# Photo-degradation Study of Dilgard (Carvedilol) using UV-Spectroscopy


Submitted By

Abida Islam Pranty

ID : 2014-1-70-027


Submitted To

Md. Anisur Rahman

Assistant Professor, Dept. of Pharmacy

East West University, Dhaka

**"A thesis report, submitted to the Department of Pharmacy, East West University, in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy"**

# DECLARATION BY THE CANDIDATE

I, Abida Islam Pranty, hereby declare that the dissertation entitled “Photosensitivity Test of Carvedilol (Dilgard)”, submitted by me to the Department of Pharmacy, East West University, in the partial fulfillment of the requirement for the degree of Bachelor of Pharmacy (Honors) with original research work carried out by me under the supervision and guidance of Md. Anisur Rahman, Assistant Professor, Department of Pharmacy, East West University, Dhaka.

**Abida Islam Pranty**

ID no: 2014-1-70-027

Department of Pharmacy

East West University, Dhaka

# CERTIFICATE BY THE SUPERVISOR

This is to certify that the dissertation entitled “Photosensitivity Test of Carvedilol (Dilgard)”, submitted to the department of pharmacy, East West University in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy was carried out by Abida Islam Pranty

(ID: 2014-1-70-027) under our guidance and supervision and that no part of the research has been submitted for any other degree. We further certify that all the sources of information and laboratory facilities availed of in this connection is duly acknowledged.

**Md. Anisur Rahman**
Assistant Professor
Dept. of Pharmacy
East West University, Dhaka

# ENDORSMENT BY THE CHAIRPERSON

This is to certified that the dissertation entitle “Photosensitivity Test of Carvedilol (Dilgard)” is a bona fide research work done by Abida Islam Pranty (ID: 2014-1-70-027) under our guidance and supervision of Md. Anisur Rahman, Assistant Professor, Department of Pharmacy, East West University, Dhaka.

---

**Prof. Chowdhury Faiz Hossain**
Professor & Chairperson
Dept. of Pharmacy
East West University, Dhaka

# ACKNOWLEDGEMENT

I express my profound gratitude and deep respect from core of my heart to Mr. Md. Anisur Rahman, Assistant Professor, Department of Pharmacy, East West University, for his expert and careful guidance, sincere help, constructive criticism, valuable time and honored suggestion, without which I would not have been able to complete this work. He had been very enthusiastic and supportive in my research.

I also express my beloved gratitude to Mr. Arif Mahbub, GTA, Depertment of Pharmacy, East West University for his friendly hand to do the lab work and help me to keep patients during my research work.

It is my great pleasure and privilege to acknowledge my deepest regards and gratitude to Dr. Shamsun Nahar Khan, Associate Professor, Department of Pharmacy, East West University, for her kind words during my troubling moments, and of course for her approval of the topic, constant inspiration and whole hearted cooperation.

I am thankful to the laboratory instructors Mrs. Razia Sultana, Mr. Sujit Kumar, Mr. Shofiq Mrs. Shipra Biswas, and Mrs. Ruchira Chakraborty for their kind support during the laboratory work.

I wish to thank my fellow researchers namely Aziza Mohammadi, Wazi Ullah, Raad Shafiq Abir, and Md. Iqbal Hossain Nayan for their endless cooperation and inspiration for preparing this work.

I also like to thank my parents and family for their support and inspiration to complete this work.

Above all, I express my gratitude to Almighty Allah for giving me the strength, energy and patients to carry out this research work.

# ABSTRACT

The study was aimed for the determination of photolytic degradation of carvedilol tartrate. The objective of this research was to determine the effect on carvedilol in various lighting conditions (control, sunlight, normal room light, 25watt & 40watt bulb). Besides, physical tests were performed for evaluation of color change, weight variation, thickness and hardness of Dilgard 6.25 mgtablets from same batch according to the specification of USP. A very insignificant fluctuation in result was observed for weight variation, hardness & thickness test.The average weight of tablets was 0.1325gm and it showed negligible fluctuation with ±0.0004. But in various lighting condition like 25watt bulb, 40watt bulb, direct sunlight and normal room light the concentration of carvedilol tartrate were decreased gradually with percent deviation 4.91%, 3.98%, 2.80%, and 23.04 % respectively. Overall this study was done by the UV- spectroscopy analytical method.So it can be said that the Dilgard 6.25 mg tabletscontaining carvedilol is light sensitive and coating alone is not sufficient to protect the drug from light. So that package should be opaque thus light cannot pass through the package.

**Keywords:** Carvedilol Tartrate, Photolytic Degradations, UV-Spectroscopy, Batch, Weight variation, Potency, USP.

# Content of Table

# Content of Figures

# Chapter 1
# Introduction

## Objective

The objective of this study is to determine the photolytic degradation of carvedilol contained in a transparent packaging and is photosensitive. The photosensitivity of carvedilol in various lighting conditions (control, sunlight, normal room light, 25watt bulb and 40watt bulb condition) was determined in this research. Only a few drugs contain opaque packaging in local market. So drugs having transparent packaging were tested for photosensitivity to evaluate coating efficiency.

## 1.1 Molecular and Cellular basis of Carvedilol: [Dulin, Abraham; 2004, Cheng,Kamia,Kodama,2001, Dunn et al,1997 ]

Carvedilol is a third-generation, Neurohormonal Antagonist with multiple activities. It blocks both β1- and β2-adrenergic receptors, enhances vasodilation via α1-adrenergic blockade, and, at high concentrations, has ion channel–blocking activities. Carvedilol lacks sympathomimetic activity. In addition to these well-known properties, Carvedilol has a number of ancillary activities, including antioxidant, anti-inflammatory, and antiapoptotic properties. Together, they contribute to the clinical efficacy of Carvedilol in a broad spectrum of patient types and may also confer a range of Cardioprotective benefits (Dulin, Abraham; 2004).

Carvedilol is a unique cardiovascular drug of multifaceted therapeutic potential. Its major molecular targets recognized to date are membrane adrenoceptors (β1,β2, and α1), reactive oxygen species, and ion channels (K+ and Ca2+). Carvedilol provides prominent hemodynamic benefits mainly through a balanced adrenoceptor blockade, which causes a reduction in cardiac work in association with peripheral vasodilation. This drug assures remarkable cardiovascular protection through its antiproliferative/atherogenic, antiischemic, antihypertrophic, and antiarrhythmic actions. These actions are a consequence of its potent antioxidant effects, amelioration of glucose/lipid metabolism, modulation of neurohumoral factors, and modulation of cardiac electrophysiologic properties. The usefulness of carvedilol in the treatment of hypertension, ischemic heart

disease, and congestive heart failure is based on a combination of hemodynamic benefits and cardiovascular protection (Cheng,Kamia,Kodama,2001).

Animal models indicate that Carvedilol confers protection against myocardial necrosis, arrhythmia and cell damage caused by oxidising free radicals, and the drug has no adverse effects on plasma lipid profiles.

Recent data have confirmed the antihypertensive efficacy of Carvedilol in patients with mild to moderate essential hypertension. Carvedilol has similar efficacy to other β-blocking agents, calcium antagonists, ACE inhibitors and hydrochlorothiazide. Carvedilol also improves exercise tolerance and ischaemic symptoms in patients with stable angina pectoris. Significant reductions in serious cardiac events after acute myocardial infarction and in frequency and severity of ischaemic events in patients with unstable angina have also been demonstrated.

Interest in the use of Carvedilol in patients with congestive heart failure (CHF) has culminated in the publication of a cumulative analysis of data from 1094 patients with mild to severe CHF who participated in the US Carvedilol Heart Failure Study Program (4 trials). After a median follow-up of 6.5 months, a significant overall reduction in mortality relative to placebo (3.2 vs 7.8%) was revealed in patients who had received carvedilol 6.25 to 50 mg twice daily (plus diuretics and ACE inhibitors). All-cause mortality, risk of hospitalization for cardiovascular reasons and hospitalization costs were also reduced significantly (by 65, 28% and 62%, respectively) in these trials. In addition, the Australia and New Zealand Heart Failure Research Collaborative Group showed a 26% reduction in the combined risk of death or hospitalization with carvedilol 12.5 to 50 mg/day relative to placebo after a mean 19-month follow-up period in 415 patients with CHF (relative risk 0.74).

Adverse events with Carvedilol appear to be less frequent than with other β-blocking agents, are dosage-related and are usually seen early in therapy. Events most commonly reported are related to the vasodilating (postural hypotension, dizziness and headaches) and the β-blocking (dyspnoea, bronchospasm, bradycardia, malaise and asthenia) properties of the drug. Carvedilol appears to date to have little effect on the incidence of

worsening heart failure. Concomitant administration of carvedilol with some medications requires monitoring.

Carvedilol is therefore likely to have a beneficial role in the management of controlled CHF, but further clinical studies are required to show the place of β-adrenoceptor blocking therapy in general in this indication, and the position of carvedilol relative to other similar agents. Carvedilol is also confirmed as effective in the management of mild to moderate hypertension and ischaemic heart disease (Dunn et al,1997).

## 1.2. Physical Profiles of Carvedilol [Brittain, 2013]

### 1.2.1. Systematic chemical name:

(2RS)-1-(9H-Carbazol-4-yloxy)-3-[[2-(2 methoxyphenoxy)ethyl]amino]propan-2-ol

2-Propanol,1-(9H-carbazol-4-yloxy)-3-[[2-(2-methoxyphenoxy)ethyl]amino]-,(+/-)-;

(+/-)-1-(Carbazol-4-yloxy)-3-[[2-(o-methoxyphenoxy)ethyl]amino]-2-propanol

### 1.2.2. Proprietary names:

Coreg (GSK), Dilatrend (Roche), Dimitone (Erco), Eucardic (Roche), Kredex (GSK), Querto (Byl Gulden).

### 1.2.3. Nonproprietary Name:

Carvedilol

## 1.3. Physical Characteristics of Carvedilol [Brittain, 2013]

### 1.3.1. Ionization Constants:

pKa = 7.8

### 1.3.2. Solubility Characteristics:

Carvedilol is reported to be freely soluble in DMSO; soluble in methylene chloride,methanol;sparingly soluble in ethanol,isopropanol;and slightly soluble in ethyl ether. The solubility of Carvedilol in ethanol (96%) is slightly soluble as reported in the British Pharmacopoeia (BP)/European Pharmacopoeia (EP).

At pH values in the pharmaceutically relevant range of 1-8, the solubility of Carvedilol in aqueous media ranges from about 0.01 to 1 mg/ml.This is consistent with the aqueous solubility profile for Carvedilol at ambient temperature throughout the physiological pH range.Carvedilol exhibitss pH-dependent solubility with its solubility increasing with decreasing pH.

Carvedilol, in various solutions of dilute aqueous acids,vary widely at pH values <5.The solubility of Carvedilol in hydrochloric acid (0.2 M) is 10 times less than that in phosphoric acid (0.1 and 0.3 M) and 400 times less than in acetic acid (0.2-0.5 M).This is due to the formation of carvedilol acetate salt that has significant higher solubility than the respective phosphate or hydrochloride salts.

### 1.3.3. Partition coefficients:

The calculated log P of Carvedilol (using ACD Labs) is 3.84 (+/-) 0.89.p

Log P (octanol/water) of Carvedilol at concentrations of (6×10^-7)M at room temperature have been reported as 3.10 (at pH 9.0), 2.74 (at pH 7.0), and 1.93 (at pH 5.0).

### 1.3.4. Hygroscopicity

Analysis of Carvedilol by Dynamic Vapor Sorption confirmed that thjs substance is non-hygroscopic based on the step isotherm. From the dry state to 95% RH, Carvedilol showed less than 0.1% increase (reversible) in weight during the absorption/desorption process.

## 1.4. Stability [Brittain, 2013]

### 1.4.1. Solid-state stability:

In the solid state, Carvedilol drug substance is very stable with respect to heat,heat/high humidity, and light.Prolonged exposure to heat (80°C,14 days),irradiation with UV-vis lught (1.3×10^6 lux h),and direct exposure to heat/high humidity (40°C,75% RH,14 days) did not result in the formation of any degradation products above 0.05%.Mass balance of all solid stress samples was about 100%.

### 1.4.2. Solution-phase stability:

Carvedilol drug substance is not susceptible to acidic, basic, thermal, and UV-visible light stress conditions. No individual degradation product >0.06% was generated under these conditions.

When exposed to oxidative solution-stress conditions (3% hydrogen peroxide, 60°C, 4 h), about 2.1% of unknown degradants were detected (multiple peaks ranging from 0.01% to 0.6%). None of the degradation products were determined to be any known compendial impurities of carvedilol. It is documented that degradation of carvedilol, under oxidative solution-stress conditions, exhibits second-order kinetics.

It has been reported in the literature that carvedilol, when exposed to much harsher alkaline stress conditions (i.e., 1N NaOH, 90°C, 6 h) or when the stress samples are exposed to 1:1 water/methanolic 0.5 N NaOH, does its alkaline degradation become noticeable.

In acidic conditions, Carvedilol is known to be stable under jarsh aqueous conditions and susceptible only in the presence of 1:1 (v/v) water/methanolic 0.5 N HCl solutions (Brittain, 2013).

## 1.5. Pharmacodynamic Properties [Dunn et al, 1997]

Carvedilol competitively blocks β1, β2 and α1 adrenoceptors, lacks sympathomimetic activity and has vasodilating properties exerted mainly through α1-blockade. Single oral doses of carvedilol 12.5 to 200mg reduce SBP and DBP with no reflex tachycardia. Significant reductions in blood pressure have been reported, starting 30 minutes after administration, with maximal decreases at 1.5 to 7 hours. Exercise-induced increases in SBP and heart rate are attenuated by the drug, and stroke index and cardiac index are preserved after single doses of 20 to 60mg in healthy volunteers. Cardiac index was reduced after carvedilol 25 mg/day for 6 to 9 months in patients with hypertension.

It has been associated with improved myocardial function, particularly improvements in afterload (left ventricular ejection fraction) and decreased left ventricular volumes in patients with left ventricular dysfunction, and regression of left ventricular hypertrophy in patients with mild to moderate essential hypertension.

Carvedilol has demonstrated protective effects against myocardial necrosis and arrhythmia and against cell damage caused by oxidizing free radicals in several animal models. The drug also does not adversely affect renal function at dosages of up to 50 mg/day, has anti-proliferative effects on tissue and does not affect plasma lipid profiles. Increased insulin sensitivity has been reported with carvedilol 25 to 50 mg/day in a 12-week study in patients with mild to moderate essential hypertension.

