2.0 REVIEW OF LITERATURE

2.1 BIOAVAILABILITY AND BIOEQUIVALENCE

United States Food & Drug Administration (USFDA) 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 (17).

The EMA guidance defines bioavailability as the rate and extent to which the active substance or active moiety is absorbed from a pharmaceutical form and becomes available at the site of action (18).

The CDSCO India defines bioavailability as the relative amount of drug from an administered dosage form which enters the systemic circulation and the rate at which the drug appears in the systemic circulation (19).

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. Where 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 (17).

A new product is said to be supra-bioavailable when it displays larger bioavailability than the approved comparative product. For these supra-bioavailable 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.

In bioequivalence studies, the primary question is to compare measures of release of drug substance between the test and reference product. Hence, bioequivalence is primarily a product quality question. Because product BA and BE are closely related, similar approaches for establishing BA and BE may be followed.

2.1.1 Historical Perspective of Bioequivalence Studies

Various guidelines and laws have been formulated and implemented by the government agencies to regulate the manufacturing process of new drug formulations by the pharmaceutical industry and conduct of clinical trials by investigators. These regulations require the mandatory assessment of safety, efficacy and quality of all the new drug formulations, before they are marketed. The fundamental mission of the drug regulatory agencies is to protect both the clinical trial participants and consumers, keeping in view both the ethics as well as legal perspectives in its purview. 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 (20)

<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 + 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 $C_{\text{max}}$ only for drug with wide safety margin</td>
</tr>
<tr>
<td>2005</td>
<td>Bioequivalence guidelines-India</td>
</tr>
</tbody>
</table>
2.1.2 Bioequivalence for First Entry Products

Bioequivalence (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.

2.1.3 Bioequivalence for Interchangeable Multi-Source Products

Bioequivalence (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 (17).

2.1.4 Bioequivalence 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 (17).

2.1.5 Types of Bioavailability

Bioavailability can be classified into four different types depending on the purpose of the study and scientific questions to be solved (21).

- **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.

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

- **Relative Bioavailability**

The relative bioavailability is the extent and rate of the bioavailability of a drug from two or more different dosage forms given by the same route of administration. For determination of relative BA, 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
- **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 per oral (po) or in part, rectal dose reaching systemic circulation $F$, under the assumption of otherwise linear kinetics can be described by following equation

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

$f_m$ - fraction of drug metabolized in liver

$LBF$ - liver blood flow

$\lambda$ - ratio of the concentration of the drug in whole blood to that in plasma

- **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 relative output BA, 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

$$\text{Relative output BA} = \frac{\text{AUC (drug + vehicle; granules; tablets)}}{\text{AUC solution}} \times 100$$

2.1.6 **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 divided into three categories (22): (a) Pharmacokinetic methods (b) Pharmacodynamic methods (c) In vitro methods

➢ **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;

(a) **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 super-imposable 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:

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

(ii) $T_{\text{max}}$: 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.

(iii) 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:

\[ F = \frac{AUC_{\text{oral}} D_{\text{iv}}}{AUC_{\text{iv}} D_{\text{oral}}} \]

(b) 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.

- **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: (a) Acute pharmacologic response (b) Therapeutic response

- **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).

2.1.7 Factors Affecting Bioavailability

The various factors affecting bioavailability of drugs can be classified as shown in table 2.2 (13).

**Table 2.2 Factors Affecting Bioavailability of Drugs**

<table>
<thead>
<tr>
<th>Pharmaceutical Factors</th>
<th>Patient Related Factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physicochemical properties of drug</td>
<td>Disintegration time</td>
</tr>
<tr>
<td>Drug solubility and dissolution rate</td>
<td>Dosage form related factors</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>Drug stability</td>
<td></td>
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<tr>
<td></td>
<td>Gastrointestinal contents:</td>
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<tr>
<td></td>
<td>Other drugs</td>
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<td></td>
<td>Food</td>
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<tr>
<td></td>
<td>Fluids</td>
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<tr>
<td></td>
<td>Other GI contents</td>
</tr>
<tr>
<td></td>
<td>Pre-systemic metabolism:</td>
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<tr>
<td></td>
<td>Luminal enzymes</td>
</tr>
<tr>
<td></td>
<td>Gut wall enzymes</td>
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<tr>
<td></td>
<td>Bacterial enzymes</td>
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<tr>
<td></td>
<td>Hepatic enzymes</td>
</tr>
</tbody>
</table>

2.1.8 Population and Individual Bioequivalence

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 (23). 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 (24). The FDA also has proposed replacing the 1992 average bioequivalence(ABE) approach with population and individual bioequivalence (25).

**Individual Bioequivalence**

In the individual bioequivalence (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 (25, 26). 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 (subject x formulation) interaction is less likely. This feature addresses a frequently expressed concern that BE studies in healthy young males lack clinical relevance (27). The re-test characteristics of replicate study design allow scrutiny of outliers.
Population bioequivalence

Population bioequivalence (PBE) approach, which evaluates the total bioavailability variances in addition to the average bioavailability values, has been proposed as a method to overcome the disadvantages of average bioequivalence approach (28). FDA has also proposed the use of population bioequivalence as a bioequivalence study which might guarantee prescribility and which is applicable in the development stages of novel drugs (25). 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 (23). 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.

2.1.9 Bioequivalence Studies: Design and Evaluation

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 design and evaluation of a well-controlled bioequivalence study requires the cooperative input from pharmacokinetic expert, statistician, clinician, bio-analytical chemist, and others.

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 a small number of subjects can be carried out before proceeding with a full-fledged 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 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 (17). Some of the designs are being discussed below

(a) 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:

<table>
<thead>
<tr>
<th>Volunteers</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>

(b) 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 in following table:
The next group of 3 volunteers will receive formulations in the same sequence as shown above.

