Reproducibility Study of the Efficiency of Packaging on Preventing Photolytic Degradation of Etnol® (Atenolol Tablet)

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In the name of

Almighty Allah,

The most Gracious

&

The most Merciful

Dedicated to My Family

DECLARATION BY THE CANDIDATE

I, Safayet Hossain Bhuiyan, hereby declare that the dissertation entitled **"Reproducibility study of the efficiency of packaging on preventing photolytic degradation of Etnol® (Atenolol Tablet)"** 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 bona fide record of original research work carried out by me during Fall 2014, under the supervision and guidance of Mohammed Faisal Bin Karim, 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.

Signature of the candidate

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CERTIFICATE BY THE CANDIDATE SUPERVISORS

This is to certify that the research **"Reproducibility study of the efficiency of packaging on preventing photolytic degradation of Etnol® (Atenolol Tablet)"** submitted to the department of pharmacy, East West University for partial fulfillment of the requirements for the degree of Bachelor of Pharmacy was carried out by 'Safayet Hossain Bhuiyan' (ID: 2011-3-70-048) 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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CERTIFICATE BY THE CHAIRPERSON

This is to certify that the thesis entitled **"Reproducibility study of the efficiency of packaging on preventing photolytic degradation of Etnol® (Atenolol Tablet)"** 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 bona fide record of original and genuine research work carried out by 'Safayet Hossain Bhuiyan' during Fall 2014 of his research in the Department of Pharmacy, East West University.

Dr. Chowdhury Faiz Hossain

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Abstract

This research work was conducted to describe the coating efficiency on photolytic degradation of Etnol® (Atenolol). The objective of this study was to determine the reproducibility of the effect of Atonolol in various lighting conditions (control, sunlight, normal room light, 25watt & 40watt bulb). UV spectrophotometer was used for measuring the absorbance. Besides, physical tests were performed for evaluation of color change, weight variation, thickness and hardness of the Etnol® tablets from same batch. Physical tests were performed according to the specification of USP and the standard deviation of weight variation; hardness and thickness were ±0.0155 mm, ±0.36253 kg & ±0.0025gm respectively. But it was also observed that the concentration of Atenolol was decreased gradually in various light condition (25watt & 40watt electrical bulb, sunlight, normal light) and at 25watt, 40watt, direct sunlight and normal room light exposure the degradation were observed 11.86%, 15.45%, 23.06% & 10.23% respectively. So, it can be said that packaging alone was not sufficient to protect the drug from light.

Chapter One

INTRODUCTION

1.1 Objective of the Research

The objective of the research project was to evaluate the reproducibility of the study that had been previously done in order to determine whether the packaging is effective to prevent the photolytic degradation of Atenolol which is a photosensitive drug. In our research we conducted experiment to determine photosensitivity of atenolol in various lightening conditions (control, sunlight, normal light, 25 watt bulb and 40 watt bulb condition). For this purpose, the available brand was chosen i.e. Etnol® of Biopharma Limited. 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 degrades in presence of light or not.

1.2 Beta blockers

1.2.1 Definition

Beta blockers, also known as beta-adrenergic blocking agents, are medications that reduce your 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).

1.2.2 Classification of Beta Blockers:

- 1) First generation (beta 1 & 2 non cardio selective):
    - Propranolol (Soliman, 2009)
- 2) Second generation (beta 1 cardio selective):
    - Atenolol
    - Metoprolol
    - Acebutolol
    - Bisoprolol
    - Betaxolol

- Celiprolol (Soliman, 2009)

- 3) Third generation:
  - Vasodilatory properties:
  - Carvedilol (beta 1&2 alpha 1)
  - Bucindolol
  - Nebivolol (beta 3) (Soliman, 2009)

1.2.3 Mode of Action of Beta Blocker

Beta blocker or beta adrenergic receptor works by blocking the endogenous catecholamines or neurotransmitters norepinephrine and epinephrine action from binding to receptors. There are three types of beta receptors. They are- beta$_1$ (β_1), beta$_2$ (β_2) and beta$_3$ (β_3).

- ⇨ β_1 - receptors are located commonly in the heart and kidneys.
- ⇨ β_2 - receptors are located mainly in the lungs, gastrointestinal tract, liver, uterus, vascular smooth muscle, and skeletal muscle.
- ⇨ β_3 - receptors are generally located in fat cells.

When the neurotransmitters or catecholamines are stopped binding to the receptors, it blocks adrenaline (epinephrine). This action allows the heart to relax and heart beat become slow thereby reducing the amount of blood that the heart can pump easily. Due to this action, it improves the pumping mechanism of the heart. (β- Blocker drug info, 2014)

1.3 Atenolol

Atenolol is in a group of drugs called beta-blockers. 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, 2014).

1.4 Physico- chemical properties of Atenolol:

1.4.1 Chemical properties:

- Chemical name: 2-[p-[2-hydroxy-3-[(isopropylamino)propoxy]phenyl]acetamide
- Molecular formula: $C_{14}H_{22}N_2O_3$
- Molecular weight : 266.34 (Drugs.com, 2014)

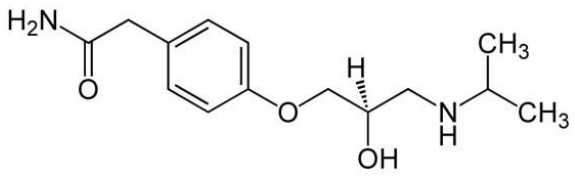


Figure 1.1: Molecular structure of atenolol

1.4.2 Physical properties:

i. Appearance: Atenolol is an odorless white powder

ii. Melting point: 152-154°C

iii. Dissociation constant: pKa-9.6 @ 24°C

iv. Partition co efficient [log P(octanol)]: 0.23

v. Enantiomers: YES R(+) and S(-) (Homepage.ntlworld.com, 2014)

1.4.3 Solubility:

i. Water: 0.3 mg/mL

ii. Ethanol: 3.4 mg/mL

iii. DMSO: 18 mg/mL

iv. Ether: Practically Insoluble (Homepage.ntlworld.com, 2014)

1.5 Pharmacokinetics:

1.5.1 Absorption, Distribution & Excretion of Atenolol:

- Absorption of an oral dose is rapid and consistent but incomplete in human. From the gastrointestinal tract approximately 50% of an oral dose is absorbed easily, the remaining amount excreted unchanged in the feces. It is shown that atenolol's peak blood levels are reached between two 2 and four 4 hours after ingestion.
- Approximately 5-15% of atenolol is bound to plasma protein.
- During pregnancy atenolol easily crosses the placenta, and has been detected in cord blood. During continuous administration, fetal serum concentrations of the drug are probably equivalent to those in maternal serum. Atenolol is distributed into milk immediately after taking the drugs.
- From the GI tract atenolol is rapidly but incompletely absorbed .From an oral dose of atenolol only about 50-60% is absorbed.
- In patients with normal renal function, atenolol has a plasma half-life (t1/2) of 6-7 hours. Children with normal renal function may exhibit a shorter elimination half-life. Approximately 40-50% of an oral dose of the drug is excreted in urine unchanged. The remainder is excreted unchanged in feces, principally as unabsorbed drug.
- Approximately 6 to 7 hours is the elimination half-life of oral atenolol, and by chronic administration there is no alteration of the kinetic profile of the drug . During the first 7 hours of administration of drug, declines from peak levels are rapid (5 to 10 fold); thereafter, plasma levels decay with a half-life similar to that of orally administered drug.
- Both beta-blocking and antihypertensive effects persist for at least 24 hours after taking oral doses of 50 mg or 100 mg of atenolol, Elimination of atenolol is closely related to the glomerular filtration rate when renal function is impaired for some reasons. (Pubchem.ncbi.nlm.nih.gov, 2014)

1.6 Pharmacodynamics:

- In standard animal or human pharmacological tests, beta-adrenoreceptor blocking activity of atenolol has been demonstrated by: (1) resting and exercise heart rate reduction and cardiac output, (2)systolic and diastolic blood pressure reduction at rest and on exercise, (3) isoproterenol inhibition that induced tachycardia, and (4) reflex orthostatic tachycardia reduction.
- By reduction of exercise tachycardia a significant beta-blocking effect of atenolol is measured, that is apparent within one hour following oral administration of a single dose. This effect of beta blocking is maximal at about 2 to 4 hours, and that is persist for at least 24 hours.
- The duration of action is dose related for both orally and intravenously administered drug, and also bears a linear relationship to the logarithm of plasma atenolol concentration.
- For a single 10 mg intravenous dose is largely dissipated by 12 hours by the effect on exercise tachycardia, whereas beta-blocking activity of single oral doses of 50 mg and 100 mg atenolol is still evident beyond 24 hours following administration. However, as has been shown for all beta-blocking agents, the antihypertensive effect does not appear to be related to plasma level.
- In asthmatic patients it has been shown that, a dose of atenolol produced a greater effect on resting heart rate. In a placebo controlled comparison of approximately equipotent oral doses of several beta blockers, atenolol produced a significantly smaller decrease of FEV1 than nonselective beta blockers such as propranolol and, unlike those agents, did not inhibit bronchodilation in response to isoproterenol.
- Atenolol Consistent with its negative chronotropic effect, this is because the beta blockade of the SA node, it increases sinus cycle length and sinus node recovery time. Conduction in the AV node is also prolonged.
- Atenolol is devoid of membrane stabilizing activity, and increasing the dose well beyond that producing beta blockade does not further depress myocardial contractility. Several studies have demonstrated a moderate (approximately 10%) increase in stroke volume at rest and during exercise.

- The dose range of atenolol is narrow and increasing the dose beyond 100 mg once daily is not associated with increased antihypertensive effect.
- The mechanisms of the antihypertensive effects of beta-blocking agents have not been established. Several possible mechanisms have been proposed and include: (1) competitive antagonism of catecholamines at peripheral (especially cardiac) adrenergic neuron sites, leading to decreased cardiac output, (2) a central effect leading to reduced sympathetic outflow to the periphery, and (3) suppression of renin activity. The results from long-term studies have not shown any diminution of the antihypertensive efficacy of atenolol with prolonged use.
- By blocking the positive chronotropic and inotropic effects of catecholamines and by decreasing blood pressure, atenolol generally reduces the oxygen requirements of the heart at any given level of effort, making it useful for many patients in the long-term management of angina pectoris.
- On the other hand, atenolol can increase oxygen requirements by increasing left ventricular fiber length and end diastolic pressure, particularly in patients with heart failure. (Tablets, 2014)

1.6.1 Atenolol Geriatric Pharmacology

In general, elderly patients present higher atenolol plasma levels with total clearance values about 50% lower than younger subjects. The half-life is markedly longer in the elderly compared to younger subjects. The reduction in atenolol clearance follows the general trend that the elimination of renal excreted drugs is decreased with increasing age. (Tablets, 2014)

1.7 Atenolol Side Effects (Drugs.com, 2014):

- **For the Consumer**

Applies to atenolol: Oral tablet

Other dosage forms: Intravenous solution

In addition to its needed effects, some unwanted effects may be caused by atenolol. In the event that any of these side effects do occur, they may require medical attention.

More common

- Blurred vision
- cold hands or feet
- confusion
- difficult or labored breathing
- dizziness, faintness, or lightheadedness
- shortness of breath
- sweating
- tightness in chest
- unusual tiredness or weakness
- wheezing

If any of the following symptoms of overdose occur while taking atenolol, get emergency help immediately:

1.8. Symptoms of overdose (Drugs.com, 2014)

- Anxiety
- coma
- cool, pale skin
- depression
- dilated neck veins
- extreme fatigue
- headache

- increased hunger
- irregular breathing
- nervousness
- nightmares
- seizures
- shakiness
- slurred speech
- unusual drowsiness, dullness, tiredness, weakness, or feeling of sluggishness

More common

- Discouragement
- feeling sad or empty
- irritability
- lack of appetite
- loss of interest or pleasure
- trouble concentrating
- trouble sleeping

1.9 Contraindications:

Depression, Anaphylactic Shock due to Allergy Shots, Acutely Decompensated HF Requiring Parenteral Inotropic Therapy, 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, and Pregnancy. (Webmd.com, 2014)

1.10 Doses of atenolol: (Drugs.com, 2014)

1.10.1 Usual Adult Dose for Hypertension

Initial dose: 50 mg orally once a day. The full effect of this dose will usually be seen within 1 to 2 weeks. If an optimal response is not achieved, the dosage should be increased to 100 mg orally once a day.

1.10.2 Usual Adult Dose for Angina Pectoris Prophylaxis

Initial dose: 50 mg orally once a day. If an optimal response is not achieved within one week, the dosage should be increased to 100 mg orally once a day. Some patients may require 200 mg once a day for optimal effect.

1.10.3 Usual Adult Dose for Angina Pectoris

Initial dose: 50 mg orally once a day. If an optimal response is not achieved within one week, the dosage should be increased to 100 mg orally once a day. Some patients may require a dosage of 200 mg once a day for optimal effect.

1.10.4 Usual Adult Dose for Myocardial Infarction

IV: 5 mg over 5 minutes followed by another 5 mg injection 10 minutes later. Oral: In patients who tolerate the full IV dose (10 mg), atenolol tablets 50 mg should be initiated 10 minutes after the last IV dose followed by another 50 mg dose 12 hours later. Thereafter either 100 mg once a day or 50 mg twice a day for 6 to 9 days.

1.10.5 Usual Adult Dose for Anxiety

Initial dose: 50 mg orally once a day. Maintenance dose: In most cases data have shown no benefit and an increased risk of fatigue with daily doses greater than 100 mg.

1.10.6 Usual Adult Dose for Esophageal Variceal Hemorrhage Prophylaxis

Initial dose: 50 mg orally once a day.

Maintenance dose: In most cases data have shown no benefit and an increased risk of fatigue with daily doses greater than 100 mg.

1.10.7 Usual Adult Dose for Migraine Prophylaxis

Initial dose: 50 mg orally once a day.

Maintenance dose: In most cases data have shown no benefit and an increased risk of fatigue with daily doses greater than 100 mg.

1.10.8 Usual Adult Dose for Alcohol Withdrawal

Initial dose: 50 mg orally once a day.

Maintenance dose: Initial oral doses may be titrated upward as needed and tolerated approximately every 7 days. The maximum recommended daily dose is 200 mg.

1.10.9 Usual Adult Dose for Supraventricular Tachycardia

Initial dose: 50 mg orally once a day.

Maintenance dose: Initial oral doses may be titrated upward as needed and tolerated approximately every 7 days. The maximum recommended daily dose is 200 mg.

1.10.10 Usual Adult Dose for Ventricular Tachycardia

Initial dose: 50 mg orally once a day.

Maintenance dose: Initial oral doses may be titrated upward as needed and tolerated approximately every 7 days. The maximum recommended daily dose is 200 mg.

1.10.11 Renal Dose Adjustments

CrCl less than 15 mL/min: Maximum dose: 25 mg orally once a day. CrCl 15 to 35 mL/min: Maximum dose: 50 mg orally once a day.

1.11 Precautions

- Patients with coronary artery disease receiving atenolol should be advised to avoid abrupt discontinuation of the drug, as severe exacerbation of angina and occurrence of myocardial infarction and ventricular arrhythmias have occurred.

- The last two complications may occur with or without preceding exacerbation of the angina pectoris.
- As with other beta-blockers, when discontinuation of atenolol is planned, the patients should be carefully monitored and advised to limit physical activity to a minimum.
- If the angina worsens or acute coronary insufficiency develops, it is advised that atenolol be promptly reinstituted, at least temporarily. Because coronary artery disease is common and may be unrecognized, it may be prudent not to discontinue atenolol treatment abruptly even in patients treated only for hypertension.
- Atenolol is contraindicated in patients with sinus bradycardia, second- or third-degree atrioventricular heart block, cardiogenic shock, untreated pheochromocytoma, and overt congestive heart failure.
- Atenolol should be used with caution in patients with heart failure, a history of heart failure, bronchospastic disease, diabetes and hypoglycemia.
- Beta-adrenergic blockade may mask certain clinical signs (e.g., tachycardia) of hyperthyroidism.
- Abrupt discontinuation of beta-blockade might precipitate a thyroid storm; therefore, patients suspected of developing thyrotoxicosis from which atenolol treatment is to be withdrawn should be closely monitored.
- Withdrawal of beta-blocker therapy prior to major surgery is controversial, as the impaired ability of the heart to respond to reflex adrenergic stimuli may augment the risks of general anesthesia and surgical procedures.
- Atenolol therapy may aggravate peripheral arterial circulatory disorders.
- Atenolol therapy should be used with caution in patients with impaired renal function.
- If bradycardia or hypotension requiring treatment or any other untoward effects occur, atenolol should be discontinued.
- Safety and effectiveness in pediatric patients have not been established. (Webmd.com, 2014)

1.12 Dialysis

Atenolol is moderately hemodialyzable (20% to 50%). Patients should be given 25 to 50 mg after each hemodialysis.

