In-vitro Comparative Dissolution Study of Different Brands (Alcet, Clarigen, Lecet, Lozin) of Levocetirizine Dihydrochloride with respect to Seasonix

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

Md. Iqbal Hossen Zahid

ID: 2014-1-70-071

Research Supervisor

Tirtha Nandi

Lecturer

Department of Pharmacy

East West University

Department of Pharmacy
East West University

Declaration by the Candidate

I, Md. Iqbal Hossen Zahid , hereby declare that the dissertation entitled ***"In-vitro* comparative dissolution study of different brands of Levocetirizine Dihydrochloride tablets 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 period 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.

_____

Md. Iqbal Hossen Zahid

ID: 2014-1-70-071

Department of Pharmacy

East West University, Dhaka.

Certificate by the Supervisor

This is to certify that the thesis entitled **"*In-vitro* comparative dissolution study of different brands of Levocetirizine Dihydrochloride tablets 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 Md. Iqbal Hossen Zahid, ID: 2014-1-70-071, during the period 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 **"*In-vitro* comparative dissolution study of different brands of Levocetirizine Dihydrochloride tablets 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 Md. Iqbal Hossen Zahid, ID: 2014-1-70-071, during the period 2017 of his research in the Department of Pharmacy, East West University.

_____

Prof. Chowdhury Faiz Hossain

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. His generous advice, constant supervision, intense support, enthusiastic encouragements and reminders during the research work not only helped shape this study but also molded me into being a better researcher.

I put forward my most sincere regards and profound gratitude to Chairperson Dr. Chowdhury Faiz Hossain, Professor & chairperson, Department of Pharmacy, East West University, for his inspiration in my study. He also paid attention for the purpose of my research work and extending the facilities to work. I want to give special thanks to Tahirarupa , Samihatamanna 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 aim of the present study was to evaluate and compare dissolution pattern of locally branded drug products of Levocetirizine Dihydrochloride available in Bangladesh with the brand of Levocetirizine Dihydrochloride (seasonix) marketed by Incepta Bangladesh Ltd. Seasonix is one of the renowned antihistamine drug of Bangladesh. Branded drugs are expensive than locally marketed drug. Substitution of drugs is very essential for the people of under developing country. Four different brands of Levocetirizine tablets which are available in Bangladesh like Alcet, Lecet, Lozin,Clarigen as well as Seasonix were collected from a reputed pharmacy store. Two tablets from each of the brands were used for the *in-vitro* dissolution study. Cumulative drug release was measured up to 50 minutes for all the brands. All the brands were compared with the reference brand (Seasonix). Differential factor,*f*1and similarity factor, *f*2 were determined. No significant difference was observed during *in-vitro* drug release pattern of brandAlcet, Lecet, Lozin, Clarigenwith the reference brand. Here it was found the values of *f*1 are 12.19, 2.9, 4.9 and 33.10 but here range is 0-15 acceptable. And the similarity factor it was seen that the values of *f*2 are 49, 73.87, 25.47 and 69.82. So it is acceptable.In conclusion, further investigations are needed to evaluate better dissolution study.

Keyword: Dissolution, Levocetirizine Dihydrochloride, Generic brand, Reference drug product, Differential Factor, Comparative dissolution, *In-vitro* drug dissolution study

List Of Contents

Chapter One: Introduction

Chapter Four : Results and Discussion

Chapter One
INTRODUCTION

1.1 Overview

Levocetirizine is a third-generation, non-sedating antihistamine, developed from the secondgeneration antihistamine cetirizine. Chemically, levocetirizine is the active levorotary enantiomer of cetirizine, also called the R-enantiomer of cetirizine. Levocetirizine is an inverse agonist that decreases activity at histamine H1 receptors. This in turn prevents the release of other allergy chemicals and increase the blood supply to the area, and provides relief from the typical symptoms of hay fever. It does not prevent the actual release of histamine from mast cells.

The manufacturers claim it to be more effective with fewer side effects than previous secondgeneration drugs; however, there have been no published studies supporting this assertion. A study part-funded by the manufacturer UCB concluded it may be more effective than some other second- and third-generation anti-histamines, but didn't compare it to cetirizine.

Figure 1.1: Levocetirizine Dihydrochloride tablet

Levocetirizine is used to treat the symptoms of seasonal and year-round allergies. It's also used to relieve itching caused by hives (patches of red, swollen, itchy skin). This drug may be used as part of a combination therapy. This means you may need to take it with other medications.

Levocetirizine works by blocking the release of a chemical called histamine from the cells in your body. This helps relieve symptoms of allergies, such as sneezing, runny nose, and red,

watery, itchy eyes. This drug also helps relieve itching caused by hives. Levocetirizine oral tablet may cause drowsiness. This occurs more often during the first few hours after you take the drug. It may also cause other side effects.

Levocetirizine oral tablet can interact with other medications, vitamins, or herbs you may be taking. An interaction is when a substance changes the way a drug works. This can be harmful or prevent the drug from working well. To help avoid interactions, your doctor should manage all of your medications carefully. Be sure to tell your doctor about all medications, vitamins, or herbs you're taking. To find out how this drug might interact with something else you're taking, talk to your doctor or pharmacist.

Levocetirizine can cause drowsiness. The use of drinks that contain alcohol raises risk of drowsiness.(Knott,2013)

Figure 1.2 : Levocetirizine Dihydrochloride tablet strip

1.2 H1 blocker:

Histamine mediates a variety of physiologic and pathologic responses in different tissues and cells and is an important chemical mediator of inflammation in allergic disease. Acting through H_1 receptors and inositol phospholipid hydrolysis, histamine plays an important part in causing smooth-muscle contraction in the respiratory and gastrointestinal tracts and in causing pruritus and sneezing by sensory-nerve stimulation. Histamine induces vascular endothelium to release nitric oxide, which stimulates guanylate cyclase and increases levels of cyclic guanosine monophosphate in vascular smooth muscle, causing vasodilation. Acting through H_1 and H_2 receptors, it causes hypotension, flushing, and headache. Activation of H_2 receptors alone increases gastric acid secretion. H_3-receptor stimulation may have negative modulatory effects.

H_1 receptors have been defined pharmacologically by the actions of their respective agonists and antagonists. Although there is little evidence that peripheral and central H_1 receptors differ, there may be isoforms of H_1 receptors or perhaps subtypes of H_1 receptors.(Knott,2013)

1.3 How H1 blocker works

H_1 antagonists, also called H_1 blockers, are a class of medications that block the action of histamine at the H_1 receptor, helping relieve allergic reactions. Agents where the main therapeutic effect is mediated by negative modulation of histamine receptors are termed antihistamines; other agents may have antihistaminergic action but are not true antihistamines. In common use, the term "antihistamine" refers only to H_1 antagonists, also known as H_1-receptor antagonists and H_1-antihistamines. It has been discovered that some H_1antihistamines function as inverse agonists, as opposed to receptor antagonists, at the histamine H_1-receptor.

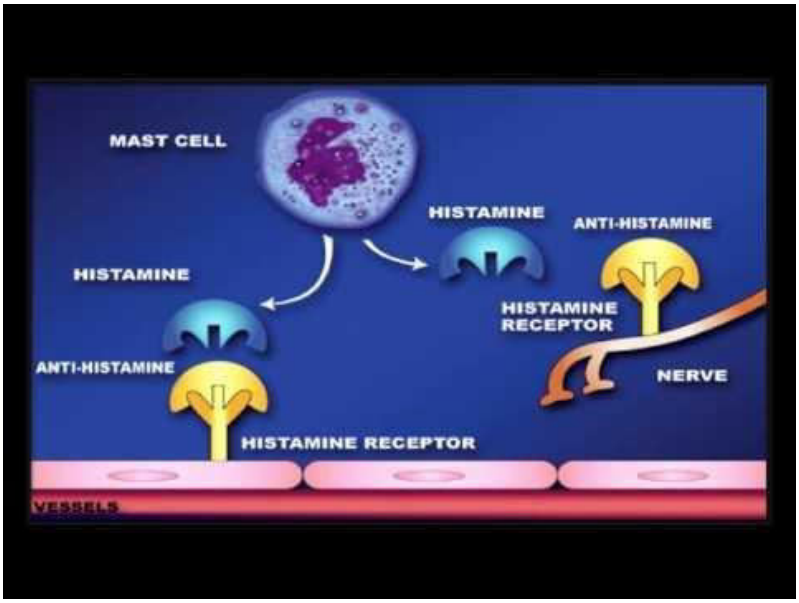

Figure 1.3: Mechanism of Antihistamine (Pubmed,2013)

In type I hypersensitivity allergic reactions, an allergen (a type of antigen) interacts with and cross-links surface IgE antibodies on mast cells and basophils. Once the mast cell-antibodyantigen complex is formed, a complex series of events occurs that eventually leads to cell degranulation and the release of histamine (and other chemical mediators) from the mast cell or basophil. Once released, the histamine can react with local or widespread tissues through histamine receptors.

Produces pruritus, vasodilation, hypotension, flushing, headache, bradycardia, bronchoconstri ction, increase in vascular permeability and potentiation of pain. While H_1-antihistamines help

against these effects, they work only if taken before contact with the allergen. In severe allergies, such as anaphylaxis or angioedema, these effects may be of life-threatening severity.

Additional administration of epinephrine, often in the form of an autoinjector (Epi-pen), is required by people with such hypersensitivities. Levocetirizine, the active enantiomer of cetirizine, is an anti-histamine; its principal effects are mediated via selective **inhibition** of H1 receptors. The antihistaminic activity of levocetirizine has been documented in a variety of animal and human models. (Anisa.1999)

1.4 Synthesis of Levocetirizine Dihydrochloride:

LEVO -015 and LEVO -016 added under the condition of triethylamine and dichloromethane in the temperature of 0-5°c.and then LEVO - 017 is found. Then LEVO -019 can be found by using the catalyst of NaOH and Toluene in 120 -130°c.Then Methanesulfonyl chloride, triethylamine and dichloromethane in the temperature of 0-5°c aid to prepare Levo-020/Then with this LEVO-004 and Toluene in 75 to 80°c produce LEVO-021. Then from that conc. HBr, H_2O in 90 to 95°c and NaOH and ethyl acetate produce Levocetirizine. And finally from that under Hcl, Acetone in 0-5°c Levocetirizine Dihydrochloride is produced.