## 1.6. Pharmacokinetic Properties [Dunn et al, 1997]

Oral carvedilol shows predominantly linear pharmacokinetics. Absolute oral bioavailability is 20 to 25% and peak plasma concentrations (Cmax) are seen 1 to 2 hours after administration. The terminal elimination half-life has ranged from 2 to 8 hours in clinical studies. There are no significant age-related variations in the pharmacokinetic properties of the drug. Carvedilol is highly lipophilic and protein bound. No dosage alterations are required in patients with renal failure. However, patients with liver

dysfunction show stereoselective alterations in metabolism of the drug; this may affect the balance between β- and α-adrenergic effects (Dunn et al, 1997).

## 1.7. Nonclinical Toxicology [Dailymed, 2011]

### 1.7.1. Carcinogenesis, Mutagenesis, Impairment of Fertility:

In 2-year studies conducted in rats given carvedilol at doses up to 75 mg/kg/day (12 times the maximum recommended human dose [MRHD] when compared on a mg/m2 basis) or in mice given up to 200 mg/kg/day (16 times the MRHD on a mg/m2 basis), carvedilol had no carcinogenic effect.

Carvedilol was negative when tested in a battery of genotoxicity assays, including the Ames and the CHO/HGPRT assays for mutagenicity and the in vitro hamster micronucleus and in vivo human lymphocyte cell tests for clastogenicity.

At doses ≥ 200 mg/kg/day (≥ 32 times the MRHD as mg/m2) carvedilol was toxic to adult rats (sedation, reduced weight gain) and was associated with a reduced number of successful matings, prolonged mating time, significantly fewer corpora lutea and implants per dam, and complete resorption of 18% of the litters. The no-observed-effect dose level for overt toxicity and impairment of fertility was 60 mg/kg/day (10 times the MRHD as mg/m2) (Dailymed, 2011).

## 1.8. Therapeutic Use [Dunn et al,1997;Krum,1995]

The antihypertensive efficacy of carvedilol 25 to 50 mg/day in patients with mild to moderate essential hypertension is well established. Recent comparative trials have confirmed earlier findings and show carvedilol to be of equivalent efficacy to other antihypertensive agents (notably atenolol, labetalol, pindolol, metoprolol, nitrendipine, hydrochlorothiazide and captopril). A 29-week comparison of carvedilol 12.5 to 50 mg/day with enalapril 10 to 40 mg/day showed similar antihypertensive response rates

and quality-of-life effects for both drugs. Enalapril reduced SBP by 5 to 10mm Hg more than carvedilol in this study.

Confirmation of the ability of carvedilol to improve ischaemic and exercise parameters in patients with stable angina pectoris has also been obtained. Carvedilol 25mg twice daily has similar efficacy to nifedipine 20mg twice daily or verapamil 120mg 3 times daily. Angina attack frequency and nitroglycerin (glyceryl trinitrate) consumption were reduced significantly in both groups in the 4-week comparison with nifedipine. In the verapamil study, mean total treadmill exercise time was increased relative to baseline by 15.3% with carvedilol and 13.5% with verapamil.

Intravenous plus oral carvedilol has been associated with significant clinical benefit in a placebo-controlled 6-month study in 151 patients with acute myocardial infarction. Significantly fewer serious cardiac events were associated with carvedilol treatment [17 vs 31 events].

Significant reductions in the frequency and severity of ischaemic events have been reported with carvedilol (50 mg/day for 48 hours) in patients with unstable angina. However, clinical efficacy of the drug in the prevention of restenosis after coronary angioplasty has not been conclusively shown.

Since the publication of the previous review in Drugs in 1993, interest in carvedilol has focused on the potential utility of the drug in congestive heart failure (CHF). A cumulative analysis of data from 1094 patients with mild to severe CHF [New York Heart Association (NYHA) classifications II to IV] who participated in a stratified programme of 4 US clinical studies (the US Carvedilol Heart Failure Study Program) has shown a significant overall reduction in mortality relative to placebo (3.2 vs 7.8%) in patients who received carvedilol 6.25 to 50mg twice daily (plus diuretics and ACE inhibitors) after the programme was terminated early (median follow-up 6.5 months) because of the clear benefit associated with carvedilol treatment. Thus, the risk of death was (significantly) reduced by carvedilol therapy (65% reduction compared with placebo). There was also a significant 28% overall reduction in the risk of hospitalisation for cardiovascular reasons across the 4 trials, and a decrease of 62% in the costs of

hospitalisation. Preliminary data from a nonblind follow-up (mean 270 days) in 899 patients from the studies suggest that these survival benefits are maintained with continuing treatment. Results from the mean 19-month follow-up period of another large trial (the Australia and New Zealand Heart Failure Research Collaborative Group's study in 415 patients with CHF secondary to ischaemic heart disease) showed a 26% reduction in the combined risk of death or hospital admission (all reasons) with carvedilol 12.5 to 50 mg/day relative to placebo.

Improvements in NYHA class were seen in 5 of the 8 published well controlled studies of carvedilol in patients with heart failure. In contrast, improved ventricular function but no improvements in exercise capacity or NYHA classification were reported after 12 months in the Australia and New Zealand study. Carvedilol had a beneficial effect on disease progression in most well controlled studies (Dunn et al,1997).

Double-Blind, Placebo-Controlled Study of the Long-term Efficacy of Carvedilol in Patients With Severe Chronic Heart Failure:

Background Clinical trials have shown that β-adrenergic blocking drugs are effective and well tolerated in patients with mild to moderate heart failure, but the utility and safety of these drugs in patients with advanced disease have not been evaluated.

Methods and Results were enrolled on 56 patients with severe chronic heart failure into a double-blind, placebo-controlled study of the vasodilating β-blocker carvedilol. All patients had advanced heart failure, as evidenced by a mean left ventricular ejection fraction of 0.16±0.01 and a mean maximal oxygen consumption of 13.6±0.6 mL · kg−1 · min−1 despite digitalis, diuretics, and an angiotensin-converting enzyme inhibitor (if tolerated). After a 3-week, open-label, up-titration period, 49 of the 56 patients were assigned (in a double-blind fashion using a 2:1 randomization) to receive either carvedilol (25 mg BID, n=33) or matching placebo (n=16) for 14 weeks, while background therapy remained constant. Hemodynamic and functional variables were measured at the start and end of the study. Compared with the placebo group, patients in the carvedilol group showed improved cardiac performance, as reflected by an increase in left ventricular ejection fraction (P=.005) and stroke volume index (P=.010) and a

decrease in pulmonary wedge pressure, mean right atrial pressure, and systemic vascular resistance (P=.003, .002, and .017, respectively). In addition, compared with placebo, patients treated with carvedilol benefited clinically, as shown by an improvement in symptom scores (P=.002), functional class (P=.013), and submaximal exercise tolerance (P=.006). The combined risk of death, worsening heart failure, and life-threatening ventricular tachyarrhythmia was lower in the carvedilol group than in the placebo group (P=.028), but carvedilol-treated patients had more dizziness and advanced heart block. So,it can be concluded that Carvedilol produces clinical and hemodynamic improvement in patients who have severe heart failure despite treatment with angiotensin-converting enzyme inhibitors (Krum,1995).

## 1.9. Tolerability [Dunn et al,1997]

Adverse events with carvedilol therapy are dosage-related, tend to be seen early in therapy, and are reported to have a lower incidence than is seen with other β-blocking agents. Events most commonly reported are postural hypotension, dizziness and headache (related to the vasodilating properties of the drug) and dyspnoea, bronchospasm, bradycardia, malaise and asthenia (related to β-blockade). From the data published to date, carvedilol appears to have little effect on the incidence of worsening heart failure.

## 1.10. Dosage and Administration [Dunn et al,1997 ; Watanabe, 2000]

A dosage of carvedilol 12.5mg once daily for 2 days, increased to 25mg daily thereafter and increased to 50mg once daily after 2 weeks if necessary, is recommended for patients with mild to moderate essential hypertension. In patients with stable angina pectoris, a dosage of 25 to 50mg twice daily appears to be appropriate. In patients with controlled CHF, the dosage should be titrated gradually upwards from 3.125 mg twice daily to a maintenance dosage of 25mg twice daily (50mg twice daily if bodyweight is >85kg). No dosage adjustment is required in patients with renal failure. The drug is contraindicated in patients with second or third degree heart block, shock, severe bradycardia, asthma,

decompensated heart failure, hepatic impairment or chronic obstructive pulmonary disease. Care should be taken when administering carvedilol to elderly patients, as they are particularly prone to orthostatic hypotension. Drug interactions with β-blocking agents are common but avoidable with regular monitoring where appropriate.(Dunn et al,1997).

The cardioprotective properties of carvedilol (a vasodilating β-adrenoceptor blocking agent) were studied in a rat model of dilated cardiomyopathy induced by autoimmune myocarditis.In that study,twenty-eight days after immunization, surviving Lewis rats (32/43=74%) were divided into three groups to be given 2 mg kg−1 day−1 (Group-C2, n=10) or 20 mg kg−1 day−1 (Group-C20, n=10) of carvedilol, or vehicle (0.5% methylcellulose, Group-V, n=12).After oral administration for 2 months, body weight, heart weight (HW), heart rate (HR), rat α-atrial natriuretic peptide (r-ANP) in blood, central venous pressure (CVP), mean blood pressure (mean BP), peak left ventricular pressure (LVP), left ventricular end-diastolic pressure (LVEDP), ±dP dt−1 and area of myocardial fibrosis were measured. Values were compared with those for normal Lewis rats (Group-N, n=10).Two out of 12 (17%) rats in Group-V died from day 28 to day 42 after immunization. No rat died in Groups-C2, -C20 and -N. Although the CVP, mean BP, LVP and ±dP dt−1 did not differ among the three groups, the HW, HR and r-ANP in Group-C2 (1.14±0.03, 339±16 and 135±31) and Group-C20 (1.23±0.04, 305±8 and 156±24) were significantly lower than those in Group-V (1.36±0.04 g, 389±9 beats min−1 and 375±31 pg ml−1, respectively). The LVEDP in Group-C2 was significantly lower than that in Group-V (7.4±1.4 and 12.2±1.2 mmHg, respectively, $P<0.05$). The area of myocardial fibrosis in Group-C2 was smaller than that in Group-V (12±1 and 31±2%, $P<0.01$).These results were the indication that a low dose of carvedilol has beneficial effects on dilated cardiomyopathy (Watanabe, 2000).

## 1.11. Dosage Forms & Strengths [Dailymed, 2011]

The white, oval, film-coated tablets are available in the following strengths: 3.125 mg, debossed with SZ on one side and 61 on the other side, 6.25 mg, debossed with SZ on one side and 62 on the other side, 12.5 mg, debossed with SZ on one side and 116 on the other side and 25 mg debossed with SZ on one side and 117 on the other side. (Dailymed, 2011).

## 1.12. Ultra Violet Spectroscopy

This technique of ultra violet spectroscopy is used in my stability test of carvedilol,which is one of most frequently employed method in pharmaceutical analysis. It involves the measurement of the amount of UV radiation (190-380 nm) or visible (380-800 nm) radiation absorbed by a substance in solution.

## 1.13. Beer–Lambert law [Pradhan,Rao,Srinivasulu,2011]

This law states that there is a logarithmic dependence between the transmission and transmissivity, T, of light through a substance and the product of the absorption coefficient of the substance, α, and the distance the light travels through the material (i.e. the path length), ℓ. The absorption coefficient can, in turn, be written as a product of either a molar absorptive (extinction coefficient) of the absorber, ε, and the concentration c of absorbing species in the material, or an absorption cross section, σ, and the (number) density N' of absorbers.

I 0 and I are the intensity of the incident light and the transmitted light.

σ is cross section of light absorption by a single particle.

N is the density (number per unit volume) of absorbing particles.

**Correlation Coefficient:**

The Correlation Coefficient "r" (X, Y) is the most useful to express the relationship of the chosen scale. To obtain the Correlation Coefficient, the covariance is divided by the product of the standard deviation of X and Y (Pradhan,Rao,Srinivasulu,2011).

## 1.14. Effect of Formulation and Manufacturing process [Aman, Thoma, 2002]

The formulation and the manufacturing process can significantly influence the photo stability of tablets. Investigations of various formulation and manufacturing parameters were done with tablets which are highly light sensitive drugs. The effect of relevant formulation factors are stated. Whereas the particle size of the drug substance and the choice of the lubricant had no effect, the drug content, the compression diluent and geometric alterations significantly affected the photo instability. Depending on the formulation drug losses varied between 30 and 55% after 12 h irradiation in a light testing cabinet (Suntest® CPS+). Manufacturing parameters like compression force and direct compression versus granulation showed less serious influences. Nevertheless, photostability changes up to 10% were registered. (Aman, Thoma, 2002).

## 1.15. Indication and Usage [Dailymed, 2011]

**I. Left Ventricular Dysfunction Following Myocardial Infarction**

Carvedilol is indicated to reduce cardiovascular mortality in clinically 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)

**II. Hypertension**

Carvedilol is indicated for the management of essential hypertension. It can be used alone or in combination with other antihypertensive agents, especially thiazide-type diuretics.

**1.16. Contraindications [**Dailymed, 2011]

Carvedilol is contraindicated in the following conditions:

I. Bronchial asthma or related bronchospastic conditions. Deaths from status asthmaticus have been reported following single doses of carvedilol

II. Second- or third-degree AV block

III. Sick sinus syndrome

IV. Severe bradycardia (unless a permanent pacemaker is in place)

V. Patients with cardiogenic shock or who have decompensated heart failure requiring the use of intravenous inotropic therapy. Such patients should first be weaned from intravenous therapy before initiating carvedilol

VI. Patients with clinically manifest hepatic impairment

VII. Patients with a history of a serious hypersensitivity reaction to carvedilol (e.g. Stevens- Johnson syndrome).

## 1.17 Warnings & Precautions [Dailymed, 2011]

**I. Cessation of Therapy**

Patients with coronary artery disease, who are being treated with carvedilol, should be advised against abrupt discontinuation of therapy. Severe exacerbation of angina and the occurrence of myocardial infarction and ventricular arrhythmias have been reported in angina patients following the abrupt discontinuation of therapy with β-blockers. The last 2 complications may occur with or without preceding exacerbation of the angina pectoris. As with other β-blockers, when discontinuation of carvedilol is planned, the patients should be carefully observed and advised to limit physical activity to a minimum. Carvedilol should be discontinued over 1 to 2 weeks whenever possible. If the angina worsens or acute coronary insufficiency develops, it is recommended that carvedilol be promptly reinstituted, at least temporarily. Because coronary artery disease is common and

may be unrecognized, it may be prudent not to discontinue therapy abruptly even in patients treated only for hypertension or heart failure.

