CHAPTER 2

LITERATURE REVIEW

2.0 REVIEW OF LITERATURE

Bioavailability is defined as the rate and extent to which the active ingredient or therapeutic moiety is absorbed from the drug product and becomes available at the site of action. For the drug products, which are not absorbed, or the drug levels in the biological matrix are too low to be reliably measured, bioavailability may be assessed by surrogate measurements that reflect the rate and extent to which the active ingredient or active moiety becomes available at the site of action.

Bioequivalence is a relative term. It is defined as the absence of 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 and under similar conditions in an appropriately designed study (CDER, 2003). 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. Some of the approaches are as follows.

2.1 Historical perspective of BE

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 1.

Table 1: List of major legislations, regulations and other milestones affecting drug development and marketing in the United States and other countries (Truman, 1992, updated).

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

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 (CDER, 1999).

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 dug 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.

2.5 Types of bioavailability

Bioavailability can be classified into four different types (Ritschel and Kearns, 1998), 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.

Absolute bioavailability = $AUC_{extravascular} \times dose_{i.v.} / AUC_{i.v.} \times dose_{extravascular}$

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.

Relative bioavailability = AUC of A / 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 other wise linear kinetics can be described by eqn.

$F = 1 - Dose_{iv} \times f_m / LBF \times AUC_{iv} \times 60 \times \lambda$

f_m - fraction of drug metabolised in liver

LBF - liver blood flow

λ - 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 $EBA_{rel.\ opt.}$, 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

$$EBA_{rel.\ opt.} = AUC_{(drug + vehicle;\ granules;\ tablets)} / AUC_{solution} \times 100$$

2.6 Different approaches used for measurement of bioavailability

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

2. t_{max}: The time of peak plasma concentration corresponds to the time required to reach maximum drug concentration after drug administration. At t_{max}, absorption is maximized and the rate of drug absorption equals the rate of drug elimination. When comparing drug products, t_{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 eqn.

$F = AUC_{oral}\ D_{iv} / AUC_{iv}\ D_{oral}$

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. $(dx_u/dt)_{max}$: 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 eqn:

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

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) (CDER, 2000). 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

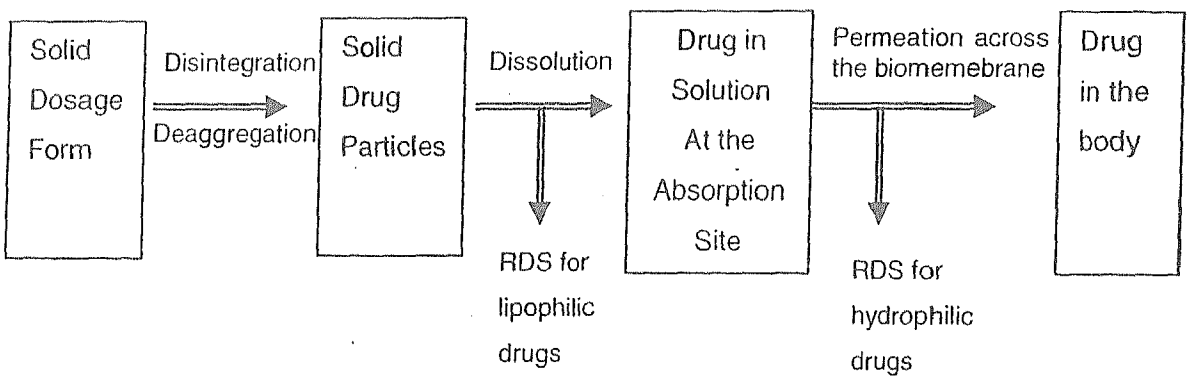
The various factors affecting bioavailability of drugs can be classified as shown in Table: 2

2.7.1 Physicochemical properties of drug substances

2.7.1.1 Drug solubility and dissolution rate

Dissolution is the rate-determining step (RDS) for hydrophobic, poorly aqueous soluble drugs like griseofulvin and spironolactone; absorption of such drugs is said to be dissolution rate-limited. If the drug is hydrophilic with high aqueous solubility e.g. cromolyn sodium or neomycin, then dissolution is rapid and the RDS in the absorption of such drugs is rate of permeation through the biomembrane. Adsorption of such drugs is said to be permeation rate limited or transmembrane rate limited. Fig.1. shows a schematic representation of this concept.

Fig. 1: The two rate-determining steps in the absorption of the drugs from orally administered formulations.



* RDS- Rate Limiting Step

Table 2: Factors affecting absorption of a drug from its dosage form (Brahmankar & Jaiswal, 1995).

