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REVIEW PAPER

Measurement uncertainty in pharmaceutical analysis and its application

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Abstract The measurement uncertainty provides complete information about an analytical result. This is very important because several decisions of compliance or non-compliance are based on analytical results in pharmaceutical industries. The aim of this work was to evaluate and discuss the estimation of uncertainty in pharmaceutical analysis. The uncertainty is a useful tool in the assessment of compliance or non-compliance of in-process and final pharmaceutical products as well as in the assessment of pharmaceutical equivalence and stability study of drug products.

1. Introduction

The current concept of Good Manufacturing Practices (GMP) emphasizes that the quality of pharmaceutical products must be constructed during the overall process cycle. Quality control department plays an important role in quality-by-design (QbD) concept, since it demands the acquisition of reliable in-process analytical data [1,2]. Many important decisions are based on the analytical data of quality control department and it is important to have indication of the quality of these results [3,4].

As a consequence of these requirements, 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. Measurement uncertainty provides additional information that may be useful for compliance or non-compliance decisions [4–7].

The evaluation of uncertainty requires a detailed study of all possible sources of uncertainty. However, it is essential that the effort expanded should not be disproportionate. A preliminary study may identify the most significant sources of uncertainty and the value obtained for the combined uncertainty may be almost entirely controlled by the major contributions [4,8].

The uncertainty on the results may arise from many possible sources, including sampling, matrix effects and interferences, environmental conditions, uncertainties of mass and volumetric

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equipment, uncertainties of spectrophotometric and chromatographic equipment, uncertainties of biological and microbiological responses, purity of reagents and chemical reference substances, method validation and random variability [9–22].

Typically, these sources of uncertainties are divided into two types: Type A – random error and Type B – systematic error. Random error arises from unpredictable variations. These random effects give rise to variations in repeated observations. The random error can usually be reduced by increasing the number of observations. Systematic error is a component of errors which remain constant or vary in a predictable way. It is independent of the number of observations. The result should be corrected for all recognized significant systematic errors [4,8,23].

The steps involved in uncertainty estimation are: (1) specification of measurand, (2) identification of uncertainty sources, (3) quantification of uncertainty components, and (4) calculation of combined and expanded uncertainty [4,24,25]. A summary of these steps is presented in Table 1.

In pharmaceutical analysis, the sources of uncertainty may arise from sampling, environment conditions, method validation, instruments, weighting and dilutions, reference materials, chemical, microbiological and others aspects. A list of the main sources of uncertainty is presented in Fig. 1.

A complete report of a measurement result should include a description of the methods used to calculate the result and its uncertainty. Usually, the result is stated together with the expanded uncertainty. The uncertainty may be a useful tool to assess compliance or the limits may be set with some allowance for measurement uncertainties [4,7].

The aim of this work was to evaluate and discuss the estimation of measurement uncertainty in pharmaceutical analysis and its application

2. Uncertainty in pharmaceutical analysis

Currently, several tests and assays are performed by quality control department of pharmaceutical industries, such as content and/or biological potency of active pharmaceutical ingredient (API), limit of organic and/or inorganic impurities, pH, microbiological and endotoxin limits. In this context, the pharmacists need to have a good understanding of several techniques, such as chemical, spectrophotometric, chromatographic and microbiological methods [26,27].

2.1. Uncertainty in spectrophotometric and chromatographic analysis

Soovälli and collaborators [17] investigated the most important uncertainty sources that affect analytical UV–vis spectrophotometric measurements. According to their results, physical sources of uncertainty often have significantly lower contributions than chemical sources. The calibration equations also have a significant contribution to the uncertainty in UV–vis spectrophotometric analysis [19].

Saviano and Lourenço [28] studied the uncertainties sources associated with linezolid determination by UV spectrophotometry. According to their results, the contributions of precision, linearity and weight of linezolid reference standard are the most significant, contributing with about 77% of the overall uncertainty. The Eurachem procedure was also compared to Monte Carlo simulation results. The final result and its uncertainty estimated by the Eurachem procedure was found to be about $93.92 \pm 2.12\%$ and the 95% confidence interval obtained from Monte Carlo simulation was $93.92 \pm 2.06\%$. Therefore, the Eurachem procedure can be considered reasonable for the estimation of measurement uncertainty of linezolid by UV spectrophotometry.

