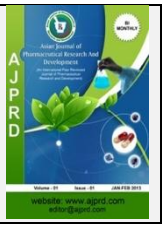
Available online on 15.6.2025 at http://ajprd.com

Asian Journal of Pharmaceutical Research and Development

Open Access to Pharmaceutical and Medical Research

© 2013-25, publisher and licensee AJPRD, This is an Open Access article which permits unrestricted non-commercial use, provided the original work is properly cited



Open Access

Review Article

A Review on: Bioanalytical Method Development and Validation

Suvarna V. Pawar[1*], Dr. Vinayak M. Gaware[2]

[1] Department of Quality Assurance, PRES's College of Pharmacy (For Women), Chincholi, Nashik.

[2] Department of Pharmaceutical Chemistry, PRES's College of Pharmacy (For Women), Chincholi, Nashik.

A B S T R A C T

The creation of selective, sensitive, and reliable bioanalytical methods for accurately quantifying drugs and their metabolites in biological samples is a critical component of successful drug development. These methods ensure that the analytical processes used are suitable for biomedical purposes. Bioanalytical method validation encompasses a series of procedures designed to confirm that a method employed for quantifying analytes in biological matrices like blood, plasma, serum, or urine is dependable and consistent for its intended applications.The data derived from such methods is essential for regulatory submissions, including investigational new drug applications (INDs), new drug applications (NDAs), abbreviated new drug applications (ANDAs), biologic license applications (BLAs), and their respective supplements. These validated methods play a vital role in human clinical pharmacology, bioavailability (BA), and bioequivalence (BE) studies, which require accurate evaluation of pharmacokinetic, toxicokinetic, or biomarker concentrations.Therefore, it is crucial to ensure that bioanalytical methods are carefully developed, thoroughly validated, and documented to meet the required standards, ensuring reliable results in drug analysis. High-performance liquid chromatography (HPLC) is a versatile analytical technique, particularly beneficial for identifying and quantifying low concentrations of drugs and metabolites in biological matrices. As such, developing and validating HPLC-based bioanalytical methods for low-dose drugs offers significant advantages.This article highlights advancements in HPLC-based bioanalytical method development and validation for various drugs, emphasizing their documentation and applications.

Key Words: Bioanalytical, Bioavailability, Bioequivalence, Documentation, HPLC, Pharmacokinetic, Toxicokinetic, Validation.

A R T I C L E I N F O: Received 19 Jan. 2025; Review Complete 24 March. 2025; Accepted 18 April 2025. ; Available online15 June. 2025

Cite this article as:

Pawar SV, Gaware VM, A Review On: Bioanayltical Method Development and Validation, Asian Journal of Pharmaceutical Research and Development. 2025; 13(3):167-179, DOI: http://dx.doi.org/10.22270/ajprd.v13i3.1578

*Address for Correspondence:

Suvarna V. Pawar, Department of Quality Assurance, PRES's College of Pharmacy (For Women), Chincholi, Nashik.

INTRODUCTION

Bioanalysis plays a significant role in the drug development process. Today, it has become an integral part of toxicological evaluations as well as pharmacokinetic and pharmacodynamic studies during drug development. (1-4)A bioanalytical method involves a series of steps, including the collection, processing, storage, and analysis of a biological matrix to detect and quantify specific chemical compounds. Bioanalytical Method Validation (BMV) is a process that ensures a quantitative analytical method is appropriate for biomedical applications. It involves performing a minimum set of validation experiments to confirm the reliability and quality of the method, yielding satisfactory results. Assessing the stability of analytes in biological samples collected during clinical studies, along with the stability of essential assay reagents, including analyte stock solutions, is a critical aspect of bioanalytical method validation. This process encompasses all procedures necessary to demonstrate that the method used for quantitative analysis of analytes in biological matrices, such as blood, plasma, serum, or urine, is dependable and reproducible for its intended purpose. (5)

1. Bioanalysis concept:

Bioanalysis involves identifying and quantifying analytes in various biological samples, including blood, plasma, serum, saliva, urine, feces, skin, hair, and organ tissues. It encompasses not only the measurement of small molecules like drugs and their metabolites but also the detection and characterization of larger molecules such as proteins and peptides.In pharmaceutical companies, bioanalysis plays a vital role in supporting drug discovery and development. It is

instrumental in conducting toxicokinetic (TK), pharmacokinetic (PK), and pharmacodynamic (PD) studies for new drug candidates. Furthermore, bioanalysis is widely utilized in clinical, preclinical, and forensic toxicology laboratories.This field holds significant importance across diverse research domains, including new drug development, forensic investigations, doping control, and the identification of biomarkers for diagnosing various diseases. As a result, bioanalysis has become an essential discipline in advancing scientific research and healthcare. The bioanalysis process involves preplanning, sample collection, sample preparation, analysis, calibration, data evaluation, and reporting. In modern bioanalysis, proper sample preparation and the use of hyphenated instrumentation are essential to ensure accurate, efficient, and reliable. (6-8).

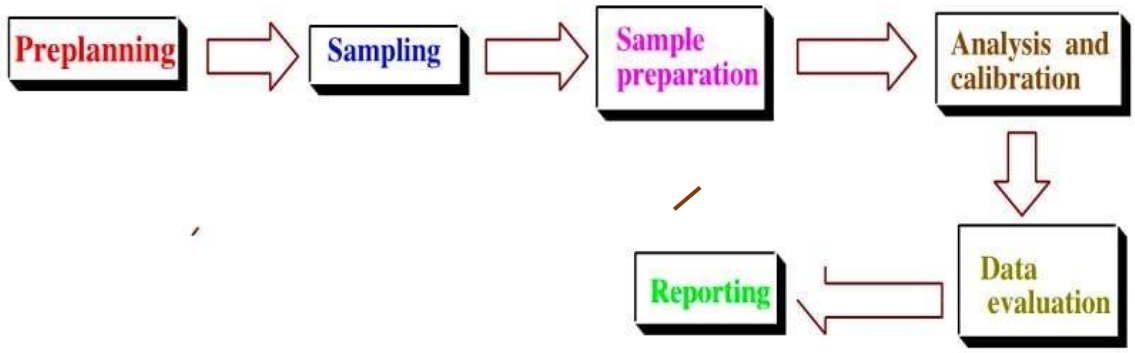


Figure 1: Bioanalytical work flow

The fundamental parameters of validation encompass all criteria that ensure the quality of data, including selectivity, sensitivity, calibration model, accuracy, precision, stability, lowerlimit of quantification (LLOQ), recovery, linearity, limit of detection, reproducibility, and ruggedness. Validated bioanalytical methods produce results used by quality control laboratories to verify the identity, purity, quality, potency, and bioavailability of drug products.

When a study involves sample analysis across multiple laboratories, it is crucial to validate the bioanalytical method at each site. Providing adequate validation data for different labs helps ensure inter-laboratory consistency and reliability. Often, existing bioanalytical techniques are adapted to meet the specific requirements of an analytical procedure. Therefore, establishing well-defined and validated bioanalytical methods is critical for obtaining reliable and interpretable results.

Biological matrices, such as plasma or urine, contain high levels of endogenous substances, while the drug concentrations are often low. Additionally, endogenous compounds may share structural similarities with the target drug, complicating the analysis. To address this, the drug must be isolated in its pure form using appropriate extraction techniques.Advances in modern analytical instruments and extraction methods have led to significant progress in bioanalytical method development and validation. High-performance liquid chromatography (HPLC) has proven to be an effective tool for identifying and quantifying drugs and their metabolites in biological fluids like plasma, serum, and urine. Successful HPLC analysis requires selecting suitable detectors, stationary phases, eluents, and proper programming for separation.