PHARMACEUTICAL FACTORS		PATIENT RELATED
Physicochemical properties of drug substances	Dosage form related factors	
Drug solubility and dissolution rate	Disintegration time	Age
Particle size and effective surface area	Dissolution time	Gastric emptying time
Polymorphism and amorphism	Manufacturing variables	Intestinal transit time
Hydrates / solvates	Pharmaceutical ingredients	GIT pH
Salt form of the drug	Nature and type of dosage form	Disease states
Lipophilicity of the drug	Product age and storage conditions	Blood flow through GIT
pKa of the drug and pH		Gastrointestinal contents: ❖ Other drugs ❖ Food ❖ Fluids ❖ Other normal GI contents
Drug stability		Presystemic metabolism by: ❖ Luminal enzymes ❖ Gut wall enzymes ❖ Bacterial enzymes ❖ Hepatic enzymes

2.7.1.2 *Particle size and effective surface area*

Particle size and surface area of a solid drug are inversely related to each other. Particle size is of importance for drugs of low solubility. The critical point seems to be if the solubility is less than 0.3 percent. With decreasing particle size, the surface area increases, thus increasing the area of solid matter being exposed to the dissolution media and, hence, dissolution rate increases. However, the actual solubility does not significantly change with particle size reduction (micronization) in the range used in pharmaceutical manufacture. The following equation describes the dissolution rate:

$$dc/dt = k.\ a.\ (C_s - C_t)$$

dc/dt = dissolution rate (amount per unit time) (Noyes Whitney equation)

a = surface area of undissolved solute

Cs = solubility of drug in solvent

Ct = concentration of dissolved drug at time t

k = constant depending on intensity of agitation, temperature, structure of solid surface and diffusion coefficient

Examples of drugs for which therapeutic differences have been found depending on particle size are: amphotericin, aspirin, bishydroxycoumarin, chloramphenicol, digoxin, acetonide, griseofulvin, meprobamate, nitrofurantoin, phenobarbital, phenothiazine, prednisolone, procaine penicillin, reserpine, spironolactone, sulfadiazine and tolbutamide (Ritschel and Kearns, 1998).

2.7.1.3 *Polymorphism and amorphism*

Polymorphism is the phenomenon that a drug may exist in different crystalline forms, polymorphs. Polymorphism exists only in solid state. The most stable form has highest stability but lowest dissolution rate. The least stable form usually has the most rapid dissolution rate. The unstable (metastable) forms convert more or less slowly into the more stable form, e.g. chloramphenicol palmitate appears in three different polymorphs, but only polymorph B is biologically active, since the other

forms do not dissolve and are not hydrolyzed. The polymorphs differ from each other with respect to their physical properties such as solubility, melting point, density, hardness and compression characteristics.

Some drugs can exist in amorphous form (i.e. having no internal crystal structure). In general, the amorphous state is more soluble and has a higher dissolution rate than the crystalline form. The crystalline form requires a higher amount of energy to free a molecule of drug from it than does the amorphous form, e.g. amorphous novobiocin and amorphous chloramphenicol esters are biologically active while their crystalline forms are inactive.

2.7.1.4 Salt form of a drug

Most drugs are either weak acids or weak bases. One of the easiest approaches to enhance the solubility and dissolution rate of such drugs is to convert them into their salt forms. At a given pH, the solubility of a drug, whether acidic/basic or its salt form, is a constant.

2.7.1.5 Drug pKa and lipophilicity and GI pH-pH Partition hypothesis

The pH Partition theory explains in simple terms, the process of drug absorption from the GIT and its distribution across all biologic membranes. The theory states that for a drug compound of molecular weight greater than 100, which are primarily transported across the biomembrane by passive diffusion, the process of absorption is governed by:

1. The dissociation constant (pKa) of the drug.
2. The lipid solubility of the unionised drug (a function of drug $K_{o/w}$).
3. The pH at the absorption site.

2.7.1.6 Lipophilicity and drug absorption

The pKa of a drug determines the degree of ionisation at a particular pH and that only the unionised drug, if sufficiently lipid soluble, is absorbed into the systemic circulation. Thus, even if the drug exists in the unionised form, it will be poorly

absorbed if it has poor lipid solubility. Ideally, for optimum absorption, a drug should have sufficient aqueous solubility to dissolve in the fluids at the absorption site and lipid solubility ($K_{o/w}$) high enough to facilitate the partitioning of the drug in the lipoidal membrane and into the systemic circulation. Hence, a perfect hydrophilic-lipophilic balance (HLB) should be there in the structure of the drug for optimum bioavailability.

2.7.2 Patient related factors

2.7.2.1 Age

In infants, the gastric pH is high and intestinal surface and blood flow to the GIT is low resulting in decreased absorption in comparison to adults. In elderly persons, causes of impaired drug absorption include altered gastric emptying, decreased intestinal surface area and GI blood flow.

2.7.2.2 Gastric emptying

Apart from dissolution of a drug and its permeation through the biomembrane, the passage from stomach to the small intestine, called as gastric emptying can also be rate limiting step in drug absorption because the major site of drug absorption is intestine. Thus generally speaking, rapid gastric emptying increases bioavailability of a drug.

Rapid gastric emptying is desired where:

1. A rapid onset of action is desired e.g. sedatives
2. Dissolution of drug occurs in the intestine e.g. enteric coated dosage forms
3. The drugs are not stable in the gastric fluids e.g. penicillin G, and erythromycin
4. The drugs is best absorbed from the distal part of the small intestine e.g. vitamin B_{12}

2.7.2.3 Intestinal Transit

Since small intestine is the major site for absorption of most drugs, long intestinal transit time is desirable for complete drug absorption. The residence time depends

upon the intestinal motility or contractions. The mixing movement of the intestine that occurs due to peristaltic contractions promotes drug absorption, firstly, by increasing the drug-intestinal membrane contact, and secondly, by enhancing the drug dissolution especially of poorly soluble drugs, through induced agitation.

