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Bioequivalence and Pharmacokinetic Comparison between Extended Release Capsules of Carvedilol Phosphate 40mg: An Open Label, Balanced, Randomized-Sequence, Single-Dose, Two-Period Crossover Study in Healthy Male Volunteers

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A B S T R A C T

This present bioequivalence study was designed to determine the pharmacokinetic, bioavailability and bioequivalence of carvedilol phosphate 40mg Extended Release Capsules in comparison with Coreg CRTM Extended Release Capsules after single dose administration under fed conditions in healthy adult male subjects. Therefore the design of an open label, balanced, randomized, two-sequence, single dose, two ways crossover study with a wash-out period of at least 7 days was used. An open-labeled, balanced, single-dose with food, 2-treatment, 2-period, 2-sequence, randomized crossover study was conducted in 18 healthy male volunteers. Each volunteer received a 40 mg capsule of the reference (or) test drug respectively. On the day of dosing, blood samples were collected before dosing and at various time points up to 50 hours after dosing. Analysis of carvedilol and its metabolite 4-Hydroxy phenyl Carvedilol concentrations was performed using a validated LC-MS/MS method. The pharmacokinetic parameters were analyzed using the non-compartmental model. Drug safety and tolerability were assessed. The primary pharmacokinetic parameters at 90% CI were within the 80 to125% interval required for bioequivalence as stipulated in the current regulations of the USFDA acceptance criteria. The geometric mean ratios (Test/Reference) between the two products of extended-release carvedilol capsule under fed condition were 114.41% (93.68% - 116.74%) and 113.15% (96.67% - 122.45%) for C_{max} ratios, 101.54% (95.73 - 104.85%) and 102.72% (95.12% - 113.35%) for AUC_{0-t} ratios and 104.56% (103.24% - 107.58%) and 105.73% (95.45% - 110.50%) for AUC_{0-inf} ratios of carvedilol and its metabolite 4-Hydroxy phenyl Carvedilol respectively. 18 volunteers had completed both treatments. There was no significant difference of the T_{max} parameter between the two formulations ($p > 0.05$). No serious adverse events related to the study drugs were found. This single dose study found that the test formulation carvedilol phosphate ER capsules is bioequivalent to the reference formulation Coreg CR™ ER capsules the extent & the rate of absorption, of 40 mg under fed condition in healthy adult male volunteers according to the USFDA regulatory guidance.

Keywords: Carvedilol, 4-Hydroxy phenyl carvedilol, Bioavailability, Bioequivalence, Intrasubject Variability

A R T I C L E I N F O

CONTENTS

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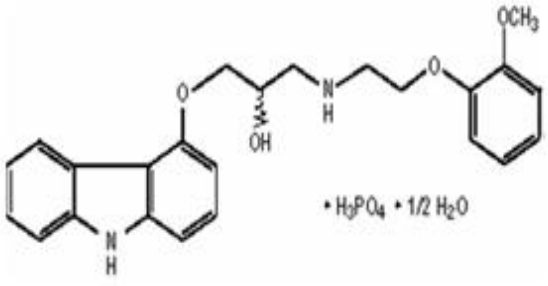
1. Introduction

Cardiovascular disease (CVD) is the leading cause of morbidity and mortality, accounting for 30% of all deaths worldwide [1]. In the United States alone, CVD accounted for more than 36% of all deaths in 2004 or 1 of every 2.8 deaths [2]. Beta-blockers have a long history in the treatment of hypertension and cardiac dysfunction, with more than 40 years of clinical use [3]. However, concerns have been raised recently from hypertension meta-analyses regarding suboptimal outcomes with use of beta-blockers, specifically atenolol, compared with outcomes for other antihypertensive drug classes [4]. Beta-blockers have also been associated with tolerability issues and concerns regarding negative effects on glucose and lipid metabolism. However, it should be noted that not all beta-blockers are identical, as differences in mechanism of action may translate into diverse efficacy and safety profiles [5]. Carvedilol phosphate is a third-generation, vasodilatory beta-blocker that nonselectively blocks both the beta 1- and beta 2-adrenergic receptors and, in addition, has alpha 1-adrenergic receptor-blocking activity. It is (2*RS*)-1-(9*H*-Carbazol-4-yloxy)-3-[[2-(2-methoxyphenoxy) ethyl] amino] propan-2-ol phosphate salt (1:1) hemihydrate. It is a racemic mixture with the following structure:



Carvedilol phosphate is a white to almost-white solid with a molecular weight of 513.5 (406.5 Carvedilol free base) and a molecular formula of $C_{24}H_{26}N_2O_4.H_3PO_4.1/2\ H_2O$. Unlike traditional beta-blockers (eg, atenolol, metoprolol, and propranolol) that lower blood pressure by reducing cardiac output[6], vasodilatory beta-blockers can lower blood pressure by reducing systemic vascular resistance (SVR) [7]. As with other beta-blockers, carvedilol has been shown to reduce sympathetic nervous system (SNS)-mediated cardiac stress and myocardial hypertrophy [8]. These activities likely contribute to the clinical benefits observed in patients treated with carvedilol for hypertension, heart failure, and post-MI left ventricular dysfunction (LVD). Moreover, the regimen of twice-daily carvedilol has been associated with a favorable side effect and tolerability profile.

In order to improve adherence to therapy and to ease the pill burden on patients, a controlled-release formulation of carvedilol (carvedilol CR) was developed and is approved for use in the same indications (ie, hypertension, heart failure, and post-MI LVD) as immediate-release (IR) carvedilol. This review presents an overview of the clinical and pharmacologic carvedilol CR data. Unlike traditional beta-blockers, carvedilol blocks norepinephrine binding to alpha 1-adrenegric receptors as well as beta 1- and beta 2-adrenegeric receptors [6,10,11], carvedilol blocks norepinephrine binding to alpha 1-adrenegric receptors as well as beta 1- and beta 2-adrenegeric receptors[12]. Alpha 1-adrenergic receptors mediate vasoconstriction. Consequently, alpha 1-blockade results in vasodilation of the peripheral arteries, decreasing SVR [6]. In addition, preclinical evidence suggests that carvedilol can also produce nitric oxide-mediated vasodilation [13].

