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Measurement Uncertainty

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ABSTRACT

The current concept of good manufacturing practices (GMP) emphasizes that the quality of pharmaceutical products should be constructed during the overall process cycle. Quality control department plays an important role in the quality-by- design (QbD) concept since it demands the acquisition of in-process reliable analytical data. Quality control department should demonstrate the quality of their results and their fitness for purpose by giving a measure of the confidence that can be placed on the results. One useful measure of this is measurement uncertainty. The present article covers the sources, reasons and evaluation of measurement uncertainty.

Keywords: Measurement uncertainty, quality by design, quality control, coverage factor, confidence level.

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INTRODUCTION[1, 4, 5]

Quantitative indication of quality is essential during the result of the measurement of a physical quantity, so that the user of the result can assess its reliability. Such an indication is indicated at best by measurement uncertainty (MU). Measurement uncertainty provides additional information that may be useful for compliance or non compliance decisions. The uncertainty on the results may arise from many possible sources, including sampling, matrix effects and interferences, uncertainties of mass and volumetric equipment, uncertainties of Spectrophotometric and chromatographic equipment, uncertainties of biological and microbiological responses, purity of reagents and chemical reference substances, method validation, random variability and matrix effects and interferences. There will be always an uncertainty about the correctness of the stated result, even when all the known or suspected components of error have been evaluated and the appropriate correction factors applied, because there will be uncertainty on these correction factors and arising from random effects.[4]When reporting the result of an analysis it is now accepted that it is essential to give some quantitative indication of this uncertainty. Without such indication it is not possible to compare measurement results nor to assess the reliability of the result and the confidence that can be placed in any decisions based on its use. Thus, it is important to know the significance of any difference between the results and this cannot be achieved if the uncertainty on the result is not available.

Measurement Uncertainty[4, 9]

The formal definition of uncertainty of measurement given in the ISO guide is A parameter associated with the result of a measurement that characterizes the dispersion of the values that could reasonably be attributed to the measured.

Note: The parameter may be, for example, a standard deviation (or a given multiple of it) or the half width of an interval having a stated level of confidence.

The uncertainty is an expression of the fact that for a given result of measurement, there is not one value but an infinite number of values dispersed about the result, with varying degree of credibility. Each component of uncertainty that adds to the measurement uncertainty is represented by an estimated standard deviation, termed as standard deviation or U. individual standard uncertainties are identified and estimated, these are combined by the square root of the sum of the squares based on the law of propagation of uncertainty. Combined standard uncertainty would be

$$UC = \sqrt{}u_1^2 + u_2^2 + u_3^2 + \ldots\ldots\ldots + u_n^2.$$

The final value of the measurement uncertainty is known as expanded uncertainty which is determined by multiplying the combined standard uncertainty by a coverage factor.

Coverage Factor[9, 3]

The coverage factor is a multiplier chosen on the basis of the desired level of confidence to be associated with the interval defined by U= k.u_c. Most frequently, k is in the range 2 to 3. When the normal distribution applies and u is a reliable estimate of the standard deviation of the measurand, U = 2.u_c defines an interval having a level of confidence of approximately 95%(more exactly, a level of confidence of 95.45%), and U = 3.u_c defines an interval having a level of confidence of approximately 99%(more exactly a level of confidence of 99.73%). Using k=2 or 3 can no longer be accepted if the combined uncertainty has too few degrees of freedom. If the effective number of degrees of freedom, veff, is too low, student distribution is the most appropriate choice for the level of confidence with u.

Coverage factor is given by the equation:

K = 2 for 95% confidence level.

K = 3 for 99% confidence level.

The measurement uncertainty in a calibration situation should be one third or less than the accuracy of the instrument under calibration.

