Comparative Dissolution Study of Four Different Brands of Clonazepam Tablets (Clonium, Disopan, Rivotril, Epiclone) With Epitra

A dissertation submitted to the Department of Pharmacy, East West University, Bangladesh, in partial fulfillment of the requirements for the Degree of Bachelor of Pharmacy.

Submitted by

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Declaration by the Candidate

I, Mahbubur Rahman Bhuiyan, hereby declare that the dissertation entitled ***"Comparative Dissolution Study of Four Different Brands of Clonazepam Tablets (Clonium, Disopan, Rivotril, Epiclone) With Epitra"*** submitted by me to the Department of Pharmacy, East West University, in the partial fulfillment of the requirement for the award of the degree Bachelor of Pharmacy, work carried out by me during the period 2016-2017 of my research in the Department of Pharmacy, East West University, under the supervision and guidance of Tirtha Nandi, Lecturer, Department of Pharmacy, East West University. The thesis paper has not formed the basis for the award of any other degree/diploma/fellowship or other similar title to any candidate of any university.

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Certificate by the Supervisor

This is to certify that the thesis entitled ***"Comparative Dissolution Study of Four Different Brands of Clonazepam Tablets (Clonium, Disopan, Rivotril, Epiclone) With Epitra"*** submitted to the Department of Pharmacy, East West University for the partial fulfillment of the requirement for the award of the degree Bachelor of Pharmacy, was carried out by Mahbubur Rahman Bhuiyan , ID: 2012-3-70-045, during the period 2016-2017 of his research in the Department of Pharmacy, East West University, under the supervision and guidance of me. The thesis has not formed the basis for the award of any other degree/diploma/fellowship or other similar title to any candidate of any university.

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Supervisor

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Endorsement by the Chairperson

This is to certify that the thesis entitled ***"Comparative Dissolution Study of Four Different Brands of Clonazepam Tablets (Clonium, Disopan, Rivotril, Epiclone) With Epitra"*** submitted to the Department of Pharmacy, East West University for the partial fulfillment of the requirement for the award of the degree Bachelor of Pharmacy, was carried out by Mahbubur Rahman Bhuiyan, ID: 2012-3-70-045, during the period 2016-2017 of his research in the Department of Pharmacy, East West University.

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Dr. Shamsun Nahar Khan

Associate Professor & Chairperson

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Dedication

This research paper is dedicated to my beloved Parents and my family members

Abstract

Clonazepamis an anticonvulsant or antiepileptic drug. It is also used to treat panic attacks. Clonazepam works by calming your brain and nerves. It belongs to a class of drugs called benzodiazepan.The aim of the present study was to evaluate the dissolution pattern of locally branded drug products of Clonazepam tablets available in Bangladesh.Here I compare the dissolution pattern of four locally marketed drugs With Epitra belongs from Square Pharmaceuticals Ltd. Four brands namely: Clonium, Rivotril, Disopan, Epiclone have been under this study. Branded drugs are expensive than locally marketed drug. Substitution of drugs is very essential for the people of under developing country. These five different brands of clonazepam tablets which are available in Bangladesh were collected from a reputed pharmacy store. Three tablets from each of the brands were used for the *in-vitro* dissolution study. Cumulative drug release was measured up to 50 minutes for all the brands.In all the cases, standard curve along with the percent release data with time was calculated.

Keyword: Clonazepam, Dissolution,*In-vitro* drug dissolution study, drug release equations.

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GABA	Gamma aminobutyric acid
GABA-A Receptor	Gamma aminobutyric acid A receptor
USP	United state pharmacopia
FDA	Food and Drug Administration
EMEA	European Medicines Agency

Chapter One

INTRODUCTION

Introduction

1.1 Sedative hypnotics

A drug that reversibly depresses the activity of the central nervous system, used chiefly to induce sleep and to allay anxiety. Barbiturates, benzodiazepines, and other sedative hypnotic have diverse chemical and pharmacological properties that share the ability to depress the activity of all excitable tissue, especially the arousal center in the brainstem. Sedative-hypnotic are used in the treatment of insomnia, acute convulsive conditions, and anxiety state sand in facilitation of the induction of anesthesia. Although sedative-hypnotics have soporific effect, they may interfere with rapid eye movement sleep associated with dreaming and when administered to patients with fever, may act paradoxically and cause excitement rather than relaxation. Sedative hypnotics may interfere with temperature regulation, depress oxygen consumption in various tissues, and produce nausea and skin rashes. In elderly patients they may cause dizziness, confusion and ataxia. Drugs in this group have a high potential for abuse that often results in physical and psychological dependence. Treatment of dependence involves gradual reduction of the dosage because abrupt withdrawal frequently causes serious disorders including convulsions. Acute reactions to an overdose of a sedative-hypnotic may be treated with an emetic, activated charcoal, gastric lavage, and measures to maintain airway patency. Buspirone, zolpidem and zaleplon are among the newer nonbarbiturate-nonbenzodiazepine sedative-hypnotic drugs.

(TheFreeDictionary.com, 2017)

1.2 Anxiolytics

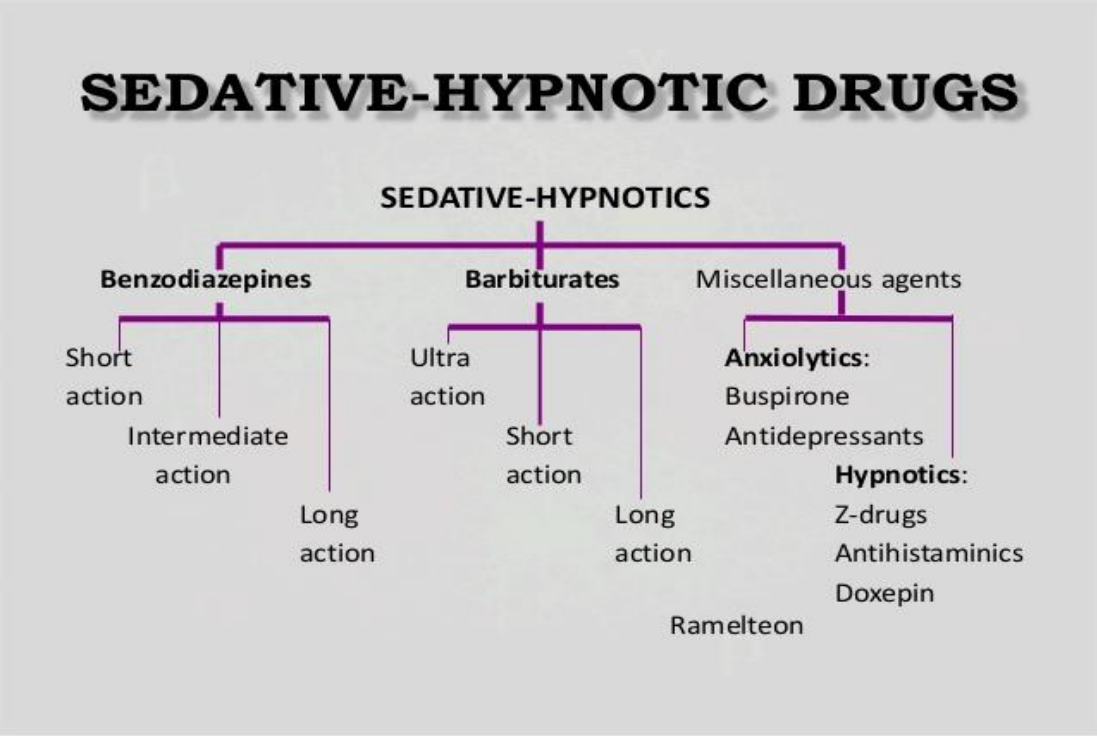
An **anxiolytic** is a medication or other intervention that inhibits anxiety. This effect is in contrast to anxiogenic agents, which increase anxiety. Together these categories of psychoactive compounds or interventions may be referred to as anxiotropic compounds or agents.

Sedatives: A drug that decreases activity and calms the recipient. E.g. Clonazepam.

Hypnotics: A drug that produced drowsiness and facilitates the onset and maintenance of a state of sleep that resembles natural sleep. E.g. Chlordiazepoxide

1.3 Classification of sedative and hypnotic drugs

Clonazepam, sold under the brand name Klonopin among others, is a medication used to prevent and treat seizures, panic disorder, and for the movement disorder known as akathisia. It is a tranquilizer of the benzodiazepine class. It is taken by mouth.



1.4 Mechanism of action of clonazepam

The precise mechanism by which clonazepam exerts its antiseizure and antipanic effects is unknown, although it is believed to be related to its ability to enhance the activity of gamma aminobutyric acid (GABA), the major inhibitory neurotransmitter in the central nervous system.

1.5 Pharmacodynamics

The precise mechanism by which clonazepam exerts its antiseizure and antipaniceffects is unknown, although it is believed to be related to its ability to enhance the activity of gamma aminobutyric acid (GABA), the major inhibitory neurotransmitter in the central nervous system. Clonazepam is a potent anticonvulsant 1,4-benzodiazepine that controls some types of

myoclonus. Its primary mode of action is to facilitate GABAergic transmission in the brain by a direct effect on benzodiazepine receptors. GABA receptors lie on the cell bodies of dorsal raphe neurons, and GABA acts to inhibit raphe cell firing, an action potentiated by benzodiazepines. Clonazepam does not alter 5-HT synthesis but decreases 5-HT utilization in brain and blocks the egress of 5-HIAA from the brain. It is not known whether the actions of clonazepam in altering 5-HT function are responsible for its antimyoclonic action, since these are observed only after large doses. Also, the effects of clonazepam are the exact opposite of those predicted from the beneficial effects of 5-HTP in human myoclonic disorders. Finally, why clonazepam, more than other benzodiazepines, is of benefit in the treatment of myoclonus is not clear. This may be due to some pharmacokinetic feature of the drug in conjunction with its potency at benzodiazepine receptors.*(RxList, 2015)*

1.6 Pharmacokinetics of Clonazepam:

1.6.1Absorption:

Clonazepam is rapidly and almost completely absorbed after oral administration of tablets. Peak plasma concentrations of clonazepam are reached in 1-4 hours. The absorption half-life is around 25 minutes. The absolute bioavailability is 90%. Plasma concentrations of clonazepam at steady state for a once-daily dosage regimen are 3-fold higher than those after a single oral dose; the predicted accumulation ratios for two times and three times daily regimens are 5 and 7, respectively. Following multiple oral doses of 2 mg three times daily steady-state pre-dose plasma concentrations of clonazepam averaged 55 ng/ml. The plasma concentration-dose relationship of clonazepam is linear. The target anticonvulsant plasma concentrations of clonazepam range from 20 to 70 ng/ml.

1.6.2 Distribution:

Clonazepam distributes very rapidly to various organs and body tissues with preferential uptake by brain structures. The distribution half-life is approximately 0.5-1 hour. The volume of distribution is 3 l/kg. The plasma protein binding is 82-86%.

1.6.3 Metabolism:

Clonazepam is extensively metabolized by reduction to 7-amino-clonazepam and by N-acetylation to 7-acetamino-clonazepam. Hydroxylation at the C-3 position also occurs. Hepatic cytochrome P-4503A4 is implicated in the nitroreduction of clonazepam to pharmacologically

inactive metabolites. The metabolites are present in urine both as free and conjugated (glucuronide and sulphate) compounds.

1.6.4 Elimination:

The mean elimination half-life is 30-40 hours. The clearance is 55 ml/min. 50-70% of the dose is excreted in the urine and 10-30% in feces as metabolites. The urinary excretion of unchanged clonazepam is usually less than 2% of the administered dose. The elimination kinetics in children are similar to those observed in adults.

(Knop, van der Kleijn and Edmunds, 1975)

1.7 Benzodiazepines

Benzodiazepines are a class of agents that work on the central nervous system, acting selectively on gamma-amino butyric acid-A (GABA-A) receptors in the brain. GABA is a neurotransmitter that inhibits or reduces the activity of nerve cells (neurons) within the brain. Benzodiazepines open GABA-activated chloride channels, and allow chloride ions to enter the neuron. This makes the neuron negatively charged and resistant to excitation. All benzodiazepines work in a similar way but there are differences in the way individual benzodiazepines act on the different GABA-A receptor sub-types. In addition, some benzodiazepines are more potent than others or work for a longer length of time. Because of this, some work better than others in particular conditions. Benzodiazepines may be used in the treatment of anxiety, panic disorder, seizures, or sleep disorders. They may also be used as a muscle relaxant, during alcohol withdrawal, or before surgery to induce relaxation and amnesia (memory loss).

(Drugs.com, 2017)

1.8 Classification of benzodiazepines

The benzodiazepines are classified into three groups:

1.Short-acting

Eg-Midazolam, Triazolam

2. Intermediate-acting

Eg- Alprazolam, estazolam, Lorazeam, Temazepam

3. Long-acting.

Eg- Clonasepam, Diazepam, Flurazepam, Clorazepate

1.9 Uses of Benzodiazepines

- Anxiety and panic
- Seizures (convulsions), and
- Insomnia or trouble sleeping.
- General anesthesia,
- Sedation prior to surgery or diagnostic procedures,
- Muscle relaxation,
- Alcohol withdrawal and drug associated agitation,
- Nausea and vomiting,
- Depression, and
- Panic attacks.

(Ogbru, 2015)

1.10 Benzodiazepines Structure

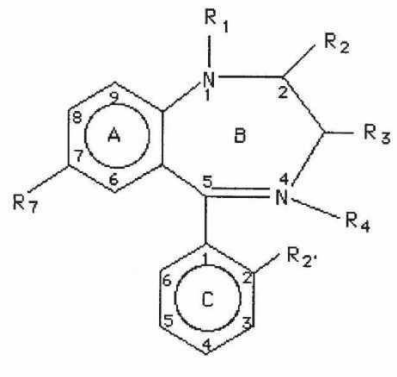


Figure 1.1-Benzodiazepines

Benzodiazepines have the same general chemical structure have been developed through the years based on chemical substitutions at two major positions on the benzodiazepine structure i.e. R and X and to some extent phenyl ring. The benzodiazepines are frequently classified into three groups:

(1) Short-acting,

(2) Intermediate-acting, and

(3) Long-acting.

