Determination of the Release Kinetics of Drug from Six Brands of Levoceterezine dihydrochloride (Alcet,Clarigen,Seasonix,Purotrol,Lecet,lozin) Available in Bangladesh

B. PHARM THESIS

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

Submitted by

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Declaration by the Candidate

I, Tahira Islam, hereby declare that the dissertation entitled ***"Determination of the Release Kinetics of six Brands of Levoceterizine 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.

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Certificate by the Supervisor

This is to certify that the thesis entitled **"*Determination of the Release Kinetics of six Brands of Levoceterezine 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 Tahira Islam, ID: 2013-3-70-040, during 2017 of her research in the Department of Pharmacy, East West University, under the supervision and guidance of me. The thesis has not formed the basis for the award of any other degree/diploma/fellowship or other similar title to any candidate of any university.

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This is to certify that the thesis entitled **"*Determination of the Release Kinetics of six Brands of Levoceterezine 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 Tahira Islam, ID: 2013-3-70-040, during the period 2017 of her research in the Department of Pharmacy, East West University.

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Dr. Chowdhury Faiz Hossain

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Table of Contents

Abstract

The purpose of this study was to determine the in vitro release kinetics of six brands of Levocetirizine tablets available in the local pharmaceutical market of Bangladesh. For this study, six widely prescribed brands Alcet, Seasonix, Purotrol, Lecet, Clarigen and Lozin were chosen. All of these brands were of 5 mg Levocetirizine 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 Alcet (R^2=0.735), Seasonix (R^2=0.915), Purotrol (R^2=0.966), Lecet (R^2=0.939), Clarigen (R^2=0.976) and Lozin (R^2=0.941) were the highest amongst the R^2 values comparing to zero order and first order values. So, this study assumes that the available Levocetirizine tablet brands available in Bangladesh generally follow the Higuchi's drug release kinetics.

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

Chapter One
Introduction

1 Introduction

1.1 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 guanylatecyclase and increases levels of cyclic guanosine monophosphate in vascular smooth muscle, causing vasodilation. Acting through H_1 and H_2 receptors, it causes hypotension, tachycardia, 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. The gene encoding the H_1receptor has been cloned from various animal species and from human leukocytes. (Anisa.1999)

1.1.1 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 relieveallergic 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. In type I hypersensitivity allergic reactions, an allergen (a type of antigen) interacts with and cross-links surface IgE antibodies onmast cells and basophils. Once the mast cell-antibody-antigen 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 and produces pruritus, vasodilation, hypotension, flushing, headache, bradycardia,bronchoconstriction, 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)

1.2 Levocetirizine dihydrochloride

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:

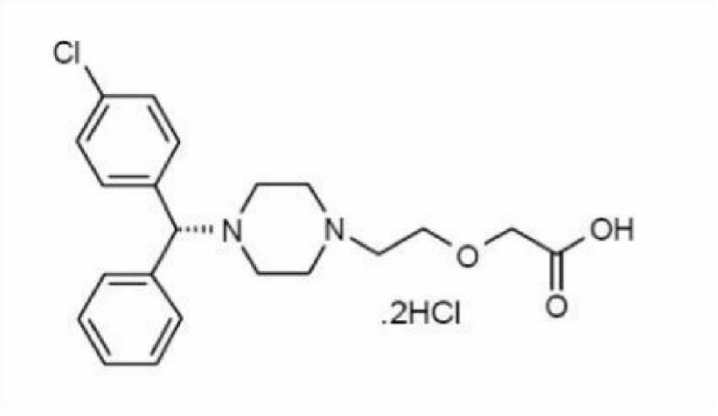

Figure 1.1: Levocetirizine dihydrochloride

Levocetirizine dihydrochloride, USP is a white or almost white powder and is freely soluble in water, practically insoluble in acetone and methylene chloride.

Levocetirizine dihydrochloride tablets, USP 5 mg are formulated as immediate release, white, film-coated, oval, scored tablets for oral administration. The tablets are debossed with “S” on the left side of bisect and “G” on the right side of the bisect and other side “1” on the left side and “36” on the right side of the bisect. Inactive ingredients are: microcrystalline cellulose, lactose monohydrate, colloidal silicon dioxide, and magnesium stearate. The film coating contains hypromellose, titanium dioxide, and polyethylene glycol. (Knott, 2010)

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 increases 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 second-generation 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. 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 belongs to a class of drugs called antihistamines. These classes of drugs are a group of medications that work in a similar way. These drugs are often used to treat similar conditions. Levocetirizine works by blocking the release of 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. (Kaplan, 2010)

1.2.1 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 LevocetirizineDihydrochloride is produced.

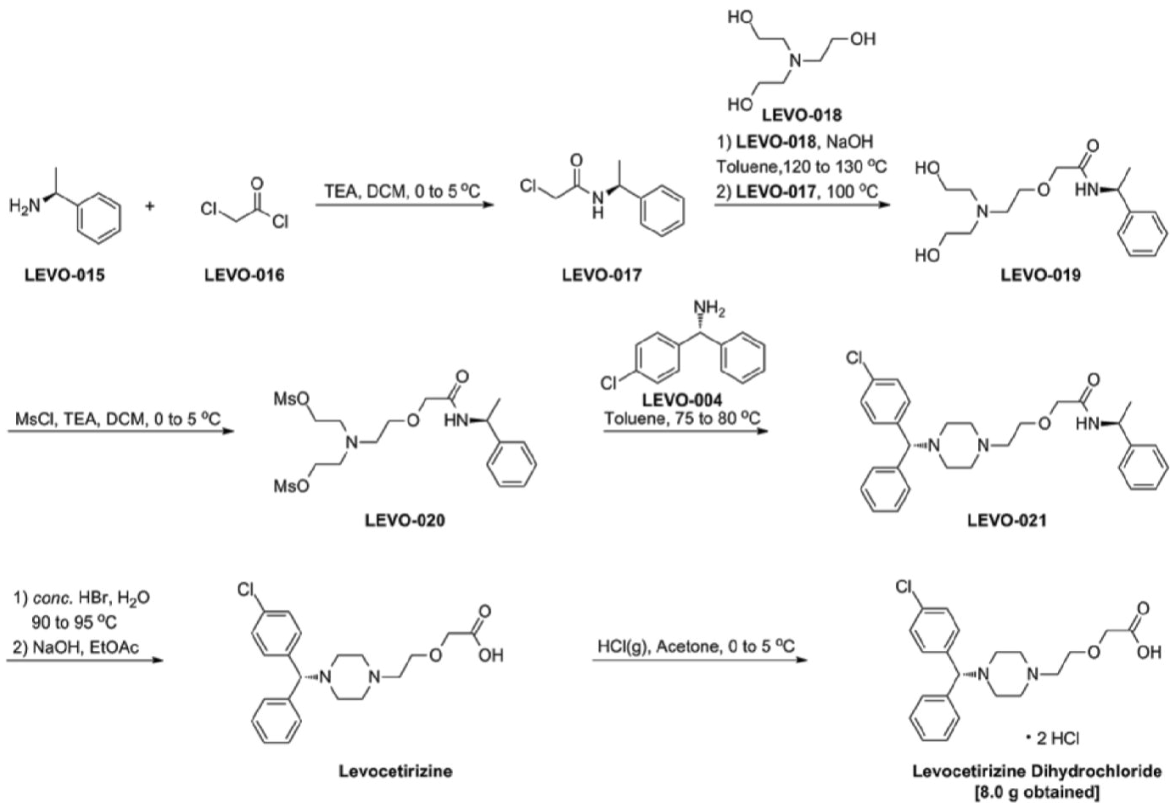

Figure 1.2: Synthesis of Levocetirizine Dihydrochloride

There are many other routes of production of this compound. But mainly this one is followed.

1.2.2 Levocetirizine dihydrochloride Information

Levocetirizinedihydrochloride 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 increases 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.

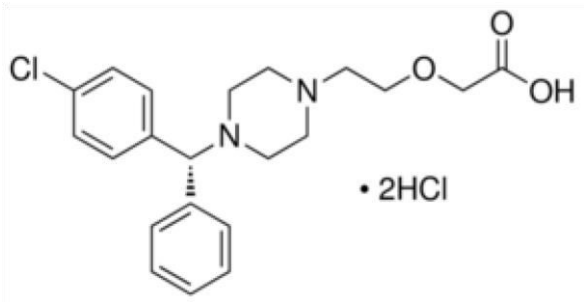

Figure 1.3: Levocetirizine Dihydrochloride

Levocetirizine is a third-generation, non-sedating antihistamine, developed from the secondgeneration antihistamine cetirizine. Biological half-life6 to 10 hours, MetabolismHepatic 14% CYP3A4 ,Formula$C_{21}H_{25}ClN_2O_3$,Drug classH1 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)

1.2.3 How it works

Levocetirizine, the active enantiomer of cetirizine, is 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**.**

1.2.4 Side Effects

Levocetirizinedihydrochloride 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 of Levocetirizinedihydrochloride

drowsiness	fatigue	weakness	tired feeling	stuffy nose	sinus pain
sore throat	cough	vomiting	diarrhea	constipation	weight gain
Confusion	Abdominal pain	Bloating	Mental cloud	Sleepiness	Anxiety

(caeolDersarkissian, 2017)

1.2.5 More common side effects

Table 1.2: Side effects of levocetirizine (according to age)

In adults and children ages 12 and older	In childrenages 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

1.2.6 Serious side effects of Levocetirizine dihydrochloride

Table 1.3: Serious side effects and their symptoms

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

1.2.7 How 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

1.2.8 Forms and strengths

Table 1.4: Forms and strengths of levocetirizine

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

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

Table 1.5: 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)
The typical dosage is one 5mg tablet once per day in the evening.	The typical dosage is one 5mg tablet once per day in the evening	The 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

1.3.1 Special considerations

Table 1.6: The typical dosages for people with kidney problems

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.

1.4 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)

1.4.1 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.

1.4.2 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).

1.4.3 Properties of the API important to dissolution

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).

1.4.4 Process involved in Dissolution process

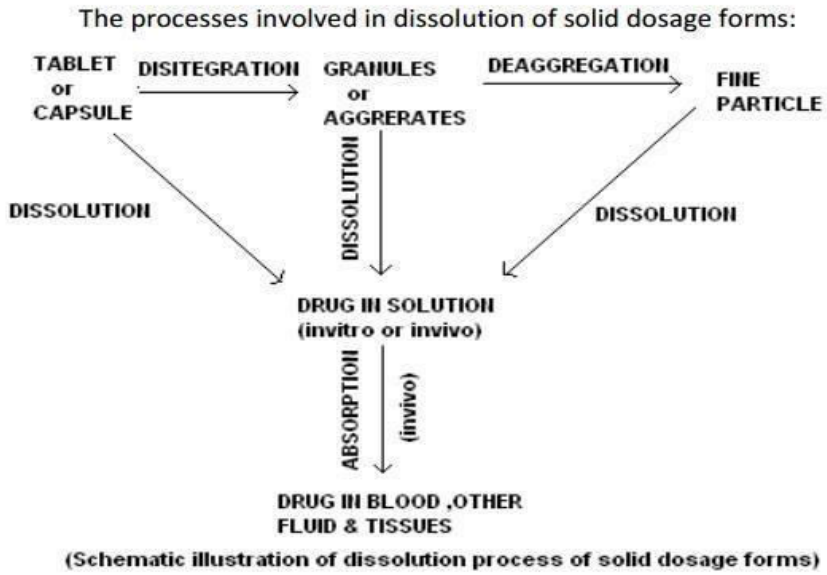

Figure 1.4: Process involved in Dissolution process

1.4.5 Applications of Dissolution in the Pharmaceutical Industry

1. As a formulation design aid (since formulation can profoundly affect dissolution behavior)

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 *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. 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).

