International Journal of Pharmaceutical Chemistry and Analysis 2024;11(4):274–280

Content available at: https://www.ipinnovative.com/open-access-journals

International Journal of Pharmaceutical Chemistry and Analysis

Journal homepage: https://www.ijpca.org/

Review Article

Extensive assessment of fundamental factors in the development and validation of bioanalytical methods: Highlighting accuracy and reliability

Sakshi S. Waikar[1]*, Manisha Raut[1], Tejashree Dugaje[1], Vasim T. Pathan[1], Atul R. Bendale[1], Anil G. Jadhav[1]

[1]*Dept. of Pharmaceutical Chemistry, Mahavir Institute of Pharmacy, Varvandi, Maharashtra, India*

ARTICLE INFO

Article history:
Received 10-10-2024
Accepted 04-11-2024
Available online 30-12-2024

Keywords:
Bioanalytical method development
Validation parameters
Documentation
application
Biological matrixes
Analyte
stability

ABSTRACT

Bioanalysis is a pivotal method employed in the pharmaceutical industry for the quantitative analysis of drugs and their metabolites within biological samples. It plays a crucial role in various aspects of pharmaceutical research, including bioequivalence, pharmacokinetic, and toxicokinetic studies. Method development, validation, and sample analysis are integral components of bioanalytical processes, ensuring the accuracy and reliability of results. Throughout each stage of analysis, careful evaluation is required to identify and mitigate any potential sources of interference, such as environmental factors, matrix effects, or procedural variations. Bioanalytical methods encompass a range of techniques utilized for the measurement of analytes within biological matrices, spanning from sample collection to data reporting. The establishment of robust bioanalytical methods is imperative throughout the drug discovery and development continuum, ultimately contributing to regulatory approval. Within this context, bioanalytical methods are extensively utilized to quantify pharmaceuticals and their metabolites within plasma matrices, facilitating both human clinical trials and non-human investigations.

1. Introduction

Bioanalysis is critical in medication development. Bioanalysis is now an integral component of toxicological evaluation, as well as pharmacokinetic and pharmacodynamics investigations during medication development. Improving methods for testing is one of the drug development hurdles, also validation of bio analytical process is critical for regard to quantifiable various types of analytes to be found in biological matrices. Sampling, preparation of sample, various analysis, diverse calibration, review of data, and to generate a report all are a part of the procedure in a workflow.[1–3]

In forensic and clinical toxicology, the reliability of analytical data is of the most crucial significance for the interpretation of toxicological findings. In addition to being subject to judicial scrutiny, unreliable outcomes may also lead to unjust legal consequences for the defendant or inadequate patient care. The significance of validation, at least for regular analytical procedures, cannot be overstated. This is especially true in the context of management in quality and accreditation, which have recently gained prominence in analytical toxicology. This also shows up in the increased technique validation requirements of reviewing by peer's scientific publications. Consequently, this problem should be extensively debated on an international level in order to acquire an accord on the scope of validation experiments and acceptability criteria for validation parameters of bioanalytical techniques in forensic (and clinical) toxicology. Similar debates that have occurred in related field of pharmacokinetic study (PK) research for pharmacological registration throughout

* *Corresponding author*.
E-mail address: sakshiwaikar12@gmail.com (S. S. Waikar).

https://doi.org/10.18231/j.ijpca.2024.040

the previous decade.[4,5]

2. Method Development

Bio analytical technique and development is a process of establishing an algorithm for recognising and measuring unknown or innovative compounds in a framework. Chemically Relevant history of the analyte, suitable concentrations of substances, examples of matrix, expenditures of the analysis method used and instruments to be, speed-time analysis, quantitative or qualitative measurement, precision, and necessary equipment are all factors to be taken into consideration while selecting an analytical method. Method development entails sample preparation, sampling, separation, detection, and assessment of the results, as well as conclusion.[6]

