde{m}^2}{E[Y]^2} \text{ (second term)} \\
& + \rho^2\frac{1-\rho^{2k_c}}{1-\rho^2}\left(\frac{1-\rho^{k_f-k_c}}{1-\rho}\right)^2\frac{\tilde{m}^2}{E[Y]^2} \text{ (third term)} \\
& + \frac{\alpha^2\tilde{w}^2}{E[Y]^2} \text{ (fourth term)} \\
& + \left\{\alpha^2\frac{1-\rho^{2k_e}}{1-\rho^2}\right. \\
& - 2\alpha\left[\frac{1-\rho^{k_f-k_c}}{1-\rho}\rho^{k_e+k_c-1}\frac{1-\rho^{-2k_c}}{1-\rho^{-2}}\right. \\
& \left.\left. + \sum_{i=1}^{k_f-k_c}\left(\frac{1-\rho^{k_f-k_c+1-i}}{1-\rho}\rho^{k_e-k_c-i}\right)\right]\right\} \\
& \frac{\tilde{m}^2}{E[Y]^2} \text{ (fifth term)} \\
& + \frac{(k_f-k_c)^2}{b}\frac{\tilde{w}^2}{E[Y]^2} \text{ (sixth term) [27]} \\
& + I^2 \text{ (seventh term)}
\end{aligned} \quad (6)$$

where $\tilde{w}$ is the S.D. of the white noise, $\tilde{m}$ and ρ are the S.D. and auto-correlation parameter of the Markov process, respectively, k_c is called cutoff point of the signal integration, k_f is filter-off point, b denotes the number of consecutive data points over which the baseline noise is averaged for the zero level, I is the injection volume error (R.S.D.), and α is described in [8]. The integration is carried out over the domain from $k_c + 1$ to k_f (for the peak height measurement, $k_c + 1 = k_f$). The zero level at the zero point is calculated based on the effect of the white noise only, because the white noise can affect the precision more seriously than the Markov process [27].

The terms of Eq. (6) denote the following stochastic contributions to the response uncertainty [8]:

first term, the error from the white noise in the integration domain ($k_f - k_c$ data points);

second term, the error from the Markov process in the integration domain;

third term, the influence of the lag time during k_c data points (if $k_c = 0$, this term is zero);

fourth term, the effect of the white noise on the oblique integration;

fifth term, the effect of the Markov process on the oblique integration;

sixth term, the error in determining the zero level at the zero point;

seventh term, the independent error (mainly originating from the injection error).

Noise parameters, $\tilde{w}$, $\tilde{m}$ and ρ, are all determined by the least-squares fitting of the theoretical power spectrum of the model process to the actual power spectrum of a baseline [6]. The theoretical power spectrum of the model (white noise + Markov process) is given in Eq. (A8) of [6]. Fig. 2 shows the power spectra $(1, 2, \ldots, n)$ of the LC baselines of 512 data points. Although the noise parameters can be estimated from each spectrum, the R.S.D. of the response S.D., σ_M, predicted from a single spectrum is relatively large (= 18.9%) [12]. In this paper, the average power spectrum ($n = 8$) is fitted by the simplex least squares for the exact prediction of the discrimination limit instead of averaging the noise parameters obtained from the individual power spectra. This is because the most critical step of the exact parametrization seems to be the simplex least squares. The average signal intensity, $E[Y]$, can be estimated from a concentrated sample. If the injection error, I, is estimated by separate experiments, we can calculate the response S.D., σ_M, or R.S.D., $\sigma_M/E[Y]$, by Eq. (6). Note that the signal parameters, k_c and k_f, have to comply with the integration mode [6].

For the area measurement, the zero line over which the intensities are integrated is drawn along the observed baseline, called the oblique integration [8]. In this paper, the zero point which is the starting point of the zero line is adjacent to the left edge of the integration domain ($\pm$ 3 s) (no lag time). The height measurement is the difference between the observed intensities at the peak center and the zero point (not the highest intensity over the domain ($\pm$ 3 s)). The zero line is drawn in the same way as that for the oblique integration. Note that the zero line varies from experiment to experiment according to the baseline trajectory.

