Comparative *In vitro* Quality Evaluation of Different Brands of Ebastine 10 mg Tablets Commercially Available in Bangladesh

By

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A thesis submitted to the Department of Pharmacy in partial fulfillment of the requirements for the degree of
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Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

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Approval

The thesis/project titled “Comparative *In vitro* Quality Evaluation of Different Brands of Ebastine 10 mg Tablets Commercially Available in Bangladesh” submitted by Saqib Rahman-15146048 of Spring, 2015 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy on August 25, 2019.

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Ethics Statement

This study does not involve any kind of trial or experiment related to animal and human.

Abstract

The main purpose of this study was to evaluate and compare the quality control parameters of twelve different brands of ebastine tablets available in Bangladesh. Different *in vitro* quality parameters including weight variation, % friability, hardness, disintegration time, dissolution profile and potency were assessed according to the compendial procedures. According to BP specification, all tablets of each brand showed % weight variation within the range. All brands showed their friability within the USP designated limit of less than 0.5%. Within 30 minutes, tablets from all brands disintegrated completely which complies with BP and JP specifications and eight out of twelve brands could not meet the first stage dissolution test. Potency was calculated using UV-spectrometric method and tablets from all brands showed potency not less than 90%. The study illustrates that all brands of ebastine tablets showed acceptable results for the experiments performed except 1st stage dissolution test for few brands.

Keywords: Ebastine; BCS class II drug; *In vitro* quality evaluation; Assay by UV-spectroscopy; Dissolution profile; Conventional oral dosage form.

Dedication

Dedicated to my Parents, Grandparents and my previous project supervisor,

Dr. Mesbah Talukder

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List of Acronyms

USP	United Sates Pharmacopeia
BP	British Pharmacopeia
IP	Indian Pharmacopeia
Ph. Int.	The International Pharmacopeia
Ph. Eur.	European Pharmacopeia
JP	Japanese Pharmacopeia
BCS	Biopharmaceutical Classification System
HCl	Hydrochloric Acid
SGF	Simulated Gastric Fluid
QTc	Corrected QT Interval
INN	International Nonproprietary Names
AUC	Area under the curve
CYP	Cytochrome P450
IFN-γ	Interferon-gamma
C_{max}	Maximum Concentration of Drug
HPLC	High-Performance Liquid Chromatography

Chapter 1

Introduction

1.1 Background

To create the required impact, medicines must be secure, efficient and of excellent quality. Ensuring these qualities involves the introduction of proficient national drug regulatory officials with the human and other resources required to regulate the manufacture, import, distribution and sale of drugs and medicines. However, these medicines can trigger impairment to health and even death. Even though the pharmaceutical companies of Bangladesh have reached the pinnacle of providing 98 percent of the country's yearly drug market and enhanced the country's trustworthiness on global markets; counterfeit, adulterated and sub-standard drugs remain a threat on the local market, though they are small in percentage (Faruque, Ononna, Al Hossain, & Ganguly, 2018). Any drug delivery system aims to provide the proper site in the body with a therapeutic quantity of medication and then retain the optimum plasma concentration over a certain period. A drug's quality is a significant factor that guarantees a patient's health and well-being. Drugs with sub-standard quality can increase the mortality and morbidity of a country (Aulton, 2005). As reported in the media, some medium and small drug manufacturers are constantly engaged in the manufacture and promotion of counterfeit, adulterated and sub-standard drugs in the lack of rigorous surveillance and efficient control by the regulatory authority. In the absence of appropriate supervisory body of countryside, these medications are on the market to exploit common people's unconsciousness. Consumers of these drugs, despite spending their hard-earned cash, never get the required therapeutic effects (Kabir, 2016). Studies have shown that some manufacturers involved in manufacturing sub-standard drugs deliberately duplicate medicines from some large pharmaceutical industries in setting trade names and developing

packages and labels with a perspective to marketing these medications at an inexpensive price on the market (Faruque et al., 2018). Occasionally, these industries only deliver these drugs to Dhaka's Mitford Market and then those imitated medicines are disseminated across the country from there, these manufacturers do not have their own division of marketing (Kabir, 2016).

Therefore, the prime objective of this investigation was to evaluate, correlate and compare the relevant *in vitro* quality control parameters of different brands of the finished drugs that are being manufactured and marketed by both multinational and local pharmaceutical companies in Bangladesh which are being used for the patients suffering from allergic rhinitis and idiopathic urticaria.

