Photolytic Degradation of Carvista® (Carvedilol): An UV Analysis

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DECLARATION BY THE CANDIDATE

I, Md. Wazi Ullah, hereby declare that the dissertation entitled "Photolytic Degradation of Carvista® (Carvedilol): An UV Analysis", submitted by me to the Department of Pharmacy, East West University, in the partial fulfillment of the requirement for the degree of Bachelor of Pharmacy (Honors) with original research work carried out by me under the supervision and guidance of Md. Anisur Rahman, Senior Lecturer, Department of Pharmacy, East West University, Dhaka.

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CERTIFICATE BY THE SUPERVISOR

This is to certify that the dissertation entitled "Photolytic Degradation of Carvista® (Carvedilol): An UV Analysis" submitted to the department of pharmacy, East West University in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy was carried out by Md. Wazi Ullah (ID: 2013-1-70-022) under our guidance and supervision and that no part of the research has been submitted for any other degree. We further certify that all the sources of information and laboratory facilities availed of in this connection is duly acknowledged.

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ENDORSMENT BY THE CHAIRPERSON

This is to certified that the dissertation entitle "Photolytic Degradation of Carvista® (Carvedilol): An UV Analysis" is a bonafide research work done by Md. Wazi Ullah (ID: 2013-1-70-022) under our guidance and supervision of Md. Anisur Rahman, Assistant Professor, Department of Pharmacy, East West University, Dhaka.

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ABSTRACT

This work was aimed for the determination of photolytic degradation of carvedilol tartrate. The objective of this study was to determine the effect on carvedilol in various lighting conditions (control, sunlight, normal room light, 25watt & 40watt bulb). Besides, physical tests were performed for evaluation of color change, weight variation, thickness and hardness of Carvista® tablets from same batch according to the specification of USP. A very insignificant fluctuation in result was observed, with 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, direct sunlight and normal room light the concentration of carvedilol tartrate were decreased gradually with percent deviation 11.19%, 12.04%, 8.26% and 19.81% respectively. So it can be said that the Carvista® containing carvedilol is light sensitive and coating alone is not sufficient to protect the drug from light. So that package should be opaque thus light cannot pass through the package.

Keywords: Presonil®, Carvedilol Tartrate, Photolytic Degradations, Batch, Weight variation, Hardness, Thickness, Potency, USP.

Serial no.	Contents	Page no
	List of figure	
	Abstract	
Chapter 1	**Introduction**	**12-24**
1.1	Stability	13
1.2	Photolytic Degradation	14
1.3	Carvedilol	14
1.3.1	Physicochemical property	14
1.3.2	Pharmacodynamics	15
1.3.3	Pharmacokinetics	15
1.3.4	Medical Uses	17
1.3.5	Side effects	17
1.3.6	Contraindications	18
1.3.7	Pregnancy and breastfeeding warnings	19
1.4	Drug-Drug interaction	19
1.5	Drug-Food interaction	21
1.6	Precautions	22
1.7	Overdose	22
1.8	Dosage form and Packaging	24
Chapter 2	**Literature Review**	**25-33**
Chapter 3	**Materials and methods**	**34-46**
3.1	Materials	35
3.1.1	Sample Collection	35
3.1.2	Samples	35
3.1.3	Reagents	36
3.1.4	Equipments & Instruments	36
3.1.5	Image of Instruments	36
3.1.6	Apparatus	37
3.2	Preparation of the solvent (0.1N H2SO4)	38

3.2.1	Determination of the Concentration of the Lab Solvent (H2SO4) in Normality (N)	38
3.2.3	Determination of λmax & Preparation of the Standard Curve of Carvedilol	39
3.2.5	Preparation of nine serial concentrations of solution for carvedilol	39
Chapter 4	**Results**	**47-53**
Chapter 5	**Discussion**	**54-55**
Chapter 6	**Conclusion**	**56-57**
Chapter 7	**References**	**58-64**

Content of Table

Table no.	Content	Page no.
3.1	Sample used in the experiment including source	35
3.2	Reagents used in the experiment including source	36
3.3	List of equipment used for the experiment	36
3.4	List of apparatus used throughout this project	37
3.5	Concentration for preparation of standard curve of Carvedilol	40
3.6	Electric bulb exposed sample lidt	44
3.7	Sunlight exposed sample list	45
3.8	Accepted percentage list for weight variation test	46
4.1	Concentration & Absorbance for standard curve of Carvedilol	48
4.2	Weight variation test of Carvedilol	49
4.3	Concentration & Absorbance of 60 days interval of Carvedilol	50
4.4	Concentration & Absorbance of 5 hour interval of Carvedilol under 25 watt	51

4.5	Concentration & Absorbance of 5 hour interval of Carvedilol under 40 watt	52
4.6	Concentration & Absorbance of 5 hour interval of Carvedilol under Sunlight	53

Content of Figure

Figure no.	Content	Page no.
1.1	Chemical structure of Carvedilol	15
3.1	Carvista® 6.25mg tablets	35
3.2	UV spectrophotometer & Electronic balance	36
4.1	Standard curve of carvedilol	49
4.2	Potency under roomlight exposure of Carvista®	50
4.3	Potency under 25 watt bulb exposure of Carvista®	51
4.4	Potency under 40 watt bulb exposure of Carvista®	52
4.5	Potency under Direct sunlight exposure of Carvista®	53

Chapter One

Introduction

Objective

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

1.1 Stability

The stability of a drug is the ability of the drug to maintain its chemical, physical, therapeutic and toxicological properties throughout its shelf life. Often times environmental and other unexplainable factors affect the stability of drugs by causing degradation of either the active ingredient of the drug product or the excipients, reducing the bioavailability of the drug or changing the physical appearance of the drug. Factors that affect drug stability include temperature, moisture, light, microorganisms, packaging materials, transportation, components of drug compositions and nature of the API. (Bahl et al, 2006)

1.2 Photolytic degradation

Photolysis is the process by which light sensitive drugs or excipient molecules are chemically degraded by light, sunlight or room light. Ultraviolet light has more harmful radiation that affects the drug. Shorter wavelengths are more damaging than longer wavelengths. For a photolytic reaction, the energy from the light radiation must be absorbed by the molecules.

Photolysis occurs in two ways-

- Firstly, the light energy must beb sufficient to activate the reaction or,
- Secondly, energy absorbed by the molecule is passed on to other which allows degradation to take place.

Several reactions take place like oxidation, polymerization or ring rearrangement. Light energy converted into thermal energy. The photolytic reaction may produce catalyst for the thermal reaction.

For this Experiment a photosensitive drug “CARVEDILOL” was taken as sample.

1.3 Carvedilol

Carvedilol belongs to a class of drugs known as alpha and beta blockers. Beta-blockers affect the heart and circulation (blood flow through arteries and veins). It is used to treat heart failure and hypertension (high blood pressure). It is also used after a heart attack that has caused your heart not to pump as well. Carvedilol may also be used for purposes not listed in this medication guide. This drug works by blocking the action of certain natural substances in your body, such as epinephrine, on the heart and blood vessels. This effect lowers your heart rate, blood pressure, and strain on your heart. (webmd.com, 2017)

Carvedilol was discovered by Fritz Wiedemann at Boehringer Mannheim and was initially approved in the U.S. in 1995. On October 20, 2006, the FDA approved an extended-release formulation. (pubmed.gov, 2017)

In this research project experiment conducted on sample which was manufactured by Incepta Pharmaceuticals Limited. (Brand name: Carvista). It is a solid dosage form which is mainly tablets of 6.25mg. Carvista (Carvedilol) is indicated for the treatment of mild or moderate heart failure of ischemic or cardiomyopathic origin, in conjunction with digitalis, diuretics and ACE inhibitor, to reduce the progression of disease as evidenced by cardiovascular death, cardiovascular hospitalization, or the need to adjust other heart failure medications.

