ISSN: 2230-7346

Kirthi et al. / JGTPS / *5(4) - (2014) 2265 - 2271*

(Review Article)

Journal of Global Trends in Pharmaceutical Sciences

Journal home page: www.jgtps.com

A REVIEW ON BIOANALYTICAL METHOD DEVELOPMENT AND VALIDATION BY RP - HPLC

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ABSTRACT

The development of sound bioanalytical method(s) is of paramount importance during the preclinical and clinical stages of drug development. Therefore, it is generally accepted that sample preparation and method validation are required to demonstrate the performance of the method and reliability. Recent years have witnessed the introduction of several high – quality review articles into the literature covering various scientific and technical aspects of bioanalysis. Now it is widely accepted that bioanalysis is an integral part of the pharmacokinetic/pharmacodynamic characterization of novel chemical entity from the time of its discovery and leading to its market authorization. Bioanalytical methods, based on a variety of physico-chemical and biological techniques such as chromatography, immunoassay and mass spectrometry, must be validated prior to and during use to give confidence in the results. Liquid chromatography-mass spectrometry is a technique that uses liquid chromatography / RP HPLC is commonly used in laboratories for the qualitative and quantitative analysis of drug substances, metabolites. The present review focused on various extraction techniques like liquid-liquid extraction, solid phase extraction and protein precipitation which play important role in sample preparation and detection by RP HPLC and consistent evaluation of the key bioanalytical method validation parameters is discussed: accuracy, precision, sensitivity, selectivity, standard curve, limits of quantification, range, recovery stability etc. These validation parameters are described, together with an example of validation methodology applied in the case of chromatographic methods used in bioanalysis.

Keywords: Bioanalytical method validation, liquid chromatography, spectrometry, extraction techniques, bioanalysis.

INTRODUCTION

A bioanalytical method is a set of procedures involved in the collection, processing, storage, and analysis of a biological matrix for a chemical compound. Bioanalytical method validation (BMV) is the process used to establish that a quantitative analytical method is suitable for biochemical applications. Reassurances as to the quality of the method and its reliability come from adopting a minimum series of validation experiments and obtaining satisfactory results. Characterization of the stability of analytes in biological samples collected during clinical studies together with that critical assay reagents, including analyte stock solutions, is recognized as an important component of biological assay validation.

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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[1]. Validation involves documenting, through the use of specific laboratory investigations, that the performance of characteristics of the method are suitable and reliable for the intended analytical applications. The increased number of biological agents used as therapeutics (in the form of recombinant proteins, monoclonal antibodies, vaccines, etc.) has prompted the pharmaceutical industry to review and redefine aspects of the development and validation of bioanalytical methods for the quantification of this therapeutics in biological matrices in support of preclinical and clinical studies. Bioanalytical method validation employed for the quantitative determination of drugs and their metabolites in biological fluids plays a significant role in the evaluation and interpretation of bioavailability, bioequivalence, pharmacokinetic, and toxicokinetic study data[2]. These studies generally support regulatory filings[3].

The quality of these studies is directly related to the quality of the underlying Bioanalytical data. It is therefore important that guiding principles for the validation of these analytical methods be established and disseminated to the pharmaceutical community. Both RP - HPLC and LCMS-MS can be used for the bioanalysis of drugs in plasma. Each of the instruments has its own merits. RP - HPLC coupled with UV, PDA or fluorescence detector can be used for estimation of many compounds. The main advantages of these chromatographic principles includes low detection limits, the ability to generate structural information, the requirement of minimal sample treatment and the possibility to cover a wide range of analytes differing in their polarities[4]. All the procedures of bioanalytical method validation includes the procedures that demonstrate that a particular method used for quantitative determination of analytes[5]. The fundamental parameters for this validation includes selectivity, accuracy, precision, linearity, limit of detection, limit of quantification, recovery, robustness, stability and range. The objective of validation of Bioanalytical procedures is to demonstrate that it is suitable for its intended purpose. The most widely accepted guidelines for method validation is the ICH guidelines Q2 (R1), which is used both in pharmaceutical and medical science[6]. Other guidelines, which are much more detailed, which require more extensive validation and which also have defined strict limits for the most of determined parameters are focused directly toward bioanalysis. They are represented by a "Guidelines on Bioanalytical Method Validation" by EMA[7] and "Guidance for Industry, Bioanalytical Method Validation" by FDA[8]. Additionally, as a matter of discussion of recent years, new parameters are required to determine within validation process including matrix effects, carryover and dilution integrity. Detailed study of the stability of analytes under various conditions during the method application is an important specific of bioanalytical methods. The guidelines provides assistance to sponsors of investigational new drug applications (INDs), new drug applications (NDAs), abbreviated new drug applications (ANDAs) and supplements in developing Bioanalytical method validation information used in clinical pharmacology, bioavailability, and bioequivalence studies requiring pharmacokinetic evaluation.