The duration of action for an individual benzodiazepine plays a major role in determining how that specific drug will be used clinically. The duration of action is dependent on two factors: (1) the half-life and (2) the metabolic fate of the benzodiazepine. The first factor, the drug half-life, is the time it takes for 50% of the drug be eliminated.The longer the half-life, the longer the duration of action. The second factor that determines the duration of action is the metabolic fate of the benzodiazepine after it enters the body. In many cases, a benzodiazepine will be metabolized by enzymes in the body to another benzodiazepine with the same pharmacodynamics effects. To summarize, the duration of action of an individual benzodiazepine is a combination of the half-life of the parent drug and the half-life of any active metabolites generated by drug metabolism.

1.11 Mechanism of actions

Benzodiazepines enhance the effect of the neurotransmitter gamma-amino butyric acid (GABA) at the $GABA_A$ receptor, resulting in sedative, hypnotic (sleep-inducing), anxiolytic (anti-anxiety), anticonvulsant, and muscle relaxant properties.

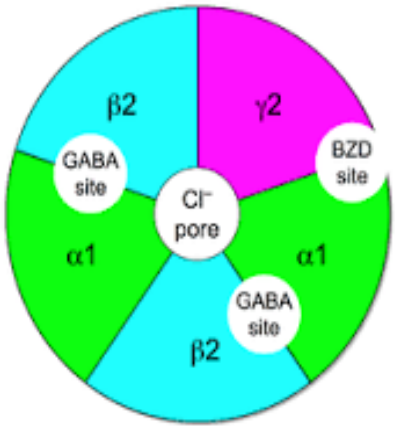


Figure 1.2- GABA receptor mechanism

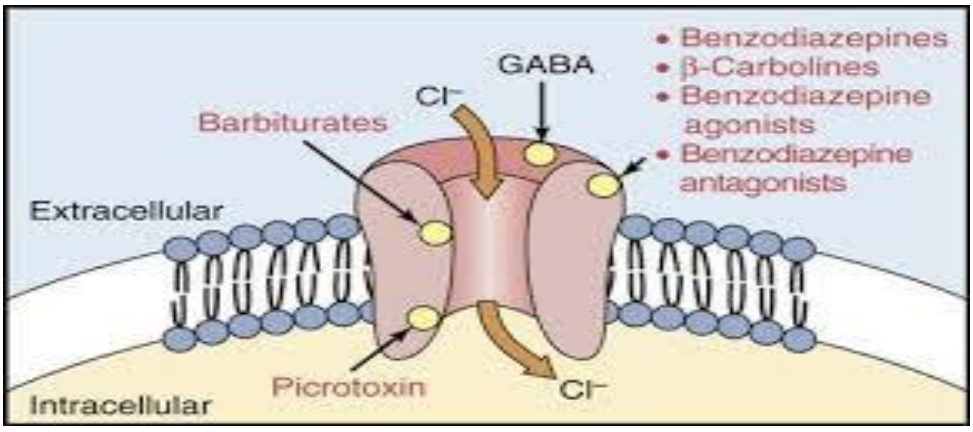


Figure 1.3- Mechanism Of action of Benzodaizepines

The molecular site of action for the benzodiazepines is at the $GABA_A$ receptors in the CNS. GABA, or gamma-amino butyric acid, is an amino acid neurotransmitter that has an inhibitory effect on neurotransmission in the CNS. Therefore, an increase in the effect of GABA results in general suppression of the CNS. When GABA binds to $GABA_A$ receptors, the result is an influx of chlorine ions into neurons through the ion channel formed by the receptor. It is the influx of chlorine that causes the negative effect on neurotransmission. On the $GABA_A$ receptors there is also a site, separate from the GABA binding site, for benzodiazepines to bind at. When both GABA and a benzodiazepine is bound to a $GABA_A$ receptor, the result is an increase in the influx of chlorine through the ion channel of the receptor. Therefore, benzodiazepines are said to increase the effect that GABA has at $GABA_A$ receptors when it binds. Finally, it should be pointed out that the benzodiazepines do not have a direct effect on the $GABA_A$ receptor; if GABA is not bound to the $GABA_A$ receptor, then benzodiazepine binding has no effect on chlorine ion influx.

1.12 List of Different Clonazepam drugs Present in Bangladesh

Band Name	Company Name
Arotil	Aristopharma Limited
Cloma	Bio Pharma Laboratories Ltd.
Clon	Globe Pharmaceuticals Ltd.
Clonapex	Apex Pharmaceuticals Ltd.
Clonapin	Popular Pharmaceuticals Ltd.
Clonatril	Healthcare Pharmaceuticals Ltd.
Clonazepam	Albion Laboratories Ltd.
Clonil	RAK Pharmaceuticals Ltd.
Clonium	ACI Limited
Clonzy	Pharmasia Ltd.
Clopam	Sharif Pharmaceuticals Ltd.
Cloron	Eskayef Bangladesh Ltd.
Denixil	Renata Limited
Depanil	Rangs Pharmaceuticals Ltd.
Disoan	Incepta Pharmaceuticals Ltd.
Epiclon	General Pharmaceuticals Ltd.
Epitra	Square Pharmaceuticals Ltd.

Epizam	Alco Pharma Limited
Band Name	Company Name
Epnil	Novartis (Bangladesh) Ltd.
Esypan	Silva Pharmaceuticals Ltd.
Leptic	Acme Laboratories Ltd.
Lonapam	Delta Pharma Limited
Lonazep	Sun Pharmaceuticals (Bangladesh) Ltd.
Myotril	IbnSina Pharmaceuticals Ltd.
Pase	OpsoninPharma Limited
Rivo	Orion Pharma Ltd.
Rivotril	Radiant Pharmaceutical Ltd.
Xetril	Beximco Pharmaceuticals Ltd.
Xiocion	Somatic Pharmaceuticals Ltd.

Table1.1- Different clonazepam Drugs present in Bangladesh

1.13 Dissolution

The dissolution of gases, liquids, or solids into a liquid or other solvent is a process by which these original states become solutes (dissolved components), forming a solution of the gas, liquid, or solid in the original solvent. Solid solutions are the result of dissolution of one solid into another, and occur, e.g., in metal alloys, where their formation is governed and described by the relevant phase diagram. In the case of a crystalline solid dissolving in a liquid, the crystalline structure must be disintegrated such that the separate atoms, ions, or molecules are released. For liquids and gases, the molecules must be able to form non-covalentintermolecular interactions with those of the solvent for a solution to form. Dissolution is of fundamental importance in all chemical processes, natural and unnatural, from the decomposition of a dying organism and return of its chemical constituents into the biosphere, to the laboratory testing of new, man-made soluble drugs, catalysts, etc. Dissolution testing is widely used in industry, including in the pharmaceutical industry to prepare and formulate chemical agents of consistent quality that will dissolve, optimally, in their target milieus as they were designed. Dissolution is the primary quality control test to determine whether a drug product can release its active pharmaceutical ingredients in a timely manner.A dissolution test is a means of identifying and proving the availability of active drug materials in their delivered form. A dissolution test simulates the availability of active substance and allows the prediction of the time for complete release of the material from the dosage form. In the pharmaceutical industry, drug dissolution testing is routinely used to provide critical in vitro drug release information for both quality control purposes, i.e., to assess batch-to-batch consistency of solid oral dosage forms such as tablets, and drug development, i.e., to predict in vivo drug release profiles. (Meyers, 2016)

1.14 Factors influence dissolution from drug products

The properties of the API,

The quality and design of the drug product,

The conditions under which the test is run and the coating material.

1.15 Effect of dissolution

Evidence is presented to show that solid dosage forms are exposed to relatively low agitation intensities after oral administration, and that it is often mandatory to use similar mild agitation conditions for predictive *in vitro* dissolution tests. Based on considerations of the effect of stirring rate on boundary layer thickness, it is demonstrated that the effective surface area of heterogeneous pellets (analogous in some respects to certain prolonged release dosage forms) may be different when such pellets are exposed to high and low agitation intensities, respectively. A proportionality between dissolution rate and the square root of stirring rate, demonstrated by others with rotating disks of inorganic salts, has been found to apply also to rotating disks of certain organic weak acids. It is shown that the ratio of dissolution rates of two or more drugs, when rates are determined by the rotating disk method, is independent of stirring rate, provided this proportionality or a similar more general relationship applies. (Levy, 1963)

1.16 Rate of dissolution

When a solute in a solvent forms a solution, it is called dissolution. A solute is the primary substance that is dissolved in a liquid called the solvent. So, what this means is that it is when something dissolves in something else. There are many factors that affect the rate at which a solute will dissolve.Solubility of A Solute Factors that Affect the Rate of Dissolving & Solubility The amount of a solute that dissolves in a given volume of solvent at a certain temperature. (Meyers, 2016)

1.17 Factors that Affect the Rate of Dissolution

There are many factors that affect the rate at which a solute will dissolve. The general rule is that 'like dissolves like.' This means that a polar substance will dissolve in another polar substance - and non-polar in non-polar. Solid substances with greater surface areas dissolve faster than solid substances with smaller surface areas. In general, solids dissolve faster with increased temperature. The solubility of gas depends on pressure and temperature.

- Like dissolves like
- Greater surface area increases dissolvability

- Temperature increases dissolvability (Meyers, 2016)

Chapter Two

LITERETURE REVIEW

Literature Review

Jorgen Neastoft,*et.al,1973 was done a study.*We have developed a gas chromatographic assay for the determination of clonazepam in human plasma, by which it is possible to follow the plasma concentrations after therapeutic doses. The kinetic pattern of clonazepam in patients is by far not fully explored. However, the usual concentrations lie between 10 and 50 microgram/1 plasma, and the half-life for the ultimate elimination phase seems to be between one or two days. The dosage per day and kg bodyweight is fairly well correlated to the peak concentration in plasma, but this in turn is not well correlated to the anti-epileptic effect in our preliminary examinations.(Jorgen Neastoft,*et.al,1973)*

Thomas R. Browne was done a study about clonazepam in 1976.Clonazepam is a new benzodiazepine anticonvulsant recently approved by the Food and Drug Administration for the treatment of typical absence, infantile myoclonic, atypical absence, myoclonic, and akinetic seizures. It is rapidly absorbed by the oral route and appears to pass quickly from blood to brain. Preliminary results indicate a biological half-life of 22 to 32 hours and a therapeutic serum concentration of 5 to 50 ng/ml. Many studies report tolerance to the anticonvulsant effects with chronic administration. Major side effects of the drug are drowsiness, ataxia, and behavior changes. They tend to be dose related, occur early in the course of therapy, and may subside with chronic administration. Accordingly, the dosage is begun at a low level and increased slowly (Thomas R. Browne,1976)

This study was performed by Chadwick D, Hallett M, Harris R , Jenner P in 1977 .Fifteen patients with a variety of myoclonic syndromes were studied clinically, pharmacologically, and physiologically. CSF tryptophan, 5HIAA, and HVA were also measured. Of these patients, 8 were improved to varying degrees by therapy with 5HTP, tryptophan in combination with MAOI (but not tryptophan alone), and clonazepam. This group included 6 cases of post-anoxic myoclonus, one case of post-traumatic myoclonus and one undiagnosed case of non-progressive focal myoclonus and epilepsy. In this group low levels of CSF 5HIAA were found compared to non-responsive cases and controls. Two cases of dysynergiacerebellarismyoclonica, 2 cases of undiagnosed aetiology, 2 cases of essential myoclonus, and one case of palatal myoclonus failed to respond to drug therapy. However, even amongst the responsive group the improvement

varied. The most dramatic responses were seen in those patients in whom physiological study suggested that myoclonus was mediated by brain-stem structures. Less dramatic responses were seen in patients in whom the myoclonus appeared to originate from cortical structures. The neurochemical basis of myoclonus responding to 5HT precursors and clonazepam is discussed. It is suggested that such myoclonus arises from a relative hypoactivity of the 5HT neuronal system which results in a release of abnormal responses to sensory stimuli which characterize this type of myoclonus.(Chadwick D, Hallett M, Harris R , Jenner P in 1977)

This study was done by Thomas R. Browne in 1978.CLONAZEPAM, an antiepileptic drug approved in 1976 by the United States Food and Drug Administration (FDA) for treatment of certain types of seizures, is a benzodiazepine structurally related to chlordiazepoxide hydrochloride, diazepam and nitrazepam. Its chemical name is 5-(2-chlorophenyl)-l,3-dihydro-7-nitro-2H-l,4-benzodiazepin-2-one . Clonazepam suppresses seizure activity in many animal models of epilepsy and many types of paroxysmal activity on the electroencephalogram of patients.1 Generalized electroencephalographic abnormalities are more readily suppressed than focal abnormalities.1 Clonazepam often limits the spread of discharge from a focal lesion while not suppressing the primary focus.(Thomas R. Browne, M.D,1978)

Herbert L. Bonkowsky and his research members conducted a study in 1980.Seizures may occur in acute intermittent porphyria or other hepatic porphyrias. Management is difficult, because barbiturates and hydantoins exacerbate the porphyric state. We studied one patient with major motor seizures and acute intermittent porphyria. The seizure disorder was exacerbated by phenytoin and did not respond to a high-carbohydrate diet or to intravenous hematin. Clonazepam was ineffective in treating the seizures and, in high doses, seemed to exacerbate the porphyria. Both clonazepam and valproate were porphyrinogenic in experimental test systems. Because both drugs may exacerbate the acute hepatic porphyrias, bromide remains the drug of choice to treat these seizures.(Herbert L. Bonkowsky,1980)

