Determination of the release kinetics of drug from five brands of Clonazepam available in Bangladesh (Cloron, Denixil, Epnil, Pase, 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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Lecturer

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Declaration by the Candidate

I, Shahadat Hossen Mozumder, hereby declare that the dissertation entitled ***"Determination of the release kinetics of drug from five brands of Clonazepam available in Bangladesh (Cloron, Denixil, Epnil, Pase, 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 year 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 **"*Determination of the release kinetics of drug from five brands of Clonazepam available in Bangladesh (Cloron, Denixil, Epnil, Pase, 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 Shahadat Hossen Mozumder, ID: 2013-3-70-012, during 2017 of his research in the Department of Pharmacy, East West University, under the supervision and guidance of me. The thesis has not formed the basis for the award of any other degree/diploma/fellowship or other similar title to any candidate of any university.

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Supervisor

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Endorsement by the Chairperson

This is to certify that the thesis entitled **"*Determination of the release kinetics of drug from five brands of Clonazepam available in Bangladesh (Cloron, Denixil, Epnil, Pase, 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 Shahadat Hossen Mozumder, ID0: 2013-3-70-012, during the period 2017 of his research in the Department of Pharmacy, East West University.

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Acknowledgement

At first, I would like to thanks the Almighty **ALLAH** the most gracious and merciful for enabling me to successfully completing my research work soundly and orderly.

I would like to express my deepest gratitude to my research supervisor, **Tirtha Nandi**, Lecturer, Department of Pharmacy, East West University, who had been always optimistic and full of passion and ideas. Her generous advice, constant supervision, intense support, enthusiastic encouragements and reminders during the research work not only helped shape this study but also moulded me into being a better researcher.

I also express my beloved gratitude to **Md. Anisur Rahman,** Assistant professor, Department of Pharmacy, East West University for his friendly hand to do the lab work and help me to keep patients during my research work. I am thankful to the laboratory instructors **Mr. Sujit Kumar**, for his kind support during the laboratory work.

I put forward my most sincere regards and profound gratitude to Chairperson **Dr. Shamsun Nahar Khan**, Associate Professor & chairperson, Department of Pharmacy, East West University, for her inspiration in my study. She also paid attention for the purpose of my research work and extending the facilities to work.

I want to give special thanks to Rafat Shahriar Islam, Kazi Shoriful Hasan, Mahbubur Rahman, and my all friends, who gave me support for my research work and for their extended cooperation for my study.

Abstract

The purpose of this experiment was to determine the release kinetics of drug from five brands of Clonazepam available in Bangladesh. For this study five widely prescribed brands Cloron, Denixil, Epnil, Pase, and Xetril were chosen. All of these brands were 0.5 mg Clonazepam with strip packaging. The dissolution was carried out using USP apparatus-II and analysis was performed with the UV spectroscopy. To find out the release kinetics K_0 (for zero order), K_1 (for first order), K_h (for Higuchi model) were determined. The R^2 value for each kinetics was also determined which indicated the linearity of the release kinetics for each brand. The study found no brand to follow the zero order and first order kinetics mostly except Higuchi's drug release profile. The brands showing different R^2 values for Higuchi drug release profile are Cloron (R^2 = 0.974), Denixil (R^2 = 0.985), Epnil (R^2 = 0.944), Pase (R^2 = 0.922), Xetril (R^2 = 0.992) was the highest amongst the R^2 values comparing to zero order and first order values. So, this study assumes that the available Clonazepam tablet brands in Bangladesh generally follow the Higuchi's drug release kinetics.

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

Dedication

This research paper is dedicated to my beloved elder brothers (Delower Hossen & Mir Hossen) and my family members

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List of Abbreviations

Short forms	**Names**
CZP	Clonazepam
BZP	Benzodiazepines
STD curve	Standard curve
Dissol. Curve	Dissolution curve
M/A	Mechanism of action
Sol.	Solution
Prep.	Preparation

Chapter One
INTRODUCTION

1.1. Introduction:

Sedative-hypnotics are drugs which depress or slow down the body's functions. Often these drugs are referred to as tranquilizers and sleeping pills or sometimes just as sedatives. Their effects range from calming down anxious people to promoting sleep. Both tranquilizers and sleeping pills can have either effect, depending on how much is taken. At high doses or when they are abused, many of these drugs can even cause unconsciousness and death.

Barbiturates and benzodiazepines are the two major categories of sedative-hypnotics. The drugs in each of these groups are similar in chemical structure. The benzodiazepines are the main class of drugs that fit into this category. Although there are more than twenty benzodiazepine derivatives, only certain ones have been approved to treat anxiety (eg, alprazolam, clonazepam, diazepam, and lorazepam), sleeplessness (insomnia) (eg, estazolam, flurazepam, quazepam, temazepam and triazolam), or panic disorder (eg, alprazolam). Barbiturates are an older class of medicine that used to be used for these indications as well; however, barbiturates have a narrow therapeutic index (window of effectiveness before toxicity occurs), and are more likely to cause respiratory depression, coma and death, and are very rarely used nowadays. The main issue with use of benzodiazepines is dependence. Benzodiazepines differ in their propensity to cause sedation and in the length of time they act for.

Sedatives: A drug that can induce depression of the central nervous system (CNS), having a calming or quieting effect, often given to reduce anxiety or to promote relaxation. E.g. Clonazepam.

Hypnotics: An agent that induces drowsiness or sleep, also known as a soporific drug that produces an uncontrollable desire to sleep. E.g. Chlordiazepoxide.

Anxiolytics: Anxiolytics or anti-anxiety drugs are used to prevent anxiety and treat anxiety related disorders. E. g. Alprazolam.

"Sedative Hypnotics Drugs". *Pharmacology2000.com*

"Medical Definition of Sedative". *MedicineNet*

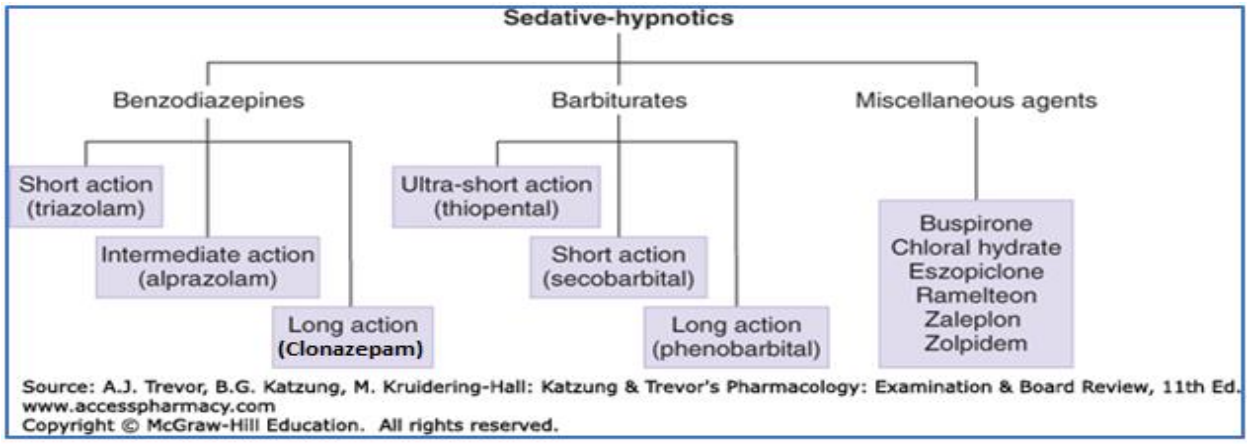


Figure 1.1. A short overview on the classification of sedative-hypnotic drugs

"List of Anxiolytics, Sedatives, And Hypnotics - Drugs.Com". *Drugs.com*

Dr Tomislav Meš trović, PhD. "List Of Sedatives". *News-Medical.net*

1.2. Benzodiazepines:

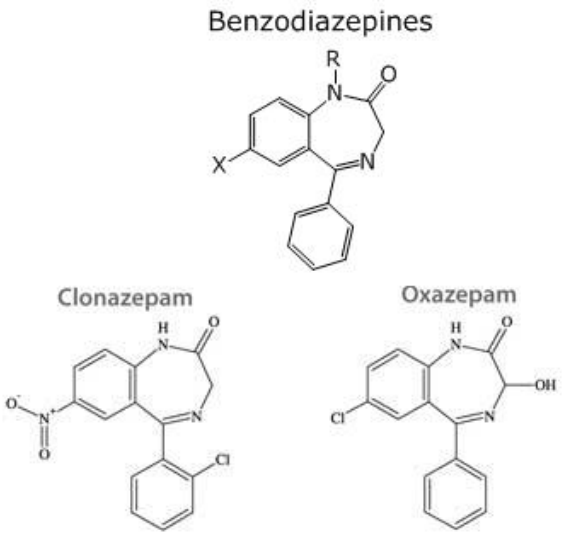


Figure 1.2. General Structure of benzodiazepines and its analogues

Benzodiazepines have the same general chemical structure have been developed through the years based on chemical substitutions at two major positions on the benzodiazepine structure i.e. R and X and to some extent phenyl ring.

