"A BIOEQUIVALENCE STUDY OF CARVEDILOL TABLETS 12.5 mg IN HEALTHY HUMAN SUBJECTS UNDER FED CONDITION"

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IN

PHARMACOLOGY

BY

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APRIL 2010

CERTIFICATE

This is to certify that ***Mr. KUSHAL H. PATEL*** *has prepared his thesis entitled "A Bioequivalence Study of Carvedilol Tablets 12.5 mg in Healthy Human Subjects under Fed Condition" in partial fulfillment for the award of M. Pharm. degree of the Nirma University, under our guidance. He has carried out the work at the Department of Pharmacology, Institute of Pharmacy, Nirma University.*

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I declare that the thesis entitled "A Bioequivalence Study of Carvedilol Tablets 12.5 mg in Healthy Human Subjects under Fed Condition" has been prepared by me under the guidance of Dr. Shital J. Panchal, Assistant Professor, and Dr. Bhoomika R. Goyal, Assistant Professor, Department of Pharmacology, Institute of Pharmacy, Nirma University. No part of this thesis has formed the basis for the award of any degree or fellowship previously.

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Date: **kushal Patel**

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CHAPTER-I

ABSTRACT

Aim & Objective: Carvedilol is a third-generation, nonselective β-blocker that also possesses α1- adrenergic blocking and antioxidants activity. Carvedilol is a multiple action oral antihypertensive drug. It is also used in left ventricular heart failure and CHF. This study was performed to compare the bioequivalence of a locally made oral tablet (test product) of carvedilol with the innovator's product (reference product) by using data from plasma carvedilol concentration.

Materials and Methods: Twelve healthy volunteers were selected after screening in the study that was of a randomized, open label, balanced, two treatment, two periods, two sequence, single dose, crossover design bioequivalence study, with a one week wash-out period. After an overnight fast, high fat high calories breakfast, which was started by subject 30 min before dosing and for at least 4 hrs post dose in each period. A single 12.5 mg carvedilol tablet of either the reference product or the test product was orally administered to each subject. A venous blood sample of five milliliters was drawn prior to dosing and at different time points intervals up to 48 hrs after dosing. LC/MS/MS was used to analyze the plasma sample for total carvedilol concentration.

Results: The results showed that the maximum carvedilol concentration (Cmax) of all subjects found 29.25 ± 11.63 and 32.30 ± 16.75 ng/ml in reference and test product, respectively. Area under the plasma concentration-time profile curve from time zero to last time (AUC_{0-t}) was 144.12 ± 41.95 and 160.38 ± 72.30 hr*ng/ml in reference and test product, respectively. Area under the plasma concentration-time profile curve from time zero to infinity time from observed (AUC_{0-inf} (observed)) was 159.08 ± 49.30 and 167.08 ± 74.31 hr*ng/ml in reference and test product, respectively. Mean time to reach maximum plasma concentration (Tmax) was 2.34 ± 0.97 and 2.30 ± 1.00 hours in reference and test product, respectively. The 90% confidence interval (CI) for logarithm transformation data of the ratio geometric mean of Cmax, AUC_{0-last} and AUC_{0-inf} (observed) between these two treatments were 92.76-123.45, 92.65-120.11 and 88.15-114.49, respectively. The mean Cmax, AUC_{0-last} and AUC_{0-inf} (observed) between the two products were not statistically different.

Conclusion: It can be concluded that the test product was bioequivalent to the reference product based on the criteria that the percent ratio of test parameters was within the range of 80.00-125.00 % with a 90 % level of confidence in terms of rate and extent of absorption. Carvedilol found to be well tolerated and no serious adverse events were found during study.

CHAPTER-II
INTRODUCTION

New drugs, like other new products, are developed under patent protection. The patent protects the investment in the drug's development by giving the company the sole right to sell the drug while the patent is in effect. When patents or other periods of exclusivity expire, manufacturers can apply to the regulatory authority to sell generic versions. The generic drug process does not require the drug sponsor to repeat costly animal and clinical research on ingredients or dosage forms already approved for safety and effectiveness. In most countries, generic manufacturers must only prove that their preparation is bioequivalent to the existing drug in order to gain regulatory approval.

In the early 1970s interest in generic drug products began to increase as various third- party groups sought to reduce the cost of prescription medication. At that time there was no formal approval process that was routinely used by the US Food and Drug Administration (FDA) to evaluate the safety and efficacy of generic drug products. In the intervening years, bioequivalence has been adopted by the FDA as a means to determine the equivalence of multisource drug products, since the introduction of generic products, the design and analysis of bioequivalence studies have continued to evolve and improve.

Studies to measure bioavailibility and/or establish bioequivalence of a product are important elements in support of INDs (Investigational New Drug), NDAs (New Drug Application), ANDAs (Abbreviated New Drug Application), and their supplements. As part of INDs and NDAs for orally administered drug products, BA studies focus on determining the process by which a drug is released from the oral dosage form and moves to the site of action. BA data provide an estimate of the fraction of the drug absorbed, as well as its subsequent distribution and elimination. BA can be generally documented by a systemic exposure profile obtained by measuring drug and/or metabolite concentration in the systemic circulation over time. The systemic exposure profile determined during clinical trials in the IND period can serve as a benchmark for subsequent BE studies. **(CDSCO Guidelines-2005, CPMP Guidelines-2001)**

BE studies are a critical component of ANDA (Abbreviated New Drug Application) submissions. The purpose of these studies is to demonstrate BE between a pharmaceutically equivalent generic drug product and the correspondence reference listed drug. Together with the determination of pharmaceutical equivalence, establishing BE allows a regulatory conclusion of therapeutic equivalence.

Studies to establish bioequivalence between two products are also important for certain changes before approval for a pioneer product in NDA and ANDA submissions and in the presence of certain post approval changes in NDAs and ANDAs. In BE studies, an applicant compares the systemic exposure profile of a test drug product to that of a reference drug product (RLD). For two orally administered drug products to be bioequivalent, the active drug ingredient or active moiety in the test product must exhibit the same rate and extent of absorption as the reference drug product. **(FDA Guidelines, 2003)**

BE information is required to ensure therapeutic equivalence between a pharmaceutically equivalent test drug product and a reference listed drug. In the process of development of a new generic formulation of any product, it is important to investigate the relative bioequivalence of new product in comparison with a market standard.

Carvedilol is a third-generation, nonselective β-blocker that also possesses α1- adrenergic blocking and antioxidant. S (-)-carvedilol contain both the β-and α- adrenoceptor activity, where as R (+)-carvedilol is only α-blocker. The racemic mixture, which is used clinically, provides the full pharmacological effect of carvedilol. **(Colin D, 1999)** Although β-blockers have been used for the treatment of hypertension for more than three decades, no study show that monotherapy with traditional β-blockers reduces morbidity and mortality compared with placebo. In most elderly patients with essential hypertension, cardiac output is low and systemic vascular resistance is elevated. Therefore, antihypertensive therapy should aim: i) to diminish vascular resistance, and ii) to preserve systemic blood flow by maintaining cardiac output. Numerous studies have confirmed that traditional non-vasodilating β-blockers lower arterial pressure by decreasing cardiac output while systemic vascular resistance remains unchanged or even increase. **(Messerli FH and Grossman E, 2004)** Carvedilol blocks both β1- and β2- adrenergic receptors, resulting in improved myocardial function and attenuation of adverse myocardial remodeling in heart failure. Carvedilol also reduces peripheral vascular

resistance via vasodilation caused by antagonism of α1-adrenergic receptor. **(Dulin B and Abraham WT, 2004)**

Carvedilol, the first β-blocker labeled in United State for the treatment of heart failure, has been shown to improve left ventricular function and may reduce mortality. Physicians treating patients with heart failure have traditionally selected agents with positive inotropic or peripheral vasodilatory effect and have avoided agents such as beta-blocker, which exert negative inotropic. Until recently, beta-blockers were contraindicated in the treatment of congestive heart failure (CHF), largely because heart failure was viewed primarily as a hemodynamic disorder. Many recent studies lend to the use of carvedilol in heart failure. The median survival of patients with chronic heart failure is now extended to nearly 8 years from the initiation of effective medical therapy that includes angiotensin-converting enzyme (ACE) inhibitor and β-blockers, especially carvedilol. **(Komajda M et al. 2004, Yancy CW, 2004)**

Carvedilol is rapidly absorbed after an oral dose, reaching maximum plasma concentration within 1 to 2 hours. Peak plasma concentrations increase linearly with dose, and absorption is not altered with repeated doses. Food has a slight effect on rate, but not extent, of carvedilol absorption. **(Morgan T, 1994)** Only few methods for determination of carvedilol in plasma can be found in the literature. Validated LC/MS/MS analytical methodology was used for determination of carvedilol and 4-hydroxyphenyl-carvedilol from the human plasma samples.

Primary Objective: To demonstrate bioequivalence between Test Product (A): Carvedilol Tablets 12.5 mg corresponding Reference Product (B): COREG® (Carvedilol) Tablets 12.5 mg manufactured by GlaxoSmithKline under fed condition in normal, healthy, adult, male, human subjects in a randomized crossover study.

Secondary Objective: To monitor the safety and tolerability of a single dose of Carvedilol Tablets 12.5 mg in normal, healthy, adult, male, human subjects.

CHAPTER-III
LITERATURE REVIEW

1. Bioavailability and Bioequivalence:

Bioavailability: (FDA guidance-2003)

Bioavailability is defined in 21 CFR 320.1 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.

Rate is defined in terms of a description of a concentration time profile. The usual approach is to estimate the time of maximum concentration, the time of the sample with the highest measured concentration. **(Holford NHG, 1994)**

The extent can be computed from the integral of the concentrations predicted in the compartment representing the systemic circulation of the area under the curve or AUC approach. **(Holford NHG, 1994)** Absolute bioavailability indicated that the bioavailability is determined by comparing the rate and extent of absorption of the drug from its administered dosage from to the data obtained following intravenous administration of the drug, as a reference preparation. Absolute bioavailability is expressed on a scale of 0-100%.

Relative bioavailability is a term that refers to the bioavailability of one drug product as compared to another standard dosage formulation with the same drug chemical entity, or to other established standards. **(Abdou HM, 1989)** If an intravenous solution cannot be administered, an intramuscular injection may be allowed as a reference standard. Also, in certain cases where parenteral administration is no advisable, the reference drug preparation is administered as an oral solution.

Bioequivalence: (FDA guidance-2003)

Bioequivalence is defined in 21 CFR 320.1 as: 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 become available at the site of drug action when administered at the same molar dose under similar conditions in an appropriately designed study.

It is commonly observed that there are several formulations of the same drug, in the same dose, in a similar dosage form and meant to be given by the same route. Substitution of one product for another can be made provided they are equally effective therapeutically as the standard accepted. In order to ensure clinical performance of such drug products, bioequivalence studies should be performed.

Equivalence: It is the relative term that compared drug products with respect to a specific characteristic or function or to a defined set of standards. There are several types of equivalences.

Chemical equivalence: It indicates that two or more drug products contain the same labeled chemical substance as an active ingredient in the same amount.

Pharmaceutical Equivalence: **(Orange book-29th edition)**

Drug products are considered to be pharmaceutical equivalence if they contain same active ingredient(s), are of same dosage form, route of administration and are identical in strength or concentration, but they may differ in characteristics such as shape, scoring configuration, release mechanism, packaging, excipients (including color, flavor and preservatives), expiration time, and with in certain limits, labeling.

Bioequivalence: It is a relative term which denotes that the drug substance in two or more identical dosage forms, reaches the systemic circulation at the same relative rate and to the same relative extent i.e. their plasma concentration-time profiles will be identical without significant statistical differences.

2. Types of Bioavailability-Bioequivalence Studies:

2.1. Study to evaluate the absolute bioavailability of an oral, topical, intramuscular, or any other dosage form. Ideally, the test dosage form should be compared with an intravenous reference dose. In reality, however, a suitable intravenous form may not be readily available, and the test dosage form is usually compared, instead, with an oral solution or suspension to determine if the former would be adequate for subsequent clinical studies. Normally, the study is conducted in **6-12 subjects** using a single dose crossover design.

2.2. Dose proportionality study to determine if bioavailability parameters [i.e., peak concentration (Cmax) and area under concentration-time curve (AUC)] are linear over the proposed dose range to be used in medical practice. Oral doses usually are given as a solution or suspension covering the therapeutic range for a single dose and tested using a three-way crossover design (low, mid, and high dose) in **12-18 subjects.**

2.3: Intra/Inter-subject variability study to determine what the variability of bioavailability parameters are at any one dose level. Oral doses at one dose level are usually given as a solution or suspension in a mock three-way crossover design.

2.4: Dosage form(s) study to determine if that used during clinical trials is bioequivalent to that proposed for marketing. This is normally a single dose crossover study evaluating the highest strength of the proposed marketed dosage form. The number of subjects to be used is dependent on available information on dose proportionality and inter- and intra-subject variability.

2.5: Dosage form proportionality study to determine if equipotent drug treatments administered as different dose strengths of the market form produce equivalent drug bioavailability. Normally, multiple strengths are evaluated by bracketing (i.e., studying the lowest and highest strengths at the same dose level in a single dose crossover design). The number of subjects again is based on dose proportionality and inter- and intra-subject variability of the drug**.**

2.6: Effect of various type of intervention studies to examine the effects of, for example, food and concomitant medication on bioavailability parameters. These are normally single and multiple dose studies conducted using the dosage form proposed for marketing.

2.7: Bioequivalence study needed as a result of changes in the formulation or manufacturing process (i.e., to show that the old and the new product are bioequivalent).

2.8: ANDA bioequivalence studies conducted for the purpose of filing an abbreviated new drug application (ANDA). The goal is to show that a generic drug is bioequivalent to the innovator's product in order to make claims of therapeutic equivalence. The three important pharmacokinetic parameters that describe the plasma level-time curve and useful in assessing the bioavailability of a drug from its formulation are;

1. Peak Plasma Concentration (Cmax)
2. Time of Peak Concentration (tmax)
3. Area Under the Curve (AUC)

3. Significance of Bioequivalence Study:

In many parts of the world, medicines are protected by patents. This means that none other than the innovator (the company which originally discovered the medicine) can market the drug. However, patents are valid only for a limited period of time, the duration depends on the country. If someone wants to sell the drug before the patent expires, they have to obtain permission from the innovator company. But after the patent expires, anyone can market the medicine. Such "copies" of innovator medicines are called Generics.

Generics are not required to replicate the extensive clinical trials that have already been used in the development of the original, brand-name drug. These tests usually involve a few hundred to a few thousand patients. Since the safety and efficacy of the brand-name product has already been well established in clinical testing and frequently many years of patient use, it is scientifically unnecessary, and would be unethical, to require that such extensive testing be repeated in human subjects for each generic drug that a firm wishes to market. Instead, generic applicants must scientifically demonstrate that their product is bioequivalent (i.e., performs in the same manner) to the pioneer drug. To be interchangeable with innovator product, a generic drug product must be not only pharmaceutically equivalent, but also bioequivalent. Bioequivalence has to be proven between the innovator medicine (called Reference formulation) and the Generic medicine (called Test formulation). Governmental agencies carefully examine the details of the findings from the bioequivalence studies.

This elaborate procedure is meant to safeguard the safety and efficacy of medicines. Due to this procedure, patients buying medicines can be confident that it will be effective without regard to the company that manufactured it. Usually, during the life of the patent, the innovator company charges a high price for the medicine. This is done so that the company can recover the expenses they put into research for developing the medicine. And they are able to do this because no one else is allowed to sell the medicine during the patent lifetime. After the patent expires, when Generic companies get into the market the price of the medicine drops due to competition among the various companies. Therefore, bioequivalence studies benefit mankind by lowering the overall cost of medicines even

though innovator products are available in market , generic products enter the market to have better product susceptibility and better Patient compliance at reasonable cost. Even though Innovator Products are available in market generic products enter the market to have better product susceptibility and better Patient compliance at reasonable cost.

Objective of Bioavailability studies:

- Bioavailability studies are important in a suitable dosage form for a new drug entity.
- Determination of influence of excipients, patient related factors and possible interaction with other drugs on the efficiency of absorption.
- Development of new formulations of the existing drugs.
- Control of quality of a drug product during the early stages of marketing in order to determine the influence of processing factors, storage and stability on drug absorption.

4: Determination of Bioavailability:

Bioavailability of drug products in human provides the most reliable method available for determining bioequivalence. The testing is normally performed in young healthy male adult volunteers under restricted dietary conditions and fixed activity levels.

Biopharmaceutics is defined as the study of factors influencing the rate and amount of drug that reaches the systemic circulation and the use of this information to optimize the therapeutic efficacy of the drug products. The process of movement of drug from its site of administration to the systemic circulation is called as absorption.

After a drug is introduced into a biological system, it is subjected to a number of processes whose rates control the concentration of drug in the elusive region known as the "site of action," thus affecting its onset, its duration of action and the intensity of the biological response. Some knowledge of these rate processes governing the fate of a drug is necessary for a full understanding of the observed pharmacological activity of the drug.

Pharmacokinetics is defined as the study of time courses of the drug ADME (Absorption, Distribution, Metabolism and Excretion) and their relation to its therapeutic and toxic effects of the drug. The use of pharmacokinetic principles in optimizing the drug dosage to suit individual patient needs and achieving maximum therapeutic utility is called as **clinical pharmacokinetics.**

4.1 Plasma drug concentration Time profile

A direct relationship exists between the concentrations of drug at the absorption site and the concentration of drug in plasma.