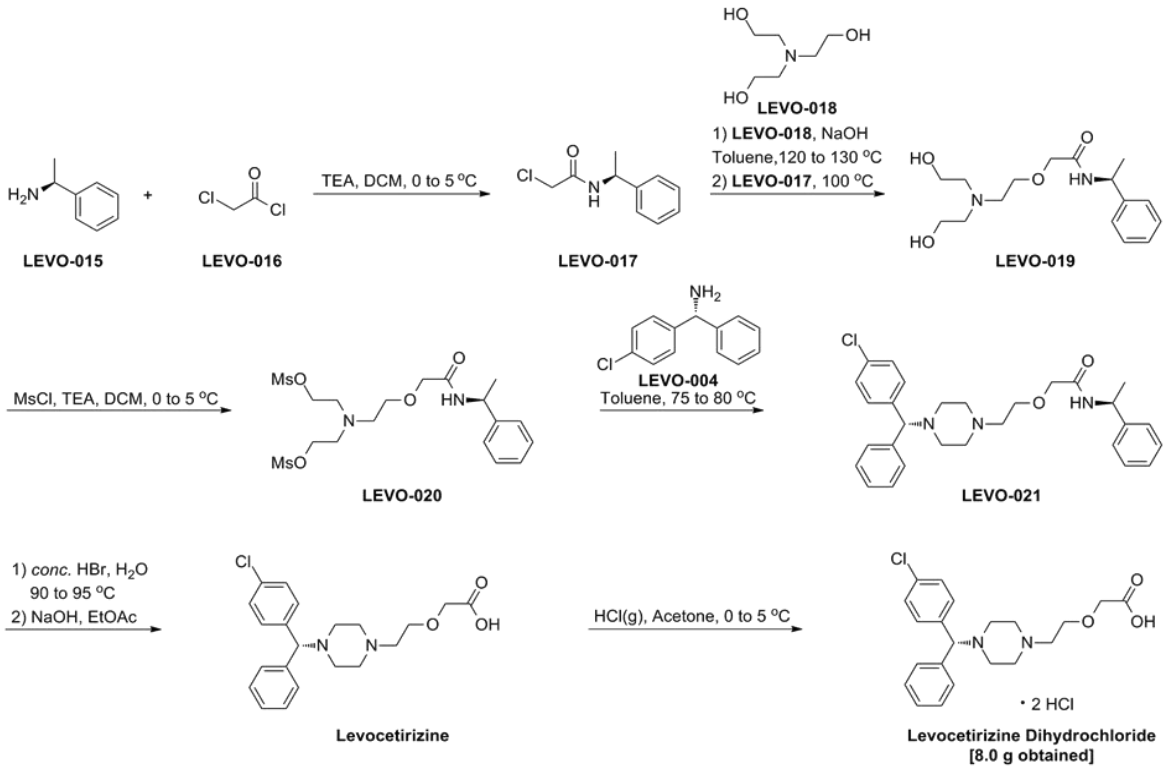

Figure 1.4: Synthesis of Levocetirizine Dihydrochloride (Pubmed,2010)

There are many other routes of production of this compound. But mainly this one is followed. Another route is given below-

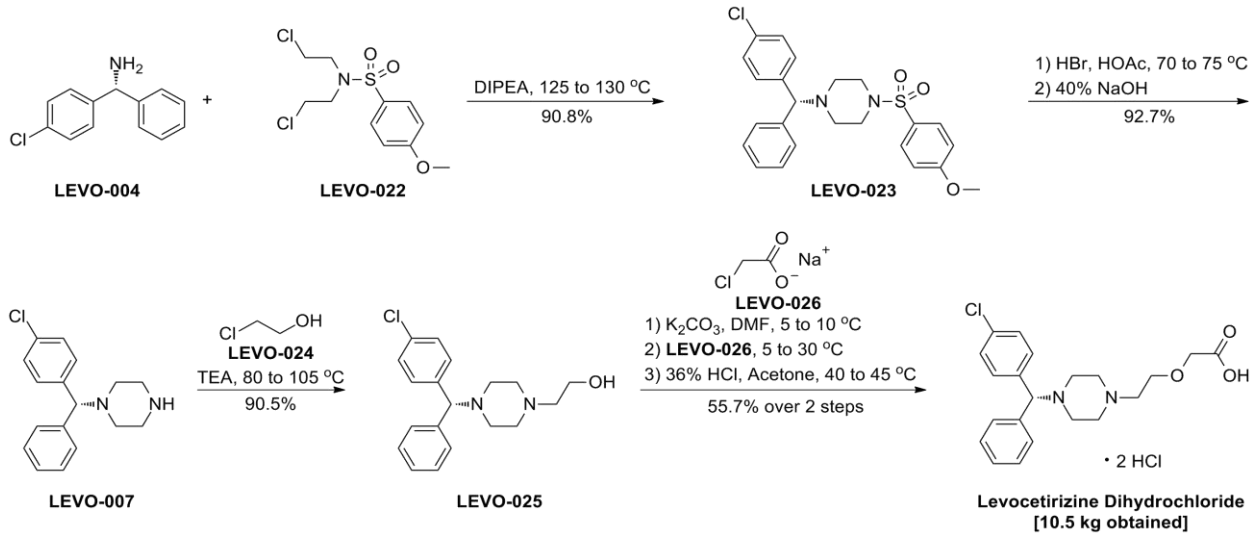

Figure 1.5: Synthesis of Levocetirizine Dihydrochloride (Pubmed,2010)

1.5 Drug Information:

Levocetirizine dihydrochloride is a third-generation, non-sedating antihistamine, developed from the second-generation antihistamine cetirizine. Chemically, levocetirizine is the active levorotary enantiomer of cetirizine, also called the *R*-enantiomer of cetirizine. Levocetirizine is an inverse agonist that decreases activity at histamine H1 receptors. This in turn prevents the release of other allergy chemicals and increase the blood supply to the area, and provides relief from the typical symptoms of hay fever. It does not prevent the actual release of histamine from mast cells.

Levocetirizine dihydrochloride, USP the active component of Levocetirizine dihydrochloride tablets, USP is an orally active H1-receptor antagonist. The chemical name is (R)-[2-[4-[(4chlorophenyl) phenylmethyl]-1-piperazinyl] ethoxy] acetic acid dihydrochloride. Levocetirizine dihydrochloride is the R enantiomer of cetirizine hydrochloride, a racemic compound with antihistaminic properties.

The empirical formula of Levocetirizine dihydrochloride is C21H25ClN2O3•2HCl. The molecular weight is 461.82 and the chemical structure is shown below:

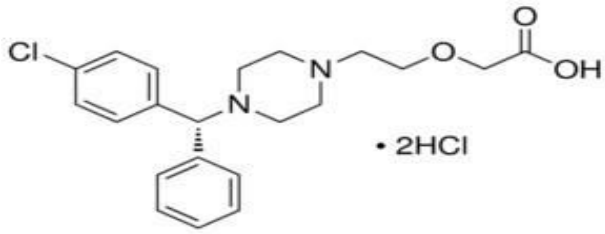

Figure 1.6 : Levocetirizine Dihydrochloride

Biological half-life 6 to 10 hours, Metabolism Hepatic 14% CYP3A4 ,Formula $C_{21}H_{25}ClN_2O_3$, Drug class H1 antagonist, Other drugs in same class Cetirizine, Desloratadine, Levocetirizine is an antihistamine that reduces the effects of natural chemical histamine in the body. Histamine can produce symptoms of sneezing, itching, watery eyes, and runny nose. Levocetirizine is used to treat symptoms of year-round (perennial) allergies in children who are at least 6 months old. Levocetirizine is also used to treat itching and swelling caused by chronic urticaria (hives) in adults and children who are at least 6 months old. (Kaplan, 2010)

1.6 When to take levocetirizine

This dosage information is for levocetirizine oral tablet. All possible dosages and drug forms may not be included here. Dosage, drug form, and how often cne take the drug will depend on:

- Age
- the condition being treated ○ how severe the condition

is ○ other medical conditions one have ○ how one react to

the first dose

Levocetirizine is an anti-histamine; its principal effects are mediated via selective **inhibition** of H1 receptors. The antihistaminic activity of levocetirizine has been documented in a variety of animal and human models. Levocetirizine is used to treat symptoms of year-round (perennial) allergies in children who are at least 6 months old. Levocetirizine is also used to

treat itching and swelling caused by chronic urticaria (hives) in adults and children who are at least 6 months old. (Kaplan, 2010)

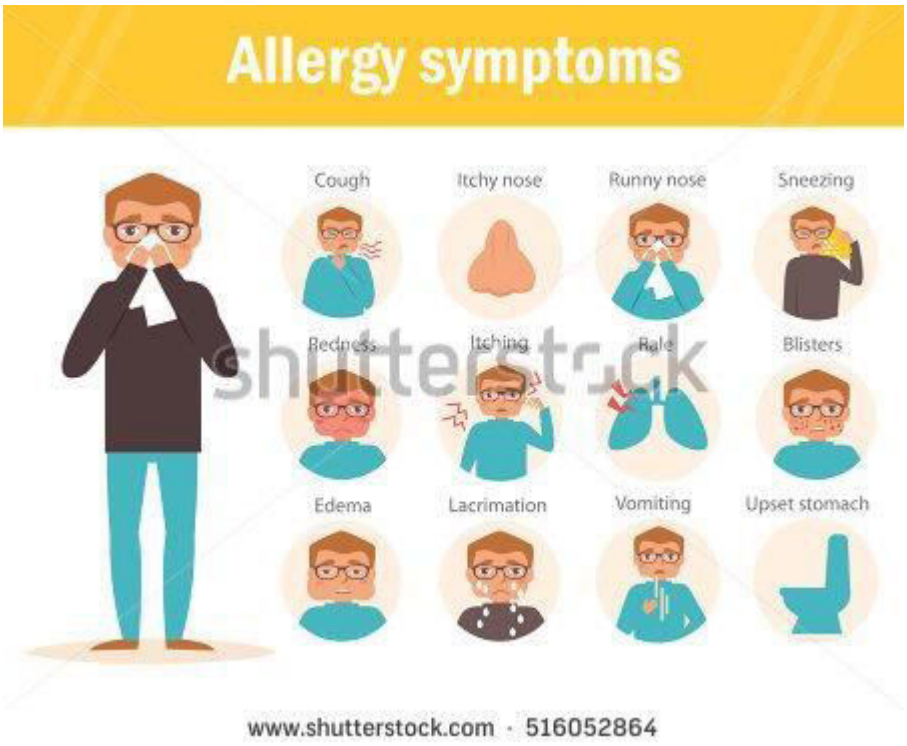

Figure 1.7: Allergy syptoms when Levocetirizine prescribed (Pubmed,2010)

1.7 Side Effects:

Levocetirizine dihydrochloride is an antihistamine indicated for the relief of symptoms associated with seasonal allergic rhinitis in adults and children 2 years of age and older. Side effects reported with the administration of this are usually include:

Table 1.1: Side Effects

drowsiness	fatigue	weakness	tired feeling	stuffy nose	sinus pain
sore throat	cough	vomiting	diarrhoea	constipation	weight gain

(Kaplan, 2010)

1.7.1 More common side effects

Table 1.2: More common side effects

In adults and children ages 12 and older	In children ages 6–11 years	In children ages 1–5 years	In children ages 6–12 months
• tiredness • dry mouth • sore throat • nasopharyngitis (redness and inflammation in the nose and throat)	• fever • cough • sleepiness • nose bleeds	• fever • diarrhoea • vomiting • ear infections	• diarrhoea • constipation

(Kaplan, 2010)

Some side effects image in children-

Figure 1.8: Nose bleed

Figure 1.9: Fever (Pubmed,2010)

1.7.2 Serious side effects

Table 1.3: Serious side effects

Allergic reactions	Kidney problems	Blurry vision
• rash • itching • hives • swelling of your lips, tongue, face, or throat	• trouble urinating • changes in the amount you urinate • blood in your urine	Different eyesight problems occur

(Kaplan, 2010)

1.8 Forms and strengths

Generic: levocetirizine Dihydrochloride

Form:	Strengths
Oral tablet	5 mg
Oral solution	2.5 mg/5 mL

Figure 1.10: Oral solution and Tablet

1.9. Available drug in Bangladesh

Brand Name	Manufacturer
ALCET	Health Care Pharmaceuticals Ltd.
CLARIGEN	Drug International Ltd.
CURIN	Beximco Pharmaceuticals Ltd.
SEASONIX	Incepta Pharmaceuticals Ltd.
LECET	Pacific Pharmaceuticals Ltd.
LECETRIN	Delta Pharma Limited
LECITIN	Zenith Pharmaceuticals Ltd
LEREX	Asiatic Laboratories Ltd.
LEVOCET	Alco Pharma Ltd.
LEVOREX	Popular Pharmaceuticals Ltd.
LINGIN	Novartis (Bangladesh) Ltd.
LISET	Syntho Pharmaceuticals Ltd.
LUPRON	Kumudini Pharma Ltd.
MEGATROL	Peoples Pharma Ltd.
VOCET	Apex Pharmaceuticals Ltd.

