EAST WEST UNIVERSITY

Photo Degradation Study of Cavelon® (Carvedilol) Using UV-Spectroscopy

A dissertation submitted to the Department of Pharmacy, East West University, in partial fulfillment of the requirement for the degree of bachelor of pharmacy.

Submitted by

Aziza Mohammadi

ID: 2014-1-70-024

Department of Pharmacy

East West University

Submitted to

Md. Anisur Rahman

Assistant Professor

Department of Pharmacy

East West University

Declaration by the Research Candidate

I, Aziza Mohammadi, hereby declare that the dissertation, entitled "**Photo Degradation Study of Cavelon® (Carvedilol) using UV-spectroscopy**" submitted by me to the Department of Pharmacy, East West University and in the partial fulfillment of the requirement for the award of the degree Bachelor of Pharmacy, under the supervision and guidance of **Md. Anisur Rahman**, Assistant Professor, Department of Pharmacy, East West University, Dhaka.

.....

AZIZA MOHAMMADI

ID: 2014-1-70-024

Department of Pharmacy

East West University

Certificate by the Supervisor

This is to certify that the thesis entitled "**Photo Degradation Study of Cavelon® (Carvedilol) using UV-spectroscopy**", submitted to the Department of Pharmacy, East west University for the partial fulfillment of the requirement for the award of the degree Bachelor of Pharmacy, is a original record and genuine research work carried out by **Aziza Mohammadi**, **ID: 2014-1-70-024** in 2017 of her research project in the Department of Pharmacy, East West University, under my supervision and guidance.

.....

MD. ANISUR RAHMAN

Assistant Professor

Department of Pharmacy

East West University

Certified by the Chairperson

This is to certify that the thesis entitled "**Photo Degradation Study of Cavelon® (Carvedilol) using UV-spectroscopy**", submitted to the Department of Pharmacy, East West University for the partial fulfillment of the requirement for the award of the degree Bachelor of Pharmacy, and is an original record and genuine research work carried out by **Aziza Mohammadi**, **ID: 2014-1-70-024** in 2017.

.....

DR. CHOWDHURY FAIZ HOSSAIN

Professor and Chairperson

Department of Pharmacy

East West University

Acknowledgement

At first, I would like to thank **Almighty Allah** for enabling me to successfully completing my research work soundly and orderly.

I would like to express my deepest gratitude to my supervisor, **Md. Anisur Rahman**, Assistant Professor, Department of Pharmacy, East West University, for his cordial support throughout this research project. His generous advice, constant supervision, intense support and enthusiastic encouragements during the research work helped me to shape this research in a better way. His motivation, support, patience and advices have made it possible for me to complete this research.

I put forward my sincere regards and profound gratitude to Chairperson, **Dr. Chowdhury Faiz Hossain**, Professor, Department of Pharmacy, East West University, for his inspiration in my study.

I express my sincere thankfulness to my parents and my sisters for their support and guidance all through my life, including that for my research project. I wish to thank my fellow researchers namely **Abida Islam Pranty, Raad Shafiq Abir** and **Md. Wazi Ullah** for their endless cooperation and inspiration for preparing this work.

I want to give special thanks to my dear friends **Shaila Nasrin**, **ABM Asikur Rahman, Iqbal Hossain Noyon** and **Md. Delowar Hossain** for their support and guidance throughout this research project. I would also like to thank the assigned graduate teaching assistant of my supervisor **Arif Mahbub Sufal** for his guidance and help throughout the research project.

I would like to thank our lab instructor **Sujit Kumer** for his support in this project and also all other lab instructors, **Ruchira Chakraborty**, **Shipra Sarkar** and **Razia Sultana** for their help and support throughout this research project. Again the help from the stuffs of Pharmacy Department cannot be neglected. My sincere thanks goes to each and every person who has contributed to complete this research work successfully.

Dedication

This research work is dedicated to my beloved parents, who are my biggest inspiration.

Table of Content

List of Tables

List of Figures

Abstract

The aim of this study is to determine the photolytic degradation of Carvedilol tartrate. The objective of the study is to determine the photolytic degradation of Cavelon® (Carvedilol) which contained in a blister packing with coating to evaluate the coating is efficient or not.. In this research, photosensitivity of carvedilol is determined in various lighting conditions (control, sunlight, normal light, 25W bulb, and 40W bulb condition). For this study UV photo spectroscope was used. Besides this, physical tests were performed for evaluation of color change, weight variation, thickness and hardness of Cavelon® tablets from same batch according to the specification of USP. A very insignificant fluctuation in result was observed, with average weight 0.0843g, standard deviation ±0.0004, ±0.05 & ±0.012 for weight variation, hardness & thickness test respectively. But in various lighting condition like 25watt bulb, 40watt bulb, and normal room light the potency of carvedilol tartrate were not decreased. But under the direct sunlight the concentration of carvedilol tartrate was decreased percent deviation 1.55%. So it can be said that the Cavelon® containing carvedilol is light sensitive and coating alone is not sufficient to protect the drug from all lights. So that package should be opaque thus no light can pass through the package.

Keywords: Carvedilol Tartrate, Photolytic Degradations, UV-spectroscopy, Weight variation, Hardness, Thickness, Potency, USP.

Chapter One

INTRODUCTION

1.1 Objective

Previously, there were number of studies that proved that Carvedilol is a photosensitive drug. But still there are some manufacturers using blister packaging for carvedilol instead of opaque packaging. The objective of the study is to determine the photolytic degradation of Cavelon® (Carvedilol) which contained in a blister packing with coating from same batch according to the specification of USP. So Cavelon® having the transparent packaging was tested for photosensitivity to evaluate coating efficiency.

In this research, Cavelon® (Carvedilol) from Drug International was taken which contained in a blister packing with coating from same batch according to the specification of USP. The strength of Cavelon® was 6.25 mg. The impact of various lighting conditions (control, sunlight, normal light, 25W bulb, and 40W bulb condition) in these tablets was observed. Then the absorbance of the sample was measured in the UV spectrophotometer at a wavelength of 241 nm and the percent of potency decrease was finally calculated.

1.2 Stability

Drug stability means the ability of the pharmaceutical dosage form to maintain the physical, chemical, therapeutic and microbial properties during the time of storage and usage by the patient. It is measured by the rate of changes that take place in the pharmaceutical dosage forms.

There are some factors that affect drug stability including temperature, conditions, light, microbes, packaging material, transportation, components of drug composition and the nature of the active ingredient. [Unit 4 drug]

1.2.1 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 for 14 days), irradiation with UV-visible light (1.3×10^6 lux h), and direct exposure to heat/high humidity (40°C and 75% RH

for 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%. [Dunn, Lea, and Wagstaff, 1997]

1.2.2 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. [Brittain, 2013]

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]

1.2.3 Factors affecting drug stability [Brittain, 2013]

1. **Temperature:**

High temperature accelerate oxidation, reduction and hydrolysis reaction which lead to drug degradation

2. **pH:**

- Acidic and alkaline pH influences the rate of decomposition of most drugs.
- Many drugs are stable between pH 4 and 8.
- Weekly acidic and basic drugs show good solubility when they are ionized and they also decompose faster when they are ionized.

So if the pH of a drug solution has to be adjusted to improve solubility and the resultant pH leads to instability then a way out of this tricky problem is to introduce a water miscible solvent into the product. It will increase stability by:

- suppressing ionization
- reducing the extreme pH required to achieve solubility
- enhancing solubility and
- reducing the water activity by reducing the polarity of the solvent

- Reactions catalyzed by pH are monitored by measuring degradation rates against pH, keeping temperature, ionic strength and solvent concentration constant. Some buffers such as acetate, citrate, lactate, phosphate and ascorbate buffers are utilized to prevent drastic change in pH.

- Sometimes pH can have a very serious effect on decomposition. As little as 1 pH unit change in pH can cause a change of ten fold in rate constant. So when we are formulating a drug into a solution we should carefully prepare a pH – decomposition profile and then formulate the solution at a pH which is acceptable physiologically and stability-wise also.

3. **Moisture:**

   a. Water catalyses chemical reactions as oxidation, hydrolysis and reduction reaction

   b. Water promotes microbial growth

4. **Light:**

Light affects drug stability through its energy or thermal effect which leads to oxidation

5. **Pharmaceutical dosage forms:**

Solid dosage forms are more stable than liquid dosage forms for presence of water.

6. **Concentration:**

Rate of drug degradation is constant for the solutions of the same drug with different concentration. So, ratio of degraded part to total amount of drug in diluted solution is bigger than of concentrated solution.

> **Stock solutions**: are concentrated solutions which diluted by using (i.e. syrup 85%) at high concentration the stability is high

7. **Drug incompatibility:**

Reactions between components of pharmaceutical dosage forms itself or between these components and cover of the container.

8. **Oxygen:**

Exposure of drug formulations to oxygen affects their stability.

1.2.4 Objectives of the Photostability Studies [Ahmad et al., 2016]

In view of the photosensitivity and photoinstability of drugs and excipients, knowledge of the photostability of these substances and their formulated products is necessary to evaluate the following:

- The intrinsic photostability characteristics.
- The physical and chemical changes upon the exposure to light.
- The photodegradation pathways and mechanisms.
- The shelf life of the products.
- The efficacy of the stabilizing agents in photostabilization.

- The need for modification of the formulation parameters.
- The need for the measures to overcome the effects of the light exposure during manufacturing, packaging, labeling, transportation, and storage.
- The light-induced biological effects.
- The primary and secondary package design.