2.7.2.4 Blood flow to the GIT

GIT is extensively supplied by blood capillary network and the lymphatic system. The absorbed drug can thus be taken by the blood or the lymph. Since the blood flow rate to the GIT (splanchnic circulation) is 500 to 1000 times (28% of cardiac output) more than the lymph flow, most drugs reach the systemic circulation via blood whereas only a few drugs, especially low molecular weight, and lipid soluble compounds are removed by lymphatic system. The high perfusion rate of GIT ensures that once the drug has crossed the membrane, it is rapidly removed from the absorption site thus maintaining the sink conditions and concentration gradient for continued drug absorption.

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 (Nakai et al, 2002). 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 (Chen et al, 2000). The FDA also has proposed replacing the 1992 average bioequivalence (ABE) approach with population and individual bioequivalence (PBE and IBE) (CDER, 1997).

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 (Williams RL, 1997; Williams RL et al, 2000). 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 (Levy G, 1996). The re-test characteristics of replicate study design allow scrutiny of outliers.

IBE can be calculated as:

$\sigma^2_D = [(\sigma^2_{BT} - \sigma^2_{BR})^2 + 2(1-\sigma)\ \sigma_{BT}\ \sigma_{BR}$

$\theta_1 = [(\mu_T - \mu_R)^2 + \sigma^2_D + \sigma^2_{WT} - \sigma^2_{WR}]/\ \sigma^2_{WR}$ when $\sigma_{WR} > 0.2$

$\theta_1 = [(\mu_T - \mu_R)^2 + \sigma^2_D + \sigma^2_{WT} - \sigma^2_{WR}]/\ 0.2^2$ when $\sigma_{WR} \leq 0.2$

Where,

μ_T = mean (test)

μ_R = mean (reference).

σ^2_{WT} = within subject variance (test)

σ^2_{WR} = within subject variance (reference)

σ^2_{BT} = between subject variance (test)

σ^2_{BR} = between subject variance (reference)

Again, σ^2_{WR} is set to 0.20 (that is, constant scaled versus reference standard) in the denominator of the formula θ_1 when the point estimate of the parameter based on the original data set falls below 0.20 (CDER, 1997).

Individual BE is demonstrated when; θ_1 (0.95) < 2.45, where θ_p (0.95) is defined as the 95th quartile of θ_1 based on the non-parametric percentile method using 2000 bootstrap samples. The bootstrap is used, as the exact distribution for the parameter θ_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 (Hauschke and Steinijans, 2000). 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 (CDER, 1997). 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 (Nakai et al, 2002). 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

The key motivation behind the proposed changes in BE criteria lie in answering more appropriate questions regarding bioequivalence. In the case of pre-marketing approval, one can formulate the bioequivalence question as "*Can a patient begin their therapy with either formulation (commercial or clinical trial) and be assured of same results in terms of safety and efficacy?*" This has been called the concept of prescribability (CDER, 1997) and is linked to PBE criteria. In case of post-marketing changes, the BE question becomes "*Can I safely and effectively switch my patient from their current formulation to another?*" This has been called the concept on switchability and is linked to the IBE criteria. PBE can be calculated as:

$\theta_p = [(\mu_T - \mu_R)^2 + \sigma^2_{TT} - \sigma^2_{TR}] / \sigma^2_{TR}$ when $\sigma_{TR} > 0.2$

$\theta_p = [(\mu_T - \mu_R)^2 + \sigma^2_{TT} - \sigma^2_{TR}] / 0.2^2$ when $\sigma_{TR} \leq 0.2$

Where,

μ_T = mean (test)

μ_R = mean (reference)

σ^2_{TT} = total variance (test)

σ^2_{TR} = total variance (reference)

θ_p is calculated in one of the two ways depending on the point estimate for σ^2_{TR} based on the original data set. When this estimate falls below 0.20, a constant scaling procedure is used. Otherwise, the scaling is proportional to σ^2_{TR}. This has been referred to as 'constant scaled' and 'reference scaled' respectively (CDER, 1997).

Population BE is demonstrated when; θ_p (0.95) < 1.75, where θ_p (0.95) is defined as the 95th quantile of θ_p based on the non-parametric percentile method using 2000 bootstrap samples. The bootstrap is used, as the exact distribution for the parameter θ_p has not yet been derived.

2.9 Design and evaluation of BE 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 design and evaluation of well-controlled bioequivalence studies require the cooperative input from pharmacokineticists, statisticians, clinicians, bio-analytical chemists, and others.

2.9.1 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 (CDER, 2003) 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 discussed below

2.9.2 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 (generally 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:

Vol. No.	Period 1	Period 2
1	A	B
2	B	A
3	A	B
4	A	B
5	B	A
6	B	A

2.9.3 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:

Vol. No.	Period 1	Period 2	Period 3
1	A	B	C
2	B	C	A
3	C	A	B

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

2.9.4 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 or 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:

Vol. No.	Period 1	Period 2
1	A	B
2	A	C
3	A	D
4	B	C
5	B	D
6	C	D
7	B	A
8	C	A
9	D	A
10	C	B
11	D	B
12	D	C

The minimum acceptable number of volunteers will be 18.

