

## Capsule review on bioanalytical method transfer: opportunities and challenges for chromatographic methods

With the globalization of drug development activities, transferring a validated bioanalytical procedure to a different site within a pharmaceutical company or to one, or multiple, contract research organizations has been dramatically increased in recent years. Undeniably, bioanalytical method transfer is the needed step prior to routine sample analysis at the receiving laboratory. It is clearly stated in the 2001 US FDA Guidance on Bioanalytical Method Validation that a partial validation is needed for method transfer between laboratories. In the current European Medicines Agency draft guidelines on Method Validation, the necessity of a method transfer is also emphasized. However, the above guidelines do not give many details on how and when a method transfer validation should be conducted. There is a need for a step-by-step deliberation on the overall strategies, procedures and even technical details, as well, for a successful bioanalytical method transfer. In this paper, we review the contemporary information available in the scientific literature on the method transfer and illustrated various bioanalytical method transfer scenarios using case studies. A 'flexible and fit for purpose' bioanalytical method transfer strategy is proposed.

Bioanalysis provides pivotal data for toxicokinetic, pharmacokinetic, bioavailability and bioequivalence studies used for regional and worldwide regulatory submission. Bioanalytical method development and validation, as well as the associated nonclinical and clinical study sample analysis, are essential components in development of therapeutic agents. With globalization of drug development, the increased bioanalytical outsourcing activities of pharmaceutical companies and merger and acquisition of pharmaceutical companies, transferring a validated bioanalytical procedure to a different site within a pharmaceutical company and/or one or multiple CROs, especially in the emerging markets (e.g., China and India), has been dramatically increased in the past several years.

The aim of bioanalytical method validation is to demonstrate that the method is suitable for the intended application(s). Guidelines have been detailed by health authorities (e.g., US FDA, European Medicines Agency [EMA]) in helping scientists worldwide to develop and validate an intended bioanalytical method. It is clearly stated in the 2001 FDA Guidance on Bioanalytical Method Validation that a **partial validation** is needed for method transfer between laboratories [101]. The current EMA draft guidelines on method validation state that a comparison is required on analytical methods

when data are to be obtained from different study sites [102]. The above guidelines address the use of the same method on different sites, modifications to the method (e.g., changes to the sample preparation procedure) or the use of a different type of method on different sites. However, there is no official guidance regarding the study design, data analysis or decision making procedures in either the FDA or EMA documents, and no further instruction was given on how and when a method transfer validation should be conducted. Apparently, this is left for the responsible bioanalytical scientists to develop an appropriate method transfer/crossvalidation protocol for the intended applications.

Method transfer might have been one of the most inconsistent practices in bioanalysis. Many procedural and technical aspects for a successful **bioanalytical method transfer** are pending on clarification for a harmonized process. Some of these are:

- What procedure should be in place for a method transfer;
- How a method transfer should be performed;
- Whether or not **quality control (QC) samples** and/or **incurred samples** should be used;
- What approach should be employed for data analysis to assess the comparability;

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**Key Terms**

**Partial validation:**

Modification of validated bioanalytical methods including transfer of the bioanalytical method to another laboratory that do not necessarily call for full revalidation.

**Bioanalytical method transfer:**

Transferring a validated bioanalytical method from a sending laboratory to a receiving laboratory.

**Quality control sample:**

A spiked sample used to monitor the performance of a bioanalytical method and to assess the integrity and validity of the results of the unknown samples analyzed in an individual batch.

**Incurred sample:** Study samples from the dosed subjects.

**Crossvalidation:** When comparison of the study data from different sites is needed, a crossvalidation of the applied analytical methods should be carried out using the quality control or incurred samples in addition to a partial validation.

**Full validation:** Establishment of all validation parameters to apply to sample analysis for the bioanalytical method for each analyte.

- What should be included as the acceptance criteria to assure comparability of a validated method between, or among, the bioanalytical laboratories;
- How a method transfer failure should be investigated;
- How method transfer data should be reported.

