Reproducibility Study of the Packaging Efficiency on Preventing Photolytic Degradation in Sedil® (Diazepam) Tablets

Submitted By

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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"

Dedication

I want to dedicate this thesis paper to my respective parents and lovely little sister.

DECLARATION BY THE CANDIDATE

I, SumiyaKhondokerMitu, hereby declare that the dissertation entitled "Evaluation of Coating Efficiency on Photolytic Degradation of Sedil® (Diazepam)", submitted by me to the Department of Pharmacy, East West University, in the partial fulfillment of the requirement for the degree of Bachelor of Pharmacy (Honors) with original research work carried out by me under the supervision and guidance of Md. AnisurRahman, Senior Lecturer, Department of Pharmacy, East West University, Dhaka.

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CERTIFICATE BY THE SUPERVISOR

This is to certify that the dissertation entitled

"Evaluation of Coating Efficiency on Photolytic Degradation of 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 bySumiyaKhondokerMitu (ID: 2011-1-70-005) 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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ABSTRACT

This work wasaimed for the determination reproducibility study of the packaging efficiency on preventing photolytic degradation of Sedil® (diazepam) which contained in a blister packaging with coating and which is photosensitive. The objective of this study was to determine the effect onDiazepam in various lighting conditions (control, sunlight, normal room light, 25watt & 40watt bulb). Besides,physical tests were performed for evaluation of color change, weight variation, thickness and hardness of Sedil® tablets from same batch according to the specification of USP. A very insignificant fluctuation in result was observed, with standard deviation ±0.001g, ±0.0009cm & ±0.2 kg for weight variation, thickness & hardness test respectively. 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%, 24.29%, 27.87%, and 31.66%. So it can be said that the Sedil® containing Diazepam is light sensitive, package should be opaque thus light cannot pass through the package.

Keywords: Sedil®, Diazepam, Photolytic Degradations, Batch, Weight variation, Hardness, Thickness, Potency, USP.

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CHAPTER ONE

INTRODUCTION

1. Overview

The objective of the study is to determine reproducibility of the packaging efficiency on preventing photolytic degradation of Sedil® (diazepam) which contained in a blister packaging with coating and which is photosensitive. In this research photosensitivity of diazepam various lightening conditions (control, sunlight, normal light, 25watt bulb and 40watt bulb condition) were determined. Only few drugs contain opaque packaging in local market. So a photosensitivity test study was performed with that of the drugs which have blister packaging to evaluating coating is efficient or not.

1,1Drug 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.

1.2 Photolytic Degradation (Kumar et al. 2013)

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

1.2.1 Photolytic Condition

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

1.2.2 Mechanism of Photolytic Degradation

Drug products are placed and exposed under the light source

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

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

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

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

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

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

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1.3 Benzodiazepines

Benzodiazepines are a class of psychoactive drugs whose core chemical structure is the fusion of a benzene ring and a diazepine ring. The first such drug, chlordiazepoxide (Librium), was discovered accidentally by Leo Sternbach in 1955, and made available in 1960 by Hoffmann–La Roche - which, since 1963, has also marketed the benzodiazepine diazepam (Valium). (Shorter E ,2005)

Benzodiazepines enhance the effect of the neurotransmittergamma-aminobutyric acid (GABA) at the $GABA_A$ receptor, resulting in sedative, hypnotic (sleep-inducing), anxiolytic (anti-anxiety), anticonvulsant, and muscle relaxant properties. High doses of many shorter-acting benzodiazepines may also cause anterograde amnesia and dissociation. These properties make benzodiazepines useful in treating anxiety, insomnia, agitation, seizures, muscle spasms, alcohol withdrawal and as a premedication for medical or dental procedures. Benzodiazepines are generally viewed as safe and effective for short-term use, although cognitive impairment and paradoxical effects such as aggression or behavioral disinhibition occasionally occur. A minority of people can have paradoxical reactions such as worsened agitation or panic. Long-term use is controversial due to concerns about adverse psychological and physical effects, decreasing effectiveness, and physical dependence and withdrawal. Due to adverse effects associated with the long-term use of benzodiazepines, withdrawal from benzodiazepines, in general, leads to improved physical and mental health. (PharmGKB, 2015)

There are variety of benzodiazepine derivatives found like alprazolam, bromazepam, clonazepam, diazepam, estazolam, flurazepam etc. Among them Diazepam is the main concern of this experiment.

1.4 Diazepam

Diazepam is a medication of the benzodiazepine type. It is commonly used to treat a range of conditions including anxiety, alcohol withdrawal syndrome, benzodiazepine withdrawal syndrome, muscle spasms, seizures, trouble sleeping, and restless legs syndrome. It may also be used to cause memory loss during certain medical procedures.

Diazepam first synthesized by Leo Sternbach. It was launched in 1963 and became the most common prescribed drug to treat wide range of conditions. (Drugs.com, 2015)

In this research project experiment conducted on sample which was manufactured by Square Pharmaceuticals Ltd. (Brand name: Sedil®). It is obtained as a solid dosage form which is mainly tablets. This diazepam containing medicine is mainly used as Tranquillizers (CNS Preparations). Besides this, it is also indicated as anxiety pain from apprehension and depression, acute and chronic stress of life, skeletal muscle spasm and strychnine poisoning

1.5Physico- chemical properties: (Inchem.org, 2015)

1.5.1 Chemical properties:

- Chemical name: 7-Chloro-1, 3-dihydro-1-methyl-5-phenyl-2H-1, 4-benzodiazepin-2-one
- Molecular formula: C16H13ClN2O
- Molecular weight : 284.7 g/mol

Figure: 1.1: Chemical structure of Diazepam

1.5.2 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

1.6Mechanism of action

Diazepam is a benzodiazepine that binds to a specific subunit on the GABA receptor at a site that is distinct from the binding site of the endogenous GABA molecule. The GABA receptor is an inhibitory channel which, when activated, decreases neuronal activity. Because of the role of diazepam as a positive allosteric modulator of GABA, when it binds to benzodiazepine receptors it causes inhibitory effects. This arises from the hyperpolarization of the post-synaptic membrane owing to the control exerted over negative chloride ions by GABAAreceptors. Benzodiazepines including diazepam however, do not have any effect on the levels of GABA in the brain. Diazepam appears to act on areas of the limbic system, thalamus and hypothalamus, inducing anxiolytic effects. Its actions are due to the enhancement of GABA activity. Benzodiazepine drugs including diazepam increase the inhibitory processes in the cerebral cortex.(Inkling.com, 2014), (Pharmacologycorner.com, 2014), (Mybwmc.org, 2014)

1.7Pharmacology: (RxList, 2015)

1.7.1 Absorption: 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

1.7.2 Distribution: 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.

1.7.3 Metabolism: 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.

1.7.4 Elimination: 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.

1.8 Indication (Nhtsa.gov, 2015), (Mybwmc.org, 2014)

Diazepam is mainly used to treat anxiety, insomnia, panic attacks and symptoms of acute alcohol withdrawal. It is also used as a premedication for inducing sedation, anxiolysis, or amnesia before certain medical procedures (e.g., endoscopy). Diazepam is the drug of choice for treating benzodiazepine dependence with its long duration of action allowing easier dose reduction. Benzodiazepines have a relatively low toxicity in overdose.

Diazepam has a number of uses including:

- Treatment of anxiety, panic attacks, and states of agitation
- Treatment of neurovegetative symptoms associated with vertigo
- Treatment of the symptoms of alcohol, opiate, and benzodiazepine withdrawal
- Short-term treatment of insomnia
- Treatment of tetanus, together with other measures of intensive treatment
- Adjunctive treatment of spastic muscular paresis (paraplegia/tetraplegia) caused by cerebral or spinal cord conditions such as stroke, multiple sclerosis, or spinal cord injury (long-term treatment is coupled with other rehabilitative measures)
- Palliative treatment of stiff person syndrome
- Pre- or postoperative sedation, anxiolysis and/or amnesia (e.g., before endoscopic or surgical procedures)
- Treatment of complications with a hallucinogen crisis and stimulant overdoses and psychosis, such as LSD, cocaine, or methamphetamine
- Preventative treatment of oxygen toxicity during hyperbaric oxygen therapy.

1.9 CONTRAINDICATIONS (Xpil.medicines.org.uk, 2015), (rochecanada, 2015)

Diazepam is contra-indicated in:

1. Patients with known hypersensitivity to benzodiazepines.
2. Patients with chronic obstructive airways disease with incipient respiratory failure.
3. Patients with severe respiratory insufficiency.
4. Patients with severe hepatic insufficiency.

5. Patients with sleep apnoea syndrome.
6. Patients with myasthenia gravis.
7. Patients with dependence on other substances including alcohol. An exception to the latter is the management of acute withdrawal reactions.

Benzodiazepines are not recommended for the primary treatment of psychotic illness.

Benzodiazepines should not be used alone to treat depression or anxiety associated with depression as suicide may occur in such patients.

1.10 Precautions: (Mybwmc.org, 2015), (RxList, 2015)

General: If Diazepam is to be combined with other psychotropic agents or anticonvulsant drugs, careful consideration should be given to the pharmacology of the agents to be employed - particularly with known compounds that may potentiate the action of diazepam, such as phenothiazines, narcotics, barbiturates, MAO inhibitors and other antidepressants. The usual precautions are indicated for severely depressed patients or those in whom there is any evidence of latent depression or anxiety associated with depression, particularly the recognition that suicidal tendencies may be present and protective measures may be necessary. Psychiatric and paradoxical reactions are known to occur when using benzodiazepines. A lower dose is recommended for patients with chronic respiratory insufficiency, due to the risk of respiratory depression. Benzodiazepines should be used with extreme caution in patients with a history of alcohol or drug abuse.

Pregnancy: An increased risk of congenital malformations and other developmental abnormalities associated with the use of benzodiazepine drugs during pregnancy has been suggested

Nursing Mothers: Diazepam passes into breast milk. Breastfeeding is therefore not recommended in patients receiving Diazepam.

Pediatric Use: Safety and effectiveness in pediatric patients below the age of 6 months have not been established.

Geriatric Use: In elderly patients, it is recommended that the dosage be limited to the smallest effective amount to preclude the development of ataxia or over sedation (2 mg to 2.5 mg once or twice daily, initially to be increased gradually as needed and tolerated).

Extensive accumulation of diazepam and its major metabolite, desmethyldiazepam, has been noted following chronic administration of diazepam in healthy elderly male subjects.

Hepatic Insufficiency: Decreases in clearance and protein binding, and increases in volume of distribution and half-life has been reported in patients with cirrhosis. In such patients, a 2- to 5- fold increase in mean half-life has been reported. Delayed elimination has also been reported for the active metabolite desmethyldiazepam. Benzodiazepines are commonly implicated in hepatic encephalopathy. Increases in half-life have also been reported in hepatic fibrosis and in both acute and chronic hepatitis

1.11 Diazepam 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 developemental 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 druf 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, 2015)

1.12 Interactions with other medicines: (Rochecanada, 2015), (Mybwmc.org, 2014)

1. The benzodiazepines, including diazepam, produce additive CNS depressant effects when coadministered with other medications which themselves produce CNS depression, e.g. barbiturates, alcohol, sedatives, anxiolytics, antidepressants including tricyclic antidepressants and non-selective MAO inhibitors, hypnotics, antiepileptic drugs, phenothiazines and other antipsychotics, skeletal muscle relaxants, antihistamines or narcotic analgesics and anaesthetics. Therefore, it should be borne in mind that the effect of these drugs may potentiate or be potentiated by the action of diazepam. Concomitant use with alcohol is not recommended due to enhancement of the sedative effect.

2. There is a potentially relevant interaction between diazepam and compounds which inhibit certain hepatic enzymes (particularly cytochrome P450IIIA). Data indicate that these

compounds influence the pharmacokinetics of diazepam and may lead to increased and prolonged sedation. Diazepam undergoes oxidative metabolism, and consequently may interact with disulfiram, cimetidine, ketoconazole, fluvoxamine, fluoxetine or omeprazole resulting in increased plasma levels of diazepam. Patients should be observed closely for evidence of enhanced benzodiazepine response during concomitant treatment with either disulfiram or cimetidine; some patients may require a reduction in benzodiazepine dosage. There have also been reports that the metabolic elimination of phenytoin is affected by diazepam.

3. Cisapride may lead to a temporary increase in the sedative effects of orally administered benzodiazepines due to faster absorption.
4. The anticholinergic effects of other drugs, including atropine and similar drugs, antihistamines
and antidepressants may be potentiated.
5. Interactions have been reported between some benzodiazepines and anticonvulsants, with changes in the serum concentration of the benzodiazepine or anticonvulsant. It is recommended that patients be observed for altered responses when benzodiazepines and anticonvulsants are prescribed together, and that serum level monitoring of the anticonvulsant be performed more frequently.

