Reproducibility Study of the Packaging Efficiency on Preventing Photolytic Degradation of Evalin®(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"

DECLARATION BY THE CANDIDATE

I, Rehnuma Ananna (ID#2011-1-70-043), declare that the dissertation entitled "Reproducibility study of the packaging efficiency on preventing photolytic degradation of Evalin® (Diazepam)" submitted to the Department of Pharmacy, East West University, Aftabnagar in partial fulfillment of the requirement for the Degree of Bachelor of Pharmacy, was carried out by me under the supervision and guidance of Md. Anisur Rahman, Senior Lecturer, and the co-advisor Faisal Bin Karim, Lecturer Dept. of Pharmacy, East West University, Dhaka.

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This is to certify that the dissertation entitled "Reproducibility study of the packaging efficiency on preventing photolytic degradation of Evalin® (Diazepam)" submitted to the department of pharmacy, East West University in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy was carried out by Rehnuma Ananna (ID: 2011-1-70-043) under our guidance and supervision and that no part of the research has been submitted for any other degree. We further certify that all the sources of information and laboratory facilities availed of in this connection is duly acknowledged.

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ENDORSMENT BY THE CHAIRPERSON

This is to be certified that the dissertation entitle "Reproducibility study of the packaging efficiency on preventing photolytic degradation of Evalin® (Diazepam)" is a bonafide research work done by Rehnuma Ananna (ID: 2011-1-70-043) under the guidance and supervision of Md. Anisur Rahman, Senior Lecturer, and the co-advisor Faisal Bin Karim, Lecturer, Department of Pharmacy, East West University, Dhaka.

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ABSTRACT

This research work was aimed to check the reproducibility of the experiment that was previously done to evaluate the packaging efficiency of a one of the photosensitive drug Diazepam. To conduct the study 700 tablets from Evalin® were taken as a sample from the same batch. To determine the photolytic effect, all tablets were exposed 3 times in various lighting conditions (control, sunlight, normal room light, 25watt & 40watt bulb). Besides this physical parameters were tested for evaluation of color change, weight variation, thickness and hardness of the Evalin® tablets. Physical tests were performed according to the specification of United States Pharmacopeia (USP) and British Pharmacopoeia (BP). The standard deviations for the weight variation, thickness and hardness are respectively ± 0.21, ± 0.14cm, ± 0.47kg and the percent variation of the decreased concentration of the samples for normal lightening condition, 25 watt & 40 watt light exposure and sunlight exposure were found respectively 20.61%, 17.56%, 26.05% & 29.02% After the observation of 60 days, it was clearly visible that the potency of the diazepam decreased gradually in various light conditions like above conditions due to photolytic degradation. So it can be concluded that the transperant packaging system is not efficient so further steps like using opaque packaging system should be done to prevent photolytic degradation.

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

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CHAPTER ONE

INTRODUCTION

Overall Objective of the Research

The objective of this research project was to determine the photolytic degradation of diazepam products. In this research, we conducted experiments to determine the photosensitivity or photostability of Diazepam under various lightening conditions (no light/control, sunlight, normal light, 25 watt bulb & 40 watt bulb). For this purpose, the available brand chosen was Evalin® from Aristopharma Ltd. In most cases, these products are available in transparent blister packaging in the market. Only few brands use opaque packaging system for this kind of drug. Since there is no published data about the photolytic degradation of diazepam, a research program was conducted to find out whether this drug is photosensitive or not.

1.1 Stability (Answers.com, 2015)

In chemistry, stability means the degree of resistance to chemical change or disintegration. Stability of a drug means the capacity of a drug to retain its properties without loss of potency for a specified length of time.

Factors affecting drug stability includes temperature, light, moisture, microbes, packaging materials, transportation, components of drug and the nature of the active ingredients.

1.2 Photolytic Degradation (Slideshare.net, 2015)

Photolytic degradation or Photolysis means the chemical decomposition of materials caused by light or other electromagnetic radiation.

It is the process by which light-sensitive drugs or excipient molecules are chemically degraded by light, room light or sunlight. Ultra violet light causes more damage than normal light. The variation of degradation depends on the wavelength of light. Shorter wavelengths are more damaging than the longer wavelengths. Before a photolytic reaction can occur, the energy from light radiation must be absorbed by the molecules.

Two ways in which photolysis can occur are,

The light energy absorbed must be sufficient to achieve the activation energy, or

The light energy absorbed by molecules is passed on to other molecules which allow degradation to take place.

In this process, the light may be the initiator while the reaction may be oxidation, polymerization or ring arrangement. The photolytic reaction can produce a catalyst for thermal reaction since light energy can be converted into heat energy.

1.3 Sedative-Hypnotic Drug

1.3.1 Definition (Nelson, 2006)

Sedative-Hypnotics are a class of drug where sedative indicates a substance that induces sedation by reducing irritability or excitement and hypnotic refers to a substance that causes drowsiness and facilitates the onset and maintenance of natural sleep.

The pharmacodynamic actions of these drugs are considered potential drug abuse, and are therefore regulated as controlled substances. In clinical therapeutics, sedative-hypnotics are useful for treatment of a variety of diseases related to the central nervous system, such as acute and chronic anxiety, anesthesia, seizure control, and insomnia. The sedative-hypnotics drugs are divided into three major groups (1) benzodiazepines (2) barbiturates (3) other class which is not included in first two classes of drugs.

1.3.2 General mode of action of Sedative-Hypnotic Drugs (Drugs.com, 2015)

In the human brain GABA is the main inhibitory neurotransmitter in the brain. In the brain there are three types of GABA (gamma – aminobutyric) receptors such as GABA-A, GABA-B, and GABA-C. Benzodiazepines acts on the central nervous system and specifically occupying certain protein areas in the brain called GABA-A receptors. Benzodiazepines improve the responses to the inhibitory neurotransmitter GABA initiating GABA-activated chloride channels and give way to the chloride ions to enter the neuron. This action permits the neuron to become negatively charged and impregnable to excitation, which leads to the various anti-anxiety, sedative, or antiseizure activity seen with these drugs. The a2 subunit of GABA-A seems to be liable for the

antianxiety effects of benzodiazepines; other subunits regulate the amnesic and sedative properties of benzodiazepines.

1.4 Benzodiazepines (PharmGKB, 2015) (Ashton, 2005)

Benzodiazepines are a class of psychoactive drugs whose core chemical structure is the fusion of a benzene ring and a diazepine ring. Benzodiazepines are a class of drugs that act on the central nervous system. That is resulting in sedative, hypnotic, anxiolytic, anticonvulsant, muscle relaxant etc. They are clinically used as a as sedatives-anxiolytic drugs therapy for epilepsies panic disorders and various other disorders such as Familial paroxysmal choreoathetosis and Hyperexplexia.

Benzodiazepine is first introduced in the 1950 and became very popular in medical fields. In the late 1970's, it became the most commonly prescribed drugs all over the world. It was used for long term therapy but by the early 1980's it was found that because of long term therapy the patients got addicted and felt difficulty of drug withdrawing. After that the doctors were advised to prescribe the drug for short term treatment in minimal dosage.

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

1.5 Diazepam (Drugs.com, 2015)

Diazepam is a benzodiazepine. It affects chemicals in the brain that may become unbalanced and cause anxiety. Diazepam is used to treat anxiety disorders, alcohol withdrawal symptoms, or muscle spasms. Diazepam is sometimes used with other medications to treat seizures. It was first synthesized by Leo Sternbach. It was launched in 1963 and became the most common prescribed drug to treat wide range of conditions.

In this research project, experiments were conducted on sample which was manufactured by Aristopharma Ltd. (Brand name: Evalin®). 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 a remedy for anxiety pain from apprehension and depression, acute and chronic stress of life, skeletal muscle spasm and strychnine poisoning.

1.5.1 Physico-Chemical Properties (Inchem.org, 2015)

1.5.1.1 Physical Properties

Color: white or yellow

State : solid-crystals

Description

Melting point : 131.5 to 134.6

Odourless and slightly bitter taste

Slightly soluble in water , soluble in alcohol and freely soluble in chloroform pH is neutral

1.5.1.2 Chemical Properties

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

Molecular weight : 284.74022 g/mol

Figure 1.1: Chemical structure of Diazepam

1.5.2 Pharmacological Properties

1.5.2.1 Pharmacodynamic Properties (Inkling.com, 2015), (Pharmacologycorner.com, 2015), (Mybwmc.org, 2015)

The GABAA receptors are ligand-gated chloride-selective ion channels that are activated by GABA, the major inhibitory neurotransmitter in the brain. Diazepam is a 1, 4-benzodiazepine, which binds with high affinity to the GABA A receptor in the brain. Binding of diazepam to this

receptor complex promotes binding of GABA and facilitates the opening of GABA activated chloride channels. This increased chloride ion influx hyperpolarizes the neuron's membrane potential. As a result, the difference between resting potential and threshold potential is increased this means less excitable state.

1.5.2.2 Pharmacokinetic Properties (Drugs.com, 2015)

Absorption

Diazepam is readily and completely absorbed from the Gastro-Intestinal tract, peak plasma concentrations occurring within about 30-90 minutes of oral administration. Diazepam is highly lipid soluble.

Distribution

Diazepam is highly bound to plasma proteins about 98%. It cross the blood –brain and placental barriers and are also found in breast milk. The volume of distribution of diazepam at steady-state is 0.8 to 1.0 L/kg. The decline in the plasma concentration-time profile after oral administration is biphasic. The initial distribution phase has a half-life of approximately 1 hour, although it may range up to 3 hours.

Metabolism

Diazepam is extensively metabolised in the liver and is N-demethylated by CYP3A4 and 2C19 to the active metabolite N-desmethyldiazepam, and is hydroxylated by CYP3A4 to the active metabolite temazepam. Both N-desmethyldiazepam and temazepam are metabolized to oxazepam. Temazepam and oxazepam are largely eliminated by glucuronidation.

Elimination

Diazepam is one of the most slowly eliminated benzodiazepines. The terminal elimination half-life of the active metabolite N-desmethyldiazepam is up to 100 hours. Diazepam is excreted mainly in the urine, predominantly as their glucuronide conjugates. The clearance of diazepam is 20 to 30 mL/min.

1.5.3 Indications (Drugs.com, 2015)

Adults

The short-term relief (2-4 weeks) only, of anxiety which is severe, disabling, or subjecting the individual to unacceptable distress, occurring alone or in association with insomnia or short-term psychosomatic, organic or psychotic illness.

Cerebral palsy.

Muscle spasm.

As an adjunct to certain types of epilepsy (e.g. myoclonus).

Symptomatic treatment of acute alcohol withdrawal.

As oral premedication for the nervous dental patient. For premedication before surgery

Children

Control of tension and irritability in cerebral spasticity in selected cases

As an adjunct to the control of muscle spasm in tetanus

Oral premedication

1.5.4 Dosage and Administration (Drugs.com, 2015)

As an anxiolytic, the lowest effective dose should be employed; dosage regimes should not exceed beyond 4 weeks and treatment should be gradually withdrawn. Patients who have received benzodiazepines for a long time may require an extended withdrawal period. Long-term chronic use is not recommended.

Adults:

Anxiety states, obsessive-compulsive neuroses, and other psychiatric disorders: 5-30mg daily in divided doses.

Insomnia associated with anxiety: 5-15mg before retiring. Cerebral palsy: 5-60mg daily in divided doses.

Upper motor neuronic spasticity: 5-60mg daily in divided doses.

Muscle spasm of varied aetiology, fibrositis, cervical spondylosis: 5-15mg daily in divided doses.

Adjunct to the management of some types of epilepsy: 2-60 mg daily in divided doses. Alcohol withdrawal: 5-20mg, repeated if necessary in 2 to 4 hours.

Oral premedication in dental patients: 5mg the night before, 5mg on waking and 5mg two hours before the appointment.

Oral Premedication before surgery: 5mg-20mg.

Children:

Alternative presentations of diazepam are recommended for pediatric usage in order to obtain suitable doses of less than 5mg.

Spastic children with minimal brain damage: 5-40mg daily in divided doses.

Oral Premedication before surgery: 2mg-10mg

Elderly and debilitated patients:

Doses should be half the above recommended doses.

Renal and hepatic impairment:

The use of diazepam in hepatic impairment may precipitate coma, therefore the dose should be reduced or an alternative drug considered. In severe renal impairment the dose should be reduced.

1.5.5 Side Effects (Drugs.com, 2015)

Applies to diazepam: oral capsule extended release, oral solution, oral tablet

More Common

Shakiness and unsteady walk

unsteadiness, trembling, or other problems with muscle control or coordination

numbed emotions

reduced alertness

confusion

headache

dizziness

muscle weakness

ataxia

Double vision

Amnesia

Applies to diazepam: injectable solution, intravenous suspension, oral capsule extended release, oral concentrate, oral solution, oral tablet, rectal kit

Nervous system: euphoria, increased agitation and hyperactivity, drowsiness, fatigue, confusion, depression, psychomotor impairment, cognitive impairment, headache, syncope, slurred speech, tremor, vertigo, dysarthria, dizziness, and ataxia.

Local: venous thrombosis, phlebitis, local irritation and swelling occur in about 8% of patients. **Psychiatric:** stimulation, restlessness, acute hyperexcited states, anxiety, agitation, aggressiveness, irritability, rage, hallucinations, psychoses, delusions, insomnia, sleep disturbances, nightmares and inappropriate behavior.

Respiratory: increased risk of aspiration, respiratory arrest.

Gastrointestinal: constipation, gastrointestinal disturbances, and nausea.

Genitourinary: sexual dysfunction, incontinence, changes in libido, and urinary retention. **Hypersensitivity:** rash, pruritus, and severe bronchospasm.

Hepatic: granulomatous hepatitis.

Endocrine: gynecomastia.

Musculoskeletal: muscle spasticity.

Cardiovascular: hypotension and possible anti-ischemic effects by reducing myocardial oxygen consumption.

Ocular: blurred vision and diplopia.

Dermatologic: skin reactions.

Other: psychosensory symptoms such as depersonalization, derealization, and perceptual distortion are a unique feature of the withdrawal syndrome.

Withdrawal symptoms: convulsions, tremor, abdominal cramps, panic attacks, depression, vomiting, anxiety, agitation, insomnia and sweating.