$n \geq \{[\sigma]^2\}/2D^2 [t\alpha+t\beta]^2 + 0.25\ t\alpha^2$

Where,

n = no. of volunteers

α = Required level of significance (0.05)

β = Required power of test (0.80)

σ^2 = Error mean sum of squares from ANOVA (estimated/guess)

D = Minimum difference between the means which if present, ought to be detected

The bioequivalence studies are conducted according to a well-defined protocol. Some elements of a bio-equivalence protocol are listed in Table 3:

Table 3: Elements of the bioavailability Protocol.

INVESTIGATORS' DECLARATION
FACILITIES
2.1 Clinical Services & Clinical Laboratory
2.2. Analytical, Pharmacokinetics & Statistical Services
OBJECTIVE
PRODUCTS TO BE EVALUATED
4.1 REFERENCE (R)
4.2 TEST (A)
4.3 TEST (B)
INTRODUCTION
PHARMACOLOGY
6.1 Absorption, Distribution, Metabolism and Excretion
6.2 Adverse Effects
6.3 Dosage
STUDY DESIGN
7.1 Summary
7.2 Number of Subjects
7.3 Admissions and Stay
7.4 Fasting/Meals
7.5 Sampling Schedule
7.6 Blood Pressure
7.7 Washout Period
RESTRICTIONS
8.1 Medications
8.2 Diet
8.3 Activity
SELECTION OF SUBJECTS
9.1 Inclusion Criteria
9.2 Exclusion Criteria
10. SCHEDULE OF ASSESSMENTS
STUDY MEDICATION
11.1 Handling, Storage and Accountability Procedures

11.2 Dose
11.3 Assignment to Treatment Sequences
11.4 Assessment of Compliance
HAEMODYNAMIC MEASUREMENTS
PHARMACOKINETICS
13.1 Blood Sampling
13.2 Analytical Procedures
13.3 Pharmacokinetic Parameters
SAFETY
14.1 Clinical Safety Measurements
HANDLING OF SAFETY PARAMETERS
15.1 Adverse Events
STATISTICAL ANALYSIS
DEVIATIONS
ETHICAL CONSIDERATION
18.1 Basic Principles
18.2 Institutional Review Board
18.3 Informed Consent
18.4 Withdrawal/Drop-out of Subjects from Study
18.5 Volunteer Compensation
TERMINATION OF THE STUDY
STUDY DOCUMENTATION
QUALITY ASSURANCE AUDIT
CONFIDENTIALITY OF DATA
ARCHIVES
PUBLICATION POLICY
REFERENCES
LIST OF APPENDICES

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, Barttlet'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. Tmax values being discrete, data on Tmax should be analysed using non-parametric methods.

2.9.6 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 (CDER, 2003).

Current DCGI requirements for bio-equivalence approval are that 90% confidence interval should be within 80-125% for log transformed C_{max} and for log transformed AUC. For narrow therapeutic index drugs, the same criterion i.e., log transformed C_{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_{max} and for log transformed AUC. For narrow therapeutic index drugs, log transformed C_{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 4 mentions the bioequivalence criteria followed by various regulatory agencies in the world.

Table 4: Criteria of bio-equivalence of various regulatory agencies.

Parameter	CPMP (EU)	USFDA	CANADIAN FDA (CEC)	DCGI
Log transformed C_{max} using 90% CI	▫ 80-125% of reference ▫ 70-143% of reference (If clinically acceptable). Tighter limits for C_{max} accepted for NTI drugs.	▫ 80-125% of reference and otherwise indicated for NTI drugs. The range is same as the wide margin drugs.	▫ 80-125% of reference ▫ 90-111% of reference for NTI drugs.	▫ 80-125% of reference ▫ 80-125% of reference for NTI drugs.
Log transformed AUC_{0-t} using 90% CI	▫ 80-125% of reference ▫ 80-125% of reference for NTI drugs.	▫ 80-125% of reference ▫ 80-125% of reference for NTI drugs.	▫ 80-125% of reference ▫ 80-125% of reference for NTI drugs.	▫ 80-125% of reference.

*NTI- Narrow Therapeutic Index

2.10 Bipolar Disorders

Bipolar disorder (BPD) is one of the most common, severe forms of mental illness, often life threatening and is characterized by swinging moods (Goodwin & Jamison, 1990; Shastry BS, 2005). It affects both sexes equally in all age groups and its worldwide prevalence is approximately 3-5%. The clinical course of illness can vary from a mild depression to a severe form of mania. The condition has a high rate of recurrence and if untreated, it has an approximately 15% risk of death by suicide. It is the third leading cause of death among people aged 15-24 years and is a burden on society and families (Shastry BS, 2005).