3. Importance and Need of Developing Bioanalytical Method

To provide accurate data that can be adequately understood, bioanalytical procedures must be well-characterized and extensively verified. It is effectively recognised that bioanalytical techniques and processes are always evolving and establishing, and in many cases, they are at the leading edge of technology. It is also pivotal to know that individual approaches have distinctive properties that vary from analyte to analyte. In these cases, separate validation criteria for each analyte may need to be defined. Furthermore, the congruous of the approach may be modified by the study's final purpose. When doing sample analysis in location of one or more for particular research, it is required to evaluate the bioanalytical method(s) at each site and provide sufficient validation information for distinct sites in order to manifest inter laboratory reliability.[7]

4. Terminologies Commonly Used in this Procedure

4.1. Validation

A specified bioanalytical technique is expected to undergo several changes throughout the curriculum of a normal development of drug program. These evolutionary changes [for example, the extra addition of a metabolite or the lowering of the lower limit of quantification (LLOQ)] necessitate multiple degrees of validation to establish the continuous an assay's performance. There are types of validation as follows, full, partial and cross-validation.

4.2. Full validation

When designing and applying a bioanalytical process for the inaugural time for a novel medicinal entity, full validation is required. If metabolites are added for quantification, complete validation of the amended assay is required for every other analyte tested.[8]

4.3. Partial validation

This verification are modifications to approved bioanalytical procedures that do not necessitate complete revalidation. Partly validation can be ranged from determining one assay's accuracy and precision to "nearly" full validation. This method transfers between numerous of laboratories or analysts, gadgets and/or programming structure changes, alter in organisms within array (e.g., rat plasma to mouse plasma), change in matrices within a species (e.g., human plasma to human urine), transpose in analytical methods (e.g., change in detection systems), and interchanging samples and rectifying procedures are examples of ordinary bioanalytical approach changes that fall into this category.

4.4. Cross validation

The procedure of distinguishing two bioanalytical methods is known as cross-validation. When collectively more than one bio analytical process is employed to pioneer data within the same research, cross-validation is required. For example, the "reference" is an original verified bioanalytical technique, while the "comparator" is an updated bioanalytical method**.** Comparisons should be made in both of the directions, analysing sample within a singular study are conducted multiple times at one site or a laboratory, cross-validation with spiked matrix and subject samples should be performed individually to establish interlaboratory reliability, and should be considered while generation of information using various analytical techniques.

5. Sample Preparation

An analyte is often found in living medium like blood, plasma, urine, serum, and so on. The blood of human volunteers or subjects is typically collected by puncturing a vein using a hypodermic syringe which is capable of holding 5-7 ml. The process involves drawing venous blood into tubes that contain an anticoagulant, often ethylenediaminetetraacetic acid or heparin. Additionally, the plasma is frequently collected after 15 minutes of spinning at 4000 revolutions per minute. Around 30-50% of the total volume is gathered. The purpose of preparing a sample is to purify the sample before analysis. Biological samples may include endogenous macromolecules, proteins, salts, small compounds, and metabolic by-products that have the potential to disrupt the analysis, chromatographic column, or detector. The sample prepping operation involves isolating the analytes from the biological matrices and transferring them to a suitable solvent for introduction into the chromatographic equipment. Sample preparation methods include liquid-liquid extraction, solid-phase extraction (SPE), protein precipitation, chromatography, and ligand binding assay (LBA).[9,10]

5.1. Parameters in validation

5.1.1. Linearity and range

A calibration curve is a connection across response and known analyte concentration. The curve should belong in the similar matrix just as the sample, and a curve for each analyte has to be constructed. The range of the technique refers to the concentration interval within which the accuracy, precision, and linearity have been verified. The calibration curve used should be the most basic model that accurately describes the connection between concentration and response. The variance from the nominal concentration of the lower limit of quantification should be no more than 20%, and no more than 15% from the other standards in the curve.