Among detectors, the UV/VIS detector is the most versatile and widely used in HPLC due to its excellent linearity and ability to provide rapid quantitative analysis against a single standard of the drug. This review highlights the development and validation of bioanalytical methods using HPLC, with a focus on efficient sample preparation techniques. (9)

2. Method development

Before initiating bioanalytical method development, several critical factors must be considered. These include the analyte's chemical structure, pKa value, solubility characteristics, stability, and adsorption tendencies to materials like plastic or glass.

Bioanalytical method development can be divided into two primary stages:

1. **Sample preparation** – ensures a clean extract with high extraction efficiency.
2. **Sample separation and detection** – Focuses on isolating and identifying the analyte effectively.

Proper sample preparation plays a vital role in achieving accurate results, as it helps eliminate impurities and improve extraction performance. The choice of detector depends on the analyte's concentration range, ensuring suitable sensitivity and precision during analysis.

Additionally, selecting an appropriate internal standard (ISTD) is crucial. An ISTD is used to compensate for matrix effects and enhance the accuracy of results. It should closely resemble the analyte in both chemical structure and properties to serve its purpose effectively.

A systematic approach to method development is essential for drug development. This process involves three interconnected components:

1. **Sample preparation** – to isolate and clean the analyte.
2. **Separation of the analyte** – to distinguish it from other components in the sample.
3. **Detection of the analyte** – to identify and quantify it accurately.

Focusing on these elements ensures the development of reliable and efficient analytical methods. (10)

- **Sample preparation:**

Sample preparation is a crucial step in bioanalytical studies for analyzing drugs and metabolites, as biological samples like blood, plasma, urine, or serum often contain proteins and various endogenous and exogenous substances that can interfere with the analyte. The primary goal of sample preparation is to isolate the analyte of interest by removing unwanted substances without causing significant analyte loss.

- **Common Biological Matrices Used in Bioanalysis:**

Measuring drug levels in urine serves as an indirect method to evaluate its bioavailability. Drug concentrations in feces can indicate either the portion of the drug that was not absorbed after oral administration or the fraction excreted through bile following systemic absorption. Salivary drug levels reflect only the free, unbound drug, as only this form diffuses into saliva. Consequently, the saliva-to-plasma drug concentration ratio is often less than 1. The most accurate way to assess a drug's pharmacokinetics is by analyzing its concentration in blood, serum, or plasma. Since drugs in plasma are generally in dynamic equilibrium with tissues, any changes in plasma drug levels typically correspond to similar changes in tissue concentrations.(11)

- **Storage and Preservation of Biological Samples:**

Biological fluids are prone to physicochemical changes due to the presence of various substances, making proper preservation essential. Since processing or purifying these samples can be time-intensive, it is crucial to establish optimal storage conditions. For samples sensitive to oxidation, airtight containers are recommended to prevent exposure to air. Similarly, moisture-sensitive drugs can be preserved effectively through freeze-drying or lyophilization, which helps prevent dehydration and maintain sample integrity.

- **Pretreatment of Serum, Plasma, and Other Biological Samples:**

If the analyte is protein-bound, pretreatment of serum and plasma samples may not be required. However, in such cases, the following methods can be used:

1. **pH Adjustment**: Adjust the sample to a pH of 9 using acids or bases with a concentration of 0.1M or higher. The resulting supernatant can then be separated and used for extraction.
2. **Protein Precipitation with Polar Solvents**: Precipitate proteins from the biological fluid by mixing with polar solvents such as acetonitrile, methanol, or acetone in a 1:2 ratio. Centrifuge the mixture and use the clear supernatant for extraction.
3. **Protein Precipitation with Acids or Salts**: Treat the sample with acids or inorganic salts, such as formic acid, perchloric acid, trichloroacetic
4. acid, ammonium sulfate, sodium sulfate, or zinc sulfate, to precipitate proteins. Adjust the pH of the supernatant, sonicate it for 15 minutes, dilute it with water or buffer, centrifuge the mixture, and use the clear supernatant for extraction.

If the analyte is not protein-bound, additional pretreatment methods are required, such as:

- **Centrifugation**: Used to separate cells from serum and plasma. Cooling centrifugation at 4°C is preferred to prevent analyte decomposition, and the clear supernatant is utilized for analysis.
- **Homogenization**: For biological samples containing insoluble proteins, such as muscle or tissue, a homogenization step with 1N hydrochloric acid may be needed before further processing.
- **Solid Sample Preparation**: Solid samples like feces can be homogenized with a small amount of methanol using a blade homogenizer or tissue homogenizer to prepare them for further analysis.
- These pretreatment steps ensure the effective extraction and analysis of analytes in various biological matrices.

- **Stages of Drug Metabolism:**

Drug metabolism involves the interaction between a drug (substrate) and an enzyme, following a sequence: **Enzyme + Drug → Enzyme-Drug Complex → Enzyme + Metabolite.**

Key enzymatic reactions in drug metabolism include oxidation, reduction, hydrolysis, and conjugation. Conjugation is a synthesis process where drugs with specific chemical structures undergo biotransformation. Each reaction produces a metabolite, which may undergo further metabolism.

Phase 0: Transport of Drugs into Hepatocytes:

Phase 0 refers to the movement of drugs from the bloodstream into liver cells (hepatocytes) via the basolateral or sinusoidal uptake processes (12)

Phase I: Functional Group Modification:

Phase I involves oxidation, reduction, and hydrolysis reactions. These reactions introduce or expose functional groups in the drug molecule, preparing it for subsequent Phase II reactions. Enzymes responsible for Phase I reactions are located in various cellular components, such as the cytoplasm, mitochondria, and endoplasmic reticulum.

Phase II: Conjugation Reactions:

Phase II involves conjugation reactions, where naturally occurring molecules in the body are added to the drug. This step often converts non-polar metabolites from Phase I into water-soluble compounds, making them easier to excrete(13). A lipid-soluble drug can enter either Phase I or Phase II directly. After Phase I, the metabolite can either become water-soluble and excreted through urine or remain non-polar and require further metabolism in Phase II to achieve water solubility. In Phase II metabolism, drugs undergo conjugation to form polar metabolites, which can be easily excreted through urine. The pharmacological effect of a drug significantly depends on these biotransformation reactions, making it essential to isolate the actual conjugates for

analysis.Samples containing conjugates like glucuronides or sulfate esters often require enzymatic or acid hydrolysis as a pretreatment step. The hydrolysis process converts these conjugates into unconjugated metabolites, which are less hydrophilic and can be more easily extracted from the biological matrix.

For non-specific acid hydrolysis, heating a biological sample with 2N to 5N hydrochloric acid at 90°C to 100°C for about 30 minutes is effective. Once cooled, the sample's pH is adjusted to a suitable level, and the resulting metabolites can be separated using solvent extraction.

Separation of analyte:

Extraction procedures for drugs and metabolites from biological samples

Extraction of analyte from biological matrix is traditionally carried out by (a) liquid-liquid extraction (LLE), (b) solid-phase extraction (SPE) and (c) precipitation of plasma proteins (PP) (14).