Epidemiologic studies reports that the life time prevalence of rate of a manic episode is 1.6% ± 0.3 for men and 1.7% ±0.3 for women in the United States (Kessler RC et al, 1994). The lifetime prevalence of bipolar I disorder is 0.4% to 1.6%; that for bipolar II disorder is 0.5% in community samples (APA, 2000; Schwartzberg AF, 1998). Bipolar Disorder has a higher genetic risk than major depressive disorders (Nathan KI et al, 1995). The exact mechanism of genetic transmission is not known and may involve multifactorial inheritance. Linkage studies suggest that loci on 4, 6, 12, 18, 21, 22, and X chromosomes may contribute to genetic susceptibility of bipolar disorders (Goodnick PJ, 1998; Pato CN, 2000).

The Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR) of American Psychiatric Association divides the bipolar disorders into four subtypes based on the identification of specific mood episodes: bipolar I, bipolar II, cyclothymic disorder, and bipolar disorder not otherwise specified. The mood states are further separated into four subcategories to differentiate the current or most recent mood episode: major depressive, manic, hypomanic, or mixed (APA 2000). Depressive episodes are usually longer in duration than manic episodes and woman tend to have more depressive episodes than do men (Goldberg JF and Harrow M, 1999).

The onset of bipolar disorder is rare before puberty, but its incidence increases during late adolescence and into early adulthood (between the age of 15 and 30) (APA, 2000; Goodnick PJ, 1998). Bipolar disorder in children and adolescent is often harder to recognize and diagnose than in a typical adult patient. Attention deficit/hyperactivity disorder (ADHD) and a manic episode have similar characteristics; thus, many children with bipolar disorder are misdiagnosed as ADHD (Goodnick PJ, 1998; Practice Parameters, 1997).

Suicide attempts occur in 25% of patients with bipolar disorder, and approximately 10% to 15% of individuals with bipolar disorder commit suicide (Goldberg JF and Harrow M, 1999; Strakowski SM et al, 1996; Jamison 1986). Suicide ideation and attempts are most likely to occur in a depressive or mixed state and in alcoholic patients. In addition to suicide, major mood disorders are also associated with many other deleterious health related effects, and the costs associated with disability and premature death represents an economic burden of tens of billions of dollars annually in the USA alone (Greenberg et al, 1993; Wyatt and Henter, 1995).

Treatment of bipolar disorder must me individualized because the clinical presentation, severity, and frequency of episodes vary widely among patients. Currently, lithium, valproic acid and carbamazapine are the only mood stabilizers recommended by the expert consensus practice guidelines for maintenance monotherapy for bipolar disorder (Hirschfeld RMA et al, 1994; Frances A et al, 1996).

Lithium, the first mood stabilizer approved by the FDA, is effective for the acute and prophylactic treatment of bipolar disorder (Bowden CL, 1998). Although lithium is traditionally the drug of choice for bipolar disorder, anticonvulsants as valproic acid and carbamazapine are accepted alternatives or adjuncts to lithium (Post RM et al, 1996; Sachs GS, 1996).

2.11 Lithium Carbonate: A Drug Profile

Lithium carbonate is an antimanic drug indicated in the treatment of manic episodes of manic-depressive illness. Lithium is the lightest element of the alkali-metals (Group Ia) together with sodium, potassium, rubidium, cesium and francium with atomic no.3, atomic weight 6.94 & an emission line at 671 nm on the flame photometer.

Due to the arrangement of its electrons and the high density of the positive charge on its nucleus, lithium is extremely reactive, and the metal never occurs free in nature. In nature it occurs in minerals and mineral waters and in trace amounts in sea water, plants and animal tissues. In animal tissues, it has no known physiological role. The salts of this monovalent cation share some characteristics with those of Na^+ & K^+ (Schou, 1968).

Lithium carbonate is a white, odourless, light alkaline powder. It is slightly soluble in water (1in 100) and practically insoluble in alcohol.

Molecular formula & Molecular weight of lithium carbonate:

Molecular formula - Li_2C0_3

Molecular weight - 73.89

2.11.1 History

Lithium was introduced into materia medica in about 1845 (Ure A, 1843) as a drug for treatment of diseases thought to be caused by gastric uric acid, i.e. 'the uric acid diathesis'. Lithium bromide was employed in that era as a sedative and as a putative anticonvulsant. Lithium citrate and lithium carbonate were incorporated in the British Pharmacopoeia in 1864. Hammond (1871) recommended a high first-day dose of lithium followed by smaller maintenance doses for acute treatment of mania and depression, and Lange (1886) recommended prophylactic lithium for periodic depression.

In 1940's lithium chloride was also employed as a salt substitute for cardiac and other chronically ill patients, which led to severe intoxication and deaths (Goodman and Gilman's, 2001). The rediscovery of the antimanic and prophylactic effects of lithium was made in 1949 by Cade and in 1963 and 1964 by Hartigan and Basstrup respectively (Hartigan, 1963; Basstrup, 1964). Cade, in Australia, while looking for toxic nitrogenous substances in the urine of mental patients for testing in guinea pigs, administered lithium salts to the animals in an attempt to increase the solubility of urates. Lithium carbonate made the animals lethargic, and, in an inductive leap, Cade gave lithium carbonate to several agitated or manic psychiatric patients as early as 1948 (Mitchell et al, 1999). In 1949, he reported that this treatment seemed to have a specific effect in mania (Cade, 1949).

