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STATISTICAL EVALUATION OF THE RESULTS OBTAINED WITH THE ANALYTICAL METHODS USED FOR THE QUALITY CONTROL OF MEDICINES

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Abstract

To improve the way by which the quality control of medicines is carried out, general statistical concepts and methods are discussed for the evaluation of the analytical results obtained for judging the adequacy of drug substances and products for Pharmacopoeia requirements.

Statistical procedures to be adopted by the producer and the controller in the determination of characteristics are suggested when the drug substance is or is not homogeneous and when the precision of the analytical method is known or unknown.

For each of these cases, detailed numerical examples are given.

INTRODUCTION

The importance of the quality of medicines has been recognized for a long time and the progress made in the general principles and practical aspects of the control of this quality has brought about the present satisfactory situation due to the understanding by all the people involved in this matter. In practice, even if the layman knows that he or she is not able to judge the quality of the medicines prescribed by the physician [as is the case when he or she purchases a commodity product such as a shirt or even a car], certainly he or she can trust the pharmacist who dispenses them. In fact, this profession is a part of a larger system producing medicines, which includes the Manufacturers and the Governmental Regulatory Authorities who have taken the responsibility for the appropriate quality. It can be said that the system has taken advantage of all the scientific knowledge and the academic and industrial technology for reaching this goal. The regulatory Authorities issue in the official Compendia, such as Pharmacopoeias, Formularies, etc., the specifications that are followed by producers of medicines before dispensing them to the public. On the whole, the present situation of the quality control of medicines can be considered satisfactory, at least in developed countries. However, improvements are always possible and in this respect the authors wish to present discussions and proposals for utilizing one of the tools that can make the quality control of medicines easier — the statistical evaluation of the results obtained with the analytical methods used for this control.

The good quality of medicines can only be achieved through good manufacturing procedures and through good analytical checks of these procedures and of the medicines themselves. For this, the analytical methods used to control the processes and products, even if in recent times they have scientifically and technologically improved very much, cannot allow us to overlook the general fact that analysis can only help us to get nearer the truth, but not to obtain it with absolute certainty. This is due to the intrinsic limitation of the sampling of the product being examined, which cannot actually be analyzed in its entirety. But we can be satisfied by the fact that we can make an estimation of the truth by analyzing limited sampling and that the halo of uncertainty can be controlled by the use of statistics. It is well known that forecasts made on the basis of sound statistical handling of the intrinsically limited information available eventually indicate the truth. For example, in political elections where, in practice, the sampling is always limited to a small number of people, can get very close to the truth because the obtained information is properly evaluated with a specialized statistical approach. This is also the case for the analytical results obtained on a given medicine under examination.

It is clear now that the improvement discussed here does not refer to the technical aspects of the production process or of the analytical control, which, as mentioned above, are at the highest and most satisfactory level, but only to the handling of the analytical results. In this way, it appears possible to achieve faster knowledge of the truth and to avoid all the consequent loss of time and money originated by the absence of this knowledge. The authors are aware that this paper cannot solve the problem completely, but they are certain it will contribute to a better understanding of this complex matter, where technical, political and economical aspects merge and create a number of controversies. In fact, a better evaluation of the results obtained with analytical methods will bring about a more certain judgement on the quality of medicines, as prescribed by the official Compendia, and a concordant approach to the task on the part of the control laboratory of the producer and of that of the governmental Agency responsible for the public's safety.

Medicines are usually administered not in the form of the active principle (the drug substance) but as a complex mixture of the active principle with various other ingredients (the excipients) required to make a precise, effective, stable and convenient dosage form (the drug product). The official Compendia give instructions for the quality control of drug substances and of drug products and both will be considered in the present paper. Nowadays, medicines are manufactured in a large scale by a series of complicated operations which, even though they take advantage of a highly sophisticated machinary, can bring about variations in the quality of each batch or of each unit of the various batches. Therefore, it is important to assure an adequate quality of the various batches of a drug substance and of the single units of a given batch of a drug product.

To this respect, this paper is divided into two parts for convenience. The first part is devoted to the evaluation of the results obtained in the determination of characteristics when the batches of the drug substance or drug product under examination are taken as homogeneous. In this case, the measurement of the characteristics is carried out on a sample representative of the batches. The judgement on the compliance of the batches under examination is based on the comparison between the analytical result and the official specifications.

The second part is devoted to the evaluation of the results obtained in the qualitative or quantitative determinations of characteristics when the batches of medicine under examination are taken as non-homogeneous, because they are formed by a large number of units. In this case, the measurement of the characteristics is carried out on a sample formed by a small and defined number of units. The judgement on the compliance of the batches under examination is based on the comparison between the number of units found to exceed the specifications and the number of the units of this type defined as acceptable by the sampling design made as a function of the official specifications.

PART I

STATISTICAL EVALUATION OF THE RESULTS OBTAINED IN THE DETERMINATION OF CHARACTERISTICS WHEN THE DRUG SUBSTANCE OR THE DRUG PRODUCT IS HOMOGENEOUS (PHYSICAL, PHYSICOCHEMICAL AND CHEMICAL METHODS)

1. INTRODUCTION

The physical, physicochemical, and chemical methods 1) described for the assays and tests 2) on drug substances and drug products 3) in the official Compendia such as Pharmacopoeias, Formularies, etc. 4), are used to determine the concentration or the quantity 5) of the active principle and of other substances, and to control the limits of foreign components, such as related substances and/or impurities.

The principles applied can be conveniently classified into two main types. The first one is that of the comparison with a reference substance so as to determine how much of a sample being examined produces the same analytical response as that of a given concentration of the reference substance. The principle is the same as that applied for biological assays already described in the Pharmacopoeia[*], where the measurements on the reference preparation and on the sample have to be carried out under strictly comparable conditions and at the same time. Analogous is the approach in the case of a physicochemical assay when the Pharmacopoeia prescribes the use of a chemical reference substance. An explanatory example can be that of an assay based on a colorimetric reaction (cortisone acetate with triphenyltetrazolium chloride).

It is worthwhile to say that in most cases the Pharmacopoeia does not prescribe the use of a chemical reference substance because the analytical response of the reference substance has already been defined in a proper way as a specific value by the Pharmacopoeia which reports it. In such a case the measurement on the sample being examined is obviously carried out in a deferred time and the Pharmacopoeia prescribes in a general way that the analyst should comply with the Good Laboratory Practices, i.e., the analyst should control the glassware and calibrate the apparatus and the instruments. An example can be that of an assay based on the absorption spectrophotometry in the ultraviolet and visible (rifampicin, 475 nm, A,1%,1cm = 187).

The second principle is that based on the knowledge of the molecular structure of the substance being examined and the Pharmacopoeia indicates the equivalence ratio between the analytical response and the concentration of the substance being examined. This basically refers to the chemical methods. An example can be the equivalent weight reported in the case of an acid-base or a red-ox titration.

2. *GENERAL STATISTICAL CONCEPTS* (Ref. 1-3)

For a proper discussion of the results obtained with the above methods, y is designated as an observed quantitative measurement of the analytical feature (*analytical response*) which is characteristic of the substance to be determined (*analyte*). The observed values are used to determine the *concen-*

From now on reference will be made only to: 1) physicochemical; 2) assays; 3) drug substances; 4) Pharmacopoeia; 5) concentration.

*) In this paper reference is made to the European Pharmacopoeia.

tration x of the analyte contained in the sample being examined and the relation between the concentration x and the quantitative measurement y is defined as the analytical function ($y = f(x)$).

The values y_i (y_1, y_2, ... y_n), due to their experimental origin, show variations caused by random and/or systematic errors, which reflect the inherent variability of the analytical response, and originate a corresponding series of values x_i (x_1, x_2, ... x_n).

Assuming that the specificity has been previously demonstrated, the quality of the analytical response (the set of the experimental values y_i) can be indicated by its precision, i.e., the closeness of agreement among the values x_i obtained by repeating the assay, and by its accuracy, i.e., the closeness of agreement between the found value $\bar{x}$ and the true value w, if known. There are two classes of experimental errors: the random and the systematic. The precision of an assay is affected by the random experimental errors that are due to any still unidentified source of variation, whereas the accuracy of an assay is affected by the systematic experimental errors that are due to the bias in the method. It should be remembered here that the identification of the source of that "random uncertainty" implies its classification as "systematic error" and eventually leads to its control.

The evaluation of the precision of an analytical method is conveniently expressed by the standard deviation (s) of the series of the n values x_i obtained and it is calculated as follows:

$$s = \sqrt{\frac{\sum_{i=1}^{n}(x_i - \bar{x})^2}{n - 1}} = \sqrt{\frac{\sum_{i=1}^{n} x_i^2 - \frac{\left(\sum_{i=1}^{n} x_i\right)^2}{n}}{n - 1}}$$

where: x_1, x_2,... x_n are the values obtained by repeating the same measurement n times and $\bar{x}$ is the arithmetic mean of the obtained values. The standard deviation s has the same units as the obtained values.

Much used is the relative standard deviation or coefficient of variation (cv %) of the series of the n obtained values, which is:

$$cv\% = \frac{s}{\bar{x}} 100$$

The evaluation of the precision of the arithmetic mean of the n obtained values x_i is given by the standard error ($s_{\bar{x}}$) which can be obtained by:

$$s_{\bar{x}} = \frac{s}{\sqrt{n}}$$

The mean $\bar{x}$ and its standard error $s_{\bar{x}}$ allow to determine an interval that certainly (i.e., at least 95 times out of 100)* includes the true concentration w of the analyte. This interval is referred to as the confidence interval of $\bar{x}$ and its limits can be calculated by the formula:

$$\bar{x} \pm t.s_{\bar{x}}$$

where: t is the value of the Student's t statistics corresponding to the probability of 0.95 (two tails test) and to n-1 degree of freedom, which can be obtained from the Table in Appendix 1. The two limits are called the lower confidence limit and the upper confidence limit.

