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Review

Methodologies for the transfer of analytical methods: A review☆

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ABSTRACT

The transfer of a method from a laboratory to a production site is an important step in the development cycle of new pharmaceutical products. Method transfers are increasingly implemented due to the economical pressure coming from the rationalization of production sites, analytical subcontracting and fusion of pharmaceutical groups. However, no official guidance regarding study design, data analysis, or decision procedures is present neither in FDA documents nor in ICH documents for method transfers. The experiments performed in such a transfer and the methodology used to accept or reject it should be fitted for purpose. In order to provide to analysts a global view of the problematic of analytical method transfer, this paper reviews the documentation available in the scientific literature about the design of transfer studies and the required sample size. Special focus is also made on the statistical methodologies available for decision making with particular emphasis on risk management. Examples of transfer of pharmaceutical, bio-pharmaceutical and biological methods published in the literature are reviewed in order to illustrate the various possibilities among the strategies for methods transfer.

Contents

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Nomenclature

$\bar{X}_{ij}$	average of the results of the series j of laboratory i; $i=1$ for the sending laboratory and $i=2$ for the receiving one.
$\bar{X}_i$	overall average of the results of the laboratory i.
X_{ijk}	kth result of the jth series of laboratory i.
J_i	number of series of laboratory i.
K_i	number of replicates of laboratory i.
MSE_i	ANOVA 1 mean square error of laboratory i.
MSM_i	ANOVA 1 mean square of the model of laboratory i.
$\hat{\sigma}^2_{w,i}$	within series or repeatability variance of laboratory i.
$\hat{\sigma}^2_{B,i}$	between-series variance of laboratory i.
$\hat{\sigma}^2_{IP,i}$	intermediate precision of laboratory i; it is the sum of the repeatability and between-series variances.
$\hat{R}$	ratio of the between-series variance over the repeatability variance.
$\hat{RSD}_{IP,i}$	intermediate precision relative standard deviation of laboratory i.
$L_{U,RSD_{IP,2}}$	one-sided upper confidence limit of the intermediate precision relative standard deviation of the receiving laboratory $i=2$.
$Q_t(df,\alpha)$	αth percentile of a Student distribution with df degrees of freedom.
$\chi^2_{\alpha,df}$	αth percentile of a chi-square distribution with df degrees of freedom.
β	coverage value of the β-expectation tolerance interval.
$\pm\lambda_T$	acceptance limits for the trueness (systematic error) criterion.
$\lambda_{RSD_{IP}}$	acceptance limit for the precision (random error) criterion.
$\pm\lambda$	acceptance limits for the accuracy (total error) criterion.
$[-\lambda_{adj}; +\lambda_{adj}]$	adjusted acceptance limits for the accuracy (total error) criterion with respect to the standard error of the mean results of the sending laboratory.

1. Introduction

An analytical method transfer is a complete process that consists in transferring a validated analytical method from a sending laboratory (called sender) to a receiving laboratory (called receiver) after having experimentally demonstrated that it also masters the method [1,2]. The transfer of a method from a laboratory to a production site is an important step in the development cycle of new pharmaceutical products. Method transfers are increasingly used due to the economical pressure coming from the rationalization of production sites, analytical subcontracting and fusion of pharmaceutical groups. The transfer of analytical method is also a part of the technology transfers of the production of drug product to different sites. Few documents or publications focusing on general principles of production transfers exist and this is also true for analytical methods transfer [1–7].

Analytical methods transfer is now fully integrated into the life cycle of an analytical method in the pharmaceutical industry. Regulatory agencies, such as the US Food and Drug Administration (FDA), require that a transfer from development to quality control laboratory, to or from contract or process laboratories should be performed to certify that the receiver is qualified to execute the methods during their future routine application. Similarly, to achieve necessary analytical throughput, bioanalytical methods are also transferred from one laboratory to another, and in some situations, the samples from a clinical study are analyzed by different laboratories. Thus, analytical methods transfer should ensure that the results obtained by the receiver will be reliable and comparable to the ones which would have been obtained by the sending laboratory in order to take the adequate subsequent decisions, such as the decision to release a batch of drug product, to evaluate pharmacokinetic studies, bioequivalence studies or to decide about the illness of a patient.

2. Aim of the analytical method transfer

Like for method validation, method transfer is the last step before the routine use of the method at the receiving laboratory. The receiver must therefore give guarantees that he has the capacity to implement the method and, above all, that he is able to obtain reliable results. The main aim of analytical method transfers is thus to give guarantees to the laboratories as well as to regulatory bodies that each future result that will be obtained during routine analysis by the receiving laboratory will be close enough to the unknown true quantity of analyte present in the assayed sample [8]. Other connected objectives linked to the method transfer can be to provide training and expertise to the receiving laboratory. Thus methods transfer should be a documented demonstration of the ability of the receiver to provide reliable results.

3. Regulatory expectations and guidelines

For method validation the general methodology is described in ICH Q2R1 guideline [9] or FDA guidance for the validation of bioanalytical methods [10]. However no detailed official guideline exists for a transfer methodology in pharmaceutical or biopharmaceutical analysis. Nonetheless, it should be reminded that according to ICH Q9 guideline [11], risk analysis and management should be integrated into a transfer process. When looking deeper in the FDA guidance for the validation of bioanalytical methods, methods transfer between laboratories is considered as partial validation. However, no guidance regarding study design, data analysis, or decision procedures is present neither in this FDA document nor in ICH documents for method transfers.

The experiments performed in such a transfer and the methodology used to accept or reject it should be fitted for purpose. Indeed, the process could take months and it is not realistic to perform such a long transfer in research or in an early development. However, when the method is transferred from development to production or when the quality control of a production is outsourced, it is highly critical to have all the guarantees that the method is mastered by the receiver in order to avoid problems in the future. To corroborate the importance of this process, the Food and Drug Administration (FDA) mentions that analytical transfers appeared frequently in their 483 observations [12] in the last years. Facing this lack of regulatory guidance, the FDA collaborated with the International Society for Pharmaceutical Engineering (ISPE) to publish a guideline on transferring expertise and technology associated with analytical methods [13,14]. Recently (April 2007), a draft guidance of the Center for Veterinary Medicine of the FDA dedicated to analytical method transfer and entitled “Protocols for the Conduct of Method Transfer Studies for Type C Medicated Feed Assay Methods” has been available [15]. However, this draft document only gives general principles for the preparation of the transfer of these particular assays and proposes a minimum sample size to analyze. No recommendations on how to decide about the acceptability of the transfer or on how to select an optimal sample size are provided in this draft guidance. Besides, a commission of the French Society for Pharmaceutical Sciences and Techniques (Société Française des Sciences et Techniques Pharmaceutiques—SFSTP) has worked

on a practical guideline to evaluate analytical method transfers dedicated to the quality control of pharmaceutical products [2]. Another SFSTP commission [16] has published a guideline about the transfer of biological methods (or bioassays) which similarly provides general guidelines for setting up methods transfer together with some statistical methodologies and examples of applications (such as the evaluation of viral activity by titration in cells, antibiotic microbiological assay by diffusion, . . .).

