Reproducibility Study of Coating Efficiency on Preventing Photolytic Degradation of Betanol® (Atenolol) Tablets

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

DEDICATION

This Research Work is dedicated to Almighty Allah And my beloved parents

Declaration by the Research Candidate

I, Irin sultana hereby declare that the dissertation entitled “Evaluation of photolytic degradation of Betanol® (Atenolol) Tablets Available in Bangladesh” 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 is a record of original research work carried out by me during 2015, under the supervision and guidance of Md.Anisur Rahman senior Lecturer, Department of Pharmacy, East West University and the thesis has not formed the basis for the award of any other degree/diploma/fellowship or other similar title to any candidate of any university.

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Certificate by the Supervisor

This is to certify that the dissertation entitled "Evaluation of Photolytic Degradation of Betanol® (Atenolol)" 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 Irin Sultana (ID: 2011-3-70-008) under your guidance and supervision and that no part of the research has been submitted for any other degree. The thesis has not formed the basis for the award of any other degree/diploma/fellowship or other similar title to any candidate of any university.

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Certificate by the Chairperson

This is to certify that the thesis entitled "Evaluation of Packaging efficiency on photolytic degradation of Betanol® (Atenolol) Tablets Available in Bangladesh" 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 record of original and genuine research work carried out by Irin Sultana during 2015 of his research in the Department of Pharmacy, East West University.

Dr. Chowdhury Faiz Hossain

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Abstract

This research work was aimed to evaluate the reproducibility of the data in a study that was previously done in order to determine whether the packaging is effective to prevent the photolytic degradation of (Betanol®). The objective of this study was to determine the effect of various lighting conditions (control, sunlight, normal room light, 25 watt & 40 watt bulb) on (Betanol®). In addition, physical tests were performed to evaluate the change of weight variation, thickness and hardness of the Betanol® tablets of same batch. Physical tests were performed according to the specification of USP and very little fluctuation in result was observed throughout the study & standard deviation. Deviations for weight variation, hardness & thickness were ±0.00135 g, ±0.47284 kg & ±0.01512 cm respectively. But when it was exposed to the above mentioned lighting conditions, the concentration of Atenolol decreased gradually. Samples which were exposed to normal light, 25 watt, 40 watt electrical bulb & direct sunlight showed 20%, 21.56%, 30.70%, 36.84% degradation respectively. So it can be said that the Betanol® containing Atenolol was light sensitive and the potency decreased after exposure to light.

Key Words: Atenolol, Betanol, Potency, Light, Hardness, Thickness, Weight Variation, Photolytic Degradation

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CHAPTER ONE

INTRODUCTION

The objective of the research project was to reproduce the data that was previously done in order to determine the photolytic degradation of Betanol® which is a photosensitive drug. In this research photosensitivity of Betanol® in various lightening conditions (control, sunlight, normal light, 25watt bulb and 40watt bulb condition) were determined. For this purpose the available brand was chosen i.e. Betanol® from Sanofi Bangladesh Ltd. for determining whether it is photosensitive or not. In most cases this product are available in transparent blister packaging system in the market. Only few brands use the opaque blister packaging system due to the photosensitive report. Since there is no published data about photolytic degradation of atenolol, a research program was operated to find whether this drug is photosensitive or not. Our goal was to find whether the concentration of Betanol® is declining due to light exposure or not because Betanol® is usually known as photosensitive drug. . Samples which were exposed to normal light, 25 watt, 40 watt electrical bulb & direct sunlight showed 20%, 21.56%,30.70% & 36.84% degradation respectively. So it can be said that the Betanol® containing atenolol was light sensitive and the potency decreased after exposure to light.

1.1 Beta blockers

1.1.1 Definition

Beta blockers, also known as beta-adrenergic blocking agents, are medications that reduce blood pressure. Beta blockers work by blocking the effects of the hormone epinephrine, also known as adrenaline. When you take beta blockers, the heart beats more slowly and with less force, thereby reducing blood pressure. Beta blockers also help blood vessels open up to improve blood flow (Mayo Clinic, 2014).

Beta-blockers are currently recommended as first-line drug therapy for hypertention when concomitant disease is present, for example , with heart failure. These drugs are efficacious but have some contraindication.

1.1.2 Mode of Action of Beta Blockers

Beta-blockers reduce blood pressure primarily by decreasing cardiac output. They may also decrease sympathetic outflow from the central nervous system(CNS) and inhibit the release of renin from the kidney, thus decreasing the formation of angiotensin II and the secretion of aldosterone.

Beta-blockers antagonize beta-1 and beta-2 receptors which are the usual targets of the sympathetic nervous system (SNS) including epinephrine and norepinephrine. This results in a decreased heart rate through decreased SA node activity and decreased AV nodal conduction as well as decreased contractility of the heart (LearntheHeart.com, 2014).

1.2 Atenolol

Atenolol is in a group of drugs called beta-blockers. Selective blockers of β1-receptors, such as Atenolol are the most commonly prescribed β-blockers. The selective β-blockers may be administered cautiously to hypertensive patients who also have asthma. Beta-blockers affect the heart and circulation (blood flow through arteries and veins). Atenolol is used to treat angina (chest pain) and hypertension (high blood pressure). It is also used to treat or prevent heart attack. Do not stop taking atenolol without first talking to your doctor. Stopping suddenly may make your condition worse. Molecular formula of Atenolol is C14H22N2O3. Its molecular weight is 266.34 g (Drugs.com, 2015).

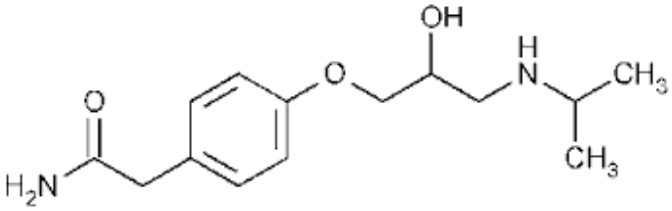

Fig 1.1: Molecular structure of Betanol®(Drugs.Com,2015)

Betanol : Betanol is a drug of atenolol generic. In this research project experiment conducted on Sample which was manufactured by Sanofi-Aventis Bangladesh Ltd. (Brand Name: Betanol®).

Physical characteristics of Betanol®:

- White in color
- Odourless
- Molecular weight 266.34 g
- Soluble in water

Examples of beta blockers(Mayo Clinic, 2015)

Some beta blockers mainly affect our heart, while others affect both our heart and our blood vessels.

Examples of Beta- blockers include:

- Acebutolol (Sectral)
- Atenolol (Tenormin)
- Bisoprolol (Zebeta)
- Metoprolol (Lopressor, Toprol-XL)
- Nadolol (Corgard)
- Propanolol(Inderal LA,Inopran XL)

1.3 Pharmacological properties of Atenolol

1.3.1 Pharmacodynamic property (eMC, 2015)

- Atenolol is a beta-blocker, which is $beta_1$-selective, (i.e. acts preferentially on $beta_1$-adrenergic receptors in the heart). Selectivity decreases with increasing dose.
- Atenolol is without intrinsic sympathomimetic and membrane-stabilizing activities and as with other beta-blockers, has negative inotropic effects (and is therefore contraindicated in uncontrolled heart failure).
- As with other beta-blockers, the mode of action of atenolol in the treatment of hypertension is unclear.
- It is probably the action of atenolol in reducing cardiac rate and contractility, which makes it effective in eliminating, or reducing the symptoms of patients with angina.
- It is unlikely that any additional ancillary properties possessed by S (-) atenolol, in comparison with the racemic mixture, will give rise to different therapeutic effects.

1.3.2 Pharmacokinetics property (eMC, 2014)

Pharmacokinetics Property include: Absorption, Distribution, Metabolism & Elimination

Absorption:

- Atenolol following oral dosing is consistent but incomplete (approximately 40–50%) with peak plasma concentrations occurring 2–4 hours after dosing.
- The bioavailability is decreased by 20% when taken with food. There is a linear relationship between dosage and plasma concentration.
- The inter-subject variability in AUC and Cmax is about 30-40%
- Atenolol penetrates tissues poorly due to its low lipid solubility and its concentration in brain tissue is low.

Distribution:

- The volume of distribution is 50 to 75 L.
- The protein binding is less than 5%.

Metabolism:

- There is no significant hepatic metabolism of atenolol and more than 90% of that absorbed reaches the systemic circulation unaltered.

Elimination:

- Most of an absorbed dose (85-100%) is excreted unchanged via the urine. The clearance is about 6 l/h and the half-life is about 6 to 9 hours.
- In elderly patients, clearance is decreased and elimination half-life increased.
- The clearance is correlated to renal function and the elimination is prolonged in patients with renal impairment.
- Impaired liver function does not influence the pharmacokinetics of atenolol

Therapeutic Use:

1. **Subsets of the hypertensive population**: The β-blockers are more effective for treating hypertension in white than in black patients and in young compared to elderly patients. (Note: conditions that discourage the use of beta-blockers , for example, severe chronic obstructive lung disease, chronic congestive heart failure, or severe symptomatic occlusive peripheral vascular disease) are more commonly found in the elderly and in diabetes.)
2. **Hypertensive patients with concomitant disease**: The beta-blockers are useful in treating conditions that may coexist with hypertention, such as supraventicular tachyarrhythmia, previous myocardial infarction, angina pectoris, chronic heart failure , and migraine headache.

1.4 Clinical Particulars of Atenolol

1.4.1 Route of administration (Mayo Clinic, 2015)

- For oral dosage form (tablets):
    - For acute heart attack:
        - Adults—50 milligrams (mg) ten minutes after the last intravenous (IV) dose followed by another 50 mg twelve hours later.
        - Then, 100 mg once a day or 50 mg two times a day for another 6 to 9 days or until discharge from the hospital.
    - For chest pain:
        - Adults—at first, 50 milligrams (mg) once a day. Doctor may increase dose if needed.
    - For high blood pressure:
        - Adults—at first, 50 milligrams (mg) once a day. Doctor may increase dose if needed.

1.4.2 Indications (Infomed, 1996)

- The efficacy of atenolol for hypertension and coronary heart disease has been well established in numerous comparative studies:
- Like antihypertensive drugs, atenolol lowers the systolic and diastolic blood pressure by 15 to 20% in a single drug treatment. But in long-term treatment it has the capability to reduce cardiovascular mortality.
- For chronic angina pectoris the frequency of heart attacks, the occurrence of silent ischemia, and the risk of an infraction are reduced.
- A long-term atenolol treatment after a myocardial infarction reduces the risk of re-infarction and the cardiovascular mortality.

1.4.3 Side effects (Drugs.com, 2015)

Side effects may occur when taking atenolol:

More common

- Blurred vision
- cold hands or feet
- confusion
- shortness of breath
- sweating

Less common

- Anxiety
- chest pain or discomfort
- chills

Rare

- Bloody urine
- decreased frequency or amount of urine
- increased blood pressure
- increased thirst

Adverse effects:

1. **Common effects:** The beta-blockers may cause bradycardia and CNS side effects such as fatigue, insomnia, and hallucinations; the drugs can also cause hypotention. The beta-blockers may decrease libido and cause impotence.
2. **Alterations in serum lipid patterns:** The beta-blockers may disturb lipid metabolism, decreasing high-density lipoprotein cholesterol and increasing plasma triacylglycerol.
3. **Drug withdrawal**: Abrupt withdrawal may induce angina , myocardial infarction, or even sudden death in patients with ischemic heart disease.

1.4.4 Contraindications

- Depression, Anaphylactic Shock due to Allergy Shots,
- Complete Heart Block,
- Second Degree Atrioventricular Heart Block,
- Sinus Bradycardia, Occasional Numbness,
- Prickling, or Tingling of Fingers and Toes,
- Asthma, Severe Chronic Obstructed Lung Disease,
- Serious Kidney Problems, Psoriasis,
- Blood Circulation Failure due to Serious Heart Condition,
- Abnormal Liver Function Tests,
- Pregnancy (WebMD, 2014).

1.4.5 Drug Interactions (WebMD, 2015)

Atenolol may interact with the following medications:

These medications may interact and cause very harmful effects.

Beta-blockers: AV node blockers/Fingolimod; Beta blockers/Fenoldopam; Beta blockers/Clonidine; Beta blockers/Epinephrine.

Moderate Interactions of atenolol:

These medications may cause some risk when taken together.

Beta blockers with Calcium channel blocker, Beta Blockers/Prazosin; Beta Blockers/Mefloquine; Quinidine; Beta Blockers/ Non-Steroidal Anti-inflammatory Drugs.

1.4.6 Pregnancy & Breastfeeding (Parker, 2012)

Pregnant Women

- The FDA classifies Betanol® as a Pregnancy Category D medication which means that it can cause harm on the unborn child when used during pregnancy. Atenolol passes the placenta which exposes the fetus to possible negative effects.

- In addition, some studies have shown that taking Betanol® during second trimester of pregnancy results in infants that are smaller for gestational age and with lower birth weight.

Fetal exposure to Betanol® during the last months of pregnancy increases the infant's risk

- Slow heart rate (bradycardia),
- Low blood sugar (hypoglycemia) and
- Low blood pressure (hypotension) immediately or several hours after delivery.

Breastfeeding Women

- Betanol® is not recommended for breastfeeding women. This medication is excreted in the human milk and can cause unwanted effects on the nursing infant.
- Betanol® tends to accumulate in the breast milk. Clinical studies reveal that breast milk contains Betanol® at a ratio of 1.5 to 6.8 when compared to drug levels in plasma.

