Reproducibility study on the Efficiency of Packaging in preventing Photolytic Degradation Of Easium® (Diazepam)Tablets

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

IN THE NAME OF ALLAH

THE MOST GRACIOUS AND THE MOST MERCIFUL

DEDICATION

This Research Project Is Dedicated to My Beloved Parents

DECLARATION BY THE CANDIDATE

I, NuzhatAhsan (ID#2011-1-70-033), declare that the dissertation entitled "Reproducibility study on efficiency of packaging on preventing photolytic degradation of Easium® (Diazepam)" submitted to the Department of Pharmacy, East West University, Aftabnagar, in partial fulfillment of the requirement for the Degree of Bachelor of Pharmacy, was carried out by me under the supervision and guidance of Md. Anisur Rahman, Senior Lecturer, and the co-advisor Faisal Bin Karim, Lecturer Dept. of Pharmacy, East West University, Dhaka.

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CERTIFICATE BY THE SUPERVISOR

This is to certify that the dissertation entitled "Evaluating Coating Efficiency on Photolytic Degradation Sedil® (Diazepam)" submitted to the department of pharmacy, East West University in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy was carried out by Nuzhat Ahsan (ID: 2011-1-70-033) under our guidance and supervision and that no part of the research has been submitted for any other degree. We further certify that all the sources of information and laboratory facilities availed of in this connection is duly acknowledged.

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ENDORSMENT BY THE CHAIRPERSON

This is to certified that the dissertation entitle "Evaluating Coating Efficiency on Photolytic Degradation Easium® (Diazepam)" is a bonafide research work done by Nuzhat Ahsan (ID: 2011-1-70-033) under the guidance and supervision of Md. Anisur Rahman, Senior Lecturer, and the co-advisor Faisal Bin Karim, Lecturer, Department of Pharmacy, East West University, Dhaka.

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ABSTRACT

This research work was aimed to evaluate the reproducibility of the data that was previously done in order to determine whether the packaging is effective to prevent the photolytic degradation of diazepam which is a photosensitive drug.To conduct the study 800 tablets Easium® from Opsonin Pharma were taken as a sample from the same batch. To determine the photolytic effect, all tablets were exposed in various lighting conditions (control, sunlight, normal room light, 25watt & 40watt bulb). Besides this physical parameters were tested for evaluation of color change, weight variation, thickness and hardness of the Easium® tablets. Physical tests were performed according to the specification of United States Pharmacopeia (USP) and British Pharmacopoeia (BP). The standard deviations for the weight variation, thickness and hardness are respectively ±0.001g, ±0.0009cm, ±0.2kg and the percent variation of the decreased concentration of the samples for normal lightening condition, 25 watt & 40 watt light exposure and sunlight exposure were found respectively 34.53%, 7.15%, 27.87%, 31.66%.

After the observation of 60 days, it was clearly visible that Easium® (diazepam) is a photosensitive drug.So it can be concluded that the Packaging system of Easium® (diazepam) is not efficient to protect the drug from light. Thus, Opaque packaging should be used for such drugs.

Keywords: Easium®, Diazepam, Weight variation, Hardness, Thickness, Potency, USP, BP.

Chapter One

INTRODUCTION

1.Introduction

The objective of the research project was to evaluate the reproducibility of the data in a study that was previously done in order to determine whether thepackaging is effective to prevent the photolytic degradation of Easium® (diazepam) which is a photosensitive drug. In this study, photosensitivity of Easium® (Diazepam) in various lightening conditions (normal light, 25watt bulb, 40watt bulb, sunlight) was determined. For this purpose, the available brand was chosen i.e. Easium® of OpsoninPharmaLimited. In most cases these products are available in transparent blister packaging system in the market. Only few brands use the opaque blister packaging system due to the photosensitive report. Since there is no published data about photolytic degradation of Diazepam, a research program was operated to find whether this drug degrades in presence of light or not.

All the tests were performed 3 times to check the reproducibility of the results that were obtained earlier.

1.1 Stability

Stability of a drug means the capacity of a drug substance or product to remain within established specifications of identity, strength quality, and purity in a specified period of time.

There are some factors that affect drug stability include temperature condition, moisture, light, microbes, packaging materials, transportation, components of drug composition and the nature of the active ingredient. (Answers.com, 2014)

1.2Photosensitivity:

During production, storage, administration and use a drug substance or drug product can be exposed to natural light or artificial light. Most of the drug substances or pharmaceutical excipients absorb possibly visible radiation and UV radiation. After absorbing radiation the compound may be participate in photochemical process and showing photoreactivity. That can result in its own decomposition or decomposition of other components of the formulation in vitro. The photoreactivity may also give rise to photosensitivity in patient like phototoxic and photoallergic reaction (kristensenet. Al 1994).

1.3Photolytic degradation

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

Two ways in which photolysis can occur.

- One is, the light energy must be sufficient to activate the energy or
- Another is, light energy which is absorbed by molecules is passed on to other which allows degradation to take place.

After initiating the energy, several reactions can take place like oxidation, polymerization, or ring rearrangement. Followed by the reaction light energy may be converted to thermal energy. The photolytic reaction may produce catalyst for the thermal reaction. (Slideshare.net, 2014)

For this experiment one the photosensitive drug diazepam which is a class of Benzodiazepine is taken as samples.

1.4 Diazepam

Overview

1.4.1 Generic Name: diazepam (dye AZ e pam)

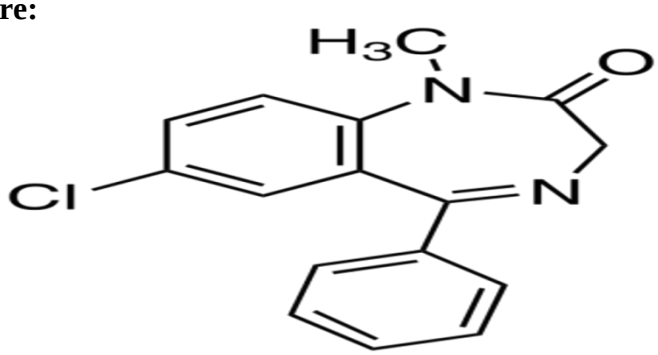
1.4.2 Brand name: Easium ®

Diazepam is an oral medication that is used to treat anxiety. It belongs to Benzodiazepine family of drugs. Diazepam and other benzodiazepines act by enhancing the effects of gamma-amino butyric acid (GABA) in the brain. GABA is a neurotransmitter (a chemical that nerve cells use to communicate with each other) that inhibits activity in the brain.

Diazepam is used for the treatment of disorders with anxiety. Diazepam also is used for the treatment of agitation, tremors, delirium, seizures, and hallucinations resulting from alcohol

withdrawal. It is used for the treatment of seizures, relief of muscle spasms in some neurological diseases, and for sedation during surgery (omudhome,ogbru, 2015)

1.4.3 Structure:



Diazepam

Figure: 1.1: Chemical structure of Diazepam

1.4.4 Physico-chemical properties

1.4.4.1 Chemical properties:

- Chemical name: 7-Chloro-1, 3-dihydro-1-methyl-5-phenyl-2H-1, 4-benzodiazepin-2-one
- Molecular formula: $C_{16}H_{13}ClN_2O$
- Molecular weight : 284.7 g/mol

1.4.4.2 Physical properties:

- Color: white or yellow
- State : solid-crystals
- Description

✓ Melting point : 131.5 to 134.6

✓ Odorless and slightly bitter taste

✓ Slightly soluble in water , soluble in alcohol and freely soluble in chloroform ✓pH is neutral (Drug.com, 2014)

1.4.5Availability:

Above 500 brands of diazepam are available throughout the world. It is found in

oral, injectable, inhalation, and rectal forms. (Drugs.com, 2014)

1.4.6 Mechanism of action:

Gamma amino butyric acid (GABA) is an inhibiting neurotransmitter that is present on human brains. As shown in the animation, gamma amino butyric acid promotes opening of a postsynaptic receptor, the GABA-A receptor. This opening leads to a increased conductance to chloride ions, which produces membrane hyperpolarization, this induces a neuronal inhibition. The binding of benzodiazepines to the GABA-A receptor increases the affinity of gamma amino butyric acid (GABA) and its receptor, thereby increasing the opening frequency of GABA-A receptor. As a consequence of this, benzodiazepines potentiate GABAergic neurotransmission.

Diazepam also acts on areas of the limbic system, thalamus, and hypothalamus, inducing anxiolytic effects. It also increases the inhibitory processes in the cerebral cortex. The anticonvulsant properties of diazepam may be in part or entirely due to binding to voltage-dependent sodium channels (Pharmacologycorner.com, 2015).

1.4.7 Pharmacokinetics:

1.4.7.1 Absorption:

1.4.7.1.1 Oral

After oral administration >90% of diazepam is absorbed and the average time to achieve peak plasma concentrations is 1 – 1.5 hours with a range of 0.25 to 2.5 hours. Absorption is delayed and decreased when administered with a moderate fat meal. In the presence of food mean lag times are approximately 45 minutes as compared with 15 minutes when fasting. There is also an increase in the average time to achieve peak concentrations to about 2.5 hours in the presence of food as compared with 1.25 hours when fasting. This results in an average decrease in Cmax of 20% in addition to a 27% decrease in AUC.

1.4.7.1.2 Intravenous:

For IV administration the onset of action is one to five minutes and the duration of peak pharmacological effects is 15 min to 1 hr.

1.4.7.1.3 Intramuscular:

The IM absorption of diazepam is slow, erratic, and incomplete. The onset of action is 15-30 min and the duration of peak pharmacological effects is same as IV.

1.4.7.1.4Distribution:

Diazepam and its metabolites are highly bound to plasma proteins (diazepam 98%). Diazepam and its metabolites cross the blood-brain and placental barriers and are also found in breast milk in concentrations approximately one tenth of those in maternal plasma (days 3 to 9 post-partum). In young healthy males, the volume of distribution at steady-state is 0.8 to 1.0 L/kg. The decline in the plasma concentration-time profile after oral administration is biphasic. The initial distribution phase has a half-life of approximately 1 hour, although it may range up to >3 hours.

1.4.7.1.5Metabolism

Diazepam is N-demethylated by CYP3A4 and 2C19 to the active metabolite N-desmethyldiazepam, and is hydroxylated by CYP3A4 to the active metabolite temazepam. N-desmethyldiazepam and temazepam are both further metabolized to oxazepam. Temazepam and oxazepam are largely eliminated by glucuronidation.

1.4.7.1.6Elimination

The initial distribution phase is followed by a prolonged terminal elimination phase (half-life up to 48 hours). The terminal elimination half-life of the active metabolite N-desmethyldiazepam is up to 100 hours. Diazepam and its metabolites are excreted mainly in the urine, predominantly as their glucuronide

conjugates. The clearance of diazepam is 20 to 30 mL/min in young adults. Diazepam accumulates upon multiple dosing and there is some evidence that the terminal elimination half-life is slightly prolonged.Range 15% to 50%) when administered with food (Drugs.com, 2015).

1.4.8 Clinical Uses:

- Anxiety disorders include generalized anxiety disorder (GAD), panic disorder, and obsessive-compulsive disorder (OCD).
- Symptoms of alcohol withdrawal known as "delirium tremens."
- Additionally, the drug can treat muscle spasms from injury, inflammation, or nerve disorders.
- Doctors sometimes prescribe Valium along with other medications to treat convulsions or seizures.
- Use before surgery or other medical procedure to reduce anxiety.
- Premedication for inducing sedation
- Insomnia
- Epileptics
- Neonatal Opiate Withdrawal.
- Labor and Delivery
- Myocardial Infarction (Everydayhealth.com, 2015).

1.4.9 Dosing Information

1.4.9.1 Adults;

Anxiety; Oral: 2 to 10 mg 2 to 4 times a day. IM or IV: 2 to 5 mg (moderate anxiety) or 5 to 10 mg (severe anxiety) for one dose. May repeat in 3 to 4 hours, if necessary.

Alcohol Withdrawal; Oral: 10 mg 3 to 4 times during the first 24 hours, then 5 mg 3 to 4 times a day as needed.

ICU Agitation; Initial dose: 0.02 to 0.08 mg/kg IV over 2 to 5 minutes every 0.5 to 2 hours to control acute agitation. Maintenance dose: 0.4 to 0.2 mg/kg/hr by continuous IV infusion.

Muscle Spasm; Oral: 2 to 10 mg 3 to 4 times a day. IM or IV: 5 to 10 mg initially, then 5 to 10 mg in 3 to 4 hours, if necessary.

Seizures; Oral: 2 to 10 mg 2 to 4 times a day. Rectal gel: 0.2 mg/kg, rounded up to the nearest available unit dose.

Status Epilepticus; IV or IM: 5 to 10 mg initially (IV preferred).

Light Anesthesia; Premedication for Anesthesia;10 mg, IM (preferred route), 1 to 2 hours before surgery (Drugs.com, 2015).

1.4.9.2 Children

For tension and irritability in cerebral spasticity: 5mg-40mg each day, in divided doses. Before an operation, the usual dose of diazepam tablet for children is 2mg-10mg.For anxiety; 1 to 12 years Oral: 0.12 to 0.8 mg/kg/day in divided doses every 6 to 8 hours as needed.
IM: 0.04 to 0.3 mg/kg every 2 to 4 hours as needed, up to a maximum of 0.6 mg/kg in 8 hours.

1.4.9.3 Elderly or Frail

In elderly patients, it is recommended that the dosage be limited to the smallest effective amount to preclude the development of ataxia or oversedation (2 mg to 2.5 mg once or twice daily, initially to be increased gradually as needed and

tolerated). The dose should not be more than half of the adult dose. If they have liver or kidney problems the dose should also be lower.

1.4.10 Side effects:

- No fear or danger
- Taking unusual risk
- Hallucination
- Confusion
- Memory loss
- Dizziness
- Muscle weakness
- Nausea
- Mild skin rash
- Double vision
- incontinence, changes in libido
- Changes in salivation, including dry mouth, hyper salivation (Drugs.com, 2015).

1.4.11 Pregnancy and Breastfeeding Warnings

Diazepam has been assigned to pregnancy category D by the FDA which has an increased risk of congenital malformations and other developmental abnormalities. There may be no teratogenic effect with the use of diazepam or benzodiazepines during pregnancy but mothers who take diazepam late in pregnancy may cause neonatal flaccidity, respiratory and feeding difficulties and hypothermia in new born babies. Besides this if mothers taking diazepam regularly then it will be difficult in case of withdrawing drugs and also seem some symptoms during post natal period. For this reason potential hazard of the drugs should be informed to the patient if diazepam is prescribed during pregnancy.

The drug should be taken carefully during labor and delivery, poor sucking, hypothermia, and moderate respiratory depression in the neonate. Diazepam is excreted into human milk. Sedation, lethargy and weight loss have been seen in nursing infants. The American Academy of Pediatrics describes diazepam as a

drug whose effect on nursing infants is unknown but may be of concern. The manufacturer states that breast-feeding is not recommended in patients receiving diazepam. (Drugs.com, 2014)

1.4.12 Contraindications:

Use of Diazepam should be avoided in these conditions;

- Alcohol Intoxication,
- Misuse or Excessive Use of Drugs,
- Depression
- Hypersensitivity to diazepam
- Myasthenia Gravis,
- Wide-Angle Glaucoma, Closed Angle Glaucoma,
- Poor Metabolizer due to Cytochrome p450 CYP2C19 Variant,
- Severe Chronic Obstructed Lung Disease
- Significant Decrease in Lung Function
- Lung Disease
- Liver Problems,
- Severe Liver Disease
- Serious Kidney Problems
- Pregnancy
- A Mother who is Producing Milk and Breastfeeding
- Low Amount of Albumin Proteins in the Blood (Webmed.com, 2015)

1.4.13 Interactions:

- Rifampin, phenytoin, carbamazepine, and phenobarbital increase the metabolism of diazepam, thus decreasing drug levels and effects. Dexamethasone and St John's wort also increase the metabolism of diazepam.