A typical plasma drug concentration time curve obtained after a single oral dose and showing various pharmacokinetic and pharmacodynamic parameters is depicted in Figure below. Such a profile can be obtained by measuring the concentration of drug in plasma samples taken at various intervals of time after administration of a dosage form and plotting the concentration of drug in plasma (Y-axis) versus the corresponding time at which the plasma sample was collected (X-axis).(Figure-2)

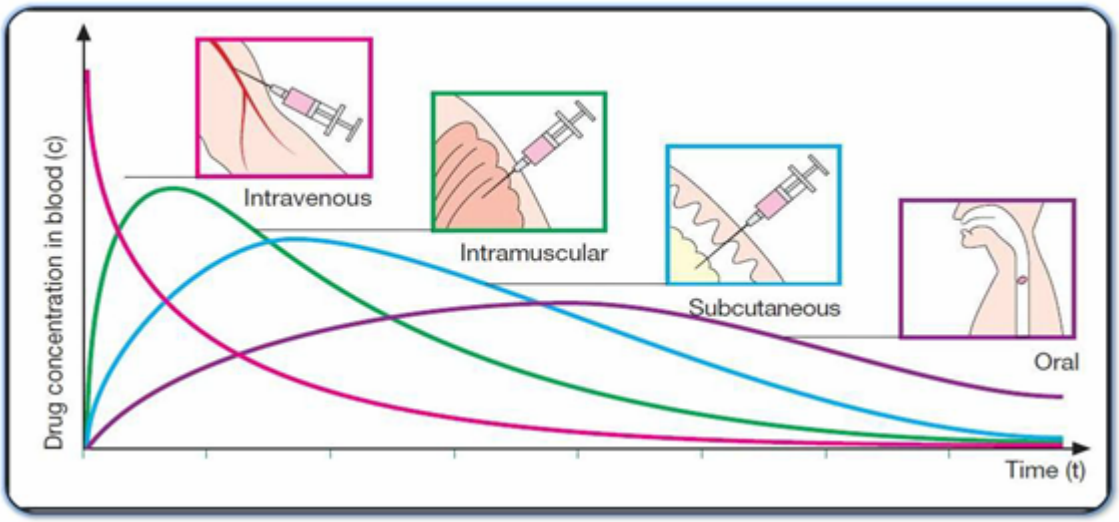


Fig 1: Pharmacokinetic profile for different routes of drug administration

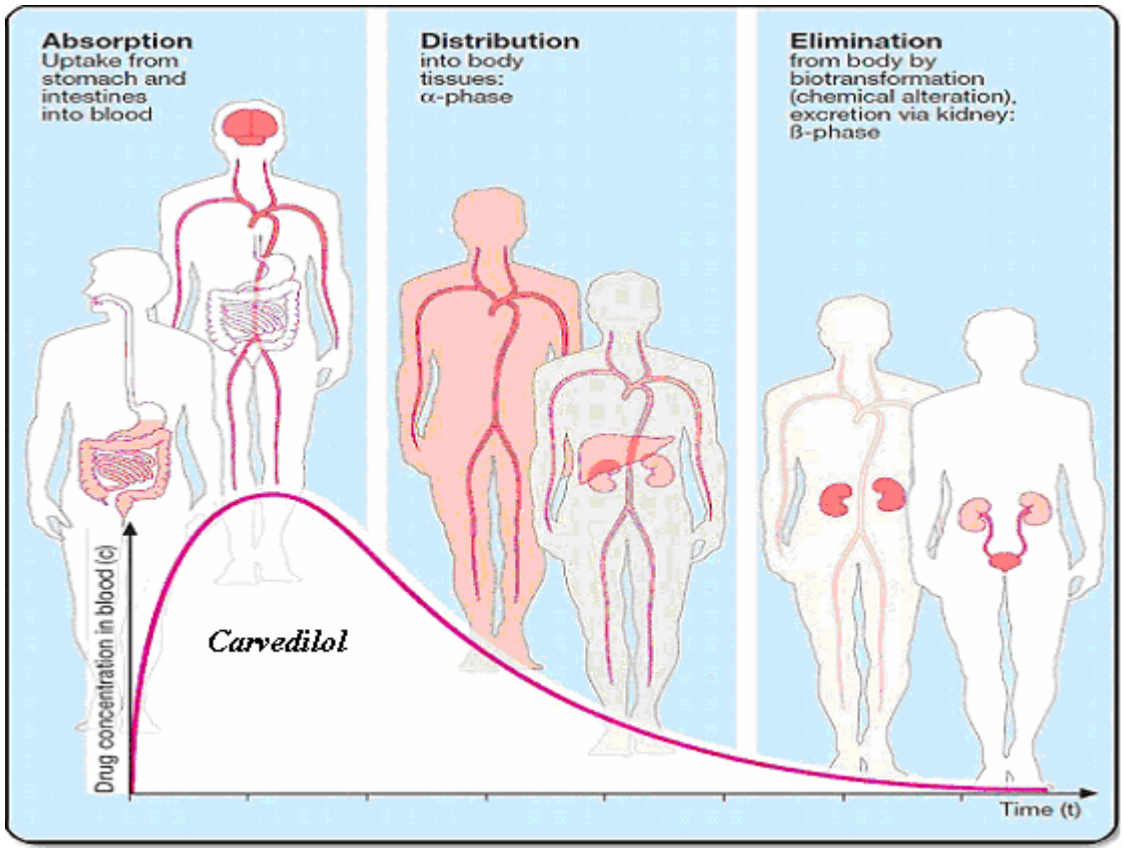


Fig 2: A typical plasma drug concentration time curve obtained after a single oral dose

4.2 Several test methods are available to assess equivalence, including:

- Comparative Bioavailability (bioequivalence) studies, in which the active drug substance or one or more metabolites is measured in an accessible biological fluid such as plasma, blood or urine.
- Comparative pharmacodynamic studies in humans.
- Comparative clinical trials.
- In-vitro dissolution tests.

4.3 The methods useful in quantitative evaluation of bioavailability can be broadly divided into two categories-pharmacokinetic methods and pharmacodynamic methods:

I. Pharmacokinetic methods:

These are very widely used and 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:

1. Plasma level-time studies.
2. Urinary excretion studies.

II. Pharmacodynamic methods:

These methods are complementary to pharmacokinetic approaches and involve direct measurement of drug effect on a pathophysiologic process as a function of time. The two pharmacodynamic methods involve determination of bioavailability from:

1. Acute pharmacologic response.
2. Therapeutic response.

Study of bioavailability can be determined in blood, urine, saliva, sweat and feces sample. Of these, blood and urine samples are mostly measured for drug concentrations, and then calculated for total area under the plasma concentration and time curve and the total amount of drug excreted in the urine, respectively.

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. The 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. With single dose study, the method requires collection of serial blood samples for a period of 2 to 3 biological half-lives after drug administration, their analysis for drug concentration and making a plot of concentration versus corresponding time of sample collection to obtain the plasma level-time profile. With i.v. dose, sampling should start within 5 minutes of drug administration and subsequent samples taken at 15 minute intervals. To adequately describe the disposition phase, at least 3 samples points should be taken if the drug follows one-compartment kinetics and 5 to 6 points if it fits two-compartment model. For oral dose at least 3 point should be taken on the ascending part of the curve for accurate determination of Ka. The points for disposition or descending phase of the cure must be taken in a manner similar to that for i.v. dose. In assessing bioavailability for blood data; three parameters usually measured in order to estimate bioequivalence as following:-

I. Maximum concentration (Cmax) represents the highest drug concentration in the systemic circulation. The peak height is related to the intensity of the biologic response and should always be above the minimum effective level or not more than the minimum toxic level of the drug.

II. Time to peak concentration (Tmax) represents the length of time required to achieve the maximum concentration of the drug in the systemic circulation. The parameter describes the onset of the peak level of the biological response and can be utilized as a rough estimation for the rate of absorption. Its value is critical in evaluation the performance of drugs used for the treatment of acute conditions such as analgesics, antispasmodics, etc.

III. Area under the curve (AUC) represents the total integrated area under the concentration/time curve. When comparing a test formulation to a reference, this parameter describes the extent of bioavailability and can be used as a rough estimation of the amount of drug absorbed.

2. Urinary excretion studies:

Urinary excretion of the unchanged drug is, in most cases, directly proportional to the plasma concentration of total drug (bound and unbound form). Therefore, if a drug is excreted, even partially, in the urine, it is possible to determine its bioavailability from the cumulative urinary excretion data.

In practice, determination of bioavailability using urinary excretion data can be conducted only if at least 20% of the dose is excreted in the urine after an intravenous dose. The fraction of drug entering the blood stream and being excreted by the kidney is assumed to remain constant. The collection of the urine must be continued until the drug has been excreted completely. This may require about five biological half-lives of drug for excretion of greater than 95% of the drug. Also, at each sample collection, total emptying of the bladder is essential or else the residual amount of urine will be erroneously added to the next point, and finally, there must be a large number of volunteers and enough sampling points to establish a reliable cumulative urinary excretion curve that reflects the blood level profile. The rate of absorption is determined from the plot of excretion rate versus time. Frequent sampling, especially at the beginning, is essential for the accurate description of the excretion curve and the precise determination of the peak time.

The utility of urinary excretion data for determining the rate of bioavailability is most applicable for drugs that are predominantly unchanged via kidney. It is also convenient for comparing the rate of absorption of drug in a standard form with other dosage forms. **(Abdou HM, 1989)** The three major parameters examined in urinary excretion rate obtained with a single oral study are:

I. (dXu/dt) max: The maximum urinary excretion rate, it is obtained from the peak of plot between rate of excretion versus midpoint time of urine collection period. It is analogous to the Cmax derived from plasma level studies since the rate of appearance of drug in the urine is proportional to its concentration in systemic circulations. Its value increases as the rate of and /or extent of absorption increases.

II. (tu)max: The time for maximum excretion rate, it is analogous to the tmax of plasma level data. Its value decreases as the absorption rate increases.

III. Xu: The cumulative amount of drug excreted in the urine, it is related to the AUC of plasma level data and increases as the extent of absorption increases. The extent of bioavailability is calculated from equations given below:

5. Factor Affecting Bioavailability:

The bioavailability of a drug can be affected by various factors such as dosage form, administration route and site, food and drug interaction. Physicochemical equivalence in drug products does not necessarily assure biological and clinical equivalency. Also, the bioavailability of a drug from dosage form can be affected by various factors. Therefore, the variation of the bioavailability may lead to failure of therapy or development of intoxication. In the present day, the marketing authorization requirement is based on the following consideration; generic product need, for the assurance of equivalent efficacy and safety, to have not only the same standard of quality but also the same bioavailability as the innovator's pharmaceutical product.

5.1 Effect of food on Bioavailability:

Different kinds of meal or food components may have marked influence on drug absorption, either quantitatively or qualitatively, by increasing or decreasing the extent of absorption, or sometimes delaying the rate of absorption. Food-induced change in the rate of gastric emptying and in the intestinal transit time, and/or in gastro-enterohepatic secretion of hydrochloric acid, bicarbonate, enzyme and bile, apparently influence the bioavailability of several drugs.

Reduced bioavailability	**Increased Bioavailability**	**Delayed absorption**	**Not affected**
Amoxicillin	Carbamazepine	Acetaminophen	Digoxin
Aspirin	Diazepam	Amoxicillin	Glibenclamide
Atenolol	Griseofulvin	Cephalexin	Glipizide
Captopril	Hydralazine	Cimetidine	Metronidazole
Cephalexin	HCTZ	Aspirin	Penicillin V
Doxycycline	Mebendazole	Diclofenac	Prednisone
Furosemide	Metoprolol	Metronidazole	Isoniacid
Phynytoin	Quinidine	Theophyline	Terbutamide
Ketoconazole	Propanolol	Theophylline	

Table 1: list of drugs whose absorption influenced by concurrent food intake.

In general, the interactions of food or food components with drugs reduce the bioavailability of drugs. The magnitude of a food-drug interaction depends on several factors, including physical and chemical nature of drug, formulation in which the drug is administered, type and size of a meal, the order in which food and drug are ingested, and time interval that elapses between meal consumption and drug administration. Mechanisms whereby drug absorption can be influenced by food are mainly the alteration in gastrointestinal motility. Table 1 lists examples of drugs whose absorption influenced by concurrent food intake. **(Kesara NB and Walther HW, 2001)**

Taking carvedilol with food, the rate of absorption is slowed, as evidenced by a delay in the time to reach peak plasma levels with no significant difference in extent of bioavailability. **(Stroe AF and Gheorghiade M 2004, GSK brochure, 2009)**

5.2 Degradation in Stomach:

A clinical study patient with peptic ulcer resistant to usual doses of proton pump inhibitor is reported. A cause of this resistance may be the degradation of proton-pump inhibitor in gastric juice due to prolonged gastric emptying rate as judged from the comparison of the plasma concentrations. Further, the ulcer is reported to be improved in the resistant patients receiving enteric coated tablets containing omeprazole, as when enteric coated granules of lansoprazole is displaced. This may be due to the difference in the degradation rate under acid condition in stomach between two dosage forms. **(Kesara NB and Walther HW, 2001)**

5.3 Influence of First-Pass Effect:

First-pass effect refers to the process by which a fraction of a drug is lost prior to reaching the systemic circulation. The process includes the inactivation of drug in the gastrointestinal lumen by gastric acid and enzyme, or transformation by enzyme of intestinal wall, microoganism, or liver. **(Kesara NB and Walther HW, 2001)**

Since carvedilol undergoes substantial oxidative metabolism, the metabolism and pharmacokinetics of carvedilol may be affected by induction or inhibition of cytochrome P450 enzymes.

In a pharmacokinetic study conducted in 8 healthy male subjects, rifampin (600 mg daily for 12 days) decreased the AUC and Cmax of carvedilol by about 70%.In a pharmacokinetic study conducted in 10 healthy male subjects, cimetidine (1000 mg/day) increased the steady-state AUC of carvedilol by 30% with no change in Cmax. **(Stroe AF and Gheorghiade M 2004)**

5.4 Effect of Dosage Form and Route of Administration

The nature of the dosage form and the route by which the formulation can be administered are closely related. It is apparent that the oral and rectal routes of drug administration have the relative highest potential for differences in rate and extent of absorption.

Many prodrug preparations containing indomethacin have been marketed. However, since the plasma concentration of indomethacin after oral administration varies largely among products, clinically their interchange can be extremely dangerous. Further, there are examples in dispensing where the grindings of dosage forms such as enteric coated and plain tablets can alter their bioavailability. In dispensing, the pharmacists may be occasionally required to alter the dosage forms, such as grinding tablets co-dispense as powders, or opening of capsules in order to dispense as powders and dissolving powders co-dispense as liquids. However, we should pay attention to the fact that the bioavailability of drug can be changed by the alteration. **(Kesara NB and Walther HW, 2001)**

5.5 Inter-Individual and Intra-Individual Variation

Attention should be paid to inter-individual and intra-individual variations in the bioavailability of drugs that have a narrow therapeutic window such as phynytoin, cyclosporin and aminoglycoside antibiotics. In case of drugs which have large inter-individual and intra-individual variation and have narrow therapeutic range, the blood concentration should be monitored to rationalize proper dosing. Plasma levels of carvedilol average about 50% higher in the elderly compared to young subjects.

Compared to healthy subjects, patients with cirrhotic liver disease exhibit significantly higher concentrations of carvedilol (approximately 4- to 7-fold) following single-dose therapy.

Although carvedilol is metabolized primarily by the liver, plasma concentrations of carvedilol have been reported to be increased in patient with renal impairment. Based on mean AUC data, approximately 40% to 50% higher plasma concentrations of carvedilol were observed in hypertensive patients with moderate to severe renal impairment compared to a control group of hypertensive patients with normal renal function. However, the range of AUC values was similar for both groups. Changes in mean peak plasma levels were less pronounced, approximately 12% to 26 higher in patients with impaired renal function. **(Stroe AF and Gheorghiade M 2004, GSK brochure, 2009)**

6. General recommendations for a standard bioequivalence study based on pharmacokinetic Measurements:

For both replicate and non-replicate, in vivo pharmacokinetic BE studies, the following general approaches are recommended, recognizing that the elements can be adjusted for certain drug substances and drug products.

6.1 Investigational Products:

Test product: Sponsor's formulation

Reference product: Reference Listed Drug in orange book

Strength: Highest marketed strength administered as a single unit. If warranted for analytical reasons, multiple units of the highest strength can be administered, providing the total single-dose remains within the labeled dose range.

Drug content: The drug content of the test product cannot differ from that of the reference listed product by more than 5 percent.

Label: The lot numbers of both test and reference listed products and the expiration date for the reference product would be stated.

6.2 Study Population:

No of subjects: 12 for pilot study

Age: 18 years or older. If the drug product is to be used predominantly in elderly, attempt to include subjects of 60 years or older age.

Gender: male and female in similar proportion unless and otherwise exception

Smokers: both smokers and non smokers

6.3 Study Conditions:

Type of the study: Under fasting and fed condition

Water restriction: Drug products should be administered with about 8 ounces (240 milliliters) of water except 1 hr pre and post dose

Food restriction: 10 hrs pre dose and 4 hrs post dose

Postural restriction: at least 2 hrs post dose

Biological matrix: Serum or plasma, in certain cases, whole blood may be more appropriate for analysis

Sampling times: 12 to 18 samples, including a pre-dose sample are collected per subject per dose.

Length of sampling: At least three or more terminal half lives. The sample collection can be spaced in such a way that the maximum concentration of the drug in the blood (Cmax) and terminal elimination rate constant (Kel) can be estimated accurately. At least three to four samples can be obtained during the terminal log-linear phase to obtain an accurate estimate of Kel from linear regression. FDA also recommend that the actual clock time when samples are drawn as well as the elapsed time related to drug administration be recorded.

Wash out period: greater than 5 half lives

Long half life drugs: AUC (0-72) used in place of AUC (0-t) or AUC (0-inf)

Subjects with predose plasma concentrations: If the predose value is greater than 5 % of Cmax, the subject be dropped from all BE study evaluations.

Data deletion due to vomiting: For immediate-release products data can be deleted from statistical analysis if vomiting occurs at or before 2 times median Tmax. In the case of modified-release products, the data from subjects who experience emesis any time during the labeled dosing interval can be deleted.

FDA recommendation for pharmacokinetic information for ANDA submission:

• Plasma concentrations and time points

• Subject, period, sequence, treatment

• AUC0-t, AUC0-inf, Cmax, Tmax, Kel, and t1/2

• Inter-subject, intra-subject, and/or total variability, if available

• Cmin (concentration at the end of a dosing interval), Cavg (average concentration during a dosing interval), degree of fluctuation [(Cmax- Cmin)/Cavg], and swing [(Cmax-Cmin)/Cmin] if steady-state studies are employed.

FDA recommendation for statistical information for pharmacokinetic parameters:

• Geometric mean

• Arithmetic mean

• Ratio of means

• Confidence intervals

Statistical criteria: For BE demonstration, 90% C.I. for log transformed pharmacokinetic parameters should be between 80% to 125%.

Rounding off of confidence interval values: Confidence interval (CI) values not be rounded off; therefore, to pass a CI limit of 80 % to125 %, the value would be at least 80.00 % and not more than 125.00 %.

6.4 Parent Drug versus Metabolites

The moieties to be measured in biological fluids collected in BA and BE studies are either the active drug ingredient or its active moiety in the administered dosage form (parent drug) and, when appropriate, its active metabolites (21 CFR 320.24(b) (1) (i)). For BE studies, measurement of only the parent drug released from the dosage form rather than the metabolite, is generally recommended. The rationale for this recommendation is that concentration-time profile of the parent drug is more sensitive to changes in formulation performance than a metabolite, which is more reflective of metabolite formation, distribution, and elimination. The following are exceptions to this general approach.

- Measurement of a metabolite may be preferred when parent drug levels are too low to allow reliable analytical measurement in blood, plasma, or serum for an adequate length of time. FDA recommend that the metabolite data obtained from these studies be subject to a confidence interval approach for BE demonstration. If there is a clinical concern related to efficacy or safety for the parent drug, FDA also recommend that sponsors and/or applicants contact the appropriate review division to determine whether the parent drug should be measured and analyzed statistically.
- A metabolite may be formed as a result of gut wall or other pre-systemic metabolism. If the metabolite contributes meaningfully to safety and/or efficacy, FDA also recommends that the metabolite and the parent drug be measured. When the relative activity of the metabolite is low and does not contribute meaningfully to safety and/or efficacy, it does not have to be measured. FDA recommend that the parent drug measured in these BE studies be analyzed using a confidence interval approach. The metabolite data can be used to provide supportive evidence of comparable therapeutic outcome.

6.5 Highly Variable Drug Product

Drugs and drug products exhibiting intra-subject variability greater than 30% CV (Coefficient of variation) in the pharmacokinetic measures, AUC and/or Cmax are considered highly variable.

To pass the conventional "goalposts", the number of subjects required for a study of these drugs or drug products can be much greater than normally needed for a typical bioequivalence study. Thus, the resource implications coupled with the ethical concern of exposing a large number of healthy subjects as suggested by sponsor to a test drug further challenges the appropriateness of the conventional bioequivalence criteria for highly variable drugs/products.

6.6 Case Report Form (CRF): (ICH-GCP-1996)

A printed, optical, or electronic document designed to record all of the protocol required information to be reported to the sponsor of each trial subject.

6.7 Trial master file (TMF): (ICH-GCP-1996)

Essential documents which individually and collectively permit evaluation of the conduct of a trial and the quality of the data produced. These documents serve to demonstrate the compliance of the investigator, sponsor and monitor with the standards of Good Clinical Practice and with all applicable regulatory requirements. These documents are audited by the sponsor's independent audit function and inspected by the regulatory authority(ies) as part of the process to confirm the validity of the trial conduct and the integrity of data collected. The various documents are grouped in three sections according to the stage of the trial during which they will normally be generated:

- before the clinical phase of the trial commences
- during the clinical conduct of the trial
- after completion or termination of the trial

Trial master files should be established at the beginning of the trial, both at the investigator/institution's site and at the sponsor's office.

