Determination of the Release Kinetics of Drug from Five Brands of Clonazepam Available in Bangladesh (Clonium, Disopan, Epitra, Rivotril, Epiclone)

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

Rafat Shahriar Islam

ID: 2013-1-70-036

Research Supervisor

Tirtha Nandi

Lecturer

Department of Pharmacy

East West University

Declaration by the Candidate

I, Rafat Shahriar Islam, hereby declare that the dissertation entitled ***"Determination of the Release Kinetics of Five Brands of Clonazepam Available in Bangladesh"*** 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.

_____

Rafat Shahriar Islam
ID: 2013-1-70-036
Department of Pharmacy
East West University, Dhaka.

Certificate by the Supervisor

This is to certify that the thesis entitled **"*Determination of the Release Kinetics of Five Brands of Clonazepam Available in Bangladesh*"** 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 Rafat Shahriar Islam, ID: 2013-1-70-036, 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.

_____

Supervisor

Tirtha Nandi

Lecturer

Department of Pharmacy

East West University, Dhaka.

Endorsement by the Chairperson

This is to certify that the thesis entitled **"*Determination of the Release Kinetics of Five Brands of Clonazepam Available in Bangladesh*"** 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 Rafat Shahriar Islam, ID: 2013-1-70-036, during the period 2017 of his research in the Department of Pharmacy, East West University.

_____

Dr. Shamsun Nahar Khan
Associate Professor & Chairperson
Department of Pharmacy
East West University, Dhaka

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, Depertment 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 Md. Wazi Ullah, Shariful Hasan, Shahdat Shimul, Mahbubur Rahman, and my all friends, who gave me support for my research work and for their extended cooperation for my study.

Dedication

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

Abstract

The purpose of this study was to determine the *in vitro* release kinetics of five brands of clonazepam tablets available in the local pharmaceutical market of Bangladesh. For this study, five widely prescribed brands Epitra, Epiclone, Rivotril, Clonium and Disopan were chosen. All of these brands were of 0.5 mg Clonazepam with strip packaging. The dissolution was carried out using USP apparatus-II and the 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 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 Clonium (R^2=0.9843), Disopan (R^2=0.9548), Epiclone (R^2=0.9726), Epitra (R^2=0.9578), Rivotril (R^2=0.9334) was the highest amongst the R2 values comparing to zero order and first order values. So, this study assumes that the available Clonazepam tablet brands available in Bangladesh generally follow the Higuchi's drug release kinetics.

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

.

Contents

Chapter 1: Introduction

List of Figures (chapter 1)

List of Tables (chapter 1)

Contents

Contents

Chapter 3: Materials and Methods

List of Figure (Chapter 3)

List of Tables (Chapter 3)

Contents

Chapter 4: Results and Discussions

4.2	Preparation of dissolution curve of Clonium	3
4.3	Prep. of Standard curve of Disopan	4
4.4	Preparation of dissolution curve of Disopan	5
4.5	Preparation of standard curve of Epiclone	6
4.6	Preparation of dissolution curve of Epiclone	7
4.7	Preparation of standard curve of Epitra	8
4.8	Preparation of dissolution curve of Epitra	9
4.9	Preparation of standard curve of Rivotril	10
4.10	Preparation of dissolution curve of Rivotril	11
4.11	Zero order drug release profile kinetics	12
4.12	First order drug release profile kinetics	13
4.13	Higuchi's drug release profile kinetics	14
4.14	Comparison of R^2 values of five brands	15
4.15	Final discussions	15

List of Figures (chapter 4)

List of Table (chapter 4)

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:

The sedative-hypnotics belong to a chemically heterogeneous class of drugs which produce dose-dependent CNS depressant effects. A major subgroup is the **benzodiazepines**, but representatives of other subgroups, including **barbiturates**, and miscellaneous agents (carbamates, alcohols, and cyclic ethers) are also under this class. Other newer drugs named as 'nonbarbiturate nonbenzodiazepine sedative-hypnotics' include the anxiolytic buspirone, several widely-used hypnotics (zolpidem, zaleplon, eszopiclone), and melatonin agonists and orexin antagonists, novel drugs used in sleep disorders like insomnia and also anxiety. (Morishita, 2010.)

Anxiolytics: A drug which reduces anxiety and causes calm and quietness in the patient. E.g. Alprazolam.

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

Hypnotics: A drug that produced drowsiness and facilitates the onset and maintenance of a state of sleep that resembles natural sleep. E.g. Chlordiazepoxide (Jenner & Pratt, 2011)

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.Most cases of severe sedative-hypnotic poisoning are deliberate (suicidal). These agents are also commonly abused as recreational drugs. (Morishita, 2012)

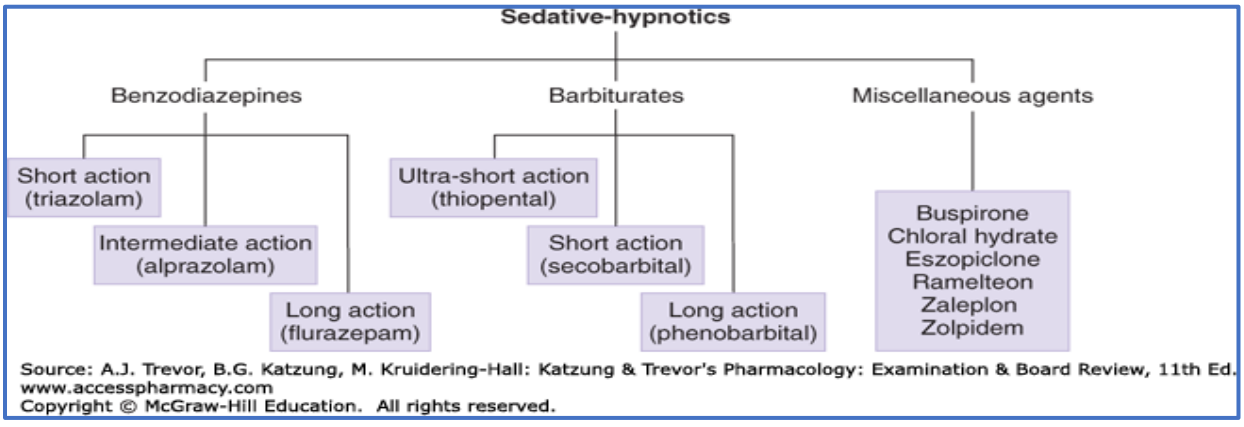


Fig 1.1. A short overview on the classification of sedative-hypnotic drugs

1.2. Benzodiazepines: The benzodiazepines are frequently classified into three groups: (1) short-acting (2) intermediate-acting, and (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 & Brown, 2012)

1.3. Benzodiazepines:

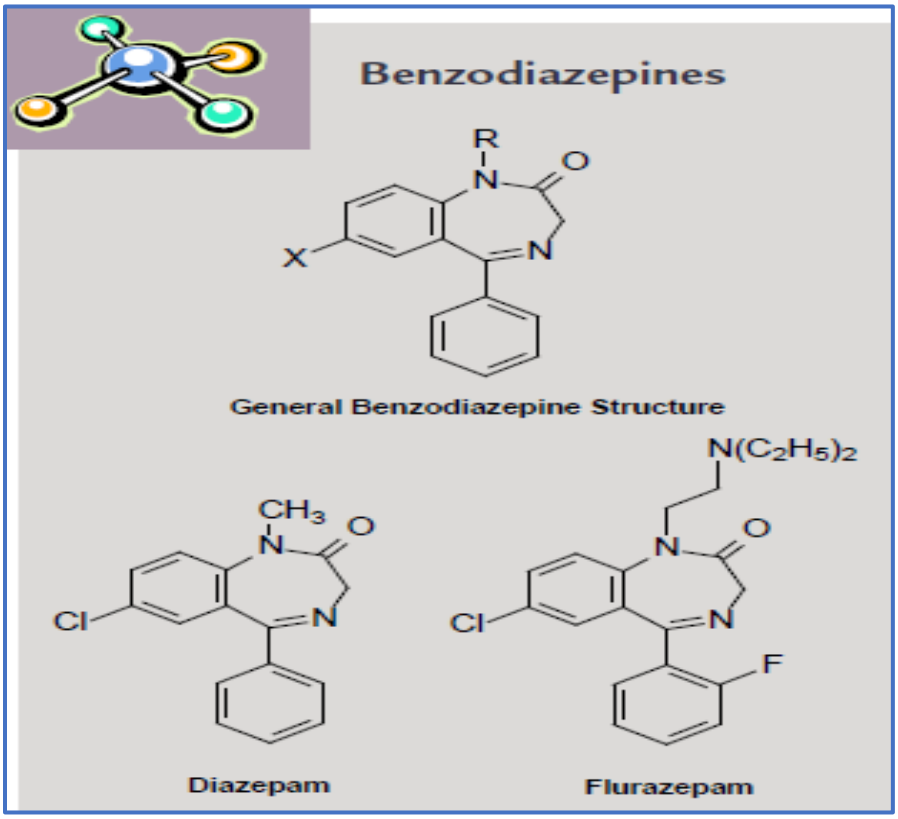


Figure 1.2. General structure of benzodiazepines and its analogues

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. (Hewlett, Vinogradov and Agras, 2011)

The benzodiazepines are frequently classified into three groups:

(1) Short-acting,

(2) Intermediate-acting, and

(3) Long-acting.

The duration of action for an individual benzodiazepine plays a major role in determining how that specific drug will be used clinically. The duration of action is dependent on two factors: (1) the half-life and (2) the metabolic fate of the benzodiazepine.

The first factor, the drug half-life, is the time it takes for 50% of the drug be eliminated. The longer the half-life, the longer the duration of action.

The second factor that determines the duration of action is the metabolic fate of the benzodiazepine after it enters the body. In many cases, a benzodiazepine will be metabolized by enzymes in the body to another benzodiazepine with the same pharmacodynamic effects.

(Woods, 2006)

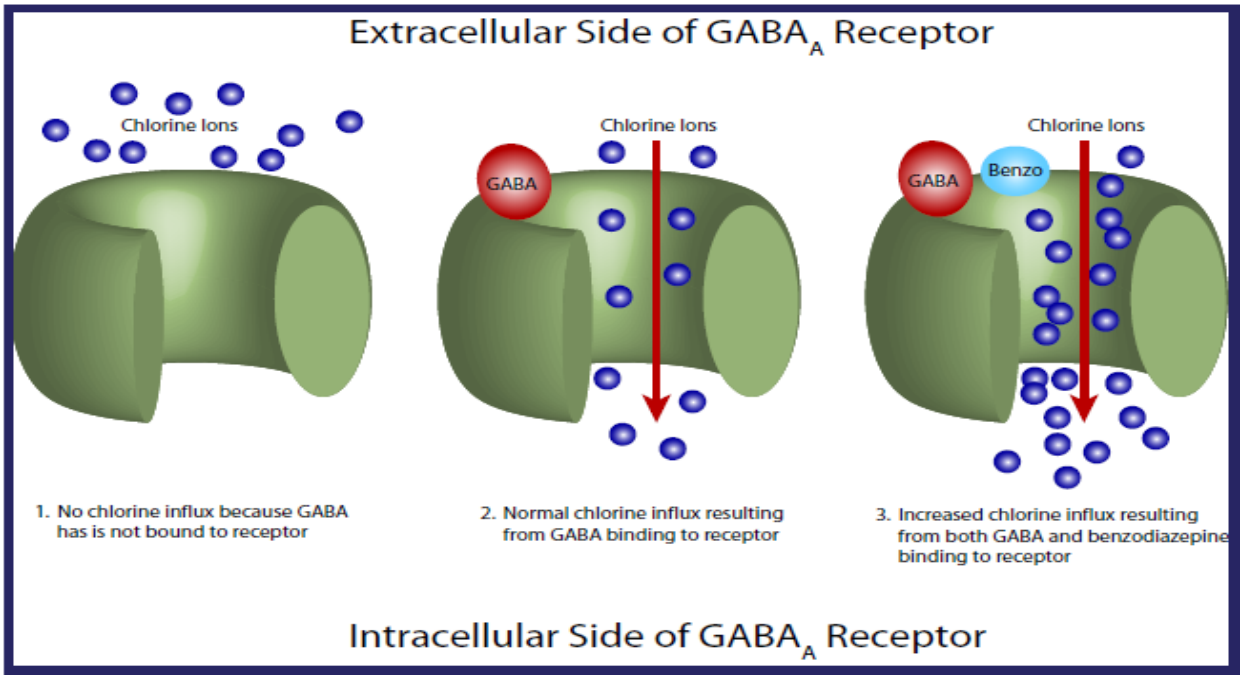


Figure 1.3. Mechanism of action of Benzodiazepines and their analogues.

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.