The valuation of the accuracy of an analytical method is conveniently expressed by the difference between the mean analytical result and the true value, known or assumed:

$$b = \bar{x} - w$$

This difference, called bias, can be a positive or a negative number. Analogously to what was said for the precision, bias can also be expressed relatively to its mean (relative bias, b%):

$$b\% = \frac{b}{\bar{x}} 100$$

The assay methods, as presented in the Pharmacopoeia, are validated methods, in which systematic errors are controlled so that the accuracy of the results is not affected.

The validation of an assay method consists in evaluating its precision and its accuracy. Before making this evaluation it is necessary to verify the exi-

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* Absolute certainty does not exist but a very likely event is considered as certain. The authors suggest that the Pharmacopoeia consider a probability of 95% (i.e., 0.95) as suitable. Then, to the word "certainly" the meaning of at least 95 times out of 100 should be conventionally attributed.

stence of a statistically significant relationship between the values x_i and y_i (regression): this relationship has already been defined as analytical function. For simplicity, only the statistical criteria that can be applied to the methods having linear and proportional responses are discussed because this is the model to which the majority of methods are amenable. The linearity and the proportionality of the response take place when, in the range of the analyte concentration, the analytical function is represented by a semi-straight line (linearity) with its origin coinciding with the origin of the reference system of Cartesian axes (proportionality). This corresponds to the assumption that the analytical function has the form $y = a + b.x$, where a is not significantly different from zero.

Other aspects contribute to the quality of an assay method with special reference to low concentrations: these are the sensitivity and the limits of detection and quantitation.

The sensitivity of the method (S) is the ratio between the variation of the observed response y and the variation of the concentration x of the analyte. This is calculated as the derivative of the analytical function, and represented by the slope of the analytical curve:

$$S = \frac{dy}{dx}$$

Then, a method is very sensitive if a small change in the analyte concentration brings about a large change in the response.

The limit of detection (x_D) of the method is the value x calculated from the smallest y value that can be detected with reasonable certainty. In practice, this is assumed to be the minimum y value that exceeds the limit y_L obtainable from:

$$y_L = \bar{y}_B + k.s_B$$

where: $\bar{y}_B$ is the arithmetic mean of the blank measures, s_B is the standard deviation of the blank measures and k is a numerical value chosen on the basis of the desired probability level. For k a value of 3 is proposed, which corresponds to a probability of about 0.1% that a measured y value higher than y_L, belongs to the set of blank measures. Then, the limit of detection results:

$$x_D = \frac{y_L - \bar{y}_B}{S} = \frac{3.s_B}{S}$$

where: S is the sensitivity of the method, which is assumed constant in the range considered. If no field blank measures can be obtained, the limit of detection is based on the amplitude of the blank noise (peak-to-peak) measured on the base line close to the analyte peak.

The limit of quantitation (x_Q) of the method is the value x calculated from the smallest y value that can be quantitatively determined with a reasonable certainty. Analogously to the procedure described for x_D, this is assumed to be the minimum y value that exceeds the limit y_L defined above. In this case for k a value of 5 is proposed, which corresponds to a probability of about 1 out of 1 million times that a measured y value, higher than y_L, belongs to the set of blank measures. It is worthwhile to mention that the limit of quantitation does not refer to the assay of the active principles but only to that of the related substances and/or impurities.

3. VALIDATION OF AN ASSAY METHOD (Ref. 4)

Analysis of the analytical function

After having decided to consider only the analytical methods having linear and proportional responses, the determination of the analytical function consists in evaluating the intercept a and the slope b (angular coefficient) of the function y = a+b.x by regression analysis.

For this purpose, k groups of samples ($k \geq 3$) having the same number (n)● are prepared. Within the i^{th} group (i = 1, 2, ... k) the samples must have the

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● The same number of samples within each k group is not a necessary condition, but it is proposed here to simplify the successive calculations. This choice is based on the reasonable hypothesis that the precision is the same in the range of the considered concentrations.

same concentration (w_i)* and the prepared groups must include the range of concentrations usually measured. By assaying the single samples, the corresponding values y_{ij} $(j = 1, 2, \ldots, n)$ of the analytical feature under examination are obtained together with the valuation of the intercept (a) and of the slope (b) of the regression straight line y against w, by means of the equations:

$$b = \frac{\sum_{i=1}^{k} \bar{w}_i \bar{y}_i - \bar{\bar{w}}.\sum_{i=1}^{k} \bar{y}_i}{\sum_{i=1}^{k} \bar{w}_i^{2} - k.\bar{\bar{w}}^{2}}$$

$$a = \bar{\bar{y}} - b.\bar{\bar{w}}$$

where:

$$\bar{w}_i = \frac{1}{n}.\sum_{j=1}^{n} w_{ij} = w_i; \qquad \bar{\bar{w}} = \frac{1}{k}.\sum_{i=1}^{k} \bar{w}_i$$

$$\bar{y}_i = \frac{1}{n}.\sum_{j=1}^{n} y_{ij}; \qquad \bar{\bar{y}} = \frac{1}{k}.\sum_{i=1}^{k} \bar{y}_i;$$

Ascertainment of the significance of the regression and linearity of the analytical function

The necessary calculations are shown in Table I.1:

In order to state that the regression of y against w is statistically significant, the value of the ratio between the variance due to the linear regression and the residual variance must exceed the value of the F test with 1 degree of freedom of the numerator and k(n-1) degree of freedom of the denominator at a 95% probability level (see Table in Appendix 2). In order to state that the analytical function is linear, the ratio between the variance due to the curvature and the residual variance must not exceed the value of

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* In this case w is used instead of x because the concentration of the prepared samples is taken to be the true one.

Table I.1

Error source	Degree of freedom	Deviance	Variance	Ratio F
Total	nk-1	$A = \sum_{i=1}^{k}\sum_{j=1}^{n} y_{ij}^{2} - n.k.\bar{\bar{y}}^{2}$	-	-
Residual	k(n-1)	$B = \sum_{i=1}^{k}\left(\sum_{j=1}^{n} y_{ij}^{2} - n\bar{y}_{i}^{2}\right)$	$\frac{B}{k(n-1)}$	-
Linear regression	1	$C = b.n \sum_{i=1}^{k} w_{i}\bar{y}_{i} - \bar{\bar{w}} \sum_{i=1}^{k} \bar{y}_{i}$	C	$\frac{C}{B} \cdot k(n-1)$
Curvature	k-2	D = A-B-C	$\frac{D}{k-2}$	$\frac{D}{B} \cdot \frac{k(n-1)}{k-2}$

the F test with k-2 degree of freedom of the numerator and k(n-1) degree of freedom of the denominator at a 95% probability level.

Ascertainment of the proportionality of the analytical function

In practice, this ascertainment consists in verifying that the analytical response observed or extrapolated for an analyte concentration equal to zero, is also equal to zero or differs in the quantity ascribable to the random error.

To ascertain that the intercept is not significantly different from zero, it is sufficient to verify that the zero is included in the range $a - t.s_{a}$ and $a + t.s_{a}$, where t is the value of the Student's t statistics corresponding to the probability of 0.95 (two tails test) and to k(n-1) degree of freedom (see Table in Appendix 1), and s_{a} is the standard error of the intercept, which can be calculated by the equation:

$$s_a = \sqrt{\left[\frac{1}{nk} + \frac{\bar{\bar{w}}^2}{n.\sum_{i=1}^{k} w_i^2 - k\bar{\bar{w}}^2}\right] \cdot \frac{B}{k(n-1)}}$$

where B is the residual deviance already calculated.

Determination of the analytical function

Once the significance, the linearity, and the proportionality of the analytical function have been ascertained, the analytical method can be considered valid and then the titer x of the analyte in the sample under examination can be calculated from the measured y value by the equation:

$$x = \frac{y}{b'}$$

where: $b' = \bar{\bar{y}}/\bar{\bar{w}}$ is the slope of the analytical function passing through the origin.

If this is not the case, the analytical method must be reexamined.

Evaluation of the precision and accuracy of the analytical method

In order to obtain the precision and the accuracy of the analytical method the values x_{ij} have to be calculated from the values y_{ij} by the equation:

$$x_{ij} = y_{ij} \cdot \frac{\bar{\bar{w}}}{\bar{\bar{y}}}$$

and then:

$$s_i = \sqrt{\frac{\sum_{j=1}^{n} x_{ij}^2 - \frac{\left(\sum_{j=1}^{n} x_{ij}\right)^2}{n}}{n-1}}$$

$$cv_i\% = \frac{s_i}{\bar{x}_i} \cdot 100$$

$$b_i = \bar{x}_i - w_i$$

$$b_i\% = \frac{d_i}{w_i} \cdot 100$$

where: $\bar{x}_i = \frac{1}{n} \sum_{j=1}^{n} x_{ij}$

In this way, k evaluations of the precision and accuracy of the analytical method are obtained individually for each group of samples.

4. STATISTICAL CRITERIA FOR JUDGING THE ADEQUACY OF PHARMACOPOEIAL DRUG SUBSTANCES AND DRUG PRODUCTS (Ref. 3,5)

The aim of Pharmacopoeia specifications is to judge from the laboratory assays if a given drug substance or product complies with the official requirements which is the reference point for the producer control laboratory as well as for that of the Agency responsible for the user's safety. There are two ways in the Pharmacopoeia to indicate the specifications: a range (i.e., an interval with both a lower, w_L, and an upper limit, w_U) or a single limit (lower limit w_L or upper limit w_U).

However, two important cases referring to the control analytical laboratory have to be considered that generate the statistical methodology to be adopted: 1) when the precision of the analytical method is known, that is predetermined; 2) when it is not known. In the case it is judged convenient to use an analytical method with known precision, this has to be predetermined with a number n of determinations on a reference substance by the method. A criterion for chosing between known precision or unknown precision can be based on the following two considerations: the method with known precision is convenient for routine analyses because, since the precision is predetermined, the many routine samples can be determined by a few or even a single analysis (this should be the usual case for the producer). The method with unknown precision is convenient for occasional analyses because it is necessary to evaluate the precision only in that given occasion (this should be the usual case for the controller who takes care of the user's safety).

