Design and Statistical Analysis of Method Transfer Studies for Biotechnology Products

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Outline

- Method development and its life cycle management
- Purpose of analytical method transfer studies
- Parameters compared in analytical method transfer studies
- Testing materials
- Analysis
- Conclusion

New Analytical Method Development/Validation

Non-Compendial Method: Non-compendial methods are validated according to the principles described under ICH Q2 (R1) document. Typical validation characteristics are:

- Specificity
- Linearity
- Accuracy
- Precision (repeatability, intermediate precision and reproducibility)
- Range
- Limits of Detection (LOD)
- Limits of Quantitation (LOQ)

Analytical Method Validation Compendial Method: Procedures used to evaluate a defined characteristic of the drug substance or drug product that are legally recognized under 21 USC 501(b) (USP/NF). Generally, will need only partial validation i.e., need to be verified under actual conditions of use (USP chapter<1225> and 1226>).

Analytical Methods Being Validated

Analytical methods that need to be validated are:

- Lot release assays
- Stability methods for defining expiration dates/holding times
- Assays for significant process related impurities (e.g., host cell proteins, residual DNA, protein A, etc.)
- Analytical in-process tests
- Excipient and raw material testing (generally compendial)

Life Cycle Management of Analytical Methods

- Including, but not limited to
  - Trend analysis on method performance at regular intervals
    - to optimize the analytical procedure
    - to revalidate all or a part of the analytical procedure
  - Development and validation of a new or alternative analytical method
    - A new impurity
  - Method transfer to a new testing site

Analytical Method Transfer

- Method Transfer should be managed under an internal transfer protocol that details the parameters to be evaluated in addition to the predetermined acceptance criteria that will be applied to the results.
- A statistically relevant number of test articles should be used by the originating and receiving laboratories (e.g., same lots of DS and/or DP lots).
- In cases where the transferred analytical method is also stability indicating; forced degradation samples or samples containing pertinent product-related impurities should be analyzed.

Qualification *vs.* Validation

- **Assay Qualification:**

  Determining whether an assay is suitable for its intended purpose

  - Limited pre-determined performance criteria

- **Assay Validation:**

  Assuring the assay is suitable for its intended purpose on a routine basis

  - Pre-defined assay performance criteria

Analytical Method Transfer Studies

- The purpose of method transfer studies
  - To determine if the two laboratories provide comparable results across the range of interest.
    - If so, then to transfer a fully validated analytical method from the originating lab to a new lab (receiving lab)
  - Once transferred, the method is suitable for its intended use and can be used to ensure process consistency and meet product specifications

Analytical Method Transfer Studies

- **How to achieve the goal?**
  - Obtaining comparative data from method transfer studies
  - Checking the receiving lab's bias (difference between the true value and the mean of the receiving lab)
  - Determining success of implementation of the fully validated analytical method in the receiving lab

Key Parameters in Method Transfer Studies

- Mean shift (often incorrectly cited as accuracy)
  - Comparing means of two labs
- Precision(repeatability and Intermediate precision, e.g., operators, different days)
  - Comparing the standard deviations of two labs
- Bias (accuracy)

Important Factors in Method Transfer Studies

- Assuming the same type of instrument, from the same manufacturer, same reagents, same experimental conditions, and same testing procedure, we investigate the following factors:
  - Operators
  - Days
  - Runs
  - Replicates
  - Lots

Testing Materials

- Is the reference standard appropriate material from which comparative data is obtained for method transfer studies?
  - No
    - Since the method is used to ensure process consistency and meet product specifications

Testing Materials

- Multiple lots of a drug product if the assay is used for drug releasing tests
- Multiple lots of a drug substance if assay is used for measuring the content in drug substance
- Forced degradation samples or samples of a drug substance or a drug product containing pertinent product-related impurities if the transferred assay is used for stability indicating

Literature Review: Statistical Analysis

- **Many proposals exist, two examples:**
  - Significance testing approach
    - Comparing the means of two labs by the p-value of rejecting H0: $\mu_R=\mu_S$
    - Comment: This hypothesis will discourage the sponsors to use a large sample size and to obtain more precise measurement
  - Quality control method
    - Checking individual values against the control limit
    - Comment: Not quantitative criteria for decision

Our Proposal: Equivalence Test for Comparing Means of Two Laboratories

- Denote the means of the response variable of interest by μ_R and μ_S , respectively, for the receiving laboratory and the sending laboratory.

$$H_0 : \mu_R - \mu_S \leq -\delta \text{ or } \mu_R - \mu_S \geq \delta$$
$$H_a : -\delta < \mu_R - \mu_S < \delta \qquad \text{(Equation 1)}$$

- Here δ is a pre-specified constant, also called an equivalence margin.

