Journal of Pharmaceutical and Biological Research

Journal Home Page: www.pharmaresearchlibrary.com/jpbr

Review Article **Open Access**

Design, Development and Validation of Bioanalytical Methods for Pharmaceutical Formulations – Review

R. Subramani[1], M. Purushothaman*[2]

[1]Alwar College of Pharmacy, Sun Rise University, Alwar, Rajasthan – 301030, India
[2]Vasavi Institute of Pharmaceutical Sciences, Kadapa-516247, India

A B S T R A C T

Any method developed for the analysis of analytes in biological fluids must yield consistent results despite the variations in conditions during the course of a project. An ideal bioanalytical method should include all of the probable effects that are going to occur during the routine analysis of study samples. Bioanalytical method validation includes all of the procedures that demonstrate that a particular method used for quantitative measurement of analytes in a given biological matrix, such as blood, plasma, serum, or urine is reliable and reproducible for the intended use. Method development for the interested component in finished product or in process tests and the sample preparation of drug product and to provide practical approaches for determining selectivity, specificity, limit of detection, limit of quantitation, linearity, range accuracy, precision, recovery solution stability, ruggedness, and robustness of liquid chromatographic methods to support the Routine, in process and stability analysis. The present review aims to study the bioanalytical method development and validation for various pharmaceutical formulations.

Keywords: Validation, Bioanalytical, Method development, Anti-hypertensive agents

A R T I C L E I N F O

CONTENTS

Article History: Received 25 October 2016, **Accepted** 21 November 2016, **Available Online** 21 December 2016

***Corresponding Author**
M. Purushothaman
Vasavi Institute of Pharmaceutical Sciences, Kadapa-516247, India
Manuscript ID: JPBR33398

PAPER-QR CODE

Citation: M. Purushothaman. Design, Development and Validation of Bioanalytical Methods for Pharmaceutical Formulations – Review. *J. Pharm. Bio. Res*., 2016, 4(2): 99-105.

1. Introduction

Bioanalysis, employed for the quantitative determination of drugs and their metabolites in biological fluids, plays a signifi cant role in the evaluation and interpretation of bioequivalence, pharmacokinetic (PK), and toxic kinetic studies. The quality of these studies, which are often used to support regulatory filings, is directly related to the quality of the underlying bioanalytical data. Method validations for these divergent methods should consider important differences including the basis of measurement, the detection modality, and whether a sample is measured directly in the matrix or extracted before analysis. The basis of measurement of LC-MS is owed to the chemical properties of the analyte, while for LBAs, the measurement depends on a high-affinity biological binding interaction between the macromolecule analyte and another macromolecule(s) in the form of 1 or more capture/detection antibodies. Detection in LC-MS methods is direct and typically results in a linear measured response, where higher concentrations of analyte have a proportional increase in response.

This methodology includes the required data for a given analytical problem, required sensitivity, required accuracy, required range of analysis and required precision to the analyst. The International Conference on Harmonization (ICH) of Technical Requirements for the Registration of Pharmaceuticals for Human Use has developed a text on the validation of analytical procedures. The United States Food and Drug Administration (USFDA) have proposed guidelines on submitting samples and analytical data for methods validation. The United States Pharmacopoeia (USP) has published specific guidelines for method validation for compound evaluation.

2. Importance of Validation

The reason for validating a bioanalytical procedure is to demonstrate the performance and reliability of a method and hence the confidence that can be placed on the results. All bioanalytical methods must be validated if the results are used to support registration of a new drug or the reformulation of an existing one. It should be noted that the initial validation is only a beginning, as a method should be monitored continually during its application to ensure that it performs as originally validated. Validation involves documenting, through the use of specific laboratory investigations, that the performance characteristics of the method are suitable and reliable for the intended analytical applications[1,2].