1.13 Dependence

Improper or excessive use of diazepam can lead to physical dependence and psychological dependence. At a particularly high risk for diazepam misuse, abuse or psychological dependence are:

- People with a history of alcohol or drug abuse or dependenc. Diazepam increases craving for alcohol in problem alcohol consumers. Diazepam also increases the volume of alcohol consumed by problem drinkers.
- People with severe personality disorders, such as borderline personality disorder

Patients from the aforementioned groups should be monitored very closely during therapy for signs of abuse and development of dependence. Therapy should be discontinued if any of these signs are noted, although if physical dependence has developed, therapy must still be

discontinued gradually to avoid severe withdrawal symptoms. Long-term therapy in these people is not recommended

People suspected of being physiologically dependent on benzodiazepine drugs should be very gradually tapered off the drug. Withdrawals can be life-threatening, particularly when excessive doses have been taken for extended periods of time. Equal prudence should be used whether dependence has occurred in therapeutic or recreational contexts. (Thomson Healthcare Micromedex ,March 2000).

1.14 Side effects: (RxList, 2015)

Side effects most commonly reported were drowsiness, fatigue, muscle weakness, and ataxia. The following have also been reported:

Central Nervous System: confusion, depression, dysarthria, headache, slurred speech, tremor, vertigo

Gastrointestinal System: constipation, nausea, gastrointestinal disturbances

Special Senses: blurred vision, diplopia, dizziness

Cardiovascular System: hypotension

Psychiatric and Paradoxical Reactions: stimulation, restlessness, acute hyperexcited states, anxiety, agitation, aggressiveness, irritability, rage, hallucinations, psychoses, delusions, increased muscle spasticity, insomnia, sleep disturbances, and nightmares. Inappropriate behavior and other adverse behavioral effects have been reported when using benzodiazepines. They are more likely to occur in children and in the elderly.

Urogenital System: incontinence, changes in libido, urinary retention

Skin and Appendages: skin reactions

Laboratories: elevated transaminases and alkaline phosphatase

Other: changes in salivation, including dry mouth, hypersalivation

Antegrade amnesia may occur using therapeutic dosages, the risk increasing at higher dosages. Amnestic effects may be associated with inappropriate behavior.

1.15 DOSAGE AND ADMINISTRATION (Xpil.medicines.org.uk, 2015)

For maximal beneficial effect, the dosage should be carefully individualised. Dosage may need to be reduced in patients with hepatic or renal disease, as the elimination half-life may be prolonged in this subgroup.

Elderly patients should be given a reduced dose. These patients should be checked regularly at the start of treatment in order to minimize the dosage and/or frequency of administration to prevent overdose due to accumulation.

Usual adult dosage: 5 to 40 mg daily.

Average dosage for ambulatory patients: 2 mg three times daily or 5 mg in the evening and 2mg once or twice during the day.

Muscle spasm: 10 to 30 mg daily.

Elderly or debilitated patients: 2 mg twice daily or half the usual adult dose.

Children: 6 months to 3 years: 1 to 6 mg daily; 4 to 14 years: 4 to 12 mg daily or calculated from 0.1 to 0.3 mg/kg bodyweight.

Benzodiazepines should not be given to children without careful assessment of the indication: the duration of treatment must be kept to a minimum.

Hospital treatment of tension, excitation, motor unrest: 10 to 15 mg three times daily until the acute symptoms subside.

1.16 Overdose: (RxList,2015)

Overdose of diazepams 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.

1.17 Dosage form and packaging

Sedil (diazepam) is available as yellow colored for solid oral dosage form containing 5mg of diazepam. Each box contains 25x20 tablets in blister packs. (Squarepharma.com.bd, 2015)

CHAPTER TWO

LITERATURE REVIEW

2.1 Literature review

In Bangladesh there are different diazepam drugs are available and they are marketed as different brands. From available brands one brand i.e. Sedil® was chosen for determining whether it is photosensitive or not. In most cases these combination products are available in transparent plastic blister packaging system in the market of Bangladesh. Since, there is no published data about photolytic degradation of diazepam, we operated a research program for reproducibility checking to find whether this drug is photosensitive or not. We think that the outcome of this study will give us the exact information about the drug and its photosensitivity which will influence the packaging system of the drug.

In 1978, the compatibility and stability of diazepam injection were studied by Morris. 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)

After some years, Smith FM and Nuessle NO studied the stability of diazepam injection repackaged in disposable glass syringes and stored at room and refrigerator temperatures in 1982. In their study thirty-nine 1.5-ml syringes were filled with 1.1 ml diazepam injection 5-mg/ml. All syringes were stored in light-resistant bags on their sides so that the solution was in contact with the rubber stoppers on both ends. Samples were assayed with a stability-indicating HPLC method for diazepam. After the experiments diazepam found decrease the concentration occurring by apparent sorption to rubber syringe components. So refrigeration is recommended as Diazepam injection is chemically stable as 5-mg doses in disposable glass syringes for 90 days when stored at 4 degrees C or 30 degrees C. (Smith and Nuessle, 1982)

In 1984, another research group (Klotz U, Reimann IW) studied pharmacokinetic and pharmacodynamic interaction of diazepam and metoprolol. 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)

In the next year three scientist H.M.Elsabbagh, M.H Elshaboury and HamdyM.Abdel-Aleem studied Physical Properties and Stability of Diazepam and Phenobarbitone Sodium Tablets Prepared with Compactrol in 1985. Compactrol is a direct compressible vehicle. It was used for the preparation of Diazepam and phenobarbitone sodium tablets. Spray dried lactose and wet granulation technique were also employed to prepare these tablets for comparison. The experiment was done at 75% RH, at two temperature levels (25° and 45°) on the physical properties of these tablets was studied for 6 weeks. After the investigation it was found that, there were an increase in tablet weight, thickness and friability per cent, while a significant decrease in hardness was observed. Compactrol prepared tablet showed no significant changes in both disintegration and dissolution times, while tablets prepared with spray dried lactose showed a marked decrease in disintegration and dissolution times. On the other hand, tablets prepared by wet granulation showed a pronounced increase in both disintegration and dissolution times. (Elsabbagh, Elshaboury and Abdel-Aleem, 1985)

In 1990, Hussey et al, 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 post infusion. 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 Cmax is because of the precipitation of diazepam in the vein. (Hussey et al., 1990)

Maidment and Upshall (1990), the injection solvent used for diazepam is likely to slow the absorption of this drug from the injection site. In order to achieve a rapid onset and to reduce the

inter-individual variability, the strategy of developing prodrug of diazepam was pursued. The water-soluble prodrug, pro-diazepam (avizafone, or lysylpeptido-aminobenzophenone diazepam) has been developed to be one component in an aqueous drug mixture with atropine and pralidoxime for the therapy of nerve agent poisoning. (Maidment and Upshall 1990)

In next year, 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)
After some year, In 1997 McDonough and Shih; It has been shown that the GABAergic agonist, diazepam, can stop seizures if injected after 5–10 min after their occurrence. Later administration of diazepam has unreliable effects, inasmuch as seizures can recur and only an incomplete neuroprotection is achieved (McDonough and Shih, 1997).

According to Gottwald et al., in 1999, he and his fellow researchers analyze about prehospital stability of diazepam and lorazepam. Injections are commonly stocked on ambulances for use by paramedics. Diazepam (5 mg/mL) and lorazepam (2 mg/mL) injectable solutions were stored for up to 210 days in clear glass syringes at three conditions: 4°C to 10°C (refrigerated); 15°C to 30°C (on-ambulance ambient temperature); and 37°C (oven-heated). High-performance liquid chromatography (HPLC) method was used for analyzing the syringe contents. Diazepam retained 90% of its original concentration for 30 days of on-ambulance storage; lorazepam retained 90% of its original concentration for 150 days. After the investigation, it is suggest that diazepam and lorazepam can be stored on ambulances. (Gottwald et al., 1999)

In 2000, Yegles, Marson and Wennig studied the influence of hair bleaching on benzodiazepines concentrations. They used hair of a dead person who died after an overdose of several illicit drugs associated with benzodiazepines drugs like diazepam or lorazepametc which was treated with bleaching product (Poly Blonde, Schwarzkopf & Henkel) for 20 min.

The extracts were obtained by solid-phase extraction on C18 columns and the derivatives were determined by GC–MS in a SIM mode. These results show that the concentrations of the entire drug detected decreased in bleached hair in comparison with non treated hair. The results found that bleaching influences the stability of entrapped benzodiazepines in hair. (Yegles, Marson and Wennig, 2000)

In the year of 2001, Sznitowska et al. studied the bioavailability of diazepam in rabbits after rectal administration. In this method three formulation used- organic-aqueous released rectal solution (containing ethanol, benzyl alcohol and propylene glycol), submicron emulsion and solid lipid nanoparticles (SLN). All formulations contained 4 mg/ml of diazepam and the dose administrated to rabbits was 2 mg/kg. Mean size of the dispersed particles are nearly same (201–206 nm) in the submicron preparations. In moderate prolongation drug release pharmacokinetic of diazepam did not alter in the submicron solution. The low relative bioavailability, 47% compared to the solution, was observed after administration of SLN. Transmission electron microscopy pictures revealed that some of diazepam is present on the surface of the SLN and this fraction was immediately absorbed, while the diffusion of the drug in the solid core was not efficient enough to allow a complete release. It may be concluded that submicron emulsion may be a good choice of an ethanol-free drug formulation, but lipid matrix, which is solid at body temperature, is not advantageous system for diazepam rectal delivery, even if delivered as a submicron dispersion. (Sznitowska et al., 2001)

In the same year Lucila M.L. Carvalho, Arício X. Linhares and José Roberto Trigo determine the drug levels and the effct of diazepam in the growth of necrophagousflies species. Two species which were reared on tissues from rabbits named Larvae of *Chrysomyaalbiceps*(Wiedemann) and *Chrysomyaputoria*(Wiedemann) (Diptera: Calliphoridae). For the determination of the drug on the ddevelopement of these two species, lethal dosage of diazepam was administered. The rabbits were given 50 mg of diazepam via ear vein infusion. From 18 to 54 h, larvae feeding on tissues containing the drug developed more rapidly than larvae from the control colony for both fly species. . The time required for pupariation and adult emergence was significantly greater for colony fed on tissues from diazepam dosed rabbits than for the control ones. The dectection of diazepam was done by gas chromatography-mass spectrometer (GC-MS) in all rabbit samples and in almost all diptera samples in this experiment. (Carvalho, Linhares and Trigo, 2001)

Anissa El Mahjoub and Christian Staub developed a method for determining benzodiazepines (clonazepam, diazepam, flunitrazepam, midazolam and oxazepam) in human hair by on line high performance liquid chromatography using a restricted access extraction column in the same year of 2001. 50 mg of powdered hair were incubated (2 h at 45°C) after sonication (1 h) in 1 ml of the following solution (methanol:ammonia, 97.5/2.5, v/v). The aliquot was centrifuged and the methanolic phase transferred to a conical tube and evaporated under a gentle stream of nitrogen. The benzodiazepines were determined by a photodiode-array detector at 254 nm, using reference data (retention time and UV spectra). The method showed excellent linearity between 0.5 and 20 ng/mg of hair for clonazepam, flunitrazepam and midazolam and between 0.5 and 100 ng/mg of hair for diazepam and oxazepam. Now this method is greatly used in forensic studies. (El Mahjoub and Staub, 2001)

In 2002,Alldredge BK, Venteicher R, Calderwood TS studied about the stability of diazepam rectal gel (Diastat) in various conditions of temperature and light exposure as might be found in ambulances. Three lots of Diastat (Xcel Pharmaceuticals, San Diego, CA) in various fill/syringe configurations were evaluated in controlled 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. After the test and configurations, concentration of diazepam always exceeded 95% of label. There was no change in excipients or physicochemical properties. Based on the results of the present study, the restocking frequency of Diastat in ambient storage conditions (eg, ambulances), could be up to 48 months in nonfreezing environments, as long as this does not exceed the labeled expiration date on the product. (Alldredge, Venteicher and Calderwood, 2002)

In the same year, Dimitrios G. Fatouros and Sophia G. Antimisiaris studied stability of prednisolone (PZ), diazepam (DZ), or griseofulvin (GF) incorporating into multilamellar liposomes. Liposome size, surface charge, and stability (in buffer and serum proteins) were measured by comparing drug-incorporating liposomes and empty liposomes. The results showed that all drugs studied drug incorporation has a substantial effect on the vesicle ζ-potential and stability. Drug-incorporating liposomes have a higher negative surface charge in membrane integrity compared with that of empty liposomes. Release of Diazepam from liposomes is induced by dilution. Summarizing, the results of this study demonstrate that the presence of PZ, DZ, or GF in liposome membranes has a significant effect on main vesicle

properties and correlates well with those obtained previously for hydrochlorothiazide and chlorothiazide. (Fatouros and Antimisiaris, 2002)

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. During pregnancy anxiety may be occurring. 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)

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)

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)