Challenge of Setting Equivalence Margin for Equivalence Approach

- Fixed margin
  - Based on the experts' knowledge
  - Different margin for a different assay
    - 1%, a reasonable margin for HPLC
      - Too stringent for bioassay
    - 2.5%, a reasonable margin for a specific bioassay
      - Too liberal for HPLC
        - » Wider than specification 2% for drug substance assay
  - ***Challenge: It is hard to define a number for the margin***

Challenge of Setting Equivalence Margin for Equivalence Approach

- Non-fixed margin: a function of assay variability
  - Unified rule for many assays
  - Based on statistical power for rejecting the null hypothesis in the equivalence hypothesis test with a limit number of observations (not exceeding hundreds)
- All margins sits well within the assay specification

How to Obtain the Assay Variability

- Long term quality control data
  - Not appropriate, e.g.,
    - If there is a stability trend over the time
    - If there is a drift from assay instruments over the time
  - Only good if there is no other confounding factor except operators, days, and runs
    - Hard to meet this criteria
- Comparative method transfer studies
  - Estimate the assay variability from studies

Statistical Analysis for The Mean Difference of Two Labs

- Hypothesis testing (1):
  - $H_0: \mu_S - \mu_R \leq -c\sigma_S$ or $\mu_S - \mu_R \geq c\sigma_S$
  - $H_a: -c\sigma_S < \mu_S - \mu_R < c\sigma_S$
    - where μ_S and μ_R are the mean responses of the sending lab and receiving lab, respectively, and $c > 0$ is the constant.
- Equivalence margin
  - $c\sigma_S$
- Value of c
  - Determined from power function of rejecting the above hypothesis if σ_S is known

Power Function for The Two One-sided Tests Procedure

- Let $P_1(\theta^*, \sigma)$ be the probability of rejecting H_0 under H_a in Hypothesis testing (1) when $\sigma_S = \sigma_R = \sigma$.
- The power function is:

$$P_1(\theta^*, \sigma) = \int_0^{\frac{(\theta_2-\theta_1)^2 \nu}{4t_\alpha^2(\nu)\sigma^2}\left(\frac{1}{n_1}+\frac{1}{n_2}\right)^{-1}} \left(\Phi\left(\frac{\theta_2 - \theta^*}{\sigma \bullet \sqrt{\left(\frac{1}{n_1}+\frac{1}{n_2}\right)}} - t_\alpha(\nu) \bullet \sqrt{\frac{x}{\nu}} \right) - \Phi\left(\frac{\theta_1 - \theta^*}{\sigma \bullet \sqrt{\left(\frac{1}{n_1}+\frac{1}{n_2}\right)}} + t_\alpha(\nu) \bullet \sqrt{\frac{x}{\nu}} \right) \right) \frac{1}{2^{\nu/2}\Gamma(\nu/2)} x^{\nu/2-1} e^{-x/2} dx$$

  - $-\theta_1 = \theta_2 = c\sigma_S$
  - n_1: # of obs. in receiving lab
  - n_2: # of obs. in sending lab
  - $\Phi(\bullet)$: normal cumulative function

Determination of $\delta=C\sigma_S$

Power function: $P\left(\theta^* = 0, \sigma, n, \delta = C\sigma, \alpha\right) = \int_0^{c_0} \left(\Phi\left(\frac{C}{2\sqrt{1/n}} - t_\alpha(\nu)\sqrt{\frac{x}{\nu}} \right) - \Phi\left(\frac{-C}{2\sqrt{1/n}} + t_\alpha(\nu)\sqrt{\frac{x}{\nu}} \right) \right) \frac{1}{2^{\nu/2}\Gamma(\nu/2)} x^{\nu/2-1} e^{-x/2} dx$

n	C	
	Power=0.80	Power=0.85
20	0.94	0.99
22	0.90	0.95
24	0.86	0.90
26	0.82	0.87
28	0.79	0.83
30	0.76	0.80

C=0.85 is reasonably chosen such that we can achieve about 85% power with a sample size in the range 20 to 30 per laboratory.

