2.0 REVIEW OF LITERATURE

The rising cost of health care has motivated governments around the world to examine methods of decreasing costs without compromising health care services. Prescription drug spending is increasing at a rate of over 10% per year. In 2001, expenditures for prescription drugs in the United States were $141 billion that rose to 188.5 billion $ in 2004. One of the methods to reduce costs, employed by many countries is the passing of regulations that encourage the use of generic drugs. Generic drugs are typically less expensive than brand-name drugs, and prices for generics have historically increased less than those for brand-name drugs. Many nations throughout the world have come to rely on low-cost, good-quality multi-source (generic) pharmaceutical products as means of controlling rising lower healthcare costs without sacrificing important public health goals.

A generic drug is the chemical equivalent of a drug that has an expired/invalidated patent. In the United States, the Food and Drug Administration (FDA) requires that all drugs, whether a brand-name drug or a generic drug, meet standards of safety, strength, purity and effectiveness. The FDA sets up guidelines and requires strict testing in order to determine which drugs act the same way in the body. For a drug to be marketed under a generic label, the manufacturer must comply with FDA standards. In order to ensure its safety and effectiveness, a generic drug undergoes intensive testing. Generic drugs that have been tested and approved by the FDA to be therapeutically equivalent to brand name drugs are published in a guide that is updated monthly and is found in most pharmacies. The guide lists drugs classified as therapeutically equivalent to each other, and gives them an "A" rating. If the FDA does not consider a drug therapeutically equivalent, it is given a "B" rating. Most pharmacies purchase drugs with an "A" rating to dispense as generic.

2.1 Historical Perspective of Bioequivalence Studies

Law often becomes a necessary control mechanism when people could be exploited and there are large potential financial gains for businesses choosing to exploit. The society, by law, has removed much of the decisions making about new drug products from the manufacturers, investigators, and physicians and vested it in the government (the Drug Regulatory Agencies). The regulations require the Regulatory Agencies to assess safety, efficacy and quality of all new drug formulations, before they are marketed. The fundamental mission of the Drug Regulatory Agencies is protection of the consumers. The historical milestones of drug law are summarized in Table 2.1.
Table 2.1: List of major legislations, regulations and other milestones affecting drug development and marketing in the United States and other countries.126

<table>
<thead>
<tr>
<th>Year</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>1902</td>
<td>Biologics Control Act</td>
</tr>
<tr>
<td>1906</td>
<td>Pure Food and Drugs Act</td>
</tr>
<tr>
<td>1912</td>
<td>Shirley Amendment to Pure Food and Drugs Act</td>
</tr>
<tr>
<td>1938</td>
<td>Elixir Sulfanilamide Disaster. FDA control over safety of new drugs</td>
</tr>
<tr>
<td>1948</td>
<td>Miller Amendment</td>
</tr>
<tr>
<td>1951</td>
<td>Durham-Humphrey Amendments</td>
</tr>
<tr>
<td>1952</td>
<td>Hench: Brand substitution case report</td>
</tr>
<tr>
<td>1962</td>
<td>Thalidomide disaster in Europe FDA; Control over both safety and efficacy of drugs-Kefauver-Harris amendment</td>
</tr>
<tr>
<td>1963</td>
<td>Initial Good Manufacturing Practices (GMP) regulations</td>
</tr>
<tr>
<td>1974</td>
<td>World Health organization, recommendations for conduct of bioavailability studies</td>
</tr>
<tr>
<td>1974</td>
<td>Dissolution test adopted as standard for in vitro comparison of bioavailability in UK</td>
</tr>
<tr>
<td>1977</td>
<td>US FDA regulations for approval of BE. The $\pm$ 20% rule with p&lt;0.05</td>
</tr>
<tr>
<td>1983</td>
<td>Orphan drug act</td>
</tr>
<tr>
<td>1984</td>
<td>ANDA for generics approval-Waxman-Hatch act (Drug price competition and patent term restoration act)</td>
</tr>
<tr>
<td>1985</td>
<td>New 80-125% for CI law for approval of generic products</td>
</tr>
<tr>
<td>1987</td>
<td>Standard 2x2 crossover test design for BE studies</td>
</tr>
<tr>
<td>1989</td>
<td>Generics scandal in USA. Concern for adequate documentation and validation of BE studies</td>
</tr>
<tr>
<td>1992</td>
<td>90-111% CI for narrow therapeutic index drugs: Canadian FDA</td>
</tr>
<tr>
<td>1995</td>
<td>EEC: 70-143% limit for C_{max} only for drug with wide safety margin</td>
</tr>
<tr>
<td>2005</td>
<td>Bioequivalence guidelines–India</td>
</tr>
</tbody>
</table>

2.2 BE for first entry products

BE studies may be useful during drug development and registration for a first entry product during the Investigational New Drug (IND) or New Drug Application (NDA) period to
establish links between (i) early and late clinical trial formulations (ii) formulations used in clinical trial and stability studies, if different (iii) Clinical trial formulations and to be marketed drug products (iv) other comparisons as appropriate. In each comparison, the new formulation or new method of manufacture is the test product and the prior formulation or method of manufacture is the reference product.

