Reproducibility Study of the Efficiency of Film Coating on Preventing Photolytic Degradation Tenoren® (Atenolol) Tablet.

A thesis report submitted to the department of pharmacy, East West University, Bangladesh, in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy

Submitted By

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Dedicated to My Parents

Declaration by the Research Candidate

I, Mahabubur Rahaman (ID: 2011-3-70-009), hereby declare that the dissertation entitled "**Reproducibility Study of the Efficiency of Film Coating on Preventing Photolytic Degradation Tenoren® (Atenolol)**" 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, under the supervision and guidance of Mohammed Faisal Bin Karim, Lecturer, Department of Pharmacy, East West University and no part of this dissertation has been or is being submitted elsewhere for the award of any Degree/Diploma.

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Thesis Certificate

This is to certify that the thesis entitled **"Reproducibility Study of the Efficiency of Film Coating on Preventing Photolytic Degradation Tenoren® (Atenolol)."** submitted to the Department of Pharmacy, East WestUniversity 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 Mahabubur Rahaman (ID: 2011-3-70-009) under my supervision and guidance and no part of this dissertation has been or is being submitted elsewhere for the award of any Degree/Diploma.

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Abstract

This research work was conducted to describe the coating efficiency on photolytic degradation of Tenoren® (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 Tenoren® 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 ±0.00020 g, ±0.36250 kg & ±0.01725 mm 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 12.55%, 15.30%, 25.96% & 9.74% respectively. So, it can be said that coating alone was not sufficient to protect the drug from light.

Keywords: Tenoren®, Atenolol, Batch, Weight variation, Hardness, Thickness, Potency, USP.

CHAPTER ONE

INTRODUCTION

1.1 Beta Blocker

1.1.1 Introduction

β-blockers or beta-adrenergic blockers are an essential class of drugs by blocking the β-receptor for the treatment of different heart diseases such as angina pectoris when the blood flow to the heart muscle is insufficient, high blood pressure, arrhythmias when the heart beat is irregular, hypertrophic cardiomyopathy means thickening of the heart muscle, and heart failure when the capability of the heart to empty or fill normally is decreased. For the treatment of migraine headache and increased pressure of the eye (glaucoma) beta-adrenergic blockers can also be used. In clinical medicine no other category of synthetic drugs has had such extensive applicability as β-blockers (Frishman, 2003).

Examples of beta blockers (Mayo Clinic, 2014).

Some beta blockers mainly affect our heart, while others affect both our heart and our blood vessels. Which one is best mainly depend on health and the condition are being treated.

Examples of beta blockers include:

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

1.1.2 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)

1.1 Overall Objective of the Research

The objective of the research project was to determine the reproducibility of results of previous research project on photolytic degradation of Atenolol and also the film coating efficiency to prevent this degradation of photosensitive drug. In this research, photosensitivity of Atenolol will be determined as same various lightening conditions (control, sunlight, normal light, 25watt bulb and 40watt bulb condition) as previous. For this purpose, an available brand is chosen i.e. Tenoren® from ACI pharmaceutical Ltd. for determining whether it is photosensitive or not.

Photolytic degradation test was done to know that the sample degrades or not in the presence of light.

The color was observed of to find any change in color. A digital camera was used to take the picture of the tablets for the comparative study. A fixed camera was maintained with fixed resolution.

Weight variation test is significant because it has a relationship with content uniformity of a solid dosage forms thus weight variation test was done to observe that whether the tablets lose their weight or not.

Variation of thickness can destroy the consumer acceptance. Thickness test was done to observe the thickness of the tablet.

Hardness test of the tablet is potentially important for tablet quality. Hardness test also performed to evaluate the ability of the tablet to withstand abrasion in packaging, handling and shipping.

If the impact is found to be responsible for degrading the drug in the presence of light exposure then the drug must be packed by those packaging system which are light protective.

1.3 Atenolol

Atenolol (2-[4-[2-hydroxy-3-(propan-2-ylamino)propoxy]phenyl] acetamide) is a β1-selective antagonist belonging to the groups of beta blocker which was introduced in 1976 and developed as a replacement for propranolol in the treatment of hypertension. Molecular weight of Atenolol is 266.33608 and chemical formula is $C_{14}H_{22}N_2O_3$. It is very hydrophilic in nature and appears to penetrate the CNS only to a limited extent. The half-life of Atenolol is somewhat longer than that of metoprolol (Westfall and Westfall, 2006).

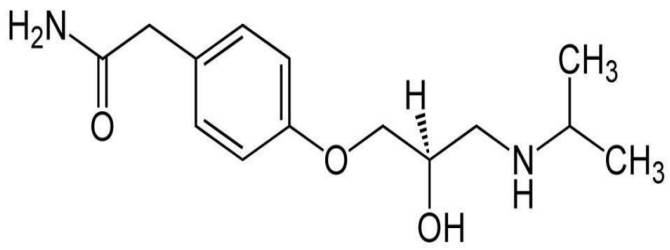

Figure1.1: Structure of Atenolol

1.3.1 Pharmaceutical form (ACI, 2015).

Film-Coated Tablets: White film coated tablet which is marked with ATL 50 or ATL 100 with or without a score line on one side and plain on the reverse. The score line is required to aid in breaking for ease of swallowing and to give the required dose.

1.4 CLINICAL Features OF ATENOLOL

1.4.1 Pharmacological Properties

1.4.1.1 Pharmacodynamic properties (EMC, 2014).

Atenolol is a beta-adrenoceptor blocking agent which is used for the management of angina pectoris and hypertension. It is one of the cardioselective beta-blocker which is selective for cardiac beta one receptors as well as has no partial agonist or membrane stabilizing activity. Still the mechanism of action of atenolol as well as other beta-blockers in the management of hypertension

is not fully understood even though its action on plasma renin and cardiac output are maybe of primary importance. It is also used to block peripheral adrenoceptors, alter baroreceptor reflex sensitivity and reduce cardiac output. It has been seen that Atenolol also decrease systolic and diastolic blood pressures approximately 15% in patients with mild to moderate hypertension. Cardiac work is reduced due to its beta-adrenoceptor antagonist properties. In anginal patients exercise tolerance is improved by this property.

1.4.1.2 Pharmacokinetic properties (EMC, 2014).

Absorption of atenolol following oral dosing is consistent but incomplete (approximately 40–50%) with peak plasma concentrations occurring 2–4 hours after dosing. The bioavailability is decreased by 20% when taken with food. There is a linear relationship between dosage and plasma concentration. The inter-subject variability in AUC and C_{max} is about 30-40%. There is no significant hepatic metabolism of atenolol and more than 90% of that absorbed reaches the systemic circulation unaltered. Atenolol penetrates tissues poorly due to its low lipid solubility and its concentration in brain tissue is low. The volume of distribution is 50 to 75 L. The protein binding is less than 5%. Most of an absorbed dose (85-100%) is excreted unchanged via the urine. The clearance is about 6 l/h and the half-life is about 6 to 9 hours. In elderly patients, clearance is decreased and elimination half-life increased. The clearance is correlated to renal function and the elimination is prolonged in patients with renal impairment. Impaired liver function does not influence the pharmacokinetics of atenolol.

1.5 CLINICAL Particulars OF ATENOLOL

1.5.1 Therapeutic indications (EMC 2014).

1. Indicated for the management of hypertension.

2. Used for the management of angina pectoris.

3.Used for the management of cardiac dysrhythmias.

4. Also used for the management of myocardial infarction.

1.5.2 Posology & method of administration (Mayo Clinic, 2014).

Route of administration: Oral

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

1.5.3 Side effects (Drugs.com, 2014).

You should check with your doctor immediately if any of these side effects occur when taking atenolol:

Most common

- Blurred vision

- cold hands or feet
- confusion
- difficult or labored breathing
- dizziness, faintness, or lightheadedness when getting up from a lying or sitting position suddenly
- shortness of breath
- sweating
- tightness in chest
- unusual tiredness or weakness
- wheezing

Less common

- Anxiety
- chest pain or discomfort
- chills
- cold sweats
- cough
- dizziness or lightheadedness
- fainting
- fast heartbeat
- leg pain
- noisy breathing
- slow or irregular heartbeat
- sudden shortness of breath or troubled breathing

Rare

- Bloody urine
- decreased frequency or amount of urine
- increased blood pressure
- increased thirst
- loss of appetite
- lower back or side pain
- nausea
- swelling of face, fingers, or lower legs
- vomiting
- weight gain

1.5.4 Contraindications (WebMD, 2015).

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.

1.5.5 Drug Interaction

Table1.1: Interaction of Atenolol with other medicinal products (EMC, 2014).

Interacting medicinal products	**Effects**
Alcohol, Aldesleukin, Alprostadil, Anaethetics	Enhanced hypotensive effect
Ampicillin	Reduces atenolol serum levels.
Analgesics particularly indomethacin	Antihypertensive effects of beta-blockers may be impaired
Antacids	Reduced absorption may occur if administered concurrently
Disopyramide, amiodarone, quinidine	Additive negative inotropic effects on the heart, with increased risk of bradycardia, hypotension, ventricular fibrillation, heart block or asystole.
Anticholinesterase agents	Increased risk of bradycardia
Antidepressants and antipsychotics such as Phenothiazines and tricyclic antidepressants and tropisetron	May increase the risk of ventricular arrhythmias

Interacting medicinal products	**Effects**
Antidiabetics	May be an enhanced hypoglycaemic effect and masking of warning signs with concurrent administration of insulin and oral antidiabetic drugs
Angiotensin-converting enzyme (ACE) inhibitors and angiotensin-II antagonists;	Enhanced hypotension;
Cardiodepressant calcium channel blocking agents such as diltiazem, nifedipine and verapamil	May induce negative inotropic effects such as severe hypotension, bradycardia, asystole and heart failure
Antimalarials	Risk of bradycardia increased with mefloquine
Anxiolytics and hypnotics	Enhanced hypotensive effect with benzodiazepines
Cardiac glycosides	Risk of marked bradycardia and AV block.
Clonidine	Increased risk of hypertension on withdrawal
Ergot alkaloids	Increased peripheral vasoconstriction
Moxisylyte	Increased risk of severe postural hypotension
Oestrogens and Progesterones	May antagonise the antihypertensive effect.
Parasympathomimetics	Increased risk of bradycardia
Sympathomimetics such as adrenaline, noradrenaline and ephedrine	Risk of severe hypertension and bradycardia
Theophylline	Atenolol antagonises bronchodilator effect
Ulcer healing drugs such as Carbenoxolone	May antagonise the hypotensive effect

1.5.6 Pregnancy & Lactation (Parker, 2012)

Pregnancy

The FDA classifies atenolol as a Pregnancy Category D medication which means that it can cause harm on the unborn child when used during pregnancy. Therefore, Atenolol should be used in pregnancy only if there are no alternatives and the perceived benefits outweigh the potential risks.

Atenolol passes the placenta which exposes the fetus to possible negative effects. Studies have shown that women who have taken atenolol (particularly during the second trimester) are at increased risk for delivering before term (less than 37 weeks). In addition, some studies have shown that taking atenolol during second trimester of pregnancy results in infants that are smaller for gestational age and with lower birth weight.