A) Liquid-Liquid Extraction (LLE):

Liquid-liquid extraction (LLE) is a technique used to separate compounds from a mixture by employing two immiscible liquids, typically water and an organic solvent. The separation is based on the partition coefficient of the analyte between these two phases, and a suitable solvent is selected for efficient extraction. LLE is widely used because it is straightforward, fast, and cost-effective compared to other extraction methods(15).

Using a continuous extraction process, up to 90% of many drugs can be effectively recovered. The process involves dissolving the sample mixture in an appropriate solvent, followed by the addition of an immiscible organic solvent. The mixture is thoroughly mixed and allowed to settle, causing the two immiscible liquids to separate into distinct layers. The analyte distributes itself between the two layers based on its partition coefficient.

Each layer is then separated, and the analyte is isolated from the respective solvent. Hydrophilic compounds remain in the aqueous layer, while hydrophobic compounds partition into the organic layer. Non-polar analytes extracted into the organic solvent can be recovered by evaporating the solvent, leaving behind a residue that can be reconstituted in a small volume of an appropriate solvent, typically the mobile phase. Polar analytes, extracted into the aqueous phase, can be directly injected into a reverse-phase (RP) column for further analysis.

In certain cases, controlling the pH of the sample enhances the efficiency of extraction. However, LLE may not be suitable for thermolabile substances because the high temperatures required during solvent evaporation can degrade heat-sensitive analytes (16).

B) Solid Phase Extraction (SPE):

Solid-phase extraction (SPE) is a widely used technique for isolating and concentrating trace amounts of analytes from various sample matrices. SPE is effective in reducing interference levels while minimizing the final sample volume, thereby improving analyte sensitivity. By using small disposable columns or cartridges packed with 0.1 to 0.5 g of sorbent, often reverse-phase (RP) materials like C18 or C8, analyte recovery can be maximized.

During SPE, the analyte of interest can either adsorb onto the solid phase or remain in the liquid phase. If adsorbed, the analyte can be selectively desorbed using a suitable solvent. Conversely, if the analyte remains in the liquid phase, it can be recovered through processes such as concentration, evaporation, or recrystallization. Although SPE provides high analyte recovery and sensitivity, it is time-consuming and less efficient for materials with high densities.

Steps in SPE for Plasma Analyte Extraction:

1. **Sample Pretreatment:**
   - The sample is diluted or its pH adjusted.
   - Filtration is performed to prevent SPE cartridge blockage and ensure better analyte adsorption.
2. **Cartridge Conditioning:**
   - For reverse-phase cartridges, preconditioning with solvents like methanol, acetonitrile, isopropyl alcohol, or tetrahydrofuran is crucial.
   - Proper conditioning ensures that the aqueous sample penetrates the pores and interacts with the entire sorbent surface. Keeping the cartridge wet is vital up to the point of sample loading.
3. **Sample Loading:**
   - The sample size must match the cartridge bed capacity.
   - A standard reverse-phase cartridge can retain up to 100 mg of strongly adsorbed substances.
4. **Washing the SPE Bed:**
   - A suitable solvent or water mixture is passed through the bed to remove contaminants while retaining the analyte.
5. **Elution:**
   - The analyte is recovered by eluting with a specific solvent or buffer, ready for subsequent analysis.
   - While SPE is a powerful technique for sample preparation, proper optimization of each step is critical to achieving reliable and reproducible results (17-19).

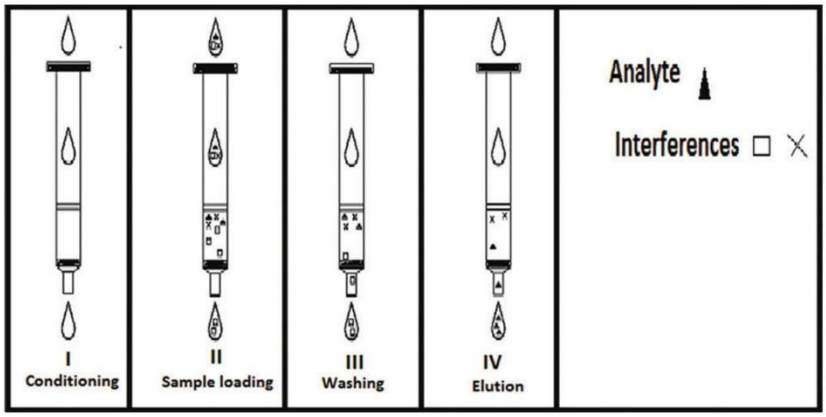


Figure 2: Steps in solid phase extraction

C) Protein Precipitation (PP)

Protein precipitation is a straightforward method for isolating analytes from blood or plasma. It relies on the analyte being readily soluble in the chosen reconstituting solvent. The process involves adding acids (e.g., trichloroacetic acid, perchloric acid), organic solvents (e.g., methanol, ethanol, acetone, acetonitrile), or salts (e.g., ammonium sulfate) to precipitate proteins from the biological sample. After precipitation, centrifugation is performed, and the analyte is collected in the clear supernatant. Among these solvents, methanol is commonly preferred as it yields a clear supernatant suitable for direct injection. This technique can extract both hydrophilic and hydrophobic compounds. However, a limitation of this method is the potential clogging of columns during analysis.

Salting Out Using Ammonium Sulfate

Ammonium sulfate is frequently used for salting out proteins due to its high solubility, strong ionic strength, and cost-effectiveness. Its solubility remains stable across temperature changes, and the density of a concentrated ammonium sulfate solution is lower than that of proteins, allowing easy centrifugation of proteins from the solution.

Solvent Precipitation

Solvent precipitation occurs when a large quantity of a water-miscible solvent like ethanol or acetone is added to a protein solution. Proteins precipitate because the solvent lowers the dielectric constant of the solution, enhancing interactions between charged groups on protein surfaces. This method is typically conducted at low temperatures, such as 0°C, or with the solvent chilled to -20°C (using an ice-salt bath) to prevent protein denaturation, which is more likely at higher temperatures.

This technique is cost-effective and versatile for sample preparation but requires careful optimization to avoid damaging the analyte or compromising the analysis (20-22).