KNOWN PRECISION

INTERVAL

In this case one batch of substance having a titer lower than the Pharmacopoeia limit w_L or higher than the Pharmacopoeia limit w_U is considered unacceptable. This corresponds to taking as optimum the titer w_O that is distant from both limits by the same value Δ :

$$\Delta = w_U - w_O = w_O - w_L$$

The true titer w of the batch under examination is unknown, so that the acceptance or refusal of the batch has to be based on the evaluation of w that can be obtained as the mean ($\bar{x}$) of the number n of determinations carried out with the prescribed analytical method.

The interest of the producer is that a batch having the best titer w_O always be accepted. In other words, the producer should be protected at level 1 (i.e., 100%) from the risk of refusal of the batch with the titer w_O (risk of the producer). This is impossible, as this requires an analytical method without errors. Then, the producer can only expect the likely certainty that the batch having the titer w_O be accepted. The control has to guarantee a protection (1−α), where α is the risk of the producer, reasonably near to 1, e.g., by the producer's choice of 0.90, 0.95 or 0.99. The Pharmacopoeia will consider as adequate a protection of 0.95 (See footnote at page 8).

On the other hand, it is to the interest of the user that a batch having a titer w lower than w_L or higher than w_U always be refused. In other words, the control should have a potency of 1 (i.e., 100%) in detecting a batch with an inadequate titer, so that the user's risk is zero. This also is impossible, and the user can only expect the likely guarantee that a batch having inadequate titer be refused. The control has to guarantee a low probability β (risk of the user), reasonably near to 0. The Pharmacopoeia will consider as adequate a value of β equal to 0.05.

Given a method of known precision and Pharmacopoeia specifications indicating the maximum deviation allowed of the titer from the optimal, the number of determinations to be carried out for guaranteeing the producer a predetermined risk α and the user a predetermined risk β can be calculated by a given methodology. However, in this way the deviation of the true titer from

w_O can bring about, for the same number of determinations, unacceptable risks for the producer or the user.

Such a situation can be overcome with the technique shown below. Suppose that the tollerance interval of the Pharmacopoeia has the lower limit w_L and the upper limit w_U. Then, two new values w'_L and w'_U inside the Pharmacopoeia tolerance interval must be defined. They are such that, if the true value w of the batch's titer is equal to one of the new limits or is inside the interval $w'_L \div w'_U$, the batch is considered good (it complies) and then has to be accepted, while if the value is outside the interval $w'_L \div w'_U$ the batch has to be considered bad (it does not comply) and then has to be rejected. As it is not possible to set up an unmistakable decision criterion based on a finite number of observations (because of the presence of the analytical error which hides the true value w of the characteristics), one has to be satisfied that the acceptance of the complying batches ($w_L \leqslant w \leqslant w_U$) takes place with a probability not lower than $(1-\alpha)$ (precisely equal to $(1-\alpha)$ for $w=w'_L$ or $w=w'_U$), while the probability that an uncomplying batch ($w < w_L$ or $w > w_U$), is accepted should not be higher than β (precisely equal to β for $w=w_L$ or $w=w_U$). α and β are the risk of the producer and of the user, respectively, established a priori ($\alpha = \beta = 0.05$, see Note page 8).

In practice the parameters necessary for evaluating the acceptance of a given batch, i.e., the operative limits L and U and the number of replications r to be carried out, are given by the following formulae:

$$L = \frac{w_L \cdot t_\alpha + w'_L \cdot t_\beta}{t_\alpha + t_\beta} \; ; \qquad U = \frac{w_U \cdot t_\alpha + w'_U \cdot t_\beta}{t_\alpha + t_\beta}$$

$$r = \frac{1}{\left[\dfrac{w'_L - w_L}{s\,(t_\alpha + t_\beta)}\right]^2 - \dfrac{1}{n}} = \frac{1}{\left[\dfrac{w_U - w'_U}{s\,(t_\alpha + t_\beta)}\right]^2 - \dfrac{1}{n}}$$

where s is the precision of the method (standard deviation), n is the number of replications carried out on the reference substance from which s has been obtained; the coefficients t_α and t_β can be obtained from the Table in Appendix 1 for the one tail test corresponding to n-1 degree of freedom. The choice of the one tail t value in the case of the interval is due to the fact that the non-compliance of the titer has to be considered from only one of the two limits. Once the three parameters L, U, and r have been obtained, the measurement of the characteristics to be controlled is repeated r times on one representative sample of the batch or the measurement is carried out on representative r samples of the batch.

Let's take $\bar{x}$ as the mean of the r measurements carried out on the sample under examination and corrected for accuracy. The decision criterion consists in accepting the batch if $\bar{x}$ is included in the interval (L ÷ U) and in rejecting the batch if $\bar{x}$ is not. By following this procedure, the risk of rejecting the batches having $w = w'_L$ or $w = w'_U$ is equal to $\alpha + \Delta\alpha$, while the risk of accepting batches having $w = w_L$ or $w = w_U$ is equal to $\beta - \Delta\beta$. The order of magnitude of $\Delta\alpha$ and $\Delta\beta$ can be obtained from Appendix 3 from values corresponding to z_α for α :

$$z_\alpha = \left[\frac{w'_U - U}{s} \right] \sqrt{r}$$

and to z_β, for β:

$$z_\beta = \left[\frac{w_U - U}{s} \right] \sqrt{r}$$

In practice, as the values of α and β are chosen equal, the resulting values $\Delta\alpha$ and $\Delta\beta$ are usually negligible and then the risks that are taken by applying the above procedure correspond to those preestablished.

It is worthwhile specifying that, having preestablished the risks α and β, a better evaluation of the precision of the analytical method, which can be obtained by increasing the number of replications on the reference substance, can lead to a decrease in the number of replications necessary for judging the acceptance of a given batch.

SINGLE LIMIT

Suppose the tolerance interval to be unilateral and having the Pharmacopoeia lower limit w_L. In this case, one has to define a value w'_L in such a way that if the value of the batch's titer is equal to or higher than w'_L the batch is considered good (it complies) and then it has to be accepted, while if this value is lower than w'_L the batch is considered bad (it does not comply) and then it has to be rejected. As it is not possible to set up an unmistakable decision criterion based on a finite number of observations (because of the presence of the analytical error which hides the true value w of the titer), one has to be satisfied that the acceptance of the complying batches ($w \geq w_L$) takes place with a probability not lower than ($1-\alpha$) (precisely equal to ($1-\alpha$) for $w=w'_L$, while the probability that a non complying batch ($w < w_L$) is accepted should not be higher than ß (precisely equal to ß for $w=w_L$); α and 1-ß are the risks of the producer and of the user, respectively, established a priori ($\alpha = ß = 0.05$, see Note page 8).

Analogously to the procedure described for the interval, the operative limit L and the number of replications r to be carried out can be calculated by the following formulae:

$$L = \frac{w_L \cdot t_\alpha + w'_L \cdot t_ß}{t_\alpha + t_ß}$$

$$r = \frac{1}{\left[\dfrac{w'_L - w_L}{s\ (t_\alpha + t_ß)}\right]^2 - \dfrac{1}{n}}$$

where s is the precision of the method (standard deviation), n is the number of measurements carried out on the reference substance from which s has been obtained and the coefficient t_α and $t_ß$ can be obtained from the Table in Appendix 1 for the one tail test corresponding to n-1 degree of freedom.

Once the two parameters L and r have been obtained, the measurement of the characteristics to be controlled is repeated r times on a representative sample of the batch or the measurement is carried out on representative r samples of the batch.

Let's take $\bar{x}$ as the mean of the r measurements carried out on the sample under examination and corrected for accuracy. The decision criterion consists in accepting the batch if $\bar{x} \geq L$ and in rejecting the batch if this is not the case. By following this procedure, the risk of rejecting the batches having the true titer $w = w'_L$ practically corresponds to the preestablished risk α, while the risk of accepting batches having $w = w_L$ practically corresponds to the preestablished risk ß.

If the tolerance interval is unilateral and there is an upper limit w_U, the parameters are:

$$U = \frac{w_U \cdot t_\alpha + w'_U \cdot t_\beta}{t_\alpha + t_\beta}$$

$$r = \frac{1}{\left[\frac{w_U - w'_U}{s\,(t_\alpha + t_\beta)}\right]^2 - \frac{1}{n}}$$

The decision criterion consists, in this case, in accepting the batch if $\bar{x} \leqslant U$ and in rejecting it if this is not the case.

UNKNOWN PRECISION

If the precision of the method is not known, the variability of the analytical response can be obtained from the n determinations carried out on the sample under examination using the confidence interval of the mean $\bar{x}$. As for the accuracy, if the procedure used considers the use of a reference chemical substance, there is no problem. In the case where the analytical procedure does refer to a specific value prescribed by the Pharmacopoeia, the behavior described under unknown precision has to take into consideration possible discrepances due to accuracy error. Consequently, in using this methodology it may be opportune, in case of controversies, to check the accuracy of the analysis.

INTERVAL

The chosen criterion is based on the comparison between the confidence limits of the mean of n replications and the specifications of the Pharmacopoeia.

Case of the producer

The producer approach to the problem should be that of having the certainty (see Note at page 8) to judge as good only those batches that are certainly good. In practice, it is established that the substance under examination corresponds to the Pharmacopoeia specifications when the confidence limits of the mean obtained by n determinations, at a 95% probability level, are inside the interval defined by the Pharmacopoeia limits, i.e., when:

$$w_L \leqslant \bar{x} - \frac{t.s}{\sqrt{n}} \quad \text{and} \quad \bar{x} + \frac{t.s}{\sqrt{n}} \leqslant w_U$$

The confidence limits of the mean are calculated utilizing the value of the Student's t statistics for the two tail test (see Table in Appendix 1).

In such a way the producer will be certain of the judgement applied to the adequacy of a given batch, but the probability still remains that good batches can be discarded. In any case, the certainty of the judgement is a function of the precision and of the number of determinations and it is up to the producer to choose the most convenient approach.