Paakkunainen and collaborators [29] studied the measurement uncertainty of lactase-containing tablets analyzed with FTIR. They reported that homogeneity of tablets was the most relevant source of uncertainty, and nearly 20 tablets had to be analyzed for a 5% uncertainty level.

Anglov and collaborators [13] performed similar studies concerning reverse phase high performance liquid chromatography (RP-HPLC). In this study, they evaluated the uncertainty contributions of repeatability of peak area, dilutions factors, reference materials and sampling. Sampling, calibration and repeatability were the most significant sources which affect combined uncertainty. As it occurs in UV–vis spectrophotometric analysis, calibration equations also have a significant contribution to the uncertainty in liquid chromatography [20].

Leito and collaborators [30] studied the influence of peak integration, nonlinearity of calibration curve and other sources of uncertainty in chromatographic assay of simvastatin in drug formulation. According to their results, uncertainty estimated for single-point calibration is only slightly larger than a five-point calibration model.

Results obtained by our research group (not yet published) indicate that accuracy, linearity and precision contribute with about 70% of overall uncertainty in determination of linezolid by RP-HPLC. According to Monte Carlo simulation results, the procedure for estimation of the measurement uncertainty can be considered appropriate. The final result and its 95% confidence interval estimated by the Eurachem procedure was found to be $95.13 \pm 2.51\%$, while Monte Carlo simulation yielded $95.14 \pm 2.46\%$.

Lecomte and collaborators [31] established the measurement uncertainty for the determination of cidofovir in human plasma by hydrophilic interaction chromatography. They compared two approaches for estimation of measurement uncertainty: from

Table 1 Steps involved in uncertainty estimation.

Step	What to do? How to do?
Specification of measurand	Define what is being measured, including a relationship between the result and the input quantities upon it depends [4].
Identification of uncertainty sources	List the possible sources of uncertainty. A cause-and-effect diagram (Fig. 1) is a very convenient way of listing the uncertainty sources and how they relate to each other [4,25].
Quantification of uncertainty components	Measure or estimate the uncertainty component associated with each potential source of uncertainty identified. These uncertainties may be obtained from method validation data, calibration certificate of instruments, purity of reagents and chemical reference substances and experimental studies [4,24].
Calculation of combined and expanded uncertainties	Express all uncertainties components as standard deviations. Two simple rules may be used to calculate the combined uncertainty. Multiply the combined standard uncertainty by a chosen coverage factor (at the required level of confidence) in order to obtain an expanded uncertainty [4].

