Austin Journal of Analytical and Pharmaceutical Chemistry

Open Access

Austin Publishing Group

Mini Review

Bio-Analytical Method Validation-A Review

Taleuzzaman M[1]*, Gilani SJ[1], Ali S[1], Hafeez A[1] and Nagarajan K[2]

[1]Glocal School of Pharmacy, Glocal University, Saharanpur, U.P.247121, India

[2]KIET School of Pharmacy, 13 Km. Stone, Ghaziabad-Meerut Road, Ghaziabad-201206, India

***Corresponding author:** Mohamad Taleuzzaman, Glocal School of Pharmacy, Glocal University, Saharanpur, U.P.247121, India

Received: November 02, 2015; **Accepted:** December 04, 2015; **Published:** December 07, 2015

Abstract

Validation is a basic requirement to ensure quality and reliability of method development in analytical and bio-analytical process. Bio-analytical method development is very important during the process of drug discovery and development for marketing approval. The purpose of this review is to discuss about the step involved in validation and provide a practical approach for determining the different parameters like selectivity, specifity, limit of detection, lower limit of quantitation, linearity, range, accuracy, precision, recovery, stability, ruggedness, and robustness to help the perfect studies of pharmacokinetic, toxic kinetic, bioavailability and bioequivalence. Bio-analysis study is for the quantitative determination of drug and their metabolites in biological fluids. Accurate and robust methods for quantitative analysis of drug and their metabolites are important for the successful conduct of pre-clinical, bio-pharmaceutics and clinical pharmacology.

Keywords: Bio-analytical method development; Validation parameters; Documentation

Introduction

Bio-analytical validation is a detailed description of different steps necessary to perform the developed methods [1-5]. Method development and validation has a great importance in the field of pharmacokinetic (PK), bioavailability (BA) and bioequivalence (BE) studies for new drug approval and investigation by regulatory bodies [6-8]. Bio-analysis of drugs and its metabolites study is very important for the drug efficacy, side effect and bioavailability of drug. A robust method also need for the forensic and toxicological interpretation. The quality of toxicological studies data can be made more accurate by following an accurate and robust method development and validation. In forensic and clinical toxicological studies bio-analysis method play a very important role to find the exact and accurate cause of concerns [9-10]. Bio-analytical method validation is performed according to the ICH guidelines which come under Q2A and Q2B [11-13]. A full and final robust method is applied for the routine analysis. It is highly true I respect of quality management and accreditation, which have become matters of increasing importance in analytical toxicology in the recent years [14-16]. This is also reflected in the increasing requirements of peer-reviewed scientific journals concerning method validation. Therefore, this topic should extensively be discussed on an international level to reach a consensus on the extent of validation experiments and on acceptance criteria for validation parameters of bio-analytical methods in forensic (and clinical) toxicology [17,18].

Demand of Bioanalytical Method Validation

Bio-analytical method validation or bio-analysis of drug has its own value which will depend upon analytes nature and technology which use for the method development and validation. A reliable and reproducible methods and techniques are always very demanding for the drugs and its metabolites studies for bioavailability (BA), bioequivalence (BE) and pharmacokinetic (PK) parameter for conducting the pre clinical studies [19-21]. A perfect approach to reach the drugs and its metabolites best efficacy and side effects value can be known by the accurate and sensitive method development and validation. In respect of economy and market demand the chief and best bio-analytical methods are adopted for routine analysis [22].

Bioanalytical Method Validation

In bio-analytical method validation different types and levels are come which must be need to understand basic requirement in the process. Here all types are defined in very specific manner.

A. Full Validation

B. Partial Validation

C. Cross Validation

A. Full Validation: After developing a new method for new drug need to validate the entire step as per the ICH guidelines. It is very important for the new drug and also if metabolites are exits with drug.