(c) Balance Incomplete Block Design

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 (BIBD). 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:
Statistical Issues in Bioequivalence Studies

The pharmacokinetic parameters, $C_{\text{max}}$, $T_{\text{max}}$ 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 $C_{\text{max}}$) 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 $C_{\text{max}}$, 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. $T_{\text{max}}$ values being discrete, data on $T_{\text{max}}$ should be analyzed using non-parametric methods.
• 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 ± 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 (17).

Current Indian regulatory 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, tighter limits have been proposed (19). As per European guidelines, for the determination of bioequivalence after single dose, the parameters need to be analyzed are $\text{AUC}_{0-t}$ or, when relevant $\text{AUC}_{0-72}$ and $C_{\text{max}}$. For these parameters the 90% confidence interval for the ratio of test and reference should be within 80.00-125.00%. In specific cases of products with a narrow therapeutic index, the acceptance interval for AUC should be tightened to 90.00-111.11%. Where $C_{\text{max}}$ is of particular importance for safety, efficacy or drug level, monitoring the 90.00-111.11% acceptance interval should also be applied this parameter (18). Table 2.3 mentions the bioequivalence criteria followed by various regulatory agencies in the world.
Table 2.3: Criteria of bio-equivalence of various regulatory agencies (17-19, 30)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CHMP (EU)</th>
<th>FDA (USA)</th>
<th>TPD (Canada)</th>
<th>DCGI (India)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log transformed C\textsubscript{max} using 90% CI</td>
<td>80-125% 70-143% for highly variable drugs 90-111% for NTI drugs</td>
<td>80-125% Same for NTI drugs otherwise indicated</td>
<td>80-125% Same for NTI drugs</td>
<td>80-125% Tighter limits for NTI drugs</td>
</tr>
<tr>
<td>Log transformed AUC\textsubscript{0-t} using 90% CI</td>
<td>80-125% 80-125% for highly variable drugs 90-111% for NTI drugs</td>
<td>80-125% Same for NTI drugs</td>
<td>80-125% 90-112% for NTI drugs</td>
<td>80-125% Tighter limits for NTI drugs</td>
</tr>
</tbody>
</table>

*NTI- Narrow Therapeutic Index

2.1.10 Need of Bioequivalence Studies (Equivalence, Interchangeability and Switchability Issues)

Regulatory authorities (FDA, CHMP) 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 bioavailability 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 bioavailability, 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 (31). 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 (32), theophylline (33), phenytoin (34), warfarin tablets (35), digoxin tablets, and levothyroxine tablets (37) have failed bioequivalence studies. Reports of the generic drugs having different in vitro profile in comparison to innovator product and substandard quality are frequent across many countries (37, 38). Nevertheless, there has always been a report of variations in the efficacy of generic drugs compared with the corresponding branded drugs (35, 39, 40).

In spite of such regulations there has been always a suspicion regarding the quality of the generics in market. This danger is of greater 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 products is of greater concern as regards to 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 branded products.

The Commissioner of Food and Drugs describes following factors to assess actual or potential bioequivalence problems of drugs/products (22):

- When there is evidence that drug products do not give comparable therapeutic effects from well-controlled clinical trials or controlled observations in patients
- When products are not bioequivalent in well-controlled BA/BE studies
➢ Narrow therapeutic ratio drugs for careful dosage titration and patient monitoring

➢ Competent medical determination that lack of bioequivalence would have a serious adverse effect if product is given for treatment or prevention of a disease or condition

➢ When physicochemical factors are important in the bioavailability of drugs like:
  - Unfavorable physicochemical properties, e.g., low solubility, instability, etc.
  - Slow dissolution rate of one or more such products
  - The particle size and/or surface area of the active drug ingredient is critical in determining its bioavailability
  - Certain physical structural characteristics of the active drug ingredient, e.g., polymorphic forms, conformers, solvates, complexes, and crystal modifications, poor dissolution which may affect absorption
  - High excipients to active ingredients ratio e.g., greater than 5 to 1
  - Specific inactive ingredients, e.g., hydrophilic or hydrophobic excipients and lubricants, either may be required for absorption of the active drug ingredient or therapeutic moiety or, alternatively, if present, may interfere with such absorption

➢ Pharmacokinetic evidence that
  - The active drug ingredient, therapeutic moiety, or its precursor is absorbed in large part in a particular segment of the gastrointestinal tract or is absorbed from a localized site
  - The degree of absorption of the active drug ingredient, therapeutic moiety, or its precursor is poor, e.g., less than 50 percent, ordinarily in comparison to an intravenous dose, even when it is administered in pure form, e.g., in solution
• There is rapid metabolism of the therapeutic moiety during the process of absorption (first-pass metabolism) so the therapeutic effect and/or toxicity of such drug product is determined by the rate as well as the degree of absorption.

• The therapeutic moiety is rapidly metabolized or excreted so that rapid dissolution and absorption are required for effectiveness.

• The active drug ingredient or therapeutic moiety is unstable in specific portions of the gastrointestinal tract and requires special coatings or formulations, e.g., buffers, enteric coatings, and film coatings, to assure adequate absorption.

• The drug product is subject to dose dependent kinetics in or near the therapeutic range, and the rate and extent of absorption are important to bioequivalence.

WHO specifies few other categories of drugs for which equivalence studies necessary (41):

- Sustained or otherwise modified release pharmaceutical products designed to act by systemic absorption
- Fixed combination products with systemic action
- Oral immediate release pharmaceutical products indicated for serious conditions requiring assured therapeutic response

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

- Product differing only in strength of the active substance it contains, provided all the following conditions hold true:
  - Pharmacokinetics is 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 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 in vitro dissolution rate is same

- 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 in vitro dissolution rates under the same test conditions are equivalent.