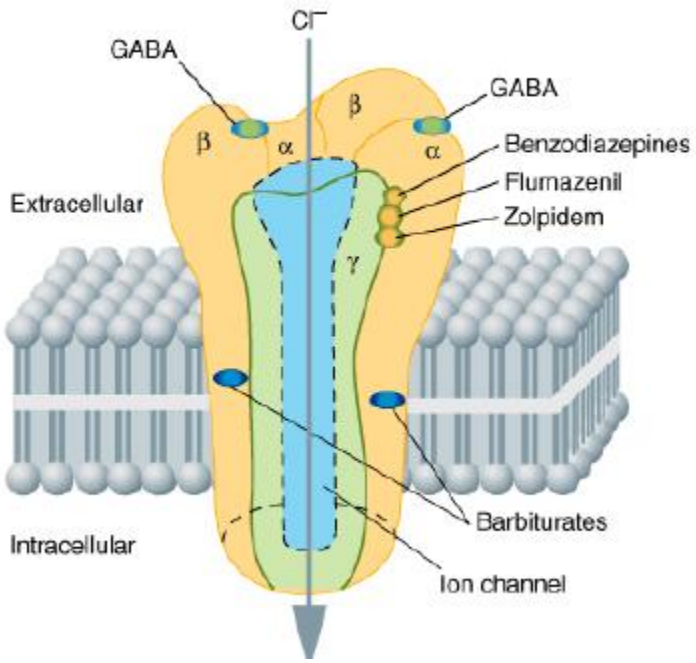


Figure 1.4. Action of Benzodiazepine antagonist (Flumazenil)

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. (Hewlett & Vinogradov, 2011)

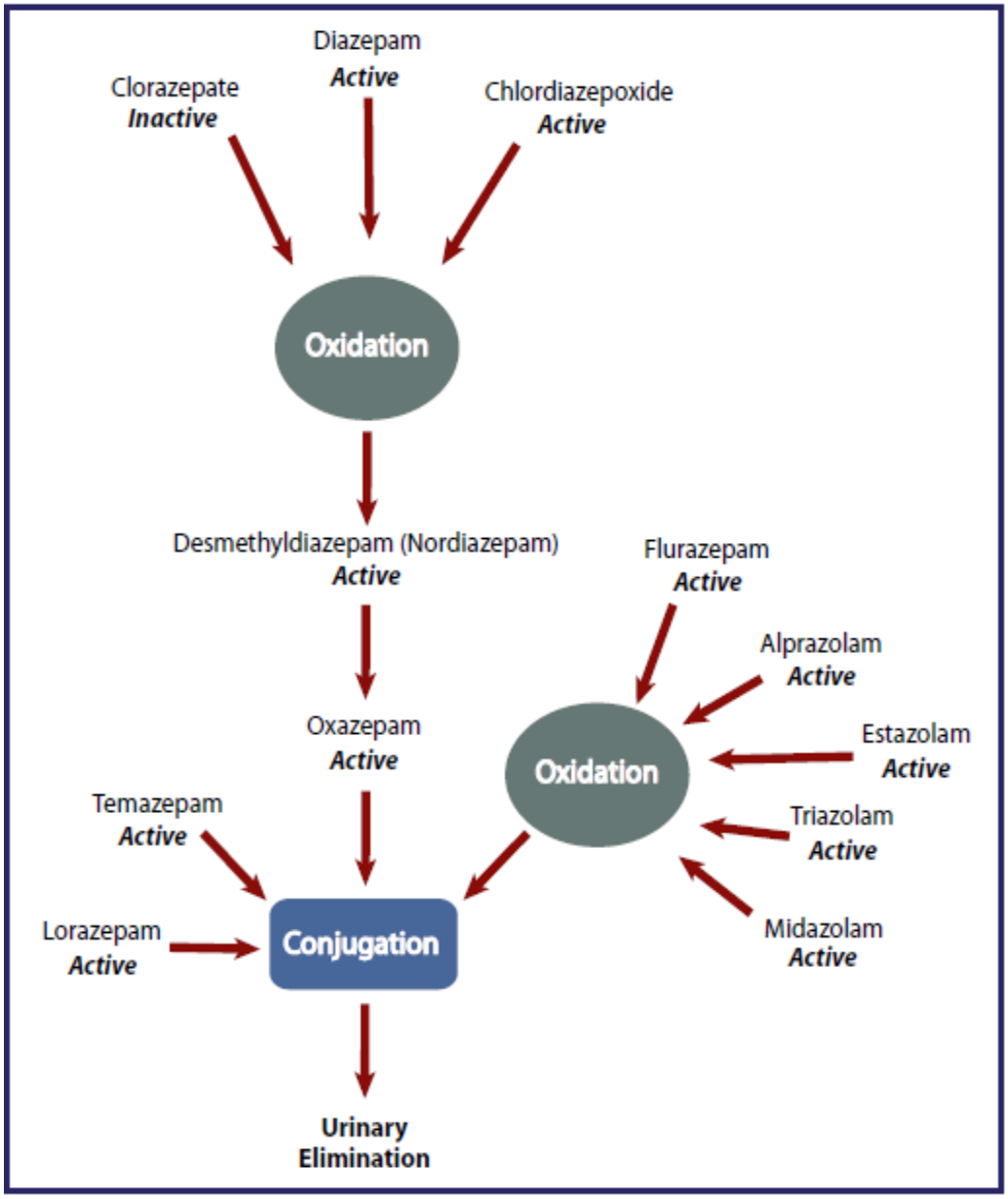


Figure 1.5. Fate of Benzodiazepines. (Spier & Tesar, 2006)

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 & Tesar, 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 & Pereira, 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, 2009)

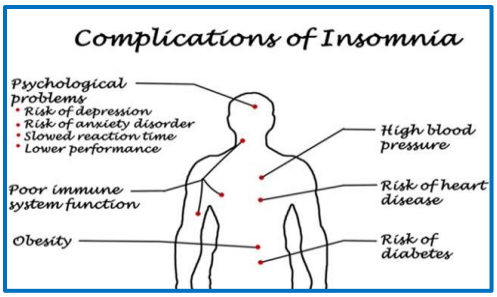


Fig1.6. Insomniac complications

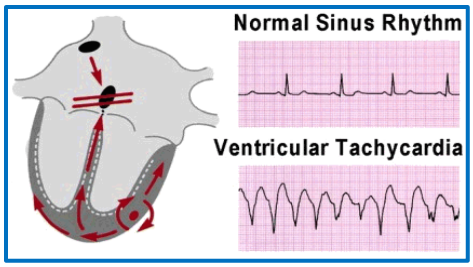


Fig1.7. Ventricular tachycardia

1.7. Dissolution:

For most dosage forms to be efficacious, the API(s) must be absorbed into the systemic circulation so that it can be transported to its site of activity. This process contributes to the bioavailability of the drug substance and involves two steps: dissolution and absorption (or permeability). Understanding the multi-step dissolution process is essential to proper in vitro method development. 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, 2005)

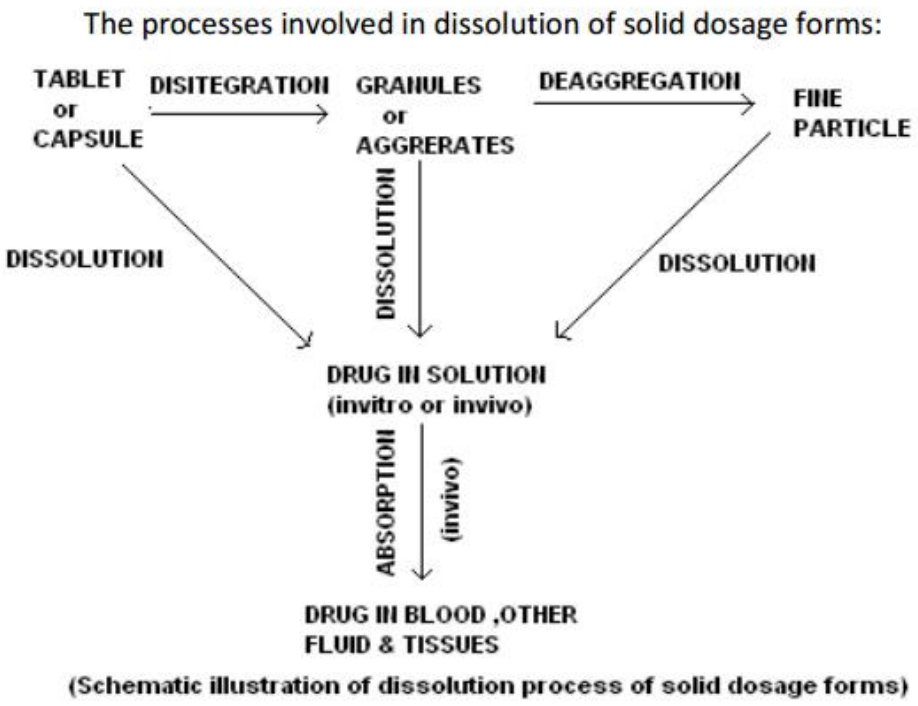


Fig 1.8. Dissolution process of solid dosage forms

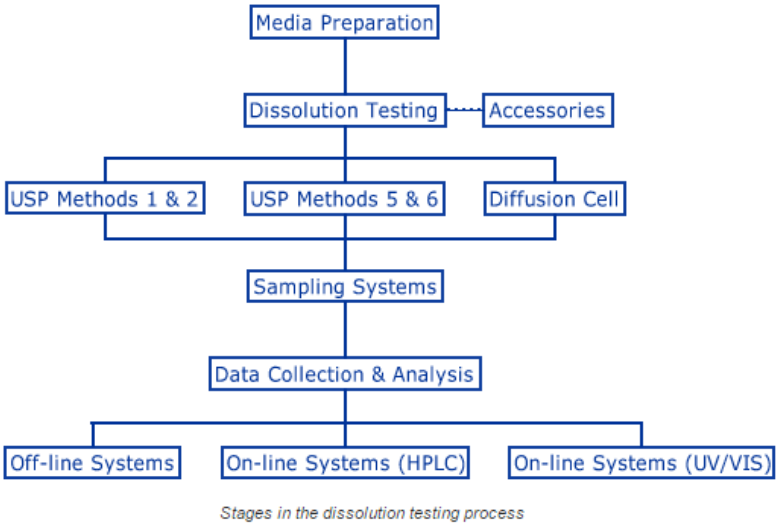


Fig 1.9. Stages in the dissolution testing process

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 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).

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 & Cheon, 2008)

Tablet Dissolution is a standardised method for measuring the rate of drug release from a dosage form. 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) (Patel and Purohit, 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 Dbioavailability or bioequivalence studies.

2. Quality assurance

D.T. 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. (Patel and Purohit, 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.

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. (Jung, et al, 2001)

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:

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

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

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, et al, 2001)

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: (Jung, et al, 2001)

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.

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. Clonazepam is used to treat certain seizure disorders (including absence seizures or Lennox-Gastaut syndrome) in adults and children. Clonazepam is also used to treat panic disorder (including agoraphobia) in adults.

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:

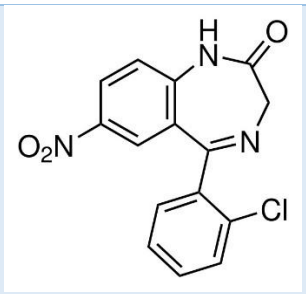
Topic	Information
Generic Name	Clonazepam
Chemical Name	5-(2-chlorophenyl)-1, 3-dihydro-7-nitro-2H-1, 4-benzodiazepin-2-one.
Molecular Formula	$C_{15}H_{10}ClN_3O_3$
Molecular Mass	315.7
Physiochemical Properties	Clonazepam is a white to yellow-white odourless fine powder. The pH of clonazepam is between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

Fig 1.10. Clonazepam 0.5 mg & 2 mg tablet

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 & Suresh, 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, Suresh and Swamy, 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, Suresh and Swamy, 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 of age 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 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 & Suresh, 2011)

Table1.2. 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.

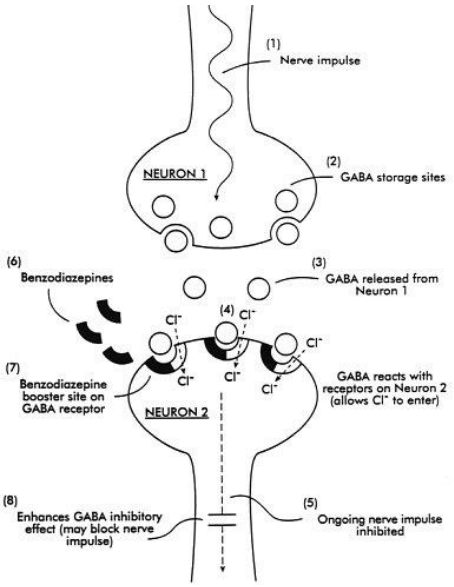


Fig 1.11. Mechanism of action of Clonazepam (Benzodiazepine drug) (Mura, 2005)

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 higher than those after a single oral dose; the predicted accumulation ratios for two times and three times daily regimens are 5 and 7, respectively. Following multiple oral doses of 2 mg three times daily steady-state pre-dose plasma concentrations of clonazepam averaged 55 ng/ml. The plasma concentration-dose relationship of clonazepam is linear. The target anticonvulsant plasma concentrations of clonazepam range from 20 to 70 ng/ml.