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.

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 collected[9].

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 byproducts[10]. 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 phase[11]. 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 technique[12]. Solid phase consists of four steps; conditioning, sample loading, washing and elution. (Fig 1)

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 A^0 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[13].

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 proteins[14]. 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[15].

Bioanalytical Method Validation (BMV): 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. In addition, Shah et al. has stated that 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[16]. 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.

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 a 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 (I) analyzing control samples spiked with analyte and (II) by comparison of the analytical method with a reference method .

Accuracy is best reported as % bias which is calculated from the expression:

Abso% 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[27]. 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-perfromance 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 braod 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[24]. 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[34]. 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 [25]. 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[26].

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

Standard Curve (calibration curve): The standard curve for 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 of the analyte in the sample i.e. calibration 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 consists of a balnk 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%.

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. The FDA guidelines on Bioanalytical method validation as well as the recent AAPS/FDA white paper require evaluating analyte stability at different stages and should be confirmed for every step of sample preparation and analysis, as well as the conditions used for long term storage[27]. 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^{0}C -70^{0}C (during storage conditions of the sample) and stability of samples on the auto-sampler[44]. 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 solution used to prepare calibration curves, in any biological matrix stored at -20^{0}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 esterase in the blood and have very short half lives, therefore to stabilize the compound an inhibitor should be added, the effectiveness of which will not to be assessed and validated. Percent stability could be calculated as follows.

% stability = mean response of stability samples/ mean response of comparison samples X 100

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 solutions. Conditions used in stability experiments should reflect situations likely to be encountered during actual sample handling and analysis. 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 solutions stability also should be assessd. Stability samples results should be within 15% of nominal concentrations[28].

Short - term stability: The stability of the analyte in biological matrix at ambient temperature should be evaluated. Three aliquots of low and high concentrations should be kept for at least 24 hours and then analysed[26].

Long - term stability: The stability of the analyte in the matrix should equal or exceed the time period between the date of first sample collections and date of last sample analysis[29].

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[29].

Bench – Top stability: Bench top stability experiments should be designed and conducted to over the laboratory handling conditions that are expected for study samples[41].

Stock Solution Stability: The stability of stock solutions of drug should be evaluated. When the solutions exists in a different state or in a different buffer composition from the certified reference standard, the stability data on this stock solutions 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 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 indications 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

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 reanalyzed 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.

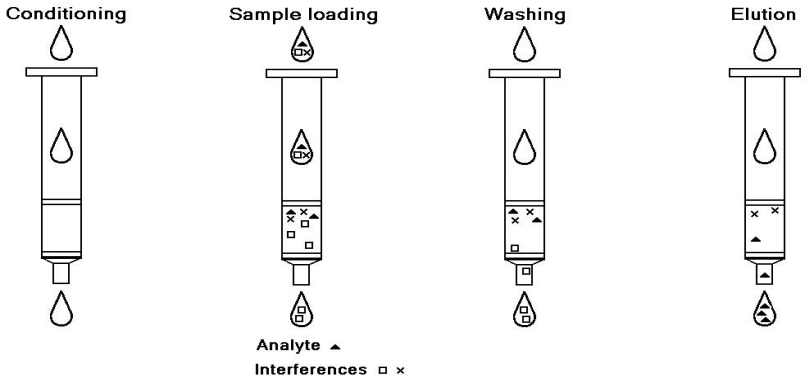

Fig 1: General Solid – Phase extraction procedure

CONCLUSION

Bioanalysis and the production of pharmacokinetic, toxicokinetic and metabolic data plays a fundamental role in pharmaceutical research, development involved in the drug discovery and development process. The relatively new concepts and recent progress made in several areas including sample preparation, separation, how to reduce matrix effect and specific recommendations for bioanalytical method validation discussed in this review and attest to the fact that RP - HPLC has been used as the technique of choice for bioanalysis of small molecules. The new concepts and guidelines covered which can be used to enhance RP - HPLC bioanalytical method development and the matrix effect caused due to the presence of unintended analytes or other interfering substances in the sample.