4. Analytical method transfer steps

An analytical method transfer can usually be composed of several steps. First, a team including representatives from the diverse sites and disciplines concerned is formed (Research and Development (R&D), Quality Control (QC), Manufacturing, Statistics). Then, the development of the analytical method transfer protocol includes [2,17]:

- The transfer of the scientific documentation,
- The reference samples to be used,
- The detailed analytical procedure(s) that will be transferred dedicated to impurity determination, assay content, dissolution, and so on,
- The statistical design, sample size, data analysis and decision procedures,
- The training of the personnel involved at the receiving site,
- Execution of the method transfer,
- Analysis of the results obtained and decision about the acceptability of the method transfer which is included in a transfer report.

General information about what to include in the protocol, which documentation to transfer and content of the report can be found in ref. [2].

5. Evaluation of the method transfer

Due to the lack of formal guidance or regulatory requirements, several approaches are possible to select the experimental design, for choosing the statistical data treatment and hence for the decision process. The success of an analytical method transfer is tested by comparing results or their summary parameters such as the means and variances of the participating laboratories obtained after analyzing similar samples. If the test results suggest the rejection of a method transfer, two possibilities may arise as shown in Table 1:

- the correct rejection of an inappropriate transfer or,
- rejecting a good method transfer (producer risk).

Table 1
Consumer and producer risk when making decision about the transfer of analytical methods. The columns show the two possible decisions made at the end of the transfer step. The lines show the reality about the transfer (never known in practice): truly acceptable or truly unacceptable.

		Transfer decision	
		Accepted	Not accepted
Transfer truly	Acceptable	Correct decision	Rejection of a satisfactory method transfer (Producer risk)
	Not acceptable	Acceptation of defective method transfer (Consumer risk)	Correct decision

For the other situation, if the results imply accepting a method transfer, two underlying possibilities are again possible (Table 1):

- the decision to accept the transfer is correct or,
- the decision to accept an inappropriate transfer (consumer risk).

This decision has to be made without the knowledge of the true situation. The probability that one of these cases arises when deciding about the acceptability of a method transfer depends on the statistical test used [8,18–20]. The consumer risk is the most important one [11]. It should be strictly controlled because its consequence would be, for instance, the release of a pharmaceutical lot based on inadequate reliability of the results obtained in pharmaceutical routine control.

The main criteria that are evaluated during the transfer of quantitative analytical methods are trueness, precision and above all accuracy. Accuracy is "*the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found*." [9,10,21–24]. It therefore refers to total measurement error. Trueness refers to "*the closeness of agreement between the average value obtained from a large series of test results and an accepted reference value*" [9,21–24]. This concept is therefore related to the systematic error of a measurement process. Finally, precision refers to "*the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions*" [9,10,21–24]. This is related to the random error of a measurement process. The ISPE and SFSTP [2,13,16] guides focus on the trueness and precision criteria to evaluate the acceptability of the method transfer. It is important to understand, as shown in Fig. 1, that if one wishes to assess method transfer by evaluating trueness and precision separately, it should not be limited to the evaluation of the trueness criteria as too frequently encountered in practice.

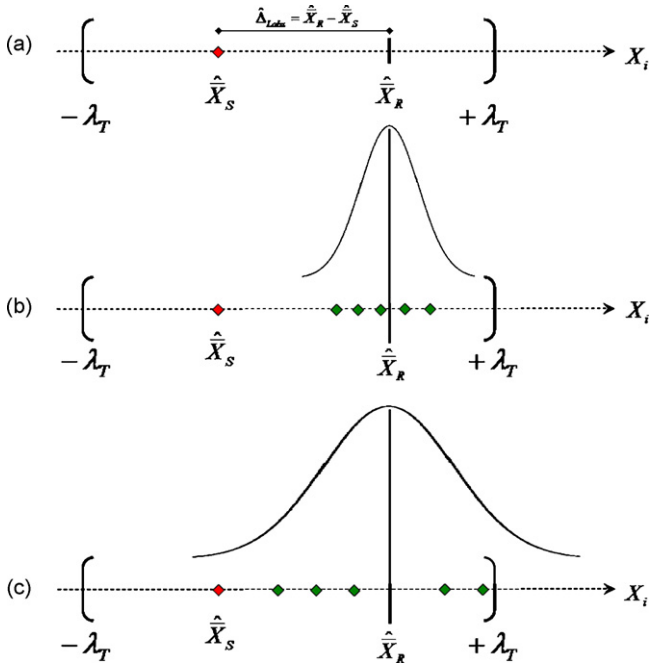

Fig. 1. Illustration of the importance to evaluate not only trueness for an analytical method transfer but to add the evaluation of the precision of the receiver. $[-\lambda_T; +\lambda_T]$ is the acceptance limits for trueness, $\hat{\bar{X}}_S$ and $\hat{\bar{X}}_R$ are the mean results for the sender and receiver, respectively, $\hat{\Delta}_{Labs}$ is the bias between the two laboratories; (a) Trueness of the transfer measured by bias between the two laboratories ($\hat{\Delta}_{Labs}$) is acceptable; (b) Trueness is acceptable and the precision (variability) of the receiver is acceptable; (c) Trueness is acceptable but the precision of the receiver is not acceptable.

Table 2
Risk management corresponding to the various statistical approaches relative to analytical methods transfer.

Statistical approach	Producer risk (transfer is erroneously rejected)	Consumer risk (transfer is erroneously accepted)	Risk of obtaining Out Of Acceptance limits (OOAc) results*
Descriptive	Not controlled	Not controlled	Not controlled
Equivalence	Sample size dependent	Strictly controlled	Not controlled
Total error	Sample size dependent	Strictly controlled	Strictly controlled and known

* If the acceptance limits are the release limits, then the OOAc risk becomes the risk to obtain Out Of Specification (OOS) results.

Indeed, if the bias between the two laboratories is acceptable as shown in Fig. 1a, two situations are possible for the dispersion of the receiver results around its mean: either it is acceptable (Fig. 1b) or it is not (Fig. 1c). Thus, when using the classical approaches both criteria (trueness and precision) should be evaluated before deciding about the acceptability of the transfer. For each of these two criteria different classical approaches are proposed:

A descriptive one which compares the point estimates of the receiving laboratory intermediate precision relative standard deviation to an acceptance limit and the point estimates of the bias between the two laboratories to another limit [2,20,25].

Another approach called the difference approach is performed and uses a Student *t* test for the bias between the two laboratories [2,20]. This approach is usually only performed for the trueness criterion. However a similar approach could be used to assess the precision one even if it is rarely used.

Finally a third approach called the equivalence approach can be used which compares the confidence interval of the bias between the two laboratories to specific acceptance limits and the upper limit of the receiving laboratory intermediate precision relative standard deviation to another limit [2,13,20,26–29]. The equivalence approach for the trueness criterion is also known as "TOST" meaning "Two One-Sided *t*-Test" [26,27]. The equivalence approach is currently a relatively frequently used approach to assess methods transfer [14,29–32].

Comparison of the behavior of the previous approaches can be found in refs. [8,18,20]. Table 2 summarizes the main conclusion between the comparisons of these approaches in terms of risk management. As can be seen from Table 2, the only classical approaches which control the consumer risk are the equivalence ones. It has also been shown that the difference approaches are definitely not fit to the objective of analytical method transfer and should be avoided to assess the transfer [18,20,26,27]. Thus no more reference to the difference approaches will be made in the remaining of this document. Finally, it can be understood that these classical approaches do not look at the final aim of quantitative analytical methods: the analytical results. Indeed, when applying those approaches, they do not give any information about the quality of the analytical results generated by the method at the receiving site [19].

