Establishing Specification Acceptance Limits
Consensus paper of the Working Group Drug Quality Control / Pharmaceutical Analytics of the German Pharmaceutical Society (DPhG)

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A proposal is made how to take the analytical variability into account in the process of establishing acceptance criteria for assays of drug substances /drug products and for impurity determinations.

The discussion started with a workshop on analytical uncertainty and rational specification setting in Frankfurt, January 31, 2002. As a conclusion of the presentations and discussion, a draft paper was published [1, 2] and accepted with some changes after a thorough discussion at the annual meeting of the Working Group Drug Quality Control / Pharmaceutical Analytics in October 2002 in Berlin [3].

1. Introduction

The quality control of drugs should be rationally based and justified, starting with an identification of potential risks. Based on these risks, the required quality, for example the maximum allowed amount of impurities, is defined. Based on this compliance with safety requirements, also manufacturing and analytical variability has to be taken into consideration for the acceptance criteria of the specification [4]. The relationship between acceptance limits and analytical variability has to be discussed separately for the assay of active ingredients in drug substances and drug products as well as for impurity determinations, because the concentration ranges and the matrix effects to be expected are different.

2. Acceptance criteria for the content determination of drug substances

It is recommended by the Working Group to apply basically the concept of Daas and Miller [5], with some adjustments.
The calculation of the acceptance limits is performed according to equation 1a and 1b [6]:

(1a) $$LAL = 100\% - \%TSI - 3 \cdot TSD$$

(1b) $UAL = 100\% + 3 \cdot TSD$

LAL and UAL: lower and upper acceptance limit, respectively. Often also the terms "specification limit" or "content limit" are used.
%TSI: Total sum of impurities (for a selective assay)
TSD: Target standard deviation from collaborative trials (as an estimate for the "true" repeatability standard deviation. As an approximation, the pooled repeatability from several series can be used.

The terms (100%-%TSI) and (100%) correspond to the lower and upper basic limits of the synthesis process of a drug substance. The 3fold of TSD describes the variability range of the analytical procedure.

The concept of a target standard deviation is generally acknowledged as a straightforward estimation of the true repeatability is generally acknowledged. Similar target standard deviations for classes of methods or sample types would allow a straightforward and rational definition of acceptance criteria. However, target standard deviations are only reliable estimates after comprehensive experimental assurance. Therefore, it is too early to define definitely general target standard deviations for classes of methods or analytes.
Alternatively, equation 2 can be used (see 3.2.). The lower basic limit LBL then corresponds to %TSI and the analytically required range is calculated from the specific prediction interval of the control test, instead of the general estimation with the threefold of the target standard deviation.

3. Acceptance criteria for the content determination of drug products

Up to now, no common concept is in place for this question. The following is proposed by the Working Group:

3.1. Standard acceptance limits 95 to 105%

For European submissions it is standard practice that the active ingredient in drug products should range between 95 and 105% of the declared content (release limits). These acceptance limits do not require an additional justification.
It is agreed, that this standard practice is a reasonable approach and suitable in most cases. However, it is also clear that there are some cases that require wider limits, with appropriate justification. Possible reasons for a higher variability not permitting the standard limits from 95 to 105% are:

- Unavoidable high batch variability caused by the manufacturing process
- Very small analytical concentrations
- Complex matrix effects
- Unavoidable high variability of the analytical procedure

3.2. Acceptance criteria in dependence on the analytical variability

Appropriate acceptance limits must consider both manufacturing and analytical variability. The latter can be described as a prediction interval of the mean:

$$(2) \qquad AL = 100\% \pm BL \pm \frac{t_{df,95\%} * RSD_R(\%)}{\sqrt{n_{assay}}}$$

AL: Acceptance limits of the active ingredient (in percent of the label claim)
BL: Basic limits, maximum variation of the manufacturing process (in %)
$RSD_R(\%)$: Reproducibility precision (relative standard deviation)
The reproducibility is obtained with the same method and the same sample in different laboratories. This precision level includes, additionally to the random variability of the measurement (including the influence of the reference standard), especially the changes by external factors (e.g. temperature, humidity, quality of reagents, qualification of the operators etc.). The intermediate precision is characterised by variation factors within the same laboratory. However, within longer terms this precision level is approaching the reproducibility, because the a.m. factors change as well Therefore, in this paper reproducibility is used as a general term, including intermediate precision.
Actually one should distinguish between reproducibility and standard deviation corresponding to reproducibility. However, because the use of reproducibility as its corresponding standard deviation is common and cannot be misunderstood, we also use reproducibility in this sense.
n_{assay}: Number of repeated, independent determinations in routine analyses (e.g. different weighings, sample preparations, etc.), as far as the mean is the reportable result, i.e. is compared to the acceptance limits. If each individual determination is defined as the reportable result, 1 has to be used.
t_{df}: t-factor for the degrees of freedom during determination of the standard deviation, correction factor for the reliability of the standard deviation
$RSD_R(\%)/\sqrt{n_{assay}}$: Standard error (in %) under reproducibility conditions, describes the variability of the mean

Usually, the acceptance range for the (release) determination of actives in drug products is symmetrical. In case of asymmetrical limits, the calculation must be performed with the tigher part of the range.