Carvedilol does not possess intrinsic sympathomimetic activity [8]. Intrinsic sympathomimetic activity induces weak stimulation of the beta-adrenergic receptors that may dampen the positive effects of beta 1-adrenergic receptor blockade [5, 10]. Of note, beta-blockers with intrinsic sympathomimetic activity have failed to demonstrate reductions in morbidity and mortality in patients with heart failure [14].

Carvedilol CR was developed to achieve sustained concentrations over a 24-hour period, allowing once-daily dosing. The pharmacokinetic and pharmacodynamic bioequivalence of carvedilol CR and IR was established through 2 clinical studies [15, 16]. In a double-blind, parallel-group, crossover study, 122 patients with essential hypertension were randomized to receive either low-dose

carvedilol CR (20 mg daily) or carvedilol IR (6.25 mg twice daily), high-dose carvedilol CR (80 mg once daily [initiated at 20 mg once daily and titrated to 40 mg and 80 mg once daily in 1-week intervals]) or carvedilol IR (25 mg twice daily [initiated at 6.25 mg twice daily and titrated to 12.5 mg and 25 mg twice daily in 1-week intervals]), or placebo[15]. After 22 days of treatment, patients were crossed over to the equivalent alternate carvedilol formulation for 8 days of treatment. Patients in the placebo treatment group continued to receive placebo throughout the study. Pharmacokinetic parameters were assessed at the end of each treatment session. The pharmacodynamic endpoint was the percentage change from baseline in exercise-induced heart rate. As carvedilol is a racemic mixture of R(+) and S(−) enantiomers[17], both forms were assessed in the pharmacokinetic analysis. The pharmacokinetic profiles of both enantiomers were equivalent between carvedilol CR and IR [15]. In addition, both formulations maintained a reduced exercise-induced heart rate over a 24-hour period [15].

In a separate 4-week study, patients with either mild to severe heart failure or with asymptomatic post-MI LVD were treated with the carvedilol IR (3.125, 6.25, 12.5, or 25 mg twice daily) for the first 2 weeks and then switched to carvedilol CR (10, 20, 40, or 80 mg once daily) for 2 weeks [6]. Trough plasma concentration, maximum plasma concentration, and area under the curve was measured for both R(+) and S(−) enantiomers after carvedilol IR and CR treatment periods. The pharmacokinetics of carvedilol IR and CR were bioequivalent in patients with heart failure and post-MI with LVD. However, the median time to maximum observed plasma concentration for carvedilol CR lagged 3 hours behind that of carvedilol IR, in accordance with the prolonged-release characteristics expected in a once-daily formulation. The pharmacodynamics of carvedilol CR were dose proportional over the dose range tested (10 mg to 80 mg).

Notably, the bioavailability of carvedilol CR is 85% that of carvedilol IR [16]. Carvedilol CR is based on carvedilol phosphate, which has a higher molecular weight than carvedilol free base and contains additional carvedilol free base compared with carvedilol IR to adjust for bioavailability. This difference contributes, in part, to slightly higher milligram dosage strengths of carvedilol CR than the “equivalent” carvedilol IR doses [16]. A model of carvedilol pharmacokinetics that takes into consideration both the IR and CR formulations has been developed and performed robustly in leverage analyses [19]. Similar to carvedilol IR, the bioavailability and pharmacokinetics of carvedilol CR are influenced by food, and both formulations are recommended to be taken with food [18]. However, the pharmacokinetics of carvedilol CR 40 mg were not affected by ethanol (38 g) intake from 2 hours before to 2 hours after dosing in 39 healthy volunteers[15].

Carvedilol is more than 98% bound to plasma proteins, primarily with albumin. The plasma protein binding is independent of concentration over the therapeutic range. Carvedilol is a basic, lipophilic compound with a steady-state volume of distribution of approximately 115 L, indicating substantial distribution into extravascular tissues.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 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 are further metabolized by conjugation via glucuronidation and sulfation. The metabolites of carvedilol are excreted primarily via the bile into the feces. Demethylation and hydroxylation at the phenol ring produce 3 active metabolites with Beta--receptor blocking activity. Based on preclinical studies, the 4'-hydroxyphenyl metabolite is approximately 13 times more potent than carvedilol for Beta-blockade. Compared to carvedilol, the 3 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 of COREG CR in healthy subjects. Apparent clearance is 90 L/h and 213 L/h for R(+)- and S(-)-carvedilol, respectively.

The rationale of this present bioequivalence study for two formulations of 40mg carvedilol phosphate extended release capsule was examined between generic drug carvedilol phosphate ER Capsules as the test product and Coreg-CR^{TM} as the reference product. This bioequivalence study could give assurance when prescribing less expensive generic drugs as alternatives with similar efficacy and safety. The study objectives of this present study are to assess the single dose bioequivalence of Carvedilol phosphate 40mg ER Capsules with Coreg-CR^{TM} in healthy, adult, human study participants under fed conditions and to monitor the clinical status, adverse events and laboratory investigations and assess relative safety and tolerance of carvedilol formulations under fed conditions.

.2. Materials and Methods

According to the USFDA Regulatory individual product recommendations, three studies (Fasting, Fed and Fed Sprinkle) to be done with 40mg carvedilol phosphate ER Capsules to obtain marketing authorization in USA. USFDA Waiver request of in-vivo testing: 10, 20, and 80 mg based on (i) acceptable bioequivalence studies on the 40 mg strength, (ii) proportionally similar across all strengths, and (iii) acceptable in vitro dissolution testing of all strengths.