1.2.5 Photolytic Degradation

Photolysis is the chemical decomposition caused by light. The materials those are sensitive to light, if exposed in light they might change and form another compound which are degraded form of the previous material. A large number of drugs are sensitive to light and therefore their formulated products may degrade during manufacturing, storage, and administration. [Moore, 2004]

1.3 Packaging Requirements

The light sensitivity of the drug substances requires the use of an effective packaging system to protect them from photochemical damage. The pharmacopoeias prescribed conditions for containers (e.g., light-resistant) and storage (e.g., protected from light) for the light-sensitive drugs and formulated products. The requirements for a packaging system for pharmaceuticals differ from product to product, that is, solid or liquid dosage form. There is a greater chance of interaction between a liquid dosage form and the container than that of the solid dosage form. The efficacy of a packaging system for a particular drug or product may be evaluated by performing photostability studies. Protection of a product from light can be achieved by the use of an opaque or amber-colored container. Amber glass is suitable for drugs absorbing in the UV region as it transmits light above about 470 nm. An opaque secondary package may also be used for this purpose. [Ashley, 1939] [Ahmad et al., 2016]

Light transmission tests may be applied to evaluate the light transmission characteristics of containers to be used for photosensitive drugs and the implications of photostability on the

manufacturing, packaging, and storage of formulated products, emphasizing the need for appropriate measures to protect photosensitive products during these processes. [Ashley, 1939]

1.3.1 Packaging for tablets

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, the tablets 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 filing 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 including in a loose condition with the tablets, it has been the practice to have it in a form which is substantially different there from. 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]

1.3.2 Different type of packaging [Butler et al., 1974]

1.3.2.1 Generally there are three types of packaging

- **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.

- **Secondary Packaging**

This is consecutive covering or package which stores pharmaceuticals packages in it for their grouping. e.g. Cartons, boxes, etc.

- **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.

1.3.2.1.1 Glass Containers

Four types of Glasses are being used in pharmaceutical industry:

a. **Type I-Borosilicate glass**

These are 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.

b. **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.

c. **Type III- Regular soda lime glass**

Untreated soda lime glass with average chemical resistance.

d. **Type IV- General Purpose soda lime glass**

Glass is not used for parenterals, used only for products intended to be used orally or topically.

1.3.2.1.2 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:

a. **Polyethylene (PE)**

It 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,

i. Stiffness,

ii. Moisture vapor transmission,

iii. Stress cracking and

iv. Clarity or translucency based on polymer density used

b. 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.

c. 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.

d. 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.

e. 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.

f. 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.

g. 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.

h. 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, 2014]

1.4 Spectrophotometric methods

The term spectrophotometry has been defined as the measurement of relative Radiant energy as a function of wavelength and the definition was given by a community of optical Society of America. The energy may come directly from an emitting source or may be transmitted, absorbed or reflected by absorbing materials. Its advantages lie in the elimination of comparison solutions, the direct calibration of instruments by a few simple measurements. [Ashley, 1939]

Spectrophotometric method is a large group of analytical methods that are based on atomic and molecular spectroscopy. Absorption promotes these particles from their normal ground state, to one or higher exited states. The absorption of light by analytes occurs by raising an electron or electrons to a higher level. Every functional group in a molecule is characterized

by the absorption of light in a definite region of the spectra and this property is used for the identification of the substances in a drug. [Pradhan, Rao, and Srinivasulu, 2011]

1.4.1 Important applications of spectrophotometer: [Pradhan, Rao, and Srinivasulu, 2011]

- Identification of many types of organic, inorganic molecules and ions.
- Quantitative determination of many biological, organic and inorganic species.
- Quantitative determination of mixtures of analytes.
- Monitoring and identification of chromatographic effluents.
- Determination of equilibrium constants.
- Determination of stoichiometry and chemical reactions.
- Monitoring of environmental and industrial process.
- Monitoring of reaction rates.

1.4.2 Ultra Violet Spectroscopy [Soderberg, 2016]

In a UV spectrophotometer the radiation of specific wavelength is passed through the sample of interest. A detector is placed that detects the light that absorbed at a specific wavelength and measures the extent of absorption.

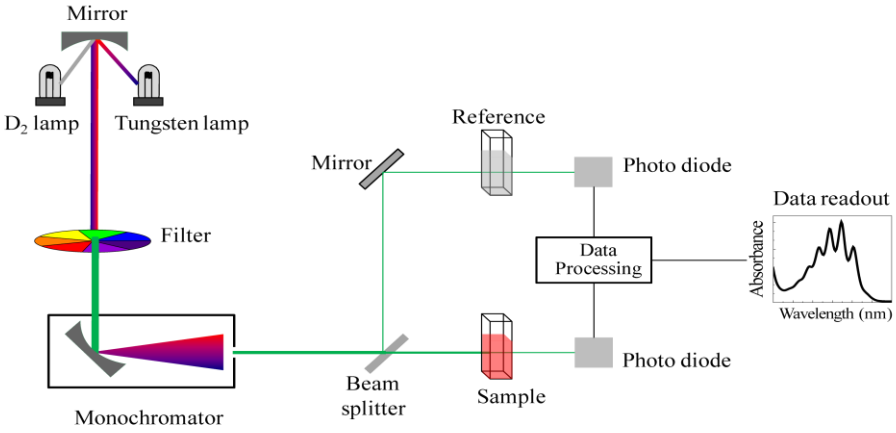

Figure 1.1: Schematic diagram for UV spectroscopy [Soderberg, 2016]

In this experiment the ultraviolet absorption of drug samples were measured by using a double beam T90+ UV/VIS spectrometer that is controlled by a computer having a specific software named 'UVWIN spectrophotometer', version 5.2.0 over a 10 mm path length. Quartz cuvettes were used as the sample holder.

1.4.3 Beer-Lambert law [Beer-Lambert Law (online)] [B.L C.LibreTexts]

The Beer-Lambert law states that, the absorbance of a solution is directly proportional to the concentration of the absorbing species in the solution and the path length. Thus, for a fixed path length, UV-spectroscopy can be used to determine the concentration of the absorber in a solution.

The Beer-Lambert law (or Beer's law) is the linear relationship between absorbance and concentration of an absorbing species.

The general Beer-Lambert law is usually written as:

A = abc

Where,

A = the measured absorbance,

a = a wavelength-dependent absorptivity coefficient,

b = the path length, and

c = the analyte concentration.

1.4.3.1 Limitations of the Beer-Lambert law [Beer-Lambert Law (online)]

The linearity of the Beer-Lambert law is limited by chemical and instrumental factors. Causes of nonlinearity include:

- deviations in absorptivity coefficients at high concentrations (>0.01M) due to electrostatic interactions between molecules in close proximity
- scattering of light due to particulates in the sample
- fluoresecence or phosphorescence of the sample
- changes in refractive index at high analyte concentration
- shifts in chemical equilibrium as a function of concentration
- non-monochromatic radiation, deviations can be minimized by using a relatively flat part of the absorption spectrum such as the maximum of an absorption band
- stray light

1.5 Effect of Formulation and Manufacturing process

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. 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 affects the photo stability. Depending on the formulation drug losses varied between 30 and 55% after 12h 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]

1.6 Carvedilol

Carvedilol is a third-generation, neurohormonal antagonist with multiple activities. It belongs to a group of medicines called non-selective beta-blockers and works by slowing down the activity of the heart. Lowering high blood pressure helps to prevent strokes, heart attacks, and kidney problems. This drug works by blocking α1 and both β-adrenergic receptors, which inhibits the action of certain natural substances in our body, such as epinephrine, on the heart and blood vessels. It stops the heart from receiving messages sent by CNS (Central Nervous System). As a result, the heart beats more slowly and with less force. The pressure of blood within the blood vessels is reduced and it is easier for the heart to pump blood around the body. This effect lowers heart rate, blood pressure, and strain on heart. Because the heart is using less energy, it also helps to reduce chest pain. [Bristow et al., 1992] [Hamed et al., 2016]

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 Ca^{2+}). 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, Kamiya, and Kodama, 2001].

Carvedilol is also used to prevent further worsening of congestive heart failure. It is also used to treat left ventricular dysfunction after a heart attack. Left ventricular dysfunction occurs when the left ventricle (the main pumping chamber of the heart) stiffens and enlarges and can cause the lungs to fill with blood. [Rao, Madhavan and Prakash, 2015]

1.6.1 Cavelon®

Carvedilol is reported as an ingredient of Cavelon® in Bangladesh.

1.6.2 Chemical Properties: [O'Neil, 2013]

- Chemical Name: 1 (±)-1-(carbazol-4-yloxy)3-[[2-(o-metoxyphenoxy)etyl]amino]-2-propanol (WHO)
- Molecular Formula: $C_{24}H_{26}N_2O_4$
- Molecular weight: 406.482 g/mol

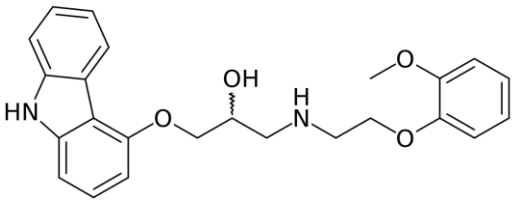

Figure 1.2: Molecular Structure of Carvedilol [Hamed et al., 2016]

1.6.3 Physical Properties: [O'Neil, 2013] [Wisler et al., 2007]

- **Color:** Colorless Crystal
- **State:** Solid
- **Description:**
  - **Melting Point:** 114.5^{o} C
  - **Odor:** Odorless
  - **Ionization Constant:** pKa = 7.8
  - **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 exhibits 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.

- **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).

Hygroscopicity:

Analysis of Carvedilol by Dynamic Vapor Sorption confirmed that this 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.

1.6.4 Mechanism of Action

Carvedilol is a racemic mixture in which nonselective beta-adrenoreceptor blocking activity is present in the S(-) enantiomer and alpha-adrenergic blocking activity is present in both R(+) and S(-) enantiomers at equal potency. Carvedilol's beta-adrenergic receptor blocking ability decreases the heart rate, myocardial contractility, and myocardial oxygen demand. Carvedilol also decreases systemic vascular resistance via its alpha adrenergic receptor blocking properties. Carvedilol and its metabolite BM-910228 (a less potent beta blocker, but more potent antioxidant) have been shown to restore the inotropic responsiveness to Ca^{2+} in OH^- free radical-treated myocardium. Carvedilol and its metabolites also prevent OH^- radical-induced decrease in sarcoplasmic reticulum Ca^{2+}-ATPase activity. Therefore, carvedilol and its metabolites may be beneficial in chronic heart failure by preventing free radical damage. [Hamed et al., 2016] [Wisler et al., 2007]

1.6.5 Indication

Carvedilol tablets USP should be taken with food to slow the rate of absorption and reduce the incidence of orthostatic effects. [Carvedilol- Davis's]

1.6.6 Dosage and Administration

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, Lea, and Wagstaff, 1997] [Watanabe et al., 2000]

1.6.7 Dosage Forms and Strengths [Coreg(Carvedilol)]

The white, oval, film-coated tablets are available in the following strengths:

- 3.125 mg
- 6.25 mg
- 12.5 mg
- 25 mg

1.6.8 Pharmacodynamics [Morgan, 1994] [Coreg(Carvedilol)]

Left Ventricular Dysfunction Following Myocardial Infarction: The basis for the beneficial effects of carvedilol in patients with left ventricular dysfunction following an acute myocardial infarction is not established.