5.1.2. Accuracy

The range of the technique refers to the concentration interval within which the accuracy, precision, and linearity have been verified. The calibration curve used should be the most basic model that accurately describes the connection between concentration and response. The variance from the nominal concentration should be no more than 20%, and no more than 15% from the other standards in the curve. The range of the technique refers to the concentration interval within which the accuracy, precision, and linearity have been verified. The calibration curve used should be the most basic model that accurately describes the connection between concentration and response. The variance from the nominal concentration should not be no more than 20%, and no more than 15% from the other standards in the curve. [11,12]

5.1.3. Bias

ISO describes bias as the disparity between predicted trial results and an accepted reference value. It could contain beyond a single systematic error component. Bias can be stated in terms of % in the allowable reference values the word trueness describes the departure wide serials of measurements' mean values from the established reference value. It is possible to express it in terms of prejudice. Due to the significant burden of processing such big series, trueness is normally confirmed after a large number of QCs during ordinary application rather than during method validation. [13]

5.1.4. Precision

When a methodology is done repeatedly too numerous to fractionalise a single identical capacity of biologic framework, the accuracy of the method indicates the closeness of individual measurements of an analyte. Precision may be assessed using at least 5 determinations per concentration. A minimum of three concentrations in the expected concentration range are required. With the exception of the LLOQ, the accuracy may be assessed at every concentration level should not be above 15 of the coefficients of variation (CV).Accuracy is further split into interday, intraday, and various analyst or repeatability, which analyses accuracy across time and may include many analysts, equipment, reagents, and laboratories. [14]

5.2. Intermediate accuracy

Intermediate precision indicates variability within different labs on various days, different analysers, equipment, and so forth. [15] The ISO defines the phrase "M-factor different intermediate precision," where M-factor represents the number of variables that range between subsequent determinations. This type of precision is known as in between-run, day, or inter-assay precision. [16]

5.3. Selectivity

The is usually used for evaluating the procedure for its capacity to discriminate and quantify the analyte in the availability of any other components present in the sample. Analyses of all blank samples of suitable biological matrix acquired from at least six sources should be performed to determine selectivity. Individually every blank sample should be checked for interference, and selectivity at the lower LOQ should be assured. [17]

5.4. Limit of detection(LOD)

The LOD is only relevant to the limit test. Under the defined experimental parameters, it is the lowest amount of analyte in the sample that can be detected but not necessarily quantified. The detection is commonly stated in terms of a percentage, parts per million, or parts per billion.

5.5. LLOQ

It is the least amount of analyte present in a sample which can be quantified with sufficient criteria of accuracy and precision. The most practical strategy is to calculate Lower Limit of Quantification based on accuracy and precision, which defines the term as the lowest concentration of the sample that can still be measured with adequate accuracy and precision. Also, it is based on the ratio of signal-to-noise ratio type that can only be employed if the baseline noise is low, as in chromatographic procedures. [18–25]

5.6. Upper limit of quantification

A greatest analyte's amount of a sample which is been measured with appropriate bias is known as the upper limit of quantification (ULOQ). In general, this corresponds to the maximum calibration standard concentration. [26]

5.7. Recovery

The recovery of an analyte's assay is an outlined difference between the detector response from a quantity of analyte injected to and extracted from bodily fluids and the detector

output from the true concentration of the pure authentic standard. The analyte recovery is not vital to be 100%, but the amount of analyte and IS recovery percentage should be correct, steady, and reproducible. Tests must be carried out by comparing the results obtained for the extracts at each of the three concentrations (lowest, medium, and maximum) with un-extracted standards that show a hundred percent recovery.[19]

5.8. Robustness

Generally termed as type of an analytical technique, according to guidelines revised by ICH recommendations, terms an estimate its ability as in to stay none affected by modest but purposeful modifications in other parameters and offers an indicator showing dependability during routine use. Secondly it is defined as the capacity to replicate any analytical procedures in diverse laboratory environments without causing any major changes in the final results.

5.9. Ruggedness

It measures of a method's sensitivity to tiny fluctuations that may happen while performing normal protocol, such as modern transitions in pH values, composition of mobile phases, temperatures, and so on. Ruggedness testing is not required for full validation, it may be highly useful during the whole development/revalidation phase, as problems which can emerge during the work are frequently recognized in earlier.[20,21]

5.10. Stability

During method of validating, the stability study of analyte with varied circumstances should also be evaluated, situations utilized along stability trials will hold similarity to that which will be experienced during real time handling of sample for analysis.[22]

5.11. Stock solution stability

The stock solution's stability must assess accordingly with normal room temperature for 6 hours.