Study drugs

Carvedilol phosphate ER Capsules and Coreg-CRTM Extended-release Capsules from GlaxoSmithKline were used as the test and the reference products respectively. Both products were prepared as Carvedilol phosphate equivalent to Carvedilol 40 mg. Both the products were stored at controlled room temperature 25˚C (77 ˚F).

Study population

The study was carried out at Actimus Biosciences Private Limited, India. The study protocol was approved by the Ethics Committee. In addition, the protocol was performed in accordance with the Declaration of Helsinki Principles [37] as outlined in the ICH-E6 Guidelines for Good Clinical Practice (GCP) [38]. All subjects were given a detailed description of the study and written informed consent was obtained prior to the enrollment. The sample size was estimated based on, Coefficient of variation (C.V.) of the drug, sufficient statistical power to detect 20% difference with the power of 0.8 in C_{max} and AUC between the test and reference product, Regulatory requirements.

Sample size was based on estimates obtained from reported literature and previous studies. Assuming a formulation ratio (T/R) ranging from 0.95-1.05 a sample of 18 subjects including dropouts would be sufficient to show bioequivalence between the two formulations with a power of at least 80%. Hence sample size of 18 subjects was enrolled in the study. 18 healthy male volunteers between the ages of 18-45 years with a body mass index between 18.5 kg/m^2 and 24.9 kg/m^2, with body weight equal to or not less than 50 kg were assessed to be in good physical condition by a complete medical screening including a medical history, physical examination, chest radiography, electro radiography, laboratory screening test for hematologic and blood biochemistry parameters and nonsmoker status. Subjects with a history of hypersensitivity to any ingredients in the carvedilol products and/or related drugs or its constituents or who were taking any medication or alcohol for a 21-day period prior to the study were excluded. Subjects who had a history of cardiovascular, hepatic, renal, gastrointestinal or hematologic disease were excluded from the study.

Study design

The study was an open-labeled, single-dose, study taken with food, two-treatment, two-period, two-sequence randomized two way crossover with at least one week washout period. Subjects were randomly allocated to two groups by the sequence of product administered [Test-Reference (TR) and Reference-Test (RT) group]. In each period, 1X40mg ER capsule of carvedilol phosphate of the test or reference product was administered 30 minutes after starting a high fat, high calorie breakfast at the same time in the morning before dosing. Subjects were housed 12 hours prior to dosing in the clinical facility from a time adequate to ensure 10 hours supervised fasting before consuming high fat breakfast and were allowed to leave the facility after 24.00 hours post-dose sample in each period. The subjects received a standard meal at about 4.0, 9.0 and 13.0 hours after dosing in each period. During housing, all meal plans were identical for all the periods. Drinking water was not allowed from one hour before dosing till one hour post-dose (except for 240 ± 02 mL of drinking water given for dosing). Before and after that, drinking water was allowed at *ad libitum*. After a minimum of 1 week washout period, the subjects were crossed over to the next treatment following the same procedure as conducted in the 1st period.

Sample collection

During dosing day in each period, 23 blood samples (6 mL each) will be collected as per the following schedule: Pre dose sample(0.00 hr) within 02 hrs prior to drug administration and the others at 0.50, 1.00, 1.50, 2.00, 2.50, 3.00, 3.50, 4.00, 4.50, 5.00, 5.50, 6.00, 6.50, 7.00, 7.50, 8.00, 10.00, 12.00, 16.00, 24.00, 36.00 and 48.00 hours post dose. The total volume collected per study participant in this study will not exceed approximately 321 mL including up to 9 mL for screening, and 7-9 mL for post clinical assessment of lab parameters and 18 mL for discarded blood sample resulting from use of intravenous cannula for 12 hours and 2-9 mL was collected for repeat/additional lab tests, if required. For separating plasma, all blood samples were centrifuged at 3800 RPM for 10 minutes at 4°C ± 2°C. Centrifugation of all samples was done as early as possible after each sample draw time point. After centrifugation, plasma samples were aliquoted into two sets in properly labeled polypropylene tubes and immediately stored at about -60°C or colder. Carvedilol and its metabolite analysis by LC-MS/MS *The* frozen CC, QC and subject samples from the deep freezer were retrieved, thawed in water bath maintained at room temperature and vortexed. The caps were removed from the polypropylene tubes. 0.100 mL (100µL) of CC, QC and subject samples was aliquoted into pre-labelled HPLC vials. 25.0 µL of Propranolol (ISTD) Dilution (100 ng/mL) was added followed by 50.0 µL of 10mM Ammonium formate buffer of pH 7.8 into HPLC vials. The HPLC vials were capped, vortexed to mix and transferred to auto sampler. The samples were analyzed by using a validated LC/MS/MS detection method [23].

The gradient program [24, 25] accomplished a Cyclone HTLC (High-throughput liquid Chromatography) column for sample extraction, elution with four pumps. TLX turbo flow on-line technique was employed for separation of analyte from sample molecules. The mechanism involved in sample preparation may be affinity. The small drug molecules bind to the HTLC (High-throughput liquid Chromatography) column, and molecules that have lower binding affinity quickly diffuse into the column particles and large sample molecules are flushed to waste, then the mobile phase elutes the analyte molecules that are bound at HTLC (High-throughput liquid Chromatography) column to analytical column, from this analytical column analytes are entered to mass detector. To achieve required chromatograms with consistency performed different combinations of the solvents and gradient system. Finally succeeded with the solution combinations as mentioned in Table 1 and analyzed more than 150 samples without overloading of the chromatographic columns with improved real throughput efficiency. Chromatograms were acquired on a TSQ tandem mass spectrometry (Thermo Finnegan, Sanjose, CA, USA) [26-30] equipped with Electrospray ionization (ESI) and connected to a PC runs with the standard software Xcalibur 2.0.7 and LC Quan 2.5.6. Mass spectroscopic detection was performed on a Triple quadrapole instrument (Thermo, TSQ Quantum Discovery Max). Robotic liquid handling system [31-35] is operated

using the software package supplied from the cohesive technologies Aria™. The calibration curve is constructed by weighted $1/x^2$ least-square linear regression analysis of the peak area ratio (drug/ISTD) vs. the concentration of drug and peak area ratio (metabolite/ISTD) vs. the concentration of metabolite. Representative chromatograms from an extract of human blank plasma spiked with internal standard and from an extract of human blank plasma spiked with drug, metabolite and internal standard are shown in Fig.1A and B.