1.5 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. 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 (Knott, 2016).

Table 1.7- The Bio pharmaceutics classification system

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

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).

1.5.1 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.

1.5.2 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).

1.5.3 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).

1.5.4 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).

1.6 Levocetirizine Dihydrochloride- Clinical Pharmacology

1.6.1 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.

1.6.2 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.

1.6.3 Pharmacokinetics

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

1.6.3.1 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 Levocetirizinedihydrochloride oral solution is bioequivalent to a 5 mg dose of Levocetirizinedihydrochloride tablets. Following oral administration of a 5 mg dose of Levocetirizinedihydrochloride oral solution to healthy adult subjects, the mean peak plasma concentrations were achieved approximately 0.5 hour post-dose.

1.6.3.2 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.

1.6.3.3 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.

1.6.3.4 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.

1.6.4 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.

1.6.4.1 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.

1.6.4.2 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 Levocetirizinedihydrochloride dose should be adjusted in accordance with renal function in elderly patients.

1.6.4.3 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.

1.6.4.4 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.

1.6.4.5 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.

1.6.4.6 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.

Chapter Two
Literature Review

2 Literature Review

Sandeep et.al. with associate professor work on dissolution profile under the department of pharmaceutics TvesLmc college of pharmacy in India at 22 November 2011 where 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 levocetirizineDihydrochloride by using HPMC, sodium CMC and PEG 400 as key excipients. (Sandeep, et.al. 2011)

M. Saeed Arayne with his research co-workers has developed a simultaneous method which is validated also for estimation of gliquidone in the presence of H1- receptor antagonists (hydrochloride, hydrochloride, and levocetirizinedihydrochloride) using reversed phase 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 levocetirizinedihydrochloride 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. (M. Saeed Arayne, Najma sultana et.al. 1 May 2010).

Mahesh along with his research mates' work on theLevoceterizine where they get fast disintegrating films of levocetirizinedihydrochloride 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. Levocetirizinedihydrochloride 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 levocetirizinedihydrochloride were improved by complexation with hydroxy propyl β-cyclodextrin and the complex could be successfully formulated into a fast disintegrating film. (Mahesh, A, et.al. January 2010)

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 levocetirizinedihydrochloride (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 levoceterizinedihydrochloride treatment successfully decrease that inflammation with improved symptoms. (Cladia et.al. 2003)

T.A popov et.al with other members give their research paper on june 2006 that is 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. (T.Apopov et.al. june 2006)

A novel, simple, sensitive and rapid spectrophotometric method has been developed for simultaneous estimation of ambroxol hydrochloride and levocetirizinedihydrochloride. 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 levocetirizinedihydrochloride, respectively. Beer's law was obeyed in the concentration range 10–50 μg/ml and 8–24 μg/ml for ambroxol hydrochloride and levocetirizinedihydrochloride respectively. Results of the method were validated statistically and by recovery studies. (S. LakshmanaPrabhu et.al. 2008)

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. . (KotaroHiraoka et.al. 3 December 2014)

Paul C Potter work for the pediatric Levocterizine study group to evaluate the effect of levocetirizine on the Total 4 Symptoms Score, the 50% response rate, the Pediatric Rhinitis QualityThelevocetirizine 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. (Paul C Potter, August 2005).

Hampel and his associates was involved in a research project on preschool childen where they saw allergic rhinitis (AR) and chronic idiopathic urticaria (CIU) are common causes of substantial illness and disability in them. 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 levocetirizinedihydrochloride 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. (Hampel, Frank, et.al. 2010).

A literature review was done to evaluate the previous scientific research works that were done on the Levocetirizine Dihydrocloride, their release kinetics as well as dissolution profile. It was observed that the studies done on the LevocetirizineDihydrocloride were not similar to this current research project. But those studies helped to find the informations that helped in the research work to a great extent and also helped to compare this research work with other research projects. Gist of some studies is listed below:

In the year 2001, Benedetti, M.S. and his associates conducted a research study and the main goal of the present study was to investigate the absorption and disposition of levocetirizine dihydrochloride, the *R* enantiomer of cetirizine dihydrochloride, following a single oral administration (5 mg) of the ^{14}C-labelled compound in healthy volunteers. Configurational stability was also investigated. Levocetirizine was rapidly and extensively absorbed: 85.4% and 12.9% of the radioactive dose were recovered 168 h post-dose in urine and faeces, respectively. Levocetirizine and/or its metabolites were not,

or only very poorly, associated with blood cells, as the blood-to-plasma ratio was 0.51 to 0.68. The mean apparent volume of distribution (V_D) was 26.9 l (0.3 l/kg) indicating that the distribution of levocetirizine is restrictive. The protein binding of radiolabelled levocetirizine was 96.1% 1 h after administration. At least 13 minor metabolites were detected in urine and represented 2.4% of the dose at 48 h. The metabolic pathways involved in levocetirizine metabolism are oxidation (hydroxylation, O-dealkylation, N-oxidation and N-dealkylation), glucuroconjugation, taurine conjugation and glutathione conjugation with formation of the mercapturic acids. There was no evidence of chiral inversion of levocetirizine in humans. This result is consistent with that obtained in preclinical studies.(Benedetti, M.S.,2001).

In the year 2008, Prabhu, S.L.et. al., conducted a study for simultaneous estimation of ambroxol hydrochloride and levocetirizine dihydrochloride through a novel, simple, sensitive and rapid spectrophotometric method. 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. (Prabhu, et. al. 2008).

This study was performed byChaudhari, P.D, *et. al*., in 2007, in order to mask the taste, by complexation technique using ion-exchange resin, Tulsion 335 (polyacrylic hydrogen with carboxylic functionality) and to formulate into an orodispersible dosage form. Formulation and in vitro evaluation of taste masked orodispersible dosage form of Levocetirizine dihydrochloride was the main concern here.Levocetrizine Dihydrochloride is an active nonsedative antihistamine. Allergic rhinitis is a significant public health concern in many developed and developing countries. Thus formulating Levocetrizine into an orodispersible dosage form would provide fast relief. The drug loading onto ion-exchange resin was optimized for concentration of resin, swelling time of resin, stirring time, pH of resin solution and stirring temperature. The tablets were evaluated for drug content, content uniformity, weight variation, hardness, friability, water absorption ratio, invitro and invivo disintegration time and invitro drug release. The tablets disintegrated invitro and invivo within 18 and 22 s respectively. Complete drug was released from tablet within 2 minutes. The results showed that Levocetrizine dihydrochloride was

successfully taste masked and formulated into an orodispersible dosage form as an alternative to conventional tablets. (Chaudhari, P.D, *et. al*. 2007).

On January 1, 2010 a research article published by Mahesh, A. et. al.,for the development of taste masked fast disintegrating films of Levocetirizine Dihydrochloride for Oral Use 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. (Mahesh, A. et. al., 2010).

Kanungo, S.,et. al. performed a research work and the 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, Tusilpinapple, Saccharine sodium, Aerosil and Magnesium stearate. MIMTs were evaluated for compliance to pharmacopoeial specifications. From in-vitro dissolution 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-vitro dissolution profile data the difference factor, the similarity factor and the indices of rescigno of model-independent approaches were determined for comparing pair of in-vitro dissolution profiles. MIMTs of levocetirizine was successfully developed complying pharmacopoeial specifications, with adequate stability at room temperature.(Kanungo, S.,et. al., 2011).

This research work deals with the formulation and evaluationof Levocetirizine hydrochloride and Montelukast sodium bilayered tablet for treating nasal allergic rhinitis effectively. By combining Levocetirizine with Montelukast gives additional benefits in comparison with either drug alone and could be considered for patients whose quality of life is impaired by persistent allergic rhinitis. Bilayered tablet may be designed for one layer for the immediate release of the drug and second layer for extended release thus maintaining a prolonged blood level. To achieve patient compliance by reduced frequency of drug administration, and reduced side effects. It was concluded that optimized bilayered Levocetirizine dihydrochloride F8 and Montelukast F5, is successful formulation and can be manufactured with reproducible characteristics from batch to batch. The optimized formulation f8, f5 was compared to the marketed product and hence found to be superior over the marketed product. (Ashrafa, S. and Khan, S.A., 2014).

The purpose of this study was to prepare and characterize levocetirizine hydrochloride loaded liposome of by film hydration technique followed by sonication. Sorbitol was added to facilitate the hydration of dried liposome into vesicles or to prepare rehydration system. The liposomes were characterized for size, shape, entrapment efficiency, invitro drug release and stability. The morphology of liposomes was characterized through a phase-contrast microscope and transmission electron microscope. On the other hand, it was observed that the drug release was decreased at higher concentration of cholesterol. The preliminary results of this study suggest that the developed multi-lamellar vesicles containing levocetirizine hydrochloride could enhance drug entrapment efficiency, reduce the initial burst release and modulate the drug release.(Visht, S. et. al.,2014)

In this study, 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 to990sec, 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 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.(Jadhav, S.D. et. al., 2012).

A research was accomplished for the simultaneous determination of simple, accurate, precise, and sensitive spectrophotometric method for estimation of Montelukast (MON) and Levocetirizine (LEV) in combined tablet dosage form have been developed and validated. Theratio derivative spectroscopic method involves measurement of first derivative amplitude of ratio spectra at 250.4 nm for MON and 238.4 nm for LEV as two wavelengths for estimation. Beer's law is obeyed in the concentration range of 4-12 and 2-6 µg/mL for MON and LEV, respectively. LOD values for MON and LEV are found to be 0.09 µg/mL and 0.178 µg/mL, respectively. LOQ values for MON and LEV are found to be 0.277µg/mL and 0.591 µg/mL, respectively. The results of analysis have been validated statistically and recovery studies carried out in the range 80-120% to confirm the accuracy of the proposed method**.**(Choudhari, V. et. al. , 2010)

A research was accomplished to see the effects of levocetirizine 2HCl .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. 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.(Hampel, F. et. al. ,2010).

