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AN APPROACH TO BIOANALYTICAL METHOD DEVELOPMENT AND VALIDATION: A REVIEW

Komal Arora and H.V. Gangadharappa *

Department of Pharmaceutics (Pharmaceutical Quality Assurance), JSS College of Pharmacy, JSS University, Sri Shivarathreeswara Nagara, Mysuru-570015, Karnataka, India

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Correspondence to Author:
H. V. Gangadharappa,

Assistant Professor
Department of Pharmaceutics,
JSS College of Pharmacy, JSS University, Sri Shivarathreeswara Nagara, Mysore 570015, India.

E-mail: gangujss@gmail.com

ABSTRACT: Bioanalytical method development is the process of creating a procedure to enable a compound of interest to be identified and quantified in a biological matrix. A compound can often be measured by several methods and the choice of analytical method involves many considerations. Analysis of drugs and their metabolites in a biological matrix is carried out using different extraction techniques like liquid-liquid extraction, solid phase extraction (SPE) and protein precipitation from these extraction methods samples are spiked with calibration (reference) standards and using quality control (QC) samples. These methods and choice of analytical method describes the process of method development and includes sampling, sample preparation, separation, detection and evaluation of the results. The developed process is then validated. These bioanalytical validations play a significant role in evaluation and interpretation of bioavailability, bioequivalence, pharmacokinetic, and toxicokinetic studies. In which different parameters like accuracy, precision, selectivity, sensitivity, reproducibility, and stability are performed.

INTRODUCTION: Analytical methods development and validation play important roles in the discovery, development and manufacture of pharmaceuticals. The official test methods that result from these processes are used by quality control laboratories to ensure the identity, purity, potency and performance of drug products [1] and includes all the procedures demonstrating particular method used for quantitative measurement of analytes in a given biological matrix, such as blood, plasma, serum, or urine, reliable and reproducible for the intended use [2, 3]. The recent studies show that sample throughput is an important part in Bioanalytical method development involving an efficient preparation [4].

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The analysis thus carried out must be verified for its alleged purpose and must be validated. An investigation should be performed during each step to determine whether the external environment, matrix or procedural variables can affect the estimation of analyte in the matrix from the time of collection up to the time of analysis [5]. Recent progress in methods development has been largely a result of improvements in analytical instrumentation.

This is true especially for chromatographs and detectors. Isocratic and gradient high-performance liquid chromatography (HPLC) have evolved as the primary techniques for the analysis of non-volatile active pharmaceutical ingredients and impurities. The emphasis on the identification of analytes and impurities has led to the increased use of hyphenated techniques such as liquid chromatography-mass spectrometry (LC-MS) and liquid chromatography-nuclear magnetic resonance spectroscopy (LC-NMR) [1]. The selective and

sensitive analytical methods for quantitative evaluation of drugs and their metabolites are critical for the successful conduct of pre-clinical and/or biopharmaceutics and clinical pharmacology studies [6].

So, in the development stage, decisions regarding choice of column, mobile phase, detectors, and method of quantitation must be addressed.

There are several valid reasons for developing new methods of analysis [7].

- There may not be a suitable method for a particular analyte in the specific sample matrix
- Existing method may be too error or contamination prone or they may be unreliable
- Existing method may be too expensive, time consuming, or energy intensive, or they may not be easily automated
- Existing method may not provide adequate sensitivity or analyte selectivity in samples of interest
- Newer instrumentation and techniques may have evolved that provide opportunities for improved methods, including improved analyte identification or detection limits, greater accuracy or precision, or better return on investment
- There may be need for an alternative method to confirm, for legal or scientific reasons, analytical data originally obtained by existing methods

Steps involved in method development:

Method development is a complex process that involves a number of steps [8], which are as follows:

- Method selection and information of sample
- Selection of initial method conditions
- Checking the analytical method in aqueous standards
- Development and optimization of sample processing method
- Checking the analytical method in biological matrix
- Pre-validation

Step 1: Method selection and information of sample:

Literature survey shall be conducted to have first-hand information on drug profile and its pharmacokinetic properties. Collection of physicochemical properties of the analytes and the related compounds are essential for the development of the analytical method. Based on the drug's physicochemical properties such as molecular size, shape, structure, functional groups, polarity, partition coefficient, solubility, dissociation constant etc., choose the internal standard having comparable molecular structure and physicochemical properties with respect to the analytes. Same molecule with different isotopes like deuterium, $C^{13}$and N^{15} will be a better alternative for internal standards.