2.3 BE for interchangeable multi-source products

BE studies are a critical component of Abbreviated New Drug Applications (ANDA). The purpose of these studies is to compare relative BA measures between a pharmaceutically equivalent multi-source test product and the corresponding reference pioneer product. The pioneer product is termed as reference listed drug (RLD). Together with the determination of pharmaceutical equivalence, demonstrating BE allows a regulatory conclusion of therapeutic equivalence and interchangeability between the test and reference product.\(^{129}\)

2.4 BE for post approval changes

Generally specifications are adequate to assure product quality on the assumption that no important change occurs post-approval. In the presence of major changes in components and composition, and/or method of manufacture of a drug product after approval, BE may need to be re-demonstrated. For approved first-entry products, the drug product after the change should be compared to the drug product before change. For approved interchangeable multi-source products, the drug product after the change should be compared to the reference listed drug.

21 CFR 320.1 defines bioavailability (BA) as “the rate and extent to which the active ingredient or active moiety is absorbed from a drug product and becomes available at the site of action. For drug products that are not intended to be absorbed into the bloodstream, bioavailability may be assessed by measurements intended to reflect the rate and extent to which the active ingredient or active moiety becomes available at the site of action”.

Bioequivalence (BE) is a relative term. Bioequivalence means the absence of a significant difference in the rate and extent to which the active ingredient or active moiety in pharmaceutical equivalents or pharmaceutical alternatives becomes available at the site of drug action when administered at the same molar dose under similar conditions in an appropriately designed study. When there is an intentional difference in rate (e.g., in certain controlled release dosage forms), certain pharmaceutical equivalents or alternatives may be
considered bioequivalent if there is no significant difference in the extent to which the active ingredient or moiety from each product becomes available at the site of drug action. This applies only if the difference in the rate at which the active ingredient or moiety becomes available at the site of drug action is intentional and is reflected in the proposed labeling, is not essential to the attainment of effective body drug concentrations on chronic use, and is considered medically insignificant for the drug (21 CFR 320.1).

A new product is said to be suprabioavailable when it displays larger bioavailability than the approved comparative product. For these suprabioavailable products, lower dosage strength should be reformulated to assure therapeutic equivalence. Finally a comparative bioavailability study of the reformulated new product with the old approved product is required for submission.

2.5 Types of Bioavailability

Bioavailability can be classified into four different types, depending on the purpose of the study and scientific questions to be solved.

2.5.1 Absolute Bioavailability

Absolute bioavailability is the ratio of the total area under the blood level - time curve upon extra vascular route of administration to the area under the blood level - time curve upon intravenous administration, corrected for the difference in the dose size.

\[
\text{Absolute bioavailability} = \frac{\text{AUC}_{\text{extravascular}} \times \text{dose}_{\text{i.v.}}}{\text{AUC}_{\text{i.v.}} \times \text{dose}_{\text{extravascular}}}
\]

2.5.2 Relative Bioavailability

The relative bioavailability is the extent (EBA) and rate (RBA) of the bioavailability of a drug from two or more different dosage forms given by the same route of administration. For determination of EBA or RBA blood level or urinary excretion data upon single or multiple dosing can be used. According to the FDA regulation the standard used in this procedure is an approved marketed drug product, a solution of the drug or suspension of the micronized drug.

\[
\text{Relative bioavailability} = \frac{\text{AUC of A}}{\text{AUC of B}}
\]

Where B is the reference standard.
2.5.3 Bioavailability in presence of first-pass effect
Drugs showing a first-pass effect may result in considerably lower blood level time curves. Even though the entire parent drug was absorbed from the site of administration, it did not reach systemic circulation in unchanged form.
The fraction of a peroral (po) or in part, rectal dose reaching systemic circulation $F$, under the assumption of otherwise linear kinetics can be described by following equation:

$$F = 1 - \frac{\text{Dose}_{iv} \times f_m}{\text{LBF} \times \text{AUC}_{iv} \times 60 \times \lambda}$$

$f_m$ - fraction of drug metabolised in liver
LBF - liver blood flow
$\lambda$ - ratio of the concentration of the drug in whole blood to that in plasma

2.5.4 Relative optimal bioavailability
This term was suggested for optimizing extent and rate of bioavailability for a drug product during the development phase. For determination of $\text{EBA}_{\text{rel. opt.}}$, the active drug is administered in aqueous solution without the addition of any further excipient by the same route which is intended for the drug product under development.

$$\text{EBA}_{\text{rel. opt.}} = \frac{\text{AUC} \ (\text{drug + vehicle; granules; tablets})}{\text{AUC}_{\text{solution}}} \times 100$$

2.6 Bioavailability Measurement
There are several direct and indirect methods for the measurement of bioavailability in humans. The selection of method depends on the purpose of the study, analytical method and nature of the drug product. The methods useful in quantitative evaluation of bioavailability can be broadly divided into two categories: (a) Pharmacokinetic methods (b) Pharmacodynamic methods

2.6.1 Pharmacokinetic Methods
These are very widely used and are based on the assumption that the pharmacokinetic profile reflects the therapeutic effectiveness of a drug. Thus, these are indirect methods. The two major pharmacokinetic methods are:
2.6.1.1 Plasma level-time studies

Unless determination of plasma drug concentration is difficult or impossible, it is the most reliable method and method of choice in comparison to urine data. This method is based on the assumption that two dosage forms that exhibit superimposable plasma level-time profiles in a group of subjects should result in identical therapeutic activity. The three parameters of plasma level-time studies, which are considered important for determining bioavailability, are:

1. $C_{\text{max}}$: The peak plasma concentration that gives an indication whether the drug is sufficiently absorbed systemically to provide a therapeutic response.