Case of the controller

The controller approach to the problem should be that of having the certainty (see Note at page 8) to judge as bad only those batches that are certainly bad. In practice, it is established that the substance under examination does not correspond to the Pharmacopoeia specifications when the upper confidence limit of the mean obtained by n determinations is lower than the lower limit of the interval defined by the Pharmacopoeia limits or when the lower confidence limit of the said mean is higher than the upper limit of the interval defined by the Pharmacopoeia limits, i.e., when:

$$\bar{x} + \frac{t.s}{\sqrt{n}} < w_L \quad \text{or} \quad \bar{x} - \frac{t.s}{\sqrt{n}} > w_U$$

The confidence limits of the mean are calculated utilizing the value of the Student's t statistics for the one tail test (see Table in Appendix 1).

In such a way the controller can be certain of the judgement given about the inadequacy of a given batch.

SINGLE LIMIT

The various considerations made on the approach of the producer and of the controller to the problem concerning the interval are obviously valid, also for the single limit and they are not repeated here.

Case of the producer

In this case the chosen criterion is based on the comparison between the lower confidence limit of the mean and the Pharmacopoeia limit, if this latter has to be reached or exceeded, or on the comparison between the upper confidence limit of the mean and the Pharmacopoeia limit, if this latter should not be exceeded. In practice, it is established that the substance under examination corresponds to the Pharmacopoeia specifications when:

$$\bar{x} - \frac{t.s}{\sqrt{n}} \geqslant w_L \qquad \text{or} \qquad \bar{x} + \frac{t.s}{\sqrt{n}} \leqslant w_U$$

The confidence limits of the mean can be calculated by utilizing the value of the Student's t statistics for the one tail test (see Table in Appendix 1).

Case of the controller

In this case the chosen criterion is based on the comparison between the upper confidence limit of the mean and the Pharmacopoeia limit, if this latter has to be reached or exceeded, or on the comparison between the lower confidence limit of the mean and the Pharmacopoeia limit, if this latter should not be exceeded. In practice, it is established that the substance under examination doe not correspond to the Pharmacopoeia specifications when:

$$\bar{x} + \frac{t.s}{\sqrt{n}} < w_L \qquad \text{or} \qquad \bar{x} - \frac{t.s}{\sqrt{n}} > w_U$$

The confidence limits of the mean can be calculated by utilizing the value of the Student's t statistics for the one tail test (see Table in Appendix 1).

PART II

STATISTICAL EVALUATION OF THE RESULTS OBTAINED IN THE DETERMINATION OF CHARACTERISTICS WHEN THE DRUG PRODUCT IS NOT HOMOGENEOUS (SAMPLING PLANS)

1. INTRODUCTION

This part of the paper deals with sampling plans useful for judging the quality of a batch of a drug product formed by a large number of single units. Practically, it is addressed to: 1) the Authors of the Pharmacopoeia as a guide to define the official sampling plans in order to state the "acceptable defects" of a given batch of a drug product, and 2) the Users of the Pharmacopoeia as a guide for choosing appropriate sampling plans in order to define the risk of making available to the market batches of drug products which would not comply on the basis of the official sampling plan.

2. GENERAL CONCEPTS (Ref. 5)

Suppose a batch formed by N units. By reference to a given characteristic, these unit can be classified as complying or uncomplying units. When the characteristics under examination can be measured (weight, diameter, concentration, etc.) the unit is considered as complying when the value of that characteristic lies within the tolerance interval, be this unilateral, i.e., limited for the lower or the upper part, or bilateral, i.e., limited both for the lower and the upper part. If this is not the case, the unit is considered as uncomplying. When the characteristic under examination cannot be measured, the classification of the complying or uncomplying units is based on considerations of qualitative nature. In both cases, the ratio p of the uncomplying units over the total ones is called the quality level of the batch and this represents, for the characteristic under examination, the parameter for deciding between complying and uncomplying batches.

To ascertain whether a batch is good or bad, the quality p of the batch has to be determined, i.e., all the N units should be analyzed. As this is obviously impossible, only a sample formed by a limited number of n units, randomly taken from the batch, is actually analyzed, and on the basis of appropriate criteria, it is decided whether to accept or to reject this batch. By this procedure based on a limited sampling, possible wrong evaluations can obviously be introduced, which, as a consequence can make bad quality batches be accepted or good quality batches be rejected.

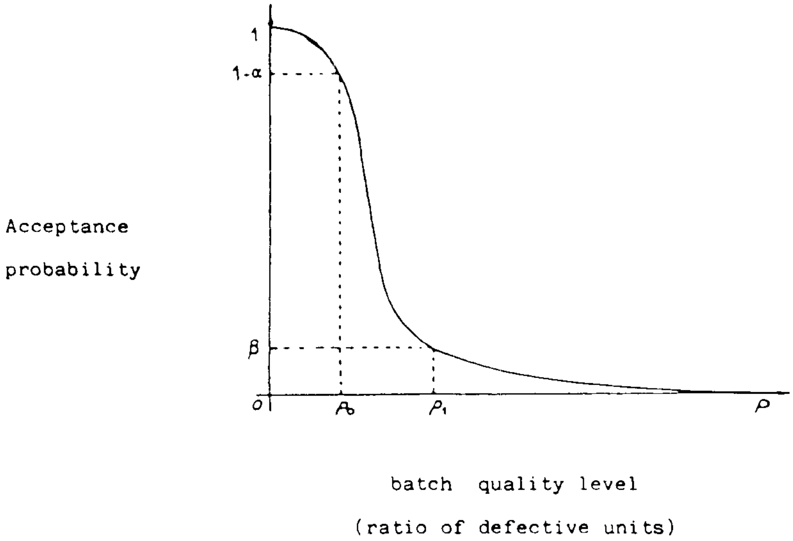
In order to minimize the damage resulting from possible wrong decisions, the following parameters: p_o, p_1, α and β are defined. The first two, p_o and p_1 ($p_o < p_1$), correspond to quality levels, so that, if $p \leqslant p_o$, the quality of the batch has to be considered good and the batch has to be accepted, if $p \geqslant p_1$, the quality of the batch has to be considered bad and the batch has to be rejected. If, finally, $p_o < p < p_1$, the quality of the batch has to be considered neither good nor bad and consequently a decision in this case results indifferent.

The number of the n units to be sampled is chosen in such a way that both the probability to reject good quality batches ($p \leqslant p_o$) and the probability to accept bad quality batches ($p \geqslant p_1$) be less or equal to the pre-established values (calculated risks). This two probabilities are referred to as α and β, respectively. More precisely, is the probability that a good quality batch ($p = p_o$) is erroneously rejected and then it represents the risk of the producer, while β is the probability that a bad quality batch ($p = p_1$) is erroneously accepted and then it represents the risk of the user.

If the estimate of the ratio of the uncomplying units in the batch is made by dividing the number d of the uncomplying units by the number n of the units of the sample, the procedure is called sampling by attributes. On the contrary, if the estimate of the ratio of the uncomplying units in the batch is made by measuring the characteristics of the n units, the procedure is called sampling by variables. The sampling by attributes can be done in every case, i.e., irrespective whether the characteristic under examination is measurable or not, while the sampling by variables can be done only if the characteristic can be measured and its measurement is normally distributed. If these two conditions are valid, the sampling by variables results more efficient than the sampling by attributes, because it decreases the size n of the sample, leaving the four parameters p_o, p_1, α and β unchanged.

Once the four parameters p_o, p_1, α and β have been determined, a sampling plan can be defined in order to fix the number n of the units of the sample to be taken and the critical number a (for the plan by attributes) or the critical ratio f% (for the plan by variables) of the defective units, so as to guarantee that the risks of the producer and of the user are actually equal to the pre-established ones, i.e., equal to α and β, respectively. After the sampling plan, i.e., the values of n and a or f% have been defined, the n units are randomly taken from the batch. The ratio of the defective units of the batch is then estimated according to the results obtained from the analysis on these n units. If this ratio is lower than the critical one (a or f%) given by the sampling plan, the batch has to be accepted. If this is not the case, the batch has to be rejected.

It is noteworthy that, if p is the true quality level (unknown) of a given batch, the probability that the batch be accepted is given by the ordinate of the operative characteristic curve (OCC) (see the graph below) in correspondence of the quality level p of the abscissa.



As can be seen, the user's risk, which corresponds to accepting batches with a quality $p = p_1$ or $p > p_1$, is β or less than β, respectively. The producer's risk, which corresponds to rejecting batches with a quality $p = p_o$ or $p < p_o$, is α or less than α, respectively.

3. STATISTICAL CRITERIA FOR JUDGING THE ADEQUACY OF DRUG PRODUCTS

Acceptable defectiveness and acceptance probability

As a general criterion, the Pharmacopoeia allows that a certain percentage of units do not comply in respect to a given characteristic. This condition is referred to as acceptable defectiveness and is indicated in the official specifications. The sampling plans that the producer has to take into consideration for the on-line and final checks, must be such that batches having the percentage of defective units equal to the acceptable defectiveness be accepted with a probability of 0.05 — in other words, defective batches certainly (see Note page 8) will not be put on the market. So that, sampling plans are suggested which satisfy this certainty. However, the producer is free to use alternative sampling plans provided they guarantee a β value not greater than 0.05.

It is worthwhile to point out that an acceptable defectiveness equal to zero is very difficult or even impossible to be achieved in pharmaceutical production. Therefore, it does not appear possible to suggest a sampling plan when this condition is officially required. In this case, the producer cannot overlook putting in practice technological devices that will guarantee the above condition of absolute absence of defectiveness.

Sampling plans by attributes and by variables

Some characteristics (e.g., sterility, pyrogens, etc.) are expressed as an attribute (complying, uncomplying), while others (e.g., weight, titer, etc.) are expressed as variable (numerical value). In this second case it is possible to apply the sampling plan by variables, which allows the decrease of the number of the units to be sampled, leaving the chosen risks equal. In any case, it is up to the producer and to the user to choose the sampling plan by attributes or by variables for checking the characteristics under examination. We can mention here that this latter plan can be applied only if the characteristic under examination is normally distributed.

The various Pharmacopoeia Monographs express the acceptable defectiveness by means of official sampling plans only by attributes, as reported in Table II.1.

