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# Bioanalysis: An Important Tool in Drug Discovery

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## ABSTRACT
In past, the major reason causing new drug failure was the lack of proper analysis of pharmacokinetic data due to absence of any high advanced techniques but recent years witnessed various advancement in the field of analytical techniques and one of these is bioanalysis. Bioanalysis emerges as an important tool during drug discovery and development. This review article focuses on the various methods used during bioanalysis and its application in various fields.

***Keywords -*** *bioanalysis, sample preparation, drug extraction, validation*

## INTRODUCTION
Bringing of new drug into market consists of several steps but it is broadly categorized into two stages i.e. discovery and development (as shown in Fig.1). Drug discovery and development is the process of finding new compounds and evaluating all their properties to determine its efficacy and therapeutic use in order to select one novel chemical entity (NCE) to become a safe and efficacious drug. Strategies in the drug discovery and drug development processes are undergoing radical change. The cost of bringing a new drug to market costs around $1 billion and it may take approximately 8-10 years. [1]

To aid in a discovery program, accurate data on pharmacokinetics and metabolism must be available as early as possible as it eventually contributes to the final success or failure of the compound. The initiation of early absorption, distribution, metabolism and excretion (ADME) screening has dramatically decreased the proportion of compounds failing in clinical trials. The main aim of preclinical ADME is to eliminate weak drug candidates in the early stages of drug development which allow resources to be focused on potential drug candidates. [2] Here comes the role of bioanalysis as it plays an important role during the process of drug discovery and development and is globally accepted. [3-6] Over the past few decades, a plethora of assays has been continuously developed for NCEs to support various stages of discovery and development, including assays for important metabolites. [7-11] Bioanalytical support plays a vital role during the lead optimization stages. The major goal of the bioanalysis is to assess the over-all ADME characteristics of the new chemical entities (NCE's). Arrays of bioanalytical methods are required to completely describe the pharmacokinetic behavior in laboratory animals as well as in humans. [12] Bioanalytical tools can play a significant role for the progress in drug discovery and development. Physiologic fluids such as blood, serum, plasma, urine and tissues are analyzed to determine the absorption and disposition of a drug candidate administered to a test animal. [13] Often the concentration of the NCE's in the biological matrix changes with time, and perhaps fall below nanogram level, therefore, quantification limits for the bioanalytical methods should be much lower than those required for analytical methods.[14] Effects from the endogenous materials (such as plasma proteins) of the biological matrix and stability issues make the accurate analysis difficult. Methods developed to analyze the pharmacokinetic study samples need complete separation of the analytes from matrix component. [15]

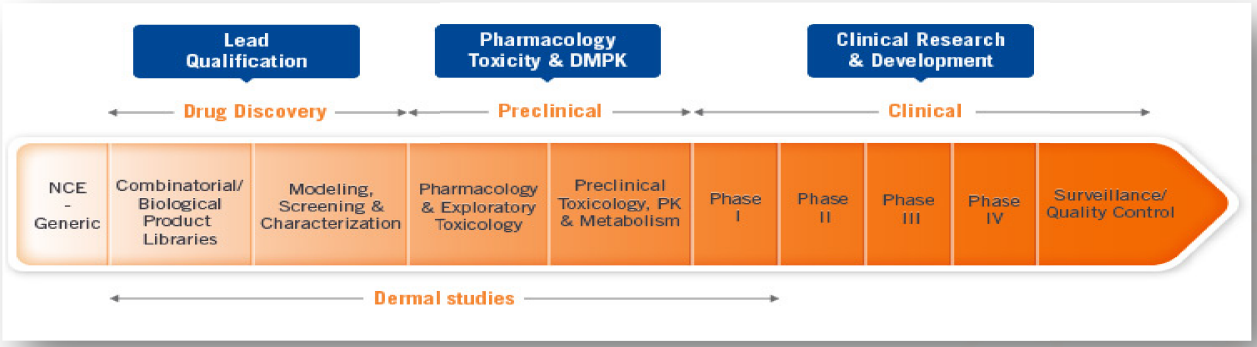


**Fig.1 Schematic flow chart of the steps involved in drug discovery**

## BIOANALYSIS:

Bioanalysis is defined as the quantitative estimation of a compound (generally drug or macro compounds such as proteins) or their metabolite in biological fluids (such as blood, plasma, serum, urine or tissue extracts).[16] Bioanalysis in the plays a critical role in pharmaceutical industry as it provide a quantitative measure of the active drug and/or its metabolite(s) in various research field such as pharmacokinetics and/ or preclinical pharmacokinetics, toxicokinetics, bioequivalence and exposure–response (pharmacokinetics/pharmacodynamics studies). Bioanalysis also applies to drugs used for illicit purposes, forensic investigations, anti-doping testing in sports, and environmental concerns. Bioanalytical assays to accurately and reliably determine these drugs at lower concentrations. [17]

Bioanalysis broadly consists of two methods which includes following:

- Preparation of samples from biological matrixes( such as blood, serum, urine etc)
- Detection and analysis of compound/drug or its metabolities.