Kintz et al., established a method in 2005 for screening and confirmatory method for benzodiazepines and hypnotis in oral fluid by LC-MS/MS. The procedure contains the screening of 17 benzodiazepines and hypnotics in oral fluid after collection with the Intercept® device by LC-MS/MS (alprazolam, 7-aminoclonazepam, 7-aminoflunitrazepam, bromazepam, clobazam, diazepam, lorazepam, lormetazepam, midazolam, nordiazepam, oxazepam, temazepam, tetrazepam, triazolam, zaleplon, zopiclone and zolpidem). The method involves extraction of 0.5 mL of oral fluid treated with 0.5 mL of phosphate buffer (pH 8.4) in the presence of 5 ng diazepam-d5 used as internal standard, with 3 mL of diethyl ether/methylene chloride (50/50). Separation is done by using liquid chromatography–tandem mass spectrometry. These results were found suitable to screen for 17 benzodiazepines in oral fluid and detect them at very low concentrations, making this method suitable for monitoring subjects under the influence. (Kintz 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 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 the same year, Kaur P and Kim K investigate the plasma pharmacokinetics and brain uptake of a lipophilic benzodiazepine anticonvulsant, diazepam. White rabbits and rats are used to evaluate the possible absorption pathways after intravenous and intranasal

administration. The formulation was prepared by dissolving diazepam and 1% sodium glycocholate into micro emulsion system composed of 15% ethyl laurate, 25% Labrasol, 37.5% Transcutol P, 12.5% ethanol, and 10% water. Diazepam was administered intravenously (1 mg/kg) or intranasal (2 mg/kg) to rats and rabbits. LC/MS method after solid phase extraction was used to determine the drug concentrations in the plasma and six different regions of the brain tissues, i.e., olfactory bulb, olfactory tract, anterior, middle, and posterior segments of cerebrum and cerebellum. Diazepam was rapidly absorbed into the systemic circulation after intranasal administered and homogeneously distributed into the different regions of brain tissues. After the investigation, the plasma pharmacokinetic and distribution studies in the two animal models clearly showed that lipophilic diazepam molecules reached the brain predominantly from the blood by crossing the blood-brain barrier after intranasal administration with no significant direct nose-to-brain transport via olfactory epithelium. (Kaur and Kim, 2008)

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 CH3 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, 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)

Again in 2012, Atanasov et al., studied the stability of diazepam in blood samples at different storage conditions and in the presence of alcohol. Diazepam is is frequently analyzed in different biological samples, especially blood samples. The diazepam stability in the sample matrices is an important factor regarding reliable data obtaining. Main object of the study is the storage of diazepam for the stability in blood samples. For evaluation of the diazepam stability the absence or presence of sodium fluoride as stabilizer as well as the influence of ethanol was used. The results of the study indicated that the temperature is the main storage factor affecting diazepam stability. In the fluoride stabilized blood samples the amount of diazepam decreases up to 85% of initial level when stored at -20° C for the period of testing (12 weeks). About 5-9% decrease in diazepam concentration showed by the Freeze-thaw experiments of whole blood samples after the first cycle. Further experiments on benzodiazepines stability at different storage conditions or in combination of different factors should be undertaken in forensic toxicology to ensure the data quality, their reliability and reproducibility. (Atanasov 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)

Also in 2013, Wang et al., determined method in which diazepam and its glucuronide metabolites in human whole blood by μ Elution 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)

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)

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).

CHAPTER THREE

MATERIALS & METHOD

3.1 Materials

3.1.1 Sample Collection

For the purpose of experimentation to observe the photolytic degradation of diazepam drug as well as to assess the packaging efficiency, 500 tablets of Sedil® (diazepam 5mg) were collected from the local drug store in Dhaka as a sample. All the tablets were from the same batch 406003. Among them 200 tablets were kept light protected for control tests and the remaining 300 tablets were subjected to various lighting conditions over certain periods of time for conducting experiments to determine their potency.

Figure 3.1: Sedil® (diazepam 5mg)

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& 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	Vernier 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.

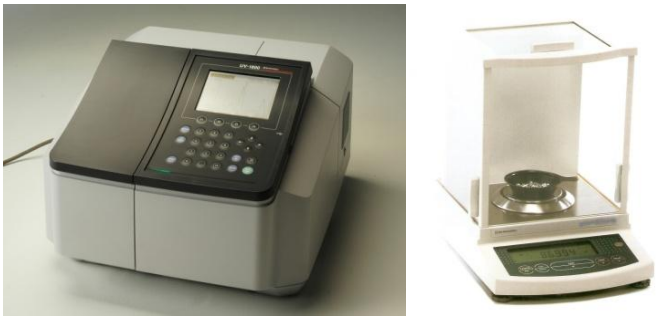

Figure 3.2: Shimadzu UV-1800 spectrophotometer and Electronic balance [Left to right]

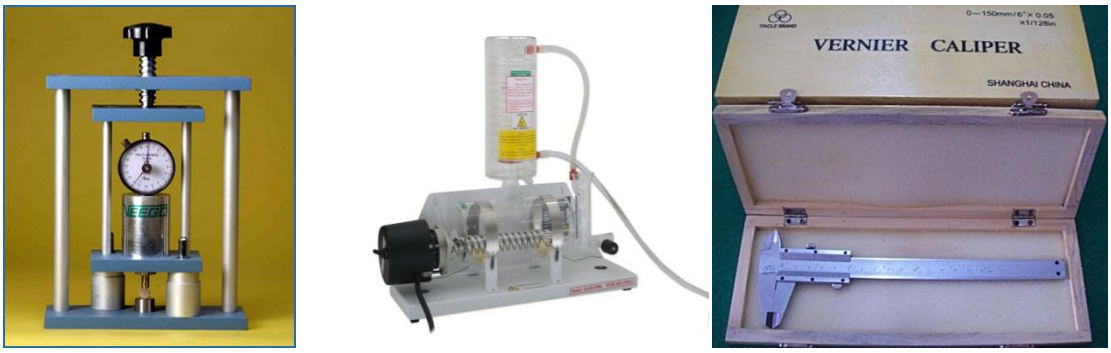

Figure 3.3: Hardness tester, Distilled water plant &Vernier calipers [Left to right]

3.1.5Apparatus

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 Method

3.2.1. Preparation of the solvent (0.1N H_2SO_4)

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

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

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

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

= 180.32gm of H_2SO_4

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

= 1803.2gm of H_2SO_4

1000 ml of stock solution contain 49gm of H_2SO_4 = 1N of H_2SO_4

1000 ml of stock contain 1803.2gm of H_2SO_4 = (1803.2/49)N of H_2SO_4

= 36.8N of H_2SO_4

3. After the determination of the concentration of the lab solvent stock solution in Normality (N), the amount of lab solvent (36.8N H_2SO_4) stock solution required to make 1000ml of 0.1N HCL solvent was calculated as below.

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

Where,

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

S_2 = Final concentration of the solvent (H_2SO_4) = 0.1N

V_1 = Volume of the lab solvent (H_2SO_4) stock solution =?

V_2 = Final volume of the solvent (H_2SO_4) = 1000ml

So that,

$V_1 = (V_2S_2) / S_1$

⇨ V_1 = (1000ml 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 a pharmaceutical company. The potency of standard compound was 99.02%.

2. The specific λmax for diazepam, at which the absorbance would be measured, was determined 240.5nm 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 250 ml of 0.1N H2SO4 (which is the solvent) in a 250 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: Concentration for Preparation of Standard Curve of diazepam

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

- ❖ $V_1= S_2V_2 / S_1$ = (0.001 x 10) / 0.02 = 0.5 ml of stock solution required to make 0.001 mg/ml concentration of the final solution of 10 ml (0.5 ml of stock solution + 9.5 ml of 0.1N H_2SO_4) of 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.

1. Then the absorbance value was measured using a UV spectrophotometer against those nine serial concentrations for Diazepam.
2. A standard curves was plotted for Diazepam.
3. From this standard curve a straight line equation was obtained which was in the form of y = mx+c, where the components of the equations are described as provided below:

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

3.2.3 Sampling, Analysis by UV-Spectrophotometry & Determination of Potency of the pharmaceutical drugs (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:

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

1. Exposure under Normal Lighting Condition

1) The tablets (Sedil®) were kept under normal lighting condition in the room for 2 month 15 days.
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:

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

c. Then the 5 tablets were crushed by using mortar and pestle.
d. Approximately the weight of 1 tablet of crushed tablet powder was taken and dissolved it in 100 ml of the solvent (0.1N H_2SO_4) for 3 times to prepare 9 samples.
e. After that 10 ml solution was filtered and 5 ml of that filtered solution was taken and dissolved in 50ml of the solvent.
f. From then 10ml of each sample was collected and kept into 3 different test-tube and wrapped it by foil paper.
g. From test-tube the solution was poured into a cuvette and was inserted into the UV spectrophotometer to observe the absorbance value.

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

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

1) 30 tablets were exposed to electric bulb lighting conditions for 6 hours at a stretch and 10 tablets were used as control.
2) After every 2 hours, 10 tablets were collected and wrapped up with foil paper to prevent any further exposure to the lighting condition and the temperature was noted using a thermometer.
3) The foil papers should be labeled to identify the intervals.
4) The tablets were then used for potency determination to see the effect of the exposure of bulb's lighting condition to drug ingredients.

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

a. First, 5 tablets from those sampled tablets were taken.
b. Then the total weight of those 5 tablets was noted using an analytical balance and the average weight was calculated using the formula :

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

c. Then the 5 tablets were crushed by using mortar and pestle. Approximately the weight of 1 tablet of crushed tablet powder was taken and dissolved it in 100 ml of the solvent (0.1N H_2SO_4) for 3 times to prepare 9 samples.
d. After that 10 ml solution was filtered and 5 ml of that filtered solution was taken and dissolved in 50ml of the solvent.
e. From then 10ml of each sample was collected and kept into 3 different test-tube and wrapped it by foil paper.
f. From test-tube the solution was poured into a cuvette and was inserted into the UV spectrophotometer to observe the absorbance value.

Table 3.4: Electric Bulb (25W & 40W) Exposed Sample List

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

6) Then the absorbance value was plotted into the standard curve to obtain the total amount of the drug that is present in one tablet.
7) Steps 5 to 6 were repeated again for another sampling hour.
8) 10 tablets were used as control and has not been exposed any of the lighting conditions.

N.B: Same procedure (steps 1 to 8) were used to determine the potency of the tablets under both exposure of 25W and 40W lighting condition for two different days for 6 hours each.

3. Under Sunlight condition

1) 30 tablets were kept in a Glass box and exposed to sunlight condition for 7.5 hours at a stretch.
2) After every 2 hours, 10 tablets were collected and wrapped up with foil paper to prevent any further exposure to the lighting condition and the temperature was noted using a thermometer.
3) The foil papers should be labeled to identify the intervals.
4) The tablets were then used for potency determination to see the effect of the exposure of sunlight condition to drug ingredients.
5) For potency determination, laboratory analysis was done by using UV spectroscopy technique:

    a. First, 5 tablets from those sampled tablets were taken.
    b. Then the total weight of those 5 tablets was noted using an analytical balance and the average weight was calculated using the formula:

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

    c. Then the 5 tablets were crushed by using mortar and pestle.
    d. Approximately the weight of 1 tablet of crushed tablet powder was taken and dissolved it in 100 ml of the solvent (0.1N H_2SO_4) for 3 times to prepare 9 samples.

e. After that 10 ml solution was filtered and 5 ml of that filtered solution was taken and dissolved in 50ml of the solvent.
f. From then 10ml of each sample was collected and kept into 3 different test-tube and wrapped it by foil paper.
g. From test-tube the solution was poured into a cuvette and was inserted into the UV spectrophotometer to observe the absorbance value.

Table 3.5: Sunlight Exposed Sample List

No. of Samples	Collected Sample	Withdrawal Intervals (Hrs)	Temparature (^{0}C)
10 (Control)	10	0	30
30	10	2	30
	10	4	31
	10	6	32

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

3.2.4 Determination of Physical parameters:

1. Color Test

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

2. Thickness Test

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

jaws and check the reading of main scale and vernier scale and calculate the values of each tablets.

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

$$\textbf{Thickness (cm)} = \textbf{Main Reading} + \left(\frac{\textbf{Vernier Reading}}{\textbf{10}} \times \textbf{Vernier Constant}\right)$$

3. Hardness Test

Hardness test was performed to determine the hardness of tablets. So the force will be applied during compression of tablet, greater the pressure applied the harder the tablet. Monsanto tablet hardness tester was used to measure the hardness of presonil®. Hardness measuring devices apply increasing pressure on the tablet until the tablet breaks (a force of about 4 kilograms is considered to be a minimum for hardness).

4. Weight Variation Test

Procedure

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

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

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

Table 3.6: Accepted Percentage List for the Weight Variation Test of Tablets

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

Calculation

Following equation was used to determine % Weight Variation of tablets

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

Where,

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

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

CHAPTER FOUR

RESULTS

4.1 Standard curve preparation

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 & Absorbance for Standard Curve of Diazepam

Concentration(mg)	Absorbance (at 221.5nm)
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.