Another methodology proposed for the evaluation of methods transfer is to use inter-laboratory tests [33,34]. This approach could be used if the method is transferred simultaneously to several different receiver laboratories and if the final aim of the study would be to analyze the samples by any of the laboratories involved. However, in order to obtain reliable estimates, guidelines and regulatory documents about inter-laboratory studies require at least five different laboratories [35]. It thus involves a relatively large amount of laboratories which is rarely met in practice for the transfer of pharmaceutical or bioanalytical methods.

Finally, an innovative and unique total error-based criterion to assess method transfer has been proposed [8,18,19,36]. This methodology considers simultaneously the systematic and random components of error, while at the same time taking into account the uncertainty of the true value estimated by the sending laboratory [8,18,19]. To achieve this, a β-expectation tolerance interval is computed, that defines a region where a defined proportion (*e.g.* 95% or 99%...) of the receiver future results is expected to fall, and compared to acceptance limits adjusted according to the uncertainty of the true value estimated from the sender results. Comparison of the risk management with the previous classical approaches is also shown in Table 2. As can be seen in this table, this last approach is the only one which allows to adequately answer the objective of an analytical method transfer by providing to the laboratories as well as to regulatory bodies the guarantee that the future individual results of the receiver will be accurate and thus reliable for decision making [19]. Table 2 shows that, in addition to the control of the consumer risk, the total error approach proposed by Dewé et al. also controls the risk to obtain Out Of Acceptance limits (OOAc) results [8,19]. Indeed, the inclusion of the β-expectation tolerance interval into the acceptance limits reveals that the probability to obtain a future results of the receiver outside these limits is at most 1-β [19,37,38]. These predictions have been shown extremely reliable using examples of analytical methods validation studies [39]. Finally, if the acceptance limits are the specification limit of the product as it is often done in practice in method transfers, then this approach controls the risk to obtain Out Of Specification (OOS) results.

5.1. The statistics to perform

Whatever the approach chosen, the first step is to perform a one way analysis of variance (ANOVA 1) on the results of each of the laboratory (i = 1–2), since it is the statistical model underlying the experiments performed. This random ANOVA 1 model with series (or runs) as random factor is as following:

$$\begin{aligned} &X_{ijk} = \mu_i + \alpha_{j(i)} + \varepsilon_{jk(i)} \\ &\alpha_{j(i)} \sim N(0, \sigma^2_{\mathrm{B},i}) \\ &\varepsilon_{jk(i)} \sim N(0, \sigma^2_{\mathrm{W},i}) \end{aligned} \quad (1)$$

where μ_i is the ith laboratory overall mean, $\mu + \alpha_{j(i)}$ is the mean in series j of laboratory i, $\varepsilon_{jk(i)}$ is the residual of the kth replicates of the jth series in the ith laboratory. $\sigma^2_{\mathrm{B},i}$ is the run-to-run variance of the ith laboratory and $\sigma^2_{\mathrm{W},i}$ is the within-run or repeatability variance of the ith laboratory.

This model allows to obtain the estimates of the laboratories means and their variance components [40], with J_i being the number of series performed at the sending (i = 1) or receiving (i = 2) laboratory and K_i is the number of replicates per series:

$$\mathrm{MSM}_i = \frac{1}{J_i - 1} \sum_{j=1}^{J} K_i (\bar{x}_{ij} - \bar{x}_i)^2 \quad (2)$$

$$\mathrm{MSE}_i = \frac{1}{J_i K_i - J_i} \sum_{j=1}^{J} \sum_{k=1}^{K} (x_{ijk} - \bar{x}_{ij})^2 \quad (3)$$

$$\text{where} \quad \bar{x}_i = \frac{\sum_{j=1}^{J} \sum_{k=1}^{K} x_{ijk}}{J_i K_i} \quad \text{and} \quad \bar{x}_{ij} = \frac{\sum_{k=1}^{K} x_{ijk}}{K_i}.$$

$$\text{If } \mathrm{MSE}_i < \mathrm{MSM}_j, \text{ then } \begin{cases} \hat{\sigma}^2_{\mathrm{W},i} = \mathrm{MSE}_i \\ \hat{\sigma}^2_{\mathrm{B},i} = \dfrac{\mathrm{MSM}_i - \mathrm{MSE}_i}{K_i} \end{cases}$$

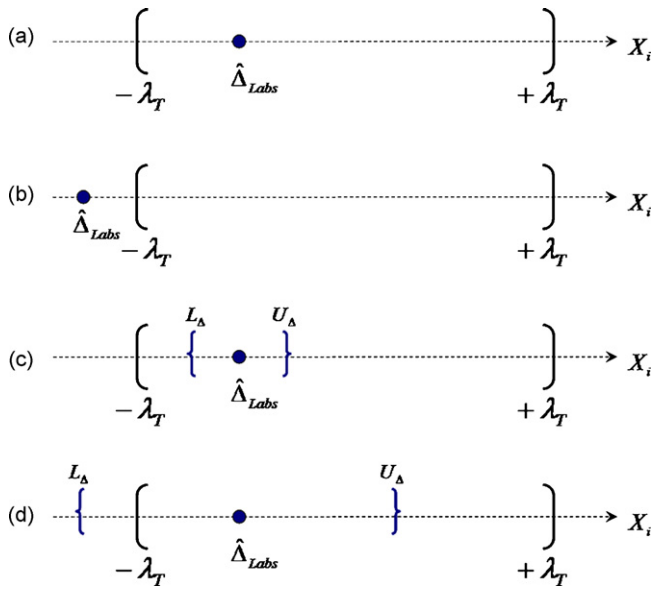

Fig. 2. Decision rule for the statistical approaches dedicated to the evaluation of the analytical method transfer trueness (bias). (a and b) The descriptive approach, where (a) is acceptable and (b) not. (c and d) The equivalence approach, where (c) leads to the acceptation of the transfer and (d) its rejection. $\hat{\Delta}_{Labs}$: Bias between the two laboratories; $[-\lambda_T; +\lambda_T]$ are the acceptance limits for trueness and $[L_\Delta; U_\Delta]$ is the confidence interval of the bias.

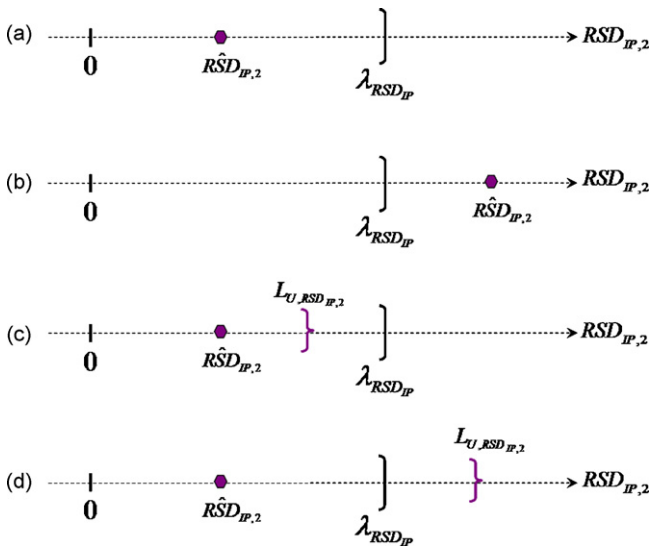

Fig. 3. Decision rule for the statistical approaches dedicated to the evaluation of the analytical method transfer precision. (a and b) The descriptive approach, where (a) is acceptable and (b) not. (c and d) The equivalence approach, where (c) concludes in the acceptation of the transfer and (d) its rejection. $\hat{RSD}_{IP,2}$: relative standard deviation (RSD) for intermediate precision (IP) of the receiving laboratory; $\lambda_{RSD_{IP}}$ is the acceptance limit for precision and $L_{U,RSD_{IP,2}}$ is the upper limit of the confidence interval of the $RSD_{IP,2}$.