Step 2: Selection of initial method conditions:

Setting the initial method conditions include diluent selection based on the solubility of the drug, drug metabolites and internal standard and compatibility with analytical method. The lowest concentration to be quantified shall be assessed using aqueous solutions during this phase. Run time and resolution between the peaks should be taken care during this phase.

Step 3: Checking the analytical method in aqueous standards:

Before going to analyze a method in biological matrix, first check the analytical method in aqueous standards. Prepare aqueous calibration curve standards, at least with four concentrations, including the highest and lowest. Concentration of the highest standard shall be based on C_{max} and lowest standard shall be tentatively fixed based on the preliminary studies. Make injections of each calibration curve standard and find the correlation

coefficient. Correlation co-efficient (r) should not be less than 0.99.

If required, adjust the mobile phase, mass spectral parameters (if applicable) and chromatographic conditions such as mobile phase constituents, buffer strength, ratio, pH, flow rate, wavelength, column, column oven temperature etc., to get the clear resolution with required sensitivity.

Step 4: Development and optimization of sample processing method

When the instrumental method is concluded with aqueous standards, prepare matrix sample. Based on the literature survey data on analyte and internal standard's physicochemical properties like structure, functional groups, pH, partition co-efficient, dissociation constant, polarity and solubility, set and optimize the sample preparation technique like protein precipitation, liquid-liquid extraction and solid phase extraction.

A. Liquid-liquid extraction (LLE): [9]

Liquid-liquid extraction is useful for separating analytes from interferences by partitioning the sample between two immiscible liquids or phases. One phase in LLE often is aqueous and second phase an organic solvent. More hydrophilic compounds prefer the polar aqueous phase; whereas more hydrophobic compounds will be found mainly in the organic solvents. Analyte extracted into the organic phase are easily recovered by evaporation of the solvent, while analytes extracted in to the aqueous phase can often be injected directly on to a reversed-phase column. The technique is simple, rapid and has relatively small cost factor per sample when compared to others. The extraction containing drug can be evaporated to dryness and the residue reconstituted in a smaller volume of an appropriate solvent (preferably mobile phase). Near quantitative recoveries (90%) of most drugs can be obtained through multiple continuous extractions.

B. Solid phase extraction (SPE):

Solid phase extraction is the most important technique used in sample pretreatment for HPLC. SPE occur between a solid phase and a liquid phase. SPE is more efficient separation process than LLE. It is easier to obtain a higher recovery of analyte. SPE employs a small plastic disposable column or cartridge, often the barrel of a medical syringe packed with 0.1 to 0.5 g of sorbent. The sorbent is commonly reversed phase material (C18-silica), and a reversed phase SPE (RP-SPE) assembles both LLE and reversed phase HPLC in its separation characteristics. In SPE, a liquid sample is added to the cartridge and wash solvent is selected so that the analyte is either strongly retained (K>>1) or un-retained (K=0). When the analyte is strongly retained, interferences are eluted or washed from the cartridge so as to minimize their presence in the final analyte fraction. The analyte is then eluted in a small volume with strong elution solvent, collected, and either

(1) Injected directly or

(2) Evaporated to dryness followed by dissolution in the HPLC mobile phase.

In the opposite case, where analyte is weakly retained, interferences are strongly held on the cartridge and the analyte is collected for the further treatment.

