1. INTRODUCTION

INTRODUCTION TO BIOAVAILABILITY & BIOEQUIVALENCE

In generic market concept of bioavailability and bioequivalence is important role in drug research and development. The sponsors file an Abbreviated New Drug Application (ANDA) to the regulatory agencies for generic approval, when a brand-name drug product is going off patent.\(^{(1)}\) The approval of generic drug products does not go through as lengthy and costly a clinical development as that of new drug product. As a result, generic drug products are relatively cheap compared to brand name drugs. It has become a public interest whether brand name drugs work as well as generic drug in term of quality and therapeutic effect \(^{(2-4)}\).

Bioequivalence trials are essential to guarantee the safety and efficacy of the generic drug products. \(^{(5)}\)

The evaluation of these studies might compare:

i. One type of dosage form with another, e.g. intravenous versus tablet dosage form

ii. Regular tablet with sustained release tablet.

iii. Two (or more) dosage forms made by two (or more) different manufacturers, e.g. innovator versus generic.

**Bioavailability**

“Bioavailability is defined as the measurement of the extent of a therapeutically active drug that reaches the systemic circulation and is available at the site of action \(^{(6)}\)."
Methods for Assessing Bioavailability:

i) *In-vivo methods:*

ii) Blood level studies:

iii) Urinary Excretion Data:

iv) Single dose Versus Multiple dose:

**Methods for assessing bioavailability and bioequivalence**

- Peak plasma drug concentration ($C_{max}$),
- Time for peak plasma (blood) concentration ($T_{max}$)
- Plasma drug concentration and Urinary drug excretion
- Area under the plasma drug concentration–time curve (AUC)
- Cumulative amount of drug excreted in the urine ($D_u$)
- Rate of drug excretion in the urine ($D_u/dt$)
- Time for maximum urinary excretion (t)
- Acute pharmacodynamic effect
- Maximum pharmacodynamic effect ($E_{max}$)
- Time for maximum pharmacodynamic effect
- Area under the pharmacodynamic effect–time curve
- Clinical observations
- In-vitro studies
- Drug dissolution

**Types of Bioavailability studies:**

There are two types
**Absolute bioavailability** is the measurement of a medication once it passes through the gut and is released into the circulatory system.

**Relative bioavailability** is a term used to compare different formulations of the same medication, for example brand name versus generic name.

**BIOEQUIVALENCE**

Bioequivalence is a comparison of the bioavailability of two or more drug products. Thus, two products or formulations containing the same active ingredient are bioequivalent if their rates and extents of absorption are the same (7).

The two products to be "therapeutic equivalents" if they each meet the following criteria

1. They are bioequivalent (demonstrated either by a bioavailability measurement or an *in vitro* standard), pharmaceutical equivalents, compliance with compendial standards for strength, quality, purity and identity, adequately labeled, and they have been manufactured in compliance with Good Manufacturing Practices as established by the FDA.

A product can be either bio-equivalent or bio-in equivalent.

**PHARMACOKINETIC MEASUREMENTS:**

Indirect (e.g., $C_{\text{max}}$, $T_{\text{max}}$, mean absorption time, mean residence time, $C_{\text{max}}$ normalized to AUC) and direct (e.g., rate constant, rate profile)

The following pharmacokinetic parameters are required for submission

- Subject, period, sequence, treatment
- Plasma concentrations and time points
• \( C_{\text{max}} \), \( \text{AUC}_{0-t} \), \( \text{AUC}_{0-\infty} \), \( T_{\text{max}} \), \( Z \), and \( t_{1/2} \).

• Intrasubject, Intersubject, and/or total variability, if available

• \( C_{\text{av}} \) (average concentration during a dosing interval),

• \( C_{\text{min}} \) (concentration at the end of a dosing interval),

• Degree of fluctuation \( [(C_{\text{max}}-C_{\text{min}})/C_{\text{av}}] \), and

• Swing \( [(C_{\text{max}}-C_{\text{min}})/C_{\text{min}}] \) if steady-state studies are employed.

The following statistical information required for \( \text{AUC}_{0-t} \), \( \text{AUC}_{0-\infty} \), and \( C_{\text{max}} \).

• Arithmetic mean

• Geometric mean

• Ratio of means

• Confidence intervals (limit of 80-125, the value should be at least 80.00 and not more than 125.00).

**Bio Pharmaceutical Analysis**

Bio-Analytical chemistry is the qualitative and quantitative analysis of drug substances in biological fluids (mainly plasma and urine) or tissue. It plays a significant role in the evaluation and interpretation of pharmacokinetic data (8). The main analytical phases comprise method development, method validation and sample analysis (method application).

**Need for pharmaceutical Analysis**

• Clinical Pharmacokinetic Studies.

• New Drug Development.

• Research in Pharmaceutical Sciences.
Analysis of Drugs from various samples

Blood, urine, feces, saliva, breath, and tissue.

Extraction Procedures for Drugs and Metabolites from Biological Samples

After pre treating biological material, the next step is usually the extraction of the drugs from the biological matrix.