1.5.6 Contraindications (Epocrates, 2008)

Use of diazepam should be avoided, when possible, in individuals with:

Ataxia

Severe hypoventilation

Acute narrow-angle glaucoma

Severe hepatic deficiencies (hepatitis and liver cirrhosis decrease elimination by a factor of two)

Severe renal deficiencies (for example, patients on dialysis)

Liver disorders

Severe sleep apnea

Severe depression, particularly when accompanied by suicidal tendencies Psychosis

Pregnancy or breast feeding

Caution required in elderly or debilitated patients

Coma or shock

Abrupt discontinuation of therapy

Acute intoxication with alcohol, narcotics, or other psychoactive substances (with the exception of some hallucinogens and/or stimulants, where it is occasionally used as a treatment for overdose)

History of alcohol or drug dependence

Myasthenia gravis, an autoimmune disorder causing marked fatiguability

Hypersensitivity or allergy to any drug in the benzodiazepine class.

1.5.7 Drug-Drug Interactions (Drugs.com, 2015)

Alcohol

Concomitant use with alcohol is not recommended due to enhancement of the sedative effect.

Antacids

Concurrent use may delay absorption of diazepam.

Sodium oxybate

Avoid concomitant use (enhanced effects of sodium oxybate).

HIV-protease inhibitors

Avoid concomitant use (increased risk of prolonged sedation)

Phenytoin

There have also been reports that the metabolic elimination of phenytoin is decreased by diazepam.

Anti-epileptic drugs

Pharmacokinetic studies on potential interactions between diazepam and antiepileptic drugs have produced conflicting results. Depression and elevation of drug levels, as well as no change, have been reported.

Phenobarbital taken concomitantly may result in an additive CNS effect. Increase risk of sedation and respiratory depression. Phenobarbital is a known inducer of CYP3A4 and increases hepatic metabolism of diazepam. Special care should be taken in adjusting the dose in the initial stages of treatment.

Compounds that affect hepatic enzymes (particularly cytochrome P450)

There is a potentially relevant interaction between diazepam and compounds which inhibit certain hepatic enzymes (particularly cytochrome P450 3A and 2C19). Data indicate that these compounds influence the pharmacokinetics of diazepam and may lead to increased and prolonged sedation. At present, this reaction is known to occur with cimetidine, ketoconazole, fluvoxamine, fluoxetine, and omeprazole.

Narcotic analgesics

Enhancement of the euphoria may lead to increased psychological dependence.

Rifamycins (rifampicin)

Rifampicin is a potent inducer of CYP3A4 and substantially increases the hepatic metabolism and clearance of diazepam. Co-administration with rifampicin gives rise to substantially decreased concentrations of diazepam.

Antihypertensives, vasodilators& diuretics:

Enhance hypotensive effect with ACEinhibitors, alpha-blockers, angiotensin–II receptor antagonists, calcium channel. blockers adrenergic neurone blockers, beta-blockers, moxonidine, nitrates, hydralazine, minoxidil, sodium nitroprusside and diuretics. Enhance sedative effect with alpha-blockers or moxonidine.

Dopaminergics

There is a possibility of antagonism effect of levodopa.

Carbamazepine

Carbamazepine is a known inducer of CYP3A4 and increases hepatic metabolism of diazepam.

This can result in up to three-fold greater plasma clearance and a shorter half-life of diazepam.

1.5.8 Drug-Food Interactions (Drugs.com, 2015)

Interactions with food have not been established. Alcohol should not take with diazepam tablets because it may increase the sedative effects if it and make people sleepy.

Caffeine

Concurrent use may result in reduced sedative and anxiolytic effects of diazepam.

Grapefruit juice

Inhibition of CYP3A4 may increase the plasma concentration of diazepam (possible increased sedation and amnesia). Cmax is increased by 1.5 times and AUC by 3.2 times. Possible increased effect of diazepam.

This interaction may have little significance in healthy individuals, but it is not clear is if other factors such as old age or liver cirrhosis increase the risk of adverse effects with concurrent use.

1.5.9 Pregnancy & Lactation (Drugs.com, 2015)

Pregnancy

For compelling medical reasons, the product is administered during the late phase of pregnancy, or during labour at high doses, effects on the neonate, such as hypothermia, hypotonia ("Floppy Infant Syndrome"), irregularities in the heart rate, poor suckling and moderate respiratory depression, can be expected, due to the pharmacological action of the compound.

Moreover, infants born to mothers who took benzodiazepines chronically during the latter stages of pregnancy may have developed physical dependence and may be at some risk for developing withdrawal symptoms in the postnatal period.

Studies in animals have shown reproductive toxicity.

Lactation

Benzodiazepines are found in the breast milk, Reports have demonstrated milk: plasma concentration ratios to vary between 0.2 and 2.7. There is therefore a risk of accumulation in the breastfeeding child. Benzodiazepines should not be given to breast feeding mothers.

1.5.10 Warnings/Precautions (RxList, 2015)

1. Special precaution should be taken in case of elderly or debilitated patients with hepatic disease including alcoholics or renal impairment because this can effect on drug metabolism. Active metabolites with extended half-lives may lead to delayed accumulation and adverse effects.

2. Parenteral administration can cause acute hypotension, muscle weakness, apnea, and cardiac arrest. Qualified personnel and appropriate resuscitative equipment should be availabe during administration and monitoring. Parenteral formulation contains propylene glycol, which create toxicity when administered in high dosages, so dose should be given carefully. Intra-arterial injection or extravasation of the parenteral formulation should be avoided.

3. Taking diazepam causes CNS depression (dose related) which results in sedation, dizziness, confusion, or ataxia which may impair physical and mental capabilities. So the patient should be informed before using it. The dosage of narcotics should be reduced by approximately 1/3 when diazepam is added. Benzodiazepine should be used with caution in patients who are suffering from traumatic injury especially elderly.

4. If the patient with severe depression like suicidal risk may present then diazepam should be prescribed very cautiously. Benzodiazepines have created dependency and show acute withdrawal symptoms on discontinuation or reduction in dose. So drug dependent patient should use it very carefully.

5. Diazepam has been associated with anterograde amnesia. Paradoxical reactions, including hyperactive or aggressive behavior, have been reported with benzodiazepines, particularly in adolescent/paediatric or psychiatric patients. So it should be used very cautiously in this type of patients. (Mybwmc.org, 2014)

6. Some agents may potentiate the action of diazepam, such as phenothiazines, narcotics, barbiturates, MAO inhibitors and other antidepressants. So if diazepam is combined with other psychotropic agents or anticonvulsant drugs, careful consideration should be given to the pharmacology of the agents.

7. A lower dose is recommended for patients with chronic respiratory insufficiency, due to the risk of respiratory depression.

8. To prevent the development of ataxia or oversedation, the dosage should be limited to the smallest effective amount in debilitated patient.

9. Repeated use of diazepam may loss of response to the effects of benzodiazepines for prolonged time.

1.5.11 Overdose (Drugs.com, 2015)

Symptoms of overdose include;

Change in consciousness

difficult or troubled breathing

irregular, fast or slow, or shallow breathing

lack of coordination

loss of consciousness

loss of strength or energy

muscle pain or weakness

pale or blue lips, fingernails, or skin sleepiness

unusual drowsiness, dullness, tiredness, weakness, or feeling of sluggishness

1.6 Dosage Form & Packaging (Aristopharma Ltd., 2015)

Evalin® is available as white colored tablet containing 5 mg of diazepam. Each box contains 50x10 tablets in blister packs.

***Figure 1.2*:** Picture of Sample – Evalin® (Diazepam

CHAPTER TWO

LITERATURE REVIEW

2.1 Literature Review

From available brands one brand i.e. Evalin® 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 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 M. E. Morris following dilution to 10 different concentrations in dextrose 5% in water, normal saline, Ringer's injection and lactated Ringer's injection. Prepared solutions were examined for clarity and pH throughout a 24-hour period. The 1:20 dilution was compatible with all four diluents and maintained acceptable potency for four hours, whereas at 1:40 dilution (5 mg in 40 ml) diazepam was stable for at least 6-8 hours in the same diluents. However, if in some circumstances it is necessary to administer diazepam as an infusion, it is recommended that it be diluted in dextrose 5% in water, normal saline, Ringer's injection or lactated Ringer's injection to a dilution of at least 1:40 and used within 6 hours or to a dilution of 1:50 and used within 24 hours. (Morris, 1978)

In 1980, the compatibility and stability of diazepam 5 mg/ml in 30% sodium salicylate, following dilution with 5% dextrose and normal saline, was investigated by L. K. El-Khordagui. No precipitation was observed when diazepam-sodium salicylate solution was injected at varying rates into the tubing of 5% dextrose and saline infusions moving at low rates. Diazepam-sodium salicylate solution induced a higher degree of haemolysis in vitro and was less bound to bovine serum albumin than a commercial diazepam injection. Further studies are necessary for the clinical evaluation of this diazepam-sodium salicylate combination. (L. K. El-Khordagui, 1980)
In 1982, the stability of diazepam injection repackaged in disposable glass syringes and stored at room and refrigerator temperatures was studied by Smith FM and Nuessle NO. 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 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 1985, Physical Properties and Stability of Diazepam and Phenobarbitone Sodium Tablets Prepared with Compactrol were studied by H. M. Elsabbagh, M. H. Elshaboury, and Hamdy M. Abdel-Aleem. Compactrol as a newly introduced direct compressible vehicle 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 effect of storage at 75% RH, at two temperature levels (25° and 45°) on the physical properties of these tablets was studied for 6 weeks. It was found that, there were an increase in tablet weight, thickness and friability per cent, while a significant decrease in hardness was observed. Tablets prepared with compactrol 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 *et al.*, 1985)

In 1985, Robert D. Caplan, Frank M. Andrews, Terry L. Conway, Antonia Abbey, David J. Abramis and John R. P. French Jr researched on ‘social effects of diazepam use: a longitudinal field study’. The study examined the effects of actual use of diazepam (Valium”) on subjective

reports of life quality, performance, stress, social support, control, coping and other variables related to mental health. Standardized interviews were conducted with 675 persons from the Detroit Metropolitan Area. Based on prescription records, diazepam users and nonusers were selected to represent a variety of sociodemopraphic characteristics rather than to be a completely random sample. They also reported consuming less alcohol when using Valium than at other times and less than non-Valium users. Although there was a modest positive cross-sectional relation between Valium use and distress. Several interpretations of the results are examined including the possibility that the effects of Valium use were short-lived rather than long-term and that Valium may have been taken in anticipation of anxiety rather than after its occurrence. (Robert D. Caplan *et al.,* 1985)

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

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 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 2002, Alldredge BK, Venteicher R, Calderwood TS did research on the stability of diazepam rectal gel in ambulance-like environments. The objective of the study was to evaluate 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. In the various configurations and tests, diazepam concentration always exceeded 95% of label, with no changes of note in excipients or physicochemical properties. The estimated shelf-life at 30 degrees C exceeds 48 months. 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 2002, Iqbal MM, Sobhan T, Aftab SR, Mahmud SZ investigate the effect after the use of diazepam during pregnancy. 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)

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)

In 2005, Marija Glavas Dodov, Katerina Goracinova, Maja Simonoska, Suzana Trajkovic – Jolevska, Jasmina Tonic Ribarska, Marija Dastevska Mitevska published a research on the 'Formulation and evaluation of diazepam hydrogel for rectal administration.' The objective of the study was to formulate and evaluate rectal hydrogels containing DZP as a drug substance in combination with suitable co-solvents and preservatives. Prepared HPMC (hydroxypropyl methylcellulose) hydrogels containing different concentrations of DZP (2, 4 and 6 mg mL–1) manifested good quality in respect to physico- -chemical parameters (pH value, drug content,

ingredients content and viscosity), antimicrobial efficiency and microbiological quality. Under the proposed HPLC conditions, satisfactory separation of DZP and the preservatives used was achieved. *In vitro* release studies have shown that the total amount of DZP was released in a period of 3 h. Prepared formulations were stable for four months at 26 °C (ambient temperature characteristic of the 2nd climate zone). (M. Glavas Dodov *et al.*, 2005)

In 2005, Maślanka A, Krzek J developed a thin-layer chromatography (TLC)-densitometry method 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. (Maslanka and Krzek, 2005)

In 2006, Effectiveness of intermittent diazepam prophylaxis in febrile seizures: long-term prospective controlled study was done by Pavlidou E, Tzitiridou M, Panteliadis C. In a prospective randomized cohort trial, 139 children (77 girls, 62 boys) who experienced a first febrile seizure were allocated to two groups: group A, which received intermittent diazepam (n = 68), and group B, which received no prophylaxis (n = 71). All children had a 3-year follow-up. The inclusion criteria were no personal history of afebrile seizures, normal neurodevelopment, no previous anticonvulsant therapy, and age between 6 months and 3 years. The 36-month recurrence rates in the no-prophylaxis group were 83% in high-risk patients, 55% in intermediate-risk patients, and 46% in low-risk patients. In the prophylaxis group, the recurrence rates were reduced in all risk groups: 38%, 35%, and 33%, respectively. Intermittent diazepam prophylaxis reduces the recurrence rate mainly in high-risk children provided that sufficient doses are given on time and adequately. (Pavlidou E, Tzitiridou M, Panteliadis C, 2006)

In 2007, a study on Pharmacodynamics of diazepam co-administered with methadone or buprenorphine under high dose conditions in opioid dependent patients was done by Nicholas Lintzeris, Timothy B. Mitchell, Alyson J. Bond, Liam Nestor, John Strang. Double-blind, randomly ordered, 2 × 2 cross-over design in which the effects of diazepam dose (0 mg versus 40 mg) and opioid dose (100% versus 150% normal dose) were examined over four sessions in

methadone- and buprenorphine-maintained patients. Four methadone- and seven buprenorphine-prescribed patients without concurrent dependence on other substances or significant medical co-morbidity were examined. It was found that; high dose diazepam significantly alters subjective drug responses and psychological performance in patients maintained on methadone and buprenorphine. (Nicholas Lintzeris *et al.*, 2007)

In 2008, Mitsushige Sugimoto, Takahisa Furuta, Akiko Nakamura, Naohito Shirai, Mutsuhiro Ikuma, Shingen Misaka, Shinya Uchida, Hiroshi Watanabe, Kyoichi Ohashi, Takashi Ishizaki, and Akira Hishida studied on 'Maintenance time of sedative effects after an intravenous infusion of diazepam: A guide for endoscopy using diazepam'. Fifteen healthy Japanese volunteers consisting of three different *CYP2C19* genotype groups underwent a critical flicker fusion test, an eye movement analysis and a postural sway test as a test for physical sedative effects, and a visual analog scale (VAS) symptom assessment method as a test for mental sedative effects during the 336 h period after the intravenous infusion of diazepam (5 mg). With the psychomotor tests, the objective sedative effects of diazepam continued for 1 h to 3 h irrespective of *CYP2C19* genotype status and the subjective sedative symptoms improved within 1 h. Up to 3 h of clinical care appears to be required after the infusion of diazepam, although patients feel subjectively improved. (Mitsushige *et al.*, 2007)