(inceptapharma.com, 2017)

1.3.1 Physico-chemical properties (pubchem.gov, 2017)

- **Chemical properties:**
  - Chemical name: 1-(9H-carbazol-4-yloxy)-3-[2-(2- methoxyphenoxy) ethylamino] propan-2-ol
  - Molecular formula: $C_{24}H_{26}N_2O_4$

- Molecular weight: 406.482 g/mol

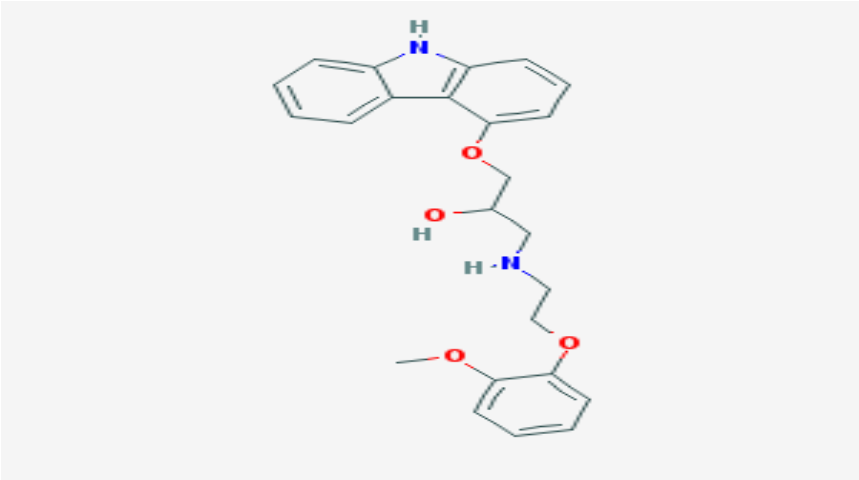

Figure1.1: Chemical structure of Carvedilol

➢ Physical properties:

- Color: Colorless crystals from ethyl acetate
- Physical description: Solid
  - ✓ Melting point 114.5°C
  - ✓ Water Solubility Practically insoluble (0.583 mg/L)
  - ✓ Freely soluble in dimethylsulfoxide; soluble in methylene chloride, methanol; sparingly soluble in ethanol, isopropanol; slightly soluble in ethyl ether
  - ✓ Odorless and slightly bitter taste
  - ✓ pH neutral (O'Neil et al, 2006)

1.3.2 Pharmacodynamics

Carvedilol is a nonselective beta-adrenergic blocking agent with alpha1-blocking activity and is indicated for the treatment of hypertension and mild or moderate (NYHA class II or III) heart failure of ischemic or cardiomyopathic origin. Carvedilol is a racemic mixture in which nonselective b-adrenoreceptor blocking activity is present in the S (-) enantiomer and a-adrenergic blocking activity is present in both R (+) and S (-) enantiomers at equal potency. Carvedilol has no intrinsic sympathomimetic activity. The effect of carvedilol's b-adrenoreceptor

blocking activity has been demonstrated in animal and human studies showing that carvedilol (1) reduces cardiac output in normal subjects; (2) reduces exercise-and/or isoproterenol-induced tachycardia and (3) reduces reflex orthostatic tachycardia. (drugbank.ca, 2017)

1.3.3 Pharmacokinetics (pubchem.gov, 2017)

- ✓ **Absorption**

Carvedilol is rapidly and extensively absorbed following oral administration, with an absolute bioavailability of approximately 25% to 35% due to a significant degree of first-pass metabolism. Absorption is slowed when administered with food; however, it does not show a significant difference in bioavailability. Taking carvedilol with food decreases the risk of orthostatic hypotension.

- ✓ **Distribution**

The majority of carvedilol is bound to plasma proteins (98%), mainly to albumin. Carvedilol is a basic, hydrophobic compound with a steady-state volume of distribution of 115 L. Plasma clearance ranges from 500 to 700 mL/min.

- ✓ **Metabolism**

The compound is metabolized by liver enzymes, CYP2D6 and CYP2C9 via aromatic ring oxidation and glucuronidation, and then further conjugated by glucuronidation and sulfation. In comparison with the parent compound, the three active metabolites exhibits only one-tenth of the vasodilating effect of the parent compound. However, the 4-hydroxyphenyl metabolite is about 13-fold more potent in ß-blockade than the parent compound. The mean half-life of carvedilol following oral administration ranges from 7 to 10 hours. Carvedilol has two enantiomers: R(+)-carvedilol and S(-)-carvedilol. R(+)-carvedilol undergoes preferential selection for metabolism, so the mean half-life of the enantiomer is about 5 to 9 hours compared with 7 to 11 hours for the S(-)-enantiomer.

- ✓ **Route of elimination**

Carvedilol is extensively metabolized. 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.

1.3.4 Medical Uses

Carvedilol is used for the treatment of mild or moderate (NYHA class II or III) heart failure of ischemic or cardiomyopathic origin. In conjunction with digitalis, diuretics and ACE inhibitor, to reduce the progression of disease as evidenced by cardiovascular death, cardiovascular hospitalization, or the need to adjust other heart failure medications. (inceptapharma.com, 2017)

Carvedilol is a unique antihypertensive medication with activity against both alpha- and beta-adrenergic receptors. Carvedilol has been linked to at least one instance of clinically apparent liver injury. (livertox.gov, 2017)

Carvedilol may be used in patients unable to tolerate an ACE inhibitor. Carvedilol may also be used in patients who are not receiving digitalis, hydralazine or nitrate therapy.

(inceptapharma.com, 2017)

1.3.5 Side effects

The most common side effects (>10% incidence) include:

- Postural hypotension
- dizziness
- headache
- fatigue
- gastro-intestinal disturbances

- bradycardia; occasionally diminished peripheral circulation
- peripheral oedema and painful extremities
- dry mouth, dry eyes, eye irritation or disturbed vision, impotence
- disturbances of micturitioni
- influenza-like symptoms, rarely angina
- AV block, exacerbation of intermittent claudication or Raynaud's phenomenon
- allergic skin reactions
- exacerbation of psoriasis
- nasal stuffiness, wheezing
- depressed mood, sleep disturbances
- paresthesia, heart failure
- changes in liver enzymes
- thrombocytopenia
- leukopenia are also reported. (inceptapharma.com, 2017)

Carvedilol is not recommended for people with uncontrolled bronchospastic disease (e.g. current asthma symptoms) as it can block receptors that assist in opening the airways. Carvedilol may mask symptoms of low blood sugar (hypoglycemia), resulting in hypoglycemia unawareness. This is termed beta blocker induced hypoglycemia unawareness. (GlaxoSmithKline, 2008)

1.3.6 Contraindication

- Bronchial asthma or related bronchospastic conditions
- Second- or third-degree AV block
- Sick sinus syndrome Severe bradycardia (unless a permanent pacemaker is in place)
- Patients with cardiogenic shock or who have decompensated heart failure requiring the use of intravenous inotropic therapy. Such patients should first be weaned from intravenous therapy
- Patients with severe hepatic impairment

- Patients with a history of a serious hypersensitivity reaction (e.g., Stevens-Johnson 83 syndrome, anaphylactic reaction, angioedema) to carvedilol.

1.3.7 Pregnancy and breastfeeding warnings

Use of beta-blockers in pregnancy has been associated with adverse effects on fetal growth, although because maternal hypertension is linked to intrauterine growth restriction, analysis is complex and any contribution of beta-blocker exposure to this outcome remains unquantified. Overall, data do not suggest that gestational beta-blocker exposure increases the risk of preterm delivery. Data on rates of spontaneous abortion, stillbirth and neurodevelopmental outcomes are too limited to permit a risk assessment. (medicinesinpregnancy.org, 2017)

This drug should be used during pregnancy only if the benefit outweighs the risk. AU TGA pregnancy category: C US FDA pregnancy category: C. Beta blockers reduce placental perfusion which may result in intrauterine fetal death and immature and premature deliveries.