5.12. Short-term temperature stability

The analyte's stable conditions in biological fluids at room temperature should be assessed. 3 portions of minimum and maximum concentrations were stored for one day before being tested.

5.13. Long-term temperature stability

The stability in the matrix should last from first day of collection to the last day of examination.

5.14. Freeze and thaw stability

After three freeze-thaw cycles sessions, the stable conditions shall be assessed. Three sections of low and high concentration should be frozen for 24 hours before being thawed at room temperature.

5.15. Post-preparative stability

Stability of an analyte will be assessed at several phases of the analytical procedure.[23,24]

6. Documentation

Laboratory studies could be used to develop and validate the validity of an analytical technique, and documentation of the successful completion of such studies shall be included in the assay of a validation report. A validated analytical technique must include general and specialized SOPs (standard operating procedures) as well as adequate record keeping. The data production used for the creation of bio analytical methods and the QCs in charge should record and make available for audit and inspection. Documentation for submission to the agency should include the following.[27]

1. Summary information,
2. Method development and establishment,
3. Bioanalytical reports on the application of any techniques to routine sample analysis, and
4. Other information pertinent to development /or routine sample analysis

6.1. Documentation for method establishment

Documentation for method development and establishment should include:

1. An operational description of the analysing method.
2. Evidence of drug standard purity and identification, as well as metabolite standards and internal standards utilized in validation experiments.[28]
3. A description of the stability studies as well as supporting data.
4. A description of the tests that were carried out to evaluate all the parameters and important data gained from these investigations.
5. Recording intra- and inter-assay precision and accuracy.
6. If appropriate, information on cross-validation study findings in NDA (new drug approval) filings.
7. Valid annotated chromatograms or spectrograms, if appropriate
8. Any variations from standard operating procedures, protocols, or (Good Laboratory Practice) GLPs (if applicable), as well as explanations for those variances.[29]

6.2. Application to routine drug analysis

The following should be included in documentation of the use of approved bio analytical technologies to routine drug analysis.

1. Proof of the purity and identification of drug standards, metabolite standards, and internal controls used in routine tests.
2. Summary tables providing sample processing and storage information: Tables should include sample identity, collection dates, storage before to shipment, shipment batch information, and storage prior to analysis. Dates, timings, sample condition, and any deviations from procedures should all be included.
3. Summary tables of clinical or preclinical sample analytical runs: Information should include assay run identification, analysis date and time, assay method, analysts, start and stop times, duration, significant equipment and material changes, and any potential issues or deviation from the established method.
4. Equations for calculating outcomes backwards.
5. Calibration curve data tables utilized in sample analysis, as well as calibration curve summary data.
6. Data on intra- and inter-assay accuracy and precision from calibration curves and QC samples utilized for accepting the analytical run, as well as summary information on intra- and inter-assay values of QC samples. In addition to raw data and summary statistics, QC graphs and trend analysis are welcomed.
7. Tables of data from clinical or preclinical sample analyses: Assay run identification, sample identification, raw data and back-calculated findings, integration codes, and/or additional reporting codes should all be included in the tables.[30]
8. Complete serial chromatograms from 5 to 20% of individuals, including with standards and quality control samples from those analytical runs: Chromatograms from 20% of serially chosen participants should be included in key bioequivalence studies for commercialization. Chromatograms from 5% of randomly selected participants in each research should be included in other investigations. Prior to the examination of any clinical samples, the subjects whose chromatograms will be submitted should be established.
9. The causes of missing samples.
10. Repeat analysis documentation: Documentation for repeat analyses should contain the initial and repeat analysis findings, the reported result, assay run identification, the cause for the repeat analysis, the requestor of the repeat analysis, and the manager authorizing reanalysis. Repeat analysis of a clinical or preclinical material should be done only in accordance with a documented SOP.[31]