This study was performed by Chouinard G, Young SN,Annable L in 1983Twelve acutely manic patients, newly admitted from the emergency room, were treated with clonazepam or lithium carbonate in a double-blind cross-over design: half the patients chosen randomly received 10 days of treatment with clonazepam followed immediately by 10 days of treatment with lithium, while the others received the same treatments in reverse order. Clonazepam was found to be

significantly more efficacious than lithium in reducing manic symptoms despite the fact that during clonazepam treatment less patients required PRNs of haloperidol. Furthermore, both the total dose of PRN haloperidol and the number of days it was needed were significantly lower during clonazepam treatment than during lithium treatment. Clonazepam had a rapid onset of action, was highly sedative, and was well tolerated at high doses. By reducing the need for neuroleptics in the treatment of acute mania, clonazepam may decrease the risk of tardive dyskinesia in manic patients.(Chouinard G, Young SN,Annable L in 1983)

The study was done by Mitler MM, Browman CP, Menn SJ, Gujavarty K,Timms RMin 1986. Clonazepam (1 mg h.s.) and temazepam (30 mg h.s.) were studied in 10 patients diagnosed as having insomnia with nocturnal myoclonus. Each subject underwent two nocturnal polysomnographic recordings while drug-free, two during treatment with clonazepam, and two during treatment with temazepam. Treatment sessions were 7 days long, and recordings were done on nights 6 and 7 of the treatment sessions. A 14-day washout period separated the treatment sessions. The order of drugs used in the first and second treatment sessions was randomized. Objective and subjective sleep laboratory data showed that both drugs improved the sleep of patients with insomnia in association with nocturnal myoclonus. Neither drug significantly reduced the number of nocturnal myoclonic events. Sleep changes were consistent with those produced by sedative benzodiazepines in general. Thus, the data support clinical reports that clonazepam, a benzodiazepine marketed for the indication of seizure, is useful in improving sleep disturbances associated with nocturnal myoclonus. Temazepam, a benzodiazepine marketed for the indication of insomnia, was found to be a suitable alternative to clonazepam in the treatment of insomnia associated with nocturnal myoclonus. The present data and other studies suggest the need for a model that explains why leg movements and sleep disturbances may wax and wane independently.(Mitler MM, Browman CP, Menn SJ, Gujavarty K,Timms RMin 1986)

Dan Boghen ,*et .al* and his group members conducted a study in 1986.The effect of clonazepam on the restless legs syndrome was studied in a group of 6 patients. Following a drug-free period, 3 patients received clonazepam for 4 weeks followed by placebo for 4 weeks thereafter and 3 patients received the same medication and for the same length of time but in reverse order. The effectiveness of the medication was evaluated by means of a self-rating system in which patients

assigned a score daily to the degree of discomfort experienced in the previous 24 hours. Three patients improved on clonazepam but 2 of these also improved on placebo. Clonazepam was not shown to be significantly more effective than placebo in the treatment of RLS.(Dan Boghen *,et .al,1986)*

The study was done by Tesar, George E.; Rosenbaum, Jerrold F.; Pollack, Mark H.; Otto, Michael W in 1991. 72 Ss with panic disorder were randomly assigned to 6 wks of treatment with either alprazolam, clonazepam, or placebo. Endpoint analysis demonstrated a significant beneficial effect of both active treatments, but not placebo treatment, on frequency of panic attacks, overall phobia ratings, and extent of disability. In general, response to active treatment occurred within 1 wk of the initiation of drug treatment and was sustained throughout the rest of the 6-wk trial. Comparison of the 2 active treatments revealed no significant differences and no consistent tendency for one agent to be favored over another, although power to detect small differences was limited. Sedation and ataxia were the most common side effects reported, but these effects were mild and transient and did not interfere with treatment outcome. The results of this double-blind, placebo-controlled trial are consistent with previous reports of clonazepam's antipanic efficacy.(Tesar , George E.; Rosenbaum, Jerrold F.; Pollack, Mark H.; Otto, Michael W in 1991)

This study was performed by Williams & Wilkins in 1993.Clonazepam and placebo were administered in a double-blind pilot study to 75 outpatients with social phobia. The mean maximum dose of clonazepam was 2.4 mg/day at endpoint (range, 0.5 to 3 mg). Treatment was continued for up to 10 weeks. The results of an intent-to-treat analysis indicated superior effects of clonazepam on most measures. Response rates for clonazepam and placebo were 78.3 and 20.0%. Drug effects were apparent on performance and generalized social anxiety, on fear and phobic avoidance, on interpersonal sensitivity, on fears of negative evaluation, and on disability measures. Significant differences were evident by week 1, 2, or 6, depending upon the rating scale used. Clonazepam was well tolerated in general, although unsteadiness and dizziness were more severe and persistent than was the case for placebo subjects.(Williams & Wilkins,1993)

This study was performed byJ Christopher Spell, James T Stewart in 1998.A stability indicating, reversed phase high-performance liquid chromatographic method utilizing a smallbore HPLC column has been developed for the determination of clonazepam in a commercial tablet dosage

form. The use of a small bore column results in a substantial solvent savings, as well as a greater mass sensitivity, especially in the identification of degradation peaks in a chromatogram. The method involves ultraviolet detection at 254 nm and utilized a 150×3.0 mm i.d. column packed with 3 μmoctyldecylsilane particles with a mobile phase of water–methanol–acetonitrile (40:30:30, v/v/v) at a flow rate of 400 μl min−1 at ambient temperature, with and without the use of 1,2-dichlorobenzene as the internal standard. The current USP method for the analysis of clonazepam using a 300×3.9 mm i.d. conventional octyldecylsilane column was utilized as a comparison to the smallbore method. The retention times for clonazepam and the internal standard on the 3.0 mm i.d. column were 4.0 and 12.5 min, respectively. The intra- and interday RSDs on the 3.0 mm i.d. column were <0.55% (n=4) using the internal standard, and <0.19% (n=4) without the internal standard at the lower limit of the standard curve, 50 μg ml−1 and had a limit of detection of 24 ng ml−1. The assay using the 3.0 mm i.d. column was shown to be suitable for measuring clonazepam in a tablet dosage form.(J Christopher Spell, James T Stewart in 1998)

Young-Il Jeongand his research members conducted a study in 1998.Block copolymers consisting of poly(γ-benzyl L-glutamate) (PBLG) as the hydrophobic block and poly(ethylene oxide) (PEO) as the hydrophilic block were synthesized and characterized. Core-shell type nanoparticles of the block copolymers (abbreviated as GE) were prepared by the diafiltration method. The particle size diameter obtained by dynamic light scattering of GE-1 (PBLG content: 60.5 mol %), GE-2 (PBLG content: 40.0 mol %), GE-3 (PBLG content: 12.4 mol %) copolymer was 309.9±160.9, 251.9±220.6 and 200.5±177.1 nm, respectively. The shape of the nanoparticles by SEM or TEM was almost spherical. The critical micelle concentration of the block copolymers obtained by fluorescence spectroscopy was dependent on the chain length of hydrophobic PBLG. The micelle structure of the copolymer nanoparticle was very stable against sodium dodecyl sulfate. Clonazepam (CZ) was loaded onto the core part of the nanoparticle as the crystalline state. Release of CZ from the nanoparticles in vitro was dependent on the drug loading contents and PBLG chain length.(Young-Il Jeong, et.al.1998)

Suresh Bandari, Rajendar Kumar Mittapalli, Ramesh Gannu was performed a study in 2000 about clonazepam. Oral drug delivery remains the preferred route for administration of various drugs. Recent developments in the technology have prompted scientists to develop orally

disintegrating tablets (ODTs) with improved patient compliance and convenience. ODTs are solid unit dosage forms, which disintegrate or dissolve rapidly in the mouth without chewing and water. Orally disintegrating tablets provide an advantage particularly for pediatric and geriatric populations who have difficulty in swallowing conventional tablets and capsules.This review describes the various formulation aspects, disintegrants employed and technologies developed for ODTs, along with various excipients, evaluation tests, marketed formulations. (SureshBandari, Rajendar Kumar Mittapalli, Ramesh Gannu,2000)

A.A Salem and his colleagues conducted a study in 2003.New spectrophotometric and fluorimetric methods have been developed to determine diazepam, bromazepam and clonazepam (1,4-benzodiazepines) in pure forms, pharmaceutical preparations and biological fluid. The new methods are based on measuring absorption or emission spectra in methanolic potassium hydroxide solution. Fluorimetric methods have proved selective with low detection limits, whereas photometric methods showed relatively high detection limits. Successive applications of developed methods for drugs determination in pharmaceutical preparations and urine samples were performed. Photometric methods gave linear calibration graphs in the ranges of 2.85–28.5, 0.316–3.16, and 0.316–3.16 μg ml−1 with detection limits of 1.27, 0.08 and 0.13 μg ml−1 for diazepam, bromazepam and clonazepam, respectively. Corresponding average errors of 2.60, 5.26 and 3.93 and relative standard deviations (R.S.D.s) of 2.79, 2.12 and 2.83, respectively, were obtained. Fluorimetric methods gave linear calibration graphs in the ranges of 0.03–0.34, 0.03–0.32 and 0.03–0.38 μg ml−1 with detection limits of 7.13, 5.67 and 16.47 ng ml−1 for diazepam, bromazepam and clonazepam, respectively. Corresponding average errors of 0.29, 4.33 and 5.42 and R.S.D.s of 1.27, 1.96 and 1.14 were obtained, respectively. Statistical Students t-test and F-test have been used and satisfactory results were obtained.(A.ASalem,*et.al,2003)*

This study was performed by Christelle Gremeau-Richard,et.al.2003. Stomatodynia is characterised by a spontaneous burning pain in the oral mucosa without known cause or recognised treatment. The purpose of this double-blind, randomised, multicentre parallel group study was to evaluate the efficacy of the topical use of clonazepam. Forty-eight patients (4 men and 44 women, aged 65±2.1 years) were included, of whom 41 completed the study. The patients were instructed to suck a tablet of 1 mg of either clonazepam or placebo and hold their saliva near the pain sites in the mouth without swallowing for 3 min and then to spit. This protocol was

repeated three times a day for 14 days. The intensity was evaluated by a 11-point numerical scale before the first administration and then after 14 days. Two weeks after the beginning of treatment, the decrease in pain scores was 2.4±0.6 and 0.6±0.4 in the clonazepam and placebo group, respectively (P=0.014). Similar effects were obtained in an intent-to-treat analysis (P=0.027). The blood concentration of clonazepam was similar whether it was measured 14 days after sucking a tablet three times a day or during the 5 h that followed sucking a single tablet (n=5). It is hypothesised that clonazepam acts locally to disrupt the mechanism(s) underlying stomatodynia.(Christelle Gremeau-Richard,et.al.2003)

MiriamGrushka,et.al.2004 with his colleagues examined The subject population consisted of 29 women and 1 man. All subjects had been symptomatic (average premorbid burning intensity, 7.0 ± 1.9 on 10-point scale) for 1 month to 12 years (mean, 3.9 ± 3.4 years; median, 2.75 years), and 16% had had burning for more than 2 years. Three groups of patients were identified: those who experienced partial to complete relief with clonazepam and who were using the medication at the last follow-up (group 1; 43%); those who found the clonazepam helpful but withdrew from the medication because of side effects—usually drowsiness (group 2; 27%); and those who did not benefit from clonazepam (group 3; 30%). Among the 3 groups, age was found to be significantly lower for group 1 than for group 2 but not significantly lower for group 1 than for group 3. Although the difference did not reach significance, the mean dose of clonazepam appeared lower for group 1 patients than for the other 2 patient groups. The number of patients with burning for less than 2 years was larger in group 1 than in the other groups.(MiriamGrushka,et.al.2004)

Yourong Fuand his colleagues was done a study about clonazepam in 2004.Fast disintegrating tablets (FDTs) have received ever-increasing demand during the last decade, and the field has become a rapidly growing area in the pharmaceutical industry. Upon introduction into the mouth, these tablets dissolve or disintegrate in the mouth in the absence of additional water for easy administration of active pharmaceutical ingredients. The popularity and usefulness of the formulation resulted in development of several FDT technologies. This review describes various formulations and technologies developed to achieve fast dissolution/dispersion of tablets in the oral cavity. In particular, this review describes in detail FDT technologies based on lyophilization, molding, sublimation, and compaction, as well as approaches to enhancing the FDT properties, such as spray-drying, moisture treatment, sintering, and use of sugar-based

disintegrants. In addition, taste-masking technologies, experimental measurements of disintegration times, and clinical studies are also discussed.(Yourong Fu,*et.al,2004)*

Matheus P. FreitasA and his colleagues done a research in 2005 .Comparison between dissolution profiles obtained by using a dissolution apparatus (conventional method) and the NIR diffuse reflectance spectra of a series of clonazepam-containing batches is reported. Ten different formulations with fixed amount of clonazepam and varying proportions of excipients were analyzed at seven dissolution times and three different media. The percentages of dissolution of each sample were correlated with the NIR spectra of three tablets of each batch, through a multivariate analysis using the PLS regression algorithm. The squared correlation coefficients for the plots of percentages of dissolution from the equipment laboratory (dissolution apparatus and HPLC determination) versus the predicted values, in the leave-one-out cross-validation, varied from 0.80 to 0.92, indicating that the NIR diffuse reflectance spectroscopy method is an alternative, nondestructive tool for measurement of drug dissolution from tablets. (Matheus P. FreitasA,*et.al,2005)*