- Diazepam increases the central depressive effects of alcohol, other hypnotics/sedatives (e.g., barbiturates), narcotics, other muscle relaxants. Also interact with certain antidepressants, sedative antihistamines,opiates, and antipsychotics, as well as anticonvulsants such as phenobarbital, phenytoin, and carbamazepine.

- Oral contraceptives significantly decrease the elimination of desmethyldiazepam,, it is a major metabolite of diazepam.

- Diazepam increases the serum levels of phenobarbital.

- Cisapride may enhance the absorption, and therefore the sedative activity, of diazepam.

- Small doses of theophylline may inhibit the action of diazepam.

- Diazepam peak concentrations are 30% lower when antacids are administered concurrently.

- Diazepam may alter digoxin serum concentrations.

- Other drugs that may have interactions with diazepam include antipsychotics

(Drugs.com, 2015).

1.4.14 Overdose

Overdose of diazepam is usually manifested by central nervous system depression ranging from drowsiness to coma. In mild cases, symptoms include;

- drowsiness,

- confusion, and

- Lethargy.

In more serious cases, symptoms may include

- ataxia,
- diminished reflexes,
- hypotonia,
- hypotension,
- respiratory depression,
- Coma (rarely) and death (very rarely).

Overdose of benzodiazepines in combination with other CNS depressants (including alcohol) may be fatal and should be closely monitored. (RxList, 2015)

1.4.15 Storage:

1.4.15.1Tablets

Kept in Tight, light-resistant containers at 15–30°C.

1.4.15.2 Solution and Solution Concentrate

Storage at 15–30°C and Protect from moisture.

1.4.15.3 Injection:

Storage at 15–30°C and Protect from light, avoid freezing.

1.4.15.4Rectal Gel:

Storage at 25°C and may be exposed to 15–30°C. (Drugs.com, 2015)

Chapter Two

LITERATURE REVIEW

2. Literature review

In 9 march 1964 Beerman, H. published a journal was published on Contolled study of Diazepam in psychiatric outpatients. The study was conducted with series of 103 patients with psychoneurotic or psychotic disorders, most of whom had been under treatment for a long period, were given diazepam (Valium) for periods ranging up to a year. The usual dosage was 5 mg. t.i.d. but the daily intake was adapted to each patient in a range of 6 to 50 mg./day, taken in 3 to 8 doses. In a few cases of severe depression isocarboxazid was used adjunctively.Results of diazepam therapy were judged good or excellent in 98 patients (95%). The agent was especially potent in relieving anxiety-tension, panic and depression, and raised the emotional tone of paients, enabling them to think more clearly and to relate better to other people and to their work (Beerman, H. 1964),

The compatibility and stability of diazepam injection were studied by Morris in 1978. In his experiment, diazepam was diluted to 10 different concentrations in dextrose 5% in water, normal saline, Ringer's injection and lactated Ringer's injection. After experiments he found that diazepam was stable for 6-8 hours at 1:40 dilution (5 mg in 40 ml). So if in some circumstances it is required to administer diazepam as an infusion, it is recommended that it be diluted in dextrose 5% in water, normal saline, Ringer's injection or lactated Ringer's injection to a dilution of at least 1:40 and used within 6 hours or to a dilution of 1:50 and used within 24 hours. (Morris, 1978)

In 1984 March, a journal was published about diazepam in panic disorder. The response to diazepam and propranolol hydrochloride was compared in 21 patients who (with one exception) met DSM-Ill criteria for panic disorder and agoraphobia. Each drug was administered for two weeks in double-blind fashion according to a crossover design. The response to diazepam was significantly superior on all measures. By observer rating, 18 patients showed at least moderate improvement with diazepam compared with seven receiving propranolol. Panic attacks and phobic symptoms responded to diazepam, but not to propranolol. The results suggest that benzodiazepines constitute effective short-term treatment for these newly defined disorders (Noyes, 1984).

In the same year another journal was published about dose response analysis of the behavioral effects of diazepam.The effects of psychomotor, cognitive and mood effects of orally administered diazepam and placebo were measured over ≃3.5 h. 120 volunteers were taken for 12 groups, each group having 10 volunteers. They are performing the combination of four treatments (placebo, 0.1, 0.2, and 0.3 mg/kg diazepam) and three testing time (7 AM, 1 PM, and 7 PM). Different types of cognitive tasks, tapping and postural stability tests and also used mood evaluation scale. For subjective evaluation, Psychomotor and cognitive functions showed consistent dose-response effects. Sedation is the only effect of dose level in the duration. The result suggests that the drug affects speed rather than accuracy. It also primarily intercepts acquisition of new information or skills. Repeated testing may be used to detect subtle drug effects. The anxiolytic effects of the drug cannot be studied in healthy volunteers. On the action of the drug there was no circadian influence (Ghoneim, Mewaldt and Hinrichs, 1984).

Klotz U, Reimann IW studied pharmacokinetic and pharmacodynamic interaction of diazepam and metoprolol in 1984. In their studies 6 normotensive, healthy male volunteers the pharmacodynamic responses (blood pressure, heart rate; sedation index, tracking test, reaction time) to metoprolol (100 mg bid orally), diazepam (0.1 mg/kg intravenously). The pharmacokinetics of diazepam was also compared with and without pre-treatment by the beta-adrenoceptor antagonist to evaluate the possibility of a drug interaction in a cross-over experiment. The investigation indicated metoprolol only slightly impaired the elimination of diazepam(18% decreases in total clearance, 25% increase in elimination half-life). But the metoprolol was not significantly altered by the bolus injection of diazepam. It is concluded that concomitant treatment with metoprolol and diazepam causes only minor and clinically irrelevant changes in drug metabolism and drug response. (Klotz and Reimann, 1984)

Scott E. Lukas,Robert D. Hienz,Joseph V. Brady were researched on effects of diazepam and published a journal In 1985 October for detecting threshold and reacting time for pure tone and white light stimuli, the adult male baboons were trained on a psychophysical procedure. Before 30 min of onset session, Intramuscular injections of diazepam or triazolam were given. Strength was randomly varied from repeated trial, and four to five allotment of sensory thresholds and reaction times were obtained throughout every session. Diazepam produced dose-related response of both auditory and visual thresholds and reaction times. After

administration effects of a single high dose of diazepam were found 4–5 days. Triazolam was nearly 100 times more potent than diazepam in elevating reaction times and visual threholds. But in auditory thresholds it did not elevate. After dosing there were no residual effects of

triazolam on the day. Form this result we found that diazepam and triazolam give qualitatively similar effects on basic psychophysical function. They can be compared on the basis of sensory modality changes and post-drug recovery time.(Lukas, Hienz and Brady, 1985

In 1987 January, the American journal of psychiatry published a journal related to diazepam which causes induce amnesia.Diazepam has great amnestic properties and its effect is selective for some psychobiologically distinct memory functions. The increasing doses of diazepam given to 10 normal volunteers in this study, who are selectively impaired anterograde episodic memory and attention while totally sparing access to information in long-term memory like semantic or knowledge memory . The volunteers are behaved like that patients who having organic amnesias and there is in sharp contrast to the pattern seen in patients with dementia. This study provides information for defining specific psychobiological determinants of cognitive failure (Wolkowitz et al., 1987).

Hussey et al., in 1990, investigated the Correlation of Delayed Peak Concentration with Infusion-Site Irritation following Diazepam Administration. Diazepam 10 mg/2 mL iv was administered undiluted over five minutes to nine healthy men on two separate occasions. Before and after each infusion, the infusion site was evaluated. The subject was assessed the pain on a severity scale of zero (none) to ten (most).). Blood samples were collected at 0, 5, 20, 30, 45, and 60 minutes, and periodically for 72 hours postinfusion. Diazepam plasma concentrations were determined by HPLC. Aftet the investigation it is found that the venous irritation associate with a low plasma concentration at the end of the infusion and a delayed $C_{max\ is}$ because of the precipitation of diazepam in the vein. (Hussey et al., 1990).

In 1991 A.M.Rabasco, J.M. Ginés, M. Fernández-Arévalo and M.A. Holgados studied dissolution rate of diazepam from polyethylene glycol 6000 solid dispersions. In this studie, the weight ratios of diazepam to polyethylene glycol 6000 and the particle size of drug in the solid dispersion have been investigated in solid dispersions. Dissolution rate is influenced by the ratio of preparation method and diazepam-polyethylene glycol 6000. Polyethylene glycol 6000 gives solubilizing effect and reduce crystal size which increase the dissolution rate.

Dissolution rate also can be increased by the intrinsic effect of the carrier. It is concluded that such a difference must be attributed to a significant reduction of the drug particle size in the carrier matrix (Rabasco et al., 1991)

In 1995 July, a journal was published which is related to the dosage form of diazepam.By using spectrophotometry we determine the imipramine HCl and diazepam in tablets is described here. The drugs is combined 0.1 M HCl and prepared a solution. The method has been applied to pure drug mixtures like commercial preparations and it was more precise and reproducible. For imipramine HC1 and 2–8 μg ml−1 for diazepam, maintaining of Beer's Law was observed in the concentration range of 10–70 μg ml−1. At the 95% confidence level,the lower limits of detection were 1.9 6 μg ml−1 for imipramine HCl and 0.21 μg ml−1 for diazepam (Umapathi, P. and Parimoo, P.1998)

In 1995 january,a journal was published about diazepam.A pre-filled injection device is invented, comprising first a barrel which is open at each end. A liquid diazepam formulation is accommodated in a sealed manner before using the device. That comprises at least one rubber sealing member to seal the proposed formulation. And the second an injection needle or a needle connection at the front end of the barrel, said sealing part being manufactured at least enough from bromobutyl rubber (Johan G. and Heuvel V.D. 1995)

In 1997, Diazepam Stability related journal was established.At different time interval the stability of flunitrazepam, flurazepam, diazepam and their metabolites in spoiled blood and plasma samples was studied by bei GC-ECD analysis up to 240 days. The method is given validation data. The plasma samples or blood were stored at 22 degrees C or 4 degrees C. The sample also exposed to global natural light irradiation or it was protected from light. From different time interval we studied that all substances considerably decreased. Diazepam and flurazepam proved to be more stable where Flunitrazepam soon degraded completely at room temperature (22 degrees C), but a clear evidance of breakdown could not befound. The result suggests that for long-term stored sample to be carefully interpreted. Further investigation and the establishment of optimal storage conditions ensured for the stability of the drug (König et al., 1996).

In 1997 December, Benzodiazepines stability related journal was published. By using gas chromatographic method the stability of benzodiazepines and their metabolites stability was determined in this study and used electron capture for detection. Validation data also required. Above 240 days the spiked blood and plasma samples were stored at 4° C and analyzed at selected times. At the end of the observation period the concentrations of all analytes had

decreased to at least 60% of the original levels. A clear method of breakdown could not be measured. From the result we found that the long-term stored samples should be explained carefully. Further investigation additional methods of identification and determination as well as the establishment of optimal storage conditions seem necessary for stability of drug in blood and plasma. (Liu, Z et al. 1997)

In 2001 september, a journal was published which help to determine the diazepam in dog plasma level.For determination of diazepam and its three metabolites, oxazepam, temazepam and desmethyldiazepam, in dog plasma a fast, sensitive and specific LC/MS/MS method is described. An automated 96-well solid phase extraction procedure and electrospray LC/MS/MS analysis involves in this method. For all compounds D5-Diazepam is used as the standard. Intra-day and inter-day examination coefficients of variations are found less than 12.7%. Based on 0.1 ml of dog plasma, the lower limit of quantitation (LLOQ) is 1 nM for each analyte. 5 min was the analytical performed time. 1–500 nM is the range of linearity observed. This method has been used for pharmacokinetic studies. (König, I et al., 2001)

Iqbal MM, Sobhan T, Aftab SR, Mahmud SZ investigate the effect after the use of diazepam during pregnancy in 2002. Benzodiazepines are mainly used for the anxiety symptoms of depression, dysthymic disorder, panic disorder, agoraphobia, obsessive-compulsive disorder, generalized anxiety disorder, eating disorder, and many personality disorders duringpregnancy anxiety may occur. In that case anxiolytic drugs benzodiazepines especially diazepam is prescribed. After the investigation it was found that ther is a potential risk of teratogenicity and direct neonatal toxicity. So it better avoiding exposure in the first trimester, especially with multidrug regimens, and prescribing the lowest dose for the shortest duration. (Iqbal et al., 2002)

In 2002 march, one American journal published about the stability of diazepam rectal gel (Diastat) in different conditions of temperature and light exposure as might be found in

ambulances. In various fill/syringe configuration, three distat(Xcel pharmaceutical, an Diego, CA) were evaluated in specific conditions of a freeze-thaw cycle, hard freeze (-30 degrees C for 72 hours), extreme light exposure (1,000 ft candles for 1 month), and long-term evaluation at either 30 degrees C or 40 degrees C. Concentration of diazepam always exceeded 95% of label in different configurations and testes without any changes of note in excipients or physicochemical properties. Approximately the shelf-life at 30 degrees C exceeds 48 months. The results suggest that in ambient storage conditions (eg, ambulances) the distat, could be stable up to 48 months in nonfreezing environments and this does not exceed the labeled expiration date on the product (Dodov M.G et al., 2005)

Next year in 2003, Seo et al. studied the dissolution rate of diazepam, preparing by melt agglomeration agglomerates containing solid dispersions of diazepam as poorly water-soluble model drug. . Lactose monohydrate was melt agglomerated with polyethylene glycol (PEG) 3000 or Gelucire® 50/13 (mixture of glycerides and PEG esters of fatty acids) as meltable binders in a high shear mixer. Different drug concentrations, maximum manufacturing temperatures, and cooling rates were investigated. After the observation it was found that it is possible to increase the dissolution rate of diazepam by melt agglomeration. A higher dissolution rate was obtained with a lower drug concentration. Gelucire 50/13 resulted in faster dissolution rates compared to PEG 3000. (Seo et al., 2003)

Again in 2003 28 November, a journal was published about potentiometric determination of diazepam.For determining diazepam, bromazepam and clonazepam 1,4-benzodiazepines in pure forms and in pharmaceutical preparations, a newly developed solid contact ion-selective electrodes (SC-ISE) have been proposed. The electrodes are mde of polyvinylchloride (PVC) membranes doped with drug-tetraphenyl borate (TPB) or drug-phosphotungestic acid (PTA) ion pair complexes as electroactive materials. The new electrodes verified they are highly selective with selectivity coefficients ranging from 10−4 to 10−6 .And the detection limits ranging from 0.1 to 0.63 µg/ml. The electrodes are used for drugs determinations in pharmaceutical preparations was obtained from this study. Average errors relatively less than or equal 1.72% and relative standard deviations less than 1.13% were obtained. Statistical student‟s t-test and Ftest showed inconsequence systematic error between measured and real values. It also helps to developed ion-selective electrodes methods and a standard HPLC method. (Salem, Barsoum and Izake, 2003)