1.27.2. Distribution:

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

1.27.3. 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 excretion of unchanged clonazepam is usually less than 2% of the administered dose. The elimination kinetics in children are similar to those observed in adults. (Mura, Nassini & Proietti, 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, Nassini & Proietti, 2004)

1.29. Different brands available in Bangladesh:

Table 1.3. Different brands available in Bangladesh

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

Disoan	Incepta Pharmaceuticals Ltd.
Epiclon	General Pharmaceuticals Ltd.
Epitra	Square Pharmaceuticals Ltd.
Epizam	Alco Pharma Limited
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

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. (Shirsand et al ,2004)

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. (de Oliveira, Neves, Soares, 2012)

A Simple, efficient and reproducible reverse phase high performance liquid chromatographic method was developed and validated for the Simultaneous determination of Escitalopram oxalate and Clonazepam in combined dosage form. The retention time of Escitalopram oxalate and Clonazepam was found to be 2.840± 0.007min and 4.007±0.006 min. Calibration curve was linear over the concentration range of 20-120µg/ml and 1-6µg/ml for Escitalopram oxalate and Clonazepam. All the analytical validation parameters were determined and found in the limit as

per ICH guidelines, which indicates the validity of the method. The developed method is also found to be precise, accurate, specific, robust and rapid for the simultaneous determination of Escitalopram oxalate and Clonazepam in tablet dosage forms. (Bhimanadhuni, 2012)

Dispersive liquid–liquid micro–extraction (DLLME) technique was successfully used as a sample preparation method for the determination of clonazepam in pharmaceutical preparations and water samples. In this method, a suitable mixture of methanol (disperser solvent) and chloroform (extraction solvent) was injected rapidly into a conical test tube that contained an aqueous solution of clonazepam. After centrifuging, phase separation was performed by sedimenting the fine droplets of the micro–extraction solvent on the bottom of a test tube (about 100 μL) and then the absorbance of the enriched extracted phase was determined at the absorption wavelength of clonazepam (307 nm). Some important parameters such as, the type and volume of extraction and dispersive solvents as well as the extraction time were investigated and optimized. (Miri, L. and Jalali, 2013)

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 nonvolatile 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. (Karmarkar, 2009)

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. Some sedative overdoses can be treated with flumazenil, a reversal agent, along with supportive care. 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. (Holgado, et al 2005)

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. (Sanka & Poienti, 2014)

Depression and anxiety disorders are distinct illnesses that often coexist. An open label comparative multicentric study was conducted to assess the efficacy and safety of therapy with fixed dose combination capsules of Paroxetine (25 mg) controlled release (CR) and Clonazepam (0.5 mg) in comparison to Paroxetine (25 mg) controlled release (CR) tablets in Indian patients suffering from co-morbid depression and anxiety. Patients between 18-65 years of age and established diagnosis of co-morbid depression and anxiety. The primary efficacy variables were the change in the score on the Hamilton Rating Scale for Depression (HAM-D) and Hamilton Rating Scale for Anxiety (HAM-A) at the end of treatment as compared to baseline. In conclusion, we found that the fixed dose combination capsules of Paroxetine (25 mg) controlled release (CR) and Clonazepam (0.5 mg) are more effective and equally well tolerated as

Paroxetine (25 mg) controlled release (CR) tablets in the management of patients with co-morbid depression and anxiety at our centre. (Weaver, 2015)

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. (Eldin & Shalaby, 2014)

Clonazepam (CLZ) is an anticonvulsant benzodiazepine widely used in the treatment of epilepsy. CLZ is a BCS Class II drug and its bioavailability is thus dissolution limited. The objective of the present study was to prepare solid dispersions (SDs) of CLZ by various techniques, using the amphiphilic carrier Gelucire 50/13 in various proportions, to increase its water solubility. Drug-polymer interactions were investigated by Fourier-transform infrared (FTIR) and Ultra-Violet (UV) spectroscopy. A phase solubility study was performed and the stability constant (Ks) was found to be 275.27, while the negative Gibbs free energy (ΔG_{tr}^o) indicated spontaneous solubilization of the drug. The dissolution study showed that the SDs considerably enhanced the dissolution rate of the drug. The FTIR and UV spectra revealed no chemical incompatibility between the drug and Gelucire 50/13. XRD patterns and the DSC profiles indicated the CLZ was in the amorphous form, which explains the improved dissolution rate of the drug from its SDs. The tablets were characterized by in-vitro disintegration and dissolution tests. In conclusion, this investigation demonstrated the potential of solid dispersions of a drug with Gelucire 50/13 for promoting the dissolution of the drug and contributed to the understanding of the effect of a superdisintegrant on mouth dissolving tablets containing a solid dispersion of a hydrophobic drug. (Jagawat, 2011)

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 long term 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. (Khan, 2013)

A Simple, efficient and reproducible reverse phase high performance liquid chromatographic method was developed and validated for the Simultaneous determination of Escitalopram oxalate and Clonazepam in combined dosage form. The separation was effected on a Hypersil ODS C18 column (250mm X 4.6mm; 5µ) using a mobile phase mixture of buffer and acetonitrile. All the analytical validation parameters were determined and found in the limit as per ICH guidelines, which indicates the validity of the method. The developed method is also found to be precise, accurate, specific, robust and rapid for the simultaneous determination of Escitalopram oxalate and Clonazepam in tablet dosage forms. (Narasimharaju and Rao, 2012.)

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. (Seshadri, 2013)

A potentiometric method is reported for clonazepam determination in biological fluid models. A simple, rapid and sensitive method for the determination of clonazepam in biological fluid models. The clonazepam selective MIP was synthesized from methacrylic acid as the functional monomer and ethylene glycol dimethacrylate as the cross-linker in methanol solution using clonazepam as the template molecule and 2, 2-azobis isobutyronitrile as the initiator. Some parameters affecting the sensor response were optimized and then the calibration curve was plotted. After optimization, the carbon past electrode constructed with a MIP exhibited a Nernstian response 29.66 ± 1.0 mVdecade−1 in a wide concentration range, from 1.0×10-7 to 1.0×10-1 M, with a low detection limit of 7.3×10-7 M and the electrode showed a response time of less than 15 s. The optimum pH values for quantitative uptake of drug were 6 and it was determined by measuring the drug content in the supernatant liquid. Finally, the proposed electrode was successfully used for potentiometric determination of clonazepam in biological fluid models and pharmaceutical samples. (Soroush & Maryam, 2016)

The ability of *in vitro* biorelevant dissolution tests to predict the *in vivo* performance of nanosized fenofibrate (Lipidil 145 ONE®) and microsized fenofibrate (Lipidil – Ter®) was evaluated in this study. *In vitro* dissolution was carried out using USP apparatus 2 (paddle method) with updated biorelevant media to simulate the pre- and postprandial states. Membrane filters with different pore sizes were evaluated for their ability to hold back undissolved, nanosized drug particles. It was shown that filters with pore sizes of 0.1 μm and 0.02 μm were able to separate molecularly dissolved drug from colloidal and undissolved particles. The

simulated plasma profiles were compared to *in vivo* data for the nanosized and the microsized formulation in the fasted and fed states. The first model approach resulted in good correlation for the microsized fenofibrate formulation, but the plasma profile of the formulation containing nanosized fenofibrate was overpredicted in the fasted state. The second model successfully correlated with *in vivo* data for both formulations, regardless of prandial state. Comparison of simulations with the two models indicates that in the fasted state, absorption of fenofibrate from the nanosized formulation is at least partly permeability-limited, while for the microsized formulation the dissolution of fenofibrate appears to be rate-determining. (Juenemann, 2011)

Evaluating an IVIVC is a desirable feature for any drug dissolution test to establish relevance and confidence in assessing the quality and safety of solid oral dosage products, such as tablets and capsules. However, success in this area has been limited. One of the reasons for this lack of success may be that the approaches described in the literature to achieve IVIVC appear to be intuitive expectations rather than an objective end-point based on scientific rationale. For example, rather than predicting an in vivo response based on in vitro results, which is the objective of IVIVC, attempts are usually made to match in vitro results with in vivo results by adjusting experimental conditions for in vitro testing. This article provides a discussion and clarification on the underlying scientific principles to help in alleviating current difficulties in developing IVIVC. Further, it provides a simpler and practical approach based on experimental studies to achieve appropriate IVIVC by predicting blood drug levels from dissolution results. (Islam, 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. (Levy, 2003)

Evidence is presented to show that solid dosage forms are exposed to relatively low agitation intensities after oral administration, and that it is often mandatory to use similar mild agitation conditions for predictive *in vitro* dissolution tests. Based on considerations of the effect of stirring rate on boundary layer thickness, it is demonstrated that the effective surface area of heterogenous pellets (analogous in some respects to certain prolonged release dosage forms) may be different when such pellets are exposed to high and low agitation intensities, respectively. A proportionality between dissolution rate and the square root of stirring rate, demonstrated by others with rotating disks of inorganic salts, has been found to apply also to rotating disks of certain organic weak acids. It is shown that the ratio of dissolution rates of two or more drugs, when rates are determined by the rotating disk method, is independent of stirring rate, provided this proportionality or a similar more general relationship applies. (Soroush, Maryam, Mohamad and Maryam, 2016.)

In this study, the aim was to apply different comparison methods to dissolution profiles of immediate release commercial film-coated tablets of naproxen sodium in order to (1) evaluate each method in terms of easy application and usefulness and (2) identify the advantages and disadvantages of each method. Dissolution testing was conducted using the USP monograph of naproxen sodium. The applied methods for the comparison of in vitro dissolution profiles are ANOVA-based methods, model-dependent methods, and model-independent methods including difference factor, f_1, and similarity factor, f_2. All the methods appear to be applicable and useful in comparing dissolution profiles. The results show that ANOVA-based methods and model-dependent methods are more discriminative than the *f*-factors. *f*-Factors seem to be easier to apply and interpret; only one value is obtained to describe the closeness of the two dissolution profiles. However, a last point for dissolution had to be determined, since the values of the *f*-factors depend on this point. The application and evaluation of model-dependent methods are more complicated; these methods present an acceptable model approach to the true relationship between percent dissolved and time variables, including statistical assumptions which could be

checked. Dissolution profiles can be tested for differences in both level and shape by ANOVA-based methods and these methods provide detailed information about dissolution data which can be useful also in formulation development to match release to a reference product. (Juenemann, Jantratid & Wagner, 2011.)

The development of a single *in vitro* dissolution rate test is described, which correlates quantitatively with the gastrointestinal absorption, in man, of a test drug (aspirin) from three markedly different types of dosage forms. The *in vitro* conditions that yield such multiple correlations may be expected to be relatively similar to dissolution conditions found *in vivo*. It is suggested that the inclusion of an increasing number of variables (different drugs and different types of dosage forms) in future efforts to develop *in vitro–in vivo* correlations will permit further refinement of the *in vitro* test. This could eventually lead to a relatively generalized test procedure suitable for development and control purposes, and for inclusion in official compendia as a test of physiologic availability. (Islam, Shahriar and Dewan, 2011)

Block copolymers consisting of poly (γ-benzyl l-glutamate) (PBLG) as the hydrophobic block and poly (ethylene oxide) (PEO) as the hydrophilic block were synthesized and characterized. Core-shell type nanoparticles of the block copolymers (abbreviated as GE) were prepared by the diafiltration method. The particle size diameter obtained by dynamic light scattering of GE-1 (PBLG content: 60.5 mol %), GE-2 (PBLG content: 40.0 mol %), GE-3 (PBLG content: 12.4 mol %) copolymer was 309.9±160.9, 251.9±220.6 and 200.5±177.1 nm, respectively. The shape of the nanoparticles by SEM or TEM was almost spherical. The critical micelle concentration of the block copolymers obtained by fluorescence spectroscopy was dependent on the chain length of hydrophobic PBLG. The micelle structure of the copolymer nanoparticle was very stable against sodium dodecyl sulfate. Clonazepam (CZ) was loaded onto the core part of the nanoparticle as the crystalline state. Release of CZ from the nanoparticles in vitro was dependent on the drug loading contents and PBLG chain length. (Levy, 1963.)