$$\text{Else} \begin{cases} \hat{\sigma}^2_{W,i} = \dfrac{1}{J_i K_i - 1} \displaystyle\sum_{j=1}^{J} \sum_{k=1}^{K} (x_{ijk} - \bar{x}_i)^2 \\ \hat{\sigma}^2_{B,i} = 0 \end{cases}$$

Then, the intermediate precision variance at each laboratory can be estimated using:

$$\hat{\sigma}^2_{IP,i} = \hat{\sigma}^2_{B,i} + \hat{\sigma}^2_{W,i}. \quad (4)$$

For the relative bias:

$$\left[100 \left\{ \left(\frac{\bar{x}_2 - \bar{x}_1}{\bar{x}_1} \right) - Q_t(\mathrm{df}, \alpha) \frac{\hat{\sigma}_{\bar{x}_2 - \bar{x}_1}}{\bar{x}_1} \right\}; \; 100 \left\{ \left(\frac{\bar{x}_2 - \bar{x}_1}{\bar{x}_1} \right) + Q_t(\mathrm{df}, \alpha) \frac{\hat{\sigma}_{\bar{x}_2 - \bar{x}_1}}{\bar{x}_1} \right\} \right] \quad (6)$$

with $\hat{\sigma}^2_{\bar{x}_2-\bar{x}_1} = \hat{\sigma}^2_{\bar{x}_1} + \hat{\sigma}^2_{\bar{x}_2} = (\hat{\sigma}^2_{B,1}/J_1 + \hat{\sigma}^2_{W,1}/J_1K_1) + (\hat{\sigma}^2_{B,2}/J_2 + \hat{\sigma}^2_{W,2}/J_2K_2)$, and $Q_t(\mathrm{df}, \gamma)$ is the γth percentile of a Student distribution with df degrees of freedom computed according to: $\mathrm{df} = (J_1 + J_1 - 2)$ or more accurately based on the Satterthwaite [43] approximation:

$$\mathrm{df} = \frac{\{(\hat{\sigma}^2_{B,1}/J_1 + \hat{\sigma}^2_{W,1}/J_1K_1) + (\hat{\sigma}^2_{B,2}/J_2 + \hat{\sigma}^2_{W,2}/J_2K_2)\}^2}{(\hat{\sigma}^2_{B,1}/J_1)^2/J_1 - 1 + (\hat{\sigma}^2_{W,1}/J_1K_1)^2/J_1K_1 - J_1 + (\hat{\sigma}^2_{B,2}/J_2)^2/J_2 - 1 + (\hat{\sigma}^2_{W,2}/J_2K_2)^2/J_2K_2 - J_2}. \quad (7)$$

5.1.1. Trueness

5.1.1.1. Descriptive approach. For the descriptive approach, the bias (or relative bias) between the sender laboratory (1) and the receiver laboratory (2) is computed: $\bar{x}_2 - \bar{x}_1$ (or $100 \times \bar{x}_2 - \bar{x}_1/\bar{x}_1$), and compared to an acceptance limit $\pm\lambda_T$. If the observed bias (relative bias) is included inside these acceptance limits then the transfer is accepted (Fig. 2a), else it is rejected as shown in Fig. 2b.

5.1.1.2. Equivalence approach. For the equivalence approach the 90% confidence interval of the bias or relative bias is computed and then compared to an acceptance limit [41,42]:

For the bias:

$$\left[(\bar{x}_2 - \bar{x}_1) - Q_t(\mathrm{df}, \alpha) \hat{\sigma}_{\bar{x}_2 - \bar{x}_1}; (\bar{x}_2 - \bar{x}_1) + Q_t(\mathrm{df}, \alpha) \hat{\sigma}_{\bar{x}_2 - \bar{x}_1} \right] \quad (5)$$

If the confidence interval of the bias (or relative bias) is fully included in the acceptance limits $[-\lambda_T; +\lambda_T]$ then the transfer is accepted (Fig. 2c), else it is rejected as shown in Fig. 2d.

5.1.2. Precision

5.1.2.1. Descriptive approach. The descriptive approach for the precision criterion is made by comparing the intermediate precision relative standard deviation of the receiving laboratory $\hat{RSD}_{IP,2} = 100 \times \hat{\sigma}^2_{IP,2}/\bar{x}_2$ to a predefined acceptance limit $\lambda_{RSD_{IP}}$. The transfer can be accepted with respect to precision if $\hat{RSD}_{IP,2}$ is smaller than this limit (Fig. 3a) else it is rejected (Fig. 3b).

5.1.2.2. Equivalence approach. For the equivalence approach dedicated to the precision evaluation, it is the one sided 95% upper confidence limit of the $RSD_{IP,2}$ ($L_{U,RSD_{IP,2}}$; [44]) that is computed and then compared to an a priori settled acceptance limit ($\lambda_{RSD_{IP}}$):

$$\text{If } \mathrm{MSE}_i < \mathrm{MSM}_i, \text{ then } L_{U,\sigma^2_{IP,2}} = \hat{\sigma}^2_{IP,2} + \sqrt{\left(\frac{J_2 - 1}{\chi^2_{\alpha, J_2 - 1}} - 1 \right)^2 \left(\frac{\mathrm{MSM}_2}{K_2} \right)^2 + \left(\frac{J_2K_2 - J_2}{\chi^2_{\alpha, J_2K_2 - J_2}} - 1 \right)^2 \left(1 - \frac{1}{K_2} \right)^2 \mathrm{MSE}_2^2} \quad (8)$$

Else $L_{U,\sigma^2_{IP,2}} = \dfrac{(J_2K_2 - 1)\,\hat{\sigma}^2_{IP,2}}{\chi^2_{\alpha,J_2K_2-1}}$,

where $\chi^2_{\theta,\gamma}$ is the θth quantile of a chi-square distribution with γ degrees of freedom.

Finally the upper limit is: $L_{U,RSD_{IP,2}} = 100\sqrt{L_{U,\sigma^2_{IP,2}}}/\bar{x}_2$. As shown in Fig. 3c, if this upper limit is smaller than the acceptance limit then the transfer is accepted else it is rejected (Fig. 3d).