"Classification of Benzodiazepine Drugs.Com - Google Search". *Google.com*

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.

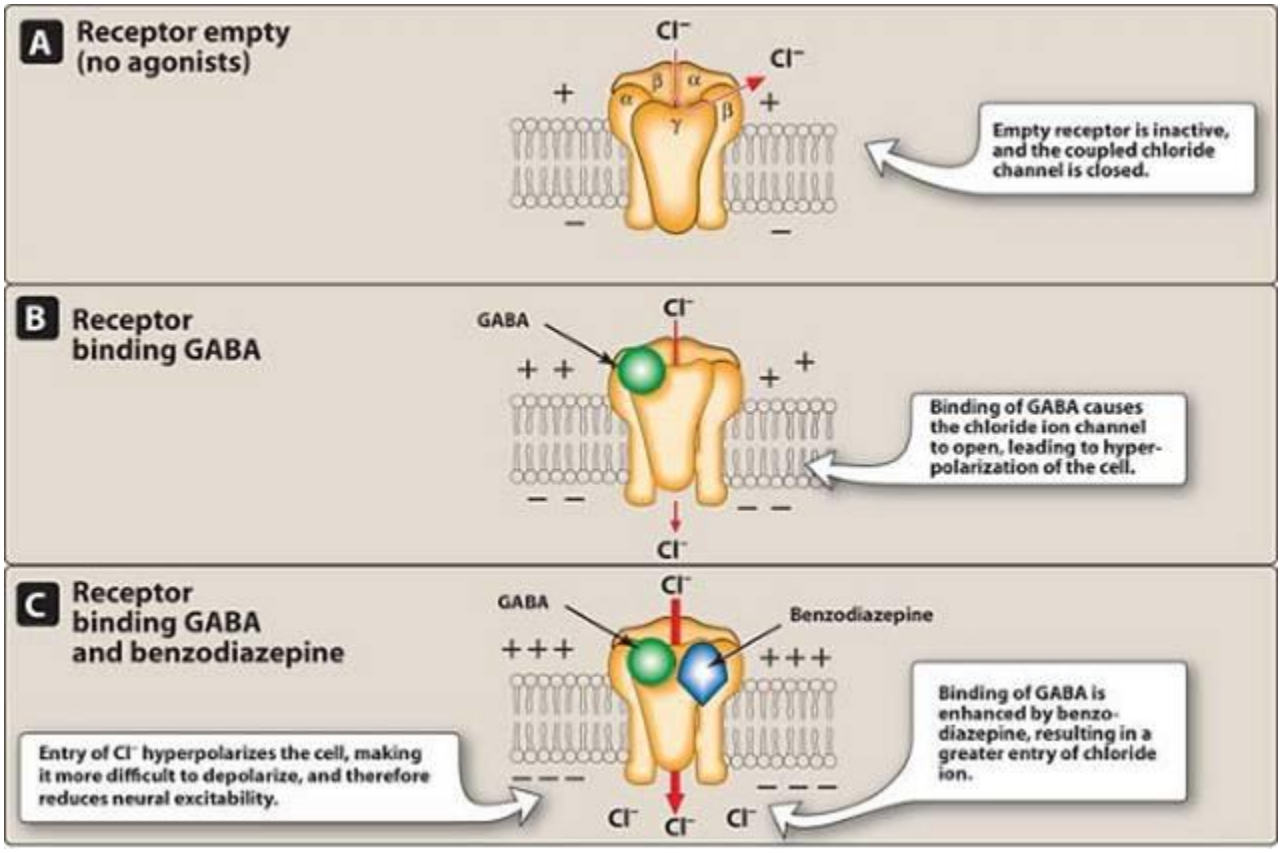


Figure 1.3. Mechanism of action of Benzodiazepines and their analogues

The molecular site of action for the benzodiazepines is at the $GABA_A$ 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 $GABA_A$ receptor, the result is an increase in the influx of chlorine through the ion channel of the receptor. Therefore, benzodiazepines are said to increase the effect that GABA has at $GABA_A$ receptors when it binds. Finally, it should be pointed out that the benzodiazepines do not have a direct effect on the $GABA_A$ receptor; if GABA is not bound to the $GABA_A$ receptor, then benzodiazepine binding has no effect on chlorine ion influx.

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

Harjot Atwal, PharmD Intern, and PharmD Intern Harjot Atwal. "List Of Sedative Hypnotic Drugs: Examples, Side Effects, Classification, Definition, And Mechanism". *RxEconsult*

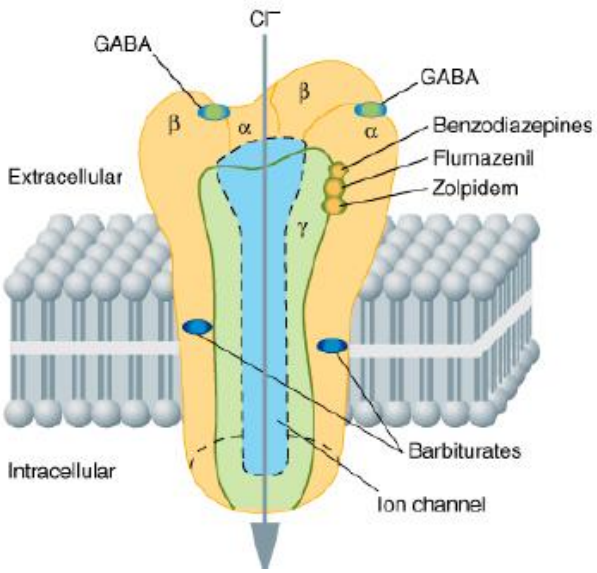


Figure 1.4. Action of Benzodiazepine antagonist (Flumazenil)

1.4. Flumazenil:

It is one of several 14- 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.

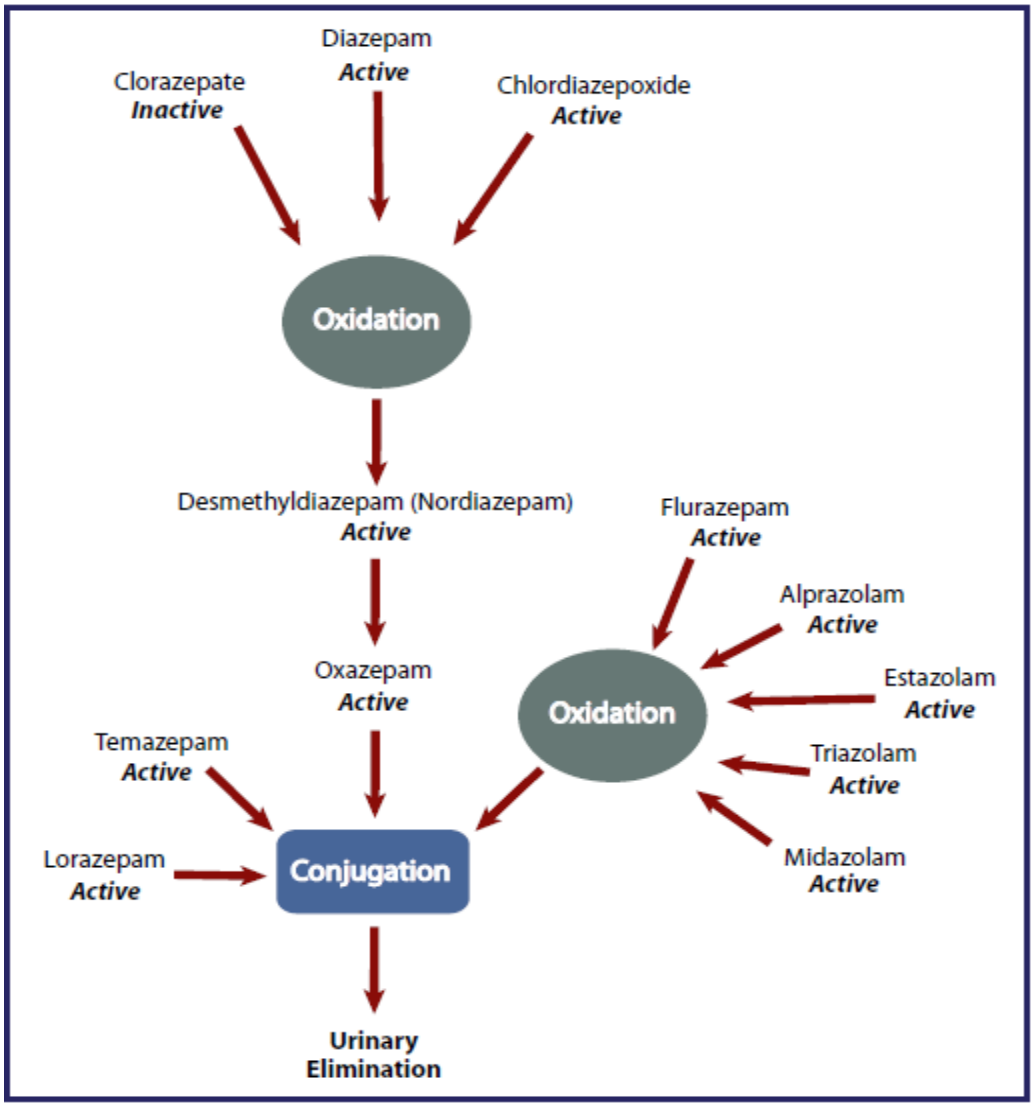


Figure 1.5. Fate of Benzodiazepines (Spier, S.A., Tesar, G.E. 2006)

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.