**II. Bradycardia**

In clinical trials, carvedilol caused bradycardia in about 2% of hypertensive patients, and 6.5% of myocardial infarction patients with left ventricular dysfunction. If pulse rate drops below 55 beats/minute, the dosage should be reduced

**III. Hypotension**

Postural hypotension occurred in 1.8% and syncope in 0.1% of hypertensive patients, primarily following the initial dose or at the time of dose increase and was a cause for discontinuation of therapy in 1% of patients.

In the CAPRICORN study of survivors of an acute myocardial infarction, hypotension or postural hypotension occurred in 20.2% of patients receiving carvedilol compared to 12.6% of placebo patients. Syncope was reported in 3.9% and 1.9% of patients, respectively. These events were a cause for discontinuation of therapy in 2.5% of patients receiving carvedilol, compared to 0.2% of placebo patients.

Starting with a low dose, administration with food, and gradual up-titration should decrease the likelihood of syncope or excessive hypotension. During initiation of therapy, the patient should be cautioned to avoid situations such as driving or hazardous tasks, where injury could result should syncope occur.

**IV. Heart Failure/ Fluid Retention**

Worsening heart failure or fluid retention may occur during up-titration of carvedilol. If such symptoms occur, diuretics should be increased and the carvedilol dose should not be advanced until clinical stability resumes. Occasionally it is necessary to lower the carvedilol dose or temporarily discontinue it. Such episodes do not preclude subsequent successful titration of, or a favorable response to, carvedilol.

**V. Non-Allergic Bronchospasm**

Patients with bronchospastic disease (e.g., chronic bronchitis and emphysema) should, in general, not receive β-blockers. Carvedilol may be used with caution, however, in patients who do not respond to, or cannot tolerate, other antihypertensive agents. It is prudent, if carvedilol is used, to use the smallest effective dose, so that inhibition of endogenous or exogenous β-agonists is minimized.

In clinical trials, patients with bronchospastic disease were enrolled if they did not require oral or inhaled medication to treat their bronchospastic disease. In such patients, it is recommended that carvedilol be used with caution. The dosing recommendations should be followed closely and the dose should be lowered if any evidence of bronchospasm is observed during up-titration.

**VI. Glycemic Control in Type 2 Diabetes**

In general, β-blockers may mask some of the manifestations of hypoglycemia, particularly tachycardia. Nonselective β-blockers may potentiate insulin induced hypoglycemia and delay recovery of serum glucose levels. Patients subject to spontaneous hypoglycemia, or diabetic patients receiving insulin or oral hypoglycemic agents, should be cautioned about these possibilities.

Studies designed to examine the effects of carvedilol on glycemic control in patients with diabetes and heart failure have not been conducted.

**VII. Peripheral Vascular Disease**

β-blockers can precipitate or aggravate symptoms of arterial insufficiency in patients with peripheral vascular disease. Caution should be exercised in such individuals.

**VIII. Deterioration of Renal Function**

Rarely, use of carvedilol in patients with heart failure has resulted in deterioration of renal function. Patients at risk appear to be those with low blood pressure (systolic blood pressure < 100 mm Hg), ischemic heart disease and diffuse

vascular disease, and/or underlying renal insufficiency. Renal function has returned to baseline when carvedilol was stopped. In patients with these risk factors it is recommended that renal function be monitored during up-titration of carvedilol and the drug discontinued or dosage reduced if worsening of renal function occurs.

**IX. Anesthesia and Major Surgery**

If treatment with carvedilol is to be continued perioperatively, particular care should be taken when anesthetic agents which depress myocardial function, such as ether, cyclopropane, and trichloroethylene, are used.

**X. Thyrotoxicosis**

β-adrenergic blockade may mask clinical signs of hyperthyroidism, such as tachycardia. Abrupt withdrawal of β-blockade may be followed by an exacerbation of the symptoms of hyperthyroidism or may precipitate thyroid storm.

**XI. Pheochromocytoma**

In patients with pheochromocytoma, an α-blocking agent should be initiated prior to the use of any β-blocking agent. Although carvedilol has both α- and β-blocking pharmacologic activities, there has been no experience with its use in this condition. Therefore, caution should be taken in the administration of carvedilol to patients suspected of having pheochromocytoma.

**XII. Prinzmetal's Variant Angina**

Agents with non-selective β-blocking activity may provoke chest pain in patients with Prinzmetal's variant angina. There has been no clinical experience with carvedilol in these patients although the α-blocking activity may prevent such symptoms. However, caution should be taken in the administration of carvedilol to patients suspected of having Prinzmetal's variant angina.

**XIII. Risk of Anaphylactic Reaction**

While taking β-blockers, patients with a history of severe anaphylactic reaction to a variety of allergens may be more reactive to repeated challenge, either accidental, diagnostic, or therapeutic. Such patients may be unresponsive to the usual doses of epinephrine used to treat allergic reaction.

**XIV. Intraoperative Floppy Iris Syndrome**

Intraoperative Floppy Iris Syndrome (IFIS) has been observed during cataract surgery in some patients treated with alpha-1 blockers (carvedilol is an alpha/beta blocker). This variant of small pupil syndrome is characterized by the combination of a flaccid iris that billows in response to intraoperative irrigation currents, progressive intraoperative miosis despite preoperative dilation with standard mydriatic drugs, and potential prolapse of the iris toward the phacoemulsification incisions. The patient's ophthalmologist should be prepared for possible modifications to the surgical technique, such as utilization of iris hooks, iris dilator rings, or viscoelastic substances. There does not appear to be a benefit of stopping alpha-1 blocker therapy prior to cataract surgery.

**1.18. ADVERSE REACTIONS [**Dailymed, 2011]

## 1.18.1. Clinical Studies Experience

Carvedilol has been evaluated for safety 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).

**I. Left Ventricular Dysfunction Following Myocardial Infarction**

Carvedilol 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 carvedilol and 980 who received placebo. Approximately 75% of the patients received carvedilol for at least 6 months and 53% received carvedilol for at least 12 months. Patients were treated for an average of 12.9 months and 12.8 months with carvedilol and placebo, respectively.

The following adverse events were reported with a frequency of > 1% but = 3% and more frequently with carvedilol: 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).

**II. Hypertension**

Carvedilol 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 carvedilol for at least 6 months. Most adverse events reported during therapy with carvedilol were of mild to moderate severity. In US controlled clinical trials directly comparing carvedilol in doses up to 50 mg (n = 1,142) to placebo (n = 462), 4.9% of patients receiving carvedilol 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 carvedilol. 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.Dyspnea and fatigue were also reported in these studies, but the rates were equal or greater in patients who received placebo.

**III. Cardiovascular:** Peripheral ischemia, tachycardia.

**IV. Central and Peripheral Nervous System:** Hypokinesia.

**V. Gastrointestinal:** Bilirubinemia, increased hepatic enzymes (0.2% of hypertension patients were discontinued from therapy because of increases in hepatic enzymes).

**VI. Psychiatric:** Nervousness, sleep disorder, aggravated depression, impaired concentration, abnormal thinking, paroniria, emotional lability.

**VII. Respiratory System:** Asthma.

**VIII. Reproductive, Male:** Decreased libido.

**IX. Skin and Appendages:** Pruritus, rash erythematous, rash maculopapular, rash psoriaform, photosensitivity reaction.

**X. Special Senses:** Tinnitus.

**XI. Urinary System:** Micturition frequency increased.

**XII. Autonomic Nervous System:** Dry mouth, sweating increased.

**XIII. Metabolic and Nutritional:** Hypokalemia, hypertriglyceridemia.

**XIV. Hematologic:** Anemia, leukopenia.

**XV. Others:** The following events were reported in ≤ 0.1% of patients and are potentially important:Complete AV block, bundle branch block, myocardial ischemia, cerebrovascular disorder, convulsions, migraine, neuralgia, paresis, anaphylactoid reaction, alopecia, exfoliative dermatitis, amnesia, GI hemorrhage, bronchospasm, pulmonary edema, decreased hearing, respiratory alkalosis, increased BUN, decreased HDL, pancytopenia, and atypical lymphocytes.

### 1.18.2. Laboratory Abnormalities

Reversible elevations in serum transaminases (ALT or AST) have been observed during treatment with carvedilol. 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 carvedilol and those treated with placebo. However, transaminase elevations, confirmed by rechallenge, have been observed with carvedilol. In a long-term, placebo-controlled trial in severe heart failure, patients treated with carvedilol had lower values for hepatic transaminases than patients treated with placebo, possibly because carvedilol-induced improvements in cardiac function led to less hepatic congestion and/or improved hepatic blood flow.

Carvedilol 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.

### 1.18.3. Postmarketing Experience

The following adverse reactions have been identified during post-approval use of carvedilol. 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. Urinary incontinence in women (which resolved upon discontinuation of the medication) and interstitial pneumonitis have been reported rarely.

## 1.19. Use in Specific Populations [Dailymed, 2011]

**I. Pregnancy**

Pregnancy Category C. Studies performed in pregnant rats and rabbits given carvedilol revealed increased post-implantation loss in rats at doses of 300

mg/kg/day (50 times the MRHD as mg/m2) and in rabbits at doses of 75 mg/kg/day (25 times the MRHD as mg/m2). In the rats,there was also a decrease in fetal body weight at the maternally toxic dose of 300 mg/kg/day (50 times the MRHD as mg/m2), which was accompanied by an elevation in the frequency of fetuses with delayed skeletal development (missing or stunted 13th rib). In rats the no-observed-effect level for developmental toxicity was 60 mg/kg/day (10 times the MRHD as mg/m2); in rabbits it was 15 mg/kg/day (5 times the MRHD as mg/m2). There are no adequate and well-controlled studies in pregnant women. Carvedilol should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

**II. Nursing Mothers**

It is not known whether this drug is excreted in human milk. Studies in rats have shown that carvedilol and/or its metabolites (as well as other β-blockers) cross the placental barrier and are excreted in breast milk. There was increased mortality at one week post-partum in neonates from rats treated with 60 mg/kg/day (10 times the MRHD as mg/m2) and above during the last trimester through day 22 of lactation. Because many drugs are excreted in human milk and because of the potential for serious adverse reactions in nursing infants from β-blockers, especially bradycardia, a decision should be made whether to discontinue nursing or to discontinue the drug, taking into account the importance of the drug to the mother. The effects of other α- and β-blocking agents have included perinatal and neonatal distress.

**III. Pediatric Use**

Pediatrics: Effectiveness of carvedilol in patients younger than 18 years of age has not been established.

In a double-blind trial, 161 children (mean age 6 years, range 2 months to 17 years; 45% less than 2 years old) with chronic heart failure [NYHA class II-IV, left ventricular ejection fraction < 40% for children with a systemic left ventricle (LV), and moderate-severe ventricular dysfunction qualitatively by echo for those

with a systemic ventricle that was not an LV] who were receiving standard background treatment were randomized to placebo or to two dose levels of carvedilol. These dose levels produced placebo-corrected heart rate reduction of 4-6 heart beats per minute, indicative of beta-blockade activity. Exposure appeared to be lower in pediatric subjects than adults. After 8 months of follow-up, there was no significant effect of treatment on clinical outcomes. Adverse reactions in this trial that occurred in greater than 10% of patients treated with carvedilol and at twice the rate of placebo-treated patients included chest pain (17% vs. 6%), dizziness (13% vs. 2%), and dyspnea (11% vs. 0%).

**IV. Geriatric Use**

Of the 975 myocardial infarction patients randomized to carvedilol in the CAPRICORN trial, 48% (468) were 65 years of age or older, and 11% (111) were 75 years of age or older.

Of the 2,065 hypertensive patients in US clinical trials of efficacy or safety who were treated with carvedilol, 21% (436) were 65 years of age or older. Of 3,722 patients receiving carvedilol in hypertension clinical trials conducted worldwide, 24% were 65 years of age or older.

With the exception of dizziness in hypertensive patients (incidence 8.8% in the elderly versus 6% in younger patients), no overall differences in the safety or effectiveness were observed between the older subjects and younger subjects in each of these populations. Similarly, other reported clinical experience has not identified differences in responses between the elderly and younger subjects, but greater sensitivity of some older individuals cannot be ruled out.

## 1.20. Overdosage [Dailymed, 2011]

Overdosage may cause severe hypotension, bradycardia, cardiac insufficiency, cardiogenic shock, and cardiac arrest. Respiratory problems, bronchospasms, vomiting, lapses of consciousness, and generalized seizures may also occur.

The patient should be placed in a supine position and, where necessary, kept under observation and treated under intensive-care conditions. Gastric lavage or pharmacologically induced emesis may be used shortly after ingestion. The following agents may be administered:for excessive bradycardia: atropine, 2 mg IV to support cardiovascular function: glucagon, 5 to 10 mg IV rapidly over 30 seconds, followed by a continuous infusion of 5 mg/hour; sympathomimetics (dobutamine, isoprenaline, adrenaline) at doses according to body weight and effect.

If peripheral vasodilation dominates, it may be necessary to administer adrenaline or noradrenaline with continuous monitoring of circulatory conditions. For therapy-resistant bradycardia, pacemaker therapy should be performed. For bronchospasm, β-sympathomimetics (as aerosol or IV) or aminophylline IV should be given. In the event of seizures, slow IV injection of diazepam or clonazepam is recommended (Dailymed, 2011).

## 1.21. Packaging for tablets [Butler et al,1974]

A novel package having two communicating chambers such that a physical separation may exist between materials situated in the respective chambers. In many fields it has been found convenient to prepare single use units in the form of a shaped object, generally referred to as a tablet. Such tablets may be prepared according to known techniques in various sizes, shapes, hardnesses, etc.