Table II.1

Sampling plan	Acceptable defectiveness	Official sampling plans for the user			
		by attributes		by variables	
		n	a	n	f%
A	zero	20	0	10	2.75
B	5.0	20	1	10	7.9
C	10.0	20	2	10	13.3
D	15.0	20	3	10	18.9
E	20.0	20	4	10	24.1

Table II.2

Sampling plan	Acceptable defectiveness	Official sampling plans for the producer			
		by attributes		by variables	
		n	a	n	%
A	zero	(not applicable)			
B	5.0	150	0	28	0.32
C	10.0	75	0	20	0.63
D	15.0	45	0	16	1.08
E	20.0	30	0	13	1.71

By inspecting Table II.1 it results that also the sampling plans by variables corresponding to the official ones by attributes are reported. The purpose is to outline the opportunness that the Pharmacopoeia Monographs refer to the acceptable defectiveness only and not to a certain sampling plan.

The following plans for the on-line and final checks are suggested by referring to the acceptable defectiveness reported in Table II.1.

These sampling plans guarantee a certainty (see Note page 8) of conformity to the official sampling plans (see Table II.1). Alternative sampling plans are reported in Table II.3, which guarantee a lower conformity to the official sampling plans, e.g., batches having an acceptance probability equal to 0.05 according to the plans reported in Table II.3 have an acceptance probability equal to 0.5 according to the official plans of Table II.1.

Table II.3

Sampling plan	Acceptable defectiveness	Official sampling plans for the producer			
		by attributes		by variables	
		n	a	n	%
A	zero	(not applicable)			
B	5.0	60	0	19	0.87
C	10.0	30	0	13	1.75
D	15.0	20	0	10	2.75
E	20.0	15	0	8	3.78

Table III.1

GROUP	w (mg)	y (absorbance)	GROUP	w (mg)	y (absorbance)	GROUP	w (mg)	y (absorbance)
1	80.0	390	2	100.0	483	3	120.0	580
	80.0	391		100.0	500		120.0	582
	80.0	384		100.0	481		120.0	584
	80.0	391		100.0	481		120.0	578
	80.0	386		100.0	478		120.0	585
	80.0	384		100.0	484		120.0	578
	80.0	376		100.0	487		120.0	576
	80.0	388		100.0	485		120.0	570
	80.0	384		100.0	479		120.0	590
	80.0	386		100.0	495		120.0	580

PART III

EXAMPLES AND APPLICATIONS

1. VALIDATION OF AN ASSAY METHOD

The validation of a spectrophotometric method for the titration of a drug is reported as an example*. By using an analytical reference substance of the

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* The data of this example were taken from: I. Setnikar, Boll.Chim.Farm., 124, 403 (1985).

drug, 3 groups, each containing 10 samples, were prepared, where all the samples of a group had the same content of drug. After each sample was properly dissolved, the absorbance of each obtained solution was determined at the proper wavelength. The obtained values are reported in Table III.1:

It is worthwhile to mention that in all the following calculations it was necessary to use a suitable number of decimals.

Analysis of the analytical function

$n = 10;\quad k = 3;\quad \bar{w}_1 = 80.0;\quad \bar{w}_2 = 100.0;\quad \bar{w}_3 = 120.0$

$$\bar{y}_1 = \frac{1}{10}\ (390+391+\ldots\ldots.+386) = 386.0$$

$$\bar{y}_2 = \frac{1}{10}\ (483+500+\ldots\ldots.+495) = 485.3$$

$$\bar{y}_3 = \frac{1}{10}\ (580+582+\ldots\ldots.+580) = 580.3$$

$$\bar{\bar{w}} = \frac{1}{3}\ (80+100+120) = 100.0$$

$$\bar{\bar{y}} = \frac{1}{3}\ (386.0+485.3+580.3) = 483.86666$$

$$b = \frac{80.0\text{x}386.0+100.0\text{x}485.3+120.0\text{x}580.3 - 100.0\text{x}(386.0+485.3+580.3)}{80.0^2+100.0^2+120.0^2 - 3\text{x}100.0^2} = 4.8575$$

$$a = 483.87 - 4.8575\text{x}100.0 = -1.88$$

Ascertainment of the significance of the regression and the linearity

$nk-1 = 10 \times 3 - 1 = 29$

$k(n-1) = 3\ (10-1) = 27$

$k-2 = 3 - 2 = 1$

$$A = 390^2+391^2+\ldots.580^2 - 10\text{x}3\text{x}483.86666^2 = 7213502-7023808 = 189694$$

$$B = (390^2+391^2+\ldots.386^2 - 10\text{x}386.0^2)+(483^2+500^2+\ldots.495^2 - 10\text{x}485.3^2) +$$
$$+ (580^2+582^2+\ldots.+580^2 - 10\text{x}580.3^2) = 182+450+268 = 900$$

$$C = 4.8575 \times 10 [80.0 \times 386.0 + 100.0 \times 485.3 + 120.0 \times 580.3 - 100 \times (386.0 + 485.3 + 580.3)] =$$

$$= 4.8575 \times 10 \times [149046 - 100 \times 1451.6] = 188762$$

$$D = 189693 - 900 - 188762 = 31$$

$$\frac{B}{k(n-1)} = \frac{900}{3(10-1)} = 33.33$$

$$\frac{D}{k-2} = \frac{31}{3-2} = 31$$

$$\frac{C}{B}\, k(n-1) = \frac{188762}{900}\, 3(10-1) = 5663$$

$$\frac{D}{B}\, \frac{k(n-1)}{k-2} = \frac{31}{900}\, \frac{3(10-1)}{3-2} = 0.93$$

Error source	Degree of freedom	Deviance	Variance	F ratio	p
Total	29	189693			
Residual	27	900	33.33		
Linear regression	1	188762	188762	5663	< 0.05
Curvature	1	31	31	0.93	N.S.

As the value of the F test relative to linear regression (5663) exceeds the value reported in the Table of Appendix 2 with 1 degree of freedom of the numerator and 27 degree of freedom of the denominator (4.21) it can be stated that the regression is significant. Moreover, as the value of the F test relative to the curvature (0.93) does not exceed the value reported in the Table of Appendix 2 with 1 degree of freedom of the numerator and 27 degree of freedom of the denominator (4.21) it can be stated that the analytical function is linear.

Ascertainment of the proportionality

$$s_a = \sqrt{\left[\frac{1}{30} + \frac{100.0^2}{10x(80.0^2+100.0^2+120.0^2-3x100.0^2)}\right] 33.33} = 6.54$$

$$a-t.s_a = -1.88-2.052x6.54 = -15.28$$

$$a+t.s_a = -1.88+2.052x6.54 = +11.52$$

As the interval $[-15.28; +11.52]$ includes the zero it can be stated that the intercept of the regression straight line is not significantly different from zero and then the analytical function is proportional.

Determination of the analytical function

$$b' = \frac{483.866}{100.0} = 4.83866$$

Evaluation of the precision and the accuracy

$$\frac{\bar{\bar{w}}}{\bar{\bar{y}}} = \frac{100.0}{483.866} = 0.2066$$

GROUP 1

$x_{1,1} = 390x0.2066 = 80.6$; $x_{1,2} = 391x0.2066 = 80.8$; $x_{1,3} = 384x0.2066 = 79.4$

$x_{1,4} = 391x0.2066 = 80.8$; $x_{1,5} = 386x0.2066 = 79.8$; $x_{1,6} = 384x0.2066 = 79.4$

$x_{1,7} = 376x0.2066 = 77.7$; $x_{1,8} = 388x0.2066 = 80.2$; $x_{1,9} = 384x0.2066 = 79.4$

$x_{1,10} = 386x0.2066 = 79.8$

$$\bar{x}_1 = (80.6+80.8+....+79.8)\,\frac{1}{10} = 79.77$$

$$s_1 = \sqrt{\frac{(80.6^2+80.8^2+....+79.8^2) - \dfrac{(80.6+80.8+....+79.8)^2}{10}}{10-1}} =$$

$$= \sqrt{\frac{63672.13-63664.44}{9}} = 0.924$$

$$cv_1\% = \frac{0.924}{79.77}\ 100 = 1.16\%$$

$$d_1 = 79.77-80.0 = -0.23$$

$$d_1\% = \frac{-0.23}{80.0}\ 100 = -0.29\%$$

GROUP 2

$x_{2,1} = 483x0.2066 = 99.8$; $x_{2,2} = 500x0.2066\ \ 103.3$; $x_{2,3} = 481x0.2066 = 99.4$

$x_{2,4} = 481x0.2066 = 99.4$; $x_{2,5} = 478x0.2066 = 98.8$; $x_{2,6} = 484x0.2066 = 100.0$

$x_{2,7} = 487x0.2066 = 100.6$; $x_{2,8} = 485x0.2066 = 100.2$; $x_{2,9} = 479x0.2066 = 99.0$

$x_{2,10} = 495x0.2066 = 102.3$

$$\bar{x}_2 = (99.8+103.3+....+102.3).\frac{1}{10} = 100.28$$

$$s_2 = \sqrt{\frac{(99.8^2+103.3^2+....+102.3^2) - \dfrac{(99.8+103.3+....+102.3)^2}{10}}{10-1}} =$$

$$= \sqrt{\frac{100579.78-100560.784}{9}} = 1.453$$

$$cv_2\% = \frac{1.453}{100.28}\ 100 = 1.45\%$$

$$d_2 = 100.28-100.0 = 0.28$$

$$d_2\% = \frac{0.28}{100.0}\ 100 = 0.28\%$$

GROUP 3

$x_{3,1} = 580x0.2066 = 119.9$; $x_{3,2} = 582x0.2066 = 120.3$; $x_{3,3} = 584x0.2066 = 120.7$

$x_{3,4} = 578x0.2066 = 119.5$; $x_{3,5} = 585x0.2066 = 120.9$; $x_{3,6} = 578x0.2066 = 119.5$

$x_{3,7} = 576x0.2066 = 119.0$; $x_{3,8} = 570x0.2066 = 117.8$; $x_{3,9} = 590x0.2066 = 121.9$