The validity of the assumption used for Eq. (5) ($d\sigma/dY < 1/k$) is verified. At low sample concentrations, the baseline noise takes predominance over the injection error and the response SD, σ_M, is independent of the signal intensity, $E[Y]$. Therefore, $d\sigma_M/dY \approx 0$. At high sample concentrations, the injection error is the major cause of the instrumental error. Therefore, $d\sigma_M/dY \approx I$ (note that $\sigma_M \approx IE[Y]$). The injection error, I, was observed to be 0.0024 in the LC apparatus [6] and is much lower than $1/k$ where $k = 1.6$.

2.3. Discrimination limit and relative discrimination limit

Substituting Eq. (6) for Eq. (3), we can obtain the discrimination limit for the LC and CE analysis:

$$\Delta Y = \sqrt{2}k\sigma_M + k^2\sigma_M \frac{d\sigma_M}{dY} \tag{7}$$

The relative discrimination limit is defined as:

$$\frac{\Delta Y}{E[Y]} = \sqrt{2}k \frac{\sigma_M}{E[Y]} + k^2 \frac{\sigma_M}{E[Y]} \frac{d\sigma_M}{dY} \tag{8}$$

where $\sigma_M/E[Y]$ is the R.S.D. of measurements. If necessary, another uncertainty equation can replace σ_M of Eqs. (7) and (8).

2.4. Probabilistic aspects of discrimination limit

Fig. 3 shows typical examples of the homo- and hetero-scedasticity. The former mimics, though roughly, LC, CE and fluorometry [6,10,20]. The discrimination limit depends entirely on the scedasticity.

For the homoscedastic situation, the discrimination limit, ΔY, is constant irrespective of the reference concentration or $E[Y]$ (see Fig. 4(A)). The positive derivative of the response S.D. in the heteroscedastic situation makes the curve of the discrimination limit steeper than the original response S.D. That is, the change in the discrimination limit is larger than the change in the response