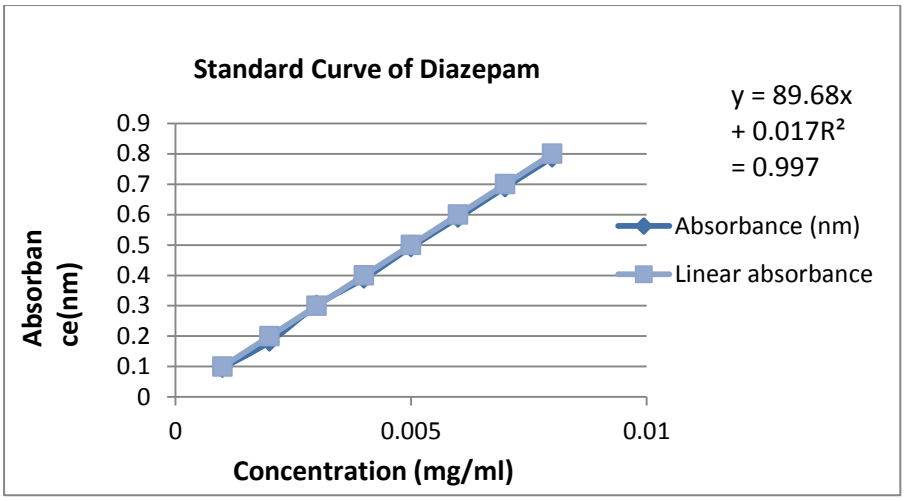

Figure: 4.1: Plot showing straight line for Absorbance (nm) with respect to Concentration (mg/ml) for Diazepam

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

R2=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

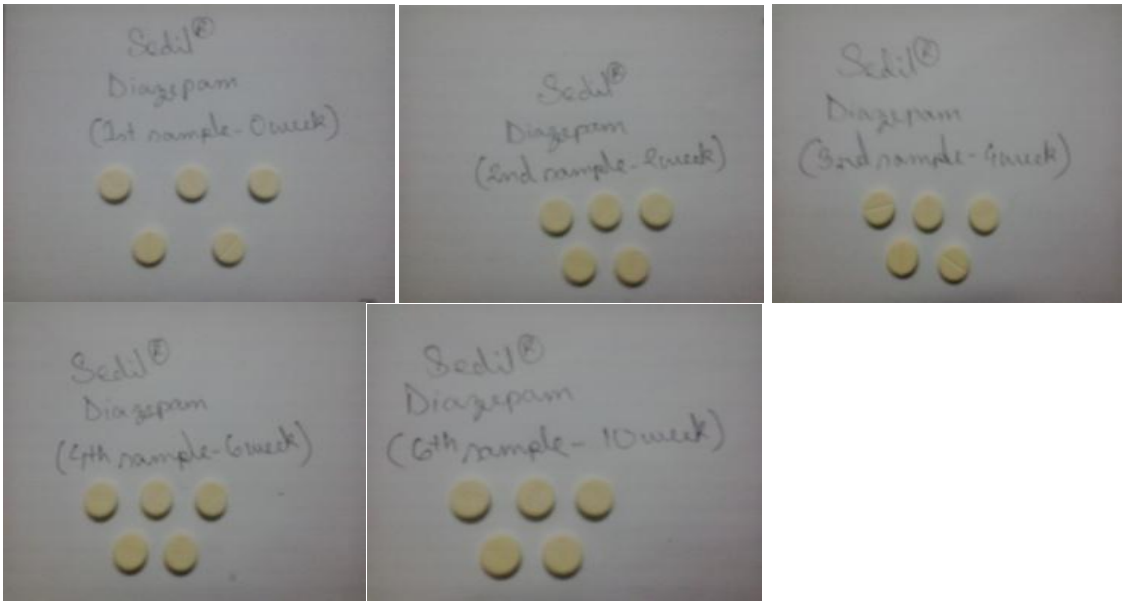

Figure 4.2: Pictures of tablets after exposure to normal light with 60 days interval

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 Diazepam

Days	**Average Weight for Particular Day, I(g)**	**Average Weight for 75 Days Intervals, A(g)**	**% Weight Variation, (A-I/A)×100 %**
Initial	0.1635	0.1626	0.053
15	0.1629		-0.368
30	0.1627		-0.053
45	0.1622		0.421
60	0.1617		-0.105

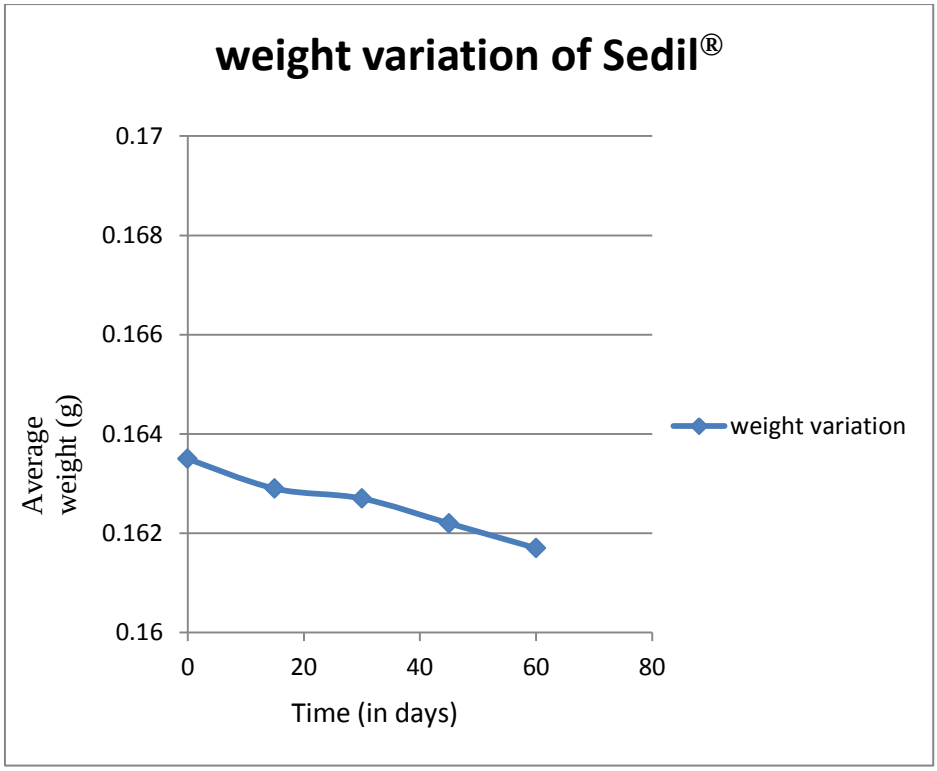

Figure 4.3: Weight variation of the sample throughout 60 days light exposure

4.2.3 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 4.3: Hardness Test of Diazepam (Sedil®)

Days	**Average Hardness of Particular Day (Kg)**
Initial	4.25
15	3.95
30	4.14
45	4.11
60	3.87

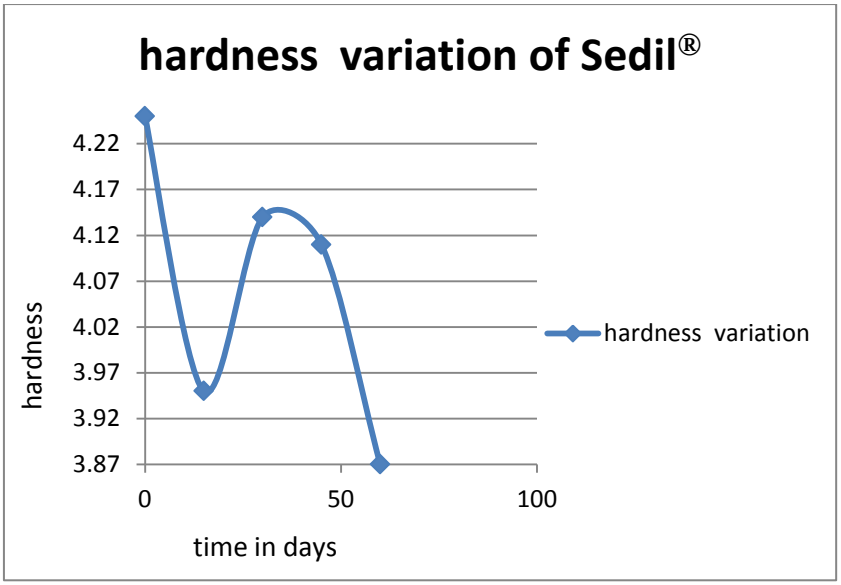

Figure 4.4: Hardness variation of the sample throughout 60 days light exposure

4.2.4 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 4.4: Thickness Test of Diazepam(Sedil®)

Days	**Average Thickness of Particular Days (cm)**
Initial	0.2164
15	0.2157
30	0.2164
45	0.2162
60	0.2152

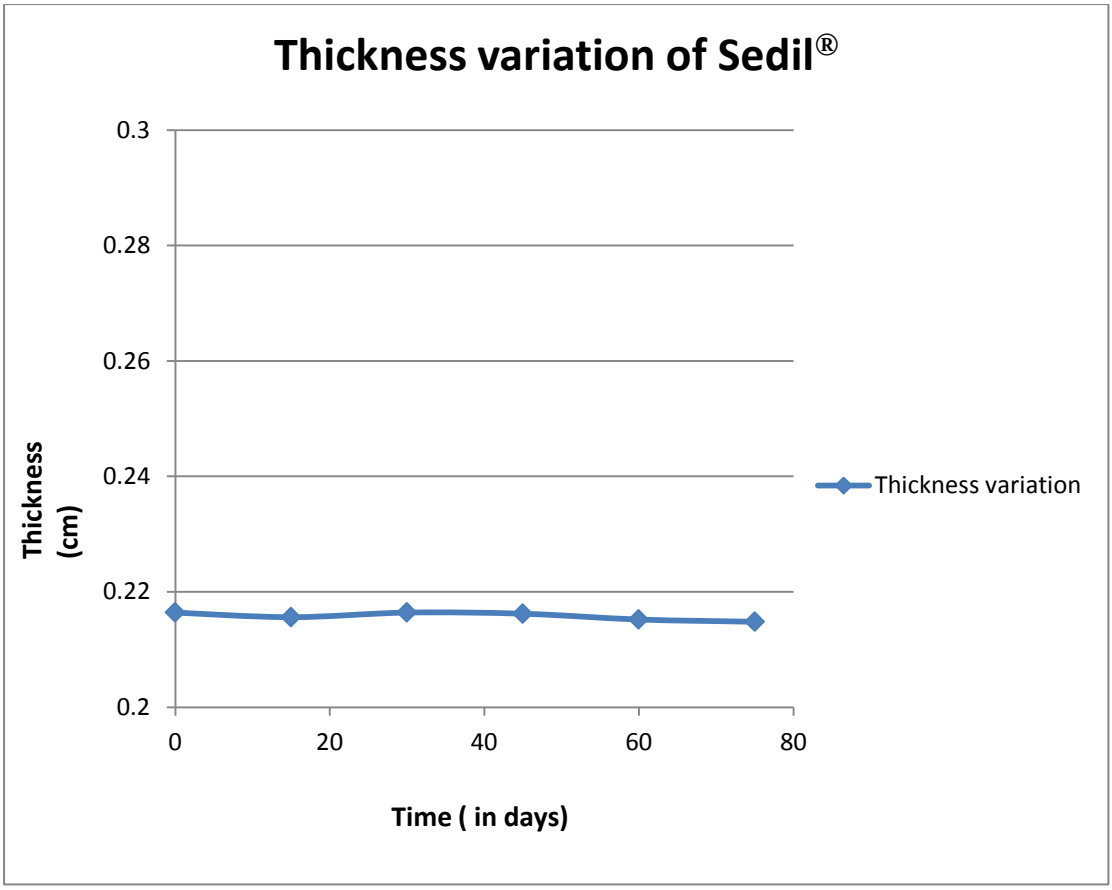

Figure 4.5: Thickness variation of sample throughout 75 days light exposure

4.3 Result from Potency Determination by UV- spectroscopy

4.3.1 Result from Sample that was exposed under Normal Lightening Condition

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

Table 4.5.1: Concentration & Absorbance of normal light exposure for Diazepam (Initial)

Time Interval (Days)	Absorbance (at 240.5nm)		Average Absorbance		Amount of Drug Present (in mg)		Potency %	
	Control	Sample	Control	Sample	Control	Sample	Control	Sample
	0.437	0.437						
	0.439	0.439	0.439	0.439	4.70	4.70	94.11	94.11
	0.439	0.437						
Initial	0.435	0.435						
	0.439	0.439	0.439	0.439	4.70	4.70	94.11	94.11
	0.440	0.440						
	0.442	0.442						
	0.435	0.435	0.439	0.439	4.70	4.70	94.11	94.11
	0.437	0.437						

Table 4.5.2: Concentration & Absorbance of normal light exposure for Diazepam (15 day)