1.2 Antihistamines (H_1-receptor antagonists)

Today, there are more than 45 H_1 antihistamines available worldwide, encompassing the largest class of medicines used in the treatment of allergic diseases and the most widely used among all medicines for the treatment of urticarial (Mittal, Godse, & Patil, 2016). H_1 antihistamines are basically competitive inverse agonists not receptor antagonists, it is known that antihistamines inhibit the effects of histamine on receptors of H_1 (Simons & Simons, 2011). Traditionally, antihistamines are not called antagonists at other histamine receptors (Waller & Sampson, 2017). H_1-antihistamines which are administered orally are well absorbed and metabolized by oxidation in the liver and removed through the kidneys only in unchanged traces (Goldstein, Weber-Schöndorfer, & Berkovitch, 2014).

1.3 Functional classification of antihistamines

Antihistamines are divided into two functional groups:

1.3.1 First (old)–generation H_1-antihistamines

Antihistamines of the first generation are lipophilic, crossing the blood-brain barrier easily leading to adverse effects of the central nervous system (CNS) for example sedation, reduced perception as well as drowsiness. They also have brief half-lives and numerous daily doses are required. They also possess central antimuscarinic effects that suppress nausea in motion sickness (Waller & Sampson, 2017). Most of the H_1-antihistamines of the first generation were introduced before any regulatory authorities existed and before any clinical pharmacology studies of new medications were needed. Examples include Azelastine, Clemastine, Cyproheptadine, Dexchlorpheniramine, Dimethindene, Hydroxyzine, Mizolastine etc. (Goldstein et al., 2014).

1.3.2 Second (new)–generation H_1-antihistamines

In order to reduce the adverse effects of drugs of the first generation, new antihistamines were introduced. With the advent of second-generation H_1-antihistamines, a significant advance in antihistamine expansion took place in the 1980s (Mittal et al., 2016). Antihistamines of the second generation are lipophobic and have low ability to cross the blood-brain barrier, decreasing sedation and cognitive impairment. For non-histamine receptors, they have a reduced affinity and a greater specificity for binding to H_1 receptors. They have longer half-lives, so they can be given as dose once or twice a day (Waller & Sampson, 2017). They have very little antimuscarinic effect as well. The latest medicines in this category are active metabolites or optical isomers of second generation for instance desloratadine and levocetirizine (Mittal et al., 2016). Some widely used second-generation H_1-receptor antagonists are desloratadine, fexofenadine, levocetirizine, bilastine, ebastine, rupatadine and olopatadine (Merlob & Weber-Schöndorfer, 2014).

1.4 General description of Ebastine

With low lipophilicity and higher molecular solubility, the second generation of antihistamines have restricted capacity to cross blood brain barriers and possess decreased side effects, frequently exhibited by the prior (first) generation (Sastre, 2008). Ebastine is a non-sedating, long-acting, second-generation antagonist of histamine receptor with an oxypiperidine-based structure that selectively binds to peripheral H_1 receptors. After oral administration, ebastine is quickly absorbed as well as go through substantial first pass metabolism and it is metabolized to carebastine, which is the active metabolite. The chemical name of ebastine is 4-(4-benzhydryloxy-1-piperidyl)-1-(4-tert-butylphenyl) butan-1- one (Hurst & Spencer, 2000). It has antihistaminic, anti-allergic activity and avoids bronchoconstriction caused by histamine. It has no major sedative or antimuscarinic actions. It is commonly prescribed against allergic conditions (Frare & Singh, 2018). The ebastine is certified in many pharmacopeias, including British pharmacopeia ("The British pharmacopoeia," 2013), European Pharmacopeia (E. Pharmacopoeia, 2019), and Japanese Pharmacopeia XVI ("Japanese Pharmacopoeia XVII," 2017). Basically raw material and impurities are defined in the first two compendiums, while the Japanese Pharmacopeia provides a monograph on active drug ingredient of ebastine, ebastine tablets, and orally disintegrating tablets of ebastine (Frare & Singh, 2018). It is available as tablets of 10 and 20 mg, fast-dissolving tablets as well as pediatric syrup (1mg/ml). Ebastine is available commercially under different brand labels worldwide (Suratiya & Pancholi, 2014).