This drug appears to present a low risk to the breastfed infant; however, because there is no published experience during breastfeeding, other agents may be preferred, especially while nursing a newborn or preterm infant.

Breastfeeding or discontinue the drug, taking into account the importance of the drug to the mother. This drug excreted into human milk. The effects in the nursing infant are unknown; however, the possibility of the consequences of alpha and beta blockade should be considered.

(drugs.com, 2017)

1.3.8 Doses

- ✓ **Adults:**

Usual Adult Dose for Congestive Heart Failure:

Immediate-release tablets:

-Initial dose: 3.125 mg orally twice a day for 2 weeks

-Titration: If tolerated, increase dosage to 6.25, 12.5, and 25 mg orally twice a day over successive intervals of at least 2 weeks

-Maximum dose: 25 mg orally twice a day in patients weighing 85 kg or less and 50 mg orally twice a day in patients weighing 85 kg or greater

Usual Adult Dose for Left Ventricular Dysfunction

Immediate-release tablets:

-Initial dose: 6.25 mg orally twice a day for 3 to 10 days

-Titration: If tolerated, increase dosage to 12.5 mg orally twice a day, then again to 25 mg orally twice a day after successive intervals of at least 3 to 10 days

-Maintenance dose: 25 mg orally twice a day

-Alternative dose: A lower starting dose may be used (3.125 mg orally twice a day) and/or the rate of up-titration may be slowed if clinically indicated (e.g., due to low blood pressure or heart rate, or fluid retention)

Usual Adult Dose for Hypertension

Immediate-release tablets:

-Initial dose: 6.25 mg orally twice a day (if this dose is tolerated, using standing systolic pressure measured about 1 hour after dosing as a guide) maintain for 7 to 14 days

-Titration: Increase to 12.5 mg orally twice a day if needed for 10 to 14 days, then to 25 mg orally twice a day if needed

-Maximum dose: 50 mg orally twice a day

Renal Dose Adjustments

Patients with underlying renal dysfunction may require extra monitoring during dosage increases; the dose should be reduced or therapy discontinued if a worsening of renal function occurs.

Liver Dose Adjustments

Severe hepatic impairment: Contraindicated (drugs.com, 2017)

1.4 Drug-Drug interaction

Possible conduction disturbance, rarely with hemodynamic compromise. Blood pressure and ECG should be monitored during concomitant use of carvedilol with diltiazem or verapamil.

Concurrent administration of myocardial depressant general anesthetics (ether, cyclopropane, trichloroethylene) has the potential to increased risk of hypotension and heart failure.

Concurrent administration of catecholamine-depleting agents (eg, reserpine, MAO inhibitors) may have potentially additive effects (eg, hypotension, bradycardia). Patients should be monitored closely for symptoms (eg, vertigo, syncope, postural hypotension).

Potential additive effects (eg, hypotension, bradycardia). If carvedilol is used concomitantly with clonidine, caution should be exercised, particularly when discontinuing therapy; carvedilol generally should be discontinued first, and clonidine continued for several days thereafter with gradual downward dosage titration.

Potential pharmacokinetic and pharmacodynamic interaction with concurrent administration of carvedilol and digoxin. Digoxin concentrations are increased by about 15% in patients receiving concomitant therapy with digoxin and carvedilol. Both cardiac glycosides and carvedilol slow AV conduction and decrease heart rate; concomitant use may increase risk of bradycardia. Digoxin therapy should be carefully monitored when carvedilol dosage is initiated, adjusted, or discontinued.

Concurrent use of paroxetine has the potential to increased plasma concentrations of R(+)-carvedilol that may result in increased alpha-adrenergic blockade effects (vasodilation).

Concurrent use of Propafenone has the potential to increased plasma concentrations of R(+)-carvedilol that may result in increased alpha-adrenergic blockade effects (vasodilation).

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.

(pubchem.gov, 2017)

1.5 Drug-Food interaction

Carvedilol and ethanol may have additive effects in lowering your blood pressure. May experience headache, dizziness, lightheadedness, fainting, and/or 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.

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. (gordon et al, 1997)

1.6 Precautions

- ✓ Patients with coronary artery disease, who are being treated with carvedilol, should be advised against abrupt discontinuation of therapy.
- ✓ If pulse rate drops below 55 beats/minute, the dosage should be reduced.
- ✓ If postural hypotention occurs, therapy should be discontinued.
- ✓ Should take caution in hepatic impairment and in heart failure monitor clinical status for 2-3 hours after initiation and after increasing each dose. Before increasing dose ensure that the renal function and heart failure are not deteriorating.
- ✓ 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.

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

1.7 Overdose

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. The following agents may be administered:

For excessive bradycardia: Atropine, 2 mg IV. (rxlist.com, 2017)

1.8 Dosage form and Packaging

Carvista 6.25 : Box containing 10 blister strips of 10 tablets.

Carvista 12.5 : Box containing 5 blister strips of 10 tablets.

Carvista 25 : Box containing 3 blister strips of 10 tablets.

Chapter Two

Literature review

2.1 Literature Review

Photosensitive drugs degrade under the exposure of normal or extreme light condition. In Bangladesh, there are different carvedilol drugs available and they r marketed as different brands. From available brands one brand that is ‘’carvista’’ was chosen for determining its photosensitivity. These products are available in blister packaging system in most case in the market of Bangladesh. To find whether this drug is photosensitive or not, we operate a research program to establish a data about photolytic degradation of carvedilol.

A high performance liquid chromatography procedure has been developed for determination of carvedilol in human plasma. The method was developed on Lichrosphere R CN column using a mobile phase of acetonitrile/20 mM ammonium acetate buffer with 0.1% triethylamine (pH adjusted to 4.5) (40/60, v/v). Using fluorescence detector (excitation wavelength 282 nm and emission wavelength 340 nm) carvedilol was extracted by liquid–liquid extraction procedure using dichloromethane. The accuracy ranges from 87.3 to 100.88% and the recovery of carvedilol was 69.90%. The stability studies showed that carvedilol in human plasma was stable during short-term period for sample preparation and analysis. This method was used to assay the carvedilol in human plasma samples obtained from subjects who had been given an oral tablet of 12.5 mg carvedilol. (Rajeshwari et al. 2007)

A study was done aimed to evaluate and correlate the physicochemical and processability properties of carvedilol. Carvedilo from three different manufacturers were characterized according to their flowability, particle size and apparent density and using microscopy, differential scanning calorimetry (DSC), thermogravimetric analysis etc were tested. The tests to evaluate flowability and compressibility were shown to be discriminative, and slight differences among the samples were noted. (Josyane et al. 2015)

As a nonselective β-adrenergic blocking agent with vasodilating activity carvedilol is used for the treatment of congestive heart failure and hypertension. During the bulk synthesis of carvedilol, they have observed six impurities: Imp-I, Imp-II, Imp-III, Imp-IV, Imp-V, and Imp-VI. The present work describes the synthesis and characterization of these impurities.