2. $T_{\text{max}}$: The time of peak plasma concentration corresponds to the time required to reach maximum drug concentration after drug administration. At $t_{\text{max}}$, absorption is maximized and the rate of drug absorption equals the rate of drug elimination. When comparing drug products, $t_{\text{max}}$ can be used as an approximate indication of the drug absorption rate.

3. AUC: The area under the plasma level-time curve that gives a measure of the extent of absorption or the amount of drug that reaches the systemic circulation.

The extent of bioavailability can be determined by equation:

$$F = \frac{\text{AUC}_{\text{oral}} \cdot D_{\text{iv}}}{\text{AUC}_{\text{iv}} \cdot D_{\text{oral}}}$$

2.6.1.2 Urinary excretion studies

This method of assessing bioavailability is based on the principle that the urinary excretion of unchanged drug is directly proportional to the plasma concentration of drug. This method is particularly useful for drugs extensively excreted unchanged in the urine. The method involves collection of urine at regular intervals for a time span equal to 7-10 biological half-lives, analysis of unchanged drug in the collected sample and determination of the amount of drug excreted in each interval and cumulative amount excreted. The three major parameters examined in urinary excretion data obtained with a single dose study are:

1. $(dxu/dt)_{\text{max}}$: The maximum urinary excretion rate, is obtained from the peak of plot between rate of excretion versus midpoint time of urine collection period. It is
analogous to $C_{max}$ derived from plasma level studies since the rate of appearance of drug in the urine is proportional to its concentration in systemic circulation.

2. $(t_u)_{max}$: The time for maximum excretion rate, is analogous to the $t_{max}$ of plasma level data. Its value decreases as the absorption rate increases.

3. $X_u$: The cumulative amount of drug excreted in the urine, is related to the AUC of plasma level data and increases as the extent of absorption increases.

The extent of bioavailability can be calculated using equation:

$$F = \frac{(X_u)_{oral} \ D_{iv}}{(X_u)_{iv} \ D_{oral}}$$

2.6.2 Pharmacodynamic Methods

These methods are complimentary to pharmacokinetic approaches and involve direct measurement of drug effect on a physiologic process as a function of time. The two pharmacodynamic methods involve determination of bioavailability from: (a) Acute pharmacologic response (b) Therapeutic response

2.6.2.1 Acute pharmacologic response

In some cases quantitative measurement of a drug is difficult, inaccurate or non reproducible. In such cases an acute pharmacologic effect such as effect on pupil diameter, heart rate or blood pressure can be a useful index of drug bioavailability. Bioavailability can be determined by construction of pharmacologic effect-time curve as well as dose-response graphs. The method requires measurement of responses for at least 3 biological half-lives of drug in order to obtain a good estimate of AUC.

2.6.2.2 Therapeutic response

Theoretically the most definite, this method is based on observing the clinical response to a drug formulation given to patients suffering from disease for which it is intended to be used. Bioequivalent drug products should have the same systemic drug bioavailability and therefore the same predictable drug response. However, variable clinical responses among individuals that are unrelated to bioavailability might be due to differences in the
pharmacodynamics of the drug. Various factors affecting pharmacodynamic drug behaviour may include age, drug tolerance, drug interactions and unknown pathophysiologic factors.

2.6.3 *In vitro* Methods

Under certain circumstances, product quality BA and BE can be documented using *in vitro* approaches. For highly soluble, highly permeable, rapidly dissolving, orally administered drug products, documentation of BE using an *in vitro* approach (dissolution studies) is appropriate based on the biopharmaceutics classification system (BCS). The preferred dissolution apparatus is USP apparatus I (basket) or II (paddle), used at compendially recognized rotation speeds (e.g., 100 rpm for the basket and 50-75 rpm for the paddle). In other cases, the dissolution properties of some ER formulations may be determined with USP apparatus III (reciprocating cylinder) or IV (flow through cell).

2.7 Factors Affecting Bioavailability

The various factors affecting bioavailability of drugs can be classified as shown in table 2.2.

**Table 2.2: Factors affecting absorption of a drug from its dosage form**

<p>| FACTORS AFFECTING BIOAVAILABILITY OF DRUGS |</p>
<table>
<thead>
<tr>
<th>Pharmaceutical Factors</th>
<th>Patient Related</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physicochemical properties of drug substances</td>
<td>Dosage form related factors</td>
</tr>
<tr>
<td>Drug solubility and dissolution rate</td>
<td>Disintegration time</td>
</tr>
<tr>
<td>Particle size and effective surface area</td>
<td>Dissolution time</td>
</tr>
<tr>
<td>Polymorphism and amorphism</td>
<td>Manufacturing variables</td>
</tr>
<tr>
<td>Hydrates / solvates</td>
<td>Pharmaceutical ingredients</td>
</tr>
<tr>
<td>Salt form of the drug</td>
<td>Nature and type of dosage form</td>
</tr>
<tr>
<td>Lipophilicity of the drug</td>
<td>Product age and storage conditions</td>
</tr>
<tr>
<td>pKa of the drug and pH</td>
<td>Gastrointestinal contents:</td>
</tr>
<tr>
<td></td>
<td>• Other drugs</td>
</tr>
<tr>
<td></td>
<td>• Food</td>
</tr>
<tr>
<td></td>
<td>• Fluids</td>
</tr>
<tr>
<td></td>
<td>• Other normal GI contents</td>
</tr>
</tbody>
</table>
### Drug stability