Margin: $\delta=0.85\sigma_S$

An Example:
Internal Transfer to A New Site

- All equipment moved to the new site
- Personnel transferred to the new site
- At least 2 lots
- >2 analysts and >2 days
- Reasonable sample size per lab: ~20-30
  - Margin: e.g., $0.85\sigma_S$
  - Power to pass equivalence test is about 85% under no true mean difference

Statistical Analysis for The Mean Difference of Two Labs

- Option 1: Treating $0.85\hat{\sigma}_S$ as a constant
  - Estimating $\hat{\sigma}_S$ from the sending lab
  - Define $T_1 = \frac{\bar{X}_R - \bar{X}_S + 0.85\hat{\sigma}_S}{\sqrt{\frac{\hat{\sigma}_R^2}{n_R} + \frac{\hat{\sigma}_S^2}{n_S}}}$ and $T_2 = \frac{\bar{X}_R - \bar{X}_S - 0.85\hat{\sigma}_S}{\sqrt{\frac{\hat{\sigma}_R^2}{n_R} + \frac{\hat{\sigma}_S^2}{n_S}}}$
  - Concluding equivalence criteria is met if $T_1 > t_\alpha(\nu)$ and $T_2 < -t_\alpha(\nu)$, where $t_\alpha(\nu)$ is the 1-α quantile of t-distribution with degrees of freedom ν, α is the nominal significance level (e.g., 0.05).
  - **Inflate both type 1 and 2 error rates**

Statistical Analysis (Continued)

- Option 2:
  - Considering 0.85 $\hat{\sigma}_S$ as a random variable
  - Define $T_1' = \dfrac{\bar{X}_R - \bar{X}_S + C\hat{\sigma}_S}{\sqrt{\hat{\sigma}_R^2/n_R + \hat{\sigma}_S^2/n_S + C^2\hat{\sigma}_S^2(C_0 - 1)}}$ and $T_2' = \dfrac{\bar{X}_R - \bar{X}_S - C\hat{\sigma}_S}{\sqrt{\hat{\sigma}_R^2/n_R + \hat{\sigma}_S^2/n_S + C^2\hat{\sigma}_S^2(C_0 - 1)}}$
  - Where $C_0 = \sqrt{\dfrac{n_S - 1}{2}}\Gamma\left(\dfrac{n_S - 1}{2}\right)\Big/\Gamma\left(\dfrac{n_S}{2}\right)$
  - Concluding equivalence criteria is met if $T_1' > Z_\alpha$ and $T_2' < -Z_\alpha$, where Z_α is the 1-α quantile of standard normal distribution, α is the nominal significance level (e.g., 0.05).

Head-to-Head Approach for Comparing Precisions Obtained in Two Labs

- Hypothesis H0: $\sigma_R \leq \sigma_S$
  - Hypothesis testing: small powers to reject H0 for small samples.
- Check the point estimate
  - $\hat{\sigma}_R \leq \hat{\sigma}_S$

Receiving Lab's Bias Verification

- Important to check bias since
  - the equivalence margin can be large enough such that 90% confidence interval in mean differences falls within the equivalence margin
  - but the receiving lab's mean fails the bias criteria

Case Study #1: Analytical Method Transfer

- Following a change in the drug substance (DS) manufacturing process and site change, the SDS-PAGE method for purity of the DS was transferred to new site.
- At the new site a different densitometer was used to quantify protein gels.
- Result on DS lots from the new site with the new densitometer showed a decrease in product purity for the non-reduced SDS-PAGE assay.

Case Study #1: Analytical Method Transfer

- To demonstrate that the decrease in product purity was not due to the process change the sponsor performed side-by-side testing as follow:
  - DS batches produced by new manufacturing process were tested at the previous analytical laboratory site and at the new analytical site.
  - DS lots from original and new manufacturing process were tested at the new analytical site.

- Results from these side-by-side comparisons showed that the apparent decrease in purity was due to the use of the new densitometer and not due to change in product quality.

Case Study #2: Analytical Method Transfer

Sponsor submitted a pre-approval supplement (PAS) for the transfer of multiple analytical methods for drug product (DP) testing. The following deficiencies were noted in the method transfer study:

- It did not include comparison on percent monomer -- a lot release and stability specification.
- The method transfer included only one analyst at the recipient site.
- The results of the DP lots used to support the transfer of the method used to detect impurities were predominantly reported as < LOQ. No data were provided to demonstrate that LOQ of the transferred method at the recipient site is comparable to the original site.