Meanwhile, fetal exposure to atenolol during the last months of pregnancy increases the infant's risk for slow heart rate (bradycardia), low blood sugar (hypoglycemia) and low blood pressure (hypotension) immediately or several hours after delivery. Close monitoring is recommended for infants who have been exposed to atenolol during the last trimester of pregnancy.

Although there are no studies concerning the use of atenolol during the first three months of pregnancy, the possibility of fetal injury is relatively high. So, if one becomes pregnant while taking atenolol, be sure to contact your healthcare provider immediately. Healthcare provider will evaluate potential hazard to the unborn child. Normally, medication will be changed.

It is important to discuss with healthcare provider if there is any plans of becoming pregnant before starting this medication. The patient and healthcare provider must evaluate the benefits and risk of using atenolol during pregnancy in order to come up with a shared decision.

Lactation

Basically, atenolol is not recommended for breastfeeding women. This medication is excreted in the human milk and can cause unwanted effects on the nursing infant. Atenolol tends to accumulate in the breast milk. Clinical studies reveal that breast milk contains atenolol at a ratio of 1.5 to 6.8 when compared to drug levels in plasma. This can result in the infant receiving potentially fatal atenolol doses. Infants who are breastfed by patients taking atenolol are at

increased risk of developing adverse effects particularly hypotension, bradycardia and hypoglycemia. There are also rare cases of infants experiencing cyanosis (lack of circulation oxygen).

Doctor should be consulted if one is or will be breast-feeding while taking atenolol. Normally, healthcare provider will substitute atenolol with another antihypertensive medication. However, if there are no alternatives and the benefits outweigh the potential risks, atenolol may be given. Regular physician visits are recommended if taking atenolol during pregnancy.

1.5.7 Precautions (Health Central, 2015).

Before taking atenolol, doctor or pharmacist should be told if one is allergic to it; or if one has any other allergies. This product may contain inactive ingredients, which can cause allergic reactions or other problems. Pharmacist should be consulted for more details.

Before using this medication, doctor or pharmacist should be told about medical history, especially of:

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

This drug may make one dizzy. One should not drive, use machinery, or do any activity that requires alertness until one is sure can perform such activities safely. Alcoholic beverages should be limited.

Before having surgery, doctor or dentist should be told about all the products one use (including prescription drugs, nonprescription drugs, and herbal products).

For patients with diabetes, this product may prevent the fast/pounding heartbeat one would usually feel when blood sugar level falls too low (hypoglycemia). Other symptoms of low blood sugar are dizziness and sweating are unaffected by this drug. This product may also make it harder to control blood sugar levels. Blood sugar levels should be checked regularly as directed by doctor. Doctor should be immediately told if one has symptoms of high blood sugar such as increased thirst/urination. Doctor may need to adjust diabetes medication, exercise program, or diet.

This medication is not recommended for use during pregnancy. It may harm an unborn baby.

This medication passes into breast milk and may have undesirable effects on a nursing infant. Doctor should be consulted before breast-feeding.

1.5.8 Overdose (EMC, 2014).

In some cases overdose of beta-blocker is uneventful, but few patients build up severe and occasionally fatal cardiovascular depression. Overdose effects are bradycardia, cardiac conduction block, hypotension, heart failure, and cardiogenic shock, Convulsions, coma, bronchospasm, respiratory depression, and bronchoconstriction. Absorption of any drug material can be prevented by gastric lavage and administration of activated charcoal which is still present in the gastrointestinal tract. In case of acute massive overdose requires hospital management and expert advice. Maintenance of a clear airway and adequate ventilation is mandatory.

1.5.9 Missed Dose (Mayo Clinic, 2014).

If you miss a dose of this medicine, take it as soon as possible. However, if it is almost time for your next dose, skip the missed dose and go back to your regular dosing schedule. Do not double doses.

1.5.10 Storage (Mayo Clinic, 2014).

Store the medicine in a closed container at room temperature, away from heat, moisture, and direct light. Keep from freezing. Keep out of the reach of children. Do not keep outdated

medicine or medicine no longer needed. Ask your healthcare professional how you should dispose of any medicine you do not use.

1.6 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.

1.6.1 Photolytic Condition

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

1.6.2 Mechanism of Photolytic Degradation

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.

1.6.3 Photo Stability Testing of Drugs (Stability testing, 1999)

The drug product is initially tested without packaging. If unacceptable changes occur, protection is added in stages starting with the primary packaging followed by the secondary packaging (commercial packaging). Depending on the result, the packaging must be improved and/or the formulation changed. The procedure is shown as follow:

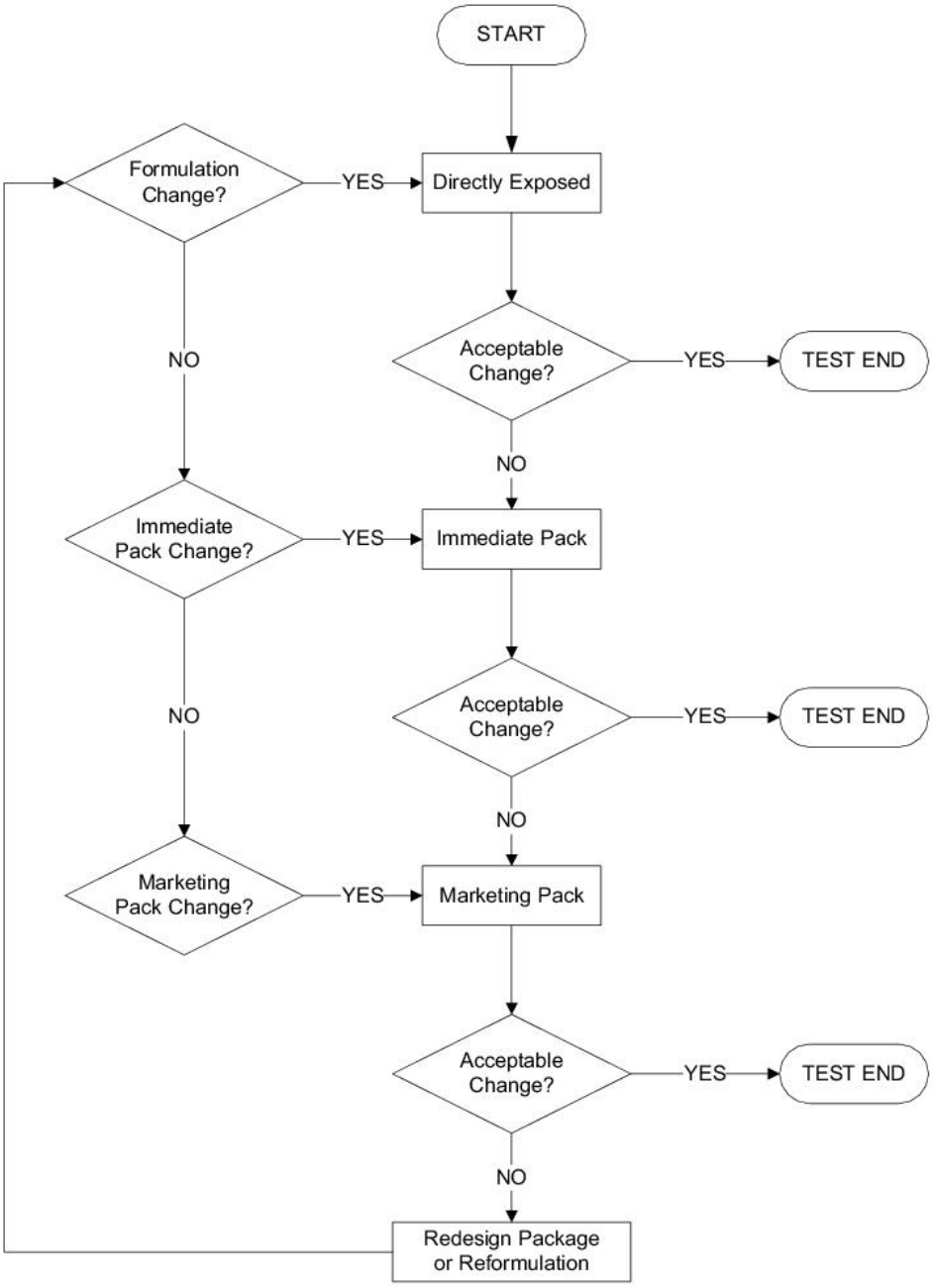

Figure 1.2: Flow chart for photo-stability testing of drug products

CHAPTER TWO

LITERATURE REVIEW

2. LITERATURE REVIEW

In December 1999, V Andrisano, R Gotti, A Leoni, and V Cavrini evaluated the photostability of the beta-blocker drug Atenolol was evaluated at pH 9, 7.4 and 4.0. The drug was exposed to UVA-UVB radiations and the photoproducts were detected by reversed phase LC methods. The photodegradation was found to increase with the pH value decreasing. The major photodegradation product at pH 7.4 was identified as 2-(4-hydroxyphenyl)-acetamide. The LC method developed for routine analyses (column: C-18 Altima; 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.

In 2003 Abreu and his colleagues (Abreu *et al.* 2003) were developed an accurate, precise, and sensitive high-performance liquid chromatography (HPLC) assay for the determination of atenolol in human plasma samples to compare the bioavailability of atenolol tablet (50 mg) formulations in 24 volunteers of both sexes. The study had an open, randomized, 2-period crossover design with a 1-week washout period. From the atenolol plasma concentration versus time curves following

pharmacokinetic parameters such as AUC-24h and C_{max} were found. The geometric mean of test/reference 50-mg tablets individual percent ratio was 102.2% for AUC 0-24h, and 101.6% for C_{max}. The 90% confidence intervals (CI) were 100.2% to 105.4% and 100.9% to 103.5%, respectively. Since the 90% CI for both C_{max} and AUC 0-24h were within the 80% to 125% interval proposed by the Food and Drug Administration, it was concluded that atenolol (50-mg tablets) test formulation was bioequivalent to the reference formulation, with regard to both the rate and extent of absorption.

In the year 2006, Krzek (Krzek *et al.* 2006) and his research team investigated 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. 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 degrees C, 60 degrees C, and 90 degrees C. The identification of degradation products was carried out by using 1H NMR. The degradation processes that

occurred in drugs under investigation are described with kinetic parameters (k, t0.1, and t0.5) and energy of activation (Ea). This study demonstrated that the stability of chosen beta-adrenergic blocking agents increases with their lipophilicity.

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

In 2007 Chan (Chan *et al*, 2007) was conducted another research 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. Atenolol 100 mg tablets 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. It was found that 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).

In July 2007, Foopa (Foopa *et al*, 2007) developed a stability-indicating to evaluate the stability of pediatric atenolol syrup. Atenolol [4-(2-hydroxy-isopropylaminopropoxy)-phenylacetamide], is a cardioselective beta1-adrenergic receptor blocking agent prescribed for treatment of hypertension, angina pectoris and cardiac arrhythmias. However, most of these medicines are not formulated for easy or accurate administration to children. Atenolol is unstable in solutions and therefore the development of a liquid dosage form is a significant challenge. Studies showed that the degradation rate of atenolol is dependent on the temperature, indicating higher stability at 4 degrees C. Atenolol syrup is stable for 9 days, with acceptable apearance. A second order model adequately described atenolol decomposition when stored as syrup.