There are only a few publications on bioanalytical method transfer and **cross-validation** [1,2]. In this paper, through reviewing the available publications on bioanalytical method transfer and several case studies of method transfers (e.g., method transfer from pharmaceutical company to CRO and from CRO to CRO), we highlighted a detailed ‘flexible and fit for purpose’ procedure for a successful chromatographic bioanalytical method transfer. This includes:

- Approach for using QC samples and/or incurred samples from clinical trials;
- Phases of method transfer;
- Preparation of a transfer protocol;
- Predefined acceptance criteria;
- Performing a method transfer;
- Investigating method transfer failures;
- Reporting data to ensure data comparability.

- Using different anticoagulant counter-ions;
- Using different strains or sex of a species;
- Moving a method between LC–MS/MS instruments.

A method transfer from one laboratory to another could be considered to be a combination of:

- Analyst crossvalidation;
- Instrumentation crossvalidation;
- Bioanalytical supporting system (e.g., balance, pipette, solvent and automation) crossvalidation.

It was recommended that when sample analyses within a single study are conducted at more than one site, or more than one laboratory, cross-validation using spiked matrix QCs should be conducted at each site, or laboratory, to establish inter-laboratory reliability. The acceptance criterion is that the difference between the results of the same set of QC samples measured by the two laboratories should not be greater than 15%. Consideration should also be taken for using a proportion of incurred samples, if they are available, to evaluate the performance of different methods. Use of incurred samples is more likely to highlight differences in the methods in a real situation [102].

**Current practice, opportunities & challenges**

Bioanalytical method transfer occurs for a number of reasons between the sending laboratories, where the method was developed and validated, and the receiving laboratories, where the method is to be transferred to prior to study sample analysis in support of various regulatory purposes.

A special issue of *Journal of Chromatography B* (Issue 23, 2009) was devoted to method validation, comparison and transfer of analytical methods with two review articles dedicated to pharmaceutical analytical and bioanalytical method transfer. Rozet *et al.* reviewed the methodologies for analytical methods transfer with a focus on the design of transfer studies, required sample size, statistical methodologies and examples of various possibilities among the strategies for method transfer [2]. Five case studies for bioanalytical method transfer were summarized in this review. Dewe reviewed the statistical methodologies used to compare (bio)assays including two one-sided *t*-tests, concordance correlation coefficient and limits of agreement, tolerance intervals and probabilistic approach [4]. In summary,

**Current regulatory perspective on a bioanalytical method transfer**

In the 2001 FDA guidance, crossvalidation is defined as ‘a comparison of validation parameters when two or more bioanalytical methods are used to generate data within the same study or across different studies.’ This appropriately covers the analysis of biological samples from a single study on multiple sites, where a method crossvalidation is mandated, the use of different analytical techniques for different studies to support the same regulatory submission or where instrument crossvalidation is required [101]. It is clear that a crossvalidation must be carried out before the study sample analysis.

More details on method crossvalidation were discussed at the 3rd American Association of Pharmaceutical Scientists/FDA Bioanalytical Workshop of 2007 [3]. Although no consensus was reached, comments on further discussion and direction were provided on crossvalidation for the following:

the current approaches for evaluating data sets obtained by analyzing the spiked QC samples [5–7] or incurred samples [1] for bioanalytical method transfer include the independent validation approach, statistical difference testing and statistical equivalence testing. Statistical approaches are based on a complete consideration of statistics and are, therefore, considered robust. However, the limited number of data sets in many bioanalytical method transfers may be a concern. Shah and Karnes proposed a practical ‘fixed’ range decision criteria (3/3/20, i.e., three independent runs with three replicates per run and a fixed criterion of  $\pm 20\%$  of the reference method mean) for transfer of bioanalytical methods considering the maximum acceptable imprecision and inaccuracy limits in the FDA bioanalytical guidance on a validated chromatographic method [8]. Tatarewicz *et al.* reported a three-step statistical approach for an immunogenicity assay (ELISA or Biacore) transfer during clinical drug development [9]. In a recently published chapter, Klump *et al.* discuss the strategies for successful transfer of ligand-binding assays and implementation in GXP environment [10].