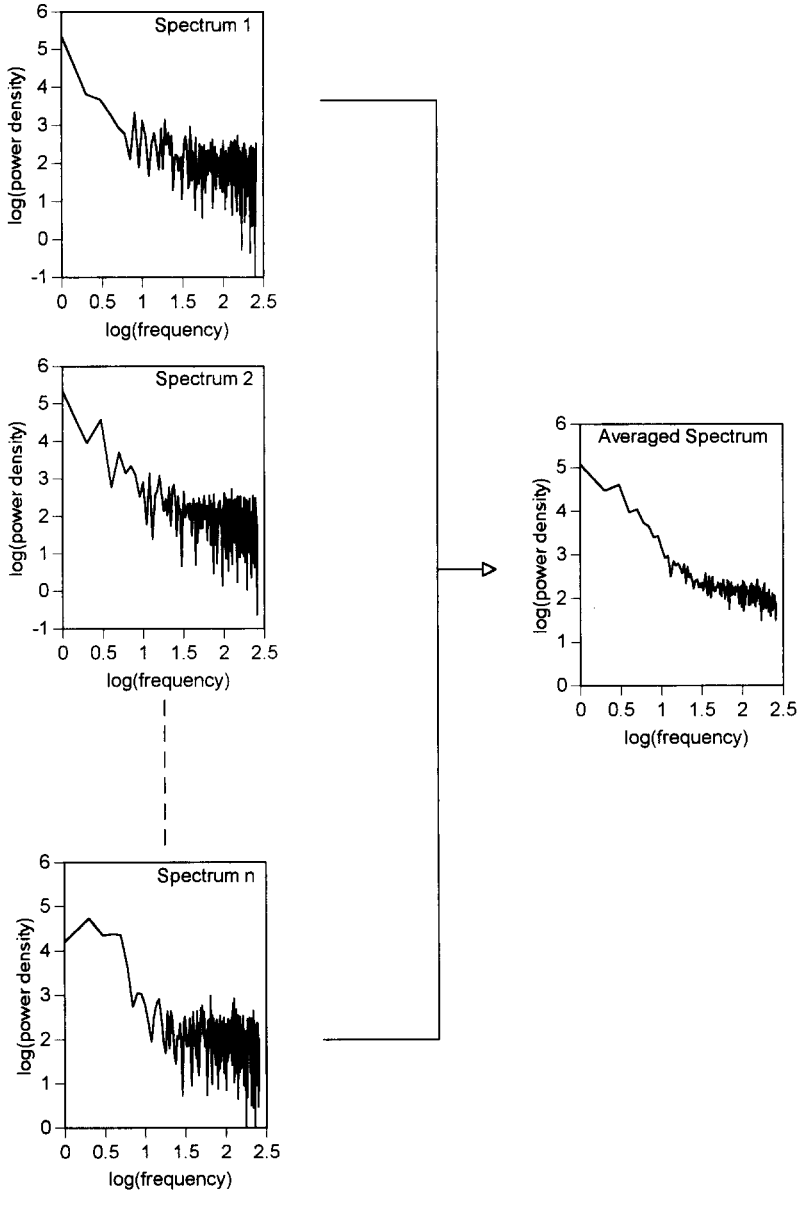

Fig. 2. Power spectral density of each baseline (512 data points) and average power spectral density. The noise parameters of the average power spectra are: $\hat{w} = 10.7$; $\hat{m} = 5.4$; $\rho = 0.99$.

S.D. We should note that $E[Y]$, ΔY and σ have the same dimension.

Fig. 4(B) demonstrates that the relative discrimination limit, $\Delta Y/E[Y]$, decreases with increasing measured quantity, whereas the discrimination limit, ΔY, is non-decreasing. These behaviors bear a close resemblance to those of S.D. and R.S.D. of measurements and the above statement is also true, if the discrimination limit and relative discrimination limit are replaced by S.D. and R.S.D., respectively [1,28]. For the homoscedastic situation of Fig. 4, when $\Delta Y/E[Y] = 0.06$ at the reference signal of 400, the sample signal ($= 424$) which is 1.06 times the reference signal can be

discriminated from the reference signal with 95% probability. However, when a smaller signal is used as a reference, the precision of the test is reduced ($\Delta Y/E[Y] = 0.23$ at $E[Y] = 100$). Now, the discrimination limit requires the signal 1.23 times the reference signal. If the probability for the right answer is reduced from 95 to 90% (or the k value is reduced), the lines of the discrimination limit and relative discrimination limit in Fig. 4 move downward and closer signals can be discriminated, but with low reliability.

Fig. 5(A), (B), (D) and (E) show the signals of reference (left) and signals for the discrimination limit (right) which are overlaid on the actual LC baseline (C). The Gaussian signals of 10% difference (A) can be discriminated with 95% reliability in the LC system. However, for the small reference signal (D), the signals of 50% difference are the minimum difference for the successful discrimination. The signals of Fig. 5 are not much larger than the baseline noise and the measurement uncertainty of the LC system is assumed to be homoscedastic ($I = 0$).

The signal processing affects the precision of measurements greatly [29]. In our LC system, the peak height measurement has been proved to be superior to the entire area integration [8]. Fig. 5(A) and (D) are based on the entire area integration. For the peak height measurement ((B) and (E)), smaller peaks can be used as standard with 95% probability of discrimination than the entire integration: ((A) and (B)) for 10% difference in signal; ((D) and (E)) 50% difference.

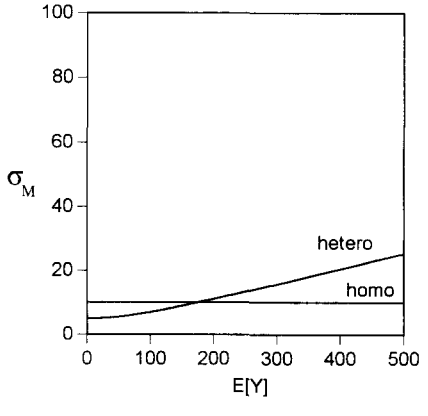

Fig. 3. Standard deviation of response as a function of mean response intensity. Homonscedasticity: $\sigma = 10$. Heteroscedasticity: $\sigma^2 = c_0 + c_1 Y^2$ where $c_0 = 25$ and $c_1 = 0.0025$.