(Spier, S.A., Tesar, G.E. 2006)

1.5. Clinical uses of sedative-hypnotics:

1. For relief of anxiety.
2. For insomnia.
3. For sedation and amnesia before and during medical and surgical procedures.
4. For treatment of epilepsy and seizure states.
5. As a component of balanced anesthesia (Intravenous administration).
6. For control of ethanol or other sedative-hypnotic withdrawal states.
7. For muscle relaxation in specific neuromuscular disorders.
8. As diagnostic aids for treatment in psychiatry.

(Izquierdo, I., Pereira, ME, 2009)

1.6. Clinical Toxicology of Sedative hypnotics:

1. **Depression of the central nervous system**: Drowsiness, impaired judgment, and diminished motor skills.
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.**

(Izquierdo, I, 2009)

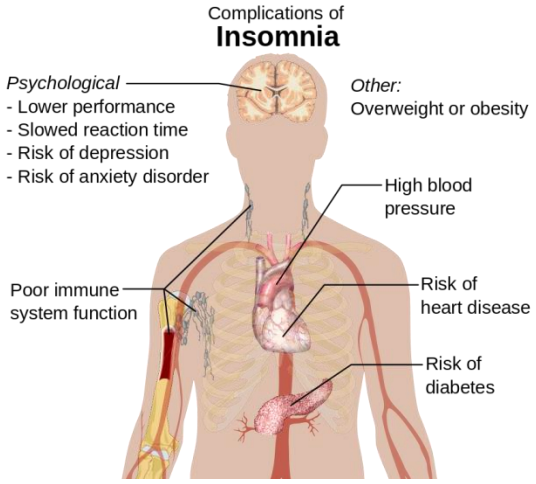


Figure 1.6. Insomniac complications

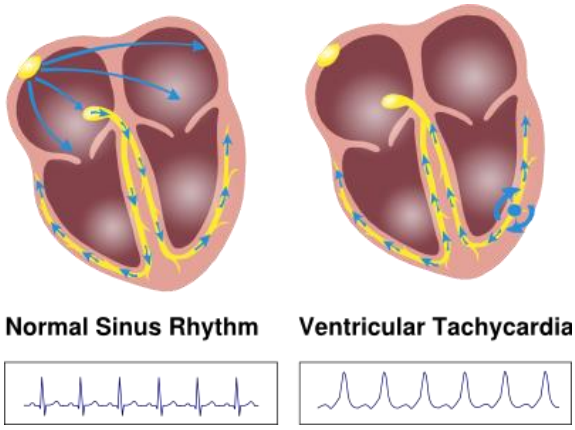


Figure 1.7. Ventricular tachycardia

"Insomnia Complications - Google Search". *Google.com*

1.7. Dissolution:

Dissolution is the process of extracting the API out of the dosage form solid-state matrix into solution within the gastrointestinal tract. Absorption is the process of transporting the drug substance from the gastrointestinal lumen into the systemic circulation.

Dissolution testing is an *in vitro* method that characterizes how an API is extracted out of a solid dosage form. It can indicate the efficiency of *in vivo* dissolution but does not provide any information on drug substance absorption. Pharmacokinetic data supplements and provides additional information regarding API absorption rate.

Selection of the appropriate *in vitro* conditions (media and hydrodynamics) that simulate the *in vivo* conditions can lead to the generation of successful IVIVC or at the very least, *in vitro-in vivo* relations (IVIVR). Conditions that are optimal for QC purposes may not be applicable for establishing IVIVC so it may be necessary to use two dissolution tests to meet different objectives such as development needs or regulatory demands.

Dissolution rate may be defined as amount of drug substance that goes in the solution per unit time under standard conditions of liquid/solid interface, temperature and solvent composition. It can be considered as a specific type of certain heterogeneous reaction in which a mass transfer results as a net effect between escape and deposition of solute molecules at a solid surface.

(Chandra, B., 2005)