In the present study a simple, accurate and precise reverse phase liquid chromatographic method has been developed for simultaneous estimation of Levocetirizine Hydrochloride and Montelukast Sodium from tablet dosage form. The detection was carried out at 225 nm. The retention time of Levocetirizine and Montelukast were found to be around 3.2 min and 4.2 min respectively. The method was validated with respect to linearity, robustness, precision and accuracy and was successfully applied for the simultaneous quantitative determination of Levocetirizine Hydrochloride and Montelukast Sodium from the tablet dosage form (Basu, A. et. al., 2011)

In the present paper, a simultaneous method has been developed and validated for estimation of gliquidone in the presence of H_1- receptor antagonists (fexofenadine hydrochloride, buclizine 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. . Thus, the proposed method is suitable for the simultaneous analysis of active ingredients in tablet dosage forms and human serum.(Arayne, M.S. et. al., 2010).

This study protocol utilized two chromatographic methods for the simultaneous determination of levocetirizine dihydrochloride and Montelukast sodium in tablets. The first method was a high performance thin layer chromatographic (HPTLC) separation followed by densitometric measurements on normal phase silica gel 60 F254. The second method was a high performance liquid chromatographic (HPLC) separation on a BDS Hypersil C18 column using disodium hydrogen phosphate buffer (0.02 M): Methanol (25: 75, v/v) pH adjusted to 7 with ortho-phosphoric acid as the mobile phase. The proposed methods were validated as per ICH guidelines and successfully applied for the determination of investigated drugs in tablets. (Rathore, A.S. et. al., 2010).

This research study investigated and compared the absorption and disposition of levocetirizine, the eutomer of cetirizine, when administered alone (10 mg) or in presence of the distomer. An additional objective was also to investigate the configurational

stability of levocetirizine in vivo in humans. The study was performed in a randomized, two-way cross-over, single-dose design with a wash-out phase of 7 days between the two periods. A total of 12 healthy male and 12 healthy female volunteers were included in the study. Bioequivalence can be concluded from the analysis of the pharmacokinetic parameters of levocetirizine when administered alone or as the racemate cetirizine. No chiral inversion occurs in humans when levocetirizine is administered, i.e. there is no formation of the distomer. When comparing the pharmacokinetic characteristics of levocetirizine and the distomer, the apparent volume of distribution of the eutomer is significantly smaller than that of the distomer (0.41 and 0.60 L/kg, respectively). For an H_1-antagonist a small distribution volume can be considered as a positive aspect, both in terms of efficacy and safety. Moreover the non-renal clearance of levocetirizine is also significantly lower than that of the distomer (9.70 and 28.70 mL/min, respectively), which constitutes an additional positive aspect particularly as far as metabolism-based drug interactions are concerned. The information collected in the present study on the pharmacokinetics of levocetirizine and the distomer provide additional reasons for eliminating the distomer and developing levocetirizine as an improvement on cetirizine. (Baltes, E.et.al, 2001).

In this research study we examined that modern H_1-antihistamines differ in their in vitro binding affinity, but their comparative in vivo bioactivity in asthmatic airways is unknown. Objectives: We compared clinically recommended doses of 3 H_1-antihistamines on airway hyperresponsiveness to AMP challenge (the primary outcome variable). Sixteen atopic patients with mild-to-moderate asthma of whom 10 were receiving inhaled corticosteroid therapy (all had positive results to house dust mite on skin prick testing) were randomized in a double-blind, placebo-controlled, cross-over fashion to receive single doses of 5 mg of desloratadine, 180 mg of fexofenadine hydrochloride (FEX), 5 mg of levocetirizine dihydrochloride (LEV), or placebo, with AMP challenge performed 12 hours after dosing. All H_1-antihistamines demonstrated significantly greater ($P < .05$) geometric mean ± SEM AMP PC_{20} values compared with that of placebo (86 ± 29 mg/mL): desloratadine, 189 ± 54 mg/mL; FEX, 176 ± 57 mg/mL; and LEV, 163 ± 48 mg/mL. There were no significant differences in either AMP PC_{20} or lung function values among the H_1-antihistamines. Conclusion: Single doses of H_1-antihistamines improved airway hyperresponsiveness and small-airways caliber to a similar degree. Data for in vitro binding affinity do not therefore translate into

commensurate differences in in vivo bioactivity at clinically recommended doses. (Lee, D.K. et.al. 2003).

In this study , some biological properties of cetirizine and levocetirizine, namely enantioselectivity in pharmacological activity and pharmacokinetic properties, with emphasis on the possibility of racemization, the compared behavior of the two enantiomers, and the potential for interactions with other drugs. The potent histamine H_1-receptor antagonist cetirizine (Zyrtec®) is a racemic mixture of levocetirizine (now available under the trademark Xyzal®) and dextrocetirizine. In this Commentary.Recent data demonstrate that the antihistaminergic activity of the racemate is primarily due to levocetirizine. Levocetirizine is rapidly and extensively absorbed, poorly metabolized, and not subject to racemization. Its pharmacokinetic characteristics are comparable after administration alone or in the racemate. Its apparent volume of distribution is smaller than that of dextrocetirizine (0.41 L kg^{-1} vs. 0.60 L kg^{-1}). Moreover, the non-renal (mostly hepatic) clearance of levocetirizine is also significantly lower than that of dextrocetirizine (11.8 mL min^{-1} vs. 29.2 mL min^{-1}). Our conclusion is that levocetirizine is indeed the eutomer of cetirizine. The evidence reviewed here confirms preclinical findings and offers a rationale for the chiral switch from the racemate to levocetirizine. (Tillement, J.P. et.al.,2003).

The aim of the present study was to determine (1) the extent of levocetirizine binding to human blood cells, plasma and individual plasma proteins; (2) the parameters for levocetirizine binding to individual plasma proteins both at their physiological concentrations and, for human serum albumin (HSA), at a lower saturating concentration; and (3) to simulate levocetirizine distribution in human blood using the information obtained at physiological haematocrit (*H*) for blood cells and at physiological concentrations for individual plasma proteins. The nature of the main binding sites of HSA, i.e. site I (warfarin) and site II (diazepam), preferentially involved in levocetirizine binding was also investigated. . In any case, at therapeutic concentrations of levocetirizine and at physiological protein concentrations, the observation that none of the levocetirizine binding proteins is saturated suggests that very little or no variation of the free fraction will occur although a different distribution of its bound forms is possible. (Bree, F. et. al., 2002).

Evidence is presented to show that a simple, accurate, and precise AUC curve spectrophotometric method was developed for simultaneous determination of Montelukast sodium (MTKT) and Levocetirizine dihydrochloride (LCTZ) in combined pharmaceutical dosage forms. The principle for AUC curve method is "the area under two points on the mixture spectra is directly proportional to the concentration of the component of interest". The area selected were 263.6 to 293.6 and 222 to 242 nm for determination of MTKT and LCTZ respectively. The two drugs follow Beer-Lambert's law over the concentration range of 5-30 µg/ml for MTKT and LCTZ. The % estimation of the drugs was found near to 100 % representing the accuracy of the method. The recovery of the MTKT and LCTZ were found near to 100 %. Validation of the proposed methods was carried out by Patel Nilam, K. and Pancholi, S.S., for its accuracy, precision, specificity and ruggedness according to ICH guidelines. The proposed methods can be successfully applied in routine work for the determination of MTKT and LCTZ in combined dosage form. (Patel Nilam, K. and Pancholi, S.S., 2011).

The prime objective of this study was to determine the better agent among rupatadinefumarate and levocetirizine dihydrochloride for seasonal allergic rhinitis. Although treating and ensuring a decent quality of life to patients is challenging, an increasing understanding of pathomechanisms has revealed the potentiality of new-generation antihistamines in the treatment of seasonal allergic rhinitis.A 2-week, single-center, randomized, open, parallel group comparative clinical study between rupatadine and levocetirizine in patients with seasonal allergic rhinitis. Setting was a tertiary care center here. llergic rhinitis (AR) is one of the most common diseases, representing approximately 20% of the general population.*Allergic rhinitis* is the general term that encompasses seasonal AR, perennial AR, and perennial AR with seasonal exacerbations. Allergic rhinitis has a relevant impact on society because of its high prevalence, association with an impaired quality of life, and the presence of comorbidities such as atopy and asthma. Rupatadine is a novel chemical entity that shows both antihistamine and anti–platelet-activating factor effects through its interaction with specific receptors and not through physiological antagonism. Levocetirizine—the R-enantiomer of cetirizine dihydrochloride with pharmacodynamically and pharmacokinetically favorable characteristics—has been proved to be safe and effective for the treatment of AR with a minimal number of adverse effects in many clinical trials. In this study, we found that

differential and absolute eosinophil counts were significantly lowered by both drugs, but rupatadine was superior. (Maiti, R. et. al., 2010)

This study was done to compare the efficacy and safety profiles of the newest second-generation antihistamines — desloratadine, fexofenadine and levocetirizine. Second-generation histamine H_1 receptor antagonists were developed to provide efficacious treatment of allergic rhinitis (AR) and chronic idiopathic urticaria (CIU) while decreasing adverse effects associated with first-generation agents. As a class, second-generation antihistamines are highly selective for the H_1 receptor. Some bind to it with high affinity, although there is marked heterogeneity among the various compounds. They have a limited effect on the CNS, and clinical studies have noted almost no significant drug drug interactions in the agents studied. No major cytochrome P450 inhibition has been reported with desloratadine, fexofenadine and levocetirizine, and the bioavailability of desloratadine is minimally affected by drugs interfering with transporter molecules. Of the second-generation antihistamines, desloratadine has the greatest binding affinity for the H_1 receptorHowever, differences among the antihistamines in relation to a lack of significant interaction with drug transporter molecules and somnolence in excess of placebo may provide some advantages for the overall profile of desloratadine compared with fexofenadine and levocetirizine..(Devillier, P. et. al., 2008)

In 2006, a clinical study was done to evaluate the additional benefits of 5 mg levocetirizine dihydrochloride in seasonal AR patients using 200 mcg fluticasone propionate nasal spray once daily. The additional effects of H1 antagonists to intranasal corticosteroid treatment of allergic rhinitis (AR) is common in clinical practice and recommended by guidelines, despite some evidence that the additive benefits are negligible. In a double-blind placebo-controlled crossover study of 27 patients, following 2 weeks without treatment, subjects used fluticasone with levocetirizine or identical placebo for 2 weeks each. Assessments were the Juniper mini Rhinoconjunctivitis Quality-of-Life Questionnaire (mini-RQLQ), domiciliary peak nasal inspiratory flow (PNIF), total nasal symptoms (TNS) scores and nasal nitric oxide concentrations. Effects were interpreted and tested against minimal clinically important differences. The results demonstrated that for the majority of patients, antihistamine add-on to effective nasal steroid treatment is inappropriate. Further work is required to confirm that this is also true in the most severe cases, and the available evidence needs to be put into guidelines and implemented. (Barnes, M.L. et. al., 2006).