Pharmacokinetic and statistical analysis [36-38]

For the purpose of Average Bioequivalence analysis C_{max}, AUC_{0-t} and AUC_{0-inf} were considered as the primary variables and T_{max}, $t_{1/2}$ and K_{el} were considered as the secondary variables. General Linear Model for analysis of variance (ANOVA) for crossover design was performed for log-transformed data and used to assess the effect of formulations, periods, sequences and subjects nested in sequence on these parameters.

The difference between two related parameters was considered statistically significant for a *p*-value equal to or less than 0.05. 90% confidence interval (CI) for the ratios of geometric mean Test/Reference (T/R) for C_{max}, AUC_{0-t} and AUC_{0-inf} was calculated based on least squares means from the ANOVA of log-transformed data. The 90% geometric CI of the ratio (T/R) of least squares means from the ANOVA of the log-transformed C_{max}, AUC_{0-t} and AUC_{0-inf} should be within 80.00% to 125.00%.

Tolerability assessment

Physical examination and measurement of vital signs (Blood Pressure, Pulse Rate and Oral Temperature) were examined at the time of Check-in, prior to administration of the each study drug (0.00 hr), 0.50, 1.00, 1.50, 2.00, 2.50, 3.00, 3.50, 4.00, 4.50, 5.00, 5.50, 6.00, 6.50, 7.00, 7.50, 8.00, 10.00, 12.00, 16.00, 24.00, 36.00 and 48.00hours post dose and during the entire study period. Adverse events were monitored throughout the study and recorded by physicians.

3. Results and Discussion

Study population

18 healthy male adults eligible for the study enrollment were randomly divided into 2 groups [Test-Reference (TR) and Reference-Test (RT)] according to the sequence of drug administration. All the subjects had completed both the periods. Thus, this study was balanced in each sequence and the results from 18 volunteers were used for pharmacokinetic and statistical analysis. Table 2 demonstrates the demographic characteristics of the volunteers.

Bioanalysis and pharmacokinetics

The LC/MS/MS system consisted of four pumps for gradient solvent delivery, and a divert valve to direct LC effluent to the mass spectrometer in the analyte elution window. The analytical column effluent is directed through the divert valve to a thermo electron TSQ quantum discovery mass spectrometer.The instrument was operated in the positive ion mode. The precursor $[M \cdot H]^+$ ions at m/z 407.113, 423.528 and 260.200 for Carvedilol, 4-Hydroxyphenyl Carvedilol and Propranolol respectively were selected by the first quadrupole (Q1). After collision-induced fragmentation in Q2, the product ions at m/z 224.503, 100.344 and 116.100 for Carvedilol, 4-Hydroxyphenyl Carvedilol and Propranolol, respectively, were monitored in Q3.A resolution of one unit (at half peak height) was used for both Q1 and Q3. The method [23] was fully validated using these Q1 and Q3 masses for both compounds with satisfactory results. *Linear calibration curves* were obtained with a coefficient of correlation (r^2) usually higher than 0.995 in range of 0.1 ng/mL to 250 ng/mL. For each calibration standard level, the concentration was back calculated from the linear regression curve equation. No significant difference was observed in any of the analyzed pharmacokinetic parameters for Carvedilol and its metabolite 4-Hydroxyphenyl Carvedilol was shown in Table 3.

Bioequivalence analysis

Ninety percent confidence interval of geometric mean ratios of bioavailability parameters between the test and reference formulation are presented in Table 4. The statistical analysis obtained from this study showed that the point estimate (90% CI) of the geometric mean ratio (GMR) (T/R) of C_{max}, AUC_{0-t} and AUC_{0-inf} was entirely within the equivalence criteria (80.00-125.00%) which was 114.41% (93.68%-116.74%) and 113.15% (96.67%-122.45%) for C_{max} ratios, 101.54% (95.73-104.85%) and 102.72% (95.12%-113.35%) for AUC_{0-t} ratios and 104.56% (103.24%-107.58%) and 105.73% (95.45%-110.50%) for AUC_{0-inf} ratios of carvedilol and its metabolite 4-Hydroxyphenyl Carvedilol respectively. In addition, no significant difference of the T_{max} parameter between the two studied formulations was observed ($p > 0.05$). Therefore, it was concluded that the two extended-release capsule formulations of carvedilol were bioequivalent in terms of rate and extent of absorption for the drug carvedilol and the metabolite data has been given as supportive evidence. The mean plasma concentration vs time profiles were given in Fig 2.

Tolerability

Almost all volunteers taking both carvedilol formulations were noted for mild adverse events. Most common events were drowsiness, nausea and loss of appetite. However, no subject had any severe adverse event or withdrew from the study because of an adverse event.