1.4.7 Precautions (Health Central, 2014)

- Before taking Betanol®, doctor or pharmacist should be told if one is allergic to it; or if one has any other allergies. Pharmacist should be consulted for more details.
- Before using this medication, doctor or pharmacist should be told about medical history, especially of:

- certain types of heart rhythm problems (such as slow heartbeat, second- or third-degree atrio-ventricular block)
- breathing problems (such as asthma, chronic bronchitis, emphysema)
- blood circulation problems (such as Raynaud's disease, peripheral vascular disease)
- kidney disease
- serious allergic reactions including those needing treatment with epinephrine
- a certain muscle disease

For patients with diabetes,

- May prevent the fast heartbeat usually feel when blood sugar level falls (hypoglycemia).
- Other symptoms of low blood sugar are dizziness and sweating are unaffected by this drug.
- This product may also make it to control blood sugar levels. Blood sugar levels should be checked regularly as directed by doctor.

1.4.8 Overdose

Beta blocker overdose occurs when someone accidentally or intentionally takes more than the normal or recommended amount of this medication.

Symptoms

- Breathing trouble
- No breathing
- Blurred vision
- Double vision
- Irregular heartbeat
- Low blood pressure
- Heart failure
- Shock

1.5 Photolytic Degradation (Kumar *et al.* 2013)

Photolytic degradation is the process by which light-sensitive drugs or excipient molecules are chemically degraded by extreme light, room light and direct sunlight.

1.5.1 Photolytic Condition

Exposure of drug molecules may produce photo degraded product. The rate of photo degradation depends upon the intensity of incident light and quantity of absorbed light by the drug molecule.Photolytic degradation is carried out by exposing the drug product to a combination of visible and UV light. The most commonly accepted wavelength of light is in the range of 300-800nm to cause the photolytic degradation.

1.5.2 Mechanism of Photolytic Degradation

1. Drug products are placed and exposed under the light source;

2. Before a photolytic degradation reaction can occur, the energy from light radiation must be absorbed by the molecules;

3. Degradation of drug occurs. Two ways in which photolytic degradation can occur are:

- Light energy absorbed must sufficient to achieve activation energy.
- Light energy absorbed by molecules is passed on to other molecules which allow degradation to take place.

4. When carrying out the test, the temperature should be carefully considered to allow the influence of light to be assessed independently;

5. After each specified time interval, the exposed drug product is collected and the physical

Parameter of the sample must be checked;

6. Finally the potency of drug must be defined by using UV spectrophotometer.

CHAPTER TWO

LITERATURE REVIEW

(Andrisano ete al, 1999) The photostability of the beta-blocker drug Atenolol was evaluated at pH 9, 7.4 and 4.0. The drug was exposed to UVA-UVB radiations and the photoproducts were detected by reversed phase LC methods. The photo degradation was found to increase with the pH value decreasing. The major photo degradation product at pH 7.4 was identified as 2-(4-hydroxyphenyl) acetamide. The LC method developed for routine analyses (column: C-18 Alltima; mobile phase: TEA acetate (pH 4; 0.01 M)-acetonitrile 96:4) was found to be suitable for the stability indicating determination of Atenolol in pharmaceutical dosage forms.

(Ceresole ete al., 2006)A reversed-phase liquid chromatographic (RP-LC) assay method, developed for the quantitative determination of atenolol in the presence of its degradation products is described. The assay involved an isocratic elution of atenolol . C_{18} column using a mobile phase consisting of acetonitrile-sodium phosphate monobasic (0.08 M, pH 3.0) (10:90, v/v). The flow rate was 1.0 mL/min and the analyte monitored at 284 nm. The assay method was found to be linear from 0.4 to 12.8 µg injected. All the validation parameters were within the acceptance range. The developed method was successfully applied to estimate the amount of atenolol in tablet.

(According to Chan, Swinden & Donyai, 2007) it was thought that if medicines were stored inside multi-compartment compliance aids (MCCAs) where the environment supports lower light protection, air and moisture, it could influence the physico-chemical stability of the medicines. The given drug was subjected to room temperature and elevated temperature and humidity storage conditions after storing in a compliance aid. Physical tests and chemical analysis by HPLC were conducted by following the standards in BP and USP. It was established that the atenolol(Betanol®) tablets exposed in the elevated conditions and moist, softer than tablets at room temperature.

In 2007 (Liu & Williams, 2007) a Related study was performed which is to improving persistence assessment of active pharmaceutical ingredients (APIs), direct aqueous photolysis of β-blockers: propranolol (hydrochloride salt), atenolol, and metoprolol (succinate salt) were investigated by exposing the samples (0.0003−10 mg L-1) to perform this study a solar irradiator (filtered xenon lamp: 290−800 nm) was used at 20−26 °C. The measured half-lifes of atenolol, and metoprolol were approximately 350 and 630 hrs. respectively. By this experiment the

measured half-lifes were related to day light surface conditions by comparing the light intensity of the lamp and the sun at different latitudes and seasons. Major direct photolysis products were identified from propranolol that led to a proposed reaction pathway, involving ring oxidation, rearrangement, and deoxygenation.

(Foppa, Murakami & Silva, 2007) Antihypertension, angina pectoris and cardiac arrhythmias. However, most of these medicines are not formulated for easy or accurate administration to children. Atenolol is unstable in solutions and therefore the development of a liquid dosage form is a significant challenge. Studies showed that the degradation rate of atenolol is dependent on the temperature, indicating higher stability at 4 °C. Atenolol syrup is stable for 9 days, with acceptable appearance. A second order model adequately described atenolol decomposition when stored as syrup. A stability-indicating method was developed and validated in order to evaluate these studies. Atenolol is a cardio selective β_1-adrenergic receptor blocking agent prescribed for treatment of antihypertension.

(According to Aryal & Skalko-Basnet, 2008) the study revealed that the bi-layer tablet formulation was more stable than the mono-layer type. Further, the stability was increased when the tablets were packed in aluminium strips as compared to PVC blisters. A study involving the effect of multi-component formulation in tablets both in mono-layer and bi-layer types containing atenolol. The tablet was packaged in blisters and stripes and tested under accelerated temperature and humidity conditions. In this study HPLC was used to compare the stability of the tablet types and also the type of packaging. The result was that there was no effect on the stability of atenolol in either mono-layer or bi-layer tablet formulation. Moreover, it was same for the packaging.

In 2008(Gonsalves ete al., 2008) A study was performed by taking the samples of atenolol encapsulated in aluminium pans sealed in contact with air were maintained at 145ºC (7ºC below the melting point) for a certain time. The samples were analyzed by HPLC-MS. Identical experiments were undertaken at 165ºC (13ºC above the melting point). In order to see the effect of the oxygen an identical plan to that described was carried out with samples handled and encapsulated in a nitrogen atmosphere. The analytical results show in all the experiments the existence of a species with 516.4 molecular weight and a decrease of the peak corresponding to atenolol. The decomposition is favored by the presence of oxygen. Infrared spectra of atenolol

after thermal treatments differ from that of the original substance. The spectral data combined with the chromatographic information indicates a thermal decomposition of acetamide group of the atenolol giving rise by molecular condensation to a higher molecular weight species.

(Liu, Cumming & Sharpe et al, 2009)In order to improve the understanding of the behaviour of pharmaceuticals in the environment there is a need to investigate in-stream depletion mechanisms, e.g. photo transformation of active pharmaceutical ingredients (APIs) in natural surface waters. In this study, abiotic and biotic degradation of selected beta-blockers was measured simultaneously in non-sterilized and sterilized river waters and deionised water (DIW) under simulated sunlight (lambda: 295-800 nm) and dark conditions, and at environmentally relevant concentrations. Results suggested that the overall degradation followed pseudo first order kinetics under the solar simulation conditions and was between two and ten times faster in river waters than in DIW. Photo transformation was the main depletion mechanism for the beta-blockers tested over a 2 to 7 day period. Slow hydrolysis was observed for metoprolol only. Loss due to biodegradation in river waters was not observed for propranolol but was found for metoprolol and atenolol at a very slow rate within the study period. Biodegradation of metoprolol was accelerated under the light conditions, implying that photo-induced intermediates could be more easily biodegraded in river waters.

(Kumar et al., 2009) A study was investigated that the compatibility of atenolol, a β1 blocker, with a variety of pharmaceutical excipients. The binary mixtures (1:1) of atenolol with the excipients were stored for 1 month at 40 degrees. The samples were directly observed for the physical changes, and also analyzed by a validated HPLC method to determine the chemical changes. The study revealed that atenolol was incompatible with ascorbic acid, citric acid and butylated hydroxyanisole. The degradation products formed in these mixtures were characterized by high resolution mass spectrometric and fragmentation analyses, using a LC-MS/TOF system. The identity of characterized structures was justified through mechanistic explanations.

According to Hapeshi ete al., 2010) was investigated that the photolytic stability of atenolol by using different samples of TiO2 for the catalytic process. The study was conducted by measuring the absorbance of the sample after it was illuminated with the UVA lamp. There were other factors that were analyzed such as the effect of catalyst loading, initial atenolol concentration, initial pH and the use of other additional oxidizing agents e.g. H2O2 on the substrate

transformation and mineralization in different aqueous environments (i.e. pure water, groundwater and treated municipal effluent). It was found that the degradation of atenolol was effected due to increasing catalyst loading, decreasing initial substrate concentration and adding H_2O_2.

(Shetty ete al., 2010) Polypill is a fixed-dose combination (FDC) containing three or more drugs in a single pill with the intention of reducing the number of tablets or capsules that need to be taken. Developing a single analytical method for the estimation of individual drugs in a Polypill is very challenging, due to the formation of drug-drug and drug-excipients interaction impurities. HPLC method for the simultaneous quantitative determination of Aspirin (ASP), Atorvastatin (ATV), Atenolol (ATL) and Losartan potassium (LST) in a polypill form in the presence of degradation products. Efficient chromatographic separation was achieved on a C18 stationary phase with simple mobile phase combination of buffer and Acetonitrile. The retention times of Atenolol, Aspirin, Losartan potassium, and Atorvastatin were 3.3, 7.6, 10.7 and 12.9 min respectively. The combination drug product were exposed to thermal, acid/base hydrolytic, humidity and oxidative stress conditions, and the stressed samples were analyzed by proposed method .Acceptable precision and accuracy were obtained for concentrations over the standard curve ranges.

(Elgawish, Mostafa,ete al, 2010) A simple, sensitive and rapid chromatographic method was developed by some chemist and validated for the simultaneous quantification of atenolol and chlorthalidone in human plasma using hydrochlorothiazide as internal standard (IS). The method utilized proteins precipitation with acetonitril as the only sample preparation involved prior to reverse phase-HPLC. The analytes were chromatographed on Shim-pack cyanopropyl column with isocratic elution with 10 mM KH_2PO_4 (pH 6.0) – methanol (70:30, v/v) at ambient temperature with flow rate of 1 mL min−1 and UV detection at 225 nm. The chromatographic run time was less than 10 min for the mixture. The method was validated in terms of accuracy, precision, absolute recovery, freeze–thaw stability, bench-top stability and re-injection reproducibility. The accuracy and precision were found to be within acceptable limits <15%. The analytes were stable after three freeze–thaw cycles (deviation <15%). The proposed method was specific for the simultaneous determination of atenolol and chlorthalidone in human plasma where there was no interference from endogenous biological substance.

In 2010(Abdelwahab,ete al, 2010) In this studies , a well-known antihypertensive drugs that are administered in combination and provide greater therapeutic effects than with either drug alone are selectively determined in the presence of their degradation products. Two chemometric methods and TLC-Densitometric one have been developed for the selective determination of Atenolol (ATE) and Chlorthalidone (CLT) along with their hydrolytic degradation products. The developed chemometric models are principal component regression (PCR) and partial least squares (PLS). These models have been updated to be used for prediction of ATE and CLT in another dosage form in which AmilorideHCl (AMH) is included. The updated models are capable of predicting the concentrations of the three components of the new dosage form with good accuracy and precision without reconstruction of the calibration set. The suggested methods have been used for the determination of the studied drugs in their pharmaceutical formulations and the results were statistically compared to the reported RP-HPLC method.

(According to YILMAZ & MERAL, 2010) the amount of atenolol present in pure and pharmaceutical preparation was determined by using spectrofluorometry method following linearity, precision, accuracy, specificity, stability, limit of detection and limit of quantification as a parameter. No significant difference was found between them.

(Isarain-Chavez ete al., 2010)In this studies investigated that the Two-electrode cells with a Pt or boron-doped diamond anode and an air-diffusion cathode for H_2O_2electro generation, and four-electrode combined cells containing the above pair of electrodes coupled in parallel to a Pt anode and a carbon-felt cathode, have been used to degrade the pharmaceutical β-blocker atenolol by electro-Fenton and photoelectron-Fenton methods. In these processes, organics are mainly oxidized with hydroxyl radical ($^{\bullet}OH$) formed simultaneously at the anode surface from water oxidation and from Fenton's reaction between added catalytic Fe^{2+} and electro generated H_2O_2.

(Amjad ete al., 2011) carried a study on the development of matrix type transdermal patches containing Atenolol that was introduced with varying amounts of HPMC (hydroxyl propyl methyl cellulose) & EC (ethyl cellulose) by solvent casting method. Interaction between drug and polymer was investigated with FTIR. Studies that evaluated physicochemical characteristics and stability studies of different formulations in transdermal patches revealed favourable physical stability.

