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Precision of the reportable result. Simultaneous optimisation of number of preparations and injections for sample and reference standard in quantitative liquid chromatography

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ABSTRACT

In pharmaceutical analysis, the precision of the reportable result, i.e. the result which is to be compared to the specification limit, is relevant for the evaluation of the suitability of the analytical procedure. But also for other applications, the precision of the result is important and an optimisation often of interest. However, increasing the number of determinations (e.g. injections or preparations) will reduce only the variability (or standard error) of the corresponding precision level. Therefore, the knowledge of the individual variance contributions, obtained from reliable precision studies is important to determine on a scientific basis which format of the (reportable) result, i.e. the number of injections and sample preparations (or even series), should be used. In case of relative analytical procedures such as LC, the calibration model and format, i.e. the number of determinations of the reference standard is one of the factors (besides instrument, operator, reagents, etc.) affecting the between-series variance contribution at intermediate precision/reproducibility level. Consequently, the precision of the reportable result is only valid for the calibration format used to obtain intermediate precision/reproducibility. Instead of repeating the whole precision study to optimize the calibration format, the present paper describes a statistical approach using variability results from the original precision study

1. Introduction

Precision is one of the most important performance characteristics of an analytical procedure, because variability is inherent and contributes to all other performance parameters. Each step of an analytical procedure contributes with its variability to the overall precision. The usual approach in pharmaceutical analysis is to combine groups of contributions linked by the procedure design and to determine the combined variability experimentally, as precision levels [1]. Basically, short-term and long-term contributions can be distinguished, with system precision and repeatability belonging to the former, intermediate precision and reproducibility to the latter. Due to the additivity of variances, each of the levels includes the lower ones (Fig. 1).

But which of the precision levels is relevant?

To answer this question, we have to look to the (intended) routine application, the suitability of which is the objective of a validation. In the long-term routine application, all sources of variability (see Fig. 1) will be included. But in addition, another important aspect must be considered: It is the final or reportable result [3] what matters, i.e. the result which is to be compared to the specification limits. For the precision of the reportable result, the knowledge of the individual variance contributions is important, because increasing the number of determinations (e.g. injections or sample preparations) will reduce only the variability (or standard error) of the corresponding level [3].

In case of relative analytical procedures such as LC, the calibration model and format, i.e. the number of determinations of the reference standard [4] is one of the factors (besides instrument, operator, reagents, etc.) affecting the between-series variance contribution at intermediate precision/reproducibility level. Therefore, this variance contribution is essentially linked to the calibration format which is applied in the experimental precision study. And consequently, the precision of the reportable result is only valid for

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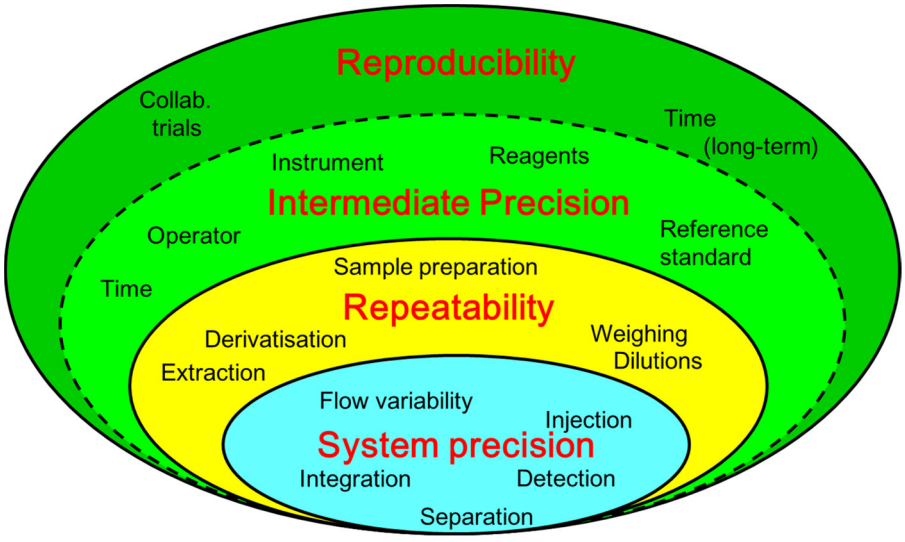

Fig. 1. Illustration of the various precision levels with (some of) their contributions for LC assay. Reprinted from [2].

this calibration format, which must then be the same in the routine application.