Venkatesh (2007) performed 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.

In 2007 Taylor and his colleagues (Taylor *et al*, 2007) were observed the degradation of atenolol by a reversed-phase liquid chromatographic (RP-LC) assay method and developed the method for the quantitative determination of atenolol in the presence of its degradation products. 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

In February 2008, Claudio Medana and others (Claudio *et al*. 2008) have studied the photocatalytic transformation of atenolol; 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. 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.

In February 2009, Hiroshi Yamamoto , Yudai Nakamura and others (Hiroshi *et al*. 2009) selected eight pharmaceuticals with relatively high potential ecological risk and high consumption—namely, acetaminophen, atenolol, carbamazepine, ibuprofen, ifenprodil, indomethacin, mefenamic acid, and propranolol—and conducted laboratory experiments to examine the persistence and partitioning of these compounds in the aquatic environment. In the results of batch sunlight photolysis experiments, three out of eight pharmaceuticals namely propranolol, indomethacin, and ifenprodil were relatively easily photodegraded (i.e., half-life < 24 h), whereas the other five pharmaceuticals were relatively stable against sunlight. The results of

batch biodegradation experiments using river water suggested relatively slow biodegradation (i.e., half-life > 24 h) for all eight pharmaceuticals, but the rate constant was dependent on sampling site and time. The determined coefficients (K_d values) were much higher for three amines (atenolol, ifenprodil, and propranolol) than for neutral compounds or carboxylic acids.

In the same year in May 2009 by Kumar V, Shah RP, Malik S and Singh S investigated compatibility of atenolol, a beta (1) blocker, with a variety of pharmaceutical excipients. The binary mixtures (1:1) of atenolol with the excipients were stored for 1 month at 40 degrees 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. The degradation/interaction products formed in these mixtures were characterized by high resolution mass spectrometric and fragmentation analyses, using a LC-MS/TOF system. The identity of characterized structures was justified through mechanistic explanations.

Again In June 2009 Liu QT and others (Liu *et al.* 2009) suggested to improve the understanding of the fate and behavior of pharmaceuticals in the environment that there is a need to investigate in-stream depletion mechanisms, e.g. photo transformation of active pharmaceutical ingredients (APIs) in natural surface waters. In this study, abiotic and biotic degradation of selected beta-blockers was measured simultaneously in non-sterilized and sterilized river waters and deionized water (DIW) under simulated sunlight (lambda: 295-800 nm) and dark conditions, and at environmentally relevant concentrations, i.e. <or= 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. Photo transformation was the main depletion mechanism for the beta-blockers tested over a 2 to 7 day period. Slow hydrolysis was observed for metoprolol only. Loss due to biodegradation in river waters was not observed for propranolol but was found for metoprolol and atenolol at a very slow rate within the study period.

In March 2010, the conversion of the antibiotic ofloxacin and the β-blocker atenolol by means of TiO_2 photocatalysis was investigated by E. Hapeshi and his colleagues. Irradiation was provided by a UVA lamp at 3.37×10^{-6} einstein/s photon flux, initial substrate concentration (5–20 mg/L),

initial pH (3–10) and the effect of H_2O_2 (0.07–1.4 mm). Conversion was assessed measuring sample absorbance at 288 and 224 nm for ofloxacin and atenolol, respectively. Degussa P25 TiO_2 was found to be more active than other TiO_2 samples for either substrate degradation, with ofloxacin being more reactive than atenolol. The effect of solution pH was substrate-specific.

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.

Shetty (2010) was developed 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.

In 2010 Belal (Belal *et al,* 2011) was developed a method for the determination of atenolol by a stability-indicating reversed-phase liquid chromatographic method. The stability-indicating capability of the method was also tested after accelerated degradation of atenolol in acidic and basic media and after freezing and heating treatments. The chromatographic assay involved the use of a C8 Column (250 mm×4.6 mm) with a simple mobile phase composed of acetonitrile:methanol:0.02 M phosphate buffer, pH 5 (20:20:60) at a flow rate of 1 ml/min and UV detection at 226 nm. Pindolol was used as an internal standard. .The proposed method was successfully applied for the analysis of atenolol in three commercial tablets with average percent recoveries of 100.14 ± 1.04, 100.20 ± 0.92, 100.00 ± 0.91 and 100.75 ± 0.67, respectively. The results were statistically compared with those obtained by the official method and were found to be in good agreement.

In 2011, H. O. Kaila and others investigated a simple, rapid, precise and accurate isocratic reversed phase stability indicating HPLC method 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.

In the same year, the significance of transformation products of pharmaceuticals resulting from the parent compounds during natural and technical photolytic processes and advanced oxidation processes has only recently started to attract the interest of the scientific community. Even though relevant studies have now started to produce important knowledge, still many gaps exist that hinder the in-depth and broad understanding of the extent of the potential problems stemming from the presence of such compounds in the environment and the applicability of such techniques for wastewater and potable water treatment. This review paper tries to highlight some of the most relevant studies performed so far and to summarize the parameters that prevent scientists from reaching comprehensive conclusions in relation to the formation, fate, and effects of transformation products of pharmaceutical compounds during photo-driven and advanced oxidation processes.

In June 2011, Arindam Basu, Bidyut Das and others (Arindam *et al.* 2011) developed and validated a simple, accurate & precise reverse phase liquid chromatographic method for simultaneous estimation of Atenolol and Indapamide from tablet dosage form. The method was developed using Waters HPLC system on a L1 column (Hypersil Gold: 250mm x 4.6 mm, 5µm) using a mixture of 0.1% Triethyl Amine in water of pH 3.0 & Methanol in the ratio 30:70 v/v as mobile phase in an isocratic elution mode at a flow rate of 1.0 ml/min, at 30°C with a load of 20µl. The detection was carried out at 240 nm. The retention time of Atenolol and Indapamide were found to be around 3.05 min and 3.93 min respectively. The method was validated with respect to linearity, robustness, precision, specificity & accuracy. The proposed method was successfully applied for the simultaneous quantitative determination of Atenolol and Indapamide from the tablet dosage form.

Again in September 2011, L.A. Ioannou, E. Hapeshi, M.I. Vasquez and others investigated the photocatalytic conversion of two β-blockers, namely atenolol and propranolol in aqueous TiO_2 suspensions. 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 H_2O_2 (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 H_2O_2 did not accelerate kinetics which seem to follow the Langmuir–Hinshelwood model.

In July 2012, photolysis behavior of atenolol (ATL) and toxicity of its photodegradation products were investigated in the presence of nitrate ions. The photodegradation was found to be dependent on nitrate concentration and increasing the nitrate from 0.5 mM L^{-1} to 10 mM L^{-1} led to the enhancement of rate constant from 0.00101 min^{-1} to 0.00716 min^{-1}. Increasing the solution pH from 4.8 to 10.4, the 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.

In July-august 2012, Zaid AN (Zaid *et al.* 2012) and his co-researchers did a research 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 tabletsfailed 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

In January 2013, an investigation was carried out by Salgado R and others (Salado *et al*. 2013) that Pharmaceutical compounds such as ketoprofen, diclofenac and atenolol are frequently detected at relatively high concentrations in secondary effluents from wastewater treatment plants. Therefore, it is important to assess their transformation kinetics and intermediates in subsequent disinfection processes, such as direct ultraviolet (UV) irradiation. The photodegradation kinetics of these compounds using a medium pressure (MP) lamp was assessed in pure water, as well as in filtered and unfiltered treated wastewater. Ketoprofen had the highest time- and effluence-based rate constants in all experiments, whereas atenolol had the lowest values, which is consistent with the corresponding decadal molar absorption coefficient and quantum yield. The fluency-based rate constants of all compounds were evaluated in filtered and unfiltered wastewater matrices as well as in pure water.

In 2013, Photo-catalytic degradation of atenolol (ATL) was investigated by Yuefei Ji, Lei Zhou and others in aqueous suspensions using TiO2 as photo-catalyst. Complete degradation of 37.6μM ATL was obtained after 60min irradiation in pH 6.8 milli-Q water in the presence of 2.0gL−1 Degussa P25 TiO2. Degradation of ATL followed pseudo-first-order reaction kinetics. Major transformation products were elucidated by high performance liquid chromatograph-mass spectrometry (HPLC–MS/MS) technique. ATL photodegradation pathways included generation of 3-(isopropyl-amino)propane-1,2-diol and p-hydroxyphenyl acetamide through ether chain cleavage, hydroxylation and the formation of 4-[2-hydroxy-3-(isopropyl amino)propoxy] benzaldehyde. Photocatalytic degradation efficiency of ATL was highly dependent on the properties of the water matrix, such as pH, the presence of organic and inorganic species (e.g., humic substance, HCO3−). River water matrix was found to play a detrimental effect on ATL photocatalytic degradation with a longer irradiation time required for complete elimination of mother compound and intermediate products.

Again in 2013, Xiaowei Liu and others described photoactivation of peroxymonosulfate (PMS) with UV (254 nm) irradiation was used to generate advanced oxidation process, which was adopted to degrade atenolol (ATL) in water. The second-order reaction rate constants of ATL with HO and $SO_4^{\cdot-}$ were determined, and the effects of operational parameters (dose of PMS,

solution pH, HCO_3^-, humic acids (HA), and N_2 bubbling) were evaluated as well. Finally the main transformation intermediates were identified and possible degradation pathways were proposed. The results showed that there was a linear positive correlation between the degradation rate of ATL and specific dose of PMS (1–16 M PMS/M ATL). Increasing solution pH from 3 to 9 promoted elimination of ATL due to the pH-dependent effect of PMS photodecomposition, while further pH increase from 9 to 11 caused slowing down of degradation because of apparent conversion of HO• to $SO_4^{\bullet-}$. 1–8 mM HCO_3^- exerted no more than 5.3% inhibition effect on ATL destruction, suggesting HCO_3^- was a weak inhibitor.

In July 2014, S. Veloutsou, E. Bizani, K. Fytianos described the degradation of atenolol and metoprolol in aqueous solutions by means of the photo-Fenton reaction. The purpose of this study was: (i) to investigate the influence of the concentrations of iron and hydrogen peroxide, by means of central composite design, (ii) to study the degradation kinetics in aqueous solutions, (iii) to evaluate the mineralization and the toxicity evolution of the target compounds and (iv) to identify the degradation products. It has been found that increase of iron and hydrogen peroxide concentration accelerate the degradation of atenolol and metoprolol. The determination of the by-products formed during the degradation using LC-MS/MS equipment and the evaluation of the toxicity of the treated solution in different stages of the process would offer significant, innovative information regarding the treatment of water and wastewater containing active pharmaceutical compounds, especially of the β-blocker group.