(Drugs.com 2016)

1.10 Dosage for seasonal and year-round allergies and chronic itching

Adult dosage (ages 18–64 years)	**Child dosage (ages 12–17 years)**	**Child dosage (ages 6–11 years)**	**Child dosage (ages 5 years and younger)**	**Senior dosage (ages 65 years and older)**
Typical dosage is one 5-mg tablet once per day in the evening.	Typical dosage is one 5-mg tablet once per day in the evening	Typical dosage is one half-tablet (2.5 mg) once per day in the evening.	Dosage for levocetirizine oral tablet hasn't been established for children younger than 6 years of age.	The kidneys of older adults may not work aand they used to. This can cause body to process drugs more slowly. As a result, more of a drug stays in your body for a longer time. This raises your risk of side effects

(Kaplan, 2010)

Table 1.4: Dosage for seasonal and year-round allergies and chronic itching

1.11 Special considerations:

Table 1.5: Special considerations

Mild kidney disease	Moderate kidney disease	Severe kidney disease	End-stage kidney disease and on hemodialysis
2.5 mg once per day.	2.5 mg once every other day.	2.5 mg twice per week (taken once every 3–4 days).	Do not take this drug.

(Kaplan, 2010)

1.12 BCS Classification

The BCS is a scientific framework for classifying a drug substance based on its aqueous solubility and intestinal permeability. It allows for the prediction of in vivo pharmacokinetics of oral immediate-release (IR) drug products by classifying drug compounds into four classes based on their solubility related to dose and intestinal permeability in combination with the dissolution properties of the dosage form. The interest in this classification system stems largely from its application in early drug development and then in.The Biopharmaceutical Classification System (BCS) is one of the experimental models that measures permeability and solubility under specific conditions.

Class	Solubility	Permeability
I	High	High
II	high	low
III	low	high
IV	low	low

Table 1.6 :The Bio pharmaceutics classification system

The main purpose of the system was to aid in the regulation of post-approval changes, providing acceptance based on in vitro data when appropriate is available. Importantly, the system was designed around on oral drug delivery since the majority of drugs is and remains orally dosed. Waivers, permission to skip *in vivo* bioequivalence studies, are kept for drug products that meet certain requirements like solubility and permeability and that are also rapidly dissolving

characters.This classification is associated with a drug dissolution and absorption model, which identifies the key parameters controlling drug absorption as a set of dimensionless numbers. Levocetirizine is in the Class III as it has high permeability and low solubility (Knott, 2016).

Class I

The drugs of this class exhibit high absorption number and high dissolution number. The ratelimiting step is drug dissolution, and if dissolution is very rapid, then the gastric-emptying rate becomes the rate-determining step. These compounds are well absorbed, and their absorption rate is usually higher than the excretion rate. Examples include metoprolol, diltiazem, verapamil, and propranolol.

Class II

The drugs of this class have a high absorption number but a low dissolution number. In vivo drug dissolution is then a rate-limiting step for absorption except at a very high dose number. The absorption for Class II drugs is usually slower than for Class I and occurs over a longer period of time. In vitro–in vivo correlation (IVIVC) is usually accepted for Class I and Class II drugs. The bioavailability of these products is limited by their solvation rates. Hence, a correlation between the in vivo bioavailability and the in vitro solvation can be found (7, 9, and 10). Examples include glibenclamide, phenytoin, danazol, mefenamic acid, nifedipine, ketoprofen, naproxen, carbamazepine, and ketoconazole (Knott, 2016).

Class III

Drug permeability is the rate-limiting step for drug absorption, but the drug is solvated very quickly. These drugs exhibit a high variation in the rate and extent of drug absorption. Since the dissolution is rapid, the variation is attributable to alteration of physiology and membrane permeability rather than the dosage form factors. Examples include cimetidine, levocetirizine, acyclovir, neomycin B, atenolol, and captopril (Knott, 2016).

Class IV

The drugs of this class are problematic for effective oral administration. These compounds have poor bioavailability. They are usually not well absorbed through the intestinal mucosa, and a high variability is expected. Fortunately, extreme examples of Class IV compounds are the

exception rather than the rule, and these are rarely developed and marketed. Nevertheless, several Class IV drugs do exist Examples include hydrochlorothiazide, taxol, and furosemide (Knott, 2016).

1.13 Levocetirizine Dihydrochloride- Clinical Pharmacology

1.13.1 Mechanism of Action

Levocetirizine, the active enantiomer of cetirizine, is an antihistamine; its principal effects are mediated via selective inhibition of H1 receptors. The antihistaminic activity of Levocetirizine has been documented in a variety of animal and human models. In vitro binding studies revealed that Levocetirizine has an affinity for the human H1-receptor 2-fold higher than that of cetirizine (Ki = 3 nmol/L vs. 6 nmol/L, respectively). The clinical relevance of this finding is unknown.

1.13.2 Pharmacodynamics

Studies in adult healthy subjects showed that Levocetirizine at doses of 2.5 mg and 5 mg inhibited the skin wheal and flare caused by the intradermal injection of histamine. In contrast, dextrocetirizine exhibited no clear change in the inhibition of the wheal and flare reaction.

Levocetirizine at a dose of 5 mg inhibited the wheal and flare caused by intradermal injection of histamine in 14 pediatric subjects (aged 6 to 11 years) and the activity persisted for at least 24 hours. The clinical relevance of histamine wheal skin testing is unknown. A QT/QTc study using a single dose of 30 mg of Levocetirizine did not demonstrate an effect on the QTc interval. While a single dose of Levocetirizine had no effect, the effects of Levocetirizine may not be at steady state following single dose. The effect of Levocetirizine on the QTc interval following multiple dose administration is unknown. Levocetirizine is not expected to have QT/QTc effects because of the results of QTc studies with cetirizine and the long post-marketing history of cetirizine without reports of QT prolongation.

1.13.3 Pharmacokinetics

Levocetirizine exhibited linear pharmacokinetics over the therapeutic dose range in adult healthy subjects.

Absorption

Levocetirizine is rapidly and extensively absorbed following oral administration. In adults, peak plasma concentrations are achieved 0.9 hour after administration of the oral tablet. The accumulation ratio following daily oral administration is 1.12 with steady state achieved after 2 days.Peak concentrations are typically 270 ng/mL and 308 ng/mL following a single and a repeated 5 mg once daily dose, respectively. Food had no effect on the extent of exposure (AUC) of the Levocetirizine tablet, but Tmax was delayed by about 1.25 hours and Cmax was decreased by about 36% after administration with a high fat meal; therefore, Levocetirizine can be administered with or without food. A dose of 5 mg (10 mL) of Levocetirizine dihydrochloride oral solution is bioequivalent to a 5 mg dose of Levocetirizine dihydrochloride tablets. Following oral administration of a 5 mg dose of Levocetirizine dihydrochloride oral solution to healthy adult subjects, the mean peak plasma concentrations were achieved approximately 0.5 hour post-dose.

Distribution

The mean plasma protein binding of Levocetirizine in vitro ranged from 91 to 92%, independent of concentration in the range of 90-5000 ng/mL, which includes the therapeutic plasma levels observed. Following oral dosing, the average apparent volume of distribution is approximately 0.4 L/kg, representative of distribution in total body water.

Metabolism

The extent of metabolism of Levocetirizine in humans is less than 14% of the dose and therefore differences resulting from genetic polymorphism or concomitant intake of hepatic drug metabolizing enzyme inhibitors are expected to be negligible. Metabolic pathways include aromatic oxidation, N- and O-dealkylation, and taurine conjugation. Dealkylation pathways are primarily mediated by CYP 3A4 while aromatic oxidation involves multiple and/or unidentified CYP isoforms.

Elimination

The plasma half-life in adult healthy subjects was about 8 to 9 hours after administration of oral tablets and oral solution, and the mean oral total body clearance for Levocetirizine was approximately 0.63 mL/kg/min. The major route of excretion of Levocetirizine and its metabolites is via urine, accounting for a mean of 85.4% of the dose. Excretion via faces accounts for only 12.9% of the dose. Levocetirizine is excreted both by glomerular filtration

and active tubular secretion. Renal clearance of Levocetirizine correlates with that of creatinine clearance. In patients with renal impairment the clearance of Levocetirizine is reduced.

Drug Interaction Studies

In vitro data on metabolite interaction indicate that Levocetirizine is unlikely to produce, or be subject to metabolic interactions. Levocetirizine at concentrations well above Cmax level achieved within the therapeutic dose ranges is not an inhibitor of CYP isoenzymes 1A2, 2C9, 2C19, 2A1, 2D6, 2E1, and 3A4, and is not an inducer of UGT1A or CYP isoenzymes 1A2, 2C9 and 3A4.

No formal in vivo drug interaction studies have been performed with Levocetirizine. Studies have been performed with the racemic cetirizine.

Pediatric Patients

Data from a pediatric pharmacokinetic study with oral administration of a single dose of 5 mg Levocetirizine in 14 children age 6 to 11 years with body weight ranging between 20 and 40 kg show that Cmax and AUC values are about 2-fold greater than that reported in healthy adult subjects in a cross-study comparison. The mean Cmax was 450 ng/mL, occurring at a mean time of 1.2 hours, weight-normalized, total body clearance was 30% greater, and the elimination half-life 24% shorter in this pediatric population than in adults. Dedicated pharmacokinetic studies have not been conducted in pediatric patients younger than 6 years of age. A retrospective population pharmacokinetic analysis was conducted in 323 subjects (181 children 1 to 5 years of age, 18 children 6 to 11 years of age, and 124 adults 18 to 55 years of age) who received single or multiple doses of Levocetirizine ranging from 1.25 mg to 30 mg. Data generated from this analysis indicated that administration of 1.25 mg once daily to children 6 months to 5 years of age results in plasma concentrations similar to those of adults receiving 5 mg once daily.