Advantages of SPE v/s. LLE

- More complete extraction of the analyte
- More efficient separation of interferences from analyte
- Reduced organic solvent consumption
- Easier collection of the total analyte fraction
- More convenient manual procedures
- Removal of particulates
- More easily automated

C. Protein Precipitation method:

Protein precipitation is the simple method of extraction as compared to the LLE and SPE. This can be carried out by using the suitable organic solvents which has good solubility of the analyte and protein precipitating properties. Acetonitrile is the first choice of solvent for protein precipitation

due to its complete precipitation of proteins and methanol is the second choice of organic precipitant provided the solubility of the analyte in these solvents. After protein precipitation the supernatant obtained can be injected directly in to the HPLC or it can be evaporated and reconstituted with the mobile phase and further cleanup of the sample can be carried out by using micro centrifuge at very high speed.

Preliminary evaluation of lower limit of quantification to be done after fixing the sample processing technique. Using the biological matrix with lowest interference, prepare at least three aliquots at each concentration level, with the concentrations of 1/20 of C_{max}, 1/30 of C_{max} and 1/40 of C_{max} of the analytes.

The wash volume and washing pattern of auto injector needle to be evaluated to avoid carryover of previous injections to next injections.

Step 5: Checking the analytical method in biological matrix:

When sensitivity of the drug is more, prefer protein precipitation and check for recovery, precision and interferences. When sensitivity of the drug is less, prefer liquid-liquid extraction and check for recovery, precision and interferences. When the recovery and reproducibility is less in liquid-liquid extraction, prefer solid phase extraction for better sensitivity, recovery, precision and low interferences.

Checking the developed bioanalytical method with matrix samples for accuracy, precision and recovery is essential before finalizing the method for pre-validation. Minimum three aliquots each of Higher Quality Control (HQC) and Lower Quality Control (LQC) and Lower Limit of Quantification (LLOQ) matrix samples are analysed with one set of extracted calibration curve standards including matrix blank and zero standard (blank with only internal standard) and the results shall be compared for recovery with aqueous quality control samples of equivalent concentration. The method is accepted if it meets the criteria of accuracy, precision and recovery. If needed, the method shall be considered for modification.

Step 6: Pre-validation:

When the method is evaluated to be reliable, prepare a brief procedure with the details of sample preparation, instrumental conditions and method conditions, to proceed for pre-validation. Selectivity, Accuracy, Precision, Recovery parameters should be evaluated in Pre-validation stage.

Parameters to be optimized:

The various parameters to be optimized during method development includes

1. Mode of separation
2. Selection of stationary phase
3. Selection of mobile phase

1. Mode of separation:

In reverse phase mode, the mobile phase is comparatively more polar than the stationary phase. For the separation of polar or moderately polar compounds, the suitable mode is reverse phase. The nature of the analyte is the primary factory in the selection of mode of separation.

2. Selection of stationary phase/column: [10]

Selection of the column is the first and the most important step in method development, because the column is the heart of separation process.

The appropriate choice of separation column includes different approaches

- Column dimensions
- Nature of packing material
- Shape of the particles
- Size of the particles
- Surface area
- Pore volume
- End capping

The optimum length of the column required for a particular separation is dictated by the number of theoretical plates needed to give the desired resolution. If the column is too short, then the clearly the column will not have enough 'resolving power' to achieve the separation and if it is too long, then analysis time is needlessely extended. The most common column lengths used in regular analytical HPLC are 10, 12.5, 15 and 25 cm, with 15 cm columns being perhaps the most popular.

Most analytical HPLC columns have internal diameters (i.d.) of around 5 mm, majority being 4.6mm.

It is generally considered that spherical forms give superior column packing properties to the non spherical forms. As the particle size of the column packing decreases, the superiority of spherical materials become more apparent and therefore spherical forms are often used for silica particles of 5µm diameter, 3µm diameter or less.

Currently, most HPLC separation are carried out with 5µm diameter packing materials. Columns with 5µm particle size give the best compromise of efficiency, reproducibility and reliability.