Presystemic metabolism by:
- Luminal enzymes
- Gut wall enzymes
- Bacterial enzymes
- Hepatic enzymes

#### 2.8 Population and Individual Bioequivalence (PBE & IBE)

The bioequivalence study in current use, so called average bioequivalence approach, judges bioequivalence between the test formulation and reference formulation by verifying that the confidence interval for the ratio of average bioavailability values of the 2 formulations is in a given acceptance range. However, the average bioequivalence approach has been indicated to be insufficient to warrant bioequivalence of the test formulation and the reference formulation, since it compares the average bioavailability values of the test and the reference formulations and does not consider differences in variance of test and reference formulation.\(^8^4\) Due to these concerns raised over the years, on the use of average bioequivalence for evaluation of comparability between formulations, scientists from academia, industry and regulatory agencies, propose the use of concepts of individual and population bioequivalence.\(^2^7\) The FDA also has proposed replacing the 1992 average bioequivalence (ABE) approach with population and individual bioequivalence (PBE and IBE).\(^1^3^0\)

#### 2.8.1 Individual bioequivalence (IBE)

In the IBE criterion, replicate designs are required, in which at least the R, and commonly both R and T drug products, are each administered on two separate occasions. The individual criteria may be utilized for equivalence questions when some change occurs in a stable dosage form. Examples include substitution of a generic for a pioneer product and, for both a pioneer and interchangeable equivalent, when re-documentation of BE is needed in the presence of specified post-approval changes in component/composition and/or method of manufacture. A regulatory objective is to encourage bioequivalent formulations over an extended period of time that clearly relate, in terms of performance, to the pivotal clinical trial material on which safety and efficacy were based. The proposed new criteria include variance as well as mean terms.\(^1^3^4,1^3^6\) The variance term for population BE is total variance, which is the sum of between and within-subject variances. For individual BE, a subject-by-formulation (S*F) interaction variance, and within-subject variance for both T and R products.
are estimated. Both PBE and IBE criteria allow scaling of the BE limit (goalpost) by R product variability.

A key concept underlying IBE criterion relates to the term switchability, which denotes the situation where a patient currently on one formulation switches to another with the expectation that the safety and efficacy of the drug will remain essentially unchanged. The criterion uses, in the aggregate, a distance concept that compares means and variances of T and R products. By expanding the variance terms, the proposed criterion offers many consumer and producer advantages, including: (i) assurance of switchability; (ii) rewards for reduction of variance in the T product; (iii) scaling for highly variable and/or narrow therapeutic range drugs.

The IBE criterion encourages BE studies in subjects more representative of the general population or even in patients for whom the drug is intended, as opposed to healthy young males where detection of S\*F interaction is less likely. This feature addresses a frequently expressed concern that BE studies in healthy young males lack clinical relevance.\(^{73}\) The re-test characteristics of replicate study design allow scrutiny of outliers.

IBE can be calculated as:

\[
\sigma^2 D = [(\sigma^2 BT - \sigma^2 BR)^2 + 2(1-\sigma) \sigma BT \sigma BR] \\
\theta_1 = [(\mu T - \mu R)^2 + \sigma^2 D + \sigma^2 WT - \sigma^2 WR] / \sigma^2 WR \text{ when } \sigma WR > 0.2 \\
\theta_1 = [(\mu T - \mu R)^2 + \sigma^2 D + \sigma^2 WT - \sigma^2 WR] / 0.22 \text{ when } \sigma WR < 0.2
\]

Where,
\(\mu T = \text{mean (test)}\)  \\
\(\mu R = \text{mean (reference)}\)  \\
\(\sigma^2 WT = \text{within subject variance (test)}\)  \\
\(\sigma^2 WR = \text{within subject variance (reference)}\)  \\
\(\sigma^2 BT = \text{between subject variance (test)}\)  \\
\(\sigma^2 BR = \text{between subject variance (reference)}\)

Again, \(\sigma^2 WR\) is set to 0.20 (that is, constant scaled versus reference standard) in the denominator of the formula \(\theta_1\) when the point estimate of the parameter based on the original data set falls below 0.20.\(^{130}\)
Individual BE is demonstrated when: $\theta_1 (0.95) < 2.45$, where $\theta_p (0.95)$ is defined as the 95$^{th}$ quartile of $\theta_1$ based on the non-parametric percentile method using 2000 bootstrap samples. The bootstrap is used, as the exact distribution for the parameter $\theta_p$ has not yet been derived.

2.8.2 Population bioequivalence (PBE)

Population bioequivalence approach, which evaluates the total bioavailability variances in the in addition to the average bioavailability values, has been proposed as a method to overcome the disadvantages of average bioequivalence approach.\textsuperscript{48} FDA has also proposed the use of population bioequivalence as a bioequivalence study which might guarantee prescribability and which is applicable in the development stages of novel drugs.\textsuperscript{130} Based on earlier published reports of bioequivalence in literature, it was concluded that population bioequivalence value was affected more extensively by the bioavailability variance rather than by the average bioavailability.\textsuperscript{84} PBE criteria aggregate the difference between the population means and variances. Both IBE and PBE criteria allow for scaling of the regulatory limits based on the variability of the reference product. Both require the use of boot strapping methodology to derive empirical distributions of the criteria, as the exact statistical distribution has not yet been established.