Recently in January 2015, Xiaowei Liu, Tuqiao Zhang and others used photoactivation of SO_3^{2-} with UV_{254} irradiation (UV/SO_3^{2-}) generate hydrated electron (e_{aq}^-)-based process to degrade atenolol (ATL) in drinking water. The decontamination mechanism of UV/SO_3^{2-}process was investigated by means of competitive kinetic analysis and identification of transformation products, followed by the assessment of potential for drinking water treatment through evaluating the influence of operational parameters (dosage of SO_3^{2-}, solution pH, HCO_3^-, dissolved organic matters (DOM), and temperature), detoxification efficiency, and technical economy. The process is pH dependent. Increasing solution pH from 5 to 9 promoted the elimination of ATL due to the pH-dependent SO_3^{2-} photoactivity. For the pH increase from 9 to 11, deprotonation of ATL caused further enhancement of ATL destruction. 1–4 mM HCO_3^- exerted little inhibition effect on the ATL destruction, while the presence of 1 and

4 mg L^{-1} L DOM deteriorate the ATL degradation due to the effective UV fluency decrease. In addition, for every 10 °C the solution temperature rose, the reaction rate increased 1.3–1.6 times.

In early 2015, Dong MM (Dong *et al*. 2015) and others examined the photochemical degradation of five pharmaceuticals in two secondary wastewater effluents. The compounds, which included atenolol, carbamazepine, meprobamate, phenytoin and primidone, were evaluated for both direct and sensitized photolysis. In the two wastewaters, direct photolysis did not lead to significant compound degradation; however, sensitized photolysis was an important removal pathway for the five pharmaceuticals. Upon solar irradiation, hydroxyl radical (HO) was quantified using the hydroxylation of benzene and singlet oxygen ($^1O_2$) formation was monitored following the degradation of furfuryl alcohol. Degradation via sensitized photolysis was observed following five-day exposures for atenolol (69-91%), carbamazepine (67-98%), meprobamate (16-52%), phenytoin (44-85%), and primidone (34-88%). Varying removal is likely a result of the differences in reactivity with transient oxidants. Averaged steady state HO concentrations ranged from 1.2 to 4.0×10^{-16}M, whereas the concentrations of $^1O_2$ were $6.0\text{-}7.6\times10^{-14}$M. Partial removal due to presence of HO indicates it was not the major sink for most compounds examined. Other transient oxidants, such as $^1O_2$ and triplet state effluent organic matter, are likely to play important roles in fates of these compounds.

Lastly in June 2015, Veronika Píšťková , Minoo Tasbihi , Milada Vávrová, Urška Lavrenčič Štangar investigated photocatalytic degradation of five β-blockers (acebutolol, atenolol, metoprolol, nadolol and propranolol) in aqueous media using immobilized TiO_2 as a photocatalyst. The analyzed parameters were pharmaceutical removal and non purgeable organic carbon. Two different types of photocatalyst incorporated in sol–gel matrix were compared; P25 exhibited a higher photocatalytic activity compared to P90 (both from Evonik Degussa). According to the results obtained, after 120 min of treatment a complete removal was achieved for all compounds (initial concentration 25 mg/L) using P25. After 240 min of irradiation a mineralization degree ranged from 75% for acebutolol to 92% for propranolol. A longer illumination time was needed for complete removal of β-blockers in river water.

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 coating efficiency, 700 tablets of Tenoren® (Atenolol 50 mg) were collected from the local drug store in Dhaka as a sample. All the tablets were from the same batch (14004). Among them 300 tablets were kept light protected for control tests and the remaining 400 tablets were subjected to various lighting conditions over certain periods of time for conducting experiments to determine their potency.

3.1.2 Samples

Table 3.1: Samples Used in the Experiment Including Source (ACI, 2015)

Sample Name	Source (Supplier Name)	Batch No.
Tenoren® Tablets	ACI Pharmaceuticals Ltd.	EO 8

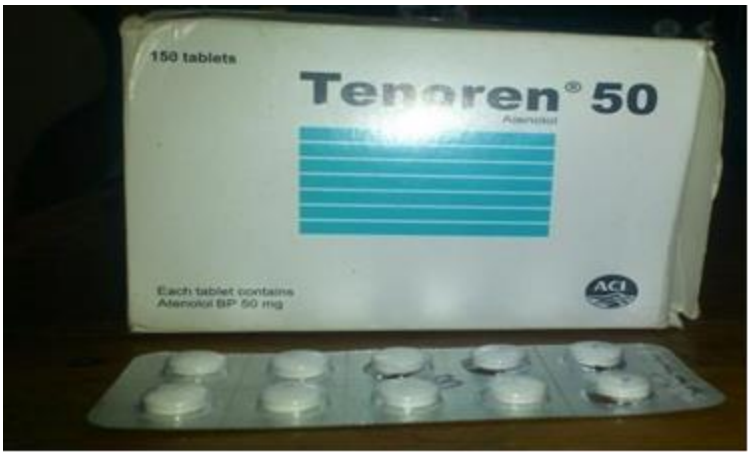

Figure 3.1: Tenoren® Tablets

3.1.3 Reagents

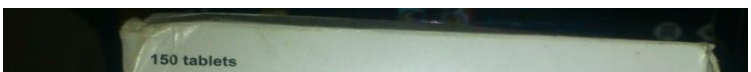

Table 3.2: Reagents Used in the Experiment Including Source

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

3.1.4 Equipments & Instruments

Table 3.3: Lists of Equipments used for the Experiment

Serial No.	Equipments	Source (Supplier Name)	Origin
1	UV-Spectrophotometer	Shimadzu UV1800	Japan
2	Distill Water Plant	Bibby Scientific W4000	United Kingdom
3	Electronic Balance	Shimadzu AY220	Japan
4	Hardness tester	Veego VTHT	India
5	Vernier Calipers	Shanghai Tricle Brand	China

3.1.5 Images of Instruments

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

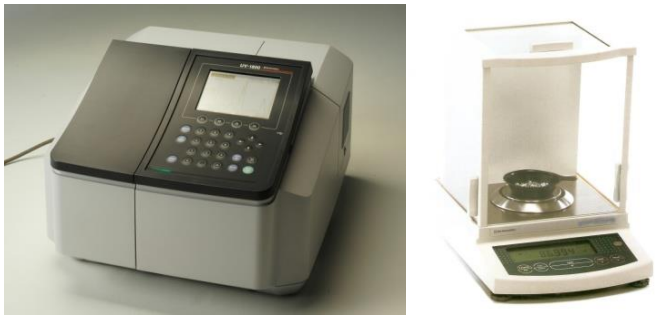

Figure 3.2: Shimadzu UV-1800 spectrophotometer and Electronic balance [Left to right]

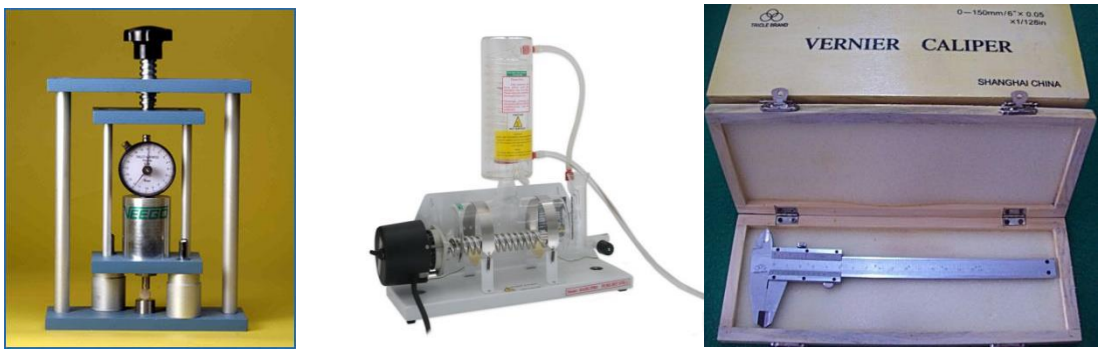

Figure 3.3: Hardness tester, Distilled water plant & Vernier calipers [Left to right]

3.1.6 Apparatus

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

Table 3.4: List of Apparatus Used throughout this Project

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

3.2 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 HCL solvent was calculated as below.

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

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,

$V_1 = (V_2S_2) / S_1$

⇨ $V_1 = (1000ml \times 0.1\ N) / 36.8N$

⇨ 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 λ_{max} & Preparation of the Standard Curve of Atenolol.

1. Standards of Atenolol was collected from the pharmaceutical company. The potency of standard compounds was 99.50%.
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.
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:

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

Thus the concentration was calculated to be:

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

= (50 / 250) mg/ml

= 0.2 mg/ml

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

So the concentration finally turned out to be:

Concentration of 2nd dilution = amount of substance added / volume
= (1 / 50) mg/ml
= 0.02 mg/ml

Preparation of nine serial concentrations of solution for atenolol:

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

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

⇨ The amount of the solution that were required from the stock solution to prepare the above concentrations were calculated using $S_1V_1=S_2V_2$ formula, where S_1= initial strength or concentration, S_2= final strength or concentration, V_1= initial volume and V_2= final volume.

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

Table 3.5: Concentration for Preparation of Standard Curve of Atenolol

Sample Name	Sample no.	Concentration (mg/ml)
Atenolol	1	0.001
	2	0.002
	3	0.003
	4	0.004
	5	0.005
	6	0.006
	7	0.007
	8	0.008
	9	0.009

- $V_1 = S_2V_2 / S_1$ = (0.001 x 10) / 0.02 = 0.5 ml of stock solution required to make 0.001 mg/ml concentration of the final solution of 10 ml (0.5 ml of stock solution + 9.5 ml of 0.1N H_2SO_4) of atenolol.
- $V_1 = S_2V_2 / S_1$ = (0.002 x 10) / 0.02 = 1 ml of stock solution required to make 0.002 mg/ml concentration of the final solution of 10 ml (1 ml of stock solution + 9 ml of 0.1N H_2SO_4) of atenolol.
- $V_1 = S_2V_2 / S_1$ = (0.003 x 10) / 0.02 = 1.5 ml of stock solution required to make 0.003 mg/ml concentration of the final solution of 10 ml (1.5 ml of stock solution + 8.5 ml of 0.1N H_2SO_4) of atenolol.
- $V_1 = S_2V_2 / S_1$ = (0.004 x 10) / 0.02 = 2 ml of stock solution required to make 0.004 mg/ml concentration of the final solution of 10 ml (2 ml of stock solution + 8 ml of 0.1N H_2SO_4) of atenolol.
- $V_1 = S_2V_2 / S_1$ = (0.005 x 10) / 0.02 = 2.5 ml of stock solution required to make 0.005 mg/ml concentration of the final solution of 10 ml (2.5 ml of stock solution + 7.5 ml of 0.1N H_2SO_4) of atenolol.
- $V_1 = S_2V_2 / S_1$ = (0.006 x 10) / 0.02 = 3 ml of stock solution required to make 0.006 mg/ml concentration of the final solution of 10 ml (3 ml of stock solution + 7 ml of 0.1N H_2SO_4) of atenolol.
- $V_1 = S_2V_2 / S_1$ = (0.007 x 10) / 0.02 = 3.5 ml of stock solution required to make 0.007 mg/ml concentration of the final solution of 10 ml (3.5 ml of stock solution + 6.5 ml of 0.1N H_2SO_4) of atenolol.
- $V_1 = S_2V_2 / S_1$ = (0.008 x 10) / 0.02 = 4 ml of stock solution required to make 0.008 mg/ml concentration of the final solution of 10 ml (4 ml of stock solution + 6 ml of 0.1N H_2SO_4) of atenolol.
- $V_1 = S_2V_2 / S_1$ = (0.009 x 10) / 0.02 = 4.5 ml of stock solution required to make 0.009 mg/ml concentration of the final solution of 10 ml (4.5 ml of stock solution + 5.5 ml of 0.1N H_2SO_4) of atenolol.