Cade's discovery was not only a major event in the history of Australian medical research but represented one of the landmark discoveries of biomedical research worldwide (Anderson & Larkins, 1998), with a 1994 article in science estimating that it had saved the USA economy alone US$ 145 billion since its introduction in 1970 (Kirschner et al, 1994).

2.11.2 Pharmacology

Lithium is well established as an efficacious treatment for bipolar disorders. Double blind controlled trials, mostly from 1960s-to the 1980s, have found lithium to be effective in acute mania, acute depression, and the prophylaxis of mood episodes in bipolar disorders (Dunner et al, 1976; Gelenberg et al, 1989; Fieve et al, 1976). Lithium is also the only mood stabilizer with FDA approved indication for both acute mania and prophylaxis.

The precise mechanism of action of lithium as a mood-stabilizing agent remains unknown, although many cellular actions of lithium have been characterized (Manji et al, 1999b). Many hypothesis have been proposed regarding the pathophysiology of mood disorders, including neurotransmitter, neuromodulator, signal transduction, neuroanatomic and physiologic abnormalities (Nathan et al, 1995; Goodwin and Jamison, 1990).—

- The serotonin hypothesis proposes that the central activity of 5-HT, which plays a critical role in modulating CNS activity (e.g., stabilization of the catecholamine system and inhibition of dopamine), is low in both mania and depression (Goodnick PJ, 1998). Lithium produces a subsensitivity of presynaptic inhibitory $5\text{-}HT_{1A}$ receptors that facilitates the release of 5-HT (Treiser SL et al, 1982) and increase post synaptic 5-HT receptor activity (Lenex RH and Hahn CG, 2000).

- Lithium attenuates nor-adrenaline stimulated c- AMP accumulation (Form and Valdecasas, 1971; Ebstein R et al, 1976) perhaps at the level of G proteins, which acts to convey the signal between receptors and adenylate cyclase (Avissar S et al 1988). Numerous studies have demonstrated that c-AMP accumulation by various neurotransmitters and hormones is inhibited by lithium at therapeutic concentrations both in vivo and in vitro (Jope, 1999; Risby et al, 1991). This may correct the imbalance in the turnover of brain monoamines.

- Lithium down regulates second messenger systems that are associated with cAMP –linked receptors. Lithium inhibits the activity of Inositol monophosphatase (Berridge et al, 1982; Wyatt and Henter, 1995; Vestergaard and Agaard, 1991). As a result the supply of free inositol is reduced for regeneration of membranes phosphatidyl-inositides, which are the sources of IP3 and DAG. The hyperactive neurons may be preferentially affected, because the supply of inositol from extra cellular sources is meager (Berridge et al, 1989). None of the above two potential actions led to the successful development of new drugs (Shaldubina A et al, 2001)

- Lithium inhibits the collapse of sensory neuron growth cones and increase growth cone area. Inositol, however, reverses the effects of the drugs on growth cones, thus implicating inositol depletion in their action (Williams RS et al 2002).
- Lithium can modify some hormonal responses mediated by adenylyl cyclase or PLC in other tissues, including the action of ADH & TSH on their peripheral target tissues (Manji et al, 1999b; Urabe et al, 1991).

- Lithium has limited effects on catecholamine –sensitive adenylyl cyclase activity or on the binding of ligands to monoamine receptors in brain tissue (Manji et al, 1999b; Turkka et al, 1992).

- In part, the actions of Li+ may reflect its ability to interfere with the activity of both stimulatory and inhibitory GTP- binding proteins (Gs and Gi) by keeping them in their less active αβγ trimer state (Jope, 1999; Manji et al, 1999b).

- Lithium treatment also leads to consistent decrease in the functioning of Protein Kinases in the brain tissues, including Ca+ activated, phospholipid dependent PKC (Jope, 1999; Lenox & Manji, 1998) particularly subtypes of ∝ & ε (Manji et al, 1999b). *These effects may alter the release of amines NTs & hormones* (Wang & Freidman, 1989; Zatz & Reisine, 1985) as well as the activity of tyrosine hydroxylase (Chen et al, 1998).

- Another important protein kinase that is inhibited by lithium is glycogen synthase kinase –3 β (GSK-3β) (Klein PS and Melton DA, 1996) which is involved in the neuronal & nuclear regulatory processes, including limiting expression of the regulatory protein β- catenin (Chen et al, 1999b; Manji et al, 1999b,).

- Other newly proposed mechanism of action of lithium includes inhibition of binding of serotonin (5HT) to 5HT1B Receptors (Massot O et al, 1999), effects on glutamate uptake and release (Dixon JF, 1998) and an increase in the levels of the neuroprotective protein bcl-2 (Manji et al, 1999b).

- Lithium decreased urinary concentrating ability (nephrogenic diabetes insipidus) with a disturbed responsiveness of the distal nephron to the action of ADH (vasopressin) is demonstrable, and the symptoms are largely reversible on cessation of lithium or reduction of the dose (Walker, 1993).