(Rao, Sitaramiah et al. 2010)

An 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. They did a Chromolith Performance (RP-18e, 100 mm × 4.6 mm) column with an isocratic mobile phase consisting of 0.01 M disodium hydrogen phosphate buffer–acetonitrile (40:60, v/v) adjusted to pH 3.5. The coefficients of variation for inter-day and intra-day assay were found to be less than 8.0%. (Zarghi et al. 2007)

A placebo-controlled trial was designed to establish the efficacy and safety of carvedilol, a "third-generation" β-blocking agent with vasodilator properties, in chronic heart failure. *Methods and Results* Three hundred forty-five subjects with mild to moderate, stable chronic heart failure were randomized to receive treatment with placebo, 6.25 mg , 12.5 mg, or 25 mg. When the three carvedilol groups were combined, the all-cause actuarial mortality risk was lowered by 73% in carvedilol-treated subjects (P<.001). Carvedilol also lowered the hospitalization rate and was generally well tolerated. (Michael, Edward et al. 1996)

Eric et al., investigate how ionization and ion-transfer efficiencies are affected by drastically reducing the flow into the MS. A postcolumn concentric flow-splitting device was constructed to allow the measurement of analyte signal and ionization suppression across a range of flow rates (0.1−200 μL/min). Using this device, the effects of flow rate on signal intensity and ionization suppression were measured in analytical experiments that included flow injection analysis MS, postcolumn addition LC−MS, and on-line LC−MS analysis of metabolites generated from rat liver microsomes. A significant improvement in concentration and mass sensitivity as well as a reduction in signal suppression is observed when the performance at 200 versus 0.1 μL/min flow rate is compared. (Eric et al. 2001)

In March 2013, Rehab N. Shamma and Mona Basha investigate the applicability of different industrially scalable techniques in the preparation of solid dispersions using a novel polyvinyl caprolactam–polyvinyl acetate–polyethylene glycol graft copolymer for preparing formulations of a poorly water-soluble BCS class II drug carvedilol. (Rehab and Mona, 2013)

Solubilities of carvedilol in binary mixtures of (ethanol + propylene glycol (PG)) at 298.2, 303.2, 308.2, and 313.2 K are reported. The modified versions of the van't Hoff and Gibbs equations were used to calculate the thermodynamic properties. Solubility data of seven drugs in (ethanol

(1) + PG (2)) at different temperatures were used to develop a quantitative structure–property relationship model for predicting solubility in solvent mixtures. In addition, enthalpy–entropy compensation using $\Delta H°$ vs $\Delta G°$ and $\Delta H°$ vs $T\Delta S°$ which explains the mechanism of cosolvency at different temperatures. (Sahel, Ali et al. 2013)

In 2013, Z. Wojnarowska, Y. Wang report the relation between ionic conductivity and structural relaxation in supercooled protic ionic liquids (PILs) under high pressure. The results of high-pressure dielectric and volumetric measurements, combined with rheological and temperature-modulated differential scanning calorimetry experiments, have revealed a fundamental difference between the conducting properties under isothermal and isobaric conditions for three PILs with different charge transport mechanisms. (Wojnarowska et al. 2013)

Hajnalka Pataki et al., real-time Raman spectroscopy is more than just an effective tool for monitoring drug crystallizations. The results verify that fibre-optic-coupled Raman spectroscopy can be used not only for monitoring processes but also for ensuring the production of the desired polymorphs by continuous feedback of the polymorph signal in the course of crystallization procedures. The aim of the control, based on Raman-signal feedback, was to ensure that the quality of the drugs was maintained in crystallization processes, despite such disturbing influences. (Hajnalka Pataki et al. 2012)

Carvedilol, a β-blocker drug used to treat hypertension, is known to exhibit polymorphism. Thus far, the crystal structure of two polymorphs (**I** and **II**) and one hydrate have been reported. In this study, three crystal modifications of carvedilol were obtained from crystallization experiments. The structure of another polymorph (**III)** was elucidated for the first time and the crystal structure of the hydrate was also determined from single-crystal diffraction data. This result showed that intermolecular interactions influence the solid-state properties of carvedilol. Moreover, polymorph **III** exhibits a higher dissolution rate than **II**, showing great potential for formulation strategies of this poorly water soluble drug. (Livia et al. 2014)

Devid et al. in 2003 studiedto investigate the chiral components of carvedilol. Further, the effects of substituent variation about a stereocenter are investigated and discussed using conformational energy as a surrogate of structure (based on the fact that energy is a function of molecular spatial orientation) to determine the energetic equivalency of prochiral and chiral structures. The

combination of prochiral and chiral potential energy surfaces, according to an equation describing two consecutive and concerted methyl substitutions, gave a practically flat, virtually zero surface, indicating that sufficiently constructed achiral structures are energetically equivalent to chiral enantiomers. (Devid et al. 2003)

Density functional theory (DFT) conformational analysis was carried out on the potential energy hypersurface (PEHS) of the carbazole-containing molecular fragment, *S*-4-(2-hydroxypropoxy)-carbazol, of the chiral cardiovascular drug molecule carvedilol. (Luca et al. 2002)

Anshu et al. investigate the variations in bioavailability of carvedilol. The purpose of the present investigation was to increase the solubility and dissolution rate of carvedilol for enhancement of oral bioavailability. The dissolution rate was substantially improved for carvedilol from its solid dispersion compared with pure drug and physical mixture. (Anshu et al. 2013)

André Talvani et al. investigate kinetic analysis under isothermal conditions using thermogravimetry of carvedilol. It was performed to determine the activation energy of CARVE through an Arrhenius plot. Differential scanning calorimetry, Fourier transform infrared spectroscopy, and optical microscopy were used to test binary mixtures and selected excipients. (André Talvani et al. 2013)

In the year of 2008, Fibele et al. forced and accelerated degradation studies of carvedilol (CAR) were carried out according to ICH guideline Q1A (R2). The drug was subjected to acid (1.0 N HCl), alkaline (1.0 N NaOH), and neutral hydrolytic conditions by refluxing at 90°C, as well as to oxidative (7.5% H_2O_2) decomposition, protected from light, at room temperature. Photolysis was carried out in solid state of the drug and in methanolic solution. The stress degradation samples were evaluated by LC and LC–MS. The kinetics of degradation were determined by the LC method, previously developed and validated for our group, that could separate the degradation products formed under various stress conditions. (Fibele et al. 2008)

Impregnation of porous SiO_2 (Sylysia) with carvedilol from acetone solution was used to improve dissolution of this poorly water-soluble drug. Solvent evaporation in a vacuum evaporator and adsorption from acetone solution were the methods used to load various amounts of carvedilol into the Sylysia pores. The impregnated carriers were characterized using nitrogen-

adsorption experiments, X-ray diffraction, wettability measurements, attenuated total reflectance FTIR spectroscopy and thermal analysis. (Odon et al. 2011)

A sensitive, selective, precise and stability-indicating, new high-performance liquid chromatographic method for the analysis of carvedilol both as a bulk drug and in formulations was developed and validated. As the method could effectively separate the drug from its degradation products, it can be employed as a stability-indicating one. The method was validated for linearity, selectivity, precision, robustness, LOD, LOQ and accuracy. This method enables the simultaneous determination of carvedilol and its degradation products, as well as stability. (Stojanović et al. 2007)

In August 2005, a rapid, sensitive and specific method to quantify carvedilol in human plasma using metoprolol as the internal standard (IS) is described. The analyte and the IS were extracted from plasma by liquid–liquid extraction using a diethyl-ether solvent. After removed and dried the organic phase, the extracts were reconstituted with a fixed volume of acetonitrile–water (50/50; v/v). The extracts were analyzed by a high performance liquid chromatography coupled to electrospray tandem mass spectrometry (HPLC–MS/MS). (Carter et al. 2005)

Olga et al. in 2008, during stability testing of CV solid dosage forms an unknown degradation product referred as UP, exceeded the identification thresholds of ICH Q3B guidelines. The HPLC analysis of the detected unknown product was performed by a newly, developed, specific and validated method, also suitable for the quantitative determination of the known CV impurities (imp B, C, E and F) and the other degradation products. The separation was achieved with an X-terra C_{18} column, using acetonitrile–phosphate buffer pH 2.5 as mobile phase. (Olga et al. 2008)