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:

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

1. Exposure under Normal Lighting Condition

1) The tablets Tenoren® were kept under normal lighting condition in the room for 4 months.
2) They were sampled after specific intervals like periodically after 15 days for determination their physical properties (like thickness, hardness & weight variation) and their potency.
3) On the sampling day, a piece of white paper was taken and all the details (brand name of the tablets, date of the sampling etc.) were written on top of the paper.
4) Now, 10 tablets were taken out and from this 10 tablets, 5 tablets were kept on over that white paper.
5) A photograph was taken of that paper showing the tablets with their appearances and those details.
6) Then from those 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, 5 tablets from those sampled tablets were taken.

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

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

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.

8) Then the absorbance value was plotted into the standard curve to obtain the total amount of the drug that is present in one tablet.
9) Steps 3 to 8 were repeated again on another sampling day.

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

1) 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 :

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

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

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

No. of Samples	Collected Sample	Withdrawal Intervals (Hrs)	Temparature (^{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.

3. 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.

Table 3.7: Sunlight Exposed Sample List

No. of Samples	Collected Sample	Withdrawal Intervals (Hrs)	Temparature (^{0}C)
10 (Control)	10	0	30
30	10	2	30
	10	4	31
	10	6	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.

3.2.4 Determination of Physical parameters:

1. 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.

2. Thickness Test

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

The equation for calculation of thickness of tablet is given below:

Total reading = Main scale reading + (Vernier scale reading × Vernier constant)

3. 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 Tenoren®. Hardness measuring devices apply increasing pressure on the tablet until the tablet breaks (a force of about 4 kilograms is considered to be a minimum for hardness).

4. Weight Variation Test

Procedure

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

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

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

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

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

Calculation

Following equation was used to determine % Weight Variation of tablets

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

Where,

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

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

CHAPTER FOUR

RESULTS

4.1 Standard Curve Preparation

For the preparation of standard curve, nine serially different concentrations were prepared for Atenolol using the standards of Atenolol obtained from SK+F 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 223.5 nm)
0.001	0.031
0.002	0.069
0.003	0.089
0.004	0.123
0.005	0.84
0.006	0.2
0.007	0.23
0.008	0.276
0.009	0.365

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

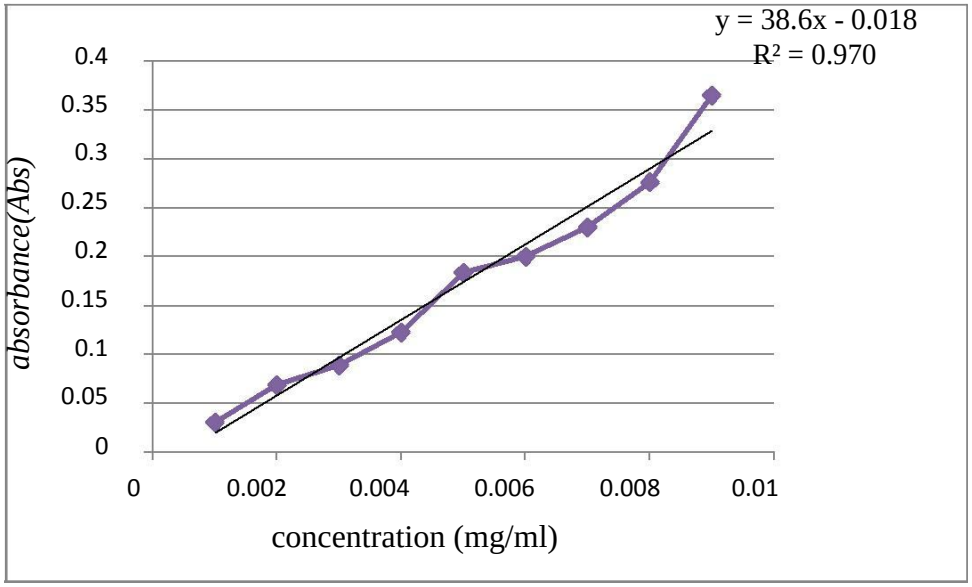

Figure 4.1: Plot showing straight line for Absorbance (Abs) with respect to concentration for Atenolol.

Here, Y = 38.6X - 0.018 & R2=0.970 Where,

Y = Absorbance (Abs)

X = Concentration of the drug (mg/ml)

The R2 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. Some of the pictures showing the color change are given below:

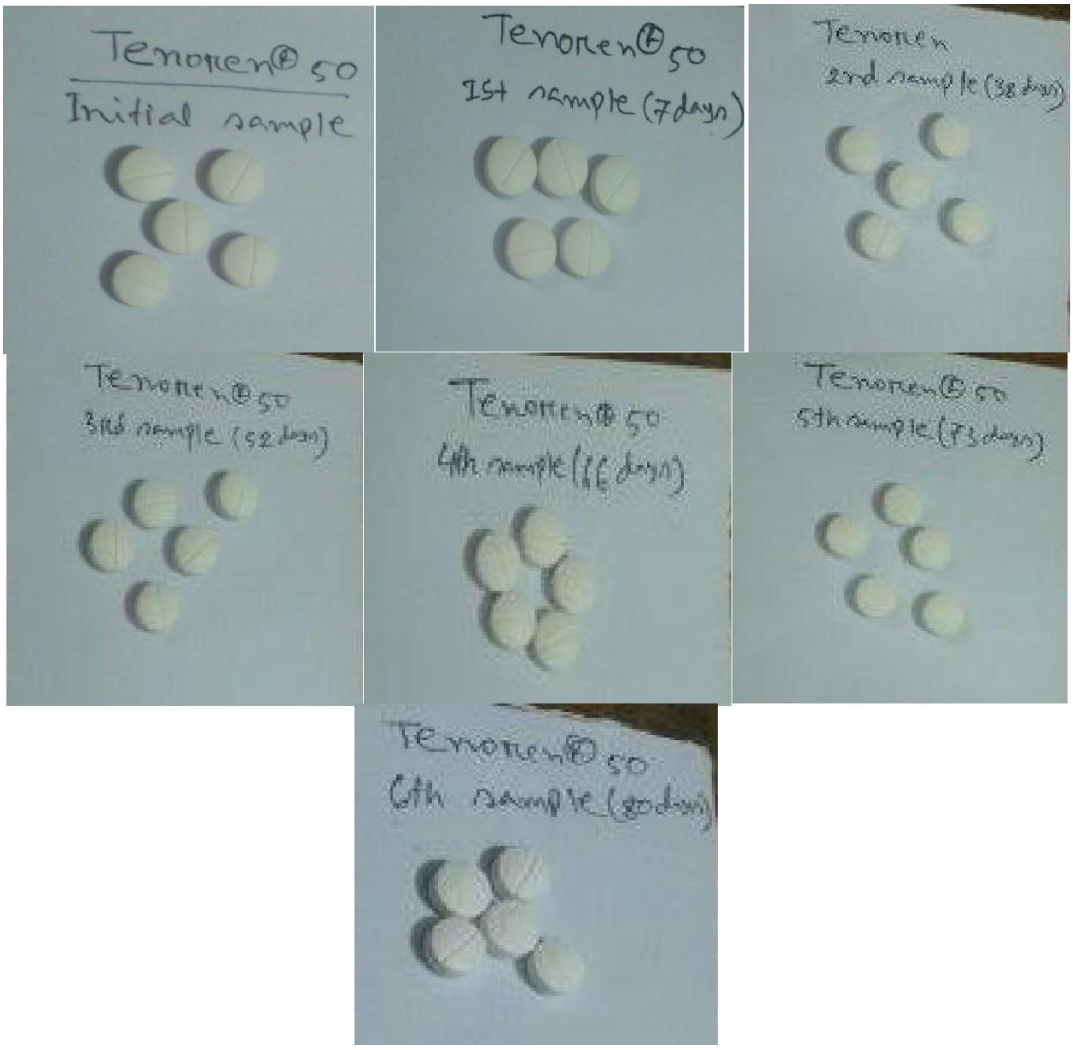

Figure 4.2: Pictures of tablets after exposure to normal light with 60 days interval

4.2.2 Weight Variation Test

4.2.2.1 Samples Exposed to Normal Light

100 sample tablets were exposed to normal light condition for 60 days. Weight variation test was conducted of 5 tablets of each day interval (0, 15, 30, 45, 60 days).Weight variation test was conducted. Data of these tests are given below:

Table 4.2: Weight Variation Test of the Sample Exposed to Normal Light

Time interval	Individual Weight, I(g)	Average weight A(g)	% Weight Variation; (A-I/A)×100 %
0	0.2136	0.2133	-0.14
15	0.2130		0.14
30	0.2132		0.04
45	0.2135		-0.09
60	0.2133		0.00

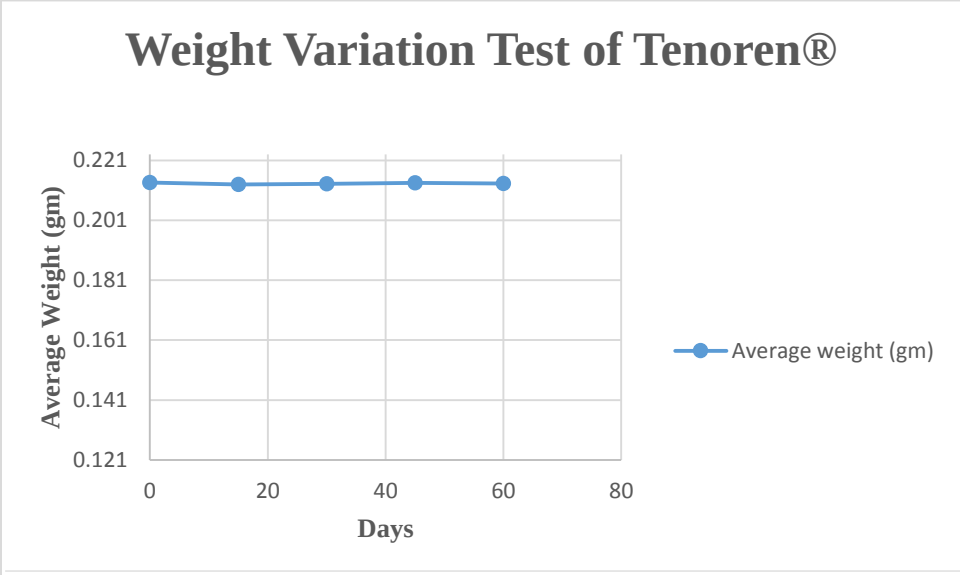

Figure 4.3: Plot showing weight variation test of samples.