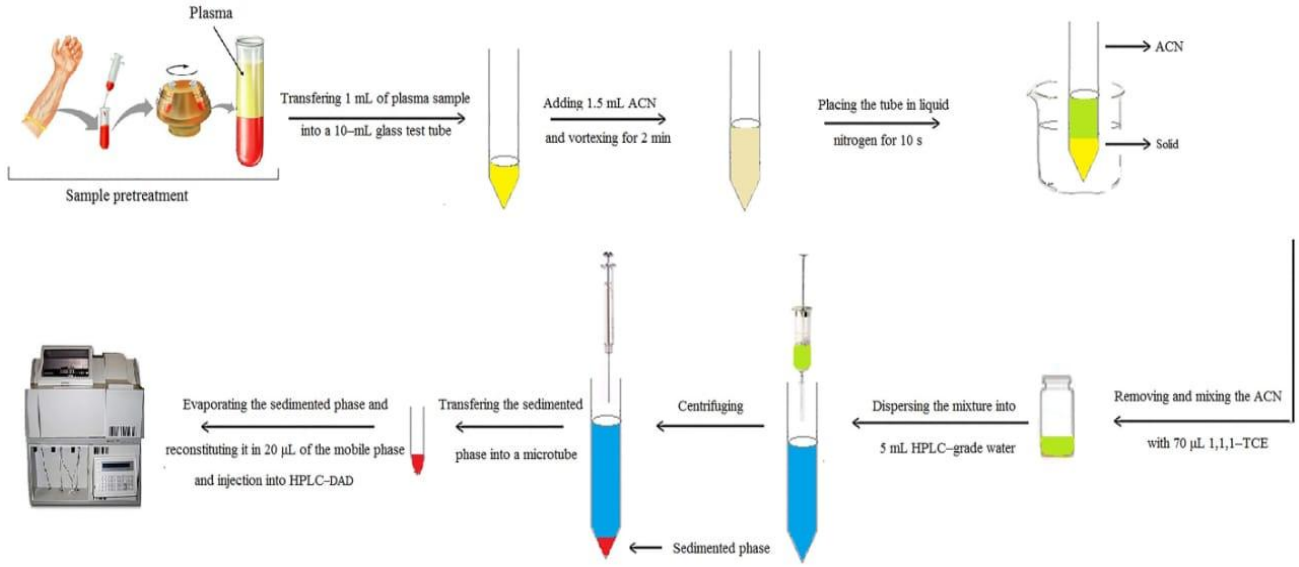


Figure 3: Protein Precipitation

Detection of analyte:

HPLC Instrumentation:

High-Performance Liquid Chromatography (HPLC) is a powerful analytical technique used in biochemistry to separate, identify, and quantify active compounds. The main components of an HPLC system include a pump, injector, column, detector, integrator, and display system.The column, considered the core of the system, is where the separation of compounds takes place. It contains a stationary phase made up of micron-sized porous particles, which necessitates the use of a high-pressure pump to drive the mobile phase through the column (23-24).The sample to be analyzed is introduced in a small volume into the stream of the mobile phase. As the analytes travel through the column, the detector measures their retention times, which represent the time taken for each compound to elute (exit) from the column. These retention times are characteristic of specific analytes and are critical for their identification and quantification.

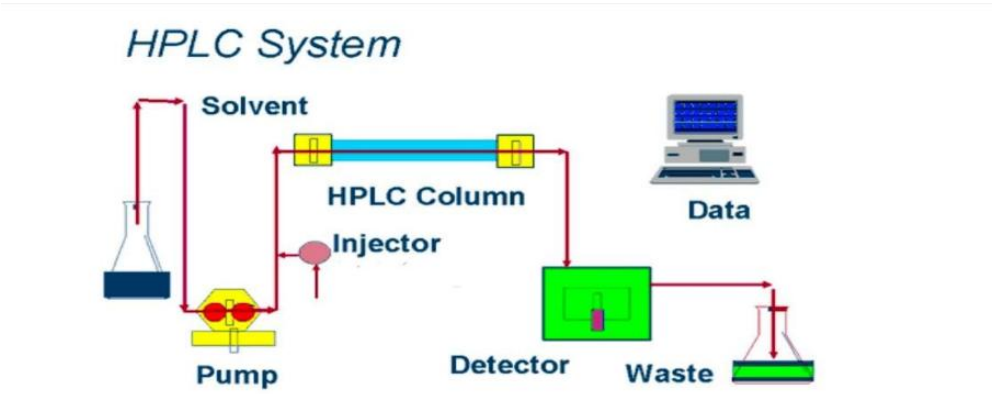


Figure 4: Schematic diagram of HPLC

Sample Injection:

Sample solutions can be introduced into the system using septum injectors, either while the mobile phase is in motion or when it is stationary. For more consistent and reproducible results, advanced rotary valve injectors equipped with sample loops are often employed. These modern injectors ensure precise sample delivery, enhancing the reliability of the analysis.

Steps involved in HPLC Method development:

Information on sample, define separation goals

↓

Need for special HPLC procedure, sample pretreatment etc

↓

Choose detector

↓

Choose LC method preliminary run, estimate best separation condition

↓

Optimize separation condition

↓

Check for problems or requirement for special procedure

↓

Recover purified Material | Quantitative calibration | Qualitative method

↓

Validate method for release to routine laboratory(25)

Bioanalytical Method Validation:

Bioanalytical method validation (BMV) is essential for accurately measuring drugs and their metabolites in biological fluids. It is crucial in assessing and interpreting data from bioavailability, bioequivalence, pharmacokinetic, and toxicokinetic studies, which are often required for regulatory submissions. The reliability of these studies depends on the precision and accuracy of the bioanalytical data (26).

Importance of Bioanalytical Method Validation:

- **Ensuring Reliable Results**: Bioanalytical methods must be well-characterized and thoroughly validated to produce dependable results that can be accurately interpreted.
- **Adaptation to Technological Advancements**: Bioanalytical methods are continuously evolving with advancements in technology, making it necessary to keep them up to date.
- **Analyte-Specific Validation**: Each bioanalytical technique has unique characteristics that can differ depending on the analyte. As such, specific validation criteria may need to be developed for each analyte.
- **Objective-Driven Approach**: The suitability of a bioanalytical technique may depend on the ultimate goal of the study, influencing method selection and validation requirements.
- **Multi-Site Validation**: When sample analysis for a study is conducted at multiple locations, it is crucial to validate the bioanalytical methods at each site. Providing comprehensive validation data ensures consistency and reliability across different laboratories (27).

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.

Types of Bioanalytical Method Validation:

Bioanalytical method validation is classified into three type:

a. Full Validation

b. Partial validation

c. Cross validation

Full validation:

Full validation involves defining and confirming all required validation parameters to ensure the bioanalytical method is appropriate for analyzing each analyte in a sample.(28-31)this comprehensive validation is essential in the following cases:

1. During the initial development and introduction of a bioanalytical method.
2. For analyzing a newly developed drug compound.
3. When adding metabolites to an existing assay for quantification, requiring thorough validation of the updated method (31-33).

This ensures the method's accuracy, consistency, and suitability for its intended purpose.

Partial validation:

Partial validation involves modifying already validated bioanalytical methods without requiring complete revalidation. It may range from conducting a single intra-assay accuracy and precision test to performing a near-complete validation. Common scenarios that necessitate partial validation include, but are not limited to:

1. Transferring a bioanalytical method between different laboratories or analysts.
2. Altering the analytical methodology, such as switching detection systems.
3. Using a different anticoagulant to collect biological fluids.
4. Changing the biological matrix within the same species, for instance, moving from human plasma to human urine.
5. Modifying the sample processing procedures.
6. Switching the species within the same matrix, such as from rat plasma to mouse plasma.
7. Adjusting the relevant concentration range of analytes.
8. Upgrading instruments or software platforms used for analysis.
9. Working with limited sample volumes, as in pediatric studies.
10. Dealing with rare or unique biological matrices.
11. Demonstrating selectivity of an analyte in the presence of other medications or specific metabolites.

Partial validation ensures that the modified method remains accurate and reliable while accommodating specific study requirements or technical changes.

Cross-Validation

Cross-validation involves comparing validation parameters when two or more bioanalytical methods are employed to generate data either within the same study or across multiple studies. It ensures consistency and reliability across different methods or laboratories.

A typical example of cross-validation arises when an originally validated bioanalytical method is used as a reference, and a revised bioanalytical method is evaluated against it. These comparisons should be conducted in both directions to ensure robustness.

- If sample analysis for a single study is performed at multiple locations or laboratories, cross-validation using spiked matrix standards and subject samples must be conducted at each site to confirm inter-laboratory reliability.