Vigdis Olsenand his group members was done a study in 2005.Sedating drugs are reported to be used in cases where people have been drugged unwittingly. In the present experiments we studied whether nine sedating medicinal drugs would dissolve in four different beverages to reach concentrations which could possibly cause impairment and whether the drugs altered the appearance and taste of the beverages. Nine sedating medicinal drugs were added separately to water, beer, Coca-Cola™ and ethanol. Drug concentrations were measured 5, 10, 20 and 40 min after spiking. The amount of drug in one swallow (50 mL) was calculated. Appearance and taste were recorded after 10 min. Flunipam®, Sobril®, Valium® and Xanor® dissolved faster than Rohypnol®, Imovane®, Somadril®, Rivotril® and Dolcontin®. Ten minutes after adding Flunipam®, Sobril®, Imovane® (in beer and Coca-Cola™), Valium® and Xanor®, the concentrations had reached more than 50% of maximum theoretical concentration. Most of the drugs caused sediment, pieces and/or turbidity in one or more of the beverages. Some of the solutions were dyed from added Rohypnol® (turquoise or green), Dolcontin® (red) and Valium® (yellow). Flunipam® and Valium® caused extensive frothing in beer. The tastes of Imovane® and Somadril® were distinct in all the beverages, while the taste of other drug solutions was less distinct. The ingestion of all solutions could probably have caused

impairment. All the nine drugs were, however, apparent to the consumer from the altered appearance and/or taste of the beverages. (Vigdis Olsen,*et.al*,2005)

Nilesh Dhavale and his research members was done a study in 2008 .A new, simple high-performance thin-layer chromatographic method has been established and validated for simultaneous determination of escitalopram oxalate and clonazepam in a combined tablet dosage form. The drugs were separated on aluminum plates precoated with silica gel 60 F254; toluene–ethyl acetate–triethylamine 7:3.5:3 (v/v) was used as mobile phase. Quantitative analysis was performed by densitometric scanning at 258 nm. The method was validated for linearity, accuracy, precision, and robustness. The calibration plot was linear over the ranges 250–2,500 and 50–500 ng band−1 for escitalopram oxalate and clonazepam, respectively. The method was successfully applied to the analysis of drugs in a pharmaceutical formulation.(Nilesh Dhavale,*et.al,2008)*

Bhalerao ,*et.al,2009 was done a study* .Recent developments in mouth dissolving/disintegrating tablets have brought convenience in dosing to elderly and children who have trouble in swallowing tablets. The objective of the present study was to prepare the mouth disintegrating tablet of clonazepam (antiepileptic). As precision of dosing and patient's compliance become important prerequisite for a long term antiepileptic treatment, there is a need to develop a formulation for this drug which overcomes problems such as difficulty in swallowing, inconvenience in administration while travelling and patient's acceptability. Hence, the present investigation was undertaken with a view to develop a fast disintegrating tablet of clonazepam which offers a new range of product having desired characteristics and intended benefits. The drug is poorly water soluble therefore to enhance the solubility and release of drug, solid dispersion of drug and PVP K30 was prepared by solvent evaporation method. Different combinations of superdisintegrants such as crosscarmellose sodium, sodium starch glycolate, crospovidone were used. Directly compressible mannitol, and aspartame were used to enhance the mouth feel and taste. Lactose was used as diluents. The tablets were prepared by direct compression technique on rotary tablet machine. The tablets were evaluated for hardness, friability, weight variation, wetting time, dispersion time and uniformity of content. Optimized formulations were evaluated by in vitro dissolution test. All the tablets had hardness 3–3.5 kg/cm2 and friability of all formulations was less than 1, weight variation and drug content were

within official limit. Amongst all formulations, formulation F10 prepared by drug:PVPK30 (1:4:)ratio and combination of 5% w/w crosscarmellose sodium and 5% w/w of sodium starch glycolate showed least dispersion time of 8s and faster dissolution.(Bhalerao ,*et.al,2009)*

DhirenderKaushik was performed a study about clonazepam in 2009.Orally disintegrating tablets (ODTs) have emerged as one of the popular and widely accepted dosage forms, especially for the pediatric and geriatric patients. To obviate the problem of dysphagia and to improve patient compliance, ODTs have gained considerable attention as preferred alternatives to conventional tablet and capsule formulations. Various scientific techniques including freeze drying, moulding, spray drying, sublimation, direct compression, cotton candy process, mass extrusion, melt granulation etc. have been employed for the development of ODTs. These techniques render the disintegration of tablet rapidly and dissolve in mouth without chewing or additional water intake. The current article is focused on ideal characteristics, significant features, patented technologies, formulation aspects including the use of superdisintegrants. Various marketed preparations along with numerous scientific advancements made so far in this avenue have also been discussed.(Dhirender Kaushik,2009)

This study was performed by A Gupta in,2010. In recent decades, a variety of pharmaceutical research has been conducted to develop new dosage forms. Among the dosage forms developed to facilitate ease of medication, the rapid disintegrating tablet (RDT) is one of the most widely employed commercial products. As our society is becoming increasingly aged, the development of Fast- or mouth dissolving tablets have been formulated for pediatric, geriatric, and bedridden patients and for active patients who are busy and traveling and may not have access to water. Such formulations provide an opportunity for product line extension in the many elderly persons will have difficulties in taking conventional oral dosage forms (viz., solutions, suspensions, tablets, and capsules) because of hand tremors and dysphagia. Swallowing problems also are common in young individuals because of their underdeveloped muscular and nervous systems. Other groups that may experience problems using conventional oral dosage forms include the mentally ill, the developmentally disabled, and patients who are uncooperative, on reduced liquid-intake plans, or are nauseated. In some cases such as motion sickness, sudden episodes of allergic attack or coughing, and an unavailability of water, swallowing conventional tablets may

be difficult. This paper summarizes the formulation methods and drug formulation coming in market (*A Gupta,2010)*

This study was done byS. B. Shirsand and research members conducted a study in 2011. Fast dissolving tablets of clonazepam were prepared by sublimation method with a view to enhance patient compliance. A 3^2 full factorial design was applied to investigate the combined effect of two formulation variables: amount of croscarmellose sodium and camphor. Croscarmellose sodium (2-8% w/w) was used as superdisintegrant and camphor (20-40% w/w) was used as subliming agent, to increase the porosity of the tablets, since it helps water to penetrate into the tablets, along with directly compressible mannitol to enhance mouth feel. The tablets were evaluated for hardness, friability, thickness, drug content uniformity, *in vitro* dispersion time, wetting time and water absorption ratio. Based on *in vitro* dispersion time (approximately 11 s); the formulation containing 5% w/w croscarmellose sodium and 40% w/w camphor was found to be promising and tested for *in vitro* drug release pattern (in pH 6.8 phosphate buffer). Short-term stability (at 40°/75% relative humidity for 3 mo) and drug-excipient interaction. Surface response plots are presented to graphically represent the effect of independent variables on the *in vitro* dispersion time. The validity of the generated mathematical model was tested by preparing two extra-design checkpoints. The optimized tablet formulation was compared with conventional commercial tablet formulation for drug release profiles. This formulation showed nearly nine-fold faster drug release ($t_{50\%}$ 1.8 min) compared to the conventional commercial tablet formulation ($t_{50\%}$ 16.4 min). Short-term stability studies on the formulation indicated that there are no significant changes in drug content and *in vitro* dispersion time (P<0.05).(S. B. Shirsand,et.al.2011)

Thakur and his colleagues done a research 2011 about An orally disintegrating tablet or orodispersible tablet (ODT) is a drug dosage form available for alimited amount of over-the-counter (OTC) and prescription medications. ODTs differ from traditional tablets in that they are designed to be dissolved on the tongue rather than swallowed whole. The ODT serves as an alternative dosage form for patients who experience dysphasia(difficulty in swallowing) or for where compliance is a known issue and therefore an easier dosageform to take ensures that medication is taken. Common among all age groups, dysphasia is observed in about 35% of the general population, as well as up to 60% of the elderly institutionalized population and 18-22%

of all patients in long-term care facilities. During the last decade, ODTs have become available in a variety of therapeutic markets, both OTC and by prescription. An additional reason to use ODTs is the convenience of a tablet that can be takenWithout water.(Thakur,*et.al,2011)*

Sams M A Sada was done a study in 2011.Clonazepam is an anticonvulsant and anxiolytic drug characterized by poor solubility and rapid absorption. The purpose of the study was to improve the solubility of clonazepam in solid dispersions by incorporating hydroxypropyl methyl cellulose (HPMC) and poloxamer 407. Solid dispersions were prepared by using polyethylene glycol 6000 as carrier in 1:20 ratio by melt granulation. The solid dispersions were compressed in to tablets by adding microcrystalline cellulose, sodium starch glycolate and talc. The solid dispersions were characterized by differential scanning calorimetry, particle size analysis, and drug contents were measured by HPLC coupled with UV detection. The in vitro dissolution of the tablets was compared with pure drug in 900 ml water using USP dissolution apparatus (type II). HPMC improved the solubility of clonazepam by decreasing its particle size and drug dissolution from the tablets also increased significantly. (Sams M A Sada,2011)

S. B. Shirsand,*et.al ,2011 a studied about dissolution of clonazepam.*Fast dissolving tablets of clonazepam were prepared by sublimation method with a view to enhance patient compliance. A 32 full factorial design was applied to investigate the combined effect of two formulation variables: amount of croscarmellose sodium and camphor. Croscarmellose sodium (2-8% w/w) was used as superdisintegrant and camphor (20-40% w/w) was used as subliming agent, to increase the porosity of the tablets, since it helps water to penetrate into the tablets, along with directly compressible mannitol to enhance mouth feel. The tablets were evaluated for hardness, friability, thickness, drug content uniformity, in vitro dispersion time, wetting time and water absorption ratio. Based on in vitro dispersion time (approximately 11 s); the formulation containing 5% w/w croscarmellose sodium and 40% w/w camphor was found to be promising and tested for in vitro drug release pattern (in pH 6.8 phosphate buffer). Short-term stability (at 40°/75% relative humidity for 3 mo) and drug-excipient interaction. Surface response plots are presented to graphically represent the effect of independent variables on the in vitro dispersion time. The validity of the generated mathematical model was tested by preparing two extra-design checkpoints. The optimized tablet formulation was compared with conventional commercial tablet formulation for drug release profiles. This formulation showed nearly nine-fold faster drug

release (t50% 1.8 min) compared to the conventional commercial tablet formulation (t50% 16.4 min). Short-term stability studies on the formulation indicated that there are no significant changes in drug content and in vitro dispersion time ($P<0.05$).(S. B. Shirsand,*et.al ,2011)*

Amos ,*et.al. in 2011 was conducted a study* .Aims: To evaluate retrospectively the efficacy of administering an anticonvulsant medication, clonazepam, by dissolving tablets slowly orally before swallowing, for the management of burning mouth syndrome (BMS). Methods: A retrospective clinical records audit was performed of patients diagnosed with BMS between January 2006 and June 2009. Patients were prescribed 0.5 mg clonazepam three times daily, and changes were made to this regimen based on their individual response. Patients were asked to dissolve the tablet orally before swallowing and were reviewed over a 6-month period. Pain was assessed by patients on an 11-point numerical scale (0 to 10). A nonparametric (Spearman) two-tailed correlation matrix and a two-tailed Mann-Whitney test were performed. Results: A total of 36 patients (27 women, 9 men) met the criteria for inclusion. The mean (± SEM) pain score reduction between pretreatment and final appointment was 4.7 ± 0.4 points. A large percentage (80%) of patients obtained more than a 50% reduction in pain over the treatment period. One patient reported no reduction in pain symptoms, and one third of the patients had complete pain resolution. Approximately one third of patients experienced side effects that were transient and mild. Conclusion: This pilot study provides preliminary evidence that the novel protocol of combined topical and systemic clonazepam..(Amos ,*et.al. in 2011)*

S.O. Alijanpour&M.J. Chaichi was performed a study about clonazepam in 2013. A novel chemiluminescence (CL) reaction, Benzodiazepines–H_2O_2–1-Ethyl-3-Methylimidazolium Ethylsulfate/copper, for determination of clonazepam and diazepam at nanogram per milliliter level in batch-type system have been described. The method relies on the catalytic effect of 1-Ethyl-3-Methylimidazolium Ethylsulfate/copper on the chemiluminescence reaction of Benzodiazepines, the oxidation of Benzodiazepines with hydrogen peroxide in natural medium. The influences of various experimental parameters such as solution pH, the ratio of 1-Ethyl-3 Methylimidazoliumethylsulfate concentration to copper ion, the type of buffer and the concentration of CL reagents were investigated. Under the optimum condition, the proposed method was satisfactorily applied for the determination of these drugs in tablets and urine without the interference of their potential impurities.(S.O. Alijanpour&M.J. ChaichI,2013)

Chapter Three

MATERIALS AND METHODS

Materials and methods

3.1 Introduction:

The study on comparative dissolution profiles of clonazepam was carried out by using dissolution method to see the release pattern of clonazepam with different time interval. The method was verified and the rotating condition of the dissolution machine is optimized before application for sample analysis. Comparative dissolution testing is a valuable tool in drug development and Characterization. In addition to serving as routine quality control tests, comparative dissolution tests have been used to support waivers for bioequivalence requirements, for approval of generic drug products and accepting product sameness under Scale-up and Post Approval (SUPAC) related changes. (Ulrich, *et. al.* 2009).

3.2 Reagents, Chemicals and Solvents:

All reagents used were of analytical reagent grade and distilled water was used for the preparation of all solutions. To observe the change in dissolution in clonazepam in dissolution media I used different brands of clonazepam tablet. I used active pharmaceutical ingredient (API) of clonazepam which was collect from Square Pharmaceuticals Ltd. (Epitra) and for the dissolution of clonazepam we used water as a solvent. Epitra is the patent drug of clonazepam. Other tablets I used to see the release pattern with different time interval like etc.