B. Partial Validation: Modification of full validation bio-analytical method that do not necessarily call for full validation. Modification require in typical bio-analytical method changes are,

- Bio-analytical method transfer between laboratories or analysts
- Change in analytical methodology
- Change in anticoagulant in harvesting biological fluid
- Change in matrix within species (e.g. human plasma to human urine)
- Change in sample processing procedure
- Change in species within matrix (e.g. rat plasma to mouse plasma)
- Change in relevant concentration range

Taleuzzaman et al. © All rights reserved

Citation: Taleuzzaman M, Gilani SJ, Ali S, Hafeez A and Nagarajan K. Bio-Analytical Method Validation-A Review. Austin J Anal Pharm Chem. 2015; 2(5): 1053.

- Change in instrument and /or software platforms
- Limited sample volume
- Rare matrices

C. Cross Validation: Comparison two bio-analytical validation method parameters within the same study or across different studies. Cross validation can be done by reference methods with revised bio-analytical methods. The comparisons should be done in both ways. Cross validation should also be done when data generated using different analytical technique (e.g. LC-MS-MS vs. ELISA) [11,23-25].

Steps of Bioanalytical Validation

1. Development Manual: Documents about company policy and essential requirements for validation should be available.
2. Selection of analytical method and instruments: Selection of analytical method and the instruments depends on the ultimate aim of analysis.
3. Installation qualification (IQ): Documents that indicate that the instrument meets the requirements of the system and ready for installation in accorandance with the standards and specification must be met.
4. Operational qualification (OQ): Verification that the installed system is suitable for intended purpose [12,13,26].

Bioanalytical Validation Parameters

Validation in Bio-analytical method is including [27,28].

1. Selectivity
2. Linearity
3. Accuracy, Precision, Recovery
4. Limit of detection (LOD)
5. Limit of quantification (LOQ)
6. Calibration Curve
7. Stability of analyte in spiked
8. Ruggedness (Robustness) (Table 1)

1. Selectivity: It is defined as that an analytical method to differentiate and quantify the analyte in the presence of other components in the sample. For this parameter practically performed as in laboratory by selecting a minimum six set of appropriate biological matrix (plasma, urine, or other matrix). Each blank sample should be tested for interference at specific retention time and should be ensured at the lower limit of quantification (LLOQ).Chance of interfering substance in a biological matrix include endogenous matrix components, metabolites, decomposition products or other substance. For method development should be select a blank sample that ensure there is no interference in at retention time of analyte.

2. Linearity: Ability of an analytical method to obtain test results which are directly proportional to the concentration of analyte in sample. In bio-analysis spiked at least five or six samples which cover entire range from lower limit to higher limit. Apart from visual observation that signal as function of the concentration special statistical calculation are recommended such as linear regression. Other parameters like slope and intercept, residual sum of squares and the and the coefficient of correlation of should be reported to better the linearity of the method.

3. Accuracy, Precision, Recovery: In Bio-analytical method development accuracy, precision and recovery is performed with a minimum of six batches with range of concentration from LOQ to highest concentration of range. Take nine samples in a one batch. Accuracy can be expressed by the following approaches, (a) Inferred from precision, linearity and specificity (b) Comparison of the results with those of a well characterized, independent procedure (c) Application to a reference material (for drug substance) (d) Recovery of drug substance spiked to placebo or drug product (e) Recovery of the impurity spiked to drug substance or drug product for impurities. The mean value should be within 15 % of the actual value except at LLOQ, where it should not deviate by more than 20%.

The precision of an analytical method expressed the closeness of individual measures of an analyte when the procedure is applied repeatedly to multiple of a single homogeneous volume of biological matrix. Precision should be measured using a minimum of five determinations per concentration. A minimum of three concentrations in the range of expected concentration is recommended .The precision determined at each level should not exceed 15 % of the coefficient of variation (CV) except for the LLOQ, where it should not exceed 20 % of the CV. Precision is further subdivided into within –run, intra-batch precision or repeatability.