6.8 Informed Consent (ICH-GCP-1996)

A process by which a subject voluntarily confirms his or her willingness to participate in a particular trial, after having been informed of all aspects of the trial that are relevant to the subject's decision to participate. Informed consent form is a written, signed and dated document.

6.9 Sample Size and Dropouts:

Regarding the number of subject needed, there is no specific recommendation, as it depends on several factors. These may include the inherent subject-to-subject variability for the drug under study, the expected magnitude of the difference between the test dosage forms and the particular statistical design of the study.

After deciding the appropriate pharmacokinetic parameters for which comparisons are conducted, the magnitude of differences among the test preparations that must be detected should be specified to the statistician. The design of the experiment, partial cross over, total cross over, Latin square, etc., should be specified and, accordingly, the statistician will be able to recommend the appropriate number of participants. A well-designed protocol is a flexible one that assesses the overall variability of the results sequentially during the progress of the study and modifies the experimental plan accordingly. Study conditions should be adhered to as rigorously as possible since they are a major source for variability.

As a rule of thumb, a minimum number of 12 healthy subjects may be employed in a crossover bioequivalence study, provided that the testing conditions are strictly standardized and the assay methodology utilized has been thoroughly validated to generate sufficiently accurate and reproducible results. The number should be increased when the patients and/or the parallel are used. **(Abdou HM, 1989)**

6.10 Pilot Study:

If the sponsor chooses, a pilot study in a small number of subjects can be carried out before proceeding with a full BE study.

Objectives of pilot study are:

- To validate analytical methodology
- To assess intra-subject variability
- To calculate sample size for pivotal study
- Assess safety parameters.
- To optimize sample collection time intervals

6.11 Pivotal Bioequivalence Studies

In larger number of subjects, replicate and non- replicate both in vivo pharmacokinetic BE studies are recommended.

6.12 Method Development (FDA guidance-2001)

The method development and establishment phase defines the chemical assay. The fundamental parameters for a bioanalytical method validation are accuracy, precision, selectivity, sensitivity, reproducibility, and stability. Measurements for each analyte in the biological matrix should be validated. In addition, the stability of the analyte in spiked samples should be determined. Typical method development and establishment for a bioanalytical method include determination of (1) selectivity, (2) accuracy, precision, recovery, (3) calibration curve, and (4) stability of analyte in spiked samples.

6.12.1 Selectivity:

Selectivity is the ability of an analytical method to differentiate and quantify the analyte in the presence of other components in the sample. For selectivity, analyses of blank samples of the appropriate biological matrix (plasma, urine, or other matrix) should be obtained from at least six sources. Each blank sample should be tested for interference, and selectivity should be ensured at the lower limit of quantification (LLOQ). Potential interfering substances in a biological matrix include endogenous matrix components, metabolites, decomposition products, and in the actual study, concomitant medication and other exogenous xenobiotics.

6.12.2 Accuracy, Precision, and Recovery:

The **accuracy** of an analytical method describes the closeness of mean test results obtained by the method to the true value (concentration) of the analyte. Accuracy is determined by replicate analysis of samples containing known amounts of the analyte. Accuracy should be measured using a minimum of five determinations per concentration. A minimum of three concentrations in the range of expected concentrations is recommended. The mean value should be within 15% of the actual value except at LLOQ, where it should not deviate by more than 20%. The **precision** of an analytical method describes the closeness of individual measures of an analyte when the procedure is applied repeatedly to multiple aliquots of a single homogeneous volume of biological matrix. Precision should be measured using a minimum of five determinations per concentration. A minimum of three concentrations in the range of expected concentrations is recommended. The precision determined at each concentration level should not exceed 15% of the coefficient of variation (CV) except for the LLOQ, where it should not exceed 20% of the CV.

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

6.12.3 Calibration/Standard Curve:

A calibration (standard) curve is the relationship between instrument response and known concentrations of the analyte. A calibration curve should be generated for each analyte in the sample. A sufficient number of standards should be used to adequately define the relationship between concentration and response. A calibration curve should be prepared in the same biological matrix as the samples in the intended study by spiking the matrix with known concentrations of the analyte. The number of standards used in constructing a calibration curve will be a function of the anticipated range of analytical values and the nature of the analyte/response relationship. Concentrations of standards should be chosen on the basis of the concentration range expected in a particular study. A calibration curve should consist of a blank sample (matrix sample processed without internal standard), a zero sample (matrix sample processed with internal standard), and six to eight non-zero samples covering the expected range, including LLOQ (Lower Limit of Quantification).

1. Lower Limit of Quantification (LLOQ):

The lowest standard on the calibration curve should be accepted as the limit of quantification if the following conditions are met:

- The analyte response at the LLOQ should be at least 5 times the response compared to blank response.
- Analyte peak (response) should be identifiable, discrete, and reproducible with a precision of 20% and accuracy of 80-120%.

2. Calibration Curve/Standard Curve/Concentration-Response:

The simplest model that adequately describes the concentration-response relationship should be used. The following conditions should be met in developing a calibration curve:

- 20% deviation of the LLOQ from nominal concentration
- 15% deviation of standards other than LLOQ from nominal concentration At least four out of six non-zero standards should meet the above criteria, including the LLOQ and the calibration standard at the highest concentration.

6.12.4 Stability:

Drug stability in a biological fluid is a function of the storage conditions, the chemical properties of the drug, the matrix, and the container system. The stability of an analyte in a particular matrix and container system is relevant only to that matrix and container system and should not be extrapolated to other matrices and container systems. Stability procedures should evaluate the stability of the analyte during sample collection and handling, after long-term (frozen at the intended storage temperature) and short-term (bench top, room temperature) storage, and after going through freeze and thaw cycles and the analytical process. All stability determinations should use a set of samples prepared from a freshly made stock solution of the analyte in the appropriate analyte-free, interference-free biological matrix. Stock solutions of the analyte for stability evaluation should be prepared in an appropriate solvent at known concentrations.

1. Freeze and Thaw Stability:

Analyte stability should be determined after three freeze and thaw cycles. At least three aliquots at each of the low and high concentrations should be stored at the intended storage temperature for 24 hours and thawed unassisted at room temperature. When completely thawed, the samples should be refrozen for 12 to 24 hours under the same conditions. The freeze–thaw cycle should be repeated two more times, and then analyzed on the third cycle. If an analyte is unstable at the intended storage temperature, the stability sample should be frozen at -70ºC during the three freeze and thaw cycles.

2. Short-Term Temperature Stability:

Three aliquots of each of the low and high concentrations should be thawed at room temperature and kept at this temperature from 4 to 24 hours (based on the expected duration that samples will be maintained at room temperature in the intended study) and analyzed.

3. Long-Term Stability:

The storage time in a long-term stability evaluation should exceed the time between the date of first sample collection and the date of last sample analysis. Long-term stability should be determined by storing at least three aliquots of each of the low and high concentrations under the same conditions as the study samples. The concentrations of all the stability samples should be compared to the mean of back calculated values for the standards at the appropriate concentrations from the first day of long-term stability testing.

4. Stock Solution Stability:

The stability of stock solutions of drug and the internal standard should be evaluated at room temperature for at least 6 hours. If the stock solutions are refrigerated or frozen for the relevant period, the stability should be documented. After completion of the desired storage time, the stability should be tested by comparing the instrument response with that of freshly prepared solutions.

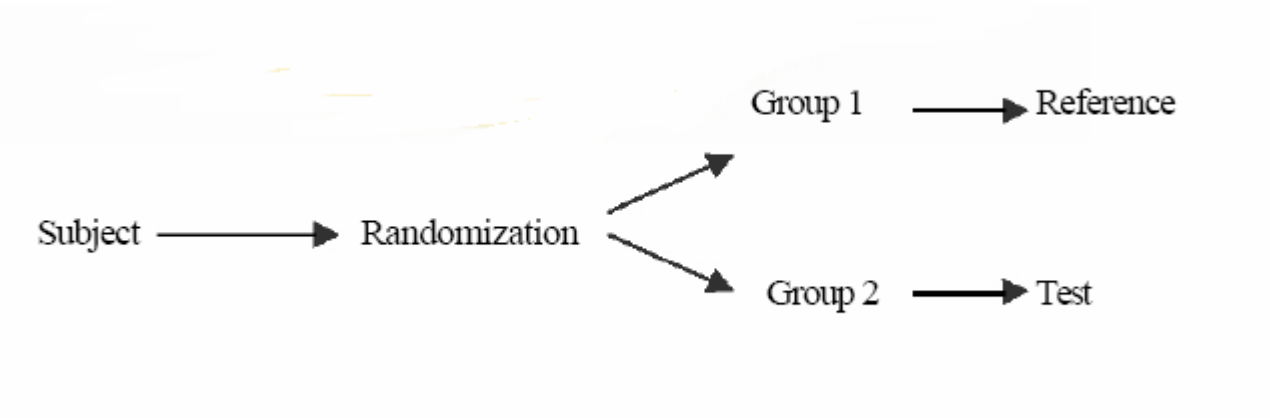
5. Post-Preparative Stability:

The stability of processed samples, including the resident time in the auto sampler, should be determined. The stability of the drug and the internal standard should be assessed over the anticipated run time for the batch size in validation samples by determining concentrations on the basis of original calibration standards. Although the traditional approach of comparing analytical results for stored samples with those for freshly prepared samples has been referred to in this guidance, other statistical approaches based on confidence limits for evaluation of analyte stability in a biological matrix can be used.

7. Design of Bioequivalence Trials:

There are two general types of designs that can be used in a bioequivalence study. There are models are the parallel and the crossover design.

7.1 Parallel design:



In the parallel-groups trial, an even number of subjects is divided randomly into two equal groups, one group receiving one formulation of the drug and the other group the second formulation. In most bioequivalence trials, one formulation will be considered as the "reference" and the other the "test" formulation; and the objective of the trial is to determine whether the test formulation is bioequivalent to the reference. The parallel-group concept can be readily generalized to more than two groups, and in this case one formulation will be the reference generally and several test formulations will be compared with it.

This type of design is not the one of choice, but may be the only alternative in certain situations with the crossover design cannot be used. For instance, if a drug with a long half-life is to be studies, the washout period needed may be too long for the crossover to be effective. The ANOVA model for the parallel design is:

$$\mathbf{X = \alpha + FORM + \varepsilon}$$

Where, X = parameter of interest

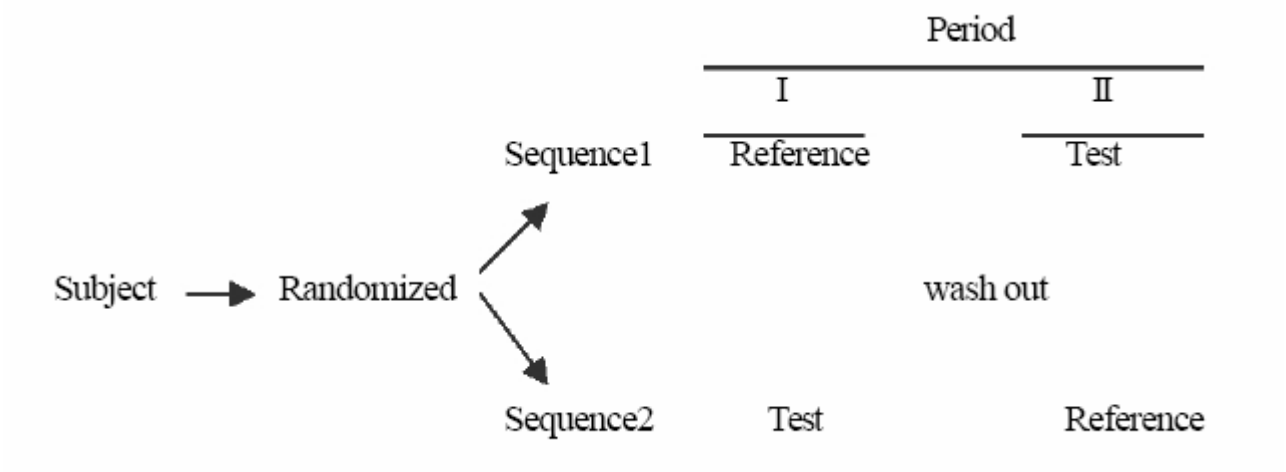
α = overall mean

Form = formulation effect

ε = between subject error

One of most striking features of bioavailability data is the enormous differences that can occur among human subjects (inter-subject variability). Not only in the size, weight and presumable blood volume, but also in the way they metabolize a drug. Consequently, in a parallel design, the error sum of squares is likely to be large and the test for a difference between formulations will be insensitive. This fact has led to a strong preference for the crossover design in bioequivalence trial. **(Wastlake WJ, 1998)**

7.2 Crossover design:



A crossover design is made up of a set of sequences that describe the order in which all or some of the formulations are to be the subjects in the periods. Subjects are randomized to one of sequences and the formulations are randomized to the letters defining the group of sequences. Each formulation is followed by a different formulation (the second period) in the same number of times. For example, in sequence 1, reference (the first period) is followed by test (the second period). The periods are separated by an adequate washout time which should be equal to at least ten elimination half-lives of the drug. **(Umesh VB, 1991)**

The basic principle behind a crossover design is that subjects generally differ less within themselves when a particular trait is measured repeatedly than they do with other subjects. The immediate consequence is that comparisons of formulations are made within subject. Recall that the intra-subject variance is usually a small component of the variance from the parallel design only. The ANOVA model for a crossover design is:

$$X = \mu + SEQ + SUB(SEQ) + PER + FORM + \varepsilon$$

Where, X = parameter of interest (AUC, C_{max}, T_{max})
μ = overall mean
SEQ = sequence effect
SUB (SEQ) = subject effect nested within sequence effect (or between subject error)
PER = period effect
FORM = formulation effect
ε = within subject error

This model assumes no interaction between the main effect of sequence, period, treatment and that the sample variances are homogenous. **(Umesh VB, 1991)**

For assessment of bioequivalence, a typical approach is to employ the standard two-sequence, two-period (2x2) crossover design. Subjects are randomly assigned to receive either sequence of RT or sequence of TR, where T and R are test product and reference product, respectively. Subjects within sequence RT receive product R during the first dosing period and product T during the second dosing period. Also, subjects within sequence TR receive product T during the first dosing period and product R during the second dosing period. Usually, dosing periods are separated by a washout of sufficient length for the drug received in the first period to be completely eliminated from the body. Note that for convenience sake, we the standard 2x2 crossover design by RT and TR.

Under the standard 2x2 crossover design, bioequivalence can be assessed using Schuirmann's two one-sided tests procedure or the method of confidence interval. In addition, a two sided tests procedure for assessment of bioequivalence in variability of bioavailability. As a result, under the standard 2x2 crossover design, average bioequivalence can be assessed.

One of major disadvantages for of using the standard 2x2 crossover design is that the sequence effect is confounded with carry-over effect which cannot be separated and estimated. As a result, if we observe a statistically significant sequence effect, it means that there is a true sequence effect, or there is true carry-over effect, or there is true formulation-by-period interaction, or there is failure of randomization. In this case, the FDA recommends that the assessment of bioequivalence (average bioequivalence) should be carefully examined. For

example, the FDA guidance indicates that bioequivalence can still be claimed when there is a statistically significant sequence effect under certain circumstances.

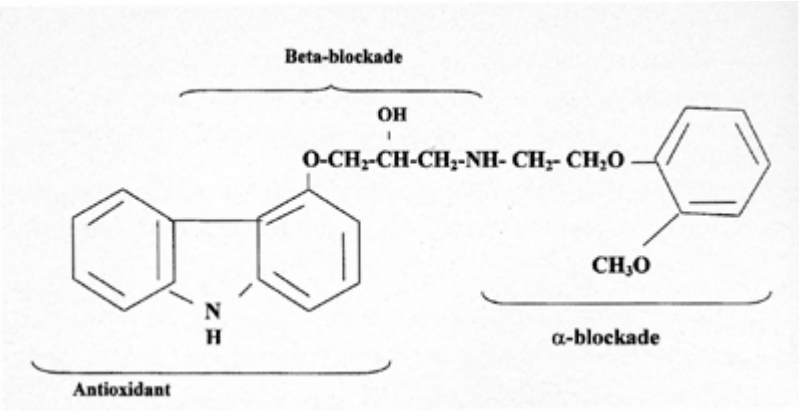
As indicated earlier, it is recognized that average bioequivalence does not guarantee drug interchangeability under current regulatory requirement because the assessment of average bioequivalence ignores intra-subject variability and subject by formulation interaction. Under the standard 2x2 crossover design, however, statistical assessment for average bioequivalence by the confidence interval approach or Schuirmann's two one-sided tests procedure is still valid even in the presence of subject-by-formulation interaction and unequal intra-subject variability between the test and reference formulation. Since each subject only receives each formulation once, the standard 2x2 crossover design can neither provide independent estimates of intra-subject variability nor give a test for the presence of the subject-by-formulation interaction.

Estimation of intra-subject variability and the subject-by-formulation interaction provide useful information for assessment of population bioequivalence for drug prescribability and for assessment of individual bioequivalence for drug switchability. As a result, this information is necessary for establishment of drug interchangeability. To provide independent estimation of intra-subject variability and/or to study the subject-by-formulation interaction, it is recommended that each formulation should be administered at least twice to each subject.