As the particle size decreases the surface area for coating increases. Generally high specific surface area will increase the retention of solutes by increasing the capacity factor.

For example: A compound A is assayed in three different columns of size, different particle size and different internal diameter. As shown in the figure.

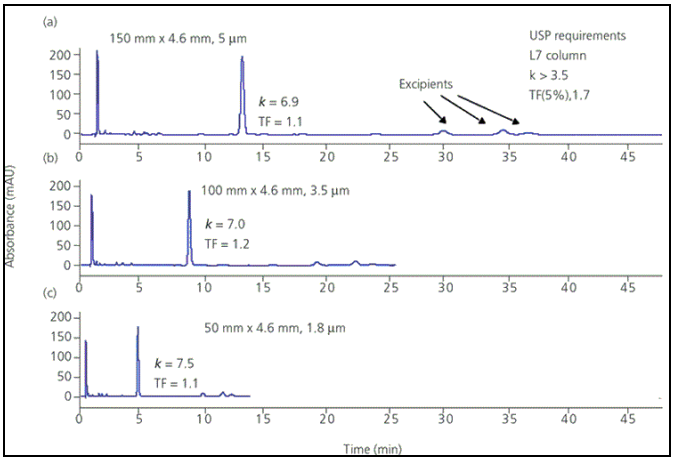

FIG.1: PARTITION CHROMATOGRAPHY

Reverse phase mode of chromatography facilitates a wide range of columns like dimethylsilane (C2), butylsilane (C4), octyl silane (C8), octadecyl silane (C18), cyanopropyl (CN), nitro, amino etc [11, 12].

3. Selection of Mobile phase:

The primary objective in selection and optimization of mobile phase is to achieve optimum separation of all the individual impurities and degradants from each other and analyte peak.

The following are the parameters to be considered during selection and optimization of mobile phase.

- Buffer
- pH of the buffer
- Mobile phase composition

Buffer and its role:

Buffer and its strength play an important role in deciding the peak symmetries and separations. The retention time depends on molar strength of buffer. Molar strength is proportional to retention time. In order to achieve better separation the strength of the buffer can be increased.

- Commonly used buffers are
- Acetic buffers includes ammonium acetate, sodium acetate.

Acetic acid buffers are prepared using acetic acid. Another important component is the influence of the pH since this can change the hydrophobicity of the analyte. For this reason most methods use a buffering agent, such as sodium phosphate, to control the pH. The buffers serve multiple purposes: they control pH, neutralize the charge on any residual exposed silica on the stationary phase and act as ion pairing agents to neutralize charge on the analyte. Ammonium formate is commonly added in mass spectrometry to improve detection of certain analytes by the formation of ammonium adducts. A volatile organic acid such as acetic acid, or most commonly formic acid, is often added to the mobile phase if mass spectrometry is used to analyze the column eluent. Trifluoroacetic acid is used infrequently in mass spectrometry

applications due to its persistence in the detector and solvent delivery system, but can be effective in improving retention of analytes such as carboxylic acids in applications utilizing other detectors, as it is one of the strongest organic acids. The effects of acids and buffers vary by application but generally improve the chromatography.

pH of buffer: [11]

pH plays an important role in achieving the chromatographic separation as it controls the elution properties by controlling the ionization characteristics. A different concentration of buffer was chosen to achieve required separations. It is important to maintain the pH of mobile phase in the range of 2.0 to 8.0 as most of the columns does not withstand out of this range [12]. As Siloxane linkages are cleaved below pH 2 and at above pH 8 silica dissolves.

Bioanalytical method validation:

Method validation is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Results from method validation can be used to judge the quality, reliability and consistency of analytical results; it is an integral part of any good analytical practice. Owing to the importance of method validation in the whole field of analytical chemistry, a number of guidance documents on this subject have been issued by various international organizations and conferences [13-18].

A. Full Validation:

- Full validation is important when developing and implementing a Bio analytical method for the first time.
- Full validation is important for a new drug entity.
- A full validation of the revised assay is important if metabolites are added to an existing assay for quantification.