Elimination is not enhanced via peritoneal dialysis. With peritoneal dialysis a supplemental dose is not needed. (Zaid, A., 2012)

1.13 Other Comments

Increasing the dosage beyond 100 mg a day is unlikely to produce any further benefit when treating hypertension.

Atenolol may be used alone or concomitantly with other antihypertensive agents including thiazide-type diuretics, hydralazine, prazosin, and alpha-methyldopa.

Compared with Caucasian patients, Black patients have a reduced blood pressure response to monotherapy with beta-blockers; however, the reduced response is largely eliminated if combination therapy that includes an adequate dose of a diuretic is instituted. (Malkieh, N.,2012)

1.14 Drug Dug interactions: (Kharoaf, M., 2012)

1.14.1 Beta-blockers (atenolol)/Fenoldopam:

Both medicines can lower your blood pressure.

1.14.2 Beta-blockers (atenolol)/Clonidine:

These two medicines are taken together to help lower the blood pressure. In some people, they may increase blood pressure. If clonidine is stopped and continue taking beta-blocker, or if stop taking them both at the same time, blood pressure may also increase.

Blood pressure may increase to a dangerous level, causing headache, nausea, vomiting, flushing, confusion, tremor, irritability with unrest, or a fast or irregular heartbeat.

1.14.3 Beta-blockers (atenolol)/Epinephrine:

Beta-blockers may block the effects of epinephrine.

Taking beta-blockers with epinephrine may cause blood pressure to be increased. Heart rate may slow down. The effect of epinephrine on severe allergic reactions may be decreased if patients are also taking beta-blockers.

1.14.4 Moderate Interactions of atenolol:

These medications may cause some risk when taken together. Consult your healthcare professional (e.g., doctor or pharmacist) for more information.

1.14.5 Selected Beta-blockers (atenolol)/Selected Calcium channel blockers:

When these two medicines are taken together, the effects of one or both medicines may increase.

An increase in the beneficial and toxic effects of one or both medicines may occur. Additional problems may develop a low heart rate. Using these medicines together may reveal an additional problem with heart.

1.14.6 Beta-blockers (atenolol)/Prazosin:

The cause of the interaction is unknown. Beta-blockers may increase the chance of a sudden decrease in blood pressure when starting taking prazosin.

A sudden decrease in blood pressure when starting prazosin, if already taking a beta-blocker. This problem may be worsened if also taking a water pill (e.g. a diuretic) or are on a low salt diet.

1.14.7 Beta-blocker (atenolol)/Mefloquine (Quinidine):

When these two medicines are taken together, body may not process beta-blocker correctly. Also, both medicines may have an additive effect on heart function.

The effects of beta-blocker may increase, and may experience more toxicities that might affect heart.

1.14.8 Beta-blocker (atenolol)/NSAID:

The cause of the interaction is not known.

The beneficial effects of beta-blocker may decrease and cause an increase in blood pressure.

1.15 Drug Food interactions

1.15.1 Atenolol & Alcohol:

Using atenolol and chlorthalidone together may lower your blood pressure and slow your heart rate. This can cause dizziness, or feeling like you might pass out, weakness, fainting, fast or irregular heartbeats, or loss of blood glucose control. If you take both medications together, tell your doctor if you have any of these symptoms.

GENERALLY AVOID: Orange juice may moderately reduce the bioavailability of atenolol by interfering with its absorption from the gastrointestinal tract. In a pharmacokinetic study, subjects ingested 200 mL orange juice 3 times daily for 3 days and twice daily on the fourth day, and took 50 mg atenolol with 200 mL orange juice on day 3. The average peak plasma concentration (Cmax) of atenolol fell by 49% and the area under the concentration-time curve (AUC) fell by 40% in comparison to subjects who drank only water. In addition, the presence of food may reduce the bioavailability of atenolol by 20%. The clinical significance is unknown.

Using atenolol together with multivitamin with minerals may decrease the effects of atenolol. (DeLima LG, 2014)

1.15.2 Licorice

Licorice and licorice tea can increase blood pressure and may counteract the effects of atenolol. Most candy known as licorice sold in the U.S. is not actually made with licorice, but anise. If you consume licorice candy on a regular basis, check the label to see if licorice is actually an ingredient. Talk with your physician regarding your licorice intake. He may advise against licorice or licorice tea use, or adjust your atenolol dosage. (Kharasch ED, 2011)

1.16 Interaction with other compounds

1.16.1 Tobacco

In a double-blind study of ten cigarette smokers with angina treated with atenolol for one week, angina episodes were significantly reduced during the nonsmoking phase compared to the smoking phase.6 People with angina taking atenolol who do not smoke should avoid starting. Those who smoke should consult with their prescribing doctor about quitting. (Butler S, 2014)

1.17 Interactions with Dietary Supplements

1.17.1 Potassium

Some beta-adrenergic blockers (called "nonselective" beta blockers) decrease the uptake of potassium from the blood into the cells,1 leading to excess potassium in the blood, a potentially dangerous condition known as hyperkalemia.2 People taking beta-blockers should therefore avoid taking potassium supplements, or eating large quantities of fruit (e.g., bananas), unless directed to do so by their doctor. (Butler S, 2014)

1.18 Interactions with Herbs

1.18.1 Pleurisy root

As pleurisy root and other plants in the Asclepius species contain cardiac glycosides, it is best to avoid use of pleurisy root with heart medications such as atenolol. (Butler S, 2014)

1.18.2 Pregnancy and Atenolol:

Atenolol is not recommended during pregnancy as it may harm an unborn child. Also, consult with your healthcare provider before breastfeeding. Atenolol has been shown to pass into breast milk and may cause harm to a nursing baby. (Butler S, 2014)

1.19 Overdose effect:

Over dosage with atenolol tablets has been reported with patients surviving acute doses as high as 5 g. One death was reported in a man who may have taken as much as 10 g acutely. (Lindholm LH, 2014)

The predominant symptoms reported following atenolol tablets overdose are lethargy, disorder of respiratory drive, wheezing, sinus pause and bradycardia. Additionally, common effects associated with over dosage of any beta-adrenergic blocking agent and which might also be expected in TENORMIN (atenolol tablets) overdose are congestive heart failure, hypotension, bronchospasm and/or hypoglycemia. Overdose symptoms may include uneven heartbeats, shortness of breath, bluish-colored fingernails, dizziness, weakness, fainting, or seizure (convulsions). (RxList, 2014)

1.20 Dosage & Packaging:

Etnol®: Atenolol 50mg & 100mg tablet form. BIOPHARMA Laboratories Limited (Bddrugs.com, 2015)

Fig: Etnol (Atenolol) tablet (Biopharmabd.com, 2015)

1.21 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.

1.21.1 Photolytic Condition

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.

1.21.2 Mechanism of Photolytic Degradation (Kumar *et al.* 2013)

Drug products are placed and exposed under the light source

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

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

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

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

After each specified time interval, the exposed drug product is collected and the physical parameter of the sample must be checked.

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

Chapter Two

LITERATURE REVIEW

In 1987-07 in a stability test Nifedipine in atenolol which is a photosensitive compound and subjected to degradation in apparent first order reaction for its methanolic solution. The reaction rate constant under diffused light is 1.6 times those under tungsten lamp. As for the influence of the initial concentration on reaction rate, kapp.5.28mg/100ml=13.28×10~ (-3) min~ (-1); kapp.42.60mg/100ml=3.81×10~ (-3) min~ (-1). (Scholar.google.com, 2007)

In 1994 Garner and his colleagues were developed a method to study the stability of atenolol in an oral liquid stored for 40 days under various conditions. By triturating 50-mg atenolol tablets with a commercially available oral diluents prepared 2 mg/ml liquid preparation of atenolol. Twelve in 12 vials of 30-mL portions were prepared where 3 vials were refrigerated at 5°C and the vials were shaken immediately before analysis, 3 were kept at room temperature 25°C and shaken, 3 were refrigerated and which are not shaken, and another 3 were kept at room temperature and not shaken. By stability-indicating high-performance liquid chromatography 1 sample from each of the vial was assayed in duplicate on days 0, 15, 30, and 40 .The result indicated that the concentration of atenolol remained above 90% of the original concentration in each vial under each of the four sets of conditions. In some cultured samples microbial growth occurred, and the pH changed minimally. (Garner *et al*, 1994)

In 1999 the photo stability test was done with β-blocker drug Atenolol and it was evaluated at pH 9, 7.4 and 4.0. At the beginning he drug (atenolol) 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. (Inform health care, 1999)

In 2000 Braza and his colleagues (Braza *et al*, 2000) were developed two liquid chromatography (LC) methods with fluorimetric detection to measure atenolol and propranolol in human plasma where the same 5 microm Nucleosil RP-18 column, extraction procedure and mobile phase such as acetonitrile, water, triethylamine and phosphoric acid, pH 3 were used. Results suggest that the linearity ranges were 25-800 ng/ml for atenolol and 3.13-100 ng/ml for propranolol.

In 2006 the main objective of this research was to investigate the relationship between the polarity of atenolol, acebutolol, and propranolol described by logP and kinetic and thermodynamic parameters characterizing their degradation process in acidic solution. For this hydrolysis was carried out in hydrochloric acid at molal concentrations of 0.1 mol/L, 0.5 mol/L, and 1 mol/L for 2 hr at 40°C, 60°C, and 90°C. For the determination of drugs under investigation chromatographic-densitometric method was used. The identification of degradation products was carried out by using 1H NMR. With the help of kinetic parameters (k, t0.1, and t0.5) and energy of activation (Ea) the degradation processes that occurred in drugs under investigation are described successfully. It has been found that the stability of drugs increases toward lipophilic propranolol in the assumed experimental model. The rate constants k decrease, contrary to t0.1, t0.5, and Ea, which vary comparably to logP, thus increasing from the most hydrophilic atenolol, through acebutolol, of lower polarity, to the most lipophilic propranolol. This study demonstrated that the stability of chosen beta-adrenergic blocking agents increases with their lipophilicity. (Inform health care, 2006)

In 2007 it was thought that there is a possibility that lower air, moisture and light protection could impact on physico-chemical stability of medicines inside multi-compartment compliance aids (MCCAs), although this has not yet been proved. The objectives of the study were to examine the physico-chemical stability of atenolol tablets stored in a compliance aid at room temperature, and at elevated temperature and humidity to simulate practice conditions. The method of this experiment was:

Atenolol 100 mg tablets were needed in 28-chamber, plastic compliance aids with transparent lids were stored for four weeks at room temperature and at 40°C with 75% relative humidity. Tablets were also stored at room temperature in original packaging and Petri dishes. Physical tests were conducted to standards as laid down in the British Pharmacopoeia 2005, and dissolution to those of the United States Pharmacopoeia volume 24. Chemical stability was assessed by a validated high-performance liquid chromatography (HPLC) method.

Results: the result was Tablets at room temperature in original packaging, in compliance aids and Petri dishes remained the same in appearance and passed physico-chemical tests. Tablets exposed to 40°C with 75% relative humidity in compliance aids passed tests for uniformity of

weight, friability and chemical stability but became pale and moist, softer (82 newtons ± 4; p< 0.0001) than tablets in the original packaging (118 newtons ± 6), more friable (0.14% loss of mass) compared with other tablets (0.005%), and failed the tests for disintegration (>15 minutes) and dissolution (only 15% atenolol released at 30 minutes). Conclusion: Although chemical stability was unaffected, storage in compliance aids at 40°C with 75% relative humidity softened atenolol tablets, prolonged disintegration time and hindered dissolution which could significantly reduce bioavailability. This formulation could be suitable for storage in compliance aids at 25°C, but not in hotter, humid weather. (Centaur.reading.ac.uk, 2007)

In 2007 another experiment of atenolol stability was performed. Most of these medicines (atenolol) are not formulated for easy or accurate administration to children. In studies it was found that 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. (Youssef, Rasha M., 2007)

In 2007 A simple, sensitive and specific reversed phase high performance liquid chromatographic (RP-HPLC) method with UV detection at 251 nm was developed for simultaneous quantitation of buparvaquone (BPQ), atenolol, propranolol, quinidine and verapamil. The method was applicable in rat in situ intestinal permeability study to assess intestinal permeability of BPQ, a promising lead compound for Leishmania donovani infections. The method was validated on a C-4 column with mobile phase comprising ammonium acetate buffer (0.02 M, pH 3.5) and acetonitrile in the ratio of 30:70 (v/v) at a flow rate of 1.0 ml/min. The retention times for atenolol, quinidine, propranolol, verapamil and BPQ were found and they were 4.30, 5.96, 6.55, 7.98 and 8.54 min, respectively. The calibration curves were linear (correlation coefficient > or =0.996) in the selected range of each analyte. The method was specific and sensitive with limit of quantitation of 15 microg/ml for atenolol, 0.8 microg/ml for quinidine, 5 microg/ml for propranolol, 10 microg/ml for verapamil and 200 ng/ml for BPQ. The validated method was found to be accurate and precise in the working calibration range. Stability studies were carried out at different storage conditions and all the analytes were found to be

stable. This method is simple, reliable and can be routinely used for accurate permeability characterization. (Venkatesh G, 2007)

In 2007 the degradation of atenolol was observed by 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 was involved an isocratic elution of atenolol in a Waters µBondapak® C18 column using a mobile phase which was consisting of acetonitrile-sodium phosphate monobasic (0.08 M, pH 3.0) (10:90, v/v). The flow rate was determined and the result was 1.0 mL/min and the analyte was monitored at 284 nm at spectophotometer. 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 tablets. (Taylor & Francis, 2007)

In 2007 a Related study was performed which is tto 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. Results suggested that direct photolysis in optically dilute solutions followed pseudo first-order kinetics. The measured half-lives of propranolol, atenolol, and metoprolol were approximately 16, 350, and 630 h, respectively. These were 3−5 orders of magnitude slower than the estimated minimum half-lives. BY this experiment the measured half-lives 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. Electron paramagnetic resonance (EPR) spectroscopy results confirmed that at least one carbon-based radical intermediate was formed during the direct photolysis of propranolol in aqueous solutions. The overall results were demonstrated that with fast direct photolysis half-lives, propranolol is unlikely to be persistent in natural waters. Further work is needed to investigate indirect photolysis of atenolol and metoprolol in surface waters in order to understand the overall persistence of these APIs in the environment. (Liu and Williams, 2007)

In 2008 A study was performed by taking the samples of atenolol encapsulated in aluminum 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 regions 4000-2000, 1800-1500, and 800-600 cm-1 are decomposed into individual vibration bands by peak fitting analysis and the effect of heat on the atenolol is shown. 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. (Gonsalves et al., 2008)

In 2008 Aryal and his colleagues (Aryal *et al,* 2008) were revealed a study that the bi-layer tablet formulation was more stable than the mono-layer type. Multi-drug tablets of amlodipine besylate and atenolol were prepared as either mono-layer (mixed matrix) or bilayer tablets containing each drug in a separate layer by using similar excipients and processing. Each tablet batch was packed in strip and blister packs and kept under accelerated temperature and humidity conditions. The stability of two tablet and packaging types was compared by HPLC analysis after 0, 1, 3 and 4.5 months and expressed as the content of intact amlodipine and atenolol. The content of atenolol did not decline regardless of tablet and packaging type. Amlodipine content in bi-layer tablets decreased to about 95 and 88% when packed in strips and blisters respectively. When prepared as mono-layer tablets, the content decreased to 72 and 32%, respectively.

In 2008 Medana and his colleagues (Medana *et al,* 2008) were studied the photocatalytic transformation of atenolol, 4-[2-hydroxy-3-[(1-methyl)amino]propoxyl]benzeneacetamide, a cardioselective β-blocking agent used to treat cardiac arrhythmias and hypertension, under simulated solar irradiation using titanium dioxide as photocatalyst. The investigation involved monitoring drug decomposition, identifying intermediate compounds, assessing mineralization, and evaluating toxicity. High-performance liquid chromatography (HPLC) coupled to high-resolution mass spectrometry (HRMS) via an electrospray ionization (ESI) interface was a powerful tool for the identification and measurement of the degradation products; 23 main species were identified. Intermediates were characterized through their chromatographic

behavior and evolution kinetics, coupled with accurate mass information. Through the full analysis of MS spectra and a comparison with parent drug fragmentation pathways, the diverse isomers were characterized. Neither atenolol nor the intermediates formed exhibit acute toxicity. All intermediates are easily degraded and no compound identified could withstand 2 h irradiation. Photomineralization of the substrate in terms of carbon mineralization and nitrogen release was rapid and, within 4 h of irradiation, organic nitrogen and carbon were completely mineralized.

