Comparative Dissolution Study of Four Different Brands of Clonazepam Tablets (Cloron, Denixil,Pase, Epnil) With Xetril

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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Research Supervisor

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Declaration by the Candidate

I am, Kazi Shoriful Hasan, hereby declare that the dissertation entitled ***"Comparative Dissolution Study of Four Different Brands of Clonazepam Tablets (Cloron, Denixil, Pase, Epnil) With Xetril"*** 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 (Cloron, Denixil, Pase, Epnil) With Xetril"** 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 Kazi Shoriful Hasan, ID: 2013-3-70-050, 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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Endorsement by the Chairperson

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Dedication

This research paper is dedicated to my beloved elder brother (Kazi Zakir Hasan) 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 Xetril belongs from BeximcoPharmaceuticals Ltd. Four brands namely: Cloron, Denixil, Epnil, Pase 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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Short forms	**Names**
CZP	Clonazepam
BZP	Benzodiazepines
STD curve	Standard curve
Dissol. Curve	Dissolution curve
M/A	Mechanism of action
Sol.	Solution
Prep.	Preparation

CHAPTRER

ONE

1.1 Introduction:

Sedative hypnotics that reversibly depresses the activity of the central nervous system, used chiefly to induce sleep and to allay anxiety. These are used in the treatment of insomnia, acute convulsive conditions, and anxiety states and in facilitation of the induction of anesthesia. Although sedative-hypnotics have a soporific effect, they may interfere with rapid eye movement sleep associated with dreaming. 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.

A major subgroup is the benzodiazepines, but other subgroups, including barbiturates and miscellaneous agents.

- Benzodiazepines (e.g.,diazepam, midazolam, clonazepam)
- Barbiturates (amobarbital, pentobarbital, thiopental)
- Miscellaneous agents (e.g. paraldehyde, meprobamate, ethchlorvynol)

("Sedative Hypnotics Drugs". *Pharmacology2000.com*)

Sedatives: A drug that calms a patient, easing agitation and permitting sleep. Sedatives generally work by modulating signals within the central nervous system. (e.g Clonazepam).

Hypnotics: A soporific drug that resembles natural sleep also induces drowsiness. (e.g Chlordiazepoxide)

Anxiolytics: Anxiolytic means preventing or lessening anxiety, and usually refers to any anti-anxiety drug. (e.g Alprazolam)

The term *sedative* describes drugs that serve to calm or relieve anxiety, the term *hypnotic* generally describes drugs whose main purpose is to initiate, sustain, or lengthen

sleep. Because these two functions frequently overlap, and because drugs in this class generally produce dose-dependent effects they are often referred to collectively as **sedative-hypnotic** drugs.

(Staff, Addiction, 2007)

Fig 1.1. A short overview on the classification of sedative-hypnotic drugs

("Classification Of Benzodiazepine Drugs.Com - Google Search". *Google.com*)

1.2 Benzodiazepines: The benzodiazepines are frequently classified into three groups:

(1) short-acting

(2) intermediate-acting

(3) long-acting.

Table 1.1. Classification of benzodiazepines

Duration of action	Names
Short Acting	Midazolam, Triazolam
Immediate acting	Alprazolam, estazolam, Lorazeam, Temazepam
Long acting	Clonasepam, Diazepam, Flurazepam, Clorazepate

(White, H.S., Brown, S.D, 2012)

1.3 Benzodiazepines:

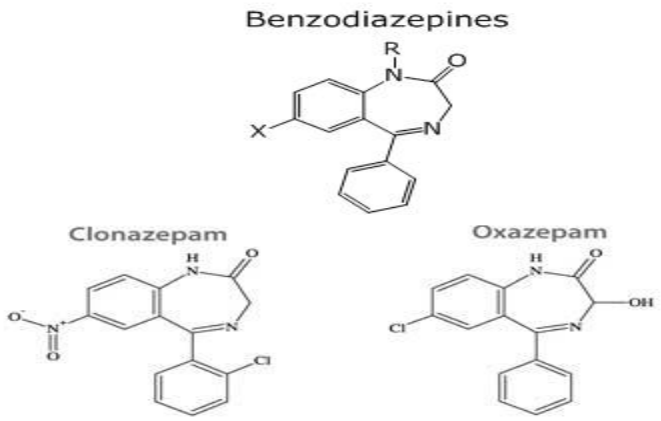


Fig 1.2 General structure of benzodiazepines and its analogues

(Action Of Benzodiazepines, 2012)

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 pharmacodynamic 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.

("Extracellular Side Of Gaba Receptor - Google Search". *Google.com*)

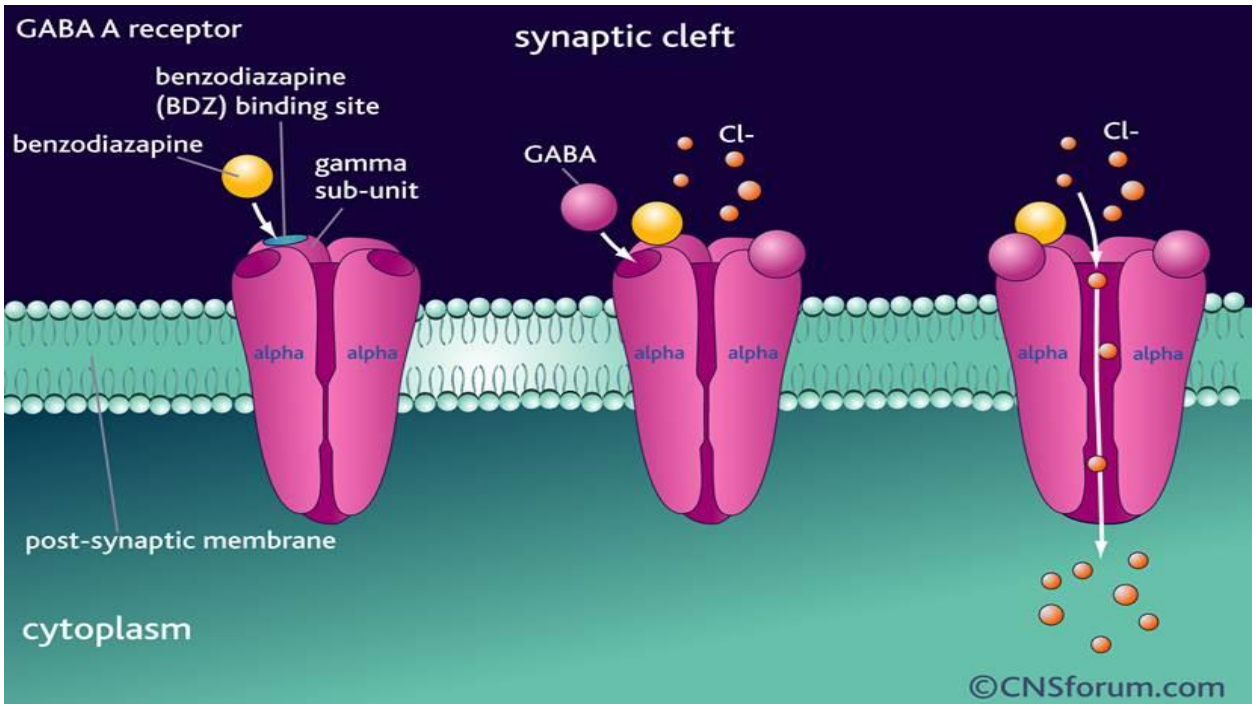


Fig1.3 Mechanism of action of Benzodiazepines and their analogues.

("Action Of Benzodiazepines - Google Search". *Google.com*)

The molecular site of action for the benzodiazepines is at the **GABA receptors** in the CNS. GABA, or **gamma-aminobutyric 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 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 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 receptor, then benzodiazepine binding has no effect on chlorine ion influx.

("Extracellular Side Of Gaba Receptor - Google Search". *Google.com*)

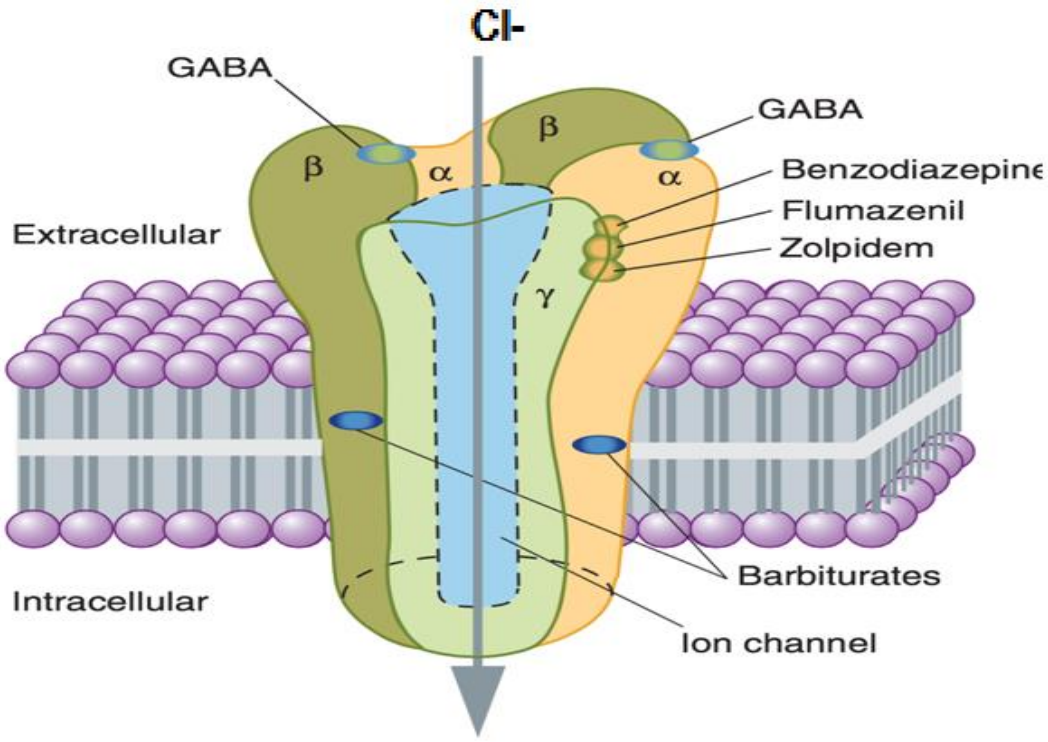


Fig1.4 Action of Benzodiazepine antagonist (Flumazenil)

1.4. Flumazenil: It is one of several 1,4- benzodiazepine derivatives with a high affinity for the benzodiazepine binding site on the GABA-A receptor that act as competitive antagonists. It blocks many of the actions of benzodiazepines, zolpidem, zaleplon, and eszopiclone, but does not antagonize the central nervous system effects of other sedative- hypnotics, ethanol, opioids, or general anesthetics. Flumazenil is approved for use in reversing the central nervous system depressant effects of benzodiazepine overdose and to hasten recovery following use of these drugs in anesthetic and diagnostic procedures.