One widely used method of forming tablets involves compressing powdered or granular compositions in punch and die sets on tableting equipment. Another known method of preparing tablets involves molding.It is also known that many tablets are friable,.and must be carefully packaged if they are to be received by a consumer in a satisfactory condition.To protect small tablets, particularly those that are easily broken, it has been a practice to insert in the top of a bottle a cushion of a flexible, soft material, such as cotton, after the bottle has been loaded with tablets.The amount of material inserted is chosen so that it fills the void space remaining after loading the bottle and exerts a light, yieldable, force upon the tablets. In this way, when the bottle is shaken during handling prior to

reaching the consumer, thetablets are not permitted to fly about and become fractured.It has been a practice with larger tablets to use as a cushion a disc of a yieldable material. This cushion is often placed in the space remaining after the filling of the bottle. In some situations, it has been found desirable to insert a cushion in the bottle prior to filling with the product as well as in the space remaining after fill- In some situations it is desirable to include within the bottle an ambient effective element. Such element may be a humidifying material, a perfume, a color signal device sensitive to heat, moisture, or breakdown of the product, or other element related to the condition of the product. When such element is include in a loose condition with the tablets, it has been the practice to have it in a form which is substantially different therefrom. One such different form is that used for desiccants.These materials are commonly placed in small packets which should be easily recognizable as not being the product. However, as in the case of a loose cushion, it has unfortunately been found that consumers endeavor, at times, to use these packets as if they were the product.Attempts have been made to physically separate these elements from the product, such as by incorporation in a cap used with the bottle. Although physical separation does alleviate the problem of the consumer improperly using this element, the cost of such packages has been prohibitively high. (Butler et al,1974)

## 1.22. Different types of packaging [Pareek, Khunteta, 2014]

### 1.22.1. Generally there are three types of packaging

**I. Primary Packaging:** This is the first packaging envelope which is in touch with the dosage form or equipment. The packaging needs to be such that there is no interaction with the drug and will provide proper containment of pharmaceuticals. E.g. Blister packages, Strip packages, etc.

**II. Secondary Packaging:** This is consecutive covering or package which stores pharmaceuticals packages in it for their grouping. E.g. Cartons, boxes, etc.

**III. Tertiary Packaging:** This is to provide bulk handling and shipping of

pharmaceuticals from one place to another. E.g. Containers, barrels, etc. Primarily two types of containers are used for packaging:

a. Glass Containers

b. Plastic Containers: These need to be chemically inert, impermeable, strong and rigid proving FDA clearance.

**1.22.2. Glass Containers:** Four types of Glasses are being used in pharmaceutical industry:

**I. Type I-Borosilicate glass:** Highly resistant and chemically inert glass. Alkali's and earth cations of glass are replaced by boron and/or aluminum and zinc. These are used to contain strong acids and alkalis.

**II. Type 2-Treated soda-lime glass:** These are more chemically inert than Type I glass. The glass surface is de-alkalized by "Sulfur treatment" which prevents blooming/weathering from bottles.

**III. Type III- Regular soda lime glass:** Untreated soda lime glass with average chemical resistance.

**IV. Type IV- General Purpose soda lime glass:** Glass is not used for parenterals, used only for products intended to be used orally or topically.

**1.22.3. Plastic Containers:** Plastic containers of high quality can be easily formed with different designs. These packages are extremely resistant to breakage and leakage. Primarily plastic containers are made from the following polymers:

**I. Polyethylene (PE):** Provides good barrier against moisture, relatively poor one against oxygen and other gases.

High density polyethylene is used with density ranging from 0.91-0.96 leading to four basic characteristics of container, (1) Stiffness, (2) Moisture vapor transmission, (3)stress cracking and(4)clarity or translucency based on polymer density used.

**II. Polypropylene (PP):** Polypropylene has features of polyethylene in addition it does not stress-crack in any condition. Hot aromatic or halogenated solvents soften the package. It has high melting point making it suitable for boilable packages and products needed to be sterilized. Brittleness at low temperature is its major disadvantages.

**III. Polyvinyl Chloride (PVC):** Can be produced with crystal clear clarity, will provide good gaseous barrier and stiffness. Reduction in residual vinyl

chloride monomers had further enhanced PVC quality. PVC is used as coating on glass bottles providing shatter resistant coating.

**IV. Polystyrene:** Rigid and crystal clear plastic. Not useful for liquid products. Polystyrene has high water and gaseous permeability also these are easily stretchable and breakable. To increase their strength and quality for permeability polystyrene is combined with rubber and acrylic compounds. Base on the composition these are classified as intermediate impact, high impact and super impact packages.

**V. Nylon (polyamide):** Many dibasic acids and amines combine to provide numerous varieties of nylon. Nylon is extremely strong and is quite difficult to be destroyed by mechanical means. Nylon provides resistance to wide range of acids and alkali only disadvantage of it is being permeable to water vapor for some amount this can also be dealt with coating of PE over the container. Not used for long term storage of products.

**VI. Polycarbonate:** Has an ability to be sterilized repeatedly. It has immense rigidity and is a possible replacement for glass, vials and syringes. It has qualities like high dimensional stability, high impact strength, resistance to strain, low water absorption, transparency, and resistance to heat and flame. Polycarbonates have impact strength five times greater than any other common packaging plastics.

**VII. Acrylic multipolymers (Nitrile Polymers):** These are polymers of acrylonitrile or methacrylonitrile monomers. These provide for packaging of those

products which are not packed in usual packages as they provide for high gas barrier, good chemical resistance, and good strength.

**VIII. Polyethylene terepthalate (PET):** Condensation polymer formed by reaction of terepthalic acid or dimethyl terepthalic acid with ethylene glycol. It has excellent strength and provides barrier for gas and aroma making it as a useful package for cosmetics, mouth washes and other products (Pareek, Khunteta, 2014).

## 1.23. Packaging of Carvedilol [Guha et al,2009;Tønnesen, H.H., 2001;Choudhary, 2015]

A stable solid oral pharmaceutical composition comprising carvedilol or a pharmaceutically acceptable salt thereof, which is packed using a suitable packaging material along with a desiccant. A process for manufacturing a stable solid oral dosage form containing carvedilol or a pharmaceutically acceptable salt thereof, which is packed in the packaging configuration comprising moisture permeation inhibitory packaging. A method of preparing a stable solid oral pharmaceutical dosage form, said method comprising, encasing a pharmaceutical dosage form comprising carvedilol or pharmaceutically acceptable salt thereof in a container comprising a desiccant. A pharmaceutical kit comprising a container impervious to moisture, wherein said container comprises a desiccant; and a solid oral pharmaceutical dosage form comprising carvedilol or a pharmaceutically acceptable salt thereof, wherein said pharmaceutical dosage form is encased in said container.(Guha et al,2009)

Exposure of a photosensitive drug like carvedilol, to irradiation can influence the stability of the formulation, leading to changes in the physicochemical properties of the product. The influence of excipients of frequently used stabilizers is often difficult to predict and, therefore, stability testing of the final preparation is important. The selection of a protective packaging must be based on knowledge about the wavelength causing the instability. Details on drug photoreactivity will also be helpful in order to minimize side-effects and/or optimize drug targeting by developing photoresponsive drug delivery

systems. This review focuses on practical problems related to formulation and stability testing of photolabile drugs. (Tønnesen, H.H., 2001).

These light sensitive APIs and pharmaceutical products should be protected from light during processing and distribution. Light sensitive products may be affected by sunlight or artificial light. Light induces the interaction between the molecules of the compound that leads to the formation of new compound known as impurity. Light has energy that can activate the molecules of drug. Photo degradation usually occurs due to absorption of short wavelength light between 500 and 300 nm. Visible blue, violet and ultraviolet light cause this degradation. For example ofloxacin remains 80% when exposed to direct light for a period of 240 hours.Some drugs are affected by light of a special wavelength that should be studied before developing the formulation and product should be protected from that light. Excipients added in formulation can also reduce the effect of light on a light sensitive drug. Formulation should be tested for photostability to determine the effect of light on formulated product and to develop the protective measures.

During manufacturing process these product should be protected from these light degradation. Lights having long wave length (more than 500 nm) are used in granulation, compression and packing areas. Brown colored light having wavelength between 500 nm and 800 nm is the best option for this purpose.

Tablets containing light sensitive products should be coated with colored film coating. It will protect the sensitive drug from degradation due to the light. These tablets should be packed in alu-alu packing or in amber colored blisters. Injections containing light sensitive drugs should be filled in amber colored vials or containers (Choudhary, 2015).

# Chapter 2

# Literature Review

In the year of 2003, high performance liquid chromatography method for the quantitation of carvedilol in human plasma was presented. The method was based on protein precipitation with methanol, concentration of the supernatant by evaporation and reversed-phase chromatography with fluorometric detection. The Precision of the experiment,expressed by relative standard deviation was less than 6% and inaccuracy does not exceed 3%. The acid was used for pharmacokinetic studies. ( Ptáçek, Macek, Klima, 2003).

In the year of 2005, ultraviolet (UV) spectrophotometric and nonaqueous volumetric methods are described for the determination of carvedilol in pharmaceutical formulations. Linearity, precision, and accuracy were evaluated according to the validation guidelines of the International Conference on Harmonization and the United States Pharmacopeia for both methods.The methods were applied to tablets and compounded capsules. Statistical analysis by analysis of variance showed no significant difference between the results obtained by the proposed methods.

In 2005, a reversed-phase high performance liquid chromatography (RP-HPLC) method has been developed for separation of carvedilol and its impurities from carvedilol tablets.Linearity,accuracy, precision, selectivity and robustness were validated and found to be satisfactory.Overall,the proposed method was found to be highly sensitive,suitable and accurate for quantitative determination of Carvedilol and its impurities in dosage forms and in raw materials.

In the year of 2006, the stability of carvedilol in extemporaneously prepared oral liquid was studied.Upon all the test results of this study, it indicated that carvedilol liquid was stable at room temperature and refrigerated for up to 8 weeks. The study findings also revealed that there was no microbial growth in either drug liquid after refrigerated storage period of 2 weeks. (Yamreudeewong, Dolence, Pahl, 2006).

In 2006, the new and rapid stability indicating ultraviolet spectroscopic methods were employed with high degree of precision and accuracy for the estimation of total drug content in two commercial tablet formulations of carvedilol drug.It was concluded that the developed

methods are accurate sensitive precise and reproducible. That can be applied directly for the estimation of drug content in pharmaceutical formulations. (Imran et al, 2006).

In the year of 2007, a simple, rapid and sensitive isocratic reversed-phase HPLC method with fluorescence detection using a monolithic column has been developed and validated for the determination of carvedilol in human plasma. The essay enables the measurement of carvedilol for therapeutic drug monitoring with a minimum quantification limit (LOQ). (Zarghi et al, 2007).

In 2007, a sensitive, selective, precise and stability-indicating,new high-performance liquid chromatographic method for the analysis of carvedilol both as a bulk drug and in formulations was developed and validated by RP-HPLC method. The absorbance was monitored with a UV detector and the linearity,reproducibility and recovery were found to be satisfactory.This method enables the simultaneous determination of carvedilol and its degradation products,as well as stability. (Stojanovic et al, 2007).

In the year of 2007, a simple, precise and sensitive high performance liquid chromatography procedure has been developed for determination of carvedilol in human plasma. The stability studies show that carvedilol in human plasma was stable during short time period for sample preparation and analysis. This method was used to assay the carvedilol in human plasma samples obtained from subjects who had been given an oral tablet of 12.5 mg carvedilol. (Rathod et al, 2007).

In 2008, the HPLC analysis of the unknown degradation product was performed by a newly, developed, specific and validated method also suitable for the quantitative determination of the known cardiovascular impurities and the other degradation products. By this study it was proved that, moisture and temperature affect the formation of unknown degradation product and its concentration in cardiovascular tablets. So appropriate modifications of the packaging of cardiovascular tablets can be made in order to reduce unknown degradation product's concentration down to the accepted levels, during the tablet's shelf life. (Galanopoulou, Rozou,Antoniadou, 2008).

2009 the Carvedilol drug was subjected to acid (1.0 N HCl), alkaline (1.0 N NaOH), and neutral hydrolytic conditions by refluxing at 90° C,as well as to oxidative decomposition,protected from light,at room temperature. The stress degradation samples were evaluated by LC and LC-MS method, that could separate the degradation products formed under various stress conditions. UV method ,weight variation, hardness ,disintegration time, friability, content and dissolution test were also performed. The drug was relatively stable under acidic, neutral and photolytic stress conditions, but showed instability under alkaline and oxidative conditions. (Lanzanova et al,2009).

In 2009, this study was done to establish a validated stability indicating LC method for essay of carvedilol and to study the degradation behaviour of the drug under different stress conditions. The drug was subjected to forced degradation and peaks of all the degradation products were well resolved from that of the pure drug, with significantly different retention times, which indicates the specificity and stability-indicating properties of the method. First order degradation kinetics of carvedilol were observed under acidic and alkaline conditions. The results of this study indicate the method can be successfully used for routine analysis of carvedilol in the bulk drug and in pharmaceutical dosage forms. (Rizwan et al, 2009).

In the year of 2009, simple a d sensitive methods for the determination of carvedilol are described here.The optimum reaction condition and other analytical parameters were evaluated.The statistical evaluations of the methods were examined by determining intra-day and inter-day precision. The methods were successfully applied to the assay of CAR in tablet formulations. The accuracy and reliability of the methods were further ascertained by parallel determination by a reference method and by calculating the Student's t-test and F-test values at 95% confidence level.

In 2010, two carvedilol aqueous solutions and one carvedilol aqueous suspension for paediatric oral use (1mg/ml) were studied to determine the stability by HPLC method. Carvedilol stayed stable in the acidic aqueous solution at the three different temperatures during the 56 days of the study. In the alkaline solution, carvedilol was stable durinh 56 days at 25℃,but only 28 days at 4 & 40℃.In the aqueous suspension, carvedilol was stable during

56 days at 4 & 25℃,but only 28 days at 40℃.All the formulations that were tested can be stored at 25℃ for at least 56 days. (Buontempo et al, 2010).

In the year of 2010, carvedilol is used for the treatment of congestive heart failure and hypertension. During the bulk synthesis of carvedilol, it has been observed six impurities. This work describes the synthesis and characterization of these impurities. (Rao et al, 2010).

In 2010, a method for the determination of carvedilol in human plasma was developed using a high-performance liq-uid chromatography with tandem mass spectrometer (HPLC-MS/MS). Plasma samples were deproteinized using acetonitrile and the supernatant was directly injected onto the HPLC column without any preparative steps.This method showed acceptable precision and accuracy, good recovery from the plasma matrix, and stability during the analytical procedures. When its application to the bio-equivalence test of two carvedilol 25 mg tablet formulations. No statistically signifi-cant difference was observed between the logarithmic transformed area under curve (AUC) and maximum plasma concentration (C max) values of the two formulations.These results suggested that the HPLC-MS/MS analysis method developed was suitable for the carvedilol analysis in human plasma.