In 2011(Kaila ete al., 2011) A simple, rapid, precise and accurate isocratic reversed phase stability indicating HPLC method was developed and validated for the simultaneous determination of atenolol and lercanidipine hydrochloride in commercial tablets. The chromatographic separation was achieved on phenomenex Gemini C18 (250×4.6 mm, 5 μm) column using a mobile phase consisting of acetonitrile and buffer (20 mM potassium dihydrogen phosphate pH 3.5) in the ratio of (55:45, v/v) at a flow rate of 1.0 ml/min and UV detection at 235 nm. The linearity of the proposed method was investigated in the range of 40-160 μg/ml (r 2 =0.9995) for atenolol and 8-32 μg/ml (r 2 =0.9993) for lercanidipine. As a result , degradation products produced stress studies did not interfere with the detection of atenolol and lercanidipine and the assay can thus be considered stability-indicating

(Zaid ete al., 2012)The purpose of this study was to formulate a 25-mg atenolol capsule starting from a commercial 100-mg atenolol tablet, given the fact that this strength is not available in Palestine and also because 50-mg atenolol tablets failed the splitting uniformity test of the European Pharmacopoeia, and to evaluate the chemical stability and dissolution behaviour of the obtained capsules so as to ensure a high-quality product. A high-performance liquid chromatographic system was used for the analysis and quantification of atenolol in the samples studied. Samples of atenolol for analysis were prepared as reported by the United States Pharmacopeia monograph. Disintegration and dissolution tests were performed according to the United States Pharmacopeia. The high-performance liquid chromatography assay indicated that the 25-mg atenolol capsules were stable for four months when stored at ambient temperature conditions. The disintegration time for all atenolol capsules was within the United States Pharmacopeia limits of 15 minutes. Atenolol release profile showed that approximately 90% of atenolol dissolved after 10 minutes. This study is important for patients who need to take one half of a 50-mg tablet, but the splitting process doesn't give equal halves, and also for modifying the dose for patients with renal or hepatic problems.

In 2012(Ji ete al., 2012) In this study the photolysis behaviour of atenolol (ATL) and toxicity of its photo degradation products were investigated in the presence of nitrate ions. The results showed that the atenolol photo degradation followed pseudo-first-order kinetics upon simulated solar irradiation. The photo degradation was found to be dependent on nitrate concentration and increasing the nitrate from 0.5 mM L−1 to 10 mM L−1 led to the enhancement of rate constant

from 0.00101 min−1 to 0.00716 min−1. Increasing the solution pH from 4.8 to 10.4, the photo degradation rate slightly decreased from 0.00246 min−1 to 0.00195 min−1, probably due to pH-dependent effect of nitrate-induced radical dotOH formation. Bicarbonate decreased the photo degradation of atenolol in the presence of nitrate ions mainly through pH effect, while humic substance inhibited the photo degradation via both attenuating light and competing radicals. The main photoproducts of atenolol(ATL) were identified by using solid phase extraction–liquid chromatography–mass spectrometry (SPE–LC–MS) techniques and possible nitrate-induced photodegradation pathways were proposed.

(Gahlawat,ete al, 2013) studies about buccal route is an excellent for systemic drug delivery because of greater bioavailability, less first pass metabolism, prolong duration of action & lower dose dependent side effect. The patch of atenolol was formed through solvent casting technique in combination with fabricated & hydrophilic polymer. From the study we observed that, drug solubility, mucoadhesive strength &vapor transmission were satisfactory.

(Ji ete al., 2013) Photocatalytic degradation of atenolol (ATL) was investigated in aqueous suspensions using TiO2 as photocatalyst. Complete degradation of atenolol was obtained 37.6μM after 60min irradiation in pH 6.8 Milli-Q water in the presence of 2.0gL−1 Degussa P25 TiO2. Degradation of ATL followed pseudo-first-order reaction kinetics. Hydroxyl radical (HO) was determined to be the predominant reactive species during photocatalysis by means of radical probes. Major transformation products were elucidated by high performance liquid chromatograph-mass spectrometry (HPLC–MS/MS) technique. Atenolol (ATL) photodegradation pathways included generation of 3-(isopropylamino) propane-1,2-diol and p-hydroxyphenylacetamide through ether chain cleavage, hydroxylation and the formation of 4-[2-hydroxy-3-(isopropylamino) propoxy] benzaldehyde. Photocatalytic degradation efficiency of ATL was highly dependent on the properties of the water matrix, such as pH, the presence of organic and inorganic species (e.g., humic substance, HCO3−).

According to (Indhumathi & Rajashekar, 2013) research unit that studied stability studies on different formulations of atenolol. The formulation was developed with different disintegrants like crospovidone, croscarmellose, sodium starch glycolate. From the study no Considerable changes were observed in drug content in any of the given formulation.

(Liu ete al., 2013) Photoactivation of peroxymonosulfate (PMS) with UV (254nm) irradiation was used to generate the SO_4 (-)-based advanced oxidation process, which was adopted to degrade atenolol (ATL) in water. The second-order reaction rate constants of ATL with HO and SO_4 (-) were determined, and the effects of operational parameters (dose of PMS, solution pH, HCO_3 (-), humic acids (HA), and N_2 bubbling) were evaluated as well. Finally the main transformation intermediates were identified and possible degradation pathways were proposed. The results showed that there was a linear positive correlation between the degradation rate of ATL and specific dose of PMS (1-16M PMS/M ATL).Absorption (or complexation) and photosensitized oxidation induced by HA improved ATL degradation during the first minute of degradation process, whereas photon competition and radical scavenging effects .

Salgado ete al., 2013) in these studies, investigating the concentration of atenolol in secondary effluents derived from wastewater treatment plants. Assessment of atenolol with regard to its photo degradation kinetics was carried out using medium pressure (MP) lamp. Samples of pure water, filtered and unfiltered treated wastewater was collected for the purpose. The study showed to have low highest time- and fluence-based rate constants in case of atenolol. Further, transformed products of atenolol were identified and the pathways leading to photo degradation was proposed. The products continued to exist due to photolysis of UV utilized in the waste water treatment plants.

(Belal ete al., 2013) attempted to determine atenolol by means of stability-indicating reversed-phase liquid chromatographic method(RP-LC). The chromatographic conditions was set up and optimized with relevant to atenolol and then assayed by using a mobile phase consisting of acetonitrile: methanol: 0.02 M phosphate buffer in the ratio of (20:20:60) having a flow rate of 1 ml/min and detected by UV at 226 nm. The proposed method was able to detect atenolol in three commercial tablets and when compared with the results obtained by official method, they had minor differences.

In 2014(Handa, Singh & Pal Singhb, 2014) In this studies shown to the use of fixed-dose combinations of drugs in the therapy of select diseases, like cardiovascular diseases, due to their multiple advantages. Though the main criterion for combining drugs in a single dosage form is the rationale, but consideration like stability of formulation is equally important, due to an added aspect of drug–drug interaction. The objective of this study was to evaluate interaction among

the drugs in an antihypertensive combination of nifedipine and atenolol. Nifedipine is a known light sensitive drug, which degrades via intra-molecular mechanisms to nitro- and nitroso-pyridine analogs, along with a few minor secondary products that are formed through inter-molecular interactions amongst primary degradation products and their intermediates.At present the study of nifedipine, atenolol and their combination to a variety of accelerated and stress conditions. HPLC studies revealed formation of a new product in the mixture of two drugs (~2%), which was also generated from nifedipine alone, but at trace levels (<0.1%). Ultra-violet, FT-IR, mass spectrometric and nuclear magnetic resonance spectroscopic studies showed that the principal photo-degradation pathway of nifedipine was modified and diverted in the presence of atenolol. A study was conducted employing two other β–blockers with similar structures to atenolol, and the same product was formed in relatively higher quantity therein also.

(According to Reddy, Reddy & Goud, 2014) did a conducted research program to develop and validate UPLC (ultra-performance liquid chromatography) method for determining atenolol in tablet form. The mobile phase composed of buffer and methanol in 70:30v/v ratio. Flow rate was maintained at 1.0mL/min and total elution time was 2 minutes. UV detection was carried at 226nm wavelength. The developed method was validated in various terms and was considered to be precise, sensitive, selective, robust and stability-indicating.

(Dong, Trenholm & Rosario-Ortiz, 2014)The photochemical degradation of five pharmaceuticals was examined in two secondary wastewater effluents. The compounds, which included atenolol, carbamazepine, meprobamate, phenytoin and primidone, were evaluated for both direct and sensitized photolysis. In the two wastewaters, direct photolysis did not lead to significant compound degradation; however, sensitized photolysis was an important removal pathway for the five pharmaceuticals. Upon solar irradiation, hydroxyl radical (HO) was quantified using the hydroxylation of benzene and singlet oxygen ($^1O_2$) formation was monitored following the degradation of furfuryl alcohol. Degradation via sensitized photolysis was observed following five-day exposures for atenolol (69-91%), carbamazepine (67-98%), meprobamate (16-52%), phenytoin (44-85%), and primidone (34-88%). Varying removal is likely a result of the differences in reactivity with transient oxidants. Averaged steady state HO concentrations ranged from 1.2 to 4.0×10^{-16}M, whereas the concentrations of $^1O_2$ were 6.0-7.6×10^{-14}M. Partial removal due to presence of HO indicates it was not the major sink .

CHAPTER THREE

MATERIALS & METHODS

3.1 MATERIALS

3.1.1 Sample Collection

For the purpose of experimentation to observe the photolytic degradation of atenolol as well as to assess the packaging efficiency, 700 tablets of Betanol® (50 mg) were collected from the local drug store in Dhaka as a sample. All the tablets were from the same batch (batch no. X 28). Among them 300 tablets were kept light protected for control tests and the remaining 400 tablets were subjected to various lighting conditions over certain periods of time for conducting experiments to determine their potency.

3.1.2 Samples

Table 3.1: Samples used in the experiment

Sample Name	**Company Name**	**Batch No.**
Betanol® tablets	Sanofi Bangladesh Limited.	X 28

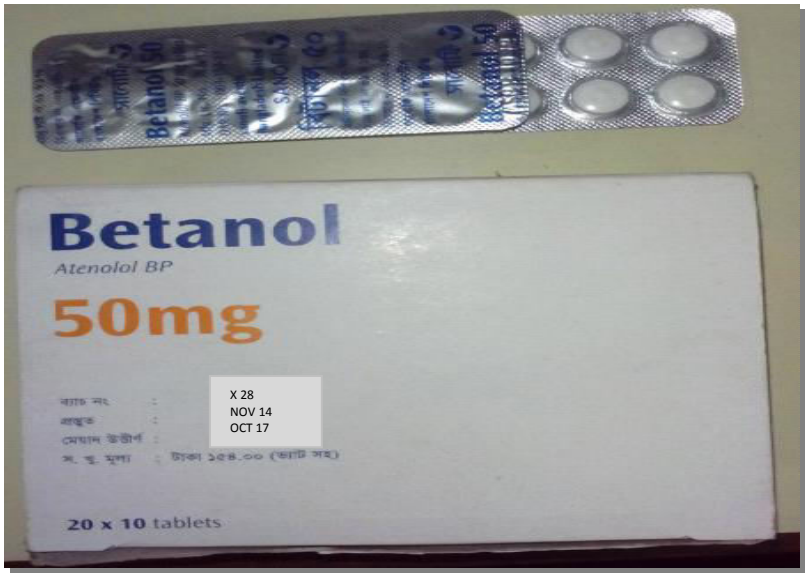

Figure 3.1: Betanol® Tablet

3.1.3 Reagents

Table 3.2: Reagents used in the experiment

Reagents Name
Concentrated H_2SO_4 (98% / 36.8N)
Distilled Water

3.1.4 Equipment & Instruments

Table 3.3: Lists of equipment used for the experiment

Equipment	Company Name	Origin
UV-Spectrophotometer	Shimadzu UV1800	Japan
Distill Water Plant	Bibby Scientific W4000	United Kingdom
Electronic Balance	Shimadzu AY220	Japan
Hardness tester	Veego VTHT	India
Venire Calipers	Shanghai Tricle Brand	China

3.1.5 Images of Instruments:These instruments were used in different tests during research work.

Figure 3.2: [Left to right] Shimadzu UV-1800 Double Beam Spectrophotometer and Electronic Balance

Figure 3.3: Hardness tester, Distilled water plant & Vernier calipers [Left to right]

3.1.6 Apparatus

Some apparatus are listed in the following table those were used throughout the experiments.

Table 3.4: List of Apparatus Used in research work.

Serial No.	Apparatus
1	Beakers
2	Test tubes
3	Volumetric Flasks (50 ml, 250 ml & 1000 ml)
4	Electric Bulb (25 Watt & 40 Watt)
5	Plastic Containers
6	Aluminium foil paper
7	Transparent Tracing Paper
8	Filter Paper
9	Mortar & Pestles
10	Spatula
11	Pipette pumper
12	Pipette (5ml & 10ml)
13	Glass & Plastic Funnel
14	Lamp
15	Funnel
16	Masking Tap
17	Thermometer

3.2 precedure

3.2.1 Preparation of the solvent (0.1N H_2SO_4)

- Lab solvent (H_2SO_4), stock solution with 98% (v/v) of strength was collected.
- Then the concentration of the lab solvent 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

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 H_2SO_4 solvent was calculated as below:

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

We know that,

$V_1 = (V_2S_2) / S_1$

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

Or, V_1 = 2.717ml (~ 2.72 ml of lab solvent H_2SO_4 stock solution)

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.

3.2.2 Determination of λ_{max} & Preparation of the Standard Curve of Atenolol

1. Standards of Atenolol was collected from a pharmaceutical company. The potency of standard compounds was 99.5%.
2. The specific λ_{max} for Atenolol at which the absorbance would be measured, was determined to be 223.5nm from the UV spectrometer by using the standard. Nine serial concentrations of the standards of Atenolol were prepared for the purpose of creating a standard curve.

Preparation of the stock solution for Atenolol using the standard :

50 mg of the standard compound, that is Atenolol 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 Atenolol solution was taken and dissolved in 50ml of 0.1N H_2SO_4. That 5ml contained 1mg of Atenolol.

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

Preparation of nine serial concentrations of solution for Atenolol:

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

- Nine serial concentrations that were prepared for Atenolol 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 $S_1V_1=S_2V_2$ 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 Atenolol as per the calculations provided below.