Geriatric Patients

Limited pharmacokinetic data are available in elderly subjects. Following once daily repeat oral administration of 30 mg Levocetirizine for 6 days in 9 elderly subjects (65–74 years of age), the total body clearance was approximately 33% lower compared to that in younger adults. The disposition of racemic cetirizine has been shown to be dependent on renal function rather than on age. This finding would also be applicable for Levocetirizine, as Levocetirizine

and cetirizine are both predominantly excreted in urine. Therefore, the Levocetirizine dihydrochloride dose should be adjusted in accordance with renal function in elderly patients.

Gender

Pharmacokinetic results for 77 patients (40 men, 37 women) were evaluated for potential effect of gender. The half-life was slightly shorter in women (7.08 ± 1.72 hr) than in men (8.62 ± 1.84 hr); however, the body weight-adjusted oral clearance in women (0.67 ± 0.16 mL/min/kg) appears to be comparable to that in men (0.59 ± 0.12 mL/min/kg). The same daily doses and dosing intervals are applicable for men and women with normal renal function.

Race

The effect of race on Levocetirizine has not been studied. As Levocetirizine is primarily renally excreted, and there are no important racial differences in creatinine clearance, pharmacokinetic characteristics of Levocetirizine are not expected to be different across races. No race related differences in the kinetics of racemic cetirizine have been observed.

Renal Impairment

Levocetirizine exposure (AUC) exhibited 1.8-, 3.2-, 4.3-, and 5.7-fold increase in mild, moderate, severe, renal impaired, and end-stage renal disease patients, respectively, compared to healthy subjects.

The corresponding increases of half-life estimates were 1.4-, 2.0-, 2.9-, and 4-fold,respectively. The total body clearance of Levocetirizine after oral dosing was correlated to the creatinine clearance and was progressively reduced based on severity of renal impairment. Therefore, it is recommended to adjust the dose and dosing intervals of Levocetirizine based on creatinine clearance in patients with mild, moderate, or severe renal impairment. In end-stage renal disease patients (CLCR < 10 mL/min) Levocetirizine is contraindicated. The amount of Levocetirizine removed during a standard 4–hour hemodialysis procedure was <10%. The dosage of Levocetirizine dihydrochloride should be reduced in patients with mild renal impairment. Both the dosage and frequency of administration should be reduced in patients with moderate or severe renal impairment.

Hepatic Impairment

Levocetirizine dihydrochloride has not been studied in patients with hepatic impairment. The non-renal clearance (indicative of hepatic contribution) was found to constitute about 28% of the total body clearance in healthy adult subjects after oral administration. As Levocetirizine is mainly excreted unchanged by the kidney, it is unlikely that the clearance of Levocetirizine is significantly decreased in patients with solely hepatic impairment. (Kaplan, 2010)

1.14 Dissolution

Dissolution is the primary quality control test to determine whether a drug product can release its active pharmaceutical ingredients in a timely manner.A dissolution test is a means of identifying and proving the availability of active drug materials in their delivered form. A dissolution test simulates the availability of active substance and allows the prediction of the time for complete release of the material from the dosage form. In the pharmaceutical industry, drug dissolution testing is routinely used to provide critical in vitro drug release information for both quality control purposes, i.e., to assess batch-to-batch consistency of solid oral dosage forms such as tablets, and drug development, i.e., to predict in vivo drug release profiles.(Kaplan,2010)

1.15 Factors influence dissolution from drug products

- The properties of the API
- The quality and design of the drug product
- The conditions under which the test is run and the coating material.

1.16 Comparative dissolution

In a dissolution test a drug product is added to media, simulating gastrointestinal fluids in a patient. At several time points the concentration of the dissolved API is determined. Drug dissolution testing is routinely used to provide critical in vitro drug release information for both drug development purposes and quality control. Dissolution testing during drug development is important to predict in vivo drug release profiles. In vitro drug dissolution data generated

from dissolution testing experiments can be related to in vivo pharmacokinetic data by means of in vitro-in vivo correlations (IVIVC). A well-established predictive IVIVC model can be very helpful for drug formulation design and post-approval manufacturing changes.

Levocetrizine is used in peptic ulcer therapy and available as several brands in the market which makes it difficult to select the safe, effective and economic one. The aim of this study is to establish similarity among the different brands of ranitidine tablets available in local market. Four different brands of (150 mg) were selected for the study. Six quality control parameters: weight variation test, hardness test, thickness, friability, disintegration test and dissolution test were carried out specified by USP. Result revealed that all brands comply within limits for hardness, weight variation, thickness, friability, disintegration and dissolution. Disintegration time for all brands was within 15 minutes complying with the USP commendation. All brands showed Q-value more than 80% within 45 minutes. .(Kaplan,2010)

A generic drug is an off-patent medication that has the same active ingredient, dose and route of administration as the original product. They are safe, effective, and cheap and thus they have many advantages from a medical and financial viewpoint as well. Since there is difficulty in the selection of generic drugs by the pharmacies or hospitals, it is important to ensure that products containing same active ingredients marketed by different pharmaceutical industries are safe, effective, high quality and clinically equivalent. Different brands of same drug would have been produced by different manufacturing methods and possibly with different excipients that may result in different bio availabilities. Different drug regulatory bodies, like Food and Drug Administration (FDA), have specified some bioequivalence requirements aimed at ensuring that similar dosage forms containing same active pharmaceutical ingredient (API) will have similar efficacy and safety. The increase in number of generic drug products from multiple sources has placed people, involved in the delivery of health care, in a position of having to select one from among several seemingly equivalent products. However, many developing countries do not have an effective means of monitoring the quality of generic drug products in the market. This results in widespread distribution of substandard and/or counterfeit drug products. Pharmaceutical equivalents are the drug products which contain the same active ingredient, are of same dosage form, route of administration and are identical in strength and concentration. Bioequivalence studies are useful in comparing the bioavailability of drug from various drug products. Once the drug products are demonstrated to be bioequivalent, then the efficacy of these products is assumed to be similar. Generic drug products must satisfy the same standards of quality, efficacy and safety as those applicable to the innovator products.

Preliminary physicochemical assessment of the products is very important and in vitro dissolution testing can be a valuable predictor of the in vivo bioavailability and bioequivalence of oral solid dosage forms. The establishment of bioequivalence is essential to interchangeability so that a patient can substitute a generic for a particular product without jeopardizing efficacy or safety. Ranitidine belongs to a class of drugs known as H2-blockers, which blocks the action of histamine on stomach cells and hence reduces stomach acid production. The H2 receptor antagonists inhibit acid production by reversibly competing with histamine binding to H2 receptors on the basolateral membrane of parietal cells in stomach the major therapeutic indications for H2 receptor antagonists are to promote healing of gastric and duodenal ulcers, to treat uncomplicated gastrointestinal esophageal reflux disease (GERD) and to prevent the occurrence of stress ulcers. This study was conducted to evaluate the pharmaceutical equivalence of different brands of Ranitidine HCl tablets that are available within the Pokhara valley from different companies of Nepal and India. Comparison of the technical quality aspects of this product will help for the selection of best brand of drug by the pharmacies or hospitals. This study aims to provide the proof of safety and effectiveness before the drugs can be used (Kerr, 2016).

1.17 Properties of the API important to dissolution include

The solubility of the API in the dissolution medium, which is usually an aqueous buffer solution (may contain surfactants as well).Whether the API is hydrophilic or hydrophobic (ease of surface wetting). The particle size of the API. Whether the API is crystalline or amorphous in the drug product. If there are polymorphs, which polymorph is present. If a salt form is used. (Kerr, 2016).

1.18 Process involved in Dissolution process

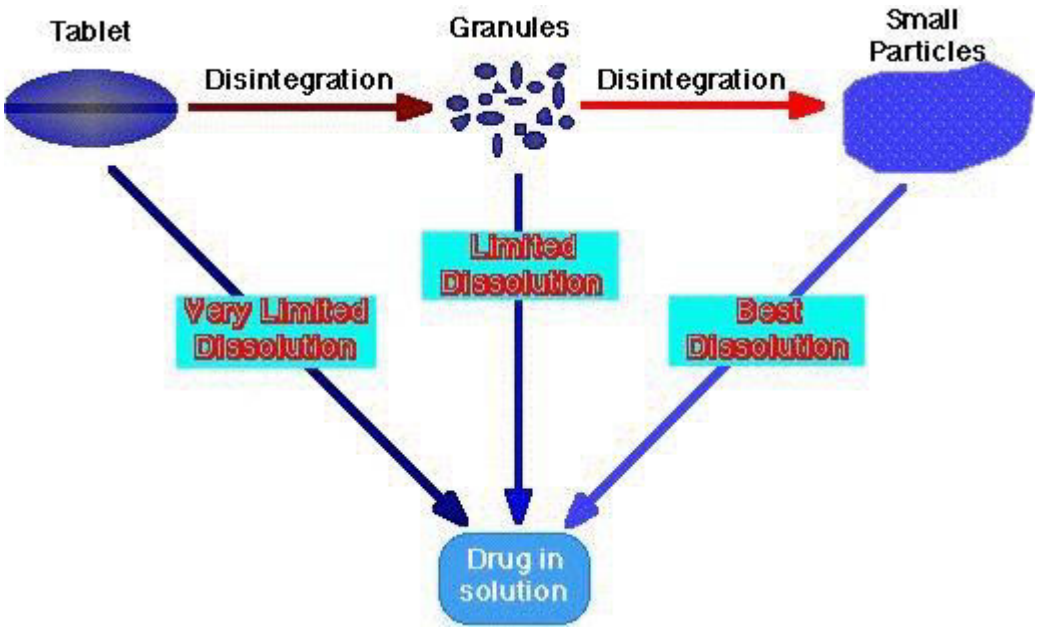

Figure 1.11: Process involved in Dissolution process (Kaplan, 2010)

1.19 Applications of Dissolution in the Pharmaceutical Industry

1. As a formulation design aid (since formulation can profoundly affect dissolution behaviour)
2. As a quality control measure immediately after production for batch release
3. As a quality control measure to check performance during the shelf life
4. To predict performance under various dosing conditions ("biorelevant" methods)
5. To verify that the quality of a product is not adversely affected when there is a change in excipients or manufacturing method (can sometimes be used instead of a pharmacokinetic study)
6. To obtain approval for a multisource drug product (''generic" version of an existing drug product) – in certain cases a pharmacokinetic study is not required

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

the tablet or capsule is therefore crucial. One of the problems facing the pharmaceutical industry is to optimize the amount of drug available to the body, i.e. its bioavailability**.** Inadequacies in bioavailability can mean that the treatment is ineffective and at worst potentially dangerous (toxic overdose).Drug release in the human body can be measured *invivo* 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. Tablet Dissolution is a standardized method for measuring the rate of drug release from a dosage form. The principle function of the dissolution test may be summarized as follows:

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

Although initially developed for oral dosage forms, the role of the dissolution test has now been extended to drug release studies on various other forms such as topical and transdermal systems and suppositories (Knott, 2016).