The most compelling reasons to optimize and validate pharmaceutical productions and supporting processes are quality assurance and cost reduction .the basic principles of quality assurance has as their goal and the production of articles that are fit for their intended use. These principles are Quality, safety, and effectiveness must be designed and built in to the product, quality cannot be inspected or tested in the finished products and each step of the manufacturing process must be controlled to maximize the probability that the finished product meets all quality and design specification[4-6]. The relationship of quality assurance and process validation goes well beyond the responsibility of any quality assurance functions, nevertheless it is fair to say that process validation is a quality assurance tool because it is establishes a quality standard for the specific process. A validated bioanalytical method must generate reproducible and accurate data to allow valid interpretation of the studies they support. In May 2001 the FDA's Center for Drug Evaluation and Research (CDER) circulated its first official guidance for bioanalytical methods, in cooperation with the FDA's Center for Veterinary Medicine (CVM). This document, titled "Guidance for Industry, Bioanalytical Method Validation," provides general recommendations for the validation of bioanalytical methods used in human clinical pharmacology, bioavailability and bioequivalence studies requiring pharmacokinetic evaluation. The guidance also applies to bioanalytical methods used for non-human pharmacology/toxicology and preclinical studies[7-9].

3. 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 satisfactorily 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 may 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 sample analysis for a given study is conducted at more than one site, it is necessary to validate the bioanalytical method(s) at each site and provide appropriate validation information for different sites to establish inter-laboratory reliability[10].

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 (excluding blank samples). 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. Reported method validation data and the determination of accuracy and precision should include all outliers; however, calculations of accuracy and precision excluding values that are

statistically determined as outliers can also be reported.

4. The stability of the analyte in biological matrix at intended storage temperatures should be established.
5. 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.
6. Reinjection reproducibility should be evaluated to determine if an analytical run could be reanalyzed in the case of instrument failure.
7. The specificity of the assay methodology should be established using a minimum of six independent sources of the same matrix[11-13]

4. Method Development

Analytical method development is the process of creating a procedure to enable a compound of interest to be identified and quantified in a matrix. A compound can often be measured by several methods and the choice of analytical method involves many considerations, such as: chemical properties of the analyte, concentrations levels, sample matrix, cost of the analysis, speed of the analysis, quantitative or qualitative measurement, precision required and necessary equipment. The analytical chain describes the process of method development and includes sampling, sample preparation, separation, detection and evaluation of the results[14-20].

Sample collection and preparation:

The biological media that contain the analyte are usually blood, plasma, urine, serum etc. Blood is usually collected from human subjects by vein puncture with a hypodermic syringe up to 5 to 7 ml (depending on the assay sensitivity and the total number of samples taken for a study being performed). The venous blood is withdrawn into tubes with an anticoagulant, e.g. EDTA, heparin etc. Plasma is obtained by centrifugation at 4000 rpm for 15 min. About 30 % to 50 % of the volume is collected9. The purpose of sample preparation is to clean up the sample before analysis and to concentrate the sample. Material in biological samples that can interfere with analysis, the chromatographic column or the detector includes proteins, salts, endogenous macromolecules, small molecules and metabolic byproducts10. A goal with the sample preparation is also to exchange the analyte from the biological matrix into a solvent suitable for injection into the chromatographic system. General procedures for sample preparation like liquid/liquid extraction, solid-phase extraction (SPE) and protein precipitation.

Liquid – Liquid extraction:

It is based on the principles of differential solubility and partitioning equilibrium of analyte molecules between aqueous (the original sample) and the organic phases. Liquid – Liquid extraction generally involves the extraction of a substance from one liquid phase to another liquid phase11. Now a day's traditional LLE has been replaced with advanced and improved techniques like liquid phase micro extraction, single drop liquid phase micro extraction and supported membrane extraction.

Solid Phase Extraction (SPE):

Solid phase extraction is selective method for sample preparation where the analyte is bound onto a solid support, interferences are washed off and the analyte is selectively eluted. Due to many different choices of sorbents, solid phase extraction is a very powerful technique12. Solid phase consists of four steps; conditioning, sample loading, washing and elution.

Conditioning:

The column is activated with an organic solvent that acts as a wetting agent on the packing material and solvates the functional groups of the sorbent. Water or aqueous buffer is added to activate the column for proper adsorption mechanisms.

Sample Loading:

After adjustment of pH, the sample is loaded on the column by gravity feed, pumping or aspirating by vacuum.

Washing:

Interferences from the matrix are removed while retaining the analyte.