In the year of 2004, Chevassus et al., studied a single dose benzodiazepines on insulin secretions, insulin sensitivity, and glucose effectiveness. The study was performed with healthy volunteers. Observation is mainly based on the effects of diazepam and clonazepam on beta-cell function, insulin sensitivity and glucose effectiveness. The study was designed as a double-blind, placebo-controlled, cross-over clinical trial. Diazepam (10 mg) and clonazepam (1 mg) were infused during 30 min to 15 male subjects with a mean age of 22 years (range: 20–29), after informed consent was given. Benzodiazepines were assayed by capillary gas chromatography with electron capture, insulin by radioimmunoassay and glucose by the enzymatic glucose oxidase method. After the tests, the result found that clonazepam may alter insulin secretion and insulin sensitivity after a single administration in healthy volunteers. No effect change with the diazepam. (Chevassus et al., 2004)

In 8November 2005, diazepam related journal was published on parenteral formulation for the waterinsoluble benzodiazepine diazepam was developed. Different cyclodextrins (CDs) suitable for parenteral injection: hydroxypropyl-β-cyclodextrin (HP-β-CD), hydroxypropyl-γ-cyclodextrin (HP-γ-CD), sulfobutylether-7-β-cyclodextrin (SBE-7-β-CD) and maltosyl-β-cyclodextrin (malt-β-CD) were used as alternatives to cosolvents to increase solubility. The increase in solubility displayed a concentration dependency for the four CDs used. Diazepam's solubility is enhanced linearly as a function of each CD concentration. The highest improvements in solubility (dissolved concentration circa 3.5 mg/ml in 40% CD) were found by adding HP-β-CD or SBE-7-β-CD. The additional use of polyvinylpyrrolidone (PVP) did not further increase the solubility of diazepam with HP-β-CD. A parenteral aqueous diazepam solution was prepared containing 10 mg diazepam/5 ml 30% HP-β-CD or SBE-7-β-CD solution. The preparations are in agreement with the requirements for parenteralia. Sterilisation by filtration is required since autoclaving degrades the active compound. The stability of the preparations, with and without pH adjustment to pH 5, was investigated during 18 months and during this period no noticeable degradation was observed. (Holvoet, C et al., 2005)

Maślanka A, Krzek J developed a thin-layer chromatography (TLC)-densitometry method in 2005 to identify and quantify psychotropic drugs like diazepam, trifluoperazine, clonazepam, and chlorpromazine. Precoated silica gel 60 F254 TLC plates were used for separation.Chromatograms were developed in various mobile phases, and 8 of 30 tested phases were selected based on spot location and developing time. Ultraviolet densitometric

measurements at chosen wavelengths were used for the identification and quantification. Under established experimental conditions, high sensitivity of the method was achieved. (Ma\'slanka and Krzek, 2005).

In February 2, 2007 a journal was published about the detection of diazepam in urine.For detection of diazepam a gas chromatography–tandem mass spectrometry method is used. The electron capture ionization and multiple reaction monitoring (MRM) involves in this method. We found that oxazepam passes through thermal degradation during chromatographic injection and it is quantified as decomposition product. The negative molecular ions do not dissociate when collision is performed under "classical" conditions (i.e. with argon as collision gas) because they are so stable. When xenon used as collision gas, two products ion diazepam and nordazepam energy transfer is sufficient and one product ion for the degradation product of oxazepam. The sample prepared as liquid/liquid extraction with TOXITUBES, a extraction tubes; it provides yields between 68 and 95% that depending on the benzodiazepine, considered with coefficients of variation less than 6% for 10 samples. The method was demonstrated on urine extracts. The method provides for 1mL of urine the quantitation limits of 0.15 ng/mL for diazepam 1.0 mg/mL for nordazepam and 1.5 ng/mL for oxazepam (Kinani, S et al., 2007)

In 2007 March, a journal was published related to spectrophotometric determination of diazepam. For it spectrophotometric determination the interaction of diazepam with picric acid (I), 3,5-dinitrobenzoic acid (II) and 2,4-dinitrobenzoic acid (III) was found to be useful . The detection was carried out at 475, 500, and 500 nm for the reaction with (I), (II) and (III), respectively. The effect of different variables on the coloring process was examined. The introduced methods have been applied successfully for the determination of diazepam in pure samples and in its pharmaceutical preparations with well accuracy and precision. The results were compared to other those are obtained by the pharmacopoeial methods. The linear ranges for maintaining Beer‟s law are up to 85.6, 180.2, and 128.6 µg/ml, Ringbom ranges are 10.0-79.0, 15.2-177.8, 17.0-83.0 µg/ml, and RSD 0.048, 0.028, and 0.026% for reaction of diazepam with I, II, and III, respectively.(El-Hawary, 2007)

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In 2008, 30 December, The adsorption of diazepam to infusion sets and plastic syringes was studied. Infusion solutions consisting of diazepam injection (Valium®) in glucose 5.5%, diazepam emulsion (Diazemuls®) in glucose 5.5%, or diazepam emulsion in a lipid emulsion (Intralipid® 10%) were infused through two different infusion sets (Transcodan L-74 and Cutter IL). It was found that, when an infusion solution with a low diazepam concentration (0.04 mg/ml) was infused slowly (4 ml/h), the diazepam adsorption was more than 80%. At a higher diazepam concentration (0.1 mg/ml) and increased infusion rate (20 ml/h) the adsorption decreased. Diazepam injection in glucose 5.5% was adsorbed to a higher degree (40–75%) than diazepam emulsion in glucose 5.5% (15–35%). When diazepam emulsion was diluted with the lipid emulsion, no diazepam adsorption to the infusion set occurred at this concentration and infusion rate. No significant difference between the two infusion sets could be found. The miscibility of diazepam emulsion with glucose 5.5%, glucose 10%, or sodium chloride 0.9% was examined. Diazepam emulsion proved to be miscible with glucose 5.5% and glucose 10%, but sodium chloride should not be used to dilute diazepam emulsion. The effect on the diazepam concentration of storing diazepam injection and diazepam emulsion in plastic syringes for up to 4h was also studied. It was found that the diazepam concentration remained unchanged during this time. (Winsnes, Jeppsson and Sjöberg, 1981)

In 2008 Majeed, n.d developed a method for screening color test for identification of

Diazepam. In this method diazepam is treated with alkaline dimethylsulfoxide produces a reddish color which gradually changes to yellow with passage of time. After adding water the color is instantly vanish attempted extraction with organic solvents, suggesting that the color is due to a transient charge-transfer complex. A choloform extraction with diazepam produces color in the expeeiment. The test is negative for other controlled substances, including other benzodiazepines, and also for various diluents and binders typically present in tablets (62 compounds were tested). (Majeed, n.d.)

In 2009, the diazepam analysis related journal was published Diazepam is commonly used as an adjuvant in antidepressant therapy. Some recent studies have suggested that the treatment with benzodiazepines could have different efficacy in depressed patients as opposite to non-depressed patient. To justify the matter, a study is carried out in rats. To obtain a more perfect set of data, the main diazepam metabolites have also been considered as nordiazepam, temazepam and oxazepam. For the simultaneous determination of these compounds in plasma and brain tissue of rats a possible and reliable HPLC method has been used. To estimate drug metabolism in various breeds,the method has been applied to "normal" rats and to genetic rat models of depression. By using an acidic phosphate buffer mixture as the mobile phase the analyte was separated on C8 revers phase. 238 nm wavelength was used for detection. An original sample pre-treatment which based on sold-phase extraction (SPE) was developed in order to eliminate endogenous interference.

The clarification of possible differences between depressed and non-depressed subjects with respect to benzodiazepine biotransformation determined from the obtained data. (Mercolini et al. 2009)

In 2011 the use of loading dose diazepam for the treatment of alcohol withdrawal was first described by Sellers *et al*.They used loading dose diazepam to treat alcohol withdrawal in in-patient de-addiction unit at JIPMER. It involved 25 consecutive admissions of alcohol use disorders between 1st August and 15thNovember, 2011. The same was used successfully in Indian patients by Manikant et al. It involvedthe administration of 20mg oral diazepam every 2 h until the patient is drowsy, but arousable. Symptoms of alcohol withdrawal are monitored using the Clinical Institutes Withdrawal Assessment for Alcohol - Revised (CIWA-Ar) scale. Further doses are withheld whenever CIWA-Ar scores fall below 8. The major advantages of this method was

faster recovery from delirium, lower total doses of diazepam and a lesser risk of complications like withdrawal seizures and arrhythmias (Bharadwaj et al., 2012)

Again in 2011, Mielcarek et al., developed a method for estimation of molecular dynamics of diazeoam-density functional theory. The molecule of the diazepam was investigated by calorimetric methods, IR absorption and NMR. The investigation of dynamics was complemented by density functional study (DFT) of vibrational frequencies and infrared intensities, calculations of steric hindrances and Monte Carlo simulations. The results indicated the occurrence of reorientation jumps of the CH_3 group and conformational motion of the benzodiazepine ring. (Mielcarek et al., 2011)

In the same year researcher Ali investigate non-invasive *in situ* identification and band assignments of diazepam, flunitrazepam and methadone hydrochloride with FT-near-infrared spectroscopy (NIR). It is a direct important and non-invasive technique in drugs analysis. Inside the USP vials two benzodiazepine derivatives, diazepam and flunitrazepam, and a synthetic opiate, methadone hydrochloride with the solid-state form of diazepam presents in tablets has been exploredin this study. The results show the potential of NIR spectroscopy for rapid, *in situ* and non-destructive identification of drugs. (Ali, 2011)

Again in 2011 Ma'slanka et al., studied the stability of diazepam along with clonazepam, haloperidol, and doxepin in acidic emvironment. Additionally kinetic and thermodynamic properties were also carried out in stability studies. Reaction rate constants (k), half-life times (t(0.1) and t(0.5)), and activation energy (Ea) were estimated for the drugs, which differed in polarity expressed with log P values. All degradation products were studied using an HPLC/electrospray ionization-MS technique in the positive ionization mode. (Ma'slanka et al., 2011)

In 2012 26th July, the chemical structure and properties related journal was published.A short description is given of the basic chemical establishment in the field of „classical‟ and „annelated‟ benzodiazepines, comparing between pro-drugs and directly acting compounds. Discussed about some properties of midazolam that are of special interest for its practical use like the basicity of its imidazole ring nitrogen, that allows water- soluble salts and well-tolerated aqueous injectable solutions to be prepared; stability of hydrolytic decomposition;

rapid metabolic inactivation, which is mainly measured by the methyl group on the imidazole ring, and which is more faster than that of classical benzodiazepines.(Gerecke, 1983).

In 2012, Rust et al., developed a detection and validated method for quantification of 21 benzodiazepines and the pharmacologically related "z-drugs" in human hair samples using liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS). The assays were found to be selective for the tested compounds (alprazolam, 7-aminoclonazepam, 7-aminoflunitrazepam, bromazepam, chlordiazepoxide, clonazepam, N-desalkylflurazepam, diazepam, flunitrazepam, flurazepam, alpha-hydroxymidazolam, lorazepam, lormetazepam, midazolam, nitrazepam, nordazepam, oxazepam, phenazepam, prazepam, temazepam, triazolam, zaleplon, zolpidem and zopiclone), all validation criteria were in the required ranges according to international guidelines, except for bromazepam. After the investigation it was found that matrix effects and process efficiencies were in the acceptable ranges evaluated using the post-extraction addition approach. It has proven that the LC–MS/MS assay is applicable for determination of the studied analytes in human hair in numerous authentic cases (n = 175) (Rust et al., 2012).

In the same year researcher Ara evaluate the quality of pharmaceutical finished dosage forms of diazepam tablets from different brands in Bangladesh. Pharmaceutical preparations take many shapes and forms and are administered through variety of routes. Oral solid dosage forms particularly the tablet dosage form is the most well known of all. Tablet dosage form of any pharmaceutical company goes through many research studies and experiments to maintain the proper quality standards. Diazepam is act on the central nervous system so it is necessary to ensure the quality of the product. Different physical parameters like hardness, thickness, friability as well as disintegration time were conducted to evaluate the quality of the tablets of different brands of diazepam. To ensure quality product a pharmaceutical industry follows the international standards. So it can be said that quality is the main theme of any product. So to maintain the proper quality, quality control parameters must be followed. (Ara, 2012)

Capra et al., developed a innovative approach for Interstitial Cystitis in the year of 2013. In their method Vaginal Pessaries were used which was loaded by diazepam. Diazepam is well known for its antispasmodic activity in the treatment of muscular hypertonus. In this method two types of formulations used which is with and without beta-glucan that was compared.

The setup of the analytical method to determine diazepam, pH evaluation, dissolution profile, and photostability assay were reported in the preparation of the pessaries. In order to determine the diazepam amount, calibration curves with good correlation coefficients were obtained, by the spectrophotometric method, using placebo pessaries as matrix with the addition of diazepam standard solution. Dissolution profiles showed a complete diazepam release just after 15 minutes, even if beta-glucanpessaries released drug more gradually. Finally, a possible drug photodegradation after exacerbated UV-visible exposition was evaluated. (Capra et al., 2013)

After the study of 2011, Ma'slanka et al., studied again to determine the stability of clonazepam, diazepam, alprazolam, haloperidol, and doxepin in basic solutions in 2013. Kinetic and thermodynamic stability indicationg parameter was assed which were compared with the lipophilicity (log *P*) of the studied drugs. The degradation products were identified using UPLC/MS/MS method. (Ma'slanka et al., 2013)

In the same year in 2013, Đorđević et al., design an experimental formulation of diazepam nanoemulsions for parenteral drug delivery. . To study the effects of the oil content, lecithin type, and the presence of diazepam as a model drug and their interactions on physicochemical characteristics of nanoemulsions. Droplet size and size distribution, surface charge, viscosity, morphology, drug-excipient interactions, and physical stability were the main concern for evaluating nanoemulsions. The result showed that the in vivo pharmacokinetic study of selected diazepam nanoemulsions with different oil content (20%, 30%, and 40%, w/w) demonstrated fast and intense initial distribution into rat brain of diazepam from nanoemulsions with 20% and 30% (w/w) oil content, suggesting their applicability in urgent situations. (Đorđević et al., 2013)

In 2013, WEang et al., determined method in which diazepam and its glucuronide metabolites in human blood by μElution solid-phase extraction and liquid Chromatography–tandem mass spectrometry. 200 μL of whole blood samples were loaded onto a Waters Oasis HLB 96-well μElution SPE plate using 75 μL of methanol as the elution solvent, and the eluents were injected into an Eclipse XDB C18 column. No hydrolysis, solvent transfer, evaporation or reconstitution was involved in the sample preparation procedures. The method was reproducible and reliable. The applicability of the method was demonstrated by analysis of several forensic cases involving diazepam and its metabolites (Wang et al., 2013).

Gautam, Sharratt and Cole, determine the stability of Benzodiazepines in Spiked Drinks Using Gas Chromatography-Mass Spectrometry recently in 2014. Benzodiazepines are detected in a significant number of drug facilitated sexual assaults (DFSA). Diazepam, flunitrazepam and temazepam used into five drinks, an alcopop (flavoured alcoholic drink), a beer, a white wine, a spirit, and a fruit based non-alcoholic drink (J2O). Blood and urine from the victim are routinely analysed. Validated GC-MS method for the simultaneous detection of these drugs in the drinks have studied the storage stability under two different storage conditions, uncontrolled room temperature and refrigerator (4°C) over a 25 day period. After the observation Diazepam was found to be stable in all of the beverages, except the J2O, under both storage conditions. The recommendations from this study are that there should be a policy change and that drinks thought to be involved in DFSA cases should be collected and analysed wherever possible to support other evidence types. (Gautam, Sharratt and Cole, 2014).