Chapter Two
LITERATURE REVIEW

- Allergic rhinitis (AR) and chronic idiopathic urticaria (CIU) are common causes of substantial illness and disability in preschool children. Antihistamines are commonly used to treat preschool children with these conditions, but their use is based mostly on extrapolated efficacy from adult populations; it is thus important to characterize the safety of antihistamines in the pediatric population. This study was designed to assess the safety of levocetirizine dihydrochloride oral liquid drops in infants and children with AR or CIU. Two multicenter, double-blind, randomized, parallel-group studies randomized infants aged 6-11 months (study 1, n = 69) and children aged 1-5 years (study 2, n = 173) to levocetirizine, 1.25 mg (q.d. or b.i.d., respectively), or placebo for 2 weeks, using a 2:1 ratio. Safety evaluations included treatment-emergent adverse events (TEAEs), vital signs, electrocardiographic (ECG) assessments, and laboratory tests. The overall incidence of TEAEs was similar between levocetirizine and placebo in both studies. Most TEAEs were mild or moderate in intensity. TEAEs prompted discontinuation of therapy in three patients receiving levocetirizine in study 1. No clinically relevant changes from baseline in vital signs or laboratory parameters were apparent in either study; changes from baseline in these evaluations were similar between groups. No significant changes were observed in ECG parameters, including corrected QT interval. Levocetirizine, 1.25 and 2.5 mg/day, was well tolerated in infants aged 6-11 months and in children aged 1-5 years, respectively, with AR or CIU. (Abo Dena, 2017)

- To evaluate the effect of levocetirizine on the Total 4 Symptoms Score, the 50% response rate, the Pediatric Rhinitis QualityThe levocetirizine group showed a significant improvement in 2-week and 4-week Total 4 Symptoms Score compared with placebo (P =.001 and P = .008, respectively). The 50% response rate for the first 2 weeks was 12.3% for the levocetirizine group compared with 3.9% for the placebo group (P = .01). The investigators' global evaluation also favored levocetirizine, because 57.1% of the children in the levocetirizine group were considered markedly or moderately improved compared with 44.7% in the placebo group. Levocetirizine also provided a significantly greater HRQL improvement than placebo at 2 weeks (P = .01), and the frequency of adverse events did not differ significantly from those seen in the placebo group. of Life Questionnaire

(PRQLQ), and investigators' global evaluation of symptom improvement. The study confirmed the efficacy of levocetirizine in relieving symptoms of perennial allergic rhinitis in children between 6 and 12 years of age. A HRQL benefit greater than placebo was shown. The treatment was well tolerated. (Abramovits, W. and Gupta, A. ,2008).

- The randomized, double-blind, placebo-controlled cross-over study compared inhibition by one 5 mg dose of levocetirizine with two 60 mg doses of fexofenadine separated by 12 h of histamine-induced wheal and flare responses in 9 Caucasian and 9 Japanese healthy male volunteers. Levocetirizine was more inhibitory than fexo fenadine on wheal, flare and pruritus ($p < 0.005$). Variability, evaluated from the standard deviation of inhibition, ranged from 14% to 23.2% for levocetirizine and 65.4% to 112.4% for fexofenadine. Levocetirizine had a faster onset of action (30–90 min versus 2 h), shorter time to maximum effect (3–4 versus 3–6 h) and longer duration of action (at least 24 h versus ~12 h) than fexofenadine. The plasma levels of levocetirizine rose more quickly, reached higher levels, were more consistent and decreased slower than those of fexofenadine. There were no clinically significant ethnic differences in responsiveness to the drugs. (Ali, O., Ismail, N. and Elgohary, R. ,2016).

- The histamine-induced wheal and flare response was used to compare quantitatively the antihistaminic potency of levocetirizine and desloratadine. All doses of levocetirizine significantly ($P < 0.0001$) inhibited both wheals and flares in a dose-related manner. Only the 10 mg dose of desloratadine achieved significant inhibition of response. ANOVA showed levocetirizine to be significantly ($P < 0.0001$) more active than desloratadine. Neither drug caused significant sedation or loss of motricity. Levocetirizine is significantly more effective than desloratadine in inhibiting wheal and flare responses to histamine in human skin *in vivo*, with 1.25 mg levocetirizine being more effective than 10 mg desloratadine. (Bachert, C. ,2005).

- A novel, simple, sensitive and rapid spectrophotometric method has been developed for simultaneous estimation of ambroxol hydrochloride and levocetirizine dihydrochloride. The method involved solving simultaneous equations based on measurement of absorbance at two wavelengths 242 nm and 231 nm, the γ max of ambroxol hydrochloride and levocetirizine dihydrochloride, respectively. Beer's law was obeyed in the concentration range 10–50 μg/ml and 8–24 μg/ml for ambroxol hydrochloride and levocetirizine dihydrochloride respectively. Results of the method were validated statistically and by recovery studies. (Bautista, A., Eisenlohr, C. and Lanz, M., 2011).

- The new generation antihistamines, such as desloratadine and levocetirizine, have provided major advances in the treatment of chronic idiopathic urticaria (CIU). There has been debate regarding the efficacy and sedative effects of desloratadine and levocetirizine, with findings from several studies indicating that levocetirizine is superior to desloratadine in terms of drug activity. However, the comparative sedative effects of the two drugs have not been well studied. In the result indicates that Levoceterizine is more efficacious and may facilitate better control than Desloratadine in that disease(Day, J., Briscoe, M. and Ratz, J ,2008).

- Allergic rhinitis is commonly treated with antihistamines. Monitoring improvement of airway inflammation noninvasively using nasal nitric oxide (nNO) would be clinically useful. To determine the anti-inflammatory effect of oral levocetirizine dihydrochloride (LC), Bautista ,Angella, Claudia etc measured nasal NO (nitric oxide) and nasal eosinophils (nEos) in perennial allergic rhinitis (PAR) subjects and the result was the oral levoceterizine dihydrochloride treatment successfully decrease that inflammation with improved symptoms. (Dena, A. and Hassan, W.,2016).

- A long-term study was performed of levocetirizine safety in young atopic children. In the randomized, double-masked Early Prevention of Asthma in Atopic Children Study, 510 atopic children who were age 12–24 months at entry received either levocetirizine 0.125

mg/kg or placebo twice daily for 18 months. The population evaluated for safety consisted of 255 children given levocetirizine and 255 children given placebo. The treatment groups were similar demographically, and with regard to number of children with: one or more adverse events (levocetirizine, 96.9%; placebo, 95.7%); serious adverse events (levocetirizine, 12.2%; placebo, 14.5%); medication-attributed adverse events (levocetirizine, 5.1%; placebo, 6.3%); and adverse events that led to permanent discontinuation of study medication (levocetirizine, 2.0%; placebo, 1.2%). The most frequent adverse events related to: upper respiratory tract infections, transient gastroenteritis symptoms, or exacerbations of allergic diseases. There were no significant differences between the treatment groups in height, mass, attainment of developmental milestones, and hematology and biochemistry tests. The long-term safety of levocetirizine has been confirmed in young atopic children. (DuBuske, L.,2007).

- Fast disintegrating films of levocetirizine dihydrochloride useful for the treatment of acute allergic rhinitis and chronic urticaria have been developed by using the taste masking ability of cyclodextrins. The fast disintegrating films were prepared by solvent casting method. The films contained water-soluble polymers such as Kollicoat IR or pullulan, aspartame and sucralose as sweeteners and pre-gelatinized starch as disintegrant. Levocetirizine dihydrochloride was incorporated into these films by in-situ complex formation with hydroxy propyl β-cyclodextrin. The optimized films were evaluated for weight variation, film thickness, folding endurance, tackiness, tensile strength, assay, content uniformity, in vitro disintegration and dissolution, in vivo disintegration and taste masking ability by human gustatory sensation test. Results revealed that the organoleptic properties of levocetirizine dihydrochloride were improved by complexation with hydroxy propyl β-cyclodextrin and the complex could be successfully formulated into a fast disintegrating film. (Gupta, V. and Matreja, P. ,2010).

- A simultaneous method has been developed and validated for estimation of gliquidone in the presence of H1- receptor antagonists (cetirizine hydrochloride, hydrochloride, and levocetirizine dihydrochloride) using reversedphase high-performance liquid chromatographic technique. A good chromatographic separation between these drugs was achieved using a mobile phase containing methanol-water (80:20 v/v) at pH 3.5 with a flow rate of 1.0 mL/min; and detection was performed at 230 nm with a UV detector. Validation of the method was performed in terms of linearity, accuracy, precision, and limit of detection and quantification. The linearity of the calibration curves for gliquidone, hydrochloride, hydrochloride, and levocetirizine dihydrochloride were found to be 0.338-50 μg/mL (r = 0.9964), 5-50 μg/mL (r = 0.9956), 0.325-50 μg/mL (r = 0.9967), and 0.553-50 μg/mL (r = 0.9950), respectively. There was no significant difference between the amount of drug spiked in serum and the amount recovered, and serum did not interfere in simultaneous estimation. Thus, the proposed method is suitable for the simultaneous analysis of active ingredients in tablet dosage forms and human serum. (Hampel, F., Ratner, P. and Haeusler, J., 2010).

- The fast dissolving oral films were designed using optimal design and numerical optimization technique was applied to find out the best formulation. Film forming agent HPMC, sodium CMC was considered as independent variables. Drug release rate from 45sec to 990sec, T50% and release exponent (n) were taken as responses. Decrease the viscosity of film former a specific limit, changes the release from zero order to Hixson-Crowell based release. The optimized formulation F1 was found superior than remaining 8 batches. Amongst all the formulation, formulation F1 releases the complete drug in 360 sec. but other formulation takes more time for complete release. The IR and DSC studies revealed that no physicochemical interaction between excipients and drug. The influence of pH and agitation intensity on the release of drug was studied and the release mechanism was through disintegration. Stability studies revealed that optimized formulation was stable. The observed independent variables were found to be very close to predicted values of most satisfactory formulation which demonstrates the feasibility of the optimization procedure in successful development of fast dissolving oral film containing levocetirizine

Dihydrochloride by using HPMC, sodium CMC and PEG 400 as key excipients. (Joshi, S., Bhatia, C., Bal, C. and Rawat, M.,2012).

- Purpose of undertaken project was to formulate crosslink polyacrilic resin based, technologically optimised, melt-in-mouth tablet (MIMT) containing 5 mg of Levocetirizine Dihydrochloride that was intended to disintegrate rapidly in the oral cavity so as to form a stabilised dispersion and possessing adequate physicochemical stability. Different grades of crosslink polyacrilic resin were utilised to prepare MIMTs; employing complexation technique; and using additives like Mannitol DC, Ac-di-sol, Avicel-pH 112, Tusil pinapple, Saccharine sodium, Aerosil and Magnesium stearate. MIMTs were evaluated for compliance to pharmacopoeial specifications. From in-vitrodissolution profile plot, values for the kinetic constant and the regression coefficient of model-dependent approaches were determined to find the best fit release kinetic model while from in-vitrodissolution profile data the difference factor, the similarity factor and the indices of rescigno of model-independent approaches were determined for comparing pair of in-vitrodissolution profiles. MIMTs of levocetirizine was successfully developed complying pharmacopoeial specifications, with adequate stability at room temperature.(Kathpalia, H. and Patil, A.,2017).