In 2008 Gotardo (Gotardo *et al*, 2008) was developed a simple analytical method for quantification of atenolol in pharmaceutical formulations by diffuse reflectance spectroscopy. The method is based on the reaction, on the filter paper surface, between the drug and p-chloranil Producing a colored compound. The best reaction conditions were obtained with 20 μl of atenolol solution and 20 μl of p-chloranil. All reflectance measurements were carried out at 550 nm and the linear range was from 1.13x10-2 to 7.88x10-2 mol L-1 (r = 0.9992).The limit of detection was 2.80 x 10-3 mol L-1. The proposed method was successfully applied to analysis of different commercial brands of pharmaceutical formulations and the results obtained by the proposed method were in good agreement with those obtained using the British Pharmacopoeia method.

In 2009 A stability-indicating UPLC method was developed for the simultaneous quantitative determination of losartan potassium, atenolol, and hydrochlorothiazide in pharmaceutical dosage forms in the presence of degradation products. The separation was achieved on a simple isocratic method (water: acetonitrile: triethyl amine: ortho phosphoric acid (60:40:0.1:0.1, v/v) at 0.7 mL min−1, a detection wavelength of 225 nm). The retention times of losartan potassium, atenolol, and hydrochlorothiazide were 2.3, 0.6 and 0.9 min. The total runtime was 3 min. Losartan potassium, atenolol, and hydrochlorothiazide was subjected to different ICH prescribed stress conditions. The method was validated with respect to linearity, accuracy, precision, robustness and ruggedness. (Durga Rao et al., 2009)

In 2009 a study was performed in order to improve the understanding of the fate and behaviour of pharmaceuticals in the environment that need to investigate in-stream depletion mechanisms,

e.g. phototransformation of active pharmaceutical ingredients (APIs) in natural surface waters. In this study, abiotic and biotic degradation of selected β-blockers was measured simultaneously in non-sterilised and sterilised river waters and deionised water (DIW) under simulated sunlight (λ: 295–800 nm) and dark conditions, and at environmentally relevant concentrations, i.e.≤ ppb levels. 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. There was a significant correlation ($p < 0.07$) between dissolved organic carbon (DOC) and overall first order degradation rate constants for the tested β-blockers ($n = 4–6$), suggesting coloured DOC triplet-induced or reactive transient mediated oxidation mechanisms in river waters. Phototransformation was the main depletion mechanism for the β-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. However, biodegradation of metoprolol was accelerated under the light conditions, implying that photo-induced intermediates could be more easily biodegraded in river waters. (Liu, Cumming and Sharpe, 2009)

In 2009 Kumer (Kumlar *et al*, 2009) another study was carried out to investigate compatibility of atenolol which is a beta (1) bocker, with a variety of pharmaceutical excipients. The binary mixtures (1:1) of atenolol with the excipients were stored for 1 month at 40 degrees C / 75% RH. 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.

In 2009 Lodhiya (Lodhiya *et al*, 2009) was carried out a study by preparing floating matrix tablets of atenolol to provide sustained drug delivery of atenolol and also to enhance the bioavailability of the drug. Atenolol was chosen as a model drug because it is better absorbed form stomach than the intestine. Tablets were prepared using direct compression technique using HPMC K15, chitosan and carbopol .The results indicated the release of atenolol from the tablets was in controlled fashion. It was observed that the release of drug followed zero order release and controlled by both diffusion and erosion mechanism. The effervescent based floating drug delivery was a promising approach to achieve in vitro buoyancy. The addition of gas generating agent sodium bicarbonate was essential to achieve in vitro buoyancy. The drug release from the

tablets depends upon the nature of gel matrix. It may thus conclude that polymer swelling play an important role in pattern and amount of drug release from the formulation.

In 2010 A study was investigated the photo catalytic degradation of three -blockers in TiO2 suspensions. The disappearance of the compounds followed pseudo-first-order kinetics according to the Langmuir–Hinshelwood model and the rate constants were 0.075, 0.072 and 0.182 min−1 for atenolol, metoprolol and propranolol, respectively. After 240 min irradiation, the reaction intermediates were completely mineralized to CO2 and the nitrogen was predominantly asNH_4+. The influence of initial pH and blocker concentration on the kinetics was also studied. From adsorption studies it appears that the photo catalytic degradation occurred mainly on the surface of TiO2. Further studies indicated that surface reaction with •OH radical was principally responsible for the degradation of these three blockers. The major degradation intermediates were identified by HPLC/MS analysis. Cleavage of the side chain and the addition of the hydroxyl group to the parent compounds were found to be the two main degradation pathways for all three blockers. (Yang et al., 2010)

In 2010 photolytic degradation study of atenolol was performed by using two-electrode cells with a Pt or boron-doped diamond anode and an air-diffusion cathode for H2O2 electro generation, and four-electrode combined cells that was 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 photoelectro-Fenton methods. In these processes, organics are mainly oxidized with hydroxyl radical (radical dotOH) formed simultaneously at the anode surface from water oxidation and from Fenton's reaction between added catalytic Fe2+ and electrogenerated H2O2. Aromatic intermediates such as 4-hydroxyphenylacetamide and p-benzoquinone and generated carboxylic acids such as maleic, fumaric, tartaric, tartronic, glycolic, formic, oxalic and oxamic are detected and quantified by high-performance liquid chromatography. Compared with the single cells, the corresponding novel four-electrode combined systems enhance strongly the mineralization rate of atenolol in electro-Fenton because of the fast Fe2+ regeneration at the carbon-felt cathode favoring: (i) the production of more amounts of radical dotOH from Fenton's reaction that destroy more rapidly aromatic pollutants and (ii) the formation of Fe(II) complexes with final carboxylic acids such as oxalic and oxamic, which are more quickly oxidized with radical dotOH. In photoelectro-Fenton,

both single and combined cells show a quite similar oxidation power giving almost total mineralization as a result of the parallel quick photolysis of Fe(III) and/or Fe(II) complexes under UVA irradiation. The efficient regeneration of Fe2+ with larger radical dotOH production in the combined cells causes a quicker atenolol decay, which always follows a pseudo first-order reaction. NH4+ and in smaller proportion NO3− are always released to the medium. (Braza *et al*, 2010)

In 2010 wahab (wahab, 2010) was performed a study where two 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 Amiloride HCl (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 developed TLC-Densitometric method depends upon quantitative densitometric separation of thin layer chromatogram of ATE, CLT, Atenolol degradation product (ATE Deg) and Chlorthalidone degradation product (CLT Deg) using silica gel plates at 227 nm and chloroform: methanol: ethyl acetate: ammonia solution (75: 28: 2: 1.6, by volume) as a developing system. 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.

In 2010 another study was observed by using Polypill which 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. Here an attempt was made to develop a new, sensitive, single stability-indicating 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. Buffer consists of 0.1% Orthophosphoric acid (pH 2.9), delivered in a gradient mode and quantitation was carried out using ultraviolet detection at 230 nm with a flow rate of 1.0 mL/min. 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 are exposed to thermal, acid/base hydrolytic, humidity and oxidative stress conditions, and the stressed samples were analyzed by proposed method. The method was validated with respect to linearity; the method was linear in the range of 37.5 to 150.0µg/mL for ASP, 5.0 to 20.0 µg/mL for ATV and 25.0 to 100.0 µg/mL for ATL(atenolol) and LST. Acceptable precision and accuracy were obtained for concentrations over the standard curve ranges. The validated method was successfully applied to the analysis of Starpill tablets constituting all the four drugs; the percentage recoveries obtained were 99.60% for ASP, 99.30% for ATV, 99.41% for ATL and 99.62% for LST. (Shetty, 2010)

In 2010 the conversion of the antibiotic ofloxacin and the β-blocker atenolol by means of TiO2 photocatalysis was investigated. Irradiation was provided by a UVA lamp at $3.37 \times 10{-}6$ einstein/s photon flux, while emphasis was given on the effect of catalyst type and loading (50–1500 mg/L), initial substrate concentration (5–20 mg/L), initial pH (3–10) and the effect of H2O2 (0.07–1.4 mM) as an additional oxidant on substrate conversion and mineralization in various matrices (i.e. pure water, groundwater and treated municipal effluent). Conversion was assessed measuring sample absorbance at 288 and 224 nm for ofloxacin and atenolol, respectively, while mineralization measuring the dissolved organic carbon. Degussa P25 TiO2 was found to be more active than other TiO2 samples for either substrate degradation, with ofloxacin being more reactive than atenolol. Conversion generally increased with increasing catalyst loading, decreasing initial substrate concentration and adding H2O2, while the effect of solution pH was substrate-specific. Reaction rates, following a Langmuir–Hinshelwood kinetic expression, were maximized at a catalyst to substrate concentration ratio (w/w) of 50 and 15 for ofloxacin and atenolol, respectively, while higher ratios led to reduced efficiency. Likewise, high concentrations of H2O2 had an adverse effect on reaction, presumably due to excessive oxidant scavenging radicals and other reactive species. The ecotoxicity of ofloxacin and atenolol to freshwater species Daphnia magna was found to increase with increasing substrate concentration

(1–10 mg/L) and exposure time (24–48 h), with atenolol being more toxic than ofloxacin. Photocatalytic treatment eliminated nearly completely toxicity and this was more pronounced for atenolol. (Durga rao et, 2010)

In 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 calibration curves were linear over the range of 0.1–10 μg mL−1. The method was validated in terms of accuracy, precision, absolute recovery, freeze–thaw stability, bench-top stability and re-injection reproducibility. The within- and between-day 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 substances. (Rogers et, 2010)

In 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. Degradation products produced as a result of stress studies did not interfere with the detection of atenolol and lercanidipine and the assay can thus be considered stability-indicating. (O et al., 2011)

In 2011 a method was developed.

Formula A: Atenolol Oral Liquid 2mg per mL: Atenolol tablets: 100mg then Roxane® Diluent to 250 mL was added after that atenolol tablets were crushed to a powder using a mortar and pestle then slowly add 50mL Roxane® Diluent and triturate to a fine paste. Again 200mL Roxane® Diluent was added to the mortar in three equal portions, thoroughly mixing after each dilution. Expiry days were 30. It's stability was for 40 days. Condition of Storage was at 5 - 25°C. Refrigeration recommended (stable).*Roxane diluent contains; ethanol 1%, saccharin 0.05% in a cherry flavored 33% polyethylene glycol 8000 base.

Formula B: Atenolol Oral Liquid 2mg per mL: Atenolol tablets 50mg were taken and then Glycerol 2 mL was added. Methylcellulose 1% to 50 mL added. After that crush the tablets and a paste was made with the glycerol before adding the vehicle. Expiry/Stability were for 30 days Storage condition was at 25°C but refrigeration recommended to retard microbial growth and it was stable. A preservative such as parabens was added. (Woods, 2011)

In 2011 the photocatalytic conversion of two β-blockers, namely atenolol and propranolol in aqueous TiO2 suspensions was investigated. Irradiation was provided by a solar simulator equipped with 1 kW Xe-OP lamp, while emphasis was given on the effect of catalyst type and loading (50–3000 mg/L), substrate concentration (5–30 mg/L), initial solution pH (3–10), and the addition of H2O2 (0.07–1.4 mM) and oxygen on degradation in two matrices (i.e. pure water and treated municipal effluent). Of the various catalysts tested, Degussa P25 was highly active yielding up to about 80% conversion after 120 min of reaction. In general, conversion was favored at lower substrate concentrations, near-neutral pH values and in the absence of other organics (i.e. in pure water), while the addition of H2O2 did not accelerate kinetics which seem to follow the Langmuir–Hinshelwood model. Toxicity to D. magna was evaluated prior to and after photocatalytic treatment. Toxicity increased during the early stages of the reaction and then progressively decreased upon the elimination of the substrate and its reaction intermediates, with propranolol being more toxic than atenolol. (Taylor & cummins, 2011)

In 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 behavior 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 atenoIol 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 for whom the splitting process doesn't give equal halves, and also for modifying the dose for patients with renal or hepatic problems. Therefore, it is possible for the community pharmacist to crush atenolol 100-mg tablets and refill them in new capsules with each containing a precise amount of atenolol, calculated according to body surface area and kidney and liver functions without affecting the chemical stability of the active ingredient nor its dissolution profile and also have a cost effective dosage form. (Zaid AN, 2012)

In 2012 Ji (Ji *et al*, 2012) was performed a study to investigate the photolysis behavior of atenolol (ATL) and toxicity of its photo degradation products in the presence of nitrate ions. The results showed that ATL photo degradation followed pseudo-first-order kinetics upon simulated solar irradiation. Hydroxyl radical was determined to play a key role in the photolysis process by using isopropanol as molecular probe. 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 .OH formation. Bicarbonate decreased the photo degradation of ATL in the presence of nitrate ions mainly through pH effect, while humic substance inhibited the photo degradation via both attenuating light and competing radicals.

In 2012 the extensive utilization of β-blockers worldwide led to frequent detection in natural water by performing this study. In this study the photolysis behavior of atenolol (ATL) and toxicity of its photo degradation products were investigated in the presence of nitrate ions. The results showed that ATL 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. Hydroxyl radical was determined to play a key role in the photolysis process by using isopropanol as molecular probe. 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 ATL in the presence of nitrate ions mainly through pH effect, while humic substance inhibited the photo degradation via both attenuating light and competing radicals. Upon irradiation for 240 min, only 10% reduction of total organic carbon (TOC) can be achieved in spite of 72% transformation rate of ATL, implying a majority of ATL transformed into intermediate products rather than complete mineralization. The main photoproducts of ATL were identified by using solid phase extraction–liquid chromatography–mass spectrometry (SPE–LC–MS) techniques and possible nitrate-induced photo degradation pathways were proposed. The toxicity of the photo transformation products was evaluated using aquatic species Daphnia magna, and the results revealed that photo degradation was an effective mechanism for ATL toxicity reduction in natural waters. (Liu and Williams, 2012)

In 2013 this study reports the photo catalytic degradation of the -blocker metoprolol (MET) using TiO2 suspended as catalyst. A series of photo experiments were carried out by a UV lamp, emitting in the 250–400 nm range, providing information about the absorption of radiation in the photo reactor wall. The influence of the radiation wavelength on the MET photo oxidation rate was investigated using a filter cutting out wavelengths shorter than 280 nm. Effects of photolysis and adsorption at different initial pH were studied to evaluate no catalytic degradation for this pharmaceutical. MET adsorption onto titania was fitted to two-parameter Langmuir isotherm. From adsorption results it appears that the photo catalytic degradation can occur mainly on the surface of TiO2. MET removed by photo catalysis was 100% conditions within 300 min, while only 26% was achieved by photolysis at the same time. TiO2 photo catalysis degradation of MET in the first stage of the reaction followed approximately a pseudo-first-order model. The major reaction intermediates were identified by LC/MS analysis such as 3-(propan-2-ylamino) propane-1,2-diol or 3-aminoprop-1-en-2-ol. Based on the identified intermediates, a photo catalytic degradation pathway was proposed, including the cleavage of side chain and the hydroxylation addition to the parent compounds. (Romero et al., 2013)

In 2013 two stability-indicating chromatographic methods are described for simultaneous determination of amiloride hydrochloride (AMI), atenolol (ATE), and chlorthalidone (CHL) in combined dosage forms. The first method was based on HPTLC separation of the three drugs followed by densitometric measurements of their bands at 274 nm. The separation was carried out on Merck HPTLC silica gel 60F254 aluminum sheets using chloroform–methanol–ammonia 27%, w/w (9 + 2 + 0.3, v/v/v) mobile phase. Analysis data was used for the linear regression graph in the range of 0.1–0.5, 0.8–5.0, and 0.3–1.5 μg/band for AMI, ATE, and CHL, respectively. The second method was based on an RP-HPLC separation of the cited drugs performed on an RP stainless steel C18 analytical column (250 × 4.6 mm id) with a gradient elution system of methanol and 0.05 M aqueous phosphate buffer adjusted to pH 4 as the mobile phase, at the flow rate of 1.0 mL/min. Quantitation was achieved with photodiode array detection at 275 nm for AMI and 225 nm for ATE and CHL. The calibration graphs for each drug were rectilinear in the range of 2–50, 25–150, and 2–100 μg/mL for AMI, ATE, and CHL, respectively. The proposed chromatographic methods were successfully applied for determination of the investigated drugs in pharmaceutical preparations. Both methods were validated in compliance with International Conference on Harmonization guidelines in terms of linearity, accuracy, precision, robustness, LOD, and LOQ. (Romero et al, 2013)

In 2014 Charde and his colleagues (Charde *et al*, 2014) were developed a force degradation profile of Atenolol (ATN) & Chlorthalidone (CTN) in combine tablet dosage form on RP-HPLC using Comosil RP-C18 (4.6 x 250mm, 5μm) in an gradient mode with mobile phase comprising of Methanol: Water (pH 3 using OPA) The flow rate was 1 mL/ min and effluent was monitored at 226 nm. The analysis of the marketed formulation shows the % RSD of 0.37 and 0.99 for ATN & CTN which fully agrees with system suitability. All the system suitability parameters were fully obeyed during generation of force degradation profile.