5.1.3. Accuracy

Recently another approach which takes into account the total measurement error (i.e. the simultaneous combination of random and systematic errors) has been proposed [8,18,19]. For data designed by a balanced one way ANOVA, Mee [45] has developed a β-expectation tolerance interval aimed at estimating the interval in which a proportion β of the measured population is expected to belong. The lower and upper β-expectation tolerance limits for the receiving laboratory ($i = 2$) results ($[L_2, U_2]$) can be computed using: $[L_2, U_2] = [\bar{x}_2 - k\hat{\sigma}_{IP,2}; \bar{x}_R + k\hat{\sigma}_{IP,2}]$ where k is calculated in order to have an expected proportion β of the population within this interval. The formula of this tolerance interval is given by:

$$k = Q_t\left(\mathrm{df}, \frac{(1+\beta)}{2}\right)\sqrt{1 + \frac{J_2\hat{R} + 1}{J_2K_2(\hat{R}+1)}}$$

$$\text{with} \quad \mathrm{df} = \frac{(\hat{R}+1)^2}{(\hat{R}+1/K_2)^2/J_2 - 1 + (1-1/K_2)/J_2K_2} \text{ [43]}$$

$$\hat{R} = \frac{\hat{\sigma}^2_{B,2}}{\hat{\sigma}^2_{W,2}} \tag{9}$$

where $Q_t(\mathrm{df}, \gamma)$ is the γth percentile of a Student distribution with df degrees of freedom. This interval is thus an interval in which it is expected that a proportion β (*e.g.* 95% or 99%) of future results that will be obtained by the receiver using the transferred method will be included in the acceptance limits ($\pm\lambda$) of the transfer. However, as pointed by Dewé et al. [8], the true value of the quantity or amount of analyte in the samples is only estimated by the sender, with uncertainty. Therefore this variability of the sender must be taken into account in order to adjust the acceptance limit. These authors have proposed to use the confidence interval $[L_1; U_1]$ of the sender mean result at a user specified confidence limit (*e.g.* 90%, 95%, ...) to perform this adjustment. The acceptance limits are thus reduced proportionally to the standard error of the sender mean result through [8]:

$$[-\lambda_{adj}; +\lambda_{adj}] = [(1-\lambda)U_1; (1+\lambda)L_1]. \tag{10}$$

The analytical transfer will be accepted if the tolerance interval of the results of the receiver is included in the adjusted acceptance limit $[-\lambda_{adj}; +\lambda_{adj}]$, else it is rejected.

5.2. Acceptance limits

Finally, as can be understood from the previous point about the statistical approaches, a main requirement to achieve a conclusion in methods transfer is to settle the acceptance limits. As stated earlier, no guidance for these acceptance limits is available from the regulatory bodies of the pharmaceutical industry such as ICH or FDA. The practical guides of the ISPE or SFSTP providing only examples of acceptance limits [2,13,16], it remains the duty of the transfer team to select the adequate one(s). For the bioanalytical world a way out is possible since in the FDA (2001) guidance for the validation of bioanalytical methods [10], method transfer is considered as partial validation and hence the limits of ±15% for trueness and 15% for precision could be used for the method transfer. Another proposition was made recently in the workshop and conference report of the AAPS/FDA conferences on *Quantitative Bioanalytical Methods Validation and Implementation: Best Practices for Chromatographic and Ligand Binding Assays* where a maximum allowable total error of ±30% has been proposed to assess the validity of bioanalytical methods [46]. A similar acceptance limit could thus be used for bioanalytical methods transfer.

When transferring quality control methods, a common practice is to settle the acceptance limits based on the conventional specification limits for a batch of product, *e.g.* 5% or 10% for drug products, 2–3% for drug substances. However care should be made when using such acceptance limits. If during the transfer, the method is already giving results close to these limits, then there is no much room left for batch or production variability. So, how to define acceptance limits? One could refer to the recent literature about setting products specifications limits where β-content γ-confidence tolerance intervals are used [47,48]. Indeed such intervals will provide the guarantee with γ% confidence (*e.g.* 95%) that β% (*e.g.* 99%) of the results provided by the sending laboratory method will fall within the calculated tolerance interval [49–52]. This interval could then be considered the acceptance limits for the transfer study. One last question remains if one wishes to apply the proposed methodology: on what data should this β-content γ-confidence tolerance intervals be computed?

If the transfer step happens early, then the only information available is the validation of the method accomplished at the sending laboratory. These acceptance limits can be computed based on these results. However, it has to be reminded that the precision of the variance components estimates obtained in validation may not be excellent due to the relative small number of experiments usually performed in validation studies. This may lead to either to tight or to loose tolerance limits and consequently acceptance limits. By opposite, if the method has previously been in use at the sending laboratory, data of the Quality Control (QC) samples used to monitor the in-study performance of the method (for *e.g.* through control charts) will provide better estimates of the method performance parameters, a more reliable β-content γ-confidence tolerance interval and thus adequate acceptance limits. Finally, a "fit-for-purpose" approach for setting acceptance limits for methods transfer (as well as for method validation) could be applied: evaluating the maximum acceptable total error of the method's results for the subsequent routine application of the analytical procedure [53].

5.3. Experimental design

Another difficult question to answer when designing a method transfer is the number of experiments to perform in each laboratory in order to take the adequate decision. Even if method transfer is usually considered to be of less workload than method validation, the number of experiments to perform should be made in order to reduce the consumer and producer risks.

In the pharmaceutical fields, an analytical transfer is usually performed with samples coming from a single batch in order to make sure that both laboratories work on the same material [8]. If the transfer is for a method dedicated to the quantification of degradation products as well as the active substance, different batches may be used in order to have one (older) batch with presence of these impurities [13]. If no batches of drug product contain the degradation product or impurities, spiking of the samples with known amount of the impurity is an acceptable practice [13]. Using different batches of products can be interesting when transferring method for the control of impurities as they may include different concentrations of the impurities of interest and thus allows

the evaluation of the method transfer over a wider range of concentration. However the statistical analysis should be realized for each batch separately as each batch will correspond to a different concentration level.

Nonetheless, if several batches are used to assess the transfer of a method dedicated to quality control of active substance as suggested in the ISPE guideline [13], it is also important to realize the statistical analysis for each batch separately, since pooling all the data together will include the batch to batch variability which may penalize the decision to accept the method transfer by including the production process variability. Indeed, for analytical methods transfer, the main aim is to assess the ability of the methods to quantify accurately, not to validate the production process. In such situations, when the transfer is rejected, the question about the source of the problem remains unanswered: is the failure of the transfer due to the analytical method or to the production process? However, performing a "by batch" analysis will inflate the risk of false rejection. Thus either a correction of the significance level could be performed in order to maintain this risk acceptable [54], or a single analysis using all the batches should be used but by removing the inter-batch variance component to apply the equivalence or total error approaches. Finally, if the batch to batch variability is null, the results of all batches could then be pooled for further analysis.

The number of series of experiments and replicates to perform in each laboratory remains the responsibility of the planner of the method transfer. However, the number of experiments is not necessarily the same for each laboratory involved. Indeed, depending on the method validation results at the sending site or on historical data, the sender can only realize a single series of experiments [8,18,19]. There should be enough analysis to perform at the sending site in order to estimate with good precision the true amount of analyte in the samples studied. Results obtained from the validation of the method can be used in order to define the minimal number of replicates and series for the sender as proposed in ref. [19] by looking at the $\hat{R}$ ratio of the between-series (or inter-run) variance over the repeatability (or intra-run) variances. If $\hat{R} \leq 1$ then only one series of at least three replicates could be performed at the sending laboratory. When available, historical data from control charts of the sending laboratory could be used similarly.

Nonetheless, it is mandatory that the receiver performs more than one series in order to evaluate its intermediate precision, even if this is too rarely done in practice. The ISPE guideline [13] proposes a fixed design of three batches of samples, analyzed by two different analysts in three replicates: i.e. a total of 18 experiments. Furthermore, other sources of variability than the operators should be investigated at the receiving site, such as day to day variability or the effect of different equipments when relevant. Indeed, it is important to determine if the receiver will be able to provide reliable results during conditions as close as possible to situations that will be encountered during routine use of the method.