Clonazepam is a new benzodiazepine anticonvulsant recently approved by the Food and Drug Administration for the treatment of typical absence, infantile myoclonic, atypical absence, myoclonic, and akinetic seizures. It is rapidly absorbed by the oral route and appears to pass quickly from blood to brain. Preliminary results indicate a biological half-life of 22 to 32 hours

and a therapeutic serum concentration of 5 to 50 ng/ml. Many studies report tolerances to the anticonvulsant effects with chronic administration. Major side effects of the drug are drowsiness, ataxia, and behavior changes. They tend to be dose related, occur early in the course of therapy, and may subside with chronic administration. Accordingly, the dosage is begun at a low level and increased slowly. (Yuksel, Kanık, and Baykara, 2000.)

Experiments were carried out using patch-clamp techniques in rat thalamic slices, maintained in vitro, to examine the effects of the benzodiazepine compound, clonazepam (CZP), on intrathalamicinhibition. Bath-applied CZP reduced the gamma-aminobutyric acid-B (GABAB) component of inhibitory postsynaptic potentials and currents (IPSPs and IPSCs, respectively) evoked in rat thalamic somatosensory relay neurons by stimulation of nucleus reticularis thalami (nRt), without consistently affecting the GABAA IPSP. Secondary IPSPs, which occur as a result of intrathalamic oscillations, were dramatically reduced. 2. Voltage-clamp experiments combined with local or bath perfusion of the GABAA antagonist bicuculline methiodide (BMI), demonstrated that nRt is a site of GABAA-mediated postsynaptic inhibition that affects inhibitory output onto relay neurons. BMI enhanced both GABAA and GABAB postsynaptic inhibition in relay neurons when applied to nRt. Focal applications in the ventrobasal relay nucleus near the recording electrode blocked the GABAA-mediated IPSP but had no effects on GABAB inhibitory potentials. 3. Results suggest that CZP acts to facilitate recurrent inhibition in nRt and decrease its inhibitory output onto relay neurons. Intra-nRt GABAA-mediated inhibition thus has an important role in controlling thalamic excitability and in the anti-absence actions of CZP. (Levy, Leonards and Procknal, 1965.)

Serotonergic reuptake inhibitors have been the primary medications for treatment of obsessivecompulsive disorder (OCD); however, other serotonergic and [alpha]2-adrenergic medications also have been reported to reduce obsessive-compulsive symptoms. In this study, we compare three medications with reported efficacy in OCD to a control medication, diphenhydramine, a medication without theoretical or demonstrated treatment benefit. The three active medications were clomipramine, a serotonergic reuptake inhibitor; clonazepam, a benezodiazepine with putative serotonergic properties; and clonidine, an [alpha]2-adrenergic agonist. Twenty-eight subjects with DSM-III-R diagnosis of OCD rotated through 6-week trials of each of the four medications in a randomized, double-blind, multiple crossover protocol.

Clomipramine and clonazepam were both effective relative to the control medication in reducing OCD symptoms. There was a significant cross-response between these two medications; however 40% of subjects failing clomipramine trials had a clinically significant response to clonazepam treatment. The control medication, diphenhydramine, itself produced a significant decrement in symptoms, whereas clonidine was ineffective in reducing OCD symptoms. Clonazepam improvement was unrelated to changes in anxiety and occurred early in treatment. Clonazepam was significantly more effective than the other medications during the first 3 weeks of treatment. The results confirm the efficacy of clomipramine in the treatment of OCD and suggest that clonazepam might be a useful alternative treatment for patients with this disorder. (Jeong, Cheon, 1998)

The 7-nitrobenzodiazepine derivative clonazepam is initially biotransformed by nitroreduction, followed by acetylation. Neither the amino- nor the acetamido- metabolites appear to have important pharmacologic activity. Clonazepam elimination half-life falls in the range of 20 to 80 hours, but means within the population and variance are not well defined. Absorption of orally administered clonazepam is 80% or more. In experimental studies, clonazepam appears to diffuse passively from plasma into brain, with a constant brain-plasma concentration ratio. The drug disappears in a parallel fashion from both brain and plasma, with no evidence of sequestration in brain tissue. Clonazepam has a relatively high molar affinity for the benzodiazepine receptor in vitro, and the fractional extent of benzodiazepine receptor occupation by clonazepam in vivo is directly and predictably related to the drug's concentration in brain tissue. Acute behavioral effects are in turn directly related to the extent of receptor occupancy. Compared to other benzodiazepines, the reportedly unique clinical properties of clonazepam are neither associated with unusual/unexpected pharmacokinetic properties or with a qualitatively different in vivo interaction with the presumed benzodiazepine binding site. (Huguenard and Prince, 1994)

A collaborative in vitro dissolution study has been performed in 5 laboratories using the flow-through method with different cell types and at various hydrodynamic conditions. The USP disintegrating prednisone calibrator tablets have been used as a test formulation. The results obtained by the flow-through method were compared with data generated using the USP XXI paddle method. The flow-through method was found to produce reproducible and corresponding

dissolution data both within and between the different laboratories. It was found that the linear flow rate in the flow-through cells is a fundamental parameter for the dissolution rate of the formulation. There was a conformity in dissolution rate between cells with different diameters when applying the same linear flow rate of the dissolution medium using the flow-through method. At low flow rates the flow-through method was found by a sensible instrument to establish differences in the disintegration properties between the various prednisone tablets examined. (Hewlett & Vinogradov, 1992)

The present work deals with the comparison of in vitro dissolution profiles of fenofibrate liquisolid tablet formulations with those of marketed fenofibrate tablets, and the application of statistical methods to evaluate each method for its usefulness. The methods used to study dissolution profile comparison include Model independent method (Similarity factor, f2); Model dependent methods (Zero order, First order, Hixson-Crowell, Matrix, Peppas, Higuchi models) and statistical methods based on ANOVA. Model independent method was found to be easier and simple to interpret. The f2 value relates closeness of dissolution profiles. Dissolution profile followed Peppas model as "best fit" model. The application and evaluation of model dependent methods are more complicated. These methods give acceptable model approach which is indication of true relationship between percent drug release and time variables, including statistical assumptions. Statistical approach is very simple and is more discriminative of dissolution profiles. The liquisolid formulation of fenofibrate serves to be an effective way to enhance dissolution rate of fenofibrate. (Greenblatt & Miller, 2006)

The release behaviour of carteolol hydrochloride matrix tablets was investigated as a function of filler nature (Emcompress®, mannitol, PEG 6000 and lactose), type of wetting liquid (Eudragit ® L 12.5% and isopropanol-acetone mixture 6:4) and mode of filler incorporation. The values of the technological parameters suggest that hardness was the most significantly affected by the three formulation factors considered. The strongest influence over the technological parameters was exerted by the mode of filler incorporation. The kinetic data conformed with the Higuchi square root equation, except for the lots containing mannitol and isopropanol-acetone mixture that conformed to a first-order plot. The lot containing Emcompress® and isopropanol-acetone mixture displayed acceptable linearity with both plots. Therefore, a non-linear regression procedure and reduced time method were used to define with precision the kinetic model

followed by this formulation. Release parameters such as the Higuchi rate constant, ts0 and dissolution efficiency were calculated. Lots containing mannitol presented more rapid release rates due to the high solubility of this filler. On the other hand, the use of PEG 6000 as diluent significantly decreased drug release. The influence of technological parameters on the release of these systems was also examined, an inverse relationship between hardness and dissolution efficiency being found. (Wennergren & Lindberg, 2007)

The release behavior of carteolol hydrochloride matrix tablets was investigated as a function of filler nature (Emcompress®, mannitol, PEG 6000 and lactose), type of wetting liquid (Eudragit ® L 12.5% and isopropanol-acetone mixture 6:4) and mode of filler incorporation. The values of the technological parameters suggest that hardness was the most significantly affected by the three formulation factors considered. The strongest influence over the technological parameters was exerted by the mode of filler incorporation. The kinetic data conformed with the Higuchi square root equation, except for the lots containing mannitol and isopropanol-acetone mixture that conformed to a first-order plot. The lot containing Emcompress® and isopropanol-acetone mixture displayed acceptable linearity with both plots. Therefore, a non-linear regression procedure and reduced time method were used to define with precision the kinetic model followed by this formulation. Release parameters such as the Higuchi rate constant, ts0 and dissolution efficiency were calculated. Lots containing mannitol presented more rapid release rates due to the high solubility of this filler. On the other hand, the use of PEG 6000 as diluent significantly decreased drug release. The influence of technological parameters on the release of these systems was also examined, an inverse relationship between hardness and dissolution efficiency being found. (Holgado & Caraballo, 2011)

The aim of the study was to select a dissolution test method for carbamazepine (CBZ) immediate release tablets, giving the best in vitro/in vivo correlations (IVIVC) and to determine the potential of this method as an estimate for bioequivalence testing. Four 200 mg CBZ products which are sold on the Dutch market, covering the innovator and three generic products, were selected. They had been tested in a randomised, fourway cross-over bioavailability study in healthy volunteers. Their dissolution rate behaviour in vitro was investigated in two dissolution media: (1) 1% sodium lauryl sulphate in water (SLS), in accordance with the United States Pharmacopeia (USP); (2) 0.1 mol/l Hydrochloric acid in water (HC). In the bioavailability study

these products had shown no large differences in the extent of absorption ($AUC_{0-\infty}$;) but large differences in absorption rate. The products now also showed large differences in dissolution rate in vitro in both dissolution media, the rank order being the same as for the absorption rate. It was concluded that the absorption rate in vivo depends on the dissolution rate in vivo. 'Level C' IVIVC according to the USP were optimised by plotting percentages dissolved on selected time points (*D* values) or their reciprocals (1/*D* values), against several pharmacokinetic parameters primarily related to the absorption phase and against $AUC_{0-\infty}$. In this way for each IVIVC the optimum *D* or 1/*D* value, was calculated. For both media, no meaningful IVIVC were obtained with $AUC_{0-\infty}$, but favourable IVIVC were obtained with the parameters primarily related to the absorption phase. In the bioavailability study indicated above it was found that, among the pharmacokinetic characteristics primarily related to the absorption phase, C_{max} is the most promising in expressing rate of absorption in bioequivalence testing in single dose studies with CBZ immediate release tablets. (Lake & Olling, 2012)

The influence of tablet hardness and density variation on *in vitro* dissolution of dye from controlled release tablets made with different types and viscosity grades of hydrophilic polymers was investigated. Tablet volumes were maintained constant and the weight varied to obtain varying degrees of hardness and density. Tablets made at varying hardness did not show markedly different release characteristics as evaluated by an *in vitro* method. Different release patterns were, however, observed with tablets prepared from different gums. (Huber and Christenson, 2008)

Valdecoxib is a new non-steroidal anti-inflammatory drug, mainly used for osteoarthritis, rheumatoid arthritis, and dysmenorrhea. The major problem with this drug, is its very low solubility in biological fluids, which results in poor bioavailability after oral administration. Therefore, solid dispersions of valdecoxib with mannitol, polyethylene glycol 4000, and polyvinyl pyrrolidone K-12, were prepared with a view to increase its water solubility. Valdecoxib solid dispersion with polyvinyl pyrolidone K-12 showed maximum drug release hence, the tablet formulation containing valdecoxib polyvinyl pyrolidone K-12 solid dispersion, was prepared with a view to improve its water solubility. The dissolution profile of best laboratory developed formulation (F1) was compared with marketed tablet products. The drug release profile was studied in 0.1 N HCL. F1 gave far better dissolution than the conventional

marketed tablet, which released only 44.3% drug and valdecoxib in b cyclodextrin, which released 53.4% drug in 20 min, while F1 exhibited almost 100% drug release in 20 min. The dissolution efficiency of F1 was compared with pure drug, conventional market tablet, and valdecoxib in b cyclodextrin. F1 showed maximum dissolution efficiency. F1 was considered better than valdecoxib in b cyclodextrin, as far as the cost of raw materials used in the product is concerned. F1 was subjected to stability studies. The formulation was found to be stable for 4 weeks at 45°, with insignificant change in the hardness, disintegration time, and in vitro drug release pattern. (Patel, 2006)

The necessity and advantages of colon-specific drug delivery systems have been well recognized and documented. In the past, the primary approaches to obtain colon-specific delivery achieved limited success and included prodrugs, pH- and time-dependent systems, and microflora-activated systems. Precise colon drug delivery requires that the triggering mechanism in the delivery system only respond to the physiological conditions particular to the colon. Hence, continuous efforts have been focused on designing colon-specific delivery systems with improved site specificity and versatile drug release kinetics to accommodate different therapeutic needs. Among the systems developed most recently for colon-specific delivery, four systems were unique in terms of achieving in vivo site specificity, design rationale, and feasibility of the manufacturing process (pressure-controlled colon delivery capsules (PCDCs), CODES™, colonic drug delivery system based on pectin and galactomannan coating, and Azo hydrogels). The focus of this review is to provide detailed descriptions of the four systems, in particular, and in vitro/in vivo evaluation of colon-specific drug delivery systems, in general. (Yang & Chu, 2007)