6.3. The following recommendations should be noted in applying a bio analytical method to routine drug analysis

1. A matrix-based standard curve should include a minimum of six standard points covering the whole range, omitting blanks (either single or duplicate).
2. *Response function:* For the standard curve inside the study, the same curve fitting, weighting, and goodness of fit specified during pre-study validation should be used. Appropriate statistical tests are used to establish the response function based on the actual standard points during each validation run. Changes in the response function connection between pre-study validation and regular run validation indicate the presence of possible issues.
3. The QC samples should be used to determine whether the run should be accepted or rejected. These QC samples have been analyte matrix spiked.[32]
4. *System suitability:* A unique SOP (or sample) should be selected based on the analyte and procedure to guarantee optimal system functioning.
5. Any needed sample dilutions should be performed using a similar matrix (e.g., human to human), eliminating the requirement for real within-study dilution matrix QC samples**.**
6. *Repeat analysis:* It is critical to develop a SOP or guideline for repeat analysis as well as acceptance criteria. This SOP or guideline should explain why sample analysis should be repeated. Repeat analyses might be performed for a variety of reasons, including regulatory purposes, inconsistent replication analysis, and samples outside of the assay range, sample processing problems, equipment failure, inadequate chromatography, and inconsistent PK results. If the sample volume allows, re-assays should be performed in triplicate. The basis for the repeat analysis, as well as its reporting, should be well recorded.
7. *Sample data reintegration:* A standard operating procedure (SOP) or a set of guidelines for sample data reintegration should be created. This SOP or guideline should describe why reintegration is necessary and how it will be carried out. The justification for reintegration should be properly stated and recorded. Data on original and reintegration should be reported.

7. Acceptance Criteria for the Run

Accepting the analytical run should take into account the following acceptance criteria.

1. Standards and QC samples can be made from the same spiking stock solution if the solution's stability and accuracy have been validated. A single matrix source may also be utilized if selectivity has been proven.

2. Within the run, standard curve samples, blanks, QCs, and research samples may be organized as needed.
3. The placement of standards and quality control samples inside a run should be tailored to identify assay drift over the course of the run.
4. Matrix-based standard calibration samples: 75%, or a minimum of six standards, when back-calculated (including ULOQ), should be within 15% of the nominal value, except for LLOQ, which should be 20%. Values that fall outside of these boundaries can be deleted as long as they do not modify the existing model.
5. For both the intra-day and intra-run experiments, specific recommendations for technique validation should be supplied.[13]
6. *QC samples:* Each run should include QC samples reproduced (at least once) at a minimum of three concentrations [one within 3 of the LLOQ (low QC), one in the midrange (middle QC), and one near the high end of the range (high QC). The findings of the QC samples are used to determine whether the run should be accepted or rejected. At least 67% (four out of six) of the QC samples must be within 15% of their respective nominal (theoretical) values; 33% of the QC samples (not all replicates at the same concentration) may be outside the 15% range. A confidence interval technique with equivalent accuracy and precision is a viable option
7. The minimum number of samples (in multiples of three) shall be at least 5% of the total number of QCs or six total unknown samples, whichever is larger. results from failed runs do not need to be documented, but the fact that a run was refused and the cause for failure should be recorded. Samples including several analyte should not be rejected based on the results from one analyte failing the acceptance criteria.[33]

8. Conclusion

Bio analysis and the production of pharmacokinetic, toxiciokinetic, and metabolic data plays a fundamental role in pharmaceutical research, development involved in the drug discovery and development process. An attempt has been made to understand and explain the bio analytical method development and validation from a quality assurance department point view. This article indicates simple to use approaches with a correct scientific background to improve the quality of the bio analytical method development and validation process. Despite the widespread availability of different bio analytical procedures for low-molecular weight drug candidates, ligand binding assay remains of critical importance for certain bio analytical applications in support of drug development such as for antibody, receptor, etc it gives an idea about which criteria Bioanalysis based on , and what it should follow to reach for proper acceptance. Applications of bioanalytical method in routine drug analysis are also taken into consideration in this article. These various essential development and validation characteristics for bioanalytical methodology have been discussed with a view to improving the standard and acceptance in this area of research.