On 26 Aug 2014 nitrate-induced photodegradation of ATL followed pseudo-first-order kinetics upon simulated solar irradiation. The photodegradation was found to be dependent on nitrate concentration and increasing the nitrate from 0.5 mmol L-1 to 10 mmol L-1 led to the enhancement of rate constant from 0.00101 min-1 to 0.00716 min-1. Hydroxyl radical was determined to play a key role in the photolysis process by using isopropanol as molecular probe. Increasing the solution pH from 4.8 to 10.4, the photodegradation rate slightly decreased from

0.00246 min-1 to 0.00195 min-1 , probably due to pH-dependent effect of nitrate-induced ·OH formation. Bicarbonate decreased the photodegradation of ATL in the presence of nitrate ions mainly through pH effect, while humic substance inhibited the photodegradation via both attenuating light and competing radicals. Upon irradiation for 240 min, only 10% reduction of total organic carbon (TOC) can be achieved in spite of 72% transformation rate of ATL, implying a majority of ATL transformed into intermediate products rather than complete mineralization. The main photoproducts of ATL were identified by using solid phase extraction-liquid chromatography-mass spectrometry (SPE-LC-MS) techniques and possible nitrate-induced photodegradation pathways were proposed. The toxicity of the phototransformation products was evaluated using aquatic species Daphnia magna, and the results revealed that photodegradation was an effective mechanism for ATL toxicity reduction in natural waters (Tel.archives-ouvertes.fr, 2014)

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, 500 tablets of Etnol® (50 mg) were collected from the local drug store in Dhaka as a sample. All the tablets were from the same batch (batch no. 067). 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 Samples

Table 3.1: Samples used in the experiment:

Sample Name	**Source (Supplier Name)**	**Batch No.**
Etnol® tablets	Biopharma Laboratories Limited	067

Figure 3.1: Etnol® 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 Equipment & Instruments

Table 3.3: Lists of equipment used for the experiment

Serial No.	**Equipment**	**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
4	Hardness tester	Veego VTHT	India
5	Venire Calipers	Shanghai Tricle Brand	China

3.1.5 Images of Instruments

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

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

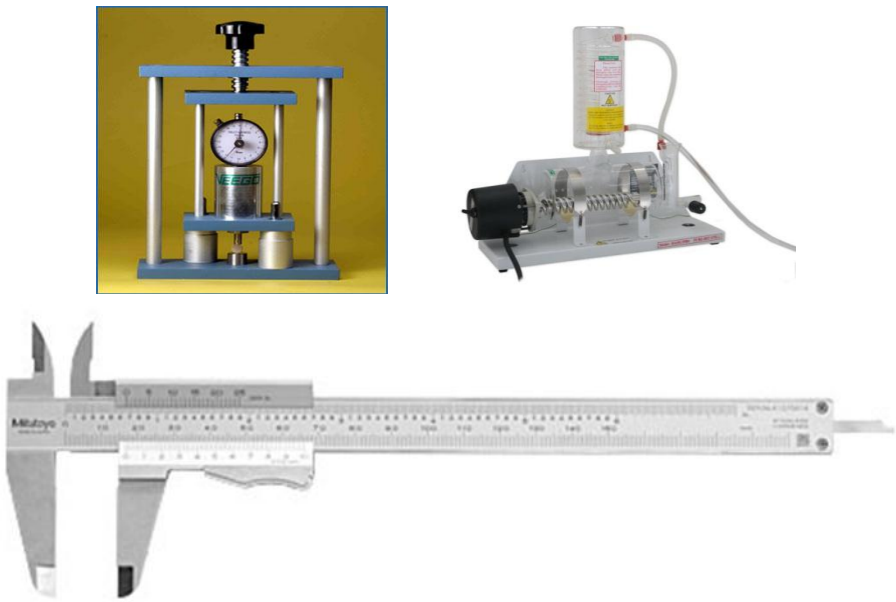


Figure 3.3: Hardness tester, Distilled water plant & Vernier calipers [Left to right]

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 throughout this project

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
18	Plastic Dropper

3.2 Method

3.2.1 Preparation of the solvent (0.1N H_2SO_4)

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

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 H_2SO_4 solvent was calculated as below.

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

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.

3.2.2 Determination of λmax & Preparation of the Standard Curve of atenolol

1. A standard of atenolol was collected from the pharmaceutical company Biopharma Laboratories Ltd. The potency of standard compounds was 99.99%.
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 that was obtained from Biopharma Laboratories Ltd.
3. 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 obtained from Eskayef Bangladesh Ltd:

- 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 $V_1S_1=V_2S_2$ 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

❖ $V_1 = V_2S_2 / S_1$ = (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.

❖ $V_1 = V_2S_2 / S_1$ = (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.

- $V_1 = V_2S_2 / S_1$ = (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.
- $V_1 = V_2S_2 / S_1$ = (0.004 x 10) / 0.02 = 2 ml of stock solution required to make 0.004 mg/ml concentration of the final solution of 10 ml (2 ml of stock solution + 8 ml of 0.1N H_2SO_4) of atenolol.
- $V_1 = V_2S_2 / S_1$ = (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.
- $V_1 = V_2S_2 / S_1$ = (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.
- $V_1 = V_2S_2 / S_1$ = (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.
- $V_1 = V_2S_2 / S_1$ = (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.
- $V_1 = V_2S_2 / S_1$ = (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.

4. Then the absorbance value was measured using a UV spectrophotometer against those nine serial concentrations for atenolol.
5. A standard curves was plotted for atenolol.
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.

3.2.3 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 (Etnol®) were kept under normal lighting condition in the room for 4 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:

a) First, Five tablets from those sampled tablets were taken.

b) Then the total weight of those five tablets was noted using an analytical balance and the average weight (which is equal to the weight for one tablet containing both active ingredients and excipients) was calculated using the formula given below:

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

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

d. Next, approximately the weight of 1 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 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 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)

1) 30 tablets were exposed to electric bulb lighting conditions for 6 hours at a stretch and 10 tablets were used as control.
2) 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.
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 :

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

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 10 ml solution was filtered and 5 ml of that filtered solution was taken and dissolved in 50ml 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 for atenolol.

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
30	10	2	27	30
	10	4	27	30
	10	6	30	32

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) Steps 5 to 6 were repeated again for another sampling hour.

8) 10 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 two different days for 6 hours each.

III. Under Sunlight condition

1) 30 tablets were kept in a Glass box and exposed to sunlight condition for 7.5 hours at a stretch.

2) 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.

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:

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

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 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 for atenolol.

Table 3.7: Sunlight Exposed Sample List

No. of Samples	**Collected Sample**	**Withdrawal Intervals (Hrs)**	**Temparature (^{0}C)**
10 (Control)	10	0	34
30	10	2	37
	10	4	38
	10	6	40

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) Steps 5 to 6 were repeated again for another sampling hour.

8) 10 tablets were used as control and has not been exposed any of the lighting conditions.

3.2.4 Determination of Physical parameters

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. Thickness Test

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.

III. 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. Monsanto tablet hardness tester was used to measure the hardness of Etnol®. Hardness measuring devices apply increasing pressure on the tablet until the tablet

IV. Weight Variation Test

Procedure

1. 10 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.

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

Calculation

Following equation was used to determine % Weight Variation of tablets

% Weight Variation = (A~I/A) ×100 %

Where,

I = Initial Weight of Tablet, in grams (gm)

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

Chapter Four

RESULTS

4.1. Standard Curve Preparation

For the preparation of standard curve, nine serially different concentrations were prepared for Atenolol using the standards of Atenolol obtained from Biopharma with a potency of 99.99%. Thus for those nine concentrations, nine absorbance (abs) values were obtained from the UV spectrophotometer machine for Atenolol. The absorbance (abs) values for those five concentrations of Atenolol are shown in the table below.

Table 4.1: Concentrations and absorbance values for standard curve

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=38.6x-0.018

R^2=0.970

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

By plotting the absorbance (abs) values against the concentrations (mg/ml) values of atenolol, a curve was obtained.

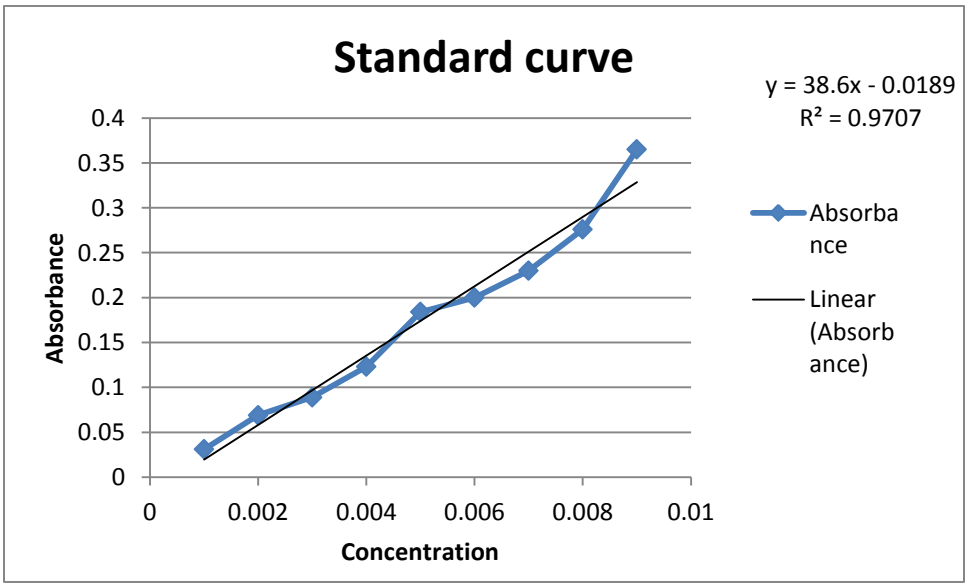


Figure 4.1: Plot showing straight line for Absorbance (Abs) with respect to Concentration (mg/ml) for atenolol.

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

Y=38.6x-0.018

R^2=0.970

Y=38.6x-0.018

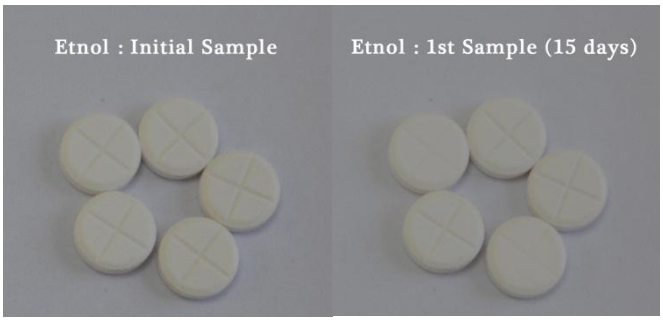
R^2=0.970

The R^2 value was found to be 0.970 which meant the graph was very linear and using this equation, provided above, the concentration from different absorbance values of the samples of atenolol were calculated

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 Hardness Test:

Four tablet strips containing 40 tablets were 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 (Etnol®**)**

Days	**Average Hardness of Particular Day (Kg)**
Initial	3.70
15	3.65
30	3.58
45	3.55
60	3.56

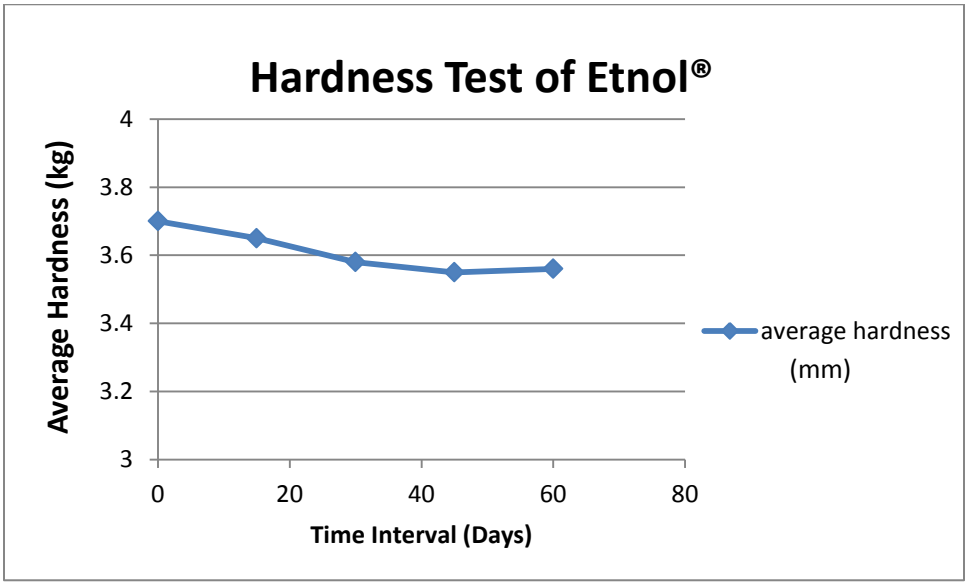


Figure 4.2: **Plot showing hardness of the sample tablets exposure to light**

4.2.3 Weight Variation Test

Four tablet strips containing 40 tablets were exposed to normal light condition for 60 days. Weight variation 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. 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 Atenolol (Etnol®)

Days	**Average Weight for Particular Day (gm)**
Initial	0.1859
15	0.1855
30	0.1852
45	0.1851
60	0.1848

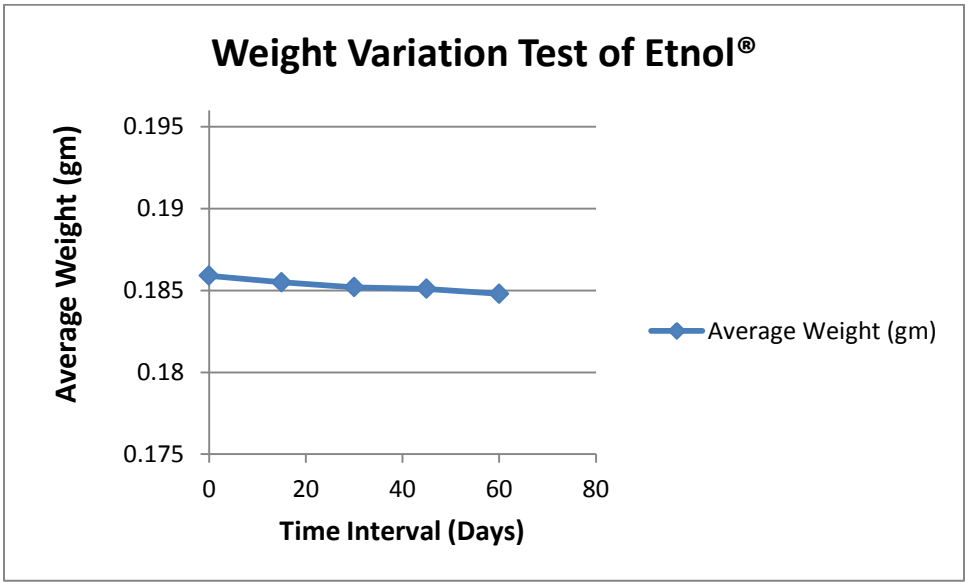


Figure 4.3: Weight variation of the sample throughout 60 days light exposure.

4.2.4 Thickness Test

Four tablet strips containing 40 tablets were 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, a tablet 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 (Etnol®**)**

Days	**Average Thickness of Particular Days (mm)**
Initial	**2.036**
15	**2.034**
30	**2.034**
45	**2.032**
60	**2.030**

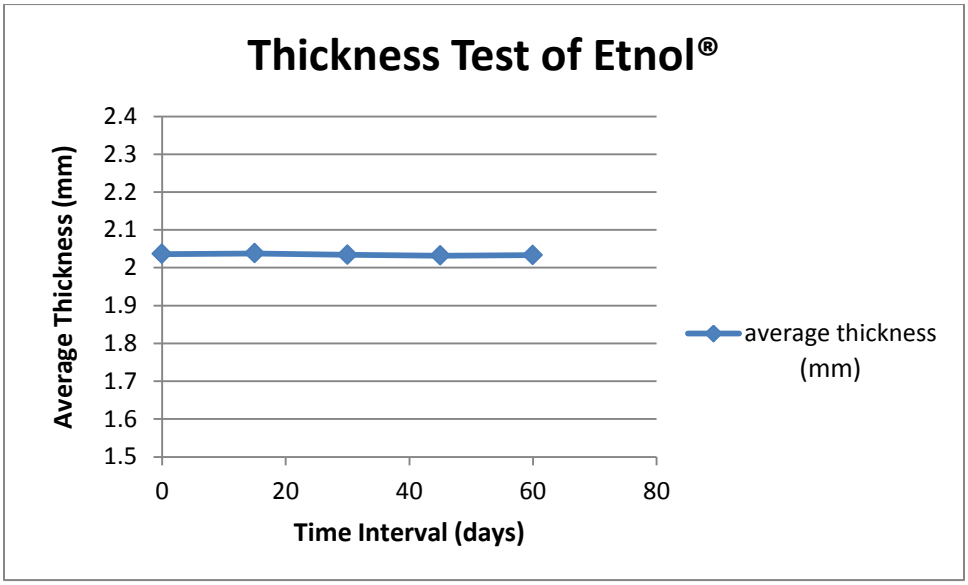


Figure 4.5: Thickness variation of sample throughout 60 days light exposure.