2.9 Bioequivalence Study

The preferred approach is an in-vivo study carried out in healthy volunteers to whom the 2 preparations (generic and innovator) are alternatively administered. The bioequivalence study consists of study design, bioanalytical method development and validation, statistical approach for data analysis and bioequivalence establishment criteria.

2.9.1 Study Design

The design of a bioavailability and/or bioequivalence study is dependent upon the drug, dosage form and study objectives. For BE studies, both the test and reference drug formulations contain the pharmaceutical equivalent drug in the same dose and are given by the same route of administration. A pilot study in small number of subjects can be carried out before proceeding with a full BE study. This study can be used to validate analytical methodology, assess variability, optimize sample collection time intervals or provide any other information. Non replicate crossover study designs are recommended by FDA\textsuperscript{127} for immediate release and modified release dosage forms. However, replicate designs can also be
used. The recommended method for analysis to establish BE is average bioequivalence. The study should be of crossover design and suitably randomized, as far as possible. Some of the designs are being discussed below:

### 2.9.1.1 Two-Period Crossover Design

In case of two formulations, an even number of subjects should be randomly divided into two equal groups. In the first period, each member of one group will receive a single dose of the test formulation and each member of the other group will receive standard formulation. After a suitable washout period (Not less than 5 half lives). In the second period, each member of the respective groups will receive a dose of an alternative formulation and the experiment will be repeated. The design can be depicted as follows:

**Two-Period Crossover Design can be depicted as:**

<table>
<thead>
<tr>
<th>Volunteer No.</th>
<th>Period 1</th>
<th>Period 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>2</td>
<td>B</td>
<td>A</td>
</tr>
<tr>
<td>3</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>4</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>5</td>
<td>B</td>
<td>A</td>
</tr>
<tr>
<td>6</td>
<td>B</td>
<td>A</td>
</tr>
</tbody>
</table>

### 2.9.1.2 Latin Square Design

In case of more than two formulations, a Latin square design should be used. For example in a bioequivalence study of 3 formulations, a group of volunteers will receive formulations in the sequence shown below:

<table>
<thead>
<tr>
<th>Volunteer No.</th>
<th>Period 1</th>
<th>Period 2</th>
<th>Period 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>2</td>
<td>B</td>
<td>C</td>
<td>A</td>
</tr>
<tr>
<td>3</td>
<td>C</td>
<td>A</td>
<td>B</td>
</tr>
</tbody>
</table>

The next group of 3 volunteers will receive formulations in the same sequence as shown above.

### 2.9.1.3 Balance Incomplete Block Design (BIBD)

In case there are more than 3 formulations, the Latin square design will not be ethically advisable, mainly because each volunteer may require the drawing of too many blood
samples. However, if each volunteer is expected to receive at least two formulations, then such a study can be carried out using Balanced Incomplete Block Design. As per this design, if there are four formulations, six possible pairs of formulations can be chosen from four formulations. Then, the first 6 volunteers will receive these six pairs of formulations and the next six volunteers will receive the same six pairs in reverse order. The design is depicted below:

<table>
<thead>
<tr>
<th>Volunteer No.</th>
<th>Period 1</th>
<th>Period 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>2</td>
<td>A</td>
<td>C</td>
</tr>
<tr>
<td>3</td>
<td>A</td>
<td>D</td>
</tr>
<tr>
<td>4</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>5</td>
<td>B</td>
<td>D</td>
</tr>
<tr>
<td>6</td>
<td>C</td>
<td>D</td>
</tr>
<tr>
<td>7</td>
<td>B</td>
<td>A</td>
</tr>
<tr>
<td>8</td>
<td>C</td>
<td>A</td>
</tr>
<tr>
<td>9</td>
<td>D</td>
<td>A</td>
</tr>
<tr>
<td>10</td>
<td>C</td>
<td>B</td>
</tr>
<tr>
<td>11</td>
<td>D</td>
<td>B</td>
</tr>
<tr>
<td>12</td>
<td>D</td>
<td>C</td>
</tr>
</tbody>
</table>

The minimum acceptable number of volunteers is 18 for bioequivalence studies.

\[ n \geq \left\lceil \frac{[\sigma^2]}{2D^2 [t_\alpha + t_\beta]^2} + 0.25 t_\alpha^2 \right\rceil \]

Where,

\( n \) = no. of volunteers
\( \alpha \) = Required level of significance (0.05)
\( \beta \) = Required power of test (0.80)
\( \sigma^2 \) = Error mean sum of squares from ANOVA (estimated/guess)
\( D \) = Minimum difference between the means which if present, ought to be detected

2.9.2 Bioanalytical Method Development

Bioanalysis, employed for the quantitative determination of drugs and their metabolites in biological fluids, plays a significant role in the evaluation and interpretation of bioequivalence, pharmacokinetic (PK), and toxicokinetic studies. The quality of these studies, which are often used to support regulatory filings, is directly related to the quality of the underlying bioanalytical data. It is essential to employ well-characterized and fully validated analytical methods to yield reliable results which can be satisfactorily interpreted as well as to
emphasize that each analytical technique has its own characteristics, which will vary from analyte to analyte.

The development of the sound bioanalytical method is of paramount importance during the process of drug discovery and development culminating in marketing approval. It is a set of various procedures, mainly used for the determination of drugs and their metabolites in biological matrices such as urine, plasma, and serum.