Chapter Three
MATERIALS & METHODS

3.1 Introduction:

The study on comparative dissolution profiles of levocetirizine dihydrochloride was carried out by using dissolution method to see the release pattern of levocetirizine dihydrochloride 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 levocetirizine dihydrochloride in dissolution media I used different brands of levocetirizine dihydrochloride tablet. I used active pharmaceutical ingredient (API) of levocetirizine dihydrochloride which was collect from Incepta Pharmaceuticals Ltd.

As the dissolution media is water for dissolution of levocetirizine dihydrochloride we used water as a solvent. xyzal is the patent drug of levocetirizine dihydrochloride.For preparing a standard curve I used seasonix tablet from Incepta pharmaceuticals. Other tablets I used to see the release pattern with different time interval like Lazine, Clarigen, Lecet, Alcet, Purotrol etc.

3.3 Methods for Comparison of Dissolution Profile Data

A simple model independent method proposed by Moore and Flanner (1996) uses fit factors to compare dissolution profile data of a pair of products under similar testing conditions. These fit

factors directly compare the difference between percent drug dissolved per unit time for a test and reference product. These factors are denoted f1 (difference factor) and f2 (similarity factor) (US FDA, 1997; Saranadasa and Krishnamoorthy 2005; Sath*, et. al.* 1996; Yuksel *et. al.* 2000).

The difference factor (f1) is a measurement of the percent difference between two dissolution curves under comparison at each time point.

It is a measure of the relative error between the two curves and is given by the formula:

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

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

3.4 Similarity factor

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

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

Where, n is the number of testing time points; Rt is the average dissolution value of the reference product units at time t and it is the average dissolution value of the test product units at time t (US FDA, 1997; Hasan, *et. al.* 2007; Shah 2001; Yuksel, *et. al.* 2000). The proviso for evaluation for similarity is availability of data for six (6) or twelve (12) units of each product, availability of three

or more dissolution time points, same conditions of testing for reference and test products and same dissolution time points for both profiles. As a further recommendation, it is suggested that only one measurement be considered after 85% dissolution of both products.

(US the US FDA and the European Medicines Agency (EMEA) for dissolution profile comparison. FDA, 1997; Hasan, *et. al.* 2007; Ochekpe, *et. al.* 2006).The similarity factor has been adopted by When two dissolution profiles are identical, f2 = 100%. An average dissolution difference of 10% at all measured time point's results in an f2 value of 50%. For this reason, the public standard for similarity of two dissolution profiles has been set at 50 - 100% (EMEA 2010d; USFDA 1997; Shah, 2001).

3.5 Dissolution Testing Methods for Levocetirizine Dihydrochloride

Dissolution media	Distilled water
RPM	50
Temperature	37ºC
Time	50 minutes
Wavelength	231nm

Table 3.1: Dissolution parameter

The release rate of levocetirizine dihydrochloride tablet was determined by using tablet dissolution tester USP XXII. The dissolution test was performed using 900ml water pH (7.4) at 37+-0.5 degree C and 50 r.p.m. At 10 , 20 and 30 min interval sample of 5 ml were withdrawn from the dissolution medium and the amount was replace by 5 ml distill water. The sample was filtered through a filter paper named Whatmaan Filter paper and diluted to a suitable concentration of distilled water. The absorbance of the solution was measured 231nm for drug levocetirizine 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 50

minutes to get simulated picture of drug release in thw in vivo condition and drug dissolve at specified time periods was plotted as percent release versus time(hours) curve (Shah,*et al.*1998).

3.6 Preparation of Standard Curve:

To prepare the standard curve, at first different concentrations (2, 4, 6, 8 and 10) µg/ml of levocetirizine dihydrochloride was prepared. For the preparation of different concentrations of levocetirizine dihydrochloride, First Seasonix (levocetirizine dihydrochloride) tablet was crushed in mortar and pestle. From the crushed tablet 5 mg was taken and was dissolved in 100 ml of distilled water. By this procedure the concentration of the stock solution became 0.05mg/ml or 50µg/ml.This solution was filtered in the volumetric flask. After that the solution was 50 times diluted and the concentrations of the solution become 5µg/ml. Then taken solution was 2 ml, 4 ml, 6 ml, 8 ml, 10 ml and added water was 8 ml, 6 ml, 4 ml, 2 ml, and 0 ml. Then spectrophotometer is turned on and 231nm 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 3.2: Concentrations of levoctirizine (Campanero, *et. al.*1998)

Serial no	**Concentration(µg/ml)**
1	**2**
2	**4**
3	**6**
4	**8**
5	**10**

3.7 Preparation for dissolution test:

3.7.1 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.

3.7.2 Method for dissolution test Levocetirizine Dihydrochloride

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 50 was set up in the dissolution machine. Then the machine was allowed to warm up until it reached at 37.5 degree C. Then 1 Zantac® tablet was placed in every vessel. After 10, 20 and 30 minutes 5 ml of solution was collected from each vessels 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 231nm. (Lawrence, *et. al.,* 2002).

3.8 Determination of physical parameters

3.8.1 Weight Variation Test

3.8.1.1 Procedure:

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

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

Table 3.3: Accepted percentage list for weight variation test of tablets

3.8.1.2 Equation:

Following equation was used to determine % weight variation of tablets

$$\% \text{ Weight Variation} = (A\text{-}I/A) \times 100$$

Where,

Initial Weight of Tablet, I (gm)

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

3.8.2 Thickness test

3.8.2.1 Procedure

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

3.8.2.2 Calculation

Following formula was used to determine thickness of tablets.

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

3.9.Equipment's:

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

No.	Equipments	Source	Origin
1	Dissolution tester USPXXII	RC-6B	CHINA
2	UV-Spectrometer	HANNA1201PC	JAPAN
3	pH meter	HANNA pH 210	PORTUGAL
4	Distill Water Plant	SMIC	CHINA

5	**Safety Pipette Filler**	**Saffron**	**ENGLAND**
6	**Filter**	**Copley Instruments**	**ENGLAND**
7	**Electronic Balance**	**Precisa XB120A**	**SWITZERLAND**
8	**Friability tester**	**VEEGO(EF-2)**	**INDIA**
9	**Vernier Slide Calipers**	**TRICLYCLE RING**	**INDIA**
10	**Hardness tester**	**Monasnto manually operating hardness tester**	**CHINA**

Table 3.4 : In the characterization of matrix tablets of Levocetirizine

3.10 Instrumentation

3.10.1 Dissolution Test Apparatus

A Dissolution tester USPXXII (source RC-6B, made in China) was used for dissolution experiments. It incorporated a clear acrylic water bath, a stirrer hood with paddle shafts, an automatic sampling unit and a control unit supported by microcontroller software with a non-volatile memory for 15 methods. The water bath incorporated an immersion circulator with an in-built thermostat for temperature control, an external temperature sensor, a water level sensor and a lid with support for eight dissolution bowls. The stirrer hood was equipped with 8 paddle shafts fitted with USP apparatus 2 and a tablet dispenser with 8 conical shaped dissolution bowl lids. The automatic sampling unit consisted of 10in-line filters, a bi-directional 12- channel peristaltic pump with tygon tubing's, a microprocessor controlled sample collector and a sample tray capable of collecting 10 x 6 sets of samples. Polycarbonate dissolution vessels with a hemispherical bottom and a capacity of 1000 ml were used for the study. Bromide (E. Merck, Darmstadt, Germany) and a manually operated hydraulic pellet press (Perking Elmer GmbH, Uberlingen, Germany).

3.10.2 Ultra- Violet Spectrophotometer

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

3.11 Samples and Chemical Reference Substances

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

3.12 Images of Instruments:

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

Figure 3.1: Dissolution apparatus

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

Figure 3.3: Distilled Water apparatus

Figure 3.5: Electronic Balance

Figure 3.6 :Rough Balance

3.13 Dissolution Efficiency

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

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

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

3.14 Apparatus:

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

Table 3.6 - Representing the apparatus (Kuss, 1992)

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

Chapter Four

RESULTS AND DISCUSSION

4.1 Standard curve of Seasonix:

For the calculation of drug release from the reference brand as well as test brands, a standard curve was prepared within the concentration range of 0-25 microgram/mL. The curve displayed sufficient linearity with a correlation coefficient (R2) value of 0.9912 and provided an equation y=0.0354x+0.0081. The standard curve is shown in figure 4.1.

Concentration (µg/ml)	**Absorbance**
0	0
2	0.079
4	0.150
6	0.233
8	0.305
10	0.343

Table 4.1: Standard curve of Seasonix

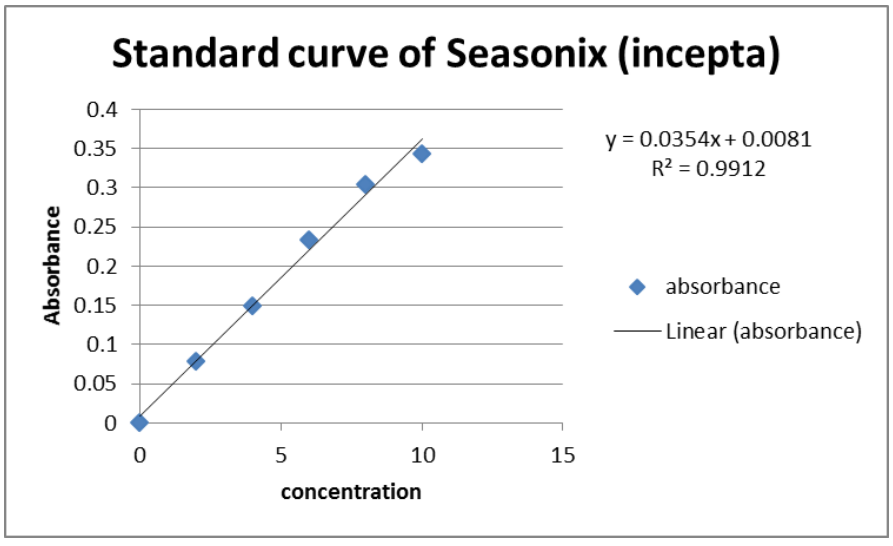

Figure 4.1: . The standard curve of Seasonix

By plotting the concentration against the absorbance of ranitidine we found a straight line. From the standard curve ranitidine, we derived an equation y=0.0354x+0.0081 & R^2=0.9912(Here, y= Absorbance and x=Concentration of drug).