Recently in 2014, Suksiriworapong et al., developed polymeric micelles for rectal adminstration of water insoluble drug diazepam. The diazepam-loaded polymeric micelles were developed by using poloxamer 407 (P407), poloxamer 188, and D-α-tocopherylpoly(ethylene glycol) 1000 succinate (TPGS). TPGS resulted in polymeric micelles with good characteristics for encapsulation of diazepam among the used polymers. Additionally, 7.5% w/v of TPGS could entirely entrap the desired concentration of diazepam (5 mg/mL). P407 also improve the physical stability upon lyophilisation, prevent aggregation and maintained chemical stability of the lyophilized powders of diazepam-loaded polymeric micelles for 3 months storage at 4°C. The concentration of TPGS determines the rate of diazepam release. In conclusion, 10% w/v TPGS and 1% w/v P407 were the optimum formulation of lyophilized diazepam-loaded polymeric micelles. (Suksiriworapong et al., 2014)

Chapter Three

MATERIALS & METHODS

3.1. Materials

3.1.1. Sample collections:

For the purpose of experimentation to observe the photolytic degradation of diazepam drug as well as to assess the packaging efficiency, 500 tablets of Easium® (diazepam 5mg) were collected from the local drug store in Dhaka as a sample. All the tablets were from the same batch.

3.1.2.Reagents:

Table 3.1: 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)

3.1.3.Equipments and instruments:

Table 3.2: 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	Verniere Calipers	Shanghai Tricle Brand	China

3.1.4.Images of instruments

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

Figure 3.1: Shimadzu UV-1800 Double Beam Spectrophotometer and Electronic Balance [Left to right]

Figure 3.2: Hardness tester and Distilled Water Plant [Left to right]

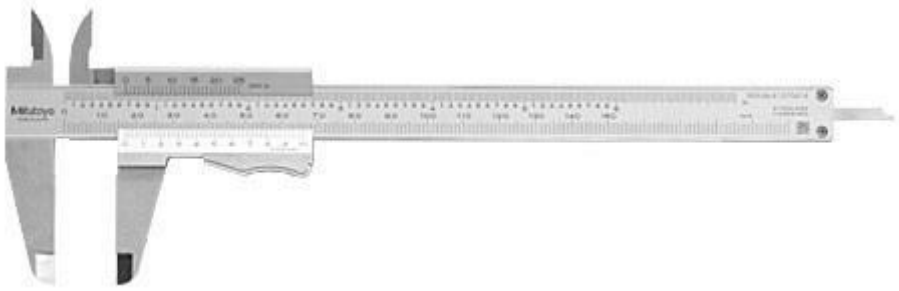

Figure 3.3: Vernier Calipers

3.1.5.Apparatus

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

- Beakers
- 50ml and 100 ml volumetric flasks
- Test tubes
- Aluminium foil paper
- Filter papers
- Mortar and pestle
- Pipette(5ml) and pipette pumper
- Spatula
- Thermometer
- 25 watt and 40watt bulb

- Lamp
- Glass rod
- Glass and plastic funnel

3.2.Methods

3.2.1.Preparation of solvent: 0.1N sulphuric acid

1. Lab solvent (HCL) stock solution was collected and its strength was found to be 98%

2. Then the concentration of the lab solvent stock solution was determined in Normality
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 x 0.1 N) / 36.8N

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

4. Then 2.72ml of 36.8N H_2SO_4 was transferred from the lab solvent stock solution to a 1000ml volumetric flask which was then filled with water up to mark to make 1000ml of

   0.1N H_2SO_4

3.2.2.Determination of λmax& Preparation of the Standard Curve of diazepam

1. Standard of diazepam was collected from Aristropharma. The potency of standard compound was 99.02%.

2. The specific λmax for diazepam, at which the absorbance would be measured, was determined 240.1nm from the UV spectrometer by using the standard.

3. Nine serial concentrations of the Standard of diazepam were prepared for the purpose of creating a standard curve.

➢**Preparation of the stock solution for diazepam using the standard**.

- 50 mg of the standard compound, that is diazepam obtained from the pharmaceutical company was weighed and dissolved in 100 ml of 0.1N H_2SO_4 (which is the solvent) in a 100 ml 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 diazepam solution was taken and dissolved in 50ml of 0.1N

  H_2SO_4. That 5ml contained 1mg of diazepam.

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 diazepam**

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

- ⇨ Nine serial concentrations that were prepared for diazepam 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 diazepam as per the calculations provided below.

Table 3.3: Concentrations for preparation of Standard Curve of diazepam

Sample Name	**Sample no.**	**Concentration (mg/ml)**
	1	0.001
Diazepam	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 diazepam.
- $V_1 = S_2V_2 / S_1$ = (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 diazepam.
- $V_1 = S_2V_2 / S_1$ = (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 diazepam.
- $V_1 = S_2V_2 / S_1$ = (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 diazepam.
- $V_1 = S_2V_2 / S_1$ = (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 diazepam.
- $V_1 = S_2V_2 / S_1$ = (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 diazepam.
- $V_1 = S_2V_2 / S_1$ = (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 diazepam.

- $V_1 = S_2V_2 / S_1$ = (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 diazepam.

- $V_1 = S_2V_2 / S_1$ = (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 diazepam.

4. Then the Absorbance values were measured using a UV spectrophotometer against those ten serial concentrations each for diazepam.

5. A standard curve was plotted.

6. From those standard curve one 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.

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

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

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

⇨ Direct Sunlight exposure

1. Exposure under Normal Lighting Condition

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

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 100 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. Fro then 10ml of each sample was collected and kept into 9 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 labelled 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.

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

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

b. 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 100 ml of the solvent (0.1N H_2SO_4) for 3 times to prepare 3 samples.

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

d. From then 10ml of each sample was collected and kept into 18 different test-tube and wrapped it by foil paper.

e. 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)	Temperature (^{0}C)
10 (Control)	10	0	25
30	10	2	27
	10	4	27
	10	6	30

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 6 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 labelled 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 100 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 18 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)	Temperature (^{0}C)
10 (Control)	10	0	25
30	10	2	27
	10	4	30
	10	6	28

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

- ✓ **Colour 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.

- ✓ **Weight Variation test**

1) The experiment has been started with 10 tablets and all tablets were weighed at one time by electronic balance.

2) Then the composite weight was divided by 10 provided in order to get an average weight.

Average weight, $X = (X_1 + X_2 + \ldots + X_3 z)/10$

3) Then each tablet selected at random was weighed individually such as X_1, X_2, X_3 …….Xz and was observed whether the individual weight are within the range or not.

4) As per BP weight variation test procedure, individual weight was compared to the average weight.

5) The tablets meet the BP test if not more than two tablets are outside the percentage limit and if no tablet differ by more than two times the percentage limit.

The equation for calculation of percentage weight variation is given below:

Percentage weight variation= (average weight - individual weight)/ individual weight x 100%

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 3.4: 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%

✓**Tablet Thickness Test**

1. Tablets have been placed between two jaws of Vernier calipers horizontally.
2. The screw of the slide calipers has been ran to hold the tablets.
3. The reading of the thickness of the tablet has been taken in cm.

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

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

✓**Hardness Test**

1. The crushing strength of the tablets was measured using a hardness tester.

2. At first 5 tablets were picked randomly from 10 tablets.

3. The sliding scale of hardness tester has been set off to zero.

4. The tablets have been placed vertically between the two jaws.

5. Force has been applied with the screw thread and spring until the tablets has been fractured.

6. A force of about 4kg is considered to be the minimum for hardness.

Chapter Four

RESULTS

4.1. Standard curve preparation

The standard was collected from a pharmaceutical company and tried to make a standard curve. For different concentration of diazepam we found different absorption.

The results are as follows:

Table 4.1: Concentration and Respective Absorbance for Standard Curve of Diazepam

Concentration(mg/ml)	Absorbance(nm)
0.001	**0.096**
0.002	**0.182**
0.003	**0.302**
0.004	**0.390**
0.005	**0.473**
0.006	**0.565**
0.007	**0.639**
0.008	**0.738**
0.009	**0.812**

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

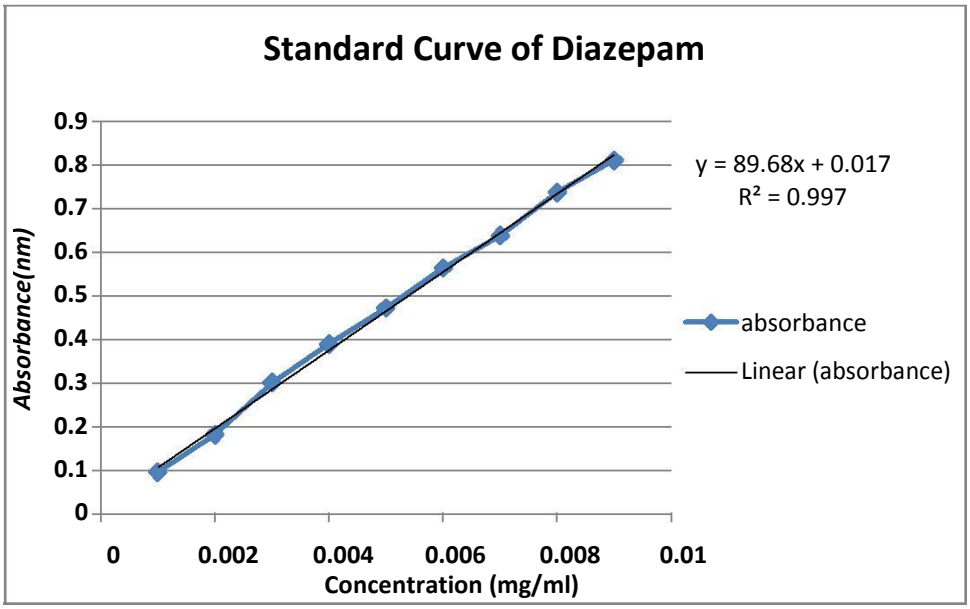

Figure: 4.1: Plot showing straight line for Absorbance (nm) with respect to Concentration (mg/ml) for Diazepam

From the Standard Curve of Diazepam (shown above by *figure 4.1*) the following

equation with a R2 value was derived which is given below.

$$Y=89.68x+0.017$$

$$R^2=0.997$$

Where, Y = Absorbance (Abs) X = Concentration of the drug (mg/ml)

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

A tablet strip containing 10 tablets was taken and 5 samples were collected for the test. Weight variation test was conducted and average weight was calculated for each day. Data of these tests are given below;

Table 4.2: Weight Variation Test of EASIUM®

Time interval(in Days)	Average Weight for Particular Day, I(g)	Average Weight for 60 daysIntervals, A(g)	% WeightVariation, (A-I/A)×100 %
0	.0660	.639	-3.6
15	.0659		-2.5
30	.0658		-0.16
45	.0655		2.2
60	.0654		4.5

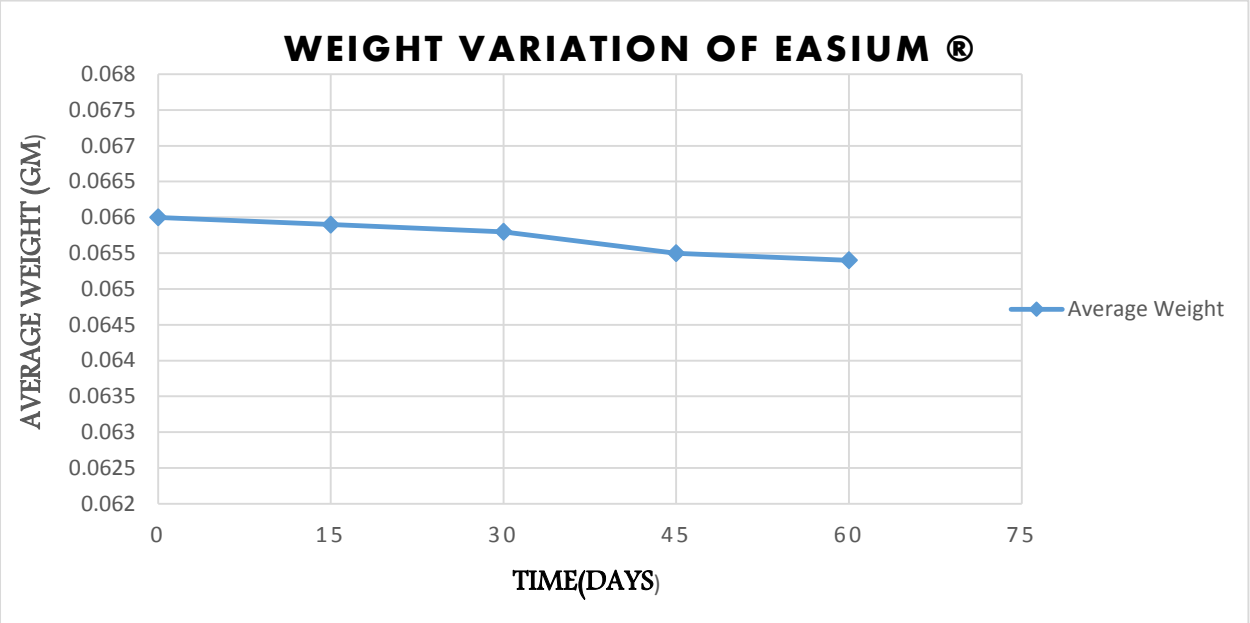

Figure 4.3: Weight variation of the sample throughout 60 days light exposure

4.2.3 Thickness test:

A tablet strip containing 10 tablets was taken and 5 samples were collected for the test. Thickness test was conducted and average weight was calculated for each day. Data of these tests are given below:

Table 4.3: Thickness Test of Easium®

Time interval(in weeks)	Average thickness of the samples(cm)
0	.645
15	.644
30	.645
45	.643
60	.644

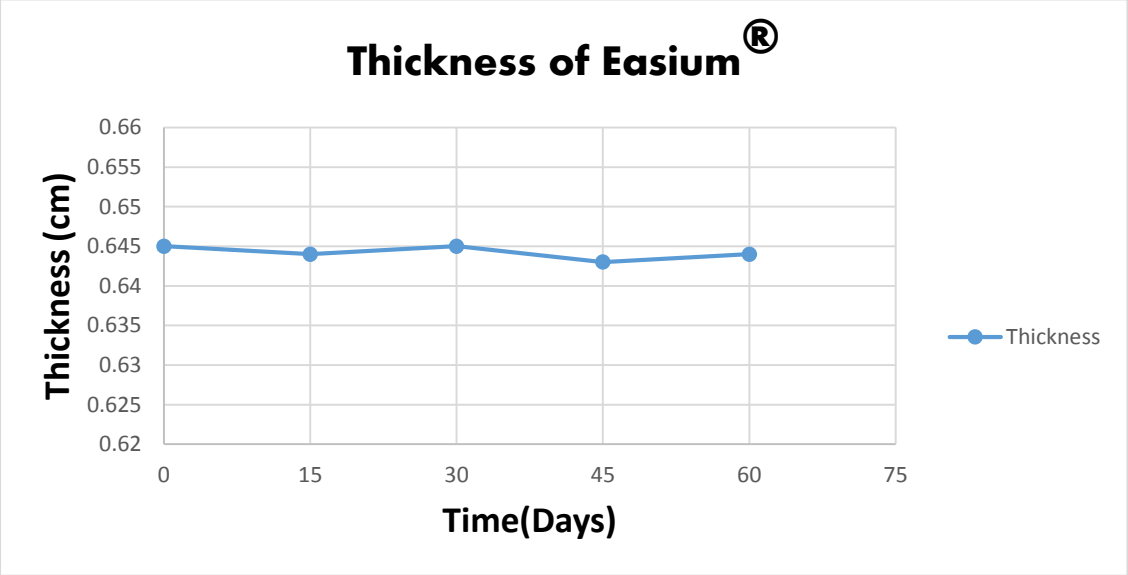

Figure 4.4:Thickness variation of sample throughout 60 days light exposure

4.2.4 Hardness Test:

A tablet strip containing 10 tablets was taken and 5 samples were collected for the test. Hardness test was conducted and average weight was calculated for each day. Data of these tests are given below:

Table 4.4: HardnessTest of Easium®

Time interval(in weeks)	Average hardness of the samples(kg)
0	5
15	4.5
30	4.8
45	4.5
60	4

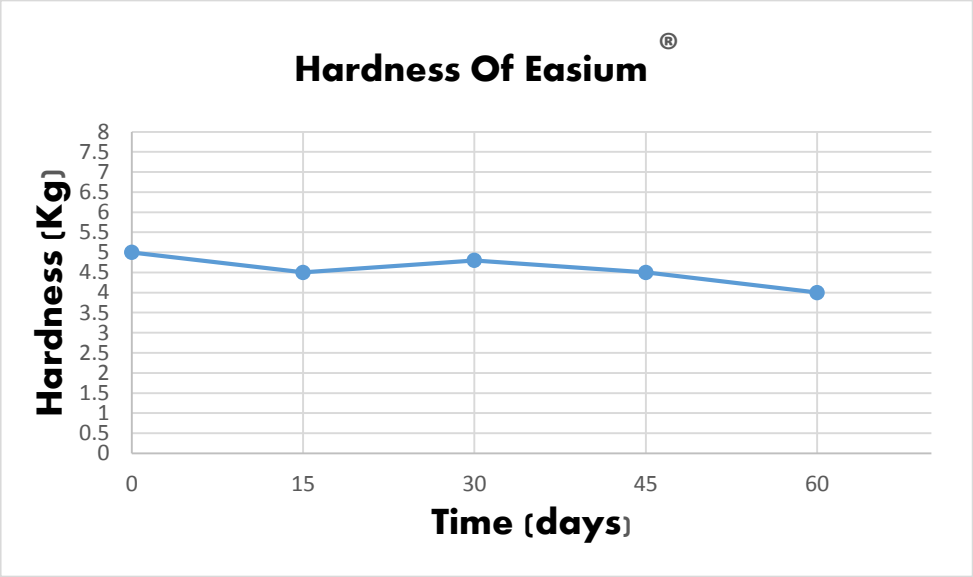

Figure 4.5: Hardness variation of the sample throughout 60 days light exposure

4.3 Result from Potency Determination by UV- spectroscopy

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

4.3.1 Result from Sample that was exposed under Normal Lightening Condition

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

Table 4.5: Concentration & Absorbance of 0 Days Interval for Easium® (Diazepam

Time Interval (Days)	Absorbance (at 240.5nm)		Average Absorbance		Amount of Drug Present (in mg/ml)		Potency (%)	
	Control	Sample	Control	Sample	Control	Sample	Control	Sample
Initial	0.458	0.458	0.456	0.458	4.895	4.895	97.9	97.9
	0.555	0.458						
	0.456	0.458						
	0.458	0.458	0.456	0.458	4.895	4.895	97.9	97.9
	0.455	0.458						
	0.456	0.458						
	0.458	0.458	0.456	0.458	4.895	4.895	97.9	97.9
	0.455	0.458						
	0.556	0.458						

Table 4.6: Concentration & Absorbance of 15 Days Interval for Easium® (Diazepam)

Time Interval (Days)	Absorbance (at 240.5nm)		Average Absorbance		Amount of Drug Present (in mg/ml)		Potency (%)	
	Control	Sample	Control	Sample	Control	Sample	Control	Sample
15	.458	.425	.456	.424	4.895	4.54	97.9%	90.8%
	.455	.426						
	.456	.423						
	.456	.422	.456	.420	4.895	4.49	97.9%	89.8%
	.458	.421						
	.455	.420						
	.458	.419	.456	.419	4.895	4.48	97.9%	89.6%
	.456	.420						
	.455	.418						

Table 4.7: Concentration & Absorbance of 30 Days Interval for Easium® (Diazepam)

Time Interval (Days)	Absorbance (at 240.5nm)		Average Absorbance		Amount of Drug Present (in mg/ml)		Potency (%)	
	Control	Sample	Control	Sample	Control	Sample	Control	Sample
30	.455	.403	.456	.403	4.895	4.30	97.9%	86%
	.456	.402						
	.458	.404						
	.455	.400	.456	.400	4.895	4.27	97.9%	85.4%
	.456	.399						
	.458	.402						
	.455	.398	.456	.398	4.895	4.24	97.9%	84.8%
	.456	.396						
	.558	.399						

Table 4.8: Concentration & Absorbance of 45 Days Interval for Easium® (Diazepam)

Time Interval (Days)	Absorbance (at 240.5nm)		Average Absorbance		Amount of Drug Present (in mg/ml)		Potency (%)	
	Control	Sample	Control	Sample	Control	Sample	Control	Sample
45	.455	.392	.456	.394	4.895	4.20	97.9%	84%
	.456	.394						
	.458	.395						
	.455	.390	.456	.389	4.895	4.148	97.9%	82.9.%
	.456	.387						
	.458	.389						
	.455	.382	.456	.384	4.895	4.09	97.9%	81.8%
	.456	.384						
	.558	.385						

Table 4.9: Concentration & Absorbance of 60 Days Interval for Easium® (Diazepam)

Time Interval (Days)	Absorbance (at 240.5nm)		Average Absorbance		Amount of Drug Present (in mg/ml)		Potency (%)	
	Control	Sample	Control	Sample	Control	Sample	Control	Sample
60	.455	.375	.456	.375	4.895	4.04	97.9%	79.8%
	.456	.377						
	.458	.373						
	.455	.370	.456	.370	4.895	3.94	97.9%	78..8%
	.456	.372						
	.458	.369						
	.455	.361	.456	.359	4.895	3.81	97.9%	76.2%
	.456	.359						
	.458	.358						

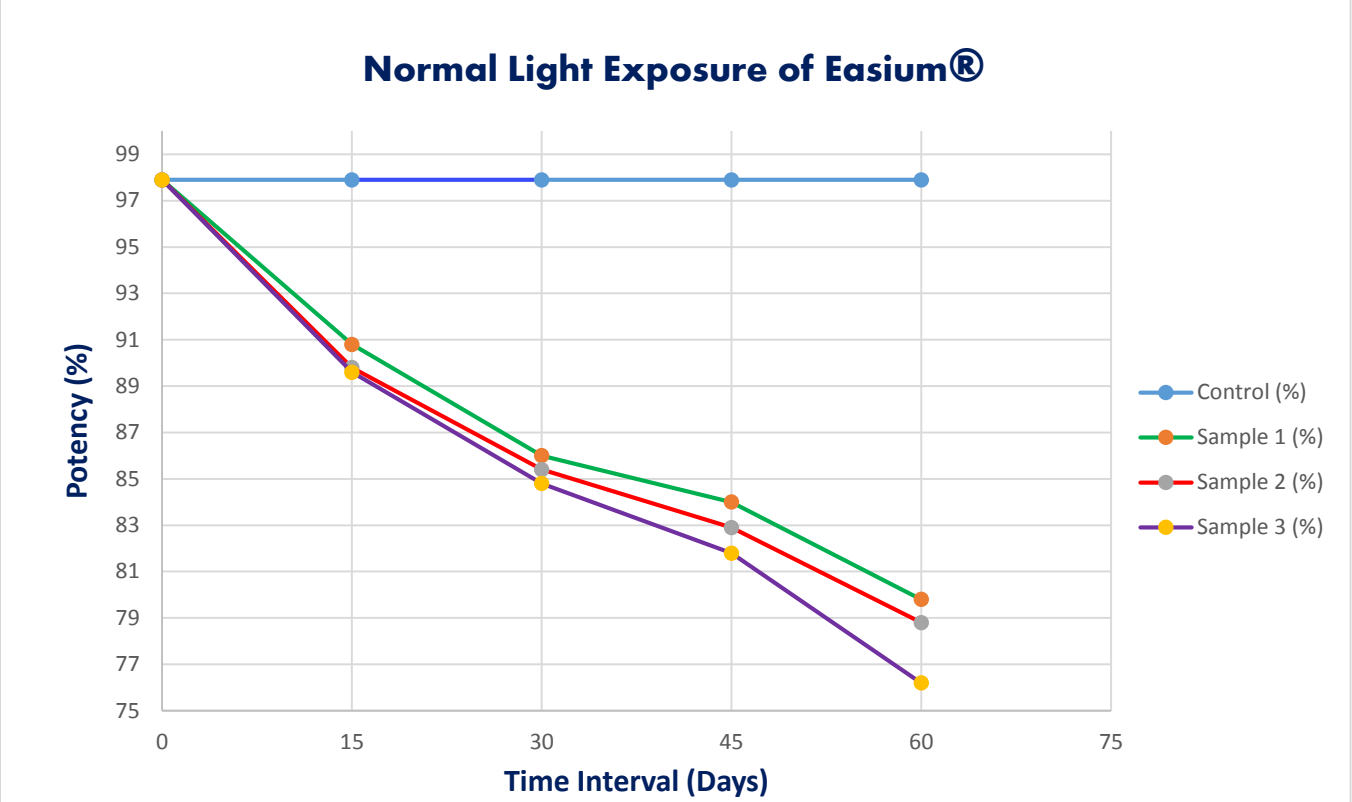

Figure 4.6: Graph showing the difference in Concentration after specific time interval for Easium® exposed under normal light

4.3.2 Result of samples that were exposed under 25W bulb

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

Table 4.10: Concentration & absorbance ofEasium®(Diazepam) for 1st time (0 hours)

Time Interval	Absorbance (at 240.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (1000 times diluted)	Amount of Drug present (in mg/ml)	Potency (%)
Control	.462	.460	0.00494	4.94	98.8
	.460				
	.459				
	.462	.460	0.00494	4.94	98.8
	.460				
	.459				
	.462	.460	0.00494	4.94	98.8
	.460				
	.459				

Table 4.11: Concentration & absorbance ofEasium®(Diazepam) for 1st time (2 hours)

Time Interval	Absorbance (at 240.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (1000 times diluted)	Amount of Drug present (in mg/ml)	Potency (%)
2 hours	.452	.453	.00486	4.86	97.2%
	.453				
	.455				
	.450	.449	.00482	4.82	96.4%
	.447				
	.451				
	.443	.445	.00477	4.77	95.4
	.446				
	.445				

Table 4.12: Concentration & absorbance of Easium® for 1st time (4 hours)

Time Interval	Absorbance (at 240.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (1000 times diluted)	Amount of Drug present (in mg/ml)	Potency (%)
4 hours	.420	.419	.00448	4.48	89.6
	.421				
	.418				
	.414	.414	.00443	4.43	88.6
	.416				
	.414				
	.413	.412	.00440	4.40	88%
	.410				
	.412				

Table 4.13: Concentration & absorbance of Easium® for 1st time (4 hours)

Time Interval	Absorbance (at 240.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (1000 times diluted)	Amount of Drug present (in mg/ml)	Potency (%)
6 Hours	.411	.409	.00437	4.37	87.4
	.408				
	.409				
	.395	.396	.00423	4.23	84.6
	.398				
	.396				
	.395	.393	.00419	4.19	83.8
	.393				
	.391				

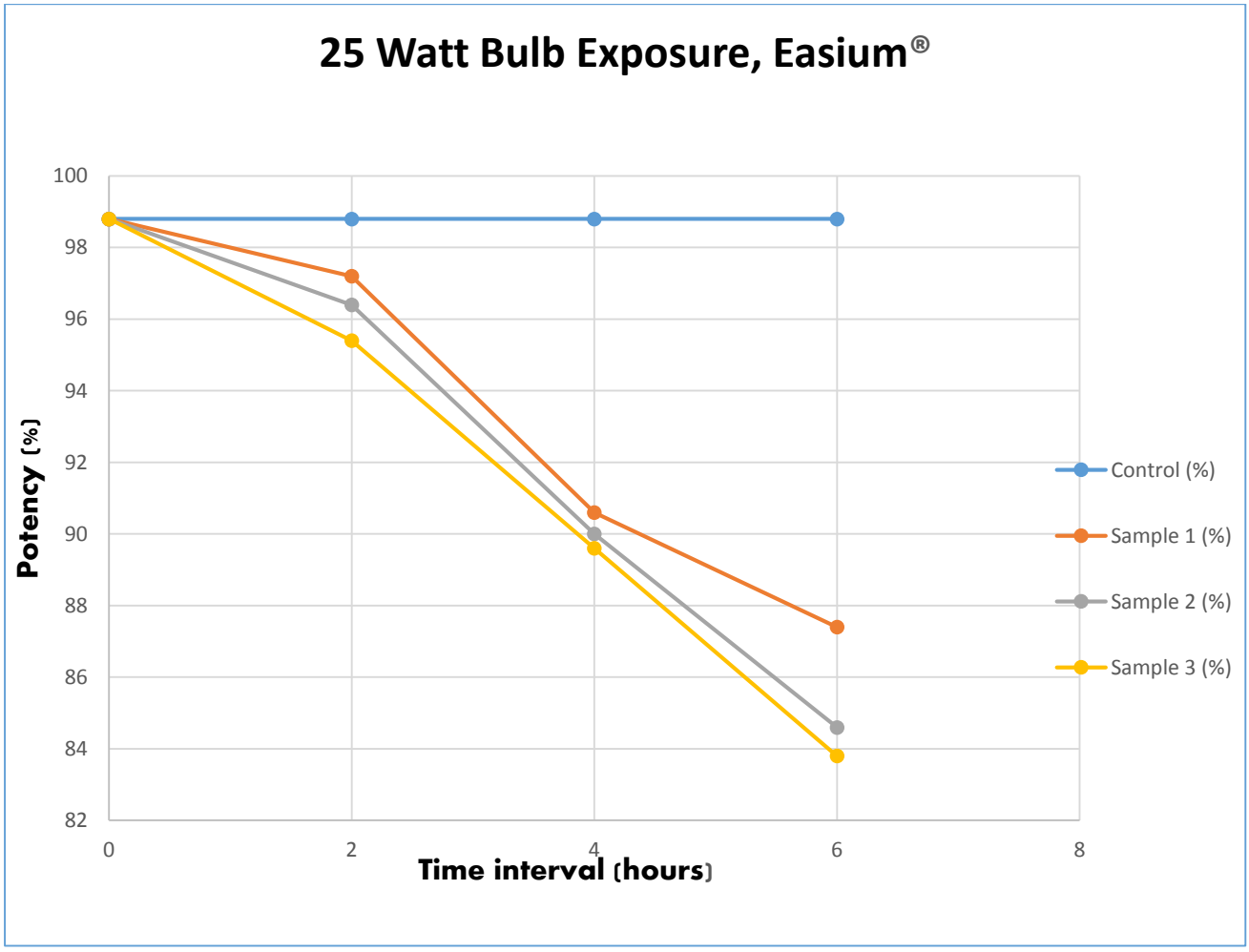

Fig 4.7: Graph showing the difference in Concentration after each 2 hour time intervalfor Easium®for 1st time[25 watt]