Hypertension: The mechanism by which β-blockade produces an antihypertensive effect has not been established. β-adrenoreceptor blocking activity has been demonstrated in animal and human studies showing that carvedilol-

- Reduces cardiac output in normal subjects;

- Reduces exercise and/or isoproterenol-induced tachycardia; and
- Reduces reflex orthostatic tachycardia

Significant β-adrenoreceptor blocking effect is usually seen within 1 hour of drug administration.

α_1-adrenoreceptor blocking activity has been demonstrated in human and animal studies, showing that carvedilol-

- Attenuates the pressor effects of phenylephrine;
- Causes vasodilation; and
- Reduces peripheral vascular resistance.

These effects contribute to the reduction of blood pressure and usually are seen within 30 minutes of drug administration.

Due to the α_1-receptor blocking activity of carvedilol, blood pressure is lowered more in the standing than in the supine position, and symptoms of postural hypotension (1.8%), including rare instances of syncope, can occur. Following oral administration, when postural hypotension has occurred, it has been transient and is uncommon when carvedilol is administered with food at the recommended starting dose and titration increments are closely followed.

In hypertensive patients with normal renal function, therapeutic doses of carvedilol decreased renal vascular resistance with no change in glomerular filtration rate or renal plasma flow. Changes in excretion of sodium, potassium, uric acid, and phosphorus in hypertensive patients with normal renal function were similar after carvedilol and placebo.

Carvedilol has little effect on plasma catecholamines, plasma aldosterone, or electrolyte levels, but it does significantly reduce plasma renin activity when given for at least 4 weeks. It also increases levels of atrial natriuretic peptide.

1.6.9 Pharmacokinetics

Carvedilol is rapidly and extensively absorbed following oral administration, with absolute bioavailability of approximately 25% to 35% due to a significant degree of first-pass metabolism. Following oral administration, the apparent mean terminal elimination half-life of carvedilol generally ranges from 7 to 10 hours. Plasma concentrations achieved are proportional to the oral dose administered. When administered with food, the rate of absorption is slowed, as evidenced by a delay in the time to reach peak plasma levels, with no significant difference in extent of bioavailability. Taking carvedilol with food should minimize the risk of orthostatic hypotension. [Dunn, Lea, and Wagstaff, 1997]

Carvedilol is extensively metabolized. Following oral administration of radiolabelled carvedilol to healthy volunteers, carvedilol accounted for only about 7% of the total radioactivity in plasma as measured by area under the curve (AUC). Less than 2% of the dose was excreted unchanged in the urine. Carvedilol is metabolized primarily by aromatic ring oxidation and glucuronidation. The oxidative metabolites are further metabolized by conjugation via glucuronidation and sulfation. The metabolites of carvedilol are excreted primarily via the bile into the feces. Demethylation and hydroxylation at the phenol ring produce 3 active metabolites with β-receptor blocking activity. Based on preclinical studies, the 4'-hydroxyphenyl metabolite is approximately 13 times more potent than carvedilol for β -blockade. [Morgan, 1994]

Compared with carvedilol, the 3 active metabolites exhibit weak vasodilating activity. Plasma concentrations of the active metabolites are about one-tenth of those observed for carvedilol and have pharmacokinetics similar to the parent. Carvedilol undergoes stereoselective first-pass metabolism with plasma levels of R(+)-carvedilol approximately 2 to 3 times higher than S(-)-carvedilol following oral administration in healthy subjects. The mean apparent terminal elimination half-lives for R(+)-carvedilol range from 5 to 9 hours compared with 7 to 11 hours for the S(-)-enantiomer. [Dailymed, 2011] [Dunn, Lea, and Wagstaff, 1997]

The primary P_{450} enzymes responsible for the metabolism of both R(+) and S(-)-carvedilol in human liver microsomes were CYP2D6 and CYP2C9 and to a lesser extent CYP3A4, 2C19, 1A2, and 2E1. CYP2D6 is thought to be the major enzyme in the 4'- and 5'-hydroxylation of carvedilol, with a potential contribution from 3A4. CYP2C9 is thought to be of primary importance in the O-methylation pathway of S(-)-carvedilol. [Dailymed, 2011] [Morgan, 1994]

Carvedilol is subject to the effects of genetic polymorphism with poor metabolizers of debrisoquin (a marker for cytochrome P450 2D6) exhibiting 2- to 3-fold higher plasma concentrations of R(+)-carvedilol compared with extensive metabolizers. In contrast, plasma levels of S(-)-carvedilol are increased only about 20% to 25% in poor metabolizers, indicating this enantiomer is metabolized to a lesser extent by cytochrome P450 2D6 than R(+)-carvedilol. The pharmacokinetics of carvedilol do not appear to be different in poor metabolizers of S-mephenytoin (patients deficient in cytochrome P450 2C19). [Dailymed, 2011]

Carvedilol is more than 98% bound to plasma proteins, primarily with albumin. The plasma-protein binding is independent of concentration over the therapeutic range. Carvedilol is a basic, lipophilic compound with a steady-state volume of distribution of approximately 115 L, indicating substantial distribution into extravascular tissues. Plasma clearance ranges from 500 to 700 mL/min. [Carvedilol- Davis's]

1.6.10 Nonclinical Toxicology

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. [Dunn, Lea, and Wagstaff, 1997]

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. [Dailymed, 2011]

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]

1.6.11 Tolerability

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. [Krum et al., 1995]

1.6.12 Drug Interactions [Coreg(Carvedilol)] [Carvedilol-WebMD]

- **CYP2D6 Inhibitors and Poor Metabolizers**

Interactions of carvedilol with potent inhibitors of CYP2D6 isoenzyme (such as quinidine, fluoxetine, paroxetine, and propafenone) have not been studied, but these drugs would be expected to increase blood levels of the R(+) enantiomer of carvedilol. Retrospective analysis of side effects in clinical trials showed that poor 2D6 metabolizers had a higher rate of dizziness during up-titration, presumably resulting from vasodilating effects of the higher concentrations of the α-blocking R(+) enantiomer.

- **Hypotensive Agents**

Patients taking both agents with β-blocking properties and a drug that can deplete catecholamines (e.g., reserpine and monoamine oxidase inhibitors) should be observed closely for signs of hypotension and/or severe bradycardia.

Concomitant administration of clonidine with agents with β-blocking properties may potentiate blood-pressure and heart rate-lowering effects. When concomitant treatment with agents with β-blocking properties and clonidine is to be terminated, the β-blocking agent should be discontinued first. Clonidine therapy can then be discontinued several days later by gradually decreasing the dosage.

- **Cyclosporine**

Modest increases in mean trough cyclosporine concentrations were observed following initiation of carvedilol treatment in 21 renal transplant patients suffering from chronic vascular rejection. In about 30% of patients, the dose of cyclosporine had to be reduced in order to maintain cyclosporine concentrations within the therapeutic range, while in the remainder no adjustment was needed. On the average for the group, the dose of cyclosporine was reduced about 20% in these patients. Due to wide interindividual variability in the dose adjustment required, it is recommended that cyclosporine concentrations be monitored closely after initiation of carvedilol therapy and that the dose of cyclosporine be adjusted as appropriate.

- **Digitalis Glycosides**

Both digitalis glycosides and β-blockers slow atrioventricular conduction and decrease heart rate. Concomitant use can increase the risk of bradycardia. Digoxin concentrations are increased by about 15% when digoxin and carvedilol are administered concomitantly. Therefore, increased monitoring of digoxin is recommended when initiating, adjusting, or discontinuing carvedilol.

- **Inducers/Inhibitors of Hepatic Metabolism**

Rifampin reduced plasma concentrations of carvedilol by about 70%. Cimetidine increased AUC by about 30% but caused no change in Cmax.

- **Amiodarone**

Amiodarone, and its metabolite desethyl amiodarone, inhibitors of CYP2C9 and P glycoprotein, increased concentrations of the S(-) enantiomer of carvedilol by at least 2 folds. The concomitant administration of amiodarone or other CYP2C9 inhibitors such as fluconazole with carvedilol may enhance the β-blocking properties of carvedilol resulting in further slowing of the heart rate or cardiac conduction. Patients should be observed for signs of bradycardia or heart block, particularly when one agent is added to pre-existing treatment with the other.

- **Calcium Channel Blockers**

Conduction disturbance (rarely with hemodynamic compromise) has been observed when carvedilol is co-administered with diltiazem. As with other agents with β-blocking properties, if carvedilol is to be administered with calcium channel blockers of the verapamil or diltiazem type, it is recommended that ECG and blood pressure be monitored.

- **Insulin or Oral Hypoglycemics**

Agents with β-blocking properties may enhance the blood-sugar-reducing effect of insulin and oral hypoglycemics. Therefore, in patients taking insulin or oral hypoglycemics, regular monitoring of blood glucose is recommended.

- **Anesthesia**

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.

1.6.13 Drug-Drug Interactions [Carvedilol-WebMD] [Coreg(Carvedilol)]

Since carvedilol undergoes substantial oxidative metabolism, the metabolism and pharmacokinetics of carvedilol may be affected by induction or inhibition of cytochrome P_{450} enzymes.

- **Amiodarone:**

In a pharmacokinetic study conducted in 106 Japanese patients with heart failure, coadministration of small loading and maintenance doses of amiodarone with carvedilol resulted in at least a 2-fold increase in the steady-state trough concentrations of S(-) carvedilol.

- **Cimetidine:**

In a pharmacokinetic study conducted in 10 healthy male subjects, cimetidine (1,000 mg/day) increased the steady-state AUC of carvedilol by 30% with no change in Cmax.