Although there are no detailed instructions in health authority guidelines, the study design, data analysis and acceptance criteria for a successful method transfer or inter-laboratory crossvalidation should, in general, remain ‘flexible’ and ‘fit for purpose’, considering various bioanalytical situations. Each party of the sending and receiving laboratories is recommended to establish its own standard operating procedure (SOP) and the method transfer protocol that serves as a bridge between the SOPs of the sending and receiving laboratories. The protocol should focus on:

- What validation parameters should be included in crossvalidation for method transfer, and how many, and what types of, samples should be evaluated for a successful method transfer;
- What constitutes acceptance for a method transfer and in what manner should crossvalidation data be analyzed to draw a valid conclusion;
- What kind of challenges might both the sending and receiving laboratories face, what action, if any, should be taken once a method of crossvalidation is completed or has failed, and how should the results of method transfer be reported.

Each of these questions is considered below.

- What validation parameters should be included in a crossvalidation for method transfer & how many, & what types of, samples should be evaluated for a successful method transfer?

Unlike a **full validation** where the specificity/selectivity, matrix effect and recovery, inter- and intra-run precision, and accuracy of QC sample results for the LLOQs, LQCs, MQCs and HQCs, dilution integrity, batch size integrity and stabilities (e.g., stock–spiking solution stability, freeze–thaw stability, bench top stability, autosampler stability and long term stability) must be fully validated according to the current guidance, method transfer from one laboratory to another might only require partial validation from as little as one intra-assay accuracy and precision determination along with necessary confirmation of matrix effect and recovery to a nearly full validation, depending on respective circumstances.

The method transfer protocol needs to be fully reviewed, discussed and agreed by both the sending laboratory and the receiving laboratory to determine whether a partial validation (one batch) or a full validation is necessary. During protocol review and discussion, it is important that both laboratories collaborate to ensure that as many method details as possible are communicated. Method-related information, such as method of pipetting, storage of reagents, analyte stock solution formulation, use of specialized equipment and plate washers can be very useful. Whenever possible, assay performance should be tested at both laboratories with multiple individuals to ensure method transferability. Similar to a full validation, QC samples at LLOQ, LQC, MQC and HQC should be analyzed in six replicates along with calibration standards in the one batch crossvalidation, the minimum requirement for method transfer/crossvalidation.

It has become a well-received practice that incurred sample reanalysis should be conducted in the receiving laboratory as a means of evaluating the comparability of the method if the comparison of the study data from different sites is needed in addition to a partial validation using QC samples. At completion of method validation, once the precision and accuracy has been established, incurred samples that have previously been analyzed in the sending laboratory are reanalyzed in the receiving laboratory. In the case where incurred samples are not readily available, a set of QC samples should be prepared to

cover the low, mid and high concentrations and tested at the sending laboratory and then sent to the receiving laboratory with QC concentrations blinded or unblinded. This practice is very similar to incurred sample reanalysis with the only difference being that the reanalysis is conducted at the receiving laboratory. The obtained analyte concentrations in the incurred samples and/or QC samples are to be evaluated for concordance along with other crossvalidation information from the receiving laboratory.

■ **What constitutes acceptance for a method transfer & in what manner should crossvalidation data be analyzed to draw a valid conclusion?**

Practically, the acceptance criteria for a crossvalidation in method transfer should not differ from well-accepted industry practice. The accuracy of a bioanalytical LC-MS/MS method crossvalidation reflects the closeness of the mean test results obtained via the method to the true values (nominal concentrations) of the analyte(s) of interest. Accuracy is determined by replicate analysis of samples (QCs) containing known amounts of the analyte. Within-run (intra-day or intra-run) accuracy should be measured using six determinations at a minimum of four concentration (e.g., LLOQ, LQC, MQC and HQC) levels. The mean measured value should be within  $\pm 15\%$  (bias) of the nominal value for all QC concentration levels except for the LLOQ, where the bias (%) should be within  $\pm 20\%$ . The precision of an LC-MS/MS method crossvalidation describes the closeness of individual measures of an analyte when the procedure is applied repeatedly to multiple aliquots of a single homogeneous volume of biological samples (QCs). Within-run (intra-run or intra-day) precision should be measured using six determinations at a minimum of four concentration (e.g., LLOQ, LQC, MQC and HQC) levels. The precision determined at each concentration level should not exceed 15% of the coefficients of variation (CV) (%) except for the LLOQ, where the CV (%) should not exceed 20%.