- Cross-validation is also necessary when regulatory submissions involve data generated using distinct analytical techniques, such as LC-MS/MS and ELISA, across different studies.

This process ensures that data generated under varying conditions or methodologies can be confidently compared and are suitable for regulatory purposes(34).

Current Validation Practices in Bioanalytical Method Validation:

In modern drug development, highly sensitive and selective analytical methods are essential for quantifying drugs in biological matrices such as blood, plasma, serum, or urine. Among these, chromatographic techniques are the most widely utilized for analyzing small molecules. The principles outlined below primarily address this type of analytical approach.The FDA's Guidance for Industry: Bioanalytical Method Validation (2001) is the standard reference for current validation practices. This guidance provides a comprehensive framework, summarized using common terminology, to ensure the accuracy, reliability, and consistency of bioanalytical methods (35).

Frequently Used Terminology in Bioanalytical Method Validation:

The terminology commonly applied in the validation of bioanalytical methods is outlined below. These terms, which are well-documented in FDA guidelines and other relevant publications, are presented here for ease of reference and better understanding.

Validation Parameters:

- **Accuracy:**

Accuracy refers to how close the observed concentration is to the known or nominal concentration of the analyte (36-38). It is commonly quantified using relative error (%RE) (39). Accuracy is an absolute measure and depends on several factors, including specificity and precision (40-41). Sometimes, accuracy is also referred to as trueness. To assess accuracy, replicate analyses of samples with known amounts of the analyte (such as quality controls) are conducted (42).Accuracy should be evaluated using at least five determinations per concentration, with a minimum of three concentrations representing the expected range of study samples. The mean value should fall within 15% of the nominal value, except at the lower limit of quantification (LLOQ), where it should not deviate by more than 20%. The deviation of the mean from the nominal value is used to evaluate accuracy. Accuracy can be determined through two main approaches:

(1) Analyzing control samples spiked with the analyte and

(2) Comparing the analytical method with a reference method (43).

Accuracy is typically expressed as percentage bias, which is calculated using a specific formula (44).

$$\text{Abso\% Bias} = \frac{\text{measured value} - \text{true value}}{\text{true value}} \times 100$$

- **Precision:**

The precision of a bioanalytical method refers to its ability to produce consistent results when the same homogeneous sample is analyzed multiple times under identical conditions. It assesses the extent of random errors by measuring how closely the results of repeated measurements align with one another. Precision is commonly expressed as the coefficient of variation (%CV) or the relative standard deviation (R.S.D.) of the repeated measurements, indicating the degree of variation in the observed values for the same sample. This ensures reliability in the method's performance(45).

$$\text{\% CV} = \frac{\text{standard deviation}}{\text{mean}} \times 100$$

Precision should be assessed using at least five measurements for each concentration, with a minimum of three different concentrations spanning the expected range. At each concentration level, the precision, expressed as the coefficient of variation (CV), should not exceed 15%, except at the limit of quantification (LOQ), where it may go up to 20%. Precision can be evaluated at three distinct levels: repeatability, intermediate precision, and reproducibility (46).

- **Repeatability:**

Repeatability refers to the consistency of analytical results when the same test is performed under identical conditions within a short time frame (within-assay, intra-assay), typically on the same instrument, by the same analyst, and on the same day. It reflects the precision of the method when conducted in the most controlled environment, ensuring minimal variability.

- **Intermediate precision:**

Intermediate precision takes into account the impact of various random factors within a laboratory, such as differences in days, analysts, or equipment, based on the intended use of the procedure. It measures how consistently the method performs, both in terms of quality and quantity, within a single laboratory, considering variations across different instruments and over multiple days (47). Essentially, it reflects the within-laboratory variation due to factors like changes in analysts, equipment, and testing days.

- **Reproducibility:**

Reproducibility refers to the consistency of a method's results when measured across different laboratories, on different days, by different analysts, and using different instruments, both for qualitative and quantitative analysis. While it is not mandatory for submission, reproducibility can be considered in the standardization of analytical procedures. It reflects the ability of the method to produce similar results for the same sample when tested on separate occasions or in different settings.

- **Linearity:**

The bioanalytical procedure should be capable of producing test results that are directly proportional to the analyte concentration in the sample within the range of the

calibration curve. The concentration range of the calibration curve must at least cover the expected concentrations in the study samples. If a single calibration curve cannot encompass the full range, two separate calibration ranges may be validated. However, it's important to note that extending the range too far, especially at the extremes, can negatively impact the accuracy and precision of the method. Linear correlation coefficients are commonly used to assess the method's linearity.

- **Selectivity and Specificity:**

The selectivity of a bioanalytical method refers to its ability to accurately measure and distinguish analytes in the presence of other components, such as metabolites, impurities, degradation products, or matrix elements that are likely to be present in the sample. Selectivity is demonstrated by the method's capacity to differentiate the target analyte from potential interfering substances. It is typically defined as "the method's ability to measure and differentiate analytes unambiguously in the presence of expected interfering components"(48).To ensure selectivity, blank samples from the relevant biological matrix (e.g., plasma, urine) must be analyzed, sourced from at least six independent origins (49). These blank samples are tested for potential interference, particularly at the lower limit of quantification (LLOQ), as interference can result from various factors, such as the biological matrix composition, individual subject characteristics (e.g., age, sex, species, developmental stage), or environmental conditions like UV exposure, temperature, and humidity.Regulatory guidance, including the FDA's recommendations for bioanalytical method validation, emphasizes using a minimum of six matrix sources to confirm selectivity. Specificity, closely related to selectivity, is defined as the method's ability to identify and measure the analyte without ambiguity in the presence of expected interferences (50-52). For instance, in HPLC-UV, specificity is achieved when a peak at a particular retention time corresponds exclusively to a single chemical entity. In LC-MS, selectivity allows accurate analyte measurement even if separation from endogenous compounds is incomplete.While there may be debate over the precise distinction between specificity and selectivity, there is general consensus that both are foundational to the reliability of any analytical method.

- **Limit of Detection (LOD)**

The limit of detection (LOD) refers to the smallest amount of analyte that can be identified, though not necessarily quantified. Its determination is subject to varying interpretations; some bioanalytical laboratories consider it as the lowest detectable amount in a reference solution, while others define it as the minimum concentration detectable in a biological sample. Despite these differences, there is a general consensus that the LOD represents the minimal concentration or amount of the analyte that can be reliably distinguished from the background noise.

- **Limit of Quantitation**

The quantitation limit of an analytical procedure is the minimum amount of analyte in a sample that can be measured accurately and precisely.

- **Quantification Range**

The range of concentrations, spanning from the lower limit of quantification (LLOQ) to the upper limit of quantification (ULOQ), represents the interval within which analytes can be measured consistently and reliably with acceptable accuracy and precision, based on a defined concentration-response relationship. According to the FDA's Bioanalytical Method Validation guidelines, LLOQ and ULOQ are specifically defined parameters.

Lower Limit of Quantification (LLOQ):

The LLOQ is the minimum concentration of an analyte in a sample that can be accurately and precisely measured.

Upper Limit of Quantification (ULOQ):

The ULOQ is the maximum concentration of an analyte in a sample that can be accurately and precisely measured.

To estimate the LLOQ, several methods are commonly used:

1. **Signal-to-Noise Ratio (S/N):**

   A ratio of 10:1 is generally deemed sufficient to distinguish the analyte signal from background noise.