4.3.3Result of samples that were exposed under 25W bulb (2nd time)

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

Table 4.14: Concentration & absorbance of Easium® (diazepam) for 2nd time (0 hours)

Time Interval	Absorbance (at 240.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (1000 times diluted)	Amount of Drug present (in mg/ml)	Potency (%)
control	.465	.463	.00497	4.97	99.4%
	.463				
	.462				
	.465	.463	.00497	4.97	99.4%
	.463				
	.462				
	.465	.463	.00497	4.97	99.4%
	.463				
	.462				

Table 4.15: Concentration & absorbance of Easium® for 2nd time (2hours)

Time Interval	Absorbance (at 240.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (1000 times diluted)	Amount of Drug present (in mg/ml)	Potency (%)
2 hours	.455	.455	0.00488	4.88	97.6%
	.453				
	.456				
	.452	.452	.00485	4.85	97%
	.454				
	.451				
	.449	.449	.00482	4.82	96.4%
	.447				
	.450				

Table 4.16: Concentration & absorbance of Easium® for 2nd time (4 hours)

Time Interval	Absorbance (at 240.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (1000 times diluted)	Amount of Drug present (in mg/ml)	Potency (%)
4 hours	.420	.420	.00449	4.49	89.98%
	.422				
	.419				
	.403	.401	.00428	4.28	85.6
	.400				
	.401				
	.395	.393	.00419	4.19	83.8
	.393				
	.391				

Table 4.17: Concentration & absorbance of Easium® for 2nd time (6 hours)

Time Interval	Absorbance (at 240.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (1000 times diluted)	Amount of Drug present (in mg/ml)	Potency (%)
6 hours	.389	.389	.00415	4.15	83%
	.390				
	.387				
	.385	.386	.00412	4.12	82.4%
	.388				
	.386				
	.379	.381	.00406	4.06	81.2%
	.384				
	.381				

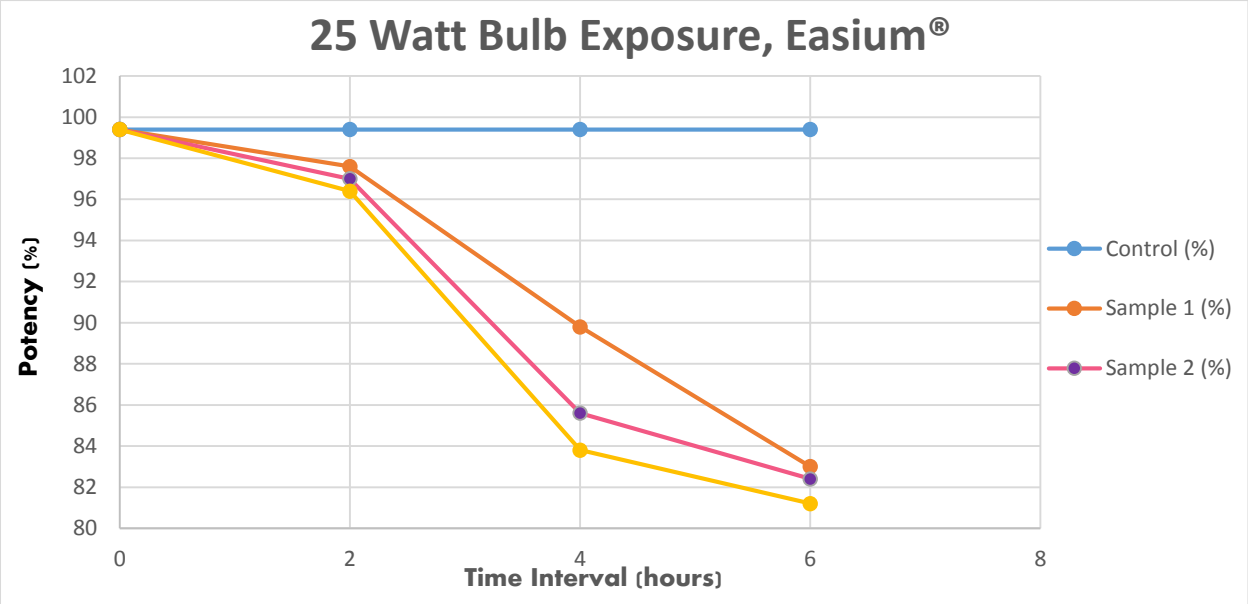

Fig 4.8: Graph showing the difference in Concentration after each 2 hour time interval for Easium®for 2nd time[25 watt]

Table 4.18: Concentration & absorbance of Easium® for 3rd time (0 hours)

Time Interval	Absorbance (at 240.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (1000times diluted)	Amount of Drug present (in mg/ml)	Potency (%)
Control	.460	.461	.00495	4.95	99%
	.461				
	.463				
	.460	.461	.00495	4.95	99%
	.461				
	.463				
	.460	.461	.00495	4.95	99%
	.461				
	.463				

Table 4.19: Concentration & absorbance of Easium® for 3rd time (2 hours)

Time Interval	Absorbance (at 240.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (1000 times diluted)	Amount of Drug present (in mg/ml)	Potency (%)
2 hours	.452	.452	.00485	4.85	97%
	.454				
	.454				
	.450	.450	.00483	4.83	96.6
	.448				
	.452				
	.445	.445	.00477	4.77	95.4
	.447				
	.445				

Table 4.20: Concentration & absorbance of Easium® for 3rd time (4 hours)

Time Interval	Absorbance (at 240.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (1000times diluted)	Amount of Drug present (in mg/ml)	Potency (%)
4 hours	.420	.419	.00448	4.48	89.6
	.421				
	.418				
	.414	.414	.00443	4.43	88.6
	.416				
	.414				
	.413				88%
	.410	**.412**	.00440	4.40	
	.412				

Table: 4.21; Concentration & absorbance of Easium® for 3rd time (6 hours)

Time Interval	Absorbance (at 240.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (1000 times diluted)	Amount of Drug present (in mg/ml)	Potency (%)
6 hours	.395	.393	.00419	4.19	83.8
	.393				
	.392				
	.390	.388	.00414	4.14	82.8
	.387				
	.389				
	.381	.381	.00406	4.06	81.2
	.382				
	.380				

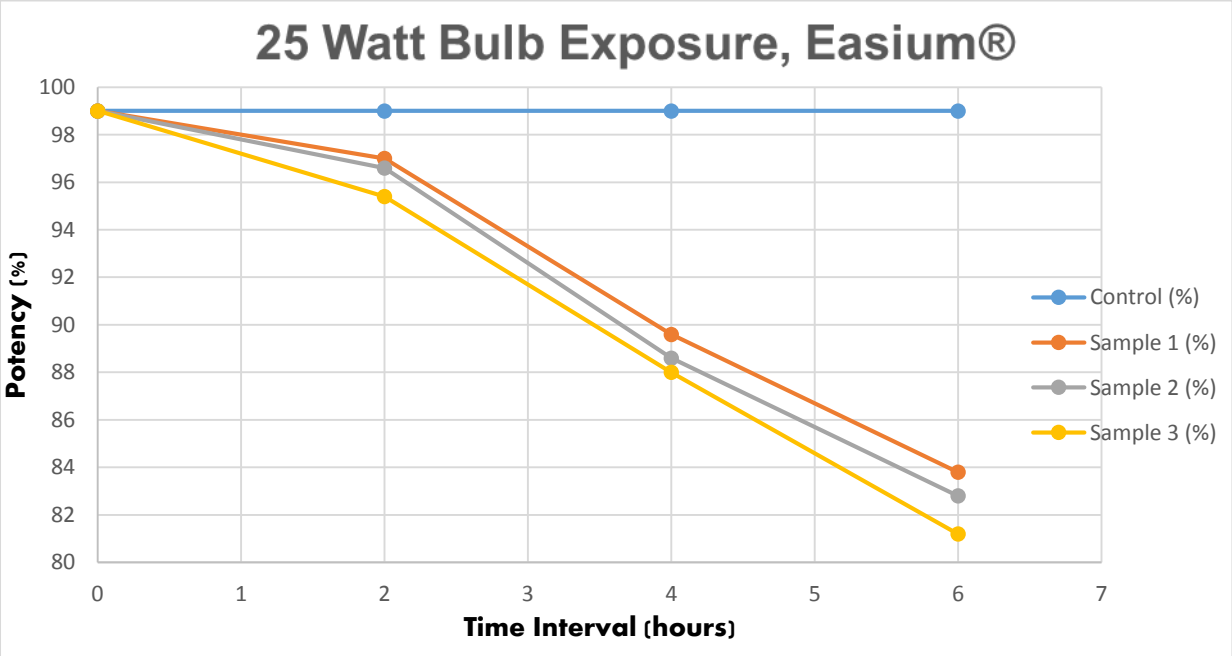

Fig 4.9: Graph showing the difference in Concentration after each 2 hour time interval for Easium®for 3rd time [25 watt]

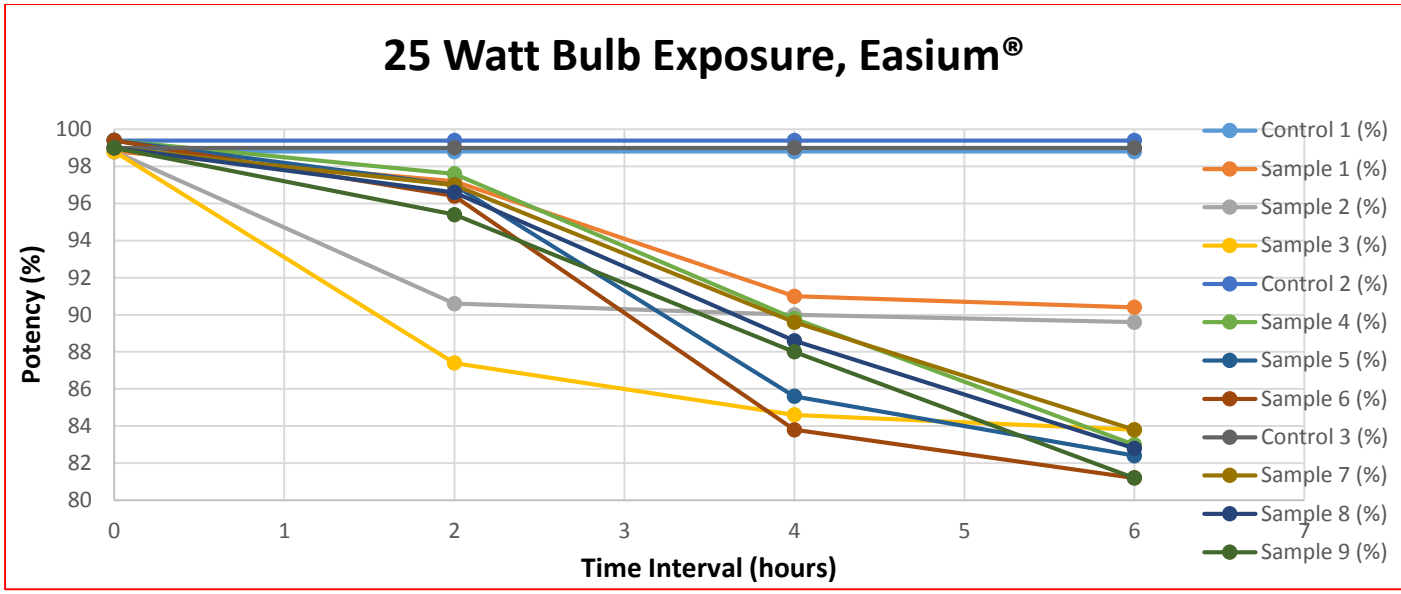

Fig 4.10: Graph showing the difference in Concentration after each 2 hour time interval for Easium®(diazepam) of the 1st, 2nd and 3rdtestunder 25W bulb exposure

4.3.4Result of samples that were exposed under 40W bulb

Three samples were exposed under the lamp (40W bulb); each for 2 hours time interval and found 36 different absorbance of diazepam and also observed that the concentration of Easium was declined in each time interval.

The results are as follows;

Table 4.22: Concentration & absorbance of Easium® for 1st time (0 hours)

Time Interval	Absorbance (at 240.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (1000 times diluted)	Amount of Drug present (in mg/ml)	Potency (%)
Control	.459	.460	00494	4.94	98.8%
	.460				
	.461				
	.459	460	00494	4.94	98.8%
	.460				
	.461				
	.459	460	00494	4.94	98.8%
	.460				
	.461				

Time Interval	Absorbance (at 240.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (1000 times diluted)	Amount of Drug present (in mg/ml)	Potency (%)
2 hours	.415	.417	.00446	4.46	89.2
	.419				
	.418				
	.413	.410	.00438	4.38	87.6
	.410				
	.408				
	.403	.401	.0042	4.20	84
	.401				
	.399				

Table 4.24: Concentration & absorbance of Easium® for 1st time (4 &6 hours)

Time Interval	Absorbance (at 240.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (1000 times diluted)	Amount of Drug present (in mg/ml)	Potency (%)
4 hours	.405	.403	00430	4.30	86
	.402				
	.401				
	.399	.397	00424	4.24	84.8
	.397				
	.396				
	.393	.390	00416	4.16	83.2
	.390				
	.388				
Time Interval	**Absorbance (at 240.5 nm)**	**Average Absorbance**	**Diluted Concentration from Samples in mg (1000 times diluted)**	**Amount of Drug present (in mg/ml)**	**Potency (%)**
6 hour	.363	.351	00372	3.72	74.4
	.367				
	.352				
	.350	.348	00369	3.69	73.8
	.348				
	.347				
	.345	.345	00366	3.66	73.2
	.348				
	.342				

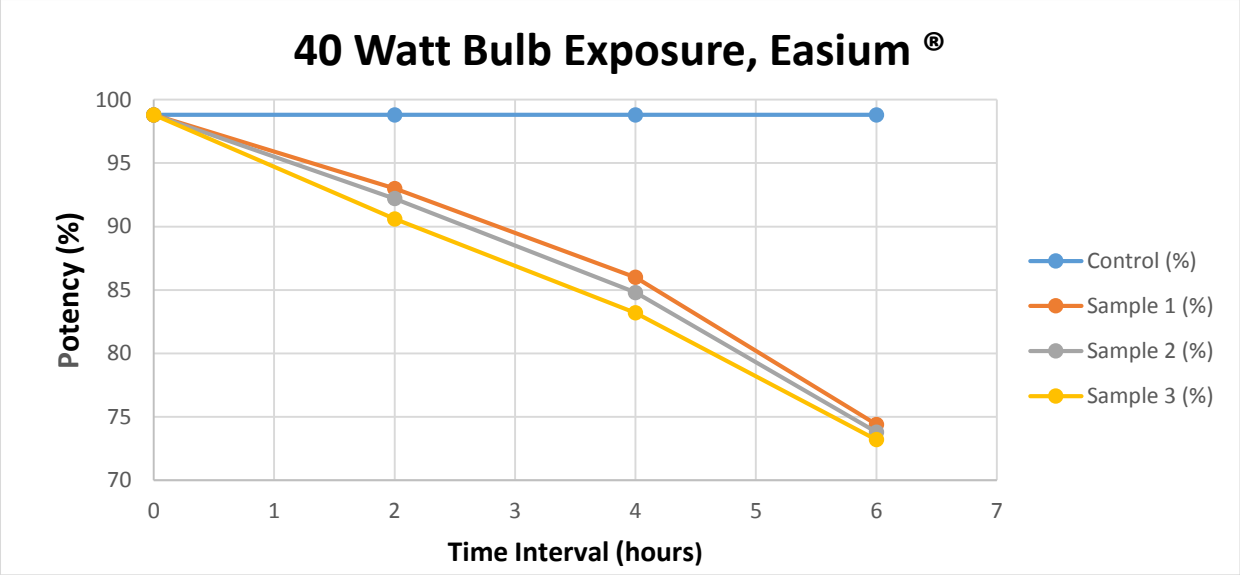

Fig 4.11: Graph showing the difference in Concentration after each 2 hour time interval for Easium®(diazepam)for 1st time [40 Watt]