In the year of 2010, in this study, new and rapid method indicating ultraviolet spectroscopic methods were developed and validated for the estimation of carvedilol in pure form and in their respective formulations. The adequate drug solubility and maximum assay sensitivity was found in methanol.The absorbance of carvedilol was measured at 241nm in the wavelength range of 200- 350 nm.The linear calibration range was found to be 50%-150%.This method was validated and applied to the determination of carvedilol in tablets. No interference was found from tablet excipients at the selected wavelength and analysis conditions.It was concluded that the developed methods are accurate, sensitive, precise, and reproducible.They can be applied directly for the estimation of drug content in pharmaceutical formulations.

In 2011, the aim of this work was to select an appropriate primary packaging and analysis of its influence on stability of carvedilol. The influence of different primary packaging materials

and doses radiation was investigated. The tablets were put in an opaque plastic container,red and white blister packs composed of polyvinyl chloride and aluminium foil. After radiation the content was estimated using a validated HPLC method. Tablets,packaged in different primary packaging materials were exposed at different doses of UV and VIS radiation. The study showed that the opaque plastic container provide better photo protection then red and white blister packs for solid oral dosage forms of carvedilol.

In the year of 2013, an ultra-performance liquid chromatography–tandem mass spectrometry (UPLC-MS/MS) method has been developed for the simultaneous determination of carvedilol and its pharmacologically active metabolite 4′-hydroxyphenyl carvedilol in human plasma using their deuterated internal standards (IS). The method was successfully applied to support a bioequivalence study of 12.5 mg carvedilol tablets in 34 healthy subjects.

In 2013, a simple, sensitive and precise reverse phase high performance liquid chromatographic method has been developed for the estimation of Carvedilol in pharmaceutical preparations. The method was found to specific for Carvedilol in presence of common excipients. Statistical analysis performed with proposed method proved it to be precise, accurate and reproducible. Hence it can be employed for routine analysis of Carvedilol both in bulk and commercial formulations.

In 2013, a novel,fast, sensitive and specific capillary electrophoresis technique coupled to a diode array detector has been developed for the separation and simultaneous determination of carvedilol. The validated capillary electrophoresis method was successfully applied to the analysis of commercial tablet dosage forms. Forced degradation studies were performed on bulk samples of the drug using thermal,photolytic,hydrolytic and oxidative stress conditions, and the stressed samples were analysed by the proposed method. Degradation products produced as a result of stress studies did not interfere with the determination of carvedilol. So,the essay could be considered stability indicating. ( Alzoman et al, 2013).

In the year of 2013, a simple and selective HPLC weight diode array detection stability-indicating method was developed for the simultaneous determination of the antihypertensive drugs carvedilol and hydrochlorothiazide in their combined formulation. Drugs were

subjected to stress conditions of acidic and alkaline hydrolysis,oxidation,photolysis and thermal degradation. The proposed method stability indicating by resolution of data from the forest degradation products. Moreover,specificity of the method was verified by resolution of drugs from more than 20 Pharmaceutical compounds of various medicinal categories. The proposed method made use of diode array detection as a tool for peak identity and purity confirmation. ( Belal et al, 2013).

In 2014, a liquid chromatography-tandem mass spectrometry method to quantify carvedilol enantiomers in human plasma was developed and validated as a measure of compliance in clinical research. Carvedilol enantiomers were extracted from human serum (0.5mL) via liquid-liquid extraction with methyl tert-butyl ether (2.5mL).Carvedilol enantiomers were quantified using a triple quadrupole mass spectrometer operated in multiple-reaction-monitoring mode using positive electrospray ionisation.The method was successfully applied to samples taken from research volunteers treated with carvedilol sustained-release tablet 18mg.

In the year of 2014, a capillary electrophoresis method was used for assay of some degradation products of carvedilol.The result of the study indicates that the proposed CE method could effectively separate carvedilol from its degradation products and can be employed as a stability indicating assay method.In addition, the presence of a nee unknown degradation product was discovered by this method.CE behavior of carvedilol in photo/force degradation conditions gave valuable information concerning the dissimilarities of their ionization.

In 2015,a simple,linear, rapid, precise and stability-indicating analytical method was developed for the estimation of related substances and degradants of carvedilol API and tablets. Carvedilol was exposed to acid, base, thermal, photolytic and oxidative stress conditions. The stressed samples were analyzed by the proposal method. The degradation of carvedilol was observed under oxidative hydrolysis, base hydrolysis, thermal and photolytic. The drug was found to be stable in all other stress conditions applied. Successful separation of the drug from organic impurities and degradation products formed under forced degradation was achieved. The developed HPLC method to determine the related substances

and assay of carvedilol can be used to evaluate the quality of regular production samples. (Rao, Madhavan, Prakash, 2015).

In the year of 2015, a novel gradient RP-HPLC method has been developed for quantitative determination of carvedilol and its four impurities and the degradant in pharmaceutical dosage forms. The drug product subjected to the stress conditions of acid,base,oxidative,hydrolytic thermal humidity and photolytic degradation. Carvedilol was found to degrade significantly under thermal stress condition.The degradation products were well resolved from carvedilol and its impurities.The peak purity test results confirmed that the Carvedilol peak was homogenous and pure in all stress samples and the mass balance was found to be more than 98%,thus proving the stability-indicating power of the method.

In 2016, thermal analytical behavior of carvedilol has been investigated using thermoanlytical techniques thermogravimetry, derivative thermogravimetry, different thermal analysis (DTA) and different scanning calorimetry (DSC). Evolved gas analysis was also performed using thermogravimetry coupled to infrared spectroscopy. Finally, a tentative mechanism for carvedilol thermal decomposition is proposed.

In the year of 2016, a study of ionic liquid based dispersive liquid-liquid microextraction with magnetic dispersive microsolid phase extraction was employed to determine carvedilol, an anti-arrhythmic drug, with fluorescence detection. The ionic liquid extract and was magnetically retrieved with unmodified nanoparticles. The method was used to determine the analyte in tablets,human plasma and urine with recoveries between 95. 04 and 106. 6%. ( Wu et al, 2016).

In 2016, the ICH Q1B guidance and additional clarifying manuscripts provide the essential information needed to conduct for the photostability testing for pharmaceutical drug products in the context of manufacturing packaging and storage. This study provides an approach for photostability testing for oral drug products. It ensures the safe and effective administration of photosensitive oral drug products by setting of practical experimental approaches. ( Allain et al, 2016).

In the year of 2016, this study describes that, carvedilol is a weak base that is substantially insoluble in water, acidic solutions and gastric and intestinal fluids; it is classified as a class || drug in the Biopharmaceutical Classical System. The solubility of carvedilol varies according to the solvent pH. This study aimed to evaluate and correlate the physicochemical and processability properties of carvedilol. In this study, it was determined that, tested samples presented the same polymorphic form, did not present good flowability, and presented different particle size distributions. (Alves, Prado, Rocha, 2016).

In 2017, electrospinning was used to produce carvedilol-loaded Soluplus polymer nanofibers using a systematic approach. Miscibility between drug and polymer was determined through calculation of the interaction parameter, and the difference between the total solubility parameters. A solubility map for Soluplus was obtained by examining different solvent systems, carrying out electrospinning, and characterizing the nanofibers formed. The use of miscibility analysis amd polymer solubility studies demonstrate great technological potential to tackle the challenge for inadequate dissolution of poorly water-soluble drugs.

So, after going through all these literatures based on carvedilol, I am doing the stability tests of carvedilol. And these stability tests are majorly the photosensitivity test of carvedilol and in some extent moisture sensitive test of carvedilol. In the photosensitivity test, I have chosen a drug named Dilgard, which is a carvedilol drug and is packed in transparent package.I am exposing my drug in room temperature, sunlight, 25 watt bulb and 40 watt bulb. After exposing the drug is tested it's potency whether it has been changed or not by UV- spectrophotometer.

# *Chapter 3*

# Materials and Method

## 3.1. Materials

### 3.1.1.Sample Collection

To observe the photolytic degradation of Carvedilol as well as to assess the coating efficiency, 500 tablets of Dilgard (Carvedilol 6.25mg) were collected from the local drug store of Dhaka as a sample. All the tablets were from the same batch (16010). Among them 200 tablets were kept light protected for control tests and the remaining 300 tablets were subjected to various lighting conditions over certain periods of time for conducting experiments to determine their potency.

### 3.1.2. Sample

**Table 3.1: Samples used in the experiment with their sources**

| Sample Name | Source (Supplier Name) | Batch No. |
|---|---|---|
| Dilgard 6.25 mg Tablets | General Pharmaceutical Ltd. | 16010 |


Figure 3.1 : Dilgard 6.25 mg tablets ( Carvedilol )

### 3.1.3. Reagents

**Table 3.2: Reagents Used in the Experiment Including Source**

| Reagent's Name | Source (Supplier's Name) |
|---|---|
| Concentrated $H_2SO_4$ (98% /36.8N) | Analar, United Kingdom |
| Distilled Water | Laboratory (East West University) |

### 3.1.4. Equipments &Instruments

**Table 3.3: Lists of Equipments Used for the Experiment**

| Serial No. | Equipments | Source (supplier name) | Origin |
|---|---|---|---|
| 1 | UV-Spectrophotometer | Shimadzu UV1800 | Japan |
| 2 | Distill Water Plant | Bibby Scientific | United Kingdom |
| 3 | Electronic Balance | Shimadzu AY220 | Japan |

### 3.1.5. Images of Instruments

Some of the important instruments those were used in different tests during research work.

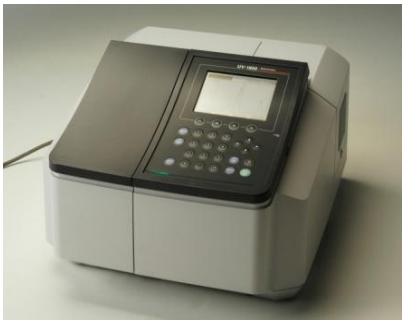

Figure 3.2: Shimadzu UV-1800 spectrophotometer and Electronic balance [Left to right]

### 3.1.6. Apparatus

Some technical equipment or machinery needed for a particular activity or research work. Apparatus may to machine, equipment and critical apparatus. Some apparatus are listed in the following table those were widely used throughout the experiments and research work.

**Table 3.4: Apparatus that were used throughout the experiments**

| Serial Number | Apparatus |
|---|---|
| 1 | Beaker |
| 2 | Test tube |
| 3 | Filter paper |
| 4 | Mortar and pestle |
| 5 | Funnel |
| 6 | Volumetric flask (250 ml, 1000 ml) |
| 7 | Pipette pumper |
| 8 | Pipette (10 ml, 2 ml) |
| 9 | Spatula |
| 10 | Lamp |
| 11 | Bulb (25 watt,40 watt) |
| 12 | Aluminium Foil paper |
| 13 | Test tube holder |
| 14 | Masking tap |
| 15 | Plastic dropper |
| 16 | Thermometer |
| 17 | Forceps |

## 3.2. Preparation of the solvent (0.1N $H_2SO_4$)

1. Lab solvent (H2SO4), stock solution with 98% (v/v) of strength was collected.

2. Then the concentration of the lab solvent stock solution was determined in normality where the specific gravity of solvent is1.84.

### 3.2.1. Determination of the Concentration of the Lab Solvent ($H_2SO_4$) in Normality(N):

100 ml of the lab solvent stock solution contains = 98ml of $H_2SO_4$

100 ml of lab solvent stock solution contains = (98 x 1.84) gm of $H_2SO_4$

= 180.32gm of $H_2SO_4$

1000 ml of stock solution contains = (180.32 x 1000)/100 gm of $H_2SO_4$

= 1803.2gm of $H_2SO_4$

= 1N of $H_2SO_4$

1000 ml of stock contain 1803.2gm of $H_2SO_4$ = (1803.2/49)N of $H_2SO_4$

= 36.8N of $H_2SO_4$

3. After the determination of the concentration of the lab solvent stock solution in Normality (N), the amount of lab solvent (36.8N $H_2SO_4$ C) stock solution required to make 1000ml of 0.1N HCL solvent was calculated as below.

### 3.2.2.Determination of the amount of 36.8N $H_2SO_4$ required to make 1000ml of 0.1N $H_2SO_4$ by using the $V_1S_1 = V_2S_2$

Where,

$S_1$ = Conc. of lab solvent ($H_2SO_4$) stock solution = 36.8 N

$S_2$ = Final concentration of the solvent ($H_2SO_4$) = 0.1N

$V_1$ = Volume of the lab solvent ($H_2SO_4$) stock solution =?

$V_2$ = Final volume of the solvent ($H_2SO_4$) = 1000ml

So that, $V_1 = (V_2S_2) / S_1$

or, $V_1$ = (1000ml x 0.1 N) / 36.8N

or, V1 = 2.717ml

(~ 2.72 ml of lab solvent $H_2SO_4$ stock solution)

4. Then 2.717 ml of 36.8N $H_2SO_4$ was transferred from the lab solvent stock solution to a 1000ml volumetric flask which was then filled with water up to mark to make 1000 ml of 0.1N $H_2SO_4$.

I. Standards of carvedilol was collected from General Pharmaceuticals Ltd. The potency of standard compounds was 99.70%.

II. The specific $\lambda_{max}$ for carvedilol, at which the absorbance would be measured, was determined to be 241 nm from the UV spectrometer by using the standard that was obtained from General Pharmaceuticals Ltd.

III. Nine serial concentrations of the standards of carvedilol were prepared for the purpose of creating a standard curve.

### 3.2.3. Preparation of the stock solution for Carvedilol using the standard obtained from General Pharmaceuticals Ltd:

- Equivalent weight of 6.25 mg of (Carvedilol) tablet was measured and then it was dissolved in 1000 ml of distilled water. By this procedure the concentration of the stock solution became 0.00625 mg/ml, which is the $1_{st}$ stock solution.

Concentration of 1st stock solution = amount of substance added / volume
= (6.25 / 1000) mg/ml
= 0.00625 mg/ml

- Then to prepare the $2_{nd}$ stock solution, equivalent weight of 6.25 mg of (Carvedilol) tablet was measured and then it was dissolved in 250 ml of 0.1N H2SO4 solvent. By this procedure the concentration of the stock solution became 0.025 mg/ml

Concentration of $2_{nd}$ stock solution = amount of substance added / volume
= (6.25 / 250) mg/ml
= 0.025 mg/ml

### 3.2.4. Preparation of nine serial concentrations of solution for Carvedilol:

a. Carvedilol had the concentration of its stock solution is 0.025 mg/ml.

b. Nine serial concentrations that were prepared for carvedilol were as follows 0.001 mg/ml, 0.002 mg/ml, 0.003 mg/ml, 0.004 mg/ml, 0.005 mg/ml, 0.006 mg/mi, 0.007 mg/ml, 0.008 mg/ml and 0.009 mg/ml for a final volume of 10 ml.

c. The amount of the solution that were required from the stock solution to prepare the above concentrations were calculated using $S_1V_1 = S_2V_2$ formula, where for the preparation of 0.001 mg/ml,

$S_1$, initial strength or concentration= 0.001

$S_2$, final strength or concentration = 0.025

$V_1$, initial volume = 10 mL

$V_2$, final volume = (0.001 x 10) / 0.025 = 0.4 mL

Thus the following concentrations were prepared as such for carvedilol as per the calculations provided below.