In the year 2005 a significant research study was accomplished on Levocetirizine. It is the latest of the H1-antihistamines indicated for adults and children (as young as 2 years old) suffering from allergic rhinitis and chronic idiopathic urticaria. Currently, it is the only therapy registered for treatment of persistent allergic rhinitis, as defined by the Allergic Rhinitis & its Impact on Asthma guidelines. Pharmacologic studies have shown levocetirizine to have a more favorable pharmacokinetic/pharmacodynamic profile than other commonly employed H1-antihistamines. This reflects its superiority in controlling the symptoms of seasonal, perennial and persistent allergic rhinitis in well-controlled trials. Clinical trials and postmarketing surveillance have indicated that levocetirizine is safe and well tolerated, and leads to clinically significant improvements in the quality of life of patients. It is also reported to reduce comorbidities as well as overall treatment costs when administered continuously over the longterm.(Bachert, C., 2005).

The aim of this study was to develop taste masked oral soluble films (OSFs) for levocetirizine dihydrochloride (LCT) and ambroxol hydrochloride (AMB) using different combination of polymers such as polyvinyl pyrrolidone (PVP) K30, propylene glycol (PG), gelatin, sodium alginate (SA), pectin, gaur gum (GG), and hydroxypropyl methylcellulose (HPMC) K15M and super disintegrants like carboxymethyl cellulose (CMC) and sodium starch glycolate (SSG). The different basic formulations were developed using solvent casting method for with and without drugs loading and prepared films were evaluated different morphological and mechanical parameters facilitated the screening of a formulation with best characteristics. The films made from HPMC K15M (42.2% w/w) and pectin (35.2% w/w) and considered as an optimized batch among the other formulations. Developed OSFs can be considered as one of the promising formulation to administer bitter drugs such as LCT and AMB especially for pediatric, geriatric, and non-cooperative patients.(Senthil, V.et. al., 2013).

In his research work, simple, fast and reliable spectrophotometric methods were developed for determination of Levocetirizine in bulk and pharmaceutical dosage forms. The solutions of standard and the sample were prepared in methanol. The quantitative determination of the drug was carried out using the zero order derivative values measured at 230 nm and the area under the curve method values measured at 227-234 nm (n=2). Calibration graphs constructed at their wavelengths of determination were linear in the concentration range of Levocetirizine using 5-25µg/ml (r^2=0.998 and r^2=0.999) for zero order and area under the curve spectrophotometric method. All the proposed methods

have been extensively validated as per ICH guidelines. There was no significant difference between the performance of the proposed methods regarding the mean values and standard deviations. Developed spectrophotometric methods in this study are simple, accurate, precise and sensitive to assay of Levocetirizine in tablets (Mali, A.D. et. al., 2015).

This study includes that levocetirizine is a second-generation nonsedative antihistaminic agent that has been demonstrated to be safe and effective for treating allergic disease. There was only one case report of levocetirizine-induced liver toxicity, but a liver biopsy was not performed. In this article, we present the first case of levocetirizine-induced liver injury with histologic findings. A 48-year-old man was hospitalized with jaundice and generalized pruritus that had developed after 2 months of therapy with levocetirizine for prurigonodularis. Laboratory findings revealed acute hepatitis with cholestasis. A liver biopsy demonstrated portal inflammation and hepatitis with apoptotic hepatocytes. The patient fully recovered 3 weeks after withdrawing levocetirizine. Although levocetirizine is safe and effective, physicians should be aware of its potential hepatotoxicity. (Jung, M.C. et. al., 2016).

This study was done to compare the potency, consistency, onset, and duration of action of levocetirizine with other popular antihistamines in 2002. Levocetirizine is the active enantiomer of cetirizine, a potent drug with little metabolism widely used for allergic rhinitis and urticarial.Levocetirizine 5 mg, ebastine 10 mg, fexofenadine 180 mg, loratadine 10 mg, mizolastine 10 mg, or placebo in single doses were given to 18 healthy male volunteers in a double-blind, crossover, randomized fashion. Wheal-and-flare responses to epicutaneous histamine dihydrochloride (100 mg/mL) challenge were measured at 0, 0.5, 1, 2, 4, 6, 8, 10, 12, and 24 hours after each dosse. The overall effect of each drug was evaluated by the area under the curve (0 to 24 hours). Levocetirizine was the most potent and consistently effective drug for inhibiting the histamine-induced wheal-and-flare surface areas. Levocetirizine, the active enantiomer of cetirizine, is more potent and consistent than other popular H_1 antihistamines for blocking the cutaneous response to histamine. These findings may predict the efficacy of this drug in treating allergic disorders. (Grant, J.A. et.al., 2002).

This reseach study contributed to the development of novel topical formulation of levocetirizine based on flexible vesicles (FVs) with an aim to have targeted peripheral antihistaminic effect in 2014.The FVs were prepared by thin film hydration method and characterized for drug content, entrapment efficiency, pH, vesicular size, spreadability, morphological characteristics and drug leakage studies. Franz diffusion cell assembly was used to carry out the *ex vivo* permeation studies through mice skin and the permeation profile of the developed FV formulation was compared with conventional formulations of levocetirizine.The *ex vivo* permeation studies revealed 1.78-fold increase in percent permeation of levocetirizine from FV formulation as compared to conventional formulations of levocetirizine in 8 h. Further, oxazolone induced atopic dermatitis murine model was selected to study the *in vivo* pharmacodynamic activity. The developed formulation was evaluated for scratching score, erythema score and histological evaluation. There was marked reduction in scratching score from 15.25 scratches/20 min with conventional levocetirizine cream to 6.75 scratches/20 min with application of levocetirizine FV formulation. Also, there was significant reduction in erythema score as well as dermal eosinophil count. Results of skin sensitivity and toxicity studies suggest that the developed formulation was dermally safe and nontoxic.A novel FVs based topical formulation of levocetirizine was successfully developed for treatment of atopic dermatitis. (Goindi, S. et. al.,2014)

In 2016, three simple, precise, accurate and validated derivative spectrophotometric methods have been developed for the simultaneous determination of levocetirizine dihydrochloride (LCD) and ambroxol hydrochloride (ABH) in bulk powder and in pharmaceutical formulations. The first method is a first derivative spectrophotometric method using a zero-crossing technique of measurement at 210.4 nm for LCD and at 220.0 nm for ABH. The second method employs a second derivative spectrophotometry where the measurements were carried out at 242.0 and 224.4 nm for LCD and ABH, respectively. In the third method, the first derivative of the ratio spectra was calculated and the first derivative of the ratio amplitudes at 222.8 and 247.2 nm was selected for the determination of LCD and ABH, respectively. The developed methods have been successfully applied to the simultaneous determination of both drugs in commercial tablet dosage form. (Ali, O.I. et. al., 2016).

Recently on 27 February, 2017, a research work investigated the formulation and dissolution of orally disintegrating films of levocetirizine dihydrochloride.To enhance the

convenience and compliance by the elderly or paediatric or bedridden and non-cooperative patients, due to its ease of administration,the present investigation was undertaken with the objective of formulating taste-masked orally-disintegrating films of the bitter levocetirizine dihydrochloride.Scope of this study was to explore the film forming properties of various film formers like modified starch, pullulan, hydroxypropyl methylcellulose and polyvinyl alcohol-polyethylene glycol based polymers. Plasticizers like glycerin, propylene glycol, sorbitol and polyethylene glycol 400 were evaluated by studying their effect on folding endurance, peelablity and in vitro disintegration time. Films were prepared by solvent casting method. The formulation developed is simple, easy to prepare and economical with great applicability during the emergency cases such as allergic reactions, whenever immediate onset of action is desired. (Kathpalia, H. and Patil, A., 2017)

Chapter Three
Materials & Method

3 Materials and methods

The study on comparative dissolution profiles of levoceterizine dihydrochloride was carried out by using dissolution method to see the release pattern of levoceterizine 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).

3.1 Reagents, Chemicals and Solvents

All reagents used were of analytical reagent grade and distilled water was used for the preparation of all solutions. For the dissolution of Levoceterizine dihydrochloride, we used water as a solvent. Xyxal is the patent drug of Levoceterizine. Other tablets I used alcet ,seasonix, lecetetcto see the release pattern with different time interval like etc.

3.2 Dissolution testing methods for Levoceterizine Dihydrochloride

Table 3.1: Parameters of dissolution of Levoceterizine

Dissolution media	Distilled water
Temperature	37ºC
RPM	50
Time	30 minutes
Wavelength	231 nm

The release rate of Levoceterizine 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 10min interval sample of 10 ml were withdrawn from the dissolution medium and the amount was replacing by 10 ml distill water. It is diluted to a suitable concentration of distilled water. The absorbance of the solution was measured 231 nm for drug Levoceterizine dihydrochloride 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 30 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).

3.3 Preparation of Standard Curve

To prepare the standard curve, at first different concentrations (0, 2, 4, 6, 8, and 10) µg/ml of Levoceterizine was prepared. Two 5mg levoceterizine dihydrochloride tablets were crushed in a mortar and pastle. Then those placed in a 100ml volumetric flask and add distill water upto 100ml. from that 10ml is taken in another volumetric flask and distill water is added upto 100ml. So the concentration is 10 times diluted. 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 231 nm wave lengths were set up. Then the spectrophotometer was adjusted for 0 and 100%. The solutions were placed on spectrophotometer to measure the absorbance. Then the absorbance was plotted against concentration. A straight line was found.

Table 3.2 Concentrations of Levoceterizine Dihydrochloride

Serial No	Concentrations (µg/ml)
1	2
2	4
3	6
4	8
5	10

3.4 Preparation for dissolution test

3.4.1 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.

3.4.2 Method for dissolution test of Levoceterizine Dihydrochloride 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 30 minutes, 50 rpm was set up in the dissolution machine. Then the machine was allowed to warm up until it reached at 37.5 degree Celsius. Then tablets were placed in every vessel. After 10, 20 and 30 minutes 10 ml of solution was collected from each vessel and no need to 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 231 nm.

3.5 Materials

3.5.1 Sample Collection

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

Table 3.3 Brand names of Levoceterizine under dissolution study

Brand Name	Source
Alcet	Healthcare Phrm Ltd.
Lozin	Phrm Ltd.
Purotrol	SquarePhrm Ltd.
Lecet	Pacific Phrm Ltd.
Clarigin	Drug IntPhrm Ltd.
Seasonix	InceptaPhrm Ltd.

3.5.2 Stock solution

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

3.6 Equipment

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

Table 3.4 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

3.7 Instrumentation

3.7.1 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).

3.7.2 Ultra- Violet Spectrophotometer

The ultra-violet absorption spectrum for Levoceterizine 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 corvettes.