4.2.3 Hardness Test

4.2.3.1 Samples Exposed to Normal Light

100 sample tablets were exposed to normal light condition for 60 days. Then 5 samples were collected after 0, 15, 30, 45, 60 days period and test was conducted. Data are given below:

Table 4.3: Hardness Test of sample exposed to normal light

Time interval	Average Hardness (kg) of particular day
0	5.8
15	5.0
30	5.8
45	6.0
60	5.6

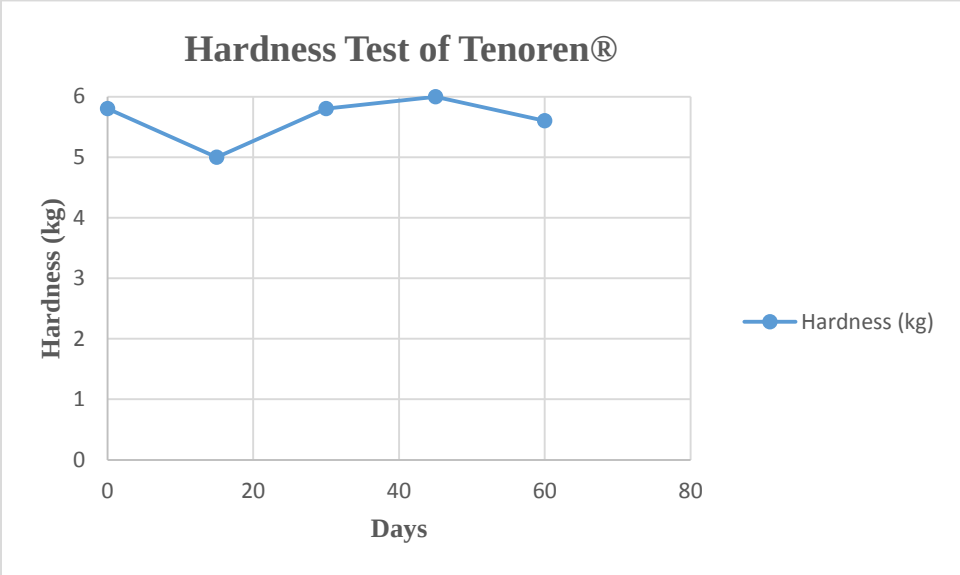

Figure 4.4: Plot showing hardness of the sample tablets.

4.2.4 Thickness test

4.2.4.1 Samples Exposed to Normal Light

100 sample tablets were exposed to normal light condition for 60 days. The 5 samples were collected after 0, 15, 30, 45, 60 days and thickness test was conducted. Data are given below:

Table 4.4: Thickness Test of sample exposed to normal light

Time interval	**Average Thickness (mm) of particular days**
0	4.410
15	4.425
30	4.450
45	4.425
60	4.450

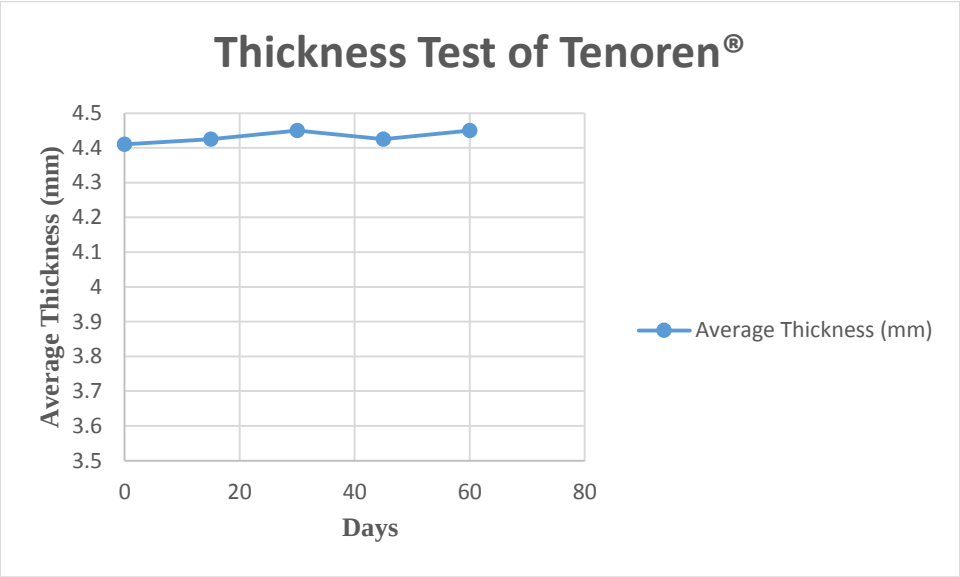

Figure 4.5: Plot showing thickness of the sample tablets.

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	0.698	0.711	0.697	47.21	46.30	94.43	92.60
	0.698	0.699						
	0.721	0.694						
	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	0.566	0.711	0.565	47.21	37.75	94.43	75.5
	0.698	0.563						
	0.721	0.567						
	0.714	0.570	0.711	0.565	47.21	37.75	94.43	75.5
	0.700	0.558						
	0.719	0.567						
	0.716	0.571	0.711	0.575	47.21	38.40	94.43	76.8
	0.700	0.576						
	0.717	0.580						

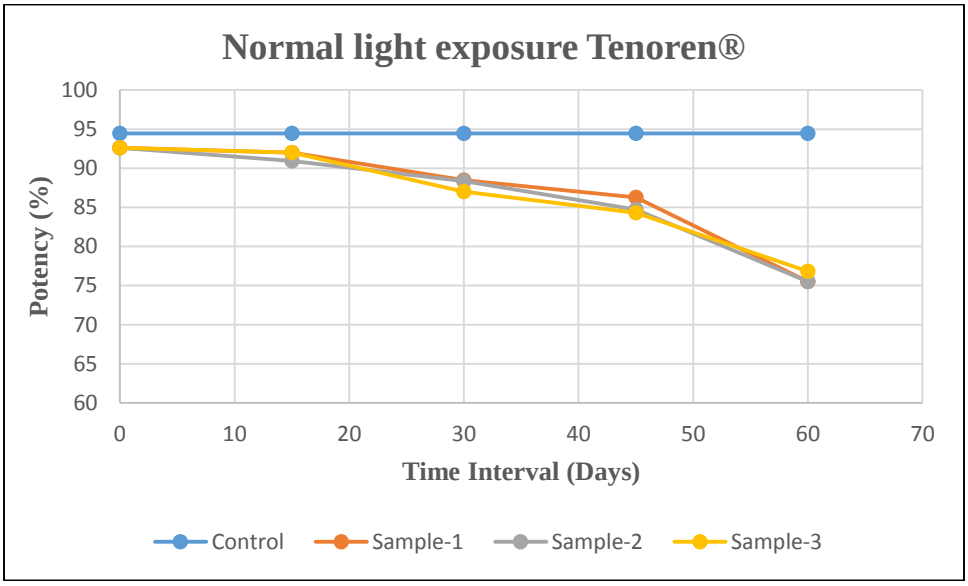

Figure 4.6: Graph showing the difference in Concentration after fixed day interval for Atenolol (Tenoren®).

4.3.2 Results 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				

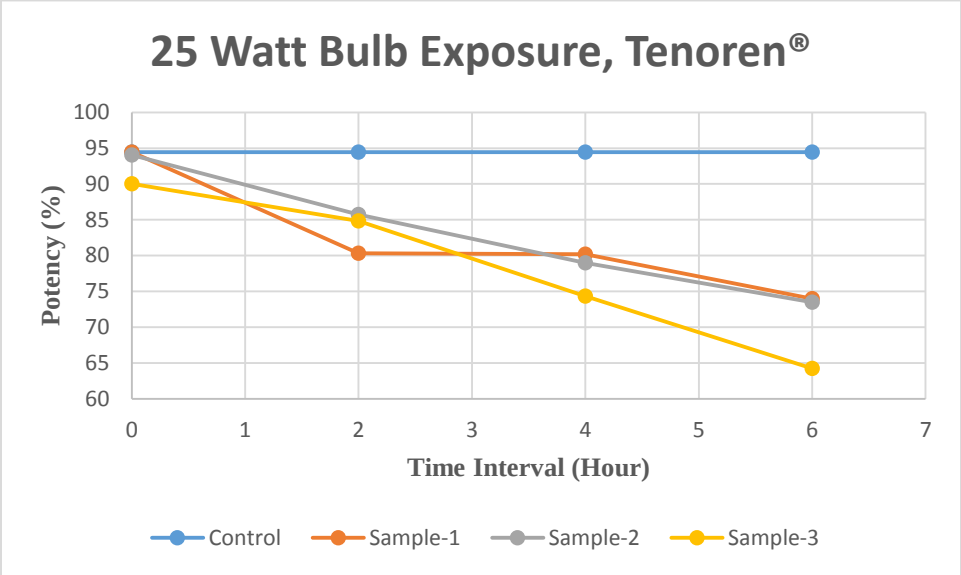

Figure 4.7: Graph showing the difference in Concentration after each 2 hour time interval for Atenolol (Tenoren®) for 1st time.

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				

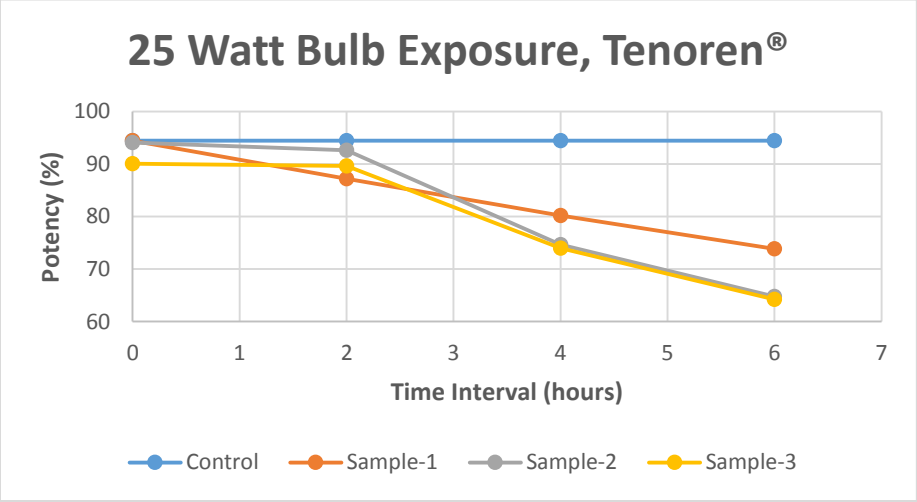

Figure 4.8: Graph showing the difference in Concentration after each 2 hour time interval for Atenolol (Tenoren®) for 2nd time.