Reasons for Estimating Uncertainty [2, 6]

There is a growing awareness that analytical data for use in any decision process must be technically sound and defensible. Limits of uncertainty are required which need to be supported but suitable documentary evidence in the form of statistical control as for some kind of ‘quality assurance.’ When a measurement process is demonstrated by such statistical control, the accuracy of the process can be implied to characterize the accuracy of all data produced by it. It is recognized fact that any chemical analysis is subject to imperfections. Such imperfection gives rise to an error in the final test result. Some of these are due to random effects, typically due to unpredictable variations of influence quantities, such as fluctuations in temperature, humidity or variability in the performance of the analyst. Other perfections are due to the practical limits to which correction can be made for systematic effects, such as offset of a measuring instrument, drift in its characteristics between calibrations, personnel bias in reading an analogue scale or the uncertainty of the value of the reference standard. Every time a measurement is taken under essentially the same conditions. Random effects gives rise to random error from various sources and this affects the measured value. Repeated measurements will show variation and a scatter of test results on both sides of the average value. Statisticians say that random errors affect the precision, or reproducibility. A number of sources may contribute to this variability, and their influence may be changing continually. They cannot be completely eliminated but can be reduced by increasing the number of replicate analysis. Systematic errors may emanate from systematic effects. They cause all the results to be in error in the same sense, i.e. either producing consistently higher or lower results than the true value. They remain unchanged when a test is repeated under the same conditions. These effects also cannot be eliminated but may be reduced or corrected with a correction factor if a systematic effect is recognized. In fact, systematic errors must be first dealt with before estimating any uncertainty in a chemical analysis. Hence, measurement uncertainty is a quantitative indication of the quality of the test result produced. It reflects how well the result represents the value of the quantity being measured. It allows the data users to assess the reliability of the result and have confidence in the comparability of results generated elsewhere on the same sample or same population of the samples. Such confidence is important in the attempt to remove barriers to trade internationally. An understanding of the measurement uncertainty helps also in the validation of a new test method or a modified test method. One can suggest additional experiments to fine tune the test method if the uncertainty of the results is found to be large. One can also optimize the critical steps in a chemical analytical procedure in order to reduce uncertainty.

Sources of Measurement Uncertainty[2]

There are many possible sources of uncertainty of measurement in testing which includes:

a. Non-representative sampling: The sample analysed may not be representative of the defined population particularly when it is not homogenous in nature.
b. Non- homogeneity nature of the sample: It leads to uncertainty in testing a sample from sub sample.
c. Incomplete definition of the measurand: It fails to specify the exact form of the analyte being determined (e.g. cr3+ and cr6+).
d. Imperfect realization of the definition of the test method. Even when the test conditions are defined clearly; it may not be possible to produce these conditions in a laboratory.
e. Incomplete extraction and pre-contamination of the test solution before analysis.
f. Contamination during sample and sample preparation; inadequate knowledge of the effects of the environmental conditions on the measurement or imperfect measurement of environmental conditions.
g. Matrix effects and interference.
h. Personal bias in reading measurements.

i. Uncertainty of weights and volumetric equipment.

j. Uncertainty in the values assigned to measurement standards and reference materials.

k. Instrument resolution, or discrimination threshold, or errors in the graduation of the scale.

l. Approximations and assumptions incorporate in the measurement method and proceure

m. Values of constants and other parameters obtained from external sources and used in the data reduction algorithm.

n. Random variations in repeated observations of the measurand under apparently identical conditions. Such random effects may be caused by short term environmental fluctuations (e.g. temperature, humidity, etc) or variability between analysts.

Procedure for Estimating Measurement Uncertainty

Steps involved in estimation of uncertainty[3, 10]

STEP 1**:** Specification of measurand.

STEP 2: Identify the possible sources of uncertainty.

STEP 3: Quantification of uncertainty components

STEP 4: Calculation of combined and expanded uncertainty.

Step 1: specification of measurand

In the context of uncertainty estimation, “specification of measurand” requires both a clear and unambiguous statement of what is being measured, and a quantitative expression relating the value of the measurand to the parameters on which it depends on. Those parameters may be either measurands or constants. It should also be clear whether a sampling step is included within the procedure or not. If it is, estimation of uncertainties associated with the sampling plan need to be considered. All of this information should be included in the standard operating procedure. In analytical measurements it is particularly important to distinguish between measurements intended to produce results which are independent of the method used, and those which are not so intended. The latter are referred as empirical methods. The distinction between the empirical and non-empirical methods is important because it affects the estimation of uncertainty.