4.3 Result from Potency Determination by UV- spectroscopy

4.3.1 Result from Sample that was exposed under Normal Lightening Condition

For 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 4.5: Concentration & Absorbance of Zero Days Interval for Atenolol

Time Interval (Days)	Absorbance (at 223.5nm)		Average Absorbance		Amount of Drug Present (in mg)		Potency (%)	
	Control	Sample	Control	Sample	Control	Sample	Control	Sample
Initial	0.714	0.697	0.711	0.701	47.21	46.57	94.43	92.60
	0.698	0.707						
	0.721	0.698						
	0.714	0.698	0.711	0.697	47.21	46.30	94.43	92.60
	0.700	0.699						
	0.719	0.694						
	0.716	0.697	0.711	0.697	47.21	46.30	94.43	92.60
	0.700	0.699						
	0.717	0.695						

Table 4.6: Concentration & Absorbance of 15 Days Interval for Atenolol

Time Interval (Days)	Absorbance (at 223.5nm)		Average Absorbance		Amount of Drug Present (in mg)		Potency (%)	
	Control	Sample	Control	Sample	Control	Sample	Control	Sample
15	0.714	0.684	0.711	0.691	47.21	45.91	94.43	92
	0.698	0.691						
	0.721	0.698						
	0.714	0.697	0.711	0.684	47.21	45.46	94.43	90.93
	0.700	0.679						
	0.719	0.676						
	0.716	0.685	0.711	0.691	47.21	45.91	94.43	92
	0.700	0.683						
	0.717	0.707						

Table 4.7: Concentration & Absorbance of 30 Days Interval for Atenolol

Time Interval (Days)	Absorbance (at 223.5nm)		Average Absorbance		Amount of Drug Present (in mg)		Potency (%)	
	Control	Sample	Control	Sample	Control	Sample	Control	Sample
30	0.714	0.654	0.711	0.665	47.21	44.23	94.43	88.47
	0.698	0.662						
	0.721	0.680						
	0.714	0.658	0.711	0.664	47.21	44.17	94.43	88.34
	0.700	0.666						
	0.719	0.668						
	0.716	0.656	0.711	0.655	47.21	43.58	94.43	87
	0.700	0.656						
	0.717	0.657						

Table 4.8: Concentration & Absorbance of 45 Days Interval for Atenolol

Time Interval (Days)	Absorbance (at 223.5nm)		Average Absorbance		Amount of Drug Present (in mg)		Potency (%)	
	Control	Sample	Control	Sample	Control	Sample	Control	Sample
45	0.714	0.655	0.711	0.648	47.21	43.13	94.43	86.26
	0.698	0.656						
	0.721	0.635						
	0.714	0.618	0.711	0.636	47.21	42.35	94.43	84.71
	0.700	0.640						
	0.719	0.650						
	0.716	0.631	0.711	0.633	47.21	42.16	94.43	84.32
	0.700	0.635						
	0.717	0.633						

Table 4.9: Concentration & Absorbance of 60 Days Interval for Atenolol

Time Interval (Days)	Absorbance (at 223.5nm)		Average Absorbance		Amount of Drug Present (in mg)		Potency (%)	
	Control	Sample	Control	Sample	Control	Sample	Control	Sample
60	0.714	0.643	0.711	0.645	47.21	42.94	94.43	75.5
	0.698	0.646						
	0.721	0.648						
	0.714	0.626	0.711	0.628	47.21	41.84	94.43	75.5
	0.700	0.628						
	0.719	0.630						
	0.716	0.571	0.711	0.575	47.21	38.40	94.43	76.8
	0.700	0.576						
	0.717	0.580						

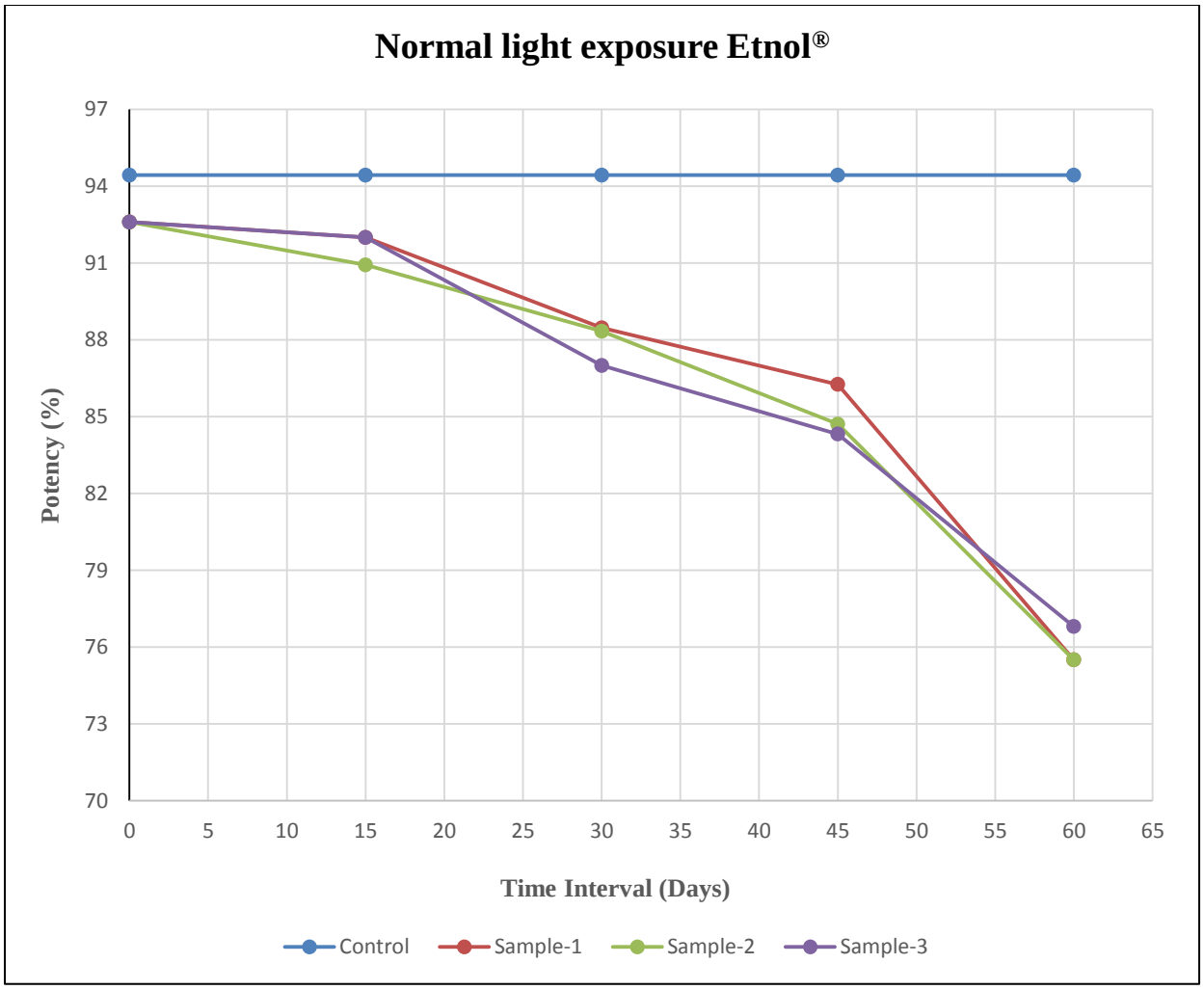


Figure 4.6: Graph showing the difference in Concentration after fixed day interval for Atenolol (Etnol®).

4.3.2 Result of samples that were exposed under 25W bulb

In each experimental day, a tablet strip containing 10 tablets was taken and 5 samples were collected for the test and observed 3 different absorbance of Atenolol for three 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.

4.3.2.1 For First experiment day: The results are given below:

Table 4.10: Concentration & absorbance of Atenolol at Zero hour

Time Interval	Absorbance (at 223.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (2500 times diluted)	Amount of Drug present (in mg)	Potency (%)
Zero (Control)	0.714	0.711	0.0188	47.21	94.43
	0.698				
	0.721				
	0.654	0.708	0.0188	47.02	94.04
	0.737				
	0.735				
	0.638	0.677	0.0180	45.01	90.02
	0.688				
	0.705				

Table 4.11: Concentration & absorbance of Atenolol after 2 hours

Time Interval	Absorbance (at 223.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (2500 times diluted)	Amount of Drug present (in mg)	Potency (%)
2	0.572	0.602	0.0160	40.15	80.31
	0.619				
	0.615				
	0.582	0.613	0.0171	42.86	85.72
	0.625				
	0.634				
	0.596	0.637	0.0169	42.42	84.84
	0.633				
	0.653				

Table 4.12: Concentration & absorbance of Atenolol after 4 hours

Time Interval	Absorbance (at 223.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (2500 times diluted)	Amount of Drug present (in mg)	Potency (%)
4	0.603	0.601	0.0160	40.09	80.18
	0.601				
	0.599				
	0.546	0.592	0.0158	39.5	79
	0.623				
	0.607				
	0.552	0.556	0.0148	37.17	74.35
	0.552				
	0.565				

Table 4.13: Concentration & absorbance of Atenolol after 6 hours

Time Interval	Absorbance (at 223.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (2500 times diluted)	Amount of Drug present (in mg)	Potency (%)
6	0.559	0.553	0.0147	36.98	74
	0.556				
	0.545				
	0.555	0.552	0.0147	36.91	73.5
	0.554				
	0.549				
	0.473	0.478	0.0128	32.12	64.24
	0.483				
	0.479				

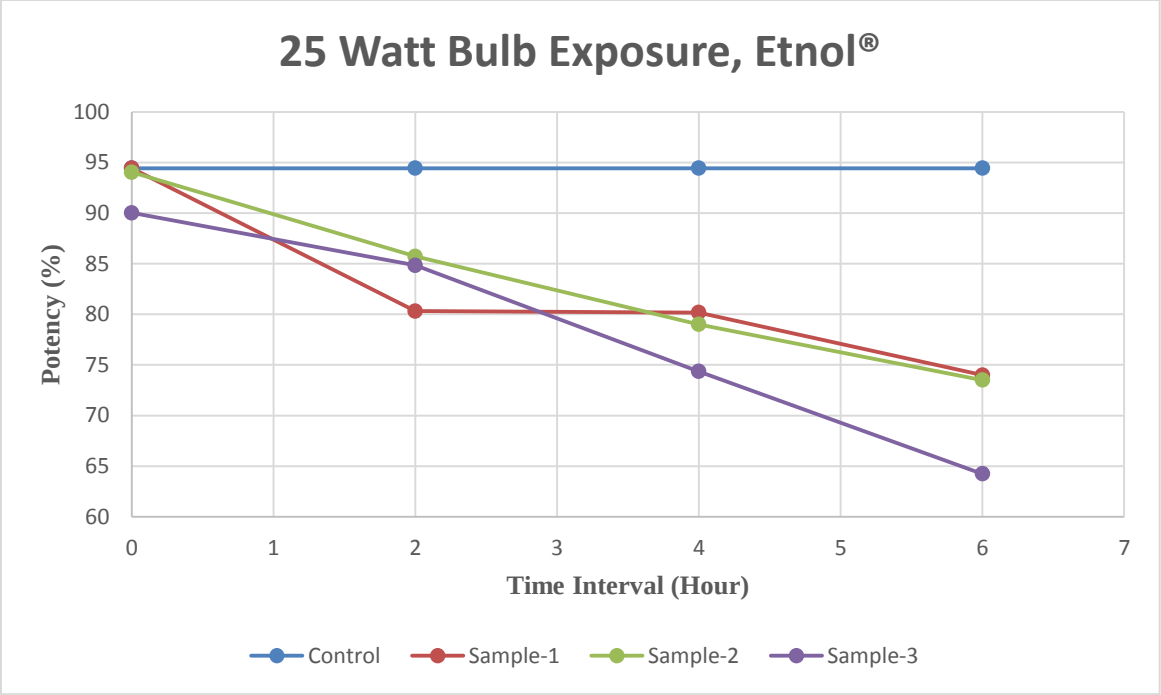


Figure 4.7: Graph showing the difference in Concentration after each 2 hour time interval for Atenolol (Etnol®) for 1st time.

4.3.2.2 For Second experiment day: The results are given below-

Table 4.14: Concentration & absorbance of Atenolol at Zero hour

Time Interval	Absorbance (at 223.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (2500 times diluted)	Amount of Drug present (in mg)	Potency (%)
Zero (Control)	0.714	0.711	0.0188	47.21	94.43
	0.698				
	0.721				
	0.654	0.708	0.0188	47.02	94.04
	0.737				
	0.735				
	0.638	0.677	0.0180	45.01	90.02
	0.688				
	0.705				

Table 4.15: Concentration & absorbance of Atenolol after 2 hours

Time Interval	Absorbance (at 223.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (2500 times diluted)	Amount of Drug present (in mg)	Potency (%)
2	0.656	0.655	0.0174	43.58	87.17
	0.656				
	0.655				
	0.698	0.697	0.0185	46.30	92.61
	0.699				
	0.694				
	0.635	0.677	0.0180	45.01	89.63
	0.713				
	0.676				

Table 4.16: Concentration & absorbance of Atenolol after 4 hours

Time Interval	Absorbance (at 223.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (2500 times diluted)	Amount of Drug present (in mg)	Potency (%)
4	0.603	0.601	0.0160	40.09	80.18
	0.601				
	0.599				
	0.552	0.558	0.0149	37.30	74.61
	0.565				
	0.559				
	0.558	0.553	0.0147	36.98	73.98
	0.556				
	0.545				

Table 4.17: Concentration & absorbance of Atenolol after 6 hours

Time Interval	Absorbance (at 223.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (2500 times diluted)	Amount of Drug present (in mg)	Potency (%)
6	0.555	0.552	0.0147	36.91	73.83
	0.554				
	0.549				
	0.483	0.482	0.0129	32.38	64.76
	0.479				
	0.485				
	0.483	0.478	0.0128	32.12	64.24
	0.473				
	0.479				

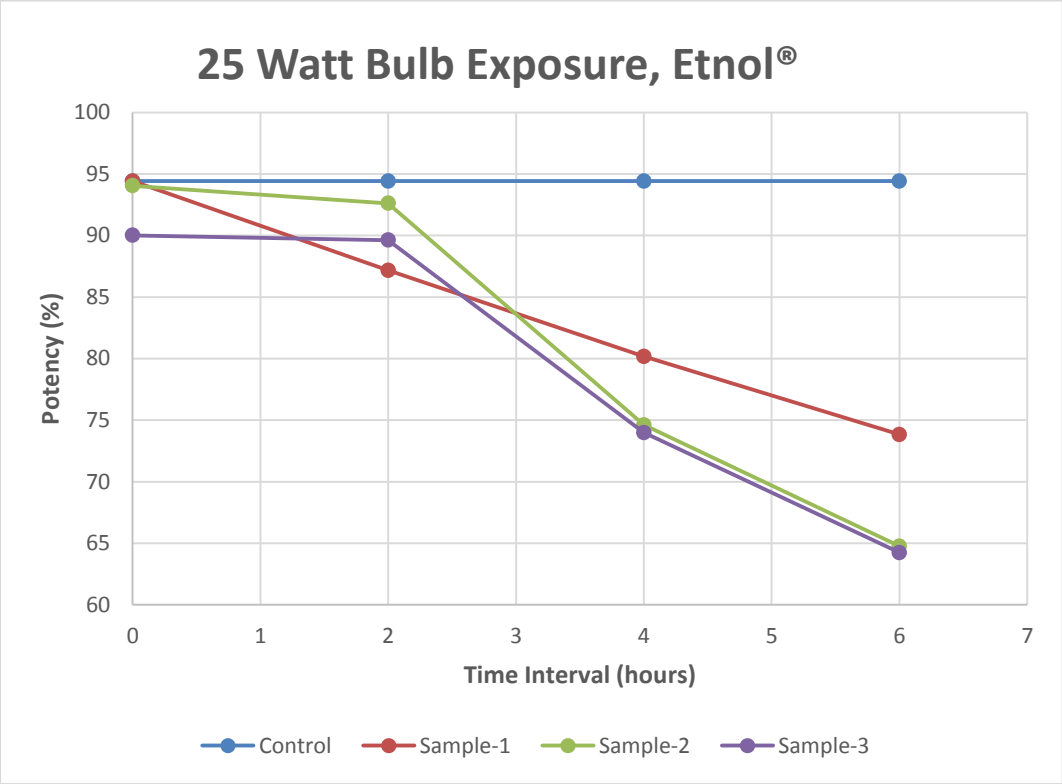


Figure 4.8: Graph showing the difference in Concentration after each 2 hour time interval for Atenolol (Etnol®) for 2nd time.