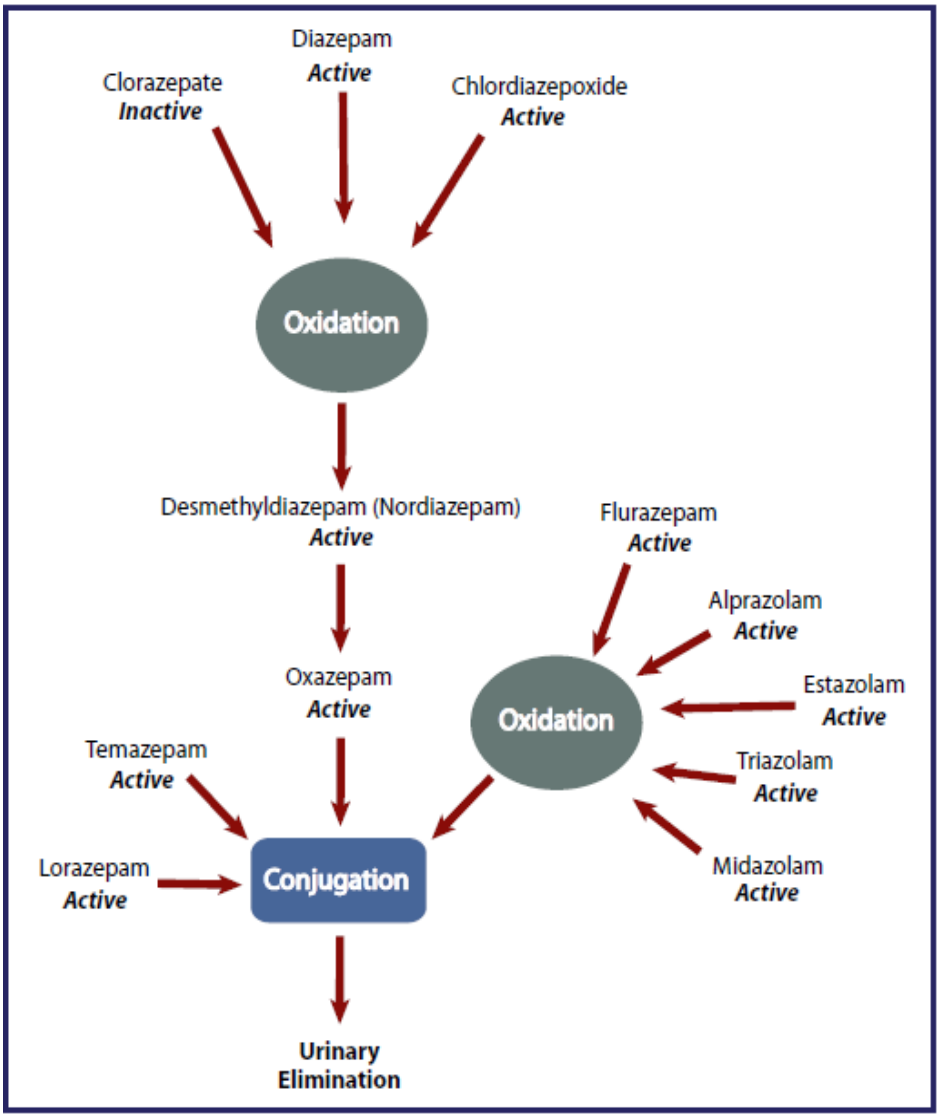


Fig 1.5. Fate of Benzodiazepines. (Spier, S.A., Tesar, G.E. 2006)

Most of the benzodiazepines undergo both oxidative metabolism (phase 1 metabolism) and conjugation to glucuronic acid, or glucuronidation (phase 2 metabolism). some benzodiazepines do notundergo significant oxidative metabolism (temazepam, oxazepam, lorazepam); there may be some benefit to using these agents in patients with liver disease or compromised hepatic function, as the majority of oxidative metabolism occurs in the liver.

1.5. Clinical uses of sedative-hypnotics:

Sedative-hypnotic agents are used to treat a variety of conditions. These include:

a. **Relief of Anxiety**. Sedative-hypnotics are effectively used to temporarily relieve anxiety associated with threatening or fearful situations (for example, anxiety that typically occurs before a surgical procedure).

b. **Treatment of Depression**. Depression is the most common manifestation of anxiety. Treatment of depression with sedative-hypnotic agents may be effective. It should be noted that major (psychotic) depressions might be intensified with sedative-hypnotics.

c. **Induction of Sleep (Hypnosis)**. Short-acting sedative-hypnotics are generally used because of less hangover or persistent effects. When used to produce sleep, sedative-hypnotics should not be administered continuously and should only be part of an overall plan of management and counseling.

d. **Anticonvulsant Therapy**. Some sedative-hypnotics (for example, phenobarbital) have been successfully used in the treatment of various types of convulsive disorders.

e. **Skeletal Muscle Relaxation**. Some sedative-hypnotics have been used to

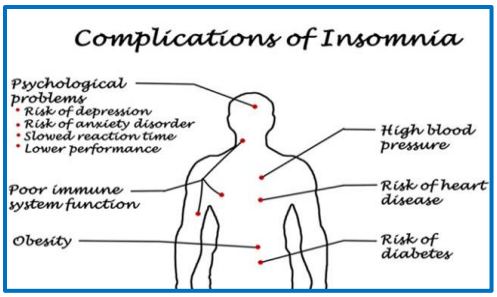
produce muscle relaxation in patients. However, the effectiveness of sedative-hypnotics for this use may be related more to their sedative properties than to their ability to produce true muscle relaxation.

f. **Anesthesia**. The ultra short-acting barbiturates (for example, thiopental) are used for surgical procedures of short duration.

(Integrated Publishing, Inc. "Clinical Uses Of Sedative-Hypnotic Agents - Pharmacology I". *Armymedical.tpub.com*)

1.6.Clinical Toxicology of Sedative hypnotics:

1. **Depression of the central nervous system**: drowsiness, diminished motor skills impaired judgment.
2. **Dose-related anterograde amnesia**: they can significantly impair ability to learn new information.
3. **Dependence:** may occur at usual doses taken beyond several weeks**.**
4. **Withdrawal:** may occur even when discontinuation is not abrupt (e.g., by 10% every 3 days). Symptoms include: tachycardia, increased blood pressure, muscle cramps, anxiety, insomnia, panic attacks, impairment of memory and concentration, perceptual disturbances, derealization, hallucinations, hyperpyrexia, seizures. May continue for months.
5. **Rebound anxiety:** return of target symptoms, with increase intensity.
6. **Respiratory or Cardiovascular depression.**



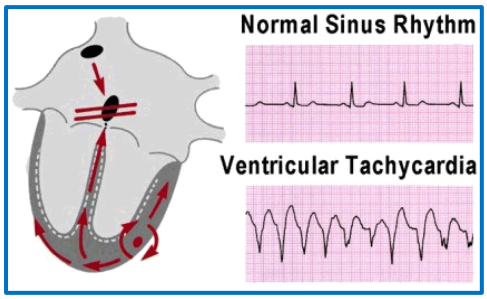


Fig1.6. Insomniac complications

Fig1.7. Ventricular tachycardia

(Integrated Publishing, Inc. "Clinical Uses Of Sedative-Hypnotic Agents - Pharmacology I". *Armymedical.tpub.com*)

1.7. Dissolution:

Dissolution is the process by which a substance forms a solution in a solvent. For the dissolution of solids, the process of dissolution can be explained as the breakdown of the crystal lattice into individual ions, atoms or molecules and their transport into the solvent.

The outcome of the process of dissolution (the amount dissolved at equilibrium, i.e., the solubility) is governed by the thermodynamic energies involved, such as the heat of solution and entropy of solution, but the dissolution itself (a kinetic process) is not. Overall the free energy must be negative for net dissolution to occur. In turn, those energies are controlled by the way in which different chemical bond types interact with those in the solvent.

The graph below shows the dissolution of 5.6 mg of dipyridamole powder in 45 mL of USP SIF buffer solution at pH 6.6. The experiment was done on the SiriusT3 instrument. Each blue point represents the weight of dipyridamole (pK_a 6.25) in solution at a specific time. The black curve fitted to the points represents a first order exponential equation that relates the weight dissolved with the extrapolated solubility and the dissolution rate constant. Very little sample is still dissolving after 30 minutes.
The weight in solution at that time, divided by the volume is equivalent to the extrapolated solubility at that pH. The value determined, 5.3 µg/mL is similar to the value interpolated from a CheqSol measurement of dipyridamole solubility vs. pH.

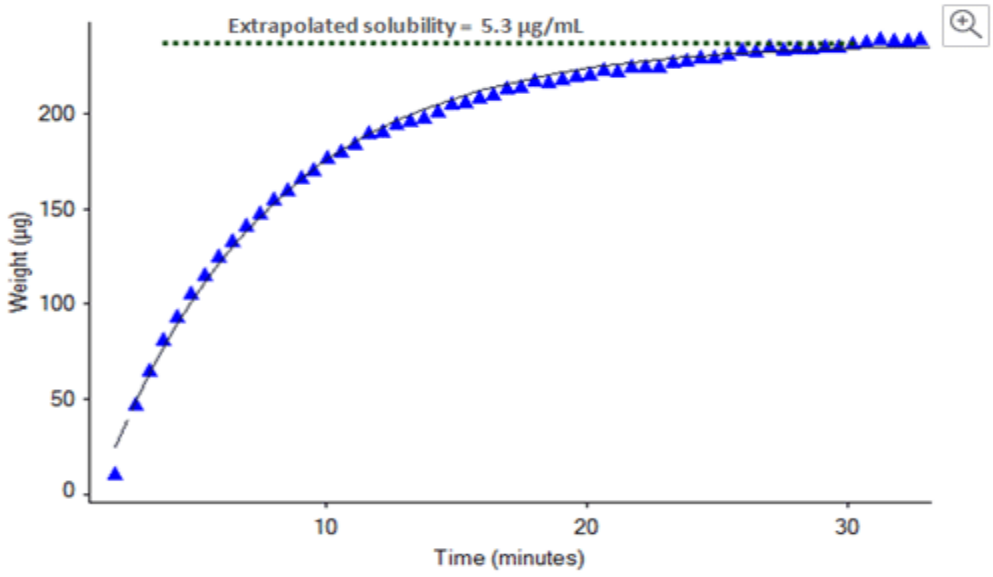


Fig 1.8. time vs weight of drug dissolved curve.