3.3 Methods for Comparison of Dissolution Profile Data

A simple model independent method proposed by Moore and Flanner (1996) uses fit factors to compare dissolution profile data of a pair of products under similar testing conditions. These fit factors directly compare the difference between percent drug dissolved per unit time for a test and reference product. These factors are denoted f1 (difference factor) and f2 (similarity factor) (US FDA, 1997; Saranadasa and Krishnamoorthy 2005; Sath, *et. al.* 1996; Yuksel *et. al.* 2000). Comparison of the dissolution profiles of clarithromycin can be satisfactorily carried out using the model independent approaches.

3.4 Difference factor

The difference factor (f1) is a measurement of the percent difference between two dissolution curves under comparison at each time point. It is a measure of the relative error between the two curves and is given by the formula:

$$f1 = \frac{\sum_{t=1}^{n} |Rt - Tt|}{\sum_{t=1}^{n} Rt} \; x\; 100$$

where, n is the number of testing time points; Rt is the average dissolution value of the reference product units at time t and Tt is the average dissolution value of the test product units at time t. Similarity of two dissolution curves is indicated by f1 values of 0 - 15% (US FDA, 1997; Hasan, *et. al.* 2007; Yuksel, *et. al.* 2000).

3.5 Similarity factor: The similarity factor (f2) is a measurement of the similarity in the percent dissolution between two dissolution curves. It is inversely proportional to the average squared difference between the two profiles. It is a logarithmic reciprocal square root transformation of the sum of squared error and is given by the formula:

$$f2 = 50.log\left[1 \Big/ \sqrt{\left\{1 + \frac{1}{n}\sum_{t=1}^{n}(Rt - Tt)^2\right\}}\; x\; 100\right]$$

Where, n is the number of testing time points; R_t is the average dissolution value of the reference product units at time t and it is the average dissolution value of the test product units at time t (US FDA, 1997; Hasan, *et. al.* 2007; Shah 2001; Yuksel, *et. al.* 2000). The proviso for evaluation for similarity is availability of data for six (6) or twelve (12) units of each product, availability of three or more dissolution time points, same conditions of testing for reference and test products and same dissolution time points for both profiles. As a further recommendation, it is suggested that only one measurement be considered after 85% dissolution of both products. (US FDA, 1997; Hasan, *et. al.* 2007; Ochekpe, *et. al.* 2006). The similarity factor has been adopted by the

US FDA and the European Medicines Agency (EMEA) for dissolution profile comparison. When two dissolution profiles are identical, f2 = 100%. An average dissolution difference of 10% at all measured time point's results in an f2 value of 50%. For this reason, the public standard for similarity of two dissolution profiles has been set at 50 - 100% (EMEA 2010; USFDA 1997; Shah, 2001).

3.6 Dissolution testing methods for Clonazepam:

Dissolution media	Distilled water
Temperature	37ºC
RPM	75
Time	60 minutes
Wavelength	273 nm

Table3.1-Dissolution testing method

The release rate of clonazepam tablet was determined by using tablet dissolution tester USP XXII. The dissolution test was performed using 900ml water pH (7.4) at 37ºC and 75 rpm at every 10-min interval sample of 10 ml were withdrawn from the dissolution medium and the amount was replacing by 10 ml distill water. The sample was filtered through a filter paper named Whitman Filter paper and diluted to a suitable concentration of distilled water. The absorbance of the solution was measured 273 nm for drug clonazepam by using a Shimadzu UV-1201 UV/visible double beam spectrophotometer (Hach, Japan). Percentage of drug release was calculated using an equation obtained from standard curve. The dissolution was continued for 60 minutes to get simulated picture of drug release in thw in vivo condition and drug dissolve at specified time periods was plotted as percent release versus time(hours) curve (Shah,*et al.*1998).

3.7 Preparation of Standard Curve:

To prepare the standard curve, at first different concentrations (8,16,24,32,40) µg/ml of clonazepam was prepared. For the preparation of different concentrations of clonazepam, first tablets were crushed in mortar and pestle. From the crushed tablet 0.5 mg was taken and was dissolved in 50 ml of distilled water. By this procedure, the concentration of the stock solution became 40 µg/ml.This solution was filtered in the volumetric flask. After that the solution was 50

times diluted and the concentrations of the solution become 40µg/ml. Then taken solution was 2 ml, 4 ml, 6 ml, 8 ml, 10 ml and added water was 8 ml, 6 ml, 4 ml, 2 ml, 0 ml. Then spectrophotometer is turned on and 273 nm wave length was set up. Then the spectrophotometer was adjusted for 0 and 100%. The solutions were placed on spectrophotometer to measure the absorbance. Then the absorbance was plotted against concentration. A straight line was found.

Serial No	Concentrations (µg/ml)
1	8
2	16
3	24
4	32
5	40

Table3.2: Concentrations of clonazepam

3.8 Preparation for dissolution test:

3.8.1 Preparation of stock solution:

Distilled water was prepared in the laboratory and was used as stock solution for dissolution test. For each batch 6L of distilled water was prepared.

3.8.2 Method for dissolution test of Clonazepam tablets

6L (6000ml) of stock solution (distilled water) was prepared. Each vessel of dissolution tester was filled with 900 ml of stock solution (distilled water) Time 1 hour; rpm 75 was set up in the dissolution machine. Then the machine was allowed to warm up until it reached at 37.5 degree Celsius. Then tablets were placed in every vessel. After 20, 40 and 60 minutes 10 ml of solution was collected from each vessel and filtered, then from that 1 ml of solution was taken in another test tube and 9 ml distilled water was added to make it 10 ml. At last UV absorbance off the solutions were taken where the wave length was 273 nm.

3.9 Determination of physical parameters

3.9.1 Weight Variation Test

3.9.1.1 Procedure:

10 Tablets were taken and weighed. The average was taken and it was considered as the standard weight of an individual tablet. All tablets were weighed individually and observed whether the individual tablets are within the range or not. N.B: The variation from the average weight in the weights not more than two tablets must not differ more than the percentage listed below:

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

Table3.3: Accepted percentage list for weight variation of tablets

3.9.1.2 Equation:

Following equation was used to determine % weight variation of tablets

% Weight Variation = (A-I/A) × 100

Where,

Initial Weight of Tablet, I (gm)

Average weight of Tablets, A (gm) **(**Dunnett, C. W., and R. Crisafio.1995)

3.9.2 Thickness test

3.9.2.1 Procedure

First the tablet was placed between the two jaws of the vernier caliper. Then the main scale reading was taken. Next vernier scale reading was taken also. The two readings were added together for multiplying with the vernier constant 0.1Cm.

3.9.2.2 Calculation

Following formula was used to determine thickness of tablets.

Thickness of the tablet = Reading of Cm scale + Reading of vernier scale × Vernier constant (0.01) + Vernier error

3.9.3 Hardness test

3.9.3.1 Procedure

The slide scale of hardness tester was made zero. One tablet was placed vertically between the two jaws of the tester. Force was applied with a screw thread and spring until tablet fractured. Reading in Kg was taken from the sliding scale (Dunnett and Crisafio, 1995).

3.9.3.2 Materials

3.9.3.2.1 Sample Collection

To observe the change in dissolution pattern of Different brands of Clonazepam tablets were collected from the local drug store in Dhaka.

Brand Name	Source
Rivotril	Radiant Phrm Ltd.
Clonium	ACI Phrm. Ltd
Epiclone	General Phrm Ltd.
Epitra	Square Phrm Ltd.
PASE	Opsonin Phrm Ltd.
Xetril	Beximco Phrm Ltd.
Epnil	Novartis Phrm Ltd.
Disopan	Incepta Phrm Ltd.
Cloron	Eskayef Phrm Ltd.
Denixil	Renata Phrm Ltd.

Table3.4-Different brands of clonazepam

3.9.3.2.2 Stock solution:

As Clonazepam is soluble in water so distilled water was prepared in the laboratory of East West University and was used as stock solution for dissolution.

3.9.3.2.3 Equipment's:

In the characterization of matrix tablets of Clonazepam (Kuss, 1992)

No.	Equipments	Source	Origin
1	Dissolution tester USPXXII	RC-6B	CHINA
2	UV-Spectrometer	HANNA1201PC	JAPAN
3	pH meter	HANNA pH 210	PORTUGAL
4	Distill Water Plant	SMIC	CHINA
5	Safety Pipette Filler	Saffron	ENGLAND
6	Filter	Copley Instruments	ENGLAND
7	Electronic Balance	Precisa XB120A	SWITZERLAND
8	Friability tester	VEEGO(EF-2)	INDIA
9	Vernier Slide Calipers	TRICLYCLE RING	INDIA
10	Hardness tester	Monasnto manually operating hardness tester	CHINA

Table3.5-Equipment used in dissolution test

3.10 Instrumentation

3.10.1 Dissolution Test Apparatus

A Dissolution tester USPXXII (source RC-6B, made in China) was used for dissolution experiments. It incorporated a clear acrylic water bath, a stirrer hood with paddle shafts, an automatic sampling unit and a control unit supported by microcontroller software with a nonvolatile memory for 15 methods. The water bath incorporated an immersion circulator with an in-built thermostat for temperature control, an external temperature sensor, a water level sensor and a lid with support for eight dissolution bowls. The stirrer hood was equipped with 8 paddle shafts fitted with USP apparatus 2 and a tablet dispenser with 8 conical shaped

dissolution bowl lids. The automatic sampling unit consisted of 10in-line filters, a bi-directional 12- channel peristaltic pump with tygon tubing's, a microprocessor controlled sample collector and a sample tray capable of collecting 10 x 6 sets of samples. Polycarbonate dissolution vessels with a hemispherical bottom and a capacity of 1000 ml were used for the study. Bromide (E. Merck, Darmstadt, Germany) and a manually operated hydraulic pellet press (Perking Elmer GmbH, Uberlingen, Germany).

3.10.2 Ultra- Violet Spectrophotometer

The ultra-violet absorption spectrum for ranitidine working standard was recorded using a double beam T90+ UV/VIS spectrometer controlled via a computer using UVWIN spectrophotometer software version 5.2.0 (HACH UV-1201 PC, JAPAN) over a 10-mm path length using quartz cuvettes.

3.11 Samples and Chemical Reference Substances

Clonazepam tablets from different manufacturers were used in the study. The samples were obtained from different private retail outlets within Bangladesh (Kuss,1992).

3.12 Images of Instruments:

Some images of important instruments those were used in different testes during research work are given below-

Figure 3.1: Dissolution apparatus

Figure 3.2: (left to right) UV-1800 Double Beam Spectrophotometer

Figure 3.3: Distilled Water apparatus

Figure 3.4: Hardness tester

Figure 3.5: Electronic Balance

3.13 Dissolution Efficiency

The dissolution efficiency is not a parameter to compare dissolution pattern between two brands. It is just a parameter to indicate drug release. It is calculated by the following equation:

$$DE = \frac{\int_{t1}^{t2} y.dt}{y100 \times (t2 - t1)} \times 100$$

In the above equation, y is the percentage of drug release. The numerator of the equation indicates the area under within the time frame. The denominator indicates the rectangle of 100% drug release from 0 times throughout the time frame. The area under the curve is calculated by the help of Microsoft Excel software (Anderson et al. 1998; Parakh and Patil 2014).

3.14 Apparatus:

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

Table 3.6- Representing the apparatus (Kuss, 1992)

Serial no	Apparatus
1	Beakers
2	Test tubes
3	Volumetric flasks
4	Filter paper
5	Spatula
6	Mortar and pestle
7	Pipette pumper
8	Pipette (1 ml & 10 ml)

Table3.6-Apparatus used in test

Chapter Four

RESULTS AND DISSCUSSION

Results& Discussion:

4.1 Preparation and method of standard curve of 'Epitra':

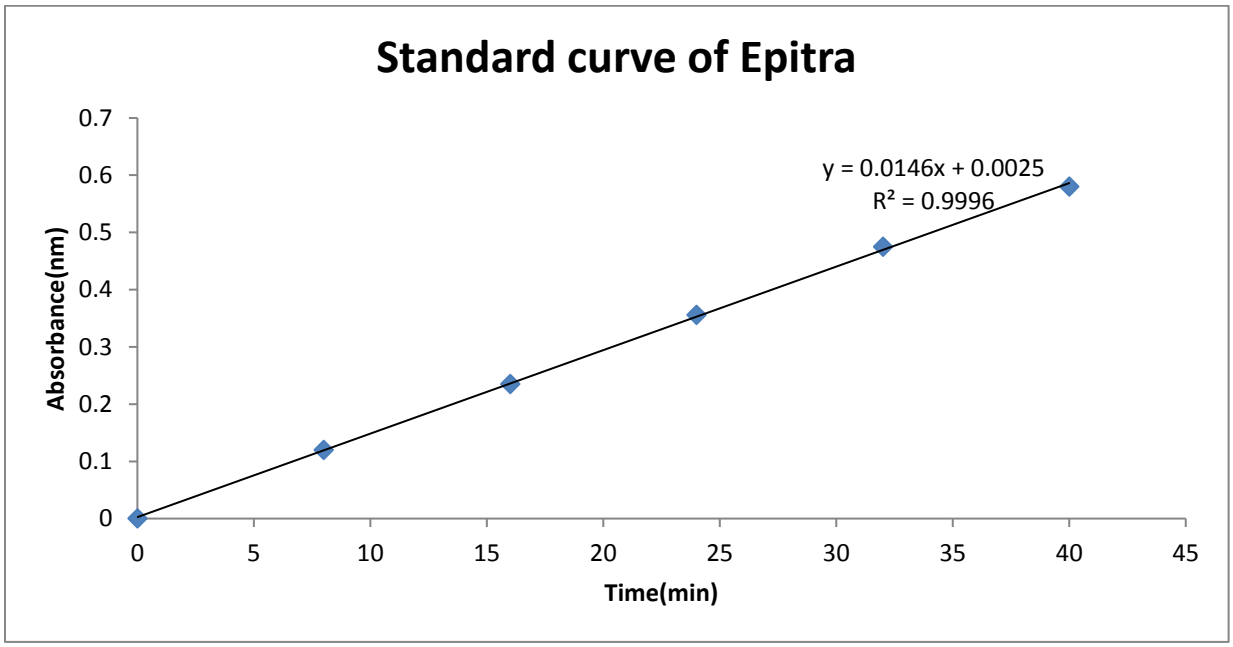


Figure 4.1: Standard curve of Epitra

4 tablets each of 0.5 mg Epitra were taken and grinded with the help of mortar and pestle to obtain a powder which was then smudged carefully and transferred into a 50ml volumetric flask. Afterwards, the grinded powder was dissolved slowly with distilled water which was made up to the 50 ml later. Here, the concentration of the stock solution after dissolving 4 tablets of clonazepam each 0.5 mg in 50 ml volumetric flask becomes 40µg/ml. Now, each of the following concentration as prepared along with the detection of their absorbance at wavelength of 273 nm to prepare the standard curve equation keeping the Time along X axis and Absorbance along Y axis.The equation**y = 0.0146x + 0.0025**helped to determine the concentration of drug release as well percent release of that drug &**R^2 = 0.9996**gave us an idea about the drug release kinetic profile.