The QC samples (low, mid and high) should be prepared and verified in six replicates for each concentration level in the sending laboratory and retested in the same manner in the receiving laboratory along with the calibration standards and QC samples prepared in the receiving laboratory to evaluate the comparability of the method. It is recommended that the difference in the QC sample results obtained from

the sending laboratory and receiving laboratory should be within  $\pm 15\%$  for all QC concentration levels tested.

As part of crossvalidation, a minimum of 20 incurred study samples should be reanalyzed at the receiving laboratory and two-thirds of results must be within  $\pm 30\%$  of the original values.

■ **What kind of challenges might both the sending & receiving laboratories face & what action, if any, should be taken once a method of crossvalidation is completed or has failed?**

Good practice, before transferring a method from the sending laboratory to the receiving laboratory, is to ensure that both parties' scientific teams communicate early and frequently about method characteristics. The sending laboratory should request a review of the receiving laboratory's method validation SOP or a summary of the method evaluations performed. If possible, a face-to-face discussion should take place and on-site method training should be provided whenever possible. Methods that were initially developed at the sending laboratory should be vigorously tested and validated to ensure the robustness. Good precision and accuracy of QC samples results from the crossvalidation and concordance of cross-QC sample check and incurred sample reanalysis at the receiving laboratory boosts the confidence for both laboratories that samples can be analyzed at either laboratory with an equivalent outcome. By contrast, a failed method transfer might be the result of one or more reasons. Listed below are the common causes that may contribute to a failed method transfer.

**Supporting system difference between the sending & receiving laboratories**

A successful method transfer will not only depend on the experience and knowledge of both sending and receiving laboratories in regulated bioanalysis, but also on communication on the characteristics of a validated method to be transferred. Seemingly irrelevant differences, such as HPLC system, MS, method of pipetting, automatic liquid-handling system, reagents, storage of reagents, method choice of siliconizing glass tubes, use of specialized equipment, plate washers, type of vortex mixers and even the fume hood air flow, between the laboratories may exaggerate inherent method variability and cause a validation failure due to unacceptable accuracy and precision. Therefore, it is important for

both the sending and receiving laboratories to collaborate to ensure that the pertinent details of the method are clearly communicated at the time of transfer.

#### Inadequate sample preparation and/or chromatographic method

Clearly, inadequate sample preparation would result in inconsistent assay recovery, instability of the analyte or metabolites, and/or chromatographic interferences for both analyte and internal standard, leading to poor reproducibility and accuracy. It is essential to ensure that both the analyte and metabolite stability are not compromised during the sample preparation and chromatography. One of the most common pitfalls is the breakdown of acyl glucuronide metabolite to the parent analyte either during the sample preparation or chromatography, leading to potential overestimation of the parent analyte concentration.

#### Inadequate assay selectivity

Ideally, assay selectivity should be evaluated by fortifying at least six individual lots of matrix with the analyte at the LLOQ of the assay to assess accuracy of the measured analyte concentrations, where the bias of within  $\pm 20\%$  of the nominal concentration values for five out of six individually spiked LLOQs is considered acceptable. For some methods, selectivity testing may have only been performed on a small number of lots, or even a single pool of matrix, before transfer. Testing in a pool of matrix obscures lot-to-lot differences and commonly results in method transfer failure. Under ideal circumstances, assay selectivity should also be tested using incurred samples to ensure no interferences are from, for example, known/unknown metabolites, dosing vehicles and co-administered medicines. Absolute and/or relative matrix effects should be evaluated. A postcolumn infusion experiment can be used to ensure that the analyte is not eluted in the proximity of any suppression/enhancement zones. It would also be useful to monitor some common agents that can cause suppression, such as phospholipids, to ensure they are not eluted after the injection cycle time to interfere with the subsequent injections.