("Dissolution Definitions". *Sirius-analytical.com*)

The processes involved in dissolution of solid dosage forms:

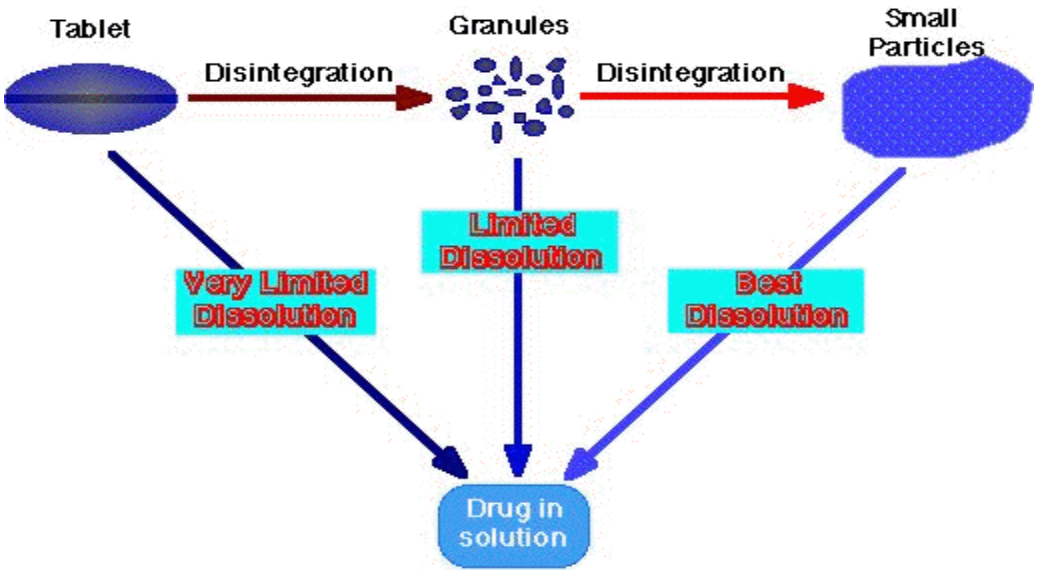


Fig 1.9. Dissolution process of solid dosage forms

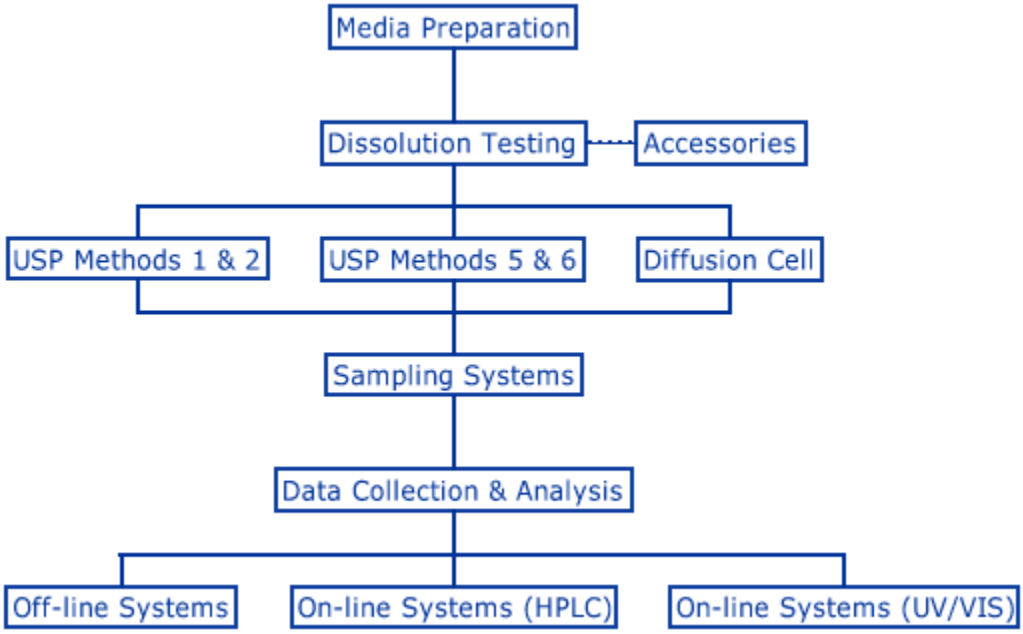


***Fig 1.10* Stages in the dissolution testing process**

("Process Of Solid Dosage Form - Google Search". *Google.com*)

1.8. What is Tablet Dissolution?

The administration of drugs via oral dosage forms is one of the most common and effective means of delivering treatments to patients. When a dosage form is swallowed, the rate at which it releases the active ingredient is critical to ensure that the drug is delivered properly. The rate at which the drug is released is called the dissolution rate.

In fact, all drug forms have a dissolution rate. Creams, skin patches and implants and others, all release their drugs so they can be taken up by the body.

One of the problems facing pharmaceutical manufacturers is to how optimise the amount of drug available to the body, i.e. its **bioavailability**. Inadequacies in bioavailability can mean that the treatment is ineffective and at worst potentially dangerous (toxic overdose). All kinds of factors affect this from the formulation of the dosage form, size, shape, excipients, bindings and other physical characteristics, to the pH, temperature and so on.

The actual drug release in the human body can be measured *in-vivo* by measuring the plasma or urine concentrations in the patient. However, there are certain obvious impracticalities involved in employing such techniques on a routine basis. These difficulties have led to the introduction of official *in-vitro* tests which are now rigorously and comprehensively defined in the respective Pharmacopoeia and recent harmonisation between the various Pharmacopoeia (notably the USP, BP, EP and JP) has lead to global standardisation in the measurement of drug release rates.

The principle function of the dissolution test may be summarised as follows:

- Optimisation of therapeutic effectiveness during product development and stability assessment.
- Routine assessment of production quality to ensure uniformity between production lots.
- Assessment of 'bioequivalence', that is to say, production of the same biological availability from discrete batches of products from one or different manufacturers.
- Prediction of *in-vivo* availability, i.e. bioavailability (where applicable).

Dissolution testing was initially developed for oral dosage forms, but the role of the test has now been extended to drug release studies on various other forms such as topical and transdermal systems and suppositories.

("About Dissolution Testing - What Is Dissolution?". *Labhut*2017)

1.9. Importance of dissolution:

1. Product development

- ✓ Important tool during development of dosage form.
- ✓ Aids in guiding the selection of prototype formulations and for determining optimum levels of ingredients to achieve drug release profiles, particularly for extended release formulations.
- ✓ Also guides in selection of a "market-image" product to be used in pivotal in-vivo bioavailability or bioequivalence studies.

2. Quality assurance

- ✓ performed on future production lots and is used to assess the lot-to-lot performance characteristics of drug product and provide continued assurance of product integrity/similarity.

3. Product stability

- ✓ In-vitro dissolution also used to assess drug product quality with respect to stability and shelf life.
- ✓ As product age, physicochemical changes to the dosage form may alter dissolution characteristics of drug product over time. For some products, polymorph transformations to more stable, and hence less soluble crystalline forms may result in reduced dissolution rates.

4. Comparability assessment

- ✓ It is also useful for assessing the impact of pre- or post- approval changes to drug product such as changes to formulation or manufacturing process. Thus, in-vitro comparability assessment is critical to ensure continued performance equivalency and product similarity.

5. Waivers of in-vivo bioequivalence requirements

- ✓ In-vitro dissolution testing or drug release testing may be used for seeking waiver of required product to conduct in-vivo bioavailability or bioequivalence studies.

("About Dissolution Testing - What Is Dissolution?". *Labhut.com*)

1.10. Drug release kinetics:

"It is a process by which a drug leaves a drug product and is subjected to ADME and eventually becoming available for pharmacological action." It involves the study of drug release rate ,dissolution /diffusion/erosion studies factors affecting release rate of drug. Drug release kinetics is application of mathematical models to drug release process.

("Definition Of Drug Release Kinetics - Google Search". *Google.com*)

1.11. Equation for Zero order kinetics:

$\boldsymbol{r = k}$; where, **r** is the reaction rate and **k** is the reaction rate coefficient with units of concentration or time.

The differential form of the rate law of zero order reaction is,

$\boldsymbol{r = -\frac{d[A]}{dT} = k;}$ **[A]** is the concentration of the chemical of interest.

1.12. Equation for first order kinetics:

The differential form of the rate law of first order reaction is,

$\boldsymbol{\frac{-dA}{dT} = r = k[A]}$; where k is the first order rate constant

The equation for half-life,

$\boldsymbol{t_{\frac{1}{2}} = \frac{ln2}{k}}$; where, $\mathbf{t_{1/2}}$is the plasma half-life of the drug.

1.13. Equation for second order kinetics:

The differential form of the rate law of second order reaction is,

$$\boldsymbol{\frac{-dA}{dt} = 2r = 2k[A]^2}$$

1.14. Equation for plasma half-life:

$$\boldsymbol{t = \frac{1}{k_{[A]}}}$$

1.15. Higuchi equation for drug release:

$\boldsymbol{Q = [D(2A - C_s)C_s t]^{1/2}}$

$\boldsymbol{\frac{dQ}{dt} = \frac{1}{2}[\frac{D(2A-C_s)C_s}{t}]^{1/2}}$

Where, **Q** is the amount of drug release in time t per unit area

$\frac{dQ}{dt}$is the rate of drug release per unit area

A is the total amount of drug in a unit volume of matrix/initial drug concentration

$C_s$is the saturation concentration solubility of the drug in the matrix

D is the diffusion coefficient of the drug in the matrix

1.16. Korsmeyer Equation for drug release:

$$F = \left(\frac{M_t}{M}\right) = k_m t^n$$

where, **F** is the fraction of drug release at time t

$M_t$is the amount of drug release at time t

M is the total amount of drug in dosage form

$k_m$is kinetic constant

n is diffusion or release exponent

t is time in hours

1.17. Hixson – crowell release equation:

The Hixson - Crowell release equation is

$\sqrt[3]{Qo - \sqrt[3]{Qt}} = K_{HC}.t$

Q_0 = Initial amount of drug. Q_t = Cumulative amount of drug release at time t. K_{HC} = Hixson Crowell release constant. t = Time in hours.

("Drug Release Kinetics". *Slideshare.net*)

1.18. Clonazepam Overview:

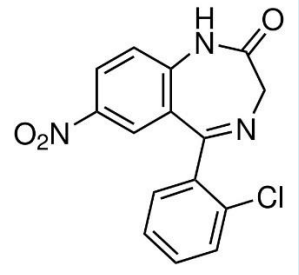
Clonazepam is a benzodiazepine. It affects chemicals in the brain that may be unbalanced. Clonazepam is also a seizure medicine, also called an anti-epileptic drug. It is used to treat certain seizure disorders (including absence seizures or Lennox-Gastaut syndrome) in adults and children. It is also used to treat panic disorder (including agoraphobia) in adults.

("Clonazepam: Drug Uses, Dosage, Side Effects - Drugs.Com". *Drugs.com*)

1.19. Product Information:

Route of administration	Dosage form/strength	Non-medical ingredients
Oral	Tablet 0.5mg	Cornstarch, iron oxide red, iron oxide yellow, lactose, magnesium stearate, potato starch and talc
Oral	Tablet 2 mg	Cornstarch, lactose, magnesium stearate and microcrystalline cellulose

1.20. Pharmaceutical Information:

Topic	Information
Generic Name	Clonazepam
Chemical Name	5-(2-chlorophenyl)-1, 3-dihydro-7-nitro-2H-1, 4-benzodiazepin-2-one.
Molecular Formula	$C_{15}H_{10}ClN_3O_3$
Molecular Mass	315.7
Physiochemical Properties	Clonazepam is a white to yellow-white odourless fine powder. The pH of clonazepam is between 5.0 and 7.0 in 1% aqueous
Composition	Each tablet contains either 0.5 mg or 2.0 mg clonazepam.
Structure	

1.21. Contraindications:

Porphyria, Having Thoughts of Suicide, Alcohol Intoxication, Misuse or Excessive Use of Drugs, Depression, Wide-Angle Glaucoma, closed angle glaucoma, Decreased Lung Function, Chronic Lung Disease, Liver Problems, Severe Liver Disease, Kidney Disease, Feeling Faint, Temporarily Stops Breathing While Sleeping, Abnormal Liver Function Tests, Abnormal Nervous System Function Affecting Mental Alertness, Susceptible to Breathing Fluid Into Lungs, Pregnancy.