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

Table 4.18: Concentration & absorbance of Atenolol (Tenoren®) 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				

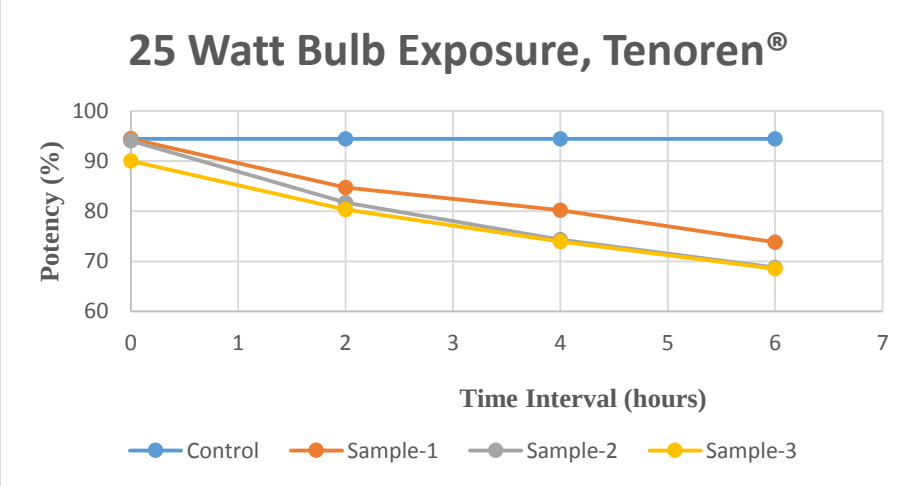

Figure 4.9: Graph showing the difference in Concentration after each 2 hour time interval for Atenolol (Tenoren®) for 3rd time.

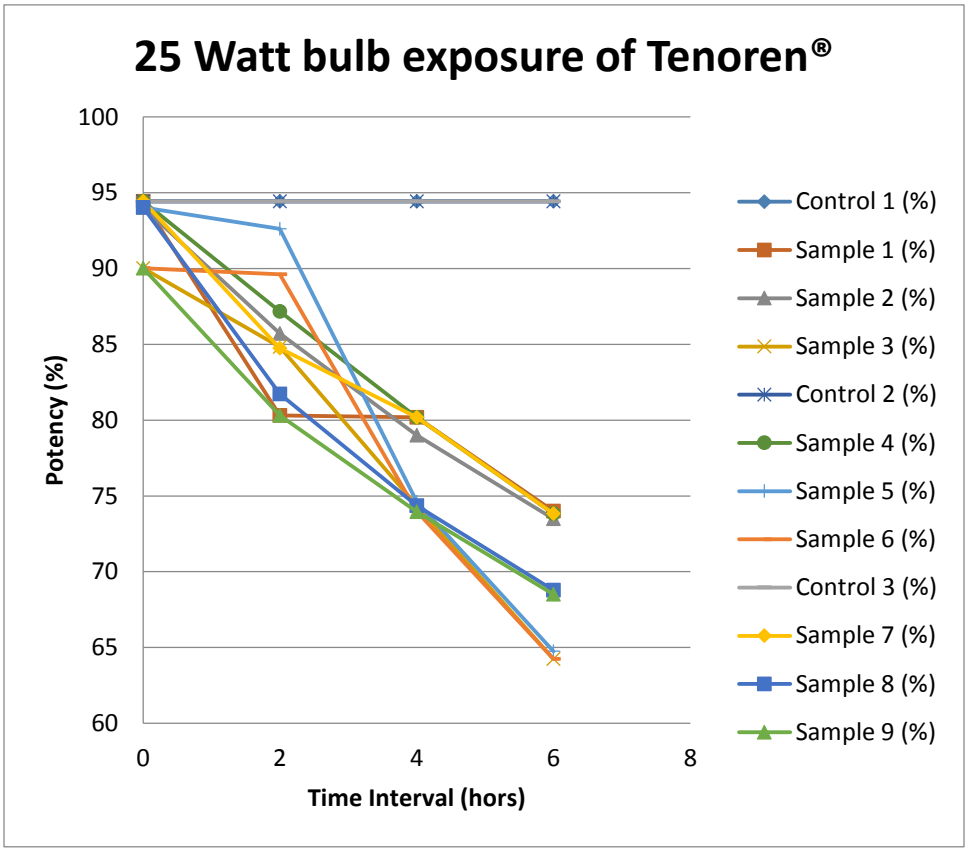

Figure 4.10: Graph showing combination in Concentration after each 2 hour time interval for Atenolol (Tenoren®) for 25W bulb

4.3.3 Results 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				

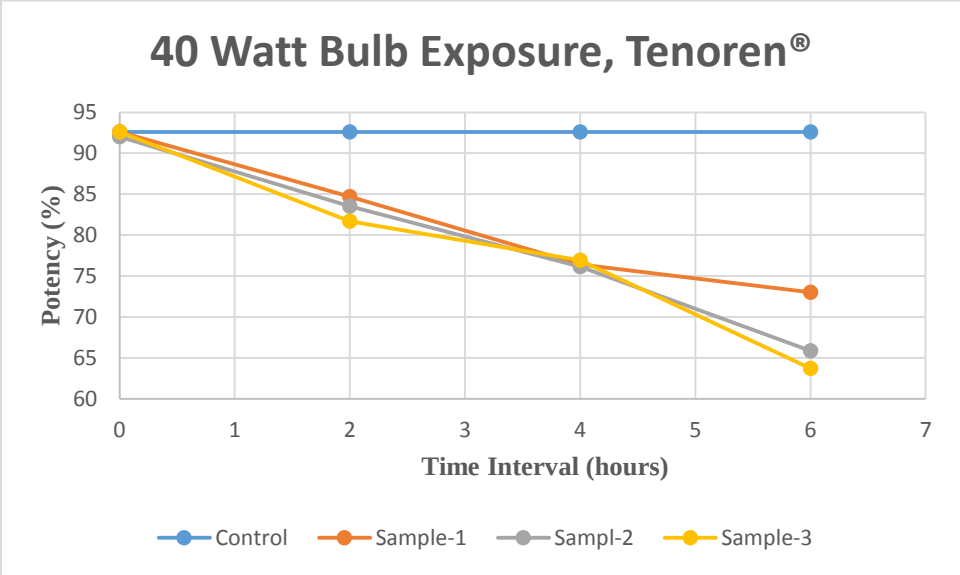

Figure 4.11: Graph showing the difference in Concentration after each 2 hour time interval for Atenolol (Tenoren®) for 1st time.

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				

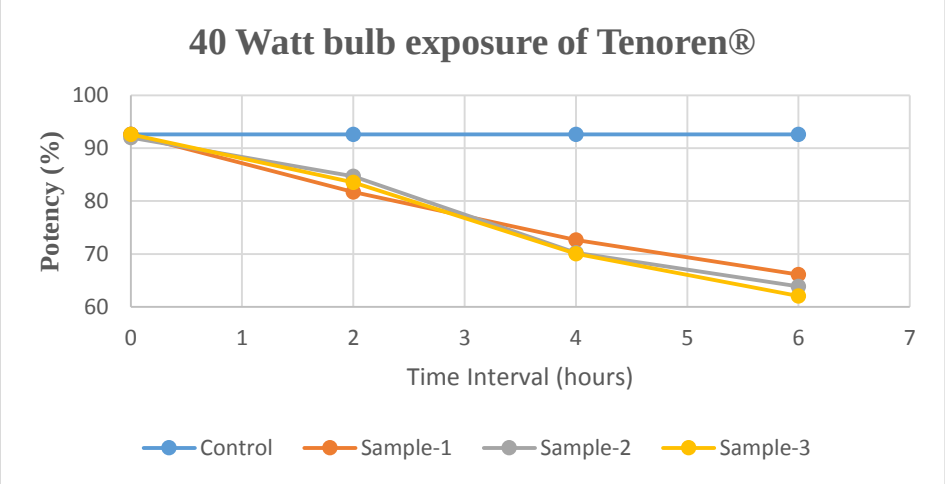

Figure 4.13: Graph showing the difference in Concentration after each 2 hour time interval for Atenolol (Tenoren®) for 2nd time.

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

Table 4.30: Concentration & absorbance of Atenolol (Tenoren®) 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				

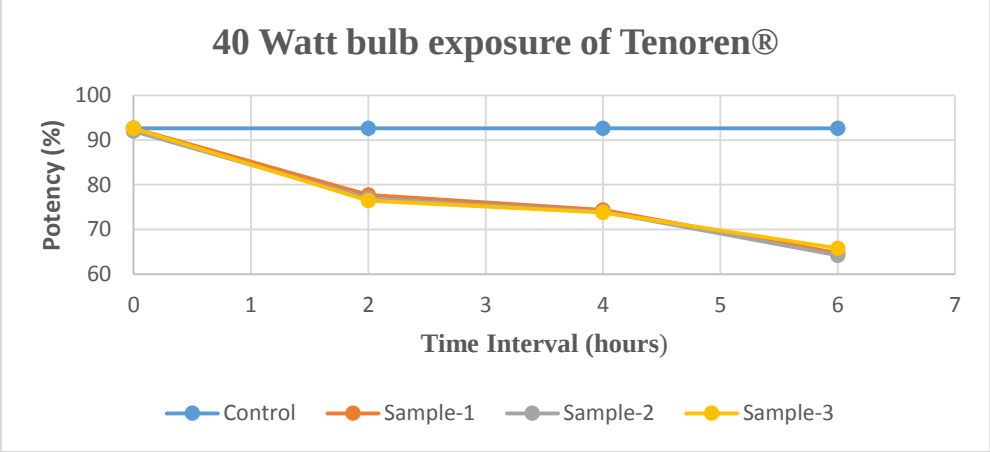

Figure 4.12: Graph showing the difference in Concentration after each 2 hour time interval for Atenolol (Tenoren®) for 3rd time.

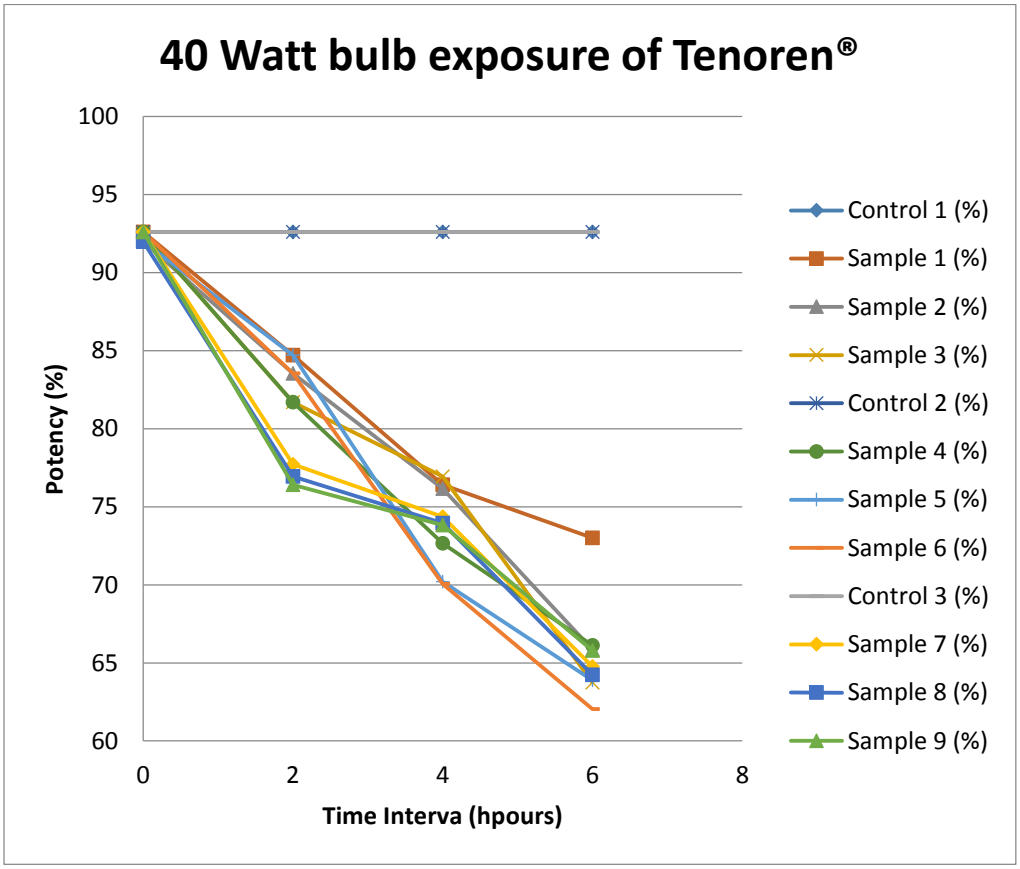

Figure 4.14: Graph showing combination in Concentration after each 2 hour time interval for Atenolol (Tenoren®) for 40W bulb.