Case Study #2: Analytical Method Transfer

Recommendations:

- To compare monomer results from both facilities.
- Method transfer should include two operators at both sites or provide data from additional testing sites to demonstrate that assay is not subject to operator bias.
- Include analysis of additional lots with detectable impurities. The analysis of spiked samples with known amount of impurities could also be used.

Summary

- Method transfer is one of the of the important aspects of life cycle management for analytical methods.
- Comparative method transfer studies should include:
  - assessments of accuracy (bias), mean shift and precision
  - forced degradation samples or samples containing relevant product related impurities (for stability indicating assays)
- Statistical analysis is a very important part of the method transfer studies.
- Overall assessment should be considered.

References

1. U.S. Food and Drug Administration. Draft Guidance on Analytical Procedures and Methods Validation for Drugs and Biologics (2014).
2. International Society for Pharmaceutical Engineering (ISPE). The Good Practice Guide: Technology Transfer. ISBN-13: 978-1931879132 (2003).
3. United States Pharmacopeia. Transfer of Analytical Procedure. 37-National Formulary 32. https://hmc.usp.org/sites/default/files/documents/HMC/GCs-Pdfs/c1224.pdf.
4. Ermer J, Limberger M, Lis K, Wätzig H. The transfer of analytical procedures. Journal of Pharmaceutical and Biomedical Analysis. 85: 262–276(2013).
5. Briggs .J, Nicholson R, Vazvaei F, et al. Method Transfer, Partial Validation, and Cross Validation: Recommendations for Best Practices and Harmonization from the Global Bioanalysis Consortium Harmonization Team. The AAPS Journal 2014. 1143-1148 (2014).
6. Wieling J. Robust, fit-for-purpose method transfer: why we should apply equivalence testing. Bioanalysis. 7(7). 807–814 (2015).
7. Lin Z, Li W, Weng N. Capsule review on bioanalytical method transfer: opportunities and challenges for chromatographic methods. Bioanalysis. 3(1). 57–66 (2011).
8. Chambers D, Kelly G, Limentani G, Lister A, Lung KR, Warner E. Analytical Method Equivalency--An Acceptable Analytical Practice. Pharmaceutical Technology. 64-80 (2005).
9. Dewé W, Govaerts B, Boulanger B, Rozet E, Chiap P, Hubert P. Using Total Error as Decision Criterion in Analytical Method Transfer. Chemometrics and Intelligent Laboratory Systems. 85.262–268 (2007).
10. Kaminski L, Schepers U, Wätzig H. Analytical method transfer using equivalence tests with reasonable acceptance criteria and appropriate effort: Extension of the ISPE concept. Journal of Pharmaceutical and Biomedical Analysis. 53. 1124–1129 (2010).
11. Frömke C, Hothorn LA, Sczesny F, Onken J, Schneider M. Analytical method transfer: Improving interpretability with ratio-based statistical approaches. Journal of Pharmaceutical and Biomedical Analysis. 74. 186– 193 (2013).
12. Zhong JL, Lee K, Tsong Y. Statistical Assessment of Analytical Method Transfer. Journal of Biopharmaceutical Statistics. 18(5). 1005-1012 (2008).
13. Schwenke JR, O'Connor DK. Design and Analysis of Analytical Method Transfer Studies. Journal of Biopharmaceutical Statistics. 18 (5). 1013-1033 (2008).
14. Altan S, Shoung JM. Block designs in method transfer experiments. Journal of Biopharmaceutical Statistics.18(5). 996-1004 (2008).
15. Agut C, Caron A, Giordano C, Hoffman D, Ségalini A. Transfer of analytical procedures: A panel of strategies selected for risk management, with emphasis on an integrated equivalence-based comparative testing approach. Journal of Pharmaceutical and Biomedical Analysis. 56. 293– 303 (2011).
16. Krause S. Validation of Analytical Methods for Biopharmaceuticals: A Guide to Risk-Based Validation and Implementation Strategies. PDA/DHI, River Grove, IL, ISBN-10: 1933722061 (2007).
17. Satterthwaite FE. An Approximate Distribution of Estimates of Variance Components. Biometrics Bulletin. 2. 110–114 (1946).
18. Chen Y, Weng Y, Dong X, Tsong Y. Wald Tests for Variance-Adjusted Equivalence Assessment with Normal Endpoints. Journal of Biopharmaceutical Statistics (2016). http://www.tandfonline.com/doi/full/10.1080/10543406.2016.1265542.
19. Okamoto M. Assay validation and technology transfer: Problems and solutions. Journal of Pharmaceutical and Biomedical Analysis. 87. 308– 312 (2014).

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