("Contraindications For Clonazepam". *WebMD*)

1.22. Major side effects:

- Body aches or pain
- chills
- cough
- difficulty breathing
- discouragement
- dizziness
- ear congestion
- feeling sad or empty
- fever
- headache
- irritability
- lack of appetite
- loss of interest or pleasure
- sleepiness or unusual drowsiness
- sore throat
- tiredness
- trouble concentrating
- trouble sleeping

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

1.23. Minor side effects:

- Being forgetful
- bladder pain
- change in speeh
- difficult, burning, or painful urination
- general feeling of discomfort or illness
- joint pain
- loss of appetite
- mood or mental changes
- muscle aches and pains
- nausea
- nervousness
- sore throat
- sweating
- vomiting

1.24. Rare:

- Burning, crawling, itching, numbness, prickling, "pins and needles", or tingling feelings
- changes in skin color
- chest pain or discomfort
- difficulty with sleeping
- dizziness, faintness, or lightheadedness when getting up suddenly from a lying or sitting position
- excessive dreaming
- excessive muscle tone
- fast, irregular, pounding, or racing heartbeat or pulse
- flu-like symptoms

("Clonazepam Side Effects In Detail - Drugs.Com". *Drugs.com*)

1.25. Dosing Information:

Children:In order to minimize drowsiness, the initial dose for infants and children (up to 10 years ofage or 30 kg of body weight) should be between 0.01 and 0.03 mg/kg/day and should not exceed 0.05mg/kg/day given in two or three divided doses. Dosage should be increased by no more than 0.25 to0.50 mg every third day until a maintenance dose of 0.1 to 0.2 mg/kg of body weight has been reached,unless seizures are controlled or side effects preclude further increase.

Adults:The initial dose for adults should not exceed 1.5 mg/day divided into three doses. Dosage maybe increased in increments of 0.5 to 1 mg every three days until seizures are adequately controlled oruntil side effects preclude any further increase. Maintenance dosage must be individualized for eachpatient depending upon response. A recommended maintenance dose for adults is 8 to 10 mg/day inthree divided doses. Dosages in excess of 20 mg/day should be administered with caution.Whenever possible, the daily dose should be divided into three equal doses. If doses are not equallydivided, the larger dose should be given before retiring.

Geriatrics: There is no clinical trial experience with RIVOTRIL in seizure disorder patients 65 yearsof age and older. In general, elderly patients should be started on low doses of RIVOTRIL andobserved closely. (Shirsand, S.B., Suresh, S., 2011)

Indicating the usages of Clonazepam according to indications

Usual Adult Dose of Clonazepam for Seizure Prophylaxis	1.5 mg orally per day divided into 3 doses; this may be increased in increments of 0.5 mg to 1 mg every 3 days until seizures are adequately controlled or until side effects preclude any further increase. Maximum dose: 20 mg orally per day
Usual Adult Dose for Panic Disorder	Initial dose: 0.25 mg orally 2 times per day Maintenance dose: 1 mg orally per day Maximum dose: 4 mg orally per day

Usual Pediatric Dose of Clonazepam for Seizure Prophylaxis	Up to 10 years of age or 30 kg of body weight: 0.01 mg/kg/day to 0.05 mg/kg/day orally administered in 2 or 3 divided doses

1.26. Mechanism of action:

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.

(Jenner P, et al. "Mechanism Of Action Of Clonazepam In Myoclonus In Relation To Effects On GABA And 5-HT. - Pubmed - NCBI". *Ncbi.nlm.nih.gov*)

1.27. Pharmacokinetics of Clonazepam:

1.27.1. Absorption:

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 around25 minutes. The absolute bioavailability is 90%. Plasma concentrations of clonazepam at steady state for a once-daily dosage regimen are 3-fold higherthan those after a single oral dose; the predicted accumulation ratios for two times and three timesdaily regimens are 5 and 7, respectively. Following multiple oral doses of 2 mg three times dailysteady-state pre-dose plasma concentrations of clonazepam averaged 55 ng/ml. The plasmaconcentration-dose relationship of clonazepam is linear. The target anticonvulsant plasmaconcentrations of clonazepam range from 20 to 70 ng/ml.

1.27.2. Distribution:

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

1.27.3 Metabolism:

Clonazepam is extensively metabolized by reduction to 7-amino-clonazepam and by N-acetylation to7-acetamino-clonazepam. Hydroxylation at the C-3 position also occurs. Hepatic cytochrome P-4503A4 is implicated in the nitro reduction of clonazepam to pharmacologically inactive metabolites.The metabolites are present in urine both as free and conjugated (glucuronide and sulphate)compounds.

1.27.4. 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 excretionof unchanged clonazepam is usually less than 2% of the administered dose. The elimination kinetics in children are similar to those observed in adults. (Mura, P., Nassini, C., Proietti, D, 2004)

1.28. Common medications checked in combination with clonazepam:

- ✓ Abilify (aripiprazole)
- ✓ Adderall (amphetamine / dextroamphetamine)
- ✓ Ambien (zolpidem)
- ✓ Aspirin Low Strength (aspirin)
- ✓ Cymbalta (duloxetine)
- ✓ Fish Oil (omega-3 polyunsaturated fatty acids)
- ✓ Lamictal (lamotrigine)
- ✓ Lexapro (escitalopram)
- ✓ Lipitor (atorvastatin)
- ✓ Lyrica (pregabalin)
- ✓ Nexium (esomeprazole)
- ✓ Norco (acetaminophen / hydrocodone)
- ✓ Prozac (fluoxetine)
- ✓ Seroquel (quetiapine)
- ✓ Singulair (montelukast)
- ✓ Synthroid (levothyroxine)
- ✓ Topamax (topiramate)
- ✓ Vitamin B12 (cyanocobalamin)
- ✓ Vitamin D3 (cholecalciferol)
- ✓ Zoloft (sertraline)

("Clonazepam - Drugs. Drug. 7 May 2017. Interactions Com". *Drugs.com*)

1.29. Different brands available 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.
Disopan	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	Ibn Sina Pharmaceuticals Ltd.
Pase	Opsonin Pharma Limited
Rivo	Orion Pharma Ltd.
Rivotril	Radiant Pharmaceutical Ltd.
Xetril	Beximco pharmaceuticals Ltd.
Xioclon	Somatic pharmaceuticals Ltd.

(DIMS, 2017)

CHAPTER

TWO

2.1. Literature Review

This study was performed by Venkateswaran, et, al. 20dec, 2013. Clonazepam is a benzodiazepine indicated for seizure disorder, panic disorder and epilepsy. Patients suffering from seizures will have difficulty in swallowing the tablets or will be reluctant to take the tablets or will spit the administered tablet. In such cases, mouth dissolving dosage forms will be an effective solution for patient compliance and efficient medicine regimen. In the present research, mouth dissolving tablet of Clonazepam was made by aqueous wet granulation process. Pearlitol Flash and Microcrystalline Cellulose were used as diluent. Crospovidone was used as disintegrant. Strawberry Flavor and Aspartame were used as flavoring and sweetening agents. Sodium Lauryl Sulphate was used as a wetting agent. Colloidal Silicon Dioxide was used as glidant. Talc and Magnesium Stearate were used as lubricants. The prepared tablets were evaluated for weight, thickness, hardness, friability, disintegration time and dissolution. Prepared tablets showed disintegration time of less than 30 seconds and drug dissolution of about 75% achieved within 30 minutes. After finalizing the composition with 2 mg strength, using the same composition 0.125 mg, 0.25 mg, 0.5 mg and 1 mg strengths were made. The prepared tablets were stability tested at 40°C / 75% RH for 3 months and were found to be stable. Prepared mouth dissolving tablets of Clonazepam 1 mg was found to be bioequivalent under fasting and fed conditions with the marketed product.

(Venkateswaran,et,al.2013)

Islam, S M AshrafulI et, al. Nov-2011. In this study five marketed brands of aceclofenac 100 mg tablets have been evaluated using dissolution test in two different media with the aim to assess bioequivalence and to select a proper dissolution medium. Other general quality parameters of these tablets like weight variation, hardness, friability, disintegration time were also determined according to established protocols. All the brands complied with the official specification for friability, uniformity of weight, disintegration time and drug content. UV spectroscopic and RP-HPLC methods were validated for the parameters like linearity, accuracy, precision and robustness. Potency was determined by using these two methods. Potency obtained from UV method and HPLC methods were found similar with paired t test. Dissolution

test results were subjected to further analysis by difference factor (f1), similarity factor (f2) and dissolution efficiency (% DE). Higher drug release was found in phosphate buffer pH 6.8 than in 0.05% sodium lauryl sulphate solution. All brands were found similar in respect of drug release in phosphate buffer pH 6.8 but they differ in respect of drug release in 0.5% sodium lauryl sulphate. So phosphate buffer pH 6.8 may be a suitable media for dissolution study of aceclofenac tablets.

(Islam, S M AshrafulI et, al. 2011)

David J.et.al, was done study in November 2005, nine healthy volunteers participated in a 3-phase clinical pharmacokinetic study of the benzodiazepine derivative clonazepam. During phases I and II, subjects received the conventional oral dosage form of clonazepam, 0.5 mg 3 times daily, for 7 days. Multiple plasma samples were drawn on day 1 and day 7 of the trial and once daily during the washout period after the final dose. Based on nonlinear regression, mean kinetic variables for clonazepam were: absorption half-life, 24 minutes; elimination half-life, 40 hours; apparent oral clearance, 72 mL/min. The extent of accumulation at steady state relative to the first day of treatment averaged 3.3-fold, and was consistent with values predicted based on the elimination half-life. This finding suggests that once-daily dosage with clonazepam would be appropriate for many patients. In phase III of the study, subjects received a single 2.7 mg subcutaneous injection of a microsphere formulation of clonazepam, designed to produce a sustained-release profile. The maximum average plasma clonazepam concentration was 3.0 ng/mL, reached at 72 hours after dosage. Thereafter, plasma concentrations fell slowly over the 13-day sampling period, remaining above 1 ng/mL for 12 days. Overall systemic availability of clonazepam from the microsphere injection, relative to the conventional oral dosage form, was 1.05. Thus, the microsphere preparation of injectable clonazepam provides complete absorption from the injection site, with the intended slow-release pharmacokinetic profile.