#### Stability issues

Industry bioanalytical method validation consensus requirements are clear that stability must be established against calibrators that are freshly prepared on the day of the evaluation. It might

not be unusual that a method is transferred under the assumption that the long-term stability of the analyte of interest, in the intended biological matrices, has been established at less than or equal to  $-15^{\circ}\text{C}$ , or less than or equal to  $-60^{\circ}\text{C}$  at the sending laboratory. The common practice, however, is to repeat some or all stability experiments at the receiving laboratory. If the stability test at the receiving laboratory shows that the analyte is not stable, the stability is further investigated in collaboration with the sending laboratory. It has often been discovered that the calibrator curve at one of the laboratories was not prepared (freshly) immediately before the stability assessment, or that stability had not been established for drug stock solutions that were used for the preparation of the fresh calibrators.

#### Common bench errors

A failed incurred sample reanalysis in a method transfer is often not due to issues of assay methodology (selectivity or stability), but due to very common bench errors, including, but not limited to:

- Inadequate sample thawing;
- Improper sample mixing prior to aliquotting;
- Improper sample dilution;
- Acknowledgement of sample ID verification.

As such, proper training of laboratory personnel is warranted. It is recommended that a procedure be in place for the investigation of a failed method transfer. Like the typical investigation procedure in the bioanalytical laboratory, the investigation should include two phases – phase one: review of the existing data and documentation and phase two: laboratory investigation, which should occur at both the sending laboratory and the receiving laboratory, if needed. The phase one investigation involves the initial evaluation of the existing data and documentation. The documentation should be reviewed with a focus on calculation errors, sample preparation mistakes, sample identification and instrument problems, and so forth. One of three determinations – assignable cause found, probable cause found, and no assignable cause found – should be made to conclude the phase one investigation. The first scenario (assignable cause found) should trigger a corrective action. Repeat analysis is normally required and additional training is necessary to prevent reoccurrence. The second scenario (probable cause found) allows for one

single test to verify the hypothesis. Following the test results, one of the two other scenarios is triggered. The third scenario (no assignable cause found) should trigger the phase two investigation, for which a close examination of the method should be made. The examination should be focused on the issues of method specificity, sample preparation procedure and unstable metabolites that are prone to conversion to the parent compound during sample process and storage. If a root cause is found and confirmed, a corrective action plan should be created to correct the existing problem, identify any deficiencies to the quality systems or personnel training programs, and repeat the method transfer. If the acceptance criteria predefined in the method transfer protocol are met, the repeated method transfer can still be considered successful.

Of course, it is ideal to have a solid method to start with for the transfer, with the goal of minimizing the failures. Method transfer should be built as an integrated part of overall bioanalytical support strategy at the onset of a program. As the drug candidate progresses to a decision point, when method transfer is likely to occur, methods should be tested vigorously for robustness and transferability including, but not limited to, intra-laboratory method transfer between analysts, standardization of instruments and automation techniques, and critique and simplification of the methods, if possible. Any additional information such as previously unnoticed metabolites or stability issues should be thoroughly reviewed and their impact on the methods should be carefully assessed. The receiving laboratory should also be identified, and appropriate method training should be conducted, which may involve an on-site visit to the sending laboratory or chemists from the sending laboratory should perform training at the receiving laboratory. Protocols/SOPs, documentations (e.g., method validation report and method) and timeline/budget relevant to transfer should be discussed and fully understood. Communication channels and expectations should be established.

### Case studies

Below are the five most common scenarios of method transfer that involves transferring a method:

- From one pharmaceutical company to another pharmaceutical company (e.g., merger and licensing drug candidate);
- From a pharmaceutical company to a CRO laboratory (outsourcing);
- From a CRO laboratory to a pharmaceutical company (program transfer);
- From one site to another site of a company (e.g., program transfer and supporting multi-site study);
- From one CRO laboratory to another CRO laboratory (program transfer).