## SAMPLE PREPARATION:

Sample preparation is a technique which is mostly used to remove any impurity or any foreign substances before analysis and/or to concentrate a sample to improve its detection. When samples are extracted from various biological fluids such as plasma, serum or urine, this technique is known as bioanalytical sample preparation.vaious techniques employed during extraction of drug are illustrated in fig2.

The qualitative estimation of drug and or its metabolities concentrations in biological fluids yields the data which are used to understand the time course of drug action, or PK, in animals and man and is an essential component of the drug discovery and development process. [18]

The two main reasons of sample preparations are: [19]

a) To remove any impurities or other endogenous compounds other than desired sample from the analyte as possible.
b) Sample preparation helps in improving the sensitivity of the systems and obtaining clear or sharp response, by enriching the sample with respect to the analyte,

### Objectives of sample preparation:

Two of the major goals of any sample pretreatment procedure are

- Quantitative recovery

- A minimum number of steps.

Successful sample preparation has a following objective:

- Free from interfering matrix elements

- At a concentration appropriate for detection and measurement

### Detection of the compound:

The detector of choice is a mass spectrometer.[20] Currently, the principle technique used in quantitative bioanalysis is high performance liquid chromatography coupled with tandem mass

spectrometry (HPLC-MS/MS) using either electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) techniques.[21] The triple quadrupole (QqQ) mass spectrometer (MS), when operated in the selected reaction monitoring (SRM) mode, offers a unique combination of sensitivity, specificity and dynamic range. Consequently, the QqQ MS has become the instrument of choice for quantitation within the pharmaceutical industry.

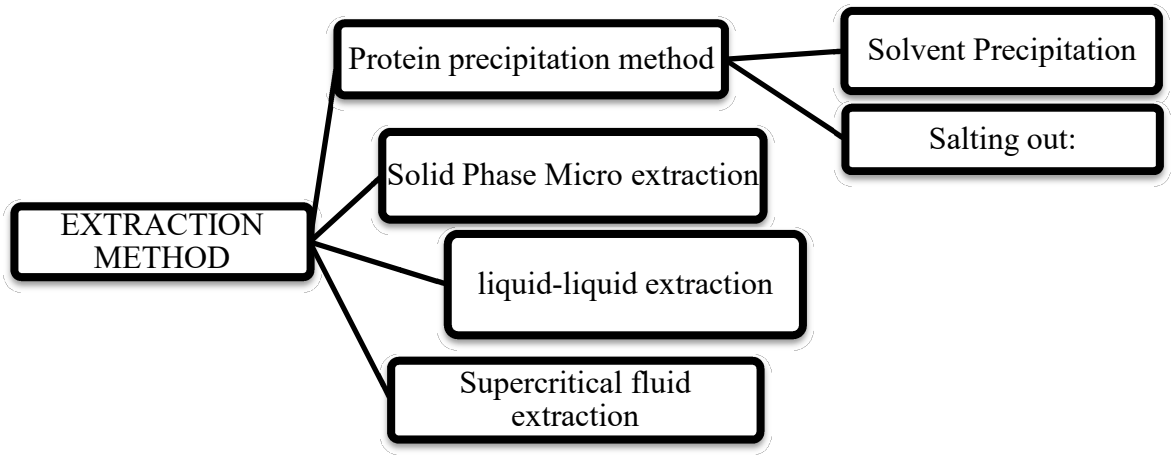


**Fig.2 Various Extraction Method used in the sample preparation.**

**Bioanalytical techniques:[22]**

Following are two different techniques which are commonly used in bioanalytical studies i.e.:

- **Hyphenated techniques:**
  - ❖ LC–MS (liquid chromatography–mass spectrometry)
  - ❖ GC–MS (gas chromatography–mass spectrometry)
  - ❖ CE–MS (capillary electrophoresis–mass spectrometry)
- **Chromatographic methods**
  - ❖ HPLC(high performance liquid chromatography)
  - ❖ Gas chromatography

**LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY (LC-MS/MS):**

Bioanalytical liquid chromatography-mass spectrometry is a technique that uses liquid chromatography with the mass spectrometry. LC-MS is commonly used in laboratories for the quantitative and qualitative analysis of drug substances, drug products and biological samples. Through LC-MS biological samples are determined throughout all phases of method development of a drug in research and quality control.