Discussion:

Cardiovascular disease (CVD) is the leading cause of morbidity and mortality, accounting for 30% of all deaths worldwide [1]. Carvedilol is a third-generation, vasodilatory beta-blocker that non selectively blocks both the beta 1- and beta 2-adrenergic receptors and, in addition, has alpha 1-adrenergic receptor-blocking activity. Carvedilol is used in the treatment of mild to moderate hypertension; angina pectoris [5], congestive heart failure [6,7,8] and possess antioxidative effects in vivo [9].Carvedilol has similar efficacy and better tolerability when compared to classical beta blockers. An open-labeled, single-dose with food, two-treatment, two-period, two-sequence randomized two way crossover design in 18 healthy adult volunteers was considered appropriate and

standard for bioequivalence evaluation of the generic and the reference products. The study simulates real life conditions including the influence of meals as well as circadian effects on the performance of the extended release product. For a safety reason, co-administration of the drug with food can reduce nausea, a common side effect of carvedilol. In general, the pharmacokinetic parameters for both formulations were similar to the pharmacokinetic parameters of carvedilol in previous published data. This study demonstrated that 90% CI of the logarithmic transformed of parameters Cmax, AUC0-t and AUC0-inf were contained in 80.00-125.00%. In addition, no significant differences of the Tmax values between the two formulations were observed ($p>0.05$). Therefore, the two extended-release capsule formulations of carvedilol are considered bioequivalent in terms of the rate and extent of absorption. Moreover, both formulations were well tolerated. Hence, the test (carvedilol) and reference (Coreg CR^{TM}) formulations of carvedilol 40mg are bioequivalent.

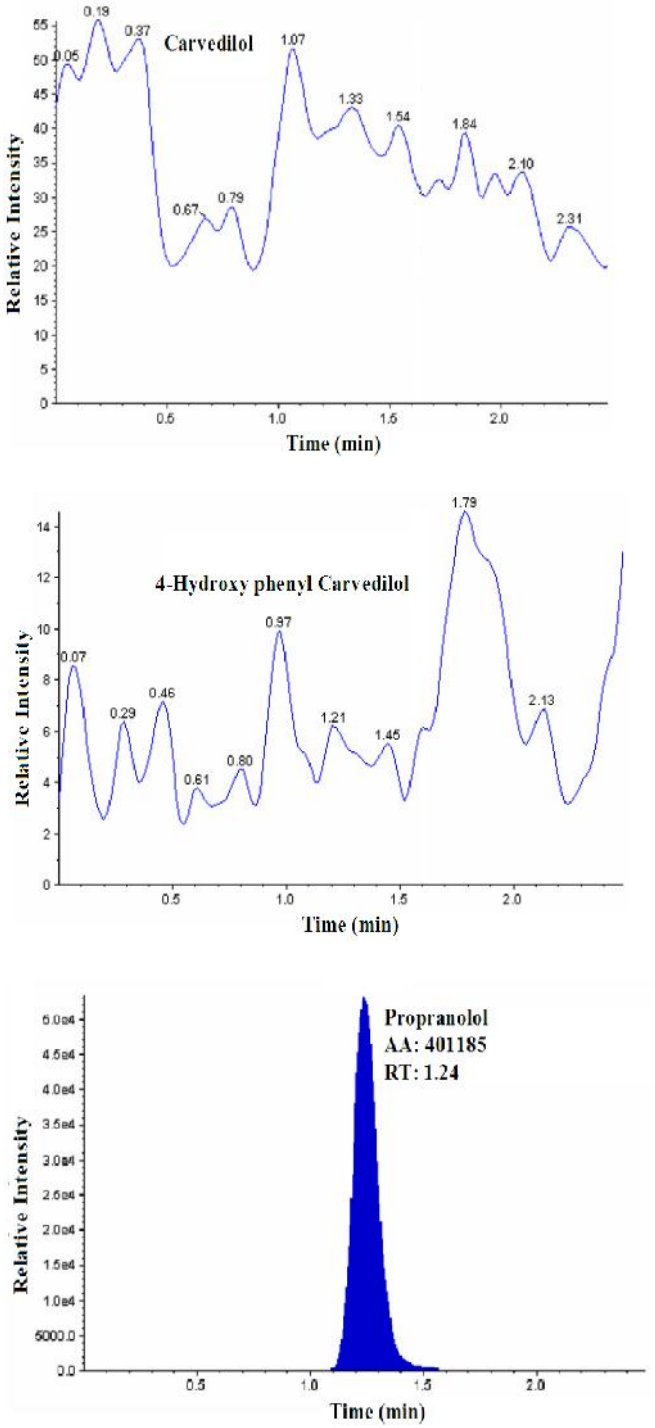


Figure 1: (A) Representative Chromatograms from an extract of Human blank plasma spiked with Propranolol as IS.