REFERENCES

1. Burhene J. Bioanalytical Method Validation. J of Anal and Bioanal Tech 3: 7. (2012)
2. Ludwing H. Validaton of Analytical Methods. Agilnet Tech 1-65 (2010)
3. Bansal S, DeStefano A. Key elementslidatin of bioanalytical method validation for small molecules AAPSJ 109-114
4. Rao R, Kalakuntla K, Kumar S. Bioanalytical Method Validation: A quality Assurance Auditor View
5. Eric Reid, Ian D. Wilson. Methodological Survey in Biochemistry and Analysis: Analysis for Drug and Metabolites, Including Anti-infective Agents.20,1-57 (1990)
6. Lang JR, Bolton S. A comprehensive method validation strategy for bioanalytical applications in the pharmaceutical industry: experimental considerations. J Pharm Biomed Anal 9:357-361, (1991)
7. Blume H, Bolton E, Brudny-Kloppel M, Grebe S, Lausecker B, et a, work shop/conference report on EMA draft guidelines on validation of Bioanalytical methods. Eur J Pharm Sci 42:300-305 (2011)
8. U.S Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research : bioanalytical method validation
9. European Medicines Agency Guidelines on Bioanalytical Method Validation: Committee for Medicinal Products for Human Use.
10. Rosing H, Man WY, Doyle E, Bult A, Beijnen JH. Bioanalytical liquid chromatographic method validation: A review on Current Practices and Procedures. J Liq Rel Technol 23; 329-354, (2000)
11. Wells DA. High throughput Bioanalytical sample preparation: Methods and automation strategies, 1st ed. Amsterdam: Elsevier Science BV (2003)
12. Kazakevich Y, Lobrutto R, HPLC for Pharmaceutical Scientists, 1st ed; John Wiley & Sons, Inc: New Jersey, 281-292. (2007)
13. Said R, Application of New Technology LC-MS/MS for determination of therapeutic drugs, Doctoral degree thesis, Department of Medicine Division of Clinical Pharmacology Karolinska Institute, Stockholm, Sweden, 1-5, (2010)
14. Thurman EM, Mils MS. Solid Phase extraction: Principles and Practice. Chemical Analysis: A series of monographs on analytical chemistry and its applications, Winefordner JD, New York Wiley & Sons Inc.147, (1998)
15. Venn RF. Principles and Practice of Bioanalysis, London: Taylor and Francis. xviii, 364. (2000)
16. Shah VP. The History of Bioanalytical Method Validation and Regulation: Evolution of a Guidance Document on Bioanalytical Method Validation. The AAPS J 9: E43-E47 (2007)
17. Buick AR, Doig MV, Jeal SC, Land GS, McDowall RD. Method Validation in the Bioanalytical laboratory. J.Pharm BioMed Anal 8: 629-637. (1990)
18. Tiwari G. Tiwari R. Bioanalytical Method Validation: An updated review: Pharm Methods 1: 25-38. (2010)
19. Causon R. Validation of chromatographic methods in Bioanalytical analysis: View point and Discussion. J Chromatogr B Biomed Sci Appl 689: 175-180. (1997)
20. 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)
21. Pandey S, Pandey P, Tiwari R. Bioanalysis in drug discovery and development. Pharm Methods1:14-24, (2010)
22. Peters FT, Dremmer OH, Musshoff F. Validation of new methods. Forensic Sci Int.165:216-224.(2007)
23. 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.
24. Sing UK, Pandey S, Pandey P, Keshri PK, et al., Bioanalytical method development and validation. Express Pharma 2008
25. Braggio, Bamaby RJ, Grossi P, Cugola M. A Strategy for Validation of Bioanalytical Methods. J Pharm Biomed Anal 1996; 14: 375-388.
26. Kames HT, Shiu G, Shah VP. Validation of Bioanalytical Methods. Pharm Res.1991; 8: 421-426.
27. 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.
28. Kalakuntla RR, Kumar KS. Bioanalytical Method Validation: A Quality Assurance Auditor View Point. J of Pharma Sci & Res. 2009; 1: 1-10.
29. Sekar V, Jayaseelan S, Subhash N, Kumar EU, Perumal P et al. Bioanalytical Method Development and Validation of Letrozole by RP – HPLC Method. Int. J of Pharma Res and Develop. 2009; 1: 1-8.

How to cite this article:

A. Kirthi*, R. Shanmugam,M. Shanti Prathyusha,D. Jamal Basha: A Review on bioanalytical method development and validation by RP – HPLC, 5(4): 2265- 2271 (2014)