**Method Development:**

Method of analysis are being routinely developed, improved, validated, collaboratively studied and applied. Chromatographic separations are mainly required which depend on the samples to be analyzed. The chromatographic procedure is important for the systemic approach to LC-MS/MS method development. In most cases as desired separation can be achieved easily with only a few experiments. In other cases a considerable amount of experimentation may be needed.

**Procedure for Method Development:**

Collect the physicochemical properties of drug molecules from the literature.

- Determine solubility profile
- MS scanning and optimization
- Mobile phase selection
- Selection of extraction method and optimization
- Selection of chromatographic method (based on solubility study, retention of compound)

**Steps in LC-MS/MS Method Development**

Proper knowledge about the sample is necessary for an effective method development. Some information regarding the analyte is necessary like [23]

- Number of compounds present
- Molecular weights of compound
- Sample Solubility
- Drug Stability
- Concentration range of compounds in samples of interest

**BIOANALYTICAL METHOD VALIDATION**:

Quantitative estimation of drug concentration in biological matrices (such as serum, plasma, blood, urine, and saliva) is an integral part of drug/biological product development. Bioanalysis play an important role in pharmaceutical industry as it helps in the quantitative estimation of low molecular weight drugs as well as high molecular weight such as proteins analysis. The result obtained from various animal toxicology studies, pharmacokinetic studies as well as preclinical pk study including bioavailability and bioequivalence study are essential to support critical decisions supporting the safety and efficacy of a medicinal drug substance or product. Therefore validation ensures that the given bioanalytical produce sensitive and reproducible data.

Types of bioanalytical validation are:

- **Full Validation**
- **Partial Validation**
- **Cross-Validation**

Bioanalytical method validation includes parameters such as Selectivity, Sensitivity, Calibration/Quality control standards, Accuracy, Precision, Recovery and Calibration curve/Linearity and stability.

- **SELECTIVITY:**

It is defined as the ability of biological method to differentiate desired analyte from other analytes or impurities present in samples.[24,25] To determine efficiency of selectivity, the response of an analyte in the biological sample at the lower limit of quantification (LLOQ) is compared with blank matrix sample. To obtain reproducible and accurate result it is recommended to take blank matrix from at least six different sources and compare it with the spiked LLOQ in the matrix.

- **SENSITIVITY:**[26]

The lowest standard (LOQ) should be accepted as the limit of quantification of the method. This test should be performed to prove the reproducibility for samples at limit of quantification level. Sensitivity should be evaluated by using at least 5 replicates of the samples at the limit of quantification. The sample used for this evaluation should be independent of calibration standards. The accepted limits for LOQ should be ±20% for accuracy and ≤20% for precision. In addition, signal to noise ratio(S/N) will also be calculated to evaluate the noise level. A minimum recommended S/N ration could be 5:1, however acceptance criteria for the signal to noise ratio depend on the individual method.

- **Calibration/Quality control standards:**

Calibration standards can be defined as a biological matrix to which a known amount of analyte has been added or spiked. Calibration standards are used to construct calibration curves, from which the concentrations of analytes in QCs and in unknown study samples are determined. In the same way, quality control standard (QC) is also a spiked sample used to monitor the performance of a bionalytical method and to assess the integrity and validity of the results of the unknown samples analyzed in an individual batch. [27] Calibration/quality control standards preparation plays an important role in the outcome of the method performance. For the preparation of master stocks, an adequately characterized standard reference material must be available, from which the other dilutions may be prepared which will be used for the spiking of calibration/QC standards.[28] Documentation of the characterization (certificate of analysis) must be available to the bionalytical laboratory when this material is used for method validation. Procedure defined in the analytical method should be followed for the preparation of calibration/QC standards.

- **Accuracy, Precision, and Recovery:**[29]

The accuracy of an analytical method describes the closeness of mean test results obtained by the method to the true value (concentration) of the analyte. Accuracy is determined by replicate analysis of samples containing known amounts of the analyte. Accuracy should be measured using a minimum of five determinations per concentration. A minimum of three concentrations in the range of expected

concentrations is recommended. The mean value should be within 15% of the actual value except at LLOQ, where it should not deviate by more than 20%.

