International Journal of Trend in Scientific Research and Development (IJTSRD)
Volume 6 Issue 7, November-December 2022 Available Online: www.ijtsrd.com e-ISSN: 2456 – 6470

Review on Bioanalytical Method Development in Human Plasma

Mayuri Gavhane, Dr. Ravindra Patil*, Tejaswini Kande

PDEA'S SUCOPS & RC Kharadi, Pune, Maharashtra, India

ABSTRACT

Bioanalytical methods are widely used to quantitate drugs and their metabolites in plasma matrices and this method are applied to study in the areas of human clinical and nonhuman study. Bioanalytical methods employed for the quantitative estimation of drugs and their metabolites plays an important role in estimation and interpretation of bioequivalence, pharmacokinetic, and toxicokinetic studies. The major bioanalytical role is method development, method validation, and sample analysis. Techniques such as high-pressure liquid chromatography (HPLC) and liquid chromatography coupled with double mass-spectrometry (LCMS-MS) can be used for bioanalytical studies.

KEYWORDS: *Method development, Analyte, Validation of bioanalysis techniques, Validation parameter*

How to cite this paper: Mayuri Gavhane | Dr. Ravindra Patil | Tejaswini Kande "Review on Bioanalytical Method Development in Human Plasma" Published in International Journal of Trend in Scientific Research and Development (ijtsrd), ISSN: 2456-6470, Volume-6 | Issue-7, December 2022, pp.1245-1251, URL: www.ijtsrd.com/papers/ijtsrd52578.pdf

IJTSRD52578

INTRODUCTION

This Bioanalytical method development is a procedure that is basically used to incorporate quantitative analysis useful in biomedical applications. Quantification of concentrations of drugs in biological matrices comprising serum, urine, plasma, saliva and blood are a relatively critical facet of development of a medicinal product; correspondingly these statistics might be a requisite for novel active substances and generics along with deviations to authorized drug products. The findings and repercussions of clinical trials and such animal toxicokinetic studies are utilized to make pivotal decisions assisting the potency and safety of a medicinal drug product. It is thereby crucial that the implemented Bioanalytical methods employed are considerably characterized, documented and completely validated to an adequate standard for the purpose of yielding trustworthy results. Bioanalytical method validation is used for the figuring out quantitative analysis of drugs and their further metabolites in biological fluid exerts substantial purpose in the elucidation and assessment of bioequivalence along with the bioavailability of the drug as well as the pharmacokinetic and toxicokinetic evidence of the study. It is paramount not only in terms of the regulatory submission but also for guaranteeing procreation of high standard data in the course of drug discovery and development. The calibre of these research studies is bluntly proportional to the quality of the fundamental data of bioanalysis. Thereby it's quite pivotal that steering principles for the validation of these methods of analysis be accustomed and distributed to the pharmaceutical society. As per the guidelines issued by globally recognized regulatory body like European Medicines Agency (EMA) and The United States Food and Drug Administration (USFDA) it is evident that methods like high-performance liquid [1,2,3,4].

Types of Bioanalytical Method Validation

Bioanalytical method validation is classified into three types

A. Full validation
B. Partial validation
C. Cross validation

Full validation

The full validation is an establishment of all validation parameters to apply to sample analysis for

the bioanalytical method for each analyte [1,15,19]. Full validation is important:

1. When developing and implementing a bioanalytical method for the first time.
2. For a new drug entity.
3. A full validation of the revised assay is important if metabolites are added to an existing assay for quantification [19,21].

Partial validation:

Partial validations are modifications of already validated bioanalytical methods or Modification of validated bioanalytical methods that do not necessarily call for full revalidation [15,16,18]. Partial validation can range from as little as one intra-assay accuracy and precision determination to a nearly full validation. Typical bioanalytical method changes that fall into this category include, but are not limited to:

1. Bioanalytical method transfers between laboratories or analysts
2. Change in analytical methodology (e.g., change in detection systems)
3. Change in anticoagulant in harvesting biological fluid
4. Change in matrix within species (e.g., human plasma to human urine)
5. Change in sample processing procedures [21].
6. Change in species within matrix (e.g., rat plasma to mouse plasma)
7. Change in relevant concentration range
8. Changes in instruments and/or software platforms
9. Limited sample volume (e.g., pediatric study)
10. Rare matrices
11. Selectivity demonstration of an analyte in the presence of concomitant medications Selectivity demonstration of an analyte in the presence of specific metabolites [1,17,19].

Cross validation:

Cross-validation is a comparison of validation parameters when two or more bioanalytical methods are used to generate data within the same study or across different studies [15,18,22].