8. Drug Profile:

8.1 Physicochemical Properties:

Carvedilol is a nonselective β-adrenergic blocking agent with α1-blocking activity. It is (+)-1-(Carbazol-4-yloxy)-3-[[2-(o-methoxyphenoxy) ethyl] amino-2-propanol. It is a racemic mixture with the following structure. (**GSK brochure, 2009**)



Carvedilol is a white to off-white powder with a molecular weight of 406.5 and a molecular formula of C24H26N2O4. It is freely soluble in dimethylsulfoxide; soluble in methylene chloride and methanol; sparingly soluble in 95% ethanol and isopropanol; slightly soluble in ethyl ether; and practically insoluble in water. **(Colin D 1999, Morgan T 1994)**

8.2 Mechanism of Action:

Carvedilol is a racemic mixture in which nonselective β-adrenoreceptor blocking activity is present in the S (-) enantiomer and α-adrenergic blocking activity is present in the both R (+) and S (-) enantiomers at equal potency. Carvedilol has no intrinsic sympathomimetic activity. (**GSK brochure, 2009**)

8.3 Adrenergic Receptor Blockade:

First generation β-blockers, such as propanolol and timolol, are nonselective β1-/β2-antagonists used for the treatment of hypertension and post-MI patients without heart failure. Second generation (β1-selective) β-blockers, including atenolol, metoprolol, and bisoprolol were developed in response to problems related to unopposed α-adrenergic activity, particularly peripheral vasoconstriction exacerbated by β2-blockade. Carvedilol is a third-generation, vasodilating β-blocker that acts at all 3 major adrenergic receptor: β1, β2 and α1. Carvedilol is

devoid of intrinsic sympathomimetic activity and does not produce a high level of inverse agonist activity. (**Borchard U 1998, Rickli H et al. 2004)**

Chronic heart failure is associated with increased activity of the sympathetic nervous system (SNS) and the renin-angiotensin-aldosterone system (RAAS) aimed at supporting cardiac output and systemic pressure. However, these short-term compensatory mechanisms may lead to long-term deterioration in cardiac function. Increased SNS activity can result in progressive left ventricular (LV) systolic impairment through direct catecholamine toxicity on cardiomyocyte, as well as the detrimental effects of increased LV after load and wall stress, promoting myocardial ischemia and oxidative stress. Chronic heart failure is also associated with selective down-regulation of myocardial β1-receptors, increasing the relative importance of β_2 and α_1 stimulation in the progressive deterioration of cardiac function. Because stimulation of all 3 adrenergic receptors may be involved in promoting myocardial toxicity, carvedilol blocks increased sympathetic activity more completely than previous β-antagonists. **(Stroe AF 2004, Reiter M 2004)**

8.4 Ancillary Properties:

Carvedilol possesses important ancillary properties that may help explain its beneficial clinical effect (antioxidant, antiarrhythmic, antiapoptotic and antiprofiferative) demonstrated in heart failure patients. It also has unique effect on carbohydrate and lipid metabolism that significantly differ from other β-blockers. **(Rickli H et al. 2004, Kowalski J 2004)**

Carvedilol acts as a potent antioxidant due to the unique carbazol moiety contained in its structure. It may directly inhibit oxidative stress by scavenging oxygen free radicals or by reducing their generation through sequestration of the ferric ions needs for the non-enzymatic production of hydroxyl radical. Carvedilol's antioxidant properties may provide a demonstrable cardio-protective effect by inhibiting apoptosis, therapy protecting against myocardial cell loss that is a part of progressive heart failure. (**Kowalski J 2004, Oliveira PJ 2004)**

8.5 Pharmacokinetic Properties:

8.5.1 Absorption:

Carvedilol is absorbed rapidly, with peak plasma concentration (Cmax) reached 1 to 2 hours post dose. The Cmax values are linearly related to the dose. Furthermore, repeated administration does not appear to result in accumulation of the drug, as shown by the area under the plasma concentration-time curve (AUC) data. The rate of absorption is impaired

by food, with the time to achieve Cmax values(Tmax) changing from 0.97 hours in fasting condition to 1.3 hours after administration of carvedilol 50 mg with food. Carvedilol undergoes extensive first-pass liver metabolism that results in an absolute bioavailability of about 25%. The S-(-)-enantiomer appear to be metabolized more rapidly than the R-(+)-enantiomer, and has an absolute bioavailability of 15% compared with the absolute bioavailability of 31% for the R-(+)-enantiomer. **(Morgan T 1994, GSK brochure, 2009)**

8.5.2 Distribution:

Because it is highly lipophilic, carvedilol distributes extensively throughout the body and has a volume of distribution (V_d) between 1.5 to 2 L/kg. The drug is highly protein bound (95%), with the R-(+)-enantiomer being more tightly bound than the S-(-)-enantiomer. Thus, the resultant exposure of the tissues to the β-blocking and α1-blocking effect of carvedilol is a complex interaction depending on the proportions of drug present in the mixture, the rate of liver metabolism and the degree of protein binding. **(Morgan T 1994, GSK brochure, 2009)**

8.5.3 Metabolism and excretion:

Carvedilol is extensively metabolism and less than 2% is secreted as unaltered drug in the urine. Metabolism occurs in the liver, with a variety of conjugated products formed. Some metabolites have pharmacological activity. Most of the metabolites are secreted into the bile and eliminated in the faeces. Only about 16% of carvedilol or its metabolites are excreted in the urine. When carvedilol was given orally, the elimination half-life (t1/2) usually varied between 4 and 7 hours. The terminal half-life of drug may be as long a 14.5 hours in a 3-compartment model analysis. Overall, carvedilol appears to exhibit relative linear pharmacokinetics, with the possibility that the lower dose (12.5 mg) is more rapidly metabolized. Carvedilol is extensively metabolized. Following oral administration of radiolabelled carvedilol to healthy volunteers, carvedilol accounted for only about 7% of the total radioactivity in plasma as measured by area under the curve (AUC). Less than 2% of the dose was excreted unchanged in the urine. Carvedilol is metabolized primarily by aromatic ring oxidation and glucuronidation. The oxidative metabolites of carvedilol are excreted primarily via the bile into the feces. Demethylation and hydroxylation at the phenol ring produce three active metabolites with β-receptor blocking activity. Based on preclinical studies, the 4'-hydroxyphenyl metabolite is approximately 13 times more potent than carvedilol for β-blockade. Compared to carvedilol, the three active

metabolites exhibit weak vasodilating activity. Plasma concentrations of the active metabolites are about one-tenth of those observed for carvedilol and have pharmacokinetics similar to the parent. Carvedilol undergoes stereo selective first-pass metabolism with plasma levels of R (+)-carvedilol approximately 2 to 3 times higher than S (-)-carvedilol following oral administration in healthy subjects. The mean apparent terminal elimination half lives for R(+)-carvedilol range from 5 to 9 hours compared with 7 to 11 for the S(-)-carvedilol. **(Morgan T 1994, GSK brochure, 2009)**

The primary P450 enzymes responsible for the metabolism of both R (+) and S (-)-carvedilol in human liver microsomes were CYP2D6 and CYP2C9 and to a lesser extent CYP3A4, 2C19, 1A2 and 2E1. CYP2D6 is thought to be the major enzyme in the 4- and 5-hydroxylation of carvedilol, with a potential contribution from 3A4. CYP2C9 is thought to be of primary importance in the O-methylation pathway of S (-)-carvedilol. (**GSK brochure, 2009**)

8.6 Indication and usage:

8.6.1 Congestive Heart Failure:

Carvedilol is indicated for the treatment of mild to severe heart failure of ischemic or cardiomyopathic origin, usually in addition to diuretics, ACE inhibitor, and digitalis, to increase survival and, also, to reduce the risk of hospitalization. **(Packer M et al. 1996, Delea TE at al. 2005)**

8.6.2 Left Ventricular Dysfunction Following Myocardial infarction:

Carvedilol is indicated to reduce cardiovascular mortality in clinical stable patients who have survived the acute phase of a myocardial infarction and have a left ventricular ejection fraction of < 40% (with or without symptomatic heart failure). **(Dargie H at el. 2001, Borrello F at el. 2003)**

8.6.3 Hypertension:

Carvedilol is also indicated for the management of essential hypertension. It can be used alone or in combination with other antihypertensive agent, especially thiazide-type diuretics. **(Jean L at el. 2004)**

8.7 Adverse reaction:

In general, carvedilol is well tolerated at doses up to 50 mg daily. Most adverse events reported during carvedilol therapy were of mild to moderate severity. In clinical trials directly comparing carvedilol monotherapy in dose < 50 mg to placebo, 4.9% of carvedilol patients discontinued for adverse events vs. 5.2% of placebo patients. The overall incidence of adverse events increased with increasing doses of carvedilol. Table 2 lists examples of adverse reaction of carvedilol. In addition to reaction listed in the table 2, chest pain, dyspepsia, headache, nausea, pain, sinusitis and upper respiratory tract infection were also reported, but rates were at least as great in placebo treated patients. **(Stroe AF and Gheorghiade M, 2004)**

Clinical Studies Experience:

COREG has been evaluated for safety in patients with heart failure (mild, moderate, and severe), in patients with left ventricular dysfunction following myocardial infarction and in hypertensive patients. The observed adverse event profile was consistent with the pharmacology of the drug and the health status of the patients in the clinical trials. Adverse events reported for each of these patient populations are provided below. Excluded are adverse events considered too general to be informative, and those not reasonably associated with the use of the drug because they were associated with the condition being treated or are very common in the treated population. Rates of adverse events were generally similar across demographic subsets (men and women, elderly and non-elderly, blacks and non-blacks).

Heart Failure:

COREG has been evaluated for safety in heart failure in more than 4,500 patients worldwide of whom more than 2,100 participated in placebo-controlled clinical trials. Approximately 60% of the total treated population in placebo-controlled clinical trials received COREG for at least 6 months and 30% received COREG for at least 12 months. In the COMET trial, 1,511 patients with mild-to-moderate heart failure were treated with COREG for up to 5.9 years (mean 4.8 years). Both in US clinical trials in mild-to-moderate heart failure that compared COREG in daily doses up to 100 mg (n = 765) to placebo (n = 437), and in a multinational clinical trial in severe heart failure (COPERNICUS) that compared COREG in daily doses up to 50 mg (n = 1,156) with placebo (n = 1,133), discontinuation rates for adverse experiences were similar in carvedilol and placebo patients. In placebo-controlled clinical trials, the only cause of discontinuation > 1%, and occurring more often on carvedilol was dizziness (1.3% on carvedilol, 0.6% on placebo in the COPERNICUS trial).

Table 2 shows adverse events reported in patients with mild-to-moderate heart failure enrolled in US placebo-controlled clinical trials, and with severe heart failure enrolled in the COPERNICUS trial. Shown are adverse events that occurred more frequently in drug-treated patients than placebo-treated patients with an incidence of > 3% in patients treated with carvedilol regardless of causality. Median study medication exposure was 6.3 months for both carvedilol and placebo patients in the trials of mild-to-moderate heart failure, and 10.4 months in the trial of severe heart failure patients. The adverse event profile of COREG observed in the long-term COMET study was generally similar to that observed in the US Heart Failure Trials.

	Mild-to-Moderate Heart Failure (HF)		Severe Heart Failure(HF)	
	COREG®(Carvedilol) n=765	**Placebo n=437**	**COREG®(Carvedilol) n=1,156**	**Placebo n=1,133**
Body as a Whole				
Asthenia	7	7	11	9
Fatigue	24	22	—	—
Digoxin level increased	5	4	2	1
Edema generalized	5	3	6	5
Edema dependent	4	2	—	—
Cardiovascular				
Bradycardia	9	1	10	3
Hypotension	9	3	14	8
Syncope	3	3	8	5
Angina pectoris	2	3	6	4
Central Nervous System				
Dizziness	32	19	24	17
Headache	8	7	5	3
Gastrointestinal				
Diarrhea	12	6	5	3
Nausea	9	5	4	3
Vomiting	6	4	1	2
Metabolic				
Hyperglycemia	12	8	5	3
Weight increase	10	7	12	11
BUN increased	6	5	—	—
NPN increased	6	5	—	—
Hypercholesterolemia	4	3	1	1
Edema peripheral	2	1	7	6
Musculoskeletal				
Arthralgia	6	5	1	1
Respiratory				
Cough increased	8	9	5	4
Rales	4	4	4	2
Vision				
Vision abnormal	5	2	—	—

Table 2: Adverse Events (%) Occurring More Frequently With COREG®(Carvedilol) than With Placebo in Patients With Mild-to-Moderate Heart Failure (HF) Enrolled in US Heart Failure Trials or in Patients With Severe Heart Failure in the COPERNICUS Trial (Incidence > 3% in Patients Treated With Carvedilol, Regardless of Causality).

Left Ventricular Dysfunction Following Myocardial Infarction:

COREG has been evaluated for safety in survivors of an acute myocardial infarction with left ventricular dysfunction in the CAPRICORN trial which involved 969 patients who received COREG and 980 who received placebo. Approximately 75% of the patients received COREG for at least 6 months and 53% received COREG for at least 12 months. Patients were treated for an average of 12.9 months and 12.8 months with COREG and placebo, respectively.

The most common adverse events reported with COREG in the CAPRICORN trial were consistent with the profile of the drug in the US heart failure trials and the COPERNICUS trial. The only additional adverse events reported in CAPRICORN in > 3% of the patients and more commonly on carvedilol were dyspnea, anemia, and lung edema. The following adverse events were reported with a frequency of > 1% but ≤ 3% and more frequently with COREG: Flu syndrome, cerebrovascular accident, peripheral vascular disorder, hypotonia, depression, gastrointestinal pain, arthritis, and gout. The overall rates of discontinuations due to adverse events were similar in both groups of patients. In this database, the only cause of discontinuation > 1%, and occurring more often on carvedilol was hypotension (1.5% on carvedilol, 0.2% on placebo).

Hypertension:

COREG has been evaluated for safety in hypertension in more than 2,193 patients in US clinical trials and in 2,976 patients in international clinical trials. Approximately 36% of the total treated population received COREG for at least 6 months. Most adverse events reported during therapy with COREG were of mild to moderate severity. In US controlled clinical trials directly comparing COREG in doses up to 50 mg (n = 1,142) to placebo (n = 462), 4.9% of patients receiving COREG discontinued for adverse events versus 5.2% of placebo patients. Although there was no overall difference in discontinuation rates, discontinuations were more common in the carvedilol group for postural hypotension (1% versus 0). The overall incidence of adverse events in US placebo-controlled trials increased with increasing dose of COREG. For individual adverse events this could only be distinguished for dizziness, which increased in frequency from 2% to 5% as total daily dose increased from 6.25 mg to 50 mg.

Table 3 shows adverse events in US placebo-controlled clinical trials for hypertension that occurred with an incidence of ≥ 1% regardless of causality, and that were more frequent in drug-treated patients than placebo-treated patients.

	COREG®(Carvedilol) n=765	**Placebo n=437**
Cardiovascular		
Bradycardia	2	—
Postural hypotension	2	—
Peripheral edema	1	—
Central Nervous System		
Dizziness	6	5
Insomnia	2	1
Gastrointestinal		
Diarrhea	2	1
Hematologic		
Thrombocytopenia	1	—
Metabolic		
Hypertriglyceridemia	1	—
*** Shown are events with rate > 1% rounded to nearest integer.**		

Table 3: Adverse Events (%) Occurring in US Placebo-Controlled Hypertension Trials (Incidence ≥ 1%, Regardless of Causality)*

Laboratory Abnormalities:

Reversible elevations in serum transaminases (ALT or AST) have been observed during treatment with COREG. Rates of transaminase elevations (2- to 3-times the upper limit of normal) observed during controlled clinical trials have generally been similar between patients treated with COREG and those treated with placebo. However, transaminase elevations, confirmed by rechallenge, have been observed with COREG. In a long-term, placebo-controlled trial in severe heart failure, patients treated with COREG had lower values for hepatic transaminases than patients treated with placebo, possibly because improvements in cardiac function induced by COREG led to less hepatic congestion and/or improved hepatic blood flow.

COREG has not been associated with clinically significant changes in serum potassium, total triglycerides, total cholesterol, HDL cholesterol, uric acid, blood urea nitrogen, or creatinine. No clinically relevant changes were noted in fasting serum glucose in hypertensive patients; fasting serum glucose was not evaluated in the heart failure clinical trials.

Post marketing Experience:

The following adverse reactions have been identified during post-approval use of COREG. Because these reactions are reported voluntarily from a population of uncertain size, it is not always possible to reliably estimate their frequency or establish a causal relationship to drug exposure.

Reports of aplastic anemia and severe skin reactions (Stevens-Johnson syndrome, toxic epidermal necrolysis, and erythema multiforme) have been rare and received only when carvedilol was administered concomitantly with other medications associated with such reactions. Rare reports of hypersensitivity reactions (e.g., anaphylactic reaction, angioedema, and urticaria) have been received for COREG and COREG CR®, including cases occurring after the initiation of COREG CR in patients previously treated with COREG. Urinary incontinence in women (which resolved upon discontinuation of the medication) and interstitial pneumonitis have been reported rarely

8.8 Dosage and Administration:

8.8.1 Congestive Heart Failure:

Dose must be individualized and closely monitored by a physician during up-titration. Prior to initiation of carvedilol, it is recommended that fluid retention be minimized. The recommended starting dose of carvedilol is 3.125 mg, twice daily for two week. Patients who tolerate a dose of 3.125 mg twice daily may have their dose increased to 6.25, 12.5, and 25 mg twice daily over successive intervals of at least two weeks. Patients should be maintained on lower doses if higher doses are not tolerated. A maximum dose of 50 mg twice daily has been administered to patient with mild to moderate heart failure weighing over 85 kg (187 lbs).

8.8.2 Left Ventricular Dysfunction Following Myocardial Infarction:

Dosage must be individualized and monitored during up-titration. Treatment with carvedilol may be started as an inpatient or outpatient and should be started after the patient is hemodynamically stable and fluid retention has been minimized. It is recommended that carvedilol be started at 6.25 mg twice daily and increased after 3 to 10 days, based on tolerability to 12.5 mg twice daily, then again to the target dose of 25 mg twice daily. A lower starting dose may be used (3.125 mg twice daily) and/or, the rate of up-titration may

be slowed if clinically indicated. Patients should be maintained on lower doses if higher doses are not tolerated.

8.8.3 Hypertension

Dosage must be individualized. The recommended starting dose of carvedilol is 6.25 mg twice daily. If this dose is tolerated, using standing systolic pressure measured about 1 hour after dosing as a guide, the dose should be maintained for 7 to 14 days, and then increased to 12.5 mg twice daily if needed, based on trough blood pressure, again using standing systolic pressure one hour after dosing as a guide for tolerance. This dose should also be maintained for 7 to 14 days and can then adjusted upward to 25 mg twice daily if tolerated and needed. The full antihypertensive effect of carvedilol is seen within 7 to 14 days. Total daily dose should not exceed 50 mg.

Carvedilol should be taken with food to slow the rate of absorption and reduce the incidence of orthostatic effect. (GSK brochure, 2009)

9. Clinical Studies of Carvedilol:

9.1 Mild-to-Moderate Heart Failure (COMET Trial) (Sweberg K at el. 2003):

In this double-blind trial, 3,029 patients with NYHA class II-IV heart failure (left ventricular ejection fraction ≤ 35%) were randomized to receive either carvedilol (target dose: 25 mg twice daily) or immediate-release metoprolol tartrate (target dose: 50 mg twice daily). The mean age of the patients was approximately 62 years, 80% were males, and the mean left ventricular ejection fraction at baseline was 26%. Approximately 96% of the patients had NYHA class II or III heart failure. Concomitant treatment included diuretics (99%), ACE inhibitors (91%), digitalis (59%), aldosterone antagonists (11%), and "statin" lipid-lowering agents (21%). The mean duration of follow-up was 4.8 years. The mean dose of carvedilol was 42 mg per day.

The study had 2 primary end points: All-cause mortality and the composite of death plus hospitalization for any reason. The results of COMET are presented in **Table 4** below. All-cause mortality carried most of the statistical weight and was the primary determinant of the study size. All-cause mortality was 34% in the patients treated with carvedilol and was 40% in the immediate-release metoprolol group (p = 0.0017; hazard ratio = 0.83, 95%CI 0.74-0.93). The effect on mortality was primarily due to a reduction in cardiovascular death. The difference between the 2 groups with respect to the composite end point was not significant (p = 0.122). The estimated mean survival was 8.0 years with carvedilol and 6.6 years with immediate-release metoprolol.

End point	Carvedilol N = 1,511	Metoprolol N = 1,518	Hazard ratio	(95% CI)
All-cause mortality	34%	40%	0.83	0.74 – 0.93
Mortality + all hospitalization	74%	76%	0.94	0.86 – 1.02
Cardiovascular death	30%	35%	0.80	0.70 – 0.90
Sudden death	14%	17%	0.81	0.68 – 0.97
Death due to circulatory failure	11%	13%	0.83	0.67 – 1.02
Death due to stroke	0.9%	2.5%	0.33	0.18 – 0.62

Table 4: Results of COMET

It is not known whether this formulation of metoprolol at any dose or this low dose of metoprolol in any formulation has any effect on survival or hospitalization in patients with heart failure. Thus, this trial extends the time over which carvedilol manifests benefits on survival in heart failure, but it is not evidence that carvedilol improves outcome over the formulation of metoprolol (TOPROL-XL®) with benefits in heart failure.