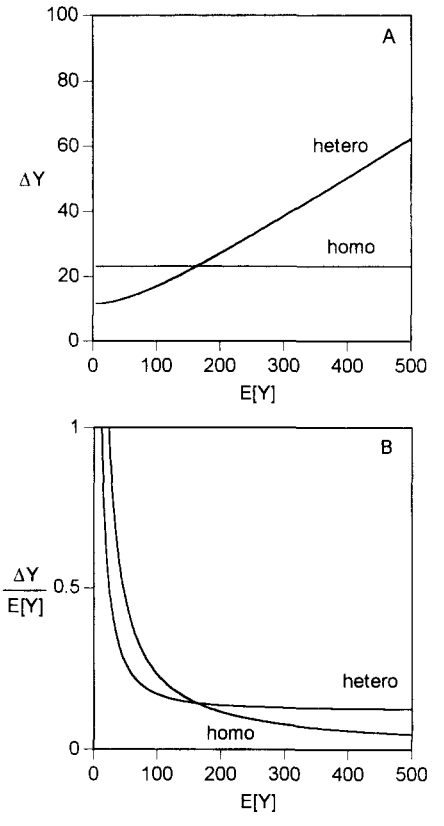

Fig. 4. Discrimination limit, ΔY (upper) and relative discrimination limit, $\Delta Y/E[Y]$ (lower) of homo- and hetero-scedasticity. $k = 1.64$. The scedasticity of Fig. 3 is used.

The Monte Carlo simulation is carried out to verify the prediction theory as follows. A peak pair of the reference and theoretical discrimination limit is overlaid on the LC baselines at random with no correlation of the measurements. The areas (or heights) for the reference and limit peaks are measured on the computer and compared. The answer of this computer experiment should be right or wrong. The above experiment with the same peak shape is repeated 5000 times on 20 baselines. The observed probability for the right answer was 94.3% for Fig. 5(A) and 94.8% for Fig. 5(B). These values are close to the theoretical value (= 95%).

The entire theory of this paper is based on the assumption that the integration results or peak

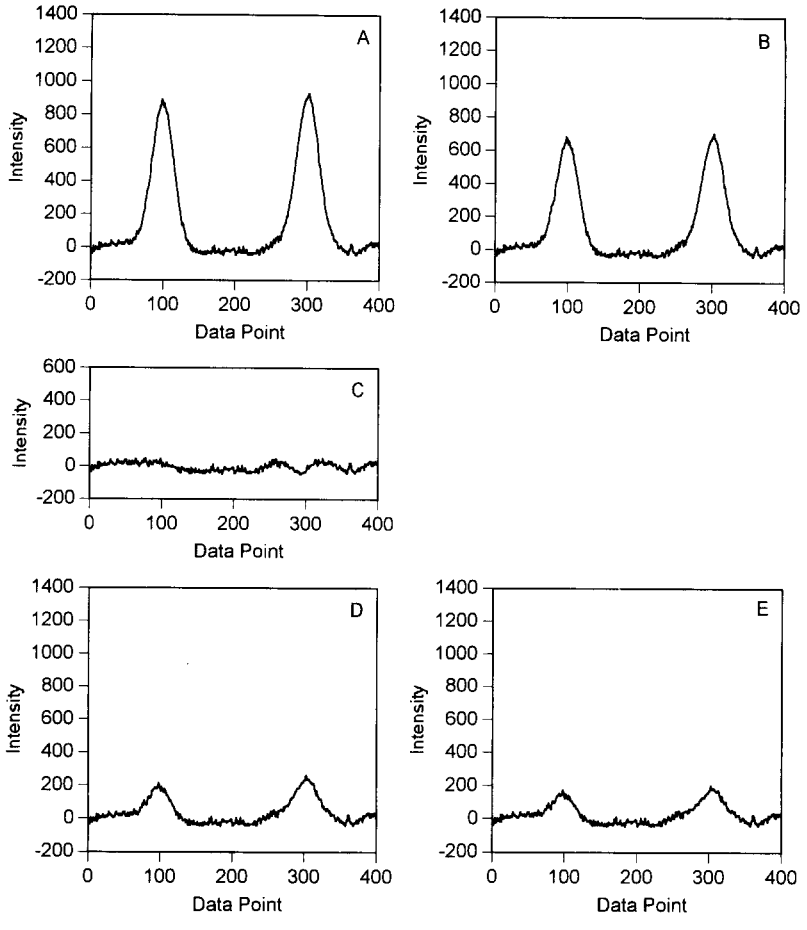

Fig. 5. Examples of discrimination limits in LC. (A), (B), (D), (E): the left peak is assumed to come from the reference material; the right peak corresponds to the signal of the discrimination limit. All the peaks are Gaussian with the width (S.D.), s, of 15 data points. (C): the baseline observed in the LC apparatus [6]. The average measurement of the right peak is 1.1 times ((A) and (B)) or 1.5 times ((D) and (E)) that of the reference peak (left) with the discriminability of 95%. (A) and (D): the average measurement is the peak area over ± 3 s around the peak center. (B) and (E): the average measurement is the peak height at the peak center. Eq. (6) and the noise parameters determined in Fig. 2 are used to predict the LC uncertainty, σ_M ($I = 0$).

height measurements have a Gaussian distribution. This assumption often appears in textbooks of analytical chemistry and in many cases, is interpretable in terms of the central limit theorem [26]. Fig. 6 provides a good evidence for the Gaussian assumption of the integrated areas in LC (a) and CE (b). Instead of the integration results, the distribution of the individual baseline intensities was recently examined in gas chromatography [30].