**Table 3.5: Concentration for Preparation of Standard Curve of Carvedilol**

| Sample name | Sample no. | Concentration |
|---|---|---|
| Carvedilol | 1 | 0.001 |
| | 2 | 0.002 |
| | 3 | 0.003 |
| | 4 | 0.004 |
| | 5 | 0.005 |
| | 6 | 0.006 |
| | 7 | 0.007 |
| | 8 | 0.008 |
| | 9 | 0.009 |

d. This 0.4 ml of stock solution was added with 9.6 ml of 0.1N $H_2SO_4$ solvent to obtain 10 ml of solution.

The same calculation was followed for the preparation of 0.002 mg/ml, 0.003 mg/ml, 0.004 mg/ml, 0.005 mg/ml, 0.006 mg/ml, 0.007 mg/ml**,** 0.008 mg/ml and 0.009 mg/ml.

For,

- 0.02 mg/ml, 0.8 ml of stock solution was added with 9.2 ml of solvent.
- 0.03 mg/ml, 1.2 ml of stock solution was added with 8.8 ml of solvent.
- 0.004 mg/ml, 1.6 ml of stock solution was added with 8.4 ml of solvent
- 0.005 mg/ml, 2.0 ml of stock solution was added with 8.0 ml of solvent.
- 0.006 mg/ml, 2.4ml of stock solution was added with 7.6 ml of solvent.
- 0.007 mg/ml, 2.8 ml of stock solution was added with 7.2 ml of solvent
- 0.008 mg/ml, 3.2 ml of stock solution was added with 6.8 ml of solvent.
- 0.009 mg/ml, 3.6 ml of stock solution was added with 6.4 ml of solvent.

e. Then the absorbance value was measured using a UV spectrophotometer against those nine serial concentrations for carvedilol.

f. A standard curves was plotted for carvedilol.

g. From this standard curve a straight line equation was obtained which was in the form of y = mx+c, where the components of the equations are described as provided below:

m = gradient value, y = absorbance values, x = concentrations and

c = y-intercept.

### 3.2.4. Sampling, Analysis by UV-Spectrophotometry & Determination of Potency of the pharmaceutical drugs (Carvedilol) under various lighting condition:

To determine the photo-stability of the drug (Carvedilol) in their packaging, the tablets were subjected to various types of light exposure, which were as follows:

1. Exposure under normal lighting conditions in the room
2. Under electric bulb exposure (25 watt & 40 watt)
3. Direct Sunlight exposure

**1. Exposure under Normal Lighting Condition**

1) The tablets (Dilgard) were kept under normal lighting condition in the room temperature in room for 4 months
2) They were sampled after specific intervals like periodically 14 for the determination of their physical properties (like thickness, hardness & weight variation) and their potency.
3) On the sampling day, a piece of white paper was taken and all the details (brand name of the tablets, date of the sampling etc.) were written on top of the paper.
4) Now, 15 tablets were taken out from those tablets, 5 tablets were kept on over that white paper. The tablets (Dilgard) were kept under normal lighting condition in the room for 4 months.
5) They were sampled after specific intervals like periodically after 14 days for determination
6) A photograph was taken of that paper showing the tablets with their appearances and those details.
7) Then from those 15 tablets, at first physical parameter was tested (weight variation test) and then these tablets will be used for potency determination. For determining the potency 5 tablets will be taken from those 15 tablets each time and the test will be done 3 times to check the reproducibility.
8) For potency determination, laboratory analysis was done by using UV spectroscopy technique:
    i. First, 5 tablets from those sampled tablets were taken.
    ii. Then the total weight of those 5 tablets was noted using an analytical balance and the average weight was calculated using the formula given below:

$$\textbf{Average weight of tablet} = \frac{\textbf{Total weight of the tablets}}{\textbf{Total number of tablets}}$$

a) Then the 5 tablets were crushed by using mortar and pestle.
b) Approximately the weight of 1 tablet of crushed tablet powder was taken and dissolved it in the solvent up to 250 ml. (0.1N H2SO4) for 3 times to prepare 3 samples.
c) After that that 250 ml solution was filtered and 2 ml solution was taken and dissolved with 8 ml of (0.1N $H_2SO_4$ ) solvent to make 10 ml solution in total.
d) Thus 10ml of each sample was prepared and kept into 3 different test-tube and wrapped it by foil paper. From test-tube the solution was poured into a cuvette and was inserted into the UV spectrophotometer to observe the absorbance value.
e) Then the absorbance value was plotted into the standard curve to obtain the total amount of the drug that is present in one tablet.
f) Steps 3 to 8 were repeated again on another sampling day.

## 2. Under electronic bulb exposure (25W & 40W)

I. 15 tablets were exposed to electric bulb lighting conditions for 5 hours at a stretch and 15 tablets were used as control.
II. After 5 hours, 15 tablets were collected and wrapped up with foil paper to prevent any further exposure to the lighting condition and the temperature was noted using a thermometer.
III. The foil papers should be labeled to identify the intervals.
IV. The tablets were then used for potency determination to see the effect of the exposure of bulb's lighting condition to drug ingredients.
V. For potency determination, laboratory analysis was done by using UV spectroscopy technique.

- First, 5 tablets from those sampled tablets were taken.
- Then the total weight of those 5 tablets was noted using an analytical balance and the average weight was calculated using the formula:

$$\text{Average weight (g)} = \frac{\text{Total weight of the tablets}}{\text{Total no. of tablets}}$$

- Then the 5 tablets were crushed by using mortar and pestle. Approximately the weight of 1 tablet of crushed tablet powder was taken and dissolved it in 250 ml of the solvent (0.1N H2SO4) for 3 times to prepare 3 samples.
- After that 2 ml of that filtered solution was taken and dissolved in 8ml of the solvent.
- Then 10ml of each sample was collected and kept into 3 different test-tube and wrapped it by foil paper.
- From test-tube the solution was poured into a cuvette and was inserted into the UV spectrophotometer to observe the absorbance value.

**Table 3.6: Electric Bulb (25W & 40W) Exposed Sample List**

| No. of Samples | Collected Sample | Withdrawal Intervals (Hrs) | Temperature (0C) | |
|---|---|---|---|---|
| | | | 25W | 40W |
| 15 (control) | 15 | 0 | 26 | 28 |
| 15 | 15 | 5 | 32 | 35 |

VI. Then the absorbance value was plotted into the standard curve to obtain the total amount of the drug that is present in one tablet.

VII. The 15 tablets, which were used as control and have not been exposed any of the lighting conditions.

[N.B: Same procedure (steps 1 to 8) were used to determine the potency of the tablets under both exposure of 25W and 40W lighting condition for three different days for 5 hours each.]

**3. Under Sunlight condition**

a) 15 tablets were exposed to sunlight condition for 5 hours at a stretch.

b) After 5 hours, 15 tablets were collected and wrapped up with foil paper to prevent any further exposure to the lighting condition and the temperature was noted using a thermometer.

c) The foil papers should be labeled to identify the intervals.

d) The tablets were then used for potency determination to see the effect of the exposure of sunlight condition to drug ingredients.

e) For potency determination, laboratory analysis was done by using UV spectroscopy technique:

i. First, 5 tablets from those sampled tablets were taken.

ii. Then the total weight of those 5 tablets was noted using an analytical balance and the average weight was calculated using the formula:

$$\text{Average weight (g)} = \frac{\text{Total weight of the tablets}}{\text{Total no. of tablets}}$$

iii. Then the 5 tablets were crushed by using mortar and pestle.

iv. Approximately the weight of 1 tablet of crushed tablet powder was taken and dissolved it in 250 ml of the solvent (0.1N H2SO4) for 3 times to prepare 3 samples.

v. After that 10 ml solution was filtered and 2 ml of that filtered solution was taken and dissolved in 10ml of the solvent.

vi. From then 10ml of each sample was collected and kept into 3 different test-tube and wrapped it by foil paper.

vii. From test-tube the solution was poured into a cuvette and was inserted

into the UV spectrophotometer to observe the absorbance value.

**Table 3.7: Sunlight Exposed Sample List**

| No. of Samples | Collected Sample | Withdrawal Intervals (Hrs) | Temperature (0C) |
|---|---|---|---|
| 15 (control) | 15 | 0 | 27 |
| 15 | 15 | 5 | 36 |

f) Then the absorbance value was plotted into the standard curve to obtain the total amount of the drug that is present in one tablet.

g) Tablets were used as control has not been exposed any of lighting conditions.

**4. Weight Variation Test**

**Procedure:**

I. 10 tablets were taken and average weight was calculated and it was considered as the standard weight of an individual tablet.

II. All the tablets were weighed individually and observed whether the individual tablets are within the range or not.

N.B: The variation from the average weight in the weights must not differ more than two tablets from the percentage listed below:

**Table 3.8: Accepted Percentage List for the Weight Variation Test of Tablets**

| Weight of tablet | Percentage difference |
|---|---|
| 130 mg or less | ±10% |
| More than 130 to 324 mg | ±5% |
| More than 324 mg | ±7.5% |

## Calculation

Following equation was used to determine % Weight Variation of tablets

$$\% \text{ Weight Variation} = (A \sim I/A) \times 100\ \%$$

Where,

I = Initial weight of tablet, in gram/grams (gm)

A = Average weight of tablet, in gram/grams (gm)

# Chapter 4

# Results

## 4.1 Standard Curve Preparation of Carvedilol ( Dilgard 6.25 mg )

- Nine serial concentration of the standards of carvedilol were prepared for the purpose of creating a standard curve.
- After preparing the desired concentrations, the spectrophotometer was turned on and 241nm wavelength was setup.
- The spectrophotometer was adjusted for 0 and 100% T(transmittance).
- The absorbance of the prepared solutions weremeasured.
- Then the absorbencies were plotted against concentrations and a straight line wasfound.

**Table 4.1: Concentration & Absorbance for Standard Curve of Carvedilol**

| Concentration(mg) | Absorbance (at 241nm) |
|---|---|
| 0.001 | 0.073 |
| 0.002 | 0.195 |
| 0.003 | 0.272 |
| 0.004 | 0.367 |
| 0.005 | 0.439 |
| 0.006 | 0.519 |
| 0.007 | 0.615 |
| 0.008 | 0.706 |
| 0.009 | 0.780 |

From this an equation was derivedwhere: This equation was used to determine the concentration of carvedilol from different samples absorbance that was found in several lighting conditions.

$y=86.65x+0.007$

$R^2=0.997$

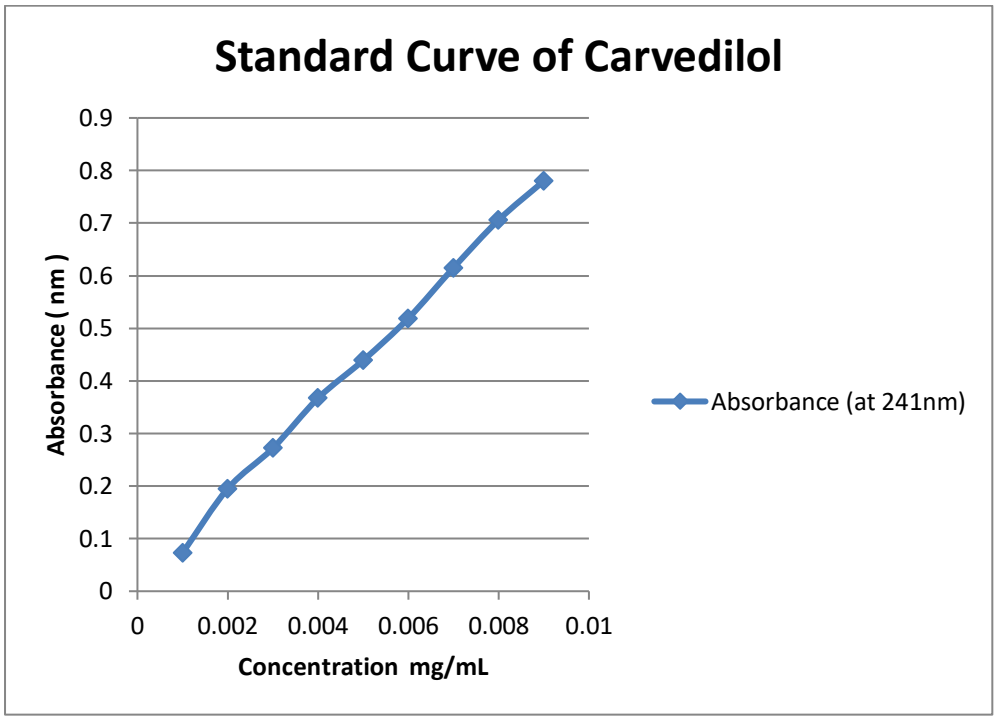


Figure 4.1: Plot showing straight line for absorbance with respect to concentration for Carvedilol

## 4.2. Physical Parameters of Room Light Exposed Samples

Eleven tablet strips containing 110 tablets were exposed to room light condition for 42 days.

Weight variation test of 5 tablets and potency determination test of 15 tablets were conducted of each day interval (0,14,28,42 days). In experimental day, a tablet strip containing 15 tablets was taken and 5 samples were collected for the test. Weight variation test was conducted and average weight was calculated for each day. Data of these tests are given below:

**Table 4.2: Weight Variation Test of Carvedilol( Dilgard)**

| Days | Average Weight for Particular Day, I(g) | Average Weight for 60 Days Intervals, A(g) | % Weight Variation, (A-I/A)×100 % |
|---|---|---|---|
| 0 | 0.1331 | 0.1325 | -0.453% |
| 14 | 0.1334 | | -0.679% |
| 28 | 0.1312 | | 0.981% |
| 42 | 0.1321 | | 0.302% |

## 4.3.Result from Potency Determination by UV-spectroscopy

### 4.3.1. Result from Sample that was exposed under Normal Lightening Condition

For this research purpose tablets were exposed to the normal room light and dispersed on top of the book shelf. Those samples were collected at specific intervals to determine its potency by UV-Spectroscopy. It has been observed that potency has been decreased after 60 days for the Dilgard 6.25 mg tablets.