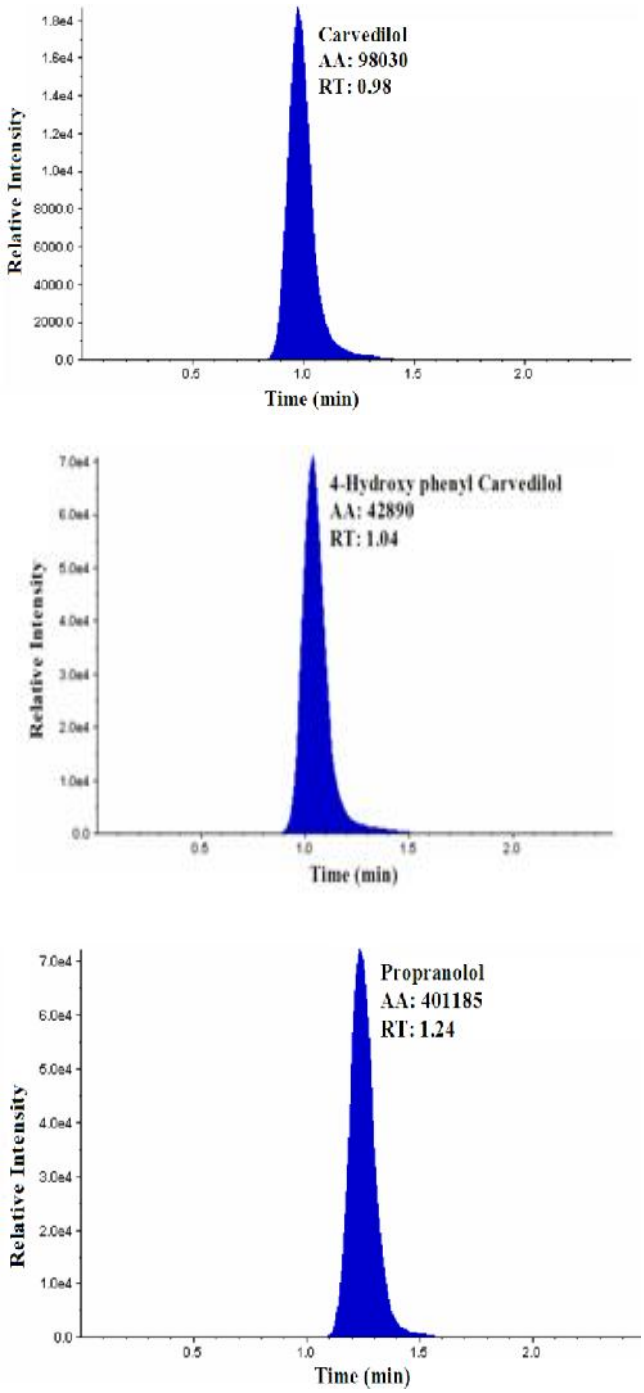


Figure 1: (B) Representative Chromatograms from an extract human blank plasma spiked with Carvedilol, 4-Hydroxyphenyl Carvedilol and Propranolol (as IS).

Table 1: Steps involved in on-line robotic method

Step	Start	Sec.	Flow	Grad	%A	%B	%C	%D	Tee	Loop	Flow	Grad	%A	%B
1	0.0	30	2.0	Step	100.0	0.0	0.0	0.0	-	Out	1.0	Step	90	10
2	0.5	30	1.0	Step	0.0	50.0	50.0	0.0	T	In	1.0	Step	90	10
3	1.0	30	2.0	Step	0.0	0.0	0.0	100.0	-	In	1.0	Ramp	90	10
4	1.5	30	2.0	Step	0.0	0.0	0.0	100.0	-	In	1.0	Step	90	10
5	2.0	30	2.0	Step	50.0	0.0	50.0	0.0	-	In	1.0	Step	90	10
6	2.5	30	2.0	Step	50.0	0.0	50.0	0.0	-	In	1.0	Step	90	10
7	3.0	30	2.0	Step	100.0	0.0	0.0	0.0	-	Out	1.0	Step	90	10

Table 2: Demographic characteristics

Category		Treatment		Total
		Test (T)	Reference (R)	
Age (years)	Mean ± SD	22.44 ± 4.37	23.84 ± 4.00	22.44 ± 4.14
	Range	19.0 – 35.0	18.0 – 35.0	18.0 – 35.0
	Median	23	23	23
	N	20	20	40
Age Groups	< 18	0	0	0
	18 – 40	20	20	40
	41 – 64	0	0	0
	65 – 75	0	0	0
	> 75	0	0	0
Gender	Female	0	0	0
	Male	20	20	40
Race	American	0	0	0
	Hispanic	0	0	0
	Caucasian	0	0	0
	Asian	20	20	40
Height (cm)	Mean ± SD	165.48 ± 4.89	163.12 ± 5.69	164.3 ± 5.57
	Range	159.0 – 176.0	155.0 – 175.0	155.0 – 176.0
	Median	168	162	165
	N	20	20	40
Weight (kg)	Mean ± SD	65.46 ± 6.43	59.76 ± 6.24	62.61 ± 6.41
	Range	52.0 – 77.0	52.0 – 70.0	52.0 – 77.0
	Median	59	58	59
	N	20	20	40
BMI (kg/m^2)	Mean ± SD	21.10 ± 1.79	22.86 ± 1.46	21.98 ± 1.62
	Range	20.0 – 24.9	20.1 – 24.8	20.0 – 24.9
	Median	21.6	22	21.8
	N	20	20	40

Table 3: Pharmacokinetic Parameters of Carvedilol & 4-Hydroxyphenyl Carvedilol for Both Formulations

Carvedilol			4-Hydroxy phenyl Carvedilol	
Pk Parameters	Test	Reference	Test	Reference
Cmax (ng/mL)	32.421	37.821	5.845	6.494
AUCt (ng.h/mL)	239.95	248.616	39.872	43.44
AUCinf (ng.h/mL)	272.713	266.912	47.440	66.141
Tmax (hr)	5.173	5.049	5.136	5.073
kel (1/h)	0.125	0.143	0.097	0.093
t1/2 (hr)	5.857	5.348	7.326	9.146

Table 4: Bioequivalence Parameters for Carvedilol & 4-Hydroxyphenyl Carvedilol

Parameter	Carvedilol			4-Hydroxyphenyl Carvedilol		
	C_{max}	AUC_t	AUC_{inf}	C_{max}	AUC_t	AUC_{inf}
90% CI Lower Limit	93.68	95.73	103.24	96.67	95.12	95.45
90% CI Upper Limit	116.74	104.85	107.58	122.45	113.35	110.50
T/R Ratio (%)	114.41	101.54	104.56	113.15	102.72	105.73
Power	0.91	1.00	1.00	0.95	0.98	0.98
Intra Subject Variability	11.34	5.10	5.07	6.62	5.08	6.82
Inter Subject Variability	28.48	52.04	51.49	29.58	30.01	28.91
ANOVA (p-Value)						
Sequence	0.1922	0.1771	0.1866	0.6120	0.1966	0.1277
Period	0.2018	0.2241	0.5355	0.0031	0.3054	0.1335
Treatment	0.9424	0.4115	0.2960	0.5610	0.9205	0.5353