Table 3.5: Concentrations for preparation of Standard Curve of Atenolol

Sample Name	Sample no.	Concentration (mg/ml)
Atenolol	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 Atenolol.
- $V_1 = S_2V_2 / S_1$ = (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 Atenolol.
- $V_1 = S_2V_2 / S_1$ = (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 Atenolol.
- $V_1 = S_2V_2 / S_1$ = (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) Atenolol.
- $V_1 = S_2V_2 / S_1$ = (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 Atenolol.
- $V_1 = S_2V_2 / S_1$ = (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 Atenolol.
- $V_1 = S_2V_2 / S_1$ = (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 H_2SO_4) of Atenolol.
- $V_1 = S_2V_2 / S_1$ = (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 Atenolol.
- $V_1 = S_2V_2 / S_1$ = (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 Atenolol.

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

4. A standard curves was plotted Atenolol.

5From this standard curve a straight 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

3.2.3 Sampling, Analysis by UV-Spectrophotometry & Determination of Potency of the pharmaceutical drugs (atenolol) under various lighting condition.

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

- Exposure to normal lighting conditions in the room
- Electric Bulb exposure (25 watt & 40 watt)
- Direct Sunlight exposure

I. Exposure under Normal Lighting Condition

1. The Tablets (Betanol®) were kept under normal lighting condition in the room for 2 months.

2. They were sampled after specific intervals like after 2 weeks (14 days) for determination their physical properties (like thickness, hardness & weight variation) and also their potency was determined after exposure to normal lighting condition.

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

4. Now 10 Tablets were taken out and from these 10 tablets, 5 tablets were kept on over that paper.

5. A photograph was taken of that paper showing the tablets and those details.

6. Then from those 10 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:

Steps:

First, Five tablets from those sampled tablets was taken;

- Then the total weight of those five tablets was noted using an analytical balance and the average weight was calculated using the formula given below:
- Average weight (in grams) = Total weight of the tablets /Total no. of tablet
- Then the five tablets were crushed by using mortar and pestle.
- Next, approximately the weight of 3 tablet of crushed tablet powder was taken and dissolved in 250 ml of the solvent (0.1N H_2SO_4) for 3 times to prepare 9 samples.
- After that 10 ml solution was filtered and 5 ml of that filtered solution was taken and dissolved in 50ml of the solvent.
- From then 10ml of each sample was collected and kept into 3 different test-tube and wrapped it by foil paper.
- These process repeated at 2 times
- From test-tube the solution was poured into a cuvette and was inserted into the UV spectrophotometer to observe the absorbance value for atenolol.

8. Then using the absorbance value obtained from UV spectrophotometer, the 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.

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

Steps:

- 30 tablets were exposed to electric bulb lighting conditions for 6 hours at a stretch and 10 tablets were used as control.
- After every 2 hours, 10 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.
- The foil papers should be labeled to identify the intervals.

- The tablets were then used for potency determination to see the effect of the exposure of bulb's lighting condition to drug ingredients.
- For potency determination, laboratory analysis was done by using UV spectroscopy technique:
- First, 5 tablets from those sampled tablets were taken.
- Then the total weight of those 5 tablets was noted using an analytical balance and the average weight was calculated using the formula :

Average weight (in grams) = Total Weight of tablets / Total no. of tablets

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

No. of Samples	**Collected Sample**	**Withdrawal Intervals (Hrs)**	**Temperature (^{0}C)**	
			25W	**40W**
10 (Control)	10	0	25	30
20	5	2	27	30
	5	4	27	30
	10	6	30	32

- Then the absorbance value was plotted into the standard curve to obtain the total amount of the drug that is present in one tablet.
- Steps 5 to 6 were repeated again for another sampling hour.
- 10 tablets were used as control and has not been exposed any of the lighting conditions and 5 tablets were used as physical parameter & 5 tablets were used as potency determination.

III. Under Sunlight condition

- 30 tablets were kept and exposed to sunlight condition for 6 hours at a stretch &10 tablets were used as control.
- After every 2 hours, 10 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.
- The foil papers should be labeled to identify the intervals.
- The tablets were then used for potency determination to see the effect of the exposure of sunlight condition to drug ingredients.
- For potency determination, laboratory analysis was done by using UV spectroscopy technique:
- First, 5 tablets from those sampled tablets were taken.
- Then the total weight of those 5 tablets was noted using an analytical balance and the average weight was calculated using the formula:

Average weight (gram) =Total weight of tablets/Total no. of tablets

- Then the 5 tablets were crushed by using mortar and pestle.
- Approximately the weight of 3 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 9 samples.
- After that 10 ml solution was filtered and 5 ml of that filtered solution was taken and dissolved in 50ml of the solvent.
- From then 10ml of each sample was collected and kept into 3 different test-tube and wrapped it by foil paper.
- These process repeated at 2 times.

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

No. of Samples	Collected Sample	Withdrawal Interval (Hrs)	Temperature(°C)
10 (Control)	10	0	30
20	5	2	31
	5	4	31
	10	6	32

- Then the absorbance value was plotted into the standard curve to obtain the total amount of the drug that is present in one tablet.
- Steps 5 to 6 were repeated again for another sampling hour.
- 10 tablets were used as control and has not been exposed any of the lighting condition.

Determination of Physical parameter:

I.Color Test

The color of tablets was observed to find any change in color. A digital camera was used to take the picture of the tablets for the comparative observation. In case of taking picture any kind of flash was not used or avoided. A fixed camera with fixed resolution was maintained.

II.Hardness Test

Hardness test was performed to determine the hardness of tablets. So the force will be applied during compression of tablet, greater the pressure applied the harder the tablet. Hardness tester was used to measure the hardness of(Betanol®). Hardness measuring devices apply increasing pressure on the tablet until the tablet breaks (a force of about 3 kilograms is considered to be a minimum for hardness).

III.Thickness Test

The thickness of tablets was measured to find the change in thickness at specific time interval. A slide calipers was used to take thickness value of tablets for the comparative observation. In case of performing the test, tablets are placed horizontally in between the fixed jaw and the moving jaw of the calipers, tighten the jaws and check the reading of main scale and vernier scale and calculate the values of each tablets.

IV. Weight Variation Test

Procedure

1. 5 tablets were taken and weighed.

2. The average was taken and it was considered as the standard weight of an individual tablet.

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

Table 3.8: Accepted percentage list for the weight variation test of tablets

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

Weight Variation: Average weight – Individual weight/Individual weight × 100%

A-I/A ×100%

Thickness: M + (V×V.C) Where, M= Main Scale result

CHAPTER FOUR

RESULT

4.1 Standard curve preparation

The standard was collected from Eskayef Bangladesh Ltd. and tried to make a standard curve. For different concentration of atenolol we found different absorption. Nine serial concentrations of the standards of atenolol were prepared for the purpose of creating a standard curve.

The results are as follows:

Table 4.1: Concentration & Absorbance for Standard Curve of atenolol

Concentration(mg)	**Absorbance (at 223.5nm)**
0.001	0.031
0.002	0.069
0.003	0.089
0.004	0.123
0.005	0.184
0.006	0.200
0.007	0.230
0.008	0.276
0.009	0.365

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

Y=29.85X+0.018

R^2=0.970

This equation was used to determine the concentration of atenolol from different samples absorbance.

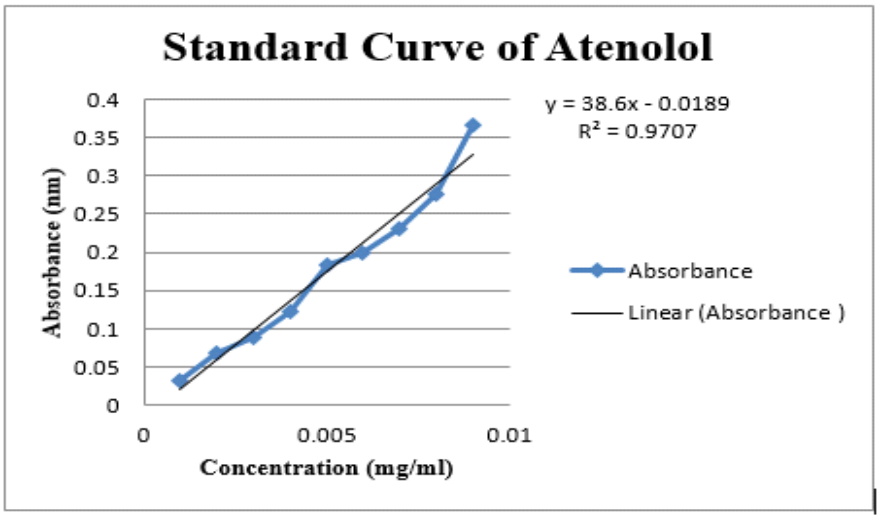

Figure 4.1: Plot showing straight line for absorbance with respect to concentration for atenolol

4.2 Physical Parameters of Normal Light Exposed Samples

4.2.1 Color Test

The color of tablets was observed to find any change in color with respect to time intervals. No significant change was observed in color of the tablet. The picture of the sample tablets of different days are showed below:

4.2.2 Weight Variation Test

Six tablet strips containing 60 tablets was exposed to normal light condition for 60 days. Weight variation test was conducted of 5 tablets of each day interval (0, 15, 30, 45, 60,days). In experimental day, a tablet strip containing 10 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 of atenolol (Betanol®)

Days	Average Weight for Particular Day (gm)
Initial	0.217
15	0.216
30	0.215
45	0.215
60	0.214

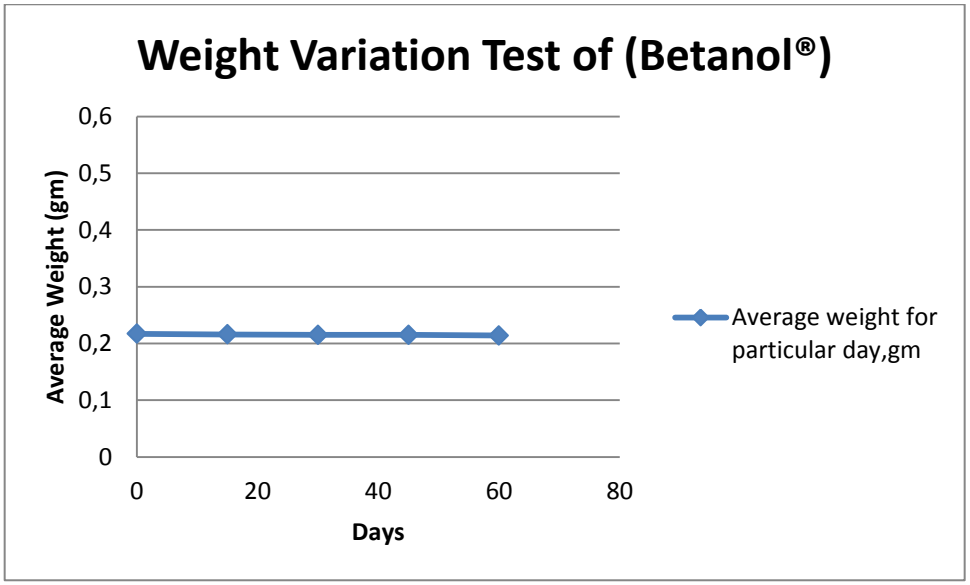

Figure 4.3: Weight variation of the sample throughout 60 days light exposure

4.2.3 Hardness Test

Four strips containing 60 tablets was exposed to normal light condition for 60 days. Hardness test was conducted of 5 tablets of each day interval (15, 30, 45, 60, days). In experimental day, a tablet strip containing 10 tablets was taken and 5 samples were collected for the test. Hardness test was conducted and average weight was calculated for each day. Data of these tests are given below:

Table 4.3: Hardness Test of Atenolol (Betanol®)

Days	Average Hardness of Particular Day (Kg-cm)
0	4.8
15	4.8
30	4.7
45	4.6
60	4.7

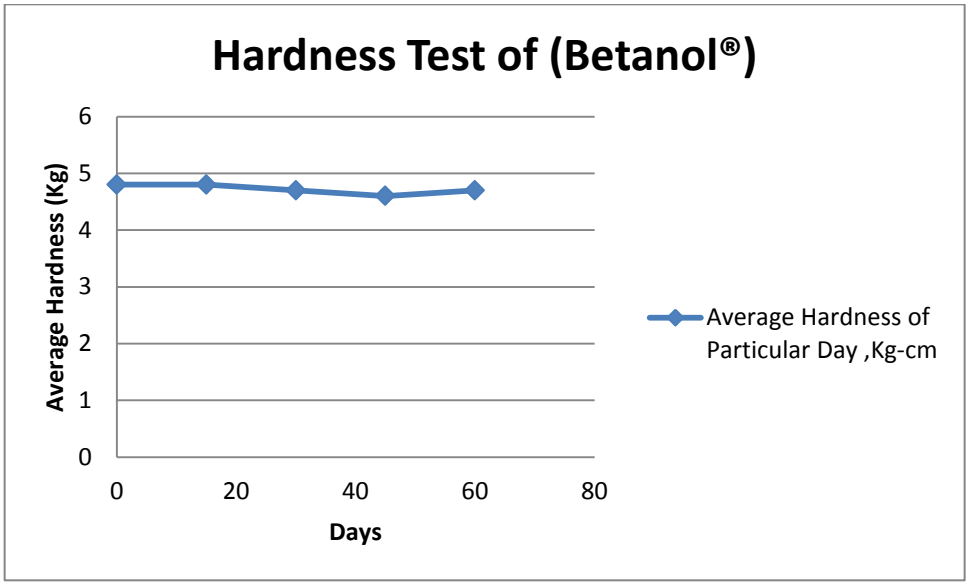

Figure 4.4: Hardness variation of the sample throughout 60 days light exposure

4.2.4:Thickness Test:

Four strips containing 60 tablets was exposed to normal light condition for 60 days. Thickness test was conducted of 5 tablets of each day interval (15, 30, 45, 60 days). In experimental day, atablet strip containing 10 tablets was taken and 5 samples were collected for the test. Thickness test was conducted and average weight was calculated for each day. Data of these tests are given below:

Table 4.4: Thickness Test of Atenolol (Betanol®)

Days	Average Thickness of Particular Days (cm)
0	0.35
15	0.35
30	0.33
45	0.32
60	0.33

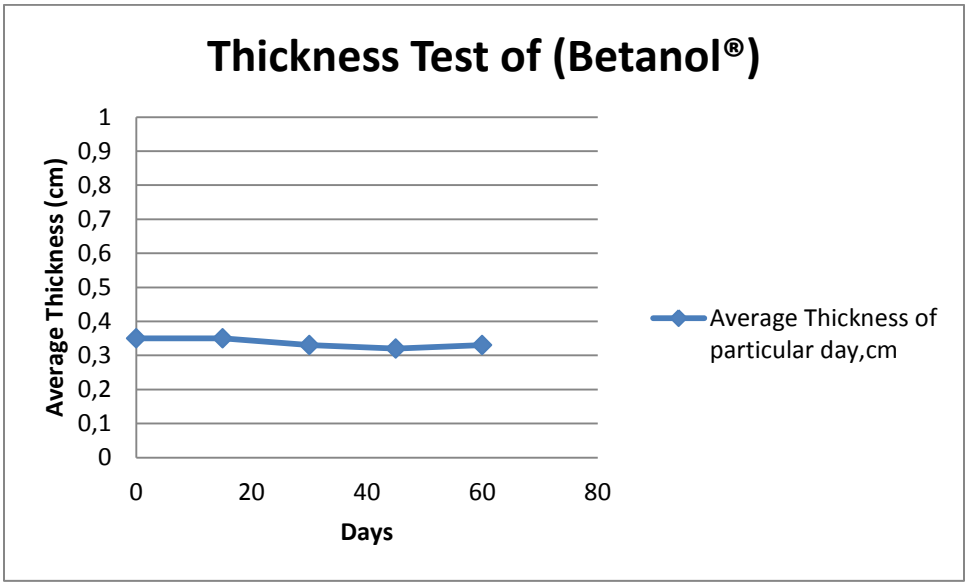

Figure 4.5: Thickness variation of sample throughout 60 days light exposure

4.3 Result from Potency Determination by UV- Spectroscopy

4.3.1 Result from Sample that was exposed under Normal Lightening Condition:

For our research purpose we have exposed tablets to the normal room light that were dispersed on top of the book shelf. We have collected those samples at specific intervals to determine its potency by UV-Spectroscopy. The results are given below;

Table 4.5: Concentration & absorbance of 60 Days interval for Atenolol (Betanol®)

Time interval	Absorbance(at 223.5 nm)	Average absorbance	Diluted conc. from sample in mg /ml (2500 times diluted)	Amount of drugs present in mg	Potency%
Control	0.794	0.781	0.0206	51.74	103.44
	0.780				
	0.770				
	0.798	0.781	0.0207	51.79	103.58
	0.779				
	0.770	0.778	0.0206	51.51	103.48
	0.794				
	0.780				
	0.760				

Table 4.6: Concentration & Absorbance of 15 Days Interval for Atenolol (Betanol®)

Time interval	Absorbance (at 223.5 nm)	Average absorbance	Diluted conc. from sample in mg /ml (2500 times)	Amount of drugs present in mg	Potency%
15 Days	0.686	0.696	0.0184	46.24	92.52
	0.696				
	0.708				
	0.684	0.690	0.0183	45.89	91.79
	0.696				
	0.692				
	0.685	0.680	0.0180	45.20	90.41
	0.671				
	0.684				

Table 4.7: Concentration & Absorbance of 30 Days Interval for Atenolol (Betanol®)

Time interval	Absorbance(at 223.5 nm)	Average absorbance	Diluted conc. from sample in mg /ml (2500 times)	Amount of drugs present in mg	Potency%
30 days	0.650	0.665	0.0177	44.25	88.58
	0.660				
	0.655				
	0.670	0.664	0.0176	44.24	88.45
	0.665				
	0.659				
	0.670	0.663	0.0176	44.10	88.24
	0.661				
	0.658				

Table 4.8: Concentration & Absorbance of 45 Days Interval for Atenolol (Betanol®)

Time interval	Absorbance (at 223.5 nm)	Average absorbance	Diluted conc. from sample in mg / ml (2500 times)	Amount of drugs present in mg	Potency%
45 Days	0.670	0.668	0.0177	44.43	88.86
	0.669				
	0.665				
	0.665	0.649	0.0172	43.19	86.39
	0.662				
	0.622				
	0.653	0.627	0.0167	41.77	83.54
	0.651				
	0.578				

Table 4.9: Concentration & Absorbance of 60 Days Interval for Atenolol (Betanol®)

Time interval	Absorbance(at 223.5 nm)	Average absorbance	Diluted conc. from sample in mg / ml (2500 times)	Amount of drugs present in mg	Potency%
60 days	0.616	0.690	0.0162	40.65	81.30
	0.610				
	0.603				
	0.610	0.603	0.0161	40.26	80.52
	0.603				
	0.598				
	0.609	0.601	0.0160	40.11	80.22
	0.603				
	0.592				

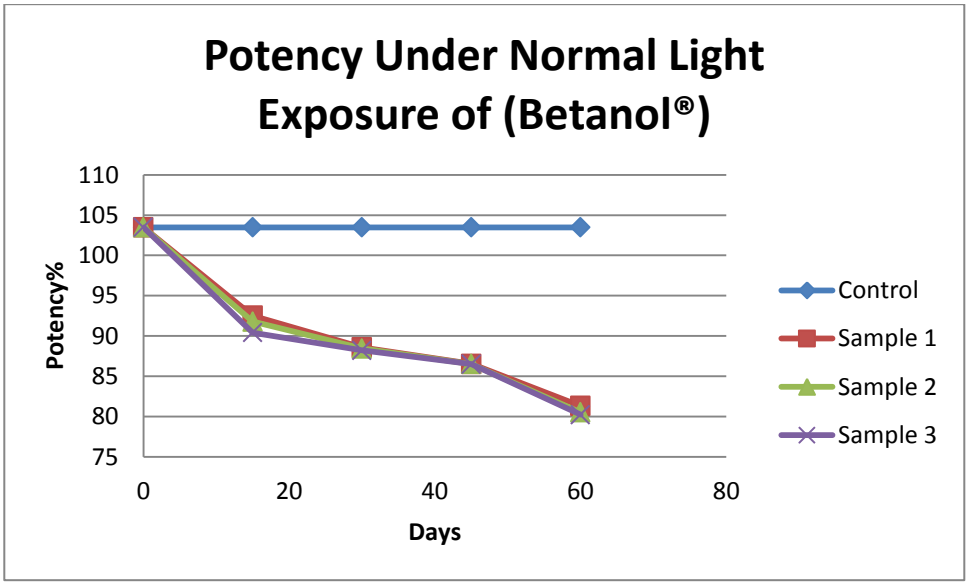

Figure4.6 **:** Graph showing the difference in Concentration after fixed day interval for Atenolol (Betanol®)

4.3.2 Result of samples that were exposed under 25W bulb

We found 27 different absorbance of atenolol for twenty seven samples exposed under the lamp (25W bulb); each for 2 hours' time interval and it was observed that the concentration of atenolol was declined in each time interval.

Table 4.10: Concentration & absorbance of Atenolol (Betanol®) for 1st time:

Time interval	Absorbance (at 223.5 nm)	Average absorbance	Diluted conc. from sample in mg / ml (2500 times)	Amount of drugs present in mg	Potency%
Control	0.686	0.696	0.0184	46.24	92.52
	0.696				
	0.708				
	0.684	0.690	0.0183	45.89	91.79
	0.696				
	0.692				
	0.685	0.680	0.0180	45.20	90.41
	0.671				
	0.684				

Table 4.10.1: Concentration & Absorbance after 2 hour Interval for Atenolol (Betanol®)

Time interval	Absorbance (at 223.5 nm)	Average absorbance	Diluted conc. from sample in mg / ml (2500 times)	Amount of drugs present in mg	Potency%
2 hours	0.670	0.668	0.0177	44.43	88.86
	0.669				
	0.665				
	0.665	0.649	0.0172	43.19	86.39
	0.662				
	0.622				
	0.653	0.627	0.0167	41.77	83.54
	0.651				
	0.578				

Table 4.10.2: Concentration & Absorbance after 4 hour Interval for Atenolol (Betanol®)

Time interval	Absorbance (at 223.5 nm)	Average absorbance	Diluted conc. from sample in mg / ml (2500 times)	Amount of drugs present in mg	Potency%
4 hours	0.611	0.602	0.0160	40.15	80.31
	0.601				
	0.595				
	0.592	0.587	0.0156	39.18	78.36
	0.577				
	0.593				
	0.542	0.567	0.0151	37.91	75.82
	0.567				
	0.593				

Table 4.10.3: Concentration & Absorbance after 6 hour Interval for Atenolol (Betanol®)

Time interval	Absorbance (at 223.5 nm)	Average absorbance	Diluted conc. from sample in mg / ml (2500 times)	Amount of drugs present in mg	Potency%
6 hours	0.638	0.603	0.0160	40.22	80.44
	0.552				
	0.620				
	0.586	0.598	0.0159	39.91	79.84
	0.579				
	0.630				
	0.670	0.593	0.0158	39.61	79.23
	0.560				
	0.551				

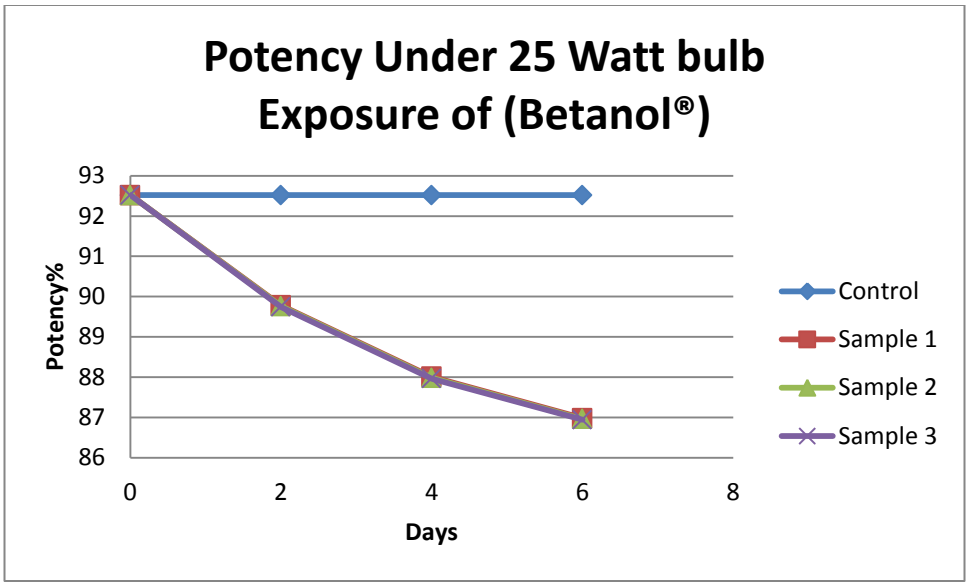

Figure 4.7 : Graph showing the difference in Concentration after each 2 hour time interval for Atenolol (Betanol®) for 1st time

Table 4.10.4: Concentration & absorbance of Atenolol (Betanol®) for Second time:

Time interval	Absorbance (at 223.5 nm)	Average absorbance	Diluted conc. from sample in mg / ml (2500 times)	Amount of drugs present in mg	Potency%
Control	0.686	0.696	0.0184	46.24	92.48
	0.696				
	0.704				
	0.698	0.689	0.0183	45.83	91.66
	0.694				
	0.677				
	0.693	0.691	0.0183	45.91	91.83
	0.691				
	0.689				

Table 4.10.5: Concentration & Absorbance after 2 hour Interval for Atenolol (Betanol®)

Time interval	Absorbance (at 223.5 nm)	Average absorbance	Diluted conc. from sample in mg / ml (2500 times)	Amount of drugs present in mg	Potency%
2 hours	0.680	0.676	0.0179	44.99	89.78
	0.677				
	0.673				
	0.670	0.665	0.0176	44.23	88.47
	0.652				
	0.673				
	0.670	0.662	0.0176	44.08	88.16
	0.666				
	0.652				

Table 4.10.6: Concentration & Absorbance after 4 hour Interval for Atenolol (Betanol®)

Time interval	Absorbance (at 223.5 nm)	Average absorbance	Diluted conc. from sample in mg / ml (2500 times)	Amount of drugs present in mg	Potency%
4 hours	0.638	0.603	0.0160	40.22	80.44
	0.552				
	0.620				
	0.586	0.598	0.0159	39.91	79.84
	0.579				
	0.630				
	0.670	0.593	0.0158	39.61	79.23
	0.560				
	0.551				

Table 4.10.7: Concentration & Absorbance after 6 hour Interval for Atenolol (Betanol®)

Time interval	Absorbance (at 223.5 nm)	Average absorbance	Diluted conc. from sample in mg / ml (2500 times)	Amount of drugs present in mg	Potency%
6 hours	0.552	0.550	0.0147	36.78	73.57
	0.548				
	0.552				
	0.525	0.540	0.0144	36.16	72.32
	0.575				
	0.521				
	0.541	0.529	0.0141	35.47	70.94
	0.527				
	0.521				