9.2 Severe Heart Failure (COPERNICUS Trial) (Colucci WS, 2004):

In a double-blind study (COPERNICUS), 2,289 patients with heart failure at rest or with minimal exertion and left ventricular ejection fraction < 25% (mean 20%), despite digitalis (66%), diuretics (99%), and ACE inhibitors (89%) were randomized to placebo or carvedilol. Carvedilol was titrated from a starting dose of 3.125 mg twice daily to the maximum tolerated dose or up to 25 mg twice daily over a minimum of 6 weeks. Most subjects achieved the target dose of 25 mg. The study was conducted in Eastern and Western Europe, the United States, Israel, and Canada. Similar numbers of subjects per group (about 100) withdrew during the titration period.

The primary end point of the trial was all-cause mortality, but cause-specific mortality and the risk of death or hospitalization (total, cardiovascular [CV], or heart failure [HF]) were also examined. The developing trial data were followed by a data monitoring committee, and mortality analyses were adjusted for these multiple looks. The trial was stopped after a median follow-up of 10 months because of an observed 35% reduction in mortality (from 19.7% per patient year on placebo to 12.8% on carvedilol, hazard ratio 0.65, 95% CI 0.52 – 0.81, p = 0.0014, adjusted) (see Figure 2).The results of COPERNICUS are shown in **Table 5.**

End point	Placebo (n = 1,133)	Carvedilol (n = 1,156)	Hazard ratio (95% CI)	% Reduction	Nominal p value
Mortality	190	130	0.65 (0.52 – 0.81)	35	0.00013
Mortality + all hospitalization	507	425	0.76 (0.67 – 0.87)	24	0.00004
Mortality + CV hospitalization	395	314	0.73 (0.63 – 0.84)	27	0.00002
Mortality + HF hospitalization	357	271	0.69 (0.59 – 0.81)	31	0.000004
Cardiovascular= CV; Heart failure = HF.					

Table 5: Results of COPERNICUS Trial in Patients with Severe Heart Failure

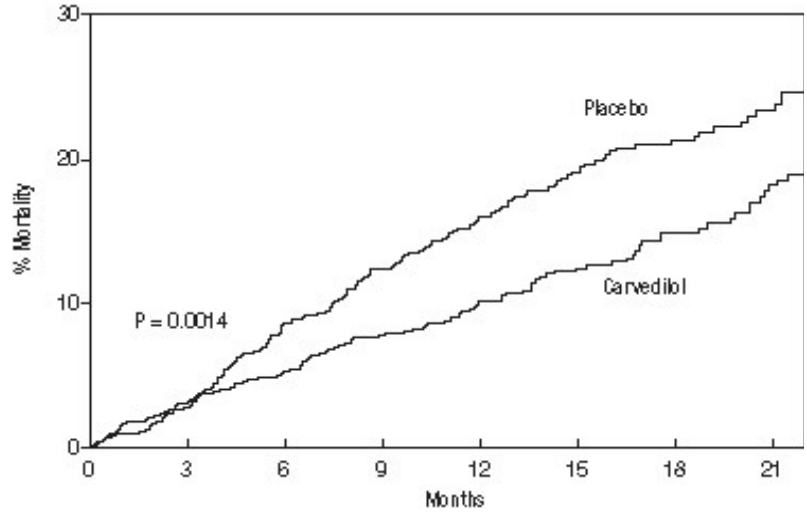


Figure 3: Survival Analysis for COPERNICUS (intent-to-treat)

The effect on mortality was principally the result of a reduction in the rate of sudden death among patients without worsening heart failure.

Patients' global assessments, in which carvedilol-treated patients were compared to placebo, were based on pre-specified, periodic patient self-assessments regarding whether clinical status post-treatment showed improvement, worsening or no change compared to baseline. Patients treated with carvedilol showed significant improvements in global assessments compared with those treated with placebo in COPERNICUS.

9.3 Left Ventricular Dysfunction Following Myocardial Infarction (CAPRICORN Trial) (Dargie H at el. 2001):

CAPRICORN was a double-blind study comparing carvedilol and placebo in 1,959 patients with a recent myocardial infarction (within 21 days) and left ventricular ejection fraction of ≤ 40%, with (47%) or without symptoms of heart failure. Patients given carvedilol received 6.25 mg twice daily, titrated as tolerated to 25 mg twice daily. Patients had to have a systolic blood pressure > 90 mm Hg, a sitting heart rate > 60 beats/minute, and no contraindication to β-blocker use. Treatment of the index infarction included aspirin (85%), IV or oral β-blockers (37%), nitrates (73%), heparin (64%), thrombolytics (40%), and acute angioplasty (12%). Background treatment included ACE inhibitors or angiotensin receptor blockers (97%), anticoagulants (20%), lipid-lowering agents (23%), and diuretics (34%). Baseline population characteristics included an average age of 63 years, 74% male, 95% Caucasian, mean blood pressure 121/74 mm Hg, 22% with diabetes, and 54% with a history of hypertension. Mean

dosage achieved of carvedilol was 20 mg twice daily; mean duration of follow-up was 15 months.

All-cause mortality was 15% in the placebo group and 12% in the carvedilol group, indicating a 23% risk reduction in patients treated with carvedilol (95% CI 2-40%, p = 0.03), as shown in Figure 4. Nearly all deaths were cardiovascular (which were reduced by 25% by carvedilol), and most of these deaths were sudden or related to pump failure (both types of death were reduced by carvedilol). Another study end point, total mortality and all-cause hospitalization, did not show a significant improvement.

There was also a significant 40% reduction in fatal or non-fatal myocardial infarction observed in the group treated with carvedilol (95% CI 11% to 60%, p = 0.01). A similar reduction in the risk of myocardial infarction was also observed in a meta-analysis of placebo-controlled trials of carvedilol in heart failure.

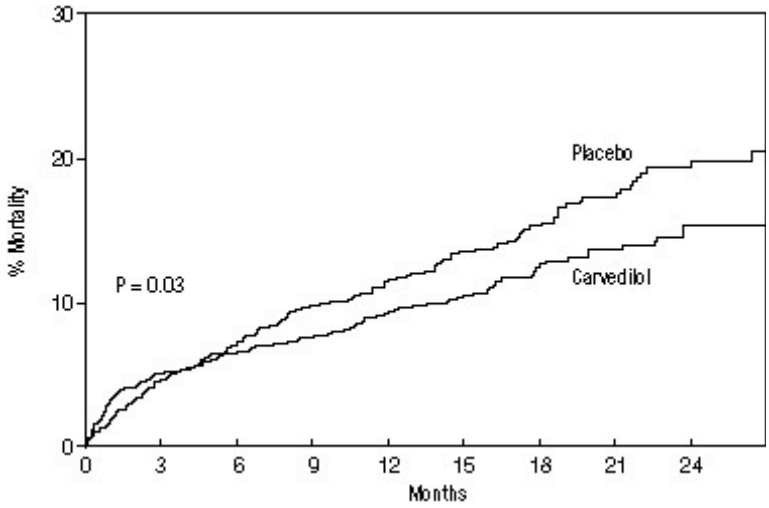


Figure 4: Survival Analysis for CAPRICORN (intent-to-treat)

9.4 Hypertension

COREG was studied in 2 placebo-controlled trials that utilized twice-daily dosing, at total daily doses of 12.5 to 50 mg. In these and other studies, the starting dose did not exceed 12.5 mg. At 50 mg/day, COREG reduced sitting trough (12-hour) blood pressure by about 9/5.5 mm Hg; at 25 mg/day the effect was about 7.5/3.5 mm Hg. Comparisons of trough to peak blood pressure showed a trough to peak ratio for blood pressure response of about 65%. Heart rate fell by about 7.5 beats/minute at 50 mg/day. In general, as is true for other β-blockers, responses were

smaller in black than non-black patients. There were no age- or gender-related differences in response.

The peak antihypertensive effect occurred 1 to 2 hours after a dose. The dose-related blood pressure response was accompanied by a dose-related increase in adverse effects.

9.5 Hypertension with Type 2 Diabetes Mellitus (GEMINI Trial):

In a double-blind study (GEMINI), COREG, added to an ACE inhibitor or angiotensin receptor blocker, was evaluated in a population with mild-to-moderate hypertension and well-controlled type 2 diabetes mellitus. The mean HbA1c at baseline was 7.2%. COREG was titrated to a mean dose of 17.5 mg twice daily and maintained for 5 months. COREG had no adverse effect on glycemic control, based on HbA1c measurements (mean change from baseline of 0.02%, 95% CI -0.06 to 0.10, p = NS).

CHAPTER-IV

MATERIALS AND METHODS

1. Protocol:

Study Design	A randomized, open label, two treatments, two periods, two sequence, single dose, crossover design study.
No. of Subjects	Total **12** normal, healthy, adult, human subjects were enrolled.
Study Duration	The duration of the clinical phase was approximately 11 days including washout period of at least 7 days between administrations of study drugs in each study period.
Pre Study Evaluation	Subjects were screened for the inclusion/exclusion criteria by the following procedure: Demographic data, clinical history, physical examination (including vital signs), 12 lead ECG, chest X-ray (if required), haemogram, biochemistry, serology (HIV, Hepatitis B and Hepatitis C) and urinalysis. Breath alcohol test was done during the screening. Any other test(s) if required was done as per the suggestion given by co-investigator or principal investigator.
During study Evaluation	Urine screen for drug of abuse and Breath alcohol test was done before check- in for each study period. Breath alcohol test was also done before each ambulatory samples in each study period. Any other test(s) if required was done as per the suggestion given by co-investigator or principal investigator.
Housing	At least 12.00 hrs prior to drug administration and until 24.00 hrs post dose.
Restrictions	Food restriction: Subjects were fasted for at least 10 hrs prior to receiving the high fat high calories breakfast, which was started by subject 30 min before dosing and for at least 4 hrs post dose in each period. Fluid Restriction: Water was not accessible to the subjects 1 hr pre dose and 1 hr post dose except 240 mL of water given during administration of the dose in each period. Postural Restriction: Subjects were remain seated for the first 2 hrs post dose and was given supine or semi-recumbent positions after 2 hrs. for at least 8 hrs after dosing except for any procedural reason. They should rise only with assistance during this period of time. When the subject experiences an adverse event appropriate position was given to the subjects.

Dose Administration	Single oral (1x 12.5 mg tablet) of test or reference product was administered as per randomization schedule in each period with 240 mL of water at ambient temperature in sitting position.
Meal	Standardized meal was given during check-in night (In such a way to maintain 10.00 hrs. fasting before high-fat and high-calorie breakfast which was started by subject exactly 30 min prior to drug administration) and at around 04.00, 08.00 and 12.00 hrs post dose.
Blood Sample Collection **(4 mL per sample)**	Total no. of blood samples: 25 per period. Sampling Hours: Pre dose (collected within 1 hr prior to dosing), 00.25, 00.50, 00.75, 01.00, 01.25, 01.50, 01.75, 02.00, 02.50, 03.00, 04.00, 05.00, 06.00, 08.00, 09.00, 10.00, 12.00, 14.00, 16.00, 18.00, 20.00, 24.00, 36.00 and 48.00 hrs post dose. Blood samples were collected in K_2-EDTA vacutainer.
Total Blood Loss	Total blood loss in this study was approximately 225.2 mL
Sample processing	All the blood samples were centrifuged under refrigeration with the machine set at 3500 RPM, 10 minutes and 5°C. Plasma samples were be placed in deep freezer maintained at -20°C ± 5°C.
Monitoring Of Subjects During Study	Physical examination & Vital examination (Blood pressure, pulse rate, temperature and respiratory rate) was done at the time of check in and check out of each study period and at last ambulatory visit of last study period. Blood pressure and pulse rate measurement and well being assessment was done at pre dose, 02.00, 05.00, 10.00, 36.00 and 48.00 hrs post dose ± 45 minutes (except for pre dose & at each ambulatory visit) of scheduled time in each study period.
Precautionary and safety measures	Since Carvedilol causes somnolence, dizziness and postural hypotension, bedside meal was provided up to 08 hrs. post dose and bedside blood sample collection, blood pressure and pulse rate measurement and well being assessment was done up to 08 hrs. post dose.
Post Study Assessment	Physical examination including vital signs, 12 lead ECG, haemogram, biochemistry and urinalysis were done at the end of study or on discontinuation of subject from the study.
Analytical Method	Plasma concentrations of Carvedilol and 4-hydroxyphenyl-carvedilol were measured by a validated LC/MS/MS analytical method as per in-house SOPs.
Pharmacokinetic parameters	C_{max}, AUC_{0-t}, AUC_{0-inf}, AUC_{0-t}/AUC_{0-inf}, T_{max}, K_{el} and $t_{1/2}$
Statistical Evaluation	Pharmacokinetic and Statistical analysis was done using SAS®

	9.2 or higher version. ANOVA was performed on log transformed pharmacokinetic parameters C_{max}, AUC_{0-t} and $AUC_{0-inf.}$ The 90% confidence interval was constructed for the ratio of geometric least square mean of the test and reference product, obtained from the log-transformed pharmacokinetic parameters. Descriptive statistics (mean, standard deviation, coefficient of variation, minimum and maximum) was computed for each pharmacokinetic parameter for the test and reference product of Carvedilol and 4-hydroxyphenyl-carvedilol. The analysis of Carvedilol was considered for statistical analysis for establishing bioequivalence. 4-hydroxyphenyl-carvedilol was considered for profiling purpose only. The drug concentrations of Carvedilol and 4-hydroxyphenyl-carvedilol in plasma for each subject, each sampling time and the each product were reported.

2. Ethics:

Independent Ethics Committee (IEC):

The Protocol and corresponding Informed Consent Forms (English and Gujarati language), Case Report Forms (Period I and II) were reviewed, discussed and approved in the IEC meeting held on 01st December, 2009. Subjects were not enrolled into the study until the IEC approved the protocol and the ICF.

Ethical Conduct of the Study:

The study was conducted according to the current version of the declaration of Helsinki (Ethical Principles for Medical Research Involving Human Subjects), Revised by WMA General Assembly, Seoul, October 2008, current ICH GCP guidelines, regulatory requirements of USFDA and CDSCO guidelines.

3. Duration of Study:

Subjects undergo the screening procedure at least 21 days before the first day of dosing. Total duration of study was of 11 days from the day of check-in of first period till the end of last period. Upon entering into study, subjects were confined in the clinical facility to ensure 10 hours overnight fasting before receiving high fat high calorie breakfast 30 minutes prior to dosing, until 24 hours post-dose sample collection in each of the periods.

Clinical Conduction:	
Period I	16th December, 09 to 19th December, 09
Period II	27th December, 09 to 30th December, 09
Date of Clinical phase Completion:	6th January, 10

Wash out Period:

This study was two period crossover study separated by more than 5 half lives of washout period between the two periods. Considering the half life of the Carvedilol, the washout period of at least 7 days was considered. Total expected duration of the study was approximately of 11 days from the day of check-in of the period-I to till the end of the period-II.

4. Volunteer Information and Consent:

A total of 12 Subjects participated in the informed consent presentation. The Informed consent form was issued to all the Subjects in vernacular (Gujarati) language. The Subjects read the ICF which summarized the discussion prior to check-in. Sufficient time was given to the Subjects to read, understand and clarify the doubts on the contents of the ICF. A total of 12 volunteer had given their consent and enrolled in the study. A copy of the signed informed consent form was given to them. (Refer Annexure G)

5. Identity of the Investigational Products (Table-6):

TEST PRODUCT	
Product name	Carvedilol Tablets 12.5 mg
Label claim	Each film coated tablet contains Carvedilol USP 12.5 mg
Dose	1x 12.5 mg tablet to be taken orally with about 240 mL of water at ambient temperature.
Dosage Form	Tablet
Batch No.	US/CART(12.5)03/09
Manufacturing date	Sept' 2009
Expiry Date	Aug' 2011
Storage condition	Store below 30°C (86°F).

REFERENCE PRODUCT	
Brand Name	COREG® (Carvedilol) Tablets 12.5 mg
Label claim	Each Tiltab® tablet contains Carvedilol 12.5 mg.
Dose	1x 12.5 mg tablet to be taken orally with about 240 mL of water at ambient temperature.
Dosage Form	Tablet
Lot No.	467 7V41
Manufacturing Date	N/A
Expiry Date	Jun' 2010
Name of Manufacturer	GlaxoSmithKline
Storage condition	Store below 30°C (86°F).

6. Procurement, Storage and Accountability Procedures for Investigational Products:

6.1 Receipt:

A total of 30 tablets of test and 30 tablets of reference drugs were received from Sponsor for fed study. The study medications were provided in a sufficient quantity for the needs of the whole study and for retention. Total 18 tablets were retained including 2 tablets which were used to check appearance.

6.2 Storage:

All drug supplies were stored at or below 30°C in accordance with the manufacturer's instructions; separately from normal practice stocks, locked and only accessible for authorized personnel. The temperature and the humidity in the storage room were continuously monitored. The storage conditions were checked by the study personnel. The study drugs were stored in a container having label bearing study code, name of product storage condition etc.

"FOR CLINICAL RESEARCH PURPOSE ONLY"	
Study Code:	Batch No.
Generic Name:	
Brand Name: Storage Condition:	
Expiry date/Use by date/Retest date:	Prepared by
No. of units received.	(Sign / Date :)

6.3 Dispensing

The study drugs were dispensed on a day of enrolment in each period. Bottles were dispensed in a labeled container by trained pharmacist under supervision of co-investigator and stored in a locked environmentally controlled (temperature at or below 30°C) area with restricted access.

The dispensed drugs were delivered to dosing area approximately 30 minutes before dosing by the Pharmacist, till that time dispensed study drugs were kept under controlled access and specified condition in pharmacy. One extra units of test and reference drug were dispensed in each period of each batch. The labels of the containers consist of two segments, a fixed and a flag segment. The dispensing record generated by pharmacist were checked by study personnel and kept in study file.

The labels, identifying the study code, study period, subject number, the treatment code (Test A or Reference B) and "For Clinical Research Use Only" were affixed on the case report form (CRF) which was signed by the assigned study personnel responsible for the activity.

For Clinical Research Use Only				For Clinical Research Use Only		
Study Code :				Study Code :		
Period:	**I**	**A**		Period:	**II**	**B**
Subject No:	**XX**			Subject No:	**XX**	

6.4 Handling of unused drugs

After completion of dosing activity, the dispensed but unused study drugs were sent back to the pharmacy. The extra dispensed study drugs were disposed by pharmacist.

6.5 Drug Accountability

The investigator was not allowed to make use of the study drugs for purposes other than specified in protocol. The drug accountability was maintained by pharmacist through out study under supervision of principal Investigator. All the study drugs (i.e. dispensed but un-dosed) returned from bio study was sent back to pharmacy and recorded.

6.6 Prior and Concomitant Medication

No subjects used any medication (Prescription or over the counter) vitamins or minerals for 14 days prior to the study and during the study. Subjects did not use any enzyme modifying drugs in the previous 28 days prior to dosing until the last sample collection of last study period or were not in any medical or surgical conditions which might significantly interfere with the functioning of gastrointestinal tract, blood-forming organs etc which was confirmed by the clinical history taken by the Assigned medical officer.

7. Randomization method:

All twelve (12) subjects were randomized to one of the treatments (Test A or Reference B) according to the randomization schedule (AB or BA), which was prepared and approved by the biostatistician prior to the conduct of the study. The subjects were assigned subject numbers serially as per their check-in time to the clinical pharmacology unit during period I, which remained the same throughout the study

The randomization was balanced and the code was kept under controlled access. Randomization generated by a statistician was kept in sealed envelop which was in the custody of pharmacist. The concerned analysts were blinded to the sequence of administration of study drugs. The randomization code was broken after completion of analysis.