1. An example of cross-validation would be a situation where an original validated bioanalytical method serves as the reference and the revised bioanalytical method is the comparator. The comparisons should be done both ways.

A. When sample analyses within a single study are conducted at more than one site or more than one laboratory, cross validation with spiked matrix standards and subject samples should be conducted at each site or laboratory to establish inter laboratory reliability.

B. Cross-validation should also be considered when data generated using different analytical techniques (e.g., LC-MS-MS vs. ELISA) in different studies are included in a regulatory submission [1,15,17,21].

IMPORTANCE OF BIOANALYTICAL METHOD VALIDATION

1. It is paramount to utilize completely verified and validated methods of Bioanalysis for showcasing dependable results which can be interpreted tolerably.
2. Such methods of bioanalysis and their sets of techniques are regularly altered and developed.
3. It is vital to highlight that every single technique of Bioanalysis has unique peculiarities that may change depending on the type of analyte, there has to be development of a particular criteria for assessment of every other analyte.
4. On top of that, the suitability of the technique can also change with respect to the aim of the study that needs to be done. For e.g., during analysis of a specific sample for defined research is carried out at multiple sites, it is essential to assess the method of Bioanalysis at every site and present relevant assessment data for various sites to set up inter-laboratory stability.

1. **Need of Bioanalytical Method Validation:**

1. It is essential to used well-characterized and fully validated bioanalytical methods to yield reliable results that can be satisfactory interpreted.
2. It is recognized that bioanalytical methods and techniques are constantly undergoing changes and improvements; they are at the cutting edge of the technology.
3. It is also important to emphasize that each bioanalytical technique has its own characteristics, which will vary from analyte to analyte, specific validation criteria ma need to be developed for each analyte.
4. Moreover, the appropriateness of the technique may also be influenced by the ultimate objective of the study. When samples analysis for a given study is conducted at more than one site, it is necessary to validate the bioanalytical methods at each site and provide appropriate validation information for different sites to establish inter-laboratory reliability 18.

Typical parameters to validate are include:
selectivity, accuracy, precision, linearity and range, limit of detection, limit of quantification, recovery, robustness and stability. General recommendation for analytical method validation. i.e. for pharmaceutical methods, can be found in the FDA guidelines or other publications.

Accuracy: The degree of closeness of the observed concentrations to the nominal or known true concentration. It is typically measured as relative error (% RE) [19]. Accuracy is an absolute measurement an accurate method depends on several factors such as specificity and precision. Accuracy is sometimes termed as trueness. Accuracy is determined by replicate analysis of samples containing known amounts of the analyte. Accuracy should be measured using minimum of five determinations per concentrations. A minimum of three concentrations in the range of expected study sample concentrations is recommended. The mean value should be within 15% of the nominal value except at LLOQ, where it should not deviate by more than 20%. The deviation of the mean from the nominal value serves as the measure of accuracy.

The two most commonly used ways to determine the accuracy or method bias of an analytical method are
1. Analysing control samples spiked with analyte and
2. by comparison of the analytical method with a reference method.

Accuracy is best reported as % bias which is calculated from the expression:
Absolute% Bias = measured value – true value/ true value X 100.

Precision: The precision of a bioanalytical method is a measure of the random error and is defined as the closeness of agreement between a series of measurement obtained from multiple sampling of the same homogenous sample under the prescribed conditions. Measurement of scatter for the concentrations obtained for replicate samplings of a homogenous sample. It is typically measured as coefficient of variation (%CV) or relative standard deviation (R.S.D.) of the replicate measurement 20% C V = standard deviation / men X 100

Repeatability: Repeatability express the analytical variability under the same operating over a short interval of time (within assay, intra assay). Repeatability means how the method performs in one lab and on one instrument, within a given day. Precision measured under the best condition possible (short period, one analyst etc.)

Reproducibility: Reproducibility is the precision between laboratories (collaborative or interlaboratory studies), is not required for submission, but can be taken into account for standardization of analytical procedures. Ability of the method to yield similar concentrations for a sample when measured on different occasions. Reproducibility refers to how that method performs from lab-to-lab, from day-to-day, from analyst-to-analyst, and from instrument-to-instrument, again in both qualitative and quantitative terms [21].

Linearity: The ability of the bioanalytical procedure to obtain test results that are directly proportional to the concentrations of analyte in the sample within the range of the standard curve. The concentrations range of the calibration curve should at least span those concentrations expected to be measured in the study samples. If the total range cannot be described by a single calibration curve, two calibration ranges can be validated. It should be kept in mind that the accuracy and precision of the method will be negatively affected at the extremes of the range by extensively expanding the range beyond necessity. Correlation coefficients were most widely used to test linearity.