**Literature survey for the physiochemical properties of drug molecules**

- Determine solubility profile
- MS Scanning and Optimisation
- Mobile phase selection
- Selection of extraction method and optimization

**Select chromatographic method** [Based on solubility study, Retention of compound]

- Reverse phase chromatography
- Normal Phase Chromatography

**Experimental trials with different chromatographic condition**

- Selection of extraction method and optimization
- Finalize the method and check the method by linearity parameter

**Validation Process**

### 2.9.3 Bioanalytical Method Validation

Bioanalytical method validation (BMV) employed for the quantitative determination of drugs and their metabolites in biological fluids plays a significant role in the evaluation and interpretation of bioavailability, bioequivalence, pharmacokinetic, and toxicokinetic study data. These studies generally support regulatory filings. Validation is a tool of quality assurance which provides confirmation of the quality in equipment system, manufacturing processes, software and testing methods. Validation assures that products with predetermined
quality characteristics and attributes can be reproduced consistently within the establish limits. Bioanalytical method validation includes all of the procedures that demonstrate that a particular method used for quantitative measurement of analytes in a given biological matrix, such as blood, plasma, serum, or urine, is reliable and reproducible for the intended use. The fundamental parameters for this validation include (1) accuracy, (2) precision, (3) selectivity, (4) sensitivity, (5) reproducibility, and (6) stability. 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.

2.9.4 Pharmacokinetic Parameters
Pharmacokinetics deals with the change of drug concentration in the drug product and changes of concentration of a drug or its metabolite(s) in the human or animal body following administration of the drug product, i.e., the changes of drug concentration in the different body fluids and tissues in the dynamic system of liberation, absorption, distribution, body storage, binding, metabolism and excretion.

The various pharmacokinetic parameters are as follows:

\( C_{\text{max}} \): Maximum measured plasma concentration over the time span specified.

\( T_{\text{max}} \): Time of the maximum measured plasma concentration. If the maximum value occurs at more than 1 time point, \( T_{\text{max}} \) is defined as the first time point with this value.

\( \text{AUC}_{0-4} \): The area under the plasma concentration versus time curve, from time zero to the last measurable concentration, as calculated by the linear trapezoidal method.

\( \text{AUC}_{0-24} \): The area under the plasma concentration versus time curve, from time zero to 24 h.

\( \text{AUC}_{0-\infty} \): The area under the plasma concentration versus time curve, from time zero to infinity. \( \text{AUC}_{0-\infty} \) is calculated as the sum of \( \text{AUC}_{0-4} \) plus the ratio of the last measurable plasma concentration to the elimination rate constant.

\( \text{AUC} \% \text{ Extrapol} \): It is the percentage of extrapolated area under the plasma concentration versus time curve from the last measurable concentration to infinity. It was calculated as \( \left( \frac{\text{AUC}_{0-\infty} - \text{AUC}_{0-4}}{\text{AUC}_{0-\infty}} \right) \times 100 \)

\( K_e \): Apparent first-order terminal elimination rate constant calculated from a semi-log plot of the plasma concentration versus time curve. The parameter was calculated by linear least-
square regression analysis using the maximum number of points in the terminal log-linear phase (e.g. three or more non-zero plasma concentrations).

$T_{1/2}$: The apparent first-order terminal elimination half-life was calculated as $0.693/K_{el}$.

### 2.9.5 Statistical issues in BE studies

The pharmacokinetic parameters, $C_{max}$, $T_{max}$ and AUC should be subjected to a three-way analysis of variance (3-way ANOVA) in order to test differences due to formulations, period and subjects. A more complex ANOVA may be appropriate in some circumstances, e.g. if treatments are replicated. The standard parametric ANOVA assumes homogeneity of variances, normality and additivity of independent variables.

In order to ensure homogeneity of variances between treatments, Bartlett’s test or a similar test should be carried out prior to performing the ANOVA. The primary comparison of interest in a bioequivalence study is the ratio of average parameter data (AUC or $C_{max}$) from the test and reference formulations rather than the difference between them. Log transformation of the data allows the general linear statistical model to draw inferences about the ratio of the two averages on the original scale. Log transformation thus achieves the general comparison based on the ratio rather than on the difference.

Moreover, plasma concentration data, including AUC and $C_{max}$, tend to be skewed and their variances tend to increase with the means. Log transformation corrects this situation and makes the variances independent of the mean.

Further, the frequency distribution skewed to the left, i.e., those with a log tail to the right is made symmetrical by log transformation.

In case no suitable transformation is available, the non-parametric method should be used. $T_{max}$ values being discrete, data on $T_{max}$ should be analysed using non-parametric methods.

#### 2.9.5.1 Two one-sided tests procedures (TOST):

This procedure is also referred to as confidence interval approach. This method is used to demonstrate if the bioavailability of the drug from the test formulation is too high or low in comparison to the reference drug product. The 90% confidence limits are estimated for the sample means. In this test, presently required by the FDA, a 90% confidence interval about the ratio of means of the two drug products must be within $\pm 20\%$ for measurement of the rate and extent of drug bioavailability. The lower 90% CI for the ratio of means cannot be
less than 0.8, and the upper 90% CI for the ratio of the means cannot be greater than 1.20. The 90% CI is a function of sample size and study variability, including inter and intra subject variability.\textsuperscript{127}

Current DCGI requirements for bio-equivalence approval are that 90% confidence interval should be within 80-125% for log transformed $C_{\text{max}}$ and for log transformed AUC. For narrow therapeutic index drugs, the same criterion i.e., log transformed $C_{\text{max}}$ and log transformed AUCs of 80-125% is applicable. No tighter limit has been proposed for NTIs. Canadian regulatory requirements for bio-equivalence approval are that 90% confidence interval should be within 80-125% for log transformed $C_{\text{max}}$ and AUC. For narrow therapeutic index drugs, log transformed $C_{\text{max}}$ should be within 90-111% and log transformed AUCs should be within 80-125.