Subject	Sequence	Period-I	Period-II
1	AB	A	B
2	BA	B	A
3	BA	B	A
4	AB	A	B
5	AB	A	B
6	BA	B	A
7	BA	B	A
8	AB	A	B
9	BA	B	A
10	AB	A	B
11	BA	B	A
12	AB	A	B

Table 7: Randomization Schedule
A= Test Product and B=Reference Product

8. Selection and Withdrawal of Subjects:

For selection into the study, the subjects undergone screening procedure within 21 days prior to the dosing of period-I and fulfill all the clauses of inclusion and none of the exclusion criteria.

8.1 Inclusion Criteria:

1. Male human subjects, age in the range of 18 – 45 years.
2. Body weight within ± 15% of ideal weight as related to height and body frame according to Life Insurance Corporation (LIC) Chart.
3. Subjects with normal findings as determined by baseline history, physical examination and vital signs (blood pressure, pulse rate, respiration rate and temperature).
4. Subjects with Clinically acceptable findings as determined by haemogram, biochemistry, serology (HIV, Hepatitis B and Hepatitis C), urinalysis, ECG and X-ray (X-ray if taken).
5. Willingness to follow the protocol requirements as evidenced by written informed consent.

6. Confirming and agreeing to, not using any prescription and over the counter medications including Vitamins and minerals for 14 days prior to study & during the course of the study.
7. Non-smokers and Non-alcoholics were included

8.2 Exclusion Criteria:

1. Known history of hypersensitivity to Carvedilol or related drugs.
2. Requiring medication for any ailment having enzyme-modifying activity in the previous 28 days, prior to dosing day.
3. Any medical or surgical conditions, which might significantly interfere with the functioning of gastrointestinal tract, blood–forming organs etc.
4. History of cardiovascular, renal, hepatic, ophthalmic, pulmonary, neurological, metabolic, hematological, gastrointestinal, endocrine, immunological or psychiatric diseases and bleeding tendency.
5. Participation in a clinical drug study or bioequivalence study within 90 days prior to present study.
6. Subjects with history of recent myocardial infarction, cardiac arrhythmias, cardiac failure and convulsions.
7. History of malignancy or other serious diseases.
8. Refusal to abstain from food for at least ten (10) hrs prior to receiving the high fat high calories breakfast, which was started by subject exactly 30 min before dosing and for at least four (4) additional hrs post dose, in each study period.
9. Any contraindication to blood sampling or difficulty in accessibility of veins.
10. Refusal to abstain from fluid for at least 1 hr prior to study drug administration and for at least 1 additional hr post dose, in each study period except 240 ml of water during administration of study drug.
11. Refusal to avoid the use of xanthine-containing food or beverages (chocolates, tea, coffee or cola drinks) or fruit juice/grapefruit juice and any alcoholic products for 48 hrs prior to dosing until the last blood sample collection of last study period.
12. Blood donation within 90 days prior to the commencement of the study.
13. Subjects with positive HIV tests or Hepatitis-B or Hepatitis-C tests.

14. Found positive in breath alcohol test done before check-in and before each ambulatory sample for each study period.

15. Found positive in urine test for drug abuse done before check-in for each study period.

16. Refusal to abstain from consumption of tobacco products 24 hrs prior to dosing until the last blood sample collection of last study period.

17. History of problem in swallowing tablets.

8.3 Pre-study (screening) and during study Evaluation

The pre-study evaluation procedure included following:

8.3.1 Demography:

Demographic information was done during screening which includes subject registration number, age, gender, height and weight of the subjects.

8.3.2 Radiological Examination:

Chest X-ray was done during screening if required by the medical officer and/or by principal investigator.

8.3.3 12-Lead Electrocardiograms:

12 lead ECG was done during screening.

8.3.4 Complete Physical Examination of subjects:

Pre-study examination (Screening) included:

- Clinical history
- Physical examination including vital signs (temperature, respiratory rate, pulse rate and blood pressure).

Physical examination & Vital examination (Blood pressure, pulse rate, temperature and respiratory rate) were done at the time of check in and check out of each study period and at last ambulatory visit of last study period.

8.3.5 Laboratory Tests:

Laboratory tests were done prior to the study for all subjects.

8.3.5.1 Blood & Urine Tests:

Pre-study blood samples were obtained for haemogram, biochemistry and serology (HIV, Hepatitis B and Hepatitis-C). Pre-study routine urine analysis was done.

Any other test(s) if required was done as per the suggestion given by co-investigator or principal investigator.

8.3.5.2 Breath alcohol test:

Breath alcohol test was done during the time of screening, before check-in and before each ambulatory samples for each study period.

8.3.5.3 Urine test for drug abuse:

Urine test for drug abuse was done before check-in for each study period.

8.4 Subject Withdrawal/ Dropout:

Any subject discontinued from the study by medical officer/ principal investigator other than personal reasons considered as withdrawn.

Subjects discontinued from the study for any of the following reasons:

1. Subjects not wishing to continue with the study, irrespective of the reason (dropout subjects).
2. Adverse event during the study.
3. Any illness requiring medication during the study.
4. Violation of the protocol by the subject.
5. The decision to withdraw the subject if vomiting occurs at or before two times of median T_{max} (documented in the literature) was taken by the principal investigator considering the nature and amount of vomitus, likely/ anticipated impact on the study outcome and the subjects' health status.

Subject was not evaluated for the post study assessment if he was discontinued from the study before dosing in period-I. Subject may also discontinued from the study for any reason beneficial to his well-being. The principal investigator, as well as the sponsor, decide to withdraw any subject's participation in the study if, in their judgment, continuation in the study may prove harmful to the subject. Such a decision may be precipitated by adverse events, including changes in vital signs, physical examination and ECG, pathological investigation etc. The principal investigator may also withdraw a subject due to poor compliance to the study protocol. An attempt was made by principal investigator to find out reason for drop out. The decision to consider the data for analytical and statistical evaluation of the subjects withdrawn/dropped out was taken by principal investigator based on the time/phase the subject has been withdrawn/dropped out from the study. If a subject was discontinued from the study any time after being assigned a subject number, the reason was recorded in the case report form ('withdrawal/dropped out'), by the medical officer/ assistant medical officer. The details of withdrawal/dropped out subjects was reported.

9. Treatment of Subjects:

Dose Administration:

The subjects were administered any of the following investigational product in sitting position, as per the randomization schedule in each study period, after receiving the high fat high calories breakfast, which was started by subject exactly 30 min before dosing.

Test Product: Carvedilol Tablets 12.5 mg

Dose: 1x 12.5 mg tablet to be taken orally with about 240 mL of water at ambient temperature

Reference Product: COREG® (Carvedilol) Tablets 12.5 mg

Dose: 1x 12.5 mg tablet to be taken orally with about 240 mL of water at ambient temperature

Dose administration was done with about 240 mL of water by trained personnel, under the supervision of the Principal investigator or Co-investigator. Subjects were instructed not to chew or crush the tablets but to consume it as a whole. The dose was administered at around 8:00 am or 9:00 am onwards, in a staggered manner to maintain subsequent blood collection schedule.

Compliance for dosing was assessed by a thorough check of the oral cavity by using a tongue depressor and torch immediately after dosing by medical officer / assistant medical

officer/trained personnel. Record of dosing for individual subject was maintained in case report form. The subjects returned to the facility, one day prior to dosing of period II.

Dosing Time (am)	Groups (Subject Numbers)	
09:00	01	02
09:02	03	04
09:04	05	06
09:06	07	08
09:08	09	10
09:10	11	12

Table 8: Dosing time of subjects in both periods

Medication

Subjects were instructed not to take any medications (either prescribed or OTC) including vitamins and minerals for at least 14 days prior and during the study. If drug therapy other than that specified in the protocol required urgently during the study or in the washout period, decisions to continue or discontinue the subject was taken by the principal investigator and/ or the sponsor, based on the following:

- Safety and well being of subject.
- Pharmacology and pharmacokinetics of the non-study medication.
- Likelihood of a drug interaction, which may affect the pharmacokinetic comparison of the study medications.
- The time of administration of the non-study medication, and likelihood of interference in bio-analysis.

9.3 Monitoring for subject Compliance

The subjects were monitored for the compliance to the following restrictions throughout the study period.

Diet: In each period all subjects required to fast for at least 10 hrs prior to receiving the high fat high calories breakfast, which was started by subject 30 min before dosing and for at least 4 hrs post dose. Standardized meal was be given during check-in night (In such a way to maintain 10.00 hrs. fasting before high-fat and high-calorie breakfast which was started by subject

exactly 30 min prior to drug administration) and at around 04.00, 08.00 and 12.00 hrs post dose.

All subjects were instructed to abstain from use of tobacco products 24 hrs prior to dosing until the last blood sample collection of last study period.

Precautionary and safety measures: Since Carvedilol causes somnolence, dizziness and postural hypotension, bedside meal was provided up to 08 hrs. post dose.

Fluid Restriction: Water was not allowed from 1 hr pre dose and 1 hr post dose except 240 mL of water given during administration of the dose in each period. At all other times drinking water was given *ad-libitum*.

Physical Activity/Posture: Subjects remain seated for the first 2 hrs post dose and given supine or semi-recumbent positions after 2 hrs. for at least 8 hrs after dosing except for any procedural reason. They should rise only with assistance during this period of time. When the subject experiences an adverse event appropriate position was given to the subjects.

9.4 Blood Samples Collection:

Blood sample collection was done using intravenous cannula. The intravenous cannula was inserted into subject's arm for collection of blood samples before pre dose blood sample and for up to 24.00 hrs post dose for each study period. If difficulty occurred in blood withdrawing or if the subject not feeling comfortable with cannula then cannula was removed before 24.00 hrs post dose and remaining blood samples were collected through fresh vein puncture or by recannulation. When meal and sample collection coincide, samples were collected before meals. Ambulatory blood samples at 36.00 and 48.00 hrs post dose were collected through fresh vein puncture.

Blood samples (4 mL) were collected in K_2-EDTA Vacutainer., at pre dose (within 1 hr prior to dosing) and at 00.25, 00.50, 00.75, 01.00, 01.25, 01.50, 01.75, 02.00, 02.50, 03.00, 04.00, 05.00, 06.00, 08.00, 09.00, 10.00, 12.00, 14.00, 16.00, 18.00, 20.00, 24.00, 36.00 and 48.00 hours post dose (25 samples), within 2 minutes of scheduled sampling time in each the study periods.

Before every blood sample collection 0.2 mL of blood present in the intravenous cannula was discarded during the use of intravenous cannula. Also after every blood sample collection, 0.2 mL of heparinised saline (by mixing 1 mL of 5000 IU / 5 mL of heparin with 500 mL of normal saline) was injected into the intravenous cannula. The actual end time of collection of each blood sample was recorded in the CRF.

Total blood loss approximately for complete study:	
Total blood samples (50 x 4.0 mL)	**: 200 mL**
Pre study screening	**: 08 mL (Up to)**
Post study evaluation	**: 08 mL (Up to)**
Discarded heparinized blood (46 x.0.2 mL)	**: 9.2 mL**
Total blood loss	**: 225.2 mL**

9.5 Sample Handling & Processing

Following centrifugation under refrigeration with the machine set at 3500 RPM, 10 minutes and 5°C, the plasma was transferred to appropriate size polypropylene screw top (previously labeled with study code and sample code) biological samples storage vials.

Plasma samples were placed in biological sample storage box/cryo box (previously labeled as per SOP for 'Labeling') and then the samples were placed in deep freezer maintained at -20°C ± 5°C. Biological samples were transferred to the sample storage area in cryo box by placing in a thermocol box containing dry ice (previously labeled as per SOP for 'Labeling') and stored in the deep freezer. The above procedures were performed in accordance with current version of SOP for 'Blood sample collection, Processing and Storage'.

10. Assessment of Safety:

The principal investigator monitored safety data throughout the course of the study. Subjects were monitored throughout the study period for occurrence of adverse events.

10.1 Safety Parameters:

Safety measurements included monitoring of AEs, SAEs, physical examination results, vital signs and clinical laboratory results.

Monitoring of Subjects during Study:

Physical examination & Vital examination (Blood pressure, pulse rate, temperature and respiratory rate) were done at the time of check in and check out of each study period and at last ambulatory visit of last study period.

Blood pressure and pulse rate measurement and well being assessment was done at pre dose, 02.00, 05.00, 10.00, 36.00 and 48.00 hrs post dose ± 45 minutes (except for pre dose & at each ambulatory visit) of scheduled time in each study period.

Precautionary and safety measures: Since carvedilol causes somnolence, dizziness and postural hypotension, bedside blood pressure and pulse rate measurement and well being assessment were done up to 08 hrs. post dose.

Post Study (End of Period II) Evaluations:

At the end of the study (48.00 hrs post dose in period II), or on discontinuation of a subject, the following procedures were completed for each subject:

- **Physical examination including vital signs**
- **12-Lead ECG was taken after last blood sample collection.**
- **Laboratory Tests (haemogram, biochemistry and urinalysis)**

Note: Physical and vital examination at the time of last ambulatory sample of period II was considered for post study evaluation if subject is not discontinued from study.

If the subject does not come for the safety evaluation on scheduled time for any reason, it was performed whenever the subject reports to the facility within the washout period.

If any subject fails to complete the study or is discontinued from the study, the reason was specified in the respective CRF and the clinical report.

The subjects were asked for their well being during the study period. The subjects may also report spontaneously any inconvenience or adverse events to the monitoring staff at any time during the conduction of study period.

10.2 Adverse Event Reporting:

All adverse events reported were properly documented on the adverse event form in the CRF. In particular the information included description of the event, details of occurrence,

frequency of adverse event, description of the severity of the event, any treatment or diagnostic steps taken in relation to the event, description of the outcome of the event, judgment by the medical officer of any relationship of the event to study medication or procedures.

All adverse events and serious adverse events whether drug related or not were reported to the sponsor and IEC by Investigator(s).

An **Adverse Event** is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. It may be an inter-current illness; a drug reaction or interaction; related to concomitant medication; an abnormal laboratory value or a significant shift from a clinically acceptable laboratory value, which is considered by the investigator to be important.

A **Serious Adverse Event** is an adverse event that results in

- Death
- Life-threatening
- Requires hospitalisation or prolongation of existing hospitalisation
- Persistent or significant disability / incapacity
- Congenital anomaly/birth defect
- A medically important event or reaction (serious or important medical events that may not be immediately life-threatening or result in a death but may require intervention to prevent one of the other outcomes listed in the definition above).

Adverse events may be classified as:

- **Mild**: Minimal interference in day-to-day activities, Special treatment may not be required to treat adverse event, Symptoms are transient.
- **Moderate:** Discomforting event, Interference in day-to-day activities, Therapeutic measures are required to treat adverse event.
- **Severe:** Severe discomfort, Day-to-day activities are impossible, Major therapeutic intervention is required to treat adverse event.

10.3 Adverse Event Follow-up:

Subjects experiencing adverse events were followed up until the events have resolved.

11. Bio-Analytical Method:

Sr. No.	Parameters	Details
1	Internal standard	Propranolol
2	Biological Matrix	Human plasma
3	Anticoagulant	K_2EDTA
4	Chromatographic Condition	LC-MS/MS
	Mobile Phase	Buffer : Acetonitrile (30 : 70 (v/v))
	Buffer	0.1% (v/v) Formic Acid in Water
	Column	Hypersil Gold (100 mm x 4.6 mm, 5µ)
	Flow Rate	0.600 mL/min
	Injection Volume	10 µL
	Column Oven Temperature	40 °C
	Polarity	Positive
	Auto sampler	10°C
	Detector	Mass Spectrometer
	Diluents	Methanol : Water (60 : 40 (v/v))
	Rinsing Solution	Strong wash :- Acetonitrile Weak wash :- Acetonitrile : Water (80 : 20 v/v)
	Mass to charge ratio (m/z)	Carvedilol : Parent Ion 407.2 amu : Product Ion 100.0 amu Propranolol : Parent Ion 260.2 amu : Product Ion 116.1 amu
5	Extraction Procedure	Solid Phase Extraction
	Sample preparation	Thaw all frozen plasma samples and vortex each plasma sample for about 10 seconds and centrifuge at 3200 RPM at 10°C for 5 minutes.
	Blank Plasma	0.200 mL Blank Plasma + 200 µL of 0.1% (v/v) Formic Acid in Water
	Zero Standard	0.200 mL Blank Plasma + 20 µL IS-2 (0.500 µg/mL) + 200 µL of 0.1% (v/v) Formic Acid in Water

	Calibration Standards & Quality Control Samples	0.200 mL Plasma containing known concentration of analyte (s) + 20 µL IS-2 (0.500 µg/mL) + 200 µL of 0.1% (v/v) Formic Acid in Water
	Subject Samples	0.200 mL Subject Sample(s) + 20 µL of IS-2 (0.500 µg/mL) + 200 µL of 0.1% (v/v) Formic Acid in Water
	Mixing	Vortex for 30 secs.
	Centrifugation	Centrifuge the samples at 14000 RPM for 5 mins at 10°C
	Cartridge	Lichrosep DVB-HL 30 mg/1mL
	Conditioning & Equilibration	1 mL Methanol followed by 1 mL 0.1% (v/v) Formic Acid in Water
	Loading	System Suitability, Blank Plasma, Zero Standard, Calibration Curve Standards, Quality Control Samples and Subject Samples on separate cartridges.
	Washing	1 mL of 10% (v/v) Methanol in Water followed by 1 mL of Water
	Elution	1 mL of Mobile phase
	Centrifugation	Centrifuge the eluted samples at 3200 RPM for 5 mins at 10°C
6	LLOQ	0.500 ng/ mL
7	Calibration Curve Range	0.500 ng/mL to 80.000 ng/mL
8	Quality Control Samples	LQC : 1.400 ng/mL MQC : 24.000 ng/mL HQC : 56.000 ng/mL
9	Calculation by	Linear regression weighted ($1/x^2$) analysis

12. Statistical Plan:

Non-compartmental pharmacokinetic analysis was performed on the observed drug concentrations in plasma for carvedilol and 4-hydroxyphenyl-carvedilol; using the statistical package SAS® 9.2.

The analysis of carvedilol was considered for statistical analysis for establishing bioequivalence. 4-hydroxyphenyl-carvedilol was considered for profiling purpose only. The data of the subject was deleted from the statistical analysis if the subject vomits during the course of the study, at or before 2 times median $T_{max.}$

If the pre-dose concentration is greater than 5% of C_{max}, the subject was dropped from the pharmacokinetic and statistical evaluations.

All concentration values below the limit of quantification (BLQ) were set to zero; for the estimation of pharmacokinetic parameters.

12.1. Pharmacokinetic Parameters:

The following parameters were calculated for each subject-over each product combination using the non-compartmental model by using statistical package SAS® 9.2:

C_{max}	Maximum observed drug concentration during the study.
AUC_{0-t}	Area under the plasma concentration - time curve measured to the last quantifiable concentration, using the trapezoidal rule.
AUC_{0-inf}	AUC_{0-t} plus additional area extrapolated to infinity, calculated using the formula $AUC_{0-t} + C_t/K_{el}$, where $C_{t\ is}$ the last measurable drug concentration and $K_{el\ is}$ the elimination rate constant.
T_{max}	Time to observe maximum drug concentration.
AUC_{0-t}/AUC_{0-inf}	Ratio of AUC_{0-t} and AUC_{0-inf}
K_{el}	Apparent first – order terminal elimination rate constant calculated from a semi-log plot of the plasma concentration versus time curve, using the method of least square regression.
$t_{1/2}$	Terminal half-life as determined by quotient $0.693/K_{el}$

Note: No value of k_{el}, $t_{1/2}$ and AUC_{0-inf} was reported for cases that do not exhibit a terminal log-linear phase in the concentration versus time profile.