4.3.2.3 For Third experiment day: The results are given below-

Table 4.18: Concentration & absorbance of Atenolol (Etnol®) at Zero hour

Time Interval	Absorbance (at 223.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (2500 times diluted)	Amount of Drug present (in mg)	Potency (%)
Zero (Control)	0.714	0.711	0.0188	47.21	94.43
	0.698				
	0.721				
	0.654	0.708	0.0188	47.02	94.04
	0.737				
	0.735				
	0.638	0.677	0.0180	45.01	90.02
	0.688				
	0.705				

Table 4.19: Concentration & absorbance of Atenolol after 2 hours

Time Interval	Absorbance (at 223.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (2500 times diluted)	Amount of Drug present (in mg)	Potency (%)
2	0.653	0.637	0.0169	42.42	84.84
	0.663				
	0.596				
	0.582	0.613	0.0163	40.86	81.73
	0.625				
	0.634				
	0.572	0.602	0.0160	40.15	80.31
	0.619				
	0.616				

Table 4.20: Concentration & absorbance of Atenolol after 4 hours

Time Interval	Absorbance (at 223.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (2500 times diluted)	Amount of Drug present (in mg)	Potency (%)
4	0.610	0.601	0.0160	40.09	80.18
	0.593				
	0.600				
	0.552	0.556	0.0148	37.17	74.35
	0.562				
	0.555				
	0.545	0.556	0.0148	36.98	73.96
	0.559				
	0.556				

Table 4.21: Concentration & absorbance of Atenolol after 6 hours

Time Interval	Absorbance (at 223.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (2500 times diluted)	Amount of Drug present (in mg)	Potency (%)
6	0.555	0.552	0.0147	36.91	73.83
	0.554				
	0.545				
	0.477	0.513	0.0137	34.39	68.78
	0.452				
	0.442				
	0.446	0.511	0.0137	34.26	68.52
	0.545				
	0.543				

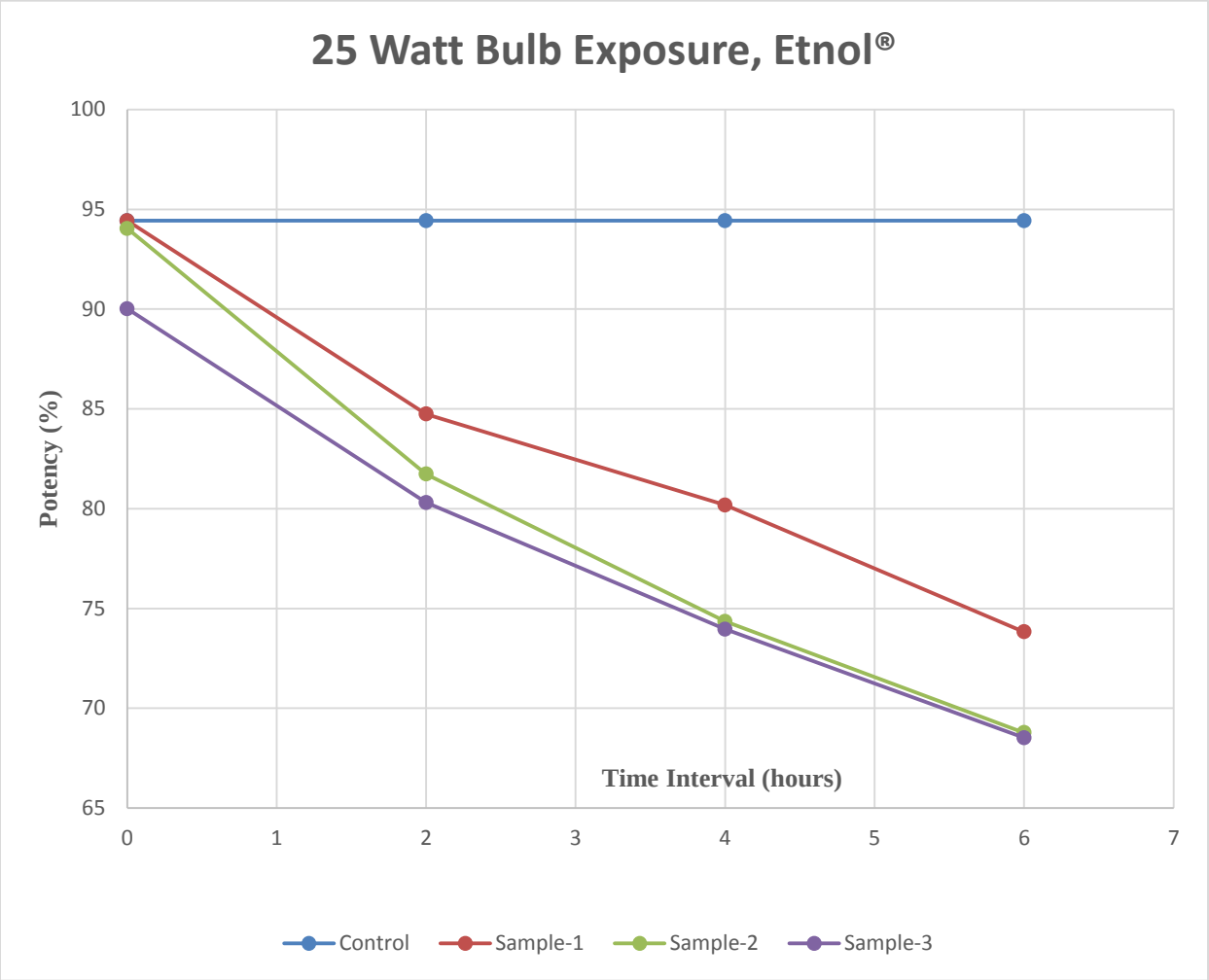


Figure 4.9: Graph showing the difference in Concentration after each 2 hour time interval for Atenolol (Etnol®) for 3rd time.

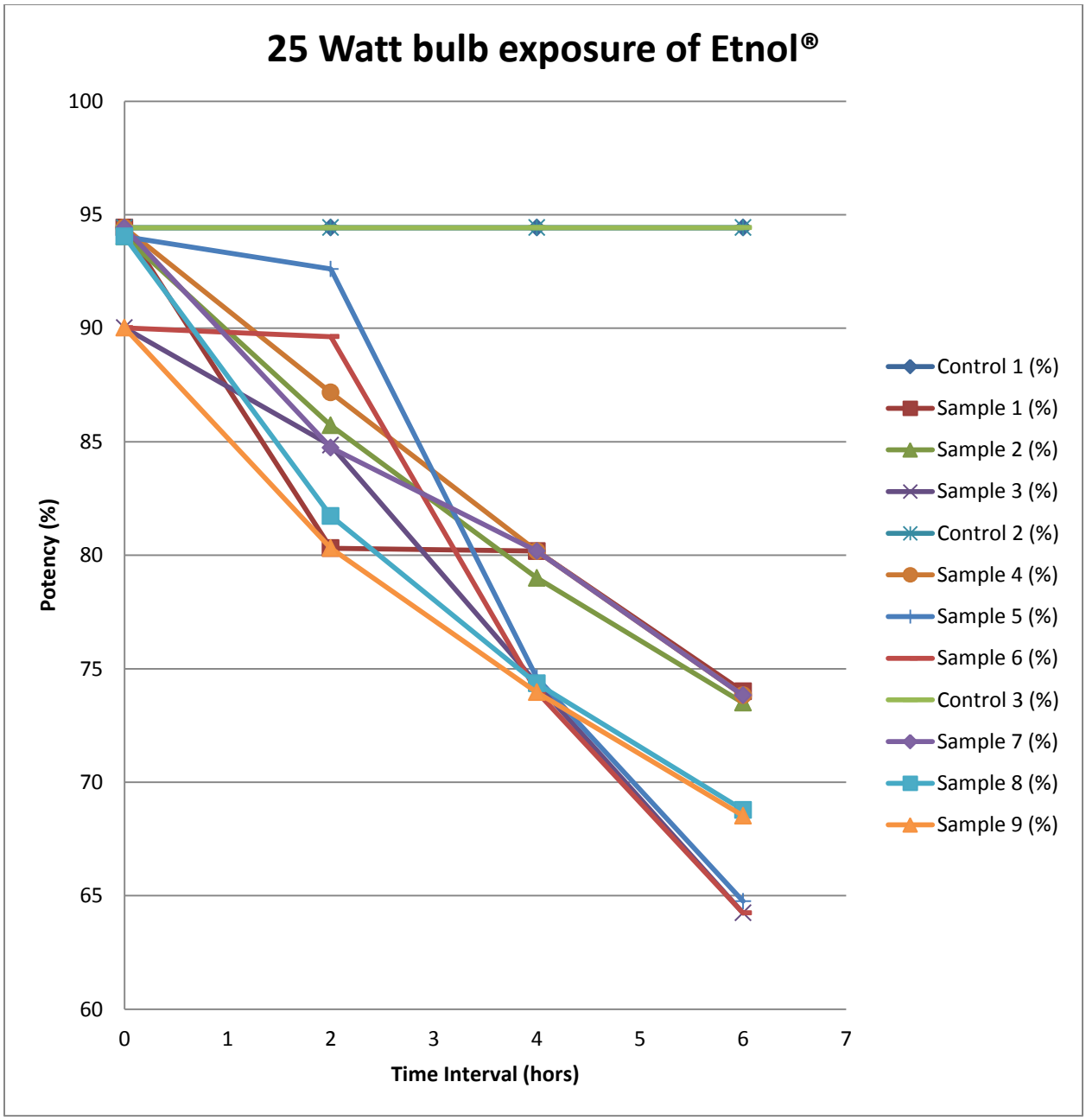


Figure 4.10: Graph showing combination in Concentration after each 2 hour time interval for Atenolol (Etnol®) for 25W bulb

4.3.3 Result of samples that were exposed under 40W bulb

In each experimental day, a tablet strip containing 10 tablets was taken and 5 samples were collected for the test and observed 3 different absorbance of Atenolol for three 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.

4.3.3.1 For First experiment day: The results are given below-

Table 4.22: Concentration & absorbance of Atenolol at Zero hour

Time Interval	Absorbance (at 223.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (2500 times diluted)	Amount of Drug present (in mg)	Potency (%)
Zero (Control)	0.698	0.697	0.0185	46.3	92.6
	0.699				
	0.694				
	0.684	0.691	0.0183	45.91	92
	0.698				
	0.691				
	0.698	0.697	0.0185	46.3	92.6
	0.697				
	0.699				

Table 4.23: Concentration & absorbance of Atenolol after 2 hours

Time Interval	Absorbance (at 223.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (2500 times diluted)	Amount of Drug present (in mg)	Potency (%)
2	0.633	0.636	0.0169	42.35	84.71
	0.634				
	0.643				
	0.611	0.627	0.0167	41.77	83.54
	0.637				
	0.634				
	0.620	0.613	0.0163	40.86	81.71
	0.622				
	0.598				

Table 4.24: Concentration & absorbance of Atenolol after 4 hours

Time Interval	Absorbance (at 223.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (2500 times diluted)	Amount of Drug present (in mg)	Potency (%)
4	0.563	0.572	0.0152	38.21	76.42
	0.577				
	0.577				
	0.532	0.570	0.0152	38.08	76.16
	0.588				
	0.591				
	0.570	0.576	0.0153	38.47	76.94
	0.578				
	0.581				

Table 4.25: Concentration & absorbance of Atenolol after 6 hours

Time Interval	Absorbance (at 223.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (2500 times diluted)	Amount of Drug present (in mg)	Potency (%)
6	0.522	0.546	0.0146	36.52	73
	0.552				
	0.565				
	0.479	0.490	0.0131	32.90	65.85
	0.491				
	0.501				
	0.476	0.474	0.0127	31.86	63.73
	0.471				
	0.476				

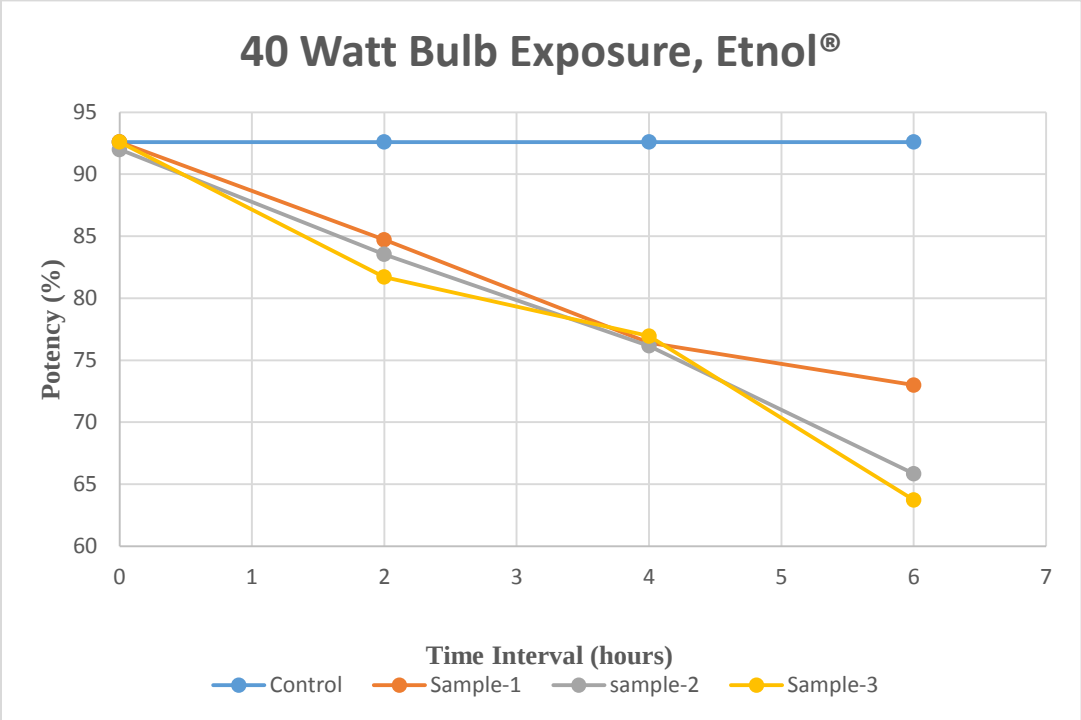


Figure 4.11: Graph showing the difference in Concentration after each 2 hour time interval for Atenolol (Etnol®) for 1st time.