Several parameters were studied for their effect on the dissolution of diclofenac sodium from Voltaren SR and hydroxypropylmethylcellulose (HPMC) based matrix tablets. The results indicate that addition of sodium or potassium chloride to the dissolution medium decreases the solubility of the drug and slows the dissolution rate, with the effect of sodium chloride being greater. The dissolution of the drug was studied in a medium which simulates the changing pH of the pathway followed by the drug as it passes from the stomach to the intestine. Dissolution was found to be inversely related to the rate at which the pH was changed. This may be caused by the deposition of an insoluble drug layer when contact is made with an acid medium. When higher

viscosity grades of HPMC are used, slower release rates result. Drug release from Voltaren SR is best described as non-Fickian in an aqueous medium irrespective of whether salt is added; however, a zero-order dependence became evident in pH-changing media. The release of diclofenac sodium from the hydrophilic HPMC matrices follows a non-Fickian transport in all media. (Sheu & Chou, 2009)

The potential of liquisolid systems to improve the dissolution properties of water-insoluble agents was investigated using hydrocortisone as the model medication. The in vitro release patterns of this very slightly water-soluble corticosteroid, formulated in directly compressed tablets and liquisolid compacts, were studied at different dissolution conditions. The new formulation technique of liquisolid compacts was used to convert liquid medications such as solutions or suspensions of hydrocortisone in propylene glycol, a nonvolatile liquid vehicle, into acceptably flowing and compressible powders by blending with selective powder excipients. Several liquisolid tablet formulations were prepared using a new mathematical model to calculate the appropriate quantities of powder and liquid ingredients required to produce acceptably flowing and compressible admixtures. Due to their increased wetting properties and surface of drug available for dissolution, liquisolid compacts demonstrated significantly higher drug release rates than those of conventionally made, directly compressed tablets containing micronized hydrocortisone. The in vitro drug dissolution rates of liquisolid tablets were found to be consistent and independent of the volume of dissolution medium used, in contrast to the plain tablets which exhibited declining drug release patterns with decreasing dissolution volumes. It has been also shown that the fraction of molecularly dispersed drug in the liquid medication of liquisolid systems is directly proportional to their hydrocortisone dissolution rates. (Spireas & Sadu, 2011)

The mechanical impact force in the paddle-beads method was determined. A manometric catheter was passed into the dissolution vessel through a hole, and the mechanical impact force was measured. In the present study, this mechanical force was evaluated as an impulse. The impulse increased with increasing number of beads added in the medium; in particular, the impulse increased markedly with more than 2500 beads in 250 ml dissolution medium. A close relationship was observed between the drug release rate and impulse. The profile of in vitro release using the paddle-beads method with rotation at 25 rpm in 250 ml of medium containing

2500 beads was similar to that of in vivo release in the fasted condition in dogs. (Aoki & Ando, 2010)

The in vitro dissolution of ciprofloxacin from commercially available tablets and capsules in China was studied using the USP apparatus I to compare the product performance from nine different manufacturers. Cumulative release greater than 75% was obtained from all of the products tested within 45 min. However, statistically significant differences were found between some of the products when in vitro data were analyzed using the Weibull function, similarity factor (f_2), and multivariate analysis of variances. (Tang and Gan, 2012)

Chapter Three

MATERIALS AND METHODS

Materials and methods

3.1. Introduction:

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

3.2. Reagents, Chemicals and Solvents:

All reagents used were of analytical reagent grade and distilled water was used for the preparation of all solutions. To observe the change in dissolution in ranitidine in dissolution media I used different brands of clonazepam tablet. I used active pharmaceutical ingredient (API) of ranitidine 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 1.4: 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 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 (8,16,24,32,40) µg/ml of clonazepam was prepared. For the preparation of different concentrations of ranitidine, 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 1.5. 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 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.

3.6. 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 1.6 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 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:

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

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

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 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 0.1Cm.

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 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 1.7. Brand names of Clonazepam under dissolution study

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

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:

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

Table. 1.8. 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 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-

Fig 3.1. Dissolution apparatus

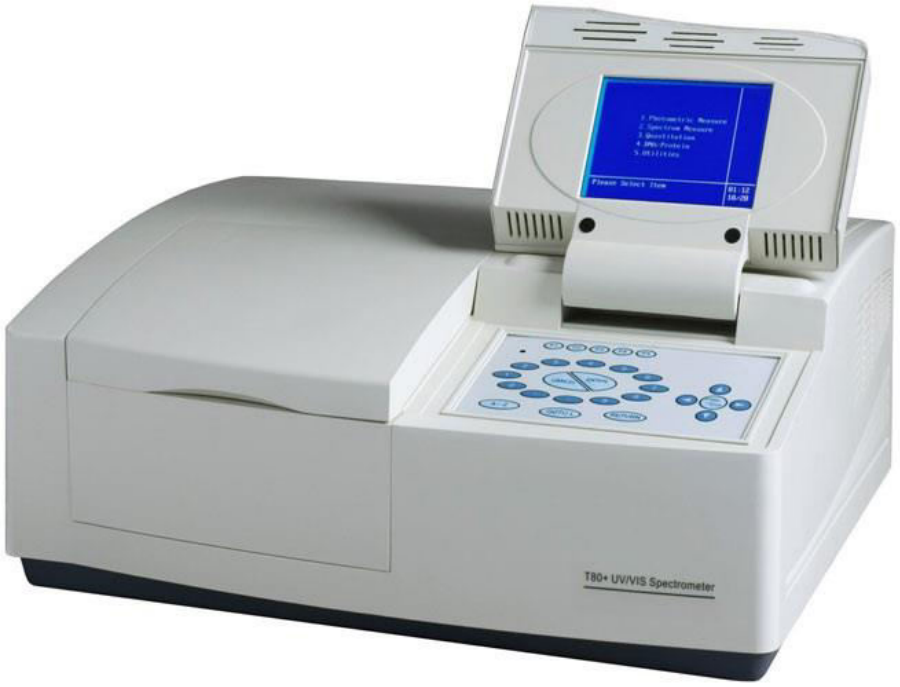


Fig 3.2. UV spectrophotometer

Fig. 3.3. Distilled water apparatus

Fig 3.4. Hardness tester

Fig 3.5. Electronic Balance

3.13 Apparatus:

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

Table 1.9 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 AND DISSCUSSION

4.0 Results & Discussion:

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

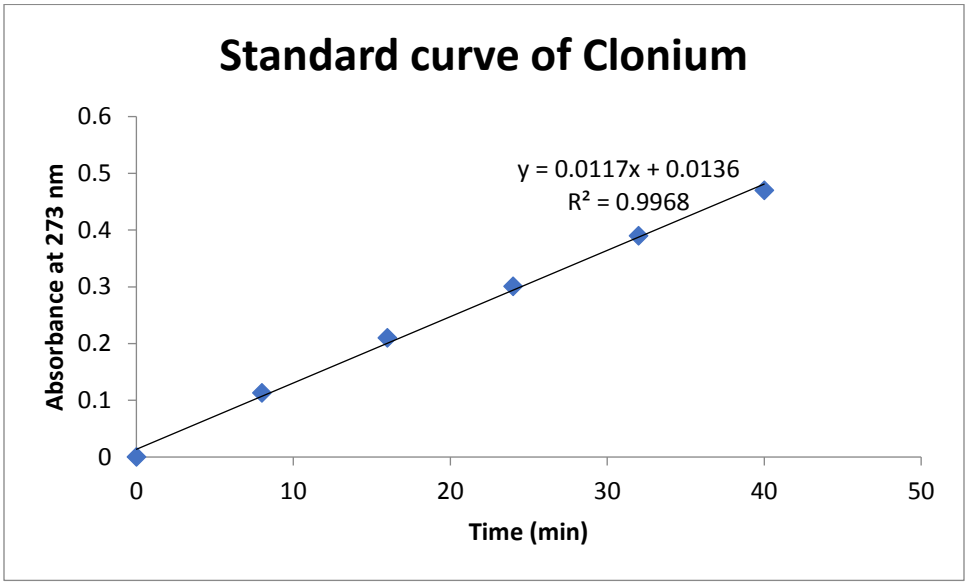


Fig 4.1 Standard curve of Clonium

4 tablets each of 0.5 mg Clonium were taken and grinded with the help of mortar and pastle to obtain a powder which was then smudged carefully and transferred into a 50ml volumetric flask. Afterwards, the grinded powder was dissolved slowly with distilled water which was made up to the 50 ml later. Here, the concentration of the stock solution after dissolving 4 tablets of clonazepam each 0.5 mg in 50 ml volumetric flask becomes 40µg/ml. Now, each of the following concentration as prepared along with the detection of their absorbance at wavelength of 273 nm to prepare the standard curve equation keeping the Time along X axis and Absorbance along Y axis. The equation **y = 0.0117x + 0.0136** helped to determine the concentration of drug release as well percent release of that drug & **R^2 = 0.9968** gave us an idea about the drug release kinetic profile.

Table 2.0 The prepared concentrations an absorbance data for preparation of standard curve

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

4.2 Preparation and method of dissolution curve of Clonium:

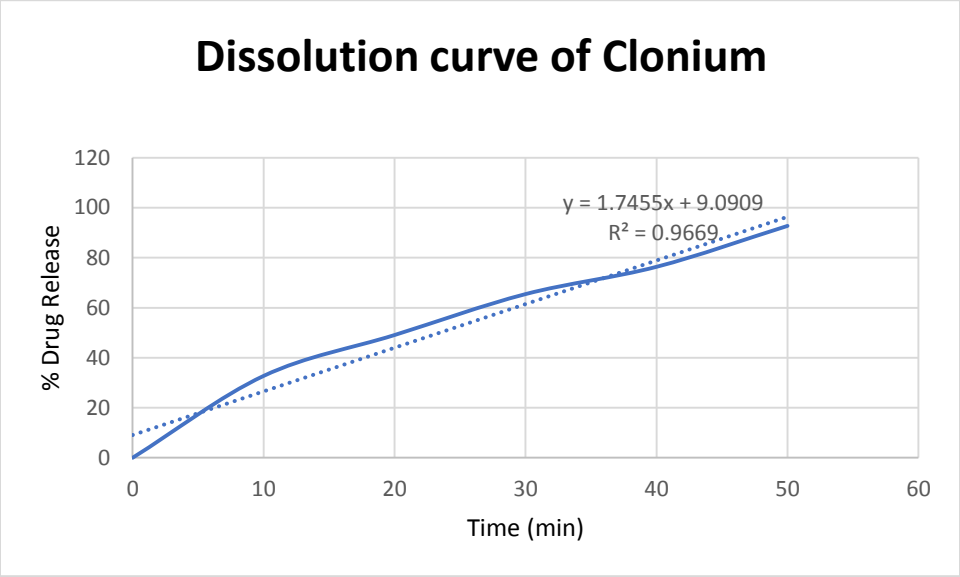


Fig 4.2 Dissolution curve of Clonium

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

Table 2.1 Data for the dissolution curve of Clonium

Time (min)	**% release of drug**
0	0
10	32.72
20	49.09
30	65.45
40	76.36
50	92.72

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

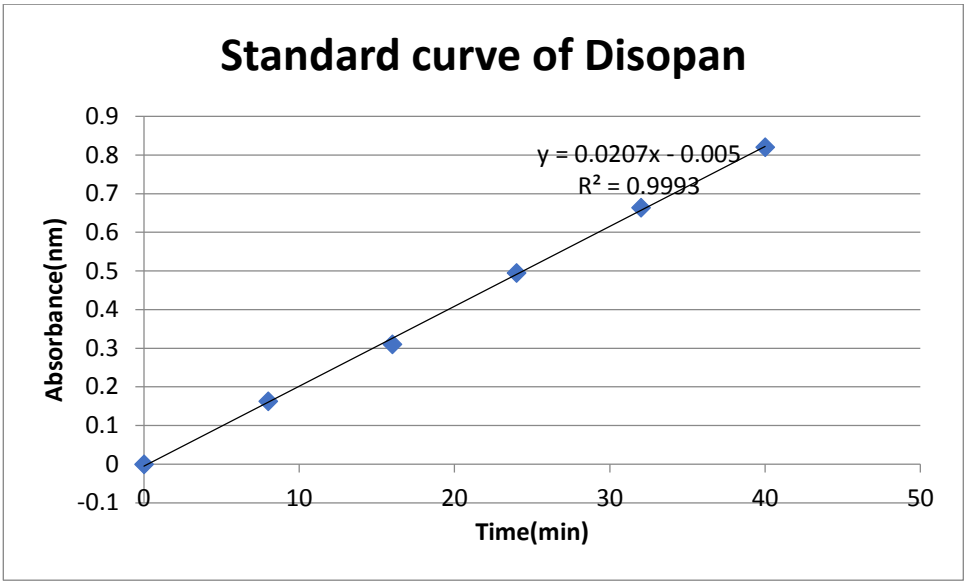


Fig 4.3 Standard curve of Disopan

4 tablets each of 0.5 mg disopan were taken and grinded with the help of mortar and pastle to obtain a powder which was then smudged carefully and transferred into a 50ml volumetric flask. Afterwards, the grinded powder was dissolved slowly with distilled water which was made up to the 50 ml later. Here, the concentration of the stock solution after dissolving 4 tablets of clonazepam each 0.5 mg in 50 ml volumetric flask becomes 40µg/ml. Now, each of the following concentration as prepared along with the detection of their absorbance at wavelength of 273 nm to prepare the standard curve equation keeping the Time along X axis and Absorbance along Y axis. The equation **y = 0.0207x - 0.005** helped to determine the concentration of drug release as well percent release of that drug & **R^2 = 0.9993** gave us an idea about the drug release kinetic profile.