12.2. Statistical Method:

Calculation of pharmacokinetic parameters and statistical analysis for establishing bioequivalence was performed using the statistical package SAS® 9.2. PROC GLM procedure in SAS was used for analysis of variance and the estimation of least square mean differences (Test-Reference) of the test and reference formulations on the log-transformed pharmacokinetic parameters C_{max}, AUC_{0-t} and AUC_{0-inf} and the corresponding standard errors of the differences were also computed.

Based on these parameters, the 90% confidence interval was constructed for the least square mean differences of log-transformed C_{max}, AUC_{0-t} and AUC_{0-inf}. The antilog (or exponential) of the limits obtained from the log-transformed data give the 90% confidence interval for the ratio of geometric means of test and reference formulations.

All the pharmacokinetic parameters were reported for each subject-over each product combination and descriptive statistics (mean, standard deviation, coefficient of variation, minimum and maximum) were computed for each pharmacokinetic parameter for each product.

12.3. Subject Population(s) for Analysis:

To estimate the intra-subject variability, 12 subjects were considered for this pilot bioequivalence study. To get the equal randomization in this randomized, open label, two treatment, two period, two sequence, single dose, crossover design, 12 subjects were randomized using statistical package SAS® 9.2.

12.4. Significance:

12.4.1 Analysis of Variance:

ANOVA was performed on log transformed pharmacokinetic parameters C_{max}, AUC_{0-t}, and AUC_{0-inf} at the level of 0.05(α). The analysis of variance model was included sequence, subjects nested within sequence, period and treatment as factors.

Each analysis of variance was also included calculation of least-square means, adjusted differences between formulation means and the standard error associated with these differences. The significance of the sequence effect was tested using the subjects nested within the sequence as the error term.

12.4.2 Confidence Interval:

Consistent with the two one-sided test for bioequivalence, 90% confidence intervals was constructed for the difference (Test – Reference) of least square means of the log-transformed C_{max}, AUC_{0-t} and $AUC_{0-inf.}$ The antilog (or exponential) of these limits give the 90% confidence interval for the ratio of geometric least square means.

12.4.2 Power of Test:

The power (i.e. probability of detecting a 20% difference relative to the reference treatment LSM at the 5% significance level using a t-test under the null hypothesis of zero difference) was calculated for log transformed C_{max}, AUC_{0-t}, $AUC_{0-inf.}$

Although if power is not sufficient due to insufficient number of subjects, the relative mean test by reference ratio estimates and intra and inter subject variability estimates are important for extrapolation forward to pivotal studies.

12.4.3 Acceptance Criteria for Bioequivalence:

The 90% geometric confidence interval of the ratio (Test/Reference) of least-squares means from the ANOVA of the ln-transformed C_{max}, AUC_{0-t} and AUC_{0-inf} fall within 80.00% to 125.00% for Carvedilol.

12.5 Accountability Procedure:

12.5.1 Treatment of Missing Values:

Missing sample values (MSV) or non-reportable values (NRV), of the plasma concentration data, were treated as 'missing values' and reasons for their missing were documented. Such missing values were represented as MSV and NRV in the plasma concentration tables. Further these missing values were arbitrarily coded as '999999' and treated as 'missing values' for statistical analysis. Data from the subjects with missing

concentrations values (missed blood draws, lost samples, samples unable to be quantified) may be used if pharmacokinetic parameters can be estimated using the remaining data points. Otherwise, concentration data from these subjects can excluded from the final analysis.

12.5.2 Missing Samples:

Missing samples can be due to withdrawal of subject and accidental spillage of samples. The clinical data identified the missing samples. The individual missing samples dealt as per case to case and the Principal Investigator and the Analytical Investigator evaluate its impact on the data.

12.6. Treatment of Time Point Deviation:

Time point deviation for any subject at any time point was taken care, while calculation of pharmacokinetic parameters (AUC_{0-t} & AUC_{0-inf}) using statistical package SAS® 9.2. Blood sampling up to 2 minutes of the schedule time was considered as an acceptable deviation. Actual blood sample collection time was considered for deviation beyond two minutes for calculation of pharmacokinetic parameters.

CHAPTER-V

RESULTS

1. Study Subjects:

1.1 Disposition of Subjects:

Twenty (20) Subjects that were most likely to meet the requirements of this study and who were willing to participate in the study were screened. Total of 12 fit and consenting subjects were enrolled in the study. These subjects were randomized to receive either of the sequence of administration of the study products.

1.2 Data Sets Analyzed:

12 subjects were enrolled into the study as per plan. 11 subjects completed the clinical phase of study and plasma samples of these 11 subjects were analyzed.

Data of these 11 subjects were considered to draw statistically conclusion.

1.3 Demographic and Other Baseline Characteristics:

Normal, Healthy, Adult Male Human, subjects between 18-50 years (both inclusive) of age were screened for Inclusion /Exclusion criteria as mentioned in the Study Protocol. Twelve (12) normal, healthy, adult, human subjects were enrolled in the study. The demographic data of 12 subject enrolled in study is given in table-9

Registration No.	Screening Date	Subject No.	Sex	Age (yrs)	Height (cms)	Weight (kg)	BMI (Kg/m^2)
009312	11/12/09	1	Male	29	182	76	22.94
007996	11/12/09	2	Male	24	173	75	25.06
011355	11/12/09	3	Male	25	167	76	27.25
011691	10/12/09	4	Male	37	164	63	23.42
011564	11/12/09	5	Male	26	168	61	21.61
005082	10/12/09	6	Male	23	171	55	18.81
001991	11/12/09	7	Male	22	174	54	17.84
007672	10/12/09	8	Male	25	177	67	21.39
010716	10/12/09	9	Male	38	164	65	24.17
012945	11/12/09	10	Male	28	165	82	30.12
011841	10/12/09	11	Male	41	168	51	18.07
011014	11/12/09	12	Male	35	173	76	25.39
Mean				**29.42**	**170.50**	**66.75**	**23.01**
SD				**6.57**	**5.55**	**10.25**	**3.73**
Median				**27.00**	**169.50**	**66.00**	**23.18**

Table-9: Demographic profile of subjects enrolled in the BE Study

Subject no. 3 was dropped out from the study before period-II. So, only 11 subjects were considered for the final analysis. Demographic data is given in table-10

Registration No.	Screening Date	Subject No.	Sex	Age (yrs)	Height (cms)	Weight (kg)	BMI (Kg/m^2)
009312	11/12/09	1	Male	29	182	76	22.94
007996	11/12/09	2	Male	24	173	75	25.06
011691	10/12/09	4	Male	37	164	63	23.42
011564	11/12/09	5	Male	26	168	61	21.61
005082	10/12/09	6	Male	23	171	55	18.81
001991	11/12/09	7	Male	22	174	54	17.84
007672	10/12/09	8	Male	25	177	67	21.39
010716	10/12/09	9	Male	38	164	65	24.17
012945	11/12/09	10	Male	28	165	82	30.12
011841	10/12/09	11	Male	41	168	51	18.07
011014	11/12/09	12	Male	35	173	76	25.39
Mean				**29.82**	**170.82**	**65.91**	**22.62**
SD				**6.74**	**5.71**	**10.31**	**3.66**
Median				**28.00**	**171.00**	**65.00**	**22.94**

Table-10: Demographic profile of subjects included in the final statistical analysis

All Subjects' general medical history, clinical examination, various Laboratory tests and 12-lead ECG recordings were conducted at the time of screening. After reviewing all the data of above mentioned tests, the physician confirmed that all values and reports were well within the clinically acceptable range and the Subjects were healthy and suitable for participation in the study.

A urine screen for drugs of abuse (amphetamine, benzodiazepines, barbiturates, cocaine, marijuana, and morphine) and alcohol breath test were performed at admission of each period and all the subjects were found fit for participation in the study.

1.4 Measurement of Treatment Compliance

Compliance for dosing was assessed by monitoring the subject till they swallow tablet and then a thorough check of the oral cavity was done by the study personnel using a torch. The duplicate label of dispensed container was then pasted on the 'Dosing' section of individual Case Report Form (CRF).

2. Handling of Dropouts or Missing Data:

11 subjects completed the study. Some of the missing data are as follows:

ub. No.	Period	No. of missing Samples	Sampling time points	Reason
No missing samples in period-I				
03	II	25	All	Sub. dropped out

Table-11: Missing Samples Record

3. Safety Evaluation:

3.1 Extent of Exposure

Total 12 subjects were administered either test or reference product as per the randomization schedule (except withdrawn). Total 11 subjects completed the clinical phase of the study and the data of these subjects were considered to draw statistical conclusion.

The duration of study was 11 days, including the washout period of 7 days between two periods. Subject no.03 was not dosed in period-II as he was dropped out.

Subject No.	Adverse event	Start Time	Relief Time	Severity	Drug Relation	Measures Taken
During Period- I						
03	**High S.G.P.T.:- 86.7 U/L**	10:36 (30/12/09)	16:07 (06/01/10)	**Mild**	**Possible**	Reassurance until resolved.
During Period- II						
No adverse event was found.						

Table 12: List of adverse events

A total of one (01) adverse event was reported during study in one subject. The adverse event was mild to moderate in nature and resolved. The event was possibly related or not related to the study drug. No serious adverse events were reported during study.

3.2 Analysis of adverse events:

The adverse events reported during the study were analyzed for onset, relationship, likelihood, severity, seriousness, duration etc. The adverse events were not life threatening or required the subjects to be hospitalized.

Adverse Event Reported	Test Product (A) N=11	Reference Product (B) N=12
Gastrointestinal tract		
Diarrhoea	00 (00.00%)	00 (00.00%)
Body as Whole		
Fever and Body ache	00 (00.00%)	00 (00.00%)
Abnormal post study parameters		
High S.G.P.T	00 (00.00%)	**01 (08.33%)**
Total	00 (00.00%)	**01 (08.33%)**

Table 13: Analysis of adverse events

3.4 Deaths, Other Serious Adverse Events, and Other Significant Adverse Events:

There were no deaths or significant adverse events during the conduct of this study.

4. Protocol Deviation:

Sampling Deviations:

All of the post-dose in-house samples and ambulatory blood samples were collected within 2 minutes and 1 hour respectively from the scheduled sampling time in both the periods of the study. Some of the deviations were observed for the same which is illustrated in below **Table-14.**

Table 14: Time Point Deviation

Sr.No.	Period	Subject No.	Sampling Hour	Schedule Time	Actual Time	Deviation (Min)	Reason	Action Taken
1	I	No deviation was found.						
2	II	10	48.00	09:18	10:05	47.00	Subject arrived	N.A
3	II	11	36.00	21:20	21:24	04.00	Subject arrived	N.A
4	II	12	48.00	09:22	09:35	13.00	Subject arrived	N.A

5. Dropout / Withdrawn Subjects:

- Subject No.03 was dropped- out from the study before check in of period –II due to his personal reason at 19:55 on 27/12/09.
- None of the subjects withdrawn from the study.

6. Plasma Concentration Profile:

Mean plasma concentration profiles of carvedilol and 4-hydroxy carvedilol under linear over the 48-hour pharmacokinetic study are presented in **figure-5 and 6** respectively for both test and reference products. Overall, mean plasma concentrations of carvedilol and its metabolite peaked rapidly and then declined in a monoexponential manner in both formulations. The mean plasma concentration-time curve of the test product and the reference drug were comparable. Both the formulations were rapidly absorbed and detected from 0.25 hour in plasma.

Figure-5: Mean Plasma Concentration of carvedilol Graph-Untransformed and Log-transformed:

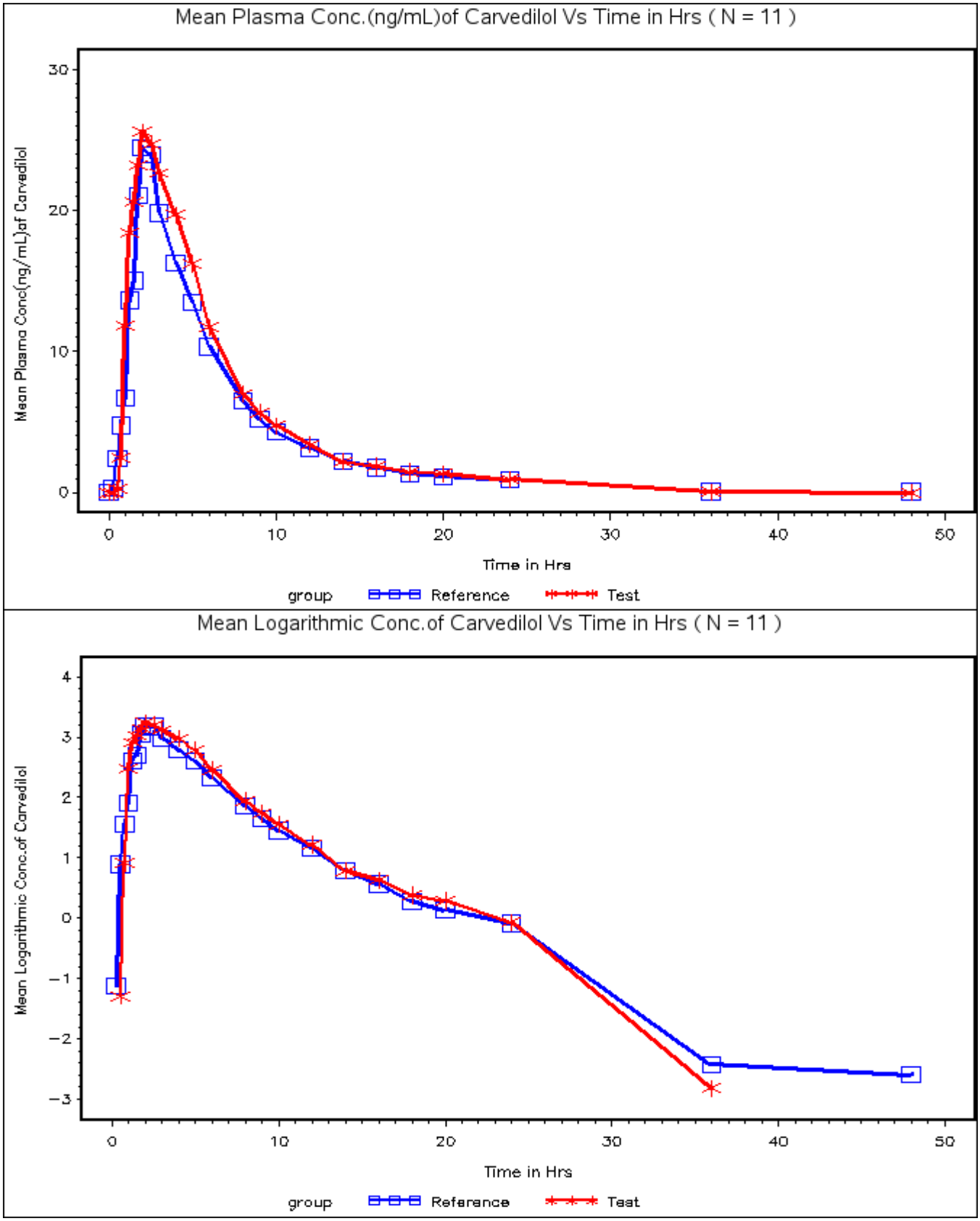
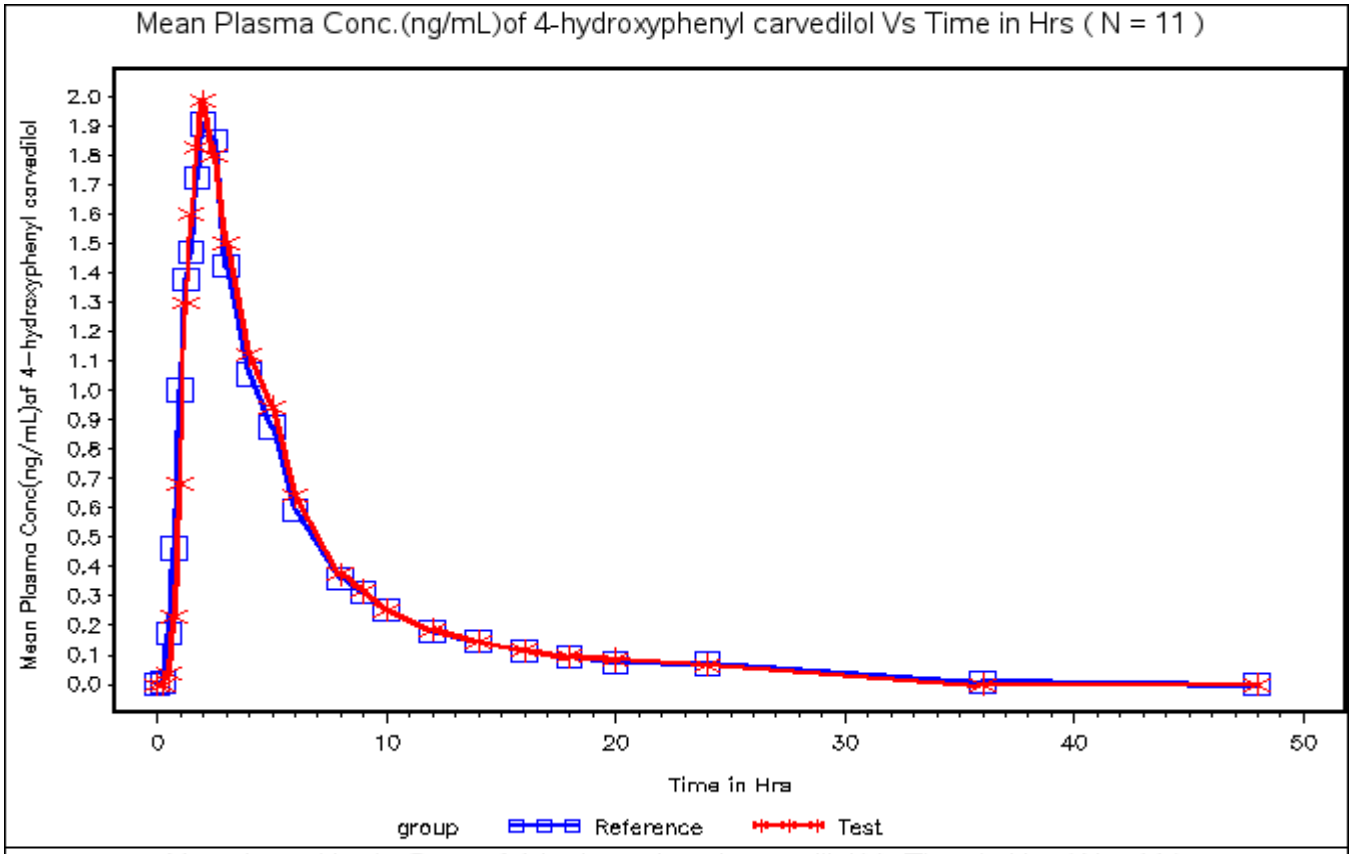
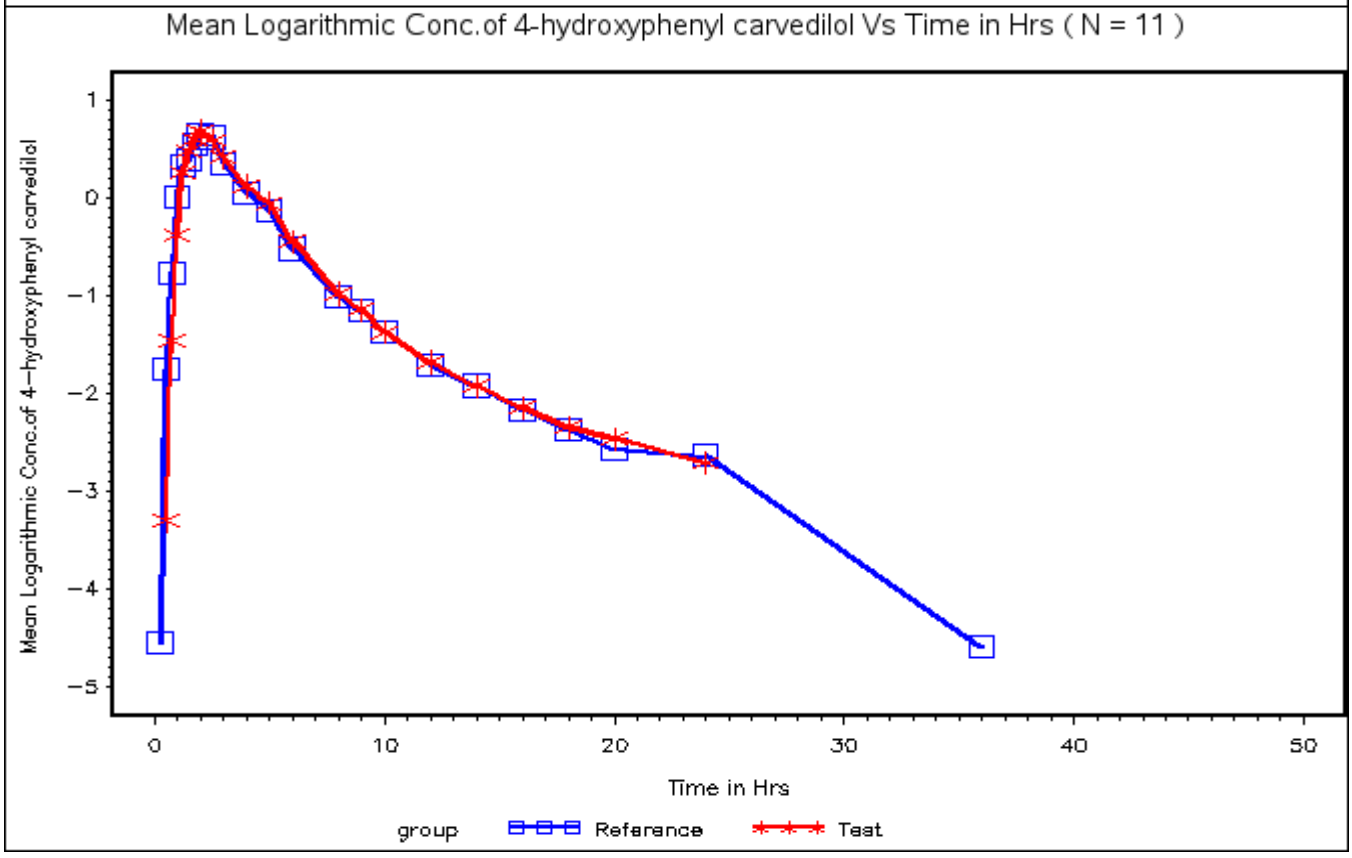