Concentration (µg/ml)	Time (min)	Absorbance (nm)
8	10	0.12
16	20	0.235
24	30	0.356
32	40	0.475
40	50	0.580

Table 4.1: Concentrations an absorbance data for preparation of standard curve of Epitra

4.2 Preparation and method of dissolution curve of Epitra:

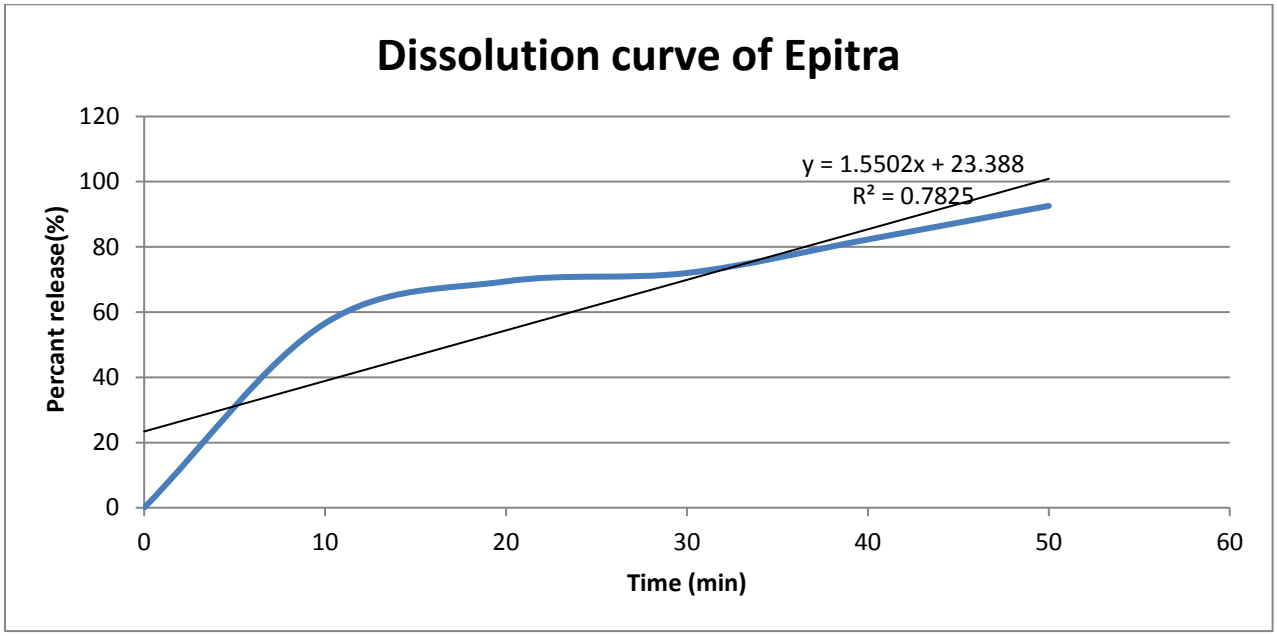


Figure4.2 : Dissolution curve of Epitra

3 tablets of Epitra were taken and they were dissolved at a rpm = 75, temperature=37± 0.5ºC with the distilled/deionized water as dissolution media and the above-mentioned concentrations were prepared for each of the tablets Afterwards, absorbance of each of the prepared concentrations for each of the tablets were measured at a wavelength of 273nm that were recorded. Thus, finally from the average of all the 3 data, a dissolution curve was prepared using the excel data sheet keeping the % drug release along Y axis and time along X axis. After 10 min and 50 min, the % release was about 56.57% and 92.57% respectivelythe equation **y = 1.5502x +**

23.388 determined the concentrations of drug release and **R^2 = 0.7825** determines the drug release kinetic profile.

Time (min)	% release of drug
0	0
10	56.57
20	69.42
30	72
40	82.28
50	92.57

Table 4.2: Data for the dissolution curve of Epitra

4.3 Preparation and method of standard curve of 'Clonium':

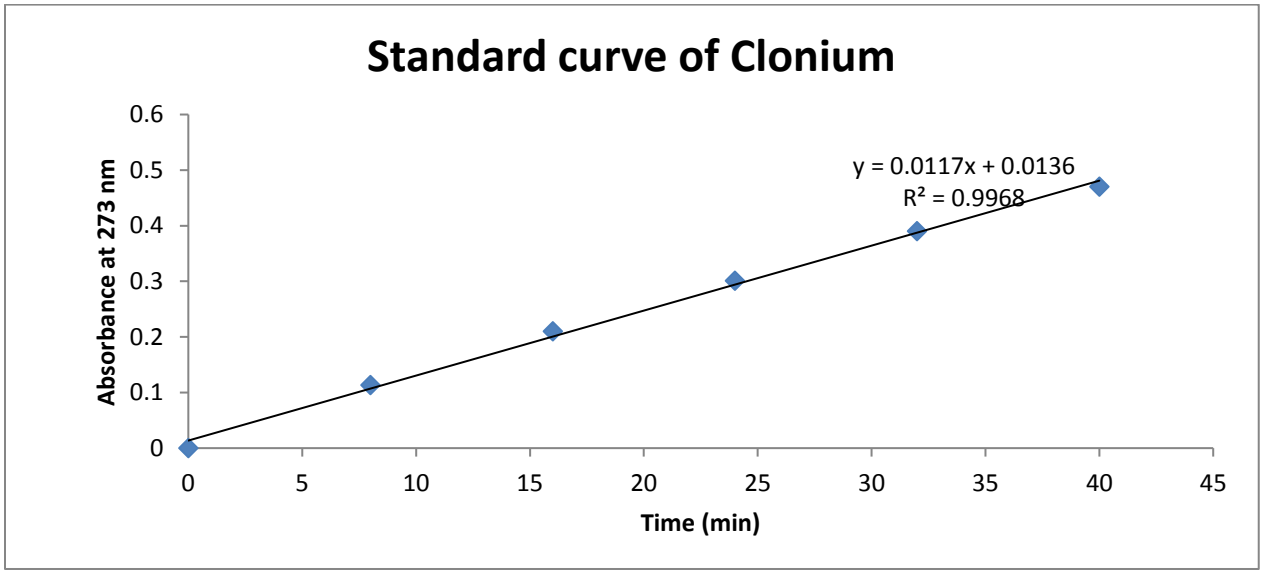


Figure4.3 : Standard curve of Clonium

4 tablets each of 0.5 mg Clonium were taken and grinded with the help of mortar and pastle to obtain a powder which was then smudged carefully and transferred into a 50ml volumetric flask. Afterwards, the grinded powder was dissolved slowly with distilled water which was made up to

the 50 ml later. Here, the concentration of the stock solution after dissolving 4 tablets of clonazepam each 0.5 mg in 50 ml volumetric flask becomes 40µg/ml. Now, each of the following concentration as prepared along with the detection of their absorbance at wavelength of 273 nm to prepare the standard curve equation keeping the Time along X axis and Absorbance along Y axis. The equation **y = 0.0117x + 0.0136**helped to determine the concentration of drug release as well percent release of that drug &**R^2 = 0.9968**gave us an idea about the drug release kinetic profile.

Concentration (µg/ml)	Time (min)	Absorbance (nm)
8	10	0.113
16	20	0.210
24	30	0.301
32	40	0.390
40	50	0.470

Table 4.3: Concentrations and absorbance data for preparation of standard curve Clonium

4.4 Preparation and method of dissolution curve of Clonium:

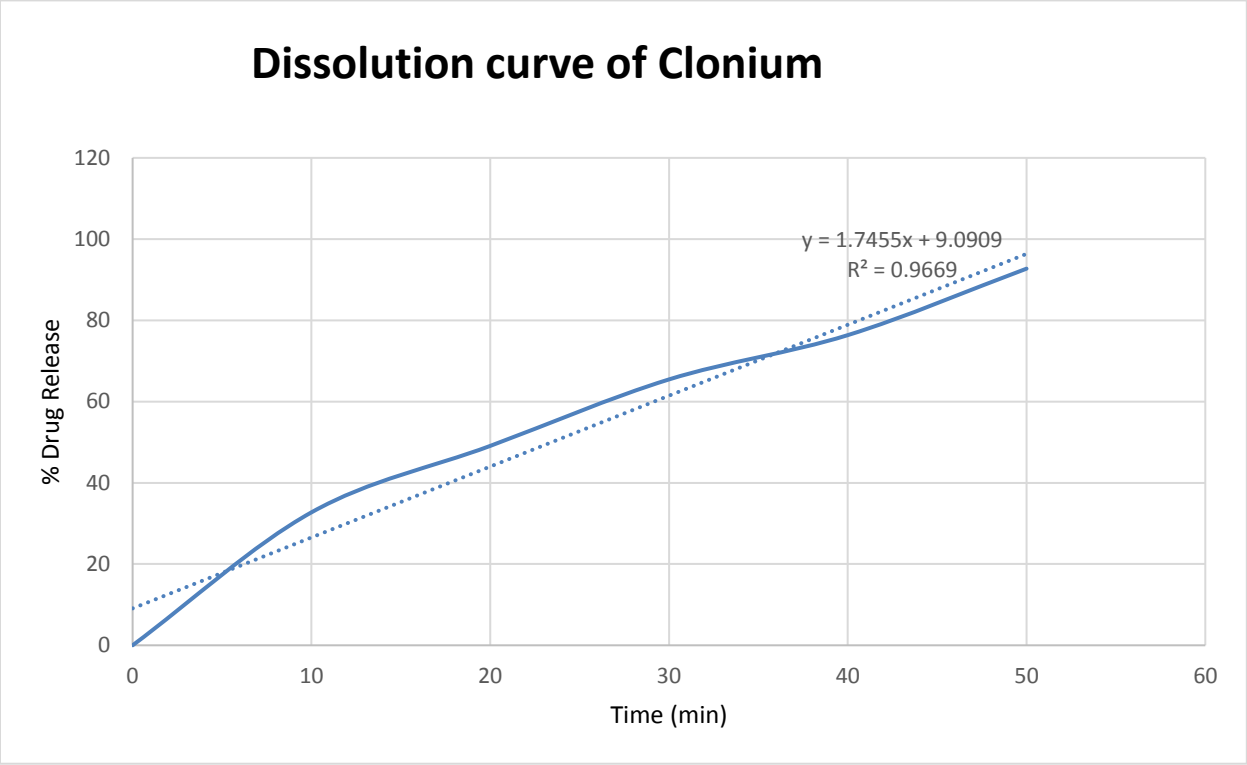


Figure 4.4 : Dissolution curve of Clonium

3 tablets of Clonium were taken and they were dissolved at a rpm = 75, temperature=37± 0.5ºC with the distilled/deionized water as dissolution media and the above-mentioned concentrations were prepared for each of the tablets Afterwards, absorbance of each of the prepared concentrations for each of the tablets were measured at a wavelength of 273nm that were recorded. Thus, finally from the average of all the 3 data, a dissolution curve was prepared using the excel data sheet keeping the % drug release along Y axis and time along X axis. After 10 min and 50 min, the % release was about 32.72% and 92.72% respectively **y = 1.7455x + 9.0909**determined the concentrations of drug release and **R^2 = 0.9669**determines the drug release kinetic profile.