- **Digoxin:**

Following concomitant administration of carvedilol (25 mg once daily) and digoxin (0.25 mg once daily) for 14 days, steady-state AUC and trough concentrations of digoxin were increased by 14% and 16%, respectively, in 12 hypertensive patients.

- **Glyburide:**

In 12 healthy subjects, combined administration of carvedilol (25 mg once daily) and a single dose of glyburide did not result in a clinically relevant pharmacokinetic interaction for either compound.

- **Hydrochlorothiazide:**

A single oral dose of carvedilol 25 mg did not alter the pharmacokinetics of a single oral dose of hydrochlorothiazide 25 mg in 12 patients with hypertension. Likewise, hydrochlorothiazide had no effect on the pharmacokinetics of carvedilol.

- **Rifampin:**

In a pharmacokinetic study conducted in 8 healthy male subjects, rifampin (600 mg daily for 12 days) decreased the AUC and C_{max} of carvedilol by about 70%.

- **Torsemide:**

In a study of 12 healthy subjects, combined oral administration of carvedilol 25 mg once daily and torsemide 5 mg once daily for 5 days did not result in any significant differences in their pharmacokinetics compared with administration of the drugs alone.

- **Warfarin:**

Carvedilol (12.5 mg twice daily) did not have an effect on the steady-state prothrombin time ratios and did not alter the pharmacokinetics of R(+) and S(-) warfarin following concomitant administration with warfarin in 9 healthy volunteers.

1.6.14 Drug-Food Interaction

Carvedilol and ethanol may have additive effects in lowering your blood pressure. May experience headache, dizziness, lightheadedness, fainting, and changes in pulse or heart rate. These side effects are most likely to be seen at the beginning of treatment, following a dose increase, or when treatment is restarted after an interruption. [Gordon, 1997] Using carvedilol together with multivitamin with minerals may decrease the effects of carvedilol. Separate the administration times of carvedilol and multivitamin with minerals by at least 2 hours. [Coreg(Carvedilol)]

1.6.15 Side effects [Coreg(Carvedilol)]

1.5.15.1 Need to seek medical advice immediately if patient develops the following symptoms:

- Allergic reactions: swelling of the face, throat or tongue, fever, difficulty in breathing, dizziness, skin rashes (exanthema); Swelling of parts of the body (edema)

- Fever, general ill feeling, itching, joint aches, multiple skin lesions (erythema multiforme)
- Severe blistering of the skin, mouth, eyes and genitals (Stevens-Johnson syndrome, toxic epidermal necrolysis)

1.6.15.2 Common side effects

- Dizziness (usually mild and most likely to occur at the beginning of treatment)
- Headache (usually mild and most likely to occur at the beginning of treatment)
- Heart failure Low blood pressure (hypotension)
- General weakness (asthenia) (usually mild and most likely to occur at the beginning of treatment)
- Tiredness, weakness or lack energy (fatigue)
- Infection causing inflammation and irritation to the main airways of the lungs (bronchitis)
- Inflammation of the lungs (pneumonia)
- Infection of the nose, sinuses and throat (upper respiratory tract infection)
- Infection of the bladder causing pain and discomfort (urinary tract infection)
- Looking pale and feeling tired (anaemia)
- Weight gain
- Very high levels of cholesterol in the blood (hypercholesterolaemia)
- Increased (hyperglycaemia) or low (hypoglycaemia) blood sugar levels in patients with diabetes

- Depression
- Visual impairment, dry eyes, eye irritation
- Slower heartbeat (bradycardia)
- Disturbances of blood flow that reaches the upper and lower extremities of the body (arms and legs) and the surface of the skin (peripheral circulation)
- Build−up of fatty deposits in the arteries causing narrowing of the arteries and restricting the blood supply to the limbs (peripheral vascular disease)
- Cramp−like pain felt in the calf, thigh or buttock during walking or other exercise (intermittent claudication)
- Poor circulation causing fingers and toes to be pale and numb (Raynaud's phenomenon)
- Difficulty in breathing (dyspnoea)
- Excess collection of watery fluid in the lungs (pulmonary oedema)
- Asthma in susceptible patients Feeling (nausea) or being (vomiting) sick
- Diarrhoea
- Indigestion (dyspepsia)
- Stomach pain
- Kidney failure and/or kidney problems in patients suffering from diffuse vascular disease (damage/disease in the coronary arteries, the major blood vessels that supply the heart) and/or underlying poor kidney function

1.6.15.3 Rare side effects (may affect up to 1 in 1000 people)

- Reduction in blood platelets, which increases risk of bleeding or bruising (thrombocytopaenia)
- Blocked nose Flu−like symptoms
- Constipation Increase in liver enzyme levels (detected by blood test)
- The hearts ability to contract may be decreased during dose adjustment
- Very rare side effects (may affect up to 1 in 10,000 people)
- A reduction in white blood cells (leukopenia)
- Dry mouth
- Increase in Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST) and Gamma− Glutamyl Transferase (GGT), blood tests typically used to detect liver disorders/diseases
- Urinary incontinence in women (should resolve when treatment is stopped)
- Other side effects (frequency not known)
- During treatment with Carvedilol latent diabetes mellitus (slow−onset diabetes) may occur, existing diabetes may be aggravated, and blood sugar levels may become disturbed.

1.6.16 Contraindications [Coreg(Carvedilol)]

Carvedilol tablets are contraindicated in the following conditions:

- If patient is allergic (hypersensitive) to Carvedilol, beta- blockers or any of the other ingredients of this medicine.

- If patient suffers from unstable heart failure (attacks suddenly come on, are more frequent, are more uncomfortable or last longer) or acute decompensated heart failure (worsening of the symptoms) requiring intravenous inotropic support (medicine administered directly into a vein to alter the force or strength of the heartbeat).
- If patient suffers from liver problems.
- If patient has a history of difficulty in breathing or wheezing (bronchospasm or asthma).
- If patient suffers from a condition where the heart beats irregularly (sick sinus syndrome) or much more slowly than normal e.g. less than 50 beats per minute whilst resting (severe bradycardia).
- If patient has a weakened heart that is unable to pump blood around the body (cardiogenic shock) or very poor blood circulation.
- If patient suffers from very low blood pressure (severe hypotension).
- If patient has an increased amount of acid in his blood (metabolic acidosis).
- If patient have chest pains when at rest (prinzmetal's angina).
- If patient has an untreated tumor of the adrenal gland (pheochromocytoma).
- If patient is taking verapamil or diltiazem, medicines used to treat high blood pressure, abnormal heart rhythms, chest pain [calcium channel blockers]

1.6.17 Warnings & Precautions [Aman, and Thoma, 2002]

- **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.

- **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.

- **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.

- **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.

- **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.

- **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.

- **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.

- **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.

- **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.

- **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.

- **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.

- **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.

- **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, accidental, diagnostic, or therapeutic. Such patients may be unresponsive to the usual doses of epinephrine used to treat allergic reaction.

- **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.

1.6.18 Specific Populations [Coreg(Carvedilol)]

- **Geriatric:**

Plasma levels of carvedilol average about 50% higher in the elderly compared to young subjects.

- **Hepatic Impairment:**

Compared to healthy subjects, patients with severe liver impairment (cirrhosis) exhibit a 4 to 7 fold increase in carvedilol levels. Carvedilol is contraindicated in patients with severe liver impairment.

- **Renal Impairment:**

Although carvedilol is metabolized primarily by the liver, plasma concentrations of carvedilol have been reported to be increased in patients with renal impairment. Based on mean AUC data, approximately 40% to 50% higher plasma concentrations of carvedilol were observed in hypertensive patients with moderate to severe renal impairment compared to a control group of hypertensive patients with normal renal function. However, the ranges of AUC values were similar for both groups. Changes in mean peak plasma levels were less pronounced, approximately 12% to 26% higher in patients with impaired renal function.

Consistent with its high degree of plasma protein-binding, carvedilol does not appear to be cleared significantly by hemodialysis.

1.6.19 Overdosage [Aman, and Thoma, 2002] [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.

Chapter Two

LITERATURE REVIEW

2.1 Literature Review

In 2003, P Ptáček et al had a study on quantitation of carvedilol in human plasma. And the method was based on protein precipitation with methanol, concentration of the supernatant by evaporation and reversed-phase chromatography with fluorimetric detection. And the assay was used for pharmacokinetic studies. [Ptáček, Macek, and Klıma, 2003]

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. [Stojanović et al., 2005]

In 2006, Weeranuj Yamreudeewong, et al had a study on stability of two extemporaneously prepared oral metoprolol and carvedilol liquids. There were signs of possible decomposition in metoprolol oral liquid after 8 weeks of storage at room temperature and also revealed that there was no microbial growth in either drug liquid after a refrigerated storage period of 2 weeks. [Yamreudeewong, Dolence and Pahl, 2006]

In 2006, M. Imran et al had a study on new and rapid stability indicating ultraviolet spectroscopic methods were developed and validated for the estimation of ezetimibe and carvedilol. And it was concluded that both of the developed methods are accurate, sensitive, precise, and reproducible. [Imran, Singh, and Chandran, 2006]

In 2007, Rajeshwari Rathod et al had a study on the Estimation of carvedilol in human plasma and done by using HPLC-fluorescence detector. The stability studies showed that carvedilol in human plasma was stable during short-term period for sample preparation and analysis. [Rathod et al., 2007]

In 2007, Jelena Stojanović et al had a study on the photochemical stability of carvedilol and its degradation products by the RP-HPLC method. By the help of this method, the simultaneous determination of carvedilol and its degradation products can be seperated. And

effectively separation of the drug from its degradation products can be obtained. [Stojanović et al.,2007]

In 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 2008, Fibele Analine Lanzanova et al had a study on degradation of carvedilol where the drug was subjected to acid, alkaline, and neutral hydrolytic conditions. Then the degradation samples were evaluated and kinetics of degradation was determined by LC-method. And LC-MS/MS method was developed and validated and that was found precise, accurate, specific, and selective. [Lanzanova et al., 2008]