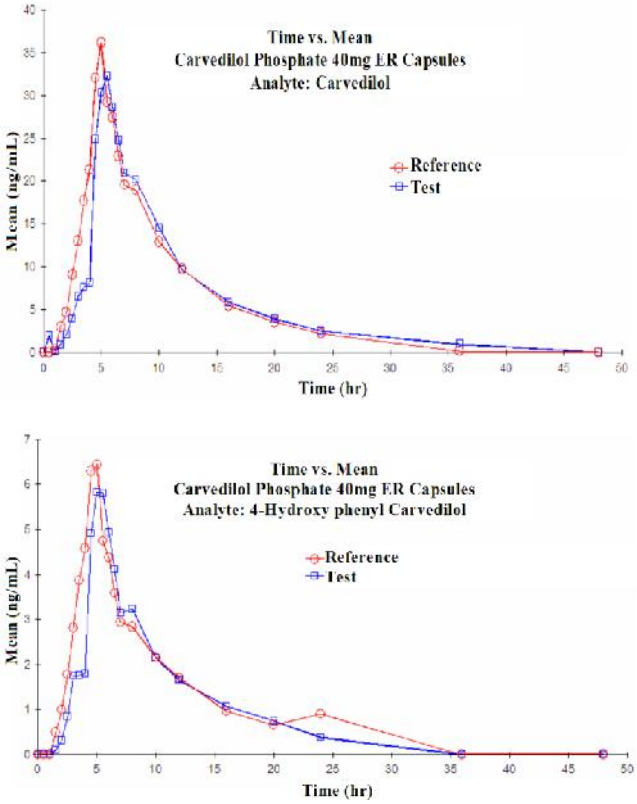


Figure 2: Time vs. Mean Plasma Concentration Graph of Carvedilol & 4-Hydroxyphenyl Carvedilol

4. Conclusion

This single dose study found that the test formulation carvedilol phosphate 40mg Extended Release Capsules is bioequivalent to the reference formulation Coreg CRTM Extended Release Capsules the extent and the rate of absorption, of 40mg under fed condition in healthy adult male volunteers according to the USFDA regulatory guidance.

5. References

1. World Health Organization Cardiovascular diseases. 2007. Fact sheet no. 317 [online]. Accessed August 4, **2008**.
2. URL: http:// www.who.int/mediacentre/factsheets /fs317 /en /index.html.
3. American Heart Association. Dallas, Texas: American Heart Association; **2007**.
4. Frishman WH. A historical perspective on the development of beta-adrenergic blockers. J Clin Hypertens. **2007**a;9(4 Suppl 3):19–27.
5. Lindholm LH, Carlberg B, Samuelsson O. Should beta blockers remain first choice in the treatment of primary hypertension. A meta-analysis. Lancet. **2005**, 366:1545–53.
6. Frishman WH. Alpha- and beta-adrenergic blocking drugs. In: Frishman WH, Sonnenblick EH, Sica DA, editors. Cardiovascular pharmacotherapeutics. 2nd ed. New York: McGraw Hill; **2003**. pp. 67–97.
7. Packer M. Beta-adrenergic blockade in chronic heart failure: principles, progress, and practice. Prog Cardiovasc Dis. **1998**, 41: 39–52.
8. Sundberg S, Tiihonen K, Gordin A. Vasodilatory effects of carvedilol and pindolol. J Cardiovasc Pharmacol. **1987**, 10(Suppl 11):S76–80.
9. Toda N. Vasodilating beta-adrenoceptor blockers as cardiovascular therapeutics. Pharmacol. Ther. 2003, 100: 215–34.
10. William H Frishman, Linda S Henderson, and Mary Ann Lukas, Controlled-release carvedilol in the management of systemic hypertension and myocardial dysfunction, Vasc Health Risk Manag. **2008** Dec; 4(6): 1387–1400.
11. Egan BM, Basile J, Chilton RJ, et al. Cardioprotection: the role of beta-blocker therapy. J Clin Hypertens (Greenwich) 2005;7:409–16.
12. Messerli FH, Grossman E. beta-Blockers in hypertension: is carvedilol different? Am J Cardiol. **2004**, 93:7B–12B.
13. Pedersen ME, Cockcroft JR. The vasodilatory beta-blockers. Curr Hypertens Rep. **2007**, 9: 269–77.
14. kozlovski VI, Lomnicka M, Chlopicki S. Nebivovol and carvedilol induce NO-dependent coronary vasodilatation that is unlikely to be

mediated by extracellular ATP in the isolated guinea pig heart. Pharmacol Rep. **2006**, 58(Suppl): 103–10.