The central limit theorem means that the sum of random variables displays a Gaussian distribution, if the random variables are mutually independent [31]. Here, the random variable represents the observed values at a data point of the baseline and the sum of the variables corresponds to the integration over the consecutive data points. Since the random variables to be summed are correlated in the instrumental analyses [6,32–36], we have to stress the fact that the central limit theorem cannot apply to the results of Fig. 6.

If the random variables (baseline noise) are independent and Gaussian with mean 0 and S.D.

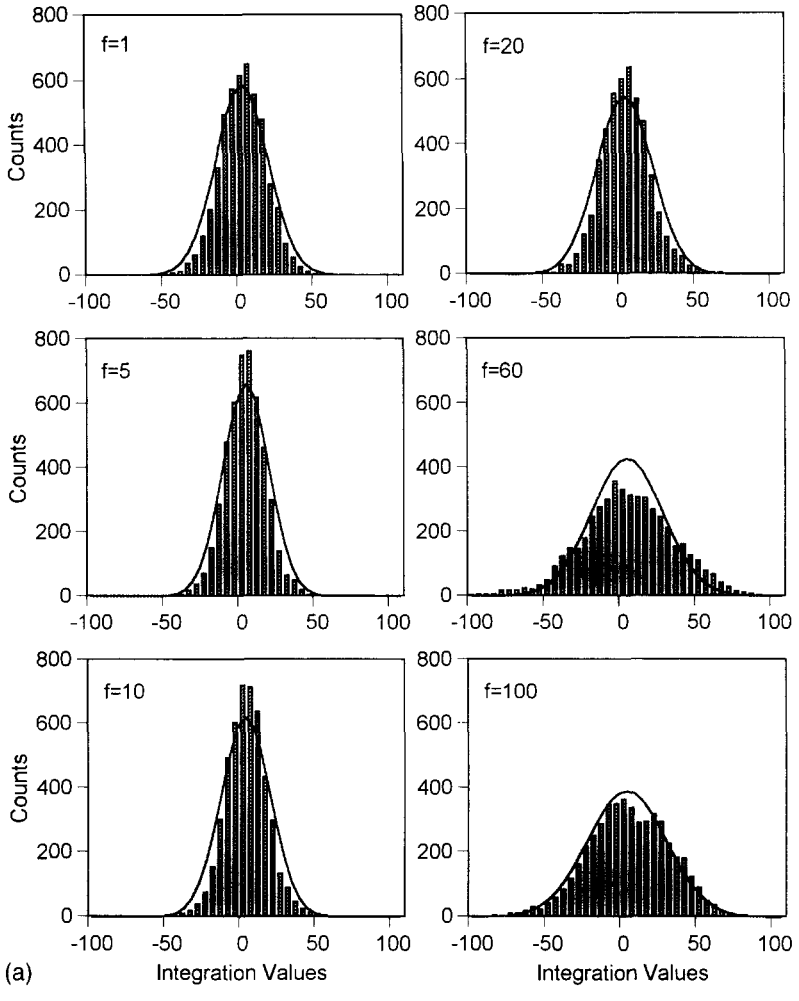

Fig. 6.

$\tilde{W}$, called white noise, the variance of the frequency of occurrence in Fig. 6 should obey the following equation (see Appendix):

$$\mathrm{Var}(X) = \left(1 + \frac{1}{f}\right)\tilde{W}^2 \tag{9}$$

where X means the sum of f intensities divided by f. Although Eq. (9) decreases with increasing f, Fig. 6 shows the disparate behaviors. For LC (Fig. 6(a)), the S.D. of the integration results first decreases ($f = 1 - 5$) and then increases ($f > 5$). This apparent anomaly can be attributed to the auto-correlation of the baseline noise. The baseline noise of the CE apparatus is mainly made up of the white noise and the statistics of the integration is different from the LC statistics. In any case, the uncertainty theory (Eq. (6)) can follow the stochastic behaviors of the LC and CE baselines (see the solid lines of Fig. 6). To recapitulate, the distribution of the integration over the LC and CE baselines seems to be Gaussian as an experimental evidence, but it cannot be explained by the central limit theorem.