The ***precision*** of an analytical method describes the closeness of individual measures of an analyte when the procedure is applied repeatedly to multiple aliquots of a single homogeneous volume of biological matrix. Precision should be measured using a minimum of five determinations per concentration. A minimum of three concentrations in the range of expected concentrations is recommended. The precision determined at each concentration level should not exceed 15% of the coefficient of variation (CV) except for the LLOQ, where it should not exceed 20% of the CV. Precision is further subdivided into within-run, intra-batch precision or repeatability, which assesses precision during a single analytical run, and between-run, inter batch precision or repeatability, which measures precision with time, and may involve different analysts, equipment, reagents, and laboratories.

The ***recovery*** of an analyte in an assay is the detector response obtained from an amount of the analyte added to and extracted from the biological matrix, compared to the detector response obtained for the true concentration of the pure authentic standard. Recovery pertains to the extraction efficiency of an analytical method within the limits of variability. Recovery of the analyte need not be 100%, but the extent of recovery of an analyte and of the internal standard should be consistent, precise, and reproducible. Recovery experiments should be performed by comparing the analytical results for extracted samples at three concentrations (low, medium, and high) with unextracted standards that represent 100% recovery.

- **Calibration curve/linearity:**[30]

The linearity of an analytical procedure is its ability to obtain test results that are directly proportional to the concentration of analyte in the sample. Test results should be evaluated by appropriate statistical methods, by calculation of a regression line like by the method of least squares. Correlation coefficient, y-intercept, slope of the regression line and residual sum of squares for which a minimum of five concentrations are recommended.

- **Stability:**

It is the chemical stability of an analyte in a given matrix under specific conditions for given time intervals. [31] The aim of a stability test is to detect any degradation of the analyte(s) of interest during the entire period of sample collection, processing, storing, preparing, and analysis. [32]All but long-term stability studies can be performed during the validation of the analytical method. Long-term stability studies might not be complete for several years after clinical trials begin. The condition under which the stability is determined is largely dependent on the nature of the analyte, the biological matrix, and the anticipated time period of storage (before analysis).

**APPLICATION OF BIOANALYSIS:**

- Bioanalysis assess in the study of in-vivo pharmacokinetic studies in animals at preclinical stages and humans at clinical stages.
- Bioanalysis play an important role in validating methods used during the analysis of sample in biological fluids.
- It helps in the screening of active new molecules by providing results obtain from ADME pharmacokinetic studies (as shown in fig. 3).

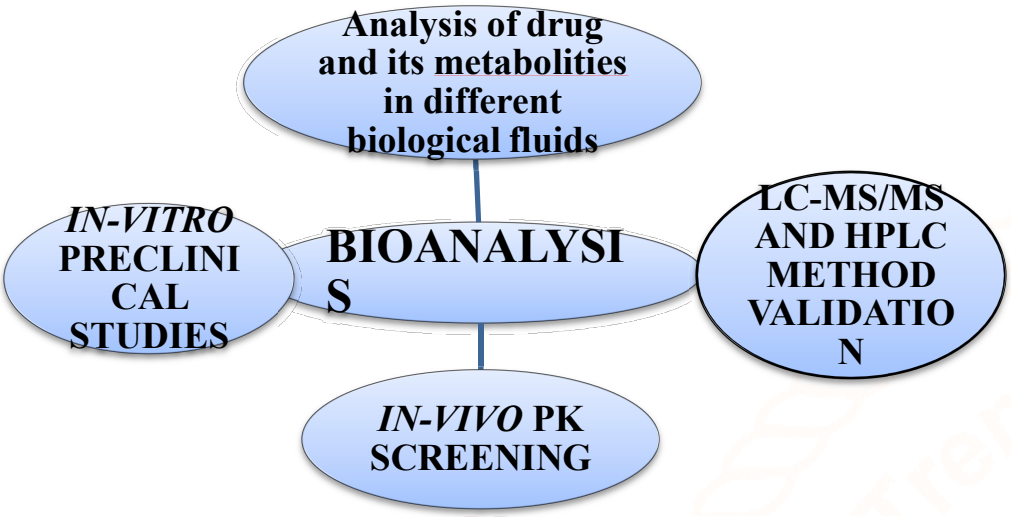


**Fig.3. Application of bioanalysis in various fields.**

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