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, 2008)

In 2008, Comparison study of buccal midazolam with rectal diazepam in the treatment of prolonged seizures in Ugandan children: a randomized clinical trial was done by Mpimbaza A, Ndeezi G, Staedke S, Rosenthal PJ, Byarugaba J. This was a single-blind, randomized clinical trial in which 330 patients were randomly assigned to receive buccal midazolam or rectal diazepam. The trial was conducted in the pediatric emergency unit of the national referral

hospital of Uganda. Consecutive patients who were aged 3 months to 12 years and presented while convulsing or who experienced a seizure that lasted >5 minutes were randomly assigned to receive buccal midazolam plus rectal placebo or rectal diazepam plus buccal placebo. The primary outcome of this study was cessation of visible seizure activity within 10 minutes without recurrence in the subsequent hour. Treatment failures occurred in 71 (43.0%) of 165 patients who received rectal diazepam compared with 50 (30.3%) of 165 patients who received buccal midazolam. Malaria was the most common underlying diagnosis (67.3%), although the risk for failure of treatment for malaria-related seizures was similar: 35.8% for rectal diazepam compared with 31.8% for buccal midazolam. For children without malaria, buccal midazolam was superior (55.9% vs 26.5%). Buccal midazolam was as safe as and more effective than rectal diazepam for the treatment of seizures in Ugandan children, although benefits were limited to children without malaria. (Mpimbaza A *et al.,* 2008)

In 2009, C Abbara, JM Rousseau, A Turcant, G Lallement, E Comets, I Bardot, P Clair and B Diquet did research on 'Bioavailability of diazepam after intramuscular injection of its water soluble prodrug alone or with atropine–pralidoxime in healthy volunteers.' The study was conducted in an open, randomized, single-dose, three-way, cross-over design. Each subject received intramuscular injections of avizafone (20 mg), diazepam (11.3 mg) or avizafone (20 mg) combined with atropine (2 mg) and pralidoxime (350 mg) using a bi-compartmental auto-injector (AIBC). Plasma concentrations of diazepam were quantified using a validated LC/MS–MS assay, and were analysed by both a non-compartmental approach and by compartmental modelling. Diazepam had a faster entry to the general circulation and achieved higher *C*max after injection of prodrug than after the parent drug. Administration of avizafone in combination with atropine and pralidoxime by AIBC had no significant effect on diazepam AUC and *C*max. (C Abbara *et al.,* 2009)

In 2009, Dong Il Noh, Kyu Nam Park, Heung Jae Chun, Chong Won Park, Ju Woong Jang, Yun Gyong Ahn studied on Compatibility of diazepam with polypropylene multilayer infusion container. Techflex®, a polypropylene-lined, multilayer infusion bag, was studied for its compatibility with diazepam, in comparison to the conventional infusion bag, Safeflex®, which is comprised of poly(vinyl chloride) (PVC). Diazepam was diluted in 0.9% sodium chloride isotonic solution and stored in the infusion bags for 24 h. To evaluate the sorption of diazepam

into the infusion bags during storage, the concentration of the drug remaining in the bag was measured using gas chromatography-mass spectroscopy. The PVC bags exhibited a marked sorption of diazepam, with a drug loss reaching up to 90% of the initial concentration after 24 h of contact, whereas Techflex® inhibited the drug sorption, showing approximately 10%, under the same conditions. The differences in the sorption behaviors of the bags are discussed in terms of solubility parameters and crystallinities of the polymers. (Dong Il Noh *et al.*, 2009)

In 2010, Khalid K. Abed, Ahmed A. Hussein , Mowafaq M. Ghareeb , and Alaa A. Abdulrasool studied on Formulation and Optimization of Orodispersible Tablets of Diazepam. Orodispersible tablets of diazepam were prepared using different types of superdisintegrants (Ac-Di-Sol, sodium starch glycolate, and crospovidone (CP)) and different types of subliming agents (camphor and ammonium bicarbonate (AB)) at different concentrations and two methods of tablets preparations (wet granulation and direct compression methods). The formulations were evaluated for flow properties, wetting time, hardness, friability, content uniformity, *in vivo* disintegration time (DT), release profiles, and buccal absorption tests. The results revealed that the tablets containing CP as a superdisintegrant have good dissolution profile with shortest DT. This study helps in revealing the effect of formulation processing variables on tablet properties. (K. Abed *et al.*, 2010)

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 2011, Lamson MJ, Sitki-Green D, Wannarka GL, Mesa M, Andrews P, Pellock J researched on Pharmacokinetics of diazepam administered intramuscularly by autoinjector versus rectal gel in healthy subjects. This was a phase I, randomized, open-label, two-part, single-dose, crossover, single-centre pharmacokinetic study in 48 healthy young adult (aged 18-40 years) male and female subjects. Part I of the study (n = 24) evaluated the dose proportionality of three strengths of the diazepam autoinjector (5, 10 and 15 mg) administered into the mid-outer thigh via a deep

IM injection. Part II (n = 24) assessed the relative bioavailability of the diazepam 10 mg autoinjector versus the diazepam 10 mg rectal gel. Results of the present study indicated that diazepam can be safely and reliably administered IM using a diazepam autoinjector. (Lamson MJ *et al.,* 2011)

In 2011, Bautitz and colleagues published their study in *Catalysis today* Photodegradation of lincomycin and diazepam in sewage treatment plant effluent by photo-Fenton process. The effect of ferrioxalate or iron nitrate on the photo-Fenton degradation efficiency of diazepam was evaluated either under black light or solar irradiation. Pharmaceuticals oxidation was not influenced by the matrix, since very similar results were obtained when compared to the experiments carried out in distilled water. DOC removal was slightly affected by matrix, due probably to the generation of recalcitrant intermediates during effluent photo degradation. The researchers concluded, "Even so, high DOC removal percentages were achieved, 80% for diazepam after 60 min irradiation." (Bautitz *et al.,* 2011)

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. 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 2012, Md. Sariful Islam Howlader et al studied on Enhancing dissolution profile of diazepam using hydrophilic polymers by solid dispersion technique. The aim of the present study was to improve the solubility and dissolution rate of a poorly water-soluble drug by a solid dispersion technique, in order to investigate the effect of these polymers on release mechanism from solid dispersions. Diazepam was used as a model drug to evaluate its release characteristics from

different matrices. The solid dispersions were prepared by solvent method. The pure drug and solid dispersions were characterized by in vitro dissolution study. Distilled water was used as dissolution media, 1000 ml of distilled water was used as dissolution medium in each dissolution basket at a temperature of 37°C and a paddle speed of 100 rpm. The very slow dissolution rate was observed for pure Diazepam and the dispersion of the drug in the polymers considerably enhanced the dissolution rate. Solid dispersions prepared with PEG-6000, Poloxamer showed the highest improvement in wettability and dissolution rate of Diazepam. Solid dispersion containing polymer prepared. (Howlader *et al.,* 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-glucan pessaries released drug more gradually. Finally, a possible drug photodegradation after exacerbated UV-visible exposition was evaluated. (Capra *et al.,* 2013)

In 2013, A simple, precise, and stability indicating high performance liquid chromatography (HPLC) method was developed and validated for the simultaneous determination of Imipramine hydrochloride and Diazepam in pharmaceutical dosage form by P.Pydiratnam1, T. Santosh Kumar , S Satyanarayana. The method involved the use of easily available inexpensive laboratory reagents. The method was successfully validated in accordance to ICH guidelines acceptance criteria for linearity, accuracy, precision, specificity, robustness. The analysis concluded that the method was selective for simultaneous estimation of Imipramine HCl and Diazepam can be potentially used for the estimation of these drugs in combined dosage form. (P.Pydiratnam *et al.,* 2013)

In 2013, A pilot study assessing the bioavailability and pharmacokinetics of diazepam after intranasal and intravenous administration in healthy volunteers was done by Agarwal SK, Kriel

RL, Brundage RC, Ivaturi VD, Cloyd JC. The primary objective of this study was to compare the bioavailability and pharmacokinetics of two novel intranasal (IN) diazepam (DZP) formulations versus intravenous (IV) administration in healthy volunteers. Twenty-four healthy volunteers were randomized into an open-label, three-way crossover study. 10mg doses of two investigational intranasal DZP formulations (solution, suspension) and a 5mg IV dose of commercially available DZP injectable, USP were given. A two-week washout period separated treatments. Plasma samples for DZP analysis were collected pre-dose and at regular intervals up to 240 h post-dose. Median time to maximum concentration (Tmax) was 1h and 1.5h for suspension and solution formulation, respectively. Both investigational intranasal formulations were well tolerated. The results of this pilot study indicate that development of an intranasal diazepam formulation with high bioavailability, reasonable variability, and good tolerability is feasible. (Agarwal SK, 2013)

In 2013 clinical study assessing the safety and tolerability of a new diazepam nasal spray for epilepsy patients with cluster seizures was done by Dr Herbert Henney, from Acorda Therapeutics. He presented his findings at the international congress of the International League Against Epilepsy (ILAE) in Montreal, Canada. The study involved 31 adults with epilepsy, with an average age of 35 years, all of whom had been admitted to a monitoring unit for pre-surgical evaluation or medical management of their condition. During their admission, patients were given a single dose of diazepam nasal spray, including ten whose dose was administered during a seizure. The exact dosage given to individual patients ranged from 12.5 to 20mg depending on their body weight. They were then observed and researchers measured the concentrations of diazepam in their blood for up to 12 hours. Analysis revealed that the diazepam was absorbed well from the nasal cavity, regardless of the timing of administration. The study also found diazepam nasal spray to be well tolerated, with the majority of adverse events that occurred following treatment being mild and localised. (Dr Herbert Henney, 2013)

In the same year in 2013, Đorđević et al., design an experimental formulation of diazepam nanoemulsions for parenteral drug delivery. . To study the effects of the oil content, lecithin type, and the presence of diazepam as a model drug and their interactions on physicochemical characteristics of nanoemulsions. Droplet size and size distribution, surface charge, viscosity, morphology, drug-excipient interactions, and physical stability were the main concern for

evaluating nanoemulsions. The result showed that the in vivo pharmacokinetic study of selected diazepam nanoemulsions with different oil content (20%, 30%, and 40%, w/w) demonstrated fast and intense initial distribution into rat brain of diazepam from nanoemulsions with 20% and 30% (w/w) oil content, suggesting their applicability in urgent situations. (Đorđević *et al.*, 2013)

In 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-tocopheryl poly(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)

In 2014, experimental studies were conducted by Jakimska A. et al to investigate the photodegradation of diazepam and sertraline, two of the most frequently used psychiatric drugs, induced by xenon lamp irradiation, which overlap the sunlight spectra, and natural sunlight. Degradation kinetics was established indicating the occurrence of autocatalytic reactions. The application of liquid chromatography coupled to quadrupole time-of-flight mass spectrometry allowed for accurate mass measurements and proper fragmentation of target compounds enabling structure elucidation of various photoproducts of diazepam and sertraline formed during the forced photolysis. As a result, phototransformation pathways were proposed including the compounds and routes of degradation described here for the first time. In the final step, the presence of parent and identified compounds was examined in various environmental water samples. Although, the analytes were non-detected it was assumed that they may undergo different process in the environment such as adsorption, dilution, advection or rapid photodegradation of all compounds. (Jakimska A *et al.*, 2014)

CHAPTER THREE

MATERIALS & METHODS

3.1 Materials

3.1.1 Sample Collection

For the purpose of experimentation to observe the photolytic degradation of diazepam as well as to assess the packaging efficiency, 700 tablets of Evalin (diazepam 5 mg) were collected from the local drug store in Dhaka as a sample. All the tablets were from the same batch (batch no. 4023). Among them 300 tablets were kept light protected for control tests and the remaining 400 tablets were subjected to various lighting conditions over certain periods of time for conducting experiments to determine their potency.

3.1.2 Sample

Table 3.1: Sample used in the experiment including source (Aristopharma, 2015).

Sample Name	Source (Supplier Name)	Batch No.
Evalin® tablets	AristopharmaLtd. Bangladesh	4023

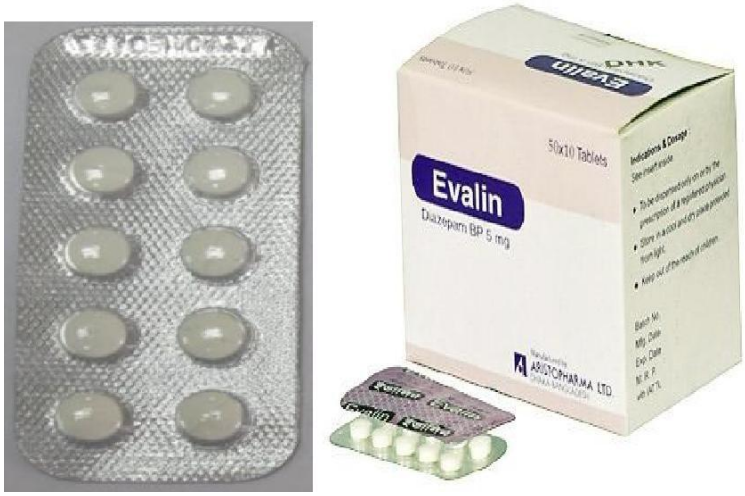

Figure 3.1 Picture of Sample – Evalin® (Diazepam)

3.1.3 Reagents

Table 3.2: Reagents used in the experiment including source

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

3.1.4 Equipments & Instruments

Table 3.3: Lists of equipments used for the experiment

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

3.1.5 Images of Instruments

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

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

***Figure 3.3*:** Hardness tester and Distilled Water Plant [Left to right]

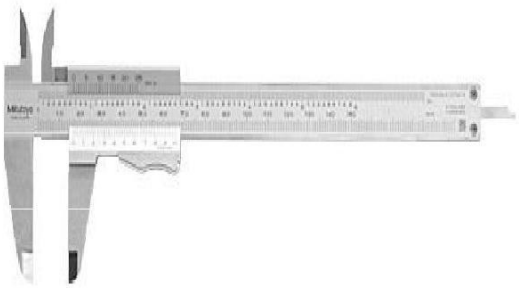

***Figure 3.4**:* Verniere Calipers

3.1.6 Apparatus

Some technical equipment or machinery needed for a particular activity or research work. Apparatus may refer to machine, equipment and critical apparatus. Here is the list.