Bioanalytical method transfers frequently occur at different stages of drug development from pre-clinical to postmarketing clinical studies.

Listed below are the three case studies that exemplify the 'flexible and fit for purpose' strategies for bioanalytical method transfer. In all three cases, method transfer protocols were in place for the procedures and acceptance criteria for the method transfer.

Case one (from pharmaceutical company to CRO using blinded QC and incurred samples). An LC-MS/MS method was fully validated and used in support of clinical studies in a global pharmaceutical company. The method was then transferred to Frontage Laboratories, PA, USA in support of the clinical study. The CRO is required to strictly follow the method procedure established at the pharmaceutical company. In addition, blinded QC samples and incurred samples were used to establish the inter-laboratory comparability. The original method involves sample extraction via protein precipitation and sample analysis on the API4000 MS/MS system coupled with a Shimadzu LC system with a dynamic range of 0.1 to 250 ng/ml for the drug candidate in human plasma. The method transfer design, data analysis, acceptance criteria and reporting were detailed in the approved method transfer protocol. The transferred LC-MS/MS method was partially validated for intra-run and inter-run precision and accuracy, linearity and dilution integrity, selectivity and LLOQ and ULOQ, matrix effect and processed sample stability and analysis of blinded QC samples. QC samples at low, mid and high QC levels (n = 6) were prepared by the sending laboratory to evaluate the comparability of the transferred method using the criterion that 12 out of 18 QC results (low, medium and high QCs) were to be within  $\pm 15.0\%$  of their nominal values and at least three results (50%) at each level to be within 15% of the nominal concentration. Crossvalidation of the method between two labs was also performed by using 36 incurred samples

from a clinical study originally analyzed in the sending laboratory. The original result from the sending laboratory was defined as the reference value. For the evaluation of the crossvalidation, the result of the analysis from the receiving laboratory was compared with the result of the original value using **EQUATION 1**:

$$\%DIFF = \frac{(\text{Receiving Lab Value} - \text{Sending Lab Value})}{(\text{Mean of Lab Value and Sending Lab Value})} \times 100$$

**EQUATION 1**

Criterion: the %DIFF at least two-thirds of all analyzed incurred samples has to be within  $\pm 30\%$ . The method transfer/crossvalidation data were reported in the partial method validation report. The data in **TABLE 1** and **FIGURE 1** were obtained during the method transfer/crossvalidation experiment using the blinded QC samples and the incurred samples, respectively. The precision and accuracy of the QC and incurred sample results met the acceptance criteria predefined in the method transfer protocol. The LC-MS/MS method was considered successfully transferred even though there was a trend of negative bias (%) for the MQC and HQC results. The method has been used in the receiving laboratory in support of the sample analysis collected from a clinical study.

Case two (from CRO site one to site two using the QC samples for method transfer and sample analysis). In order to support a Phase III multisite clinical trial, a total of nine LC-MS/MS methods were transferred from Frontage Laboratories, USA, to Frontage Laboratories, Shanghai, China,