- It has some insulin like actions on glucose metabolism. Lithium ion, like insulin, activated adipocyte glycogen synthase with or without glucose in the medium. Effect of lithium ion was much greater than that of insulin. . The effect of lithium ion on glycogen synthase was rapid (Cheng K, 1983).

- Leucocyte count is increased by lithium therapy. Lithium leads to a release of hematopoietic growth factors (CSF) and therefore to proliferation of neutrophil granulocytes quite significantly and, to a lesser extent, the number of eosinophil granulocytes and lymphocytes as well. An effective and very cost-effective alternative for treating neutropenia is to administer lithium carbonate (Hager ED, 2002).

- Lithium causes thyroid enlargement (Schou et al 1968) and thus impairs thyroid functions (Lubey et al, 1971). It is reported that the around 4 % patients receiving lithium develop hypothyroidism (with a female: male ratio of 9:1) (Mannisto, 1980). Lithium appears to exert its effect on the thyroid primarily by inhibiting thyroid hormone release, resulting in a compensatory elevation of TSH levels (Salata and Klein, 1987).

2.11.3 Pharmacokinetics

- Lithium is absorbed readily & almost completely from the gastro–intestinal tract. Less than one percent of the lithium given as lithium carbonate leaves the body with the feces (Hullin et al, 1966). Complete absorption occurs in about 6-8 hours, with peak concentration in plasma 2-4 hours after immediate release and 3-5 hours after sustained release of an oral dose (Goodman and Gilman, 2001). The optimal place for absorption of lithium is small intestine.

- With repeated administration, lithium excretion increases during first 5-6 days until steady- state is reached between ingestion & excretion.

- Lithium initially is distributed in the extra cellular fluid & then gradually accumulates in various tissues. The final Vd (0.7-0.9 l / kg) approaches that of total body water.

- After the peak, a biphasic fall occurs. The initial phase lasts about 1-1.5 hrs and is followed by a slower phase with a half-life ranging from 8-45 hrs with a mean of 24 hrs (Amdisen A and.Carson SW, 1986). Elimination can be simulated by a two compartment model (Nielsen-Kudsk F and Amdisen A, 1979).

Table 5: Pharmacokinetic parameters of lithium (Dollery, 1999).

Pharmacokinetic Parameter	Value
Oral absorption	>97%
Presystemic metabolism	nil
Plasma half life range	8-45 h
Volume of distribution	0.8 l/kg
Plasma protein binding	< 10%

- The ion does not bind appreciably to plasma protein & does not get metabolized.
- Passage through the blood brain barrier is slow & at steady-state the concentration in CSF is 40-50 % of the concentration in plasma.
- Approximately 95 % of a single dose of lithium is eliminated in urine, 33-66 % of an acute dose is excreted during a 6-12 hours initial phase of excretion, followed by slower excretion.
- 80 % of filtered lithium is reabsorbed by proximal renal tubules; clearance of lithium by kidney is about 20 % of that for creatinine ranging between 15-30 ml/minutes (Lower in elderly- 10 to 15 ml/minute).
- Loading with Na+ produces a small enhancement of lithium excretion, but Na+ depletion promotes a clinically important degree of retention of lithium.

- The concentration of lithium in blood usually is measured at a trough of the oscillation that results from repetitive administration, but the peaks can be 2 or 3 times higher at steady-state. When the peaks are reached, intoxication may result, even when concentrations in morning samples of plasma are in the acceptable range of around 1 mEq / l.
- Nevertheless, because of low margin of safety of lithium & short T1/2 during initial distribution, divided daily doses are used, & even slow release formulation is given twice.
- Less than 1 % of ingested lithium leaves the human body in feces & 4-5 % in sweat. Lithium is also secreted in saliva in concentration about twice those in plasma, while its concentration in tears is about equal to that in plasma. Since the ion also is secreted in human milk, women receiving lithium should not breast –feed infants (Goodman and Gilman, 2001).

2.11.4 Therapeutic Indications

- Prophylactically in bipolar and unipolar manic depressive illness for attenuation of both manic and depressive episodes (Johnson GF, 1987).
- Prophylactically in the attenuation of schizo-affective disorders (Tyrer SP, 1988).
- Acute treatment of mania and depression (Tyrer SP, 1985).
- Treatment of endogenous depression and pathological aggression (Wickham EA and Reed JV, 1987; Tyrer SP, 1988): - patients with unstable character disorders and episodic aggressiveness and assaultive behavior, violent psychopaths.
- Use in other non-psychiatric conditions: – acute thyrotoxicosis, cluster headache, Meniere's disease.

2.11.5 Contraindications

- Kidney disorders
- Heart disease
- Disturbed electrolyte balance
- Salt poor diets
- Major surgeries
- *Pregnancy, delivery and lactation*
- Psoriasis
- Acne form eruptions

2.11.6 Adverse Effects

The occurrence and severity of adverse reactions are generally directly related to serum lithium concentrations as well as to individual patient sensitivity to lithium, and generally occur more frequently and with greater severity at higher concentrations. Adverse reactions may be encountered at serum lithium levels below 1.5 mEq/L. Mild to moderate adverse reactions may occur at levels from 1.5 to 2.5 mEq/L and moderate to severe reactions may be seen at levels of 2.0 mEq/L and above.