Time Interval (Days)	Absorbance (at 240.5nm)		Average Absorbance		Amount of Drug Present (in mg)		Potency %	
	Control	Sample	Control	Sample	Control	Sample	Control	Sample
	0.437	0.402						
	0.439	0.401	0.439	0.402	4.70	4.29	94.11	85.86
	0.439	0.400						
15 day	0.435	0.400						
	0.439	0.401	0.439	0.400	4.70	4.28	94.11	85.11
	0.440	0.398						
	0.442	0.402						
	0.435	0.399	0.439	0.399	4.70	4.21	94.11	84.89
	0.437	0.396						

Table 4.5.3: Concentration & Absorbance of normal light exposure for Diazepam (30 day)

Time Interval (Days)	Absorbance (at 240.5nm)		Average Absorbance		Amount of Drug Present (in mg)		Potency %	
	Control	Sample	Control	Sample	Control	Sample	Control	Sample
	0.437	0.396						
	0.439	0.394	0.439	0.393	4.70	4.19	94.11	83.85
	0.439	0.395						
30 day	0.435	0.388						
	0.439	0.393	0.439	0.392	4.70	4.17	94.11	83.60
	0.440	0.395						
	0.442	0.393						
	0.435	0.396	0.439	0.390	4.70	4.15	94.11	83.18
	0.437	0.391						

Table 4.5.4: Concentration & Absorbance of normal light exposure for Diazepam (45 day)

Time Interval (Days)	Absorbance (at 240.5nm)		Average Absorbance		Amount of Drug Present (in mg)		Potency %	
	Control	Sample	Control	Sample	Control	Sample	Control	Sample
	0.437	0.356						
	0.439	0.360	0.439	0.356	4.70	3.78	94.11	75.60
	0.439	0.359						
45 day	0.435	0.362						
	0.439	0.366	0.439	0.366	4.70	3.89	94.11	77.83
	0.440	0.364						
	0.442	0.364						
	0.435	0.360	0.439	0.366	4.70	3.89	94.11	77.83
	0.437	0.366						

Table 4.5.5: Concentration & Absorbance of normal light exposure for Diazepam (60 day)

Time Interval (Days)	Absorbance (at 240.5nm)		Average Absorbance		Amount of Drug Present (in mg)		Potency %	
	Control	Sample	Control	Sample	Control	Sample	Control	Sample
	0.437	0.318						
	0.439	0.315	0.439	0.314	4.70	3.31	94.11	66.24
	0.439	0.316						
60 day	0.435	0.321						
	0.439	0.321	0.439	0.320	4.70	3.35	94.11	66.88
	0.440	0.319						
	0.442	0.318						
	0.435	0.316	0.439	0.314	4.70	3.31	94.11	66.24
	0.437	0.312						

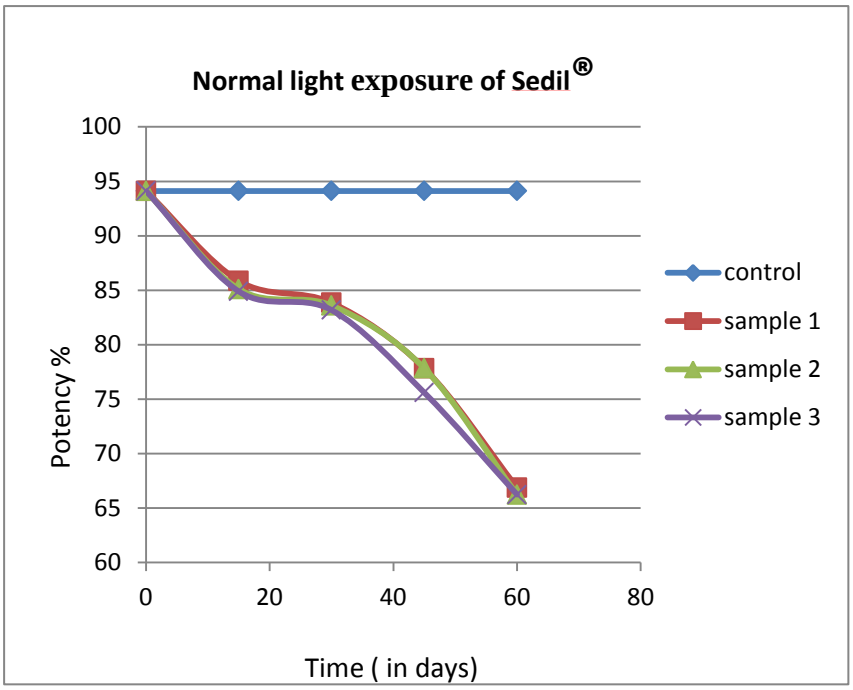

Figure 4.6: Graph showing the difference in Concentration after fixed day interval for Diazepam (Sedil®)

4.3.2 Result of samples that were exposed under 25W bulb

In experimental day, a tablet strip containing 10 tablets was taken and 5 samples were collected for the test and observed 3 different absorbance of Diazepam for three samples exposed under the lamp (25W bulb); each for 2 hours time interval and it was observed that the concentration of Diazepam was declined in each time interval.

Table 4.6.1: Concentration & absorbance of Diazepam (Sedil®) under 25 W bulbfor 1st time

Time Interval	Absorbance (at 240.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (1000 times diluted)	Amount of Drug present (in mg)	Potency (%)
Control	0.437	0.437	0.0047	4.65	92.23
	0.439				
	0.440				
	0.438	0.439	0.0047	4.70	94.11
	0.442				
	0.437				
	0.441	0.435	0.0047	4.60	91.13
	0.440				
	0.438				

Table 4.6.2: Concentration & absorbance of Diazepam (Sedil®) under 25 w bulbfor first time

Time Interval	Absorbance (at 240.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (1000 times diluted)	Amount of Drug present (in mg)	Potency (%)
2 hour	0.418	0.418	0.0045	4.47	89.43
	0.420				
	0.417				
	0.416	0.414	0.0042	4.14	82.80
	0.415				
	0.414				
	0.414	0.414	0.0042	4.14	82.80
	0.415				
	0.416				

Table 4.6.3: Concentration & absorbance of Diazepam (Sedil®) under 25 w bulb for first time

Time Interval	Absorbance (at 240.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (1000 times diluted)	Amount of Drug present (in mg)	Potency (%)
4 hour	0.412	0.411	0.0038	3.80	76.05
	0.410				
	0.411				
	0.412	0.413	0.0037	3.78	73.67
	0.413				
	0.414				
	0.411	0.411	0.0038	3.80	76.05
	0.410				
	0.409				

Table 4.6.4: Concentration & absorbance of Diazepam (Sedil®) under 25w bulb for first time

Time Interval	Absorbance (at 240.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (1000 times diluted)	Amount of Drug present (in mg)	Potency (%)
6 hour	0.406	0.407	0.0035	3.55	70.92
	0.405				
	0.407				
	0.406	0.406	0.0034	3.45	70.76
	0.405				
	0.407				
	0.405	0.404	0.0033	3.38	70.03
	0.406				
	0.404				

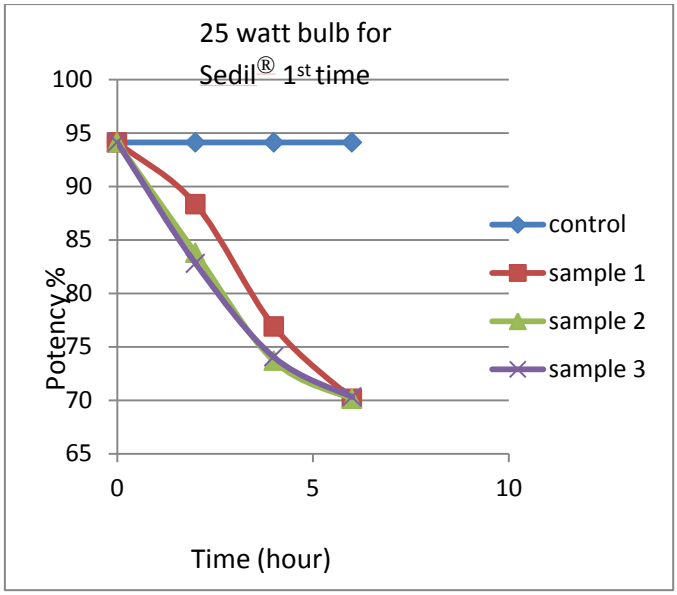

Figure 4.7.1 : Plot showing linear decrease in Potency (%) over time period (in hours) for diazepam under 25 W bulb light (1st time)

Table 4.6.5: Concentration & absorbance of Diazepam (Sedil®) under 25 W bulbfor 2nd time

Time Interval	Absorbance (at 240.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (1000 times diluted)	Amount of Drug present (in mg)	Potency (%)
Control	0.437	0.438	0.0047	4.68	93.91
	0.439				
	0.440				
	0.438	0.437	0.0047	4.65	93.23
	0.442				
	0.437				
	0.441	0.439	0.0047	4.70	94.11
	0.440				

Table 4.6.6: Concentration & absorbance of Diazepam (Sedil®) under 25 w bulbfor 2nd time

Time Interval	Absorbance (at 240.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (1000 times diluted)	Amount of Drug present (in mg)	Potency (%)
2 hour	0.418	0.419	0.0044	4.42	89.88
	0.420				
	0.417				
	0.416	0.415	0.0042	4.14	82.86
	0.415				
	0.414				
	0.414	0.414	0.0042	4.14	82.84
	0.415				
	0.416				

Table 4.6.7: Concentration & absorbance of Diazepam (Sedil®) under 25 w bulb for 2nd time

Time Interval	Absorbance (at 240.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (1000 times diluted)	Amount of Drug present (in mg)	Potency (%)
4 hour	0.414	0.413	0.0038	3.89	74.09
	0.413				
	0.414				
	0.411	0.412	0.0039	3.92	75.13
	0.410				
	0.416				
	0.411	0.411	0.0038	3.80	76.05
	0.410				
	0.409				

Table 4.6.8: Concentration & absorbance of Diazepam (Sedil®) under 25w bulb for 2nd time

Time Interval	Absorbance (at 240.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (1000 times diluted)	Amount of Drug present (in mg)	Potency (%)
6 hour	0.410	0.409	0.0036	3.65	72.17
	0.408				
	0.407				
	0.406	0.406	0.0034	3.45	70.79
	0.405				
	0.407				
	0.405	0.404	0.0033	3.38	70.30
	0.406				
	0.404				

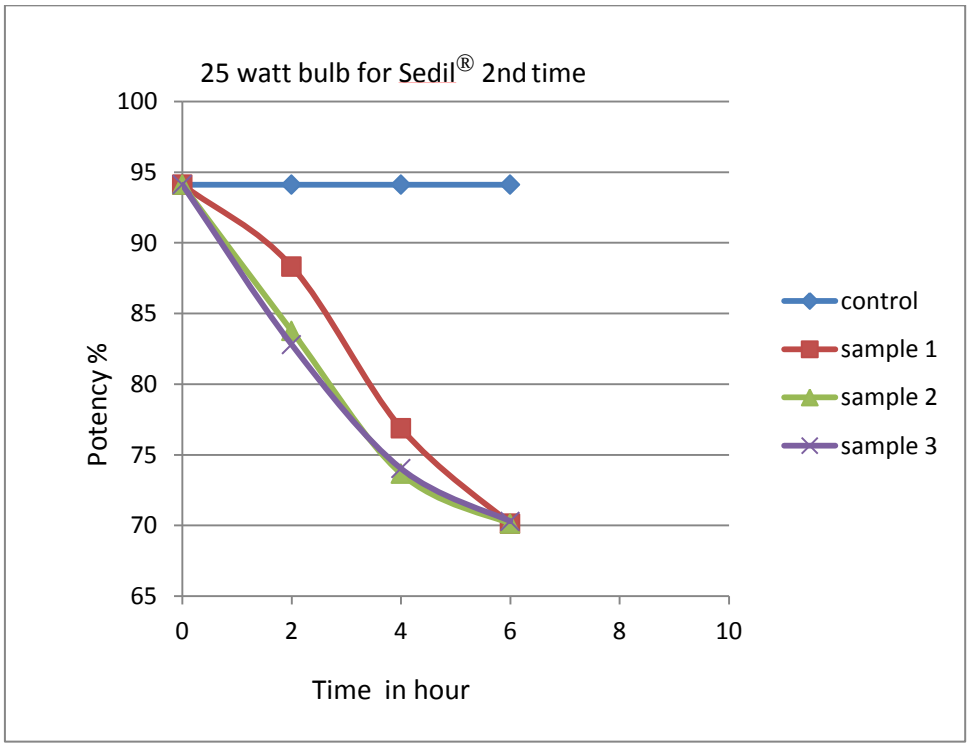

Figure 4.7.2 : Plot showing linear decrease in Potency (%) over time period (in hours) for diazepam under 25 W bulb light (2nd time)

Table 4.6.9: Concentration & absorbance of Diazepam (Sedil®) under 25 W bulbfor 3rd time