In 2008, Olga Galanopoulou et al had a study on HPLC analysis, isolation and identification of a new degradation product in carvedilol tablets. And the process was done with the help of HPLC analysis. During stability testing of carvedilol solid dosage forms an unknown degradation product, which was detected as carvedilol impurity and the separation of that product was achieved by using semi-preparative chromatography method. [Galanopoulou et al., 2008]

In 2009, simple a d sensitive method 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. [Sreevidya, and Narayana, 2009]

In 2009, Mohammad Rizwan wt al had a study to establish a validated stability-indicating LC method for assay of carvedilol and to study the degradation behaviour of the drug under different ICH-recommended stress conditions. And the method then successfully used for

routine analysis of carvedilol in the bulk drug and in pharmaceutical dosage forms. [Rizwan et al., 2009]

In 2009, Somisetti Narender Rao et al had a study on Synthesis and Characterization of Potential Impurities of Carvedilol. . During the bulk synthesis of carvedilol, they had observed six impurities. [Rao et al., 2009]

In 2010, F. Buontempo et al had a study on Carvedilol stability in paediatric oral liquid formulations by using HPLC. All the formulations that were tested can be stored at room temperature for at least 56 days. [Buontempo et al., 2010]

In 2010, Soo-Hwan Kim et al had a study on the determination of carvedilol in human plasma by using high-performance liquid chromatography with tandem mass spectrometer (HPLC-MS/MS). These results suggested that the HPLC-MS/MS analysis method development was suitable for the carvedilol analysis in human plasma. [Kim et al., 2010]

In 2010, 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 200-350nm.This method was validated and applied to the determination of carvedilol in tablets. It was concluded that the developed methods are accurate, sensitive, precise, and reproducible. [Theivarasu, Ghosh, and Indumathi, 2010]

In 2011, Ivan M. Savic et al had a study to select an appropriate primary packaging and analysis of its influence on stability, of tablets containing Carvedilol using a validated HPLC method. [Savic et al., 2011]

In 2012, P. Ajit et al had a study on the determination of Carvedilol & its impurity by using an isocratic reversed-phase high performance liquid chromatographic (RP-HPLC) method which was found to be accurate, precise, linear, specific, sensitive, rugged, robust, and stability-indicating. [Ajit et al., 2012]

In 2013, Alzoman Nourah Z. et al had a study on validated stability-indicating capillary electrophoresis method for the separation and determination of a fixed-dose combination of carvedilol and hydrochlorothiazide in tablets. Forced degradation were performed on bulk samples of the two drugs using thermal, photolytic, hydrolytic, and oxidative stress conditions, and the stressed samples. As a result of stress degraded products produced and did not interfere with the determination of CRV and HCT. [Alzoman et al., 2013]

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. [Hossain et al., 2013]

In 2013, Daxesh Patel et al had a study on the simultaneous determination of carvedilol and its pharmacologically active metabolite 4'-hydroxyphenyl carvedilol in human plasma by using an ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) method. And the method was successfully applied to support a bioequivalence study of 12.5 mg carvedilol tablets in 34 healthy subjects. [Patel et al., 2013]

In 2013, C. Purna Chander et al had a study on carvedilol and its stress degradation products by developing an isocratic liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS) method for the separation and identification of stress degradation products (DPs) of carvedilol (CAR), a non-selective beta-blocker with vasodilatory properties. [Chander et al., 2013]

In 2013, Tarek S. Belal et al had a study on validated stability test by using HPLC method to determine the antihypertensive binary mixture of carvedilol and hydrochlorothiazide in tablet dosage forms. In this study an effective chromatographic separation was achieved. And the proposed method made use of DAD as a tool for peak identity and purity confirmation. [Belal et al., 2013]

In 2014, Juanjuan Jiang et al had a study on the determination of carvedilol in human plasma using chiral stationary-phase column and reverse-phase liquid chromatography with tandem mass spectrometry. And this method was successfully applied to samples taken from research volunteers treated with carvedilol sustained-release tablet 18mg. [Jiang et al., 2014]

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. [Jouyban, Hasanzadeh, and Shadjou, 2014]

In 2015, Hao Wu et al had a study on ‘Magnetic retrieval of ionic liquids: high sensitivity fluorescence determination of carvedilol in tablets, plasma, and urine’ by Ionic liquid-based dispersive liquid–liquid microextraction with magnetic dispersive micro-solid phase extraction. The method was then used to determine the analyte in tablets, human plasma, and urine with recoveries between 95.04 and 106.6%. [Wu et al., 2015]

In 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 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%. [Raju, 2015]

In 2015, L. Samba Siva Rao et al had a study on Development and validation of stability indicating method for the quantitative determination of carvedilol and its related impurities in pharmaceutical dosage forms using RP HPLC. It’s also used to test the stability samples of Carvedilol. [Rao, Madhavan and Prakash, 2015]

In 2016, Leonardo Allain et al had a study on photostability test for pharmaceutical oral drug products, in which ICH-defined light sources was used to derive a set of practical experimental approaches to support the safe and effective administration of photosensitive oral drug products. [Allain et al., 2016]

In 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, and Rocha, 2016]

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. [Gallo et al., 2016]

In 2017, Olivera Kaljević et al had a study on application of miscibility analysis and determination of Soluplus solubility map for development of carvedilol-loaded nanofibers by using electrospinning. And miscibility between drug and polymer was determined through calculation of the interaction parameter. [Kaljević et al., 2017]

So, after going through all these literatures based on carvedilol, I have done the photosensitivity test of carvedilol and in some extent moisture sensitivity test of carvedilol. In the photosensitivity test, I have chosen a drug named Cavelon®, which is a carvedilol drug and is packed in transparent package. I have exposed my samples in room temperature, sunlight, 25 watt bulb and 40 watt bulb. After exposing, the drug was tested by UV-spectrophotometer to determine whether its potency has been changed or not.

Chapter Three

METHOD AND MATERIALS

3.1 Sample Collection:

To observe the impact of different exposure, as well as to assess the coating efficiency to carvedilol which contained in a blister packing with coating, 500 tablets of Cavelon® (6.25 mg) were collected from local drug store in Dhaka. All the tablets were from the same batch. 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 and determine their potency.

Table 3.1: Samples used in the experiment with their sources

Name of Sample	Source (Supplier)	Batch No.
Cavelon®	Drug International Ltd.	0616

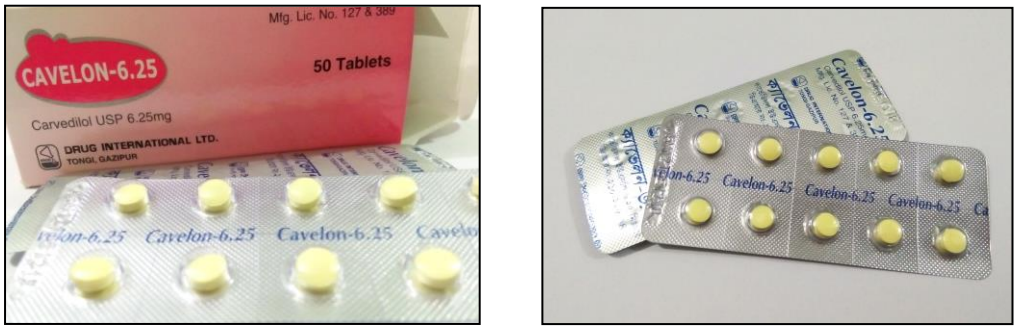

Figure 3.1: Cavelon® 6.25 mg (Carvedilol) (Drug International Ltd, 2016)

Table 3.2: Reagents used in the experiment

Reagent Name	Source (Supplier)
Distilled water	East West University Laboratory
Concentrated H_2SO_4 (98% / 36.8N)	Analar, United Kingdom

Table 3.3: Instruments used in the experiment

Serial No.	Equipments	Source (Name of supplier)	Origin
01	UV Spectrophotometer	Shimadzu UV-1800	Japan
02	Electronic balance	Shimadzu AY220	Japan

Table 3.4: Apparatus that were used throughout the experiments

Serial No.	Apparatus
1	Beaker (250 ml)
2	Test tube
3	Filter paper
4	Mortar and pestle
5	Funnel
6	Measuring Cylinder
7	Pipette (10 ml, 5ml, 2 ml)
8	Pipette pumper
9	Spatula
10	Aluminum foil paper
11	Filter Papers
12	Masking Tape
13	Thermometer
14	Electric Bulb (25 Watt & 40 Watt)
15	Plastic Dropper
16	Table Lamp
17	Quartz Cuvette

3.2 Important Instruments

Some important images of instruments that were used during different steps of experiments:

Figure 3.2.1: UV Spectrophotometer

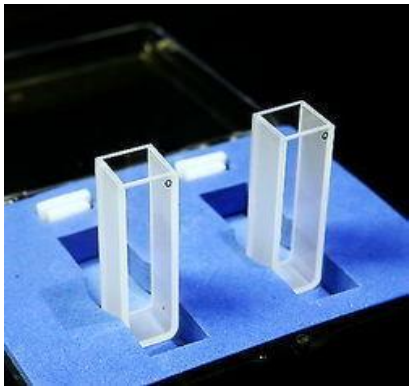

Figure 3.2.2: Quartz cuvette

Figure 3.2.3: Electric analytical balance

3.3 Method:

3.3.1 Preparation of Dissolution Medium for Standard Curve:

Carvedilol is a water insoluble drug. So, for preparing standard curve of Carvedilol Solvent (concentrated H_2SO_4) was used.

3.3.1.1 Preparation of the solvent (0.1N H_2SO_4)

1. Lab solvent (H_2SO_4), stock solution with 98% (v/v) of strength was collected.
2. Then the concentration of the stock solution was determined in normality where the specific gravity of solvent is 1.84.

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

1000 ml of stock solution contain 49gm 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) stock solution required to make 1000 ml of 0.1N HCL solvent was calculated as below.

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

Here,

S_1 = Concentration of lab solvent (H_2SO_4) stock solution = 36.8N

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, $V_1 = (V_2S_2) / S_1$

- ⇨ V_1 = (1000ml x 0.1 N) /36.8N
- ⇨ V_1 = 2.717ml (~ 2.72 ml of lab solvent H_2SO_4 stock solution)

4. Then 2.72ml 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 the mark to make 1000ml of 0.1N H_2SO_4 solvent.

    a. A standard of carvedilol was collected from the pharmaceutical company Drug International Ltd. The potency of standard compounds was 99.56%.

b. The specific λ_{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 Drug International Ltd.