B. Partial Validation:

Partial validations are modifications of already validated bio analytical methods. Partial validation can range from as little as one intra-assay accuracy and precision determination to a nearly full validation. Typical bio analytical method changes that fall into this category include, but are not limited to:

- Bio analytical method transfers between laboratories or analysts
- Change in analytical methodology (e.g., change in detection systems)
- Change in anticoagulant in harvesting biological fluid
- Change in matrix within species (e.g., human plasma to human urine)
- Change in sample processing procedures
- Change in species within matrix (e.g., rat plasma to mouse plasma)
- Change in relevant concentration range
- Changes in instruments and/or software platforms
- Limited sample volume (e.g., pediatric study)
- Rare matrices
- Selectivity demonstration of an analyte in the presence of concomitant medications
- Selectivity demonstration of an analyte in the presence of specific metabolites

C. Cross-Validation:

Cross-validation is a comparison of validation parameters when two or more bio analytical methods are used to generate data within the same study or across different studies. An example of cross-validation would be a situation where an original validated bio analytical method serves as the reference and the revised bio analytical method is the comparator. The comparisons should be done both ways. 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.

The Fundamental parameters involved in bio analytical validation are:

1. Selectivity
2. Sensitivity
3. Linearity
4. Accuracy
5. Precision
6. Recovery
7. Matrix effect
8. Dilution integrity
9. Stability

1. Selectivity:

Selectivity is defined as, "the ability of an analytical method to differentiate and quantify the analyte in the presence of other components in the sample. The definition of selectivity is quite similar to the definition of specificity "the ability to assess unequivocally the analyte in the presence of components which might be expected to be present. Selectivity is evaluated by injecting extracted blank plasma and comparing with the response of extracted LLOQ samples processed with internal standard. There should be no endogenous peak present within 10% window of the retention time of analyte and an internal standard. If any peak is present at the retention time of analyte, its response should be 20% of response of an extracted Lower calibration standard i.e. LLOQ standard If any peak is present at the retention time of an internal standard, its response should be 5% of the response of an extracted internal standard at the concentration to be used in study.

2. Sensitivity:

Sensitivity is measured using Lower Limit of Quantification (LLOQ) is the lowest concentration of the standard curve that can be measured with acceptable accuracy and precision. The LLOQ should be established using at least five samples independent of standards and determining the coefficient of variation and appropriate confidence interval. The LLOQ should serve the lowest concentration on the standard curve and should not be confused with limit of detection and low QC sample. The highest standard will define the upper limit of quantification (ULOQ) of an analytical method.

3. Accuracy:

Accuracy of an analytical method describes the closeness of mean test results obtained by the method to the true value (concentration) of the analyte. This is sometimes termed as trueness. The two most commonly used ways to determine the accuracy or method bias of an analytical method, are (i) analysing control samples spiked with analyte and (ii) by comparison of the analytical method with a reference method.

4. Precision:

It is the closeness of individual measures of an analyte when the procedure is applied repeatedly to multiple aliquots of a single homogenous volume of biological matrix. There are various parts to precision, such as repeatability, intermediate precision, and reproducibility ruggedness). Repeatability means how the method performs in one lab and on one instrument, within a given day. Intermediate precision refers to how the method performs, both qualitatively and quantitatively, within one lab, but now from instrument to-instrument and day to-day. Finally, 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.

The duration of these time intervals are not defined. Within/intraday, assay, run and batch are commonly used to express the repeatability. Expressions for reproducibility of the analytical method are between/interday, assay, run and batch. The expressions intra/within-day and inter/between- day precision are not preferred, because a set of measurements could take longer than 24 hours or multiple sets could be analysed within the same day.

5. Linearity:

According to the ICH-definition 'the linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. 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. Although the correlation coefficient is of benefit for demonstrating a high degree of relationship between concentration-response data, it is of little value in establishing linearity. Therefore, by assessing an acceptable high correlation coefficient alone the linearity is not guaranteed and further tests on linearity are necessary, for example a lack-of-fit test.