9. Source of Funding

None.

10. Conflict of Interest

None.

11. Abbreviations

Limit of detection (LOD), Upper limit of quantification (ULOQ), Lower Limit of Quantification (LLOQ)

References

1. Eeckhaut AV, Lanckmans K, Sarre S, Smolders I, Michotte Y. Validation of bioanalytical LC-MS/MS assays: evaluation of matrix effects. *J Chromatogr*. 2009;877(23):2198–207.
2. González O, Blanco ME, Iriarte G, Bartolome L, Maguregui MI, Alonso RM. Bioanalytical chromatographic method validation according to current regulations, with a special focus on the non-well defined parameters limit of quantification, robustness and matrix effect. *J Chromatography*. 2014;1353:10–27.
3. Buick AR, Doig MV, Jeal SC, Land GS, Mcdowall RD. Method validation in the bioanalytical laboratory. *J Pharm Biomed Anal*. 1990;8:629–37.
4. Thompson M, Ellison S, Wood R. Harmonised Guidelines for Single Laboratory Validation of Method of Analysis. *Pure Appl Chem*. 2008;74(5):835–55.
5. Liu L, Zheng T, Morris MJ, Wallengren C, Clarke AL, Reid CA. The mechanism of carbamazepine aggravation of absence seizures. *J Pharmacol Exp Ther*. 2006;319(2):790–8.
6. Puluido A, Ruusanches I, Boque R, Rius FX. Uncertainty of results in routine qualitative analysis in analytical chemistry. *J Pharm Biomed Anal*. 2005;22:647–54.
7. Wood R. How to Validate Analytical Methods. *Trends Analyt Chem*. 2005;18:624–132.
8. Mcdowall RD. The Role of Laboratory Information Management Systems LIMS in Analytical Method Validation. *Anal Chim Acta*. 2007;54:149–58.
9. Kallner A. Quality specifications based on the uncertainty of measurement. *Scand J Clin Lab Invest*. 1999;59(7):513–9.
10. Rao KR, Kumar KS. Bioanalytical method validation-A quality assurance auditor view point. *J Pharm Sci Res*. 2009;1(3):1–10.
11. Kringle RO. An assessment of the 4-6-20 rule of acceptance of analytical runs in bioavailability, bioequivalence, and pharmacokinetic studies. *Pharm Res*. 1994;11(4):556–60.
12. Wieling J, Hendriks G, Tamminga WJ, Hempenius J, Mensink CK, Oosterhuis B. Rational experimental design for bioanalytical methods validation. Illustration using an assay method for total captopril in plasma. *J Chromatogr A*. 1996;730(1-2):381–94.
13. Kringle R, Hoffman D. Stability methods for assessing stability of compounds in whole blood for clinical bioanalysis. *Drug Inf J*. 2001;35(4):1261–70.
14. Viswanathan CT, Bansal S, Booth B, Destefano AJ, Rose MJ, Sailstad J, et al. Quantitative bioanalytical methods validation and implementation: best practices for chromatographic and ligand binding assays. *Pharm Res*. 2007;24(10):1962–73.