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

Preparation of the stock solution for carvedilol using the standard obtained from Drug International Ltd:

1. 50 mg of the standard compound, that is carvedilol was weighed and dissolved in 250 ml of 0.1N H_2SO_4 (which is the solvent) in a 250ml volumetric flask for the 1st dilution.

Thus the concentration was calculated to be:

Concentration of 1st dilution = amount of substance added / volume

= (50 / 250) mg/ml

= 0.2 mg/ml

2. Then 5ml of that 0.2 mg/ml carvedilol solution was taken and dissolved in 50ml of 0.1N H_2SO_4. That 5ml contained 1mg of carvedilol.

So the concentration finally turned out to be:

Concentration of 2nd dilution = amount of substance added/ volume

= (1 / 50) mg/ml

= 0.02 mg/ml

3.3.1.2 Preparation of nine serial concentrations of solution for carvedilol:

1. Carvedilol had the concentration of its stock solution is 0.02mg/ml.
2. 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 10ml.
3. 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 S_1= initial strength or concentration, S_2= final strength or concentration, V_1= initial volume and V_2= final volume.

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

Table 3.5: Concentration for Preparation of Standard Curve of Carvedilol

Sample name	Sample no.	Concentration (mg/ml)
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

- $V_1 = S_2V_2 / S_1$ = (0.001 x 10) / 0.02 = 0.5 ml of stock solution required to make 0.001 mg/ml concentration of the final solution of 10 ml (0.5 ml of stock solution + 9.5 ml of 0.1N H_2SO_4) of carvedilol.

- $V_1 = S_2V_2 / S_1$ = (0.002 x 10) / 0.02 = 1 ml of stock solution required to make 0.002 mg/ml concentration of the final solution of 10 ml (1 ml of stock solution + 9 ml of 0.1N H_2SO_4) of carvedilol.

- $V_1 = S_2V_2 / S_1$ = (0.003 x 10) / 0.02 = 1.5 ml of stock solution required to make 0.003 mg/ml concentration of the final solution of 10 ml (1.5 ml of stock solution + 8.5 ml of 0.1N H_2SO_4) of carvedilol.

- $V_1 = S_2V_2 / S_1$ = (0.004 x 10) / 0.02 = 2 ml of stock solution required to make 0.004 mg/ml concentration of the final solution of 10 ml (2 ml of stock solution + 8 ml of 0.1N H_2SO_4) of carvedilol.

- $V_1 = S_2V_2 / S_1$ = (0.005 x 10) / 0.02 = 2.5 ml of stock solution required to make 0.005 mg/ml concentration of the final solution of 10 ml (2.5 ml of stock solution + 7.5 ml of 0.1N H_2SO_4) of carvedilol.

- $V_1 = S_2V_2 / S_1$ = (0.006 x 10) / 0.02 = 3 ml of stock solution required to make 0.006 mg/ml concentration of the final solution of 10 ml (3 ml of stock solution + 7 ml of 0.1N H_2SO_4) of carvedilol.

- $V_1 = S_2V_2 / S_1$ = (0.007 x 10) / 0.02 = 3.5 ml of stock solution required to make 0.007 mg/ml concentration of the final solution of 10 ml (3.5 ml of stock solution + 6.5 ml of 0.1N H2SO4) of carvedilol.

- $V_1 = S_2V_2 / S_1$ = (0.008 x 10) / 0.02 = 4 ml of stock solution required to make 0.008 mg/ml concentration of the final solution of 10 ml (4 ml of stock solution + 6 ml of 0.1N H_2SO_4) of carvedilol.

- $V_1 = S_2V_2 / S1$ = (0.009 x 10) / 0.02 = 4.5 ml of stock solution required to make 0.009 mg/ml concentration of the final solution of 10 ml (4.5 ml of stock solution + 5.5 ml of 0.1N H_2SO_4) of carvedilol.

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

5. A standard curves was plotted for carvedilol.

6. From this standard curve a straight line equation was obtained which was in the form of

$$\mathbf{y = mx+c}$$

where the components of the equations are described as provided below:

m = gradient value,

y = absorbance values,

x = concentrations and

c = y-intercept.

3.3.1.3 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 & 40watt)
3. Direct Sunlight exposure

1. Exposure under Normal Lighting Condition

a) The tablets (Cavelon®) were kept under normal lighting condition in the room for 2months.

b) They were sampled after specific intervals like periodically after 14 days for determination their physical properties (like thickness, hardness & weight variation) and their potency.

c) Then from those 15 tablets, 5 tablets were used for physical parameter test and the rest 5 tablets for potency determination.

d) 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:

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

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 H_2SO_4) for 3 times to prepare 3 samples.

v. After that 10 ml solution was filtered and 5 ml of that filtered solution was taken and dissolved in 50ml 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.

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

ix. Steps 3 to 8 were repeated again on another sampling day.

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

a) 15 tablets were exposed to electric bulb lighting conditions for 5 hours at a stretch and 15 tablets were used as control.

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 bulb's lighting 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:

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

iii. 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 H_2SO_4) for 3 times to prepare 3 samples.

iv. After that 2 ml of that filtered solution was taken and dissolved in 8ml of the solvent.

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

vi. 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)	Temparature (^{0}C)	
			25W	40W
15 (control)	15	0	26	28
15	15	5	32	35

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) 15 tablets were used as control and has 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 kept in a Glass box and exposed to sunlight condition for 7.5 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:

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

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 H_2SO_4) 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)	Temparature ($_0$C)
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

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

b) 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 not more than two tablets must not differ more than 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

$$\textbf{\% Weight Variation} = \left(\frac{A - I}{A}\right) \times 100\ \%$$

Where,

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

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

Chapter Four

RESULTS

4.1 Standard curve preparation

The samples were collected from Drug International Ltd. and tried to make a standard curve. For different concentration of carvedilol different absorption were recorded. Nine serial concentrations of the standards of carvedilol were prepared for the purpose of creating a standard curve.

Here,

Volume of solution, **v** = 10 ml

Concentration of Stock solution, **c'** = $\frac{6.25 \text{ mg}}{250 \text{ ml}}$

= 0.025 mg/ml

Table 4.1: Calculation of Nine Serial Concentration, Stock & Solvent

Concentration (mg)	**Stock Solution $\frac{conc \times v}{c'}$ (ml)**	**Solvent (ml)**
0.001	0.4	9.6
0.002	0.8	9.2
0.003	1.2	8.8
0.004	1.6	8.4
0.005	2.0	8.0
0.006	2.4	7.6
0.007	2.8	7.2
0.008	3.2	6.8
0.009	3.6	6.4

Then the absorbance was taken for each concentration for the purpose of making a standard curve. The results are as follows:

Table 4.2: Concentration & Absorbance for Standard Curve of Carvedilol

Concentration (mg/ml)	Absorbance (at 241nm)
0.001	0.082
0.002	0.147
0.003	0.225
0.004	0.326
0.005	0.398
0.006	0.454
0.007	0.546
0.008	0.629
0.009	0.694

By plotting the absorbance against the concentration of carvedilol a straight line was found.

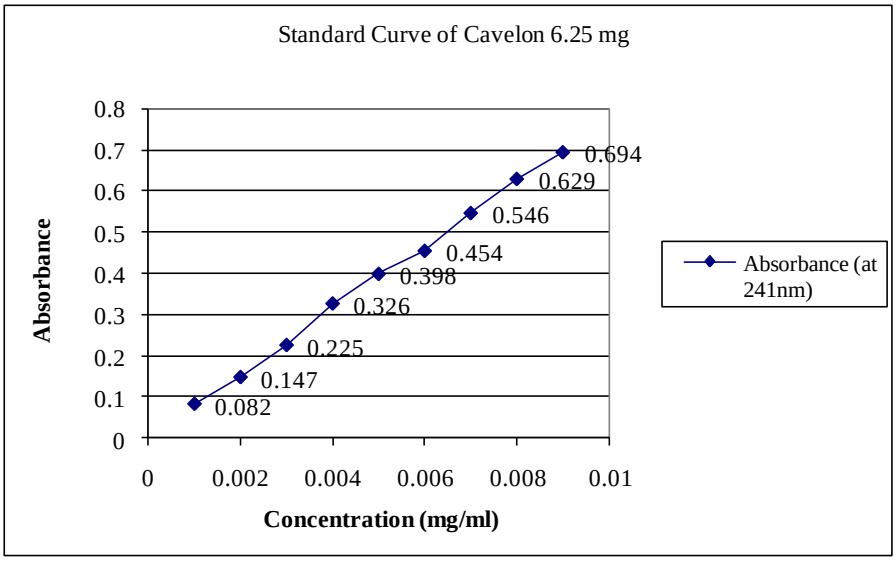

Figure 4.1: Graph showing straight line for absorbance with respect to concentration for Carvedilol

From this an equation was derived where:

$$y=77.7333x+0.0003$$

$$R^2=0.998$$

4.2 Physical Parameters of Normal Light Exposed Samples

Six tablet strips containing 60 tablets was exposed to normal light condition for 80 days. Weight variation test was conducted of 5 tablets of each day interval (0,15,30,45 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 4.3: Weight Variation Test of Carvedilol (Cavelon®)

Days	**Average Weight for Particular Day, I(g)**	**Average Weight for 42 Days Intervals, A(g)**	**Weight Variation; % Deviation, $\left(\frac{A - I}{A}\times 100\right)$ %**
Initial	0.0841	0.0843	0.237
14	0.0845		-0.237
28	0.0842		0.118
42	0.0845		-0.237

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. The results are given below:

Table 4.4: Concentration & Absorbance for Carvedilol (Cavelon®) under Normal Room Lightening Condition

Sample	Time Interval (Days)	Potency (%)	Potency Decrease (%)	Mean Potency Decrease (%)
Control A	0 Days	100%	0%	0%
Sample A1		100%	0%	
Sample A2		100%	0%	
Sample A3		100%	0%	
Control B	14 Days	100%	0%	
Sample B1		100%	0%	
Sample B2		100%	0%	
Sample B3		100%	0%	
Control C	28 Days	100%	0%	
Sample C1		100%	0%	
Sample C2		100%	0%	
Sample C3		100%	0%	
Control D	42 Days	100%	0%	
Sample D1		100%	0%	
Sample D2		100%	0%	
Sample D3		100%	0%	

By plotting the Potency against the days interval of for Cavelon® under exposure of normal room light a straight line was found.