Table 4.25: Concentration & absorbance of Easium® for 2nd time (0 hours)

Time Interval	Absorbance (at 240.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (1000 times diluted)	Amount of Drug present (in mg/ml)	Potency (%)
Control	.463	.465	.00491	4.91	98.2
	.465				
	.466				
	.463	.465	.00491	4.91	98.2
	.465				
	.466				
	.463	.465	.00491	4.91	98.2
	.465				
	.466				

Table 4.26: Concentration & absorbance of Easium® for 2nd time (2 hours)

Time Interval	Absorbance (at 240.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (1000 times diluted)	Amount of Drug present (in mg/ml)	Potency (%)
2 hours	.438	.439	00470	4.70	94%
	.440				
	.441				
	.439	.437	00468	4.68	93.6
	.436				
	.435				
	.432	.430	00461	4.61	92.2
	.430				
	.429				

Table 4.27: Concentration & absorbance of Easium® for 2nd time (4 hours)

Time Interval	Absorbance (at 240.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (1000 times diluted)	Amount of Drug present (in mg/ml)	Potency (%)
4 hours	.400	.398	00425	4.25	85
	.398				
	.395				
	.391	.389	.00415	4.15	83
	.389				
	.387				
	.384	.381	.00406	4.06	81.2
	.382				
	.379				

Table 4.28: Concentration & absorbance of Easium® for 2nd time (6 hours)

Time Interval	Absorbance (at 240.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (1000 times diluted)	Amount of Drug present (in mg/ml)	Potency (%)
6 hours	.348	.345	.00366	3.66	73.2
	.345				
	.342				
	.339	.339	.00359	3.59	71.8
	.337				
	.340				
	.332	.332	.00351	3.51	70.2
	.335				
	.330				

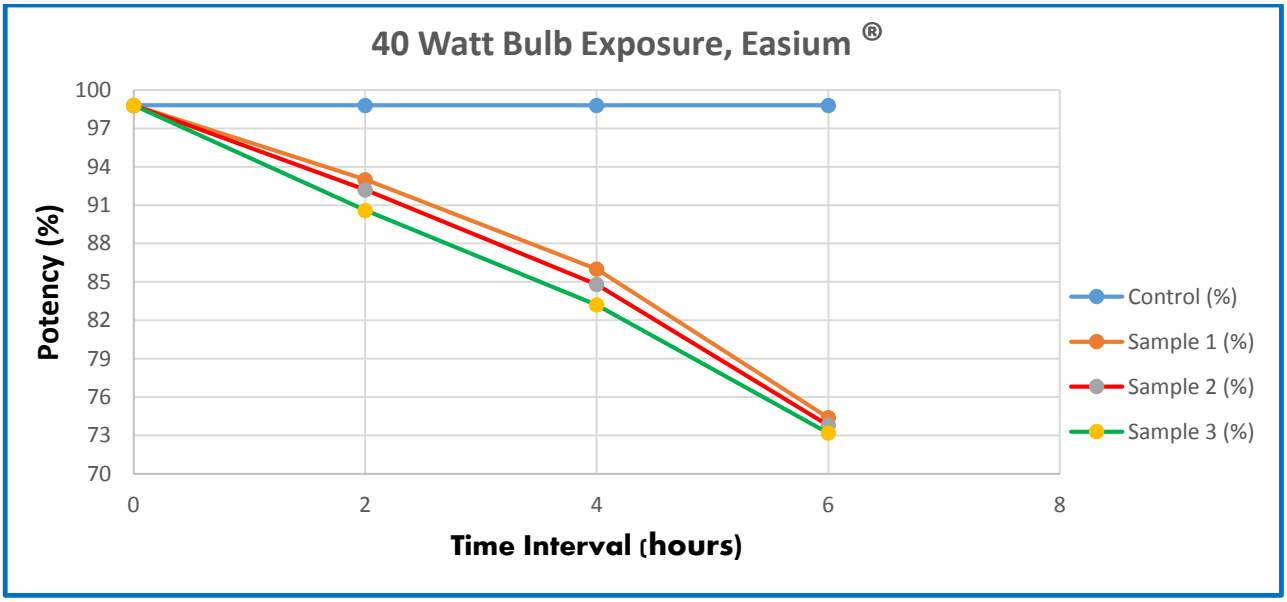

Fig 4.12: Graph showing the difference in Concentration after each 2 hour time interval for Easium®(diazepam)for 2nd time[40 watt]

Table4.29: Concentration & absorbance of Easium® for 3rd time (0 hours)

Time Interval	Absorbance (at 240.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (1000 times diluted)	Amount of Drug present (in mg/ml)	Potency (%)
Control	.466	.466	.00501	5.01	100.2
	.468				
	.463				
	.466	.466	.00501	5.01	100.2
	.468				
	.463				
	.466	.466	.00501	5.01	100.2
	.468				
	.463				

Table 4.30: Concentration & absorbance of Easium® for 3rd time (2 hours)

Time Interval	Absorbance (at 240.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (1000 times diluted)	Amount of Drug present (in mg/ml)	Potency (%)
2 hours	.450	.450	.00483	4.83	96.6
	.453				
	.449				
	.445	.443	.00475	4.75	95
	.441				
	.443				
	.438	.438	.00469	4.69	93.8
	.440				
	.435				

Table 4.31: Concentration & absorbance of Easium® for 3rd time (4 hours)

Time Interval	Absorbance (at 240.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (1000 times diluted)	Amount of Drug present (in mgml)	Potency (%)
4 hours	.412	.410	.00438	4.38	87.6
	.410				
	.408				
	.403	.400	.00427	4.27	85.4
	.400				
	.399				
	.393	.391	.00417	4.17	83.4
	.391				
	.389				

Table 4.32: Concentration & absorbance of Easium® for 3rd time (6 hours)

Time Interval	Absorbance (at 240.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (1000 times diluted)	Amount of Drug present (in mg/ml)	Potency (%)
6 hours	.369	.368	.00391	3.91	78.2
	.370				
	.365				
	.362	.360	.00382	3.82	76.4
	.360				
	.359				
	.350	.350	.00371	3.71	74.2
	.348				
	.352				

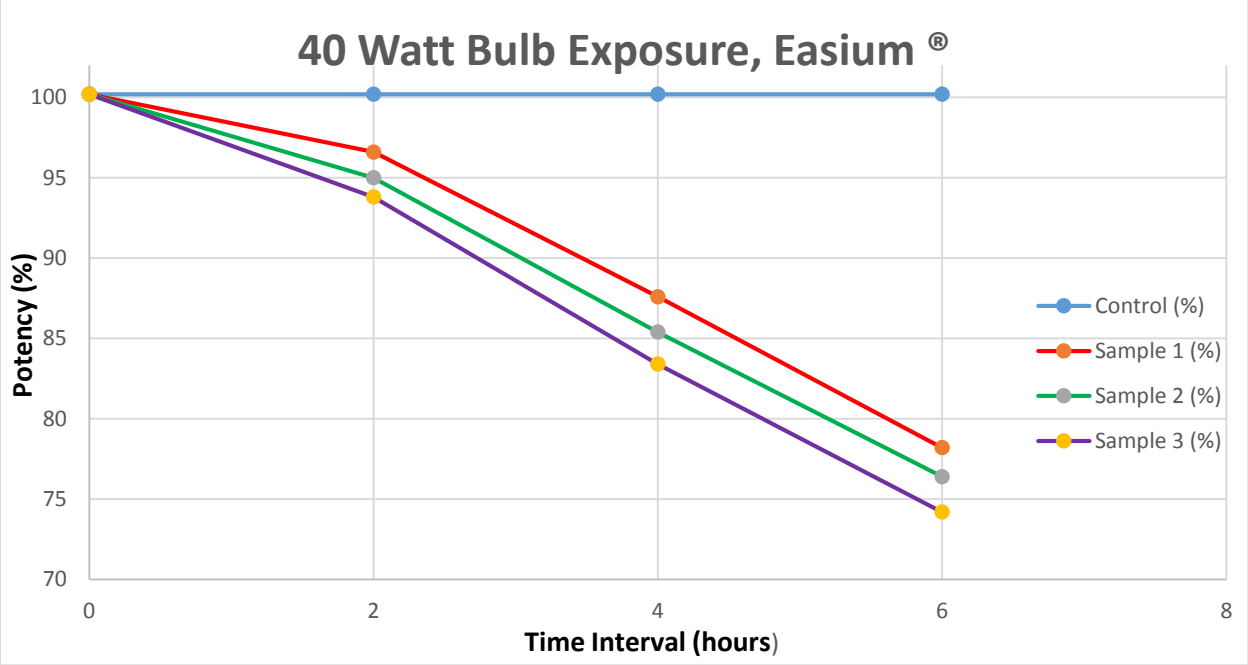

Fig 4.13: Graph showing the difference in Concentration after each 2 hour time interval for Easium® of the 3rdtestunder 40W bulb exposure

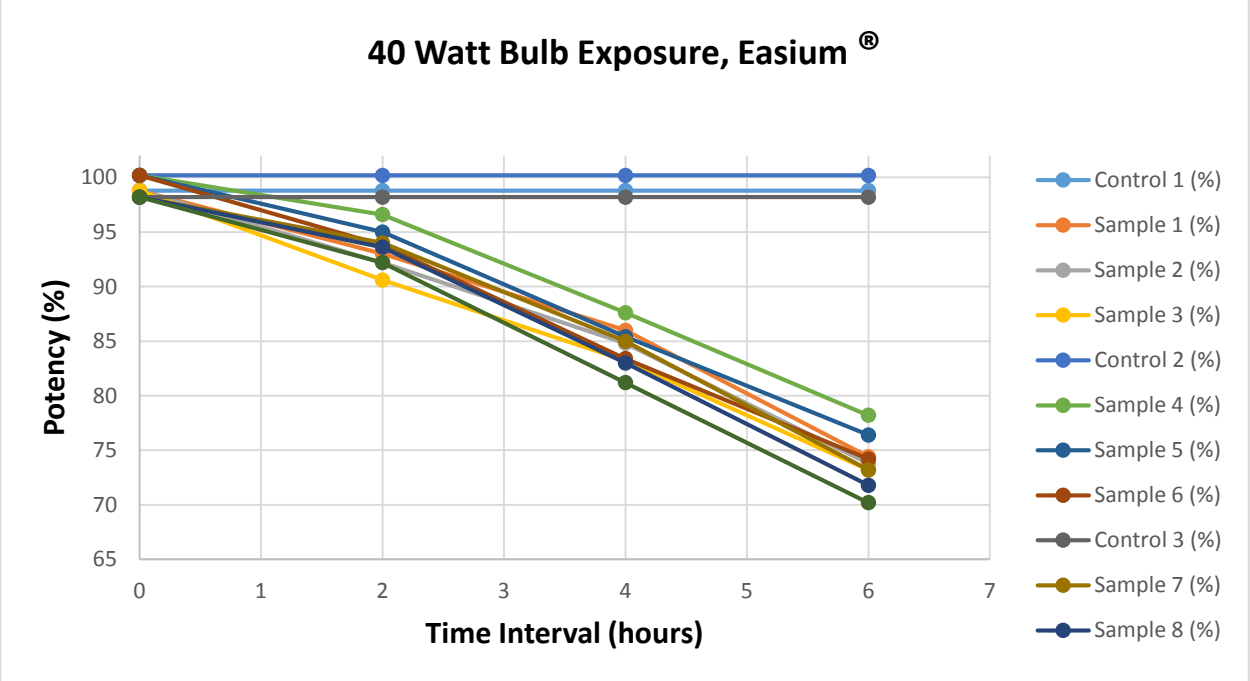

Fig 4.14: Graph showing the difference in Concentration after each 2 hour time interval for (Easium®diazepam) of the 1st, 2nd and 3rdtestunder 40W bulb exposure

4.3.5 Result of samples that were exposed under direct sunlight

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

Table 4.33: Concentration & absorbance for Easium®of the 1st test under direct sunlight exposure

Time Interval	Absorbance (at 240.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (1000 times diluted)	Amount of Drug present (in mg/ml)	Potency (%)
Control	.455	.455	.00488	4.88	97.6
	.458				
	.452				
	.455	.455	.00488	4.88	97.6
	.458				
	.452				
	.455	.455	.00488	4.88	97.6
	.458				
	.452				

Table 4.34: Concentration & absorbance for Easium®of the 1st test under direct sunlight

Time Interval	Absorbance (at 240.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (1000 times diluted)	Amount of Drug present (in mg/ml)	Potency (%)
2 hours	.415	.417	.00446	4.46	89.2
	.419				
	.418				
	.413	.410	.00438	4.38	87.6
	.410				
	.408				
	.403	.401	.0042	4.20	84
	.401				
	.399				

Table4.35: Concentration & absorbance for Easium®of the 1st test under direct sunlight

Time Interval	Absorbance (at 240.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (1000 times diluted)	Amount of Drug present (in mg/ml)	Potency (%)
4 hours	.391	.388	.00414	4.14	82.8
	.389				
	.384				
	.387	.384	.00409	4.09	81.8
	.385				
	.381				
	.379	.377	.00401	4.01	80.2
	.375				
	.376				

Time Interval	Absorbance (at 240.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (1000 times diluted)	Amount of Drug present (in mg/ml)	Potency (%)
6 hours	.349	.350	.00371	3.71	74.2
	.352				
	.350				
	.340	.340	.00360	3.60	72
	.341				
	.338				
	.335	.332	.00351	3.51	70.2
	.332				
	.333				
	.331				

Table 4.36: Concentration & absorbance for Easium®of the 1st test under direct sunlight