4.2 Percent (%) release of Seasonix Tablets samples:

Time	Absorbance	Conc.	DR(mg)	release	% release
0	0	0	0	0	0
10	0.308	8.571429	7714.286	4060.15	81.20301
20	0.351	9.8	8820	4642.105	92.84211
30	0.354	9.885714	8897.143	4682.707	93.65414

Table 4.2: Percent (%) release of Seasonix samples

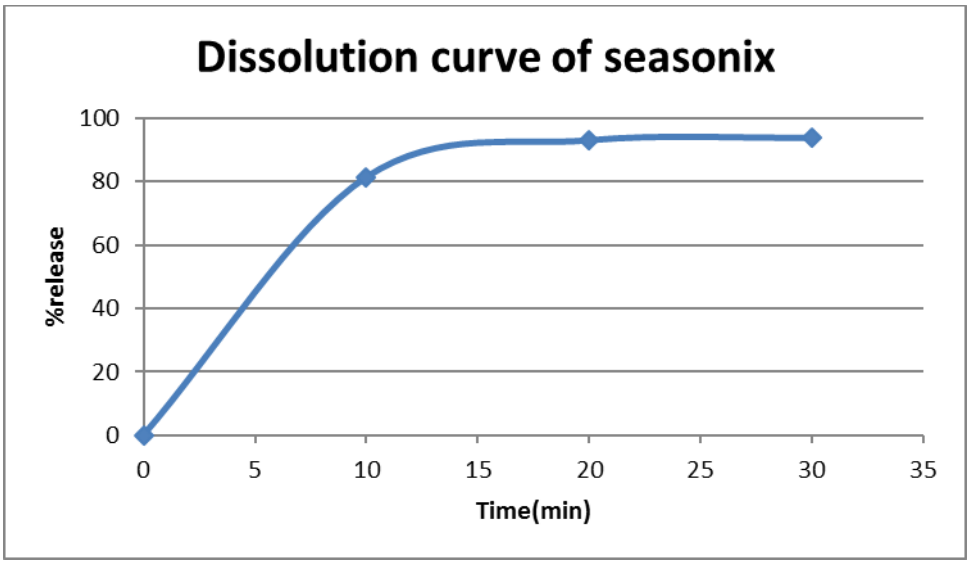

Figure 4.2: Time Vs Drug Release (%) of Seasonix samples.

Here the graph shows that, the release pattern of Seasonix is increasing with time. So, the dissolution pattern is increased with time.

4.3 Percent (%) release of Alcet tablet

Time	Absorbance	Conc.	DR(mg)	release	% release
0	0	0	0	0	0
10	0.437	11.02632	9923.684	4961.842	99.23684
20	0.384	9.631579	8668.421	4334.211	86.68421
30	0.378	9.473684	8526.316	4263.158	85.26316

Table 4.3: Percent (%) release of Alcet tablet

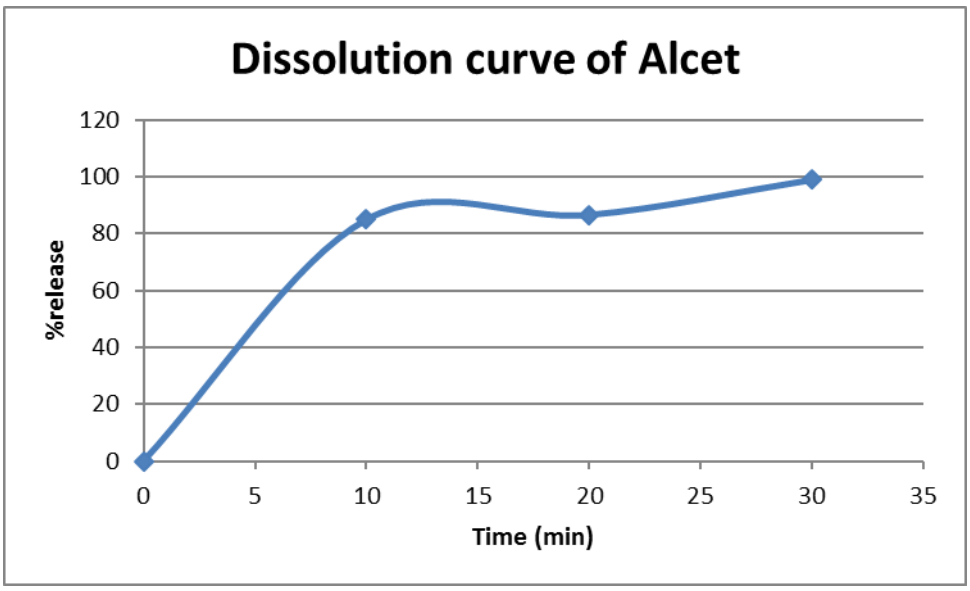

Figure 4.3: Time Vs Drug release (%) of Alcetsamples.

Here the graph shows that, the release pattern of Alcet is increasing with time. So, the dissolution pattern is increased with time.

4.4 Percent (%) release of Lecet tablets samples

Time	Absorbance	Conc.	DR(mg)	release	% release
0	0	0	0	0	0
10	0.182	5.457143	4911.429	4092.857	81.85714
20	0.205	6.114286	5502.857	4585.714	91.71429
30	0.224	6.657143	5991.429	4992.857	99.85714

Table 4.4: Percent (%) release of Lecet tablet

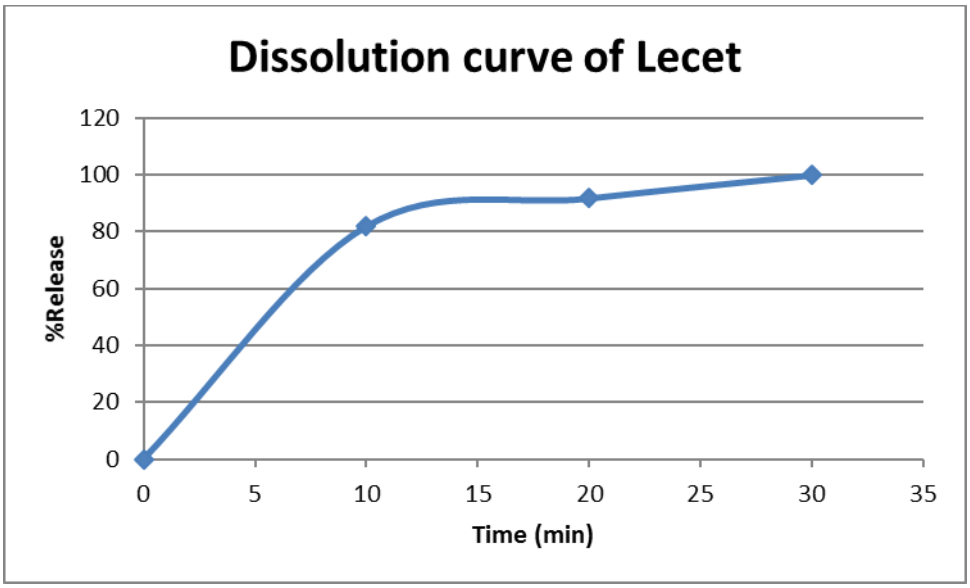

Figure 4.4: Time Vs Drug release (%) of Lecetsamples.

Here the graph shows that, the release pattern of Lecet is increasing with time. So, the dissolution pattern is increased with time.

4.5 Percent release of Clarigen tablets samples

Time	Absorbance	conc	DR(mg)	%release
0	0	0	0	0
10	0.114	2.016949	1815.254	36.30508
20	0.216	3.745763	3371.186	67.42373
30	0.242	4.186441	3767.797	75.35593

Table 4.5: Percent (%) release of Clarigentablet

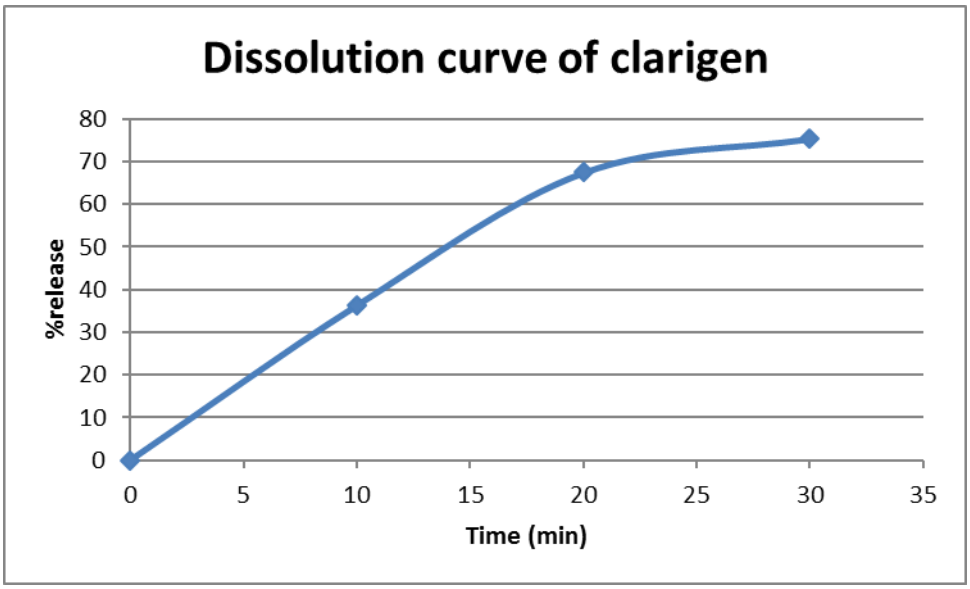

Figure 4.5: Time Vs Drug release (%) of Clarigensamples.

Here the graph shows that, the release pattern of Clarigen is increasing with time. So, the dissolution pattern is increased

4.6 Percent release of Lozin tablet samples

Time	Absorbance	Conc.	DR(mg)	release	%release
0	0	0	0	0	0
10	0.388	10.21053	9189.474	3828.947	76.57895
20	0.498	13.10526	11794.74	4914.474	98.28947
30	0.49	12.89474	11605.26	4835.526	96.71053

Table 4.6 : Percent (%) release of Lozintablet

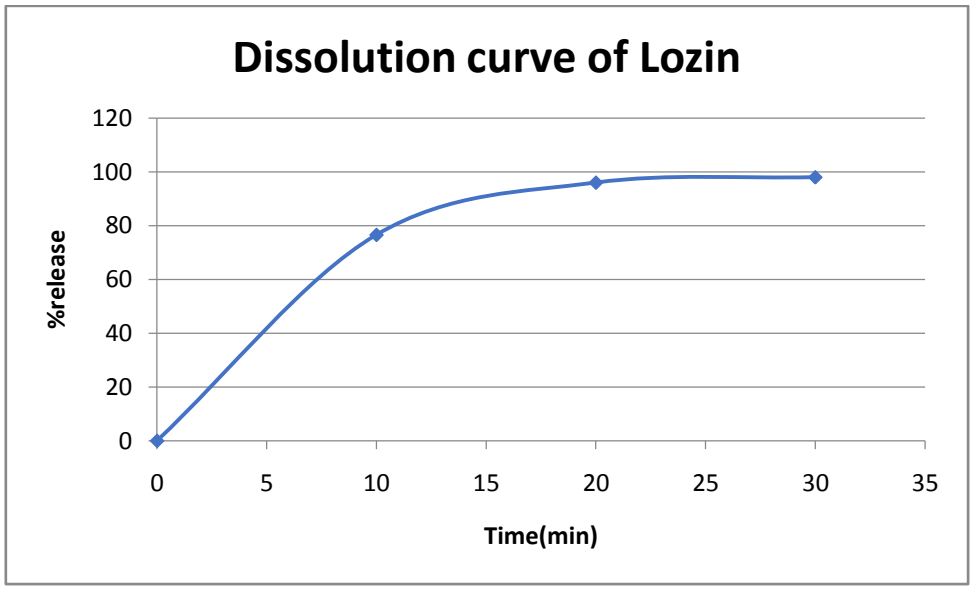

Figure 4.6: Time Vs Drug release (%) of Lozinsamples.