In 2009, Mohammad Rizwan et al. set an investigation were 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. Chromatographic separation was achieved on a C_{18} column with 55:45 (%, *v*/*v*) acetonitrile–0.02 M phosphate buffer, pH 3.5, as mobile phase at a flow rate of 1.0 mL min^{-1}; detection was by UV absorbance at 242 nm. The method was validated for linearity, precision, accuracy, robustness, specificity, and sensitivity, with the bulk drug. (Rizwan et al. 2009)

Two carvedilol aqueous solutions and one carvedilol aqueous suspension for paediatric oral use (1 mg/ml) were studied to determine their stability. All samples were stored at 4, 25 and 40° C. Carvedilol content of each of the three formulations was tested using high performance liquid chromatography (HPLC). Each sample was analysed in triplicate at 0, 3, 7, 14, 28 and 56 days. Carvedilol stayed stable in the acidic aqueous solution at the three different temperatures during the 56 days of the study. In the alkaline solution, carvedilol was stable during 56 days at 25° C, but only 28 days at 4 and 40° C. In the aqueous suspension, carvedilol was stable during 56 days at 4 and 25° C, but only 28 days at 40° C. (Buontempo et al. 2010)

In 2010 V. Raju AND K. V. R. Murthy studied carvedilol. The objective of the present study was to develop and validate a discriminative dissolution method for evaluation of carvedilol tablets. Different conditions such as type of dissolution medium, volume of dissolution medium and rotation speed of paddle were evaluated. (Raju et al. 2010)

A capillary electrophoresis method was used for assay of some degradation products of carvedilol. The optimized parameters were as; running buffer 80 mM acetate dissolved in methanol/ethanol mixture (65:35 v/v), applied voltage of 19 kV, temperature is 20 ºC and the wavelength range of 200-350 nm. The results indicate that the proposed CE method could effectively separate carvedilol from its degradation products and can be employed as a stability indicating assay method. (Abolghasem et al. 2014)

In 2016 Rania et al. investigate the pH-dependent solubility and dissolution of weakly basic Biopharmaceutical Classification Systems (BCS) class II drugs, characterized by low solubility and high permeability, using carvedilol, a weak base with a pK_a value of 7.8, as a model drug. A series of solubility and *in vitro* dissolution studies was carried out using media that simulate the gastric and intestinal fluids and cover the physiological pH range of the GI from 1.2 to 7.8. The effect of ionic strength, buffer capacity, and buffer species of the dissolution media on the solubility and dissolution behavior of carvedilol was also investigated. (Rania et al. 2016)

In this study, new and rapid stability indicating ultraviolet spectroscopic methods were developed and validated for the estimation of ezetimibe and carvedilol in pure form and in their respective formulations. Since both the drugs are poorly water soluble, 20% v/v acetonitrile in

triple distilled water was selected as the solvent system for both the drugs. This ensured adequate drug solubility and maximum assay sensitivity. (Imran, M. et al. 2006)

In 2009 Enas A. Mahmoud conducted a research on carvedilol. The purpose of this study was to combine the advantages of self-nanoemulsifying drug delivery systems and tablets as a conventional dosage form emphasizing the excipients' effect on the development of a new dosage form. Prepared self-nanoemulsifying tablet produced acceptable properties of immediate-release dosage forms and expected to increase the bioavailability of carvedilol. (Enas et al. 2009)

Vijay et al. in 2009 investigate to design and evaluate controlled release tablets of carvedilol employing synthetic polymers like polyethylene oxides, of different molecular weights as release retarding materials and to select the optimized formulation based on the pharmacokinetics of carvedilol. Matrix tablets each containing 80 mg of carvedilol were formulated employing PEO N60 K, PEO 301, and PEO 303 as release-retarding polymers and β Cyclodextrin and HP β cyclodextrin as release modulators from the matrix. carvedilol release from the formulated tablets was very slow. Hence the release was modulated with the use of cyclodextrins. The dissolution from the matrix tablets was spread over more than 24 hours and depended onthe type of polymer, its concentration and the type of cyclodextrin used. (Vijay et al. 2009)

Burra et al. in 2012 attempt to enhance the dissolution profile, absorption efficiency and bioavailability of water insoluble drugs, such as Carvedilol. A novel "Powder Solution Technology" involves absorption and adsorption efficiency, which makes use of liquid medications, admixed with suitable carriers, coating materials and formulated into a free flowing, dry looking, non-adherent and compressible powder forms. Based upon a new mathematical model expression, improved flow characteristics and hardness of the formulation have been achieved. (Burra et al. 2012)

Azharuddin et al. studied to design and evaluate fast dissolving carvedilol tablets using β-Cyclodextrin and superdisintegrants adopting sublimation technique. Tablets were prepared by direct compression method. Tablets were evaluated for their physico chemical properties, wetting time, disintegration, in-vitro release and stability studies. SEM analysis was carried out to determine the surface characteristics of solid dispersions. Precompressional studies revealed

good micromeritic properties of powder blend for direct compression. The hardness (3.9-4.3 kg/cm2), friability (0.35-0.51), drug content (96.58-99.43 %) and disintegration time (44.05-66.21 sec) of fast dissolving tablets were found uniform and reproducible.

(Azharuddin et al. 2012)

In 2006, Sherry Wang, Matthew Cyronak and Eric Yang did Several experiments, including dilution of the extract with LC mobile phase and post-column infusion of the carvedilol solution followed by the injection of extracted blank plasma, have indicated that a high level of matrix suppression affected the ionization of the carvedilol-*S* enantiomer and its deuterated internal standard differently in these two lots of plasma. For the first time, it was clearly demonstrated that a slight difference in retention time between the analyte and the SIL internal standard, caused by deuterium isotope effect, has resulted in a different degree of ion suppression between these two analogues. This difference was significant enough to change the analyte to internal standard peak area ratio and affect the accuracy of the method. (Sherry et al. 2006)

Chapter Three

Materials &Methods

3.1 Materials

3.1.1 Sample Collection

For the purpose of experimentation to observe the photolytic degradation of carvedilol as well as to assess the coating efficiency, 500 tablets of Carvista® (carvedilol 6.25mg) were collected from the local drug store in Dhaka as a sample. All the tablets were from the same batch (16027). Among them 200 tablets were kept light protected for control tests and the remaining 300 tablets were subjected to various lighting conditions over certain periods of time for conducting experiments to determine their potency.

3.1.2 Sample

Table 3.1: Samples Used in the Experiment Including Source (Incepta, 2012)

Sample Name	Source (Supplier Name)	Batch No.
Carvista 6.25mg Tablets	Incepta Pharmaceutical Ltd.	16027

Figure3.1: Carvista® 6.25mg Tablets

3.1.3 Reagents

Table 3.2: Reagents Used in the Experiment Including Source

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

3.1.4 Equipments & Instruments

Table 3.3: Lists of Equipments Used for the Experiment

Serial No.	Equipments	Source (supplier name)	Origin
1	UV-Spectrophotometer	Shimadzu UV1800	Japan
2	Distill Water Plant	Bibby Scientific W4000	United Kingdom
3	Electronic Balance	Shimadzu AY220	Japan

3.1.5 Images of Instruments

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

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

3.1.6 Apparatus

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

Table 3.4: List of Apparatus Used throughout this Project

Serial No.	Apparatus
1	Funnel
2	Spatula
3	Beakers
4	Forceps
5	Test tubes
6	Glass Rod
7	Table Lamp
8	Pipette (10 ml, 5 ml, 2 ml)
9	Filter Papers
10	Masking Tap
11	Thermometer
12	Pipette pumper
13	Plastic Dropper
14	Test tube Holder
15	Mortar & Pestles
16	Plastic Containers
17	Aluminum foil paper
18	Electric Bulb (25 Watt & 40 Watt)
19	Volumetric Flasks (50 ml, 250ml & 1000 ml)

3.2 Preparation of the solvent (0.1N H2SO4)

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

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

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

100 ml of lab solvent stock solution contains = (98 x 1.84)gm of H_2SO_4
= 180.32gm of H_2SO_4

1000 ml of stock solution contains = (180.32 x 1000)/100 gm of H_2SO_4
= 1803.2gm of H_2SO_4

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 1000ml of 0.1N HCL solvent was calculated as below.