3.7.3 Samples and Chemical Reference Substances

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

3.7.4 Images of Instruments

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

Figure 3.1: Dissolution apparatus

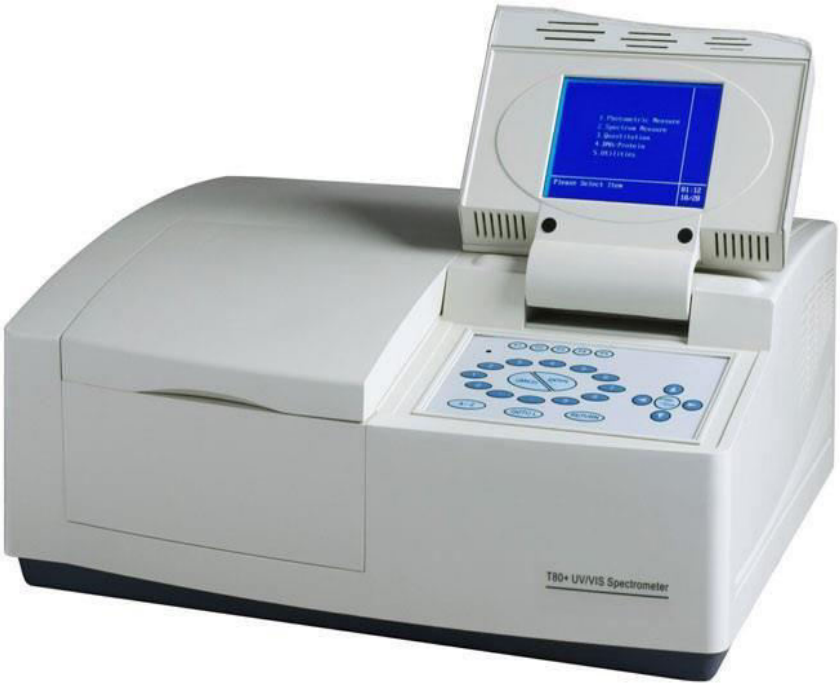

Figure 3.2: UV spectrophotometer

Figure 3.3: Distilled water apparatus

Figure 3.4: Hardness Tester

Figure3.5: Electronic Balance

3.8 Apparatus

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

Table 3.5: Representing the apparatus (Kuss, 1992)

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

Chapter Four
Results
&
Discussion

4 Results & Discussion

4.1 Preparation and method of standard curve of 'Alcet'

2 tablets each of 5 mg Alcet were taken and grinded with the help of mortar and pastle to obtain a powder which was then smudged carefully and transferred into a 100 ml volumetric flask. Afterwards, the grinded powder was dissolved slowly with distilled water which was made up to the 100ml later. Here, the concentration of the stock solution after dissolving 2 tablets of levocetrizine each 5 mg in 100 ml volumetric flask becomes 10µg/ml. Now, each of the following concentration as prepared along with the detection of their absorbance at wavelength of 231 nm to prepare the standard curve equation keeping the Time along X axis and Absorbance along Y axis. The equation **y = 0.038x + 0.018**helped to determine the concentration of drug release as well percent release of that drug &**R^2 = 0.991** gave us an idea about the drug release kinetic profile.

Table 4.1.A: Data for the standard curve of Alcet

Concentration (µg/ml)	Time (min)	Absorbance (nm)
2	10	0.113
4	20	0.18
6	30	0.243
8	40	0.315
10	50	0.398

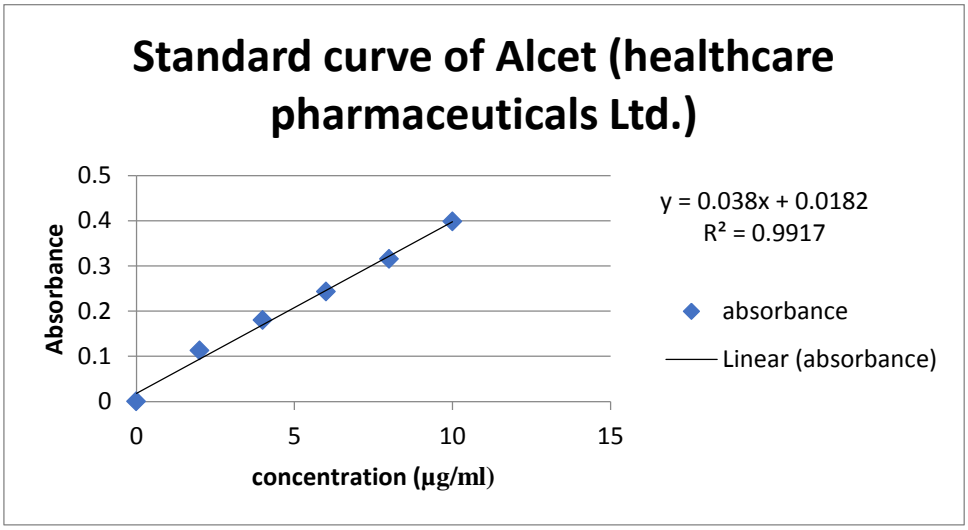

Figure 4.1.a: Standard curve of Alcet

4.1.1Preparation and method of dissolution curve of Alcet

2 tablets of Alcet were taken and they were dissolved at a rpm = 50, 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 231 nm that were recorded. Thus, finally from the average of all the 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 30 min, the % release was about 99.23% and 85.26% respectively determined the concentrations of drug release and **y = 2.432x + 31.31**&**R^2 = 0.473** determines the drug release kinetic profile.

Table 4.1.1.A: Data for the dissolution curve of Alcet

Time (min)	% release of drug
0	0
10	9.9E+01
20	8.7E+01
30	8.5E+01

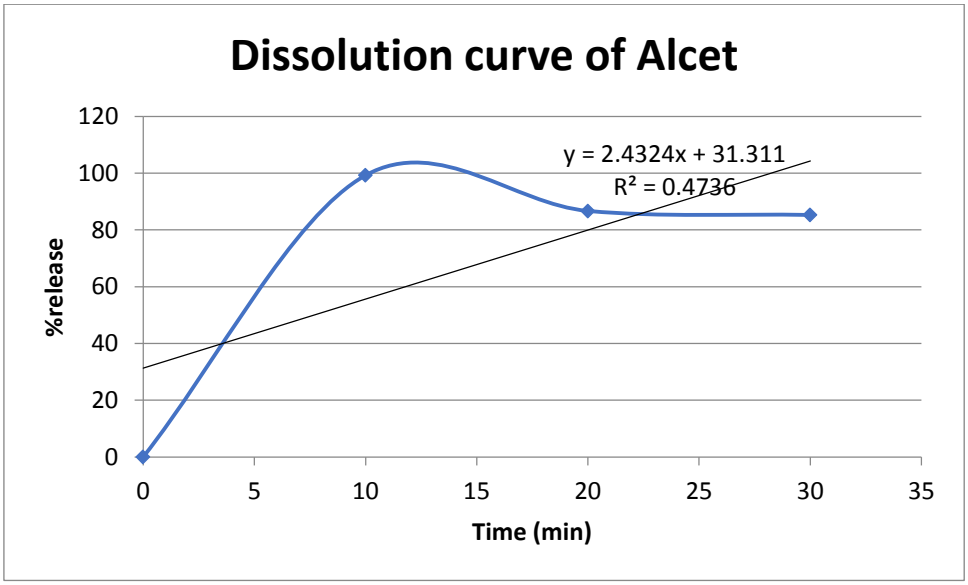

Figure 4.1.1.a: Dissolution curve of Alcet

4.2 Preparation and method of standard curve of 'Seasonix'

2 tablets each of 5 mg Seasonix were taken and grinded with the help of mortar and pastle to obtain a powder which was then smudged carefully and transferred into a 100ml volumetric flask. Afterwards, the grinded powder was dissolved slowly with distilled water which was made up to the 100 ml later. Here, the concentration of the stock solution after dissolving 2 tablets of Levocetirizine each 5 mg in 100 ml volumetric flask becomes 10µg/ml. Now, each of the following concentration as prepared along with the detection of their absorbance at wavelength of 231 nm to prepare the standard curve equation keeping the Time along X axis and Absorbance along Y axis. The equation **y = 0.035x + 0.008**helped to determine the concentration of drug release as well percent release of that drug &**R^2 = 0.991** gave us an idea about the drug release kinetic profile.

Table 4.2.A: Data for the standard curve of Seasonix

Concentration (µg/ml)	Time (min)	Absorbance (nm)
2	10	0.079
4	20	0.15
6	30	0.233
8	40	0.305
10	50	0.343

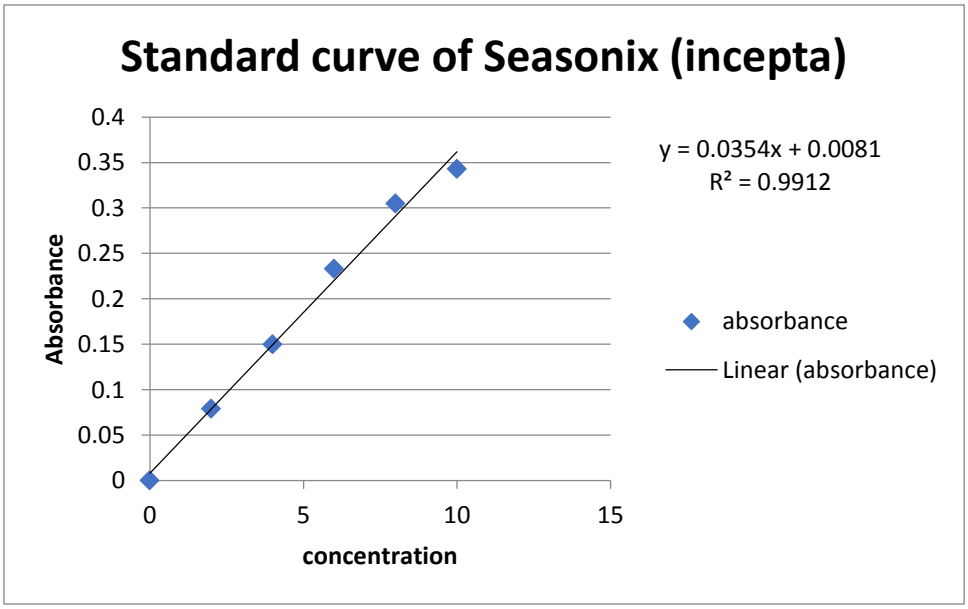

Figure 4.2.a: Standard curve of Seasonix

4.2.1 Preparation and method of dissolution curve of Seasonix

2 tablets of Seasonix were taken and they were dissolved at a rpm = 50, 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 231nm that were recorded. Thus, finally from the average of all the 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 30 min, the % release was about 81.20% and 93.65% respectively. The equation**y = 2.926x + 23.03**determined the concentrations of drug release and **R^2 = 0.705**determines the drug release kinetic profile.