(David J.et.al,2005)

This study was perfored by Ivana Kacirova, et.al, in 2016 Mar 7. Clonazepam is long-acting benzodiazepine agonist used in short-acting benzodiazepine withdrawal; however, recent observations suggest the existence of its abuse. We demonstrate a 40-year-old man with a 20-year history of psychiatric care with recently benzodiazepine dependence (daily intake of ∼60 mg of clonazepam and 10 mg of alprazolam). High serum levels of both drugs were analyzed 3 weeks before admission to hospitalization (clonazepam 543.9 ng/mL, alprazolam 110 ng/mL) and at the time of admission (clonazepam 286.2 ng/mL, alprazolam 140 ng/mL) without any signs of benzodiazepine intoxication. Gradual withdrawal of clonazepam with monitoring of its serum levels and increase of gabapentin dose were used to minimize physical signs and symptoms of clonazepam withdrawal. Alprazolam was discontinued promptly. Clinical consequences of the treatment were controllable tension, intermittent headache, and rarely insomia. It is the first case report showing utilization of therapeutic drug monitoring during withdrawal period in the patient with extreme toleration to severe benzodiazepine dependence.

(Ivana Kacirova, et.al,2016)

Sheng-Min Wang, and his research members conducted a study in 2016 May 31, This study compared the efficacy and tolerability of clonazepam with other benzodiazepines in patients with anxiety disorders. The present study suggests that clonazepam is as efficacious as other benzodiazepines for the treatment of various anxiety disorders. Furthermore, the safety profile of clonazepam was superior to the other benzodiazepines in this study. Anxiety disorders are chronic, recurrent and serious mental illnesses that result in functional impairment and are associated with significant social costs.

(Sheng-Min Wang2016)

Ivan Miziara, was done a study about clonazepam in 2014 july 9. Topical clonazepam showed good short-term results for the relief of pain, although this was not presented as a definitive cure. Similarly, α-lipoic acid showed good results, but there are few randomized controlled studies that showed the long-term results and complete remission of symptoms. On the other hand, cognitive therapy is reported as a good and lasting therapeutic option with the advantage

of not having side effects, and it can be combined with pharmacologic therapy. treatment, stomatodynia, burning mouth syndrome.

(Ivan Miziara,2014)

André S. and his research members was done a study about clonazepam in 4 july 2014. Long-term sedative use is prevalent and associated with significant morbidity, including adverse events such as falls, cognitive impairment, and sedation. The development of dependence can pose significant challenges when discontinuation is attempted as withdrawal symptoms often develop. We conducted a scoping review to map and characterize the literature and determine opportunities for future research regarding deprescribing strategies for long-term benzodiazepine and Z-drug (zopiclone, zolpidem, and zaleplon) use in community-dwelling adults.

(André S.2014)

The study was done by Carlo Marchesi et, al. in 2008 Feb; 4 Panic disorder (PD) is a disabling condition which appears in late adolescence or early adulthood and affects more frequently women than men. PD is frequently characterized by recurrences and sometimes by a chronic course and, therefore, most patients require long-term treatments to achieve remission, to prevent relapse and to reduce the risks associated with comorbidity. Pharmacotherapy is one of the most effective treatments of PD. In this paper, the pharmacological management of PD is reviewed. Many questions about this effective treatment need to be answered by the clinician and discussed with the patients to improve her/his collaboration to the treatment plan: which is the drug of choice; when does the drug become active; which is the effective dose; how to manage the side effects; how to manage nonresponse; and how long does the treatment last. Moreover, the clinical use of medication in women during pregnancy and breastfeeding or in children and adolescents was reviewed and its risk-benefit balance discussed.

(Carlo Marchesi, 2008)

participated in a 3-phase clinical pharmacokinetic study of the benzodiazepine derivative During phases I and II, subjects received the conventional oral dosage form of clonazepam, 0.5

mg 3 times daily, for 7 days. Multiple plasma samples were drawn on day 1 and day 7 of the trial and This study was performed by David J Greenblatt; et.al at 2005 Nov. Nine healthy volunteers once daily during the washout period after the final dose. Based on nonlinear regression, mean kinetic variables for clonazepam were: absorption half-life, 24 minutes; elimination half-life, 40 hours; apparent oral clearance, 72 mL/min. The extent of accumulation at steady state relative to the first day of treatment averaged 3.3-fold, and was consistent with values predicted based on the elimination half-life. This finding suggests that once-daily dosage with clonazepam would be appropriate for many patients. In phase III of the study, subjects received a single 2.7 mg subcutaneous injection of a microsphere formulation of clonazepam, designed to produce a sustained-release profile. The maximum average plasma clonazepam concentration was 3.0 ng/mL, reached at 72 hours after dosage. Thereafter, plasma concentrations fell slowly over the 13-day sampling period, remaining above 1 ng/mL for 12 days. Overall systemic availability ofclonazepam from the microsphere injection, relative to the conventional oral dosage form, was 1.05. Thus, the microsphere preparation of injectableclonazepam provides complete absorption from the injection site, with the intended slow-release pharmacokinetic profile.

(Greenblatt; et.al,2005)

S. B. Shirsand, . 2011 Sep-Oct; was done study.Fast dissolving tablets of clonazepam were prepared by sublimation method with a view to enhance patient compliance. 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. 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.

(S. B. Shirsand, . 2011)

Ana Carolina de Oliveira Neves and his research members 5 jan, 2012.was done study.This work utilized the near-infrared spectroscopy (NIRS) and multivariate calibration to measure the percentage drug dissolution of clonazepam. Each spectrum was the average of 50 scans obtained in the diffuse reflectance mode. The dissolution test, which was initially carried out in 900 mL of 0.1 N hydrochloric acid at 37 ± 0.5 °C, was used to determine the percentage a drug that dissolved from each tablet measured at the same time interval (45 min) at pH 6.8. The correlation coefficient (R^2) for the HPLC determination versus predicted values (NIRS) ranged from 0.88 to 0.98. The root-mean-square error of prediction (RMSEP) obtained from PLS models were 9.99%, 8.63%, 8.57% and 9.97% for isoniazid, rifampicin, ethambutol and pyrazinamide, respectively, indicating that the NIR method is an effective and non-destructive tool for measurement of drug dissolution from tablets.

(Ana Carolina de Oliveira Neves[a],2012)

Krishna Sanka in April 2014.The liquisolid powder compacts (LSPCs) proved to be the potential solubility improvement strategy for efficient oral delivery of BCS class II and IV drugs. Henceforth, an attempt was made to improve the oral delivery of BCS class II drug clonazepam (CLZ) by formulating into a novel LSPCs. Solubility studies were conducted in different liquid vehicles, namely propylene glycol, span 20 and span 80. The LSPCs were formulated using propylene glycol as non volatile solvent. The effect of different formulation variables on LSPCs performance was evaluated using 3^2 factorial design. LSPCs of CLZ formulated with propylene glycol at optimum drug concentration produced high dissolution profile with acceptable tablet properties. Fourier transform infra-red spectroscopy (FTIR) studies revealed that there was no interaction between drug and polymers, differential scanning calorimetry (DSC) and X-Ray Diffraction (XRD) indicated conversion of crystalline to amorphous form of the CLZ. Further the permeation studies carried out in isolated rat intestine revealed that potential of LSPCs for enhanced permeation of CLZ across rat intestinal barrier. The increase in permeation of

clonazepam from LSPCs formulation across rat intestine suggests the potential of LSPC formulation for improved oral delivery of CLZ.

(Krishna Sanka, 2014)

Ahmed Badr Eldin[a] et,al. was done study in 18 November 2014. Clonazepam contains one benzodiazepine ring in its chemical structure which makes it vulnerable to degradation. In this study, green analytical chemistry approach was applied in attempts for the development of validated stability indicating RP-HPLC method for determining clonazepam and its related substances in pharmaceutical formulation. Validation has been performed according to ICH guidelines. HPLC method allowed good resolution between the peaks that corresponded to the active pharmaceutical ingredients and its degradation products with good linearity, precision, accuracy, specificity, LOD and LOQ. The expanded uncertainty (0.33%) of the method was also estimated from method validation data. This analytical technique is not only ecofriendly but also faster than the conventional liquid chromatographic system official in the USP-36.

(Ahmed Badr Eldin et,al.2014)

P. Mura,et,al.was done study 5 November 2016,Two kinds of mucoadhesive buccal tablets of clonazepam (CLZ) were developed to provide, a prolonged local or systemic delivery respectively. Tablets prepared by direct compression of combinations of different polymers were tested for swelling, erosion and residence time properties. Carbopol 971P/hydroxypropylmethylcellulose and Poloxamer/chitosan mixtures were the best and were selected for drug loading. The effect of CLZ complexation with different cyclodextrins was investigated. Randomly-methylated-βCD (RAMEßCD) was the most effective, allowing 100% drug released increase from local-delivery buccal tablets. In vitro permeation studies from coated-tablets showed that CLZ loading as RAMEßCD-coground enabled a 5-times increase in drug flux and permeability. Therefore, complexation with RAMEßCD was a successful strategy to improve the CLZ performance from buccal tablets for both local or systemic action.

(P. Mura,2016)

MD Amer Khan *1 et,al.4, sep 2013 was done study. Clonazepam is a benzodiazepine drug having anxiolytic, anticonvulsant, muscle relaxant, sedative, and hypnotic properties and is metabolized by CYP3A iso-enzyme. Fluconazole is an antifungal used in the treatment and prevention of superficial and systemic fungal infections and is known to inhibit the CYP 3A iso-enzymes. Pharmacokinetic studies have established that fluconazole inhibits clonazepam metabolism which may lead to toxicity when these two drugs are given concurrently. One needs to be aware that this drug combination predictably causes adverse side effects hence, closely monitoring should be done in patient receiving longterm clonazepam therapy. We report a case of respiratory depression induced by the concurrent administration of clonazepam and fluconazole. Naranjo's causality assessment algorithm was used to assess the adverse effect and it indicated that concurrent use of clonazepam and fluconazole as probable cause of respiratory depression. Although information is available regarding an interaction between clonazepam and fluconazole, there are no large randomized controlled studies reporting this interaction. This is the first report of clonazepam and fluconazole interaction causing respiratory depression. Hence further detailed pharmacokinetic and pharmacogenetic studies are needed before one a truly determine the possible effects of this interaction.

(MD Amer Khan 1 et, al 2013)

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.A Salem,*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)

Miriam Grushka,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.

(Miriam *Grushka*,et.al.2004)

Yourong Fu and 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. Freitas A 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. Freitas A,*et.al,2005)*

Vigdis Olsen and 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)

CHAPTER

THREE

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 Beximco Pharmaceuticals Ltd. (Xetril) and for the dissolution of clonazepam we used water as a solvent. klonapin is the patent drug of ranitidine. 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.

(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 beenadopted 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:

Table: Parameters of dissolution of clonazepam

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

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.

Table: Concentrations of clonazepam

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

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

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 notdiffer more than the percentage listed below:

Table: Accepted percentage list for weight variation of tablets

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

3.9.2. Equation:

3.9.2.1. Equation for Zero order kinetics:

$\boldsymbol{r = k}$; where, **r** is the reaction rate and **k** is the reaction rate coefficient with units of concentration or time.