Table 3.4: List of Apparatus used throughout this project

Serial No.	Apparatus
1	Funnel
2	Spatula
3	Beakers
4	Forceps
5	Test tubes
6	Glass Rod
7	Table Lamp
8	Pipette (5 ml)
9	Filter Papers
10	Masking Tap

11	Thermometer
12	Pipette pumper
13	Plastic Dropper
14	Test tube Holder
15	Mortar & Pestles
16	Plastic Containers
17	Aluminum foil paper
18	Electric Bulb (25 Watt & 40 Watt)
19	Volumetric Flasks (50 ml, 100ml & 1000 ml)

3.2 Methods

3.2.1. Preparation of solvent: 0.1N Sulphuric acid

1. Lab solvent (HCL) stock solution was collected and its strength was found to be 98%
2. Then the concentration of the lab solvent stock solution was determined in Normality
3. 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
4. After the determination of the concentration of the lab solvent stock solution in Normality (N), the amount of lab solvent (36.8N H_2SO_4) stock solution required to make 1000ml of 0.1N 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)

5. Then 2.72ml of 36.8N H_2SO_4 was transferred from the lab solvent stock solution to a 1000ml volumetric flask which was then filled with water up to mark to make 1000ml of 0.1N H_2SO_4

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

1. Standard of diazepam was collected from a pharmaceutical company. The potency of standard compound was 99.99%.
2. The specific λ_{max} for diazepam, at which the absorbance would be measured, were determined from the UV spectrometer by using the standard.
3. Nine serial concentrations of the Standard of diazepam were prepared for the purpose of creating a standard curve.

Preparation of the stock solution for diazepam using the standard.

- 50 mg of the standard compound, that is diazepam obtained from the pharmaceutical company was weighed and dissolved in 100 ml of 0.1N H_2SO_4 (which is the solvent) in a 100 ml volumetric flask for the 1st dilution.
  Thus the concentration was calculated to be:

Concentration of 1st dilution = amount of substance added / volume
= (50 / 250) mg/ml
= 0.2 mg/ml

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

So the concentration finally turned out to be:

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

Preparation of nine serial concentrations of solution for diazepam

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

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

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

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

Table 3.5: Concentrations 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.

4. Then the Absorbance values were measured using a UV spectrophotometer against those ten serial concentrations each for diazepam.
5. A standard curve was plotted.

6. From those standard curve one straight line equation was obtained which was in the form of y = mx+c, where the components of the equations are described as provided below: m = gradient value, y = absorbance values, x = concentrations and c = y-intercept.

Preparation of the stock solution for diazepam using Evalin tablets

Five tablets were weighed in an analytical balance and determine the average weight.

Five tablets were then crushed by mortar and pestle and made a powder of it.

Then using an analytical balance the average weight of the powdered Evalin® tablet was weighed three times and taken.

This average weighted powder of Evalin® tablet containing 5 mg diazepam was then dissolved three times in 100 ml of 0.1N H_2SO_4 (which is the solvent) in a 100 ml volumetric flask for the 1st dilution.

Then from this solution 10 ml solution is then filtered in a beaker and from this 5ml of the filtered solution is taken into 50ml volumetric flask and dissolved with 0.1N H_2SO_4 three times.

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:

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

Direct Sunlight exposure

1. Exposure under Normal Lighting Condition

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

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

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

c. Then the 5 tablets were crushed by using mortar and pestle.
d. Approximately the weight of 1 tablet of crushed tablet powder was taken and dissolved it in 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 9 different test-tube and wrapped it by foil paper.

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

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

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

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

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

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

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

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

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

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

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

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 six different days for 6 hours each.

3. Under Sunlight condition

1) 30 tablets were kept in a Glass box and exposed to sunlight condition for 6 hours at a stretch.
2) After every 2 hours, 10 tablets were collected and wrapped up with foil paper to prevent any further exposure to the lighting condition and the temperature was noted using a thermometer.
3) The foil papers should be 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 Total Weight of the 5 tablets was noted using an analytical balance and the average weight was calculated using the formula:

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

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

Table 3.7: Sunlight Exposed Sample List

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

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

N.B: Same procedures (steps 1 to 8) were used to determine the potency of the tablets under exposure of Sunlight condition for three different days for 6 hours each.

3.2.4 Determination of Physical parameters

Colour test :

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

Weight Variation test

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

2) Then the composite weight was divided by 10 provided in order to get an average weight. Average weight, $X= (X_1 +X_2 +X_3 +\ldots+ X_z)/10$

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

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

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

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

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

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

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

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

Tablet Thickness Test

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

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

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

Hardness Test

1. The crushing strength of the tablets was measured using a hardness tester.
2. At first 5 tablets were picked randomly from 10 tablets.
3. The sliding scale of hardness tester has been set off to zero.
4. The tablets have been placed vertically between the two jaws.
5. Force has been applied with the screw thread and spring until the tablets has been fractured.
6. A force of about 4kg is considered to be the minimum for hardness.

CHAPTER FOUR

RESULT

4.1 Results

4.1.1. Standard Curve Preparation

For the preparation of standard curve, nine serially different concentrations were prepared for diazepam using the standards of diazepam from Aristopharma with a potency of 99.99%. The absorbance (abs) values for those nine concentrations of diazepam are show in the table below:

Table 4.1: Diazepam Standard Curve

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

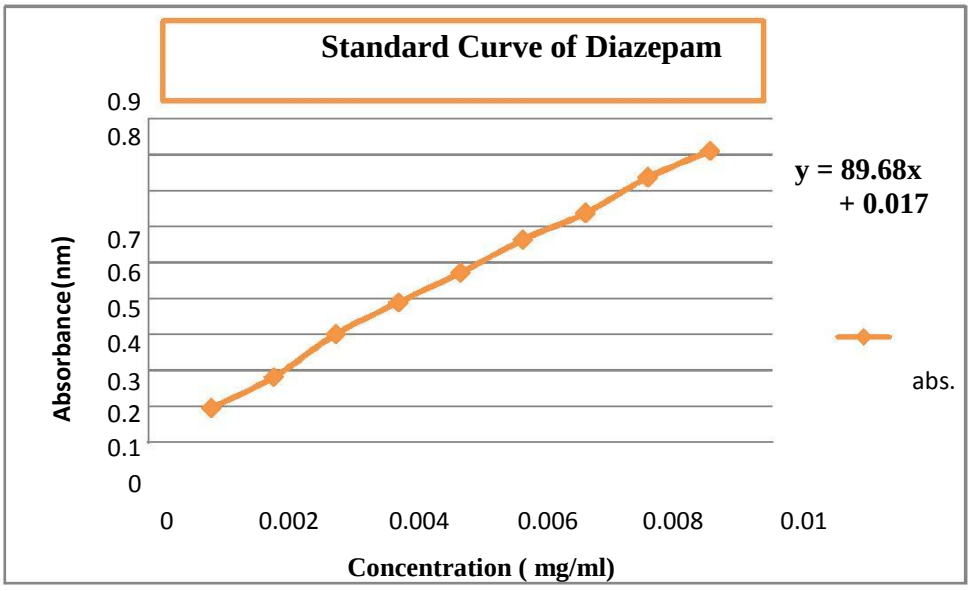

Figure 4.1: Plot showing straight line for Absorbance (Abs) with respect to Concentration (mg/ml) for diazepam.

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

y=89.68x+0.017

This equation was used to determine the concentration of diazepam from different sample's absorbance that was found in several lighting conditions.

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:

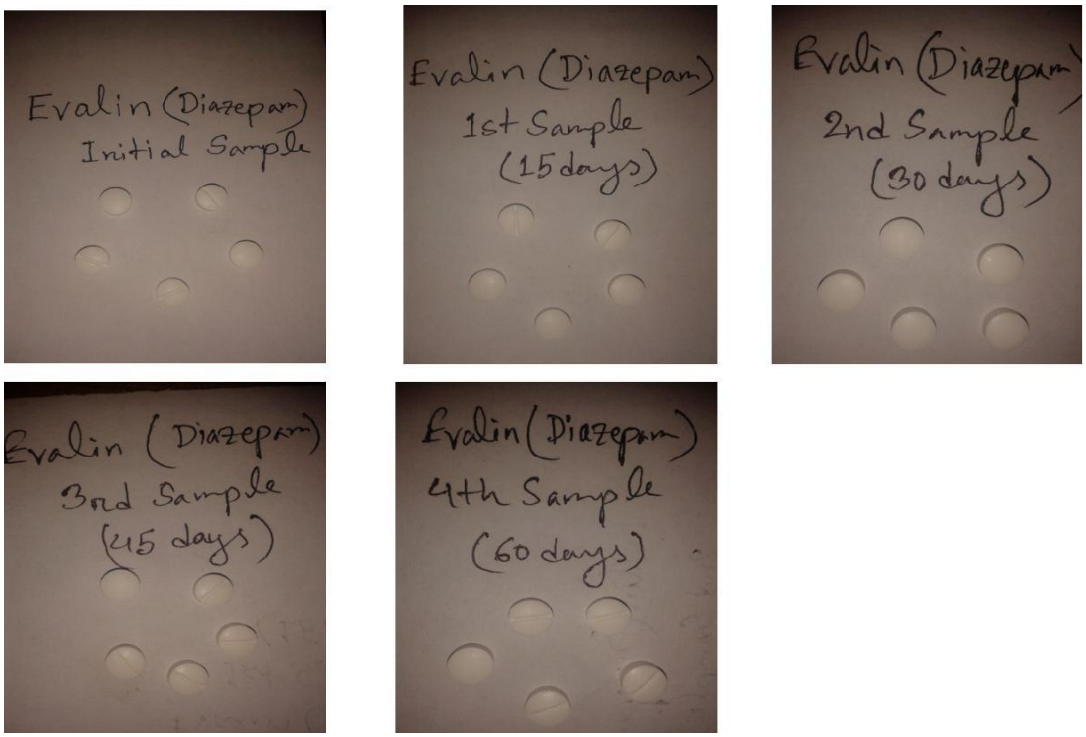

Figure 4.2: Pictures of Tablets after exposure to normal light with 60 days interval.

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 (Evalin®)

Days	Average Weight for Particular Day I(g)	Average Weight for 60 Days A(g)	% Weight Variation, (A-I/A)×100 %
0	0.1023		-0.2941
15	0.1021		-0.0980
30	0.1024	0.1020	-0.3922
45	0.1018		0.1961
60	0.1016		0.3922

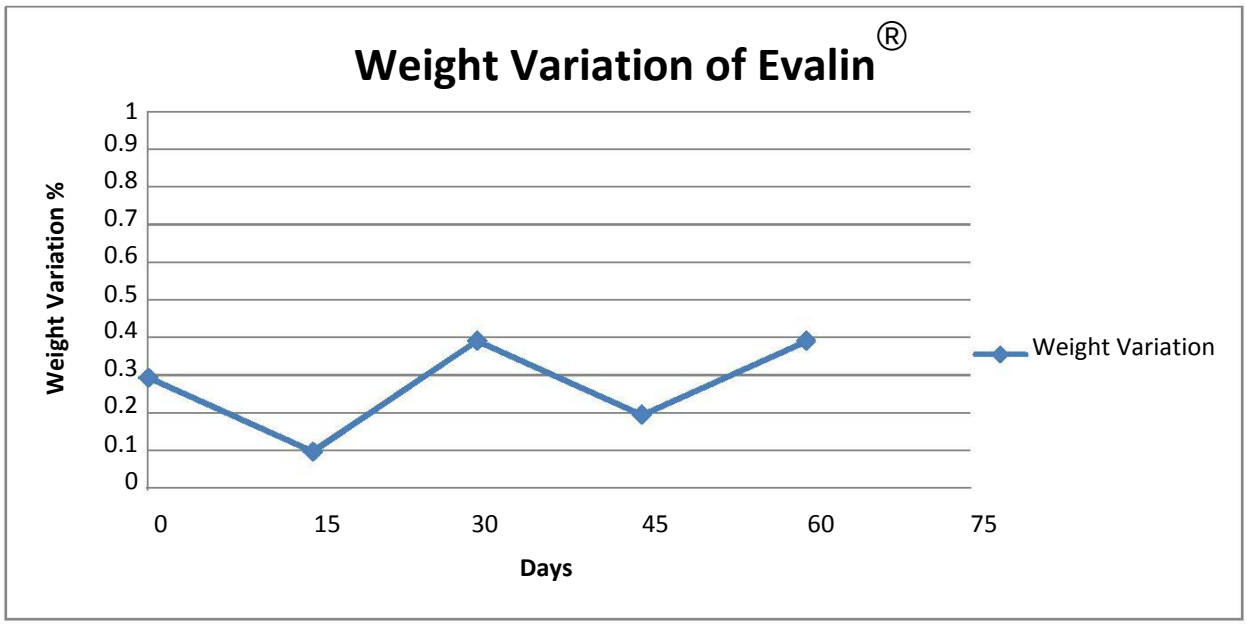

Figure 4.3: Weight variation of the sample throughout 60 days light exposure

4.2.3 Thickness test

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

Table 4.3: Thickness Test of Diazepam (Evalin®)

Days	Average Thickness of Particular Days (cm)
0	0.240
15	0.234
30	0.231
45	0.241
60	0.231

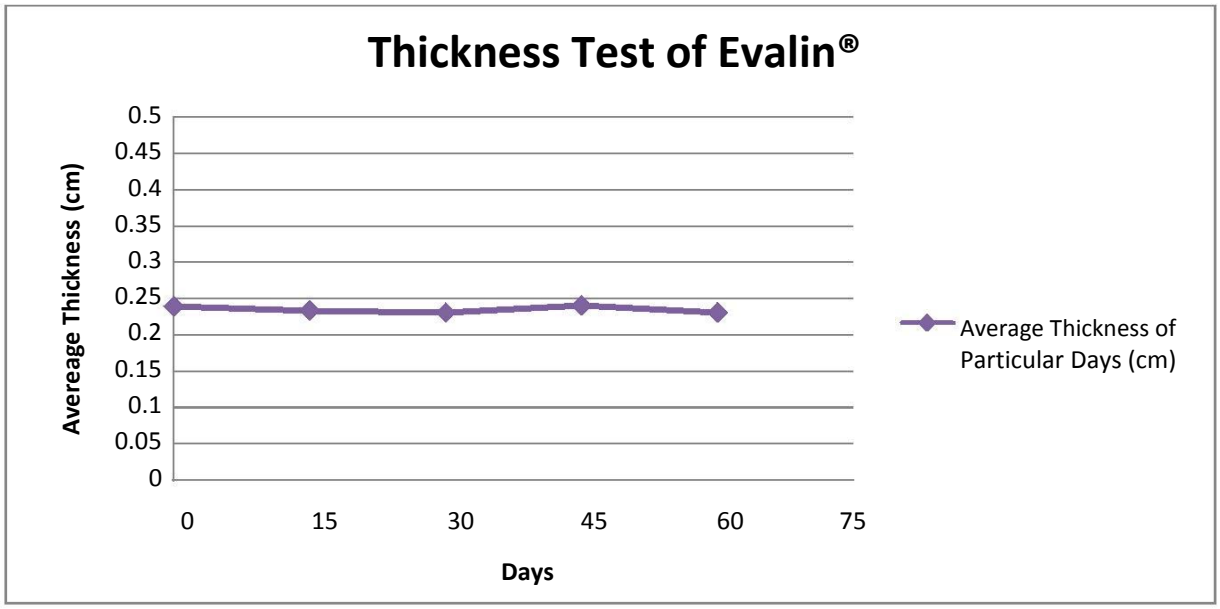

Figure 4.4: Thickness variation of sample throughout 60 days light exposure.