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

Where,

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

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 mark to make 1000ml of 0.1N H_2SO_4.

1. Standards of carvedilol was collected from the pharmaceutical company Incepta Pharmaceuticals Ltd. The potency of standard compounds was 99.56%.
2. The specific λmax 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 Incepta Pharmaceuticals Ltd.
3. Nine serial concentrations of the standards of carvedilol were prepared for the purpose of creating a standard curve.

3.2.4 Preparation of the stock solution for carvedilol using the standard obtained from Incepta Pharmaceuticals Ltd:

⇨ 50 mg of the standard compound, that is carvedilol was weighed and dissolved in 250ml 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

⇨ 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

3.2.5 Preparation of nine serial concentrations of solution for carvedilol:

- Carvedilol had the concentration of its stock solution is 0.02 mg/ml.

- Nine serial concentrations that were prepared for carvedilol were as follows 0.001 mg/ml, 0.002 mg/ml, 0.003 mg/ml, 0.004 mg/ml, 0.005 mg/ml, 0.006 mg/mi, 0.007 mg/ml, 0.008 mg/ml and 0.009 mg/ml for a final volume of 10 ml.
- The amount of the solution that were required from the stock solution to prepare the above concentrations were calculated using S1V1=S2V2 formula, where S1= initial strength or concentration, S2= final strength or concentration, V1= initial volume and V2= final volume.

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

Table 3.5: Concentration for Preparation of Standard Curve of Carvedilol

Sample name	Sample no.	Concentration (mg/ml)
Carvedilol	1	.001
	2	.002
	3	.003
	4	.004
	5	.005
	6	.006
	7	.007
	8	.008
	9	.009

- V1= S2V2 / S1 = (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 H2SO4) of carvedilol.
- V1= S2V2 / S1 = (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 H2SO4) of carvedilol.
- V1= S2V2 / S1 = (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 H2SO4) of carvedilol.

- V1= S2V2 / S1 = (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 H2SO4) of carvedilol.
- V1= S2V2 / S1 = (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 H2SO4) of carvedilol.
- V1= S2V2 / S1 = (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 H2SO4) of carvedilol.
- V1= S2V2 / S1 = (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.
- V1= S2V2 / S1 = (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 H2SO4) of carvedilol.
- V1= S2V2 / 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 H2SO4) 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 $y = mx+c$, where the components of the equations are described as provided below:

m = gradient value, y = absorbance values, x = concentrations and c = y-intercept.

3.2.6 Sampling, Analysis by UV-Spectrophotometry & Determination of Potency of the pharmaceutical drugs (metoprolol tartrate) under various lighting condition:

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

1. Exposure under normal lighting conditions in the room

2. Under electric bulb exposure (25 watt & 40 watt)

3. Direct Sunlight exposure

1. Exposure under Normal Lighting Condition

1) The tablets (Carvista®) were kept under normal lighting condition in the room for 4 months.

2) They were sampled after specific intervals like periodically after 15 days for determination their physical properties (like thickness, hardness & weight variation) and their potency.

3) On the sampling day, a piece of white paper was taken and all the details (brand name of the tablets, date of the sampling etc.) were written on top of the paper.

4) Now, 5 tablets were taken out and from this 15 tablets, 5 tablets were kept on over that white paper.

5) A photograph was taken of that paper showing the tablets with their appearances and those details.

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

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

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

b. 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:

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

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

d. 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.

e. After that 10 ml solution was filtered and 5 ml of that filtered solution was taken and dissolved in 50ml of the solvent.

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

g. From test-tube the solution was poured into a cuvette and was inserted into the UV spectrophotometer to observe the absorbance value.

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

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

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

1) 15 tablets were exposed to electric bulb lighting conditions for 5 hours at a stretch and 15 tablets were used as control.

2) 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.

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

4) The tablets were then used for potency determination to see the effect of the exposure of bulb's lighting condition to drug ingredients.

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

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

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

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

c. 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.

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

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

f. 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

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

7) 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

1) 15 tablets were kept in a Glass box and exposed to sunlight condition for 7.5 hours at a stretch.

2) 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.

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

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

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

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

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

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

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

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

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

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

g. 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

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

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

4. Weight Variation Test

Procedure

1) 10 tablets were taken and average weight was taken and it was considered as the standard weight of an individual tablet.

2) 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

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

Where,

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

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

Chapter Four

Results

4.1 Standard curve preparation

The standard was collected from Incepta Pharmaceuticals 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.

The results are as follows:

Table 4.1: Concentration & Absorbance for Standard Curve of Carvedilol

Concentration(mg)	Absorbance (at 241nm)
0.001	0.097
0.002	0.186
0.003	0.233
0.004	0.336
0.005	0.409
0.006	0.496
0.007	0.576
0.008	0.796
0.009	0.725

By plotting the absorbance against the concentration of carvedilol a straight line was found. From this an equation was derived where:

y=86.476x-0.0041

R^2=0.9644

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

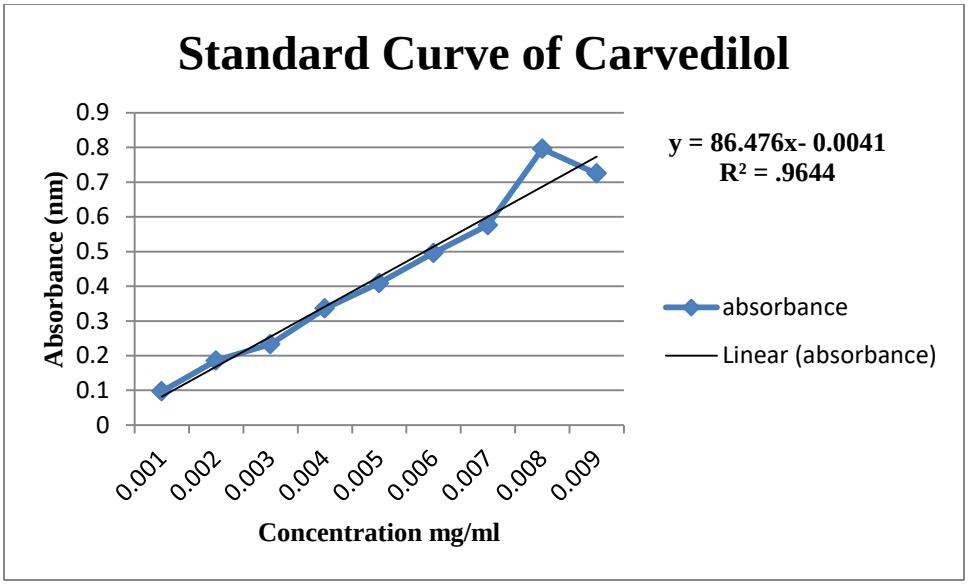

Figure 4.1: Plot showing straight line for absorbance with respect to concentration for Carvedilol

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 4.2: Weight Variation Test of Carvedilol (Carvista®)

Days	Average Weight for Particular Day, I(g)	Average Weight for 60 Days Intervals, A(g)	% Weight Variation, (A-I/A)×100 %
Initial	0.176	0.178	0.011
15	0.180		-0.011
30	0.177		0.005
45	0.179		-0.005