The recovery of an analyte in bio-analytical method is the detector response obtained from an amount of the analyte added to and extracted from biological matrix, compared to the detector response obtained for the true concentration of the pure authentic standard. Recovery pertains to extraction efficiency of an analytical method within the limits of variability. Analyte recovery need not be 100% but the extent of recovery of an analyte and of the internal standard should be consistent, precise and reproducible. Recovery experiments should be performed by comparing the analytical results for extracted samples at three concentrations (Low, Medium and High).

4. Limit of Detection (LOD): The lowest concentration of sample that can be differentiating signal from background noise. Use a standard solution of analyte and signal to noise ratio not less than 3.0. The lowest concentration of sample which can be quantitavely determined with suitable precision and accuracy. Various approaches are applied for the determinations of LOD, (a) Visual definition (b) calculation from single to noise ratio (c) calculation from the standard deviation of the blank (d) calculation from the calibration line at low concentration.

5. Limit of Quantification (LOQ): It is the lowest concentration of analyte at which the analyte can not only be reliably detected but at which some predefined goals for bias and imprecision are met.

6. Calibration Curve: A standard curve or calibration curve is a relationship between instrument response and analyte concentration. A calibration curve covers a range from lower concentration to higher concentration with eight different concentration values. Calibration

Table 1: Overview of Validation Parameter and Stability Criteria.

S.No.	Validation Parameter	Minimum Experiments	Performance Criteria
1.	Selectivity	Matrix blanks: 6 lots, n=1 for each lot Matrix blank fortified with IS: 6 lots, n=1 for each lot LLOQ Selectivity Sample: 6 lots, fortified with analyte at LLOQ level and IS. n=3 for each lot	At least 5 out of the 6 lots must meet the following criteria: Response for the analyte in matrix blanks or matrix blank fortified with IS must be ≤20% of the mean analyte response in the acceptable LLOQ calibration standards Response for IS in matrix blanks must be ≤5% of the mean IS response in the acceptable LLOQ calibration standards At least two-thirds of the selectivity LLOQ replicates for each lot must meet accuracy acceptance limit, and the mean accuracy must be within ±20.0% of the nominal concentration
2.	Cross-analyte Interference	Each analyte at ULOQ evaluated separately. IS at the level of use evaluated separately	Interference must be ≤ 20% of the mean analyte peak response or ≤ 5% of the mean IS response of the acceptable LLOQ calibration Standards
3.	Linearity	Minimum of 6 non zero calibration standard (CS) levels.	(R^2) ≥0.985
4.	Calibration Standards: Accuracy	Injected at the beginning and end of the analytical run	Minimum 6 non-zero (or 75% of total) CS must be within ±15.0% RE of nominal (exception: LLOQ within ±20.0 %RE)
5.	QC Samples Core Validation	Three concentration levels: Low, Mid, High; n=6 at each level	Minimum 50% of the QC replicates at each level and 66.7% of all QCs must be within 15.0% RE of nominal Mean inter- and intra-assay accuracy within ±15.0% RE of nominal; Precision ≤15.0% RSD.
6.	LLOQ Samples (Sensitivity)	n=6, ≥ 1 run	Mean accuracy within ±20.0%RE of nominal; precision ≤20.0% RSD.
7.	Recovery	Analyte at low, medium and high levels, and IS at the level of use: pre extraction spiked samples (n=6) are compared with mean response of post-extraction spiked matrix samples (n=6)	Recovery for analyte and IS must be relatively consistent across all QC levels.
8.	Matrix Effect	Post-extraction spiked samples (n=6, at each QC low, mid and high level) are compared with mean response of 6 injections of analyte or IS in solvent.	MF will be calculated and reported for the analyte and for the IS
9.	Ruggedness	Minimum of two variables over the course of validation (e.g. different column, instrument and/or analyst)	Mean inter- & intra-assay accuracy within ±15.0% RE of nominal; precision ≤15.0% RSD.

curve should be prepared same matrix as used in bio-analysis of drug. The number of standard for the calibration curve will be a function of the anticipated range of analytical values and the nature of the analyte response. Concentration of standard should be chosen on the basis of concentration range on particular study. A calibration curve should consist of blank sample (matrix sample without internal standard), zero sample (matrix with internal standard) and six to eight non-zero samples covering the expected range including LLOQ.