4.2.4 Hardness Test

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

Table 4.4: Hardness Test of Diazepam (Evalin®)

Days	Average Hardness of Particular Day (Kg)
0	4.0
15	4.0
30	3.5
45	4.0
60	3.0

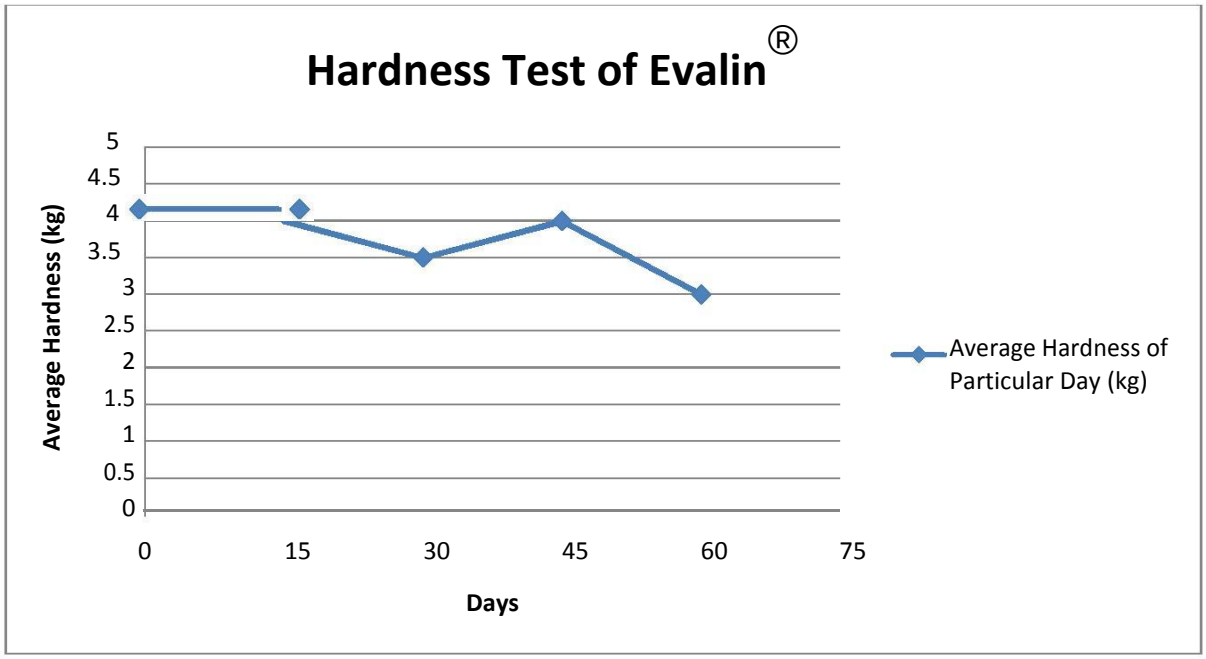

Figure 4.5: Hardness variation of the sample throughout 60 days light exposure.

4.3 Result of Potency Determination by UV- spectroscopy

4.3.1 Result of Samples that were exposed under Normal Lightening Condition

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

Table 4.5: Initial Concentration & Absorbance for Diazepam (Evalin®)

Time Interval (Days)	Absorbance (at 240.5nm)		Average Absorbance		Amount of Drug Present (in mg)		Potency (%)	
	Control	Sample	Control	Sample	Control	Sample	Control	Sample
Initial	0.459	0.459	0.461	0.461	4.95	4.95	99.00	99.00
	0.464	0.464						
	0.459	0.459						
	0.457	0.457	0.461	0.461	4.95	4.95	99.00	99.00
	0.459	0.459						
	0.466	0.466						
	0.464	0.464	0.461	0.461	4.95	4.95	99.00	99.00
	0.461	0.461						
	0.457	0.457						

Table 4.6: Concentration & Absorbance at 15 days interval for Diazepam (Evalin®)

Time Interval (Days)	Absorbance (at 240.5nm)		Average Absorbance		Amount of Drug Present (in mg)		Potency (%)	
	Control	Sample	Control	Sample	Control	Sample	Control	Sample
15	0.459	0.445	0.461	0.448	4.95	4.81	99.00	96.20
	0.464	0.448						
	0.459	0.450						
	0.457	0.440	0.461	0.439	4.95	4.71	99.00	94.20
	0.459	0.439						
	0.466	0.439						
	0.464	0.436	0.461	0.440	4.95	4.72	99.00	94.40
	0.461	0.441						
	0.457	0.444						

Table 4.7: Concentration & Absorbance at 30 days interval for Diazepam (Evalin®)

Time Interval (Days)	Absorbance (at 240.5nm)		Average Absorbance		Amount of Drug Present (in mg)		Potency (%)	
	Control	Sample	Control	Sample	Control	Sample	Control	Sample
30	0.459	0.411	0.461	0.415	4.95	4.44	99.00	88.80
	0.464	0.416						
	0.459	0.418						
	0.457	0.418	0.461	0.418	4.95	4.47	99.00	89.40
	0.459	0.419						
	0.466	0.416						
	0.464	0.433	0.461	0.425	4.95	4.55	99.00	91.00
	0.461	0.423						
	0.457	0.419						

Table 4.8: Concentration & Absorbance at 45 days interval for Diazepam (Evalin®)

Time Interval (Days)	Absorbance (at 240.5nm)		Average Absorbance		Amount of Drug Present (in mg)		Potency (%)	
	Control	Sample	Control	Sample	Control	Sample	Control	Sample
45	0.459	0.402	0.461	0.404	4.95	4.32	99.00	86.40
	0.464	0.406						
	0.459	0.403						
	0.457	0.401	0.461	0.402	4.95	4.29	99.00	85.80
	0.459	0.402						
	0.466	0.405						
	0.464	0.397	0.461	0.396	4.95	4.23	99.00	84.60
	0.461	0.394						
	0.457	0.398						

Table 4.9: Concentration & Absorbance at 60 days interval for Diazepam (Evalin®)

Time Interval (Days)	Absorbance (at 240.5nm)		Average Absorbance		Amount of Drug Present (in mg)		Potency (%)	
	Control	Sample	Control	Sample	Control	Sample	Control	Sample
60	0.459	0.380	0.461	0.382	4.95	4.07	99.00	81.40
	0.464	0.382						
	0.459	0.384						
	0.457	0.372	0.461	0.375	4.95	3.99	99.00	79.80
	0.459	0.374						
	0.466	0.380						
	0.464	0.371	0.461	0.369	4.95	3.93	99.00	78.60
	0.461	0.369						
	0.457	0.367						

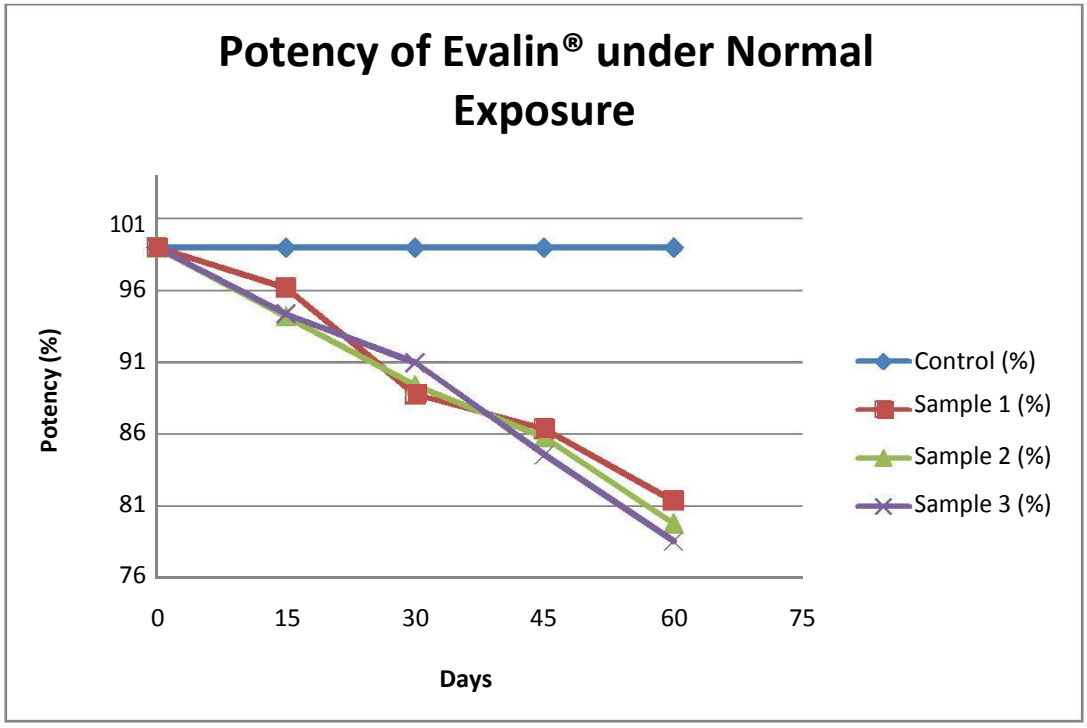

***Figure 4.6*:** Difference in potency of Evalin® after specific days interval under normal exposure

4.3.2 Result of samples that were exposed under 25W bulb

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

Table 4.10: Concentration & Absorbance of Diazepam (Evalin®) under 25W bulb (1st time)

Time Interval (Hours)	Absorbance (at 240.5nm)		Average Absorbance		Amount of Drug Present (in mg)		Potency (%)	
	Control (Initial)	Sample (2hrs)	Control	Sample	Control	Sample	Control	Sample
Initial and 2 hours	0.459	0.452	0.461	0.451	4.95	4.84	99.00	96.80
	0.464	0.453						
	0.459	0.448						
	0.461	0.445	0.461	0.448	4.95	4.81	99.00	96.20
	0.460	0.448						
	0.462	0.450						
	0.466	0.438	0.461	0.440	4.95	4.72	99.00	94.40
	0.457	0.441						
	0.459	0.442						

Table 4.11: Concentration & Absorbance of Diazepam (Evalin®) (1st time)

Time Interval (Hours)	Absorbance (at 240.5 nm)	Average Absorbance	Amount of Drug present (in mg)	Potency (%)
4	0.410	0.411	4.39	87.80
	0.411			
	0.412			
	0.401	0.404	4.32	86.40
	0.405			
	0.406			
	0.402	0.402	4.29	85.80
	0.404			
	0.400			

Table 4.12: Concentration & Absorbance of Diazepam (Evalin®) (1st time)

Time Interval (Hours)	Absorbance (at 240.5 nm)	Average Absorbance	Amount of Drug present (in mg)	Potency (%)
6	0.384	0.383	4.08	81.60
	0.384			
	0.382			
	0.388	0.387	4.13	82.60
	0.387			
	0.386			
	0.381	0.381	4.06	81.20
	0.381			
	0.382			

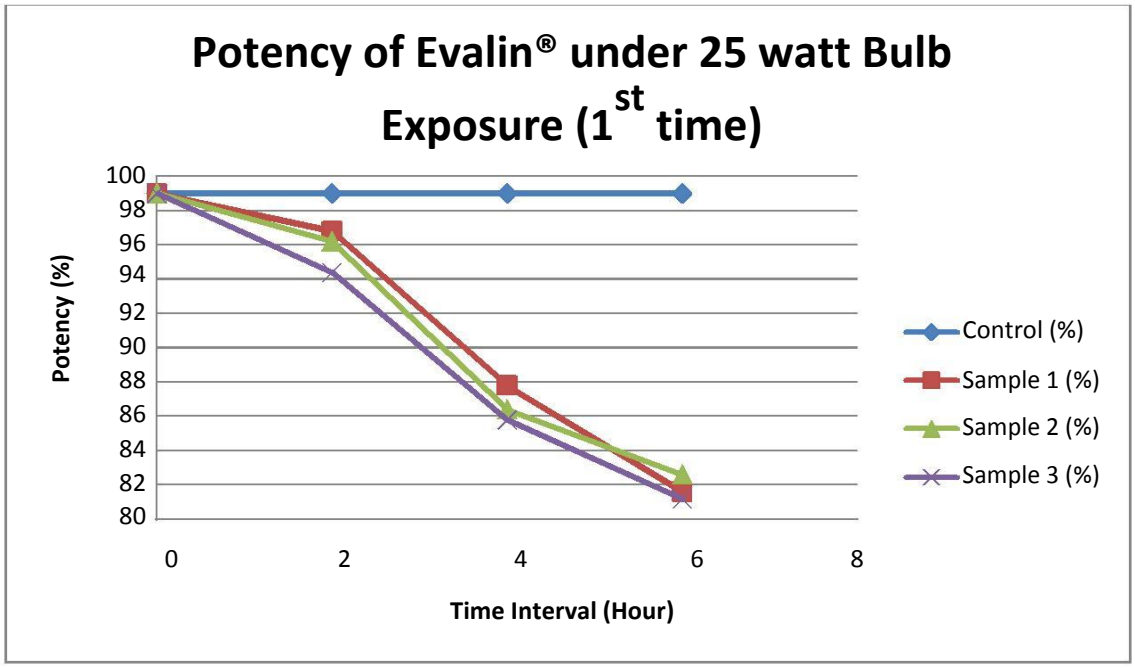

Figure 4.7: Difference in potency of Evalin® after specific time interval under 25 watt bulb (1st time)

Table 4.13: Concentration & Absorbance of Diazepam (Evalin®) under 25W bulb (2nd time)

Time Interval (Hours)	Absorbance (at 240.5nm)		Average Absorbance		Amount of Drug Present (in mg)		Potency (%)	
	Control (Initial)	Sample (2hrs)	Control	Sample	Control	Sample	Control	Sample
Initial and 2 hours	0.459	0.430	0.453	0.432	4.86	4.63	97.20	92.60
	0.453	0.434						
	0.448	0.432						
	0.458	0.435	0.453	0.432	4.86	4.63	97.20	92.60
	0.448	0.434						
	0.454	0.426						
	0.452	0.437	0.453	0.437	4.86	4.68	97.20	93.60
	0.450	0.432						
	0.456	0.442						