Time Interval	Absorbance (at 240.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (1000 times diluted)	Amount of Drug present (in mg)	Potency (%)
Control	0.437	0.439	0.0047	4.70	94.11
	0.439				
	0.440				
	0.438	0.437	0.0047	4.65	93.23
	0.442				
	0.437				
	0.441	0.440	0.0048	4.75	95.09
	0.440				
	0.438				

Table 4.6.10: Concentration & absorbance of Diazepam (Sedil®) under 25 w bulbfor 3rd time

Time Interval	Absorbance (at 240.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (1000 times diluted)	Amount of Drug present (in mg)	Potency (%)
2 hour	0.418	0.417	0.0044	4.44	88.33
	0.420				
	0.417				
	0.416	0.415	0.0042	4.24	83.76
	0.415				
	0.414				
	0.414	0.414	0.0042	4.14	82.80
	0.415				
	0.416				

Table 4.6.11: Concentration & absorbance of Diazepam (Sedil®) under 25 w bulb for 3rd time

Time Interval	Absorbance (at 240.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (1000 times diluted)	Amount of Drug present (in mg)	Potency (%)
4 hour	0.412	0.412	0.0038	3.82	76.89
	0.410				
	0.411				
	0.412	0.411	0.0037	3.71	73.67
	0.413				
	0.414				
	0.411	0.410	0.0038	3.66	73.03
	0.410				
	0.409				

Table 4.6.12: Concentration & absorbance of Diazepam (Sedil®) under 25w bulb for 2nd time

Time Interval	Absorbance (at 240.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (1000 times diluted)	Amount of Drug present (in mg)	Potency (%)
6 hour	0.406	0.406	0.0034	3.49	70.13
	0.405				
	0.407				
	0.406	0.406	0.0034	3.49	70.13
	0.405				
	0.407				
	0.405	0.404	0.0033	3.38	70.03
	0.406				
	0.404				

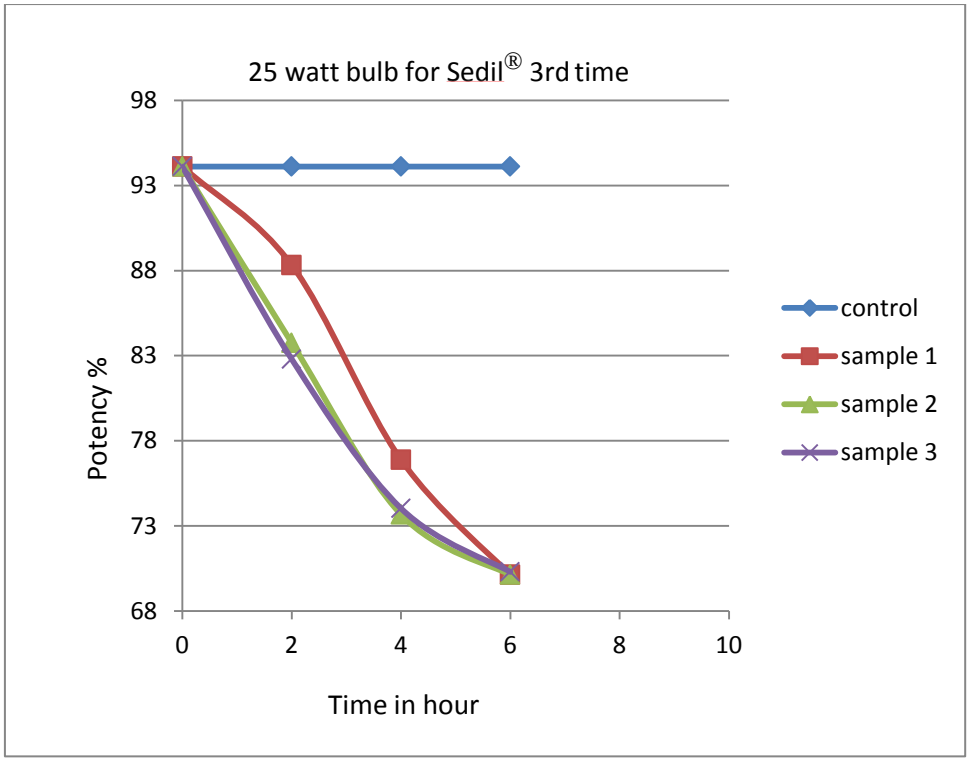

Figure 4.7.3 : Plot showing linear decrease in Potency (%) over time period (in hours) for diazepam under 25 W bulb light (3rd time)

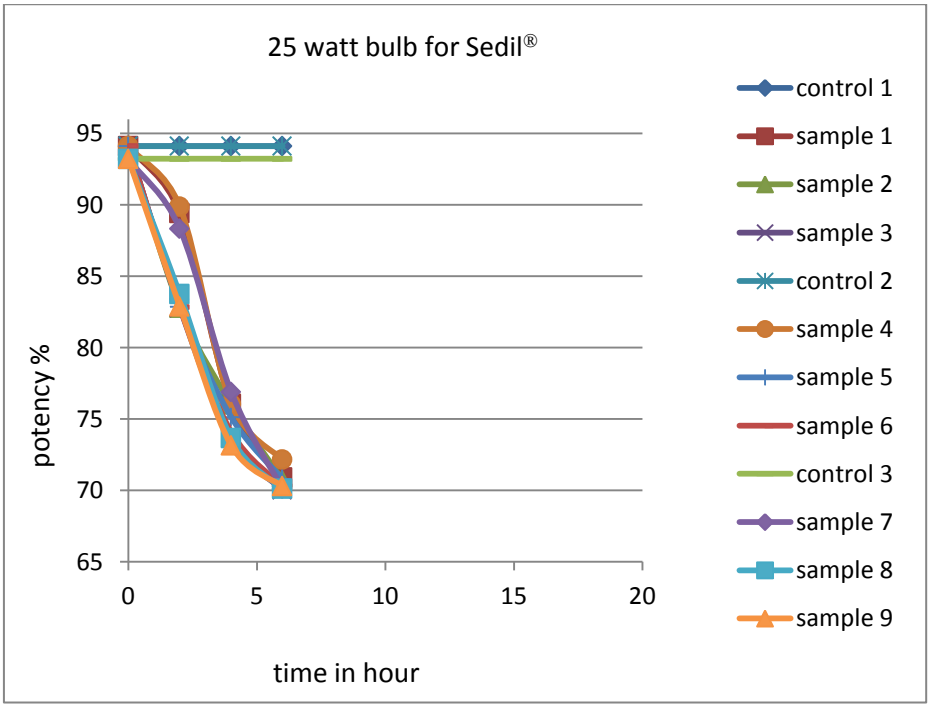

Figure 4.7.4 : Plot showing linear decrease in Potency (%) over time period (in hours) for diazepam under 25 W bulb light

4.3.3 Result of samples that were exposed under 40W bulb

In experimental day, a tablet strip containing 10 tablets was taken and 5 samples were collected for the test and observed 3 different absorbance of Diazepam for three samples exposed under the lamp (40W bulb); each for 2 hour time interval and it was observed that the concentration of Diazepam was declined in each time interval.

Table 4.7.1: Concentration & absorbance of Diazepam (Sedil®) under 40 w bulbfor 1st time

Time Interval	Absorbance (at 240.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (1000 times diluted)	Amount of Drug present(mg)	Potency (%)
Control	0.437	0.439	0.0047	4.70	94.11
	0.439				
	0.440				
	0.438	0.439	0.0047	4.70	94.11
	0.442				
	0.437				
	0.441	0.439	0.0047	4.70	94.11
	0.440				
	0.438				

Table 4.7.2: Concentration & absorbance of Diazepam (Sedil®) under 40w bulb for first time

Time Interval	Absorbance (at 240.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (1000 times diluted)	Amount of Drug present (in mg)	Potency (%)
2 hour	0.393	0.391	0.0042	4.18	83.18
	0.392				
	0.390				
	0.398	0.393	0.0042	4.22	83.98
	0.393				
	0.390				
	0.391	0.390	0.0042	4.16	82.80
	0.390				
	0.392				

Table 4.7.3: Concentration & absorbance of Diazepam (Sedil®) under 40 w bulb for 1st time

Time Interval	Absorbance (at 240.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (1000 times diluted)	Amount of Drug present (in mg)	Potency (%)
4 hour	0.332	0.334	0.0035	3.54	70.27
	0.335				
	0.333				
	0.335	0.333	0.0035	3.51	70.01
	0.331				
	0.332				
	0.332	0.335	0.0036	3.61	71.65
	0.334				
	0.335				

Table 4.7.4: Concentration & absorbance of Diazepam (Sedil®) under 40w bulb for first time

Time Interval	Absorbance (at 240.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (1000 times diluted)	Amount of Drug present (in mg)	Potency (%)
6 hour	0.322	0.320	0.0033	3.38	67.55
	0.321				
	0.320				
	0.323	0.322	0.0034	3.45	67.96
	0.322				
	0.321				
	0.321	0.320	0.0033	3.38	67.55
	0.320				
	0.320				

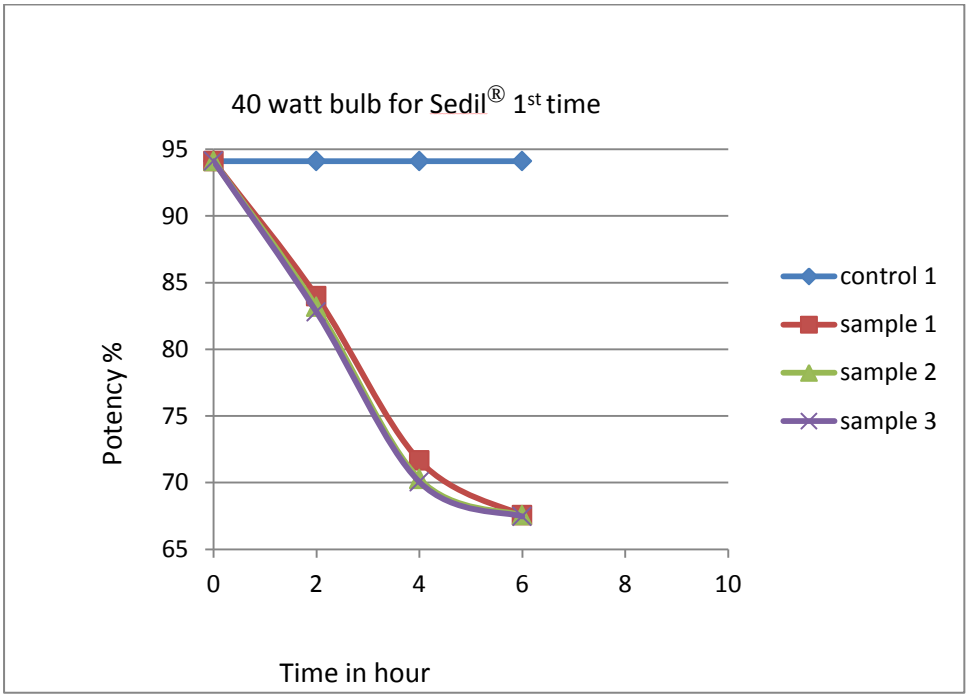

Figure 4.8.1 : Plot showing linear decrease in Potency (%) over time period (in hours) for diazepam under 40 w bulb (1st time)

Table 4.7.5: Concentration & absorbance of Diazepam (Sedil®) under 40 W bulbfor 2nd time

Time Interval	Absorbance (at 240.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (1000 times diluted)	Amount of Drug present (in mg)	Potency (%)
Control	0.438	0.435	0.0046	4.60	92.91
	0.433				
	0.437				
	0.438	0.439	0.0047	4.70	94.11
	0.442				
	0.437				
	0.441	0.437	0.0047	4.65	93.23
	0.440				
	0.438				

Table 4.7.6: Concentration & absorbance of Diazepam (Sedil®) under 40 w bulbfor 2nd time

Time Interval	Absorbance (at 240.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (1000 times diluted)	Amount of Drug present (in mg)	Potency (%)
2 hour	0.390	0.392	0.0042	4.19	83.88
	0.392				
	0.391				
	0.390	0.391	0.0042	4.17	83.47
	0.393				
	0.391				
	0.390	0.390	0.0042	4.16	83.18
	0.394				
	0.388				

Table 4.7.7: Concentration & absorbance of Diazepam (Sedil®) under 40 w bulb for 2nd time

Time Interval	Absorbance (at 240.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (1000 times diluted)	Amount of Drug present (in mg)	Potency (%)
4 hour	0.334	0.332	0.0035	3.51	70.25
	0.330				
	0.332				
	0.332	0.334	0.0036	3.63	70.57
	0.334				
	0.334				
	0.332	0.332	0.0035	3.51	70.25
	0.334				
	0.332				

Table 4.7.8: Concentration & absorbance of Diazepam (Sedil®) under 40w bulb for 2nd time

Time Interval	Absorbance (at 240.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (1000 times diluted)	Amount of Drug present (in mg)	Potency (%)
6 hour	0.320	0.322	0.0034	3.41	67.76
	0.324				
	0.324				
	0.321	0.320	0.0034	3.38	67.57
	0.320				
	0.325				
	0.320	0.322	0.0034	3.41	67.76
	0.322				
	0.322				