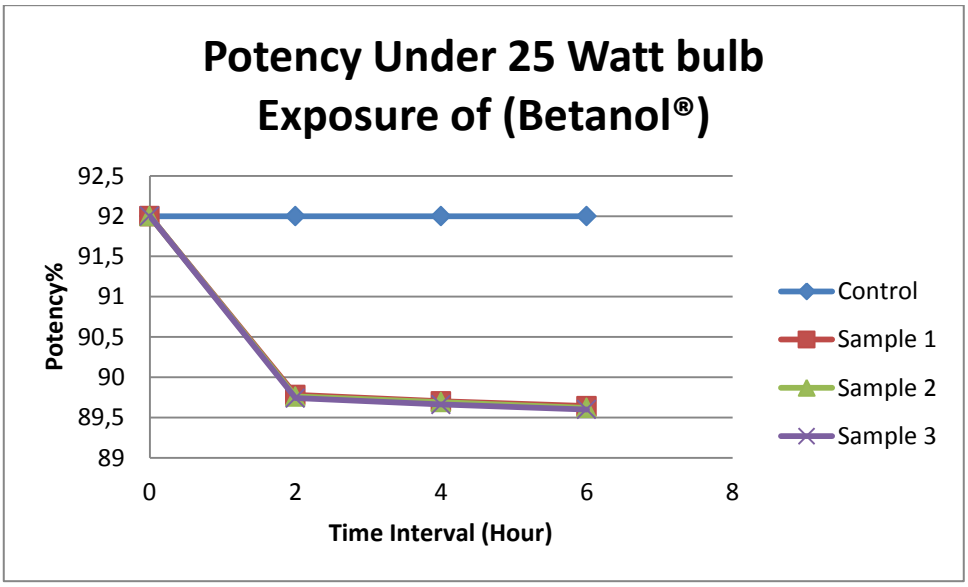

Figure 4.8 : Graph showing the difference in Concentration after each 2 hour time interval for Atenolol (Betanol®) for Second time

Table 4.10.8 : Concentration & absorbance of Atenolol (Betanol®) for Third time:

Time interval	Absorbance (at 223.5 nm)	Average absorbance	Diluted conc. from sample in mg / ml (2500 times)	Amount of drugs present in mg	Potency%
Control	0.686	0.696	0.0184	46.24	92.52
	0.696				
	0.708				
	0.694	0.693	0.0184	46.04	92.09
	0.689				
	0.697				
	0.685	0.680	0.0180	45.20	90.41
	0.671				
	0.684				

Table 4.10.9: Concentration & Absorbance after 2 hour Interval for Atenolol (Betanol®)

Time interval	Absorbance (at 223.5 nm)	Average absorbance	Diluted conc. from sample in mg / ml (2500 times)	Amount of drugs present in mg	Potency%
2 hours	0.671	0.653	0.0173	43.45	86.91
	0.603				
	0.685				
	0.671	0.648	0.072	43.15	86.31
	0.589				
	0.685				
	0.671	0.641	0.0170	42.72	85.44
	0.665				
	0.589				

Table 4.10.10: Concentration & Absorbance after 2 hour Interval for Atenolol (Betanol®)

Time interval	Absorbance(at 223.5 nm)	Average absorbance	Diluted conc. from sample in mg / ml (2500 times)	Amount of drugs present in mg	Potency%
4 hours	0.584	0.590	0.0157	39.37	78.7
	0.596				
	0.592				
	0.565	0.556	0.0148	37.17	74.34
	0.551				
	0.554				
	0.523	0.557	0.0148	37.24	74.48
	0.571				
	0.579				

Table 4.10.11: Concentration & Absorbance after 4 hour Interval for Atenolol (Betanol®)

Time interval	Absorbance (at 223.5 nm)	Average absorbance	Diluted conc. from sample in mg / ml (2500 times)	Amount of drugs present in mg	Potency%
6 hours	0.503	0.551	0.0147	36.85	73.70
	0.571				
	0.579				
	0.536	0.545	0.0145	36.48	72.97
	0.551				
	0.549				
	0.550	0.543	0.0145	36.33	72.66
	0.549				
	0.530				

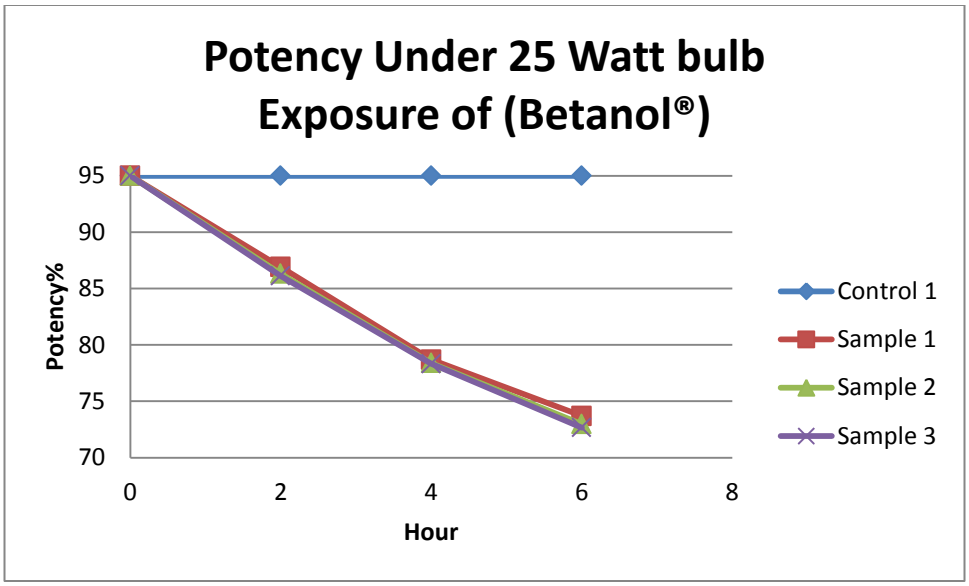

Figure 4.9 : Graph showing the difference in Concentration after each 2 hour time interval forAtenolol (Betanol®) for Third time

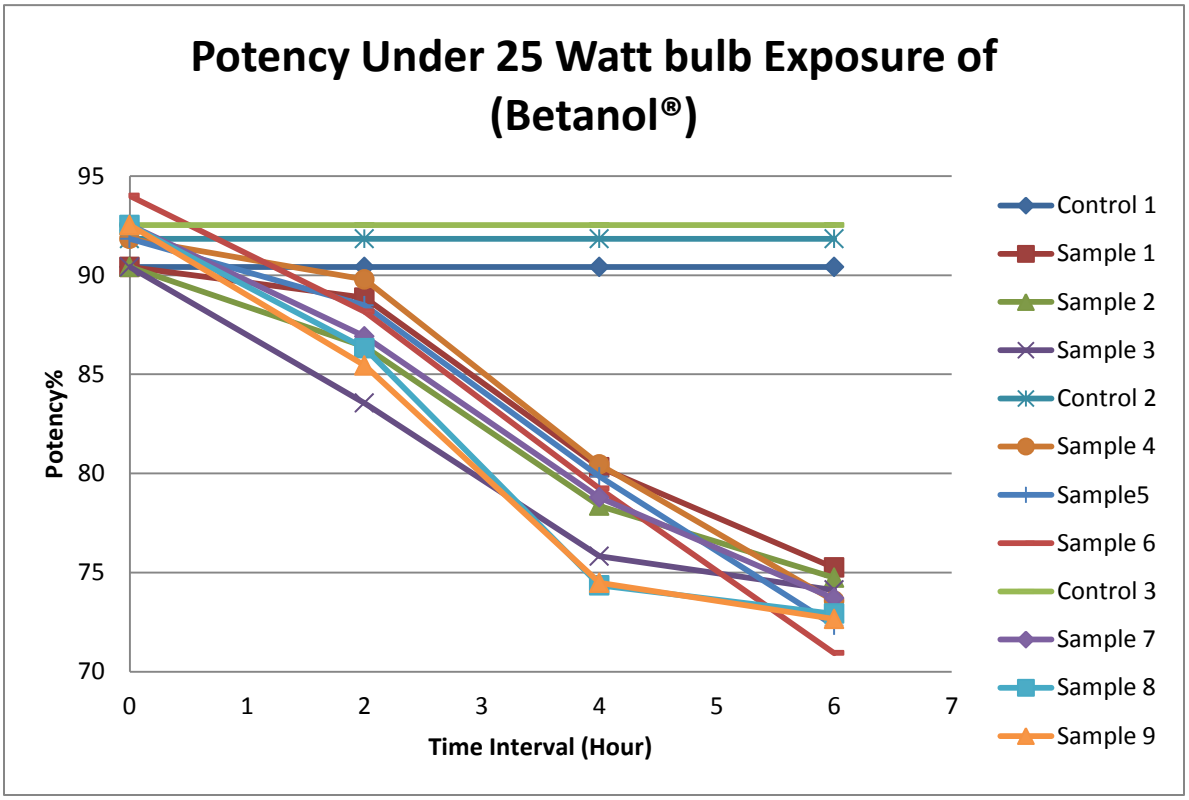

Figure 4.10 : Graph showing the difference in Concentration after each 2 hour time interval for Atenolol (Betanol®) for first time, second time, third time.

4.3.3 Result of samples that were exposed under 40W bulb

We found 27 different absorbance of atenolol for twenty seven samples exposed under the lamp (40W bulb); each for 2 hours time interval and it was observed that the concentration of atenolol was declined in each time interval.

Table4.10.12: Concentration & absorbance of Atenolol (Betanol®) for 1st time

Time interval	Absorbance (at 223.5 nm)	Average absorbance	Diluted conc. from sample in mg / ml (2500 times)	Amount of drugs present in mg	Potency%
Control	0.686	0.696	0.0184	46.24	92.52
	0.696				
	0.708				
	0.694	0.693	0.0184	46.04	92.09
	0.689				
	0.697				
	0.685	0.680	0.0180	45.20	90.41
	0.671				
	0.684				

Table 4.10.13: Concentration & Absorbance after 2 hour Interval for Atenolol (Betanol®)

Time interval	Absorbance (at 223.5 nm)	Average absorbance	Diluted conc. from sample in mg / ml (2500 times)	Amount of drugs present in mg	Potency%
2 hours	0.664	0.625	0.0166	41.66	83.33
	0.623				
	0.589				
	0.636	0.619	0.0165	41.27	82.55
	0.613				
	0.609				
	0.607	0.600	0.0160	40.06	80.13
	0.598				
	0.597				

Table 4.10.14: Concentration & Absorbance after 4 hour Interval for Atenolol (Betanol®)

Time interval	Absorbance (at 223.5 nm)	Average absorbance	Diluted conc. from sample in mg / ml (2500 times)	Amount of drugs present in mg	Potency%
4 hours	0.560	0.547	0.0146	36.63	73.27
	0.549				
	0.534				
	0.577	0.546	0.0146	35.57	73.14
	0.562				
	0.578				
	0.548	0.546	0.0146	35.55	73.10
	0.550				
	0.541				

Table 4.10.15: Concentration & Absorbance after 6 hour Interval for Atenolol (Betanol®)

Time interval	Absorbance (at 223.5 nm)	Average absorbance	Diluted conc. from sample in mg / ml (2500 times)	Amount of drugs present in mg	Potency%
6 hours	0.526	0.525	0.0140	35.16	70.33
	0.526				
	0.523				
	0.526	0.522	0.0140	35.01	70.03
	0.523				
	0.519				
	0.520	0.511	0.0137	34.26	69.52
	0.519				
	0.494				

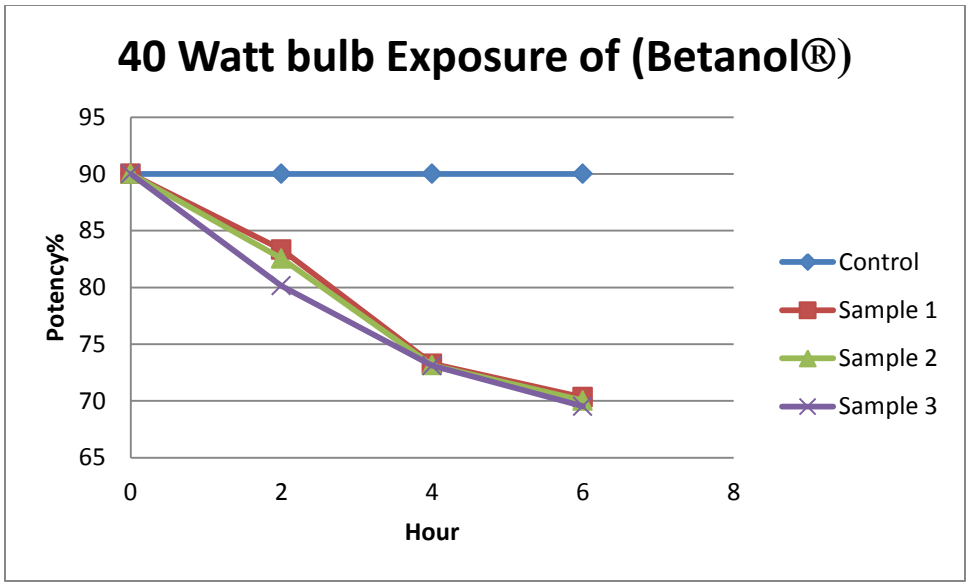

Figure: 4.11:Graph showing the difference in Concentration after each 2 hour time interval for Atenolol (Betanol®) for 1st time

Table 4.10.16:Concentration & absorbance of Atenolol (Betanol®) for Second time

Time interval	Absorbance (at 223.5 nm)	Average absorbance	Diluted conc. from sample in mg / ml (2500 times)	Amount of drugs present in mg	Potency%
Control	0.686	0.696	0.0184	46.24	92.52
	0.696				
	0.708				
	0.694	0.693	0.0184	46.04	92.09
	0.689				
	0.697				
	0.685	0.680	0.0180	45.20	90.41
	0.671				
	0.684				