4.3.4 Result of samples that were exposed under Direcr 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				

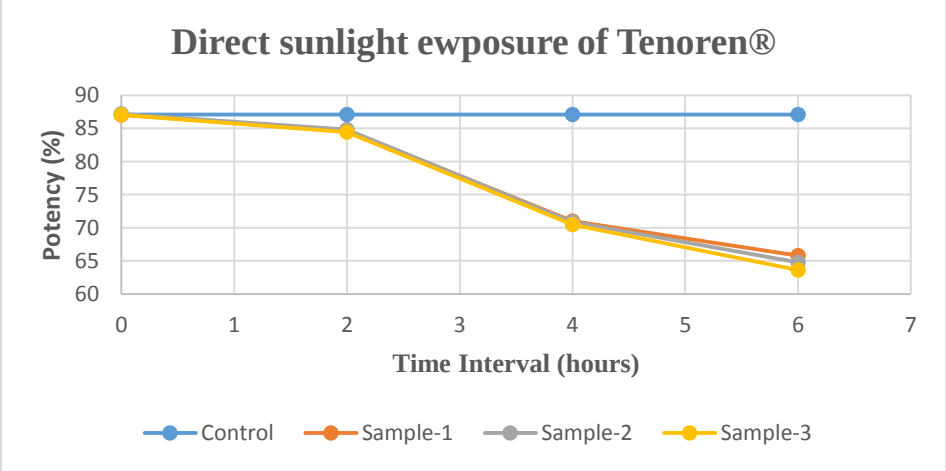

Figure 4.15: Graph showing the difference in Concentration after each 2 hour time interval for Atenolol (Tenoren®) for 1st time.

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

Table 4.38: Concentration & absorbance of Atenololat 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				

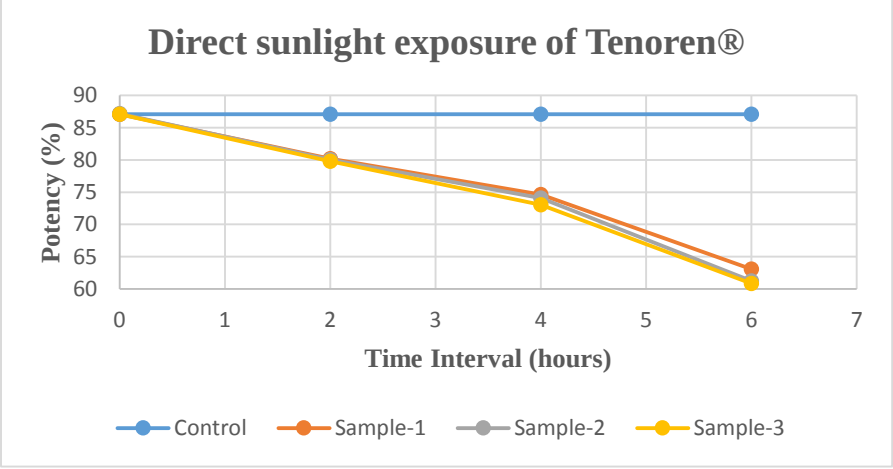

Figure 4.16: Graph showing the difference in Concentration after each 2 hour time interval for Atenolol (Tenoren®) for 2nd time.

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

Table 4.42: Concentration & absorbance of Atenolol (Tenoren®) 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				

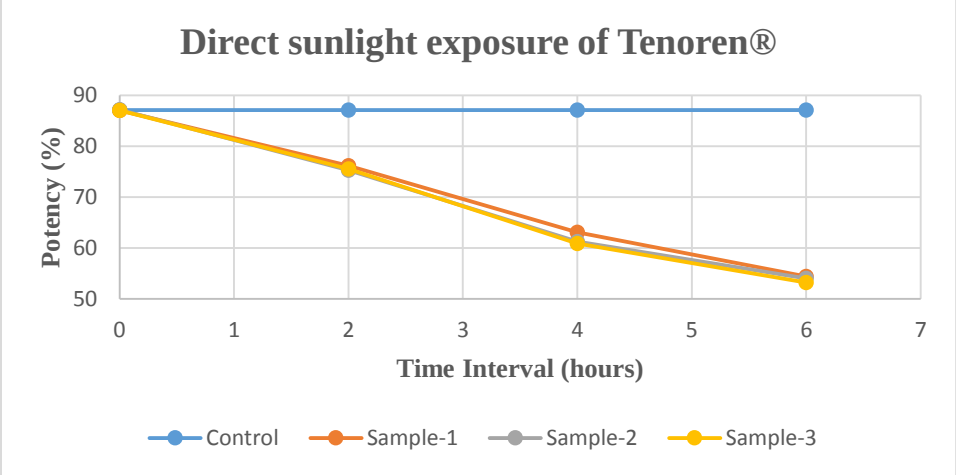

Figure 4.17: Graph showing the difference in Concentration after each 2 hour time interval for Atenolol (Tenoren®) for 3rd time.

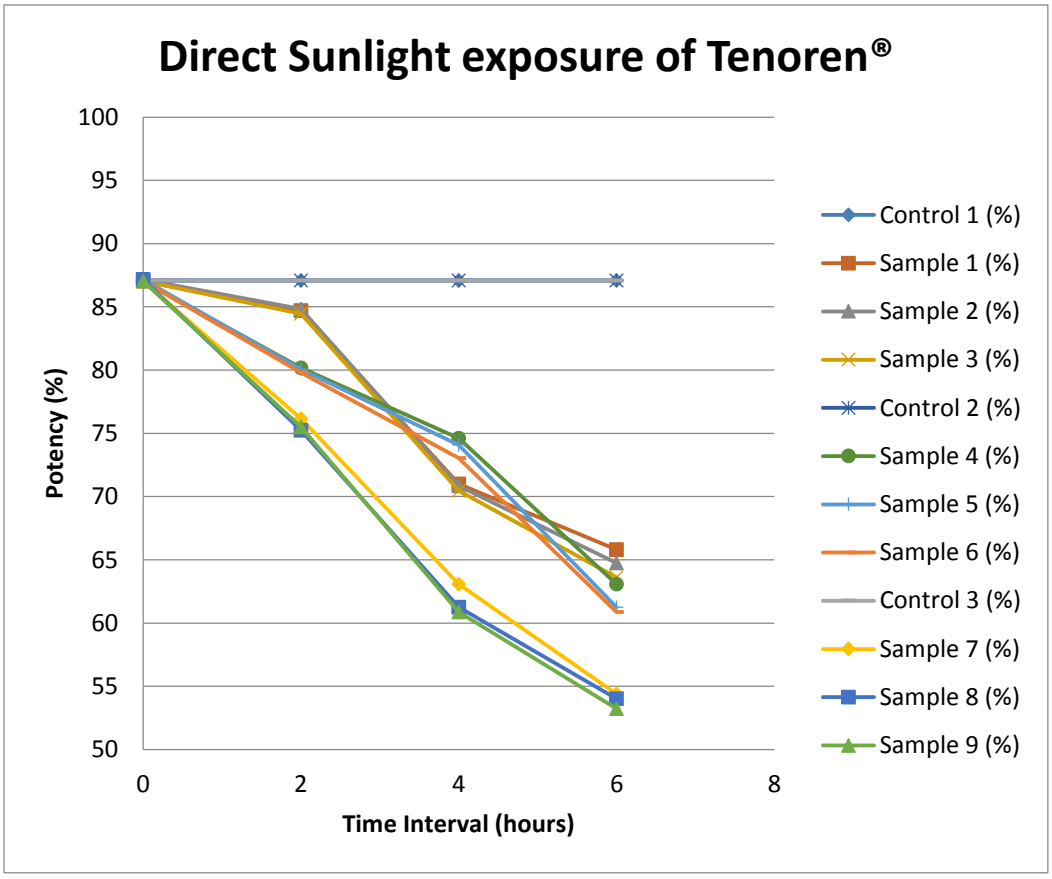

Figure 4.18: Graph showing combination in Concentration after each 2 hour time interval for Atenolol (Tenoren®) for Direct sunlight.

CHAPTER FIVE

DISCUSSION

5 DISCUSSION

The research was conducted to determine the reproducibility study on photolytic degradation of Tenoren® (Atenolol) in various lightening condition. It was found that the concentration of Atenolol was decreased gradually in every lightening condition (normal light, direct sun light, 40w and 25w bulb),when sample tablets (Tenoren®) 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 experiments were 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 12.55%, 15.30%, 25.96% & 9.74% respectively. It was established from the research that coating alone was not sufficient to protect the drug from light as samples degraded precisely every time.

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

In the study it was found that the weights of the tablet fluctuate with a very short range but results were very close within the total 60 days period. The standard deviation was observed only ±0.00020 g. The percentage of Weight Variation of the Tenoren® was within the accepted range (Weight of tablet 130 mg or less then = ±10%). According to U.S.P. if no more than 2 tablets are outside the percentage limit and if no tablet 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 60 days interval works. The standard deviation was observed only ±0.36250 kg. Even the average hardness value was also very close to each other. So the hardness of Atenolol was not affected by different lighting conditions.

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 ±0.01725 mm .So the effects of light dose not influence the thickness of Atenolol.

CHAPTER SIX

CONCLUSION

6 CONCLUSION

Atenolol is a drug developed in the 1976s. But it is still widely in use. It is developed as a replacement for propranolol in the treatment of hypertension. It is also used for angina pectoris, cardiac dysrhythmias & myocardial infarction. In this experiment it was seen that the samples meet most of the official specifications but large variation of potency observed under different lightening condition every time. From the research project we can conclude with a decision that photosensitive product must be packed in an opaque container or blister where light can't pass.

CHAPTER SEVEN

REFERENCES

7. References

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