2. **Standard Deviation and Slope Method:**

This involves the formula: **LLOQ = 10σ/S**, where:

σ = standard deviation of the response.

S = slope of the calibration curve.

1. **Relative Standard Deviation (RSD) Method:**

In this method, the RSD is plotted against concentrations near the anticipated LLOQ to determine the threshold at which acceptable precision is achieved.

These approaches ensure that the LLOQ is determined reliably for accurate quantification.

Standard Curve (Calibration Curve)

A standard curve, also known as a calibration curve, is a representation of the relationship between the instrument's response (e.g., area under the curve, peak height, or absorption) and the known concentrations of the analyte within a specified range. This relationship provides a foundation for quantifying the analyte in a sample.

The calibration curve should ideally be described by a simple, monotonic response function (either strictly increasing or decreasing) that ensures reliable, accurate, and reproducible measurements. It is prepared using the same biological matrix as the study samples, with the matrix spiked with known concentrations of the analyte.

A complete calibration curve should include:

- **Blank Sample:** A matrix sample processed without the internal standard.

- **Zero Sample:** A matrix sample processed with the internal standard but without the analyte.
- **Six to Eight Non-Zero Samples:** These samples cover the expected concentration range, including the lower limit of quantification (LLOQ).

The lowest concentration on the calibration curve can be considered the limit of quantification if:

- The analyte response is at least five times higher than the blank response.
- The analyte response is identifiable, distinct, and reproducible.
- The precision is within 20%, and the accuracy ranges between 80% and 120%.
- This systematic approach ensures that the calibration curve is robust and suitable for reliable analyte quantification in bioanalytical studies.

- **Recovery**

The extraction efficiency of an analytical process is expressed as a percentage of the known amount of analyte successfully extracted and processed through the method's steps. Recovery reflects the efficiency of the method within acceptable variability limits. While 100% recovery is not mandatory, the recovery of both the analyte and the internal standard must be consistent, precise, and reproducible.

Recovery experiments are conducted by comparing the analytical results of extracted samples at three concentration levels (low, medium, and high) with unextracted.6+ standards that represent 100% recovery. (53).

Recovery can also be expressed as **absolute recovery**, (54).

Calculated using the formula:

Absolute Recovery (%) = (Response of analyte spiked into processed matrix / Response of pure unprocessed analyte standard) × 100

This ensures the method's efficiency and reliability for analytical purposes.

- **Stability:**

The stability of an analyte refers to its chemical or physical integrity in a specific matrix under defined conditions and time intervals. Stability testing is conducted to identify any degradation of the analyte during the processes of sample collection, preparation, storage, and analysis. The stability conditions depend on the analyte's properties, the biological matrix used, and the expected storage duration before analysis.According to FDA guidelines and the AAPS/FDA white paper on bioanalytical method validation, analyte stability must be evaluated at different stages (55). Stability should be confirmed during all steps of sample preparation, analysis, and storage, including:

1. **Freeze-thaw stability** (through multiple freeze-thaw cycles).
2. **Bench-top stability** (under sample preparation conditions).
3. **Long-term storage stability** (e.g., at -20°C or -70°C).
4. **Auto-sampler stability** (stability of processed samples in the auto-sampler).(56)

Stability testing should include at least two concentration levels (low and high) using blank biological matrix samples spiked with the analyte. The evaluation must cover all matrices and species where the analyte will be quantified.

Additionally, the analyte's stability must be investigated under various conditions, such as:

- In standard solutions used for calibration curve preparation.
- In biological matrices stored at -20°C or at room temperature before analysis.
- In the final extracted sample awaiting analysis.

For analytes prone to rapid degradation, such as those metabolized by enzymes (e.g., esterases in blood), stabilizers like inhibitors may be added. The effectiveness of these stabilizers must also be validated.

The percentage stability can be calculated as (57):

% Stability = (Mean response of stability samples ÷ Mean response of comparison samples) × 100

Stability samples should be compared to freshly prepared calibrators or quality controls (QCs). At least three replicates for both low and high concentrations should be tested. Stability assessments must use samples from freshly prepared stock solutions, and the experimental conditions should mirror real-world sample handling and analysis scenarios, such as short-term and long-term storage, room temperature exposure, and freeze-thaw cycles.

If storage conditions during a study deviate from those evaluated during method validation, additional stability tests under the new conditions are required. Furthermore, stock solution stability should also be assessed, and stability sample results should be within 15% of the nominal concentration to ensure reliability.

Short-term stability:

The analyte's stability in the biological matrix at room temperature should be assessed by storing three aliquots of both low and high concentrations for a minimum of 24 hours, followed by analysis

Long-term stability:

The analyte's stability in the matrix should be at least as long as the duration between the first sample collection and the final sample analysis (58).

Freeze and Thaw Stability:

In freeze/thaw stability testing, the freezing and thawing processes for the stability samples should replicate the conditions that will be used during sample analysis. Stability should be evaluated over at least three freeze-thaw cycles.

Bench-Top stability:

Bench top stability studies should be planned and carried out to simulate the laboratory handling conditions anticipated for the study samples.

Stock solution stability:

The stability of drug stock solutions should be assessed. If the stock solution differs in state (e.g., solution vs. solid) or buffer composition (commonly for macromolecules) from the certified reference standard, stability data for the stock solution must be generated to validate its storage duration.

Processed Sample Stability:

The stability of processed samples, including the duration up to the completion of analysis, should be evaluated.

- **Range:**

The range of an analytical method refers to the span between the highest and lowest concentrations of an analyte in a sample, including these limits, where the method has been proven to deliver acceptable precision, accuracy, and linearity. Similarly, the range of a bioanalytical assay is the concentration range within which the analyte can be reliably measured with suitable precision and accuracy (59).

- **Robustness:**

According to ICH guidelines, robustness is the ability of an analytical method to remain unaffected by small, intentional variations in method parameters, indicating its reliability during routine use. It reflects the method's consistency in delivering accurate results when applied in different laboratories or under varying conditions. Robustness testing involves conducting experiments to assess the method's stability and reliability under such conditions.

- **Ruggedness**

Ruggedness refers to the reproducibility of test results for the same samples when analyzed under varying normal test conditions. This includes factors such as different analysts, laboratories, columns, instruments, and sources of reagents, chemicals, or solvents. The ruggedness of the method can be evaluated by altering experimental conditions, such as(60):

a) Using a different column of the same type.

b) Performing the analysis with different operators within the same laboratory.

Guidelines for Validating Bioanalytical Methods

1. To validate a bioanalytical method, accuracy and precision should be assessed using at least five measurements per concentration level (excluding blank samples). The average value must be within 15% of the theoretical value. Alternative methods that meet these accuracy and precision criteria may also be considered acceptable.
2. The method should demonstrate its ability to accurately and precisely determine known concentrations of the analyte in the biological matrix. This can be achieved by analyzing replicate sets of known concentration QC samples from a similar biological matrix.
3. Method validation data should include all outliers, but calculations of accuracy and precision excluding statistically identified outliers may also be reported.
4. The stability of the analyte in the biological matrix at the intended storage temperatures should be determined.
5. The stability of the analyte at ambient temperature should be evaluated over a period equivalent to the usual sample preparation, handling, and analytical processing times.
6. Reinjection reproducibility should be tested to ensure that the analytical run can be reanalyzed if there is an instrument failure.
7. The specificity of the assay method should be confirmed by using at least six independent sources of the same matrix.