4.3 Result from Potency Determination by UV- spectroscopy

4.3.1Result 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 4.3: Concentration & Absorbance of 60 Days Interval for Carvedilol

Test Type	Initial potency %	Potency after 60 days	Potency Decrease %	Mean Potency decrease of each formulation	Standard deviation+/- of each formulation	Mean potency decrease
Sample 1A	98.72	98.00	0.72	1.00	0.28	1.05
Sample 2A	99.02	98.00	1.02			
Sample 3A	100.00	98.72	1.28			
Sample 1B	100.00	99.20	0.80	1.03	0.32	
Sample 2B	99.62	98.72	0.90			
Sample 3B	99.40	98.00	1.40			
Sample 1C	100.20	98.00	2.20	1.13	0.94	
Sample 2C	100.00	99.2	0.80			
Sample 3C	100.00	99.60	0.40			

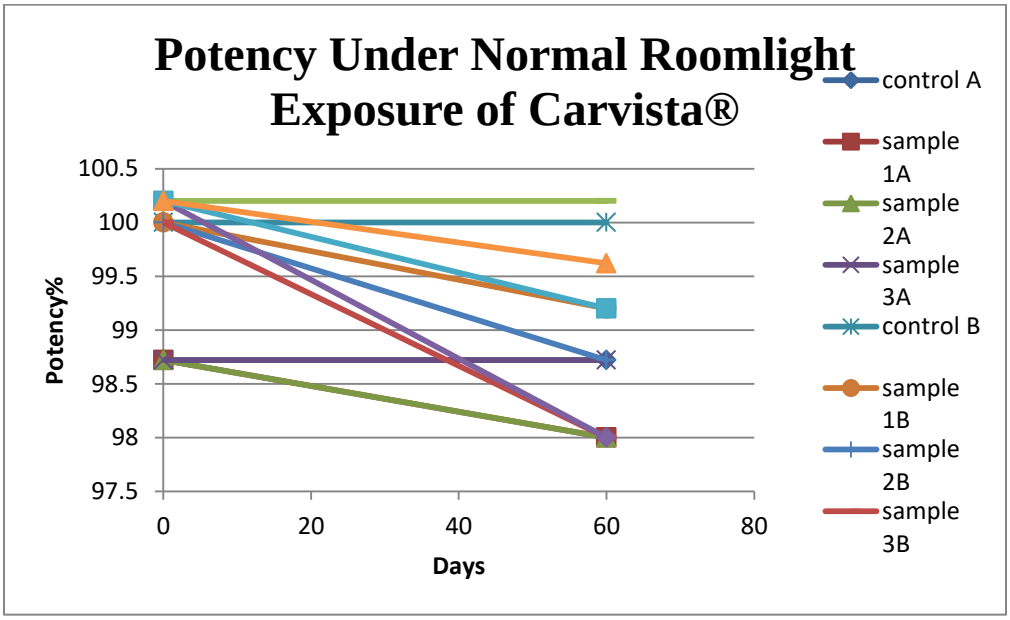

Figure 4.2: Difference in concentration after 5 hours time interval for carvedilol

4.3.2 Result of samples that were exposed under 25W bulb

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 4.4: Concentration & Absorbance for Carvedilol (Carvista®)

Test Type	Initial potency %	Potency after 5 hours %	Potency Decrease %	Mean Potency decrease of each formulation	Standard deviation+/- of each formulation	Mean potency decrease
Sample 1A	99.50	95.55	3.95	3.84	0.23	3.32
Sample 2A	99.00	95.00	4.00			
Sample 3A	100.00	96.42	3.58			
Sample 1B	100.00	96.45	3.55	3.41	1.16	
Sample 2B	100.20	98.02	2.18			
Sample 3B	100.00	95.50	4.50			
Sample 1C	99.62	96.42	3.20	2.72	0.46	
Sample 2C	99.62	97.33	2.29			
Sample 3C	100.02	97.33	2.69			

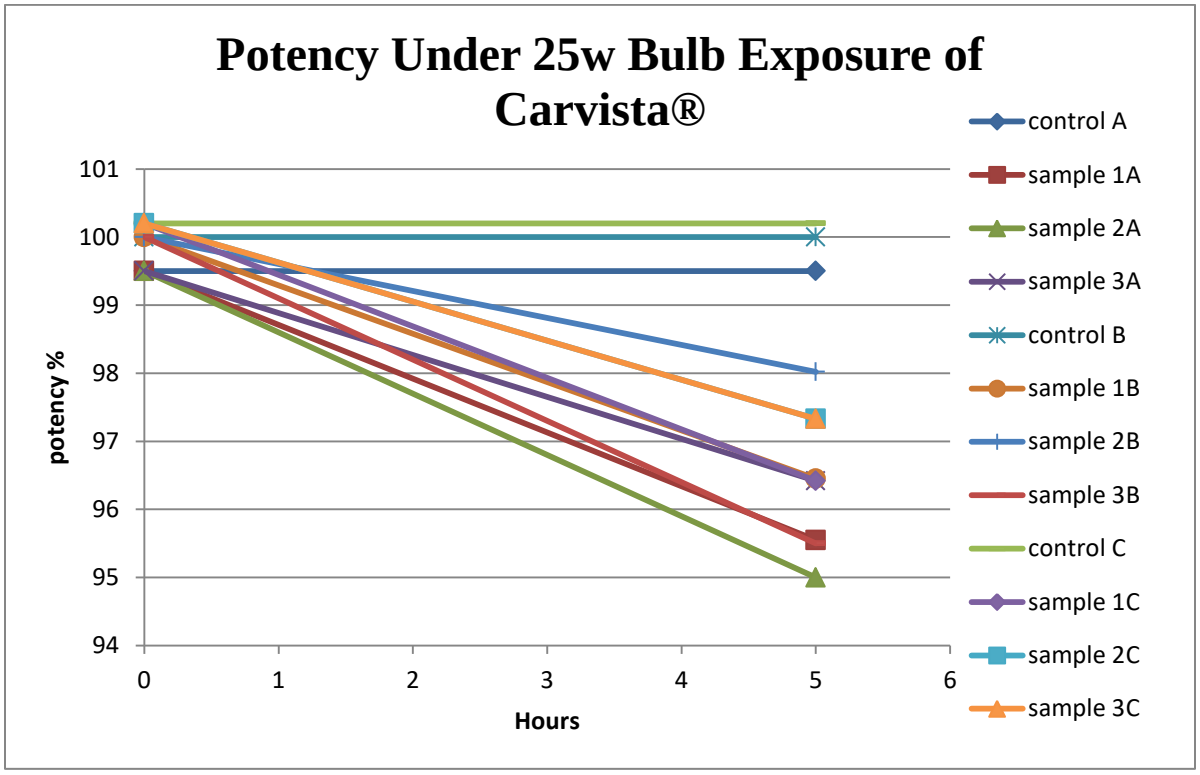

Figure 4.3: Difference in concentration after 5 hours time interval for carvedilol

4.3.3 Result of samples that were exposed under 40W bulb

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 (40W 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 (Carvista®)

Test Type	Initial potency %	Potency after 5 hours %	Potency Decrease %	Mean Potency decrease of each formulation	Standard deviation+/- of each formulation	Mean potency decrease
Sample 1A	100.00	91.66	8.44	7.84	1.33	6.76
Sample 2A	100.00	93.68	6.32			
Sample 3A	99.62	90.84	8.78			
Sample 1B	98.02	93.50	4.52	5.04	0.50	
Sample 2B	98.72	93.65	5.07			
Sample 3B	99.02	93.50	5.52			
Sample 1C	99.62	90.00	9.62	7.40	1.98	
Sample 2C	100.00	93.20	6.80			
Sample 3C	100.02	94.20	5.80			