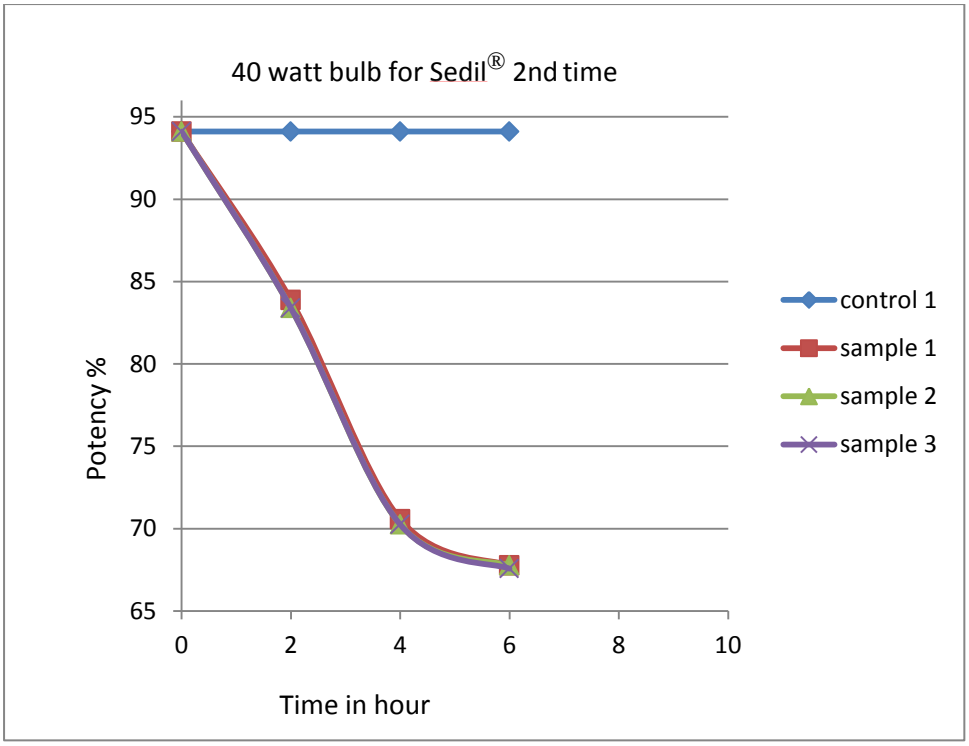

Figure 4.8.2 : Plot showing linear decrease in Potency (%) over time period (in hours) for diazepam under 40W bulb light (2nd time)

Table 4.7.9: Concentration & absorbance of Diazepam (Sedil®) under 40 W bulbfor 3rd time

Time Interval	Absorbance (at 240.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (1000 times diluted)	Amount of Drug present (in mg)	Potency (%)
Control	0.437	0.439	0.0047	4.70	94.11
	0.439				
	0.440				
	0.438	0.438	0.0047	4.68	93.97
	0.442				
	0.437				
	0.441	0.439	0.0047	4.70	94.11
	0.440				
	0.438				

Table 4.7.10: Concentration & absorbance of Diazepam (Sedil®) under 40w bulb for 3rd time

Time Interval	Absorbance (at 240.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (1000 times diluted)	Amount of Drug present (in mg)	Potency (%)
2 hour	0.390	0.392	0.0042	4.18	83.78
	0.392				
	0.391				
	0.390	0.391	0.0042	4.17	83.47
	0.393				
	0.391				
	0.390	0.390	0.0042	4.16	83.18
	0.394				
	0.388				

Table 4.7.11: Concentration & absorbance of Diazepam (Sedil®) under 40 w bulb for 3rd time

Time Interval	Absorbance (at 240.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (1000 times diluted)	Amount of Drug present (in mg)	Potency (%)
4 hour	0.334	0.331	0.0035	3.51	70.30
	0.330				
	0.332				
	0.332	0.334	0.0036	3.63	70.87
	0.334				
	0.334				
	0.332	0.332	0.0035	3.51	70.25
	0.334				
	0.332				

Table 4.7.12: Concentration & absorbance of Diazepam (Sedil®) under 40w bulb for 3rd time

Time Interval	Absorbance (at 240.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (1000 times diluted)	Amount of Drug present (in mg)	Potency (%)
6 hour	0.320	0.322	0.0034	3.41	67.71
	0.324				
	0.324				
	0.321	0.320	0.0034	3.38	67.59
	0.320				
	0.325				
	0.320	0.322	0.0034	3.41	67.73
	0.322				
	0.322				

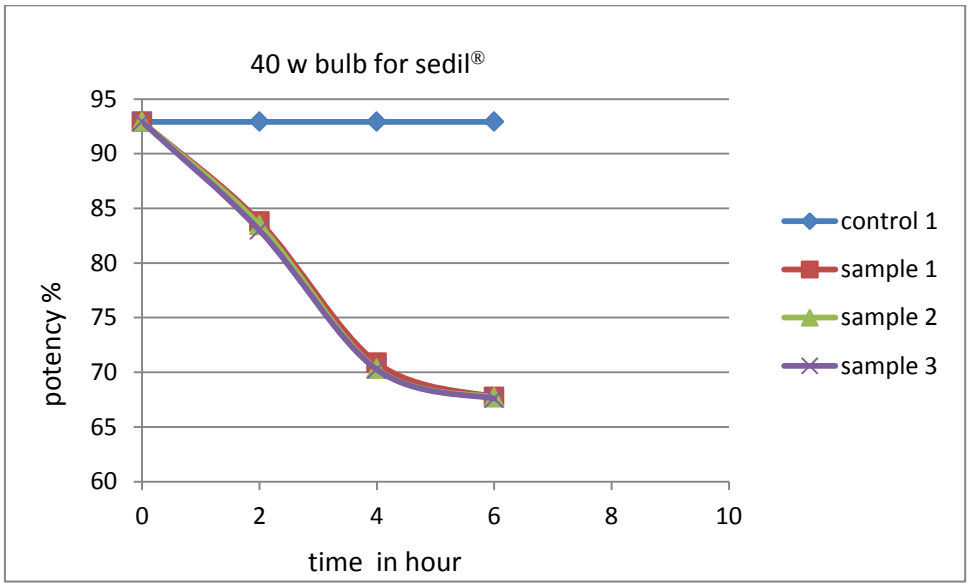

Figure 4.8.3 : Plot showing linear decrease in Potency (%) over time period (in hours) for diazepam under 40 W bulb (3rd time)

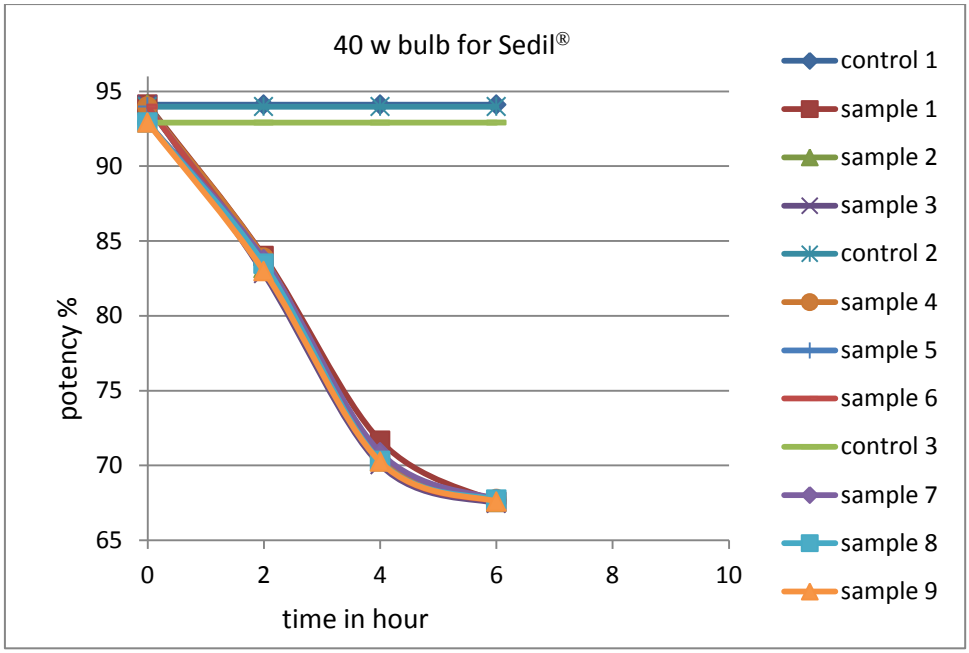

Figure 4.8.4 : Plot showing linear decrease in Potency (%) over time period (in hours) for diazepam under 40 W bulb

4.3.4 Result of samples that were exposed under direct sunlight

In experimental day, a tablet strip containing 10 tablets was taken and 5 samples were collected for the test and observed 3 different absorbance of metoprolol tartrate for three 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 4.7.1: Concentration & absorbance of Diazepam (Sedil®) under sunlight for 1st time

Time Interval	Absorbance (at 240.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (1000 times diluted)	Amount of Drug present (in mg)	Potency (%)
Control	0.437	0.439	0.0047	4.70	94.11
	0.439				
	0.440				
	0.438	0.439	0.0047	4.70	94.11
	0.442				
	0.437				
	0.441	0.439	0.0047	4.70	94.11
	0.440				
	0.438				

Table 4.7.2: Concentration & absorbance of Diazepam (Sedil®) under sunlight for first time

Time Interval	Absorbance (at 240.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (1000 times diluted)	Amount of Drug present (in mg)	Potency (%)
2 hour	0.325	0.324	0.0035	3.53	68.88
	0.325				
	0.322				
	0.327	0.325	0.0042	3.39	68.68
	0.325				
	0.327				
	0.326	0.323	0.0042	3.49	67.98
	0.336				
	0.324				

Table 4.7.3: Concentration & absorbance of Diazepam (Sedil®) under sunlight for 1st time

Time Interval	Absorbance (at 240.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (1000 times diluted)	Amount of Drug present (in mg)	Potency (%)
4 hour	0.323	0.321	0.0034	3.39	67.23
	0.321				
	0.321				
	0.318	0.318	0.0032	3.23	66.76
	0.318				
	0.319				
	0.320	0.320	0.0032	3.29	67.39
	0.321				
	0.319				

Table 4.7.4: Concentration & absorbance of Diazepam (Sedil®) under sunlight for first time

Time Interval	Absorbance (at 240.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (1000 times diluted)	Amount of Drug present (in mg)	Potency (%)
6 hour	0.305	0.305	0.0032	3.20	63.78
	0.304				
	0.305				
	0.308	0.306	0.0032	3.18	64.03
	0.302				
	0.304				
	0.304	0.305	0.0033	3.20	63.63
	0.304				
	0.306				

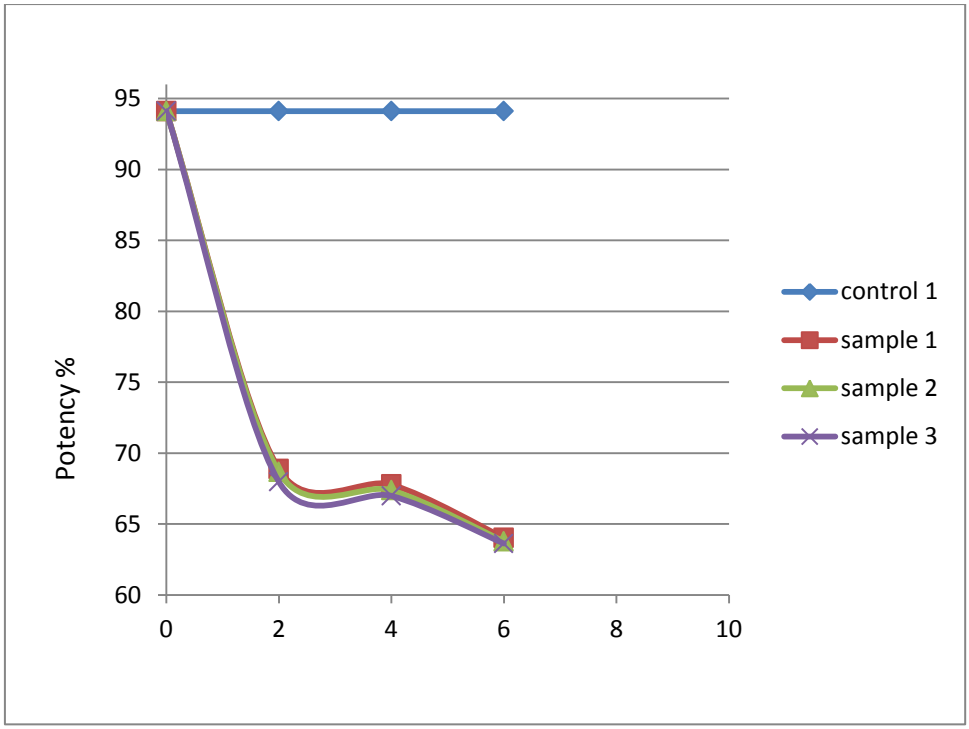

Figure 4.9.1 : Plot showing linear decrease in Potency (%) over time period (in hours) for diazepam under sunlight (1st time)