Table 2.2 The prepared concentrations an absorbance data for preparation of standard curve

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

4.4 Preparation and method of dissolution curve of Disopan:

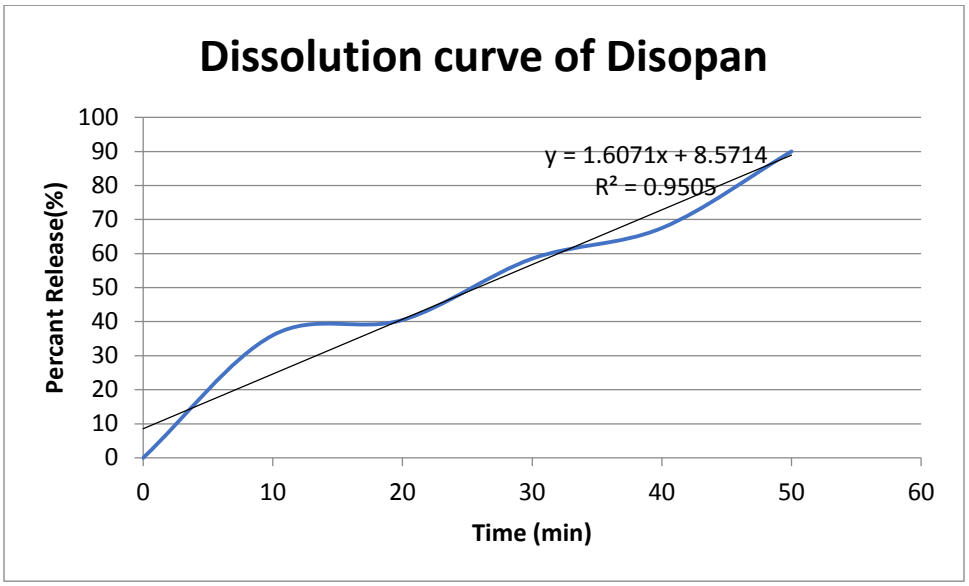


Fig 4.4 Dissolution curve of Disopan

3 tablets of Disopan were taken and they were dissolved at a rpm = 75, temperature=37± 0.5ºC with the distilled/deionized water as dissolution media and the above-mentioned concentrations were prepared for each of the tablets Afterwards, absorbance of each of the prepared concentrations for each of the tablets were measured at a wavelength of 273nm that were recorded. Thus, finally from the average of all the 3 data, a dissolution curve was prepared using the excel data sheet keeping the % drug release along Y axis and time along X axis. After 10 min and 50 min, the % release was about 36% and 90% respectively. The equation **y = 1.6071x + 8.5714** determined the concentrations of drug release and **R^2 = 0.9505** determines the drug release kinetic profile.

Table 2.3 Data for the dissolution curve of Disopan

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

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

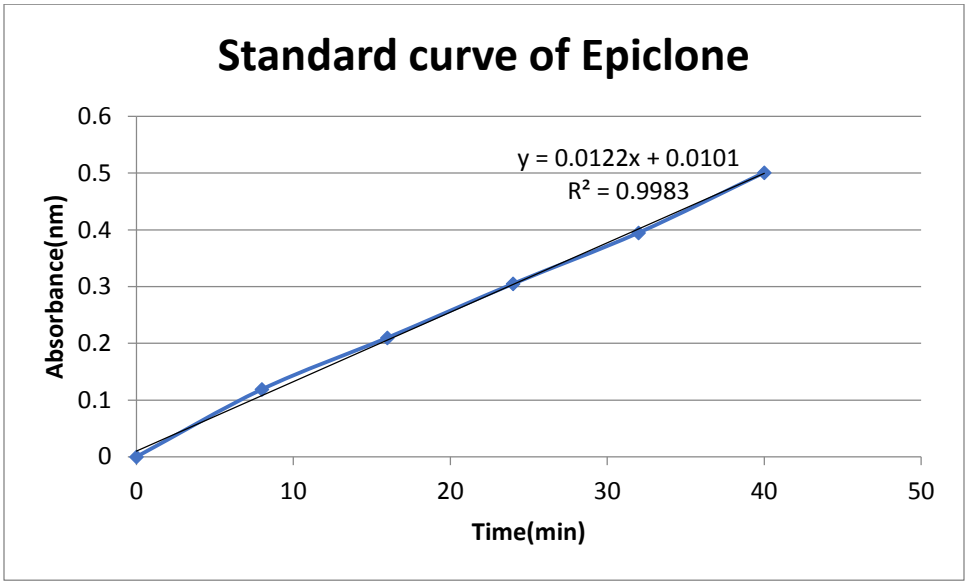


Fig 4.5 Standard curve of Epiclone

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

Table 2.4 The prepared concentrations an absorbance data for preparation of standard curve

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

4.6 Preparation and method of dissolution curve of Epiclone:

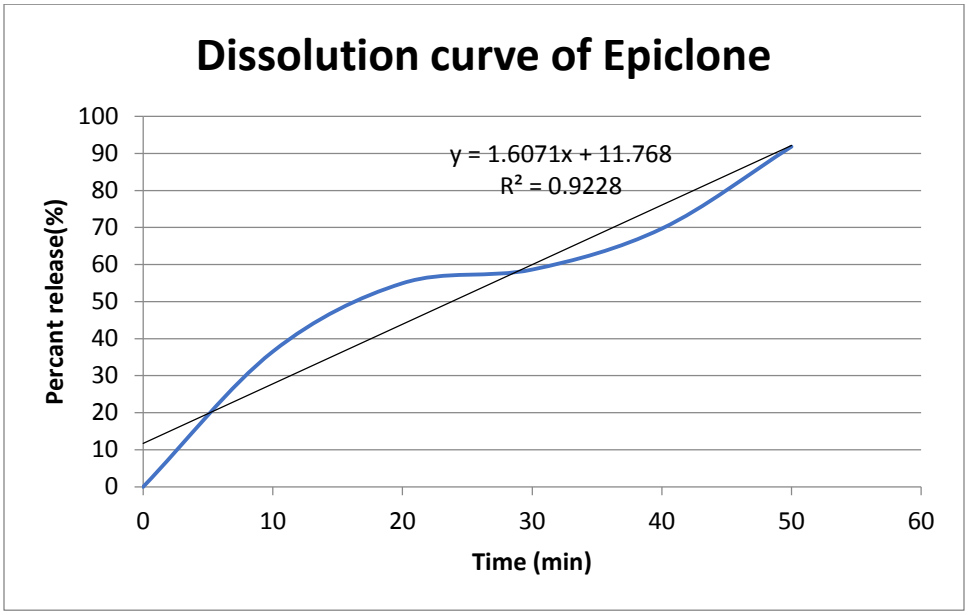


Fig 4.6 Dissolution curve of Epiclone

3 tablets of Epiclone were taken and they were dissolved at a rpm = 75, temperature=37± 0.5ºC with the distilled/deionized water as dissolution media and the above-mentioned concentrations were prepared for each of the tablets Afterwards, absorbance of each of the prepared concentrations for each of the tablets were measured at a wavelength of 273nm that were recorded. Thus, finally from the average of all the 3 data, a dissolution curve was prepared using the excel data sheet keeping the % drug release along Y axis and time along X axis. After 10 min and 50 min, the % release was about 36.51% and 69.71% respectively. The equation **y = 1.6071x + 11.768** determined the concentrations of drug release and **R^2 = 0.9228** determines the drug release kinetic profile.

Table 2.5 Data for the dissolution curve of Epiclone

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

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

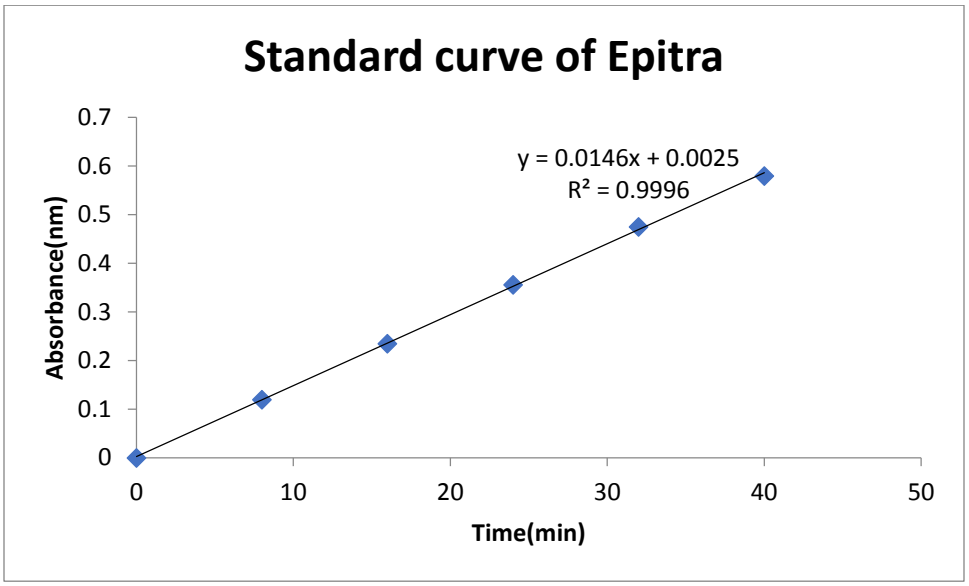


Fig 4.7 Standard curve of Epitra

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

Table 2.6 The prepared concentrations an absorbance data for preparation of standard curve

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

4.8 Preparation and method of dissolution curve of Epitra:

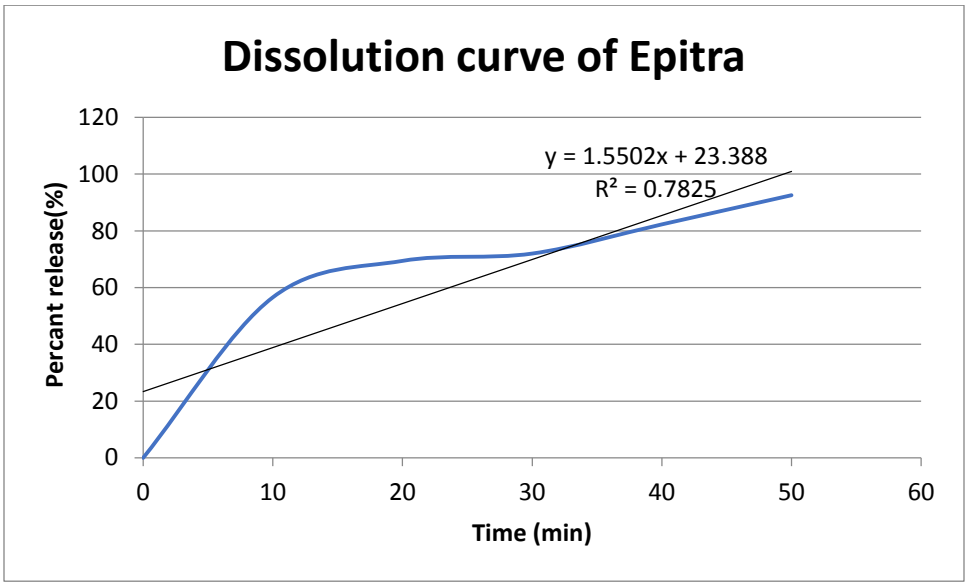


Fig 4.8 Dissolution curve of Epitra

3 tablets of Epitra were taken and they were dissolved at a rpm = 75, temperature=37± 0.5ºC with the distilled/deionized water as dissolution media and the above-mentioned concentrations were prepared for each of the tablets Afterwards, absorbance of each of the prepared concentrations for each of the tablets were measured at a wavelength of 273nm that were recorded. Thus, finally from the average of all the 3 data, a dissolution curve was prepared using the excel data sheet keeping the % drug release along Y axis and time along X axis. After 10 min and 50 min, the % release was about 56.57% and 92.57% respectively the equation **y = 1.5502x + 23.388** determined the concentrations of drug release and **R^2 = 0.7825** determines the drug release kinetic profile.