Table 4.10.17: Concentration & Absorbance after 2 hour Interval for Atenolol (Betanol®)

Time interval	Absorbance (at 223.5 nm)	Average absorbance	Diluted conc. from sample in mg / ml (2500 times)	Amount of drugs present in mg	Potency%
2 hours	0.648	0.656	0.0174	43.65	87.30
	0.663				
	0.658				
	0.663	0.655	0.0174	43.58	87.17
	0.650				
	0.652				
	0.670	0.655	0.0174	43.60	87.21
	0.649				
	0.647				

Table 4.10.18: Concentration & Absorbance after 4 hour Interval for Atenolol (Betanol®)

Time interval	Absorbance(at 223.5 nm)	Average absorbance	Diluted conc. from sample in mg / ml (2500 times)	Amount of drugs present in mg	Potency%
4 hours	0.584	0.590	0.0157	39.37	78.78
	0.596				
	0.592				
	0.570	0.586	0.0156	39.11	78.23
	0.596				
	0.592				
	0.590	0.585	0.0156	39.05	78.01
	0.581				
	0.584				

Table 4.10.19: Concentration & Absorbance after 6 hour Interval for Atenolol (Betanol®)

Time interval	Absorbance (at 223.5 nm)	Average absorbance	Diluted conc. from sample in mg / ml (2500 times)	Amount of drugs present in mg	Potency%
6 hours	0.526	0.523	0.0140	35.03	70.07
	0.523				
	0.520				
	0.521	0.521	0.0139	34.93	69.86
	0.526				
	0.517				
	0.517	0.517	0.0138	34.65	69.95
	0.511				
	0.520				

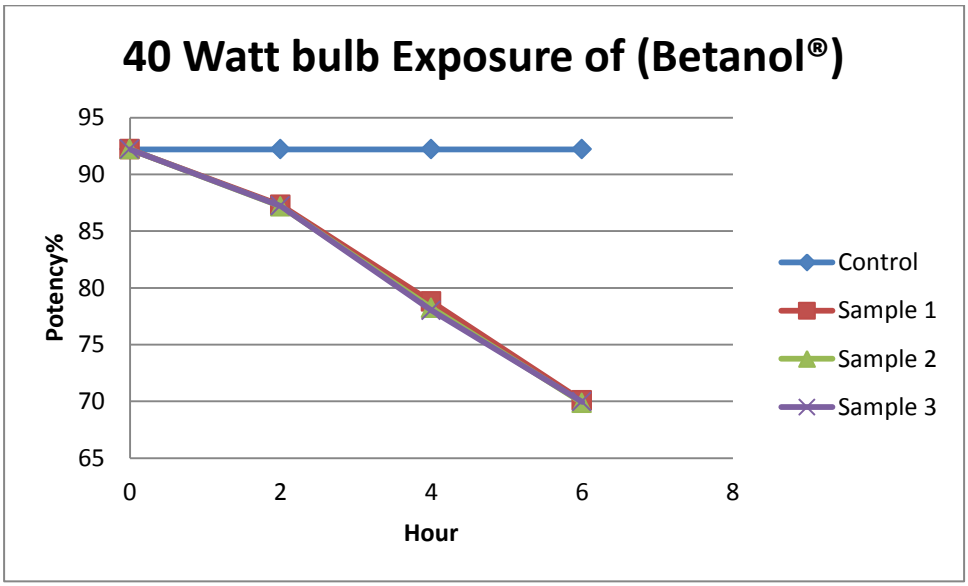

Figure4.12:Graph showing the difference in Concentration after each 2 hour time interval for Atenolol (Betanol®) for Second time

Table4.10.19:Concentration & absorbance of Atenolol (Betanol®) for Second time

Time interval	Absorbance (at 223.5 nm)	Average absorbance	Diluted conc. from sample in mg / ml (2500 times)	Amount of drugs present in mg	Potency%
Control	0.686	0.696	0.0184	46.24	92.52
	0.696				
	0.708				
	0.684	0.690	0.0183	45.89	91.79
	0.696				
	0.692				
	0.685	0.680	0.0180	45.20	90.41
	0.671				
	0.684				

Table 4.10.20: Concentration & Absorbance of after 2 hour Interval for Atenolol (Betanol®)

Time interval	Absorbance (at 223.5 nm)	Average absorbance	Diluted conc. from sample in mg / ml (2500 times)	Amount of drugs present in mg	Potency%
2 hours	0.678	0.667	0.0177	44.40	88.81
	0.671				
	0.654				
	0.671	0.663	0.0176	44.12	88.25
	0.668				
	0.651				
	0.670	0.660	0.0175	43.93	87.86
	0.661				
	0.650				

Table 4.10.21: Concentration & Absorbance after 4 hour Interval for Atenolol (Betanol®)

Time interval	Absorbance (at 223.5 nm)	Average absorbance	Diluted conc. from sample in mg / ml (2500 times)	Amount of drugs present in mg	Potency%
4 hours	0.581	0.575	0.0153	38.44	76.89
	0.576				
	0.570				
	0.571	0.567	0.0152	37.88	75.77
	0.568				
	0.562				
	0.548	0.546	0.0146	35.55	73.10
	0.550				
	0.541				

Table 4.10.22: Concentration & Absorbance after 6 hour Interval for Atenolol (Betanol®)

Time interval	Absorbance (at 223.5 nm)	Average absorbance	Diluted conc. from sample in mg / ml (2500 times)	Amount of drugs present in mg	Potency%
6 hours	0.530	0.526	0.0141	35.25	70.50
	0.528				
	0.521				
	0.530	0.526	0.0140	35.25	70.46
	0.527				
	0.521				
	0.530	0.524	0.0140	35.14	70.29
	0.525				
	0.519				

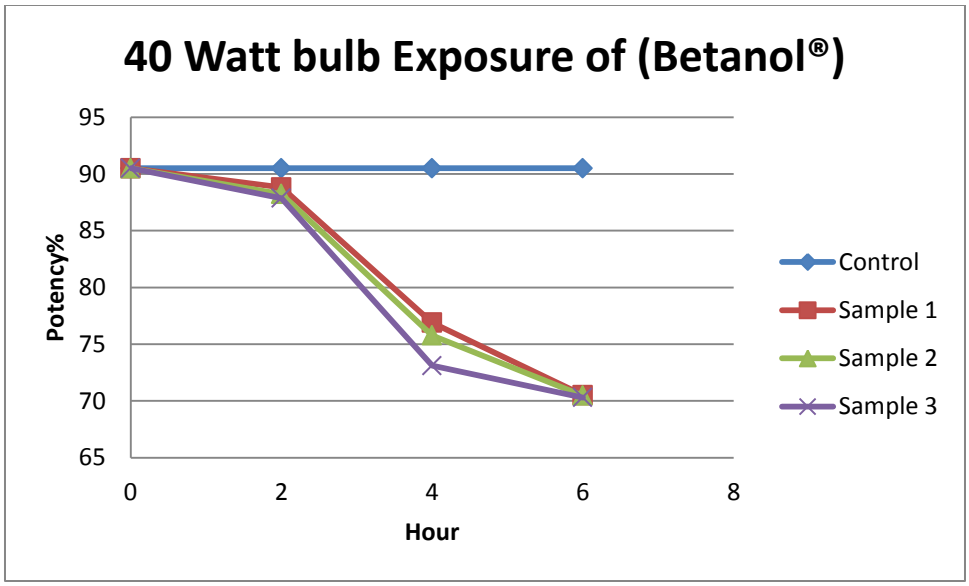

Figure 4.13:Graph showing the difference in Concentration after each 2 hour time interval for Atenolol (Betanol®) for Third time

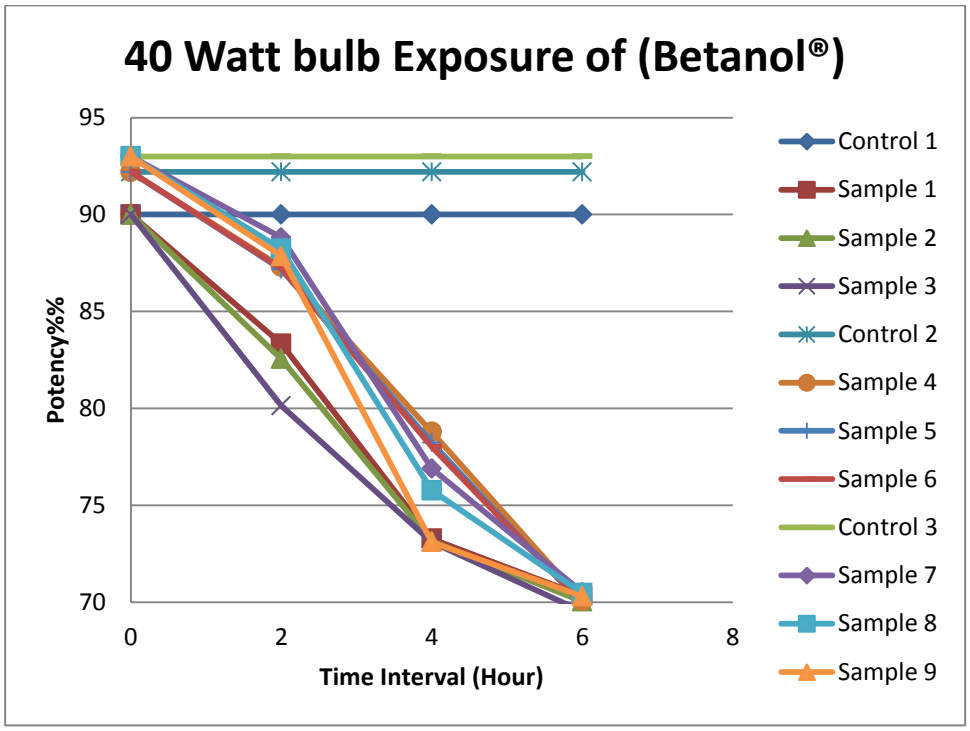

Figure 4.14:Graph showing the difference in Concentration after each 2 hour time interval for Atenolol (Betanol®) for First time, Second time, third time

4.3.4 Result of samples that were exposed under direct sunlight

We found 27 different absorbance of atenolol for twenty seven samples exposed under the direct sunlight, each for 2 hours time interval and it was observed that the concentration of atenolol was declined in each time interval.

Table4.10.23:Concentration & absorbance of Atenolol (Betanol®) for 1st time

Time interval	Absorbance (at 223.5 nm)	Average absorbance	Diluted conc. from sample in mg/ ml (2500 times)	Amount of drugs present in mg	Potency%
Control	0.686	0.696	0.0184	46.24	92.52
	0.696				
	0.708				
	0.684	0.690	0.0183	45.89	91.79
	0.696				
	0.692				
	0.685	0.680	0.0180	45.20	90.41
	0.671				
	0.684				

Table 4.10.24: Concentration & Absorbance after 2 hour Interval for Atenolol (Betanol®)

Time interval	Absorbance (at 223.5 nm)	Average absorbance	Diluted conc. from sample in mg / ml (2500 times)	Amount of drugs present in mg	Potency%
2 hours	0.628	0.603	0.0160	40.22	80.44
	0.620				
	0.552				
	0.579	0.568	0.0151	37.95	75.90
	0.568				
	0.560				
	0.552	0.556	0.0148	37.17	74.35
	0.552				
	0.548				

Table 4.10.25: Concentration & Absorbance after 4 hour Interval for Atenolol (Betanol®)

Time interval	Absorbance (at 223.5 nm)	Average absorbance	Diluted conc. from sample in mg / ml (2500 times)	Amount of drugs present in mg	Potency%
4 hours	0.543	0.541	0.0144	36.20	72.40
	0.542				
	0.539				
	0.531	0.527	0.0141	35.31	70.63
	0.530				
	0.521				
	0.521	0.515	0.0138	34.56	69.12
	0.516				
	0.510				

Table 4.10.26: Concentration & Absorbance after 6 hour Interval for Atenolol (Betanol®)

Time interval	Absorbance (at 223.5 nm)	Average absorbance	Diluted conc. from sample in mg / ml (2500 times)	Amount of drugs present in mg	Potency%
6 hours	0.515	0.510	0.0136	34.21	68.43
	0.509				
	0.507				
	0.501	0.497	0.0133	33.37	66.75
	0.497				
	0.494				
	0.490	0.487	0.0130	32.70	65.45
	0.487				
	0.484				

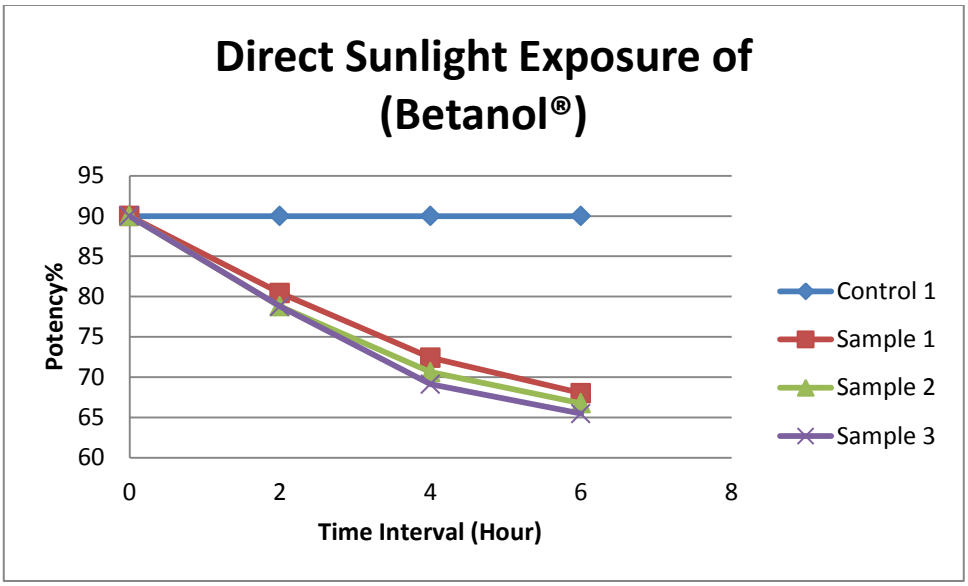

Figure 4.15Graph showing the difference in Concentration after each 2 hour time interval for Atenolol (Betanol®) for 1st time