Schwenke and O'Connor [29] describe three types of designs depending on how samples used for the method transfer are shared among the two laboratories and the analysts involved. They typically include days and analysts as major sources of variability. The simplest design being independent sets of samples being analyzed at each laboratory by each analyst on different days, and the most complex one being similar sets of samples shared among the two laboratories. They also show that increasing the number of days of analysis increase the power of the statistical analysis. To corroborate this, recommendations from the literature state that only one batch of product is enough and that the number of series (for *e.g.* days) to perform should be at least four to five with a minimum of three replicates per series [8,20,25].

These values of number of experiments to perform are only minimum values and it has to be evaluated in a case by case basis. To define the number of experiments to perform, general formulas are available for the determination of the sample size for the transfer.

Table 3
Summary of the statistical approaches, experimental designs and acceptance limits used in the examples of analytical methods transfer from the scientific literature.

Cases	Number of replicates/series		Number of series		Statistical approach	Acceptance limits	Reference
	Sender	Receiver	Sender	Receiver			
Pharmaceutical methods							
1	3	3	5	5	Equivalence for trueness	[−2%; +2%]	
					Equivalence for precision	<2.5%	[20]
2	3	3	8	8	Equivalence for trueness	[−1%; +0.5%]	
					Equivalence for precision	<1%	
3	4	4	4	4	Equivalence for trueness	[−17%; +17%]	
					Descriptive for precision	<6%	[30]
4	3	3	6	6	Equivalence for trueness	[−15 mg; +15 mg]	[29]
5	6	6	1	4	Total error	[−5%; +5%]	[8]
6	3	3	1	5	Total error	[−5%; +5%]	
7	3	3	1	5	Total error	[−10%; +10%]	[18]
Bioanalytical methods							
8	3	3	5	5	Total error	[−15%; +15%]	
9	3	3	5	5	Total error	[−15%; +15%]	[19]
10	>1	>1	>1	>1	Equivalence for trueness	[−10%; +10%]	[56]
11	≥6	≥6	>1	>1	Descriptive for trueness	[−15%; +15%]	[55]
					Descriptive for precision	[−15%; +15%]	
12	20[a]	20[a]	1	1	Difference for trueness	Not significant (α = 5%)	[62]
					Descriptive for precision	[5.7%; 15.5%]	
Biological methods							
13	3	3	3	3	Equivalence for trueness	[−0.30; +0.30][b]	
					Difference for precision	Not significant (α = 5%)	
14	3	3	3	3	Equivalence for trueness	[−5%; +5%]	
					Difference for precision	Not significant (α = 5%)	
15	3	3	3	3	Equivalence for trueness	[−0.50; +0.50][c]	[16]
					Difference for precision	Not significant (α = 5%)	

[a] Twenty different incurred samples were analyzed.
[b] In $\log_{10}$ $CCID_{50}$/mL.
[c] In g/L.

For instance using the equivalence approach for the trueness criterion, Kringle et al. [20] have proposed two formulas. However, care should be made when using such formulas where various assumptions are made, the main one being that all the parameters that are required are supposed to be the true ones, such as the true bias between the laboratories or the true components of variance of the sending and receiving laboratories. Indeed, these true values are never known and only estimated. Thus several scenarios should be studied in order to evaluate the impact on the sample size of various true values of variances and biases. Another more general approach, for example when no explicit formula exists, is to perform statistical simulations of the transfer: random data are generated for different combination of true bias, between-series and repeatability variances, number of replicates and series [8,18–20,28]. The transfer is then simulated a great number of times (>1000). Then the decision methodology that will be used for the real method transfer is applied to each of the simulations and the proportion of successful transfers can be computed which allows the selection of the most adequate number of series and repetitions in order to take adequate decision with a high probability.

For bioanalytical applications the transferability of the method should be evaluated over the whole range studied. Samples coming from routine analysis such as incurred samples could be used if available. If, for examples incurred samples do not cover the validated range of the method or when the study has not already been launched, spiked samples of the matrix under investigation could be used [19,55,56]. In this condition, a minimum of three concentration levels should be evaluated to assess the method transfer: a low (close to the lower limit of quantification), middle and high ones (close to the upper limit of quantification) in order to cover the working range [19,55,56].

6. Examples of methods transfer

Several publications can be found in the literature where demonstration of the transferability of a method between two laboratories is made. In the sequel of this document a review of these publications is proposed. A distinction between three groups of method is made. First, analytical methods dedicated to the evaluation of pharmaceutical drug substances and drug products i.e. methods for the determination of active substances, impurities and degradation products or excipients. These are mainly physico-chemical methods like spectrophotometric methods (*e.g.* UV, NIRS) chromatographic or electrophoretic ones. The second group of methods includes also physico-chemical ones but used for the determination of substances in biofluids. These substances could be active ingredient of pharmaceutical products or their metabolites as well as endogenous compounds [57–59]. Finally the last group of methods concerns the biological methods or bioassays [60,61]. They differ from the others in that the detection and quantification is based on biological reactions. Examples of such bioassays can be immunoassays (*e.g.* ELISA), microbiological assays, cell based assays, and so on.

6.1. Pharmaceutical analytical methods

The published quantitative pharmaceutical methods transfers are for methods relative to the quality control of active ingredients in drug substances [20] or in pharmaceutical drug products [8,18,20,29], for methods dedicated to the control of degradation products of the active substance in pharmaceutical formulations [18] and for the control of excipients in pharmaceutical products [30]. They are examples of transfer from R&D laboratory to QC laboratories [18,20,30] or to contract research organization (CRO) [8]. These transfers involved HPLC–UV methods [8,18] and a reversed-phase ion pair chromatographic (RP-IPC) method with evaporative light scattering detection (ELSD) [30]. The experimental designs, the statistical methodologies used and the selected acceptance limits are given in Table 3.

6.2. Bio-pharmaceutical methods

For methods dedicated to the quantification of substances in biofluids few examples are available from the literature. In one publication two cases studies are described, one involves the transfer of a LC method coupled on-line with solid phase extraction (SPE) using electrochemical detection (ECD) for the determination of three catecholamines in human urine, while the other one describes the transfer of an on-line SPE–LC method with fluorimetric detection for the determination of *N*-methyl-laudanosine in human plasma [19]. A short example is also proposed in ref. [56], about the transfer of a LC-MS-MS method but with no details about the compounds and matrix. Gansser [55] and Gilbert et al. [62] also present applications of methods transfer but here also no analytical details are available in these examples. Table 3 summarizes the methodologies