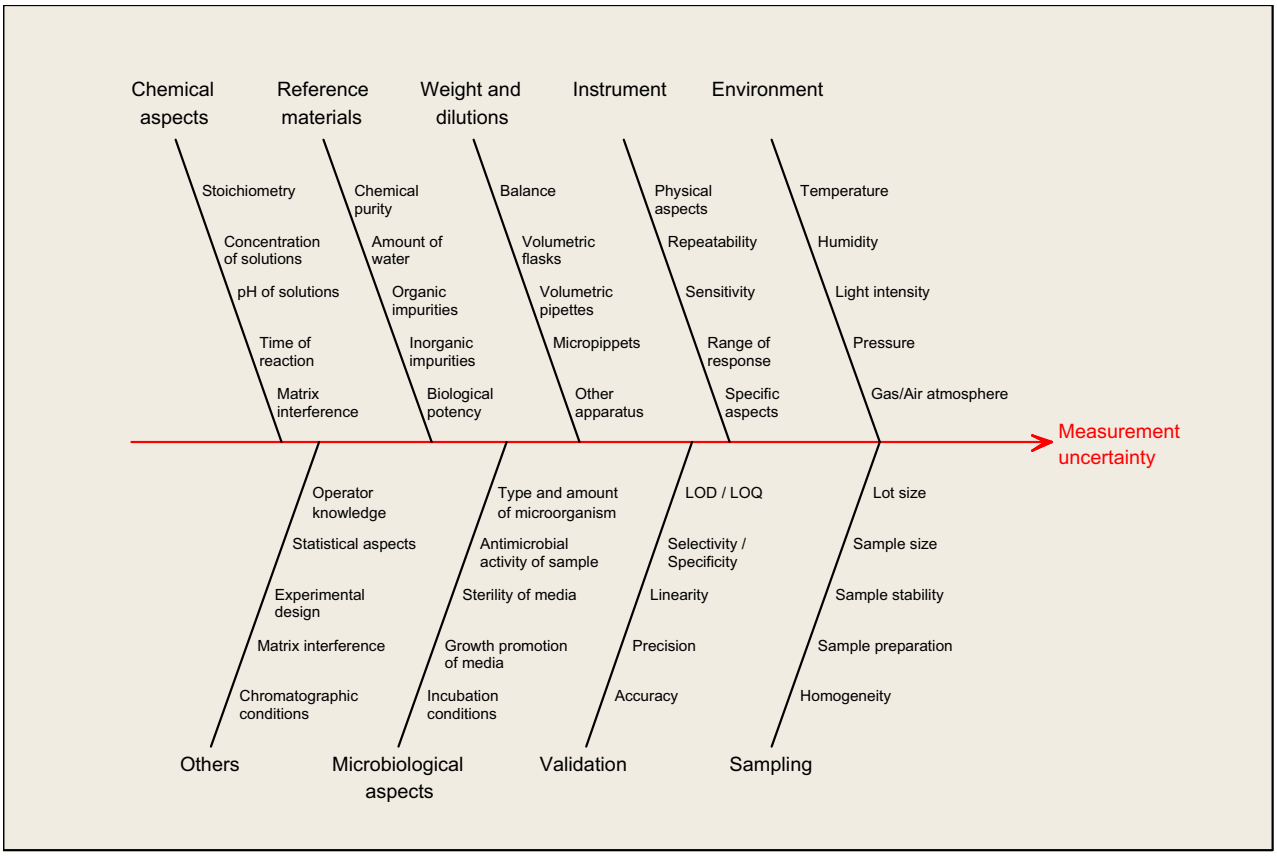

Fig. 1 Main sources of uncertainties affecting measurement uncertainty in pharmaceutical analysis [4,25].

method validation and from routine application. They found that estimations obtained during method validation underestimated those obtained from routine applications and that this magnitude of underestimation was related to cidofovir concentration.

2.2. *Uncertainty in microbiological analysis*

The most significant sources of uncertainty in microbiological assay were studied by Lourenço and collaborators [18] and Lourenço [20]. The uncertainty was estimated based on the method validation data [18], which included precision and accuracy. The uncertainty may be estimated based on the variability of inhibition zone diameter within and between dishes [20]. In another study, Lourenço [32] evaluated the uncertainties associated with reference and standard weighing and dilutions, such as balance, volumetric flasks and pipettes; and uncertainty estimated based on the variability of inhibition zone diameters of a validated microbiological assay for apramycin in soluble powder [33]. According to the results, the variability of inhibition zone diameters (within and between plates) was the most significant source of uncertainty.

In microbiological limits the result is obtained by counting the colony forming units (CFU). Traditionally, microbiological uncertainty estimates have been based on replicate measurements and on the Poisson theory. However, other aspects such as antimicrobial activity of product, growth promotion of culture media and specific characteristics of microorganisms should be considered as potential sources of uncertainty [9,15,34].

Lourenço and collaborators [16] established a procedure to estimate uncertainty of LAL gel-clot test. The usual form of estimating and informing the uncertainty in qualitative analysis ('pass/fail') is the use of false-negative and false-positive responses [35,36]. Temperature, time of incubation, pH, sensitivity of LAL and interference of product were evaluated as sources of uncertainty [16,21].