Table 4.14: Concentration & Absorbance of Diazepam (Evalin®) (2nd time)

Time Interval (Hours)	Absorbance (at 240.5 nm)	Average Absorbance	Amount of Drug present (in mg)	Potency (%)
4	0.403	0.401	4.28	85.60
	0.400			
	0.401			
	0.404	0.398	4.25	85.00
	0.401			
	0.388			
	0.400	0.403	4.30	86.00
	0.401			
	0.407			

Table 4.15: Concentration & Absorbance of Diazepam (Evalin®) (2nd time)

Time Interval (Hours)	Absorbance (at 240.5 nm)	Average Absorbance	Amount of Drug present (in mg)	Potency (%)
6	0.391	0.388	4.14	82.80
	0.388			
	0.385			
	0.386	0.386	4.11	82.20
	0.387			
	0.384			
	0.389	0.382	4.07	81.40
	0.378			
	0.389			

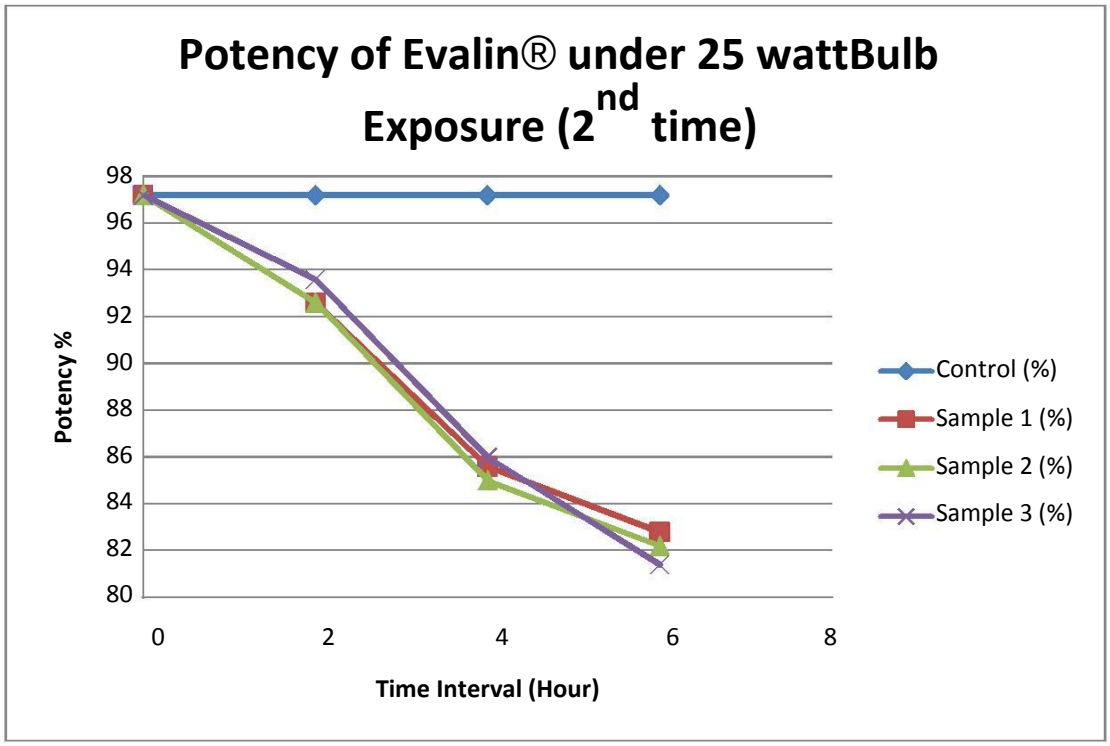

Figure 4.8: Difference in potency of Evalin® after specific time interval under 25 watt bulb (2nd time)

Table 4.16: Concentration & Absorbance of Diazepam (Evalin®) under 25W bulb (3rd time)

Time Interval (Hours)	Absorbance (at 240.5nm)		Average Absorbance		Amount of Drug Present (in mg)		Potency (%)	
	Control (Initial)	Sample (2hrs)	Control	Sample	Control	Sample	Control	Sample
Initial and 2 hours	0.451	0.430	0.451	0.429	4.84	4.59	96.80	91.80
	0.453	0.429						
	0.449	0.429						
	0.448	0.427	0.451	0.428	4.84	4.58	96.80	91.60
	0.451	0.428						
	0.454	0.428						
	0.451	0.427	0.451	0.426	4.84	4.56	96.80	91.20
	0.451	0.424						
	0.450	0.428						

Table 4.17: Concentration & Absorbance of Diazepam (Evalin®) (3rd time)

Time Interval (Hours)	Absorbance (at 240.5 nm)	Average Absorbance	Amount of Drug present (in mg)	Potency (%)
4	0.404	0.406	4.34	86.80
	0.405			
	0.408			
	0.410	0.411	4.39	87.80
	0.411			
	0.412			
	0.405	0.405	4.33	86.60
	0.404			
	0.405			

Table 4.18: Concentration & Absorbance of Diazepam (Evalin®) (3rd time)

Time Interval (Hours)	Absorbance (at 240.5 nm)	Average Absorbance	Amount of Drug present (in mg)	Potency (%)
6	0.390	0.387	4.13	82.60
	0.384			
	0.386			
	0.383	0.384	4.09	81.80
	0.385			
	0.385			
	0.378	0.375	3.99	79.80
	0.375			
	0.372			

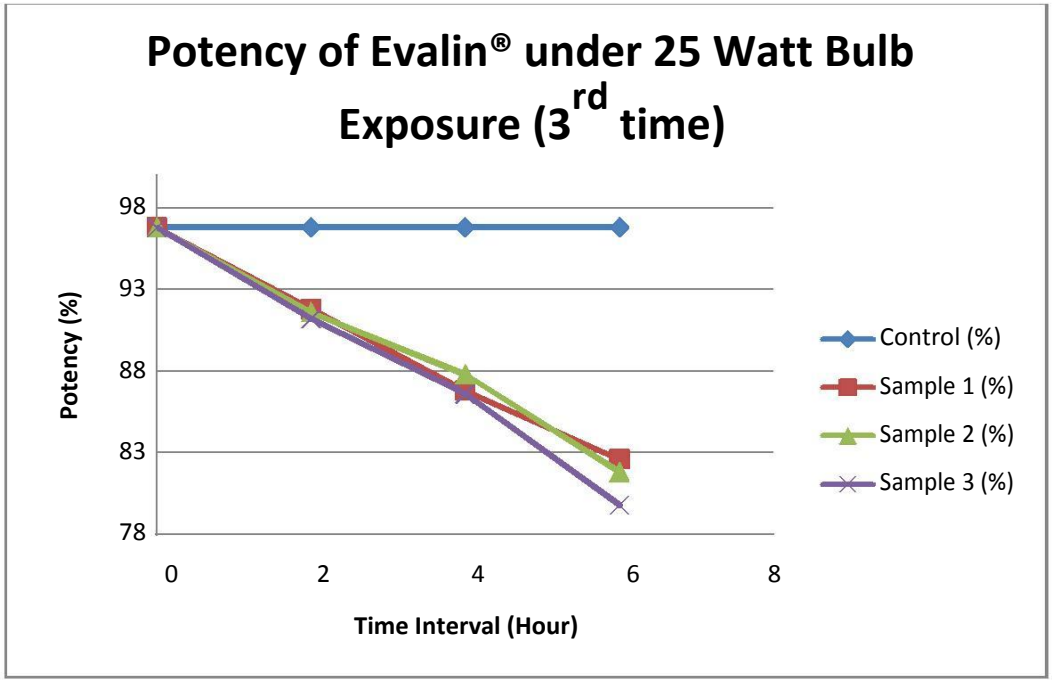

Figure 4.9: Difference in potency of Evalin® after specific time interval under 25 watt bulb (3rd time)

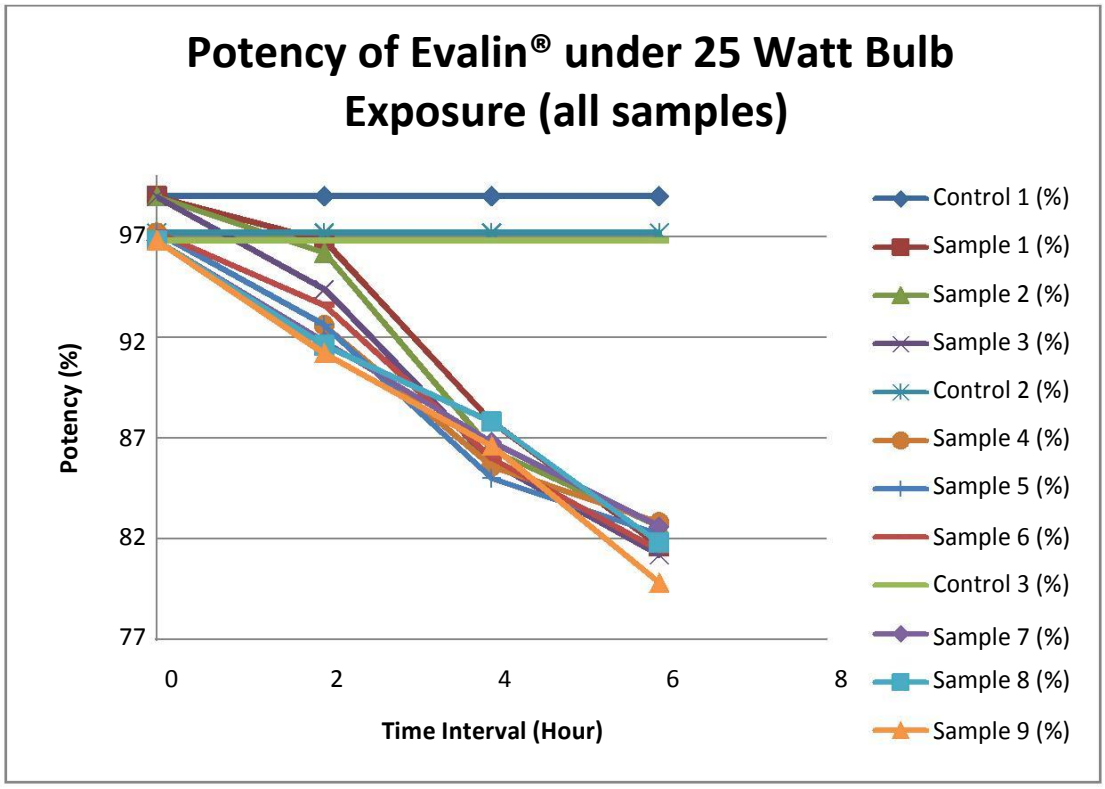

Figure 4.10: Difference in potency of Evalin® under 25 watt bulb exposures

4.3.3 Result of samples that were exposed under 40W bulb

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

Table 4.19: Concentration & Absorbance of Diazepam (Evalin®) under 40W bulb (1st time)

Time Interval (Hours)	Absorbance (at 240.5nm)		Average Absorbance		Amount of Drug Present (in mg)		Potency (%)	
	Control (Initial)	Sample (2hrs)	Control	Sample	Control	Sample	Control	Sample
Initial and 2 hours	0.459	0.446	0.458	0.448	4.92	4.81	98.40	96.20
	0.458	0.450						
	0.458	0.448						
	0.459	0.444	0.458	0.439	4.92	4.71	98.40	94.20
	0.452	0.438						
	0.464	0.436						
	0.462	0.440	0.458	0.445	4.92	4.77	98.40	95.40
	0.454	0.450						
	0.459	0.445						

Table 4.20: Concentration & Absorbance of Diazepam (Evalin®) (1st time)

Time Interval (Hours)	Absorbance (at 240.5 nm)	Average Absorbance	Amount of Drug present (in mg)	Potency (%)
4	0.409	0.408	4.36	87.20
	0.407			
	0.408			
	0.401	0.404	4.32	86.40
	0.405			
	0.406			
	0.407	0.402	4.29	85.80
	0.401			
	0.398			

Table 4.21: Concentration & Absorbance of Diazepam (Evalin®) (1st time)

Time Interval (Hours)	Absorbance (at 240.5 nm)	Average Absorbance	Amount of Drug present (in mg)	Potency (%)
6	0.376	0.375	3.99	79.80
	0.374			
	0.375			
	0.385	0.387	4.13	82.60
	0.391			
	0.386			
	0.381	0.381	4.06	81.20
	0.381			
	0.382			

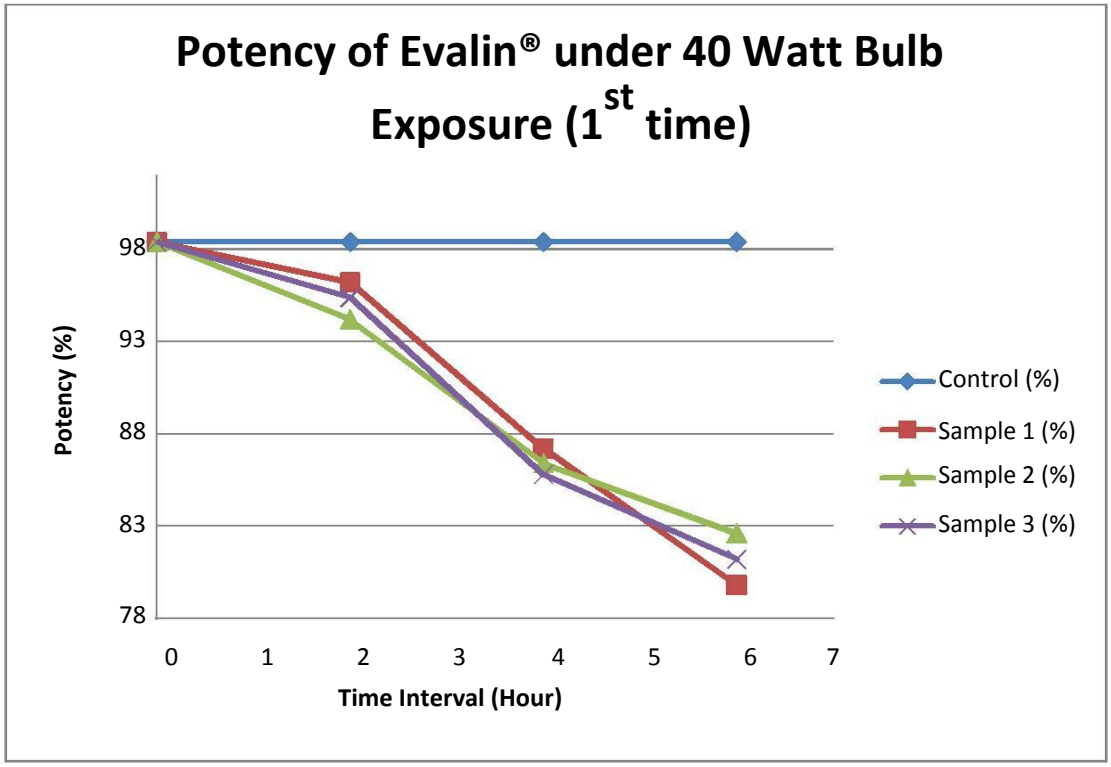

Figure 4.11: Difference in potency of Evalin® after specific time interval under 40 watt bulb (1st time)