The differential form of the rate law of zero order reaction is,

$\boldsymbol{r = -\frac{d[A]}{dT} = k;}$ **[A]** is the concentration of the chemical of interest.

3.9.2.2 Equation for first order kinetics:

The differential form of the rate law of first order reaction is,

$\boldsymbol{\frac{-dA}{dT} = r = k[A]}$; where k is the first order rate constant

The equation for half-life,

$\boldsymbol{t_{\frac{1}{2}} = \frac{ln2}{k}}$; where, $\mathbf{t_{1/2}}$is the plasma half-life of the drug.

(Jung, H., et al, 2001)

3.9.2.3 Equation for second order kinetics:

The differential form of the rate law of second order reaction is,

$\boldsymbol{\frac{-dA}{dt} = 2r = 2k[A]^2}$

3.9.2.4 Equation for plasma half-life:

$\boldsymbol{t = \frac{1}{k_{[A]}}}$

3.9.2.5 Higuchi equation for drug release:

$$Q = [D(2A - C_s)C_s t]^{1/2}$$

$$\frac{dQ}{dt} = \frac{1}{2}\left[\frac{D(2A-C_s)C_s}{t}\right]^{1/2}$$

Where, **Q** is the amount of drug release in time t per unit area

$\frac{dQ}{dt}$is the rate of drug release per unit area

A is the total amount of drug in a unit volume of matrix/initial drug concentration

$C_s$is the saturation concentration solubility of the drug in the matrix

D is the diffusion coefficient of the drug in the matrix.

(Jung, H., et al, 2001)

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)

3.9.3. Thickness test

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.

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

3.9.4. Hardness test

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).

3.10. Materials

3.10.1. Sample Collection:

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

Table: Brand names of Clonazepam under dissolution study

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

3.10.2. 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.

3.11. Equipment's:

In the characterization of matrix tablets of Clonazepam.

(Kuss, 1992)

Table Details about equipment

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

3.12. Instrumentation

3.12.1. 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).

3.12.2. 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.

3.12.3. 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).

3.12.4. Images of Instruments:

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

Fig 3.1. Dissolution apparatus

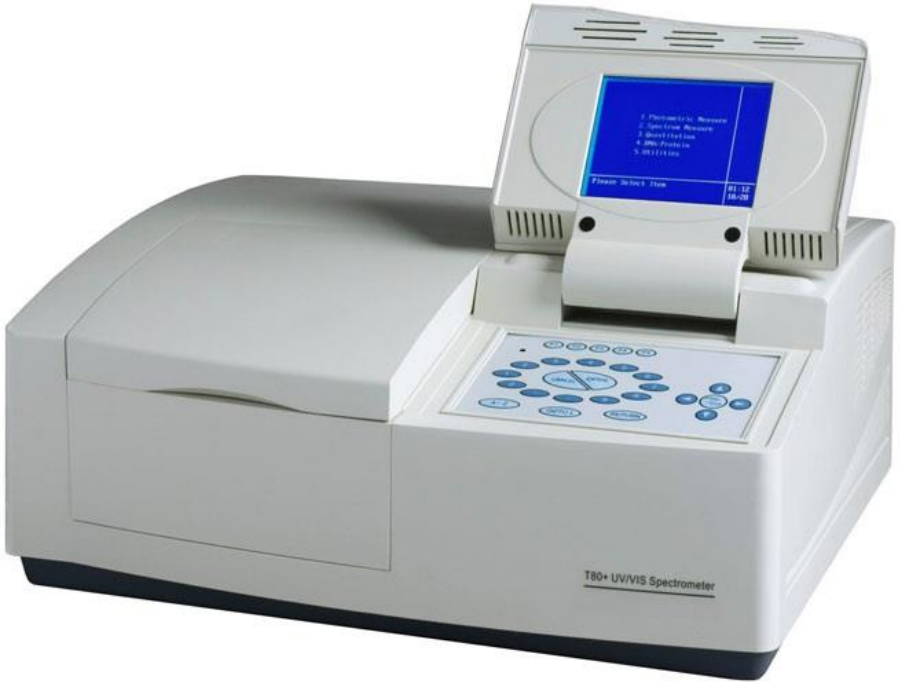


Fig 3.2 UV spectrophotometer

Fig.3.3. Distilled water apparatus

Fig.3.4. Hardness tester

Fig. 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 equationindicates 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 bythe help of Microsoft Excel software

(Anderson et al. 1998; Parakh and Patil 2014).

3.14. Apparatus:

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

Table Representing the apparatus (Kuss, 1992)

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

CHAPTER FOUR

Results & Discussion

4.1 Preparation and method of standard curve of Cloron:

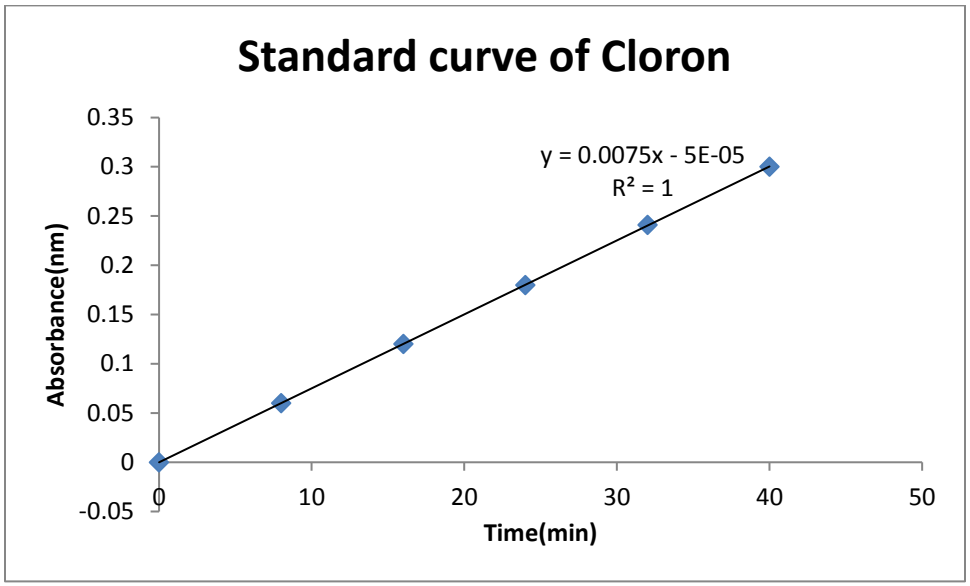


Fig. Standard curve of Cloron

4.1.1 Method of preparation: 4 tablets each of 0.5 mg Cloron were taken and grinded with the help of mortar and pestle to obtain a powder which was then smudged carefully and transferred into a 50 ml 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 = 0.007x - 5E-05** helped to determine the concentration of drug release as well percent release of that drug & **R^2 = 1** gave us an idea about the drug release kinetic profile.

Table: The prepared concentrations an absorbance data for preparation of standard curve

Concentration (µg/ml)	Time (min)	Absorbance (nm)
8	10	0.06
16	20	0.12
24	30	0.18
32	40	0.241
40	50	0.30

4.2 Preparation and method of dissolution curve of Cloron:

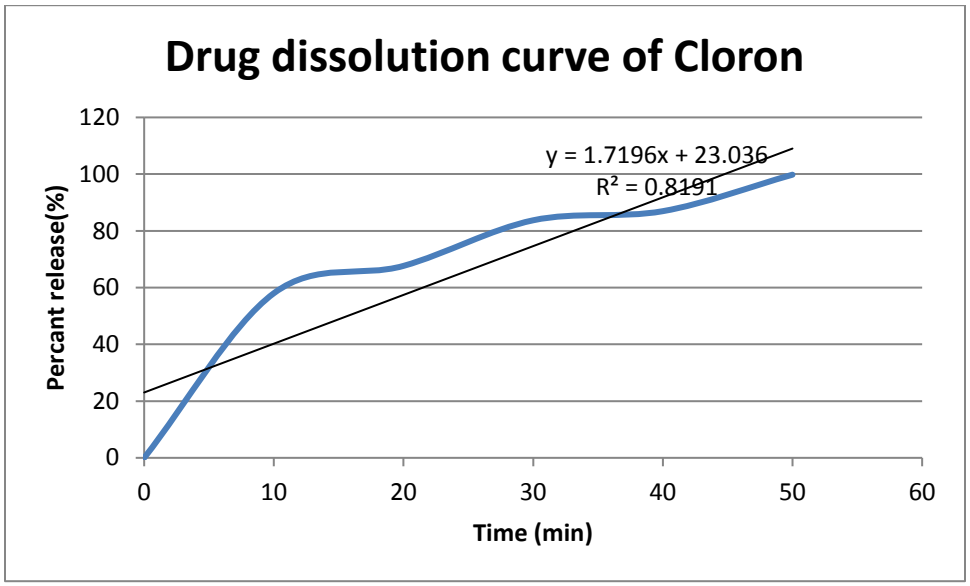


Fig. Dissolution curve of Cloron

4.2.1 Method of preparation: 3 tablets of Cloron 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 58.02% and 99.80% respectively **y = 1.719x+23.03** determined the concentrations of drug release and **R^2 = 0.819** determines the drug release kinetic profile.

Table: Data for the dissolution curve of Cloron

Time (min)	% release of drug
0	0
10	58.02
20	67.66
30	83.73
40	86.95
50	99.80

4.3 Preparation and method of standard curve of Denixil:

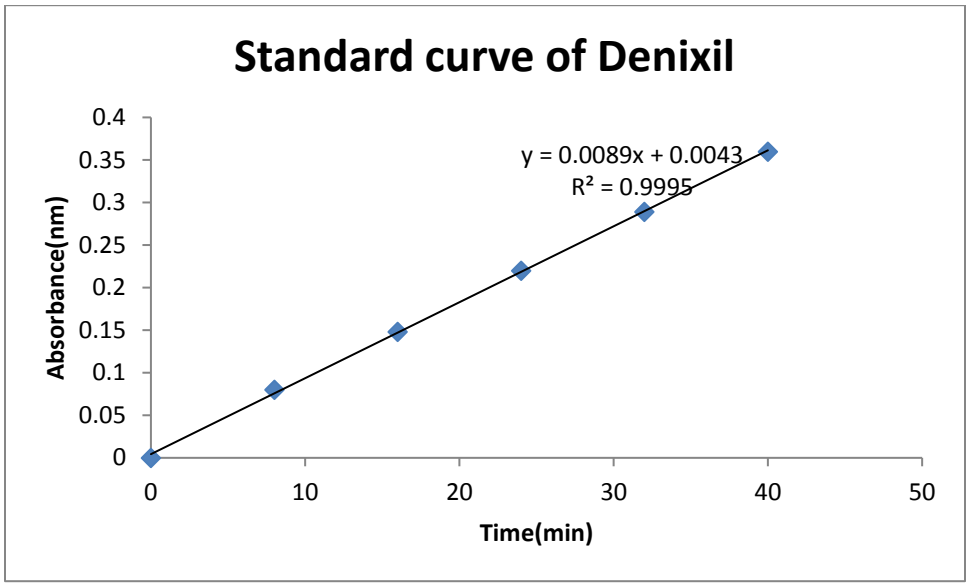


Fig. Standard curve of Denixil

4.3.1 Method of preparation: 4 tablets each of 0.5 mg Denixil 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 = 0.008x + 0.004** helped to determine the concentration of drug release as well percent release of that drug & **R^2 = 0.999** gave us an idea about the drug release kinetic profile.