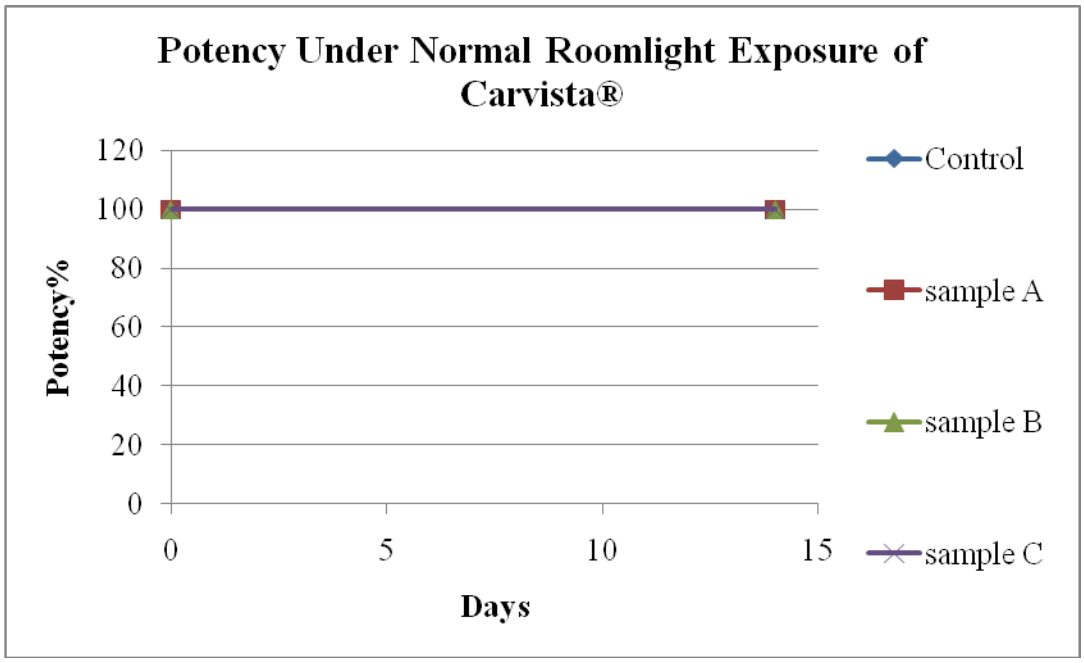

Figure 4.2: Graph showing straight line for Potency with respect to 14 days interval for Cavelon® under exposure of normal room light.

4.3.2 Result of samples that were exposed under 25Wbulb

In experimental day, a tablet strip containing 15 tablets was taken and 5 samples 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.

Table 4.5: Concentration & Absorbance for Carvedilol (Cavelon®) exposed under 25Wbulb

Sample	Initial Potency (%) (0 hours)	Potency (%) (after 5 hours)	Potency Decrease (%)	Mean Potency Decrease (%)
Control A	100%	100%	0%	0%
Sample A1	100%	100%	0%	
Sample A2	100%	100%	0%	
Sample A3	100%	100%	0%	
Control B	100%	100%	0%	
Sample B1	100%	100%	0%	
Sample B2	100%	100%	0%	
Sample B3	100%	100%	0%	
Control C	100%	100%	0%	
Sample C1	100%	100%	0%	
Sample C2	100%	100%	0%	
Sample C3	100%	100%	0%	

By plotting the Potency against the days interval of for Cavelon® under exposure of 25W bulb a straight line was found.

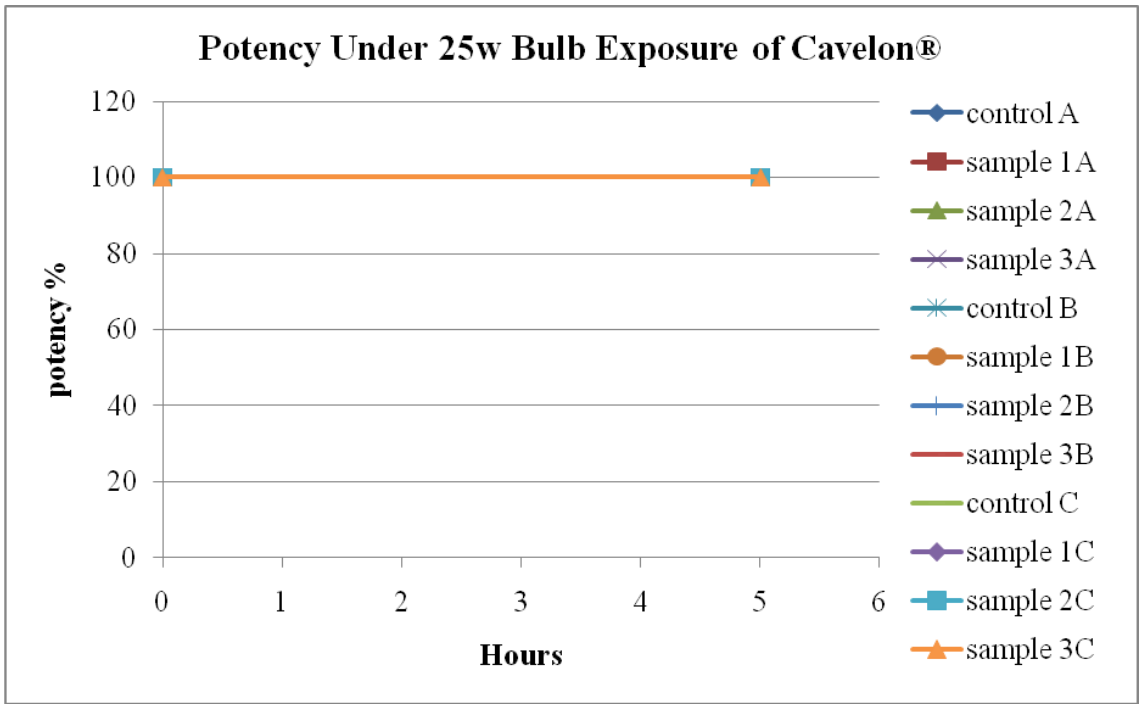

Figure 4.3: Graph showing straight line for Potency with respect to sample numbers for Cavelon® under exposure of 25W bulb.

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 time interval. The results are given below.

Table 4.6: Concentration & Absorbance for Carvedilol (Cavelon®) under 40Wbulb

Sample	Initial Potency (%) (0 hours)	Potency (%) (after 5 hours)	Potency Decrease (%)	Mean Potency Decrease (%)
Control A	100%	100%	0%	0%
Sample A1	100%	100%	0%	
Sample A2	100%	100%	0%	
Sample A3	100%	100%	0%	
Control B	100%	100%	0%	
Sample B1	100%	100%	0%	
Sample B2	100%	100%	0%	
Sample B3	100%	100%	0%	
Control C	100%	100%	0%	
Sample A1	100%	100%	0%	
Sample A2	100%	100%	0%	
Sample A3	100%	100%	0%	

By plotting the Potency against the days interval of for Cavelon® under exposure of 40W bulb a straight line was found.

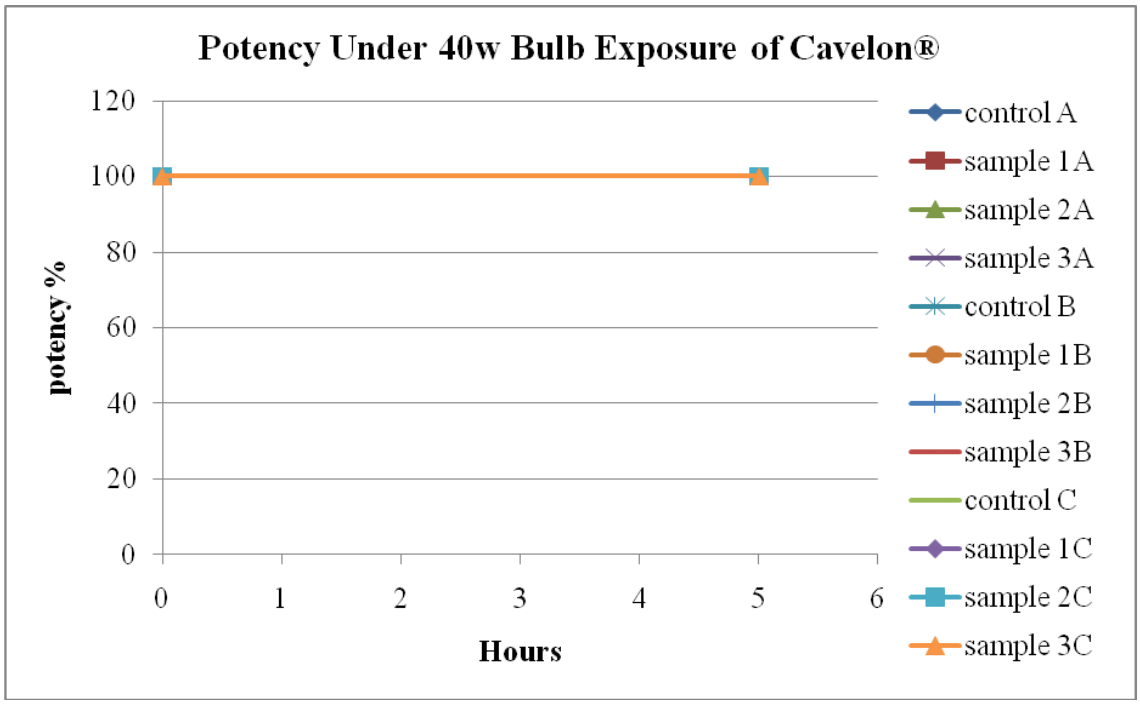

Figure 4.4: Graph showing straight line for Potency with respect to sample numbers for Cavelon® under exposure of 40W bulb.