Protein Precipitation or Denaturation

Protein denaturation procedures include the use of tungstic acid, ammonium sulfate, heat, alcohol, trichloroacetic acid and perchloric acid.

Methanol and acetonitrile frequently have been used as protein denaturants of biological samples (9).

Liquid-Liquid Extraction

It is most widely used technique because

- The technique is simple, rapid, and has a relatively small cost factor per sample.
- It is depended on the partition or distribution coefficient of the immiscible liquid phases (9).

Solid Phase Extraction

Factors governing the adsorption and elution of drugs from the resin column include solvent polarity, flow rate of the solvent through the column, and the degree of contact the solvent has with the resin beads.

In the adsorption process, the hydrophobic portion of the solute that has little affinity for the water phase is preferentially adsorbed on
the resin surface while the hydrophilic portion of the solute remains in the aqueous phase (9).

Alteration in the lipophilic/hydrophilic balance within the solute or solvent mix and not within the resin affects adsorption of the solute.

**Method development and validation**

Method development involves evaluation and optimization of the various stages of chromatographic separation, sample preparation, detection and quantification (10).

Prior to method development of selected drug it is important for extensive literature survey regarding:

1. Choice of the instrument which is suitable for the analyte such as UV, NMR, HPLC, GC, LC-MS-MS
2. Choice of the chromatographic conditions such as Column, Mobile Phase, Flowrate, injection volume, Autosampler conditions
3. Choice of extraction method
5. Choice of regression methods.

The method development and establishment for a analytical method include determination of selectivity, accuracy, precision, recovery, calibration curve, and stability of analyte in spiked samples (11).

**Selectivity**

It is the ability of an analytical method to differentiate and quantify the analyte in the presence of other components in the sample.
Accuracy

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.

Precision

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.

i. Within-run or intra-batch precision: ii. Between-run or inter-batch precision:

Recovery

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

Calibration/Standard Curve

A calibration (standard) curve is the relationship between instrument response and known concentrations of the analyte. It should be generated for each analyte in the sample. A sufficient number of standards should be used to adequately define the relationship between concentration and response (12).

A calibration curve should consist of

i. A blank sample (matrix sample processed without internal standard)
ii. A zero sample (matrix sample processed with internal standard)

iii. Six to eight non-zero samples covering the expected range, including LLOQ.

**Calibration Curve/Standard Curve-Concentration-Response**

The simplest model that adequately describes the concentration-response relationship should be used. Selection of weighting and use of a complex regression equation should be justified. The following conditions should be met in developing a calibration curve:

* Deviation of the LLOQ was 20% from nominal concentration.
* Deviation of standards other than LLOQ were 15% from nominal concentration.
* At least four out of six non-zero standards should meet the above criteria, including the LLOQ and the calibration standard at the highest concentration.
* The standards when excluded should not change the model used.

**Stability in a Biological Fluid**

Stability procedures should evaluate the stability of the analytes during sample collection and handling, after long-term (frozen at the intended storage temperature) and short-term (bench top, room temperature) storage, and after going through freeze and thaw cycles and the analytical process (13).

**Freeze and Thaw Stability**

Analyte stability should be determined after three freeze and thaw cycles. When completely thawed, the samples should be refrozen for 12 to 24 hours under the same conditions. The freeze-thaw cycle should be
repeated two more times, and then analyzed on the third cycle. If an analyte is unstable at the intended storage temperature, the stability sample should be frozen at -70°C during the three freeze and thaw cycles.

**Short-Term Temperature Stability**

Three aliquots of each of the low and high concentrations should be thawed at room temperature and kept at this temperature from 4 to 24°C.

**Long-Term Stability**

The storage time in a long-term stability evaluation should exceed the duration between the date of first sample collection and the date of last sample analysis.

**Stock Solution Stability**

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

**Post-preparative Stability/Autosampler Stability**

The stability of processed samples, including the resident time in the auto sampler, should be determined.

Validation is categorized into full validation, partial validation, and cross validation (14).

**Full Validation**

Full validation is important when developing and implementing a bioanalytical method for the first time.
**Partial Validation** (14).

Partial validations are modifications of already validated analytical methods. Typical bioanalytical method changes that fall into this category include, but are not limited to:

- Change in analytical methodology (e.g., change in detection systems).
- Change in anticoagulant in harvesting biological fluid.
- Bioanalytical method transfers between laboratories or analysts.
- Change in matrix within species (e.g., human plasma to human urine).
- Change in species within matrix (e.g., rat plasma to mouse plasma).
- Change in sample processing procedures.
- Change in relevant concentration range.
- Changes in instruments and/or software platforms.
- Limited sample volume (e.g., pediatric study).
- Selectivity demonstration of an analyte in the presence of concomitant medications.
- Rare matrices.
- Selectivity demonstration of an analyte in the presence of specific metabolites.
**Cross-Validation** (14).

Cross-validation is a comparison of validation parameters when two or more analytical methods are used to generate data within the same study or across different studies.

**Application of Validated Method to Routine Drug Analysis**

Assay and analysis of all samples in a biological matrix should be completed within the time period for which stability data are available (15,16).