$x_{3,10} = 580x0.2066 = 119.9$

$$\bar{x}_3 = (119.9+120.3+...+119.9)\ \frac{1}{10} = 119.94$$

$$s_3 = \sqrt{\frac{(119.9^2+120.3^2+\ldots.+119.9^2) - \dfrac{(119.9+120.3+\ldots.+119.9)^2}{10}}{10-1}} =$$

$$= \sqrt{\frac{143867.36-143856.086}{9}} = 1.120$$

$$cv_3\% = \frac{1.120}{119.94}\ 100 = 0.93\%$$

$$d_3 = 119.94-120.0 = -0.06$$

$$d_3\% = \frac{-0.06}{120.0}\ 100 = -0.05\%$$

2. CRITERIA FOR JUDGING THE ADEQUACY OF PHARMACOPOEIAL SUBSTANCES AND PRODUCTS

KNOWN PRECISION

INTERVAL

Suppose that the titer of a batch of trimethoprim has to be checked. The Pharmacopoeia limits are:

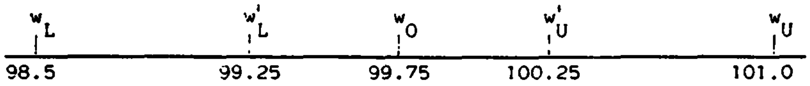
$$w_L = 98.5\% \quad \text{and} \quad w_U = 101\%$$

The analytical methodology used to determine the titer has an error that was estimated by n = 10 determinations on a reference sample with the titer w = 99.75%. The results were: 99.3; 99.7; 100.1; 99.9; 100.1; 100.1; 99.6; 100.1; 99.6; 99.3. The mean of these 10 measurements resulted: $\bar{x}$ = 99.78% and the standard deviation:

$$s = \sqrt{\frac{\sum_{i=1}^{10}(x_i - \bar{x})^2}{9}} = 0.326\%$$

Then, the evaluation of the accuracy is given by: b = 99.78% - 99.75% = 0.03% and the evaluation of the precision by: s = 0.326%.

Now, suppose the two internal limits were chosen as w'_L = 99.25% and w'_U = 100.25%, according to the following configuration:



Having chosen $\alpha = \beta = 0.05$ and considering that the degrees of freedom are:

$$n - 1 = 10 - 1 = 9$$

the parameters L, U and r are given by:

$$L = \frac{w_L \cdot t_\alpha + w'_L \cdot t_\beta}{t_\alpha + t_\beta} = \frac{98.5 \times 1.833 + 99.25 \times 1.833}{1.833 + 1.833} = 98.875$$

$$U = \frac{w_U \cdot t_\alpha + w'_U \cdot t_\beta}{t_\alpha \bullet t_\beta} = \frac{101 \times 1.833 + 100.25 \times 1.833}{1.833 + 1.833} = 100.625$$

$$r = \frac{1}{\left[\dfrac{w_U - w'_U}{s\,(t_\alpha + t_\beta)}\right]^2 - \dfrac{1}{n}} = \frac{1}{\left[\dfrac{101 - 100.25}{0.326(1.833+1.833)}\right]^2 - \dfrac{1}{10}} = 3.4$$

For guaranteeing the chosen limits four determinitions (approximation by excess) on the batch under examination are then necessary. If the mean of these four determinations (corrected for the accuracy error, which is equal to 0.03%) is included whithin the limits 98.875% and 100.625%, the batch has to be judged as complying. If this is not the case, the batch has to be judged as uncomplying.

When the r value is not compatible with the capability of the analytical laboratory, the w'_L, w'_U, α and β values have to be modified, or the n number of the measurements to be carried out on the reference for obtaining s has to be increased. Suppose that 20 measurements were obtained on the reference as follows: 99.3; 99.7; 100.1; 99.9; 100.2; 100.1; 99.6; 100.1; 99.6; 99.3; 99.7; 99.3; 100.1; 99.9; 100.1; 99.6; 100.1; 99.5; 100.1; 99.3.

$$\bar{x} = 99.78\% \qquad s = 0.327\%$$

$$L = \frac{w_L \cdot t_\alpha + w'_L \cdot t_\beta}{t_\alpha + t_\beta} = \frac{98.5 \times 1.729 + 99.25 \times 1.729}{1.729 + 1.729} = 98.875$$

$$U = \frac{w_U \cdot t_\alpha + w'_U \cdot t_\beta}{t_\alpha + t_\beta} = \frac{101 \times 1.729 + 100.25 \times 1.729}{1.729 + 1.729} = 100.625$$

$$r = \frac{1}{\left[\dfrac{w_U - w'_U}{s\,(t_\alpha + t_\beta)}\right]^2 - \dfrac{1}{n}} = \frac{1}{\left[\dfrac{101 - 100.25}{0.327(1.729+1.729)}\right]^2 - \dfrac{1}{20}} = 2.6$$

For guaranteeing the chosen limits, three determinations (approximation by excess) on the batch under examination are then necessary. If the mean of these three determinations (corrected for the accuracy error, which is equal to 0.03%) is included within the limits 98.875% and 100.625%, the batch has to be judged as complying. If this is not the case, the batch has to be judged as uncomplying.

SINGLE LIMIT

Suppose that the titer of a batch of cefaloridine has to be checked. The Pharmacopoeia specifications state that the titer must not be lower than w_L = 95%.

The error of the prescribed analytical methodology was evaluated by n = 10 measurements on a reference with the titer 99%.

98.7; 98.0; 98.9; 98.6; 99.1; 98.1; 98.3; 99.2; 98.0; 98.9.

The mean results: x = 98.58%, and the standard deviation:

$$s = \sqrt{\frac{\sum_{i=1}^{10} (x_i - \bar{x})^2}{9}} = 0.45\%$$

The internal limit w'_L was chosen as 97% and the parameters L and r were calculated by the following formulae:

$$L = \frac{w_L \cdot t_\alpha + w'_L \cdot t_\beta}{t_\alpha + t_\beta} = \frac{95\% \times 1.833 + 97\% \times 1.833}{1.833 + 1.833} = 96\%$$

$$r = \frac{1}{\left[\dfrac{w'_L - w_L}{s\,(t_\alpha + t_\beta)}\right]^2 - \dfrac{1}{n}} = \frac{1}{\left[\dfrac{97 - 95}{0.45(1.833+1.833)}\right]^2 - \dfrac{1}{10}} = 0.73$$

For guaranteeing the limit prescribed by the Pharmacopoeia one single determination is sufficient and if the result of this determination, corrected for the accuracy error given by:

$$b = 98.6\% - 99.0\% = -0.6\%$$

is higher than 96%, the batch has to be judged as complying; if this is not the case, the batch has to be rejected.

UNKNOWN PRECISION

INTERVAL

Producer's case

Suppose a producer has to check a batch of trimethoprim; the Pharmacopoeia limits are:

$$w_L = 98.5\% \quad \text{and} \quad w_U = 101\%$$

Five determinations carried out on a sample representative of the batch yielded the following results: 100.3; 99.2; 100.2; 99.3; 99.9

$$\bar{x} = \frac{100.3 + 99.2 + 100.2 + 99.3 + 99.9}{5} = 99.78$$

$$s = \sqrt{\frac{100.3^2+99.2^2+100.2^2+99.3^2+99.9^2 - \dfrac{(100.3+99.2+100.2+99.3+99.9)^2}{5}}{4}} = 0.51$$

Then, the confidence limits of the mean are:

$$99.78 \pm 2.776 \frac{0.51}{\sqrt{5}} \text{ ,i.e., } 99.15 \text{ and } 100.41$$

which are included in the Pharmacopoeia limits and allow the batch to be accepted.

Controller's case

Now, suppose a controller carries out three analyses on a sample representative of the same batch obtaining the following results: 97.5; 97.8; 99.7.

$$\bar{x} = \frac{97.5 + 97.8 + 99.7}{3} = 98.33$$

$$s = \sqrt{\frac{97.5^2+97.8^2+99.7^2 - \dfrac{(97.5+97.8+99.7)^2}{3}}{2}} = 1.19$$

Then, the confidence limits of the mean are:

$$98.33 \pm 2.92 \frac{1.19}{\sqrt{3}} \text{ ,i.e., } 96.32 \text{ and } 100.34$$

Thus, the controller cannot reject the batch because either the lower confidence limit of the mean (96.32) does not exceed the Pharmacopoeia upper limit (101) or even the upper confidence limit of the mean (100.37) is lower than the Pharmacopoeia lower limit (98.5).

LOWER SINGLE LIMIT

The Pharmacopoeia specifications state that the titer of the cefaloridine must not be lower than 95%.

Producer's case

Suppose a producer has to check a batch of cefaloridine. Four determinations carried out on a sample representative of the batch yielded the following results: 98.4; 97.0; 97.1; 99.5

$$\bar{x} = \frac{98.4 + 97.0 + 97.1 + 99.5}{4} = 98.0$$

$$s = \sqrt{\frac{98.4^2 + 97.0^2 + 97.1^2 + 99.5^2 - \dfrac{(98.4+97.0+97.1+99.5)^2}{4}}{3}} = 1.19$$

Then, the confidence limit of the mean is:

$$98.0 - 2.353\ \frac{1.19}{\sqrt{4}} = 96.6$$

which is higher than the Pharmacopoeia limit and allows the batch to be accepted.

Controller's case

Suppose a controller carries out two analyses on a sample representative of the same batch obtaining the following results: 93.0 and 96.5

$$\bar{x} = \frac{93.0 + 96.5}{2} = 94.75$$

$$s = \sqrt{\frac{93.0^2 + 96.5^2 - \dfrac{(93.0 + 96.5)^2}{2}}{1}} = 2.48$$

The upper confidence limit of the mean results:

$$94.75 + 6.314\ \frac{2.48}{\sqrt{2}} = 105.82$$

This limit is higher than the Pharmacopoeia limit and the controller is to accept the batch.