Table 4.22: Concentration & Absorbance of Diazepam (Evalin®) under 40W bulb (2nd time)

Time Interval (Hours)	Absorbance (at 240.5nm)		Average Absorbance		Amount of Drug Present (in mg)		Potency (%)	
	Control (Initial)	Sample (2hrs)	Control	Sample	Control	Sample	Control	Sample
Initial and 2 hours	0.449	0.436	0.449	0.436	4.82	4.67	96.40	93.40
	0.454	0.435						
	0.445	0.438						
	0.450	0.430	0.449	0.427	4.82	4.57	96.40	91.40
	0.444	0.426						
	0.454	0.425						
	0.452	0.434	0.449	0.434	4.82	4.65	96.40	93.00
	0.446	0.435						
	0.449	0.433						

Table 4.23: Concentration & Absorbance of Diazepam (Evalin®) (2nd time)

Time Interval (Hours)	Absorbance (at 240.5 nm)	Average Absorbance	Amount of Drug present (in mg)	Potency (%)
4	0.390	0.390	4.16	83.20
	0.395			
	0.385			
	0.393	0.396	4.23	84.60
	0.402			
	0.394			
	0.385	0.386	4.11	82.20
	0.386			
	0.386			

Table 4.24: Concentration & Absorbance of Diazepam (Evalin®) (2nd time)

Time Interval (Hours)	Absorbance (at 240.5 nm)	Average Absorbance	Amount of Drug present (in mg)	Potency (%)
6	0.369	0.369	3.93	78.60
	0.371			
	0.367			
	0.364	0.367	3.90	78.00
	0.367			
	0.370			
	0.361	0.361	3.84	76.80
	0.358			
	0.363			

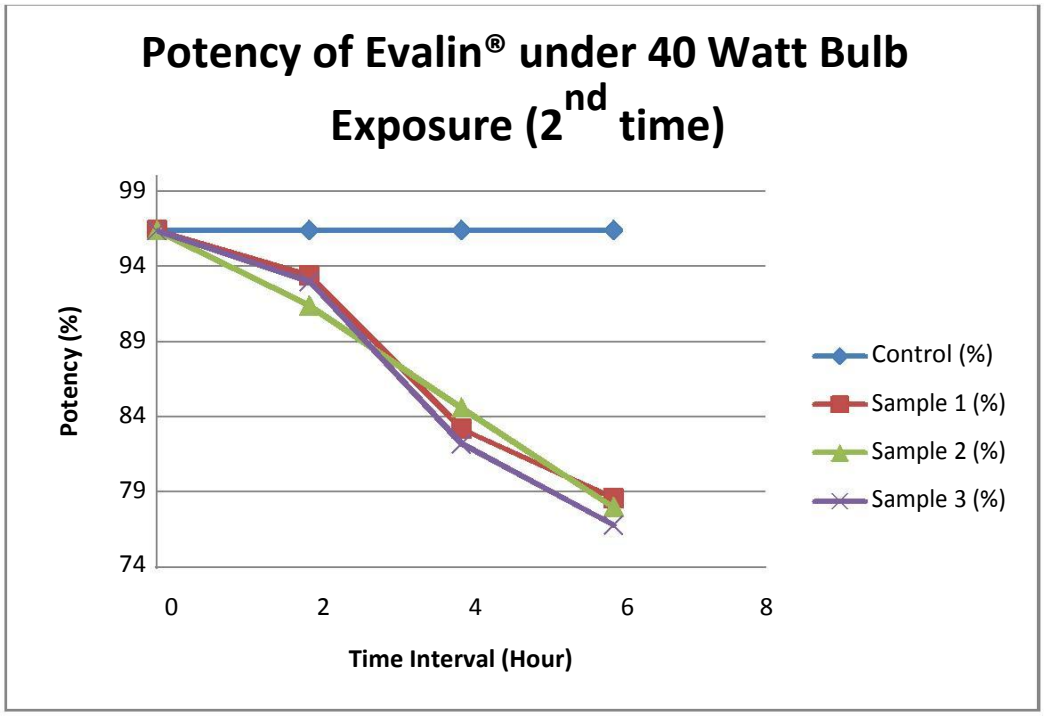

Figure 4.12: Difference in potency of Evalin® after specific time interval under 40 watt bulb (2nd time)

Table 4.25: Concentration & Absorbance of Diazepam (Evalin®) under 40W bulb (3rd time)

Time Interval (Hours)	Absorbance (at 240.5nm)		Average Absorbance		Amount of Drug Present (in mg)		Potency (%)	
	Control (Initial)	Sample (2hrs)	Control	Sample	Control	Sample	Control	Sample
Initial and 2 hours	0.449	0.416	0.444	0.425	4.76	4.55	95.20	91.00
	0.444	0.429						
	0.439	0.429						
	0.440	0.434	0.444	0.432	4.76	4.63	95.20	92.60
	0.450	0.425						
	0.442	0.436						
	0.444	0.428	0.444	0.427	4.76	4.57	95.20	91.40
	0.445	0.426						
	0.444	0.428						

Table 4.26: Concentration & Absorbance of Diazepam (Evalin®) (3rd time)

Time Interval (Hours)	Absorbance (at 240.5 nm)	Average Absorbance	Amount of Drug present (in mg)	Potency (%)
4	0.392	0.390	4.16	83.20
	0.391			
	0.386			
	0.380	0.377	4.01	80.20
	0.374			
	0.376			
	0.400	0.91	4.17	83.40
	0.387			
	0.387			

Table 4.27: Concentration & Absorbance of Diazepam (Evalin®) (3rd time)

Time Interval (Hours)	Absorbance (at 240.5 nm)	Average Absorbance	Amount of Drug present (in mg)	Potency (%)
6	0.356	0.350	3.71	74.20
	0.350			
	0.345			
	0.345	0.348	3.69	73.80
	0.348			
	0.350			
	0.334	0.333	3.52	70.40
	0.330			
	0.334			

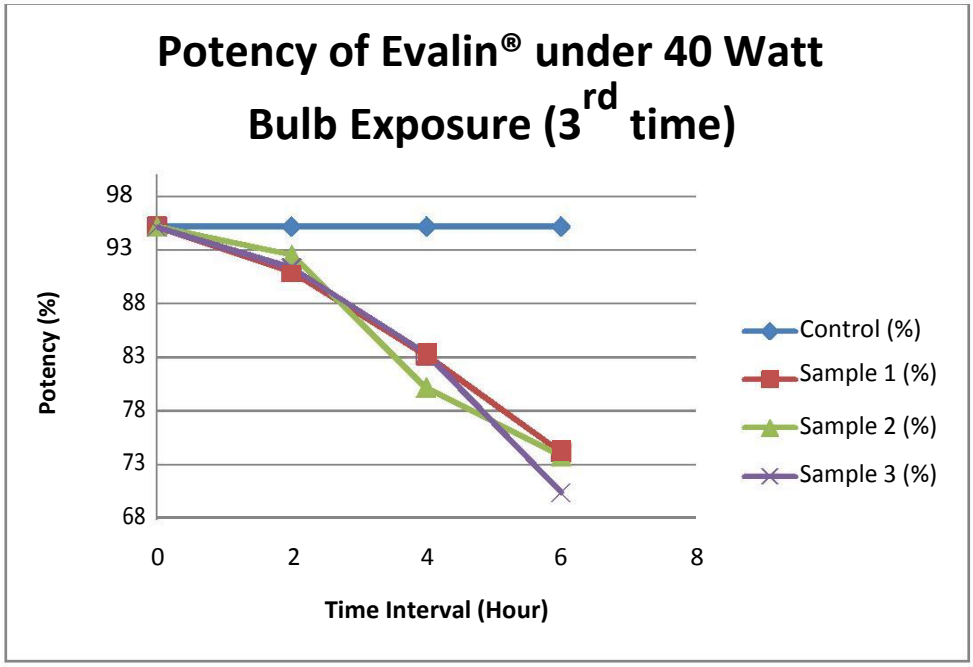

Figure 4.13: Difference in potency of Evalin® after specific time interval under 40 watt bulb (3rd time)

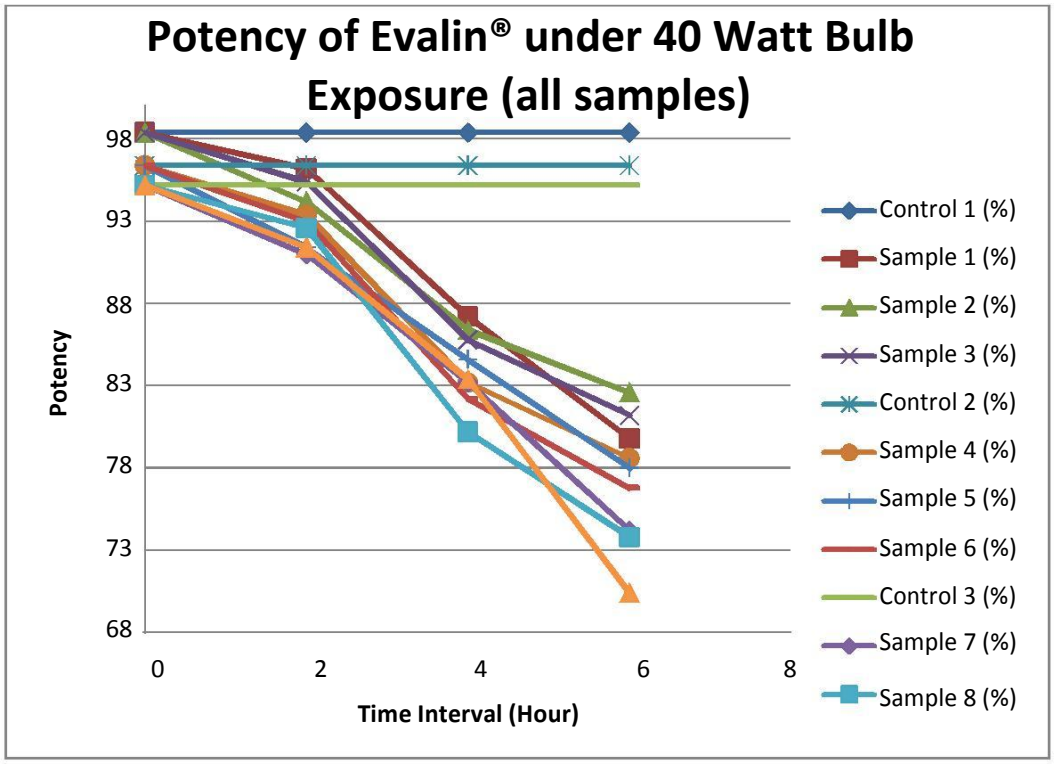

Figure 4.14: Difference in potency of Evalin® under 40 watt bulb exposures

4.3.4 Result of samples that were exposed to sunlight

We found 27 different absorbance of Diazepam (Evalin®) for twenty seven samples exposed to sunlight; each for 2 hours time interval and it was observed that the concentration of Diazepam declined in each time interval.

Table 4.28: Concentration & Absorbance of Diazepam (Evalin®) under direct sunlight (1st time)

Time Interval (Hours)	Absorbance (at 240.5nm)		Average Absorbance		Amount of Drug Present (in mg)		Potency (%)	
	Control (Initial)	Sample (2hrs)	Control	Sample	Control	Sample	Control	Sample
Initial and 2 hours	0.457	0.451	0.460	0.448	4.94	4.81	98.80	96.20
	0.464	0.450						
	0.459	0.444						
	0.459	0.451	0.460	0.448	4.94	4.81	98.80	96.20
	0.460	0.449						
	0.460	0.445						
	0.462	0.447	0.460	0.445	4.94	4.77	98.80	95.40
	0.461	0.443						
	0.458	0.445						

Table 4.29: Concentration & Absorbance of Diazepam (Evalin®) (1st time)

Time Interval (Hours)	Absorbance (at 240.5 nm)	Average Absorbance	Amount of Drug present (in mg)	Potency (%)
4	0.414	0.415	4.44	88.80
	0.415			
	0.415			
	0.411	0.412	4.40	88.00
	0.415			
	0.409			
	0.409	0.406	4.34	86.80
	0.404			
	0.405			

Table 4.30: Concentration & Absorbance of Diazepam (Evalin®) (1st time)

Time Interval (Hours)	Absorbance (at 240.5 nm)	Average Absorbance	Amount of Drug present (in mg)	Potency (%)
6	0.367	0.365	3.88	77.60
	0.364			
	0.365			
	0.363	0.367	3.90	78.00
	0.367			
	0.371			
	0.361	0.361	3.84	76.80
	0.357			
	0.364			

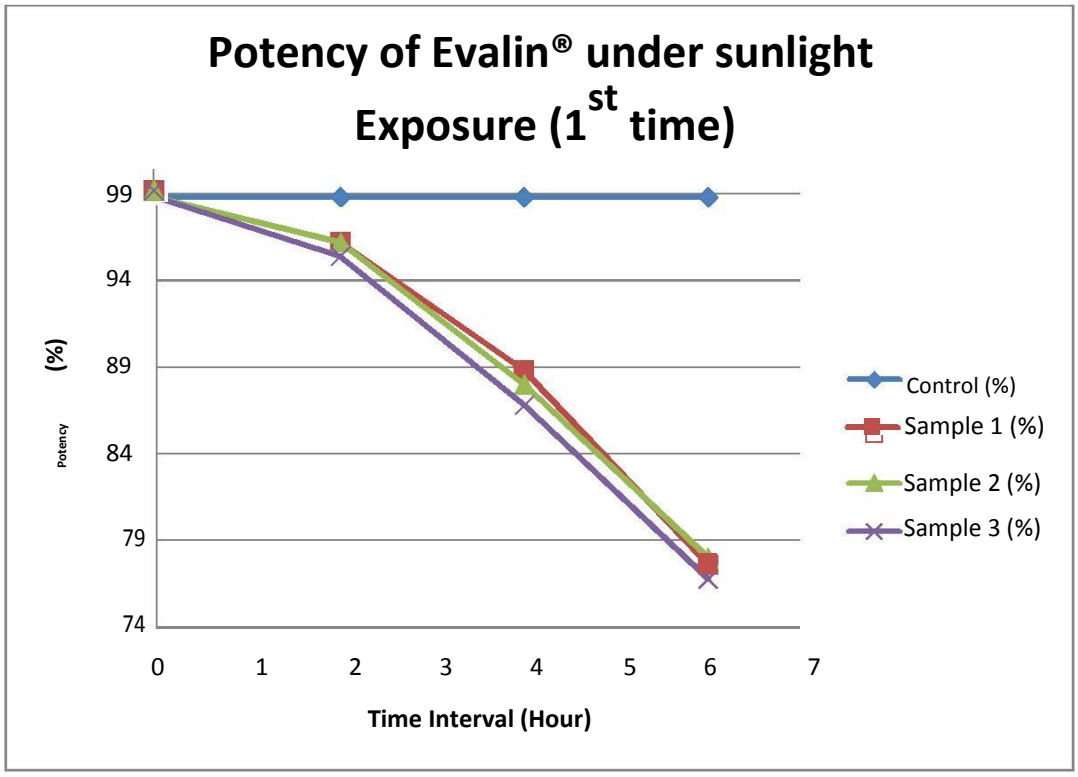

Figure 4.15: Difference in potency of Evalin® after specific time interval under sunlight exposure (1st time)