Elution:

Distribution of analyte – sorbent interactions by appropriate solvent, removing as little of the remaining interferences as possible**.** Typically, sorbents used in SPE consists of 40 µm diameter silica gel with approximately 60 A0 pore diameters. To this silica gel, functional groups are chemically bonded, for different mode of actions. The most commonly used format is a syringe barrel that contains a 20 µm frit at the bottom of the syringe with the sorbent material and another frit on top, referred to as packed columns. Extractions disks are placed in syringe barrels. These disks consists of 8 -12 µm particles of packing material imbedded into an inert matrix. Disks are conditioned and used in a similar way as packed columns. The major advantage of disks compared to packed columns is that higher flow rates can be applied. Analytes can be classified into four categories; basic, acid, neutral and amphoteric compounds. Amphoteric analytes have both basic and acid functional groups and can therefore functions as cations, anions or zwitterions, depending on pH

Protein Precipitation

Protein precipitation is often used in routine analysis to remove proteins. Precipitation can be induced by the addition of an organic modifier, a salt or by changing the pH which influence the solubility of the proteins14. The samples are centrifuged and the supernatant can be injected into the HPLC system or be evaporated to dryness and thereafter dissolved in a suitable solvent. A concentration of the sample is then achieved. There are some benefits with precipitation method as clean-up technique compared to SPE. It is less time consuming, smaller amounts of organic modifier or other solvents are used. But there are also disadvantages; the samples often contain protein residues and it is a no-selective sample cleanup method, there is a risk that endogenous compounds or other drugs may interfere in the RP- HPLC - system. However, the protein precipitation technique is often combined with SPE to produce clean extract. Methanol is generally preferred solvent amongst the organic solvents as it can produce clear supernatant which is appropriate for direct injection into

HPLC. Salts are other alternative to acid organic solvent precipitation. This technique is called as salt induced precipitation. As the salt concentration of a solution is increased, proteins aggregate and precipitate from the solution.

Validation[21-33]

The common terms used in bioanalytical method validation is given as follows, these are available in FDA guidance or other publications, but are provided here for convenience.

Accuracy

The degree of closeness of the observed concentration to the nominal or known true concentration. It is typically measured as relative error (%RE). Accuracy is an absolute measurement and 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 (i.e., QCs). Accuracy should be measured using a minimum of five determinations per concentration. 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 (I) analyzing control samples spiked with analyte and (II) by comparison of the analytical method with a reference method.

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 measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Measurement of scatter for the concentrations obtained for replicate samplings of a homogeneous sample. It is typically measured as coefficient of variation (%CV) or relative standard deviation (R.S.D.) of the replicate measurements. Precision should be measured using a minimum of five determinations per concentration. A minimum of three concentrations in the range of expected concentrations is recommended. The precision determined at each concentration level should not exceed 15% coefficient of variation (CV) except for the LOQ where it should not exceed 20% CV. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility.

Repeatability

Repeatability expresses the analytical variability under the same operating conditions over a short interval of time (within-assay, intraassay). 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.).

Intermediate precision

It includes the influence of additional random effects within laboratories, according to the intended use of the procedure, for example, different days, analysts or equipment, etc. (betweenassay, inter-assay). Intermediate precision refers to how the method performs, both qualitatively and quantitatively, within one lab, but now from instrument-to-instrument and from day-to-day. Precision measure of the within laboratory variation due to different days, analysts, equipments, etc.

Reproducibility

Reproducibility is the precision between laboratories (collaborative or interlaboratorystudies), is not required for submission, but can be taken into account for standardisation of analytical procedures. Ability of the method to yield similar concentration 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.

Linearity

The ability of the bioanalytical procedure to obtain test results that are directly proportional to the concentration of analyte in the sample within the range of the standard curve. The concentration 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 method 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. Selectivity is the documented demonstration 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”. Analyses of blank samples of the appropriate biological matrix (plasma, urine, or other 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). These interferences may arise from the constituent of the biological matrix under study.

They may depend on characteristics of the individual 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). The actual FDA guidance for bioanalytical method validation requires the use of at least six independent sources of matrix to demonstrate methods selectivity. 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 (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 (LC-MS) 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. The calculation of the LOD is open to is 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 the biological sample. 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 procedures is the lowest amount of analyte in a sample, which can be quantitatively determined with suitable precision and accuracy.