Step 2: Identify the Possible Sources of Uncertainty

A comprehensive list of relevant sources of uncertainty should be assembled. At this stage, the main aim is to completely clear about what should be considered. In forming the list of uncertainty sources it is usually convenient to start with the basic expression used to calculate the measurand from intermediate values. All the parameters in this expression may have an uncertainty associated with their value and are therefore potential sources of uncertainty. In case of analytical measurements uncertainties associate with the overall method performance and bias measured with respect to appropriate reference materials should be considered.

Typical sources of uncertainty

Sampling:

Sampling forms the part of the specified procedure, effects such as random variations between different samples and any potential for bias in the sampling procedure forms the components of uncertainty affecting the final result.

Storage conditions:

Test items which are stored for any period prior to analysis, the storage conditions may affect the results. In those cases the duration of storage and conditions during storage should be considered as sources of uncertainty.

Instrument effects:

Instrument effects may include the limits of accuracy on the calibration of an analytical balance, a temperature controller that may maintain a mean temperature which differs from its indicated set-point, an auto-analyser that could be subject to carry-over effects.

Sample effects:

The recovery of an analyte from a complex matrix, or an instrument response, may be affected by composition of the matrix. Analyte speciation may further compound this effect. The stability of a sample or analyte may change during analysis because of a changing thermal regime or photolytic effect.

Step 3: Quantification of Uncertainty Components[8]

After identification of the uncertainty sources the next step us to quantify the uncertainty arising from these sources. This can be done by:

- Evaluating the uncertainty arising from each individual source.
- Directly determining the combined contribution to the uncertainty on the result from some or all of these sources using method performance data.
- The procedure used for estimating the overall uncertainty depends on the data available for method performance.
- The list of uncertainty sources should be examined to see which sources of uncertainty are accounted for by the available data.

- Sampling should include a collaborative studies include a sampling plan. If the method involves sub-sampling, or the measurand is estimating a bulk property from a small sample, then the effects of sampling should be investigated and their effects included.
- Pretreatment of the samples should be done. Samples should be homogenized and stabilized before distribution. It may be necessary to investigate and add the effects of the particular pre-treatment procedures applied.
- Method bias is often examines prior to or during inter laboratory study, where possible by comparison with reference methods or materials. Where the bias itself, the uncertainty in the reference values used and the precision associated with the bias are checked.
- The precision should be estimated over an extended time period and chosen to allow natural variation of all factors affecting the result.
- The standard deviation of results for a typical sample analysed several times over a period of time, using different analysts and equipment where possible.
- The standard deviation obtained from the replicate analyses performed on each of several samples.

Step 4: Calculation of Combined and Expanded Uncertainty

The uncertainty component was evaluated experimentally from the dispersion of repeated measurements; it can readily be expressed as standard deviation. For the contribution to uncertainty in single measurements the standard uncertainty is simply the observed standard deviation. Where an uncertainty estimate is derived from previous results and data, it may already be expressed as standard deviation. A confidence interval is given with a level of confidence. The next stage is to calculate the combined standard uncertainty. Two simple rules for combining standard uncertainties are given here:

Rule 1: For models involving only a sum or difference of quantities, the combined uncertainty u_c is given by

$$u_c\ (p, q) = \sqrt{}u(p)^2 + u(q)^2 + \ldots..$$

Rule 2: For models involving only a product or quotient, the combined standard uncertainty u_c is given by

$$U_c = y\sqrt{}(u(p)^2/p)^2 + (u(q)^2/q)^2 + \ldots\ldots..$$

- Where u(p)/p is the uncertainty expressed as relative standard deviation.
- The final stage is to multiply the combined standard uncertainty by the chosen coverage factor. The expanded uncertainty is required to provide an interval which may be expected to

encompass a large fraction of the distribution of values which could reasonably be attributed to the measurand.

Evaluation of Measurement Uncertainty[2]

The ISO Guide 98, ISO/TS 21748:2004 and the EURACHEM document have all adopted the approach of grouping uncertainty components into two categories based on their method of evaluation, i.e., Type A and Type B evaluation methods. This categorization based on the method of evaluation rather than on the components themselves, applies to uncertainty and is not substitutes for the words “random” and “systematic”. It avoids certain ambiguities – a random component of uncertainty in one measurement may become a systematic component in another measurement that has, as its input, the result of the first measurement.

