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Relationship between HPLC precision and number of significant figures when reporting impurities and when setting specifications

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Abstract

The rounding of an analytical result is a process that should take into account the uncertainty of the result, which is in turn assessed during the validation exercise. Rounding rules are known in physical and analytical chemistry since a long time, but are often not used or misused in pharmaceutical analysis. The paper describes the theoretical background of the most common rules and their application to fix the rounding of results and specifications. The paper makes use of uncertainty values of impurity determination acquired during studies of reproducibility and intermediate precision with regards to 22 impurities of drug substances or drug products. As a general rule, authors propose the use of sound and well-established rounding rules to derive rounding from the results of the validation package.

Keywords: Rounding; Significant digits; Impurity content; Validation

1. Introduction

The number of papers related to the validation of analytical methods is absolutely enormous and reflects the huge amount of work that industrial and academic laboratories as well as regulatory agencies spend in this kind of work. Concerning the pharmaceutical domain, the guidelines of the International Conference of Harmonisation (ICH) describe the way to present data in the pharmaceutical dossier aimed to be submitted to health authorities. These regulations are applicable in the three regions belonging to the ICH process, Europe, Japan and United States, but are also accepted in other countries. ICH guidelines Q2A and Q2B describe definitions [1] and methodology [2] of analytical validations, respectively. According to the key definition of Q2A, "the objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose". Thresholds for reporting, identification and toxicological qualification of impurities are defined in ICH guidelines Q3A [3] and Q3B [4], covering drug substances and drug products, respectively. First of all, the *intended purpose* of the impurity determination is to ensure that no drug substance or drug product is released if the level of any specified impurity exceeds the specification limit assessed by toxicological and/or clinical studies or if the level of any unspecified impurity exceeds the threshold accepted by convention as the identification (in most cases 0.10%) or qualification limit (in most cases 0.15%). But the *intended purpose* is also to allow the applicant and the authorities to detect trends in the quality during manufacturing or during the storage of the products. We should expect an absolute coherence between thresholds and reporting limits introduced in the ICH impurity guidelines and data stemming from validation studies. Surprisingly, the final revision of Q3A (for the drug substance, DS) and Q3B (for the drug product, DP) states: "below 1.0%, the results should be reported to two decimal places; at and above 1.0% the results should be reported to one decimal place... the use of two decimal places for thresholds does not necessarily indicate the precision of the analytical procedure used for routine quality control purposes". This triggered by the authors the question: should not the rounding of any analytical data reflect its uncertainty? The authors of the present paper think that the quoted sentence can be understood as a "practical" compromise, but should trigger further scientific considerations

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on the relationship between analytical method performance and rounding of the final result, in order to achieve a full scientific consistency.

It is worth stressing that rounding of the final result has not only a pure scientific value, because the pharmacopoeias rule is: "round, then compare to the specification limits" [6]. According to this rule, in case of a test result of 0.21% the test does pass if the limit is "≤0.2%", whereas it does not if the limit is "≤0.20%". Rounding has therefore an impact on the final decision about the batch conformity, and not only on the numerical result.

In 2001, at the occasion of a symposium organized by the European Pharmacopoeia in Cannes, one of the authors tackled this topic but without entering into any theoretical and experimental considerations [7]. The purpose of the present paper is to support the claim made at that time and to show how the number of significant figures in reporting impurity levels can be related to the true precision of the method as assessed by validation studies. This paper has been intended as an effort to follow good scientific practices and not to design new rules: "as scientists, we are compelled to adhere to the fundamental conventions of mathematics or the chaos will be complete", as written by Bunnell [8] in one of the very rare publications dedicated to the reporting of quantitative analytical results.

It is common to find between pharmaceutical analysts rather confusing practices in assessing significant figures, but this should not be very surprising, taking into account that these simple concepts are not always taught and practised even in the academic and scientific worlds [9,10].

Results of several validation studies on impurity determination will be presented in order to show real examples of application of the rules. This paper only covers HPLC methods, that are, to a great extent, the most common analytical methods in pharmaceutical impurity determinations.

2. Theoretical considerations

Every physicochemical measurement is always affected by random error and may be affected by systematic error. Therefore, a result is fully expressed only if its uncertainty is also given. This is of course also true for the HPLC determination of impurities contained in drug substances and drug products.

As for any physical measure, forgetting the systematic error and only focusing on the random error, the impurity level I (assumed to follow a normal distribution) from p independent determinations results ($x_i(\%)$, $i = 1, \ldots, p$), obtained for any individual impurity, could normally be reported in percentage (m/m) with regard to the active substance as (1):

$$I(\%) = \bar{x}(\%) \pm \frac{s}{\sqrt{p}} t_{p-1,\alpha} \quad (1)$$

$$s^2 = \frac{\sum(\bar{x} - x_i)^2}{p-1} \quad (2a)$$

and

$$\mathrm{CV} = 100\frac{s}{\bar{x}} \quad (2b)$$

where $\bar{x}$ is the arithmetic mean value of the p independent determinations x_i; s the estimate of the standard deviation; $t_{p-1,\alpha}$ the Student parameter for $p-1$ degrees of freedom at a level of risk α (generally 0.05) and CV is the coefficient of variation (%).

The right part of the second term of Eq. (1) is the limit of error (or "confidence limit" in the statistical jargon), or, according to another terminology, the expanded uncertainty, that is the standard uncertainty (standard error in the present case) multiplied by a fixed number k [11] or an appropriate distribution coefficient (Student's t, most often). In all the discussion below, the term "uncertainty", symbolized by u, will be used to mean expanded uncertainty, because this is the definition that better complies with this concept.

In an equivalent manner, an individual impurity determination x_0 from a validated analytical method should be reported, in an equivalent manner, as:

$$x_0 \pm u \quad (3)$$

u represents the expanded uncertainty, of which a mathematical expression can be written as:

$$u = ks = k\frac{x_0 \mathrm{CV}}{100} \quad (4)$$

where k is the coverage factor; s the standard uncertainty (standard deviation) of the analytical method and CV is the precision coefficient of variation of the analytical method (s and CV are estimated in the scope of the method validation).

If the measurand is known to be normally distributed with known standard deviation, a coverage factor of $k = 3$, of common use in statistical process control [12], ensures a 99.7% confidence level. If the distribution is not known, but can be assumed as unimodal, recent developments of the Bienaymé–Tchebychev theorem (cf. Vysochanskii and Petunin [13]) enable us to propose approximate coverage factors (in general, 3 for a 95% confidence interval). These arguments will justify the use of the coverage factor of $k = 3$ throughout the manuscript.

In the common practice of the pharmaceutical analysis, we mean in general routine quality control, uncertainty is not evaluated routinely for each impurity determination; the uncertainty value obtained during validation studies is considered as the reference indicator of the precision of the method. In passing, we recall that suitability parameters have to be introduced in the QC monograph; they should support the performance of the method as assessed in the validation package. Two main arguments support the relevance of the uncertainty estimate from the validation unit. Firstly, the operating conditions (instruments performance, balances and volumetric apparatuses) are strictly controlled according to common standards. Secondly, when performed in accordance with the guidelines in force [2,5], validation studies include, as a minimum, intermediate precision and, often also, reproducibility that capture all the sources of variability (day, operators, instruments and laboratories). Two practical factors, amongst the others, have an insidious impact on method reproducibility. The first is the effect of the "integration method", that is the algorithm and the set of parameters used to integrate the chromatogram: a different threshold can lead a laboratory to systematically increase or decrease impurity peak