15. Mark H. Application of improved procedure for testing linearity of analytical method to pharmaceutical analsysis. *J Pharm Biomed Anal*. 2003;33(1):346–53.
16. Tiwari G, Tiwari R. Bioanalytical method validation: An updated review. *Pharm Methods*. 2010;1(1):25–38.
17. Heyden YV, Nijhuis A, Verbeke JS, Vandeginste BG, Massart DL. Guidance for robustness/ruggedness tests in method validation. *J Pharm Biomed Anal*. 2001;24(5-6):723–53.
18. Singh PS, Shah G. Analytical method development and validation. *J Pharm Res*. 2011;4(5):2330–2.
19. Wood R. How to validate analytical methods. *Trends Analyt Chem*. 2005;18(9-10):624–56.
20. Dadgar D, Burnett PE. Issues in evaluation of bioanalytical method selectivity and drug stability. *J Pharm Biomed Anal*. 1995;14(1-2):1634–5.
21. Miller KJ, Bowsher RR, Celniker A, Gibbons J, Gupta S, Lee JW. Workshop on bioanalytical methods validation for macromolecules: Summary report. *Pharm Res*. 2001;18(9):1373–83.
22. Hubert H, Chiap P, Crommen J, Boulanger B, Chapuzet E, Mercier N. The SFSTP guide on the validation of chromatographic methods for drug analysis: From the Washington Conference to the laboratory. *Anal Chim Acta*. 1999;391(2):45–55.
23. Timm U, Wall M, Dell D. A new approach for dealing with the stability of drugs in biological fluids. *J Pharm Sci*. 1985;74(9):972–9.
24. Nowatzke W, Woolf E. Best practices during bioanalytical method validation for the characterization of assay reagents and the evaluation of analyte stability in assay standards, quality controls, and study samples. *AAPS J*. 2007;9(2):117–39.
25. Zhoua S, Songb Q, Tangb Y, Weng N. Critical Review of Development, Validation, and Transfer for High Throughput Bioanalytical LC-MS/MS Methods. *Curr Pharm Anal*. 2005;55:3–14.
26. Mohammad AT, Leung HF. Reversed-phase high-performance liquid chromatography method for the analysis of nitro-arginine in rat plasma and urine. *J Chromatogr*. 1988;235:567–73.
27. Shah VP, Midha KK, Dighe S, Mcgilveray IJ, Skelly JP. Analytical methods validation: Bioavailability, bioequivalence and pharmacokinetic studies. *Eur JDrug Metab Pharmacokinetics* . 1991;16:249–55.
28. Yacobi A. Analytical methods validation: bioavailability, bioequivalence and pharmacokinetic studies. *Conference report Pharm Res*. 2009;9:588–92.
29. Penninckx W, Hartmann C, Massart DL, Smeyers-Verbeke J. Validation of the Calibration Procedure in Atomic Absorption Spectrometric Methods. *J Anal At Spectrom*. 1998;11:237–83.
30. Wieling J, Hendriks G, Tamminga WJ, Hempenius J, Mensink CK, Oosterhuis B. Rational experimental design for bioanalytical methods validation. Illustration using an assay method for total captopril in plasma. *J Chromatogr*. 2006;730(1-2):381–94.
31. Shah VP, Midha KK, Dighe SV, Mcgiveray IJ, Skelly JP, Yacobi A. Analytical methods validation: Bioavailability, bioequivalence, and pharmacokinetic studies. *J Pharm Sci*. 1988;81(3):309–21.
32. Findlay JW, Smith WC, Lee JW, Nordblom GD, Das I, Desilva BS. Validation of Immunoassays for bioanalysis: A pharmaceutical industry perspective. *J Pharm Biomed Anal*. 2008;21(6):244–51.
33. Rodbard D, Feldman Y, Jaffe M. Kinetics of Two-Site Immuno radiometric (Sandwich) Assays-II. *Immunochem*. 1955;15:90046–55.

Author's biography

Sakshi S. Waikar, Assistant Professor https://orcid.org/0009-0003-1631-8902

Manisha Raut, Assistant Professor https://orcid.org/0009-0003-5526-0432

Tejashree Dugaje, Assistant Professor https://orcid.org/0009-0001-2786-164X

Vasim T. Pathan, Associate Professor https://orcid.org/0000-0001-8743-8141

Atul R. Bendale, Professor https://orcid.org/0000-0002-3219-0377

Anil G. Jadhav, Professor https://orcid.org/0000-0003-3336-6503

Cite this article: Waikar SS, Raut M, Dugaje T, Pathan VT, Bendale AR, Jadhav AG. Extensive assessment of fundamental factors in the development and validation of bioanalytical methods: Highlighting accuracy and reliability. *Int J Pharm Chem Anal* 2024;11(4):274-280.