Table 2.7 Data for the dissolution curve of Epitra

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

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

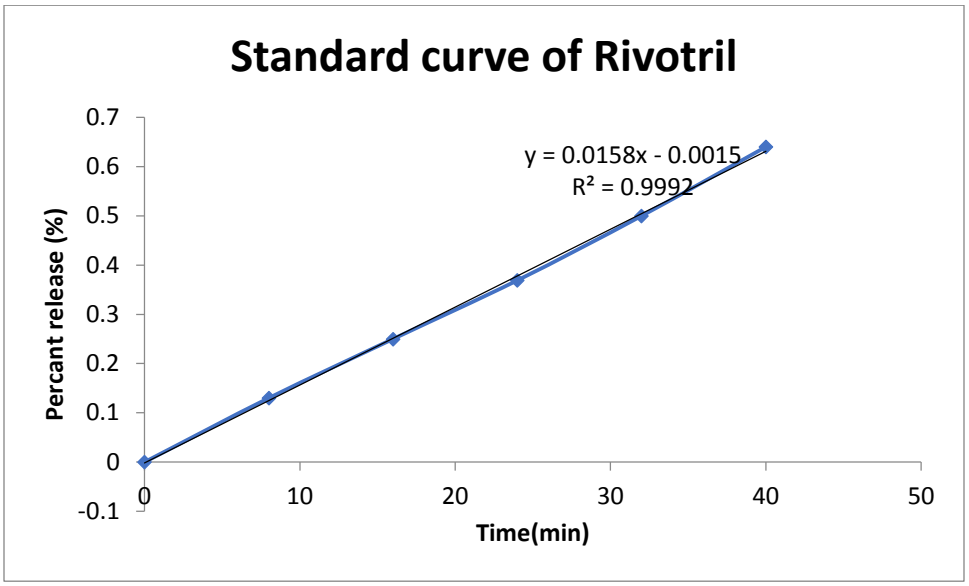


Fig 4.9 Standard curve of Rivotril

4 tablets each of 0.5 mg Rivotril were taken and grinded with the help of mortar and pastle to obtain a powder which was then smudged carefully and transferred into a 50ml volumetric flask. Afterwards, the grinded powder was dissolved slowly with distilled water which was made up to the 50 ml later. Here, the concentration of the stock solution after dissolving 4 tablets of clonazepam each 0.5 mg in 50 ml volumetric flask becomes 40µg/ml. Now, each of the following concentration as prepared along with the detection of their absorbance at wavelength of 273 nm to prepare the standard curve equation keeping the Time along X axis and Absorbance along Y axis. The equation **y = 0.0158x - 0.0015** helped to determine the concentration of drug release as well percent release of that drug & **R^2 = 0.9992** gave us an idea about the drug release kinetic profile.

Table 2.8 The prepared concentrations an absorbance data for preparation of standard curve

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

4.10 Preparation and method of dissolution curve of Rivotril:

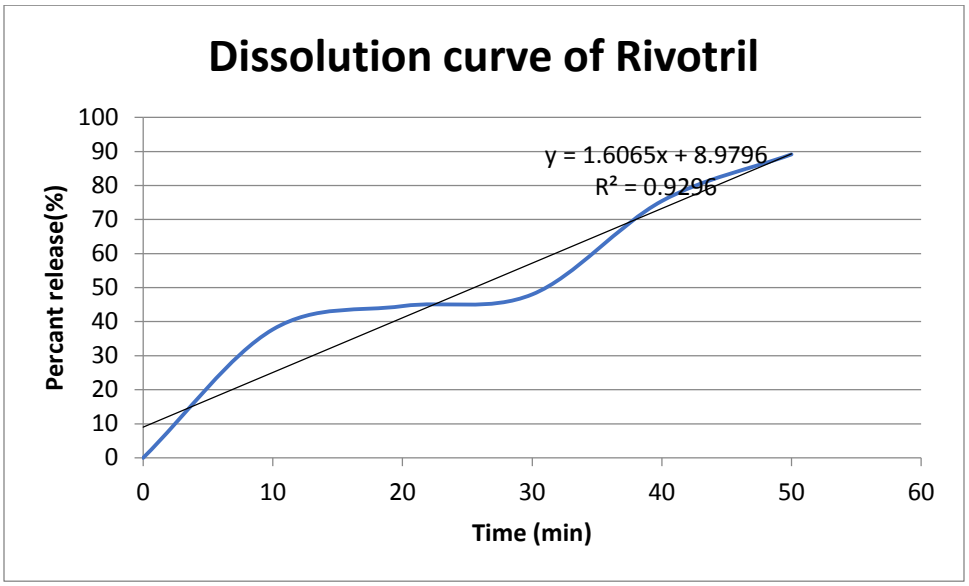


Fig 4.10 Dissolution curve of Rivotril

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

Table 2.9 Data for the dissolution curve of Rivotril

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

4.11 Zero order drug release profile:

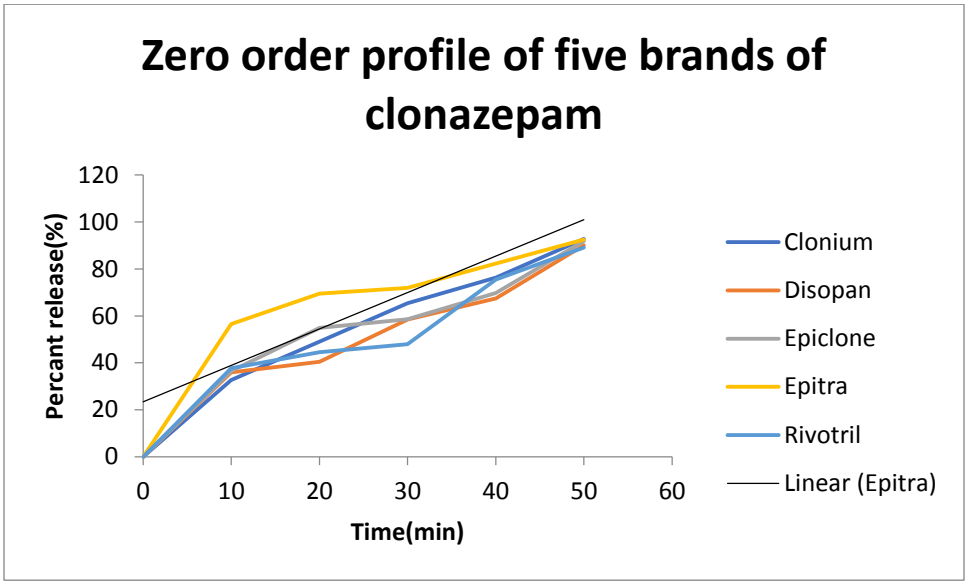


Fig 4.11 Zero order drug release profile of five brands of Clonazepam

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 brands 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 3.0 $\mathbf{R^2}$ values of Five different brands of Clonazepam for zero order drug release profile

Name	**R^2 values**
Clonium	0.9669
Disopan	0.9505
Epiclone	0.9228
Epitra	0.7825
Rivotril	0.9296

4.12 Zero order drug release profile:

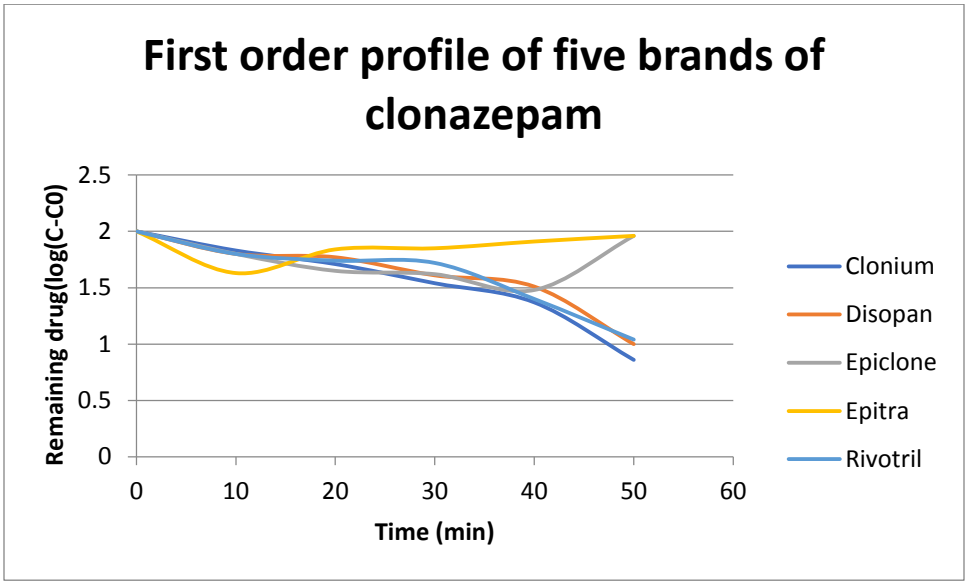


Fig 4.12 First order drug release profile of five brands of Clonazepam

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 brands 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 3.1 **R^2** values of Five different brands of Clonazepam for first order drug release profile

Name	**R^2 values**
Clonium	0.9212
Disopan	0.8719
Epiclone	0.0968
Epitra	0.0707
Rivotril	0.8836

4.13 Higuchi drug release profile:

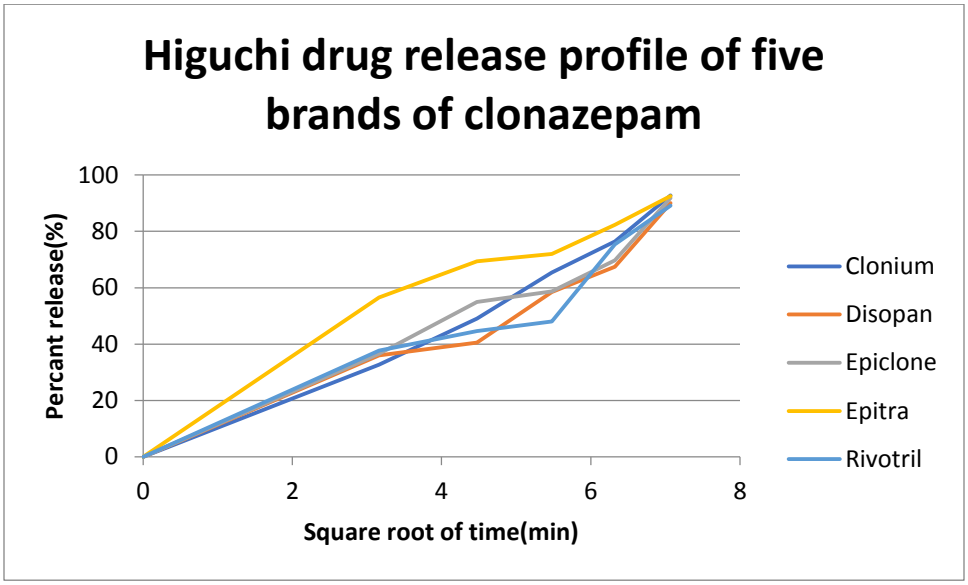


Fig 4.13 Higuchi drug release profile of five brands of Clonazepam

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 brands 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. sqrt 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 3.2 $\mathbf{R^2}$ values of Five different brands of Clonazepam for Higuchi's drug release profile

Name	**R^2 values**
Clonium	0.9843
Disopan	0.9548
Epiclone	0.9726
Epitra	0.9578
Rivotril	0.9334

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
Clonium	0.9669	0.9212	0.9843
Disopan	0.9505	0.8719	0.9548
Epiclone	0.9228	0.0968	0.9726
Epitra	0.7825	0.0707	0.9578
Rivotril	0.9296	0.8836	0.9334

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 Clonium, the highest R^2 value=0.9843 is for Higuchi's equation so Clonium will be released from its solid matrix in the dissolution media following Higuchi's equation presumably. Similarly, the highest R^2 values for Disopan, Epiclone, Epitra, Rivotril are 0.9548, 0.9726, 0.9578 and 0.9334 that clearly indicated that all of the rest brands of Clonazepam also followed Higuchi's equation profile of release kinetics.