Time (min)	% release of drug

0	0
10	32.72
20	49.09
30	65.45
40	76.36
50	92.72

Table 4.4: Data for the dissolution curve of Clonium

4.5 Preparation and method of standard curve of 'Disopan':

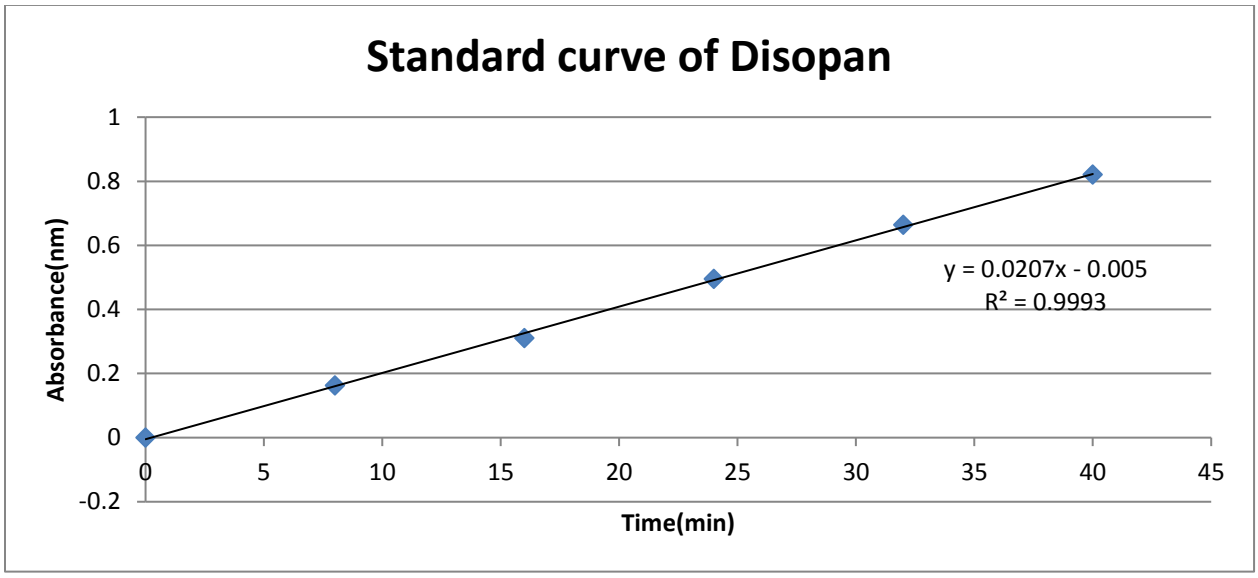


Figure 4.5: Standard curve of Disopan

4 tablets each of 0.5 mg disopan were taken and grinded with the help of mortar and pestle to obtain a powder which was then smudged carefully and transferred into a 50ml volumetric flask. Afterwards, the grinded powder was dissolved slowly with distilled water which was made up to the 50 ml later. Here, the concentration of the stock solution after dissolving 4 tablets of clonazepam each 0.5 mg in 50 ml volumetric flask becomes 40µg/ml. Now, each of the following concentration as prepared along with the detection of their absorbance at wavelength of 273 nm to prepare the standard curve equation keeping the Time along X axis and Absorbance along Y axis. The equation**y = 0.0207x - 0.005** helped to determine the concentration of drug

release as well percent release of that drug &**R² = 0.9993**gave us an idea about the drug release kinetic profile.

Concentration (µg/ml)	Time (min)	Absorbance (nm)
8	10	0.163
16	20	0.310
24	30	0.495
32	40	0.664
40	50	0.821

Table 4.3: Concentrations and absorbance data for preparation of standard curve of Disopan

4.6 Preparation and method of dissolution curve of Disopan:

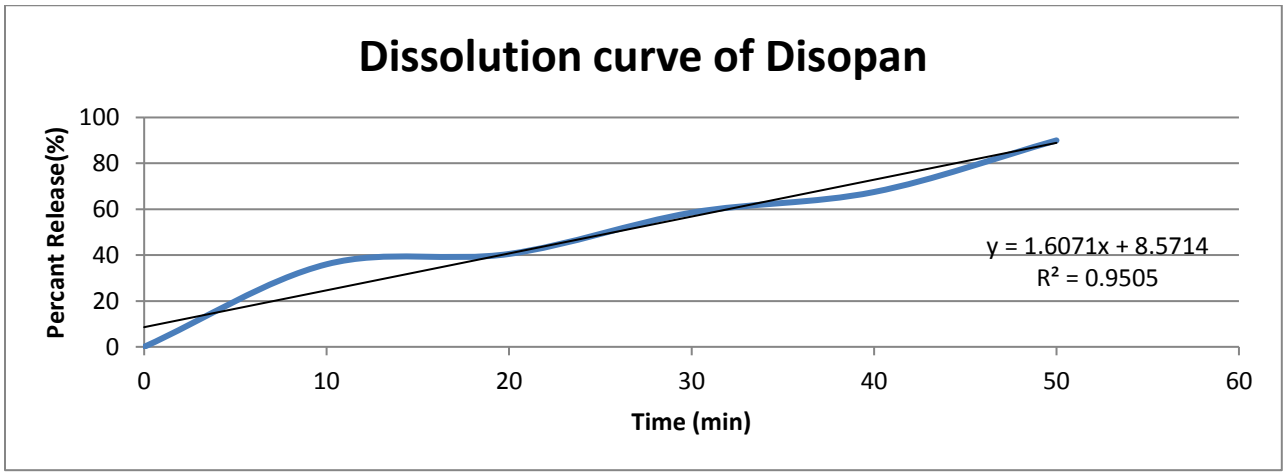


Figure 4.6: Dissolution curve of Disopan

3 tablets of Disopan were taken and they were dissolved at a rpm = 75, temperature=37± 0.5ºC with the distilled/deionized water as dissolution media and the above-mentioned concentrations were prepared for each of the tablets Afterwards, absorbance of each of the prepared concentrations for each of the tablets were measured at a wavelength of 273nm that were recorded. Thus, finally from the average of all the 3 data, a dissolution curve was prepared using

the excel data sheet keeping the % drug release along Y axis and time along X axis. After 10 min and 50 min, the % release was about 36% and 90% respectively. The equation**y = 1.6071x + 8.5714**determined the concentrations of drug release and **R^2 = 0.9505** determines the drug release kinetic profile.

Time (min)	% release of drug
0	0
10	36
20	40.5
30	58.5
40	67.5
50	90

Table 4.6: Data for the dissolution curve of Disopan

4.7 Preparation and method of standard curve of 'Epiclone':

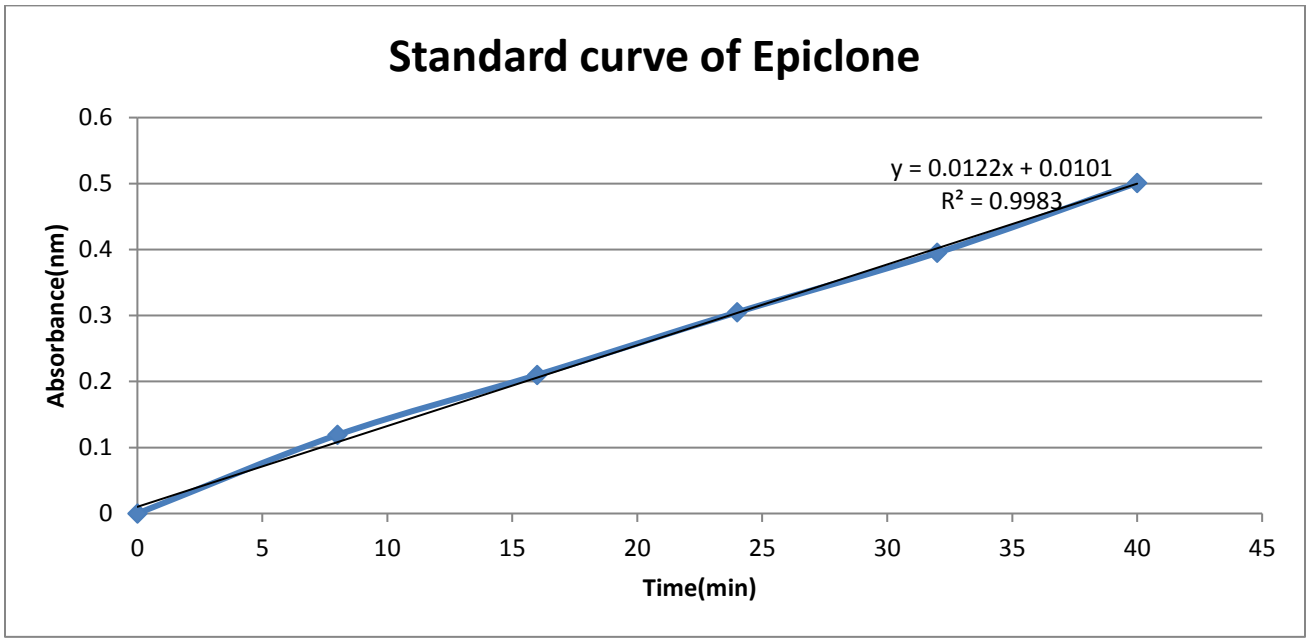


Figure 4.7: Standard curve of Epiclone

4 tablets each of 0.5 mg Epiclone were taken and grinded with the help of mortar and pastle to obtain a powder which was then smudged carefully and transferred into a 50ml volumetric flask. Afterwards, the grinded powder was dissolved slowly with distilled water which was made up to the 50 ml later. Here, the concentration of the stock solution after dissolving 4 tablets of clonazepam each 0.5 mg in 50 ml volumetric flask becomes 40µg/ml. Now, each of the following concentration as prepared along with the detection of their absorbance at wavelength of 273 nm to prepare the standard curve equation keeping the Time along X axis and Absorbance along Y axis. The equation**y = 0.0122x + 0.0101**helped to determine the concentration of drug release as well percent release of that drug &**R^2 = 0.9983**gave us an idea about the drug release kinetic profile.

Concentration (µg/ml)	Time (min)	Absorbance (nm)
8	10	0.119
16	20	0.210
24	30	0.305
32	40	0.395
40	50	0.501

Table 4.7: Concentrations and absorbance data for preparation of standard curve of Epiclon

4.8 Preparation and method of dissolution curve of Epiclone:

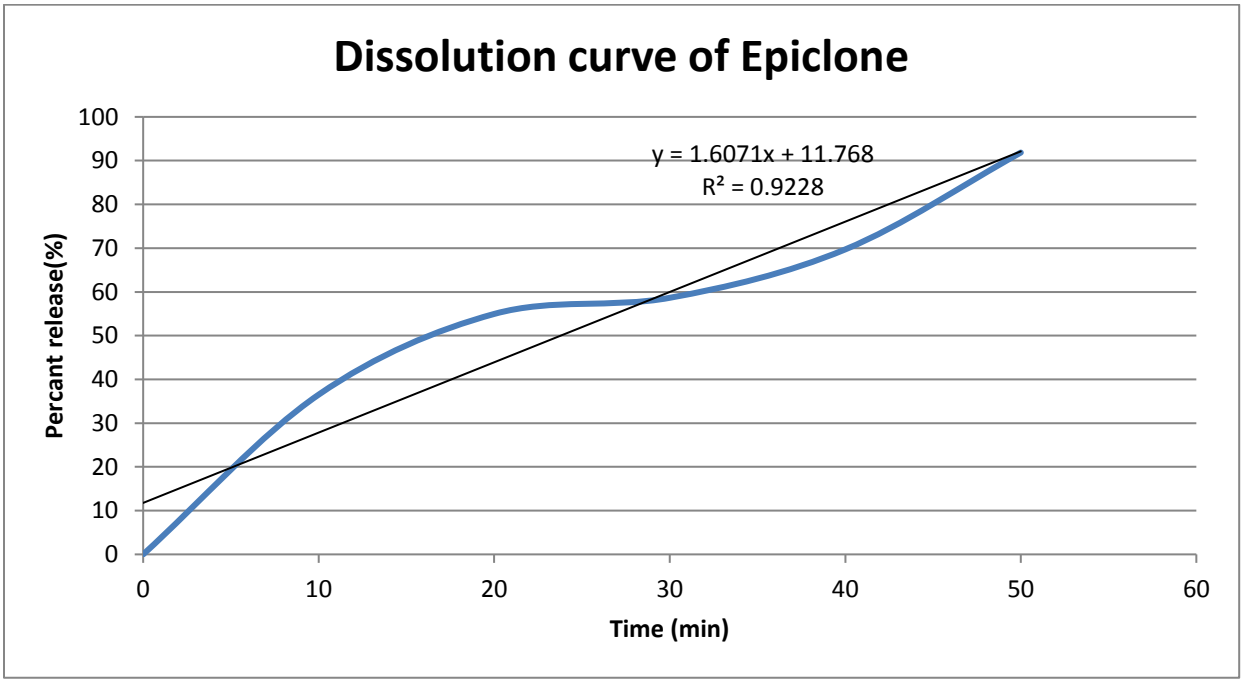


Figure 4.8 : Dissolution curve of Epiclone

3 tablets of Epiclone were taken and they were dissolved at a rpm = 75, temperature=37± 0.5ºC with the distilled/deionized water as dissolution media and the above-mentioned concentrations were prepared for each of the tablets Afterwards, absorbance of each of the prepared concentrations for each of the tablets were measured at a wavelength of 273nm that were recorded. Thus, finally from the average of all the 3 data, a dissolution curve was prepared using

the excel data sheet keeping the % drug release along Y axis and time along X axis. After 10 min and 50 min, the % release was about 36.51% and 69.71% respectively.The equation **y = 1.6071x + 11.768** determined the concentrations of drug release and **R^2 = 0.9228** determines the drug release kinetic profile.