The T/R ratio should be as close as possible to 95-105%. Intra subject CV should be as low as possible (<15%). Table 2.3 mentions the bioequivalence criteria followed by various regulatory agencies in the world.

\subsection{2.9.6 Bioequivalence establishment criteria}

In order for different formulations of the same drug substance to be considered bioequivalent, they must be equivalent with respect to the rate and extent of drug absorption. Thus, the two predominant issues involved in the assessment of bioequivalence are: the pharmacokinetic parameters that best characterize the rate and extent of absorption and, the most appropriate method of statistical analysis of the data.

With regard to the choice of the appropriate pharmacokinetic characteristics, Westlake suggests comparisons of the formulations should be made with respect to only those parameter(s) of the blood level profile that possess some meaningful relation to the therapeutic effect of the drug. Since the AUC is directly proportional to the amount of drug absorbed, this pharmacokinetic parameter is most commonly used to characterize the extent of absorption, both in single- and multiple- dose studies. The choice of an appropriate pharmacokinetic characteristic for the rate of absorption is still being discussed with considerable controversy. Although a broad array of methods exists for calculating absorption rates (e.g. moment analysis, deconvolution procedures and curve-fitting), the most commonly used parameters are peak concentration ($C_{\text{max}}$) and time to peak concentration ($t_{\text{max}}$).
Table 2.3: Criteria of bio-equivalence of various regulatory agencies

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CPMP (EU)</th>
<th>USFDA</th>
<th>CANADIAN FDA (CEC)</th>
<th>DCGI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log transformed $C_{\text{max}}$ using 90% CI</td>
<td>□ 80-125% of reference □ 70-143% of reference (If clinically acceptable). Tighter limits for $C_{\text{max}}$ accepted for NTI drugs.</td>
<td>□ 80-125% of reference and otherwise indicated for NTI drugs. The range is same as the wide margin drugs.</td>
<td>□ 80-125% of reference □ 90-111% of reference for NTI drugs.</td>
<td>□ 80-125% of reference □ 80-125% of reference for NTI drugs.</td>
</tr>
<tr>
<td>Log transformed $\text{AUC}_{0-t}$ using 90% CI</td>
<td>□ 80-125% of reference □ 80-125% of reference for NTI drugs.</td>
<td>□ 80-125% of reference □ 80-125% of reference for NTI drugs.</td>
<td>□ 80-125% of reference □ 80-125% of reference for NTI drugs.</td>
<td>□ 80-125% of reference.</td>
</tr>
</tbody>
</table>

*NTI- Narrow Therapeutic Index

2.10 Need of Bioequivalence Studies (Equivalence, interchangeability and Switchability Issues):

Regulatory authorities (FDA, CPMP) insists that generic products should compulsorily be "essential similar" (Composition, formulation and bioequivalence) with that of reference product in order to exclude any clinically significant difference. When two formulations of the same drug present similar bioavailabilities to the extent that they are considered bioequivalent by prescribed criteria, it is assumed that when administered in the same molar dose, they will provide the same therapeutic effect (Therapeutically equivalent). The use of generic drugs is of increasing importance, in terms of efficiency, in the selection of therapeutic alternatives. But their use in clinical practice depends not only on their, but mostly on the conviction of their interchangeability with their reference counterparts.

Despite many advantages for consumers and health care providers, with low-priced generic formulation, these are not always as safe or effective as their counterpart.\textsuperscript{22,79,122} Issue of the impurities in manufacturing generic drugs had been addressed by US Office of Generic Drugs and draft guideline for industry has been proposed. In the USA itself, many important drugs like diclofenac sodium,\textsuperscript{121} theophylline,\textsuperscript{13} phenytoin,\textsuperscript{106} warfarin tablets,\textsuperscript{54} digoxin
tablets, and levothyroxine tablets\textsuperscript{119} have failed bioequivalence studies. Reports of the generic drugs having different \textit{in vitro} profile in comparison to innovator product and substandard quality are frequent across many countries.\textsuperscript{70,87,88} National and international regulatory authorities are highly concerned with the substandard quality of drugs in society. Nevertheless, there has always been a report of variations in the efficacy of generic drugs compared with the corresponding brand-name drugs.\textsuperscript{10,54,64}

In spite of such regulations there has been always a suspicion regarding the quality of the generics in market.\textsuperscript{29} This danger is of much concern in the developing countries like India where quality of the drugs is always questionable and also there is not much data available on the bioequivalence studies of marketed drugs. The risk of non-bioequivalent product is of much concern with toxic drugs, narrow therapeutic drugs, potent drugs, modified release products and drugs used for longer duration of treatment like in diabetes, cancer etc. Thus, a system must be in place to ensure that generics will have the same level of safety, efficacy and quality as the brand products. Hence, publication of the comparative bioavailabilities of test and reference formulations is significant for the knowledge and appropriate assessment by the scientific community of what they are dealing with.

