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ScienceDirect
Journal of Pharmaceutical and Biomedical Analysis 43 (2007) 708–710
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
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Short communication

Comparison of the recovery spread in analytical development and routine quality control—Based on the ICH quality guideline Q2B

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Received 17 March 2006; received in revised form 3 July 2006; accepted 3 July 2006
Available online 9 August 2006

Abstract

In the present study, a simulation was performed for the ICH Q2B guideline for assessing the accuracy. By means of an experimental data set a permutation has been performed to investigate in which interval experimental mean recovery can be expected to scatter just by random effects. A good agreement has been found between the experimental intervals obtained by means of a permutation and the statistically derived confidence intervals. These findings could be confirmed with additionally generated virtual data sets with a true mean of 100% and a true standard deviation of 0.7%.

Keywords: ICH Q2B; Accuracy; Permutation; Confidence interval; Quality control

1. Introduction

The demonstration of suitability of analytical procedures for their intended purpose is of great importance to ensure quality, safety and efficacy of pharmaceuticals. Consequently, analytical validation has been in the focus of regulatory requirements for a long time. However, a sensible validation is also essential from a business perspective, because analytical data are the basis of many decisions such as batch release, establishment and verification of shelf life, etc.

The ICH Guideline Q2B details basic requirements for the various validation characteristics and some methodological recommendations. With respect to accuracy, "a minimum of nine determinations over a minimum of three concentration levels covering the specified range (e.g. three concentrations/three replicates)" should be used [1]. Taking the minimum working range required for an assay of a drug product, this leads to "synthetic mixtures of the drug product components" to which 80, 100 and 120% of the nominal content of the drug substance has been added (three times each), also called spiking [1]. These samples are then analyzed, applying the quantitation mode described in the intended analytical procedure. For smaller working ranges (factor between upper and lower limit less than 10), preferably the ratio between added and found amount of analyte is calculated, as percent recovery [1,2]. The mean recovery is compared to the theoretical result of 100% and evaluated whether or not the deviation is of an acceptable size. Additionally, the variability of the data can be calculated.

The objective of accuracy investigation is to identify systematic errors, i.e. a deviation beyond random variability. However, in case of a limited number of data, the mean result can be strongly influenced by random effects. These influences can then be interpreted as deviations.

The ICH-design is investigated for the influence of random effects on the mean recovery, in order to provide orientation to better define acceptance criteria for accuracy.

In the present work, according to the method of a more comprehensive real study of precision and recovery over a wide concentration range [3], for each of the concentrations 80, 100 and 120% six spiked samples were analyzed. From this 6×3 data matrix, all possible combinations of 3×3 data sets (according to the ICH requirement) were generated and the mean recoveries were calculated.

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doi:10.1016/j.jpba.2006.07.012

Afterwards a simulation was performed for the ICH 3×3 design assuming a true mean of 100% and a true standard deviation of 0.7% in order to confirm the results obtained by the experimental data.

2. Method

2.1. Experimental HPLC-data

Using the HPLC-method and the experimental set up as described in Ref. [3] the concentration levels 80, 100 and 120% of the article have been determined again to get values close to 100%. Thus, 18 new single values (6 of each concentration) for recovery have been obtained.

In Ref. [3] the main compound glibenclamide has been determined as a drug substance next to a tablet matrix by means of a reversed-phase method. Six standard solutions and six samples were measured. A concentration of 100% corresponded to 0.2 mg/mL.

Further experimental details were: the mobile phase (acetonitrile–buffer, 45:55, v/v) at a pH of 3 was prepared by dissolving 650 mg sodium dihydrogen phosphate dihydrate in 550.0 g water and adding two drops of phosphoric acid 85% and 351.5 g acetonitrile to 1000 mL. For the sample solvent (acetonitrile–buffer, 80:20, v/v) 190.0 g water, 10.0 g of 71 mM phosphate buffer pH 7 and 625.0 g acetonitrile were dissolved to 1000 mL. Phosphate buffer pH 7 was prepared by dissolving 0.88 g potassium dihydrogenphosphate and 1.82 g disodium hydrogenphosphate dihydrate in 250 mL water. The tablet matrix was mainly consisted of lactose monohydrate.

2.2. Generation of data sets

In order to obtain similar data to the performed HPLC-method given in Ref. [3] several virtual normally distributed data sets of the same structure (standard deviation of 0.7% and mean of 100%) have been generated by means of Microsoft Excel for the concentration levels 80, 100 and 120%. Six single values of the recovery of the three concentration levels were the basis of the subsequent calculations yielding 18 values.