LOWER LIMIT OF QUANTIFICATION (LLOQ): For preparation of calibration curve LLOQ standard analyte response must be five times response compared to blank response. Analyte response should be identifiable and reproducible with a precision of 20% and accuracy 80-120%. Standard curve response of the standard must be met the following, that LLOQ deviation not more than 20% and other standard deviation not more than 15 %. A minimum of eighty percent of sample must within the acceptance criteria then the standard curve will be passing.

7. Stability of spiked sample: Drug stability in biological fluid is related to the chemical properties of analyte, the storage condition, the matrix and the container system. Stability procedure should evaluate the stability of the analyte during sample collection and handling. Stability study performed by the following parameters –Freeze and thaw stability, Short term temperature stability, Long term stability, Stock solution stability and Post –preparative stability [29] (Table 2).

(A) Freeze and thaw stability: Perform by studying the three freeze and thaw cycles. At least three aliquots are analyzed from lowest and highest concentration standards of the sample. Three-three sample of each concentration are stored at intended storage temperature and after 24 hours thawed at room temperature. After completely thaw the sample should be re-frozen for 12- 24 hours for the same condition.

(B) Short – Term temperature stability: Three aliquots of each sample from lowest and highest concentration should be thawed at room temperature for 4- 24 hrs. And then analyzed.

(C) Long –Term Stability: Storage time for the long term stability is from the time between date of sample collection and the date of last sample analysis in laboratory.

(D) Stock Solution stability: The stability of stock solution and the internal standard should study at room temperature for at least six hours. After completion of the desired storage time the stability should be tested by comparing the instrument response with that of freshly prepared solution [1-5].

8. Ruggedness (Robustness): Ruggedness explain the susceptibility of a method to small changes that might be occurring during small changes in routine analysis like small changes of pH values, composition of mobile phase, temperature, flow rate of mobile phase etc. Ruggedness of a developed method shows perfectness of the method.

Basic Principal of Bio-analytical Method Validation and its Establishment

i. The basic fundamental parameter for the bio-analytical method development are accuracy, precision, selectivity, sensitivity, reproducibility and stability should be ensure the acceptability as per the ICH guidelines.

ii. The specific detailed should be written in the form of protocol, study plan or SOP.

Table 2: Overview of Validation Stability and Criteria.

S.No.	Validation Parameter	Minimum Experiments	Performance Criteria
1.	Stock Solution	n ≥6; long term at typical storage conditions; bench top at conditions representing typical processing conditions for ≥6 hours.	Precision of area response or relative response must be ≤15.0% RSD; RD within 7.0% for analytes, 20.0% for internal standards
2.	Bench top	≥ 4 hours n ≥6 at QC Low and High levels	Mean accuracy within ±15.0% RE of nominal; precision ≤15.0% RSD
3.	Freeze/Thaw	3 freeze/thaw cycles n ≥6 at QC Low and High levels	Mean accuracy within ±15.0% RE of nominal; precision ≤15.0% RSD
4.	Long term	n ≥ 6 at QC Low and High levels at -10 to -30°C or -50 to -90°C for at least 1 and 4 months	Mean accuracy within ±15.0% RE of nominal; precision ≤15.0% RSD
5.	Reinjection Reproducibility	Calibrations standards (CS) and QCs (n=6 at each level) reinjected from an acceptable validation batch run, maintained at autosampler temperature for ≥72 hours.	Mean accuracy within ±15.0% RE of nominal; precision ≤15.0% RSD; calculated using calibration standards from re-injected run
6.	Extract Stability	Stored extracts at QC Low, Mid, and High levels (n=6) maintained at autosampler temperature for ≥72 hours.	Mean accuracy within ±15.0% RE of nominal; precision ≤15.0% RSD; calculated using freshly extracted curves or back calculated using the original curves from the batch the aged extracts were originally extracted and injected

iii. Each step in the method should be investigated the effect of environmental, matrix, and procedural effect from time of collection material up to and including the time of analysis.