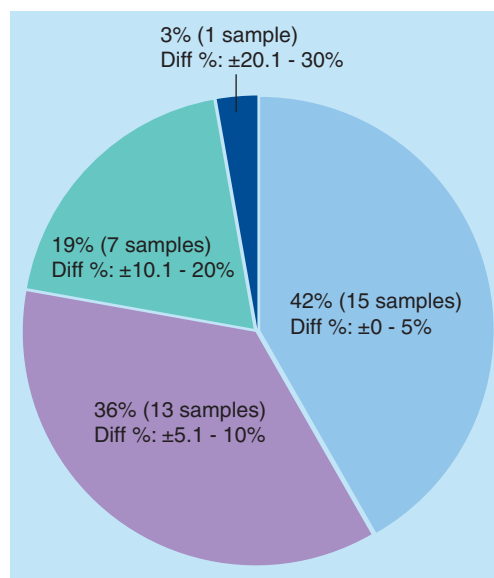
in support of the analysis of the study samples generated from the trial locally. The instrument settings and operation procedures in the sending and receiving laboratories are identical and there was no need for a change of the method. It was agreed between the sponsor and Frontage Laboratories that the QC samples prepared in the US laboratory (the sending laboratory) would be used for the method transfer/crossvalidation and study sample analysis in the Chinese laboratory (the receiving laboratory). The method transfer protocol described the procedures and acceptance criteria for the evaluation of the validation parameters including specificity, sensitivity, intra-day accuracy and precision of QC sample results and dilution integrity using the criterion of 12 out of 18 QC results (low, medium and high QCs) to be within  $\pm 15.0\%$  of the nominal values and at least three QC sample results (50%) at each concentration level to be within  $\pm 15\%$  of the nominal values. The CV (%) of QC sample results were no more than 15% at each concentration level. An example of the precision and accuracy of QC samples for one of the nine method transfers is listed in **TABLE 2**. A total of nine LC-MS/MS methods were successfully transferred/cross-validated in the receiving laboratory and were used to support the sample analysis for the Chinese clinical trial of the Phase III study.

Case three (from CRO to CRO using the fortified QC samples with different assay range). An LC-MS/MS method needed to be transferred from CRO A to CRO B due to the closing of the CRO A, where the method was originally developed and validated to support a clinical study. The original method was fully

**Table 1. Precision and accuracy of blinded quality control samples analyzed in the receiving laboratory.**

	QC low (0.264 ng/ml)	Bias (%)	QC medium (5.28 ng/ml)	Bias (%)	QC high (192 ng/ml)	Bias (%)
	0.283	7.2	4.95	-6.3	183	-4.7
	0.296	12.1	5.37	1.7	177	-7.8
	0.281	6.4	5.03	-4.7	180	-6.3
	0.277	4.9	5.07	-4.0	172	-10.4
	0.272	3.0	5.25	-0.6	179	-6.8
	0.286	8.3	5.19	-1.7	180	-6.3
Intra-run mean	0.283		5.14		179	
SD	0.00822		0.155		3.73	
%CV	2.9		3.0		2.1	
% bias	7.2		-2.7		-6.8	
n	6		6		6	

Run date: 3rd February 2009.  
CV: Coefficient of variation; QC: Quality control; SD: Standard deviation.



**Figure 1.** Accuracy of incurred sample analysis in the receiving laboratory.

validated with the stability data for the drug substance, but the calibration dynamic range (10–2000 ng/ml) was different from what the sponsor requested (2–400 ng/ml) for the continuation of sample analysis support for the ongoing clinical study. Therefore, the original method was modified and fully validated in the receiving laboratory. In order to determine the equivalency of the original method and the modified method at the receiving laboratory, human plasma QC samples at three concentration levels (30, 600 and 1500 ng/ml), provided by the sending laboratory were evaluated using the modified method with a freshly prepared standard curve in the receiving laboratory. The

QC samples prepared in the sending laboratory, in four replicates for each concentration level due to the limited available sample volume, were diluted five times with blank human plasma. The results for the QC samples are summarized in **TABLE 3**. The acceptance criteria for the QC samples, outlined in the method transfer/cross-validation protocol, specified that 8 out of 12 QC-results (low, medium and high), must be within 15% of the respective nominal values and at least two results at each concentration level must be within 15% of the nominal concentration. The QC sample results met the acceptance criteria and the method transfer was considered successful to establish the equivalency of the LC–MS/MS methods.