And what about changing the calibration format?

A repetition of the whole precision study for investigating new calibration formats would be very expensive and time consuming. The present paper describes a statistical approach to optimize the calibration format using standard variability results.

The described approach to define the reportable result on the basis of a thorough understanding of the performance of the analytical procedure is well aligned with current initiatives to apply quality-by-design principles to pharmaceutical analytics [5]. The precision of the reportable result is an important aspect of the Analytical Target Profile, where the performance requirements of the intended measurement are established [6]. It should be noted that additional contributions may influence the reportable result, for example the uncertainty of the declared content of the reference standard used. However, this is beyond the scope of this article.

2. Background: Precision levels

2.1. System precision

The variability of the measurement itself is addressed in system precision, also termed instrument/injection precision, or injection repeatability by repeated analysis of the same sample (solution). Although in LC the contribution from the injection system is normally the dominating one (at least at higher concentrations), there are additional contributions from the pump (short-term flow constancy, relevant for peak area measurements), the separation process, the noise, etc. (see Fig. 1, Eq. (1)).

$$s_{sys}^2 = s_i^2 + s_f^2 + s_n^2 + s_{sep}^2 + \cdots \quad (1)$$

Variances of injection (i), flow-constancy (f), noise (n), separation (sep).

Although unfortunately not described in the ICH Guideline Q2, system precision provides valuable information about the variability of the analytical system, mainly the instrument. Therefore, it is an important parameter for equipment qualification and for System Suitability Tests [7,8]. However, due to the strong concentration dependence of the variance contributions to system precision, the analyte concentration needs to be sufficiently above the quantitation limit (at least 100 times) to reflect mainly the performance of the instrument (injection/application system). Otherwise, the contributions of the noise variability will dominate [9].

2.2. Repeatability

Repeatability includes, in addition to system precision, the contributions from the sample preparation, such as homogenization, weighing, aliquoting, dilution, extraction, etc, depending on the analytical procedure (see Fig. 1, Eq. (2)).

$$s_r^2 = s_p^2 + s_{sys}^2 \quad (2)$$

Variance of system (sys), sample preparation (p).

Therefore, it is essential to apply the *whole sample preparation* (as described in the analytical procedure) and to use *authentic samples* [1] as far as possible, because only for these the analytical procedure can be performed exactly as in the routine application. There may be exceptions, but these should be appropriately justified. For example, analysing impurities near the quantitation limit, where the variance contribution of the sample preparation can be neglected, injection precision and repeatability are identical [9] (see also Fig. 4). If artificially prepared (i.e. spiked) samples are used, the impact on the sample preparation variance (compared to the routine procedure) should be small with respect to the intended application.

2.3. Intermediate precision/reproducibility

For intermediate precision, factors which can be expected to vary in long-term applications within the same laboratory, such as calibration, operator, instrument, reagents, should be investigated. Several independent series of applications of the whole analytical procedure to preferably authentic, identical samples must be performed. Often, these factors are not investigated individually, but combined, which results in a confounded variance contribution ("between-series", see Eq. (3)).

$$s_R^2 = s_b^2 + s_r^2 = s_b^2 + s_p^2 + s_{sys}^2 \quad (3)$$

Variances: between series (b), repeatability (r), sample preparation (p), and system (sys).

Reproducibility addresses strictly variation of factors between laboratories. However, at least for applications within the same company, under the same Quality System, both sub-level merge long-term.

The ICH guideline [1] provides no guidance on the number of determinations nor series for the estimation of intermediate precision/reproducibility. The simplest approach is to perform further