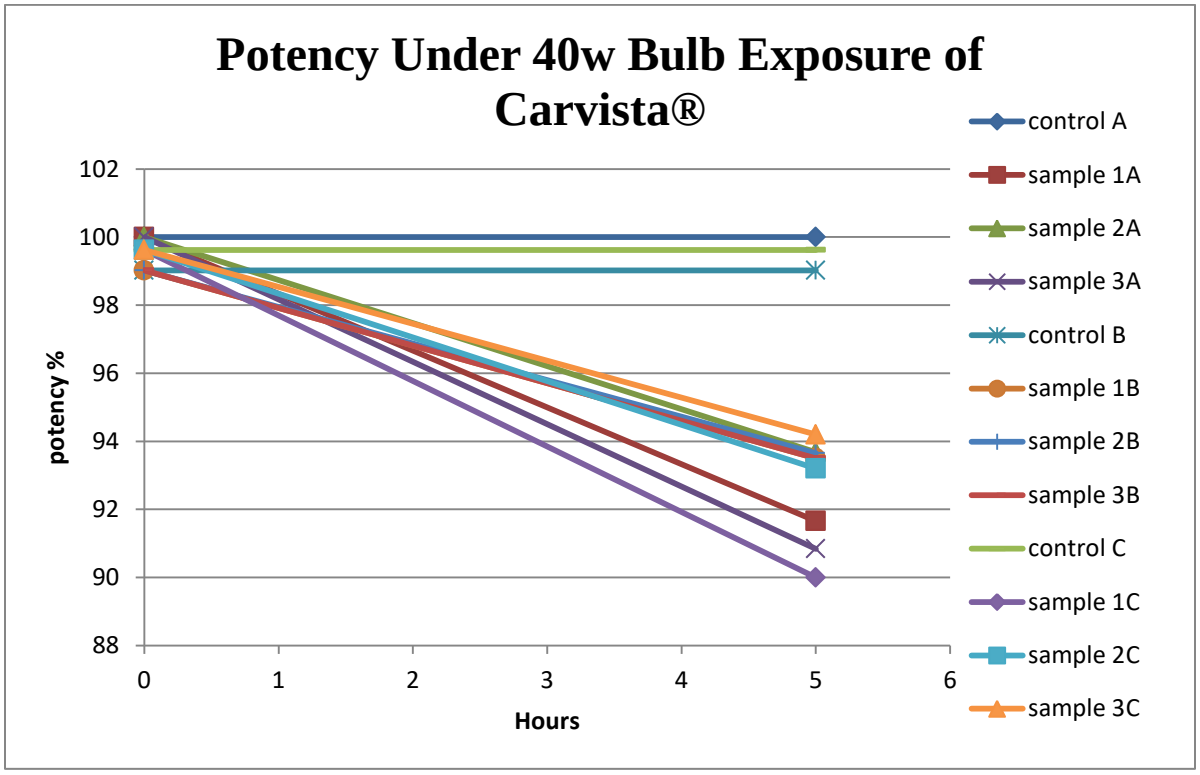

Figure 4.4: Difference in concentration after 5 hours time interval for carvedilol

4.3.4 Result of samples that were exposed under direct sunlight

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 direct sunlight, each for 5 hours' time interval and it was observed that the concentration of carvedilol was declined in each time interval.

Table 4.6: Concentration & Absorbance for Carvedilol (Carvista ®)

Test Type	Initial potency %	Potency after 5 hours %	Potency Decrease %	Mean Potency decrease of each formulation	Standard deviation +/- of each formulation	Mean potency decrease
Sample 1A	100.20	84.33	15.73	13.57	2.15	14.75
Sample 2A	100.00	86.45	13.55			
Sample 3A	100.00	88.66	11.44			
Sample 1B	99.62	83.50	16.12	15.82	0.42	
Sample 2B	100.00	84.00	16.00			
Sample 3B	99.60	84.26	15.34			
Sample 1C	100.00	84.26	15.74	14.87	0.76	
Sample 2C	100.00	85.50	14.50			
Sample 3C	99.96	85.60	14.36			

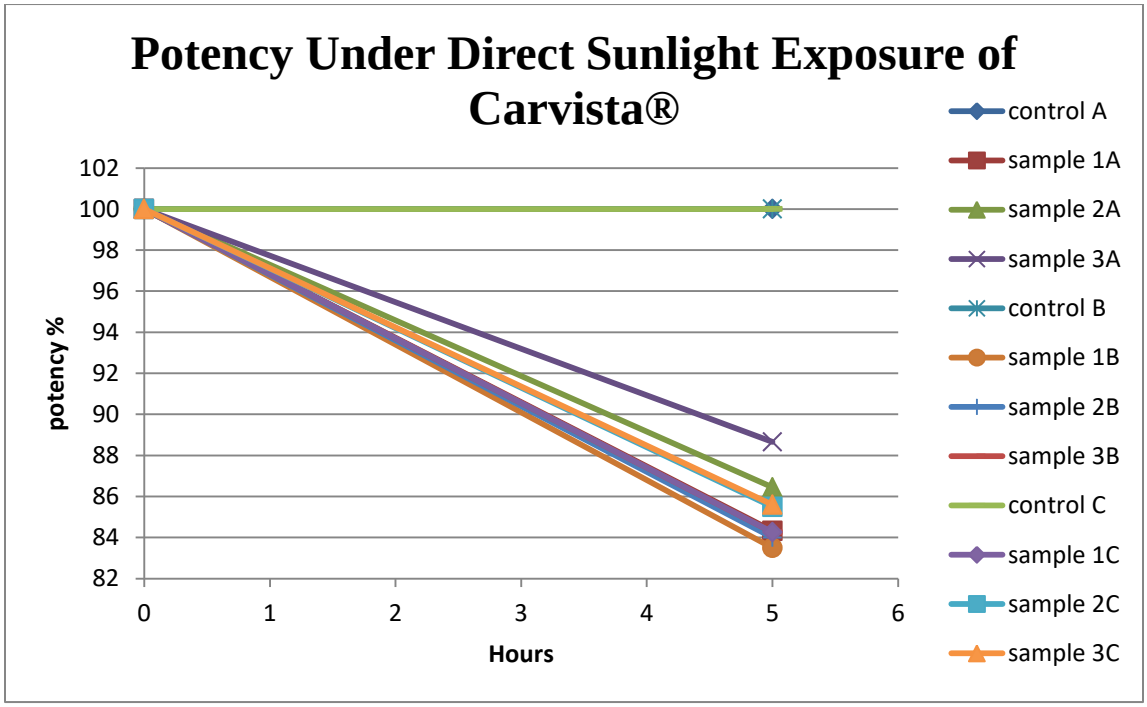

Figure 4.5: Difference in concentration after 5 hours time interval for carvedilol

Chapter Five

Discussions

It was found that the concentration of carvedilol was decreased gradually in every ovation of light exposure. When sample tablets (Carvista®) were kept under the electrical bulb (25 watt & 40 watt) and tested every 5 hour light exposed, it was found that the concentration of carvedilol was decreased gradually. Same results were found for direct sunlight exposed sample tablets and for the tablets which were kept on normal room light conditions, sunlight exposed tablets were degraded much. So that, in normal room light, 25watt bulb, 40watt bulb and direct sunlight the concentration of carvedilol were decreased gradually with percent deviation 1.92%, 4.00%, 6.08% and 24% respectively. From this research project it can be conclude with a decision that, there should be a change in the packaging system of the carvedilol. Now in local market most of the available brand of this drug is packaged in plastic transparent blister strip. This package should be opaque thus the light cannot pass through the package.

Chapter Six

Conclusion

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

Chapter Seven

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