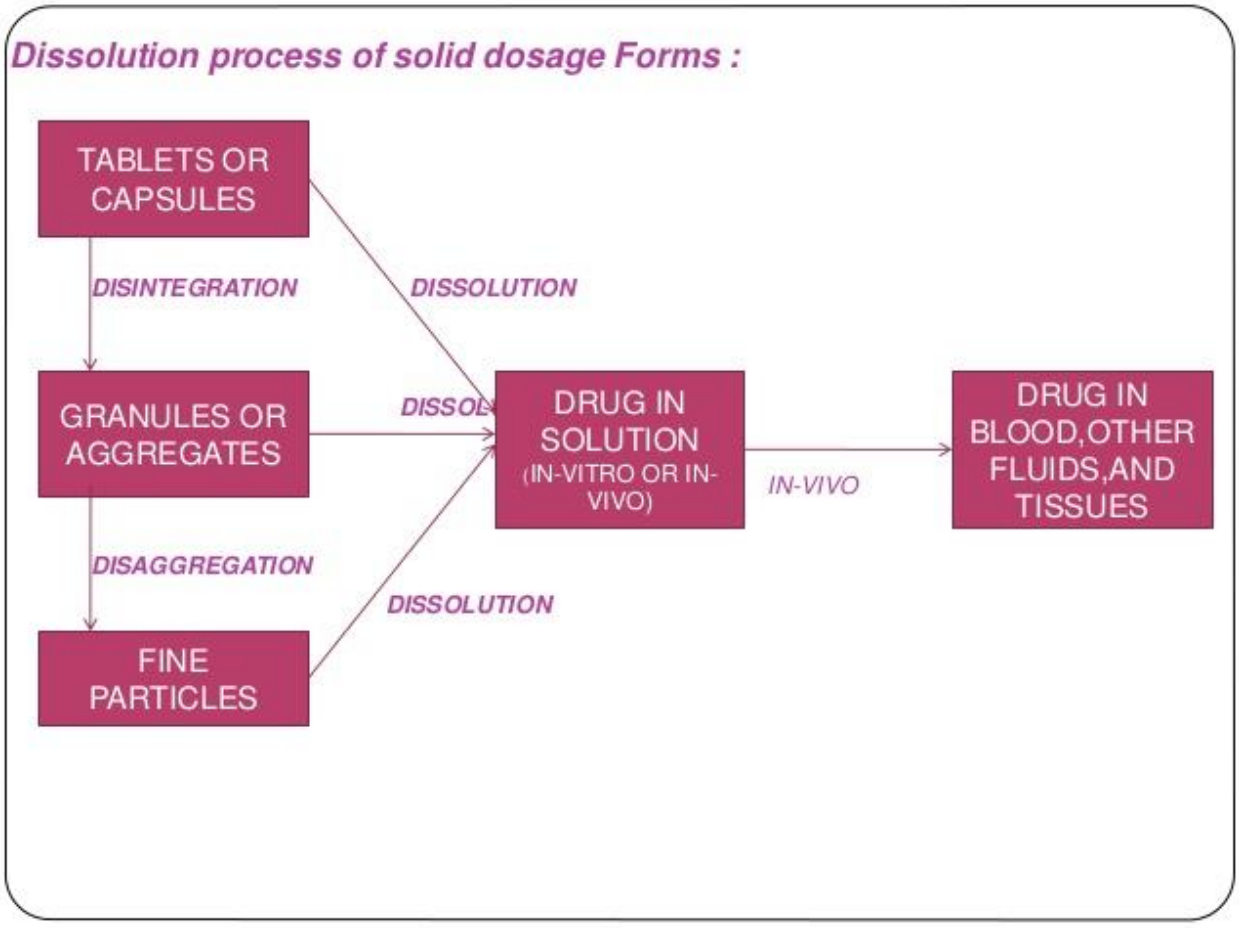


Figure 1.8. Dissolution process of solid dosage forms

Schematic Illustration of Dissolution Process - Google Search". Google.com

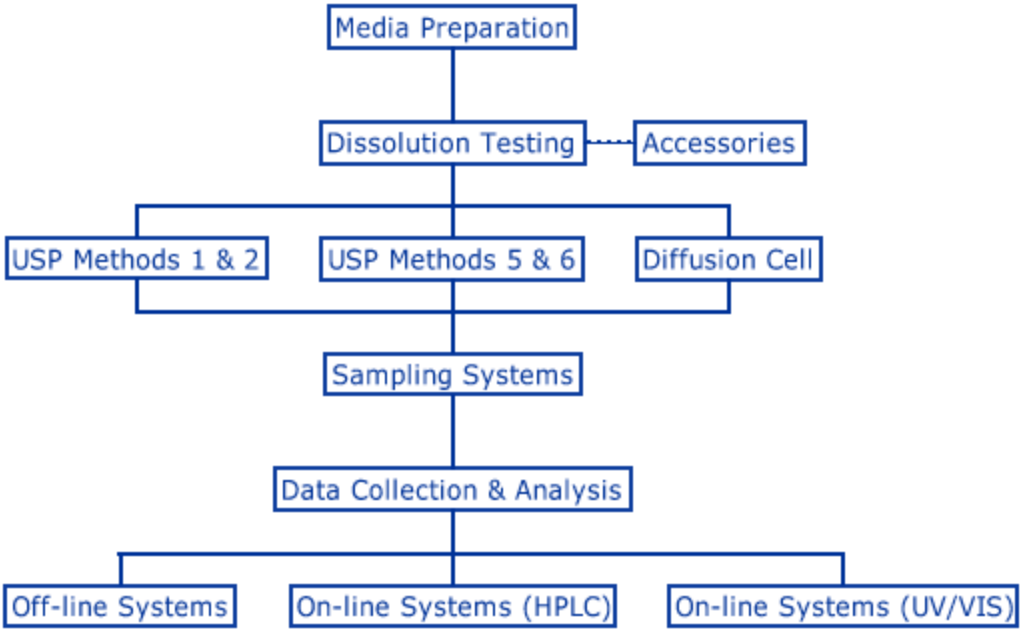


Figure 1.9. Stages in the dissolution testing process

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

1.8. What is Tablet Dissolution?

Tablets or capsules taken orally remain one of the most effective means of treatment available. The effectiveness of such dosage forms relies on the drug dissolving in the fluids of the gastrointestinal tract prior to absorption into the systemic circulation. The rate of dissolution of the tablet or capsule is therefore crucial.

One of the problems facing the pharmaceutical industry is to optimize 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).

Drug release in the human body can be measured *in-vivo* by measuring the plasma or urine concentrations in the subject concerned. 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. (Jeong, Y.I., Cheon, J.B, 2008)

Tablet Dissolution is a standardized method for measuring the rate of drug release from a dosage form. The principle function of the dissolution test may be summarized as follows:

- Optimization 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).

(Patel, R. and Purohit, N., 2009)

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

- Dissolution testing 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 shelflife.

- 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.

(Patel, R. and Purohit, N., 2009)

1.10. Drug release kinetics:

"Drug release" refers to the process in which drug solutes migrate from the initial position in the polymeric system to the polymer's outer surface and then to the release medium. This seemingly simple process is affected by multiple complex factors such as the physicochemical properties of the solutes, the structural characteristics of the material system, release environment, and the possible interactions between these factors.

"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,

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

The equation for half-life,

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

(Jung, H., et al, 2001)

1.13. Equation for second order kinetics:

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

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

1.14. Equation for plasma half-life:

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

1.15. Higuchi equation for drug release:

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

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

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 (Jung, H., et al, 2001)

1.16. Korsmeyer Equation for drug release:

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

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

$\mathbf{M_t}$ is the amount of drug release at time t

M is the total amount of drug in dosage form

$\boldsymbol{k_m}$ is kinetic constant

n is diffusion or release exponent

t is time in hours

(Jung, H., et al, 2001)

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.

(Jung, H., et al, 2001)

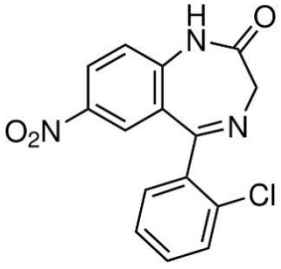
1.18. Clonazepam Overview:

Clonazepam is in a group of drugs called benzodiazepines. Benzodiazepines are central nervous system (CNS) depressants, which are medicines that slow down the nervous system. Clonazepam affects chemicals in the brain that may become unbalanced and cause anxiety. It is used to treat seizure disorders or panic disorder. Clonazepam is also a seizure medicine, also called an anti-epileptic drug. Clonazepam is a controlled substance drug. It's available as an oral tablet or an orally disintegrating (dissolving) tablet.

1.19. Product Information:

Route of Administration	**Dosage Form / Strength**	**Non-medicinal Ingredients**
Oral	Tablet 0.5 mg	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. Indications and clinical use:

Clonazepam has been found useful when used alone or as an adjunct in the management of myoclonic and akinetic seizures and petit mal variant (Lennox-Gastaut syndrome). Clonazepam may be of some value in patients with absence spells (petit mal) who have failed to respond to succinimides. Up to nearly one-third of the patients in some studies have shown a loss of anticonvulsant activity, often within the first three months of administration of RIVOTRIL. In some cases, dosage adjustment may re-establish efficacy. (Shirs, S.B., Suresh, S. 2009)

1.22. Contraindications:

Narrow-angle glaucoma
Severe liver disease
A history of allergic reaction to any benzodiazepine, such as diazepam (Valium), alprazolam (Xanax), lorazepam (Ativan), chlordiazepoxide, flurazepam, and others.
Clonazepam can pass into breast milk and may harm a nursing baby. No breast-feeding while using this medicine.
Clonazepam is not approved to treat panic disorder in anyone younger than 18 years old.

(Shirs, S.B., Suresh, S. and Swamy, P.V., 2009)

1.23. Major side effects:

Body aches or pain	Chills
Cough	difficulty breathing
Dizziness`	Ear congestion
Feeling sad or empty	Fever
Lack of appetite	Loss of interest or pleasure
Loss of voice	Runny nose
Shakiness and unsteady walk	Sleepiness or unusual drowsiness
Sneezing	Sore throat
Tiredness	Trouble concentrating
Trouble sleeping	Problems with muscle control or coordination

(Shirs, S.B., Suresh, S. and Swamy, P.V. 2009)

1.24. Minor side effects:

Difficulty with swallowing	Dryness or soreness of throat
Heavy bleeding	Hives

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 or until side effects preclude any further increase. Maintenance dosage must be individualized for each patient depending upon response. A recommended maintenance dose for adults is 8 to 10 mg/day in three 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 equally divided, the larger dose should be given before retiring.

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

Table 1.1. 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.

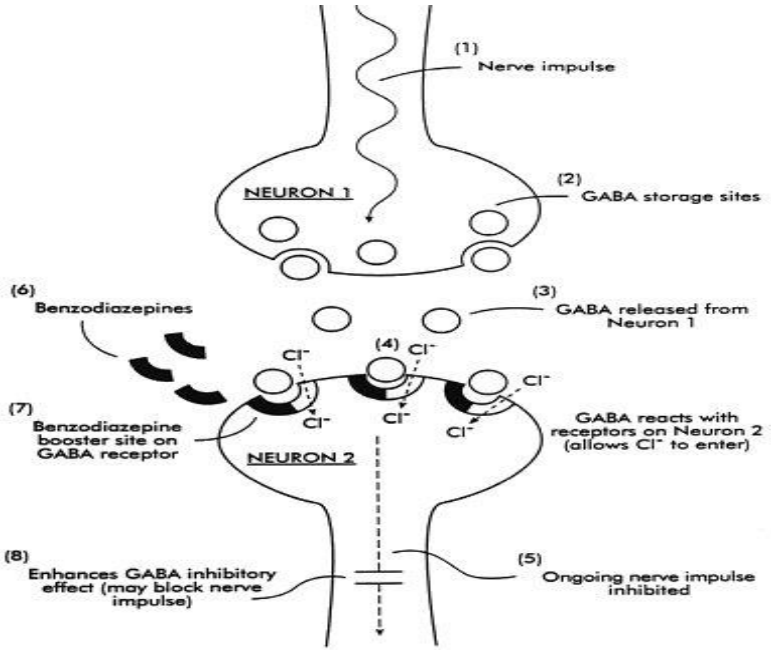


Figure 1.10. Mechanism of action of Clonazepam (Benzodiazepine drug) (Mura, P. 2005)

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 are 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

1. Abilify (aripiprazole)	2. Adderall (amphetamine / dextroamphetamine)
3. Ambien (zolpidem)	4. Aspirin Low Strength (aspirin)
5. Cymbalta (duloxetine)	6. Fish Oil (omega-3 polyunsaturated fatty acids)
7. Lamictal (lamotrigine)	8. Lexapro (escitalopram)
9. Lipitor (atorvastatin)	10. Lyrica (pregabalin)
11. Nexium (esomeprazole)	12. Norco (acetaminophen / hydrocodone)
13. Prozac (fluoxetine)	14. Seroquel (quetiapine)
15. Singulair (montelukast)	16. Synthroid (levothyroxine)

(Mura, P., Nassini, C., Proietti, D, 2004)

1.29. Different brands available in Bangladesh:

Table 1.2. 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.

Brand Name	Company Name
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
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.