Chapter Five

CONCLUSION

The aim of this thesis was to elucidate the release kinetics of five brands of clonazepam (Rivotril, Epitra, Epiclone, Disopan & Clonium) available in Bangladeshi pharmaceutical market. The R^2 value is the indicative of the release kinetics for the different brands. For Clonium, the highest R^2 value=0.9843 is for Higuchi's equation so Clonium will be released from its solid matrix in the dissolution media following Higuchi's equation presumably. Similarly, the highest R^2 values for Disopan, Epiclone, Epitra, Rivotril are 0.9548, 0.9726, 0.9578 and 0.9334 that clearly indicated that all of the rest brands of Clonazepam also followed Higuchi's equation profile of release kinetics. Future research is highly recommended using the international brands including innovator brand of Clonazepam.

Reference

Aoki, S., Ando, H., Tatsuishi, K., Uesugi, K. and Ozawa, H., 2010. Determination of the mechanical impact force in the in vitro dissolution test and evaluation of the correlation between in vivo and in vitro release. *International journal of pharmaceutics*, *95*(1-3), pp.67-75.

Bhimanadhuni, C.N., Garikapati, D.R. and Usha, P., 2012. Development and validation of an RP-HPLC method for the simultaneous determination of Escitalopram Oxalate and Clonazepam in bulk and its pharmaceutical formulations. *International Current Pharmaceutical Journal*, *1*(8), pp.193-198.

Brown, S.D., Woodhead, J.H., Skeen, G.A. and Wolf, H.H., 2000. Topiramate Modulates GABA-Evoked Currents in Murine Cortical Neurons by a Nonbenzodiazepine Mechanism. *Epilepsia*, *41*(s1), pp.17-20.

BENZODIAZEPINE. *Indo American Journal of Pharmaceutical Research*, *3*(12), pp.1457-1474.

Chandra, B., 2005. The use of clonazepam in the treatment of tic douloureux (a preliminary report). *Proceedings of the Australian Association of Neurologists*, *13*, pp.119-122.

de Oliveira Neves, A.C., Soares, G.M., de Morais, S.C., da Costa, F.S.L., Porto, D.L. and de Lima, K.M.G., 2012. Dissolution testing of isoniazid, rifampicin, pyrazinamide and ethambutol tablets using near-infrared spectroscopy (NIRS) and multivariate calibration. *Journal of pharmaceutical and biomedical analysis*, *57*, pp.115-119.

Eldin, A.B., Shalaby, A., Abdallah, M.S., Shaldam, M.A. and Abdallah, M.A., 2014. Applying green analytical chemistry (GAC) for development of stability indicating HPLC method for determining clonazepam and its related substances in pharmaceutical formulations and calculating uncertainty. *Arabian Journal of Chemistry*.

Greenblatt, D.J., Miller, L.G. and Shader, R.I., 2006. Clonazepam pharmacokinetics, brain uptake, and receptor interactions. *The Journal of clinical psychiatry*, *48*, pp.4-11.

Hewlett, W.A., Vinogradov, S. and Agras, W.S., 2011. Clomipramine, clonazepam, and clonidine treatment of obsessive-compulsive disorder. *Journal of clinical psychopharmacology*, *12*(6), pp.420-430.

Huber, H.E. and Christenson, G.L. 2008. Utilisation of hydrophilic gums for the control of drug substance release from tablet formulations II. Influence of tablet hardness and density on dissolution behavior. *Journal of pharmaceutical sciences*, *57*(1), pp.164-166.

Huguenard, J.R. and Prince, D.A., 1994. Clonazepam suppresses GABAB-mediated inhibition in thalamic relay neurons through effects in nucleus reticularis. *Journal of Neurophysiology*, *71*(6), pp.2576-2581.

Holgado, M. A., et al. "Influence of diluents and manufacturing method on the in vitro dissolution of carteolol hydrochloride matrix tablets." *International journal of pharmaceutics* 118.2 (2005): 151-160.

Hewlett, W.A., Vinogradov, S. and Agras, W.S., 1992. Clomipramine, clonazepam, and clonidine treatment of obsessive-compulsive disorder. *Journal of clinical psychopharmacology*, *12*(6), pp.420-430.

Holgado, M.A., Caraballo, I., Alvarez-Fuentes, J., Fernández-Hervás, M.J., Fernández-Arévalo, M. and Rabasco, A.M., 2011. Influence of diluents and manufacturing method on the in vitro dissolution of carteolol hydrochloride matrix tablets. *International journal of pharmaceutics*, *118*(2), pp.151-160.

Islam, S.M., Islam, S., Shahriar, M. and Dewan, I., 2011. Comparative in vitro dissolution study of Aceclofenac Marketed Tablets in Two Different Dissolution Media by Validated Analytical Method.

Izquierdo, I., Pereira, M.E. and Medina, J.H., 1990. Benzodiazepine receptor ligand influences on acquisition: suggestion of an endogenous modulatory mechanism mediated by benzodiazepine receptors. *Behavioral and neural biology*, *54*(1), pp.27-41.

Jenner, P., J. A. Pratt, and C. D. Marsden. "Mechanism of action of clonazepam in myoclonus in relation to effects on GABA and 5-HT." *Advances in neurology* 43 (2011): 629-643.

Jeong, Y.I., Cheon, J.B., Kim, S.H., Nah, J.W., Lee, Y.M., Sung, Y.K., Akaike, T. and Cho, C.S., 1998. Clonazepam release from core-shell type nanoparticles in vitro. *Journal of Controlled Release*, *51*(2), pp.169-178.

Jeong, Y.I., Cheon, J.B., Kim, S.H., Nah, J.W., Lee, Y.M., Sung, Y.K., Akaike, T. and Cho, C.S., 2008. Clonazepam release from core-shell type nanoparticles in vitro. *Journal of Controlled Release*, *51*(2), pp.169-178.

Jagawat, T., 2011. A Comparative Study to Assess the Efficacy and Safety of Combination Capsules of Paroxetine and Clonazepam in comparison to Paroxetine in patients suffering from Co-morbid Depression and Anxiety.

Jung, H., et al. "Bioequivalence study of carbamazepine tablets: in vitro/in vivo correlation." *International journal of pharmaceutics* 152.1 (1997): 37-44.

Juenemann, D., Jantratid, E., Wagner, C., Reppas, C., Vertzoni, M. and Dressman, J.B., 2011. Biorelevant in vitro dissolution testing of products containing micronized or nanosized fenofibrate with a view to predicting plasma profiles. *European journal of pharmaceutics and biopharmaceutics*, *77*(2), pp.257-264.

Karmarkar, A.B., Gonjari, I.D., Hosmani, A.H., Dhabale, P.N. and Bhise, S.B., 2009. Dissolution rate enhancement of fenofibrate using liquisolid tablet technique. Part II: Evaluation of in vitro dissolution profile comparison methods. *Lat Am J Pharm*, *28*(4), pp.538-43.

Khan, M.A., Nematullah, K., Omer, M., Aamer, K., Javeedullah, M. and Nasir, M., 2013. Respiratory depression due to clonazepam and fluconazole interaction: case report and discussion. *Indo American Journal of Pharmaceutical Research*, *3*(9), pp.7140-7145.

Levy, G., 2003. Effect of certain tablet formulation factors on dissolution rate of the active ingredient I. Importance of using appropriate agitation intensities for In vitro dissolution rate measurements to reflect In vivo conditions. *Journal of pharmaceutical sciences*, *52*(11), pp.1039-1046.

Levy, G., Leonards, J.R. and Procknal, J.A., 1965. Development of in vitro dissolution tests which correlate quantitatively with dissolution rate-limited drug absorption in man. *Journal of Pharmaceutical Sciences*, *54*(12), pp.1719-1722.

Lake, O.A., Olling, M. and Barends, D.M., 2012. In vitro/in vivo correlations of dissolution data of carbamazepine immediate release tablets with pharmacokinetic data obtained in healthy volunteers. *European journal of pharmaceutics and biopharmaceutics*, *48*(1), pp.13-19.

Morishita, S., 2010. Clonazepam as a therapeutic adjunct to improve the management of depression: a brief review. *Human Psychopharmacology: Clinical and Experimental*, *24*(3), pp.191-198.

Morishita, S., 2012. Clonazepam as a therapeutic adjunct to improve the management of depression: a brief review. *Human Psychopharmacology: Clinical and Experimental*, *24*(3), pp.191-198.

Mura, P., Nassini, C., Proietti, D., Manderioli, A. and Corti, P., 2004. Effect of presence and type of lipophilic compound on the in vitro diffusion of clonazepam from hydrophilic ointment bases. *Pharmaceutica Acta Helvetiae*, *70*(2), pp.175-180.

Miri, L. and Jalali, F., 2013. Dispersive liquid-liquid micro-extraction as a sample preparation method for clonazepam analysis in water samples and pharmaceutical preparations. *Journal of Reports in Pharmaceutical Sciences (J. Rep. Pharm. Sci.)*, *2*(2), pp.103-110.

Narasimharaju, C. and Rao, D., 2012. Development and validation of an RP-HPLC method for the simultaneous determination of Escitalopram Oxalate and Clonazepam in bulk and its pharmaceutical formulations.

Patel, R. and Purohit, N., 2009. Physico-Chemical Characterization and In Vitro Dissolution Assessment of Clonazepam—Cyclodextrins Inclusion Compounds. *AAPS PharmSciTech*, *10*(4), p.1301.

Patel, M.M. and Patel, D.M., 2006. Fast dissolving valdecoxib tablets containing solid dispersion of valdecoxib. *Indian journal of pharmaceutical sciences*, *68*(2).

Spier, S.A., Tesar, G.E., Rosenbaum, J.F. and Woods, S.W., 2006. Treatment of panic disorder and agoraphobia with clonazepam. *Journal of Clinical Psychiatry*.

Shirs, S.B., Suresh, S. and Swamy, P.V., 2009. Formulation design and optimization of fast dissolving clonazepam tablets. *Indian journal of pharmaceutical sciences*, *71*(5), p.567.

Shirsand, S.B., Suresh, S., Kusumdevi, V. and Swamy, P.V., 2011. Formulation design and optimization of fast dissolving clonazepam tablets by sublimation method. *Indian journal of pharmaceutical sciences*, *73*(5), p.491.

Shirsand, S. B., et al. "Formulation design and optimization of fast dissolving clonazepam tablets by sublimation method." *Indian journal of pharmaceutical sciences* 73.5 (2004): 491.

Sanka, K., Poienti, S., Mohd, A.B. and Diwan, P.V., 2014. Improved oral delivery of clonazepam through liquisolid powder compact formulations: in-vitro and ex-vivo characterization. *Powder Technology*, *256*, pp.336-344.

Seshadri, V.C., Manohari, P.J. and Kunchithapatham, J., 2013. FORMULATION AND CHARACTERIZATION OF MOUTH DISSOLVING TABLETS CONTAINING White, H.S.,

Soroush, S., Maryam, B., Mohamad, S.T. and Maryam, M., 2016. Potentiometric Determination of Clonazepam Using Carbon Paste Electrode Based on Molecular Imprinted Polymer (MIP) in Solution and in a Biological Fluid Model. *Pharm Anal Acta*, *7*(499), p.2.

Sheu, M.T., Chou, H.L., Kao, C.C., Liu, C.H. and Sokoloski, T.D., 2009. Dissolution of diclofenac sodium from matrix tablets. *International journal of pharmaceutics*, *85*(1-3), pp.57-63.

Spireas, S., Sadu, S. and Grover, R., 2011. In vitro release evaluation of hydrocortisone liquisolid tablets. *Journal of pharmaceutical sciences*, *87*(7), pp.867-872.

Tang, Y. and Gan, K., 2012. Statistical evaluation of in vitro dissolution of different brands of ciprofloxacin hydrochloride tablets and capsules. *Drug development and industrial pharmacy*, *24*(6), pp.549-552.

Weaver, M.F., 2015. Focus: Addiction: Prescription Sedative Misuse and Abuse. *The Yale journal of biology and medicine*, *88*(3), p.247.

Wennergren, B., Lindberg, J., Nicklasson, M., Nilsson, G., Nyberg, G., Ahlgren, R., Persson, C. and Palm, B., 2007. A collaborative in vitro dissolution study: comparing the flow-through method with the USP paddle method using USP prednisone calibrator tablets. *International Journal of Pharmaceutics*, *53*(1), pp.35-41

Yuksel, N., Kanık, A.E. and Baykara, T., 2000. Comparison of in vitro dissolution profiles by ANOVA-based, model-dependent and-independent methods. *International journal of pharmaceutics*, *209*(1), pp.57-67.

Yang, L. Chu, J.S. and Fix, J.A., 2007. Colon-specific drug delivery: new approaches and in vitro/in vivo evaluation. *International Journal of Pharmaceutics*, *235*(1), pp.1-15.