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 hour's time interval and it was observed that the concentration of carvedilol was declined in each time interval. The results are given below.

Table 4.7: Concentration & Absorbance of Carvedilol at sunlight exposure under direct sunlight

Sample	Initial Potency (%) (0 hours)	Potency (%) (after 5 hours)	Potency Decrease (%)	Mean Potency Decrease (%)
Control A	100%	100%	0%	**1.55%**
Sample A1	100%	97.5%	2.5%	
Sample A2	100%	98%	2%	
Sample A3	100%	98%	2%	
Control B	100%	100%	0%	
Sample B1	100%	98.2%	1.8%	
Sample B2	100%	97.5%	2.5%	
Sample B3	100%	98%	2%	
Control C	100%	100%	0%	
Sample C1	100%	97.5%	2.5%	
Sample C2	100%	98.2%	1.8%	
Sample C3	100%	98.5%	1.5%	

By plotting the Potency against the days interval of for Cavelon® under exposure of Sunlight for 5 hours.

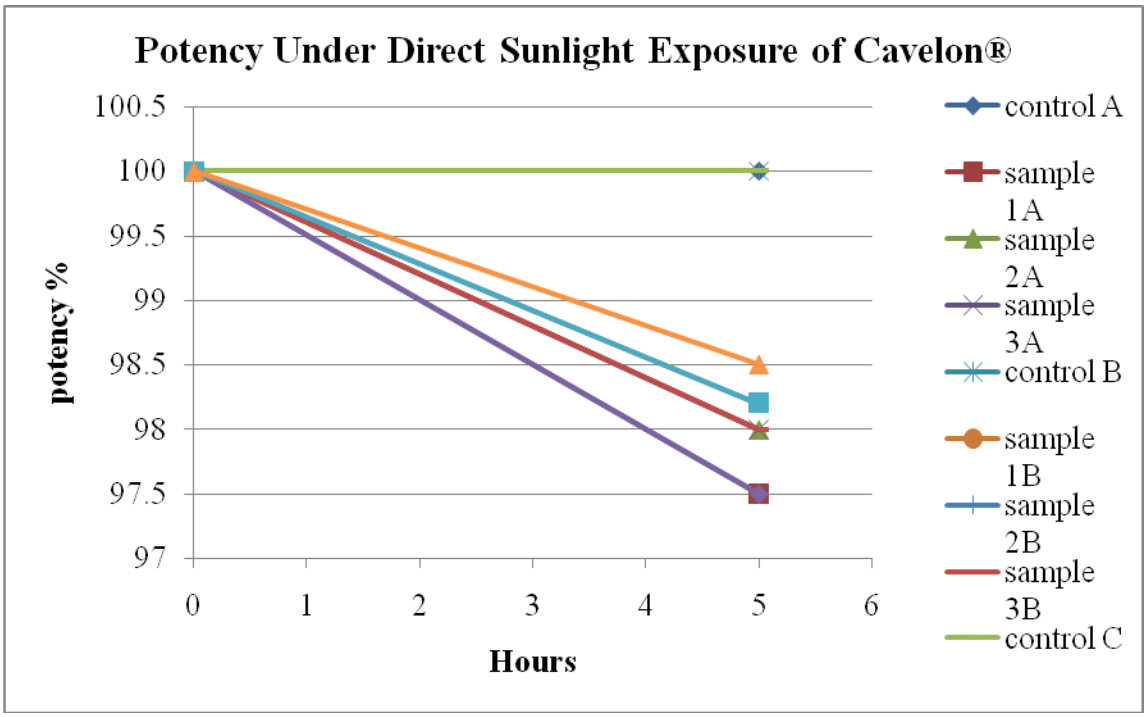

Figure 4.5: Graph showing straight line for Potency with respect to sample numbers for Cavelon® under exposure of Sunlight for 5 hours.

Chapter Five

DISCUSSION

5.1 Discussion

It was found that the concentration of carvedilol has not decreased in any of the light exposure (sunlight, normal light, 25W bulb, and 40W bulb condition). When sample tablets (Cavelon® 6.25 mg) were kept under the electrical bulb (25W & 40W) and tested after 5 hours light exposure, it was found that the potency of carvedilol hasn't decreased. Same results were found for the tablets which were kept under normal room light conditions. But under the sunlight exposure, the sample tablets were degraded in a little amount. So that, in normal room light, 25 watt bulb, and 40 watt bulb the potency of carvedilol haven't decreased. And under sunlight the sample tablets were decreased with percent deviation **1.55%**.

From this research project it can be conclude with a decision that, the packaging system of the carvedilol can be a transparent packaging but it will also be unsafe when those transparent blister strips kept in sunlight exposure. Now in local market most of the available brand of this drug is packaged in plastic transparent blister strip. But for the safety purpose, this package should be opaque thus the light cannot pass through the package.

Chapter Six

CONCLUSION

6.1 Conclusion

According to this experiment, it was observed that the physical parameters like weight variation have passed the USP and BP specification. And there were no remarkable changes in concentration/potency. The concentration of carvedilol has not decreased after it has been exposure under electrical bulb (25W & 40W) light condition, and normal light exposure (room temperature) condition. But under the direct sunlight exposure condition the potency of sample tablets has decreased in a little amount. So it can say that the Cavelon® (carvedilol) which is light sensitive but the coating can protect the concentration/potency after light exposure. But it doesn't mean coating alone is sufficient to protect the drug from light. So that packaging should be opaque thus light cannot pass through the package of the drug.

Chapter Seven

REFERENCE

7.1 References

Ahmad, I., Ahmed, S., Anwar, Z., Sheraz, M.A. and Sikorski, M., 2016. Photostability and Photostabilization of Drugs and Drug Products. *International Journal of Photoenergy*, 2016.

Ajit, P., Hate, M., Godwin, L., Sudesh, B., Amjad, A., Janardhan, T. and Vrushali, P., 2012. Method development and validation of carvedilol and its impurities by RP-HPLC. *Int J Pharm Sci*, *4*, pp.1908-15.

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.

Ashley, S.E.Q., 1939. Spectrophotometric methods in modern analytical chemistry. *Industrial & Engineering Chemistry Analytical Edition*, *11(2)*, pp.72-79.

Beer-Lambert Law [Online] Available at http://life.nthu.edu.tw/~labcjw/BioPhyChem/Spectroscopy/beerslaw.htm [Accessed 13th May 2017]

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.

Bristow, M.R., Larrabee, P., Müller-Beckmann, B., Minobe, W., Roden, R., Skerl, L., Klein, J., Handwerger, D. and Port, J.D., 1992. Effects of carvedilol on adrenergic receptor pharmacology in human ventricular myocardium and lymphocytes. *Journal of Molecular Medicine, 70,* pp.S105-S113

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.

Carvedilol-Davis's Drug Guide [Online] Available at https://www.drugguide.com/ddo/view/Davis-Drug-Guide/51134/all/carvedilol [Accessed 12th May 2017]

Carvedilol-WebMD [Online] Available at http://www.webmd.com/drugs/2/drug-5574-5168/carvedilol/details/list-interaction-medication [Accessed 13th May 2017]

Chander, C.P., Raju, B., Sulthana, A. and Srinivas, R., 2013. LC–ESI–MS/MS study of carvedilol and its stress degradation products. *Analytical Methods, 5(17),* pp.4330-4335.

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.

Coreg (Carvedilol) Drug Information: Clinical Pharmacology-RxList (the internet drug index) [Online] Available at http://www.rxlist.com/coreg-drug/clinical-pharmacology.htm [Accessed 12th May 2017]

Dailymed, (2011). Carvedilol- carvedilol tablet,film coated' [Online] Available at: https://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?setid=577b23c1-bd6d-41a4-a7c5ebe7b92e37bd (Accessesed: 13 May, 2017).

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.

Hamed, R., Awadallah, A., Sunoqrot, S., Tarawneh, O., Nazzal, S., AlBaraghthi, T., Al Sayyad, J. and Abbas, A., 2016. pH-dependent solubility and dissolution behavior of carvedilol—case example of a weakly basic BCS class II drug. *AAPS PharmSciTech*, *17(2)*, pp.418-426

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.

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.

Moore, D.E., 2004. Photophysical and photochemical aspects of drug stability. *Photostability of drugs and drug formulations,* pp.17-19.

Morgan, T., 1994. Clinical pharmacokinetics and pharmacodynamics of carvedilol. *Clinical pharmacokinetics, 26(5),* pp.335-346.

O'Neil, M.J., 2013. *The Merck index: an encyclopedia of chemicals, drugs, and biologicals*. RSC Publishing.

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.

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.

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

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.

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.

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.

Soderberg, T. (2016) UV and Visible Spectroscopy. *Chemistry Libretexts* [Online] Available at: http://chem.libretexts.org/Textbook_Maps/Organic_Chemistry_Textbook_Maps/Map%3A_Organic_Chemistry_With_a_Biological_Emphasis_(Soderberg)/Chapter_04%3A_Structure_De termination_I/4.3%3A_Ultraviolet_and_visible_spectroscopy [Accessed 12th May 2017]

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

The Beer-Lambert Law - Chemistry LibreTexts [Online] Available at https://chem.libretexts.org/Core/Physical_and_Theoretical_Chemistry/Spectroscopy/Electronic_Spectroscopy/Electronic_Spectroscopy_Basics/The_Beer-Lambert_Law [Accessed 13th May 2017]

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.

Tonnesen, H.H. ed., 2004. Photostability of drugs and drug formulations. CRC Press.

Unit 4 Drug Stability, (P-75, 76) [Online] Available at http://cst-kh.edu.ps/staff/mabujamee/wp-content/uploads/2010/10/Unit-4-Drug-Stability.pdf [Accessed 12th May 2017]

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.

Wisler, J.W., DeWire, S.M., Whalen, E.J., Violin, J.D., Drake, M.T., Ahn, S., Shenoy, S.K. and Lefkowitz, R.J., 2007. A unique mechanism of β-blocker action: carvedilol stimulates β-arrestin signaling. *Proceedings of the National Academy of Sciences*, *104(42)*, pp.16657-16662

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.