Use of Validated Method for Regular Drug Analysis

In general, biological samples can be analyzed with a single determination without the need for duplicate or replicate analysis, provided the assay method has an acceptable level of variability, as demonstrated by validation data. This applies to procedures where precision and accuracy consistently fall within acceptable tolerance limits. When applying a bioanalytical method to routine drug analysis, the following recommendations should be considered:

1. A matrix-based standard curve should include at least six standard points (excluding blanks), covering the full range of concentrations.
2. Response Function: The curve fitting, weighting, and goodness of fit used during pre-study validation should also be applied to the standard curve in the study. The response function is determined through statistical tests based on the actual standard points in each validation run. Any differences between pre-study validation and routine run validation may indicate potential issues.
3. Quality control (QC) samples should be used to determine whether the run is acceptable or needs to be rejected. These QC samples are matrix-spiked with the analyte.
4. System suitability: A specific SOP (or sample) should be established based on the analyte and technique to ensure the system is operating optimally.
5. Any necessary sample dilutions should use a similar matrix (e.g., human to human), eliminating the need for actual within-study dilution matrix QC samples (61).
6. Repeat Analysis: An SOP or guideline should be created for repeat analyses, specifying the reasons for repeating

sample analysis, such as for regulatory purposes, inconsistent replicate analyses, samples outside the assay range, sample processing errors, equipment failure, poor chromatography, or inconsistent pharmacokinetic data (62).

7. Sample Data Reintegration: An SOP or guideline should be established for data reintegration, explaining the reasons for reintegration and the procedure for performing it (63-64).

CONCLUSION

Bioanalysis, along with the generation of pharmacokinetic, toxicokinetic, and metabolic data, is essential in pharmaceutical research and development, particularly in the drug discovery and development process. Therefore, the data must adhere to the scientific standards and specifications set by regulatory agencies worldwide. Bioanalytical methods must undergo validation to ensure their suitability for the intended use. This article outlines the specific recommendations and applications of bioanalytical methods in routine drug analysis for drug discovery and development. It can serve as a guide for developing bioanalytical methods for routine analysis and various biological processes. Additionally, it offers insights into bioavailability, bioequivalence, and therapeutic drug monitoring studies.

Authors' contributions:- Every author involved in this study has declared that they have no conflicts of interest for publication of work.The paper has been read and approved by all authors.

Acknowledgements:- Not Applicable

REFERNCES

1. Moein MM, El Beqqali A, Abdel-Rehim M. Bioanalytical method development and validation: Critical concepts and strategies. J chromatography B. 2017 Feb 1;1043:3-11.
2. Van Eeckhaut A, Lanckmans K, Sarre S, Smolders I, Michotte Y. Validation of bioanalytical LC–MS/MS assays: evaluation of matrix effects. J chromatography B. 2009 Aug 1;877(23):2198-207.
3. González O, Blanco ME, Iriarte G, Bartolomé 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 A. 2014 Aug 1;1353:10-27.
4. Zimmer D. Introduction to quantitative liquid chromatography-tandem mass spectrometry (LC-MS-MS). Chromatographia. 2003 Jan;57(Suppl):S325-32.
5. Sonawane LV, Poul BN, Usnale SV, Waghmare PV, Surwase LH. Bioanalytical method validation and its pharmaceutical application—a review. Pharm Anal Acta. 2014 Apr;5(288):2.
6. Sankar PR, Geethika AS, Rachana G, Babu PS, Bhargavi J. Bioanalytical method validation: A comprehensive review. Int J Pharm Sci Rev Res. 2019;9:50-8.
7. Findlay JW, Smith WC, Lee JW, Nordblom GD, Das I, DeSilva BS, et al. Validation of immunoassays for bioanalysis: a pharmaceutical industry perspective. J Pharm Biomed Anal. 2000 Jan 1;21(6):1249-73.
8. Nakashima K. High-performance liquid chromatographic analysis of drugs of abuse in biologic samples. J Health Sci. 2005;51(3):272-7.
9. Pushpa Latha E, Sailaja B. Bioanalytical method development and validation by HPLC: a review. J Appl Pharm. 2013 Dec;1:1-9.
10. Chandramouli R, Kumar P, Bibhishan KV. Analytical method development and validation for pre-clinical analysis. J Pharm Sci Res. 2010 Dec 1;2(12):795.
11. Bonate PL, Howard DR, editors. Pharmacokinetics in drug development: regulatory and development paradigms. New York: Springer Science & Business Media; 2004 Dec 5.
12. Ishikawa T. The ATP-dependent glutathione S-conjugate export pump. Trends Biochem Sci. 1992 Nov 1;17(11):463-8.
13. Kwon Y. Handbook of essential pharmacokinetics, pharmacodynamics and drug metabolism for industrial scientists. New York: Springer Science & Business Media; 2001 Dec 31.
14. Watt AP, Morrison D, Evans DC. Approaches to higher-throughput pharmacokinetics (HTPK) in drug discovery. Drug Discov Today. 2000 Jan 1;5(1):17-24.
15. Wal P, Kumar B, Bhandari A, Rai AK, Wal A. Bioanalytical method development–Determination of drugs in biological fluids. J Pharm Sci Technol. 2010;2(10):33.
16. Rahman A. Bioanalytical method development, validation and techniques used for pharmacokinetic studies using LC-MS/MS. Contemp Investig Observ Pharm. 2012;1(2):63-71.
17. Krishnan TR, Ibraham I. Solid-phase extraction technique for the analysis of biological samples. J Pharm Biomed Anal. 1994 Mar 1;12(3):287-94.
18. Murugan S, Elayaraja A, Chandrakala K, Ramaiah P, Vulchi C. A review on method development and validation by using HPLC. Int J Novel Trends Pharm Sci. 2013 Jul 10;3(4):78-81.
19. LK T, NT R, UN M. A review on bioanalytical method development and validation. Asian J Pharm Clin Res. 2016;9(3):6-10.
20. Bakes D. Strategy for the development of quantitative analytical procedures. In: Lee JW, editor. Principles and Practice of Bioanalysis. Boca Raton: CRC Press; 2003 Jun 22. p. 359-75.
21. Wells DA. High throughput bioanalytical sample preparation—methods and automation strategies. Prog Pharm Biomed Anal. 2003.
22. Kupiec T. Quality-control analytical methods: High-performance liquid chromatography. Int J Pharm Compd. 2004 May 1;8:223–7.
23. Khairnar KV, Mahamuni AP, Babalsure AV, Udbale SV, Panchal CV. A review on HPLC method development and validation.
24. Snyder LR, Kirkland JJ, Glajch JL. Practical HPLC method development. New York: John Wiley & Sons; 1997 Dec 3.
25. Shah VP. The history of bioanalytical method validation and regulation: evolution of a guidance document on bioanalytical methods validation. AAPS J. 2007 Mar;9(1):E43.
26. Karnes HT, Shiu G, Shah VP. Validation of bioanalytical methods. Pharm Res. 1991 Apr; 8:421–6.
27. U.S. Department of Health and Human Services, Food and Drug Administration. Guidance for industry: Bioanalytical method validation. Center for Drug Evaluation and Research (CDER); Center for Veterinary Medicine (CVM).
28. Tiwari G, Tiwari R. Bioanalytical method validation: An updated review. Pharm Methods. 2010 Oct 1;1(1):25–38.
29. Shah VP, Midha KK, Findlay JW, Hill HM, Hulse JD, McGilveray IJ, et al. Bioanalytical method validation—a revisit with a decade of progress. Pharm Res. 2000 Dec;17:1551–7.
30. Gao L, Li J, Kasserra C, Song Q, Arjomand A, Hesk D, et al. Precision and accuracy in the quantitative analysis of biological samples by accelerator mass spectrometry: application in microdose absolute bioavailability studies. Anal Chem. 2011 Jul 15;83(14):5607–16.
31. James CA, Breda M, Frigerio E. Bioanalytical method validation: a risk-based approach?. J Pharm Biomed Anal. 2004 Jun 29;35(4):887–93.
32. [No authors listed]. SOP 12: Validation of bioanalytical methods. Onkologie. 2003;26 Suppl 6:52–5.
33. Gilbert MT, Barinov-Colligon I, Miksic JR. Cross-validation of bioanalytical methods between laboratories. J Pharm Biomed Anal. 1995 Apr 1;13(4-5):385–94.
34. Rozet E, Dewé W, Morello R, Chiap P, Lecomte F, Ziemons E, et al. Risk-based approach for the transfer of quantitative methods: Bioanalytical applications. J Chromatogr A. 2008 May 2;1189(1-2):32–41.
35. Patil S, Pandurang ND, Kuchekar BS. Bioanalytical method development and validation: Guidelines latest reviews. Pharmainfo.net. 2009;7:1–8.
36. Singh UK, Pandey S, Pandey P, Keshri PK, Wal P. Bioanalytical method development and validation. Express Pharma. 2008;2:1–8.
37. Hartmann C, Massart DL, McDowall RD. An analysis of the Washington Conference Report on bioanalytical method validation. J Pharm Biomed Anal. 1994 Nov 1;12(11):1337–43.
38. Causon R. Validation of chromatographic methods in biomedical analysis: viewpoint and discussion. J Chromatogr B Biomed Sci Appl. 1997 Feb 7;689(1):175–80.
39. Buick AR, Doig MV, Jeal SC, Land GS, McDowall RD. Method validation in the bioanalytical laboratory. J Pharm Biomed Anal. 1990 Jan 1;8(8-12):629–37.
40. Tur F, Tur E, Lentheric I, Mendoza P, Encabo M, Isern B, et al. Validation of an LC–MS bioanalytical method for quantification of