Quantification Range

The range of concentration, including the LLOQ and ULOQ that can be reliably and reproducibly quantified with suitable accuracy and precision through the use of a concentration response relationship The FDA Bioanalytical Method Validation document defines the lower limit of quantification (LLOQ) and the upper limit of quantification (ULOQ) as following,

Lower limit of quantification (LLOQ)

The lowest concentration of an analyte in a sample that can be quantitatively determined with an acceptable precision and accuracy.

Upper limit of quantification (ULOQ)

The highest amount of an analyte in a sample that can be quantitatively determined with an acceptable precision and accuracy. Several approaches exist in order to estimate the lower limit of quantification (LLOQ). A first approach is based on the well-known signal-to-noise (S/N) ratio approach. A 10:1 S/N is considered to be sufficient to discriminate the analyte from the background noise. The other approaches are based on the "Standard Deviation of the Response and the Slope". The computation for LLOQ is: LLOQ = 10σ/S, Where σ is the standard deviation of the response and S = the slope of the calibration curve. Another approach to estimate the LLOQ is to plot the RSD versus concentrations close to the expected LLOQ.

Standard Curve (Calibration Curve)

The standard curve for a bioanalytical procedure is the existing relationship, within a specified range; between the response (signal, e.g., area under the curve, peak height, absorption) and the concentration (quantity) of the analyte in the sample i.e. Calibration (standard) curve is the relationship between instrument response and known concentrations of the analyte. It is also called as calibration curve. This standard or calibration curve should be described preferably by a simple monotonic (i.e. strictly increasing or decreasing) response function that gives reliable measurements, i.e. accurate results as discussed thereafter. A calibration curve should be prepared in the same biological matrix as the samples in the intended study by spiking the matrix with known concentrations of the analyte. A calibration curve should consist of a blank sample (matrix sample processed without internal standard), a zero sample (matrix sample processed with internal standard), and six to eight non-zero samples covering the expected range, including LLOQ. The lowest standard on the calibration curve should be accepted as the limit of quantification if the analyte response is at least five times the response compared to the blank response and if the analyte response is identifiable, discrete, and reproducible with a precision of 20% and accuracy of 80 to 120%.

Recovery

The extraction efficiency of an analytical process, reported as a 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 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.

Stability

The chemical or physical 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 analytes of interest during the entire period of sample collection, processing, storing, preparing, and analysis. 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). The FDA guidelines on bioanalytical method validation as well as the recent AAPS/FDA white paper require evaluating analyte stability at different stages. Stability should be confirmed for every step of sample preparation and analysis, as well as the conditions used for long-term storage. They also include the evaluation of the analyte stability in the biological matrix through several freeze–thaw cycles, bench-top stability (i.e. under the conditions of sample preparation), long term stability at for example -20°C or -70°C (i.e. during storage conditions of the samples) and stability of samples on the auto-sampler. Generally, stability should be evaluated at least at two concentration levels, using blank biological matrix matched samples spiked at a low and high concentration level.

It should be assessed in each matrix and species in which the analyte will be quantified. Also the stability of the analyte must be investigated under various conditions: in the standard solutions used to prepare calibration curves, in any biological matrix stored at -20°C and at room temperature prior to analysis and also in the final extract awaiting analysis. There may also be the need to investigate the stability of the analyte between the sample being taken and stored: some compounds are metabolized by esterases in the blood and have very short half-lives, therefore to

stabilize the compound an inhibitor should be added, the effectiveness of which will need to be assessed and validated. Stability samples should be compared to freshly made calibrators and/or freshly made QCs. At least three replicates at each of the low and high concentrations should be assessed. Assessments of analyte stability should be conducted in the same matrix as that of the study samples. All stability determinations should use samples prepared from a freshly made stock solution. Conditions used in stability experiments should reflect situations likely to be encountered during actual sample handling and analysis (e.g., short-term, long-term, bench top, and room temperature storage; and freeze-thaw cycles). If, during sample analysis for a study, storage conditions changed and/or exceed the sample storage conditions evaluated during method validation, stability should be established under the new conditions. Stock solution stability also should be assessed. Stability sample results should be within 15% of nominal concentrations.