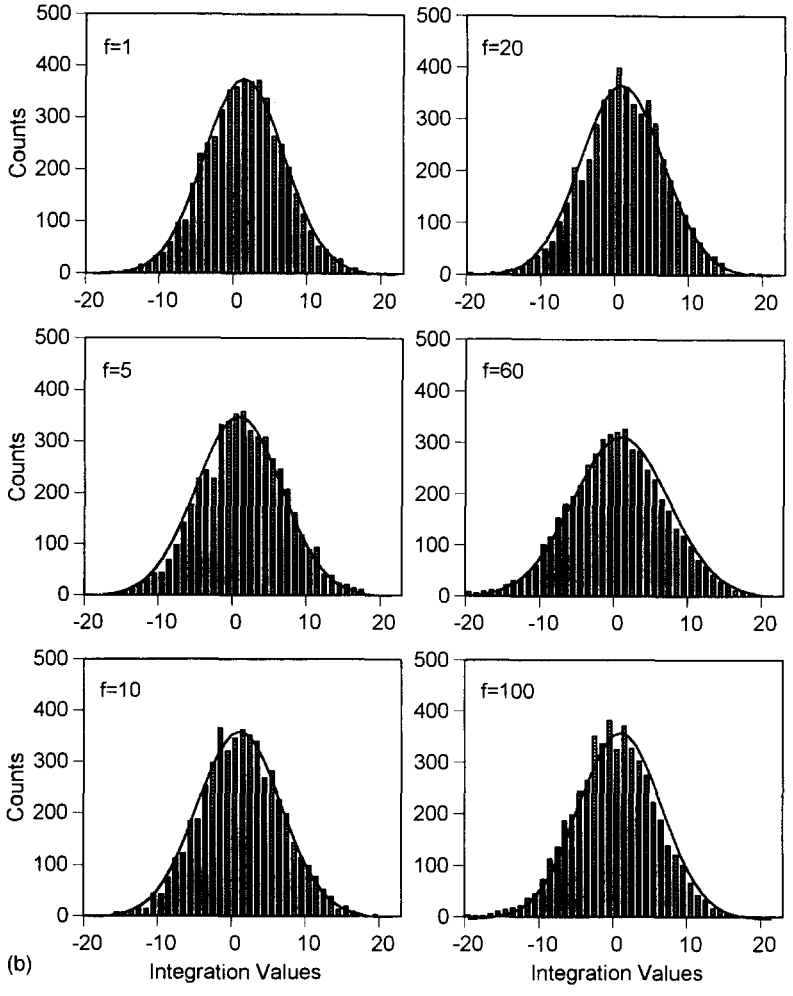

Fig. 6. Distributions of integrated areas over the baselines of LC (a) and CE (b) f denotes the number of data point integrated (if $f = 1$, the peak height measurement is carried out). The discrete frequency of occurrence comes from the integration over the real baselines (the Monte Carlo experiment uses the real baselines but without any signals; the number of repetition is 5000). The smooth lines are drawn according to Eq. (6): (a) $\tilde{w} = 10.7$; $\tilde{m} = 5.4$; $\rho = 0.99$, $I = 0$; (b) $\tilde{w} = 27.8$; $\tilde{m} = 0.4$; $\rho = 0.999$, $I = 0$. The wavelength is 254 nm for LC and 220 nm for CE. The integration starts with the data point adjacent to the zero point (no lag time). The sixth term of Eq. (6) is omitted for the LC. The detailed experimental conditions of LC and CE were described previously [6,20].

3. Discussion

The values of the discrimination limit determined in this paper can apply only to the analytical system of our laboratory. In general, analysts should know the stochastic properties of their own apparatus for this purpose as follows:

1. Fourier-transform a baseline (e.g. 2048 data points) of an instrument to obtain the power spectrum;
2. least-squares fit the baseline model (Eq. (A8) of [6]) to the power spectrum to acquire the parameters, $\tilde{w}$, $\tilde{m}$ and ρ;
3. determine the integration mode (k_c and k_f);
4. determine the injection error of the system, I, if necessary;
5. observe the signal intensity, $E[Y]$, for the reference;
6. calculate the S.D. of the false areas with Eq. (6) over a sufficiently wide region around the reference intensity;

7. use Eq. (7) (8) to calculate the discrimination limit or relative discrimination limit (the derivative of the response uncertainty, $d\sigma/dY$, can also be given numerically).