Table4.10.27:Concentration & absorbance of Atenolol (Betanol®) for Second time

Time interval	Absorbance (at 223.5 nm)	Average absorbance	Diluted conc. from sample in mg / ml (2500 times)	Amount of drugs present in mg	Potency%
Control	0.699	0.696	0.0184	46.24	92.48
	0.697				
	0.692				
	0.690	0.688	0.0183	45.76	91.53
	0.689				
	0.687				
	0.680	0.676	0.0179	44.96	90.10
	0.677				
	0.672				

Table 4.10.28: Concentration & Absorbance after 2 hour Interval for Atenolol (Betanol®)

Time interval	Absorbance (at 223.5 nm)	Average absorbance	Diluted conc. from sample in mg / ml (2500 times)	Amount of drugs present in mg	Potency%
2 hours	0.648	0.615	0.0164	41.04	82.08
	0.620				
	0.579				
	0.577	0.576	0.0153	38.16	76.33
	0.576				
	0.575				
	0.552	0.556	0.0148	37.17	74.35
	0.552				
	0.548				

Table 4.10.29: Concentration & Absorbance after 4 hour Interval for Atenolol (Betanol®)

Time interval	Absorbance (at 223.5 nm)	Average absorbance	Diluted conc. from sample in mg / ml (2500 times)	Amount of drugs present in mg	Potency%
4 hours	0.546	0.541	0.0144	36.22	72.45
	0.540				
	0.538				
	0.538	0.534	0.0143	35.79	71.58
	0.536				
	0.530				
	0.523	0.519	0.0139	34.77	69.55
	0.520				
	0.516				

Table 4.10.30: Concentration & Absorbance after 6 hourInterval for Atenolol (Betanol®)

Time interval	Absorbance (at 223.5 nm)	Average absorbance	Diluted conc. from sample in mg / ml (2500 times)	Amount of drugs present in mg	Potency
6 hours	0.506	0.504	0.0135	33.80	67.61
	0.503				
	0.503				
	0.498	0.497	0.0133	33.35	66.70
	0.497				
	0.496				
	0.490	0.485	0.0130	32.62	65.24
	0.487				
	0.480				

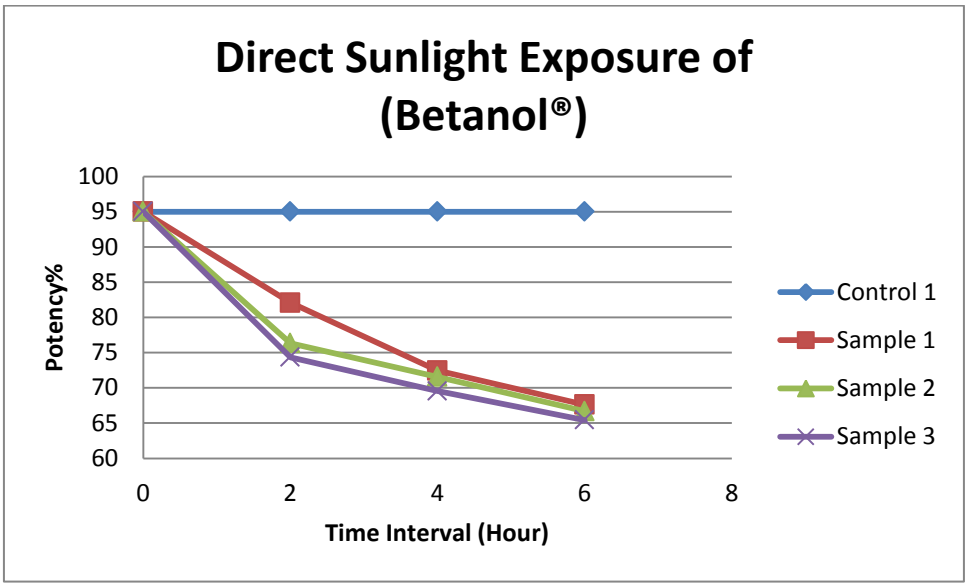

Figure 4.16:Graph showing the difference in Concentration after each 2 hour time interval for Atenolol (Betanol®) for Second time

Table 4.10.30:Concentration & absorbance of Atenolol (Betanol®) for Third time

Time interval	Absorbance (at 223.5 nm)	Average absorbance	Diluted conc. from sample in mg / ml (2500 times)	Amount of drugs present in mg	Potency%
Control	0.686	0.696	0.0184	46.24	92.52
	0.696				
	0.708				
	0.684	0.690	0.0183	45.89	91.79
	0.696				
	0.692				
	0.685	0.680	0.0180	45.20	90.41
	0.671				
	0.684				

Table 4.10.31: Concentration & Absorbance After 2 hour Interval for Atenolol (Betanol®)

Time interval	Absorbance (at 223.5 nm)	Average absorbance	Diluted conc. from sample in mg / ml (2500 times)	Amount of drugs present in mg	Potency%
2 hours	0.648	0.615	0.0164	41.04	82.08
	0.620				
	0.579				
	0.577	0.576	0.0153	38.16	76.33
	0.576				
	0.575				
	0.552	0.556	0.0148	37.17	74.35
	0.552				
	0.548				

Table 4.10.32: Concentration & Absorbance after 4 hour Interval for Atenolol (Betanol®)

Time interval	Absorbance (at 223.5 nm)	Average absorbance	Diluted conc. from sample in mg / ml (2500 times)	Amount of drugs present in mg	Potency
4 hours	0.546	0.541	0.0144	36.22	72.45
	0.540				
	0.538				
	0.538	0.534	0.0143	35.79	71.58
	0.536				
	0.530				
	0.523	0.519	0.0139	34.77	69.55
	0.520				
	0.516				

Table 4.10.33: Concentration & Absorbance After 6 hour Interval for Atenolol (Betanol®)

Time interval	Absorbance (at 223.5 nm)	Average absorbance	Diluted conc. from sample in mg / ml (2500 times)	Amount of drugs present in mg	Potency
6 hours	0.515	0.510	0.0136	34.21	68.43
	0.509				
	0.507				
	0.501	0.497	0.0133	33.37	66.75
	0.497				
	0.494				
	0.490	0.487	0.0130	32.70	65.45
	0.487				
	0.484				

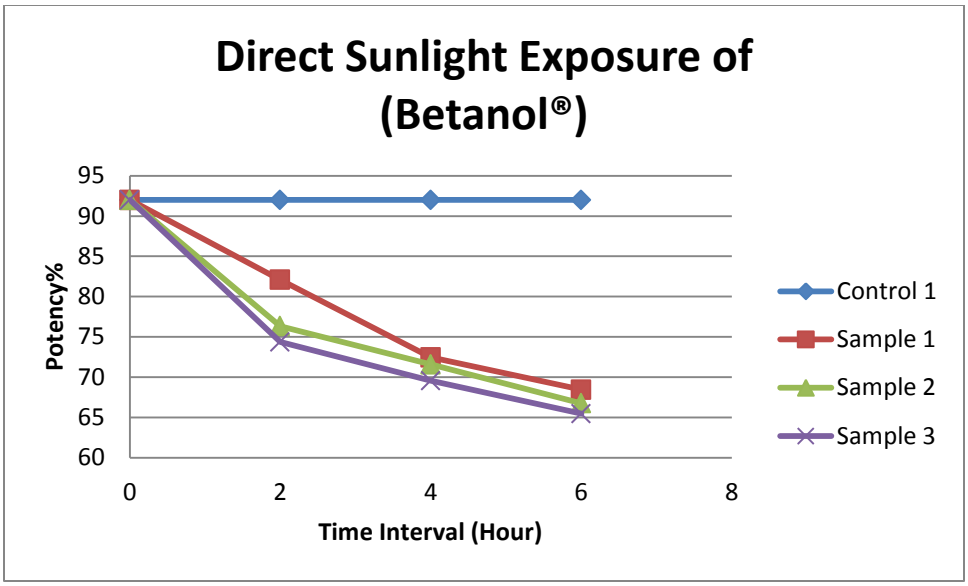

Figure 4.17: Graph showing the difference in Concentration after each 2 hour time interval for Atenolol (Betanol®) for Third time

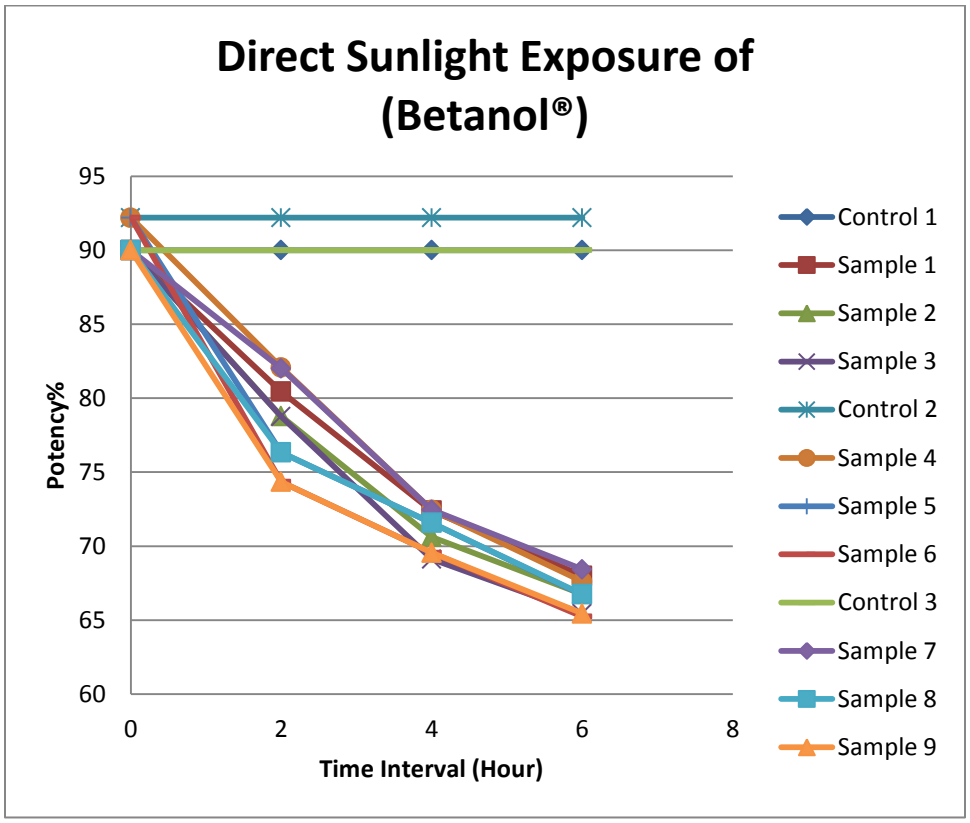

Figure 4.18: Graph showing the difference in Concentration after each 2 hour time interval for Atenolol (Betanol®) for first time, Second time, Third time.

CHAPTER FIVE

DISSCUSSION

In this study it was observed that the hardness of the sample tablets fluctuated with in a very short range. The results were very close within the total 60 days of work. Even the average hardness values were also very close to each other. The standard deviation for hardness was ±0.47284 kg. So the hardness of atenolol tablet was not affected by different lighting conditions.

It was found that the Weight Variation of the sample tablets were within the specified range (Weight of tablet 130 mg or less then = ±10%). According to U.S.P. if no more than 2 tablets are outside the percentage limit and if no tablet varies by more than 2 times from the specified percentage limit, the tablets pass the test. The standard deviation for weight variation was ±0.00135 g. Therefore, it is clear that the light has no effect on weight of the atenolol tablets.

The thickness of the sample tablets were also very close to each other. Little fluctuation was observed with the periodic work. After each days interval the thickness remained constant or close to constant. The standard deviation of thickness was ±0.0152. So the effects of light dose not influence the thickness of atenolol.

It was found that the concentration of atenolol decreased gradually in every innovation of light exposure. When sample tablets (Betanol®) were kept under the electrical bulb (25 watt & 40 watt) and direct sunlight and was exposed for 2 hours, it was found that the concentration of atenolol decreased gradually. The tablet samples which were exposed 4 hours on light had less concentration of atenolol than the 2 hour exposed samples. Moreover, samples exposed for 6 hours had less concentration of atenolol than 2 hour and 4 hour light exposed samples. It was observed that the direct sunlight exposed samples which were degradation rate higher compare to normal room light, 25 watt bulb and 40 watt bulb.

From this research work it can be concluded that, there should be a change in the packaging system of the atenolol tablets.

CHAPTER SIX

CONCLUSION

Conclusion

In this experiment it was concluded that the physical parameters like weight variation, hardness, thickness have complies with the USP and BP specifications. As a result, there were remarkable changes in concentration or potency. The concentration of atenolol in the samples decreased gradually after exposure to electrical bulb (25 watt and 40 watt) light condition, direct sunlight and normal light exposure (room temperature) condition. It can be established that the Betanol® containing atenolol is light sensitive and the concentration/potency decreases after exposure to light. Therefore packaging of this drug should be done in such a way that it does not come with the contact of light.

CHAPTER SEVEN

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