Table 4.2.1.A: Data for the dissolution curve of Seasonix

Time (min)	**% release of drug**
0	0
10	8.1E+01
20	9.3E+01
30	9.4E+01

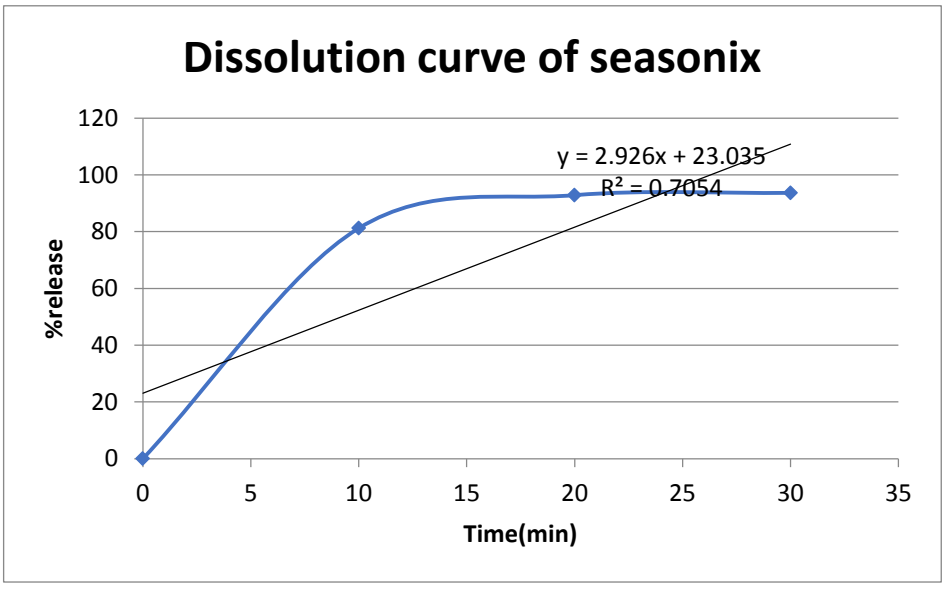

Figure 4.2.1.a: Dissolution curve of Seaspnix

4.3 Preparation and method of standard curve of 'Purotrol'

2 tablets each of 5 mg Purotrol were taken and grinded with the help of mortar and pastle to obtain a powder which was then smudged carefully and transferred into a 100ml volumetric flask. Afterwards, the grinded powder was dissolved slowly with distilled water which was made up to the 100 ml later. Here, the concentration of the stock solution after dissolving 2 tablets of Levocetirizine each 5 mg in 100 ml volumetric flask becomes 10µg/ml. Now, each of the following concentration as prepared along with the detection of their absorbance at wavelength of 231 nm to prepare the standard curve equation keeping the Time along X axis and Absorbance along Y axis. The equation**y = 0.033x - 0.009**helped to determine the concentration of drug release as well percent release of that drug &**R^2 = 0.991** gave us an idea about the drug release kinetic profile.

Table 4.3.A: Data for the standard curve of Purotrol

Concentration (µg/ml)	Time (min)	Absorbance (nm)
2	10	0.06
4	20	0.115
6	30	0.174
8	40	0.25
10	50	0.337

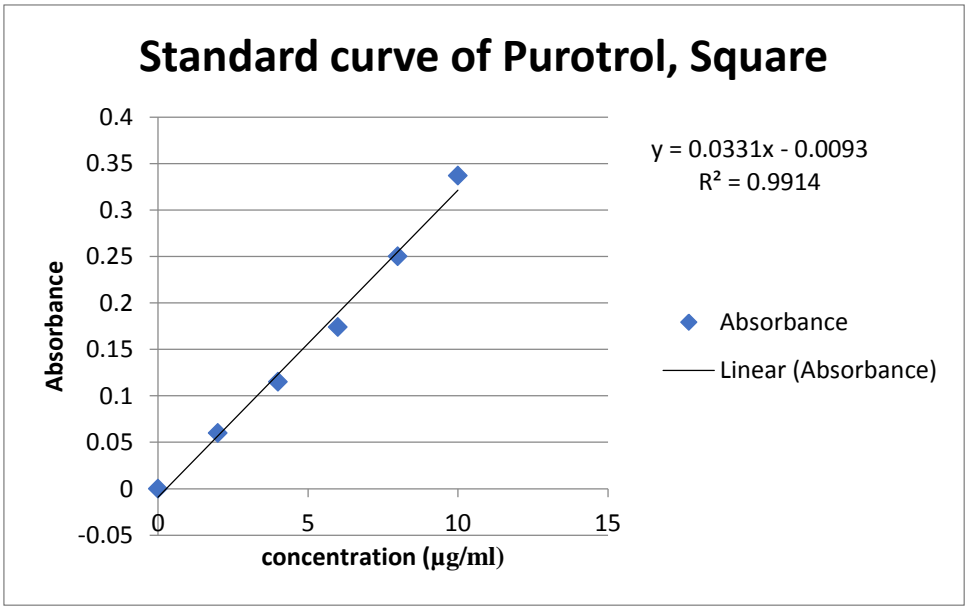

Figure 4.3.a: Standard curve of Purotrol

4.3.1 Preparation and method of dissolution curve of Purotrol

2 tablets of Purotrol were taken and they were dissolved at a rpm = 50, 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 231 nm that were recorded. Thus, finally from the average of all the 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 30 min, the % release was about 52.02% and 93.98% respectively.The equation**y = 3.230x + 11.32**determined the concentrations of drug release and **R^2 = 0.882**determines the drug release kinetic profile.

Table 4.3.1.A: Data for the dissolution curve of Purotrol

Time (min)	**% release of drug**
0	0
10	5.2E+01
20	9.3E+01
30	9.4E+01

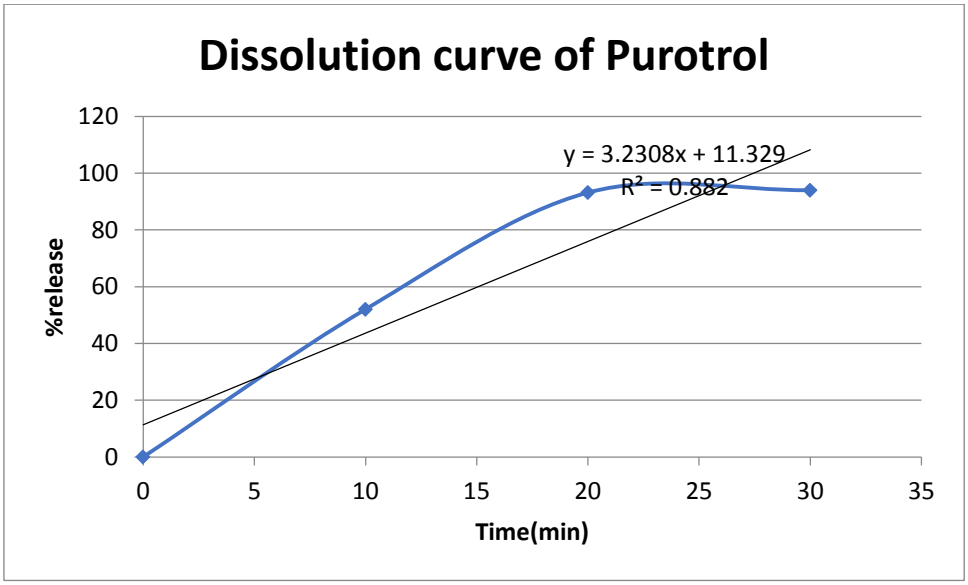

Figure 4.3.1.a: Dissolution curve of Purotrol

4.4 Preparation and method of standard curve of 'Lecet'

2 tablets each of 5 mg Lecet were taken and grinded with the help of mortar and pastle to obtain a powder which was then smudged carefully and transferred into a 100ml volumetric flask. Afterwards, the grinded powder was dissolved slowly with distilled water which was made up to the 100 ml later. Here, the concentration of the stock solution after dissolving 2 tablets of Levocetirizine each 5 mg in 100 ml volumetric flask becomes 10µg/ml. Now, each of the following concentration as prepared along with the detection of their absorbance at wavelength of 231 nm to prepare the standard curve equation keeping the Time along X axis and Absorbance along Y axis.The equation**y = 0.035x - 0.009**helped to determine the concentration of drug release as well percent release of that drug &**R^2 = 0.994** gave us an idea about the drug release kinetic profile.

Table 4.4.A: Data for the standard curve of Alcet

Concentration (µg/ml)	Time (min)	Absorbance (nm)
2	10	0.066
4	20	0.121
6	30	0.194
8	40	0.276
10	50	0.359

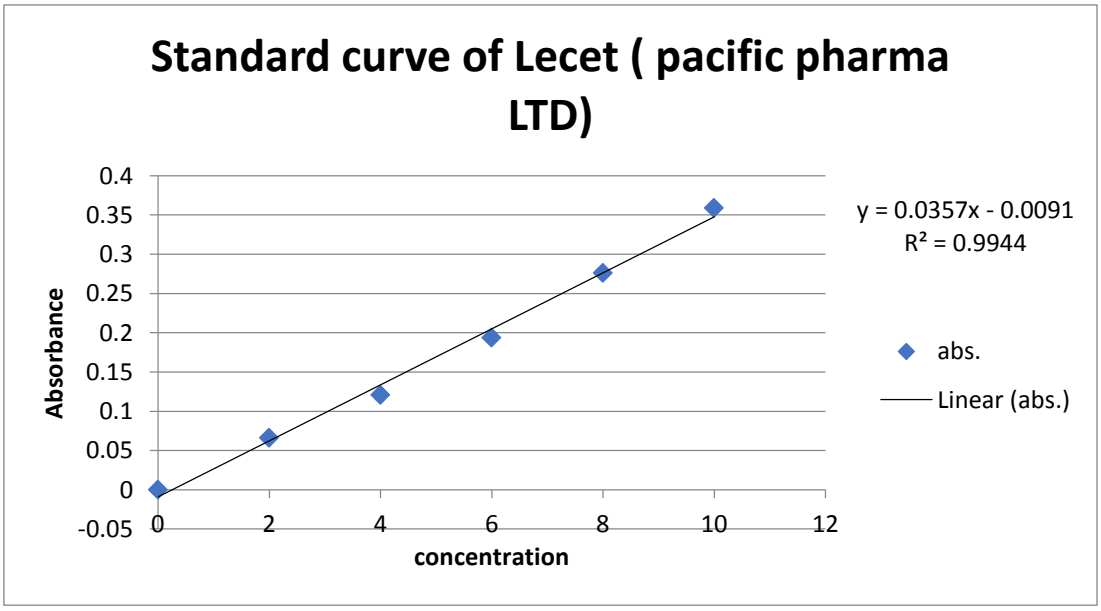

Figure 4.4.a: Standard curve of Lecet

4.4.1 Preparation and method of dissolution curve of Lecet

2 tablets of Lecet were taken and they were dissolved at a rpm = 50, 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 231 nm that were recorded. Thus, finally from the average of all the 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 30 min, the % release was about 81.85% and 99.85% respectively the equation**y = 3.094x + 21.94**determined the concentrations of drug release and **R^2 = 0.748**determines the drug release kinetic profile.