- 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.

- 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.

- 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.
2.2 BIOANALYTICAL METHODOLOGY

2.2.1 Chromatography

Chromatography comprises of a group of methods used for separating molecular mixtures that depends on the differential affinities of the solutes between two miscible phases (42). There are different types of chromatographic techniques like reverse phase chromatography, normal phase chromatography, ion exchange chromatography etc.

➢ Analytical Methodologies

The analytical methodologies include infrared spectrometry, mass spectrometry, NMR, HPLC etc. In bioanalytical laboratory, following instruments are mainly used for the analysis of biological samples

- High performance liquid chromatography (HPLC)
- Liquid chromatography mass spectrometry (LCMS)
- Gas chromatography (GC)
- Gas chromatography mass spectrometry

Triple quadrapole LCMSMS is the most preferred instrument used in bioanalytical laboratories. The reason being shorter run times, selectivity of the analyte and sensitivity is easily attained etc.

➢ Principle of Mass Spectrometry

The mass spectrometer is an instrument designed to separate gas phase ions according to their m/z (mass to charge ratio) value. The "heart" of the mass spectrometer is the analyzer. This element separates the gas phase ions. The analyzer uses electrical or magnetic fields, or
combination of both, to move the ions from the region where they are produced, to a
detector, where they produce a signal which is amplified. Since the motion and separation of
ions is based on electrical or magnetic fields, it is the mass to charge ratio, and not only the
mass, which is of importance. The analyzer is operated under high vacuum, so that the ions
can travel to the detector with a sufficient yield. The components of mass spectrometer are:

- A vacuum system
- Tools to introduce the sample (LC, GC)
- Tools to produce the gas phase ions from the sample molecules
- Tools to fragment ions in order to obtain structural information/get more selective
detection
- A detection system
- Software and computing

MS/MS is done:

- Either by coupling multiple analyzers (of the same or different kind) or
- With an ion trap, by doing the various experiments within the trap

LCMS is a hyphenated technique, combining the separation power of HPLC, with the
detection power of mass spectrometry. Even with a very sophisticated MS instrument, HPLC
is still useful to remove the interferences from the sample that would impact the ionization.

2.2.2 Analysis of Drugs in Biological Matrices

- Sample Collection and Storage

The biological samples most often collected for the determination of drug levels are plasma,
serum and urine. When the samples cannot be analyzed immediately, the collected samples
are usually stored frozen at or below -15°C or -50°C under the conditions that drugs and metabolites are stable for the duration of the storage before the samples are analyzed (43, 44).

➢ Preparation of Biological Samples for Analysis

Biological samples are very complex multi-component mixtures. Drugs are often present in these samples as minor components. Also detection of drug or its metabolite in biologic media is usually complicated by the matrix effects. Because of this, various types of cleanup procedures involving solvent extraction and chromatography are employed to effectively separate the drug components from endogenous biologic material (43, 45). It may also be desirable to concentrate the analyte and/or derivatize them for improved detection or better separation.

Protein precipitation, liquid-liquid extraction and solid phase extraction are the frequently used sample processing techniques. Protein precipitation is a simple technique and is economical in nature. Solid phase extraction is expensive, but the final sample is clean and increases the life time of column and instrument. Liquid-liquid extraction involves multiple steps, but is less expensive than solid phase extraction. Even though solid phase extraction is expensive, it is most preferred technique used worldwide. By using the proper sorbent and controlled pH, clean samples with better recoveries are obtained. Now a days, ion exchange cartridges are being used which helps in reducing the matrix effect. The other procedures used are ultra-filtration, hydrolysis of conjugates etc. (43, 45-47).
2.2.3 Method Validation

In today’s drug development environment, highly sensitive and selective methods are required to quantify drugs in matrices such as blood, plasma, serum, or urine. These methods, for the quantitative evaluation of drugs and their metabolites (analytes), are critical for the successful conduct of preclinical and/or biopharmaceutics and clinical pharmacology studies. 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 the validation include selectivity, sensitivity, matrix effect, limit of detection, linearity, precision and accuracy, recovery, ruggedness, stability, dilution integrity, haemolysis effect, carryover effect and reproducibility.

Analysis of drugs and their metabolites in a biological matrix is carried out using samples spiked with calibration (reference) standards and using quality control (QC) samples. The purity of the reference standard used to prepare spiked samples can affect study data. For this reason, an authenticated analytical reference standard of known identity and purity should be used to prepare solutions of known concentrations (48-53). The reason for validating a bioanalytical procedure is to demonstrate the performance and reliability of a method and hence the confidence that can be placed on the results. All bio-analytical methods must be validated if the results are used to support registration of a new drug or the reformulation of an existing one. It should be noted that the initial validation is only a beginning, as a method should be monitored continually during its application to ensure that it performs as originally validated (54, 55).
2.3 DRUG DELIVERY SYSTEM

2.3.1 Definitions

➢ **Immediate Release Dosage Form** (Conventional Release dosage form): Preparations showing a release of the active ingredient which is not deliberately modified by special formulation and/or manufacturing method. In case of a solid dosage form, the dissolution profile of the active ingredient depends essentially on the intrinsic properties of the active ingredient (56).

➢ **Modified Release Dosage Forms**: Preparations where the rate and/or place of release of the active ingredient(s) is different from that of the conventional dosage form administered by the same route. This deliberate modification is achieved by special formulation design and/or manufacturing method. Modified release dosage forms include prolonged release, extended release (controlled release), sustained release, delayed release, pulsatile release and accelerated release dosage forms (56).