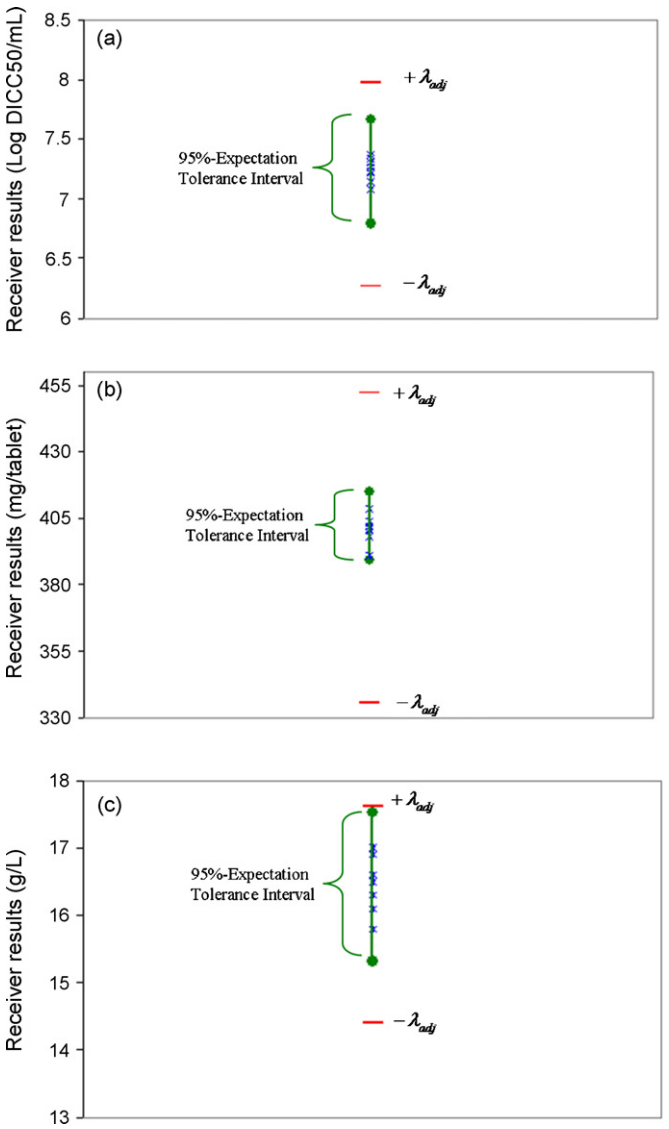

Fig. 4. Application of the total error statistical approach to the three biological methods transfer of ref. [16]. (a) Transfer of a viral activity measurement method by titration in cells; (b) Transfer of a microbiological assay by diffusion; (c) Transfer of a method for the determination of an active substance by weighing. The acceptance limits were settled at ±15%. ✻: Receiver results; ●—●: 95% β-expectation tolerance interval; ▬: adjusted acceptance limits ($[-\lambda_{adj}; +\lambda_{adj}]$).

used in these case studies in terms of number of experiments, statistical decision methodologies and acceptance limits.

6.3. Bioassays

A single document presents applications of quantitative biological methods (bioassays) transfer [16]. They include the transfer of a viral activity measurement assay by titration in cells, an antibiotic microbiological assay and a method for the determination of an active substance by weighing [16]. The experimental designs, decision methodologies and acceptance limits for methods transfer are presented in Table 3. Furthermore as the data used in these examples are available, Fig. 4 presents the results obtained using the total error statistical approach in order to demonstrate its applicability to these special kinds of methods.

7. Conclusion

Analytical methods transfer is more and more performed for pharmaceutical and bio-pharmaceutical applications in order to attain efficiency and productivity in a highly competitive industrial sector. However the lack of regulatory documents and the existence of only non-binding guidance allow personal interpretations on how to design, assess and conclude about the acceptability or rejection of an analytical method transfer. The method transfer study should be precisely planned, communication between the two laboratories involved in the transfer should be frequent and the relevant documentation exchanged. The statistical methodology used to decide about the acceptability of the transfer should be clearly detailed, keeping in mind its ability to manage the risks linked to the transfer. The total error approach is the only one providing actually the best risk management as it looks at the reliability of the results that will be obtained by the receiving laboratory during the future routine application of the transferred method. This original and powerful methodology has been shown to improve considerably the reliability of the decision about the transferability of analytical methods. Use of the descriptive or equivalence approaches for decision making in methods transfer should be taken cautiously, knowing the risks linked to their application. The number of experiments to perform should also be carefully evaluated. Realizing different series of experiments at the receiving site is essential in order to evaluate its intermediate precision variability. A general tendency is thus to increase the number of series rather than the number of repetitions. Performing simulations of the method transfer is the best way to define the optimal experimental design in terms of series and repetitions per series. Still, one point remains highly problematical, but is a mandatory step before launching the transfer study: the definition of the acceptance limits. Some further work should be performed to provide tools and guidelines on how to adequately define them with regard to the final aim of the transferred methods.

Analytical methods transfer requires non-negligible amount of work in order to make the adequate decision about the acceptability or rejection of the transfer. Extreme care should be made when choosing one strategy or another as the transferred method will daily be used by the receiving laboratory to make highly critical decisions such as batch releases or the evaluation of pharmacokinetic, bioequivalence and clinical studies.