4.3.3.2 For Second experiment day: The results are given below-

Table 4.26: Concentration & absorbance of Atenolol at Zero hour

Time Interval	Absorbance (at 223.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (2500 times diluted)	Amount of Drug present (in mg)	Potency (%)
Zero (Control)	0.698	0.697	0.0185	46.3	92.6
	0.699				
	0.694				
	0.684	0.691	0.0183	45.91	92
	0.698				
	0.691				
	0.698	0.697	0.0185	46.3	92.6
	0.699				
	0.697				

Table 4.27: Concentration & absorbance of Atenolol after 2 hours

Time Interval	Absorbance (at 223.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (2500 times diluted)	Amount of Drug present (in mg)	Potency (%)
2	0.598	0.613	0.0163	40.86	81.71
	0.620				
	0.622				
	0.633	0.636	0.0169	42.35	84.71
	0.634				
	0.643				
	0.611	0.627	0.0167	41.77	83.54
	0.637				
	0.634				

Table 4.28: Concentration & absorbance of Atenolol after 4 hours

Time Interval	Absorbance (at 223.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (2500 times diluted)	Amount of Drug present (in mg)	Potency (%)
4	0.539	0.543	0.0145	36.33	72.66
	0.548				
	0.543				
	0.521	0.524	0.0140	35.10	70.20
	0.530				
	0.522				
	0.519	0.523	0.0140	35.03	70.07
	0.523				
	0.529				

Table 4.29: Concentration & absorbance of Atenolol after 6 hours

Time Interval	Absorbance (at 223.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (2500 times diluted)	Amount of Drug present (in mg)	Potency (%)
6	0.432	0.446	0.0120	30.05	66.11
	0.452				
	0.456				
	0.454	0.466	0.0125	31.34	63.88
	0.470				
	0.474				
	0.458	0.461	0.0124	31.02	62.04
	0.455				
	0.470				

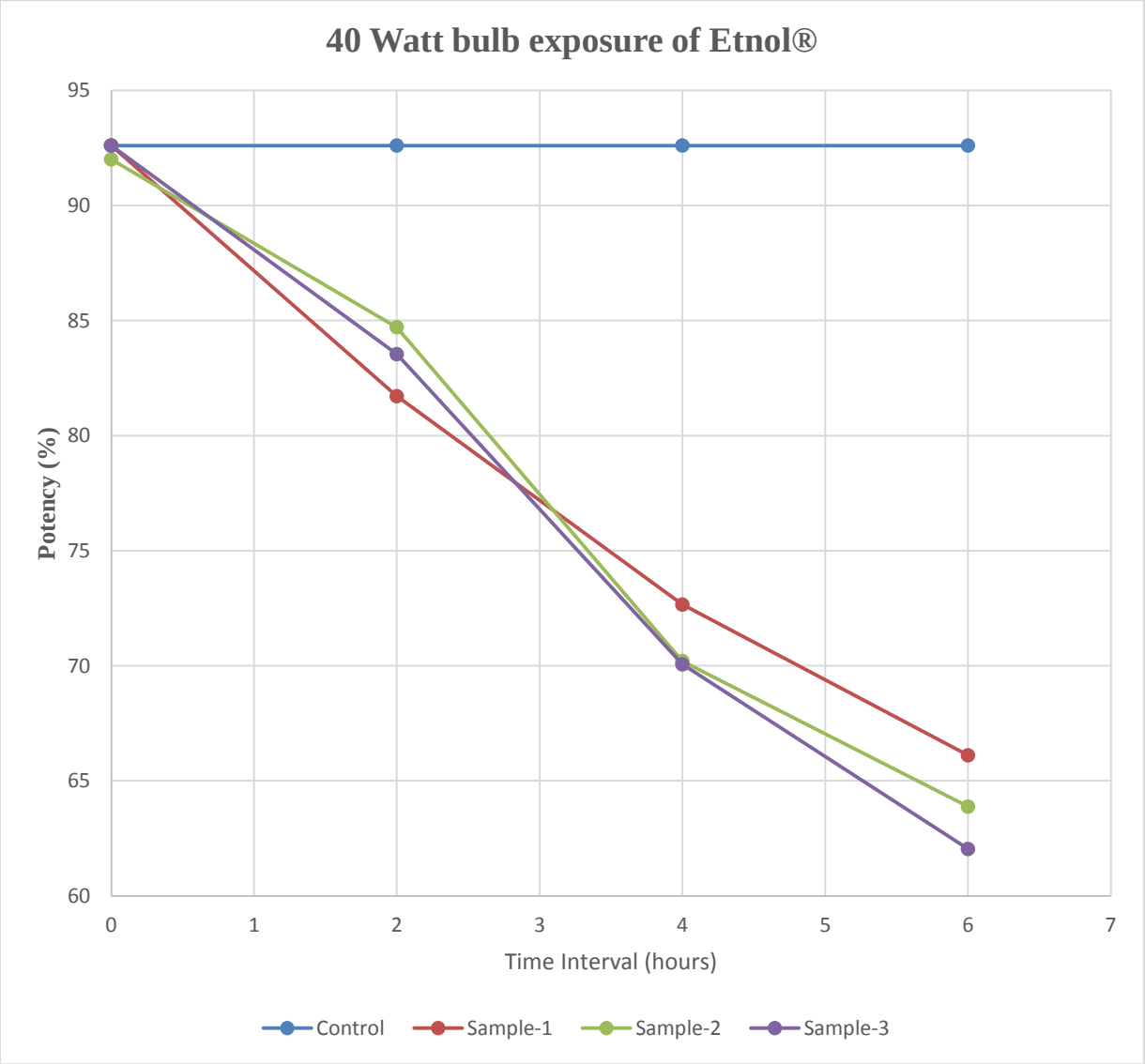


Figure 4.13: Graph showing the difference in Concentration after each 2 hour time interval for Atenolol (Etnol®) for 2nd time.

4.3.3.3 For Third experiment day: The results are given below-

Table 4.30: Concentration & absorbance of Atenolol (Etnol®) at Zero hour

Time Interval	Absorbance (at 223.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (2500 times diluted)	Amount of Drug present (in mg)	Potency (%)
Zero (Control)	0.698	0.697	0.0185	46.3	92.6
	0.699				
	0.694				
	0.684	0.691	0.0183	45.91	92
	0.698				
	0.691				
	0.698	0.697	0.0185	46.3	92.6
	0.699				
	0.697				

Table 4.31: Concentration & absorbance of Atenolol after 2 hours

Time Interval	Absorbance (at 223.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (2500 times diluted)	Amount of Drug present (in mg)	Potency (%)
2	0.577	0.582	0.0155	38.86	77.72
	0.590				
	0.579				
	0.570	0.576	0.0153	38.47	76.94
	0.578				
	0.581				
	0.577	0.572	0.0152	38.21	76.42
	0.563				
	0.577				

Table 4.32: Concentration & absorbance of Atenolol after 4 hours

Time Interval	Absorbance (at 223.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (2500 times diluted)	Amount of Drug present (in mg)	Potency (%)
4	0.552	0.556	0.0148	37.17	74.35
	0.555				
	0.562				
	0.550	0.553	0.0147	36.98	73.96
	0.565				
	0.545				
	0.555	0.552	0.0146	36.91	73.83
	0.553				
	0.550				

Table 4.33: Concentration & absorbance of Atenolol after 6 hours

Time Interval	Absorbance (at 223.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (2500 times diluted)	Amount of Drug present (in mg)	Potency (%)
6	0.483	0.482	0.0129	32.38	64.76
	0.479				
	0.485				
	0.473	0.478	0.0127	32.12	64.24
	0.483				
	0.479				
	0.479	0.490	0.0131	32.90	65.80
	0.491				
	0.501				

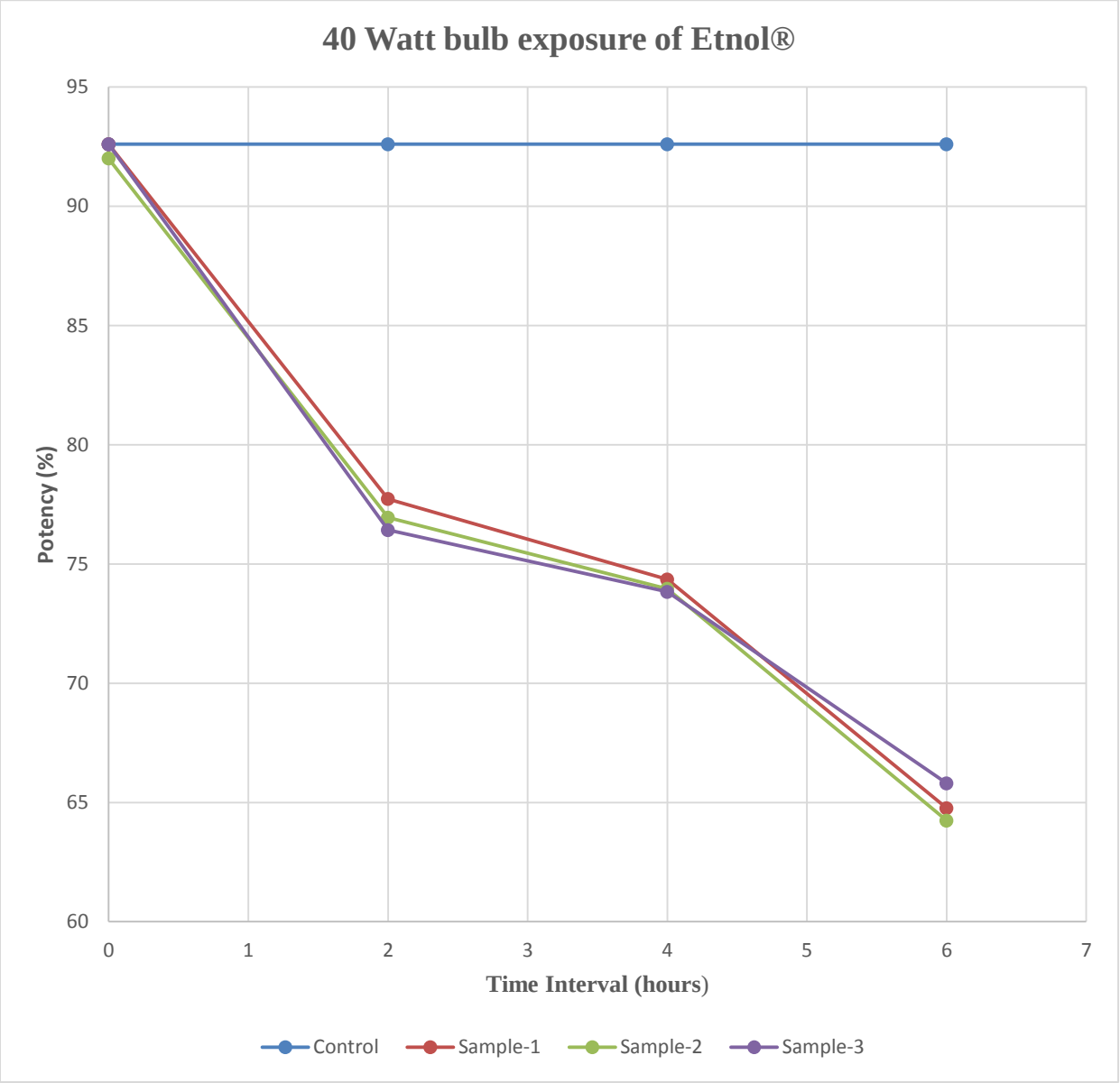


Figure 4.12: Graph showing the difference in Concentration after each 2 hour time interval for Atenolol (Etnol®) for 3rd time.

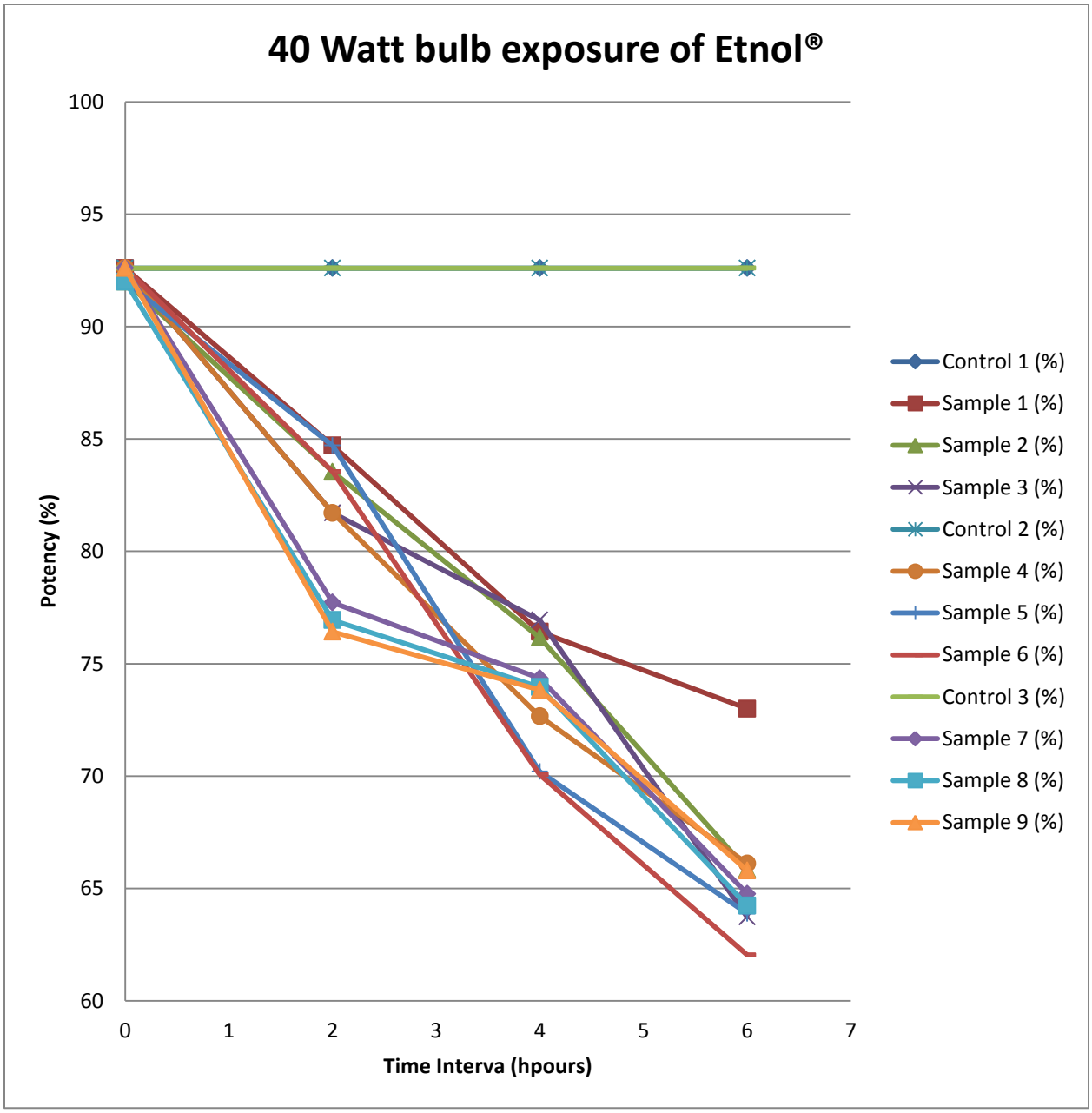


Figure 4.14: Graph showing combination in Concentration after each 2 hour time interval for Atenolol (Etnol®) for 40W bulb.

4.3.4 Result of samples that were exposed under Sunlight.

In each experimental day, a tablet strip containing 10 tablets was taken and 5 samples were collected for the test and observed 3 different absorbance of Atenolol for three 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.

4.3.4.1 For First experiment day: The results are given below-

Table 4.34: Concentration & absorbance of Atenolol at Zero hour

Time Interval	Absorbance (at 223.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (2500 times diluted)	Amount of Drug present (in mg)	Potency (%)
Zero (Control)	0.649	0.654	0.0174	43.52	87.04
	0.703				
	0.612				
	0.610	0.655	0.0174	43.58	87.17
	0.651				
	0.704				
	0.650	0.654	0.0174	43.52	87.04
	0.657				
	0.655				

Table 4.35: Concentration & absorbance of Atenolol after 2 hours

Time Interval	Absorbance (at 223.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (2500 times diluted)	Amount of Drug present (in mg)	Potency (%)
2	0.640	0.636	0.0169	42.35	84.72
	0.652				
	0.616				
	0.620	0.737	0.0169	42.42	84.84
	0.644				
	0.648				
	0.650	0.634	0.0168	42.22	84.45
	0.645				
	0.608				

Table 4.36: Concentration & absorbance of Atenolol after 4 hours

Time Interval	Absorbance (at 223.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (2500 times diluted)	Amount of Drug present (in mg)	Potency (%)
4	0.532	0.530	0.0142	35.5	71
	0.532				
	0.527				
	0.522	0.529	0.0141	35.42	70.85
	0.530				
	0.535				
	0.532	0.526	0.0140	35.23	70.46
	0.520				
	0.528				

Table 4.37: Concentration & absorbance of Atenolol after 6 hours

Time Interval	Absorbance (at 223.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (2500 times diluted)	Amount of Drug present (in mg)	Potency (%)
6	0.470	0.490	0.1316	32.90	65.80
	0.500				
	0.501				
	0.485	0.482	0.0129	32.38	64.76
	0.483				
	0.479				
	0.476	0.473	0.0127	31.80	63.60
	0.481				
	0.462				

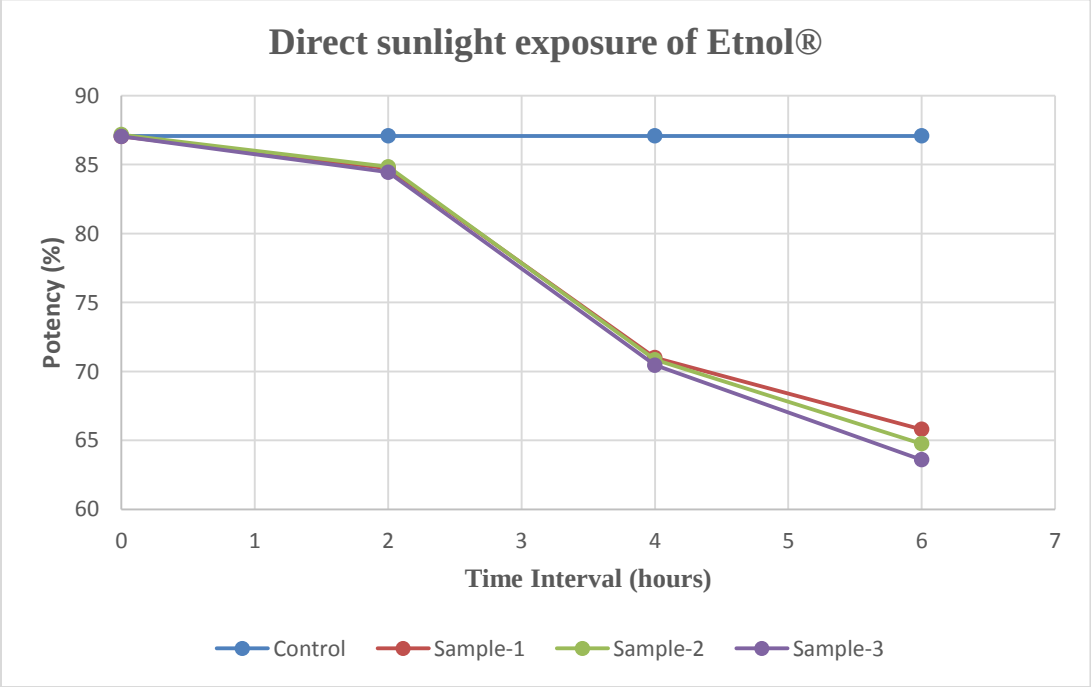


Figure 4.15: Graph showing the difference in Concentration after each 2 hour time interval for Atenolol (Etnol®) for 1st time.