Table 4.31: Concentration & Absorbance of Diazepam (Evalin®) under direct sunlight (2nd time)

Time Interval (Hours)	Absorbance (at 240.5nm)		Average Absorbance		Amount of Drug Present (in mg)		Potency (%)	
	Control (Initial)	Sample (2hrs)	Control	Sample	Control	Sample	Control	Sample
Initial and 2 hours	0.449	0.436	0.451	0.436	4.84	4.67	96.80	93.40
	0.454	0.435						
	0.449	0.438						
	0.448	0.443	0.451	0.440	4.84	4.72	96.80	94.40
	0.455	0.438						
	0.449	0.440						
	0.452	0.437	0.451	0.437	4.84	4.68	96.80	93.60
	0.451	0.435						
	0.450	0.439						

Table 4.32: Concentration & Absorbance of Diazepam (Evalin®) (2nd time)

Time Interval (Hours)	Absorbance (at 240.5 nm)	Average Absorbance	Amount of Drug present (in mg)	Potency (%)
4	0.400	0.403	4.30	86.00
	0.403			
	0.406			
	0.380	0.377	4.01	84.20
	0.374			
	0.376			
	0.400	0.91	4.17	83.40
	0.387			
	0.387			

Table 4.33: Concentration & Absorbance of Diazepam (Evalin®) (2nd time)

Time Interval (Hours)	Absorbance (at 240.5 nm)	Average Absorbance	Amount of Drug present (in mg)	Potency (%)
6	0.357	0.351	3.72	74.40
	0.351			
	0.346			
	0.344	0.348	3.69	73.80
	0.348			
	0.351			
	0.344	0.345	3.66	73.20
	0.345			
	0.346			

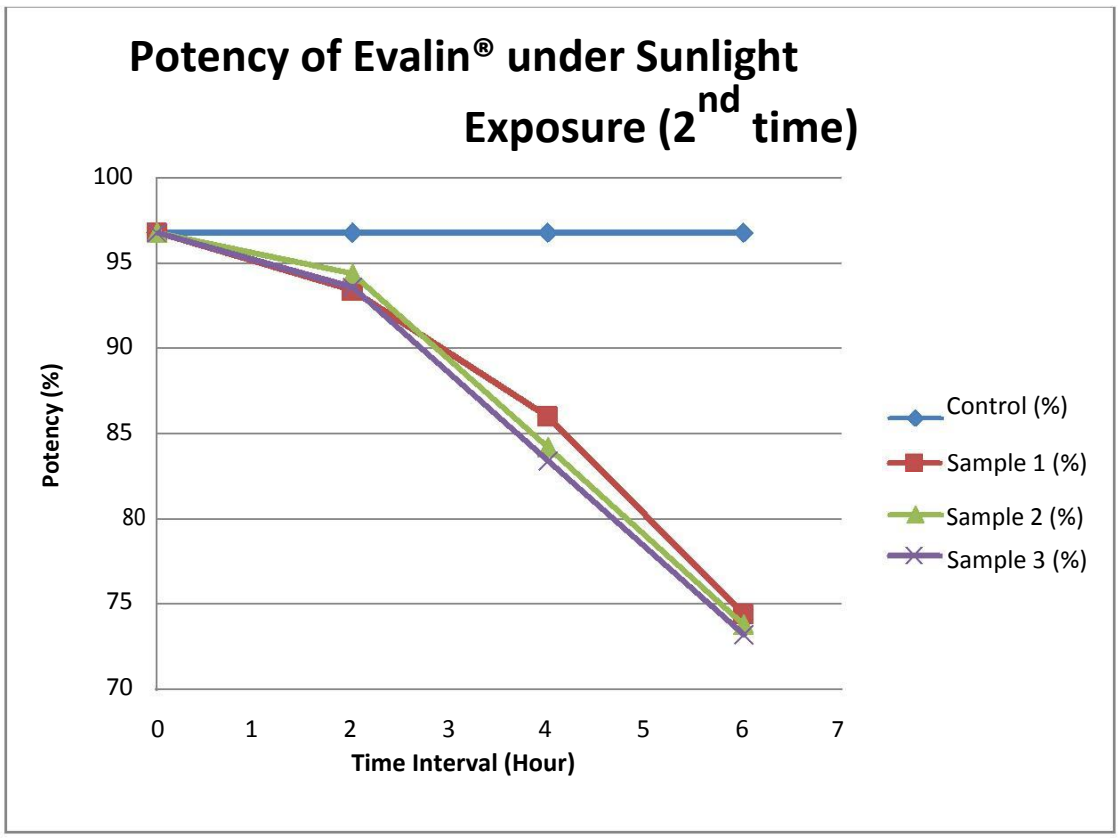

Figure 4.16: Difference in potency of Evalin® after specific time interval under sunlight exposure (2nd time)

Table 4.34: Concentration & Absorbance of Diazepam (Evalin®) under direct sunlight (3rd time)

Time Interval (Hours)	Absorbance (at 240.5nm)		Average Absorbance		Amount of Drug Present (in mg)		Potency (%)	
	Control (Initial)	Sample (2hrs)	Control	Sample	Control	Sample	Control	Sample
Initial and 2 hours	0.440	0.416	0.440	0.425	4.72	4.55	94.40	91.00
	0.440	0.429						
	0.439	0.429						
	0.440	0.432	0.440	0.432	4.72	4.63	94.40	92.60
	0.442	0.428						
	0.438	0.436						
	0.444	0.428	0.440	0.427	4.72	4.57	94.40	91.40
	0.442	0.426						
	0.434	0.428						

Table 4.35: Concentration & Absorbance of Diazepam (Evalin®) (3rd time)

Time Interval (Hours)	Absorbance (at 240.5 nm)	Average Absorbance	Amount of Drug present (in mg)	Potency (%)
4	0.380	0.386	4.11	82.20
	0.391			
	0.386			
	0.380	0.377	4.01	80.20
	0.374			
	0.376			
	0.369	0.368	3.91	78.20
	0.368			
	0.368			

Table 4.36: Concentration & Absorbance of Diazepam (Evalin®) (3rd time)

Time Interval (Hours)	Absorbance (at 240.5 nm)	Average Absorbance	Amount of Drug present (in mg)	Potency (%)
6	0.320	0.324	3.42	68.40
	0.324			
	0.328			
	0.334	0.332	3.51	70.20
	0.328			
	0.333			
	0.316	0.317	3.35	67.00
	0.316			
	0.320			

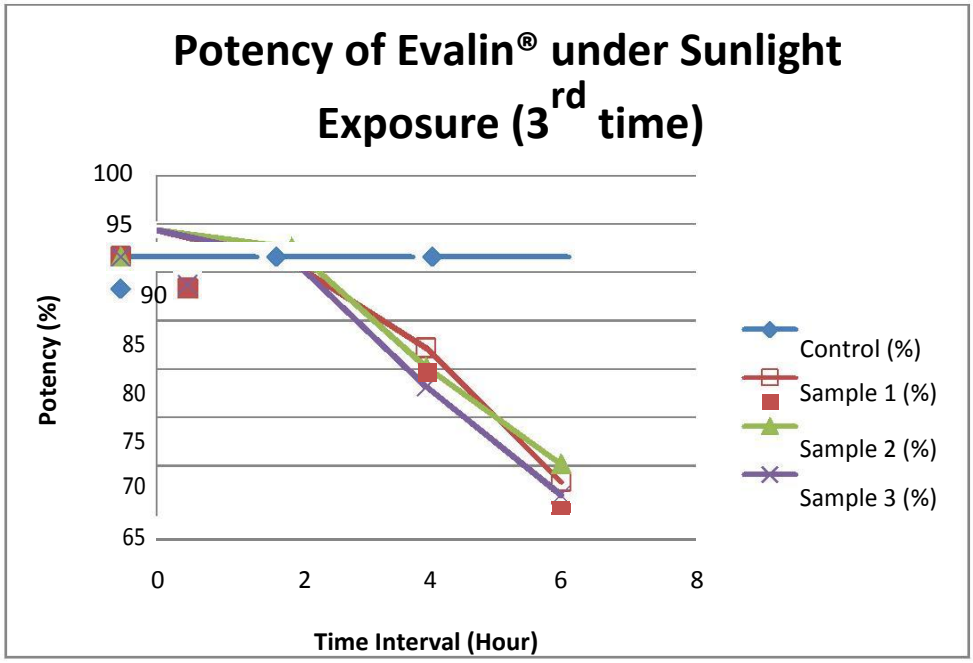

Figure 4.17: Difference in potency of Evalin® after specific time interval under sunlight exposure (3rd time)

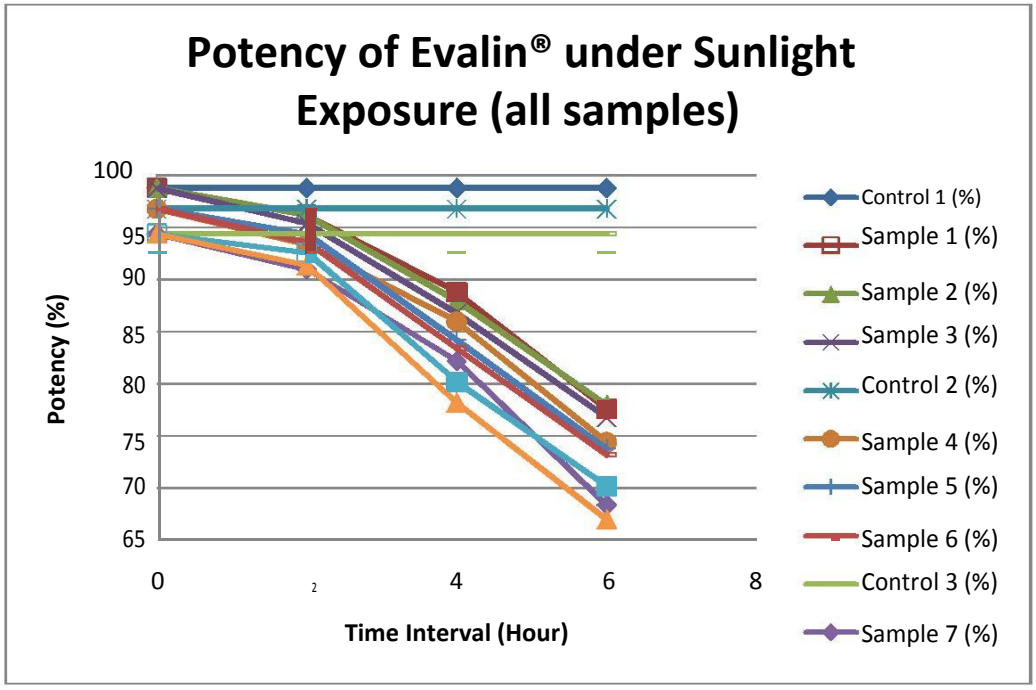

Figure 4.18: Difference in potency of Evalin® under sunlight exposures

CHAPTER FIVE

DISCUSSION

5.1 Discussion

In this research work it was observed that the percentage of Weight Variation of the sample tablets were within the accepted range (Weight of tablet 5 mg or less then % error = ±10%) as according to U.S.P. if no more than 2 tablets are outside the percentage limit and if no tablet differs by more than 2 times the percentage limit the tablet pass the test. The standard deviation of weight variation of Evalin® is± 0.21. So, it can be concluded that, the light has no effect on weight of the Evalin®.

In our experiment, we observed that the hardness of the sample tablets was within the accepted range though the result was fluctuated within 60 days. The standard deviation of hardness of Evalin® is ± 0.47.

Again, we checked the thickness of the diazepam tablet along with the other experiment. The thickness of the drug was not changed within the time limit of our research project rather the thickness was close together to the every sample which was taken in every intervals time for the experiment. The standard deviation of Evalin® for thickness was ± 0.14.

In the experiments that were conducted, we saw that the potency of Evalin® decreased steadily in every case of light exposure (in sunlight and electric bulb and normal light exposure) for Evalin tablets.

The sample tablets which were Evalin tablets, when exposed under the electrical bulb (25and 40 watts) and sampled after every 2 hour light exposure was experienced that the concentration of diazepam decreased steadily. The tablet sample which were exposed at four hour on light had a reduced amount of concentration of Diazepam than the two hour exposed sample tablet had and we also found that six hour exposed sample tablets have even less concentration of Diazepam than two and four hour light exposed sample. We also experienced that the same result for the sunlight exposed sample tablets. Here standard deviation of 25watt, 40watt, direct sunlight and normal room light were observed 17.56%, 26.05%, 29.02% & 20.61% respectively.

In summary, we can come to the final decision that there should be a change in the packaging system of the Diazepam drug product by the company. Recently most of the available brands of this drug in local market are packaged in plastic transparent blister strip. This package should be opaque thus the light would not be able to pass through the package.

CHAPTER SIX

CONCLUSION

6.1 Conclusion

It is clearly visible from the study that the physical parameters such as weight variation, hardness, thickness have passed the USP and BP specification. But there have been remarkable change in concentration/potency. The concentration of Diazepam decreased gradually after exposure in 25 and 40 watt electrical light condition, sunlight and normal light exposure (room temperature) condition. In this study we also observed that hardness of six and nine hours 40 watt exposure sample do not meet the USP specification. So we can conclude that Evalin® is photosensitive and the transparent blister packaging is not efficient to prevent degradation and decrease in the potency of Evalin® (Diazepam).

CHAPTER SEVEN

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