iv. Variability of the matrix effect due to physiological nature of the sample. In case of LC-MS-MS based procedure appropriate step should be taken to ensure the free from the effect of matrix effect.

v. A Bio-analytical method should be validated for the intended use or application.

vi. The stability of analyte (Drug or Metabolite product) in matrix during the collection process and also in bio-analysis should be assessed preferable prior to sample analysis.

vii. Whenever possible use the same biological matrix in method development and validation both.

viii. The accuracy, precision, reproducibility, response function and selectivity of the method developed for intended biological matrix use in method development as well as validation study should be same acceptance parameter.

ix. The standard curve first developed in aqueous medium than this range of concentration may be some time modified to extend the range to prepare six to eight non-zero standard concentration.

x. The linearity of standard curve depends upon the concentration and response relationship. The response of a particular concentration should be continuous and reproducible. Standard curve linearity developed and validated by performing three batch of blank sample, zero standards and none zero standard samples.

xi. Sufficient number QC samples should be analyzed as per the run size of the batch. The number of QC samples to ensure the proper control of the analysis.

xii. To ensure the bio-analytical method valid must be specific acceptance criteria for the QC sample over range of standard [30].

References

1. Thompson M, Ellison SLR, Wood R. Harmonized Guidelines for Single Laboratory Validation of Method of Analysis. Pure Appl Chem. 2008; 74: 835–855.

2. Wood R. How to Validate Analytical Methods. Trends Analyt Chem. 2005; 18: 624–632.

3. McDowall RD. The Role of Laboratory Information Management Systems LIMS in Analytical Method Validation. Anal. Chim. Acta. 2007; 54: 149–158.

4. Vander Heyden Y, Nijhuis A, Smeyers-Verbeke J, Vandeginste BG, Massart DL. Guidance for robustness/ruggedness tests in method validation. J Pharm Biomed Anal. 2001; 24: 723-753.

5. Puluido A, Ruusanches I, Boque R, Rius FX. Uncertainty of results in routine Qualitative Analysis in Analytical Chemistry. J Pharm Biomed Anal. 2005; 22: 647–654.

6. Jhang JS, Chang CC, Fink DJ, Kroll MH. Evaluation of linearity in the clinical laboratory. Arch Pathol Lab Med. 2004; 128: 44-48.

7. Mark H. Application of an improved procedure for testing the linearity of analytical methods to pharmaceutical analysis. J Pharm Biomed Anal. 2003; 33: 7-20.

8. Trullols E, Ruisanchez I, Rius FX. Trends in Analytical Chemistry. J Lab Invest. 2003; 23: 137–145.

9. Valcarcel M, Cardenas S, Gallego M. Sample screening system in analytical chemistry. Trends Analyt Chem. 1999; 18: 685-694.

10. Ye C, Liu J, Ren F, Okafo N. Design of experiment and data analysis by JMP (SAS institute) in analytical method validation. J Pharm Biomed Anal. 2000; 23: 581-589.

11. Lindner W, Wainer IW. Requirements for initial assay validation and publication in J. Chromatography B. J Chromatogr B Biomed Sci Appl. 1998; 707: 1-2.

12. Shah VP, Midha KK, Dighe S, McGilveray IJ, Skelly JP, Yacobi A, et al. Analytical methods validation: bioavailability, bioequivalence and pharmacokinetic studies. Conference report. Pharm Res. 2009; 9: 588–592.

13. Penninckx W, Hartmann C, Massart DL, Smeyers-Verbeke J. Validation of the Calibration Procedure in Atomic Absorption Spectrometric Methods. J Anal At Spectrom. 1998; 11: 237–246.

14. Nowatzke W, Woolf E. Best Practices during Bioanalytical Method Validation for the Characterization of Assay Reagents and the Evaluation of Analyte Stability in Assay Standards, Quality Controls, and Study Samples. AAPS J. 2007; 9: E117–122.