Table 4.7.5: Concentration & absorbance of Diazepam (Sedil®) under sunlight for 2nd time

Time Interval	Absorbance (at 240.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (1000 times diluted)	Amount of Drug present (in mg)	Potency (%)
Control	0.437	0.439	0.0047	4.70	94.11
	0.439				
	0.440				
	0.438	0.437	0.0047	4.65	93.23
	0.442				
	0.437				
	0.441	0.439	0.0047	4.70	94.11
	0.440				
	0.438				

Table 4.7.6: Concentration & absorbance of Diazepam (Sedil®) under sunlight for first time

Time Interval	Absorbance (at 240.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (1000 times diluted)	Amount of Drug present (in mg)	Potency (%)
2 hour	0.325	0.324	0.0035	3.53	68.58
	0.325				
	0.322				
	0.327	0.325	0.0042	3.39	68.58
	0.325				
	0.327				
	0.326	0.321	0.0042	3.49	67.88
	0.336				
	0.324				

Table 4.7.7: Concentration & absorbance of Diazepam (Sedil®) under sunlight for 2nd time

Time Interval	Absorbance (at 240.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (1000 times diluted)	Amount of Drug present (in mg)	Potency (%)
4 hour	0.323	0.320	0.0034	3.36	67.69
	0.321				
	0.321				
	0.318	0.317	0.0032	3.21	66.91
	0.316				
	0.319				
	0.320	0.317	0.0032	3.19	66.98
	0.321				
	0.319				

Table 4.7.8: Concentration & absorbance of Diazepam (Sedil®) under sunlight for first time

Time Interval	Absorbance (at 240.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (1000 times diluted)	Amount of Drug present (in mg)	Potency (%)
6 hour	0.305	0.305	0.0032	3.20	63.78
	0.304				
	0.305				
	0.308	0.305	0.0032	3.20	63.78
	0.302				
	0.304				
	0.304	0.303	0.0033	3.19	63.53
	0.304				
	0.306				

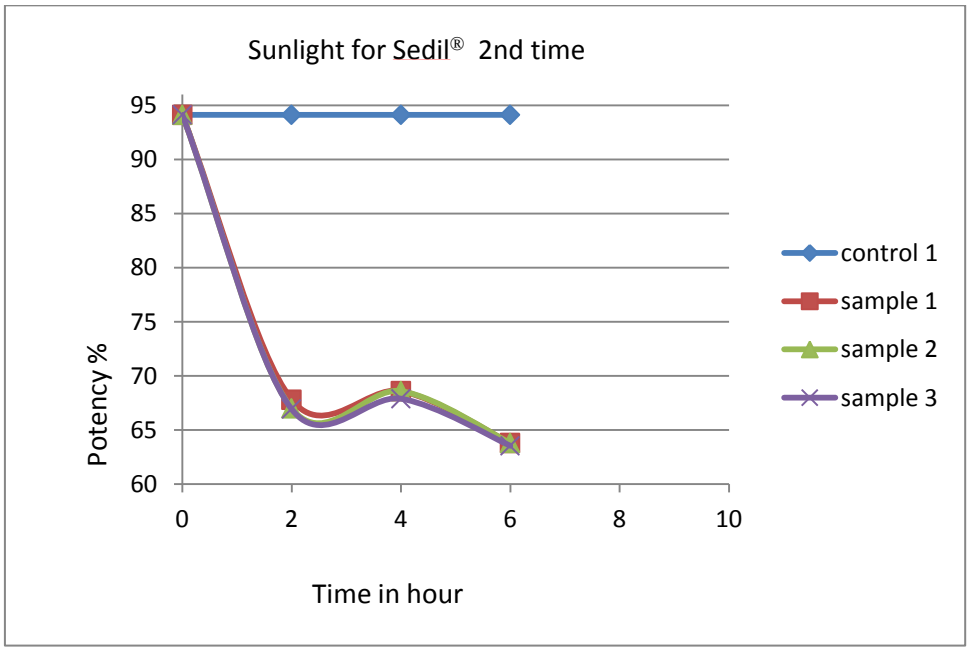

Figure 4.9.2 : Plot showing linear decrease in Potency (%) over time period (in hours) for diazepam under sunlight (2nd time)

Table 4.7.9: Concentration & absorbance of Diazepam (Sedil®) under sunlight for 3rd time

Time Interval	Absorbance (at 240.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (1000 times diluted)	Amount of Drug present (in mg)	Potency (%)
Control	0.437	0.438	0.0047	4.68	93.91
	0.439				
	0.440				
	0.438	0.439	0.0047	4.70	94.11
	0.442				
	0.437				
	0.441	0.437	0.0047	4.65	93.23
	0.440				
	0.438				

Table 4.7.10: Concentration & absorbance of Diazepam (Sedil®) under sunlight for 3rd time

Time Interval	Absorbance (at 240.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (1000 times diluted)	Amount of Drug present (in mg)	Potency (%)
2 hour	0.325	0.324	0.0035	3.53	68.88
	0.325				
	0.322				
	0.327	0.325	0.0042	3.39	68.68
	0.325				
	0.327				
	0.326	0.323	0.0042	3.49	67.98
	0.336				
	0.324				

Table 4.7.11: Concentration & absorbance of Diazepam (Sedil®) under sunlight for 3rd time

Time Interval	Absorbance (at 240.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (1000 times diluted)	Amount of Drug present (in mg)	Potency (%)
4 hour	0.323	0.321	0.0034	3.39	67.48
	0.321				
	0.321				
	0.318	0.318	0.0032	3.23	66.93
	0.318				
	0.319				
	0.320	0.320	0.0032	3.29	67.38
	0.321				
	0.319				

Table 4.7.12: Concentration & absorbance of Diazepam (Sedil®) under sunlight for 3rd time

Time Interval	Absorbance (at 240.5 nm)	Average Absorbance	Diluted Concentration from Samples in mg (1000 times diluted)	Amount of Drug present (in mg)	Potency (%)
6 hour	0.305	0.305	0.0032	3.20	63.88
	0.304				
	0.305				
	0.308	0.306	0.0032	3.18	64.05
	0.302				
	0.304				
	0.304	0.305	0.0033	3.20	63.83
	0.304				
	0.306				

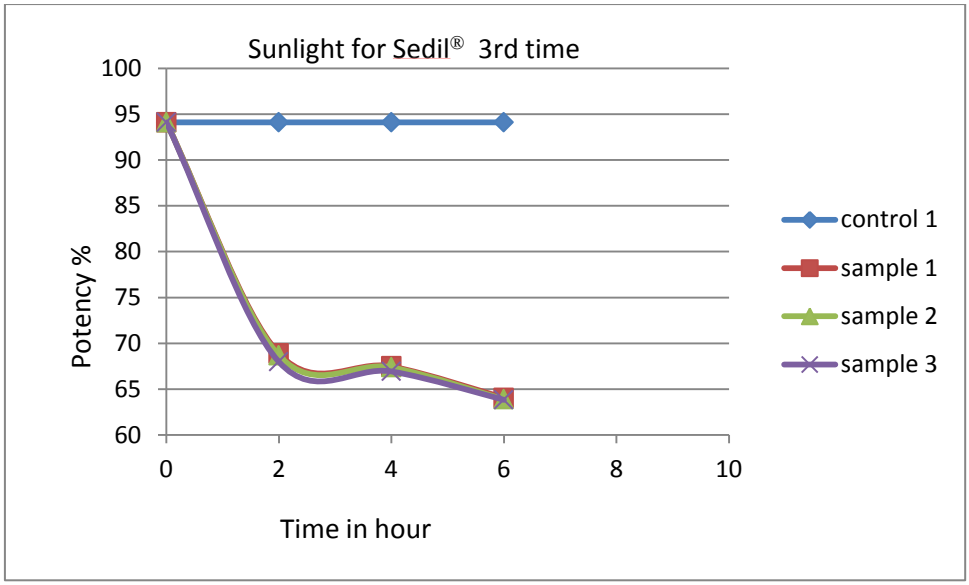

Figure 4.9.3 : Plot showing linear decrease in Potency (%) over time period (in hours) for diazepam under sunlight (3^{rd} time)

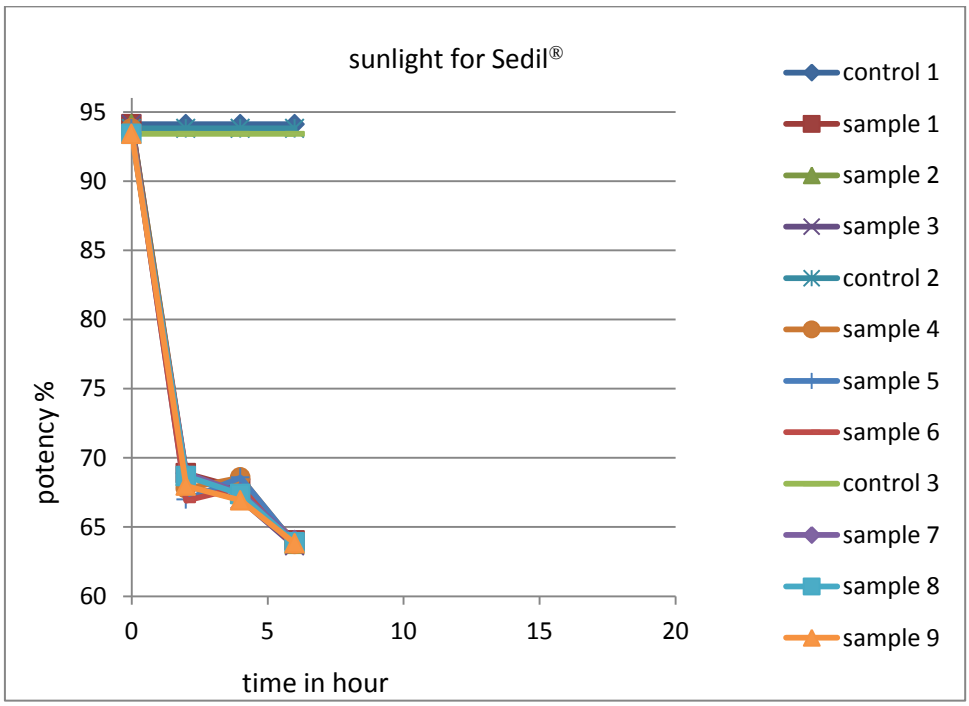

Figure 4.9.4 : Plot showing linear decrease in Potency (%) over time period (in hours) for diazepam under sunlight

CHAPTER FIVE

DISCUSSIONS

5.1 Discussions

In this experiment it was found that the percentage of Weight Variation of the sample tablets was within the accepted range (Weight of tablet 130 mg or less then = ±10%) with standard deviation ±0.0004. According to U.S.P. if no more than 2 tablets are outside the percentage limit and if no tabletdiffers by more than 2 times the percentage limit, the tablet pass the test. So, it is clear that, the light has no effect on weight of the Diazepam. Even the color of the tablets remains unchanged throughout the periodic work.

In this study it was observed that the hardness of the sample tablets was fluctuated with a very short range with standard deviation ±0.05within the total 80 days interval works. Even the average hardness value wasalso very close to each other. So the hardness of Diazepam was not affected by different lighting conditions.

According to this experimental overview the thickness of the sample tablets was also very close to each other or a very insignificant fluctuation with standard deviation ±0.012throughout periodic work. After each days interval the thickness remains constant or close to constant. So the effects of light dose not influencethethickness of Diazepam.

After completing the study, it was found that the concentration of diazepam was decreased gradually in every ovation of light exposure. When sample tablet (Sedil®) was kept under the electrical bulb (25 watt & 40 Watt) and test every two hour light exposed sample tablet it was found that the concentration of diazepam was decreased gradually. The tablet sample which were exposed 4 hours on light had less concentration of diazepam than the 2 hour exposed sample tablet had and also found that 6 hour exposed sample tablets have even less concentration 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 12 weeks for the normal lightening and room temperature condition also found that the concentrations of the samples were decreased gradually. 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%, 24.29%, 27.87%, and 31.66%. So it was found that the coating system alone is not efficient. Further steps like packaging system could be change to prevent photolytic degradation.

CHAPTER SIX

CONCLUSION

6.1 Conclusion

From the above study it was observed that the physical parameter like weight variation, hardness and thickness have passed the USP and BP specification. But there have remarkably changed in concentration/potency. The concentration of diazepam was decreased gradually after exposure in electrical light condition, sunlight and normal light exposure (room temperature) condition. So it can be said that the Sedil® containing diazepam is light sensitive and the potency is decreased after light exposure.

After this study, it can be concluded with a decision that, the packaging system could be change to prevent photolytic degradation in Sedil®. This blister packaging system could be changed into opaque package thus the light cannot pass through the package.

CHAPTER SEVEN

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