It is quite obvious from this equation, that the standard acceptance criteria (95 to 105%) can be met in most cases:
With an assumed (standard) manufacturing variability of 2.5% and an intermediate precision obtained with two series and six values each, a standard error of approximately 1% is required.

$$(3) \qquad AL = 100\% \pm 2.5\% \pm \frac{2.23 * RSD_R(\%)}{\sqrt{n_{assay}}}$$

The value $t_{0.05,\ 10}$ is 2.23 for a two-sided test. The number of degrees of freedom corresponds to the sum of df in the two series, that this 2*(6-1)=10.

Consequently the standard limits can be met with a relative reproducibility of 1% using single determinations, or with a value of 1.7% with triplicates.

At the time of submission, the basic limits are often not exactly known. However, the assumption of consuming half of the acceptance range by the manufacturing process should be realistic for standard processes. However, any other estimate for the manufacturing variability according to previous experience or data can be used.

If either the manufacturing or the analytical variability is much larger than discussed in the example, equation 2 allows an estimation of suitable individual acceptance criteria. Assuming a manufacturing variability of 2.5%, wider acceptance criteria are required if the standard error results in a value of more than about 1% (performing a reasonable number of repeated determinations).

For example, it is a well known, that the relative standard deviation increases with decreasing concentration (seen 3.3 [7-9]). Due to the increasing analytical variability, wider acceptance criteria are required:

Table 1: Lower (LAL) and upper (UAL) acceptance limits calculated according to equation 3 for a range of analyte concentrations, based on the best estimate for the reproducibility RSD_R(%) from the Horwitz-function (i. e. 50% of the target reproducibility [9]). The number of repetitions in the assay was kept fixed at 3.

c (mg/g)	RSD_R(%)	n	analytical variability	LAL	UAL
100	1,4	3	1,8	95,7	104,3
10	2	3	2,6	94,9	105,1
1	2,9	3	3,7	93,8	106,2
0,1	4	3	5,1	92,4	107,6

In order to maintain the standard limits of 95 to 105%, the increasing analytical variability might be compensated for by increasing the number of determinations in the routine application. However, this quickly leads to a very high number of determinations (see Table 2).

Table 2: Number of repetitions in the assay required to obtain acceptance limits of about 95 to 105%, calculated according to equation 3. The reproducibility RSD_R(%) for a given analyte concentrations corresponds to the best estimate of the Horwitz-function (i. e. 50% of the target reproducibility [9]).

c (mg/g)	RSD_R(%)	n	analytical variability	LAL	UAL
100	1,4	3	1,8	95,7	104,3
10	2	4	2,2	95,3	104,7
1	2,9	7	2,4	95,1	104,9
0,1	4	13	2,5	95	105

The same reasoning can be applied for otherwise caused higher analytical variability, such as matrix effects from the placebo, or a trend over longer time periods. Even if the scattering of individual analytical results is very high, it is theoretically possible to keep the variability of the mean low. However, this already requires 100 repeated determinations for a reproducibility of 10 percent. Such number of repetitions is usually unacceptable unless the applied analytics is extremely fast and cheap. Therefore, the Working Group recommends not

to go beyond three determinations, unless tighter acceptance criteria are required by safety considerations, or an increase in the number of repetitions is easily possible.

These considerations show, that wider acceptance limits should be taken into consideration if the reproducibility exceeds approximately 2%.

3.3. How to estimate the analytical variability?

There are only few publications concerning the reproducibility in pharmaceutical analyses. Therefore, a reliable estimation of the ranges of analytical variability or a generalisation is not possible. During method validation, the intermediate precision is determined for each analytical procedure. This value can be used as an estimate of the reproducibility and for the establishment or verification of acceptance limits, as far as those factors relevant for the future applications of the analytical procedures were a taken into consideration during validation. However, besides the reliability of these estimates, the question arises about how predictive they are for future long-term applications.

The Working Group has recognised further need for a general investigation of repeatability and reproducibility precisions to obtain information about usual ranges for individual precisions and the ratio between the precision levels, if classification for methods or types of drug products are possible, etc. Therefore, the Working Group has started a compilation of respective data. If the reader is interested to participate or for further information, please contact the authors. The obtained information will be summarised regularly and jointly published with the participants in an appropriate manner.

A rather simple relationship between analytical variability and the analyte concentrations was found by Horwitz, based on a comprehensive data compilation, mainly from food and environmental analysis. The relative reproducibility was mainly dependent on the concentration of the analyte in the original sample (concentration fraction, in g/g) [7-9] and relatively independent on the applied analytical method and the sample preparation (see Table 1).
It is obvious that the Horwitz-function should be used rather as orientation than as prediction for a specific (type of) drug product. It is a general relationship for a variety of analytical methods resulting in target reproducibilities for large concentration ranges. However, the Horwitz-function is very useful as a general orientation and to justify which precision is not realistic for a certain concentration. In many cases of small analyte concentrations, it can be concluded that a standard error of 1% is only possible with an unreasonable high number of determinations (see Table 2).

4. Acceptance criteria for impurities: A proposal based on ICH Q6A

Acceptance limits for impurities can be justified in several ways. The Working Group suggests a possible approach based on the ICH-guideline Q6A.

The (upper) acceptance limit AL for an impurity is defined according to the following equation:

(4) $$AL = \bar{x} + 3 \cdot \hat{\sigma}$$

The obtained values are rounded to one decimal place. Mean and standard deviation should be determined for at least five representative, if possible subsequent batches from clinical phases II and III. Of course, the limit thus obtained has to be qualified toxicologically.

For the total sum of impurities, a recommendation has been avoided on purpose. Here, a case-by-case approach has to take into account individual aspects.

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