Advantages of extended release over conventional dosage form are

- Improved patient convenience and compliance due to less frequent drug administration
- Reduction in fluctuation in steady state levels and therefore better control of disease condition and reduce intensity of local or systemic side effects
- Increase safety margin of high potency drugs due to better control plasma levels
- Maximum utilization of drug enabling reduction in total amount of dose administered
• Reduction in health care cost through improved therapy, shorter treatment period, less
  frequency of dosing and reduction in personnel time to dispense, administer and
  monitor patients

Disadvantages of extended release over conventional dosage form are

• Decrease systemic availability in comparison to immediate release conventional
dosage forms; this may be due to incomplete release, increase first pass metabolism,
increase instability, insufficient residence time or complete release, site specific
absorption, pH-dependent solubility, etc.

• Poor in vitro/in vivo correlation

• Possibility of dose dumping due to food, physiologic or formulation variables or
  chewing or grinding of oral formulation by the patient and thus, increase risk of
toxicity

• Retrieval of drug is difficult in case of toxicity, poisoning or hypersensitive reactions

• Reduced potential for dosage adjustment of drugs normally administered in varying
  strengths

• Higher cost of formulation

2.3.2 Design of Controlled Drug Delivery Systems

The basic rationale of a controlled drug delivery system is to optimize the biopharmaceutics,
pharmacokinetic and pharmacodynamic properties of a drug. It is done in such a way that it’s
utility is maximize through reduction in side effects and cure or control of condition in the
shortest possible time by using smallest quantity of drug administered by the most suitable
route (57).
Biopharmaceutics Characteristics of the Drug

The performance of a drug presented as a controlled release system depends upon it’s

- Release from the formulation
- Movement within the body during its passage to the site of action

The former depends upon the fabrication of the formulation and the physicochemical properties of drug while the latter element is dependent upon pharmacokinetics of the drug. In comparison to conventional dosage form where the rate-limiting step in drug availability is usually absorption through the bio-membrane, the rate-determining step in the availability of a drug from controlled delivery system is the rate of release of drug from the dosage form which is much smaller than the intrinsic absorption rate for the drug.

The desired biopharmaceutical properties of a drug to be used in controlled drug delivery systems are

- Molecular weight of the drug: Drugs with large molecular size are poor candidate for oral controlled release systems for e.g. peptides and proteins
- Aqueous solubility of the drug: A drug with good aqueous solubility, especially if Ph- independent, serves as a good candidate for controlled release dosage forms
- Apparent partition coefficient of the drug: Greater the apparent partition coefficient of the drug greater is it’s rate and extent of absorption
- Drug pKa and Ionization at Physiologic pH: Drugs existing largely in ionized forms are poor candidates for controlled delivery e.g. hexamethonium
- Drug stability: Drugs unstable in GI environment cannot be administered as oral controlled release formulation because of bioavailability problems e.g. nitroglycerine
• Mechanism and site of Absorption: Drugs absorbed by carrier-mediated transport processes and those absorbed through a window are poor candidates for controlled release systems e.g. several B vitamins

➢ Pharmacokinetic Characteristics of the Drug

• Absorption rate: For a drug to be administered as controlled release formulation, its absorption must be efficient since the desired rate-limiting step is rate of drug release. A drug with slow release will result in a pool of unabsorbed drug e.g. iron

• Elimination half-life: Smaller the T_{1/2}, larger amount of drug to be incorporated in the controlled release dosage form. Drugs with half-life in the range 2 to 4 hours make good candidates for such a system e.g. propranolol

• Rate of metabolism: A drug which is extensively metabolized is suitable for controlled release system as long as the rate of metabolism is not too rapid

• Dosage form index: Since the goal of controlled release formulation is to improve therapy by reducing the dosage form index while maintaining the plasma levels within the therapeutic window, ideally it’s value should be as close to one as possible.

➢ Pharmacodynamic Characteristics of a Drug

• Therapeutic Range: A candidate drug for controlled delivery system should have a therapeutic range wide enough such that variations in the release rate do not result in a concentration beyond this level.

• Therapeutic Index: The release rate of a drug with narrow therapeutic index should be such that the plasma concentration attained is within the therapeutically safe and effective range. This is necessary because such drugs have toxic concentration nearer to their therapeutic range.
• Plasma Concentration-Response Relationship: Drugs such as reserpine whose pharmacologic activity is independent of its concentration are poor candidates for controlled release systems.

2.3.3 Oral Controlled Release Systems

Oral route has been the most popular and successfully used for controlled delivery of drugs because of convenience and ease of administration, greater flexibility in dosage form design (possible because of versatility of GI anatomy and physiology) and ease of production and low cost of such a system. Depending upon the manner of drug release, these systems are reclassified as follows:

➢ **Continuous Release Systems:** These systems release the drug for a prolonged period of time along the entire length of GIT (especially up to the terminal region of small intestine) with normal transit of the dosage form. The various systems under this category
  • Dissolution controlled release systems
  • Diffusion controlled release systems
  • Dissolution and diffusion controlled release systems
  • Ion-exchange resin-drug complexes
  • Slow dissolving salts and complexes
  • Ph-dependent formulations
  • Osmotic pressure controlled systems
  • Hydrodynamic pressure controlled systems

➢ **Delayed Release Systems:** The design of such systems involves release of drug only at a specific site in the GIT. The drugs contained in such a system are those that have following qualities:
• Destroyed in the stomach or by intestinal enzymes
• Known to cause gastric distress
• Absorbed from a specific intestinal site
• Meant to exert local effect at a specific GI site

The two types of delayed release systems are

• Intestinal release systems
• Colonic release systems

➢ **Delayed Transit and Continuous Release Systems:** The gastric emptying time (GET) in humans is normally 2-3 hrs through the major absorption zone, i.e., stomach and upper part of the intestine. It can result in incomplete drug release from the drug delivery system leading to reduced efficacy of the administered dose (58). Therefore, control of placement of a drug delivery system (DDS) in a specific region of the GI tract offers advantages for a variety of important drugs (59). The advantages of gastro retentive drug delivery systems are:

• Enhanced bioavailability
• Sustained drug delivery/reduced frequency of dosing
• Targeted therapy for local ailments in the upper GIT
• Reduced fluctuations of drug concentration
• Improved selectivity in receptor activation
• Extended time over critical (effective) concentration
• Minimized adverse activity at the colon
• Site specific drug delivery
2.3.4 Work on Extended Release Dosage Form

- Basak et al (60) designed floatable gastro retentive tablet of metformin hydrochloride using a gas-generating agent and gel-forming hydrophilic polymer. The formulation was optimized on the basis of floating ability and in vitro drug release. The in vitro drug release test of these tablets indicated controlled sustained release of metformin hydrochloride and 96-99% released at the end of 8 h.