Fine hand tremor, polyuria, and mild thirst may occur during initial therapy for the acute manic phase, and may persist throughout treatment. Transient and mild nausea and general discomfort may also appear during the first few days of lithium administration.

Lithium toxicity may be assessed on the basis of symptoms, and if these are present treatment should be stopped immediately (Amdisen A, 1988; Hansen HE and Amdisen A, 1978).

Diarrhea, vomiting, drowsiness, muscular weakness, and lack of coordination may be early signs of lithium intoxication, and can occur at lithium levels below 2.0 mEq/L. At higher levels, ataxia, giddiness, tinnitus, blurred vision, and a large output of dilute urine may be seen. Serum lithium levels should not be permitted to exceed 2.0 mEq/L during the acute treatment phase (Eskalith, 2003). A vicious circle may

also be initiated by temporarily reduced renal function due to kidney disorders, dehydration due to fever, vomiting, diarrhea, salt poor diets and heavy sweating (residence in a hot climate, occupational, etc.) (Hestbech J et al, 1977; Olsen S, 1976).

Serious effects on central nervous system are characterized by mental confusion, hyperreflexia, gross tremor, dysarthria, seizures, and cranial nerve and focal neurological signs, progression to coma and death; some times neurological damage may be irreversible. Other toxic effects are cardiac arrhythmias, hypotension and albuminuria (Baldessarini et al, 1996b).

2.11.7 Drug Interactions:

- Haloperidol and Lithium: An encephalopathic syndrome-weakness, lethargy, confusion, tremors, leucocytosis, followed by irreversible brain damage in some patients. Should be monitored for early evidence of symptoms, although no casual relationship in this interaction (Amdisen A, 1982; Cohen WJ and Cohen NH, 1974).
- Neuromuscular blocking agents: Succinylcholine and pancuronium produces prolonged paralysis in lithium treated patients (Amdisen A, 1982).
- Indomethacin, Piroxicam other NSAIDS -increases steady-state plasma level of lithium-toxicity in some cases (Amdisen A, 1988).
- Diuretics or Angiotensin converting enzyme inhibitors – because sodium loss may reduce the renal clearance of lithium and hence risk of lithium toxicity (Amdisen A, 1982).
- Noradrenaline: Lithium reduces the presser responses to nor-adrenaline.
- Insulin / Sulfonylurea: Lithium tends to enhance insulin / sulfonylurea induced hyp
- Antidepressants: Lithium augments antidepressants in cases of unsatisfactory responses, a potentially useful interaction (Heninger GR et al, 1983).

2.11.8 Dosage and Administration

Because the therapeutic ratio is narrow and both efficacy and toxicity are related to concentration, therapeutic monitoring of serum lithium concentrations is universal (Amdisen A and Nielsen-Kudsk F, 1986). The relationship between the desired effects and serum concentration of lithium have not been fully validated and there is substantial inter individual variations (Amdisen A, 1987). The therapeutic range is usually stated to be from 0.75 -1.25 mEq/l although a lower range of 0.5 - 0.8 mEq/l has also been proposed (Baldessarini, 1985; Vestergaard P and Thomsen K, 1981). All lithium preparations are administered orally.

- 0.6 -1.25 mEq/l — effective and safe
- 0.9 -1.1 mEq/l — for acute mania and hypomanic patients.
- 0.6 - 0.75 mEq/l — for long-term use or for recurrent manic-depressive illness.
- 0.5 - 0.6 mEq/l — no relapse in some patients. Lower levels are better tolerated (Maj et al, 1986; Tondo et al, 1998a).
- Above concentrations obtained at 10-12 hours after the last dose.
- The above conc. can be attained by doses of 900-1500 mg of lithium carbonate per day.
- Elderly: use with caution. Adjust serum levels to the lower ends of the above ranges.
- Children: not recommended for use in children under 12 years of age.

2.12 Summary of various BE studies of lithium

Very few BE studies of Lithium have been carried out by various investigators. Some are listed in Table 6.

Table 6: Different bioequivalence studies conducted on Lithium reported in the literature

S. No.	Study	Conclusion	Ref.
1.	Absorption of lithium following administration of slow-release and conventional preparations.	There was no difference in the rate of absorption. (CI not applied)	Tyrer S et al, 1976
2.	Bioavailability of lithium carbonate: in vivo comparison of two products.	The two formulations were bioinequivalent (CI not applied)	Meinhold et al, 1979
3.	Steady-state lithium blood level fluctuations in man following administration of a lithium carbonate conventional and controlled release dosage form.	The two dosage forms of lithium carbonate were bioequivalent and tablet (controlled release) produced smoother serum curve than capsule (conventional). (CI applied)	Caldwell HC et al, 1981
4.	Single-dose bioavailability of two extended-release lithium carbonate products.	The two marketed brands of extended-release lithium carbonate were bioinequivalent. (CI applied)	Kirkwood CK et al, 1994
5.	Bioavailability of immediate and controlled release formulations of lithium carbonate after single and multiple doses.	The two brands of lithium carbonate after single dose were bioequivalent; however, they were not after multiple doses. (CI applied)	Luciana et al, 2002