Table 4.4.1.A: Data for the dissolution curve of Lecet

Time (min)	**% release of drug**
0	0
10	8.2E+01
20	9.2E++01
30	1.0E+02

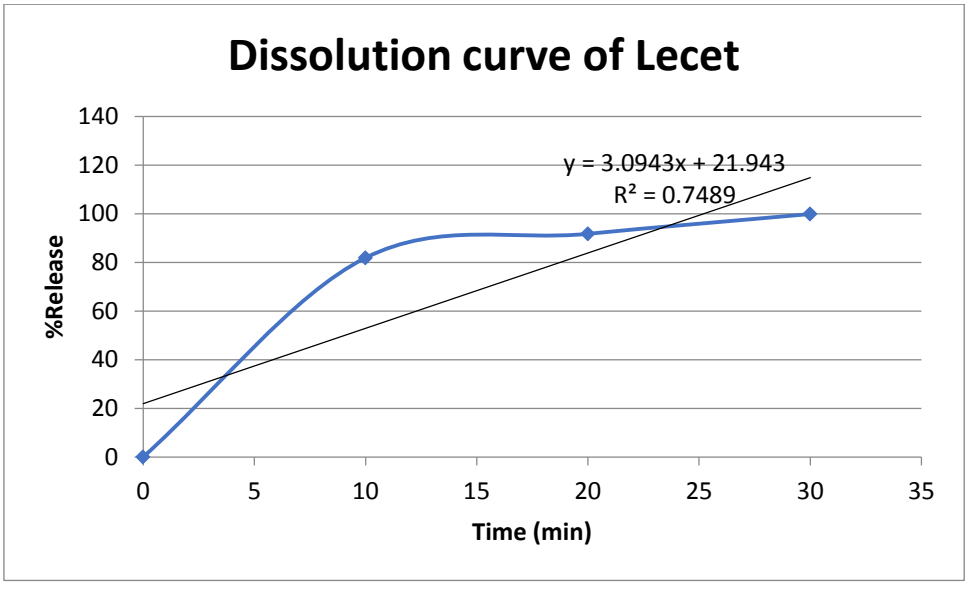

Figure 4.4.1.a: Dissolution curve of Lecet

4.5 Preparation and method of standard curve of 'Clarigen'

2 tablets each of 5 mg Clarigen were taken and grinded with the help of mortar and pastle to obtain a powder which was then smudged carefully and transferred into a 100 ml volumetric flask. Afterwards, the grinded powder was dissolved slowly with distilled water which was made up to the 100 ml later. Here, the concentration of the stock solution after dissolving 2 tablets of Levocetirizine each 5 mg in 100 ml volumetric flask becomes 10µg/ml. Now, each of the following concentration as prepared along with the detection of their absorbance at wavelength of 231 nm to prepare the standard curve equation keeping the Time along X axis and Absorbance along Y axis. The equation**y = 0.059x - 0.005**helped to determine the concentration of drug release as well percent release of that drug &**R^2 = 0.991** gave us an idea about the drug release kinetic profile.

Table 4.5.A: Data for the standard curve of Alcet

Concentration (µg/ml)	Time (min)	Absorbance (nm)
2	10	0.088
4	20	0.236
6	30	0.38
8	40	0.484
10	50	0.569

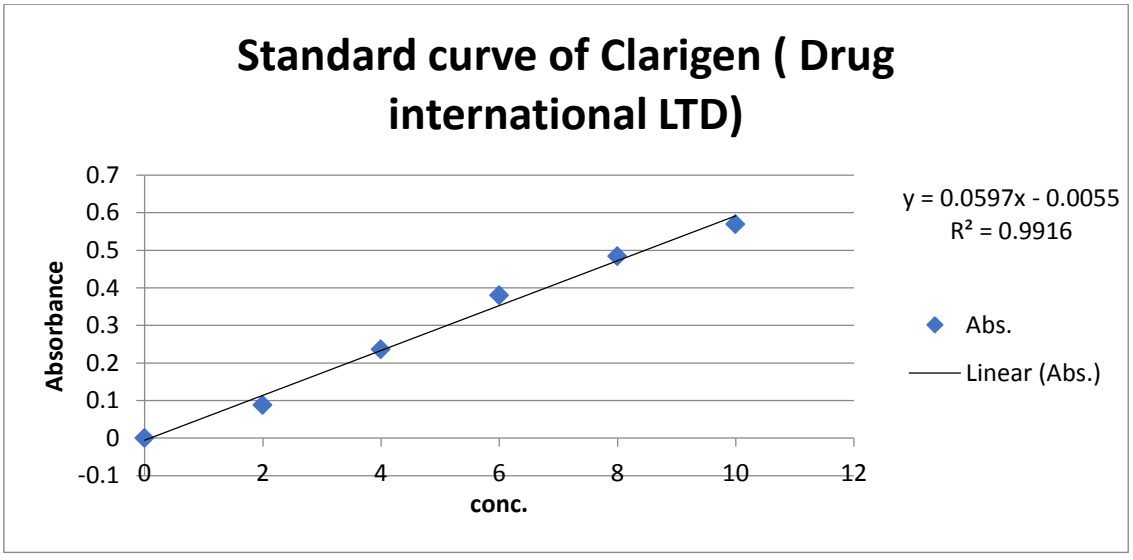

Figure 4.5.a: Standard curve ofClarigen

4.5.1 Preparation and method of dissolution curve of Clarigen

2 tablets of Clarigen were taken and they were dissolved at a rpm = 50, 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 231 nm 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 30 min, the % release was about 36.30% and 75.35% respectively. The equation**y = 2.571x + 6.193**determined the concentrations of drug release and **R^2 = 0.938** determines the drug release kinetic profile.

Table 4.5.1.A Data for the dissolution curve of Clarigen

Time (min)	**% release of drug**
0	0
10	3.6E+01
20	6.7E+01
30	7.5E+01

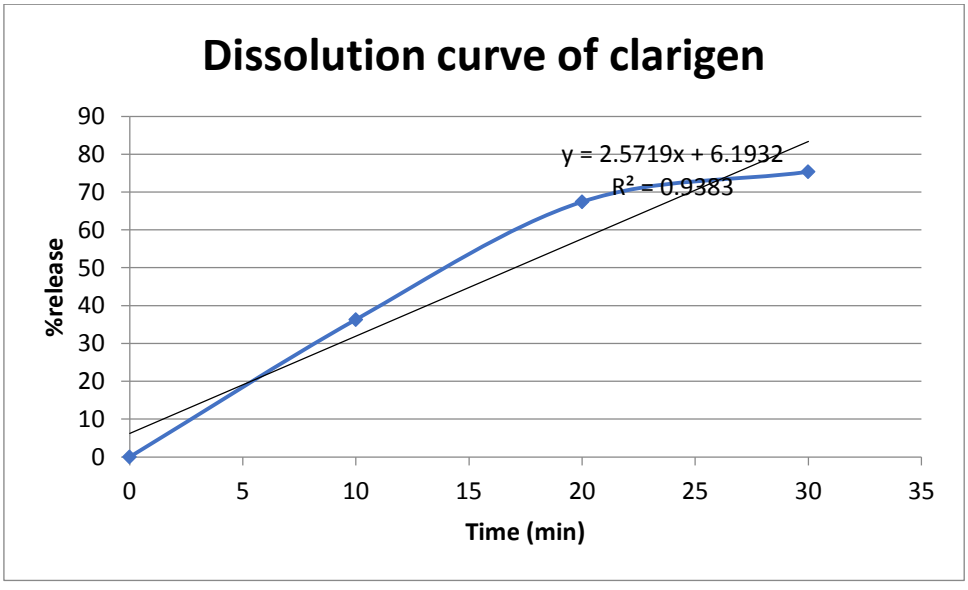

Figure 4.5.1.a: Dissolution curve of Clarigen

4.6 Preparation and method of standard curve of Lozin

2 tablets each of 5 mg Lozin were taken and grinded with the help of mortar and pastle to obtain a powder which was then smudged carefully and transferred into a 100 ml volumetric flask. Afterwards, the grinded powder was dissolved slowly with distilled water which was made up to the 100 ml later. Here, the concentration of the stock solution after dissolving 2 tablets of Levocetirizine each 5 mg in 100 ml volumetric flask becomes 10µg/ml. Now, each of the following concentration as prepared along with the detection of their absorbance at wavelength of 231 nm to prepare the standard curve equation keeping the Time along X axis and Absorbance along Y axis. The equation**y = 0.038x + 0.000**helped to determine the concentration of drug release as well percent release of that drug &**R^2 = 0.996** gave us an idea about the drug release kinetic profile.

Table 4.6.A: Data for the standard curve of Alcet

Concentration (µg/ml)	Time (min)	Absorbance (nm)
2	10	0.088
4	20	0.151
6	30	0.218
8	40	0.315
10	50	0.394

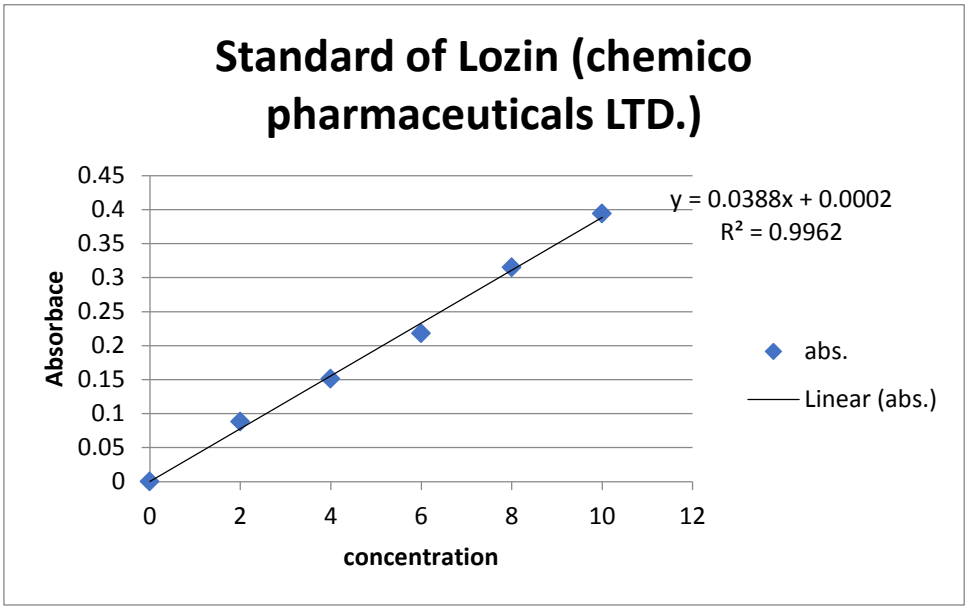

Figure 4.6.a: Standard curve of Lozin

4.6.1 Preparation and method of dissolution curve of Lozin

2 tablets of Lozin were taken and they were dissolved at a rpm = 50, 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 231 nm 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 30 min, the % release was about 36.30% and 75.35% respectively. The equation**y = 3.118x + 21.11**determined the concentrations of drug release and **R^2 = 0.755**determines the drug release kinetic profile.