2.3. *Uncertainty in chemical and physical analysis*

The estimation of uncertainty in routine pH measurement was evaluated by Leito and collaborators [12]. The study included physical and chemical sources of uncertainty. According to the results, the uncertainty was the lowest near pH 7 and increased when moving to pH 2 or 11 [12]. Chui and collaborators [11] studied the precision of Karl Fischer for water determination. They identified kinetic and stoichiometric factors, matrix interference and mass of sample as potential sources of uncertainty [11]. Paakkunainen and collaborators [37] studied the uncertainties associated with dissolution test of drug release. According to their results, uncertainties associated with sampling error, total analytical error and the error arising from the heterogeneous samples were the main sources of uncertainty. Further studies should be performed in order to study the uncertainty sources associated with disintegration, friability, hardness and other tests and assays employed in pharmaceutical analysis.

3. Applications of uncertainty in pharmaceutical analysis

Decisions of compliance or non-compliance in pharmaceutical industry are based on analytical results obtained by quality control

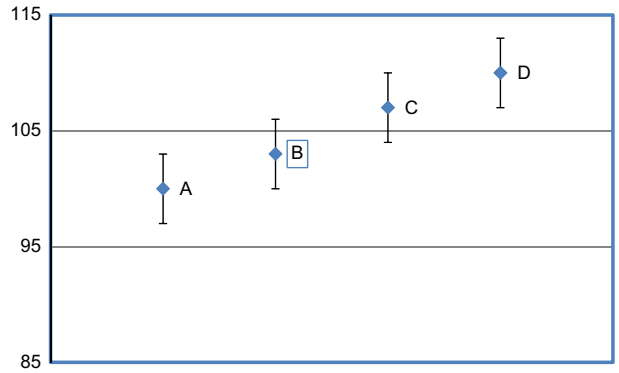

Fig. 2 Evaluation of compliance or non-compliance based on analytical result and its uncertainty (specification limits from 95% to 105%) [4,7].

department. Uncertainty is a useful indicator of quality of the results and should be considered when testing a sample against legal specifications. It can be a critical situation when the result is so close to the limits that its uncertainty affects decision [4,7].

Typically, we have four situations that must be considered: result and its uncertainty within specification interval (Fig. 2A); result within the specification interval, but its uncertainty out of specification (Fig. 2B); result out of specification, but its uncertainty within the specification interval (Fig. 2C); and result and its uncertainty out of specification (Fig. 2D) [4,7].

This approach is very useful in the assessment of compliance or non-compliance of in-process and final pharmaceutical products. Uncertainty cannot replace method validation, but it is a way to provide complete information about a sample. If the uncertainty is unknown, the information is not complete; therefore this decision might be impossible. Uncertainty may be useful in the development, validation and comparison of analytical methods [3,4,22,38].

3.1. Uncertainty in pharmaceutical equivalence assessment

Pharmaceutical equivalence is an important step to confirm similarity and interchangeability in pharmaceutical products, particularly regarding those that will not be tested for bioequivalence/relative bioavailability. Lourenço and collaborators [39] compared *t*-student difference test and two one-side equivalence tests in the assessment of pharmaceutical equivalence. Uncertainty may be an alternative to assess pharmaceutical equivalence, once it provides information to compare reference and test (similar or generic) products.

Okamoto and collaborators [40] established a procedure to estimate the measurement uncertainty for metronidazole quantification by RP-HPLC and applied it in the assessment of pharmaceutical equivalence. The measurement uncertainty approach was compared to the two one-sided tests (TOST) for equivalence [40]. According to their results, both TOST and uncertainty approaches allow us to assess pharmaceutical equivalence among test (generic or similar) and brand-name drugs [40].

3.2. Uncertainty in stability studies

Uncertainty can also be useful for investigating out of specification results in stability study of pharmaceutical products [41]. Use of warning and action lines—time values around the established shelf life that takes into account the measurement uncertainty—may be helpful when the measured property is increasing or decreasing with time [38]. This approach may provide additional information regarding shelf life of product and estimation of the producer's and customers' risk of the established shelf life [41].

In another study, Magari [42] evaluated how the number of lots and replicates analyzed in stability study affect the estimation of shelf life and its measurement uncertainty. According to his conclusions, the number of lots and replicates would be defined based on measurement uncertainty, experimental design and other practical considerations.

4. Conclusions

We conclude that uncertainty provides complete information about an analytical result and it plays an important role in decision of compliance or non-compliance of in-process and final pharmaceutical products as well as in the assessment of pharmaceutical equivalence and stability study of drug products.

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