- Jaimini et al. (61) prepared floating tablets of famotidine employing two different grades of methocel K100 (HPMC K100) and methocel K15 (HPMC K15) by an effervescent technique. These grades were evaluated for their gel-forming properties. The tablets with methocel K100 were found to float for a longer duration compared with the formulation containing methocel K15M. Decrease in the citric acid level increased the floating lag time. The drug release from the tablets was sufficiently sustained and non-Fickian transport of the drug from tablets was confirmed.

- Badve et al. (62) developed hollow calcium pectinate beads for floating-pulsatile release of diclofenac sodium intended for chronopharmacotherapy. Floating pulsatile concept was applied to increase the gastric residence of the dosage form having lag phase followed by a burst release. This approach suggested the use of hollow calcium pectinate micro particles as promising floating pulsatile drug delivery system for site- and time-specific release of drugs for chronotherapy of diseases.

- Chavanpatil et al (63) developed a new gastro retentive sustained release delivery system of ofloxacin with floating, swellable and bioadhesive properties. Various release retarding polymers such as psyllium husk, HPMC K100M and a swelling agent, crosspovidone, in combinations were tried and optimized to obtain release profile over 24
hours. The in vitro drug release followed Higuchi kinetics and the drug release mechanism was found to be non-Fickian.

- Rahman et al (64) established a bilayer-floating tablet (BFT) for captopril using direct compression technology. HPMC K-grade and effervescent mixture of citric acid and sodium bicarbonate formed the floating layer. The release layer contained captopril and various polymers such as HPMC-K15M, PVP-K30 and carbopol 934, alone or in combination with the drug. The formulation followed the Higuchi release model and showed no significant change in physical appearance, drug content, floatability or in vitro dissolution pattern after storage at 45 °C/75% RH for three months.

- Xiaoqiang et al (65) developed a sustained release tablet for phenoprolamine hydrochloride because of its short biological half-life. Three floating matrix tablets based on a gas-forming agent were prepared. HPMC K4M and carbopol 971P were used in formulating the hydrogel system. Incorporation of sodium bicarbonate into the matrix resulted in the tablets floating over simulated gastric fluid for more than 6 hours. The dissolution profile of all the tablets showed non-Fickian diffusion in simulated gastric fluid.

A list of marketed modified release oral dosage forms is given in table 2.4.
### Table 2.4: Proprietary modified release oral dosage form in the market