2.3. Calculation of the statistical parameters

Using the maximum statistical information from all of the 18 values of the data sets the mean recovery (1), the standard deviation (2), the 95%-confidence interval for the mean (3) and the 95%-prediction interval for the mean (4) have been calculated as follows [4]:

$$\bar{x} = \sum_{i=1}^{18} x_i \tag{1}$$

$$\hat{\sigma} = \sqrt{\frac{\sum (x_i - \bar{x})^2}{n-1}} \tag{2}$$

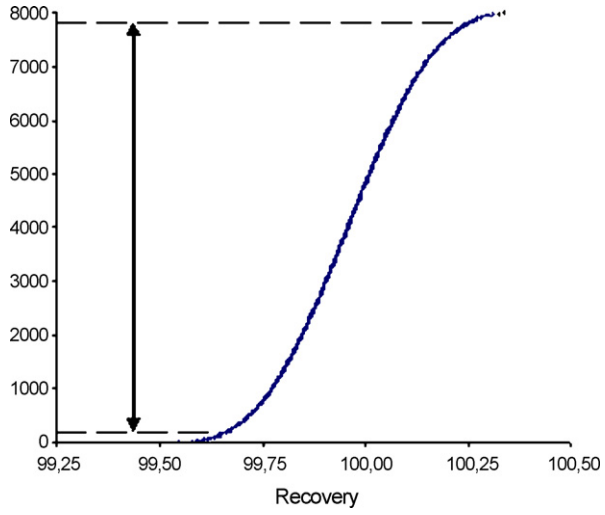

Fig. 1. Graphical overview of the 8000 mean recoveries sorted in ascending order obtained by permutation. The interval where 95% of all values can be found is marked by the arrow.

$$\mathrm{cnf}(\bar{x}) = \bar{x} \pm t_{17,0.975} \cdot \hat{\sigma} \cdot \sqrt{\frac{1}{18}} \tag{3}$$

$$\mathrm{prd}(\bar{x}) = \bar{x} \pm t_{17,0.975} \cdot \hat{\sigma} \cdot \sqrt{\frac{1}{18} + \frac{1}{9}} \tag{4}$$

Here $t_{17,0.975}$ is the t-value with d.f. $= n - 1 = 17$ degrees of freedom and an error probability.

2.4. Permutations

The permutation of a data set was performed in the following way: every possible combination of taking three out of six single values from each of the three concentrations 80, 100 and 120% was created (3×3). So the total number of combinations yield $20^3 = 8000$ different 3×3 data sets.

2.5. Calculation of the distribution of the permutation

Then the mean recoveries for all 3×3 data sets were calculated according to Eq. (1). These parameters were sorted in ascending order as depicted in Fig. 1.

From this array the lower and upper limits of the interval, where 95% of the values can be found, have been obtained by choosing the 200th and the 7801st of the 8000 values.

3. Results and discussion

Comparing the calculated statistical 95%-confidence intervals for the mean values of the data sets (marked with (2) in Fig. 2) with the 95%-intervals obtained by means of the permutation (marked with (1)), it can be seen, that for all cases the 95%-interval of the permutation is completely within the statistical 95%-confidence interval (see Fig. 2).

The very similar size of these intervals is just a coincidence due to the chosen number of data. The 95%-prediction inter-

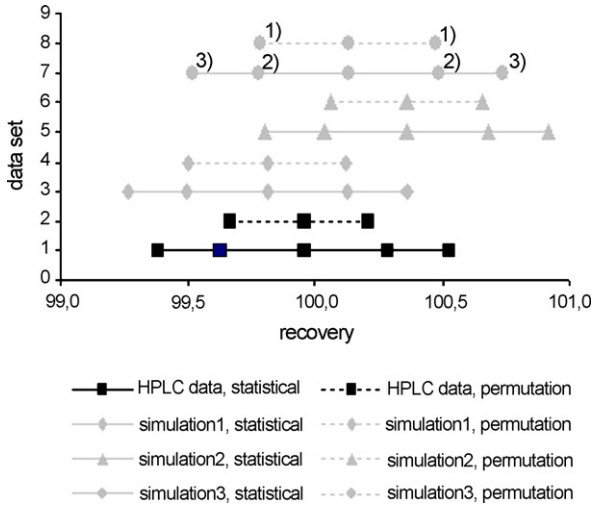

Fig. 2. The figure shows all calculated intervals for all data sets and its permutations. In rows (1) and (2) the intervals of the HPLC-data and from rows (3–8) the intervals of three further generated data sets are shown.

val (marked with (3)) is systematically a bit larger than the 95%-interval of the permutation. The real HPLC-data as well as the data of the simulations give the same results. The 95%-confidence interval and the 95%-interval of the permutation have a comparable size, whereas the prediction interval produces broader intervals. It is not possible to get an idea for future results by means of building the spread with a permutation. One can conclude, that the information of the permutation is not larger than the statistical information given by the full $n = 18$ data set.

Acknowledgment

We are grateful to Heidi Köppel for critically reading the manuscript.

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