Selectivity and specificity: The ability of the bioanalytical methods to measure and differentiate the analytes in the presence of components that may be expected to be present. These could include metabolites, impurities, degradants or matrix components [22]. Selectivity is the documented demonstrations of the ability of the Bioanalytical procedure to discriminate the analyte from interfering components. It is usually defined as the ability of the bioanalytical method to measure unequivocally and to differentiate "the analytes in the presence of components, which may be expected to be present" [22]. Analysis of blank samples of the appropriated biological matrix should be obtained from at least six sources. Each blank sample should be tested for interference and selectivity should be ensured at the lower limit of quantification (LLOQ) [23]. These interference may arise from the constituents of the biological matrix under study, be it an animal (age, sex, race, ethnicity etc.) or a plant (development stage, variety, nature of the soil, etc.) or they could also depend on environmental exposure (climatic conditions such as UV –light, temperature and relative humidity). Specificity is the ability to assess unequivocally the analyte in the presence of components that may be expected to be present. For example, in high- performance liquid chromatography with UV detection (RP-HPLC-UV), a classic chromatographic method, the method is specific if the assigned peak at a given retention time belongs only

to one chemical entity; in liquid chromatography with mass spectrometry detection the detector could measure selective an analyte, even if this is not fully separated from endogenous compounds etc. Despite this controversy, there is a broad agreement that specificity/ selectivity is the critical basis of each analytical procedure.

Limit of Detection (LOD): The lowest amount of analyte that can be detected but not quantified [21]. The calculation of the LOD is open to mis interpretation as some bioanalytical laboratories just measure the lowest amount of a reference solution that can be detected and others the lowest concentration that can be detected in biological sample [23]. There is an overall agreement that the LOD should represent the smallest detectable amount or concentration of the analyte of interest.

Limit of Quantitation: The quantitation limit of individual analytical procedure is the lowest amount of analyte in a sample, which can be quantitatively determined with suitable precision and accuracy [23]. LLOQ is the lowest amount of analyte in a sample that can be quantitatively determined with suitable precision and accuracy. Determining LLOQ on the basis of precision and accuracy is probably the most practical approach and defines the LLOQ as the lowest concentration of the sample that can still be quantified with acceptable precision and accuracy. LLOQ based on signal-to-noise ratio (S/N) can only be applied only when there is baseline noise, for example to chromatographic methods. A 10:1 S/N is considered to be sufficient to discriminate the analyte from the background noise. Upper limit of quantification (ULOQ) is the maximum analyte concentration of a sample that can be quantified, with acceptable precision and accuracy. The ULOQ is identical with the concentration of the highest calibration standards.

Quantification Range: The range of concentration, including the LLOQ and ULLOQ that can be reliably and reproducibly quantified with suitable accuracy and precision through the use of a concentration response relationship.

Recovery: The extraction efficiency of an analytical process, reported as percentage of the known amount of an analyte carried through the sample extraction and processing steps of the method. Recovery pertains to the extraction efficiency of an analytical method within the limits of variability. Recovery of the analyte need not to 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) with unextracted standards that represent 100% recovery. It also be given by absolute recovery [21].

Absolute recovery =response of analyte spiked into matrix (processed)/response of analyte of pure standard (unprocessed) X 100

Specific Recommendation for Bioanalytical Method Validation:

1. For validation of the bioanalytical method, accuracy and precision should be determined using a minimum of five determinations per concentration level. The mean value should be within 15% of the theoretical value. Other methods of assessing accuracy and precision that meet these limits may be equally acceptable.
2. The accuracy and precision with which known concentrations of analyte in biological matrix can be determined should be demonstrated. This can be accomplished by analysis of replicate sets of analyte samples of known concentrations QC samples from an equivalent biological matrix.
3. The stability of the analyte in biological matrix at intended storage temperature should be established.
4. The stability of the analyte in matrix at ambient temperature should be evaluated over a time period equal to the typical sample preparation, sample handling and analytical run times.
5. Reinjection reproducibility should be evaluated to determine if an analytical run could be reanalysed in the case of instrument failure.
6. The specificity of the assay methodology should be established using a minimum of six independent source of the same matrix.

Processed sample stability: The stability of processed samples, including the time until completion of analysis, should be determined.

Range: The range of analytical procedures is the interval between the upper and lower concentrations of analyte in the sample for which it has been demonstrated that the analytical procedures has a suitable level of precision, accuracy and linearity. The range of a bioanalytical assay is the concentration interval over which an analyte can be measured with acceptable precision and accuracy.