Table 4.6.1.A: Data for the dissolution curve of Lozin

Time (min)	**% release of drug**
0	0
10	7.7E+01
20	9.8E+01
30	9.7E+01

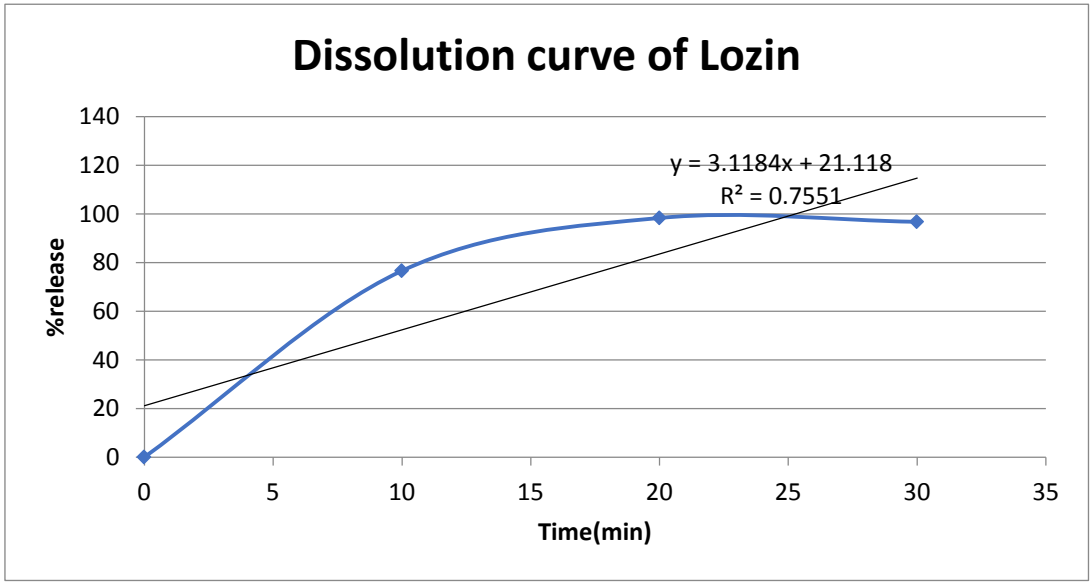

Figure 4.6.1.a: Dissolution curve of Lozin

4.7 Zero order drug release profile

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

Table 4.7.A: R^2values of Six different brands of Levocetirizine for zero order drug release profile

Name	R^2values
Alcet	0.473
Seasonix	0.705
Purotrol	0.882
Lecet	0.748
Clarigen	0.938
Lozin	0.755

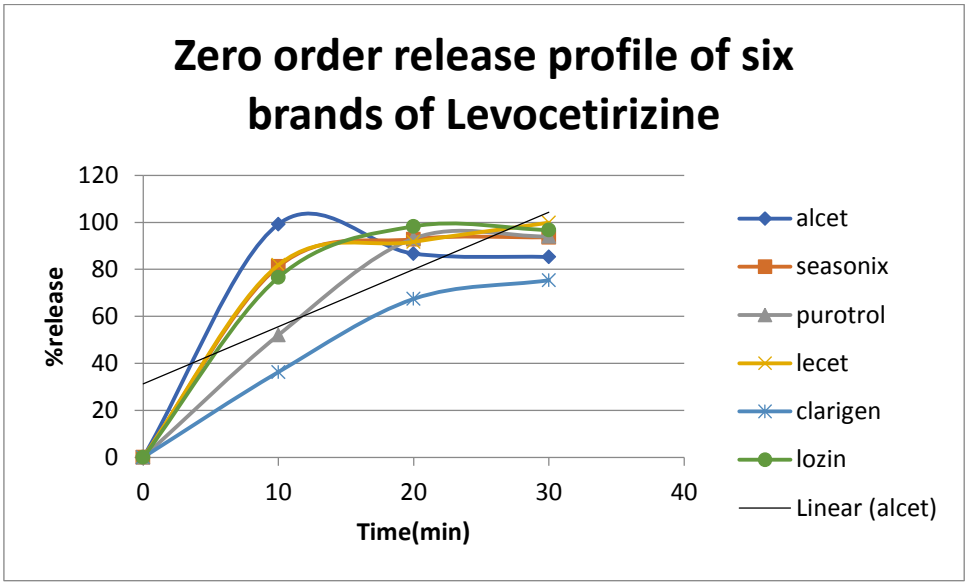

Figure 8.7.a: Zero order drug release profile of six brands of Levocetirizine

4.8 First order drug release profile

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

Table4.8.A:R^2values of Six different brands of Levocetirizine for first order drug release profile

Name	R^2values
Alcet	0.860
Seasonix	0.008
Purotrol	0.027
Lecet	0.022
Clarigen	0.004
Lozin	0.023

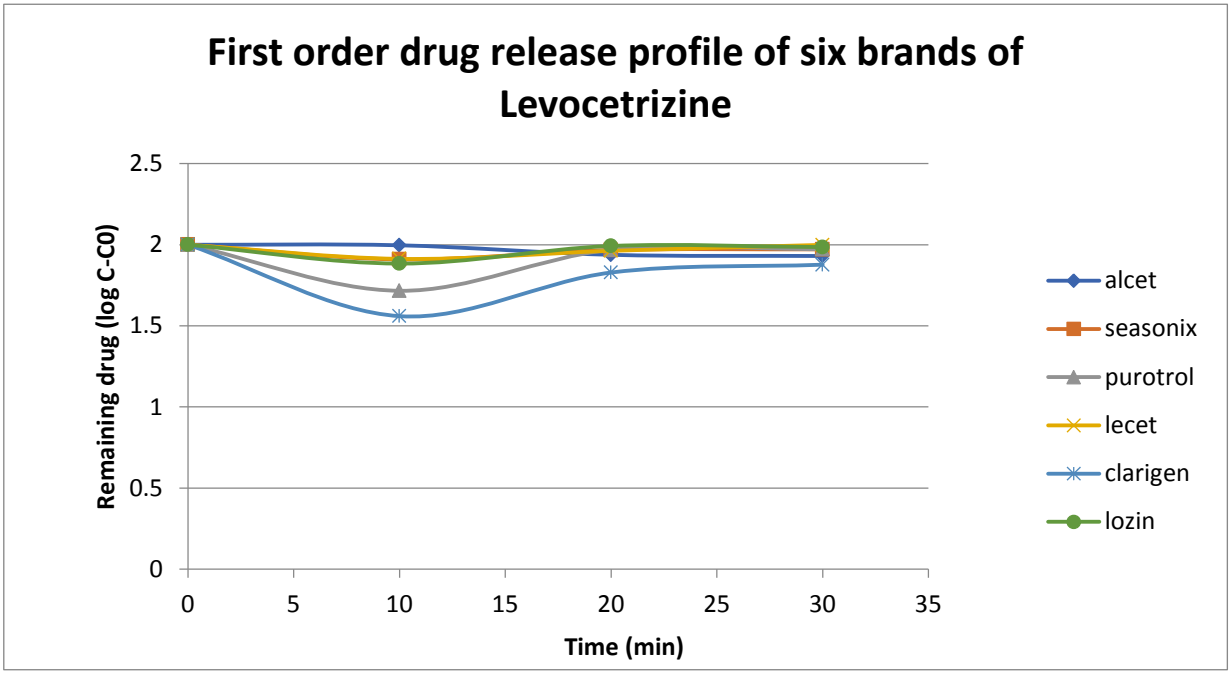

Fig 4.8.a: First order drug release profile of six brands of Levocetirizine

4.9 Higuchi drug release profile

During the determination of dissolution curve, the % release data that were used have now been used cumulatively to determine the Higuchi's drug release profile of each brand at a time. From the Higuchi's equation, $\boldsymbol{Q = [D(2A - C_s)C_s t]^{1/2}}$we can observe that square root of time (min) will be along the x axis and % release along the Y axis. From this cumulative %release vs. 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 4.9.A: R^2values of Six different brands of Levocetirizine for Higuchi's drug release profile

Name	R^2values
Alcet	0.735
Seasonix	0.915
Purotrol	0.966
Lecet	0.939
Clarigen	0.976
Lozin	0.941

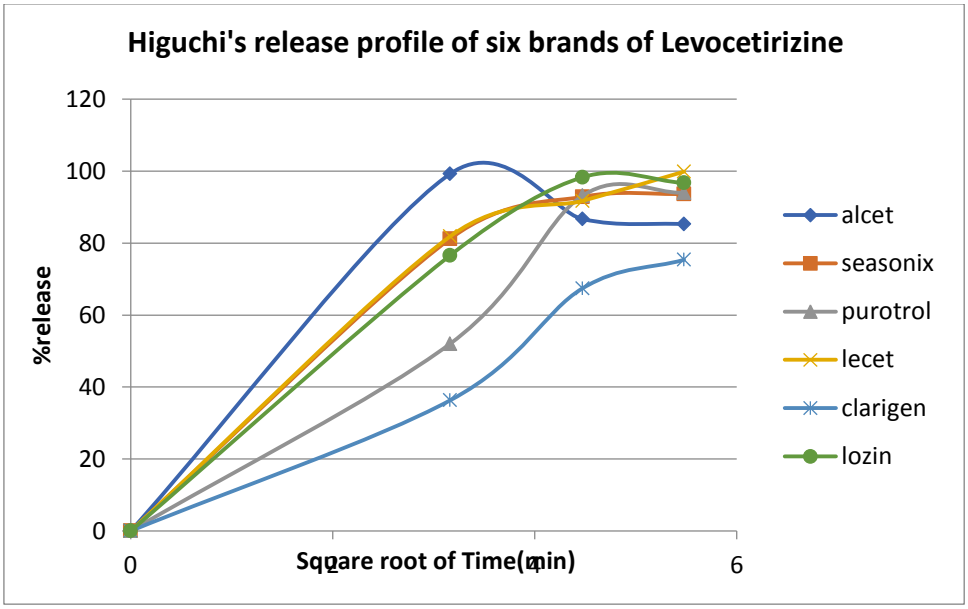

Figure 4.9.a: Higuchi drug release profile of six brands of Levocetirizine

4.10 Comparison among different R^2 values and final discussion on drug release profile

Table 4.10: 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
Alcet	0.473	0.860	0.735
Seasonix	0.705	0.008	0.915
Purotrol	0.882	0.027	0.966
Lecet	0.748	0.022	0.939
Clarigen	0.938	0.004	0.976
Lozin	0.755	0.023	0.941

Chapter Five
Conclusion

5 Conclusion

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 Alcet, the highest R^2 value=0.735 is for Higuchi's equation so Alcet will be released from its solid matrix in the dissolution media following Higuchi's equation presumably. Similarly, the highest R^2 values for Seasonix, Purotrol, Lecet, Clarigen and Lozinare 0.915, 0.966, 0.939, 0.976 and 0.941 that clearly indicated that all of the rest brands of Levocetirizine also followed Higuchi's equation profile of release kinetics.

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