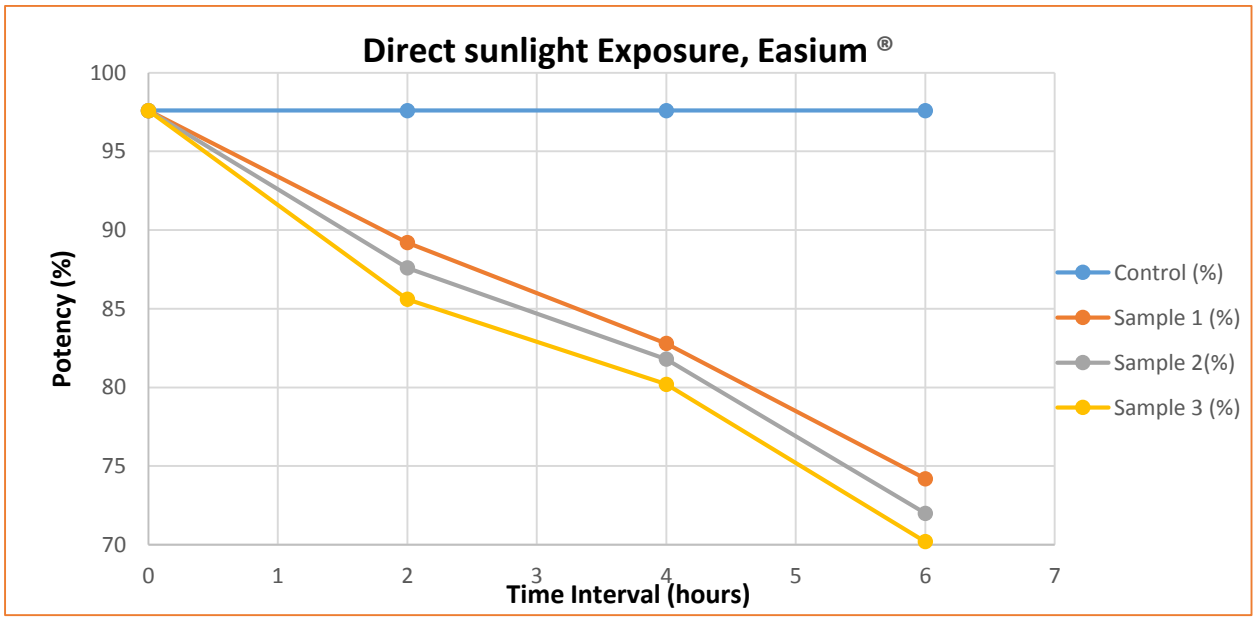

Fig 4.15: Graph showing the difference in Concentration after each 2 hour time interval for Easium® of the 1sttestunder direct sunlight exposure

Table 4.37: Concentration & absorbance for Easium®of the 2nd test under direct sunlight

Time Interval	Absorbance (at 240.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (1000 times diluted)	Amount of Drug present (in mg/ml)	Potency (%)
Control	.465	.463	.00497	4.97	99.4%
	.463				
	.462				
	.465	.463	.00497	4.97	99.4%
	.463				
	.462				
	.465	.463	.00497	4.97	99.4%
	.463				
	.462				

Table 4.38: Concentration & absorbance for Easium®of the 2nd test under direct sunlight

Time Interval	Absorbance (at 240.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (1000 times diluted)	Amount of Drug present (in mg/ml)	Potency (%)
2 hours	.412	.410	.00438	4.38	87.6
	.410				
	.408				
	.407	.406	.00434	4.34	86.8
	.405				
	.407				
	.400	.400	.00427	4.27	85.4
	.398				
	.402				

Table 4.39: Concentration & absorbance for Easium®of the 2nd test under direct sunlight

Time Interval	Absorbance (at 240.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (1000 times diluted)	Amount of Drug present (in mg/ml)	Potency (%)
4 hours	.348	.345	.00366	3.66	73.2
	.342				
	.345				
	.343	.343	.00363	3.63	72.6
	.346				
	.342				
	.340	.338	.00358	3.58	71.6
	.337				
	.338				

Table 4.4: Concentration & absorbance for Easium®of the 2nd test under direct sunlight

Time Interval	Absorbance (at 240.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (1000 times diluted)	Amount of Drug present (in mg/ml)	Potency (%)
6 hours	.333	.331	.00350	3.50	70
	.331				
	.330				
	.327	.325	.00343	3.43	68.6
	.324				
	.325				
	.323	.323	.00341	3.41	68.2
	.325				
	.320				

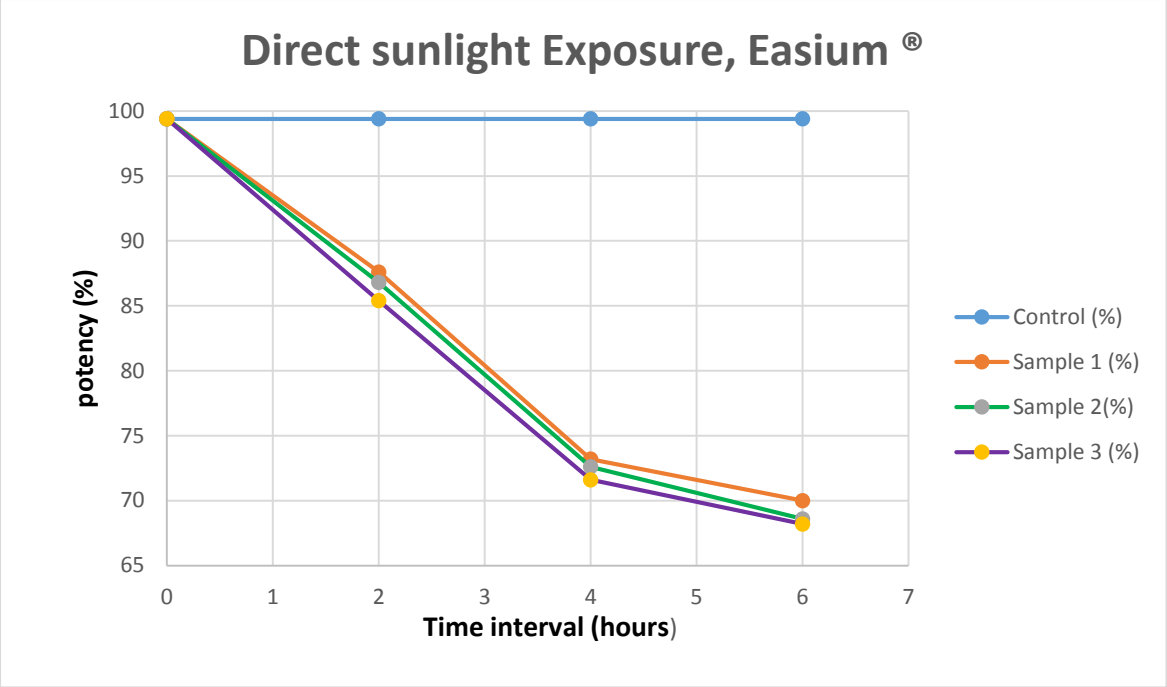

Fig 4.16: Graph showing the difference in Concentration after each 2 hour time interval for Easium® of the 2nd testunder direct sunlight exposure

Table 4.41: Concentration & absorbance for Easium®of the 3rd test under direct sunlight

Time Interval	Absorbance (at 240.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (1000 times diluted)	Amount of Drug present (in mg/ml)	Potency (%)
Control	.460	.460	.00494	4.94	98.8
	.462				
	.459				
	.460	.460	.00494	4.94	98.8
	.462				
	.459				
	.460	.460	.00494	4.94	98.8
	.462				
	.459				

Table 4.42: Concentration & absorbance for Easium®of the 3rd test under direct sunlight

Time Interval	Absorbance (at 240.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (1000 times diluted)	Amount of Drug present (in mg/ml)	Potency (%)
2 hours	.413	.412	.00440	4.40	88
	.410				
	.412				
	.412	.409	.00437	4.37	87.4
	.408				
	.407				
	.405	.402	.00429	4.29	85.8
	.401				
	.400				

Table4.43: Concentration & absorbance for Easium®of the 3rd test under direct sunlight

Time Interval	Absorbance (at 240.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (1000 times diluted)	Amount of Drug present (in mg/ml)	Potency (%)
4 hours	.341	.343	.00364	3.64	72.8
	.345				
	.343				
	.340	.342	.00362	3.62	72.4
	.343				
	.342				
	.339	.341	.00361	3.61	72.2
	.343				
	.341				

Table 4.45: Concentration & absorbance for Easium®of the 3rd test under direct sunlight

Time Interval	Absorbance (at 240.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (1000 times diluted)	Amount of Drug present (in mg/ml)	Potency (%)
6 hours	.331	.333	.00352	3.52	70.04
	.334				
	.333				
	.329	.329	.00348	3.48	69.6
	.327				
	.330				
	.325	.322	.00340	3.40	68
	.321				
	.319				

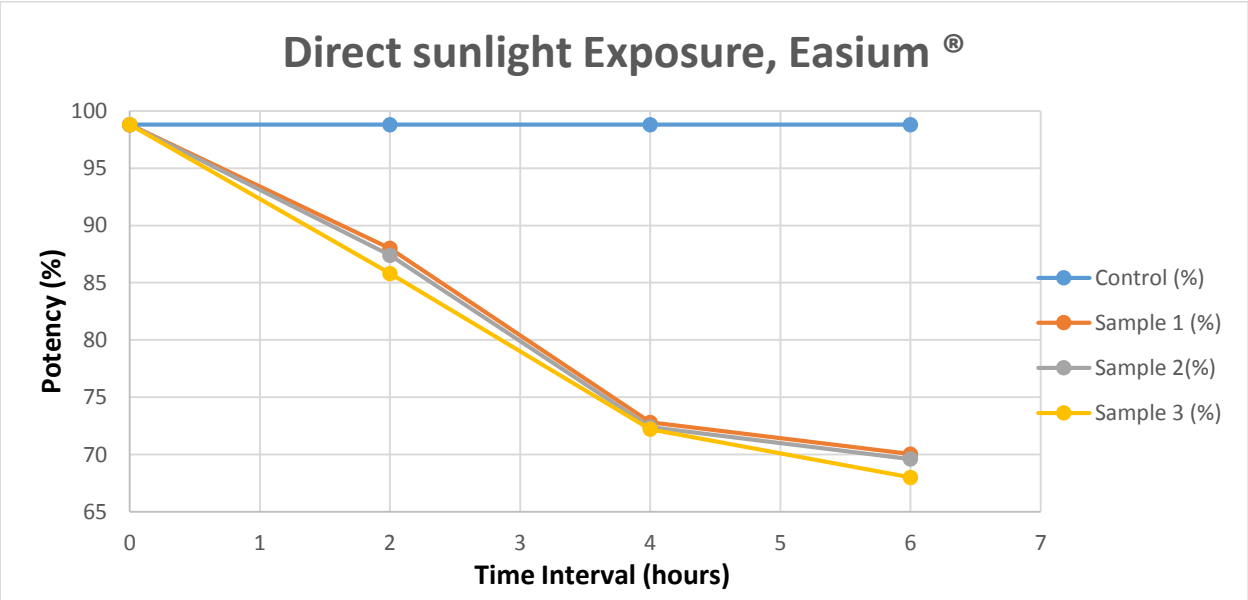

Fig 4.17: Graph showing the difference in Concentration after each 2 hour time interval for Easium® (diazepam) of the 3rdtestunder direct sunlight exposure

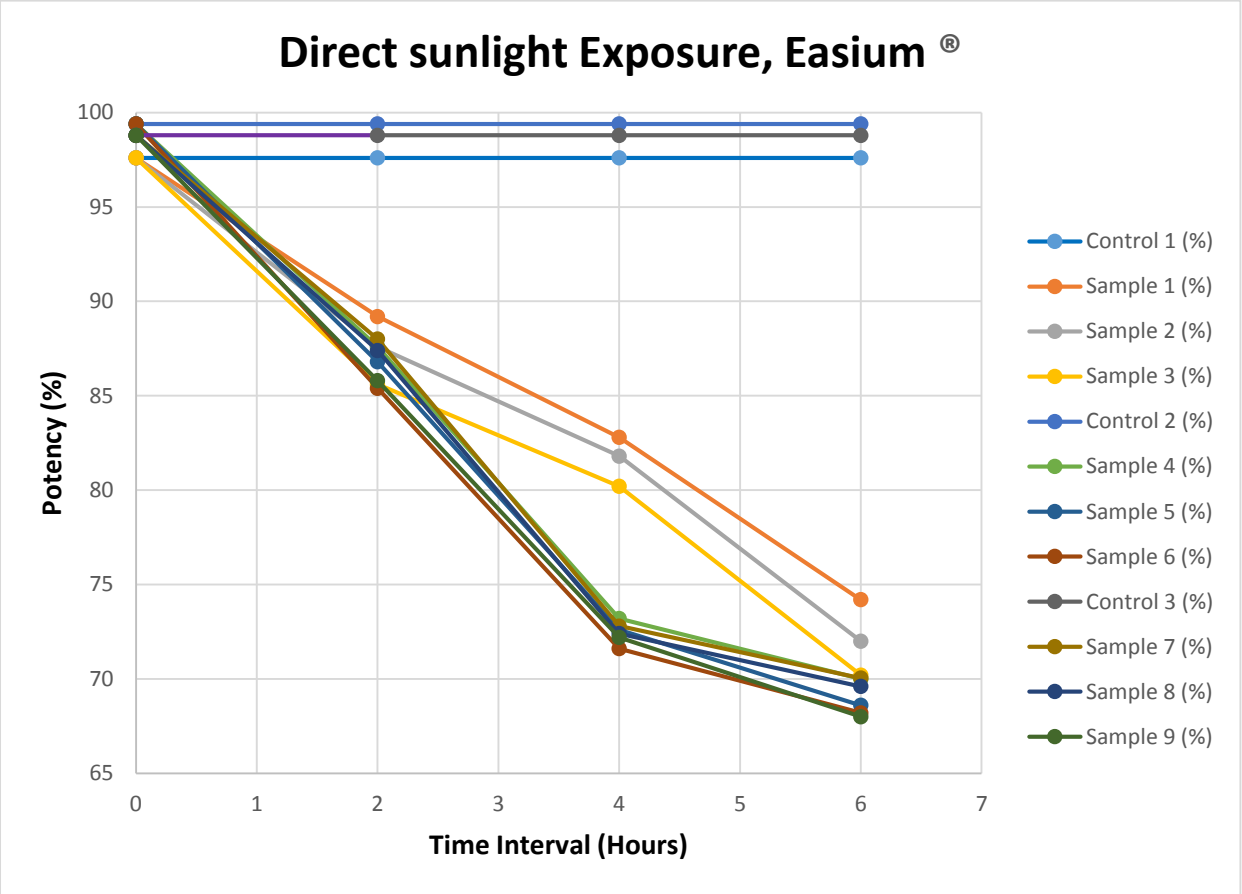

Figure 4.18: Graph showing the difference in Concentration after each 2 hour time interval for Easium® (diazepam) of the 1st, 2nd and 3rdtestunder direct sunlight exposure

Chapter Five

Discussion

In this experiment, it was found that the measured physical parameters- color test, weight variation, hardness and thickness- did not change significantly throughout the course of the study. Average weight, hardness and also thickness of the tablets were close to each other. The standard deviation of weight variation , hardness and thickness was ±0.001g cm, ±±0.2kg. kg&±0.0009cmgm respectively.So, it can be said that light has little or no effect on the color, weight, hardness and thickness of Easium® (Diazepam).

After completing the study, it was found that the potency of diazepam was remarkably decreased y in every ovation of light exposure. When sample tablet (Easium®) was kept under the electrical bulb (25 watt & 40 Watt) every two hour light exposed sample tablet was tested and it was found that the concentration of diazepam was decreased gradually. The tablet sample which were exposed 4 hours on light had less potency than the 2 hour exposed sample tablet and we had also found that 6 hour exposed sample tablets have even less potency of diazepam than 2 hour and 4 hour light exposed sample. The same result was found for the sunlight exposed sample tablets. The study of 60 days for the normal lightening and room temperature condition also found that the concentrations of the samples were decreased gradually.

Potency test was performed by UV spectroscopy at 240.5 nm wavelength which showed gradual decline in potency of the tablet. In various lighting condition like 25watt bulb, 40watt bulb, direct sunlight and normal room light,the percent variation in potency was13.66%, 22.3%, 26.01% and 20.01% respectively.

So from this study it is verified that only blister packaging of Easium®containing (Diazepam) may not protect it from photolytic degradation since storage condition is different throughout the country. Therefore, to prevent this photolytic degradation protective opaque packaging should be used.

Chapter Six

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