6. Recovery:

It can be calculated by comparison of the analyte response after sample workup with the response of a solution containing the analyte at the theoretical maximum concentration. Therefore absolute recoveries can usually not be determined if the sample workup includes a derivatisation step, as the derivatives are usually not available as reference substances. Nevertheless, the guidelines of the Journal of Chromatography require the determination of the recovery for analyte and internal standard at high and low concentrations. Recovery does not seem to be a big issue for forensic and clinical toxicologists as long as precision, accuracy (bias), LLOQ and especially LOD are satisfactory. However, during method development one should of course try to optimize recovery.

7. Matrix effect:

Matrix effect is the effect on bio analytical method caused by all other components of the sample except the specific compound to be quantified. It happens due to ion suppression/enhancement by the others ions present in the biological matrix which might get ionized during detection and will give false results. Matrix effect studied by comparing the response of extracted samples spiked before extraction with response of the blank matrix sample to which analyte has been added at the same nominal concentration just before injection. Matrix effect is done in LCMS-MS to find out if there is any ion suppression or enhancement effect by the matrix.

8. Dilution integrity:

Dilution integrity is performed in order to check the validity of method incase the sample needs to be diluted during analysis. It is done by spiking analyte working standard in drug free and interference free plasma to get concentration of 2xULOQ. Two and four fold dilution made of the original concentration using screened and pooled plasma and analysed against a fresh calibration curve. The concentration will be calculated using the dilution factor.

Stability in a biological fluid:

Drug stability in a biological fluid is a function of the storage conditions, the chemical properties of the drug, the matrix, and the container system. The stability of an analyte in a particular matrix and container system is relevant only to that matrix and container system and should not be extrapolated to other matrices and container systems. Stability procedures should evaluate the stability of the analytes during sample collection and handling, after long-term (frozen at the intended storage temperature) and short-term (bench top, room temperature) storage, and after going through freeze and thaw cycles and the analytical process. Conditions used in stability experiments should reflect situations likely to be encountered during actual sample handling and analysis. The procedure should also include an evaluation of analyte stability in stock solution.

Freeze and Thaw Stability:

Analyte stability should be determined after three freeze and thaw cycles. At least three aliquots at each of the low and high concentrations should be stored at the intended storage temperature for 24 hours and thawed unassisted at room temperature. When completely thawed, the samples should be refrozen for 12 to 24 hours under the same

conditions. The freeze-thaw cycle should be repeated two more times, then analyzed on the third cycle. If an analyte is unstable at the intended storage temperature, the stability sample should be frozen at -70C during the three freeze and thaw cycles.

Short-Term Temperature Stability:
Three aliquots of each of the low and high concentrations should be thawed at room temperature and kept at this temperature from 4 to 24 hours (based on the expected duration that samples will be maintained at room temperature in the intended study) and analyzed.

Long-Term Stability:
The storage time in a long-term stability evaluation should exceed the time between the date of first sample collection and the date of last sample analysis. Long-term stability should be determined by storing at least three aliquots of each of the low and high concentrations under the same conditions as the study samples. The volume of samples should be sufficient for analysis on three separate occasions. The concentrations of all the stability samples should be compared to the mean of back-calculated values for the standards at the appropriate concentrations from the first day of long-term stability testing.

Processed sample Stability:
The stability of processed samples, including the resident time in the autosampler, 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. Although the traditional approach of comparing analytical results for stored samples with those for freshly prepared samples has been referred to in this guidance, other statistical approaches based on confidence limits for evaluation of analyte stability in a biological matrix can be used.

Bench top Stability:
The stability of stock solutions of drug and the internal standard should be evaluated at room temperature for at least 6 hours. 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.