<table>
<thead>
<tr>
<th>Drug product &amp; form</th>
<th>Manufacturer</th>
<th>Marketed Name</th>
<th>Drug Name</th>
<th>Characteristics</th>
<th>Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delayed release Tablets</td>
<td>Knoll</td>
<td>E-Mycin</td>
<td>Erythromycin</td>
<td>Tablets enteric coated with cellulose acetate phthalate, carnauba wax, and cellulose polymer.</td>
<td>Antibiotic</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>tablet</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delayed release Tablets</td>
<td>Procter &amp; Gamble</td>
<td>Asacol</td>
<td>Mesalamine</td>
<td>Tablets coated with Eudragit S (methyl acrylate acid copolymer B), a resin that bypasses the stomach dissolves in the ileum and beyond.</td>
<td>Ulcerative colitis</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>tablet</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delayed release capsules</td>
<td>Astra-Merck</td>
<td>Prilosec</td>
<td>Omeprazole</td>
<td>Enteric coated granules of omeprazole placed in capsules</td>
<td>Duodenal ulcer</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>capsules</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extended-Release coated</td>
<td>Astra-Merck</td>
<td>Toprol-XL</td>
<td>Metoprolol</td>
<td>Drug pellets coated with cellulose polymers compressed into tablets</td>
<td>Hypertension</td>
</tr>
<tr>
<td>particles and Beads</td>
<td></td>
<td></td>
<td>succinate tablets</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extended-Release coated</td>
<td>Astra-Merck</td>
<td>IndocinSR</td>
<td>Indomethacin</td>
<td>Coated pellets for SR formulation includes polyvinyl acetate-crotonic acid copolymer and hydroxyl propyl methyl cellulose.</td>
<td>Analgesic - Anti-</td>
</tr>
<tr>
<td>particles</td>
<td></td>
<td></td>
<td>capsule</td>
<td></td>
<td>inflammatory</td>
</tr>
<tr>
<td>Extended-Release coated</td>
<td>Smithline Beecham</td>
<td>compazine</td>
<td>prochlorperazine</td>
<td>Coated pellets in capsule formulated to release initial dose promptly with addition drug for prolonged release</td>
<td>Anti-nausea, Anti-</td>
</tr>
<tr>
<td>particles</td>
<td></td>
<td></td>
<td>capsule</td>
<td>Drug impregnated in an inert, porous, Plastic matrix, drug leaches out as it passes slowly through the GI tract.</td>
<td>vomiting</td>
</tr>
<tr>
<td>Extended-Release Inert</td>
<td>Abbott</td>
<td>Desoxyn</td>
<td>Methamphetamine HCl tablet</td>
<td></td>
<td>Attention deficient disorder</td>
</tr>
<tr>
<td>Matrix</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extended-Release Inert</td>
<td>Parke-Davis</td>
<td>Procanbid</td>
<td>Procanamid</td>
<td>Extended-release tablets with core tablets of a no erodible wax matrix coated with cellulose polymers</td>
<td>Ant arrhythmic</td>
</tr>
<tr>
<td>Matrix</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drug &amp; product form</td>
<td>Manufacturer</td>
<td>Marketed Name</td>
<td>Drug Name</td>
<td>Characteristics</td>
<td>Use</td>
</tr>
<tr>
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<td>---------------------------------------------------------------------------------</td>
<td>----------------------------</td>
</tr>
<tr>
<td>Extended-Release</td>
<td>Robins</td>
<td>quinidex</td>
<td>Quinidine sulfate</td>
<td>Extended-release provided by hydrophilic matrix that swells and slowly erodes</td>
<td>Ant arrhythmic</td>
</tr>
<tr>
<td>Hydrophilic/Eroding Matrix</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extended-Release</td>
<td>Roxane</td>
<td>Ormorph SR</td>
<td>Morphine sulfate</td>
<td>Sustained-release hydrophilic matrix system, based on polymer hydroxypropyl methylcellulose</td>
<td>Analgesic for severe pain</td>
</tr>
<tr>
<td>Hydrophilic/Eroding Matrix</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extended-Release</td>
<td>Key</td>
<td>K-Dur</td>
<td>Potassium chloride</td>
<td>Immediately dispersing drug micro encapsulated with ethyl cellulose and hydroxypropyl cellulose</td>
<td>Potassium depletion</td>
</tr>
<tr>
<td>microencapsulated</td>
<td></td>
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<td></td>
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<tr>
<td>K-Dur Microburst</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>release system</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extended-Release</td>
<td>Pfizer</td>
<td>Glucotrol XL</td>
<td>Glipizide tablet</td>
<td>Controlled-release GIT, osmotic system. Ingredients include polyethylene oxide, hydroxypropyl cellulose, cellulose acetate</td>
<td>Ant hyperglycemic</td>
</tr>
<tr>
<td>osmotic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Searle</td>
<td>Covera-HS</td>
<td>Verapamil HCl</td>
<td>A COER osmotic system</td>
<td>Antihypertensive, Antianginal</td>
</tr>
<tr>
<td>Extended release</td>
<td>ultramer@ER</td>
<td>Tramadol HCL</td>
<td></td>
<td></td>
<td>Analgesic</td>
</tr>
<tr>
<td></td>
<td></td>
<td>tablet</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extended release film coat</td>
<td>Mylan</td>
<td>Ansaid</td>
<td>Flurbiprofen tablet</td>
<td>Tablets film coated, Enteric coated with, camauca wax, and microcrystalline cellulose polymer.</td>
<td>Analgesic</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Anti inflammatory</td>
</tr>
<tr>
<td>Extended release film coat</td>
<td>Merck</td>
<td>Relafen</td>
<td>Nabumetone tablet</td>
<td>Tablet film coated with microcrystalline cellulose polymer.</td>
<td>Analgesic</td>
</tr>
<tr>
<td>Drug product &amp; form</td>
<td>Manufacturer</td>
<td>Marketed Name</td>
<td>Drug Name</td>
<td>Characteristics</td>
<td>Use</td>
</tr>
<tr>
<td>---------------------------</td>
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<td>-------------</td>
<td>-------------------------------------------------------------------------------</td>
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</tr>
<tr>
<td>Extended release(sodas)</td>
<td>GlaxoSmithKline</td>
<td>Innopran XL</td>
<td>Propranolol HCL</td>
<td>Microencapsulation (beads)(sodas) sperosal oral drug absorption system.</td>
<td>Hypertension</td>
</tr>
<tr>
<td>Extended Release Hydrophilic/Eroding Matrix</td>
<td>Merck</td>
<td>Dolobid</td>
<td>Diflunisal tablet</td>
<td>Tablet hydrophilic matrix system, based on polymer hydroxypropyl methylcellulose</td>
<td>Analgesic</td>
</tr>
<tr>
<td>Extended Release coated particle beads</td>
<td>Noven</td>
<td>Ritalin LA</td>
<td>Methylphenidate hydrochloride capsule</td>
<td>Extended-Release Microencapsulation(beads)</td>
<td>CNS stimulant</td>
</tr>
<tr>
<td>Extended Release film coat</td>
<td>AstraZeneca</td>
<td>Seroquel XR</td>
<td>Quetiapine fumarate</td>
<td>Tablets film coated with microcrystalline cellulose polymer</td>
<td>Psychotropic agent</td>
</tr>
</tbody>
</table>
2.3.5 Advantages of Sustained Release Dosage Form

Olopatadine is available as an immediate release (IR) formulation in tablet and is administered to patients two times a day.

Patients receiving olopatadine or other drugs which are administered twice or thrice daily would likely to benefit from once daily dosing. The advantages of sustained or extended release dosage form are:

- **Improves Patient Compliance**

To maintain therapeutic activity, frequent administration of conventional formulations of many drugs with short half-life is necessary. By maintaining a constant plasma drug concentration for a prolonged period, extended-release dosage forms maximize the therapeutic effect. Another undoubted advantage of extended-release formulation is improved patient compliance (66). Compliance improves dramatically as prescribed dose frequency decreases (8-11).

Kadian™/Kapanol™ (K), extended release capsule formulation of morphine designed for 12- or 24-hourly dosing. In a double-blind study comparing the efficacy and safety of morphine capsule every 24 hr to morphine every 12 hr and MS Contin® tablets (MSC) every 12 hr, it was found that there were no statistically significant differences among the treatments for any morphine-related side effects when adjusted for baseline. K had efficacy and safety profiles similar to MSC every 12 hr but had the advantage of 12- or 24-hourly administration that ultimately leads to increased patient compliance (67).