phytate levels in rat, dog and human plasma. J Chromatogr B. 2013 Jun 1;928:146–54.
41. Gil J, Cabrales A, Reyes O, Morera V, Betancourt L, Sánchez A, et al. Development and validation of a bioanalytical LC–MS method for the quantification of GHRP-6 in human plasma. J Pharm Biomed Anal. 2012 Feb 23;60:19–25.
42. Lang JR, Bolton S. A comprehensive method validation strategy for bioanalytical applications in the pharmaceutical industry—1. Experimental considerations. J Pharm Biomed Anal. 1991 Jan 1;9(5):357–61.
43. Causon R. Validation of chromatographic methods in biomedical analysis viewpoint and discussion. J Chromatogr B Biomed Sci Appl. 1997 Feb 7;689(1):175–80. Rozet E, Marini RD, Ziemons E, Boulanger B, Hubert P. Advances in validation, risk and uncertainty assessment of bioanalytical
44. methods. J Pharm Biomed Anal. 2011 Jun 25;55(4):848–61.
45. Boulanger B, Rozet E, Moonen F, Rudaz S, Hubert P. A risk-based analysis of the AAPS conference report on quantitative bioanalytical methods validation and implementation. J Chromatogr B. 2009 Aug 1;877(23):2235–43.
46. Pandey S, Pandey P, Tiwari G, Tiwari R. Bioanalysis in drug discovery and development. Pharm Methods. 2010 Oct 1;1(1):14–24.
47. Nováková L. Challenges in the development of bioanalytical liquid chromatography–mass spectrometry method with emphasis on fast analysis. J Chromatogr A. 2013 May 31;1292:25–37.
48. Peters FT, Drummer OH, Musshoff F. Validation of new methods. Forensic Sci Int. 2007 Jan 17;165(2-3):216–24.
49. McPolin O. Validation of analytical methods for pharmaceutical analysis. Morrisville: Lulu.com; 2009 May 1.
50. DeSilva B, Smith W, Weiner R, Kelley M, Smolec J, Lee B, et al. Recommendations for the bioanalytical method validation of ligand-binding assays to support pharmacokinetic assessments of macromolecules. Pharm Res. 2003 Nov;20:1885–900.
51. Kelley M, DeSilva B. Key elements of bioanalytical method validation for macromolecules. AAPS J. 2007 Jun;9:E156–63.
52. Braggio S, Barnaby RJ, Grossi P, Cugola M. A strategy for validation of bioanalytical methods. J Pharm Biomed Anal. 1996 Feb 1;14(4):375–88.
53. Karnes HT, Shiu G, Shah VP. Validation of bioanalytical methods. Pharm Res. 1991 Apr;8:421–6.
54. Blume H, Brendel E, Brudny-Klöppel M, Grebe S, Lausecker B, Rohde G, et al. Workshop/conference report on EMA draft guideline on validation of bioanalytical methods. Eur J Pharm Sci. 2011 Feb 14;42(3):300–5.
55. Chau CH, Rixe O, McLeod H, Figg WD. Validation of analytic methods for biomarkers used in drug development. Clin Cancer Res. 2008 Oct 1;14(19):5967–76.
56. Kalakuntla RR, Kumar KS. Bioanalytical method validation: A quality assurance auditor viewpoint. J Pharm Sci Res. 2009 Sep 1;1(3):1.
57. Dadgar D, Burnett PE, Choc MG, Gallicano K, Hooper JW. Application issues in bioanalytical method validation, sample analysis and data reporting. J Pharm Biomed Anal. 1995 Feb 1;13(2):89–97.
58. Singtoroj T, Tarning J, Annerberg A, Ashton M, Bergqvist Y, White NJ, et al. A new approach to evaluate regression models during validation of bioanalytical assays. J Pharm Biomed Anal. 2006 Apr 11;41(1):219–27.
59. Sekar V, Jayaseelan S, Subash N, Udhaya KE, Perumal P, Venkatesh RP. Bioanalytical method development and validation of letrozole by RP-HPLC method. Int J Periodont Restor Dent. 2009;1:1–7.
60. Dawes ML, Bergum JS, Schuster AE, Aubry AF. Application of a design of experiment approach in the development of a sensitive bioanalytical assay in human plasma. J Pharm Biomed Anal. 2012 Nov 1;70:401–7.
61. Goncalves D, Alves G, Soares-da-Silva P, Falcao A. Bioanalytical chromatographic methods for the determination of catechol-O-methyltransferase inhibitors in rodents and human samples: A review. Anal Chim Acta. 2012;710:17–32.
62. Naudiyal R, Kumar P, Kothiyal P. Int J Univ Pharm Bio Sci.
63. Somthane PN, Shaikh IA, Bavage NB, Gali V, Bavage SB. Bioanalytical method validation and its pharmaceutical application—a review.
64. Behnisch PA, Hosoe K, Sakai SI. Bioanalytical screening methods for dioxins and dioxin-like compounds—a review of bioassay/biomarker technology. Environ Int. 2001 Nov 1; 27(5):413–39.