**Situations when bioequivalence studies are not recommended/required (exemptions):**

1. Product differing only in strength of the active substance it contains, provided all following conditions hold:
   – pharmacokinetics are linear;
   – the qualitative composition is the same;
   – the ratio between active substance and the excipients is the same, or (in the case of small strengths) the ratio between the excipients is the same;
   – both products are produced by the same manufacturer at the same production site;
   – a bioavailability or bioequivalence study has been performed with the original product;
   – under the same test conditions, the dissolution rate in vitro is the same.
2. Product has been slightly reformulated or the manufacturing method has been slightly modified by the original manufacturer in ways that can convincingly be argued to be irrelevant for the bioavailability. The bioavailability of original product has been investigated and the dissolution rates in vitro under the same test conditions are equivalent.
3. Product is to be parenterally administered as a solution and contains the same active substance(s) and excipients in the same concentrations as a medicinal product currently approved.
4. Product is a liquid oral form in solution (elixir, syrup, etc.) containing the active substance in the same concentration and form as currently approved medicinal product, not containing excipients that may significantly affect gastric passage or absorption of the active substance.

5. When an acceptable in vivo and in vitro dissolution rate correlation has been shown and in vitro dissolution rate of the new product is equivalent with that of the already approved medicinal product under the same test conditions.

Parkinson's disease is the second most common neurodegenerative disorder and the most common movement disorder. It is characterized by progressive loss of muscle control, which leads to trembling of the limbs and head while at rest, stiffness, slowness, and impaired balance. As symptoms worsen, it may become difficult to walk, talk, and complete simple tasks. A substance called dopamine acts as a messenger between two brain areas - the substantia nigra and the corpus striatum - to produce smooth, controlled movements. Most of the movement-related symptoms of Parkinson's disease are caused by a lack of dopamine due to the loss of dopamine-producing cells in the substantia nigra. When the amount of dopamine is too low, communication between the substantia nigra and corpus striatum becomes ineffective, and movement becomes impaired; the greater the loss of dopamine, the worse the movement-related symptoms. Other cells in the brain also degenerate to some degree and may contribute to non-movement related symptoms of Parkinson's disease. Age is the largest risk factor for the development and progression of Parkinson's disease. Most people who develop Parkinson's disease are older than 60 years of age. Men are affected about 1.5 to 2 times more often than women.

The goal of medical management of Parkinson disease is to provide control of signs and symptoms for as long as possible while minimizing adverse effects. Studies demonstrate that a patient's quality of life deteriorates quickly if treatment is not instituted at or shortly after diagnosis. Pharmacologic treatment of Parkinson disease can be divided into symptomatic and neuroprotective (disease modifying) therapy. At this time, there is no proven neuroprotective or disease-modifying therapy.

Levodopa, coupled with carbidopa, a peripheral decarboxylase inhibitor (PDI), remains the gold standard of symptomatic treatment for Parkinson disease. Carbidopa inhibits the decarboxylation of levodopa to dopamine in the systemic circulation, allowing for greater levodopa distribution into the central nervous system. Levodopa provides the greatest antiparkinsonian benefit for motor signs and symptoms, with the fewest adverse effects in the short term; however, its long-term use is associated with the development of motor
fluctuations ("wearing-off") and dyskinesias. Once fluctuations and dyskinesias become problematic, they are difficult to resolve.\textsuperscript{42}

Monoamine oxidase (MAO)-B inhibitors can be considered for initial treatment of early disease. These drugs provide mild symptomatic benefit, have minor adverse effect and according to a Cochrane review, have improved long-term outcomes in quality-of-life indicators by 20-25 %.\textsuperscript{23}

Rasagiline mesylate is an irreversible, monoamine oxidase type B inhibitor (MAO-B). Monoamine oxidase (MAO) is a flavin-containing enzyme and is classified into two major molecular species A and B and is localized in liver and intestinal mucosa. MAO-B inhibition elicits additional primary pharmacodynamic effect due to elevation of brain monoamine levels and dopamine in particular. These include protection against MPTP-induced neurotoxicity and amelioration in the performance of the motor tasks and cognition that has been impaired neural injury. Rasagiline mesylate is indicated for the treatment of idiopathic Parkinson’s disease as initial monotherapy and as adjunct therapy to Levodopa.\textsuperscript{24} In a multi centre 26 weeks parallel group randomized double blind placebo controlled trial rasagiline was effective as monotherapy in patients with early Parkinson disease (PD). Rasagiline offers an advantage over the first-generation MAO-B inhibitor selegiline in its lack of amphetamine metabolites, which may interfere with neuroprotection.\textsuperscript{86}

Rasagiline is well absorbed after oral administration and readily crosses the BBB. The bioavailability of rasagiline is about 35% and it exerts linear absorption at doses of 1–10 mg/day. Maximal plasma concentration (C\textsubscript{max}) obtained after 1 mg and 2 mg oral dose is 2.5 ng/mL and 4.9 ng/mL, respectively. The time to reach maximal concentration (T\textsubscript{max}) is 0.5–1 hours and is unaffected by food intake. High-fat meal decreases the area under the curve (AUC) of rasagiline by 20%, which is considered clinically insignificant; therefore, rasagiline can be administered independently of food intake. The volume of distribution (Vd) of rasagiline varies between 87 to 243 L, according to different reports. Plasma albumin binding is considered to be 60%–70% \textsuperscript{5}, although a higher plasma protein binding value was reported. The half-life of rasagiline is 1.5–3.5 h and may be dose-dependent.\textsuperscript{110}