4.3.4.2 For Second experiment day: The results are given below-

Table 4.38: Concentration & absorbance of Atenolol at Zero hour

Time Interval	Absorbance (at 223.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (2500 times diluted)	Amount of Drug present (in mg)	Potency (%)
Zero (Control)	0.649	0.654	0.0174	43.52	87.04
	0.703				
	0.612				
	0.610	0.655	0.0174	43.58	87.17
	0.651				
	0.704				
	0.650	0.654	0.0174	43.52	87.04
	0.657				
	0.655				

Table 4.39: Concentration & absorbance of Atenolol after 2 hours

Time Interval	Absorbance (at 223.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (2500 times diluted)	Amount of Drug present (in mg)	Potency (%)
2	0.603	0.601	0.0160	40.09	80.18
	0.601				
	0.599				
	0.600	0.600	0.0160	40.02	80.05
	0.612				
	0.589				
	0.598	0.598	0.0159	39.89	79.79
	0.599				
	0.599				

Table 4.40: Concentration & absorbance of Atenolol after 4 hours

Time Interval	Absorbance (at 223.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (2500 times diluted)	Amount of Drug present (in mg)	Potency (%)
4	0.559	0.558	0.0149	37.30	74.61
	0.552				
	0.565				
	0.560	0.554	0.0148	37.04	74.09
	0.553				
	0.550				
	0.546	0.546	0.0146	36.52	73.05
	0.544				
	0.550				

Table 4.41: Concentration & absorbance of Atenolol after 6 hours

Time Interval	Absorbance (at 223.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (2500 times diluted)	Amount of Drug present (in mg)	Potency (%)
6	0.450	0.469	0.0126	31.54	63.08
	0.421				
	0.438				
	0.440	0.455	0.0122	30.63	61.26
	0.445				
	0.480				
	0.478	0.452	0.0121	30.44	60.88
	0.446				
	0.434				

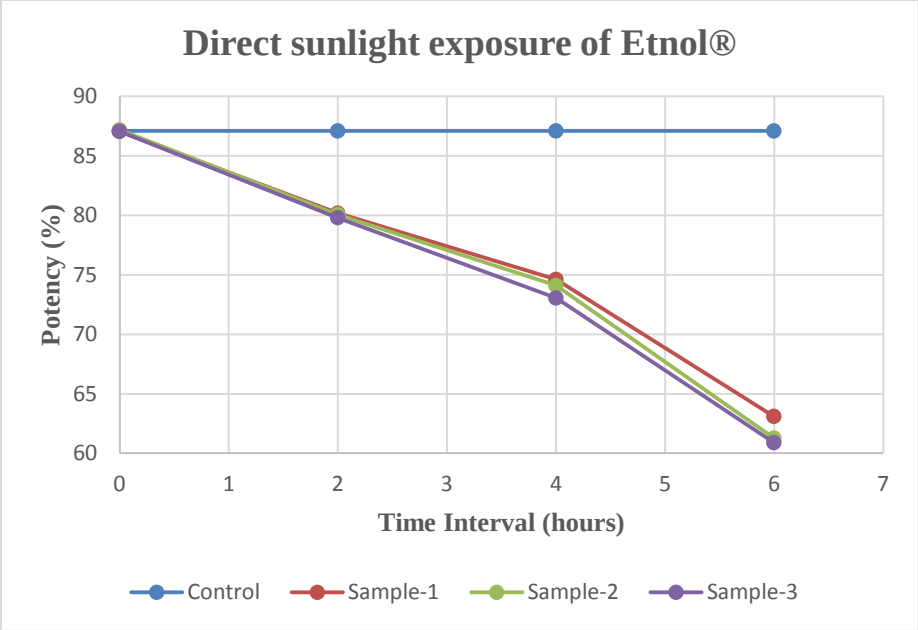


Figure 4.16: Graph showing the difference in Concentration after each 2 hour time interval for Atenolol (Etnol®) for 2nd time.

4.3.4.3 For Third experiment day: The results are given below-

Table 4.42: Concentration & absorbance of Atenolol (Etnol®) at Zero hour

Time Interval	Absorbance (at 223.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (2500 times diluted)	Amount of Drug present (in mg)	Potency (%)
Zero (Control)	0.649	0.654	0.0174	43.52	87.04
	0.703				
	0.612				
	0.610	0.655	0.0174	43.58	87.17
	0.651				
	0.704				
	0.650	0.654	0.0174	43.52	87.04
	0.657				
	0.655				

Table 4.43: Concentration & absorbance of Atenolol after 2 hours

Time Interval	Absorbance (at 223.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (2500 times diluted)	Amount of Drug present (in mg)	Potency (%)
2	0.588	0.570	0.0152	38.08	76.16
	0.530				
	0.593				
	0.590	0.563	0.0150	37.62	75.25
	0.581				
	0.520				
	0.535	0.566	0.0151	37.75	75.50
	0.537				
	0.627				

Table 4.44: Concentration & absorbance of Atenolol after 4 hours

Time Interval	Absorbance (at 223.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (2500 times diluted)	Amount of Drug present (in mg)	Potency (%)
4	0.450	0.469	0.0126	31.54	63.08
	0.421				
	0.438				
	0.440	0.455	0.0122	30.63	61.26
	0.445				
	0.480				
	0.478	0.452	0.0121	30.44	60.88
	0.446				
	0.434				

Table 4.45: Concentration & absorbance of Atenolol after 6 hours

Time Interval	Absorbance (at 223.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (2500 times diluted)	Amount of Drug present (in mg)	Potency (%)
6	0.403	0.402	0.0110	27.72	54.40
	0.404				
	0.400				
	0.393	0.393	0.0108	27.00	54.01
	0.403				
	0.401				
	0.392	0.393	0.0106	26.61	53.23
	0.399				
	0.389				

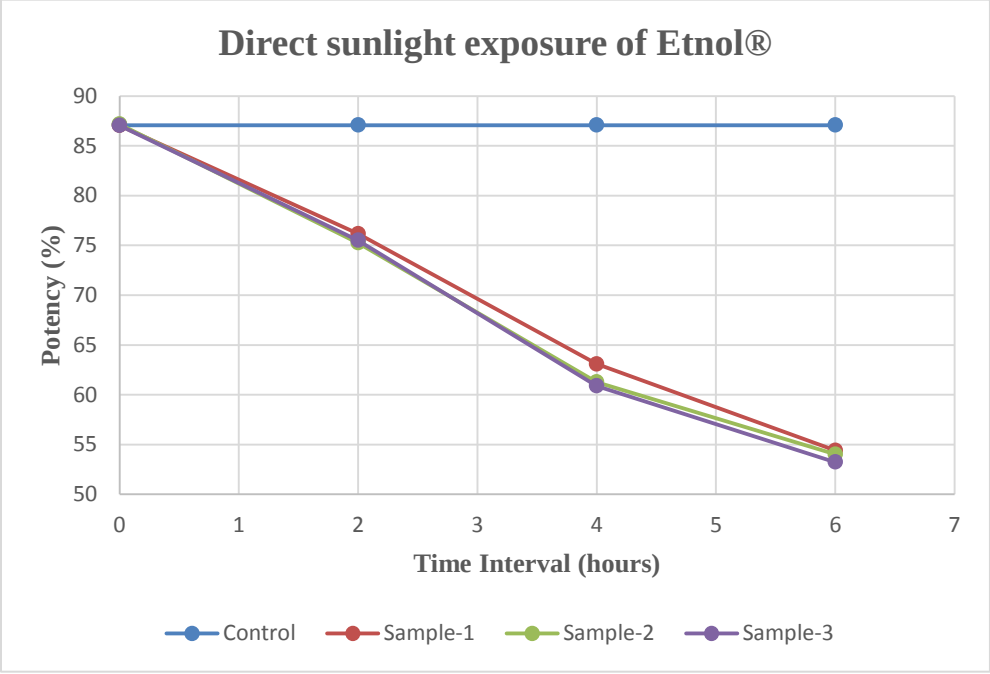


Figure 4.17: Graph showing the difference in Concentration after each 2 hour time interval for Atenolol (Etnol®) for 3rd time.

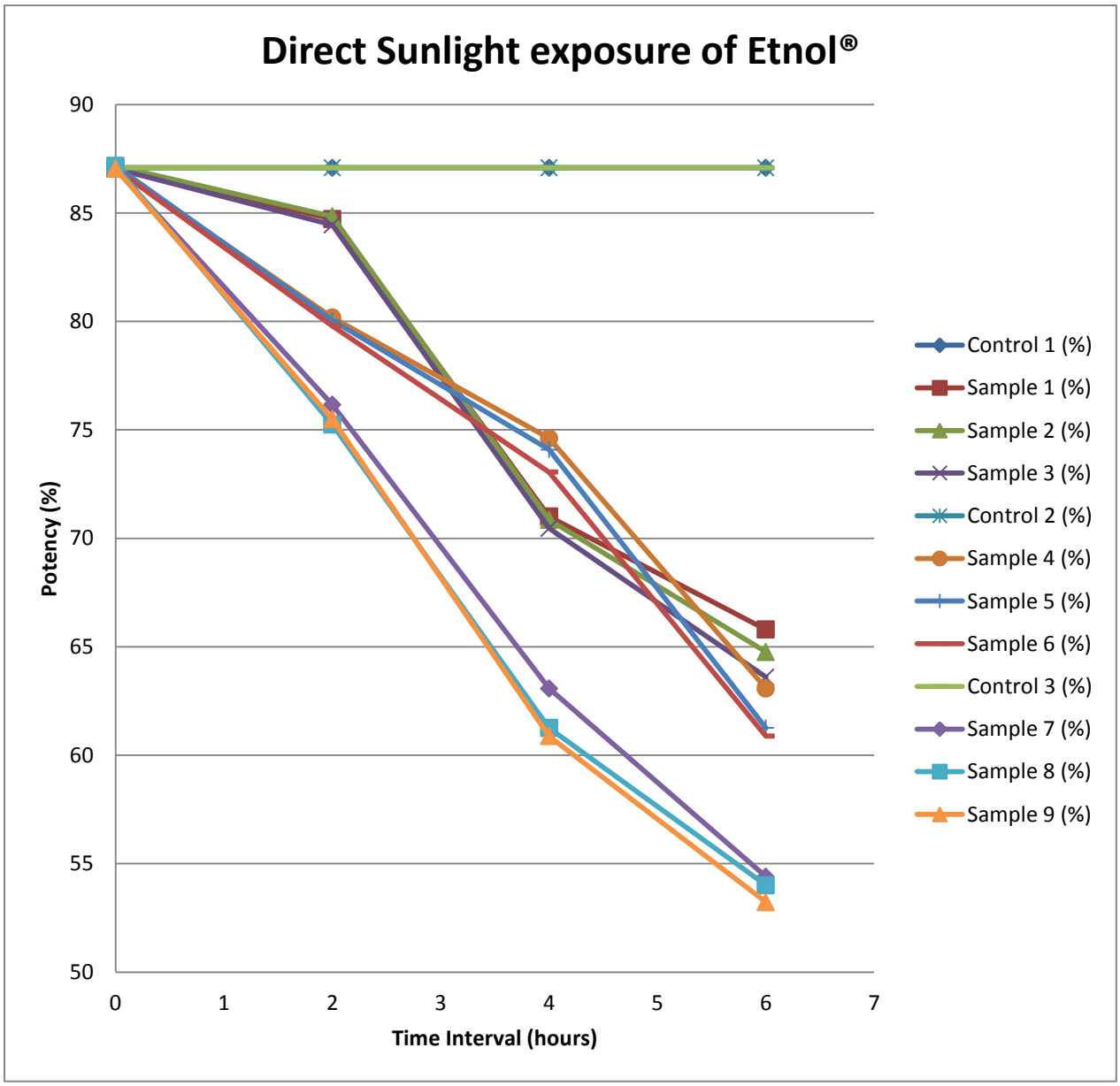


Figure 4.18: Graph showing combination in Concentration after each 2 hour time interval for Atenolol (Etnol®) for direct sunlight.

Chapter Five

Discussion

In this experiment, it was found that the measured physical parameters - color test, weight variation, hardness and thickness did not change significantly throughout the course of the study. Average weight, hardness and also thickness of the tablets were close to each other. The standard deviation of the thickness, hardness and weight variation was ±0.0155 mm, ±0.36253 kg & ±0.0025gm respectively. So, it can be said that light has little or no effect on the color, weight, hardness and thickness of Etnol® (atenolol).

But there were remarkable changes in potencies. The potency of Etnol® (atenolol) was decreased gradually in every lightening condition (normal light, direct sun light, 40w and 25w bulb),when sample tablets (Etnol®) were kept under the electrical bulb (25 watt & 40 watt) and tested every 2 hour light exposed. The tablet samples which were exposed 4 hours on light had less concentration of Atenolol than the 2 hour exposed sample tablet had. Even for 6 hour exposed sample tablets had less concentration of Atenolol than 2 hour and 4 hour light exposed sample. Same result was found for direct sunlight exposed sample tablets and for the tablets which were kept on normal room light conditions. Every experiment was also done 3 times to observe reproducibility of the result. The degradation of Atenolol at 25watt, 40watt, direct sunlight and normal room light were observed 11.86%, 15.45%, 23.06% & 10.23% respectively.

Each time of testing the potency of exposed sample, the potency of the control was also tested which was kept in dark and found that controlled sample retained its potency over the period of the study.

Potency test was performed by UV spectroscopy at 223.5 nm wavelength showed gradual decline in potency of the tablet. So from this study it is verified that only packaging of the Etnol® containing (atenolol) may not protect it from photolytic degradation since storage condition is different throughout the country. Therefore, to prevent this photolytic degradation protective opaque package should be used.

From physical parameter test it was found that the appearance of the tablet was changed slightly after exposure under different lightening condition.

From the thickness test it was obtained that the thickness of the sample tablets was also very close to each other or a very little fluctuation with the periodic work. After each days interval the

thickness remains constant or close to constant. The standard deviation was observed only ±0.0155 mm .So the effects of light dose not influence the thickness of Atenolol.

In the study of weight variation the standard deviation was observed only ±0.0002 g. The percentage of Weight Variation of the Etnol® was within the accepted range (Weight of tablet 185 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 differs by more than 2 times the percentage limit, the tablet pass the test. So, it is clear that, the light has no effect on weight of the Atenolol.

From the hardness test it was found that hardness of the sample tablets was fluctuated with a very short range but the results were very close within the total 80 days interval works. The standard deviation was observed only ±0.36253 kg. Even the average hardness value was also very close to each other's. So the hardness of Atenolol was not affected by different lighting conditions.

Chapter Six

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