Type A Evaluation

Type A evaluation of uncertainty is based on any valid statistical methods in analysis of a series of repeated observations. The statistical estimated standard uncertainty is called Type A standard uncertainty. Component of type A evaluation of standard uncertainty arises from random effect. The Gaussian or normal law of error forms the basis of the analytical study of the random effects. It is a fact that the mean of the sample of measurements provides us with an estimate of the true value, μ of the quantity we are trying to measure. Since, however, the individual measurements are distributed about the true value with a spread which depends on the precision; it is most unlikely that the mean of the sample is exactly equal to true value of the population. For this reason, it is more useful to give a range of values within which are almost lies in the true value. The width of the range depends on two factors. The first is the precision of the individual measurements, which in turn depends on the variance of the population. The second is the number of replicates made in the sample. The very fact that we repeat measurements implies that we have more confidence in the mean of several values than in the single one. Most people will feel that the more measurements we make, the more reliable our estimate of μ, the true value. In most cases, the best available estimate of the expected value of a measurand quantity x that varies randomly is the arithmetic mean x for n number of replicates.

$$X = \Sigma x_i / n \ldots\ldots\ldots\ldots\ldots (1)$$

The experimental standard deviation S is used to estimate the distribution of x as:

$$S = \sqrt{} [\Sigma (x_i - x) 2/ (n-1)]$$

Alternatively, it can be simplified to the following equation form.

$$S = \sqrt{} [(\Sigma (x_i^2) / (n-1)) - (\Sigma(x_i^2)/n(n-1))]$$

The experimental standard deviation of mean, or standard error of the mean, σ_x, or a distribution of sample means has an exact mathematical relationship between it and the standard deviation, σ, of the distribution of the individual measurements, which is independent of the way in which they are distributed. If N is the sample size, this relationship is

$$\sigma_x = \sigma/\sqrt{N}.$$

From the equation (4) above it is noted that the larger N is the smaller the spread of the sample means about μ. This universally used term, the standard error of the mean, might mislead us into thinking that $\sigma/\sqrt{N}$ gives the difference between x and μ. This is not so. The $\sigma/\sqrt{N}$ gives a measure or confidence involved in the estimating μ from x.

Type B Evaluation

- Type B evaluation is by means other than used for type A evaluation such as:
  - From data provided in calibration certificates and other reports;
  - From previous measurement data;
  - From experience with, or general knowledge of the behavior of the instruments.
  - From manufacturers specifications.
  - From all other relevant information.
- Components evaluated using type B methods are also characterized by estimated standard uncertainty.
- When we are considering Type B uncertainty, we have to convert the quoted uncertainty to a standard uncertainty expressed as standard deviation. We can convert a quoted uncertainty that is a stated multiple of an estimate standard deviation to a standard uncertainty by dividing the quoted uncertainty by the multiplier.

Benefits of Uncertainty Evaluation[4, 7]

Evaluation of uncertainty is clearly quite a challenge in the case of quality control and quality assurance. It includes the following

- Improves understanding of the measurement.
- Provides tool for monitoring the quality of sampling.
- Provides the opportunity to save on costs.
- Provides information for making compliance judgments.
- Increases the acceptability of the results.
- Gives a measure of the quality of a result that enables the user of the result to make an objective assessment of the reliability that can be placed in its value.

Applications of Measurement Uncertainty[1, 2]

- The uncertainty in microbiological analysis was estimated based on the method validation data which includes precision, accuracy. The uncertainty may be estimated based on the variability of inhibition zone diameter within and between dishes.
- Measurement uncertainty is a useful indicator of quality of the results.
- It is very useful in the assessment of compliance or non compliance of in-process and final pharmaceutical products.
- Measurement uncertainty may be useful in the development, validation and comparison of analytical methods.
- It provides additional information regarding the shelf life of product and estimation of the producer's and customer's risk of the established shelf life.
- Measurement uncertainty may be an alternative to assess the pharmaceutical equivalence.

CONCLUSION

Measurement uncertainty is a useful tool in the evaluation of quality of analytical procedure. By evaluating measurement uncertainty one can reduce the maximum possible sources of errors. It plays an important role in the decision of compliance or non-compliance of in-process and final pharmaceutical products as well as in the assessment of pharmaceutical equivalence, development of analytical methods and stability study of drug products.

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