Robustness: According to ICH guidelines, the robustness of an analytical procedure is the measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provide an indication of its reliability during normal usage. Robustness can be described as the ability to reproduce the method in different laboratories or

under different circumstances without the occurrence of unexpected differences in the obtained results and a robustness test as an experimental set-up to evaluate the robustness of a method.

Ruggedness: This includes different analysts, laboratories, columns, instruments, sources of reagents, chemicals, solvents. Ruggedness of an analytical method is the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of normal test condition. The ruggedness of the method was studied by changing the experimental condition such as, Changing to another column of similar type and Different operations in the same laboratory.

Bioanalysis in the Drug Discovery and Development Lifecycle:

The lead optimization / selection, confirmation, and testing process for new drug candidates is well defined as a series of activities. Broadly, these can be split into discovery, lead optimization and preclinical development, through to clinical elevation (phases 1 to 40). Each stage places different requirements on the bioanalytical assay used to provide information. The use of LC/MS/MS assays gives the specificity, flexibility, and sensitivity to enable fast and effective decision-making at each stage.

pk and bioanalysis in drug discovery:

Full pk characterization in the drug discovery phase is not required; however, in conjunction with in vitro techniques, the ability to assess the bioavailability of a compound through bioanalysis provides a good indication of suitability for advancement to development.

Some analytical priorities are:
Fast pass/fail determination of pk parameters.
Medium-sensitivity assay.
Minimum assay development.
High specificity for the compounds of interest.
pk and bioanalysis in efficacy and safety studies.

Phase I: First time in to humans.

The key requirements for this stage are that the assay must completely characterize the absorption and elimination phases of the plasma concentration-time curve. All metabolites must be fully resolved, identified, and quantified. Adverse effects of a drug (toxicokinetic, TK) are investigated and need accurate measurement of AUC and C-max after single and multiple doses. in the way the "no-toxic-effect dose level" can be established, a key parameter when dosing in first-time-into-human and further trails. The demands placed on the bioanalytical assay are for:

- High sensitivity to ensure that the lowest effective doses can be identified.
- High sensitivity to identify and quantitate metabolites.
- Moderative throughput; sample groups are small.
- Full validation is required.

Phase II a: proof of concept

The drug compound id tested in small groups of patients to assess efficacy in treating the disease state.pk analysis is employed to assess the dose/exposure response(pk/pd).this is another key stage in deciding whether the drug should progress further through clinical trials and therefore incur the investment required.

Phase II b:

Dose ranging studies are carried out on patients to establish effective doses for phase 2 trails. Analytical priorities include:

- High sensitivity assays.
- High specificity assays for drug compound and metabolites.
- Fast turnaround of samples.

Phase III: Long Term Studies

Large numbers of patients take part in phase3 clinical trials with the objective of showing efficacy across a wide range of populations. vast numbers of samples must be handled and analysed with a bioanalytical assay that is specific, robust, and fast.

- Assay specific to very few analytes.
- Robust to variations in matrix.
- Ability to process very large volumes of data.

Stability

It is the chemical stability of an analyte in a given matrix under specific conditions for given time intervals. The aim of a stability test is to detect any degradation of the analyte(s) of interest, during the entire period of sample collection, processing, storage, preparation, and analysis. All but long term stability studies can be performed during the validation of the analytical method. Long term stability studies might not be complete for several years after clinical trials begin. The condition under which the stability is determined is largely dependent on the nature of the analyte, the biological matrix, and the anticipated time period of storage (before analysis) [4].

A. Freeze-thaw stability:

The influence of freeze/thaw cycles on analyte stability should be determined after at least 3cycles at 2 concentrations in triplicate. At least three aliquots at each of the low and high concentrations should be stored at intended storage temperature for 24 hours and thawed at room temperature. When completely thawed, refreeze again for 12-24 hours under the

same conditions. This cycle should be repeated two more times, then analyse on 3rd cycle. All stability determinations should use a set of samples prepared from a freshly made stock solution of the analyte in the appropriate blank, interference-free biological matrix. Standard deviation of error should be <15%. If analyte unstable freeze at -70°C for three freeze-thaw cycles.

B. Short-term stability:

Three aliquots of each of the low and high concentrations should be thawed at room temperature and kept at this temperature for 4-24 hours and analyse. % Deviation should be <15%.

C. Long-term stability:

At least three aliquots of each of low and high concentrations should be thawed at room temperature and kept at this temperature for 4-24 hours and analyse. Analyse on three separate occasions. Storage time should exceed the time between the date of first sample collection and the date of last sample analysis.