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References

[1] M. Galiay, STP Pharma Pratiques 12 (2002) 258.
[2] F. Minois-Offroy, Y. Appriou, V. Brousset, E. Chapuzet, G. de Fontenay, W. Dewé, E. Dumas, C. Ellie, M. Galiay, N. Lefebvre, P. Mottu, M.P. Quint, F. Schoeffter, STP Pharma Pratiques 12 (2002) 337.
[3] D. Sixsmith, Pharm. Technol. Eur. (1999) 50.
[4] D. Sixsmith, Pharm. Technol. Eur. (2000) 26.
[5] L. Pisarik, P.Y. Blais, P. Carraud, F. Douere, A. Froissant, S. Koeberle-Ramond, V. Laugel, F. Muffat, V. Proteau-Chabrat, STP Pharma Pratiques 10 (2000) 61.
[6] S. Scypinski, D. Roberts, M. Oates, J. Etse, Pharm. Technol. (March) (2002) 84.
[7] J. Ermer, J.H.McB. Miller, Method Validation in Pharmaceutical Analysis, Wiley-VCH Verlag, Weinheim, Germany, 2005, p. 281.
[8] W. Dewé, B. Govaerts, B. Boulanger, E. Rozet, P. Chiap, Ph. Hubert, Chemometr. Intell. Lab. Syst. 85 (2007) 262.
[9] International Conference on Harmonization (ICH) of Technical Requirements for registration of Pharmaceuticals for Human Use, Topic Q2 (R1): Validation of Analytical Procedures: Text and Methodology, Geneva, 2005.
[10] Guidance for industry: Bioanalytical Method Validation, US Department of Health and Human Services, US Food and Drug Administration (FDA), Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER), Rockville, May 2001.
[11] International Conference on Harmonization (ICH) of Technical Requirements for registration of Pharmaceuticals for Human Use, Topic Q9: Quality Risk Management, Geneva, 2005.
[12] Y. Johnson, An FDA Perspective on Method Transfer of Chromatographic Analytical Methods in the Pharmaceutical Industry, in: The Chromatography Forum of Delaware Valley, October meeting, 2004.
[13] International Society for Pharmaceutical Engineering (ISPE), Analytical Procedure/Technology Transfer, ISPE Guideline, 2003, p. 23.
[14] U. Schepers, H. Wätzig, J. Pharm. Biomed. Anal. 39 (2005) 310.
[15] FDA, Guidance for Industry: Protocols for the Conduct of Method Transfer Studies for Type C Medicated Feed Assay Methods, U.S. Department of Health and Human Services, Food and Drug Administration (FDA), Center for Veterinary Medicine (CVM), April 2007.
[16] B. Ramond, A. Gaillandre, N. Gibelin, C. Michalski, P. Nabet, C. Niewiaski, D. Poirault, H. Rigal, STP Pharma Pratiques 17 (2007) 67.
[17] H.J. Mortko, Pharm. Technol. 23 (1999) 30.
[18] E. Rozet, B. Mertens, W. Dewe, A. Ceccato, B. Govaerts, B. Boulanger, P. Chiap, B. Streel, J. Crommen, Ph. Hubert, J. Pharm. Biomed. Anal. 42 (2006) 64.
[19] E. Rozet, W. Dewé, R. Morello, P. Chiap, F. Lecomte, E. Ziemons, K.S. Boos, B. Boulanger, J. Crommen, Ph. Hubert, J. Chromatogr. A 1189 (2008) 32.
[20] R. Kringle, R. Khan-Malek, F. Snikeris, P. Munden, C. Agut, M. Bauer, Drug Inf. J. 35 (2001) 1271.
[21] ISO 5725-1, Application of the statistics—Accuracy (trueness and precision) of the results and methods of measurement. Part 1. General principles and definitions. International Organization for Standardization (ISO), Geneva, 1994.
[22] Ph. Hubert, J.-J. Nguyen-Huu, B. Boulanger, E. Chapuzet, P. Chiap, N. Cohen, P.-A. Compagnon, W. Dewé, M. Feinberg, M. Lallier, M. Laurentie, N. Mercier, G. Muzard, C. Nivet, L. Valat, J. Pharm. Biomed. Anal. 36 (2004) 579.
[23] International Vocabulary of Basic and General Terms in Metrology, ISO, Geneva, 1993.
[24] E. Rozet, A. Ceccato, C. Hubert, E. Ziemons, R. Oprean, S. Rudaz, B. Boulanger, P. Hubert, J. Chromatogr. A 1158 (2007) 111.
[25] G. de Fontenay, J. Pharm. Biomed. Anal. 46 (2008) 104.
[26] C. Hartmann, D.L. Massart, R.D. McDowall, J. Pharm. Biomed. Anal. 12 (1994) 1337.
[27] D.J. Schuirmann, J. Pharmacol. Biopharm. 15 (1987) 657.
[28] U. Schepers, H. Wätzig, J. Pharm. Biomed. Anal. 41 (2006) 290.
[29] J.R. Schwenke, D.K. O'Connor, J. BioPharm. Stat. 18 (2008) 1013.
[30] S.A. Rodriguez, R. Kubiak, M. Mulcey, T. Tamblyn, T. Tougas, Am. Lab. 37 (2005) 9.
[31] D. Chambers, G. Kelly, G. Limentani, A. Lister, R. Lung, E. Warner, Pharm. Technol. (September) (2005) 64.
[32] S. Feng, Q. Liang, R.D. Kinser, K. Newland, R. Guilbaud, Anal. Bioanal. Chem. 385 (2006) 975.
[33] J. Vial, A. Jardy, P. Anger, A. Brun, J.M. Menet, J. Chromatogr. A 815 (1998) 173.
[34] J. Vial, A. Jardy, Chromatographia 53 (2001) S-141.
[35] ISO 5725-2, International Organization for Standardization (ISO), Statistical methods for quality control, accuracy (trueness and precision) of measurement methods and results. Part 2. Basic method for the determination of repeatability and reproducibility of a standard measurement method, vol. 2, 4th ed., Geneva, ISO, 1994.
[36] Y. Tsong, J. Zhong, K. Lee, Analytical Method Transfer, in: 1st ISBS Symposium, Shanghai, China, June 30–July 2, 2008.
[37] B. Boulanger, W. Dewé, A. Gilbert, B. Govaerts, M. Maumy-Bertrand, Chemometr. Intell. Lab. Syst. 86 (2007) 198.
[38] B. Govaerts, W. Dewé, M. Maumy, B. Boulanger, Qual. Reliab. Eng. Int. 24 (2008) 667.
[39] E. Rozet, C. Hubert, A. Ceccato, W. Dewé, E. Ziemons, F. Moonen, K. Michail, R. Wintersteiger, B. Streel, B. Boulanger, Ph. Hubert, J. Chromatogr. A 1158 (2007) 126.
[40] S.R. Searle, G. Casella, C.E. McCulloch, Variance Components, Wiley, 1992.
[41] D.J. Schuirmann, J. Pharmacokinet. Biopharm. 15 (1987) 657.
[42] C. Hartmann, J. Smeyers-Verbeke, W. Penninckx, Y. Vander Heyden, P. Vankeerberghen, D. Massart, Anal. Chem. 67 (1995) 4491.

[43] F. Satterthwaite, Psychometrika 6 (1941) 309.
[44] R. Burdik, F. Graybill, Confidence Interval on Variance Components, Marcel Dekker, Inc., New York, NY, 1992.
[45] R.W. Mee, Technometrics 26 (1984) 251.
[46] C.T. Viswanathan, S. Bansal, B. Booth, A.J. DeStefano, M.J. Rose, J. Sailstad, V.P. Shah, J.P. Skelly, P.G. Swann, R. Weiner, AAPS J. 9 (2007) E30, http://www.aapsj.org.
[47] R. Burdick, T. Gleason, S. Rausch, J. Seely, BioPharm. Int. 20 (2007) 40.
[48] T. Orchard, BioPharm. Int. 19 (2006) 22.
[49] A. Wald, J. Wolfowitz, Ann. Math. Stat. 17 (1946) 208.
[50] D. Hoffman, R. Kringle, J. Biopharm. Stat. 15 (2005) 283.
[51] K.R. Eberhardt, R.W. Mee, C.E. Reeve, Commun. Stat.-Simulation Comput. 18 (1989) 397.
[52] C.T. Liao, T.Y. Lin, H. Iyer, Technometrics 47 (2005) 323.
[53] A. Jullion, B. Boulanger, Fit-for-purpose limits and Tolerance intervals: connecting the assay performance to the clinical trial, in: Non Clinical Statistics Conference, Leuven, Belgium, September 23–25, 2008.
[54] C. Hartmann, J. Smeyers-Verbeke, D.L. Massart, R.D. McDowall, J. Pharm. Biomed. Anal. 17 (1998) 193.
[55] D. Gansser, Chromatographia 55 (2002) S-71.
[56] D.C. Muirhead, T.S. Smart, Chromatographia 52 (2000) S-72.
[57] N.C. van de Merbel, Trend Anal. Chem. 27 (2008) 924.
[58] D. Tsikas, B. Schubert, F.M. Gutzki, J. Sandmann, J.C. Frölich, J. Chromatogr. B 798 (2003) 87.
[59] E. Rozet, R. Morello, F. Lecomte, G.B. Martin, P. Chiap, J. Crommen, K.S. Boos, Ph. Hubert, J. Chromatogr. B 844 (2006) 251.
[60] J.W.A. Findlay, W.C. Smith, J.W. Lee, G.D. Nordblom, I. Das, B.S. DeSilva, M.N. Khan, R.R. Bowsher, J. Pharm. Biomed. Anal. 21 (2000) 1249.
[61] J.W. Lee, R.S. Weiner, J.M. Sailstad, R.R. Bowsher, D.W. Knuth, P.J. O'Brien, J.L. Foucroy, R. Dixit, L. Pandite, R.G. Pietrusko, H.D. Soares, V. Quarmby, O.L. Vesterqvist, D.M. Potter, J.L. Witliff, H.A. Fritche, T. O'Learly, L. Perlee, S. Kadam, J.A. Wagner, Pharm. Res. 22 (2005) 499.
[62] M.T. Gilbert, I. Barinov-Collignon, J.R. Miksic, J. Pharm. Biomed. Anal. 13 (1995) 385.