Short-term stability

The stability of the analyte in biological matrix at ambient temperature should be evaluated. Three aliquots of low and high concentration should be kept for at least 24 hours and then analyzed.

Long-term stability

The stability of the analyte in the matrix should equal or exceed the time period between the date of first sample collection and the date of last sample analysis.

Freeze and Thaw Stability

During freeze/thaw stability evaluations, the freezing and thawing of stability samples should mimic the intended sample handling conditions to be used during sample analysis. Stability should be assessed for a minimum of three freeze-thaw cycles.

Bench-Top stability

Bench top stability experiments should be designed and conducted to cover the laboratory handling conditions that are expected for study samples.

Stock solution stability

The stability of stock solutions of drug should be evaluated. When the stock solution exists in a different state (solutions vs. solid) or in a different buffer composition (generally the case for macromolecules) from the certified reference standard, the stability data on this stock solution should be generated to justify the duration of stock solution storage stability.

Processed Sample Stability

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

Range

The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure 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 provides an indication of its reliability during normal usage. Robustness can be described as the ability to reproduce the (analytical) method in different laboratories or under different circumstances without the occurrence of unexpected differences in the obtained result(s), 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
- Different operation in the same laboratory

5. Conclusion

In pharmacokinetic studies, bioanalytical method validation is crucial to minimizing random error and systematic bias, which ensures quality of analytical results. Bioanalytical method validation is a controlled procedure that comprises all the vital steps to establish that a certain method is capable of producing accurate, dependable and reproducible results that are appropriate for a specific analytical application. Currently, there is a strong emphasis on incurred sample reanalysis (ISR), which serves to further validate sample reproducibility and accuracy of the reported analytical results. Thus it is very important that guiding principles for the validation of bioanalytical methods are established and circulated in the scientific community.

6. References

[1] Shah VP, Midha KK, Findlay JW, Hill HM, Hulse JD, McGilveray IJ, McKay G, Miller KJ, Patnaik RN, Powell ML, Tonelli A, Viswanathan CT, Yacobi A, Bioanalytical method validation--a revisit with a decade of progress, Journal of Pharma research. 2000; 17:P 1551-1557.

[2] U.S.Department of Health and Human Services and Food and Drug Administration. Guidance for Industry-Bioanalytical Method Validation.2001; fda.gov/cder/guidance/index.htm.

[3] International Conference on Harmonization (ICH). Validation of Analytical Methods: Definitions and Terminology. 1994; ICH Q2 A.

[4] International Conference on Harmonization (ICH), Validation of Analytical Methods: Methodology. 1996; ICH Q2 B.

[5] US EPA. Validation and peer review of u.s. environmental protection agency chemical methods of analysis.2005.

[6] Tiwari G, Tiwari R, “Bioanalytical Method Validation: A Updated Review”, Pharmaceutical Methods, 1(1) 2010, 25-38.

[7] Murugan. S., Pravallika. N., Sirisha. P., “A review on bioanalytical method development and

validation by using LC-MS/MS", *Journal of Chemical and Phamaceutical Sciences*, Department of Pharmaceutical Analysis, Andhra Pradesh, India., Issue: 1, Vol: 6, March 2013.

[8] U.S Department of Health and Human Services, Food and Drug Administration, Guidance for Industry, Bioanalytical Method Validation, May 2001.

[9] Ludwig. H., "Validation of Analytical Methods". *Agilent Technology*, 1-65. March 2010.

[10] Lalit. V. Sonawane, Bhagwat. N. Poul, Sharad. V. Usnale, "Bioanalytical Method Validation and Its Pharmaceutical Application- A Review", *Pharmaceutica Analytica Acta*, Issue: 3, Vol: 5, 2014. Sharma. G., Jain. A, "Bioanalytical Technologies: A Review To Method Validation", *International Journal of Pharmaceutical Research and Development*, Vol: 3, May 2011.

[11] Putheti RR, Okigbo RN, Patil SC, Advanapu MS, Leburu R, "Method Development and Validations: Characterization of Critical Elements in the Development of Pharmaceuticals", *International Journal of Health Research*, 1(1), june 2008, pg: 11-20.

[12] Wells DA. "High throughput bioanalytical sample preparation: methods and automation strategies, Progress in pharmaceutical and biomedical analysis", Amsterdam, London, Elsevier, Oct 2003, pg: 610.