Especially for elderly patients and for patients taking multiple medications poor compliance is a recognized factor related to the inadequate control. Numerous studies have demonstrated
that poor medication compliance poses a significant impediment to the effective treatment of a wide variety of illnesses.

➢ **Reduction in Fluctuation in Steady State Levels**

The sustained release formulations draw attention in search for improved patient compliance and decreased incidence of adverse drug reactions. Under ideal conditions, a sustained-release formulation maintains therapeutic blood level of a drug for a specific period of time. Oral controlled-release dosage forms have been developed and studied to restrict these systems to specific regions of the gastrointestinal tract as well as to improve the pharmacological activity and to reduce toxic effects (2, 3).

Side effects for sustained release formulations are often less prominent because controlled-release formulations exhibit lower peak plasma drug concentrations when compared with immediate-release formulations. Venlafaxine extended-release (XR), bupropion sustained-release (SR) and paroxetine controlled-release (CR) are 3 commonly utilized controlled-release antidepressants that have demonstrated improvement over their immediate-release predecessors in reducing certain adverse effects (4, 5).

Sustained release (SR) mode of drug administration has certain features that have an important impact on the magnitude of the pharmacologic response: (a) it minimizes fluctuation in blood drug concentrations (i.e. between peak and trough). However, due to the pronounced non-linear relationship between drug concentration and pharmacologic effect (i.e. pharmacodynamics) the impact of this property differs considerably as a function of the shape of the pharmacodynamic profile and the position of the specific range of concentrations on the curve of this profile; (b) it produces a slow input rate which tends to minimize the body's counteraction to the drug's intervening effect on regulated physiological
processes; and (c) it provides a continuous mode of drug administration. For many drugs with non-concentration-dependent pharmacodynamics, the exposure time, rather than the AUC, is the relevant parameter and it can therefore be optimized by SR preparations (1).

In a study comparing sustained-release tablets with ordinary tablets of procaine amide, ten patients with acute myocardial infarction were recruited. During treatment it was found that the average fluctuation of plasma concentrations was 3.5±0.1 µg/ml with sustained-release tablets (dosage interval 8 h) and 4.2±0.4 µg/ml with ordinary tablets (dosage interval 4 h), i.e. it was 20% greater during treatment with the conventional preparation. There was no difference between the two preparations in recovery of the drug from urine (sustained-release tablets 85.4±3.0%; and conventional tablets 90.3±5.4%). Thus, the new sustained-release preparation of procaine amide administered 3 times daily produced the same range of plasma levels as the identical dose of conventional tablets given 6 times a day (68).

➢ Other Advantages of Sustained Release Dosage Form

Number of days for the treatment can be reduced by using sustained release formulation in comparison to conventional immediate release formulation as shown in a study on clarithromycin. A total of 539 patients, aged 12–75 years, were randomized to receive either clarithromycin extended-release (ER) 500 mg once daily for 5 days or penicillin V 500 mg three times daily for 10 days in this multicenter, double-blind, parallel-group trial. Bacterial eradication was sustained in both treatment groups at the follow-up visit 88% (135/153) vs. 91% (112/123) respectively; 95% CI for difference (−10.0, 4.4)). Clinical cure was achieved in ≥ 94% of patients in each treatment group. So it is confirmed that clarithromycin ER 500 mg once daily for 5 days is equally effective as penicillin V 500 mg three times daily for 10 days in the treatment of with streptococcal tonsillopharyngitis (69).
Sustained release formulations can enhance the bioavailability of the drugs which have poor bioavailability because of the narrow absorption window in the upper part of the gastrointestinal tract as seen in the below mentioned examples. Menon et al developed the monolithic sustained release (SR) dosage form of furosemide with prolonged gastric residence time and the bioavailability was increased. AUC obtained with the SR tablets was approximately 1.8 times those of conventional furosemide tablets (79). Similarly, Ichikawa et al developed a multiparticulate system that consisted of floating pills of a drug (p- amino benzoic acid) having a limited absorption site in the gastrointestinal tract. It was found to have 1.61 times greater AUC than the control pills (71).

Sustained drug delivery system is also beneficial for the site specific drug action. In case of amoxicillin conventional immediate release formulation which has short residence time in stomach and amoxicillin being degraded by gastric acid resulting in lesser concentration requiring frequent dosing for treatment of Helicobacter pylori in stomach. This delivery system enabled the release of the drug in stomach for longer period with sustained high levels in gastric blood required for Helicobacter pylori eradication (72). Similarly Yang et al has shown sustained formulation of triple drug regimen (Tetracycline, Metronidazole, Clarithromycin) for Helicobacter pylori associated peptic ulcers has caused sustained delivery of tetracycline and metronidazole over 6-8 hours and concluded that this system has the potential to increase the efficacy of therapy and patient compliance (73).
2.4 ALLERGIC RHINITIS AND URTICARIA

2.4.1 Allergic Rhinitis

Rhinitis is defined as the presence of at least one of the following: congestion, rhinorrhea, sneezing, nasal itching, and nasal obstruction. Other reported symptoms include throat clearing, headaches, facial pain, ear pain, itchy throat and palate, snoring, and sleep disturbances. Allergic rhinitis is present when these symptoms are triggered by an allergen. Perennial allergic rhinitis is most often attributed to dust mites, mold spores, and animal dander, whereas seasonal allergic rhinitis is attributed to a large variety of pollens that varies based on geographical region. Treatment includes avoidance to the exposure of allergens and pharmacological therapies such as use of antihistaminic drugs, steroids, decongestant, cromones, anticholinergic drug (Ipratropium bromide) and leukotriene antagonist etc. (74).