The results are given below:

**Table 4.3: Concentration & Absorbance of 42 Days Interval for Carvedilol at room temperature**

| **Test Type** | **Exposure Interval (Days)** | **Potency After exposure %** | **Potency decrease %** | **Mean potency decrease of 42 days %** |
|---|---|---|---|---|
| Control | 0 | 100.00 | 0 | |
| Sample A1 | 14 | 100.00 | 0.0 | |
| Sample A2 | 14 | 100.00 | 0.0 | |
| Sample A3 | 14 | 100.00 | 0.0 | |
| Sample A1 | 28 | 100.00 | 0.0 | 4.91 |
| Sample A2 | 28 | 94.56 | 5.44 | |
| Sample A3 | 28 | 97.44 | 2.56 | |
| Sample A1 | 42 | 94.08 | 5.92 | |
| Sample A2 | 42 | 85.12 | 14.88 | |
| Sample A3 | 42 | 84.64 | 15.36 | |

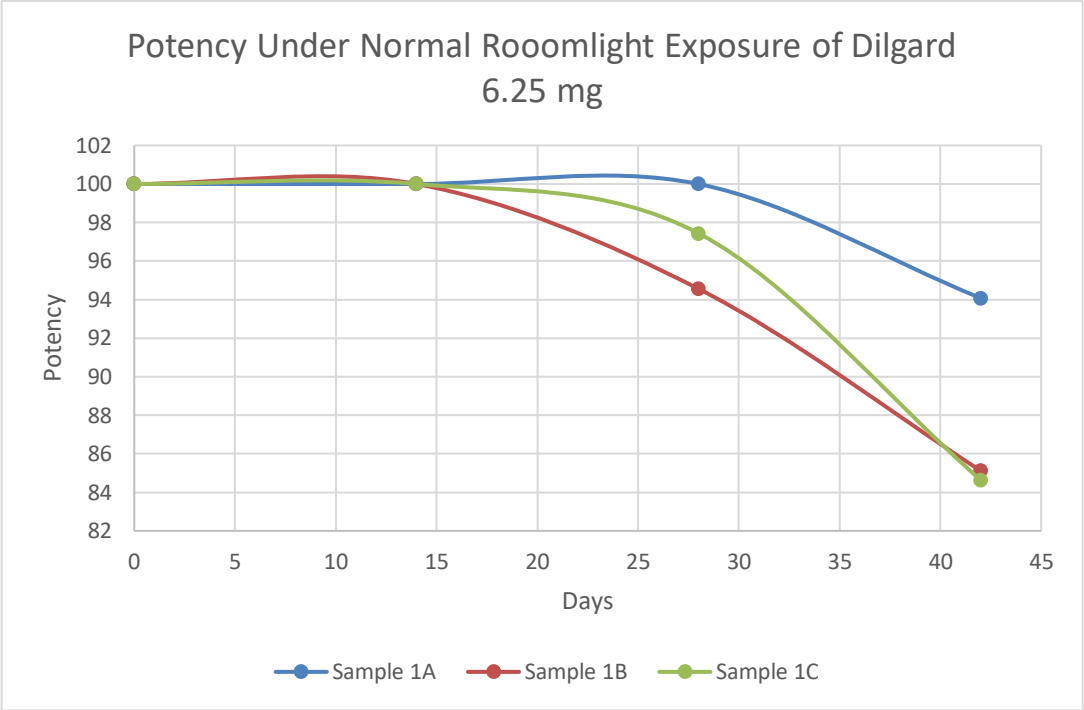


Figure 4.2: Potency Under Exposure of Dilgard at Normal Room Temperature

### 4.3.2.Result of samples that were exposed under 25W bulb

In experimental day, 30 tablets were taken from the tablet strips. 15 tablets have been used for control test and the rest 15 tablets have been used after 5 hours 25 watt bulb exposure. 5 tablets were collected for the test and observed 3 different absorbance of carvedilol for three samples exposed under the lamp (25W bulb); each for 5 hours' time interval and it was observed that the concentration of carvedilol was declined in each time interval. The results are given below:

**Table 4.4: Concentration & Absorbance of Carvedilol at 25 watt bulb exposure**

| Test type | Initial potency % (0 hr) | Potency After exposure % (5 hrs) | Potency decrease % | Mean potency decrease after exposure % |
|---|---|---|---|---|
| Sample 1A | 100.00 | 93.76 | 6.24 | |
| Sample 1B | 100.00 | 95.52 | 4.48 | |
| Sample 1C | 100.00 | 97.6 | 2.4 | |
| Sample 2A | 100.00 | 98.72 | 1.28 | |
| Sample 2B | 100.00 | 99.2 | 0.8 | 3.98 |
| Sample 2C | 100.00 | 92.96 | 7.04 | |
| Sample 3A | 100.00 | 98.72 | 1.28 | |
| Sample 3B | 100.00 | 90.88 | 9.12 | |
| Sample 3C | 100.00 | 96.8 | 3.2 | |

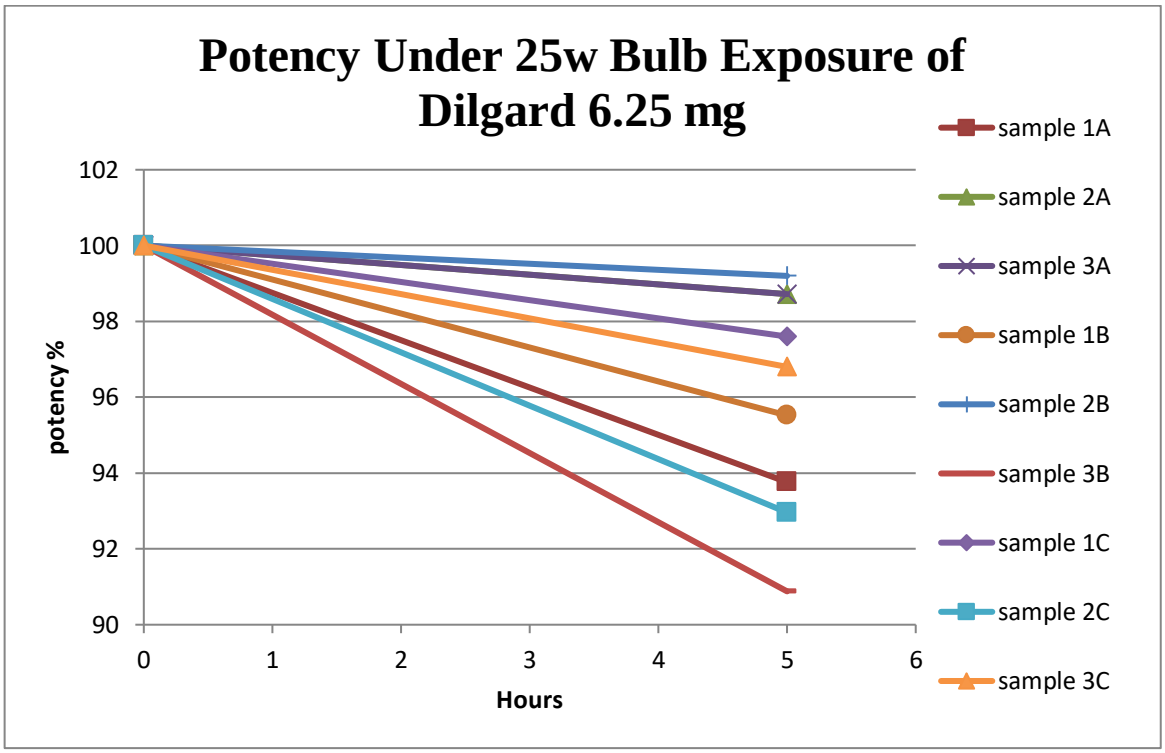


Figure 4.3: Potency Under 25 Watt Bulb of Dilgard

### 4.3.3 Result of samples that were exposed under 40Wbulb

In experimental day, 30 tablets were taken.15 tablets were used for control test and the rest 15 tablets were exposed to 40 watt bulb for 5 hours. 5 samples were collected for the test and observed 3 different absorbance of carvedilol for three samples exposed under the lamp (40W bulb); each for 5 hours' time interval and it was observed that the concentration of carvedilol was declined in each timeinterval.

The results are given below.

**Table 4.5: Concentration & Absorbance of Carvedilol at 40 watt bulb exposure**

| Test type | Initial potency % (0 hr) | Potency After exposure % (5 hrs) | Potency decrease % | Mean potency decrease after exposure % |
|---|---|---|---|---|
| Sample 1A | 100.00 | 92.0 | 8.0 | |
| Sample 1B | 100.00 | 98.24 | 1.76 | |
| Sample 1C | 100.00 | 99.04 | 0.06 | |
| Sample 2A | 100.00 | 98.9 | 1.1 | |
| Sample 2B | 100.00 | 98.2 | 1.8 | 2.80 |
| Sample 2C | 100.00 | 97.12 | 2.88 | |
| Sample 3A | 100.00 | 97.6 | 2.4 | |
| Sample 3B | 100.00 | 96.0 | 4.0 | |
| Sample 3C | 100.00 | 96.82 | 3.18 | |

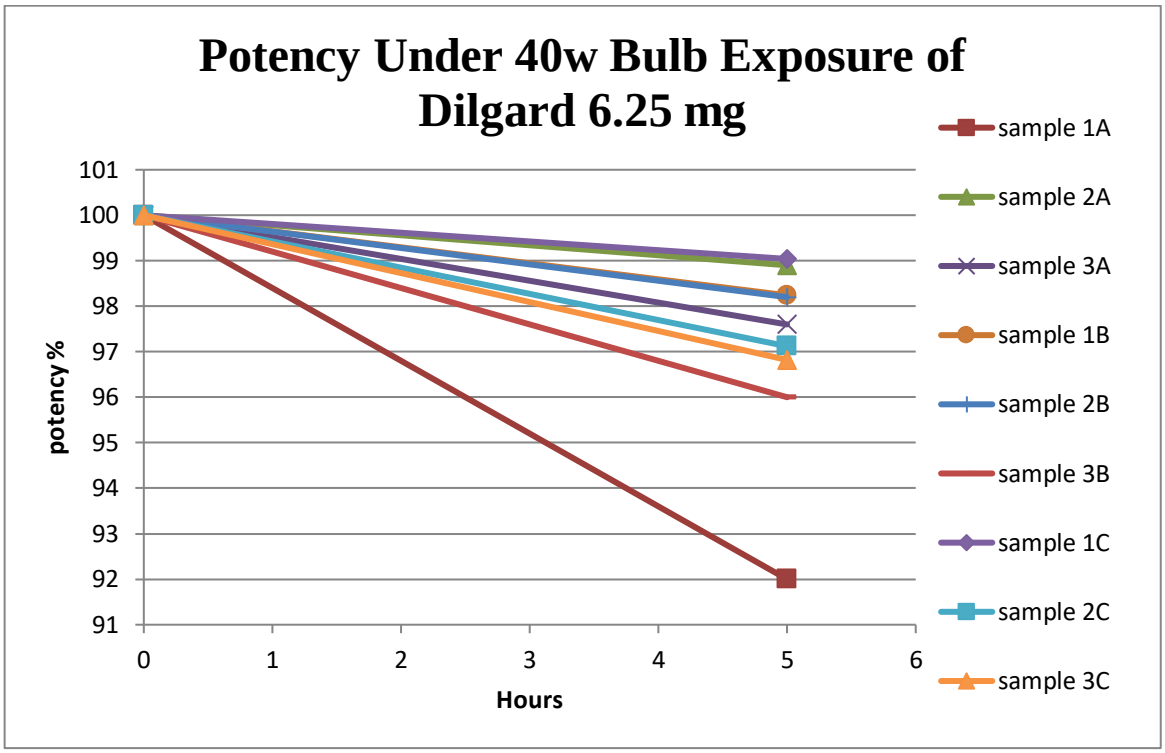


Figure 4.4: Potency Under 40 Watt Bulb of Dilgard

### 4.3.4.Result of samples that were exposed under direct sunlight

In experimental day, 30 tablets were taken.15 tablets were used for control test and the rest 15 tablets were used for the sunlight exposure testing. 5 samples were collected for the test and observed 3 different absorbance of Carvedilol for three samples exposed under the direct sunlight, each for 5 hours' time interval and it was observed that the concentration of Carvedilol was declined in each time interval.

The results are given below.