[13] Silvia I, Laurian V, Daniela LM (2008) Bioanalytical method validation. Revista Romana de Medicina de Laborator 10: 13-21.

[14] Blume H1, Brendel E, Brudny-Klöppel M, Grebe S, Lausecker B, et al. (2011) Workshop/conference report on EMA draft guideline on validation of bioanalytical methods. Eur J Pharm Sci 42: 300-305.

[15] Shah VP (2007) The History of Bioanalytical Method Validation and Regulation: Evolution of a Guidance Document on Bioanalytical Methods Validation. The AAPS J 9: E43-E47.

[16] Buick AR1, Doig MV, Jeal SC, Land GS, McDowall RD (1990) Method validation in the bioanalytical laboratory. J Pharm Biomed Anal 8: 629-637.

[17] Yadav AK, Singh SK, Yashwant, Verma S (2012) Bioanalytical Method Validation –How, How Much and Why: A Reaseach Perspective. Int J of Nat Prod Sci 1: 123.

[18] Patil S, Pandurang ND, Kuchekar BS (2009) Bioanalytical method development and validation: Guidelines Latest reviews. pharmainfo.net 7: 1-8.

[19] Tiwari G1, Tiwari R (2010) Bioanalytical method validation: An updated review. Pharm Methods 1: 25-38.

[20] Silvia I, Laurian V, Daniela LM (2008) Bioanalytical method validation. Revista Romana de Medicina de Laborator 10: 13-21.

[21] Shah VP1, Midha KK, Findlay JW, Hill HM, Hulse JD, et al. (2000) Bioanalytical method validation--a revisit with a decade of progress. Pharm Res 17: 1551-1557.

[22] Wal P, Kumar B, Bhandari A, Rai AK, Wal A (2010) Bioanalytical Method Development–Determination of Drugs in Biological Fluids. J of Pharma Sci and Tech 2: 333-347.

[23] Gao L1, Li J, Kasserra C, Song Q, Arjomand A, et al. (2011) 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.

[24] James CA1, Breda M, Frigerio E (2004) Bioanalytical method validation: a riskbased approach. J Pharm Biomed Anal 35: 887-893.

[25] Murugan S, Pravallika N, Sirisha P, Chandrakala K (2013) A Review on Bioanalytical Method Development and Validation by Using LC-MS/MS. J of Chem and Pharma Sci 6: 41-45.

[26] Hartmann C1, Massart DL, McDowall RD (1994) an analysis of the Washington Conference Report on bioanalytical method validation. J Pharm Biomed Anal 12: 1337-1343.

[27] Causon R (1997) Validation of chromatographic methods in biomedical analysis. Viewpoint and discussion. J Chromatogr B Biomed Sci Appl 689: 175-180.

[28] Tur F1, Tur E, Lentheric I, Mendoza P, Encabo M, et al. (2013) Validation of an LC-MS bioanalytical method for quantification of phytate levels in rat, dog and human plasma. J Chromatogr B Analyt Technol Biomed Life Sci 928: 146-154.

[29] Gil J1, Cabrales A, Reyes O, Morera V, Betancourt L, et al. (2012) Development and validation of a bioanalytical LC-MS method for the quantification of GHRP-6 in human plasma. J Pharm Biomed Anal 60: 19-25.

[30] Rozet E1, Marini RD, Ziemons E, Boulanger B, Hubert P (2011) Advances in validation, risk and uncertainty assessment of bioanalytical methods. J Pharm Biomed Anal 55: 848-858.

[31] Boulanger B1, Rozet E, Moonen F, Rudaz S, Hubert P (2009) A risk-based analysis of the AAPS conference report on quantitative bioanalytical methods validation and implementation. J Chromatogr B Analyt Technol Biomed Life Sci 877: 2235-2243.

[32] Pandey S1, Pandey P, Tiwari G, Tiwari R (2010) Bioanalysis in drug discovery and development. Pharm Methods 1: 14-24.

[33] Nováková L (2013) Challenges in the development of bioanalytical liquid chromatography-mass spectrometry method with emphasis on fast analysis. J Chromatogr A 1292: 25-37.