2.4.2 Urticaria

Urticaria affects 10% to 25% of the population at some point in their life. It is characterized by short-lived swellings of the skin, mouth, and genitalia related to a transient leakage of plasma from small blood vessels into the surrounding connective tissues. Urticaria may present with superficial swellings of the dermis called wheals or deeper swellings of the dermis, subcutaneous, or submucosal tissues known as angioedema. Wheals are typically itchy with a pale center due to edema, maturing into pink superficial plaques that usually resolve within 24 hours without sequelae. Areas of angioedema tend to be pale and painful, and last longer than wheals, and may also affect the mouth and rarely the bowel. Pharmacological therapies include antihistaminics, corticosteroids, NSAIDs, immunosuppressive therapy, doxepine (a tricyclic antidepressant) and montelukast (75).
2.5 DRUG DESCRIPTION

Olopatadine hydrochloride (olopatadine, 11-[(Z)-3-(dimethylamino) propyldiene]-6,11-dihydrodibenz[b,e]oxepin- 2-acetic acid monohydrochloride) is a tricyclic compound. Its molecular formula is C21H23NO3·HCL=373.87(14). Olopatadine hydrochloride occurs as a white crystal or crystalline powder. It is odorless and has a bitter taste. It is very soluble in formic acid, sparingly soluble in water and very slightly soluble in ethanol (99.5). Melting point is about 250°C (decomposition) and partition coefficient logP'OCT=0.3 (measured by Flask-shaking method using n-octanol/pH 7.4 buffered solution) (16).

Figure 2.1: Chemical and molecular structure of olopatadine
Olopatadine is a novel antiallergic/histamine H1-receptor antagonistic with multiple mechanisms of action against multiple allergic conditions. It is a potent histamine H1-receptor antagonist and a specific mast cell stabilizer, with additional anti-inflammatory properties (15). Olopatadine hydrochloride principally acts as a selective histamine H1 receptor antagonist. This drug also inhibits the production and release of chemical mediators (leukotriene, thromboxane, PAF, etc.) and the release of the neurotransmitter tachykinin. Olopatadine is indicated for allergic rhinitis, urticaria, itching resulting from skin diseases (eczema/dermatitis, prurigo, pruritus cutaneous, psoriasis vulgaris, multiform exudative erythema) (16).

For detailed drug profile, refer to monograph of the drug (Annexure 1).

2.5.2 Bioequivalence/Bioavailability Studies of Olopatadine

- Ohmori K et al (14) reported that after single oral administration of olopatadine to healthy male volunteers at doses of 5, 10, 20, 40, and 80 mg under fasting conditions, olopatadine was absorbed rapidly, reached $C_{\text{max}}$ values at 0.5-2.0 hrs, and decreased thereafter biexponentially. The elimination half-lives ($t_{\frac{1}{2}}$) were 7.13-9.36 hrs within this dose range. The $C_{\text{max}}$ and $\text{AUC}_{0-\infty}$ values increased in proportion to the dose administered. Therefore, the pharmacokinetics of olopatadine was considered to be linear at the doses from 5 to 80 mg in healthy volunteers. In contrast to many other antiallergic drugs that are eliminated by hepatic clearance, olopatadine was eliminated by the renal clearance. After oral administration under the non-fasting condition, a delayed $T_{\text{max}}$ (by about 0.3 hrs), decreased $\text{AUC}_{0-\infty}$ (by about 16%) and decreased ratio of cumulative urinary excretion within 48-h post-dose (by about 9%) was observed compared to those parameters under fasting conditions. The renal clearance was found to be constant under both fasting and non-fasting conditions. The food
effect on the absorption of olopatadine was not considered to be remarkable. During repeated oral administration of olopatadine to healthy male volunteers at doses of 10 and 20 mg twice daily, the plasma concentration reached the steady state until day 4 after starting the repeated administration. The repeated administration at a dose of 10 mg twice a day caused the increase of $C_{\text{max}}$ by 1.14 fold. The repeated administration at a dose of 20 mg twice a day caused the increases of $C_{\text{max}}$ by 1.19 fold. The plasma concentrations of olopatadine during and after repeated administration agreed with those predicted from the plasma concentrations after single administration, demonstrating that the effects of the repeated administration on the pharmacokinetics are small. It has been demonstrated that the $C_{\text{max}}$ and $\text{AUC}_{0-\infty}$ values in healthy male volunteers after single oral administration of olopatadine at a dose of 20 mg increase by 1.7 and 1.9 times, respectively, compared to those values after administration at a dose of 10 mg, while there were no risk in the safety.

- Chu NN et al (76) conducted a pharmacokinetic study of orally administered single and multiple dose olopatadine in 12 healthy Chinese subjects. The pharmacokinetic parameters for olopatadine following a single dose were: $C_{\text{max}}$ (69.98±20.87 ng/mL), $T_{\text{max}}$ (1.02±0.34 h), $t_{1/2}$ (5.87±4.24 h), $\text{AUC}_{\text{last}}$ (266.00±143.95 ng*h/mL) and $\text{AUC}_{0-\infty}$ (283.46±152.96 ng*h/mL). The pharmacokinetic parameters of olopatadine after multiple doses were similar to those after single dose. In both studies, significantly higher $\text{AUC}_{\text{last}}$, $\text{AUC}_{\infty}$ and $C_{\text{max}}$ and longer $t_{1/2}$ (single dose only) were observed in female subjects compared with male subjects after single and multiple dosing. The study showed that there was no change in absorption and elimination of olopatadine following multiple doses, compared with a single dose and no accumulation was found in plasma, which is consistent with the reported results in Japanese subjects by Ohmori et al (14).