**Table 4.6: Concentration & Absorbance of Carvedilol at sunlight exposure**

| Test Type | Initial potency % (0 hr) | Potency After exposure % (5 hrs) | Potency decrease % | Mean potency decrease % |
|---|---|---|---|---|
| Sample 1A | 100.00 | 79.84 | 20.16 | |
| Sample 1B | 100.00 | 75.52 | 24.48 | |
| Sample 1C | 100.00 | 84.32 | 15.68 | |
| Sample 2A | 100.00 | 70.88 | 29.12 | |
| Sample 2B | 100.00 | 77.60 | 22.4 | 23.04 |
| Sample 2C | 100.00 | 73.12 | 26.88 | |
| Sample 3A | 100.00 | 73.92 | 26.08 | |
| Sample 3B | 100.00 | 78.08 | 21.92 | |
| Sample 3C | 100.00 | 79.36 | 20.64 | |

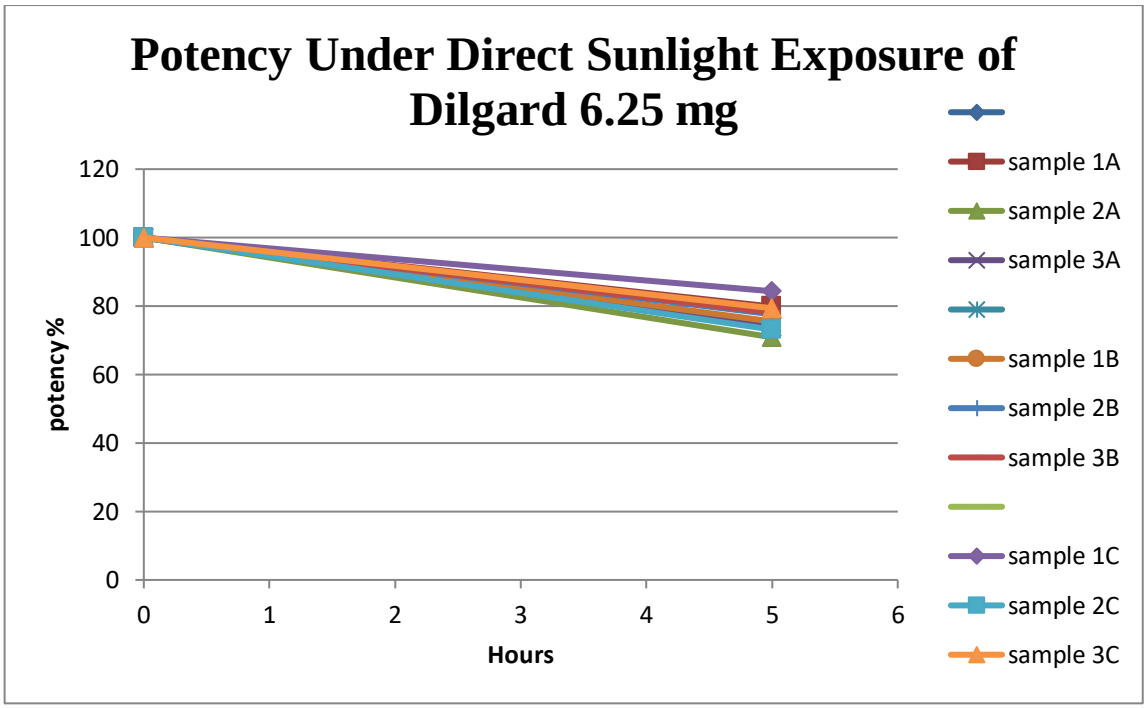


Figure 4.5 : Potency Under Direct Sunlight Exposure of Dilgard 6.25 mg

# Chapter 5
# Discussion

From different type of previous studies it has been claimed that carvedilol is a photosensitive drug. So the packaging of this drug should not be transparent. But still it is found in the market that many pharmaceuticals are using transparent packaging with different coating or without coating even. In this research, 500 carvedilol tablets were taken from same batch and exposed in different lighting condition to see that if there is any change in potency or not. This work was done by UV-Spectroscopy method. Different physical parameter tests were also done and in this case the fluctuation was insignificant. It was found that the concentration of carvedilol was decreased gradually in every ovation of light exposure. When sample tablets (Dilgard 6.25 mg) were kept under the electrical bulb (25 watt & 40 watt) and tested every 5 hour light exposed, it was found that the concentration of carvedilol was decreased gradually. Same results were found for direct sunlight exposed sample tablets and for the tablets which were kept on normal room light conditions, sunlight exposed tablets were degraded much. So that, in normal room light, 25watt bulb, 40watt bulb and direct sunlight the concentration of carvedilol were decreased gradually with percent deviation 4.91%, 3.98%, 2.80% and 23.04% respectively. From this research project it can be conclude with a decision that, there should be a change in the packaging system of the carvedilol. Coating is not sufficient to protect the drug and maintain its potency. Now in local market most of the available brand of this drug is packaged in plastic transparent blister strip. This package should be opaque thus the light cannot pass through the package.

# Chapter 6

# Conclusion

According to this experiment it was observed that the physical parameters like weight variation have passed the USP and BP specification. But there were remarkable changes in concentration/potency. The concentration of carvedilol was decreased gradually after exposure in electrical bulb light condition, direct sunlight and normal light exposure (room temperature) condition. So it can say that the Dilgard 6.25 mg tablets containing carvedilol is light sensitive and the concentration/potency is decreased after light exposure. It means coating alone is not sufficient to protect the drug from light. So that package should be opaque thus light cannot pass through the package.

# Chapter 7

# Reference

Allain, L., Baertschi, S.W., Clapham, D., Foti, C., Lantaff, W.M., Reed, R.A., Templeton, A.C. and Tønnesen, H.H., 2016. Implications of In-Use Photostability: Proposed Guidance for Photostability Testing and Labeling to Support the Administration of Photosensitive Pharmaceutical Products, Part 3. Oral Drug Products. *Journal of pharmaceutical sciences*, 105(5), pp.1586-1594.

Alves, J.M.V., Prado, L.D. and Rocha, H.V.A., 2016. Evaluation and correlation of the physicochemical properties of carvedilol. *Pharmaceutical development and technology*, 21(7), pp.856-866.

Alzoman, N.Z., Sultan, M.A., Maher, H.M., Al-Shehri, M.M. and Olah, I.V., 2013. Validated stability-indicating capillary electrophoresis method for the separation and determination of a fixed-dose combination of carvedilol and hydrochlorothiazide in tablets. *Journal of AOAC International*, 96(5), pp.951-959.

Aman, W. and Thoma, K., 2002. The influence of formulation and manufacturing process on the photostability of tablets. *International journal of pharmaceutics*, 243(1), pp.33-41.

Belal, T.S., Shaalan, R.A., El Yazbi, F.A. and Elonsy, S.M., 2013. Validated stability-indicating HPLC–DAD determination of the antihypertensive binary mixture of carvedilol and hydrochlorothiazide in tablet dosage forms. *Chromatographia*, 76(23-24), pp.1707-1720.

Brittain, H.G. ed., 2013. *Profiles of drug substances, excipients and related methodology* (Vol. 38). Academic Press.

Buontempo, F., Bernabeu, E., Glisoni, R.J., Quiroga, E., Bregni, C. and Chiappetta, D.A., 2010. Carvedilol stability in paediatric oral liquid formulations. *Farmacia Hospitalaria*, 34(6), pp.293-297.

Butler, A., Debroka, R., Moyer, H. and Yinger, R., Miles Lab, 1974. *Package for tablets*. U.S. Patent 3,826,358.

Cheng, J., Kamiya, K. and Kodama, I., 2001. Carvedilol: molecular and cellular basis for its multifaceted therapeutic potential. *Cardiovascular Therapeutics*, 19(2), pp.152-171.

Choudhary Ankur, (2015). 'Protection of Light Sensitive Products' [Online], Available at:http://www.pharmaguideline.com/2015/02/protection-of-light-sensitive-products.html?m=1 (Accesses: 13 May, 2017).

Dailymed, (2011). '*Carvedilol- carvedilol tablet,film coated'* [Online] Available at:https://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?setid=577b23c1-bd6d-41a4-a7c5-ebe7b92e37bd (Accessesed: 13 May, 2017).

Dulin, B. and Abraham, W.T., 2004. Pharmacology of carvedilol. *The American journal of cardiology*, 93(9), pp.3-6.

Dunn, C.J., Lea, A.P. and Wagstaff, A.J., 1997. *Carvedilol. Drugs*, 54(1), pp.161-185.

Galanopoulou, O., Rozou, S. and Antoniadou-Vyza, E., 2008. HPLC analysis, isolation and identification of a new degradation product in carvedilol tablets. *Journal of pharmaceutical and biomedical analysis*, 48(1), pp.70-77.

Gallo, R.C., Ferreira, A.P.G., Castro, R.E.A. and Cavalheiro, E.T.G., 2016. Studying the thermal decomposition of carvedilol by coupled TG-FTIR. *Journal of Thermal Analysis and Calorimetry*, 123(3), pp.2307-2312.

Guha, A., Metkar, B. and Avachat, M.K., 2009. *Stable pharmaceutical compositions of carvedilol*. U.S. Patent Application 12/921,261.

Hossain, M.A., Kundu, D.R., Sharmin, T., Maleque, M. and Chowdhury, S.R., 2013. A simple reversed phase High Performance Liquid Chromatography method development and validation for determination of Carvedilol in pharmaceutical dosage forms. *International Journal of Advances in Pharmaceutical Analysis*, 3(3), pp.68-71.

Ieggli, C.V.S., Cardoso, S.G. and Belle, L.P., 2005. Validation of UV spectrophotometric and nonaqueous titration methods for the determination of carvedilol in pharmaceutical formulations. *Journal of AOAC International*, 88(5), pp.1299-1303.

Imran, M., Singh, R.S.P. and Chandran, S., 2006. Stability indicating ultraviolet spectroscopic method for the estimation of ezetimibe and carvedilol. *Die Pharmazie-An International Journal of Pharmaceutical Sciences*, 61(9), pp.766-769.

Jiang, J., Tian, L., Huang, Y., Yan, Y. and Li, Y., 2014. Enantioselective and sensitive determination of carvedilol in human plasma using chiral stationary-phase column and

reverse-phase liquid chromatography with tandem mass spectrometry. *Journal of Chromatography B*, 960, pp.92-97.

Jouyban, A., Hasanzadeh, M. and Shadjou, N., 2014. Non-aqueous electromigration analysis of some degradation products of carvedilol. *Iranian Journal of Pharmaceutical Research*, 13(2), pp.471-486.

Kaljević, O., Djuris, J., Čalija, B., Lavrič, Z., Kristl, J. and Ibrić, S., 2017. Application of miscibility analysis and determination of Soluplus solubility map for development of carvedilol-loaded nanofibers. *International Journal of Pharmaceutics*.

Kim, S.H., Lee, S.H. and Lee, H.J., 2010. Rapid and sensitive carvedilol assay in human plasma using a high-performance liquid chromatography with mass/mass spectrometer detection employed for a bioequivalence study. *American Journal of Analytical Chemistry*, 1(03), p.135.

Krum, H., Sackner-Bernstein, J.D., Goldsmith, R.L., Kukin, M.L., Schwartz, B., Penn, J., Medina, N., Yushak, M., Horn, E., Katz, S.D. and Levin, H.R., 1995. *Double-blind, placebo-controlled study of the long-term efficacy of carvedilol in patients with severe chronic heart failure*. Circulation, 92(6), pp.1499-1506.

Lanzanova, F.A., Argenta, D., Arend, M.Z., Junior, L.B. and Cardoso, S.G., 2009. LC and LC-MS evaluation of stress degradation behavior of carvedilol. *Journal of Liquid Chromatography & Related Technologies*, 32(4), pp.526-543.

Patel, D.P., Sharma, P., Sanyal, M., Singhal, P. and Shrivastav, P.S., 2013. UPLC-MS/MS assay for the simultaneous quantification of carvedilol and its active metabolite 4′-hydroxyphenyl carvedilol in human plasma to support a bioequivalence study in healthy volunteers. *Biomedical Chromatography*, 27(8), pp.974-986.

Pareek, V.I.K.A.S. and Khunteta, A., 2014. Pharmaceutical packaging: current trends and future. *International journal of pharmacy and pharmaceutical Sciences*, 6(6), pp.480-485.

Pradhan, K.K., Rao K, R. and Srinivasulu, P., 2011. Analytical Method Development and Stability Studies of Carvedilol.

Ptáček, P., Macek, J. and Klıma, J., 2003. Liquid chromatographic determination of carvedilol in human plasma. *Journal of Chromatography B*, 789(2), pp.405-410.

Rao L.S.S., Madhavan P. and Prakash V.K., 2015. Development and validation of stability indicating method for the quantitative determination of carvedilol and its related impurities in pharmaceutical dosage forms using RP HPLC. *Journal of Chemical and Pharmaceutical Research*, 2015, 7(9):144-154.

Rao, S.N., Sitaramaiah, D., Srimannarayana, K., Rao, C.N., Rao, P.S. and Babu, K.S., 2010. Synthesis and Characterization of Potential Impurities of Carvedilol, *An Antihypertensive Drug. Synthetic Communications*, 41(1), pp.85-93.

Raju, T.R., 2015. Development and Validation of Stability-Indicating Impurity Profiling Method for the Carvedilol in API and Pharmaceutical Formulation. *International Journal of Pharmacology*, 2(6), pp.22-31.

Rathod, R., Prasad, L.P.C., Rani, S., Nivsarkar, M. and Padh, H., 2007. Estimation of carvedilol in human plasma by using HPLC-fluorescence detector and its application to pharmacokinetic study. *Journal of Chromatography B*, 857(2), pp.219-223.

Rizwan, M., Aqil, M., Azeem, A., Sultana, Y., Talegaonkar, S. and Ali, A., 2009. Study of the degradation kinetics of carvedilol by use of a validated stability-indicating LC method. *Chromatographia*, 70(7-8), p.1283.

Savic, I., Marinkovic, V., Savic, I. and Sibinovic, P., 2011. Study of the influence of primary packaging on photostability of tablets containing carvedilol. *Indian Journal of Pharmaceutical Education and Research*, 45(4), pp.353-359.

Sreevidya, T.V. and Narayana, B., 2009. Simple and facile methods for the determination of carvedilol in pharmaceuticals.

Stojanović, J., Marinković, V., Vladimirov, S., Veličković, D. and Sibinović, P., 2005. Determination of carvedilol and its impurities in pharmaceuticals. *Chromatographia*, 62(9-10), pp.539-542.

Stojanović, J., Vladimirov, S., Marinković, V., Veličković, D. and Sibinović, P., 2007. Monitoring of the photochemical stability of carvedilol and its degradation products by the RP-HPLC method. *Journal of the Serbian Chemical Society*, 72(1), pp.37-44.

Theivarasu, C., Ghosh, S. and Indumathi, T., 2010. UV spectrophotometric determination of carvedilol in pharmaceutical formulations. *Asian Journal of Pharmaceutical and Clinical Research*, 3(4), pp.64-68.

Tønnesen, H.H., 2001. Formulation and stability testing of photolabile drugs. *International Journal of pharmaceutics*, 225(1), pp.1-14.

Watanabe, K., Ohta, Y., Nakazawa, M., Higuchi, H., Hasegawa, G., Naito, M., Fuse, K., Ito, M., Hirono, S., Tanabe, N. and Hanawa, H., 2000. Low dose carvedilol inhibits progression of heart failure in rats with dilated cardiomyopathy. *British journal of pharmacology*, 130(7), pp.1489-1495.

Wu, H., Zhang, L., Gao, N., Li, Y., Wang, H., Liu, Y., Tian, L. and Du, L.M., 2016. Magnetic retrieval of ionic liquids: high sensitivity fluorescence determination of carvedilol in tablets, plasma, and urine. *Analytical Letters*, 49(2), pp.319-330.

Yamreudeewong, W., Dolence and Pahl, D., 2006. Stability of two extemporaneously prepared oral metoprolol and carvedilol liquids. *Hospital Pharmacy*, 41(3), pp.254-259.

Zarghi, A., Foroutan, S.M., Shafaati, A. and Khoddam, A., 2007. Quantification of carvedilol in human plasma by liquid chromatography using fluorescence detection: application in pharmacokinetic studies. *Journal of pharmaceutical and biomedical analysis*, 44(1), pp.250-253.