Figure-6: Mean Plasma Concentration of 4-Hydroxyphenyl Carvedilol Graph-Untransformed and Log-transformed:





7. Pharmacokinetic and Statistical Results:

7.1 Pharmacokinetic Parameters

The pharmacokinetic parameters Cmax, AUC_{0-t}, $AUC_{0-\infty}$, T_{max}, K_{el}, $t_{1/2}$ and AUC ratio were calculated using non-compartmental model by SAS® 9.2 for carvedilol with the data obtained from 11 subjects.

The pharmacokinetic parameters were calculated for both carvedilol and its active metabolite 4-hydroxyphenyl carvedilol.

7.2 Comparison of Pharmacokinetic Parameters:

From the graphical representation of statistical comparison (Fig.7-9) of pharmacokinetic parameters like Cmax, AUC_{0-t} and AUC_{o-inf} of Carvedilol, it was found that there was no significant difference between parametric values of test and reference formulations.

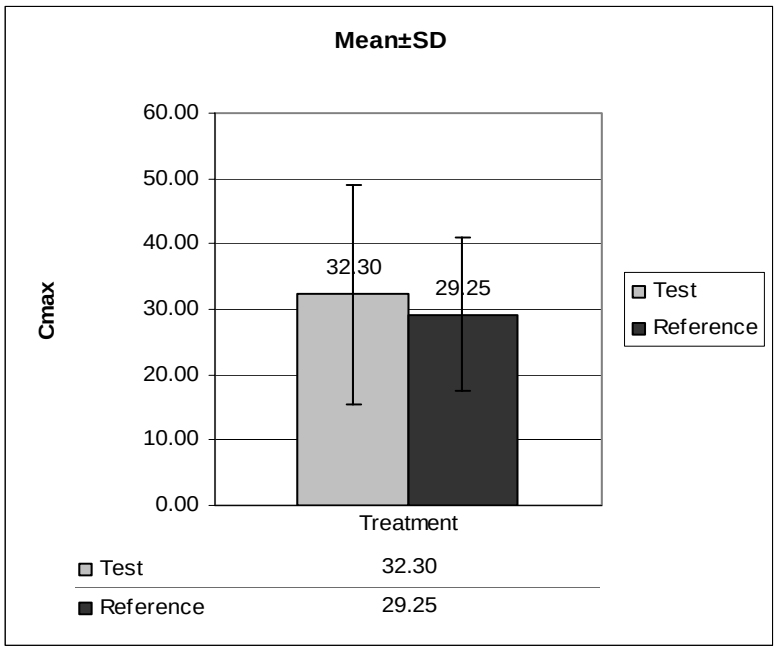


Figure-7: Comparison of pharmacokinetic Parameter C_{max} (Mean ± SD) for Carvedilol

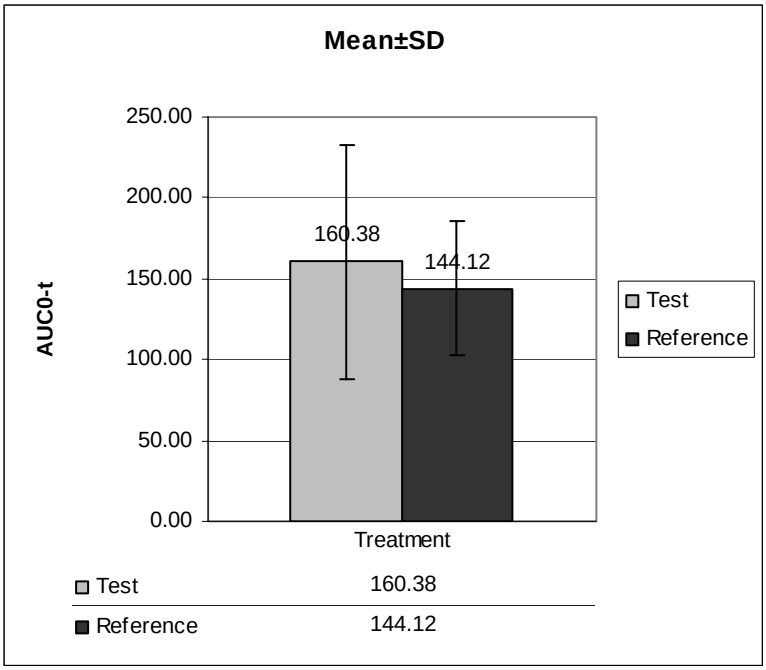


Figure-8: Comparison of pharmacokinetic Parameter AUC_{0-t} (Mean ± SD) for Carvedilol

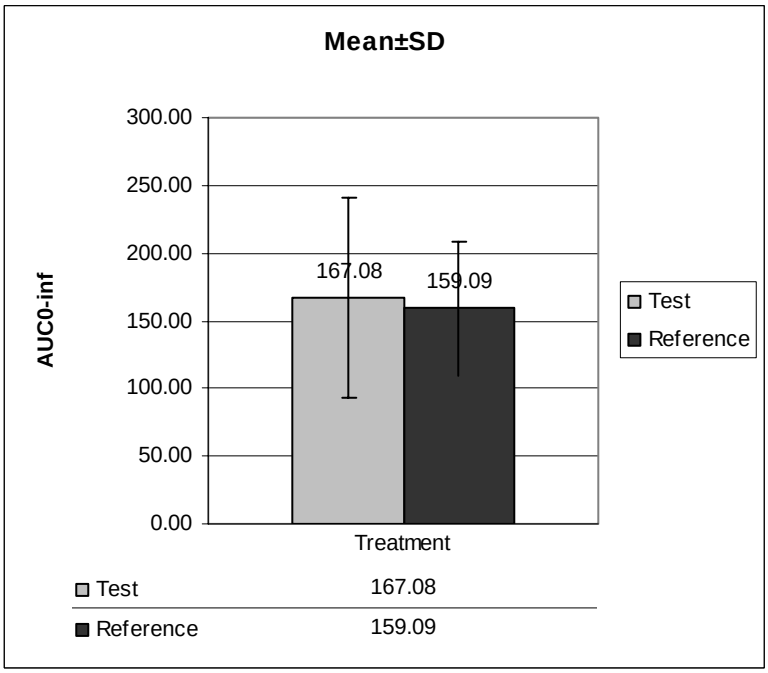


Figure-9: Comparison of pharmacokinetic Parameter AUC_{0-inf} (Mean ± SD) for Carvedilol

7.3 Statistical Calculations:

The geometric least square mean for log-transformed Cmax was found 29.48 ng/mL for Test Product and 27.55 ng/mL for Reference Product. The geometric least square mean ratio of Test and Reference Products was found 107.01%. The 90% confidence interval for log-transformed data for Cmax (as a measure of rate of absorption) of Test Product compared to that of the Reference Product was found 92.76-123.45 %. **(Table -15)**

The geometric least square mean for log-transformed AUC_{0-t} was found 145.41 ng*hr/mL for Test Product and 137.84 ng*hr/mL for Reference Product. The geometric least square mean ratio of Test and Reference Products was found 105.49%. The 90% confidence interval for log-transformed data for AUC_{0-t} (as a measure of extent of absorption) of Test Product compared to that of the Reference Product was found 92.65-120.11 %. **(Table -15)**

The geometric least square mean for log-transformed AUC_{0-inf} was found 151.56 ng*hr/mL for Test Product and 150.86 ng*hr/mL for Reference Product. The geometric least square mean ratio of Test and Reference Products was found 100.46%. The 90% confidence interval for log-transformed data for AUC_{0-inf} (as a measure of extent of absorption) of Test Product compared to that of the Reference Product was found 88.15-114.49 %. **(Table -15)**

Comparison of Test Treatment 'A' with Reference Treatment 'B' (Table -15)

Obs. Name	Geomean A	Geomean B	Ratio	Power	intra_cv	inter_cv	Lower Limit	Upper Limit
Ln C_{max}	29.48	27.55	107.01	83.47	18.36	38.72	**92.76**	**123.45**
Ln AUC_{0-t}	145.41	137.84	105.49	89.01	16.65	34.49	**92.65**	**120.11**
LnAUC_{0-inf}	151.56	150.86	100.46	88.63	16.77	34.66	**88.15**	**114.49**

The power of the confidence Interval based on Schuirmann's two one-sided test procedures for Log-transformed pharmacokinetic parameter Cmax, AUC0-t and AUC0-inf was found greater than 80% which is very much acceptable. **(Table -15)**

The mean Tmax was found 2.30 ± 1.00 hrs and 2.34 ± 0.97 hrs for the Test and Reference Product respectively.

The mean half life ($t_{1/2}$) was found 4.96 ± 1.95 hrs and 10.79 ± 10.12 hrs for the Test and Reference Product respectively.

The mean elimination rate constant (K_{el}) was found 0.15 ± 0.09 hrs^{-1} and 0.10 ± 0.06 hrs^{-1} for the Test and Reference Product respectively.

The results of ANOVA of Carvedilol for log-transformed data showed p value greater than 0.05. So, we can say that there is no statistically significant variation for treatment, sequence and period factors of Cmax, AUC_{0-t} and AUC_{0-inf} for the Reference and Test products. Also, the subject within the sequence effect was found to be significant ($p<0.05$) for Log-transformed pharmacokinetic parameter ($C_{max,}$ AUC_{0-t} and AUC_{0-inf}) for Carvedilol. This statistical difference is not likely to have any clinical significance, as significant subject effect always be present. It simply tells that subjects do differ from each other.

Wilcoxon Test for Tmax suggest that null hypothesis of equality of Tmax was accepted as the p-value was found to be > 5% using the Nonparametric Wilcoxon- test for Carvedilol. So, there was no significant difference in designed time frame for T_{max} because P value is greater than 0.05.

7.4 Safety Evaluation (During study vital parameters):

To assure effect of drugs on vital parameter, blood pressure and pulse rate were measured during the study. It has been found that test and reference product have not any clinical significance effect on vital parameters at different vital time points. Statistical evaluation (paired t-test) suggests that no formulation factor play important role by which treatment difference (it has been found that p value is greater than 0.05). So, two pharmaceutically equivalent Carvedilol formulations are therapeutic equivalent in terms of effect on vital parameters (safety)**.** So both products are safe.

8. Sample Size Calculation:

Based on the pilot study data, the maximum intra-subject variability observed was 18.36 % for Cmax. **(Table-15)** Considering this intra-subject variability and the actual Test/Reference ratio which was 1.07 % and assuming 5 % level of significant at 80% power the calculated sample size was found to be 18. Again considering certain drop-outs/withdrawal a sample size of 26 subjects is suggested for the pivotal study. **(Table -16)**

Two-Sample Equivalence
Multiplicative Model Lower Bound = 0.80 Upper Bound = 1.25
Coefficient of Variation = 0.1836 Alpha = 0.05

Null Ratio	Power	N per Group
0.95	0.800	15
	0.850	18
	0.900	21
1.00	0.800	13
	0.850	14
	0.900	16
1.05	0.800	15
	0.850	17
	0.900	20
1.07	**0.800**	**18**
	0.850	**21**
	0.900	**25**

Table-16: sample size calculation

CHAPTER-VI
DISCUSSION

Randomized, balanced, two way cross over bioequivalence study of carvedilol tablets 12.5 mg under fed condition was conducted in compliance with the current ICH GCP, USFDA and GLP guidelines, all requirements of the current version of the declaration of Helsinki and fulfilled the objectives of the pilot study including safety, efficacy, time point determination and determination of sample size to conduct pivotal study. No changes of the protocol have been carried out after start of the study and no major deviations from the protocol were observed.

Carvedilol is a third generation nonselective β-adrenergic blocking agent with α1-blocking activity and used in treatment of hypertension and congestive heart failure. According to **Morgan T, 1994,** when carvedilol is administered with food, the rate of absorption is slowed, as evidenced by a delay in the time to reach peak plasma levels, with no significant difference in extent of bioavailability. In this study oral administration of carvedilol tablet has no effect on overall pharmacokinetic due to presence of feed except slight effect on rate (Cmax), but not extent of carvedilol absorption.

It is reported that oral administration of carvedilol tablet causes somnolence, dizziness and postural hypotension. **(GSK patient information brochure, 2009)** Hence, during study bed side blood sample collection, blood pressure and pulse rate measurement, ECG and bed side meal were provided up to 8 hrs post dose for each study period for safety/precautionary measures.

Tomlinson B. et al. has reported that there is no effect of carvedilol (12.5 mg) on vital parameters like blood pressure and pulse rate in healthy human subjects. So, in our study to assure effect of drugs on vital parameter like temperature, blood pressure and pulse rate, they were measured during the study at regular interval after dosing. From results, it has been found that test and reference product have not any clinical significance effect on vital parameters at different vital time points.

During clinical trial of COREG **(COPERNICUS and COMET)** reversible elevations in serum SGPT and SGOT have been observed during treatment with COREG. Rates of transaminase elevations (2 to 3 times the upper limit of normal) observed during controlled clinical trials have generally been similar between patients treated with COREG and those treated with placebo. In our study only 01 (08.33%) adverse event was reported during study in one subject that was elevation of plasma SGPT level (86.7 U/L) and it was found in reference product treated subject. Carvedilol tablet 12.5 mg was well tolerated by the subjects. The adverse event was mild to moderate in nature and resolved. The event was possibly related or not related to the study drug.

None of the reported AEs was considered serious by the investigators. Potential recall bias of AEs in this study was not likely because only one dose of each formulation was administered during each treatment period, subjects were under medical surveillance in the clinical unit, and the duration of the washout period was only 07 days.

During this study, one subject (subject 3) was dropped out before check in of period-II, because he did not follow the study completely until the last blood sampling due to his personal reason.

Plasma concentrations were presented with mean, standard deviation & percentage coefficient of variation for each sampling time point for both the formulations of carvedilol. Descriptive statistical analysis were presented for all primary (Cmax, AUC0-t, AUC0-inf) and secondary (AUC0-t/AUC0-inf, Tmax, Kel, and t1/2) pharmacokinetic parameters.

The quantification of carvedilol and its active metabolite 4-hydroxyphenyl carvedilol in plasma samples was performed by validated LC-MS-MS methodology which allowed specific and sensitive determination of carvedilol and its active metabolite in plasma.

McPhillip et al, 1988 reported a randomized crossover study conducted on 44 healthy volunteers to compare bioavailibility of carvedilol 12.5 mg tablets. He found Cmax value 39 ng/L and AUCo-t value 180 ng/L*h.

Von Mollendroff et al, 1987 studied bioavailibility of carvedilol 12.5 mg tablets in 20 subjects. The Cmax, AUCo-t and Tmax was found 21 ng/L, 157 ng/L*h and 1.47 hrs respectively. He also reported the elimination half life ($t_{1/2}$) of carvedilol which was 2 to 4.7 hrs.

From this we can say that values of Cmax, AUCo-t, Tmax and half life ($t_{1/2}$) are comparable to that of the above studies, and they were found within the range.

Morgan et al, 1990 studied bioavailibility of carvedilol 12.5 mg tablets in 8 elderly subjects. The Cmax, AUC0-t and Tmax was 58 ng/L, 225 ng/L*h and 1.3 hrs respectively. Here, value of Cmax is very high compare to our study. **W. J. Louis et al.** and **CAPRICORN** clinical trials showed that plasma levels of carvedilol average about 50% higher in the elderly compared to young subjects and they are more sensitive to carvedilol compare to adult subjects. Our study was performed on adult subjects only that might be the reason for low value of Cmax compare to Morgan et al. study.

In the present study, the intra subject coefficient of variance (% CV) obtained from the ANOVA for carvedilol was 18.36%, it means that the study only required a sample size of less than 25 subjects. Therefore this study had an adequate power to confirm a statistical conclusion.

The results of our study suggest that the test and reference formulations of carvedilol were not statistically different in terms of their pharmacokinetic parameters (Cmax and AUC). Considering that all 90% confidence intervals of the ratios of the pharmacokinetic parameters (Cmax and AUC) were found to be within the predetermined range (80% -125%) and the Schuirmann two one-sided *t* test procedure (probability of exceeding limits of acceptance) found all probability values <0.05, the hypothesis that the estimated parameters exceeded limits of acceptance was rejected.

Based on the accepted regulatory requirements (USFDA), this study suggests that the test formulation was bioequivalent to the reference formulation.

CHAPTER-VII

CONCLUSION

Based on the statistical analysis Test Products A: Carvedilol Tablets 12.5 mg of sponsor's formulation is bioequivalent to Reference Product B: COREG® (Carvedilol) 12.5 mg tablet of GlaxoSmithKline in terms of rate and extent of absorption under fed condition.

Carvedilol is well tolerated in healthy subjects and adverse events were mild in severity and resolved during the study and on follow up.

CHAPTER-VIII

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