(DIMS, 2017)

Chapter Two

LITERETURE REVIEW

2.1. Literature Review

This study was performed 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 insomnia. 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)

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 co morbidity. 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 nonresponsive; 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, et.al, 2008)

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 Wang 2016)

This study was performed by Michael F. Weaver, et.al in 2015 Sep. 3. Sedatives are widely prescribed for anxiety or insomnia and include benzodiazepines, selective benzodiazepine receptor subtype agonists (z-drugs), and barbiturates. These sedatives are controlled substances due to their potential for misuse and abuse. Misuse is often self-medication (chemical coping) of psychological symptoms in ways unauthorized by the prescriber, usually as dose escalation leading to requests for early refills. Sedatives are abused for euphoric effects, which may have dangerous consequences. Sedative withdrawal syndrome is treated by tapering the sedative and may require hospitalization. Long-term treatment of sedative addiction requires counseling, often with the help of an addiction-treatment professional.

(Michael F. Weaver, et.al 2015)

Ivan Miziara, et.al, 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, et.al, 2014)

This study was done by S. B. Shirsand and research members conducted a study in 2011. Fast dissolving tablets of clonazepam were prepared by sublimation method with a view to enhance

patient compliance. A 32 full factorial design was applied to investigate the combined effect of two formulation variables: amount of croscarmellose sodium and camphor. Croscarmellose sodium (2-8% w/w) was used as superdisintegrant and camphor (20-40% w/w) was used as subliming agent, to increase the porosity of the tablets, since it helps water to penetrate into the tablets, along with directly compressible mannitol to enhance mouth feel. The tablets were evaluated for hardness, friability, thickness, drug content uniformity, *in vitro* dispersion time, wetting time and water absorption ratio. Based on *in vitro* dispersion time (approximately 11 s); the formulation containing 5% w/w croscarmellose sodium and 40% w/w camphor was found to be promising and tested for *in vitro* drug release pattern (in pH 6.8 phosphate buffer). Short-term stability (at 40°/75% relative humidity for 3 mo) and drug-excipient interaction. Surface response plots are presented to graphically represent the effect of independent variables on the *in vitro* dispersion time. This formulation showed nearly nine-fold faster drug release ($t_{50\%}$ 1.8 min) compared to the conventional commercial tablet formulation ($t_{50\%}$ 16.4 min). Short-term stability studies on the formulation indicated that there are no significant changes in drug content and *in vitro* dispersion time ($P<0.05$).

(S. B. Shirsand,et.al. 2011)

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*)

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)

André S. Pollmann, Andrea L. Murphy,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. Pollmann, et.al, 2014)

This study was performed by David J Greenblatt; Philip D Blaskovich; E S Nuwayser; Jerold S Harmatz; at 2005 Nov. Nine healthy volunteers 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 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 clonazepamconcentration 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 Greenblatt; Philip D Blaskovich; E S Nuwayser; Jerold S Harmatz; 2005)

This study was performed by Venkateswaran, et,al. 20 dec,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)

Ana Carolina de Oliveira Nevesa 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, 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 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 and systemic action.

(P. Mura, 2016)

MD Amer Khan 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, 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 characterized by a spontaneous burning pain in the oral mucosa without known cause or recognized treatment. The purpose of this double-blind, randomized, 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. FreitasA and his colleagues done a research in 2005 .Comparison between dissolution profiles obtained by using a dissolution apparatus (conventional method) and the NIR diffuse reflectance spectra of a series of clonazepam-containing batches is reported. Ten different formulations with fixed amount of clonazepam and varying proportions of excipients were analyzed at seven dissolution times and three different media. The percentages of dissolution of each sample were correlated with the NIR spectra of three tablets of each batch, through a multivariate analysis using the PLS regression algorithm. The squared correlation coefficients for the plots of percentages of dissolution from the equipment laboratory (dissolution apparatus and HPLC determination) versus the predicted values, in the leave-one-out cross-validation, varied from 0.80 to 0.92, indicating that the NIR diffuse reflectance spectroscopy method is an alternative, nondestructive tool for measurement of drug dissolution from tablets.

(Matheus P. FreitasA, *et.al, 2005)*

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. Klonopin is the patent drug of Clonazepam. Other tablets I used to see the release pattern with different time interval like etc.

3.3. Dissolution testing methods for Clonazepam:

Table 3.1. 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 5 ml were withdrawn from the dissolution medium and the amount was replacing by 5 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 the in vivo condition and drug dissolve at specified time periods was plotted as percent release versus time(hours) curve. (Shah,*et al.*1998)

3.4. Preparation of Standard Curve:

To prepare the standard curve, at first different concentrations (8, 16, 24, 32 and 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 100 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 and 0 ml. Then spectrophotometer is turned on and 273 nm wave lengths were 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 3.2. Concentrations of Clonazepam

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

3.5. Preparation for dissolution test

3.5.1. 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.

3.5.2 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 10, 20, 30, 40, 50 and 60 minutes 5 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.

3.6. Determination of physical parameters

3.6.1 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 not differ more than the percentage listed below:

Table 3.3. 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%

3.7. Equation:

3.7.1 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.

3.7.2 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)

3.7.3 Equation for second order kinetics:

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

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

3.7.4 Equation for plasma half-life:

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

3.7.5 Higuchi equation for drug release:

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

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

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)

3.8. 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 0.1 cm.

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. 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 3.4. 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. Equipments:

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

Table 3.5. 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 10 in-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 Clonazepam 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-

Figure 3.1. Dissolution apparatus

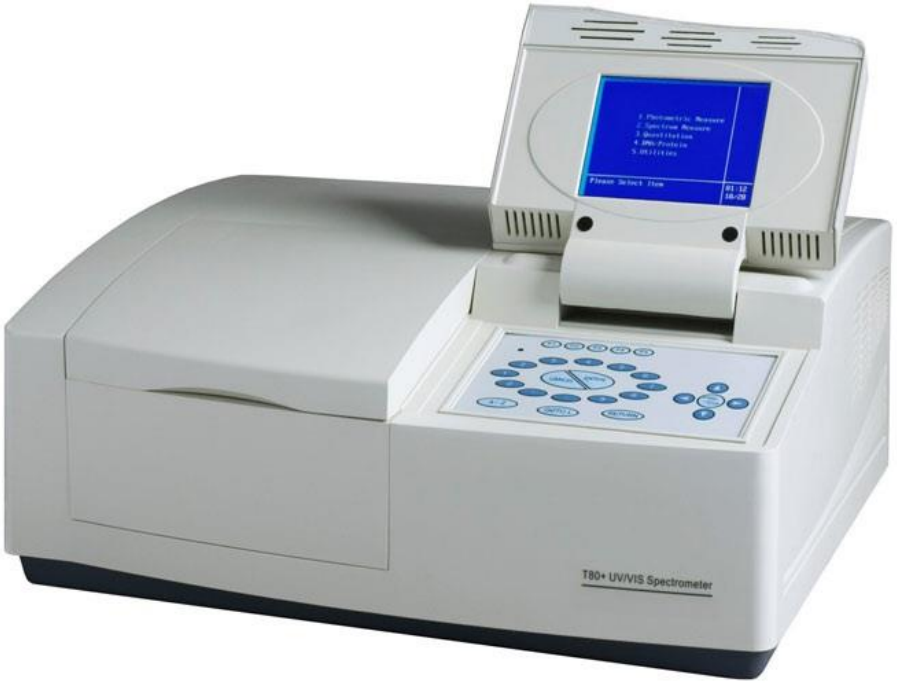


Figure 3.2. UV spectrophotometer

Figure 3.3. Distilled water apparatus

Figure 3.4. Hardness tester

Figure 3.5. Electronic Balance

3.13. Apparatus:

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

Table 3.6. Representing the apparatus (Kuss, 1992)

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

Chapter Four

RESULTS AND DISSCUSSION

4. Results & Discussion

4.1. Preparation and method of standard curve of Cloron:

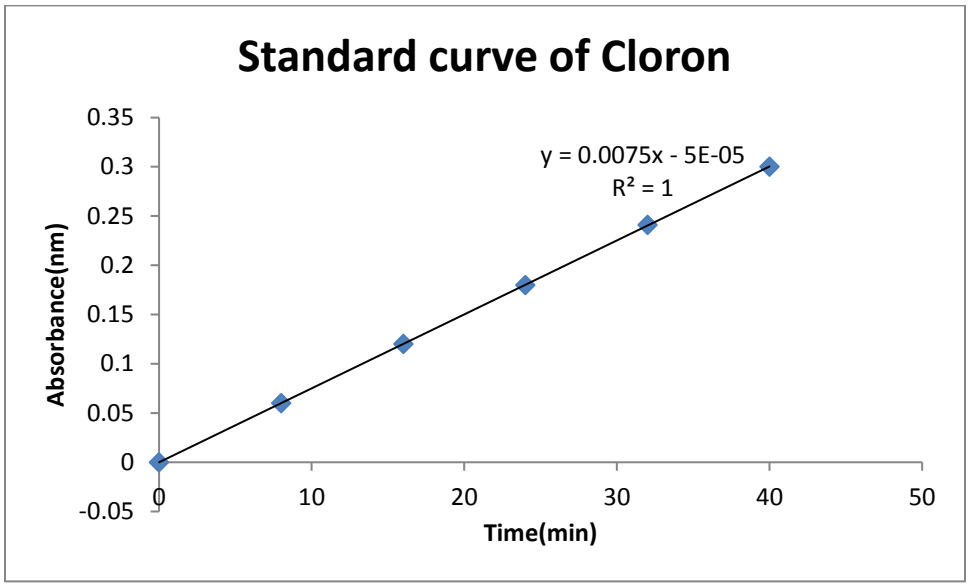


Figure.4.1 Standard curve of Cloron

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 4.1. 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:

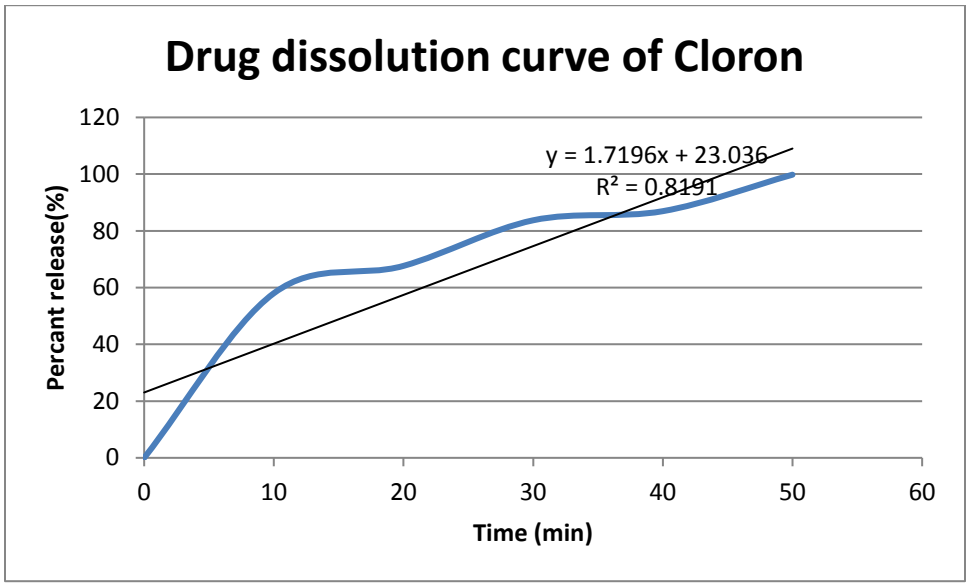


Figure.4.2 Dissolution curve of Cloron

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 4.2. 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:

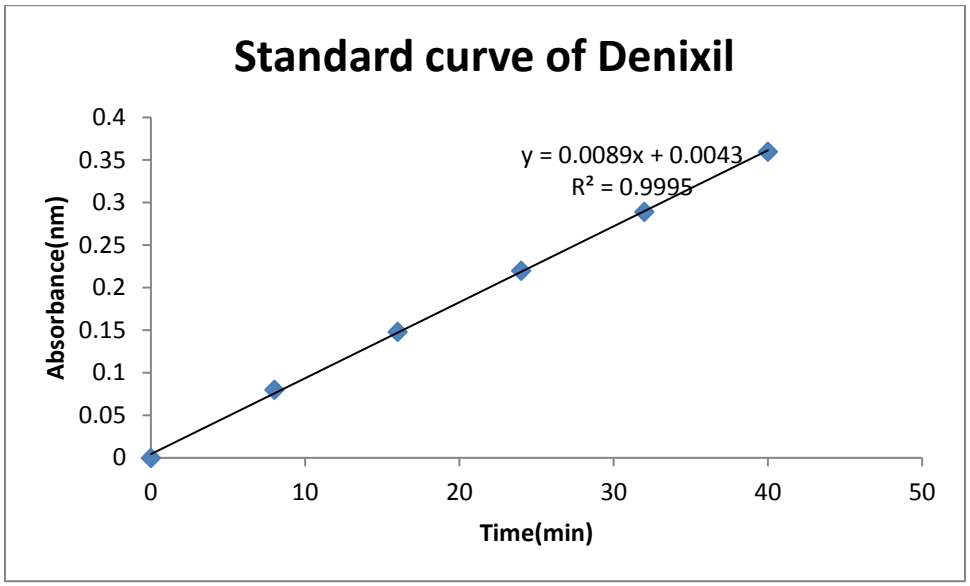


Figure.4.3 Standard curve of Denixil

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 4.3. 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:

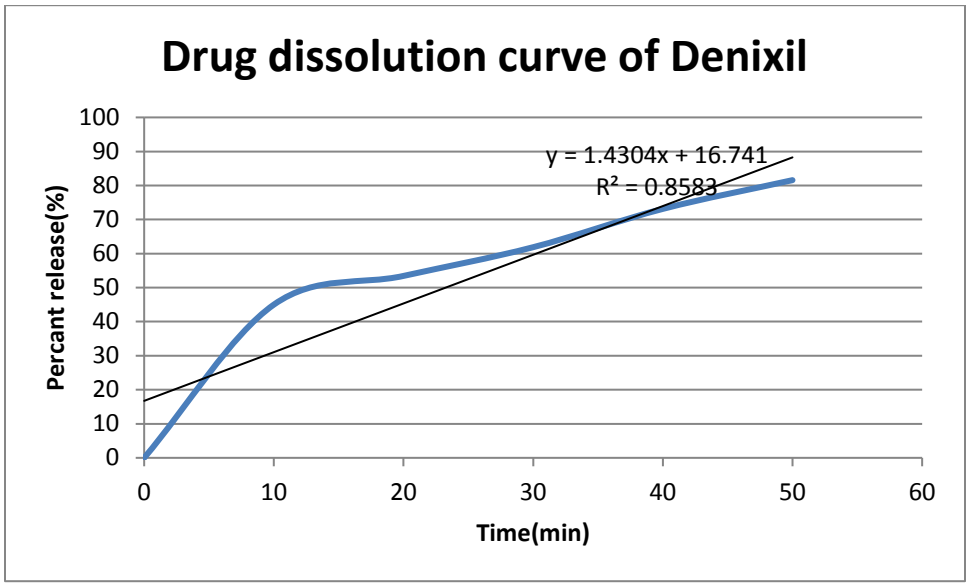


Figure.4.4 Dissolution curve of Denixil

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 4.4. 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:

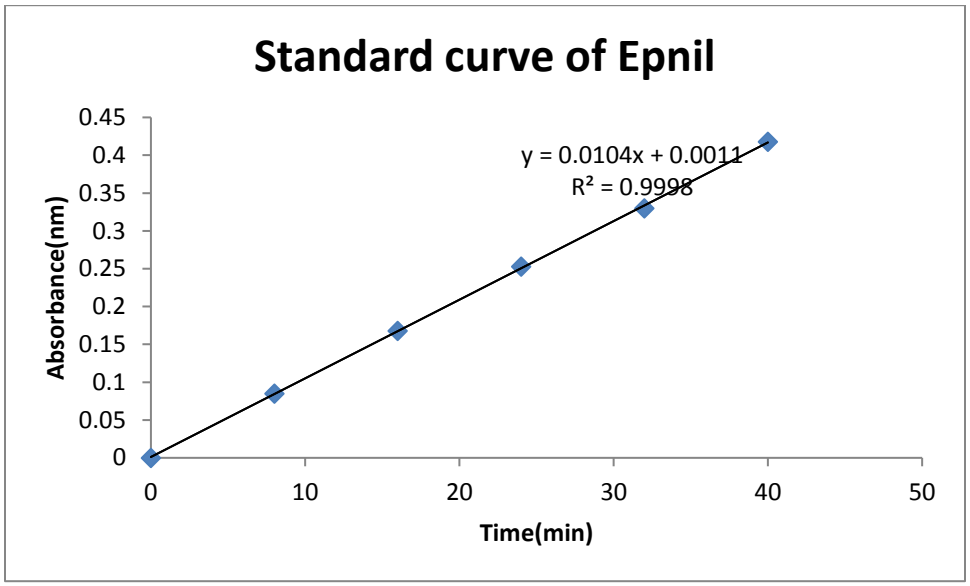


Figure.4.5 Standard curve of Epnil

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 4.5. 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:

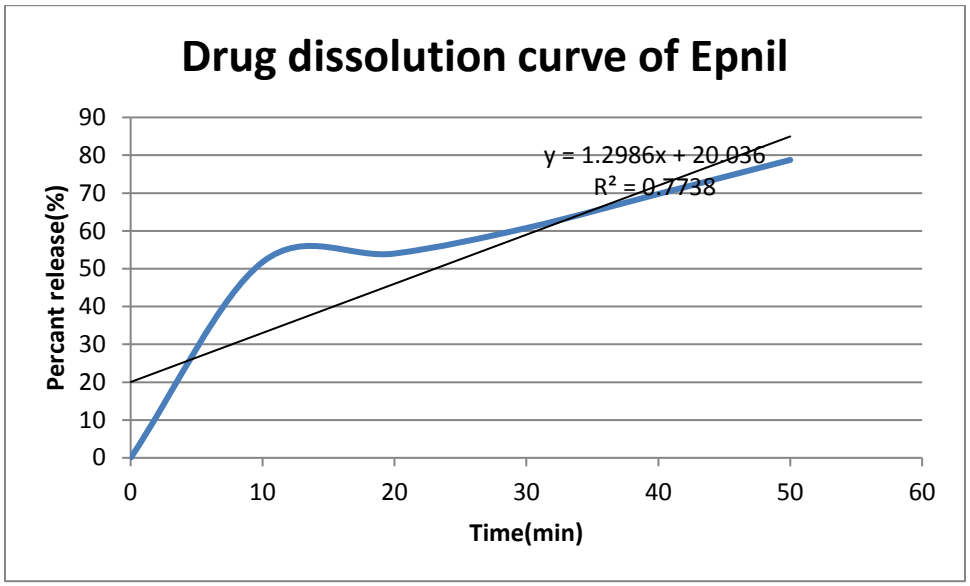


Figure.4.6 Dissolution curve of Epnil

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 4.6. 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:

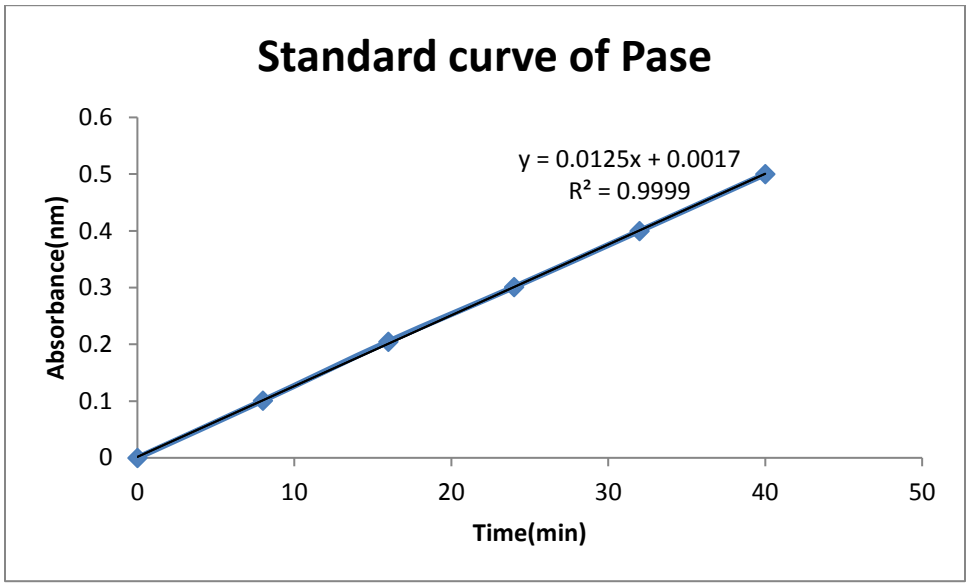


Figure.4.7 Standard curve of Pase

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 4.7. 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:

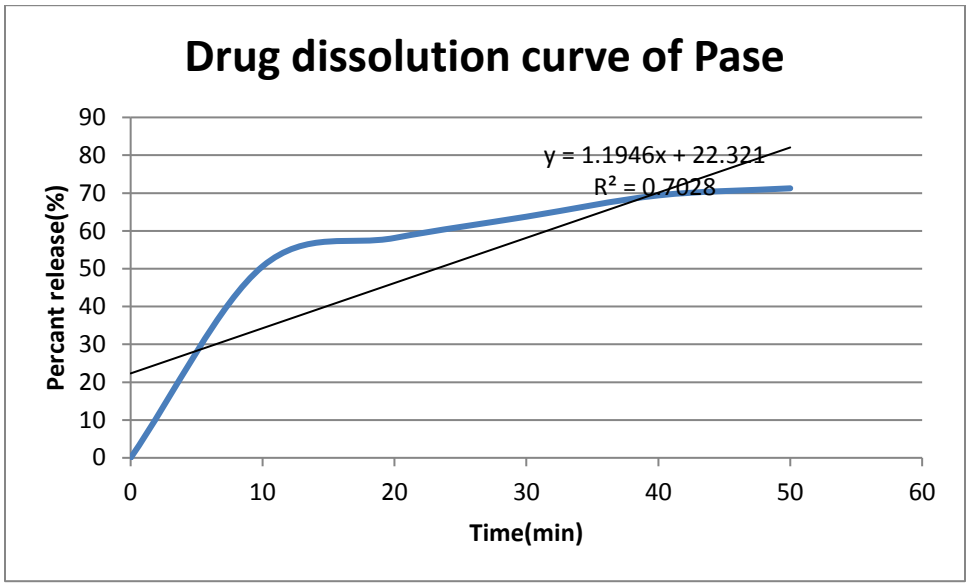


Figure.4.8 Dissolution curve of Pase

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 4.8. 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:

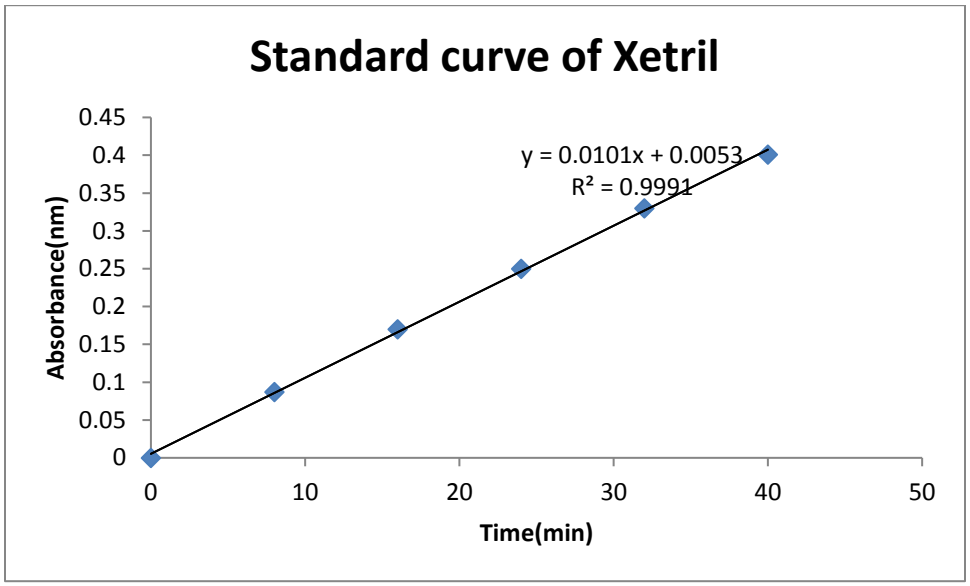


Figure.4.9 Standard curve of Xetril

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 4.9. 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:

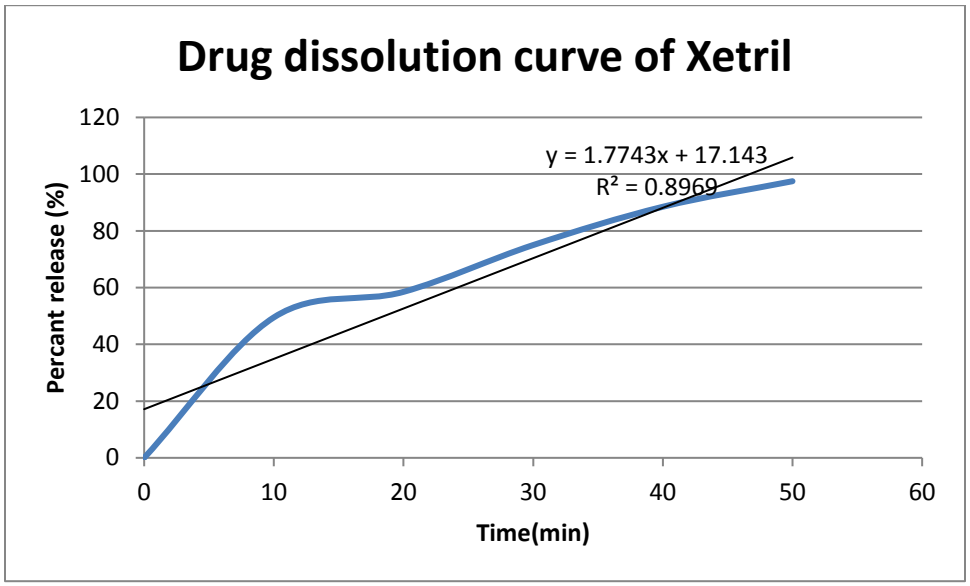


Figure.4.10 Dissolution curve of Xetril

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 4.10. 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. Zero order drug release profile:

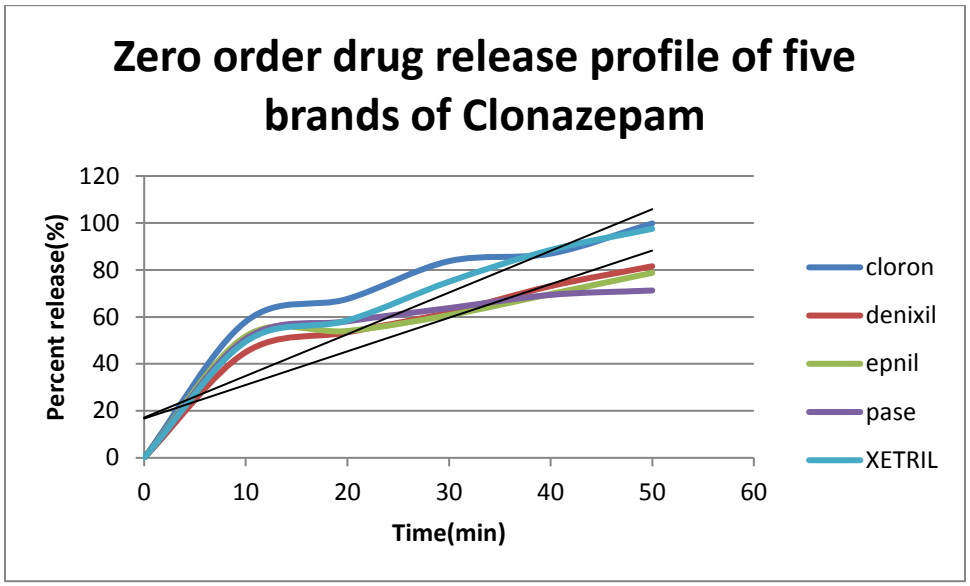


Figure.4.11 Zero order drug release profile of five brands of Clonazepam

Method of analysis: During the determination of dissolution curve, the % release data that were used have now been used cumulatively to determine the zero-order drug release profile of each brand at a time. From the integrated zero order equation, $\mathbf{C=K_0.t}$ we can observe that time (min) will be along the x axis and % release along the Y axis. From this cumulative %release vs. time (min) graph, we can calculate the $\mathbf{R^2}$ values of five brands that lead us to the final discussion about drug release kinetic profile.

Table 4.11. $\mathbf{R^2}$ values of five different brands of Clonazepam for zero order drug release profile

Name	$\mathbf{R^2}$ values
Cloron	0.819
Denixil	0.858
Epnil	0.773
Pase	0.702
Xetril	0.896

4.12. First order drug release profile:

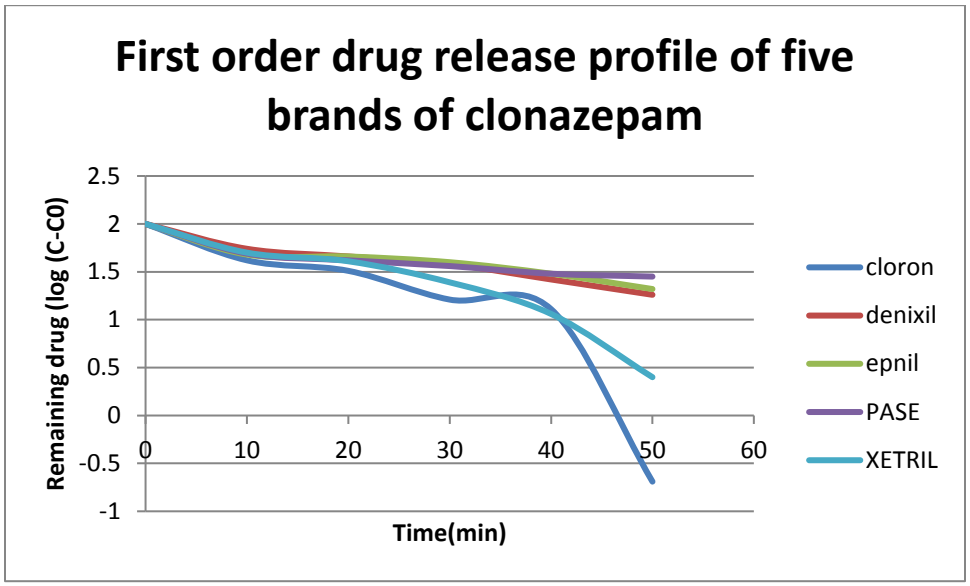


Figure.4.12 First order drug release profile of five brands of Clonazepam

Method of analysis: During the determination of dissolution curve, the % release data that were used have now been used cumulatively to determine the first-order drug release profile of each brand at a time. From the integrated first order equation, **log (C-C_0) =K_0.t** we can observe that time (min) will be along the x axis and % release along the Y axis. From this cumulative %release vs. time (min) graph, we can calculate the **R^2** values of five brands that lead us to the final discussion about drug release kinetic profile.

Table 4.12. R^2 values of five different brands of Clonazepam for first order drug release profile

Name	**R^2** values
Cloron	0.747
Denixil	0.969
Epnil	0.907
Pase	0.843
Xetril	0.917

4.13. Higuchi drug release profile:

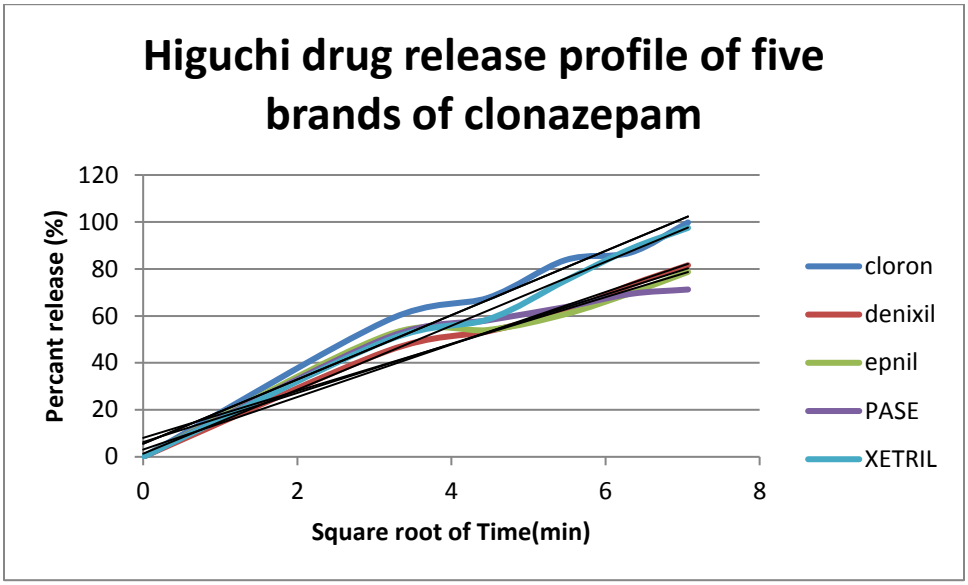


Figure.4.13 Higuchi drug release profile of five brands of Clonazepam

Method of analysis: During the determination of dissolution curve, the % release data that were used have now been used cumulatively to determine the Higuchi's drug release profile of each brand at a time. From the Higuchi's equation, $\boldsymbol{Q = [D(2A - C_s)C_s t]^{1/2}}$we can observe that square root of time (min) will be along the x axis and %release along the Y axis. From this cumulative %release vs. square of time (min) graph, we can calculate the $\mathbf{R^2}$ values of five brands that lead us to the final discussion about drug release kinetic profile.

Table 4.13. $\mathbf{R^2}$ values of five different brands of Clonazepam for Higuchi's drug release profile

Name	$\mathbf{R^2}$ values
Cloron	0.974
Denixil	0.985
Epnil	0.944
Pase	0.922
Xetril	0.992

4.14. Comparison among different R^2 values and final discussion on drug release profile:

Brands	**R^2** values zero order	**R^2** values first order	**R^2** values Higuchi's
Cloron	0.819	0.747	0.974
Denixil	0.858	0.969	0.985
Epnil	0.773	0.907	0.944
Pase	0.702	0.843	0.922
Xetril	0.896	0.917	0.992

4.15. Final discussion:

From the above chart, we can conclude by saying that, the highest R^2 value for a particular brand of any release kinetic profile that may follow either zero/first/Higuchi's drug release profile will be assumed to have been released following that particular release kinetic equation. For Xetril the highest R^2 value = 0.992 is for Higuchi equation so Xetril will be released from its solid matrix in the dissolution media following Higuchi equation presumably. Similarly, the highest R^2 values for Pase, Epnil, Cloron, Denixil are 0.922, 0.944, 0.974, 0.985 that clearly indicated that all of the rest brands of Clonazepam also followed the Higuchi equation.

Conclusion:

We can conclude by saying that, the highest R^2 value for a particular brand of any release kinetic profile that may follow either zero/first/Higuchi's drug release profile will be assumed to have been released following that particular release kinetic equation. For Xetril the highest R^2 value = 0.992 is for Higuchi equation so Xetril will be released from its solid matrix in the dissolution media following Higuchi equation presumably. Similarly, the highest R^2 values for Pase, Epnil, Cloron, Denixil are 0.922, 0.944, 0.974, 0.985 that clearly indicated that all of the rest brands of Clonazepam also followed the Higuchi equation.

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