UPPER SINGLE LIMIT

The Pharmacopoeia specifications state that the water content, determined by semi-micro method, of the delta form of cefaloridine must not be higher than 3%.

Producer's case

Suppose a producer has to check the content of water in a batch of cefaloridine. Four determinations carried out on a sample representative of the batch yielded the following results: 1.3; 2.2; 1.6; 2.2

$$\bar{x} = \frac{1.3 + 2.2 + 1.6 + 2.2}{4} = 1.83$$

$$s = \sqrt{\frac{1.3^2 + 2.2^2 + 1.6^2 + 2.2^2 - \dfrac{(1.3 + 2.2 + 1.6 + 2.2)^2}{4}}{3}} = 0.45$$

Then, the upper confidence limit of the mean is:

$$1.83 + 2.353 \frac{0.45}{\sqrt{4}} = 2.36$$

This limit is lower than the Pharmacopoeia limit and the controller is to accept the batch.

Controller's case

Suppose a controller carries out two analyses on a sample representative of the same batch obtaining the following results: 2.5 and 3.7

$$\bar{x} = \frac{2.5 + 3.7}{2} = 3.10$$

$$s = \sqrt{\frac{2.5^2 + 3.7^2 - \dfrac{(2.5 + 3.7)^2}{2}}{1}} = 0.85$$

Then, the lower confidence limit of the mean is:

$$3.10 - 6.314 \frac{0.85}{\sqrt{2}} = -0.695$$

This limit is lower than the Pharmacopoeia limit and the controller will accept the batch.

3. APPLICATION OF SAMPLING PLANS BY VARIABLE

Suppose a producer has to check the weight uniformity of a drug product in tablets with a theoretical weight of 500 mg. The Pharmacopoeia Monograph indicates for this product the following limits: L1 = ± 5% of the mean weight with an acceptable defectiveness equal to ± 10% and L2 = ± 10% of the mean weight with an acceptable defectiveness equal to zero.

The sampling plan by variables suggested for the two limits L1 is plan C in Table II.2, which indicates to sample 20 units and an acceptance criterion equal to 0.63%. The sampling plan by variables suggested for the two limits L2 is plan A in Table II.2, which is not applicable since the required acceptance defectiveness is equal to zero.

The weight measurements of the 20 units randomly sampled from the batch yielded the following results in mg:

487, 488, 491, 494, 496, 496, 498, 498, 499, 500, 501, 501, 503, 503, 504, 507, 507, 508, 510, 514

the corresponding mean and standard deviation are:

$\bar{x} = 500.25$; $s = 7.12$.

The limits L1 (L2) are:

$$L_1\ (L_2) = \bar{x} \pm \frac{k.\bar{x}}{100}$$

where: $\bar{x}$ = sample mean; k = 5 for L1; k = 10 for L2

therefore: $L1_L = 475.24$ and $L1_U = 525.26$

$L2_L = 450.225$ and $L2_U = 550.275$

The values of the standard normal deviate z corresponding to the two limits L1 are calculated:

$$z = \frac{\bar{x} - L1_L}{s} = \frac{L1_U - \bar{x}}{s} = 3.51$$

By means of the Table in Appendix 3 (standard normal deviate) the z value is transformed in the corresponding f value, which multiplied by 100, represents the defectiveness of each L1 limit:

$$f = 0.00023 = 0.023\%.$$

Therefore, the total defectiveness is:

$$f\ (\text{tot.}) = 2f = 0.00023 \times 2 = 0.00046 = 0.05\%.$$

As the estimated total defectiveness (0.05%) is lower than the acceptance criterion of the adopted sampling plan (0.63%) the batch under examination can be considered complying by reference to the limits L1. As none out of the 20 measured values is out of the limits L2 the batch under examinations can be considered complying, also by reference to the limits L2. However, no considerations can be made by reference to the conformity of the batch because no sampling plan can guarantee the absolute absence of the defectiveness.

PART IV

GLOSSARY AND STATISTICAL TABLES

1. GLOSSARY

y = analytical response

x = analyte concentration

$y=f(x)$ = analytical function

w = true value of the analyte concentration

n = number of replications of a measurement

$\bar{x}$ = arithmetic mean of the n values of x (x_i)

s = standard deviation

cv% = relative standard deviation or coefficient of variation

$s_{\bar{x}}$ = standard error of the mean

$x \pm t.s_{\bar{x}}$ = limits of the confidence interval of $\bar{x}$

t = Student's t test (Appendix 1)

b = bias

b% = relative bias

S = sensitivity

x_D = limit of detection

x_Q = limit of quantitation

a = estimate of the intercept of the analytical function

b = estimate of the slope of the analytical function

k = number of groups of samples used for the validation

$\bar{w}$ = arithmetic mean of the w values for the samples of the same group

$\bar{y}$ = arithmetic mean of the y values for the samples of the same group

$\bar{\bar{w}}$ = arithmetic mean of the w values for all the samples used in the validation

$\bar{\bar{y}}$ = arithmetic mean of the y values for all the samples used in the validation

F = ratio of two independent variance estimates (Fisher's F test Appendix 2)

s_a = standard error of the intercept a

b' = estimate of the slope of the analytical function for $a=0$

w_L = Pharmacopoeia lower limit

w_U = Pharmacopoeia upper limit

w_O = optimal titer of a batch

α = producer's risk

β = user's risk

w'_L = lower limit by producer's choice

w'_U = upper limit by producer's choice

L and U = calculated operative limits for judging the acceptance of a batch

r = number of replications for judging the acceptance of a batch

N = total number of units of a batch

n = number of sampled units of a batch

p = quality level of a batch

p_o = acceptable quality level

p_1 = unacceptable quality level

d = number of defective units

a = critical number (sampling plan by attributes)

$f\%$ = critical ratio (sampling plan by variables)

$L1_L$ and $L2_L$ = Pharmacopoeia lower limits for sampling plans by attributes

$L1_U$ and $L2_U$ = Pharmacopoeia upper limits for sampling plans by attributes

z, z_α, z_β = standard normal deviates.

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Appendix 1

Values of the Student's t random variable

Degree of freedom	$t_{0.95}$ 2 tails	1 tail
1	12.706	6.314
2	4.303	2.920
3	3.182	2.353
4	2.776	2.132
5	2.571	2.015
6	2.447	1.943
7	2.365	1.895
8	2.306	1.860
9	2.262	1.833
10	2.228	1.813
11	2.201	1.796
12	2.179	1.782
13	2.160	1.771
14	2.145	1.761
15	2.131	1.753
16	2.120	1.750
17	2.110	1.740
18	2.101	1.734
19	2.093	1.729
20	2.086	1.725
21	2.080	1.721
22	2.074	1.717
23	2.069	1.714
24	2.064	1.711
25	2.060	1.708
26	2.056	1.706
27	2.052	1.703
28	2.048	1.701
29	2.045	1.699
30	2.042	1.697
∞	1.960	1.645