The signal taken here is so small that the stochastic properties of the LC and CE systems are considered homoscedastic with neglecting the injection error, I. The practical examples of this paper reaffirm the reliability of the uncertainty prediction (Eq. (6)), rather than it focus only on the wide applicability of the prediction theory of the discrimination (Eq. (8)). Nevertheless, the heteroscedastic situations are common in analytical chemistry [11,15,17,18,21,22,25,37–40] and cannot be neglected in many situations. Consider the situation where the risk for the wrong decision is set at a low value (e.g. 0.13% and $k=3$) and the independent error (R.S.D.) produced during the sample preparation and measurement is relatively high (e.g. $d\sigma_M/dY=10\%$ R.S.D.). Eq. (7) can take the form: $\Delta Y=\sqrt{2}k\sigma_M[1+k/\sqrt{2}(d\sigma_M/dY)]$. Therefore, the derivative term of the above equation greatly affects the discrimination limit: $\Delta Y=\sqrt{2}k\sigma_M(1+0.21)$ where $0.21\approx 3/1.414\times 0.1$.

As mentioned above, the prediction error of the discrimination is as small as 1%. In the probability–theoretic approach of this paper, therefore, the heteroscedasticity, $d\sigma_M/dY$, about 0.01 or more, will be rather significant in determining the discrimination limit. Many CE apparatus will show appreciable scedasticity because of the large injection error as compared with modern LC systems (e.g. $d\sigma_M/dY\approx I\approx 0.02$ for CE). The actual treatment of the heteroscedasticity will be our next subject.

The prediction of the measurement uncertainty is also useful for another critical problems in analytical chemistry. The detection limit can be defined as the lowest amount of analyte which yields negative measurements with a negligibly small probability [28]. The starting equation of this problem is that $k\{\sigma+(d\sigma/dY)\Delta Y\}=\Delta Y$. The detection limit, ΔY, takes the form:

$$\Delta Y=\frac{k\sigma}{1-k\dfrac{d\sigma}{dY}} \tag{10}$$

If $d\sigma/dY=0$, Eq. (10) becomes the well-known equation for the limit of detection [21,25,26].

Consider the situation where a legitimate drug meets the requirement of contamination. The corresponding equation for the discrimination limit represents the highest amount of contaminant that passes the purity test with a probability or the risk for the test to mistake the drug as illicit (for the corresponding equation, see Appendix). This situation can also be treated in a similar way.

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Appendix A

Discrimination limit

Eq. (1) can be written as:

$$\left\{1-k^2\left(\frac{d\sigma}{dY}\right)^2\right\}\Delta Y^2-2\sigma k^2\frac{d\sigma}{dY}\Delta Y-2\sigma^2k^2=0 \tag{A1}$$

The solution of this equation is:

$$\Delta Y=\left[k^2\sigma\frac{d\sigma}{dY}\pm\sqrt{k^4\sigma^2\left(\frac{d\sigma}{dY}\right)^2+2k^2\sigma^2\left\{1-k^2\left(\frac{d\sigma}{dY}\right)^2\right\}}\right]\Big/\left\{1-k^2\left(\frac{d\sigma}{dY}\right)^2\right\} \tag{A2}$$

We can easily obtain Eq. (2) from Eq. (A2). If ΔY is positive and if $d\sigma/dY=0$, the sign of the square root term in the numerator of Eq. (2) should be positive. If ΔY is negative and if $d\sigma/dY=0$, the sign should be negative.

Integration

Let W_i be the white noise at data point i. The average of the integrated white noise, X, with the white noise, W_0, at the zero point as a standard is written as:

$$X=\frac{W_1+\cdots+W_f-fW_0}{f} \tag{A3}$$

where f denotes the number of integrated data points. The variance of the integration results takes the form:

$$\mathrm{Var}(X) = \frac{1}{f^2}(\tilde{W}_1^2 + \cdots + \tilde{W}_f^2 + f^2\tilde{W}_0^2) \qquad \text{(A4)}$$

If the S.D. of the white noise is constant, $\tilde{W}$, we can obtain Eq. (9).

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