Specific Recommendations for Method Validation: [19]

- ✓ The matrix-based standard curve should consist of a minimum of six standard points, excluding blanks, using single or replicate samples. The standard curve should cover the entire range of expected concentrations.
- ✓ Standard curve fitting is determined by applying the simplest model that adequately describes the concentration-response relationship using appropriate weighting and statistical tests for *goodness of fit.*
- ✓ LLOQ is the lowest concentration of the standard curve that can be measured with acceptable accuracy and precision. The LLOQ should be established using at least five samples independent of standards and determining the coefficient of variation and/or appropriate confidence interval. The LLOQ should serve as the lowest concentration on the standard curve and should not be confused with the limit of detection and/or the low QC sample. The highest standard will define the upper limit of quantification (ULOQ) of an analytical method.
- ✓ 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, except at LLOQ, where it should not deviate by more than ±20%. The precision around the mean value should not exceed 15% of the CV, except for LLOQ, where it should not exceed 20% of the CV. Other methods of assessing accuracy and precision that meet these limits may be equally acceptable.

- ✓ 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 C QC samples C from an equivalent biological matrix. At a minimum, three concentrations representing the entire range of the standard curve should be studied: one within 3x the lower limit of quantification (LLOQ) (low QC sample), one near the center (middle QC), and one near the upper boundary of the standard curve (high QC).

- ✓ 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.

- ✓ The stability of the analyte in biological matrix at intended storage temperatures should be established. The influence of freeze-thaw cycles (a minimum of three cycles at two concentrations in triplicate) should be studied.

- ✓ 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.

- ✓ Reinjection reproducibility should be evaluated to determine if an analytical run could be reanalyzed in the case of instrument failure.

- ✓ The specificity of the assay methodology should be established using a minimum of six independent sources of the same matrix. For hyphenated mass spectrometry-based methods, however, testing six independent matrices for interference may not be important. In the case of LC-MS and LC-MS-MS-based procedures, matrix effects should be investigated to ensure that precision, selectivity, and sensitivity will not be compromised. Method selectivity should be evaluated during method development and throughout method validation and can continue throughout application of the method to actual study samples.

- ✓ Acceptance/rejection criteria for spiked, matrix-based calibration standards and validation QC samples should be based on the nominal (theoretical) concentration of analytes. Specific criteria can be set up in advance and achieved for accuracy and precision over the range of the standards, if so desired.