### Summary & future perspective

In summary, successful bioanalytical method transfer has played an increasingly important role in drug development. It involves multiple scientists from multiple institutes (or sites). Successful transfer depends on not only the scientific capabilities of both the sending and receiving laboratories, but also their communication skills. Many factors can contribute to the success or failure of the method transfer. Sharing of common processes and expectations is essential. Methods that were initially developed at the sending laboratory need to be vigorously tested to ensure that methods meet not only the acceptance criteria, but also scientific rigor. Potential risks (e.g., breakdown of unstable metabolites, differential recovery between incurred and QC samples and lack of suitable internal standards) of failing method transfer are carefully considered, resolved and/or mitigated during the initial method development and validation in order to avoid any delays resulting from unexpected or inconclusive results. Methods must be operated by any bioanalytical chemists with adequate professional training instead of only limited experts who may know the ‘ins and outs’ of the methods but fail to put them on paper. Method details must be carefully described and procedures verified. It cannot be overemphasized that the trivial details left out of the method procedures can literally cause the failure of the method transfer. The receiving laboratories also play a pivotal role. They should ensure the full understanding of the method transfer and ask for clarification if there are any questions regarding the method itself, instruments, reference standards, storage stability, and so forth. They should not be afraid of making suggestions for method

**Table 2.** Precision and accuracy of quality control samples from one of the method transfers in the receiving laboratory.

	QC low (50 ng/ml)	QC medium 1 (500 ng/ml)	QC medium 2 (4000 ng/ml)	QC high (14000 ng/ml)
	51.2	497	4068	13388
	50.3	506	4123	13551
	52.3	500	4129	13290
	52.8	533	4092	13715
	47.9	534	4032	13469
	49.9	529	4058	13559
Intra-run mean	50.7	516	4084	13495
SD	1.80	17.4	37.9	148
%CV	3.5	3.4	0.9	1.1
% bias	1.5	3.3	2.1	-3.6
n	6	6	6	6

Run date: 17th September 2009.

CV: Coefficient of variation; QC: Quality control; SD: Standard deviation.

improvement, but at the same time understand what the working limitations for the transfer are. Some methods can be fully revamped, while others need to stay as close as possible to the original methods, particularly within the same study. Method transfer should be built as an integral part of drug discovery/development with adequate resources and budget. Protocols, or SOP, pertinent to method transfer should be established ahead of the transfer. Since there are different scenarios for method transfer, goal and acceptance criteria of the transfer should be fully understood by all parties. Frequent communications and even on-site visits/training are essential for the success of transfers, particularly for difficult methods, all parties involved in the transfer should keep an open mind, and any information/discussion should be transparent. There will be unavoidable pitfalls and setbacks during the method transfer, but it is extremely important to keep a positive attitude and avoid accusation. Timely communication of any issue with focus on issue resolution, and an open and flexible mind to drive towards the common goal will go a long way. Successful transfer not only reduces drug discovery/development cost but also builds long-lasting relationships.

**Table 3. Precision and accuracy of quality control samples from the sending laboratory and analyzed in the receiving laboratory.**

Sample number	QC samples from sending laboratory (drug concentration: ng/ml)		
	30	600	1500
1	30.5	594	1568
2	29.2	580	1507
3	31.6	596	1562
4	33.6	602	1554
Intra-run mean	31.0	594	1538
SD	1.7	8.9	32.2
%CV	5.5	1.5	2.1
% bias	3.4	-0.9	2.6

CV: Coefficient of variation; QC: Quality control; SD: Standard deviation.

#### Financial & competing interests disclosure

*The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.*

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#### Executive summary

- Method transfers are increasingly used due to globalization of pharmaceutical development, increased bioanalytical outsourcing and the merger or acquisition of pharmaceutical companies.
- There is a need to crossvalidate bioanalytical methods between different laboratories to ensure that the pharmacokinetic and safety data are reliable and comparable.
- Method transfer between laboratories is considered as partial validation. However, no guidance regarding study design, data analysis or decision procedure is present in the current guidance. There are insufficient criteria to assure comparability and the procedure for performing appropriate method comparison or transfer program still require clarification. Method transfer might have been one of the most inconsistent practices in bioanalysis.
- A 'flexible and fit for purpose' strategy using quality control samples and incurred samples for method crossvalidation with the fixed acceptance criteria is proposed.
- To ensure a successful method transfer, protocols/standard operating procedure, documentations (e.g., method validation report and method) and timeline/budget relevant to transfer should be discussed and fully understood. Communication channel and expectation should be established.

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- **Draft EMA guidelines on the validation of bioanalytical methods with recommended procedure and acceptance criteria for method crossvalidation.**