15. Maack C, Cremers B, Flesch M, et al. Different intrinsic activities of bucindolol, carvedilol and metoprolol in human failing myocardium. Br J Pharmacol. **2000**, 130:1131–9.
16. Henderson LS, Tenero DM, Baidoo CA, et al. Pharmacokinetic and pharmacodynamic comparison of controlled-release carvedilol and immediate-release carvedilol at steady state in patients with hypertension. Am J Cardiol. **2006**, 98:17L–26L.
17. Packer, Lukas MA, Tenero DM, et al. Pharmacokinetic profile of controlled-release carvedilol in patients with left ventricular dysfunction associated with chronic heart failure or after myocardial infarction. Am J Cardiol. **2006**, 98:39L–45L.
18. Nichols AJ, Sulpizio AC, Ashton DJ, et al. The interaction of the enantiomers of carvedilol with alpha 1- and beta 1-adrenoceptors. Chirality. **1989**, 1: 265–70.
19. Tenero DM, Henderson LS, Baidoo CA, et al. Pharmacokinetic properties of a new controlled-release formulation of carvedilol. Am J Cardiol. **2006**, 98: 5L–16L.
20. Othman ,Tenero DM, Boyle DA, et al. Population pharmacokinetics of S(−)-carvedilol in healthy volunteers after administration of the immediate-release (IR) and the new controlled-release (CR) dosage forms of the racemate. Aaps J. **2007**, 9: E208–18.
21. World Medical Association Declaration Of Helsinki, Ethical Principles For Medical Research Involving Human Subjects, 59th WMA General Assembly, **2008.**
22. International Conference On Harmonisation Of Technical Requirements For Registration Of Pharmaceuticals For Human Use, Guideline For Good Clinical Practice, E6, **1996.**
23. U.S. Department of Health and Human Services Food and Drug Administration, Bioanalytical Method Validation, **2001**.
24. I.Sarath Chandiran, K. N. Jayaveera and Raghunadha Reddy. S, High-Throughput Liquid Chromatography – Tandem Mass Spectrometric Method for Simultaneous Quantification of Carvedilol and Its Metabolite 4-Hydroxyphenyl Carvedilol in Human Plasma and Its Application to Bioequivalence Study, Journal of Chemical and Pharmaceutical Research, **2011**, 3(2):341-353.
25. Koteswara Rao.Divi, I. Sarath Chandiran, K.N. Jayaveera, Y.K. Naidu, M.P. Kalyan Reddy. Development and validation of high-throughput liquid chromatography tandem mass spectrometric method for simultaneous quantification of Clopidogrel and its metabolite in human plasma, Journal of Chromatography B, Elsevier Publications, February- **2010**, 878(3-4): 502–508.
26. Y.K.N, Koteswara Rao Divi, Penchala Kalyan Reddy Mule, Sarath Chandiran I and Jayaveera K N. Simultaneous Quantification of Alverine And Its Metabolite P-Hydroxy Alverine In Human Plasma With Robotic Liquid-Liquid Extraction By Using Fully Validated LC-MS/MS and Its Application To A Bioequivalence Study, Journal Of Pharmacy Research, **2010**, 3(6): 1407-1411.
27. K.N. Jayaveera, Koteswara Rao.Divi, I.Sarath chandiran and RReddy.S. Quantification of Artemether in human plasma with liquid- liquid extraction by using fully validated high performance liquid Chromatography–Tandem mass spectrometric method, Journal of Pharmacy Research, August-**2010**, 3(8).
28. I. Sarath Chandiran, K. N. Jayaveera and RRSeelam. Development and Validation of High-Throughput Liquid Chromatography-Tandem Mass Spectrometric Method for Quantification of Itraconazole and its Metabolite in Human Plasma. Scholars Research Library, Der Pharmacia Lettre, **2011**, 3(2): 316-328.
29. SRR, I. Sarath Chandiran, K. N. Jayaveera and Koteswara Rao. Divi. Quantification of Ursodeoxy Cholic acid in human plasma by using High performance liquid chromatography–tandem mass spectrometric method and its applications in pharmacokinetics, Journal of Chemical and Pharmaceutical Research, **2010**, 2(3): 59-69.
30. Sarath Chandiran I, Koteswara Rao Divi, Jayaveera K. N., Development and Validation of High Performance Liquid Chromatography-Tandem Mass Spectrometric Method for Simultaneous Quantification of Telmisartan in Human Plasma, International journal of pharmaceutical sciences and Drug Research, **2010**, 2(3): 188-192.
31. RR Seelam, K.N. Jayaveera and Koteswara Rao.Divi. Quantification of ibuprofen in human plasma by using high throughput liquid chromatography–tandem mass spectrometric method and its applications in pharmacokinetics, scholars research library, archives of applied research, **2010**, 2(3):101-111.
32. Raghunadha Reddy, Koteswara Rao.Divi, Y.K.Naidu, I.Sarath Chandiran, k.N. Jayaveera and M.P.Kalyan Reddy. Development and validation of high-performance liquid chromatography tandem mass spectrometric method for quantification of Clonidine in human plasma, journal of chemical and pharmaceutical sciences, April – June **2010**, 3(2).
33. Reddy.S, M.P. Kalyan Reddy, Y.K.Naidu, Koteswara Rao.Divi, I.Sarath Chandiran and K.N. Jayaveera. Development and Validation of High Performance Liquid Chromatography–Tandem Mass Spectrometric Method for Quantification of Lumefantrine in Human Plasma with Precipitation, International Journal of Pharma Research and Development [IJPRD], Apr -**2010**, 2(2):1-9

34. YK.N, SRR. Koteswara Rao Divi, M.P. Kalyan Reddy, I. Sarath Chandiran and K.N. Jayaveera. Quantification of Levetiracetam in Human Plasma with Precipitation Extraction by Using Fully Validated LC-MS/MS and Its Application to a Bioequivalence Study, Research J. Pharm. and Tech., July-Sept **2010**, 3(3): 847-853.
35. Kalyan Reddy MP, Jaya Chandra Reddy P, RRS, Jayaveera KN. Development and validation of bio-analytical method for Quantification of Pantoprazole in human plasma using LC-MS/MS, Journal of Pharmacy and Chemistry, July - September **2010**, 4(3): 80-86.
36. Naidu YK, Koteswara Rao Divi, Ravikiran V, Sandhya Rani Y, Kalyan Reddy MP. Development and Validation of High Performance Liquid Chromatography Tandem Mass Spectrometric Method for Quantification of Aceclofenac in Human Plasma, Journal of Pharmacy and Chemistry, July - September **2010**, 4(3): 89-95.
37. U.S. Department of Health and Human Services Food and Drug Administration, Bioavailability and Bioequivalence Studies for Orally Administered Drug Products General Considerations, **2003**.
38. I.S. Chandiran and K.N. Jayaveera, Pharmacokinetic and Bioequivalence Comparison Between Extended Release Capsules of Venlafaxine Hydrochloride 150mg: An Open Label, Balanced, Randomized-Sequence, Single-Dose, Two-Period Crossover Study In Healthy Indian Male Volunteers, International Research Journal of Pharmacy. **2011**, 2(3): 262-269.
39. U.S. Department of Health and Human Services Food and Drug Administration, Statistical Approaches to Establishing Bioequivalence, **2001**.