15. Braggio S, Barnaby RJ, Grossi P, Cugola M. A strategy for validation of bioanalytical methods. J Pharm Biomed Anal. 1996; 14: 375-388.

16. James CA, Breda M, Frigerio E. Bioanalytical method validation: a risk-based approach? J Pharm Biomed Anal. 2004; 35: 887-893.

17. Nakashima K. High-Performance Liquid Chromatography of drug of abuse in biological samples. J Health Sci. 2009; 51: 272–277.

18. Boulanger B, Chiap P, Dewe W, Crommen J, Hubert P. An analysis of the SFSTP guide on validation of chromatographic bioanalytical methods: progress and limitations. J Pharm Biomed Anal. 2003; 32: 753-765.

19. Causon R. Validation of chromatographic methods in biomedical analysis. Viewpoint and discussion. J Chromatogr B Biomed Sci Appl. 1997; 689: 175-180.

20. Hartmann C, Smeyers-Verbeke J, Massart DL, McDowall RD. Validation of bioanalytical chromatographic methods. J Pharm Biomed Anal. 1998; 17: 193-218.

21. Hubert P, Chiap P, Crommena J, Boulanger B, Chapuzet EN, Laurentie M, et al. The SFSTP guide on the validation of chromatographic methods for drug bioanalysis: from the Washington Conference to the laboratory. Anal Chim Acta. 1999; 391: 135–148.

22. Zhoua S, Songb Q, Tangb Y, Weng N. Critical Review of Development, Validation, and Transfer for High Throughput Bioanalytical LC-MS/MS Methods. Curr Pharm Anal. 2005; 55: 3–14.

23. Tabrizi-Fard MA, Fung HL. Reversed-phase high-performance liquid chromatography method for the analysis of nitro-arginine in rat plasma and urine. J Chromatogr B Biomed Appl. 1996; 679: 7-12.

24. Dadgar D, Burnett PE. Issues in evaluation of bioanalytical method selectivity and drug stability. J Pharm Biomed Anal. 1995; 14: 23-31.

25. Hartmann C, Massart DL, McDowall RD. An analysis of the Washington Conference Report on bioanalytical method validation. J Pharm Biomed Anal. 1994; 12: 1337-1343.

26. Wieling J, Hendriks G, Tamminga WJ, Hempenius J, Mensink CK, Oosterhuis B, et al. Rational experimental design for bioanalytical methods validation. Illustration using an assay method for total captopril in plasma. J Chromatogr. 2006; 730: 381–394.

27. Hubert H, Chiap P, Crommen J, Boulanger B, Chapuzet E, Mercier N, et al. The SFSTP guide on the validation of chromatographic methods for drug analysis: from the Washington Conference to the laboratory. Anal Chim Acta. 1999; 391: 45–55.

28. Miller KJ, Bowsher RR, Celniker A, Gibbons J, Gupta S, Lee JW, et al. Workshop on bioanalytical methods validation for macromolecules: summary report. Pharm Res. 2001; 18: 1373-1383.

29. Kringle R, Hoffman D. Stability methods for assessing stability of compounds in whole blood for clinical bioanalysis. Drug Info J. 2001; 35: 1261–1270.

30. Dighe S, Shah VP, Midha KK, McGilveray IJ, Skelly JP, Yacobi A, et al. Analytical methods validation: bioavailability, bioequivalence and pharmacokinetics studies. Conference Report. Eur J Drug Metabol Pharmacokinetics. 1998; 16: 249–255.

Austin J Anal Pharm Chem - Volume 2 Issue 5 - 2015
ISSN : 2381-8913 | www.austinpublishinggroup.com
Taleuzzaman et al. © All rights reserved

Citation: Taleuzzaman M, Gilani SJ, Ali S, Hafeez A and Nagarajan K. Bio-Analytical Method Validation-A Review. Austin J Anal Pharm Chem. 2015; 2(5): 1053.