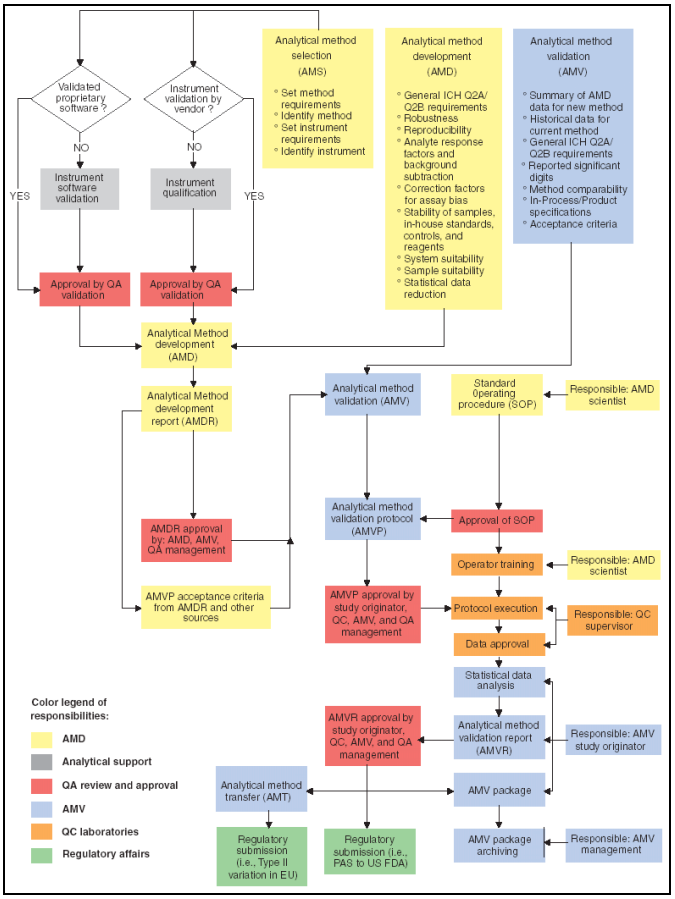

FIG.2: OVERVIEW OF METHOD DEVELOPMENT AND VALIDATION

CONCLUSION: The efficient development and validation of analytical methods are critical elements in the development of pharmaceuticals. In bioanalytical method development should not be restricted to pure and neat analyte solutions. The objective of this paper is to review the sample preparation of drug in biological matrix and to provide practical approaches for determining

selectivity, specificity, lower limit of quantitation, linearity, accuracy, precision, recovery, stability, ruggedness of chromatographic methods to support pharmacokinetic, toxicokinetic, bioavailability, and bioequivalence studies. An attempt has been made to understand and explain the development of bioanalytical method and its validation from basic point of view.

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REFERENCES:

1. Analytical Method Development and Validation, a report by Jay Breaux , Kevin Jones and Pierre Boulas, AAI Development Services, United States.
2. U.S .Department of Health and Human Services, Food and Drug Administration., Guidance for Industry, Bioanalytical Method Validation, May 2001.
3. Xu RN, Fan L, Rieser M, El-Shourbagy TA, Recent advances in high-throughput quantitative bioanalysis by LC–MS/MS. Journal. Pharm. Biomed. Anal. 2007: 44(2): 342–355.
4. Pranay Wal et al. Method Development –Determination of Drugs in Biological Fluids. J. Pharm Sci and Tech. 2010: 2 (10), 333-347.
5. Singh U K, Pandey S, Pandey P, Keshri P K, Wal P, Bioanalytical method development and validation, Expresspharma online, 2008: 16: 1-3.
6. Rashmin B P, Mrunali R P, An Introduction To Analytical Method Development For Pharmaceutical Formulations, Pharma info, 2008:6(4).
7. Sethi PD. HPLC Quantitative Analysis of Pharmaceutical Formulations. 1st ed. New Delhi: CBS Publishers & Distributors; 2001
8. How do I develop an HPLC method. Available from: www.sge.com
9. Snyder LR, Kirkland JJ, Glajch JL. Practical HPLC Method Development. 2nd ed. USA: John Wiley & Sons; 2001
10. Christain GD. Analytical Chemistry. 6th ed. USA: John Wiley & Sons Inc; 2001.
11. Separations Solutions: Mobile Phase pH, U.D. Neue, American Laboratory, March 1999, p. 60.
12. Nledner W, Karsten M, Stelner F, Swart R. Automating Method Development with an HPLC System Optimized for Scouting of Columns, Eluents and Other Method Parameters. Pittcon presentation; 2008.
13. International Conference on Harmonization (ICH), Validation of analytical methods definitions and terminology, ICH Q2 A, 1994.
14. International Conference on Harmonization (ICH), Validation of analytical methods: methodology, ICH Q2 B, 1996.
15. EURACHEM, The Fitness for Purpose of Analytical Methods—A Laboratory Guide to Method Validation and Related Topics, 1998.
16. Lalit VS, Bhagwat NP, Sharad VU, Pradeepkumar VW and Laxman HS Bioanalytical Method Validation and Its Pharmaceutical Application- A Review. Pharm Anal Acta 2014, 5:3.
17. Thompson M, Ellison SLR, Wood R, Harmonized guidelines for single-laboratory validation of methods of analysis (IUPAC technical report). Pure Appl. Chem 2002;74: 835–855.
18. Anhang C: Anforderungen an die Durchfu¨hrung von Analysen, Validierung. Toxi chem Krimtech 2004;74:146–154.
19. US FDA Guidelines for Bioanalytical Validation, 2001: 4-10.

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