Here the graph shows that, the release pattern of lozin is increasing with time. So, the dissolution pattern is increased

4.7 *f*1 Calculation for different Levocetirizine tablets

Difference Factor, *f*1 is the average difference between all the points of sampling between two brands e.g. reference brand and one of the two test brands. Acceptable range of *f*1 is between 0-15.

*f*1 value greater than 15 means significant difference between two brands which is not accepted (Lokhandwala et al. 2013; Parakh and Patil 2014; Patel,*et. al*. 2015; Qazi et al. 2013).

Table 4.7.1- *f*1 Calculation for Alcet tablets

Time	Seasonix (R)	Alcet (T)	R-T	IR-TI	*f 1*
0	0	0	0	0	
10	81.203	99.236	18.033	18.033	
20	92.842	86.684	-6.158	6.158	12.15
30	93.654	85.263	8.391	8.391	
	267.699			32.582	

Table 4.7.1 - *f*1 Calculation for Alcet tablets

Table 4.7.2- *f*1 Calculation for Lecet tablets

Time	Seasonix (R)	Lecet (T)	R-T	IR-TI	*f 1*
0	0	0	0	0	
10	81.203	81.857	-0.654	0.654	
20	92.842	91.714	1.128	1.128	2.9
30	93.654	99.857	-6.221	6.221	
	267.699			8.003	

Table 4.7.3- *f*1 Calculation for Clarigen tablets

Time	Seasonix (R)	Clarigin (T)	R-T	IR-TI	*f 1*
0	0	0	0	0	
10	81.203	36.305	44.898	44.898	
20	92.842	67.423	25.419	25.419	33.10
30	93.654	75.355	18.299	18.299	
	267.699			88.616	

Table 4.7.4- *f*1 Calculation for Lozin tablets

Time	Seasonix (R)	Lozin (T)	R-T	IR-TI	*f 1*
0	0	0	0	0	
10	81.203	76.578	4.625	4.625	
20	92.842	98.289	-5.447	5.447	4.9
30	93.654	96.710	-3.056	3.056	
	267.699			13.128	

Acceptable range of *f*1 is between 0-15. *f*1 value greater than 15 means significant difference between two brands which is not accepted. From the table 4.9 and 4.10 we see that the values of *f*1 are 12.15 , 2.9 , 33.10 , 4.9.

4.8*f*2 Calculation for differentlevocetirizine tablets:

Similarity Factor, *f*2 Similarity factor is calculated to determine significant similarity between two brands. The range of the *f*2 value is between 0 to 100. If the value remains between 50 to 100, it is acceptable (Lokhandwala et al. 2013; Parakh and Patil 2014; Patel et al. 2015; Qazi et al. 2013).

Table 4.8.1- *f*2 Calculation for Seasonix and Alcet tablets

Time	Seasonix (R)	Alcet (T)	R-T	IR-TI	IR-TI^2	*f 2*
0	0	0	0	0	0	
10	81.203	99.236	18.033	18.033	325.895	
20	92.842	86.684	-6.158	6.158	37.922	49.00
30	93.654	85.263	8.391	8.391	70.408	
	267.699			32.582	434.225	

Table 4.8.2. - *f*2 Calculation for Seasonix and Lecet Tablets

Time	Seasonix (R)	Lecet (T)	R-T	IR-TI	IR-TI^2	*f 2*
0	0	0	0	0	0	
10	81.203	81.857	-0.654	0.654	0.427	
20	92.842	91.714	1.128	1.128	1.272	73.87
30	93.654	99.857	-6.221	6.221	38.702	
	267.699			8.003	40.399	

Table 4.8.3- *f*2 Calculation for Seasonix and Clarigen Tablets

Time	Seasonix (R)	Clarigen (T)	R-T	IR-TI	IR-TI^2	*f 2*
0	0	0	0	0	0	
10	81.203	36.305	44.898	44.898	2015.830	
20	92.842	67.423	25.419	25.419	646.125	25.47
30	93.654	75.355	18.299	18.299	334.854	
	267.699			88.616	3825.978	

4.8.4 - *f*2 Calculation for Seasonix and Lozin Tablets

Time	Seasonix (R)	Lozin (T)	R-T	IR-TI	IR-TI^2	*f 2*
0	0	0	0	0	0	
10	81.203	76.578	4.625	4.625	21.390	
20	92.842	98.289	-5.447	5.447	29.669	69.82
30	93.654	96.710	-3.056	3.056	9.339	
	267.699			13.128	60.398	

The range of the *f*2 value is between 0 to 100. If the value remains between 50 to 100, it is acceptable. From the table 4.11 and 4.12 we see that the values of *f*2 are 49.00, 73.87, 25.47 , 69.82 .so it is acceptable.

Chapter Five
CONCLUSION

Conclusion

Levocetirizine Dihydrochloride is classified as a Class III drug (high solubility and low permeability) by the BCS. Dissolution tests are essential for the prognosis of dosage form oral absorption and bioequivalence of drugs. In this study we have compared the dissolution profile of four local brands Alcet, Lecet, Clarigen and Lozin with Seasonix (referance drug of levocetirizine). It was found that the difference factor of Alcet with Seasonix is 12.15, Lecet with seasonix is 2.9, ,Lozin with seasonix 4.9, Clarigen with Seasonix 34.10. Here the result of Clarigen show significant difference with the Seasonix. Similarity factor of Alcet, Lecet , Clarigen, Lozin with Seasonix is 49, 73.87, 25.47, 69.82 respectively. The similarity factor (except clarigen) and Difference factors of these four brands was in the acceptable range. In conclusion, further investigations are needed to find out the better dissolution profile for these four brands.

Chapter Six
REFERENCE

Abo Dena, A. and Abdel Gaber, S. (2017). In vitro drug interaction of levocetirizine and diclofenac: Theoretical and spectroscopic studies. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, [online] 181, pp.239-248. Available at: https://www.ncbi.nlm.nih.gov/pubmed/28371723.

Abramovits, W. and Gupta, A. (2008). Xyzal (levocetirizine dihydrochloride). *SKINmed*, [online] 7(2), pp.84-85. Available at: https://www.ncbi.nlm.nih.gov/pubmed/18327014.

Ali, O., Ismail, N. and Elgohary, R. (2016). Validated derivative and ratio derivative spectrophotometric methods for the simultaneous determination of levocetirizine dihydrochloride and ambroxol hydrochloride in pharmaceutical dosage form. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 153, pp.605-611.

Bachert, C. (2005). Levocetirizine: a modern H1-antihistamine for the treatment of allergic rhinitis. *Expert Review of Clinical Immunology*, 1(4), pp.495-510.

Bautista, A., Eisenlohr, C. and Lanz, M. (2011). Nasal nitric oxide and nasal eosinophils decrease with levocetirizine in subjects with perennial allergic rhinitis. *American Journal of Rhinology and Allergy*, 25(6), pp.383-387.

Day, J., Briscoe, M. and Ratz, J. (2008). Efficacy of levocetirizine compared with montelukast in subjects with ragweed-induced seasonal allergic rhinitis in the Environmental Exposure Unit. *Allergy and Asthma Proceedings*, [online] 29(3), pp.304-312. Available at: https://www.ncbi.nlm.nih.gov/pubmed/18387222.

Dena, A. and Hassan, W. (2016). Experimental and quantum mechanical studies on the ion–pair of levocetirizine and bromocresol green in aqueous solutions. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, [online] 163, pp.108-114. Available at: https://www.ncbi.nlm.nih.gov/pubmed/27038582.

DuBuske, L. (2007). Levocetirizine: The latest treatment option for allergic rhinitis and chronic idiopathic urticaria. *Allergy and Asthma Proceedings*, 28(6), pp.724-734.

Gupta, V. and Matreja, P. (2010). Efficacy of Montelukast and Levocetirizine as Treatment for Allergic Rhinitis. *Journal of Allergy & Therapy*, [online] 01(01). Available at: https://www.ncbi.nlm.nih.gov/pubmed/20112841.

Hampel, F., Ratner, P. and Haeusler, J. (2010). Safety and tolerability of levocetirizine dihydrochloride in infants and children with allergic rhinitis or chronic urticaria. *Allergy and*

Asthma Proceedings, [online] 31(4), pp.290-295. Available at: https://www.ncbi.nlm.nih.gov/pubmed/20819318.

Joshi, S., Bhatia, C., Bal, C. and Rawat, M. (2012). Quantization of dextromethorphan and levocetirizine in combined dosage form using a novel validated RP-HPLC method. *Indian Journal of Pharmaceutical Sciences*, [online] 74(1), p.83. Available at: https://www.ncbi.nlm.nih.gov/pubmed/23204629.

Kathpalia, H. and Patil, A. (2017). Formulation and Evaluation of Orally Disintegrating Films of Levocetirizine Dihydrochloride. *Indian Journal of Pharmaceutical Sciences*, [online] 79(02). Available at: https://www.ncbi.nlm.nih.gov/pubmed/20169858.

Kim, M., Jo, E., Chang, Y., Cho, S., Min, K. and Kim, S. (2013). A case of levocetirizine-induced fixed drug eruption and cross-reaction with piperazine derivatives. *Asia Pacific Allergy*, [online] 3(4), p.281. Available at: https://www.ncbi.nlm.nih.gov/pubmed/24260733.

Lee, C., Sun, H., Lu, K., Ku, M. and Lue, K. (2009). The comparison of cetirizine, levocetirizine and placebo for the treatment of childhood perennial allergic rhinitis. *Pediatric Allergy and Immunology*, [online] 20(5), pp.493-499. Available at: https://www.ncbi.nlm.nih.gov/pubmed/19175892.

Liu, X., Du, Y., Sun, W., Kou, J. and Yu, B. (2009). Study on the interaction of levocetirizine dihydrochloride with human serum albumin by molecular spectroscopy. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, [online] 74(5), pp.1189-1196. Available at: https://www.ncbi.nlm.nih.gov/pubmed/19857990.

Mahesh, A., Shastri, N. and Sadanandam, M. (2010). Development of Taste Masked Fast Disintegrating Films of Levocetirizine Dihydrochloride for Oral Use. *Current Drug Delivery*, [online] 7(1), pp.21-27. Available at: http://ttps://www.ncbi.nlm.nih.gov/pubmed/19863484h.

Patel, J. and Modi, A. (2012). Formulation, optimization and evaluation of levocetirizine dihyrochloride oral thin strip. *Journal of Pharmacy and Bioallied Sciences*, [online] 4(5), p.35. Available at: https://www.ncbi.nlm.nih.gov/pubmed/23066198.

Prabhu, P., Malli, R., Koland, M., Vijaynarayana, K., D′Souza, U., Harish, N., Shastry, C. and Charyulu, R. (2011). Formulation and evaluation of fast dissolving films of levocitirizine di hydrochloride. *International Journal of Pharmaceutical Investigation*, 1(2), p.99.

Yoruk, O., Seven, B., Varoglu, E., Ucuncu, H., Sahin, A., Ayan, A., Sutbeyaz, Y. and Tozoglu, S. (2010). Evaluation of salivary gland function in allergic rhinitis patients by [99mTc] pertechnetate salivary gland scintigraphy. *Open Medicine*, 5(2).