Time (min)	% release of drug
0	0
10	36.51
20	54.95
30	58.64
40	69.71
50	91.84

Table 4.8: Data for the dissolution curve of Epiclone

4.9 Preparation and method of standard curve of 'Rivotril':

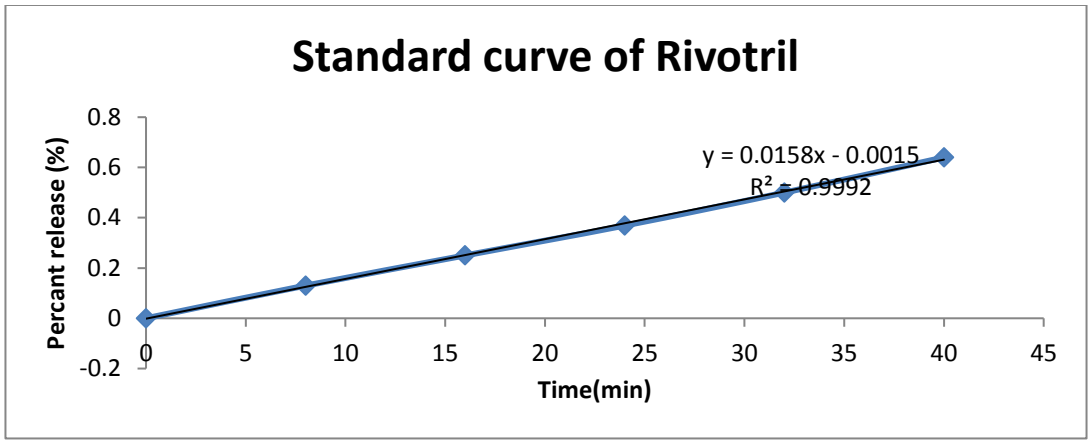


Figure 4.9: Standard curve of Rivotril

4 tablets each of 0.5 mg Rivotril were taken and grinded with the help of mortar and pastle to obtain a powder which was then smudged carefully and transferred into a 50ml volumetric flask. Afterwards, the grinded powder was dissolved slowly with distilled water which was made up to the 50 ml later. Here, the concentration of the stock solution after dissolving 4 tablets of clonazepam each 0.5 mg in 50 ml volumetric flask becomes 40µg/ml. Now, each of the

following concentration as prepared along with the detection of their absorbance at wavelength of 273 nm to prepare the standard curve equation keeping the Time along X axis and Absorbance along Y axis. The equation**y = 0.0158x - 0.0015**helped to determine the concentration of drug release as well percent release of that drug &**R^2 = 0.9992**gave us an idea about the drug release kinetic profile.

Concentration (µg/ml)	Time (min)	Absorbance (nm)
8	10	0.13
16	20	0.25
24	30	0.369
32	40	0.5
40	50	0.64

Table 4.9: Concentrations and absorbance data for preparation of standard curve of Rivotril

4.10 Preparation and method of dissolution curve of Rivotril:

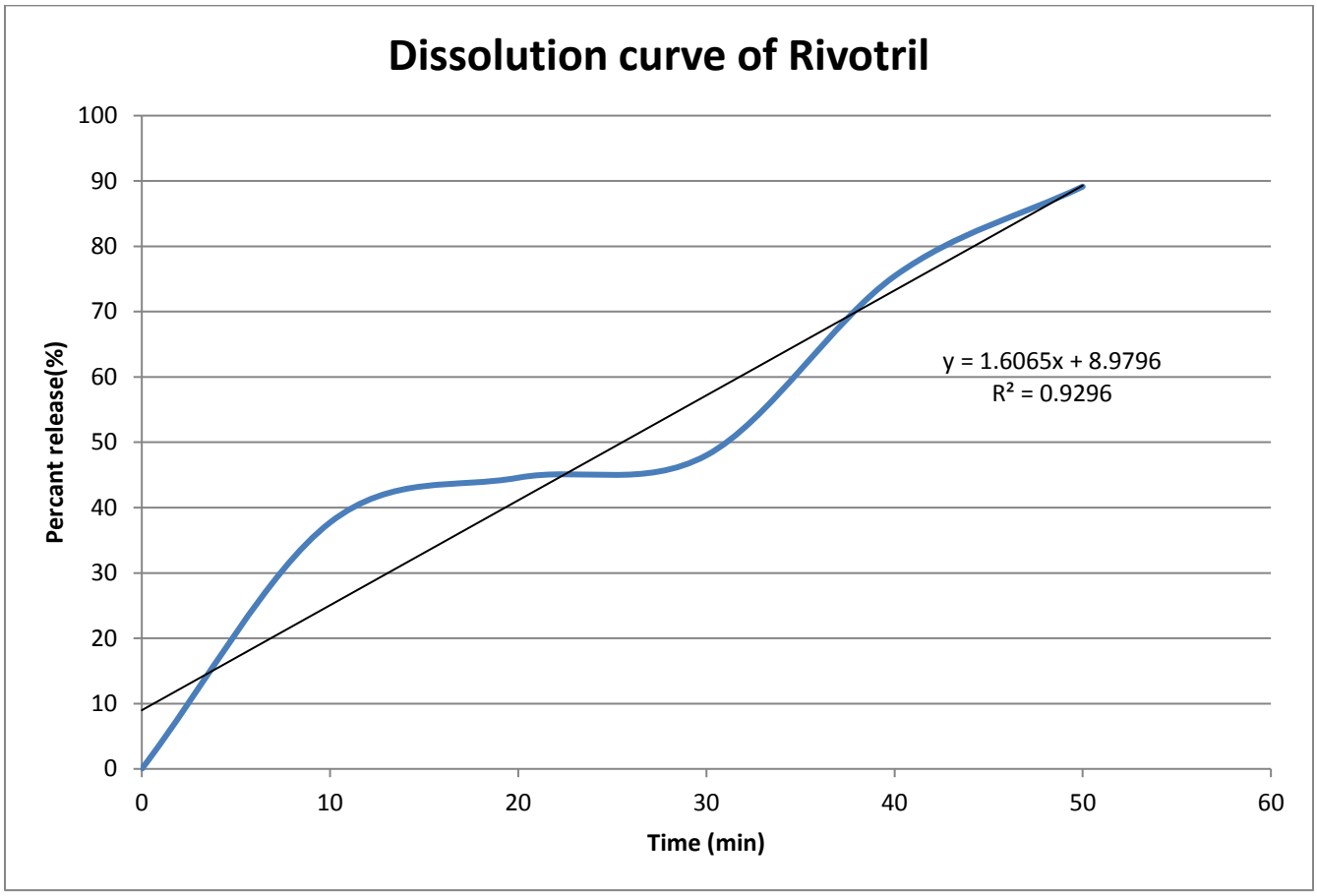


Figure 4.10: Dissolution curve of Rivotril

3 tablets of Rivotril were taken and they were dissolved at a rpm = 75, temperature=37± 0.5ºC with the distilled/deionized water as dissolution media and the above-mentioned concentrations were prepared for each of the tablets Afterwards, absorbance of each of the prepared concentrations for each of the tablets were measured at a wavelength of 273nm that were recorded. Thus, finally from the average of all the 3 data, a dissolution curve was prepared using the excel data sheet keeping the % drug release along Y axis and time along X axis. After 10 min and 50 min, the % release was about 37.71% and 89.14% respectively. The equation **y = 1.6065x + 8.9796** determined the concentrations of drug release and **R^2 = 0.9296** determines the drug release kinetic profile

Time (min)	% release of drug
0	0
10	37.71
20	44.57
30	48
40	75.42
50	89.14

Table 4.10: Data for the dissolution curve of Rivotril

4.11 f1 Calculation

Difference Factor, *f*1 the difference factor f1 is the average difference between all the points of sampling between two brands e.g. reference brand and one of the two test brands. The equation of *f*1 is given below:

$$f1 = \frac{\sum_{t=1}^{n} |Rt - Tt|}{\sum_{t=1}^{n} Rt} x\ 100$$

Rt is the percentage of drug release from the reference drug product and it is the percentage of drug release from the test drug product at t time. Acceptable range of *f*1 is between 0-15. *f*1 value greater than 15 means significant difference between two brands which is not accepted (Lokhandwala et al. 2013; Parakh and Patil 2014; Patel et al. 2015; Qazi et al. 2013).

4.11.1 f1 Calculation for Epiclone

Time (Minutes)	EPITRA Drug release (%) (R)	EPICLON Drug release (%) (T)	R-T	| R-T |	*f*1(%)
10	56.57143	36.51639	20.06	20.06	
20	69.42857	54.95902	14.47	14.47	
30	72	58.64754	13.36	13.36	12.52
40	82.28571	69.71311	12.57	12.57	
50	92.57143	91.84426	0.73	0.73	
Total	488.57			61.19	

Table 4.11: f1 calculation for Epiclone

4.11.2 f1 Calculation for Rivotril

Time (Minuets)	EPITRA Drug release (%) (R)	RIVOTRIL Drug release (%) (T)	R-T	|R-T |	*f*1(%)
10	56.57143	37.71429	18.86	18.86	
20	69.42857	44.57143	24.85	24.85	
30	72	48	24	24	15.96
40	82.28571	75.42857	6.86	6.86	
50	92.57143	89.14286	3.43	3.43	
Total	488.57			78	

Table 4.12: *f*1 calculation for Rivotril

4.11.3 f1 calculation for Disopan

Time (Minutes)	EPITRA (R)	DISOPAN (T)	R-T	| R-T |	*f*1(%)

10	56.57143	36	20.57	20.57	
20	69.42857	40.5	28.92	28.92	
30	72	58.5	13.5	13.5	16.38
40	82.28571	67.5	14.78	14.78	
50	92.57143	90	2.57	2.57	
Total	488.57			80.34	

Table 4.13: f1 calculation for Disopan

4.11.4 f1 calculation for Clonium

Time (Minuets)	EPITRA Drug release (%) (R)	CLONIUM Drug release (%) (T)	R-T	|R-T |	*f*1(%)
10	56.57143	32.72727	23.85	23.85	
20	69.42857	49.09091	20.33	20.33	
30	72	65.45455	6.55	6.55	14.53
40	82.28571	76.36364	5.92	5.92	
50	92.57143	92.72727	-13.98	13.98	
Total	488.57			70.63	

Table 4.14: f1 calculation for clonium

Here the values of f1 for epiclone and clonium are within the range means it is below the 15. Both of the brands can be accepted as well manufactured. On the other hand, Disopan and Rivotril were out of this range due to instrumental errors while manufacturing.

F2 calculation:

4.12.1 f2 calculation for Epiclone

Time (Minutes)	EPITRA Drug release (%) (R)	EPICLON Drug release (%) (T)	R-T	| R-T |	$\|R-T\|^2$	*f*2

10	56.57143	36.51639	20.06	20.06	402.40	
20	69.42857	54.95902	14.47	14.47	209.38	
30	72	58.64754	13.36	13.36	178.48	43
40	82.28571	69.71311	12.57	12.57	158	
50	92.57143	91.84426	0.73	0.73	0.54	
Total	488.57			61.19	948.8	

Table 4.15 : f2Calculation for Epiclone

4.12.2 f2 calculation for Rivotril

Time	EPITRA Drug release (%) (R)	RIVOTRIL Drug release (%) (T)	R-T	|R-T |	$\|R-T\|^2$	*f*2
10	56.57143	37.71429	18.86	18.86	355.69	
20	69.42857	44.57143	24.85	24.85	617.52	
30	72	48	24	24	576	37.28
40	82.28571	75.42857	6.86	6.86	47.05	
50	92.57143	89.14286	3.43	3.43	11.76	
Total	488.57			78	1608.94	

Table4.16: f2 calculation for Rivotril

4.12.3 f2 calculation for Disopan

Time (Minutes)	EPITRA Drug release (%) (R)	DISOPAN Drug release (%) (T)	R-T	| R-T |	$\|R-T\|^2$	*f*2
10	56.57143	36	20.57	20.57	432.12	
20	69.42857	40.5	28.92	28.92	836.36	
30	72	58.5	13.5	13.5	182.25	36.84
40	82.28571	67.5	14.78	14.78	218.44	
50	92.57143	90	2.57	2.57	6.6	
Total	488.57			80.34	1675.07	

Table 4.17: f2 calculation for Disopan

4.12.4 f2 calculation for Clonium

Time	EPITRA Drug release (%) (R)	CLONIUM Drug release (%) (T)	R-T	|R-T |	|R-T |^2	*f*2
10	56.57143	32.72727	23.85	23.85	568.82	
20	69.42857	49.09091	20.33	20.33	413.30	
30	72	65.45455	6.55	6.55	42.90	39.96
40	82.28571	76.36364	5.92	5.92	35.04	
50	92.57143	92.72727	-13.98	13.98	195.44	
Total	488.57			70.63	1255.5	

Table 4.18 : f2 calculation for Clonium

Here, none of the brands met the required range which is 50-100% for the calculation of *f2*. So, this problem arose due to the manufacturing problem or instrumental error while manufacturing the tablets. As a result, these values of *f2* cannot be accepted.

4.13 General Discussion:

In this study, comparisons of dissolution profiles of Clonazepam tablets oral formulations were made between four generic products namely Clonium, Epiclone, Disopan, Rivotril with Epitra. Comparison of the dissolution profiles was carried out by calculation of the similarity factor and difference factor. The criteria for similarity were taken as *f1*= (0 -15) an *f*2 value of 50 - 100 for the tablets. The study was carried out at pH 7 normal range and with the media water and then it was calculated for the values of factors. It was ran for 50 minutes with the intervals of 10 minutes and found the results provided previous discussion. The influence of pH was ignored in this study. The extreme variations in the API release profiles for Clonazepam tablets reflect differences in the quality of manufacturing. This could be due to differences in the source and quality of coating, formulation factors like the coating process, relative composition of the content of the polymers and other excipients. According to the result, Though Epitra has the value approved by the FDA which is a standard one but Rivotril and Disopan is more than the desired value. According to the FDA approval rule Clonium and Epiclone has the legal value in *f*1 calculation. On the other hand, while calculating *f2,* none of the four tablets that is Clonium, Epiclone, Rivotril and Disopan did not meet the desired value *f2*=(50-100) which clearly indicated that these values of difference factors cannot be accepted at all and the manufacturing tech facilities needs to be well calibrated for accurate measurement.

Generally, the similarity factor patterns observed in this study indicate that assay and single point dissolution tests are not sufficient to prove efficacy or pharmaceutical equivalence of the products tested. Lack of comparative dissolution data for pharmaceutical equivalence and subsequently, bioequivalence raises questions of product quality. These have impacts on efficacy of the products raising further concerns about the effect of sub-therapeutic outcomes and repercussions of treatment failures especially for Clonazepam tablets.

Chapter Five

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