D. Stock-solution stability:

The stability of stock solutions of drug and the internal standard should be evaluated at room temperature for at least 6 hours. % Deviation should be <15%. If the stock solutions are refrigerated or frozen for the relevant period, the stability should be documented. After completion of the desired storage time, the stability should be tested by comparing the instrument response with that of freshly prepared solutions.

E. Post-Preparative Stability:

The stability of processed samples, including the resident time in the auto sampler, should be determined. The stability of the drug and the internal standard should be assessed over the anticipated run time for the batch size in validation samples by determining concentrations on the basis of original calibration standards. SOPs should clearly describe the statistical method and rules used. Additional validation may include investigation of samples from dosed subjects.

Reference:

[1] Validation: An updated review: Pharm Methods 1: 25-38. (2010)

[2] Causon R. Validation of chromatographic methods in Bioanalytical analysis: View point and Discussion. J. Chromatogr B Biomed Sci Appl 689: 175-180. (1997)

[3] Chau CH, Rixe O, McLeod H, Figg WD. Validation of Analytic Methods for Biomarkers Used in Drug Development. Clin Cancer Res. 2008; 14: 5967-5976.

[4] Kalakuntla RR, Kumar KS. Bioanalytical Method Validation: A Quality Assurance Auditor View Point. J of Pharma Sci & Res. 2009; 1: 1-10.

[5] Sekar V, Jayaseelan S, Subhash N, Kumar EU, Perumal Petal. Bioanalytical Method Development and Validation of Letrozole by RP –HPLC Method. Int. J of Pharma Res and Develop. 2009; 1: 1-8.

[6] Braggio S, Barnaby RJ, Grosi P, Cugola A, strategy for validation of bioanalytical methods, J Pharm Biomed Anal, 14, 1996, 375–88.

[7] James CA, Breda M, Frigerio E, Bioanalytical method validation: A risk-based approach, J Pharm Biomed Anal, 35, 2004, 887–9

[8] Hartmann C, Smeyers VJ, Massart DL, Validation of bioanalytical chromatographic methods, J Pharm Biomed Anal, 17, 1999, 193–218.

[9] Kelley M, DeSilva B, Key Elements of Bioanalytical Method Validation for Macromolecules, AAP J, 9, 2007, 156–63.

[10] http://www.fda.gov/downloads/Drugs/Guidances/ucm070107.pdf.

[11] S. Karger GmbH and Freiburg. SOP 12: Validation of Bioanalytical methods, Standard Operating Procedures for Clinical Trials of the CESAR, Oncology, 2003; 26(suppl 6): 52-55

[12] Rozet E, Marini RD, Ziemons E, Boulanger B, Hubbert P. Advances in Validation, Risk and Uncertainity assessment of Bioanalytical Methods. J Pharm Biomed Anal 55: 848-858. (2011)

[13] Pandey S, Pandey P, Tiwari R. Bioanalysis in drug discovery and development. Pharm Methods1: 14-24, (2010)

[14] Peters FT, Dremmer OH, Musshoff F. Validation of new methods. Forensic Sci Int. 165: 216-224. (2007)

[15] Gao L, Li J, Kassera C, Song Q, Arjomand A, et al. Precision and accuracy in the quantitative analysis of biological samples by accelerator mass spectrometry: application in microdose absolute bioavailability studies. Anal Chem 83: 5607-5616.

[16] Sing UK, Pandey S, Pandey P, Keshri PK, et al., Bioanalytical method development and validation. Express Pharma 2008

[17] Braggio, Bamaby RJ, Grossi P, Cugola M. A Strategy for Validation of Bioanalytical

Methods. J Pharm Biomed Anal 1996; 14: 375-388.

[18] Kames HT, Shiu G, Shah VP. Validation of Bioanalytical Methods. Pharm Res. 1991; 8: 421- 426.

[19] Chau CH, Rixe O, McLeod H, Figg WD. Validation of Analytic Methods for Biomarkers Used in Drug Development. Clin Cancer Res. 2008; 14: 5967-5976.

[20] Kalakuntla RR, Kumar KS. Bioanalytical Method Validation: A Quality Assurance Auditor View Point. J of Pharma Sci & Res. 2009; 1: 1-10.

[21] Sekar V, Jayaseelan S, Subhash N, Kumar EU, Perumal Petal. Bioanalytical Method Development and Validation of Letrozole by RP – HPLC Method. Int. J of Pharma Res and Develop. 2009; 1: 1-8.

[22] Causon R. Validation of chromatographic methods in Bioanalytical analysis: View point and Discussion. J Chromatogr B Biomed Sci Appl 689: 175-180. (1997)

[23] 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, 9, 2007, 117–9.