Appendix 2

Values of the Snedecor's F (Fisher) random variable for $1-\alpha = 0.95$

Degrees of freedom of the numerator

Degrees of freedom of the denominator

	1	2	3	4	5	6	7	8	9	10	12	16	20	24	30	40	50	60	75	100	∞
1	161	200	216	225	230	234	237	239	241	242	244	246	248	249	250	251	252	252	253	253	254
2	18·51	19·00	19·16	19·25	19·30	19·33	19·35	19·37	19·38	19·40	19·41	19·43	19·45	19·45	19·46	19·47	19·47	19·48	19·48	19·49	19·50
3	10·13	9·55	9·28	9·12	9·01	8·94	8·89	8·84	8·81	8·78	8·74	8·69	8·66	8·64	8·62	8·59	8·58	8·57	8·57	8·56	8·53
4	7·71	6·94	6·59	6·39	6·26	6·16	6·09	6·04	6·00	5·96	5·91	5·84	5·80	5·77	5·74	5·72	5·70	5·69	5·68	5·66	5·63
5	6·61	5·79	5·41	5·19	5·05	4·95	4·88	4·82	4·77	4·74	4·68	4·60	4·56	4·53	4·50	4·46	4·44	4·43	4·42	4·40	4·36
6	5·99	5·14	4·76	4·53	4·39	4·28	4·21	4·15	4·10	4·00	4·00	3·92	3·87	3·84	3·81	3·77	3·75	3·74	3·72	3·71	3·67
7	5·59	4·74	4·35	4·12	3·97	3·87	3·79	3·73	3·68	3·64	3·57	3·49	3·44	3·41	3·38	3·34	3·32	3·30	3·29	3·28	3·23
8	5·32	4·46	4·07	3·84	3·69	3·58	3·50	3·44	3·39	3·35	3·28	3·20	3·15	3·12	3·08	3·04	3·03	3·00	3·00	2·98	2·93
9	5·12	4·26	3·86	3·63	3·48	3·37	3·29	3·23	3·18	3·14	3·07	2·98	2·94	2·90	2·86	2·82	2·80	2·79	2·77	2·76	2·71
10	4·96	4·10	3·71	3·48	3·33	3·22	3·14	3·07	3·02	2·98	2·91	2·82	2·77	2·74	2·70	2·66	2·64	2·62	2·61	2·59	2·54
11	4·84	3·98	3·59	3·36	3·20	3·09	3·01	2·95	2·90	2·86	2·79	2·70	2·65	2·61	2·57	2·53	2·50	2·49	2·47	2·45	2·40
12	4·75	3·88	3·49	3·26	3·11	3·00	2·91	2·85	2·80	2·75	2·69	2·60	2·54	2·50	2·47	2·42	2·40	2·38	2·36	2·35	2·30
13	4·67	3·80	3·41	3·18	3·02	2·92	2·83	2·77	2·71	2·67	2·60	2·51	2·46	2·42	2·38	2·34	2·32	2·30	2·28	2·26	2·21
14	4·60	3·74	3·34	3·11	2·96	2·85	2·76	2·70	2·65	2·60	2·53	2·44	2·39	2·35	2·31	2·27	2·24	2·22	2·21	2·19	2·13
15	4·54	3·68	3·29	3·06	2·90	2·79	2·71	2·64	2·59	2·54	2·48	2·39	2·33	2·29	2·25	2·20	2·18	2·16	2·15	2·12	2·07
16	4·49	3·63	3·24	3·01	2·85	2·74	2·66	2·59	2·54	2·49	2·42	2·33	2·28	2·24	2·19	2·15	2·13	2·10	2·09	2·07	2·01
17	4·45	3·59	3·20	2·96	2·81	2·70	2·61	2·55	2·49	2·45	2·38	2·29	2·23	2·19	2·15	2·10	2·08	2·06	2·04	2·02	1·96
18	4·41	3·55	3·16	2·93	2·77	2·66	2·58	2·51	2·46	2·41	2·34	2·25	2·19	2·15	2·11	2·06	2·04	2·02	2·00	1·98	1·92
19	4·38	3·52	3·13	2·90	2·74	2·63	2·54	2·48	2·42	2·38	2·31	2·21	2·16	2·11	2·07	2·03	2·00	1·98	1·96	1·94	1·88
20	4·35	3·49	3·10	2·87	2·71	2·60	2·51	2·45	2·39	2·35	2·28	2·18	2·12	2·08	2·04	1·99	1·96	1·95	1·92	1·90	1·84
21	4·32	3·47	3·07	2·84	2·68	2·57	2·49	2·42	2·37	2·32	2·25	2·15	2·10	2·05	2·01	1·96	1·93	1·92	1·89	1·87	1·81
22	4·30	3·44	3·05	2·82	2·66	2·55	2·46	2·40	2·34	2·30	2·23	2·13	2·07	2·03	1·98	1·94	1·91	1·89	1·87	1·84	1·78
23	4·28	3·42	3·03	2·80	2·64	2·53	2·44	2·37	2·32	2·27	2·20	2·10	2·05	2·00	1·96	1·91	1·88	1·86	1·84	1·82	1·76
24	4·26	3·40	3·01	2·78	2·62	2·51	2·42	2·36	2·30	2·25	2·18	2·09	2·03	1·98	1·94	1·89	1·86	1·84	1·82	1·80	1·73
25	4·24	3·38	2·99	2·76	2·60	2·49	2·40	2·34	2·28	2·24	2·16	2·06	2·01	1·96	1·92	1·87	1·84	1·82	1·80	1·77	1·71
26	4·22	3·37	2·98	2·74	2·59	2·47	2·39	2·32	2·27	2·22	2·15	2·05	1·99	1·95	1·90	1·85	1·82	1·80	1·78	1·76	1·69
27	4·21	3·35	2·96	2·73	2·57	2·46	2·37	2·30	2·25	2·20	2·13	2·03	1·97	1·93	1·88	1·84	1·80	1·78	1·76	1·74	1·67
28	4·20	3·34	2·95	2·71	2·56	2·44	2·36	2·29	2·24	2·19	2·12	2·02	1·96	1·91	1·87	1·82	1·78	1·77	1·75	1·72	1·65
29	4·18	3·33	2·93	2·70	2·54	2·43	2·35	2·28	2·22	2·18	2·10	2·00	1·94	1·90	1·85	1·80	1·77	1·75	1·73	1·71	1·64
30	4·17	3·32	2·92	2·69	2·53	2·42	2·33	2·27	2·21	2·16	2·09	1·99	1·93	1·89	1·84	1·79	1·76	1·74	1·72	1·69	1·62
40	4·08	3·23	2·84	2·61	2·45	2·34	2·25	2·18	2·12	2·08	2·00	1·90	1·84	1·79	1·74	1·69	1·66	1·64	1·61	1·59	1·51
50	4·03	3·18	2·79	2·56	2·40	2·29	2·20	2·13	2·07	2·02	1·95	1·85	1·78	1·74	1·69	1·63	1·60	1·58	1·55	1·52	1·44
60	4·00	3·15	2·76	2·52	2·37	2·25	2·17	2·10	2·04	1·99	1·92	1·81	1·75	1·70	1·65	1·59	1·56	1·53	1·50	1·48	1·39
70	3·98	3·13	2·74	2·50	2·35	2·23	2·14	2·07	2·01	1·97	1·89	1·79	1·72	1·67	1·62	1·56	1·53	1·50	1·47	1·45	1·35
80	3·96	3·11	2·72	2·48	2·33	2·21	2·12	2·05	1·99	1·95	1·88	1·77	1·70	1·65	1·60	1·54	1·51	1·48	1·45	1·42	1·32
100	3·94	3·09	2·70	2·46	2·30	2·19	2·10	2·03	1·97	1·92	1·85	1·75	1·68	1·63	1·57	1·51	1·48	1·45	1·42	1·39	1·28
150	3·91	3·06	2·67	2·43	2·27	2·16	2·07	2·00	1·94	1·89	1·82	1·71	1·64	1·59	1·54	1·47	1·44	1·41	1·37	1·34	1·22
200	3·89	3·04	2·65	2·41	2·26	2·14	2·05	1·98	1·92	1·87	1·80	1·69	1·62	1·57	1·52	1·45	1·42	1·39	1·35	1·32	1·19
∞	3·84	3·00	2·60	2·37	2·21	2·09	2·01	1·94	1·88	1·83	1·75	1·64	1·57	1·52	1·46	1·39	1·35	1·32	1·28	1·24	1·00

Appendix 3

Standard normal distribution

$\Delta\alpha$, $\Delta\beta$, f	$z\alpha$, $z\beta$, z	$\Delta\alpha$, $\Delta\beta$, f	$z\alpha$, $z\beta$, z	$\Delta\alpha$, $\Delta\beta$, f	$z\alpha$, $z\beta$, z
0.200	0.842	0.165	0.974	0.130	1.126
0.199	0.845	0.164	0.978	0.129	1.131
0.198	0.849	0.163	0.982	0.128	1.136
0.197	0.852	0.162	0.986	0.127	1.141
0.196	0.856	0.161	0.990	0.126	1.146
0.195	0.860	0.160	0.994	0.125	1.150
0.194	0.863	0.159	0.999	0.124	1.155
0.193	0.867	0.158	1.003	0.123	1.160
0.192	0.871	0.157	1.007	0.122	1.165
0.191	0.874	0.156	1.011	0.121	1.170
0.190	0.878	0.155	1.015	0.120	1.175
0.189	0.882	0.154	1.019	0.119	1.180
0.188	0.885	0.153	1.024	0.118	1.185
0.187	0.889	0.152	1.028	0.117	1.190
0.186	0.893	0.151	1.032	0.116	1.195
0.185	0.896	0.150	1.036	0.115	1.200
0.184	0.900	0.149	1.041	0.114	1.206
0.183	0.904	0.148	1.045	0.113	1.211
0.182	0.908	0.147	1.049	0.112	1.216
0.181	0.912	0.146	1.054	0.111	1.221
0.180	0.915	0.145	1.058	0.110	1.227
0.179	0.919	0.144	1.063	0.109	1.232
0.178	0.923	0.143	1.067	0.108	1.237
0.177	0.927	0.142	1.071	0.107	1.243
0.176	0.931	0.141	1.076	0.106	1.248
0.175	0.935	0.140	1.080	0.105	1.254
0.174	0.938	0.139	1.085	0.104	1.259
0.173	0.942	0.138	1.089	0.103	1.265
0.172	0.946	0.137	1.094	0.102	1.270
0.171	0.950	0.136	1.098	0.101	1.276
0.170	0.954	0.135	1.103	0.100	1.282
0.169	0.958	0.134	1.108	0.099	1.287
0.168	0.962	0.133	1.112	0.098	1.293
0.167	0.966	0.132	1.117	0.097	1.299
0.166	0.970	0.131	1.122	0.096	1.305

Appendix 3 (follows)

$\Delta\alpha$, $\Delta\beta$, f	$z\alpha$, $z\beta$, z	$\Delta\alpha$, $\Delta\beta$, f	$z\alpha$, $z\beta$, z	$\Delta\alpha$, $\Delta\beta$, f	$z\alpha$, $z\beta$, z
0.095	1.311	0.060	1.555	0.025	1.960
0.094	1.317	0.059	1.563	0.024	1.977
0.093	1.323	0.058	1.572	0.023	1.995
0.092	1.329	0.057	1.580	0.022	2.014
0.091	1.335	0.056	1.589	0.021	2.034
0.090	1.341	0.055	1.598	0.020	2.054
0.089	1.347	0.054	1.607	0.019	2.075
0.088	1.353	0.053	1.616	0.018	2.097
0.087	1.359	0.052	1.626	0.017	2.120
0.086	1.366	0.051	1.635	0.016	2.144
0.085	1.372	0.050	1.645	0.015	2.170
0.084	1.379	0.049	1.655	0.014	2.197
0.083	1.385	0.048	1.665	0.013	2.226
0.082	1.392	0.047	1.675	0.012	2.257
0.081	1.398	0.046	1.685	0.011	2.290
0.080	1.405	0.045	1.695	0.010	2.326
0.079	1.412	0.044	1.706	0.009	2.366
0.078	1.419	0.043	1.717	0.008	2.409
0.077	1.426	0.042	1.728	0.007	2.457
0.076	1.433	0.041	1.739	0.006	2.512
0.075	1.440	0.040	1.751	0.005	2.576
0.074	1.447	0.039	1.762	0.004	2.652
0.073	1.454	0.038	1.774	0.003	2.748
0.072	1.461	0.037	1.787	0.002	2.878
0.071	1.468	0.036	1.799	0.001	3.090
0.070	1.476	0.035	1.812	0.0009	3.121
0.069	1.483	0.034	1.825	0.0008	3.156
0.068	1.491	0.033	1.838	0.0007	3.195
0.067	1.499	0.032	1.852	0.0006	3.239
0.066	1.506	0.031	1.866	0.0005	3.291
0.065	1.514	0.030	1.881	0.0004	3.353
0.064	1.522	0.029	1.896	0.0003	3.432
0.063	1.530	0.028	1.911	0.0002	3.540
0.062	1.538	0.027	1.927	0.0001	3.719
0.061	1.546	0.026	1.943	0.0000	∞