Table: The prepared concentrations an absorbance data for preparation of standard curve

Concentration (µg/ml)	Time (min)	Absorbance (nm)
8	10	0.08
16	20	0.148
24	30	0.22
32	40	0.289
40	50	0.36

4.4 Preparation and method of dissolution curve of Denixil:

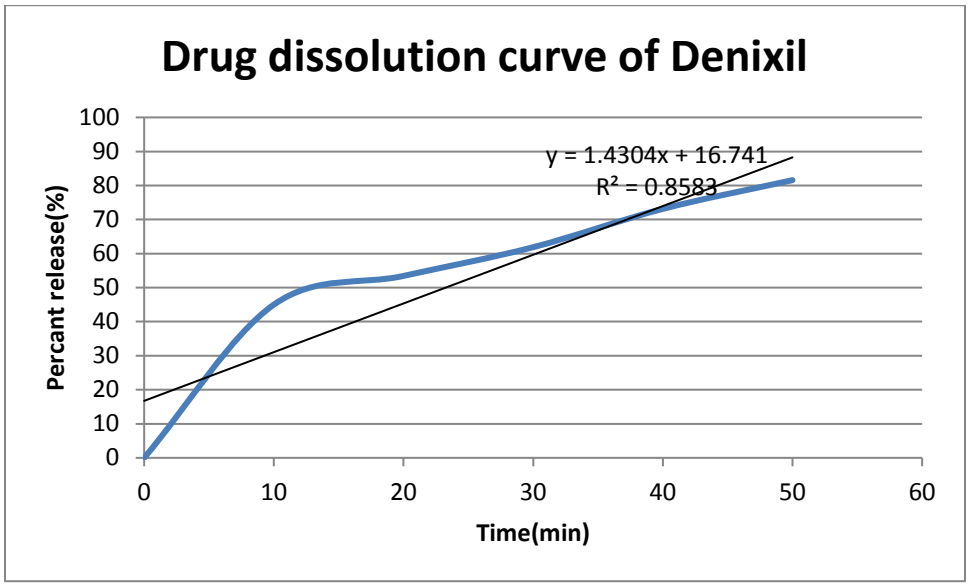


Fig. Dissolution curve of Denixil

4.4.1 Method of preparation: 3 tablets of Denixil 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 45% and 81.562% respectively. The equation **y = 1.430x + 16.74** determined the concentrations of drug release and **R^2 = 0.858** determines the drug release kinetic profile.

Table: Data for the dissolution curve of Denixil

Time (min)	% release of drug
0	0
10	45
20	53.437
30	61.875
40	73.125
50	81.562

4.5 Preparation and method of standard curve of Epnil:

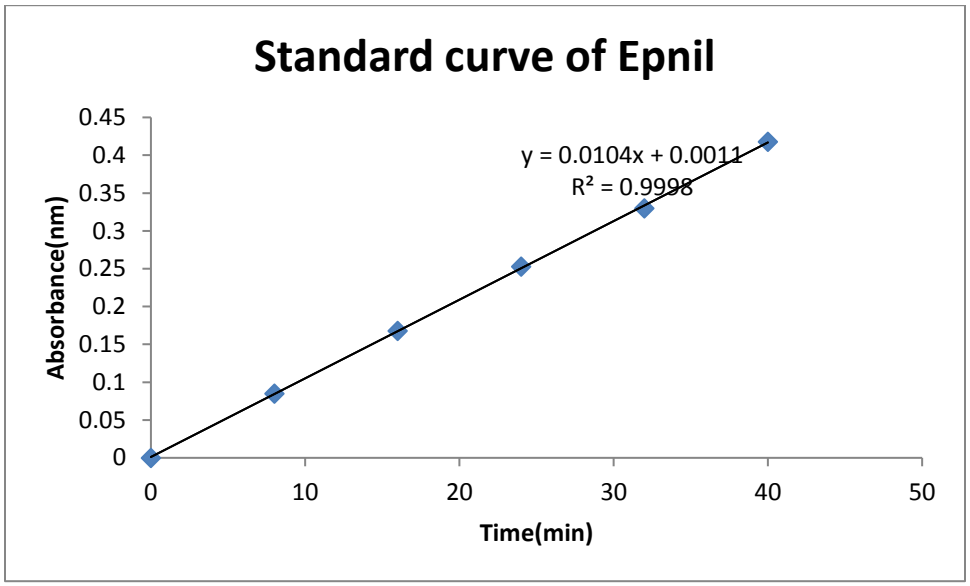


Fig. Standard curve of Epnil

4.5.1 Method of preparation: 4 tablets each of 0.5 mg Epnil 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= 0.010x+0.001** helped to determine the concentration of drug release as well percent release of that drug & **R^2 = 0.999** gave us an idea about the drug release kinetic profile.

Table: The prepared concentrations an absorbance data for preparation of standard curve

Concentration (µg/ml)	Time (min)	Absorbance (nm)
8	10	0.085
16	20	0.168
24	30	0.253
32	40	0.33
40	50	0.418

4.6 Preparation and method of dissolution curve of Epnil:

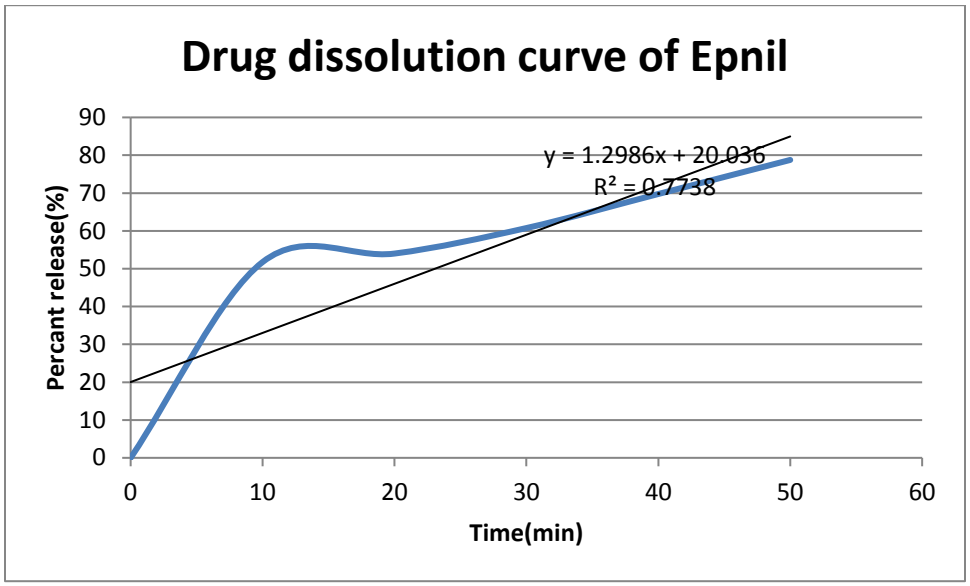


Fig. Dissolution curve of Epnil

4.6.1 Method of preparation: 3 tablets of Epnil 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 51.75% and 78.75% respectively.The equation **y = 1.298x + 20.03** determined the concentrations of drug release and **R^2 = 0.773** determines the drug release kinetic profile.

Table: Data for the dissolution curve of Epnil

Time (min)	% release of drug
0	0
10	51.75
20	54
30	60.75
40	69.75
50	78.75

4.7 Preparation and method of standard curve of Pase:

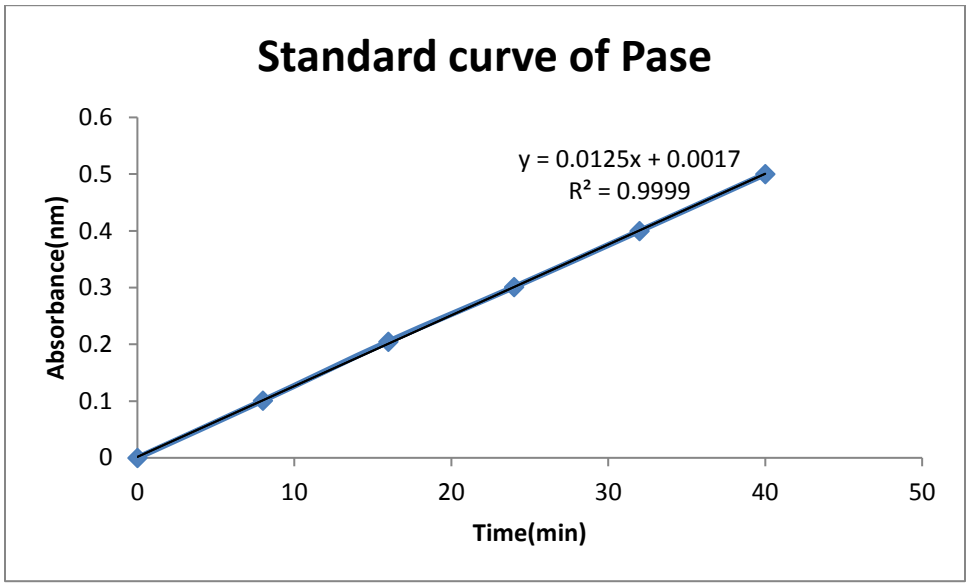


Fig. Standard curve of Pase

4.7.1 Method of preparation: 4 tablets each of 0.5 mg Pase 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 = 0.012x+0.001** helped to determine the concentration of drug release as well percent release of that drug & **R^2 = 0.999** gave us an idea about the drug release kinetic profile.

Table: The prepared concentrations an absorbance data for preparation of standard curve

Concentration (µg/ml)	Time (min)	Absorbance (nm)
8	10	0.101
16	20	0.205
24	30	0.301
32	40	0.4
40	50	0.5

4.8 Preparation and method of dissolution curve of Pase:

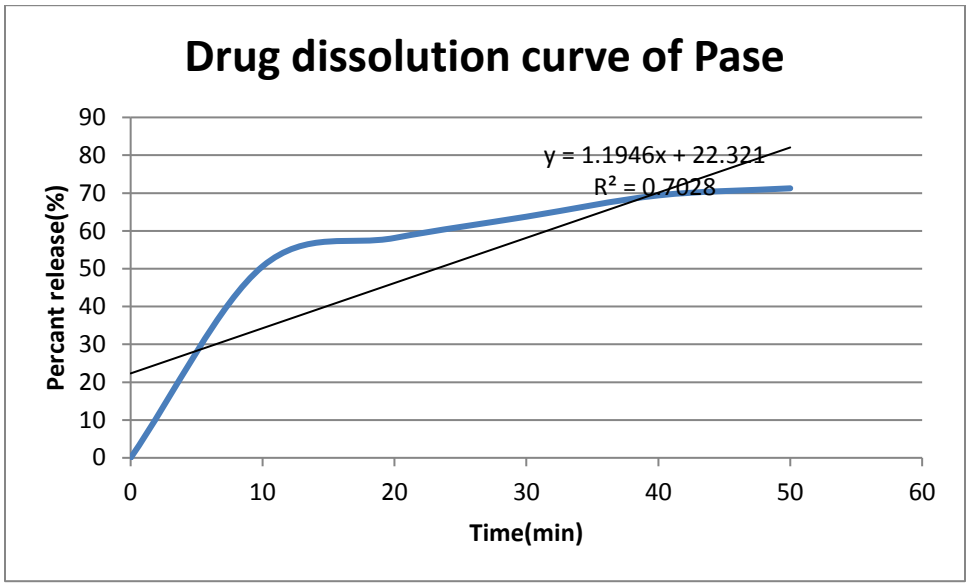


Fig. Dissolution curve of Pase

4.8.1 Method of preparation: 3 tablets of Pase 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 50.625% and 71.25% respectivelythe equation **y = 1.194x+22.32** determined the concentrations of drug release and **R^2 = 0.702** determines the drug release kinetic profile.

Table: Data for the dissolution curve of Pase

Time (min)	% release of drug
0	0
10	50.625
20	58.125
30	63.75
40	69.375
50	71.25

4.9 Preparation and method of standard curve of Xetril:

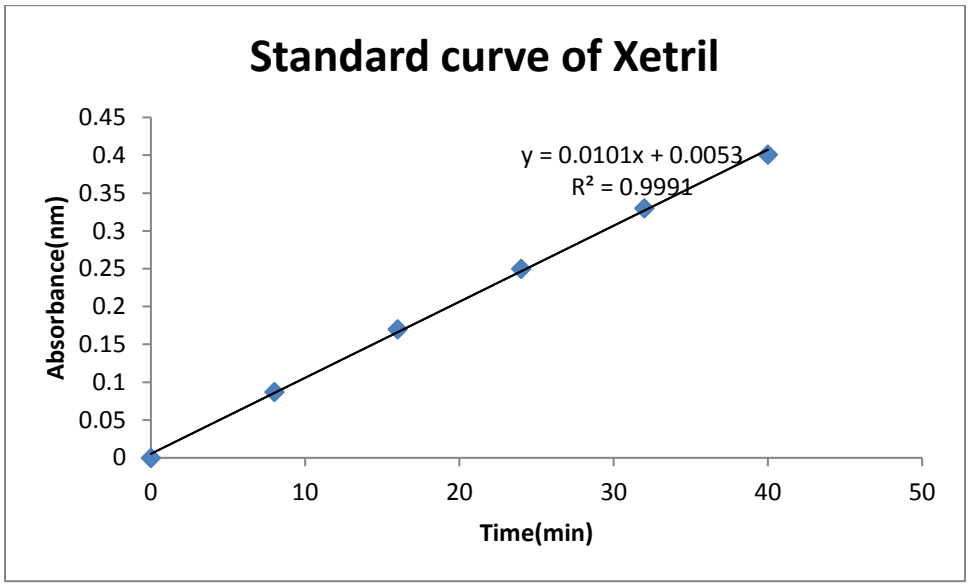


Fig. Standard curve of Xetril

4.9.1 Method of preparation: 4 tablets each of 0.5 mg Xetril 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 = 0.010x+0.005** helped to determine the concentration of drug release as well percent release of that drug & **R^2 = 0.999** gave us an idea about the drug release kinetic profile.

Table: The prepared concentrations an absorbance data for preparation of standard curve

Concentration (µg/ml)	Time (min)	Absorbance (nm)
8	10	0.087
16	20	0.17
24	30	0.25
32	40	0.33
40	50	0.401

4.10 Preparation and method of dissolution curve of Xetril:

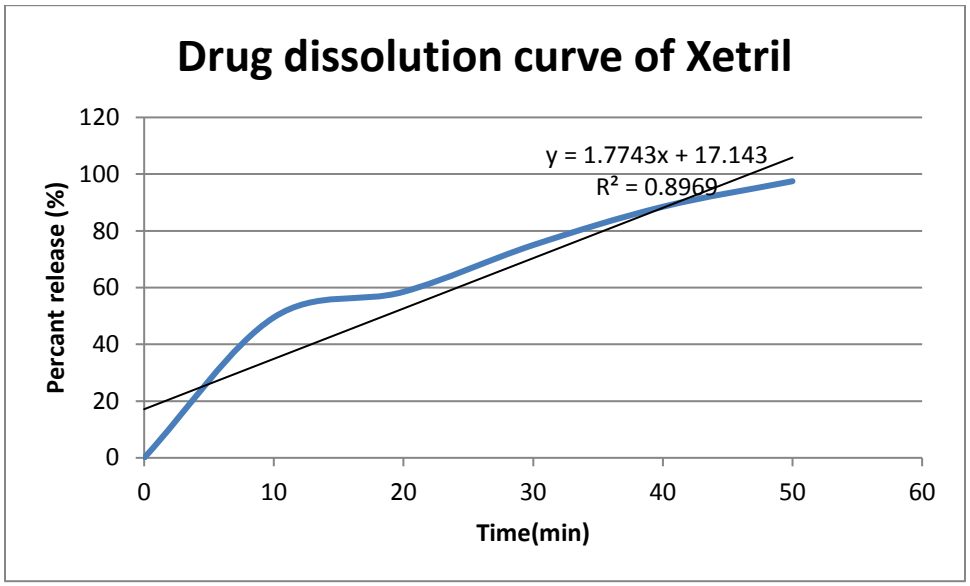


Fig. Dissolution curve of Xetril

4.10.1 Method of preparation: 3 tablets of Xetril 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 49.5% and 97.5% respectively. The equation **y = 1.774x+17.14** determined the concentrations of drug release and **R^2 = 0.896** determines the drug release kinetic profile.

Table: Data for the dissolution curve of Xetril

Time (min)	% release of drug
0	0
10	49.5
20	58.5
30	75
40	88.5
50	97.5

*4.11 f*1 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 f*1 calculation for EPNIL

Time (Minutes)	XETRIL (R)	EPNIL (T)	R-T	| R-T |	*f*1
10	49.5	51.75	-2.25	2.25	
20	58.5	54	4.5	4.5	
30	75	60.75	14.25	14.25	15.85
40	88.5	69.75	18.75	18.75	
50	97.5	78.75	18.75	18.75	
Total	369			58.5	

*4.11.2 f*1 calculation for CLORON

Time (Minuets)	XETRIL Drug release (%) (R)	CLORON Drug release (%) (T)	R-T	|R-T|	*f*1
10	49.5	58.01	-8.51	8.51	
20	58.5	67.66	-9.16	9.16	
30	75	83.73	-8.73	8.73	8.2
40	88.5	86.94	1.56	1.56	
50	97.5	99.80	-2.3	2.3	
Total	369			30.26	

*4.11.3 f*1 calculation for DENIXIL

Time (Minutes)	XETRIL (R)	DENIXIL (T)	R-T	| R-T |	*f*1
10	49.5	45	4.5	4.5	
20	58.5	53.43	5.07	5.07	
30	75	61.8	13.2	13.2	14.65
40	88.5	73.12	15.38	15.38	
50	97.5	81.56	15.94	15.94	
Total	369			54.09	

*4.11.4 f*1 calculation for PASE

Time (Minuets)	XETRIL Drug release (%) (R)	PASE Drug release (%) (T)	R-T	|R-T|	*f*1
10	49.5	50.62	-1.12	1.12	
20	58.5	58.12	0.38	0.38	
30	75	63.75	11.25	11.25	15.75
40	88.5	69.37	19.13	19.13	
50	97.5	71.25	26.25	26.25	
Total	369			58.13	

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

4.12 f2 calculation:

*4.12.1 f*2 calculation for EPNIL

Time (Minutes)	XETRIL Drug release (%) (R)	EPNIL Drug release (%) (T)	R-T	| R-T |	|R-T |^2	*f*2
10	49.5	51.75	-2.25	2.25	5.06	
20	58.5	54	4.5	4.5	20.25	
30	75	60.75	14.25	14.25	203.06	43.18
40	88.5	69.75	18.75	18.75	351.56	
50	97.5	78.75	18.75	18.75	351.56	
Total	369			58.5	931.49	

*4.12.2 f*2 calculation for CLORON

Time	XETRIL Drug release (%) (R)	CLORON Drug release (%) (T)	R-T	|R-T |	|R-T |^2	*f*2
10	49.5	58.01	-8.51	8.51	72.42	
20	58.5	67.66	-9.16	9.16	83.90	
30	75	83.73	-8.73	8.73	76.21	57.73
40	88.5	86.94	1.56	1.56	2.43	
50	97.5	99.80	-2.3	2.3	5.29	
Total	369			30.26	240.25	

*4.12.3 f*2 calculation for DENIXIL

Time (Minutes)	XETRIL Drug release (%) (R)	DENIXIL Drug release (%) (T)	R-T	| R-T |	|R-T |^2	*f*2
10	49.5	45	4.5	4.5	20.25	
20	58.5	53.43	5.07	5.07	25.70	
30	75	61.87	13.2	13.2	174.24	46.10
40	88.5	73.12	15.38	15.38	236.54	
50	97.5	81.56	15.94	15.94	254.08	
Total	369			54.09	710.81	

*4.12.4 f*2 calculation for PASE

Time	XETRIL Drug release (%) (R)	PASE Drug release (%) (T)	R-T	|R-T|	|R-T|^2	*f*2
10	49.5	50.62	-1.12	1.12	1.25	
20	58.5	58.12	0.38	0.38	0.144	
30	75	63.75	11.25	11.25	126.56	41.55
40	88.5	69.37	19.13	19.13	265.95	
50	97.5	71.25	26.25	26.25	689.06	
Total	369			58.13	1082.96	

Here, Cloron met the required range which is 50-100% for the calculation of *f2* and Pase, Denixil, Epnil didn't 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* for Cloron is accepted but the values of Pase, Denixil,Epnil 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 Pase, Denixil, Epnil, Cloron with Xetril 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 Xetril has the value approved by the FDA which is a standard one but Epnil and pase is more than the desired value. According to the FDA approval rule Cloron and Denixil has the legal value in *f*1 calculation. On the other hand, while calculating *f2*, Cloron met the required range which is (50-100%) for the calculation of *f2* and Pase, Denixil, Epnil didn't 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* for Cloron is accepted but the values of Pase, Denixil,Epnil cannot be accepted. Three tablets that is Pase, Denixil and Epnil 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.

Conclusion:

comparisons of dissolution profiles of Clonazepam tablets oral formulations were made between four generic products namely Pase, Denixil, Epnil, Cloron with Xetril 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 Xetril has the value approved by the FDA which is a standard one but Epnil and pase is more than the desired value. According to the FDA approval rule Cloron and Denixil has the legal value in *f*1 calculation. On the other hand, while calculating *f2*, Cloron met the required range which is (50-100%) for the calculation of *f2* and Pase, Denixil, Epnil didn't 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* for Cloron is accepted but the values of Pase, Denixil,Epnil cannot be accepted. Three tablets that is Pase, Denixil and Epnil 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.