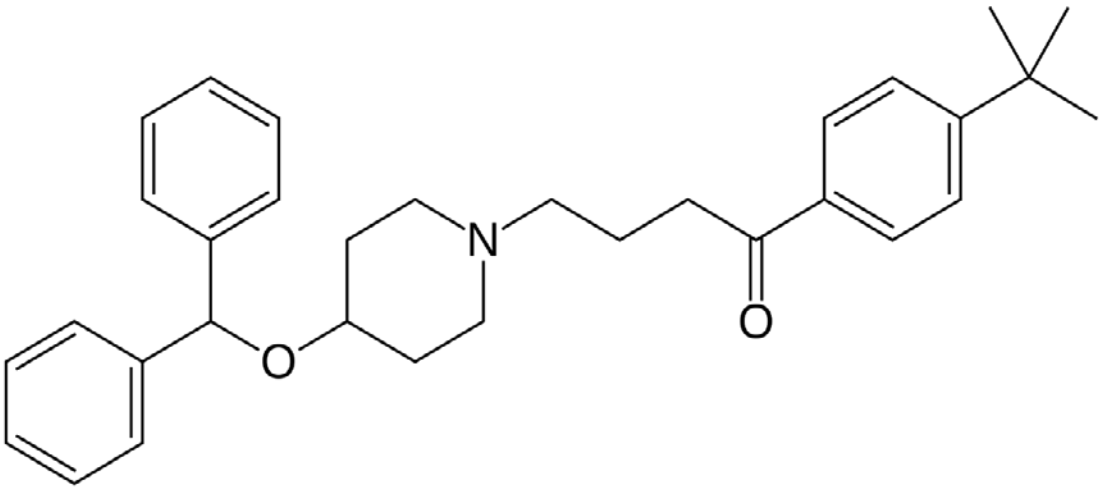

Figure 1: Chemical configuration of Ebastine (Frare & Singh, 2018).

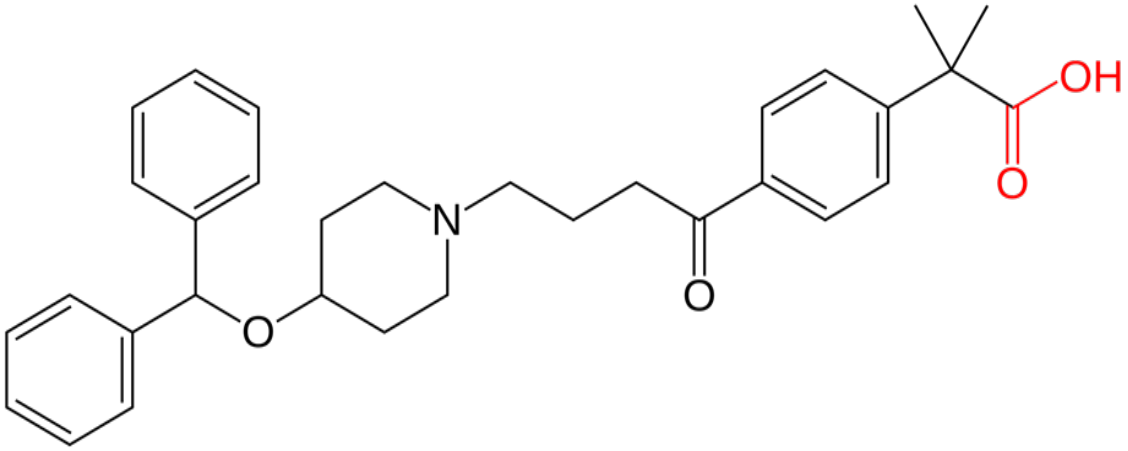

Figure 2: Chemical configuration of Carebastine (Sastre, 2008).

1.5 Drug profile of Ebastine

1.5.1 Therapeutic class

Second generation non-sedating, long-lasting antihistamine (Ciprandi, 2010).

1.5.2 Mechanism of action

Ebastine is a piperidine H_1-antihistamine of second generation. H_1-antihistamines interfere with histamine's agonistic action in the H_1 receptor and are used to mitigate inflammatory processes to treat illnesses for example allergic rhinitis, allergic conjunctivitis and urticaria. Ebastine improves the production of IFN-γ in patients with chronic allergic rhinitis and improves the prognosis of allergic diseases. Reducing the activity of the NF-κB immune reaction transcription factor through the signaling pathways of phospholipase C and phosphatidylinositol (PIP2) also reduces the existence and activity of cell adhesion molecules, pro-inflammatory cytokines and chemotactic factors. Furthermore, reducing the concentration of calcium ion contributes to enhanced stability of the mast cell, which further decreases the release of histamine (Simons & Simons, 2011).

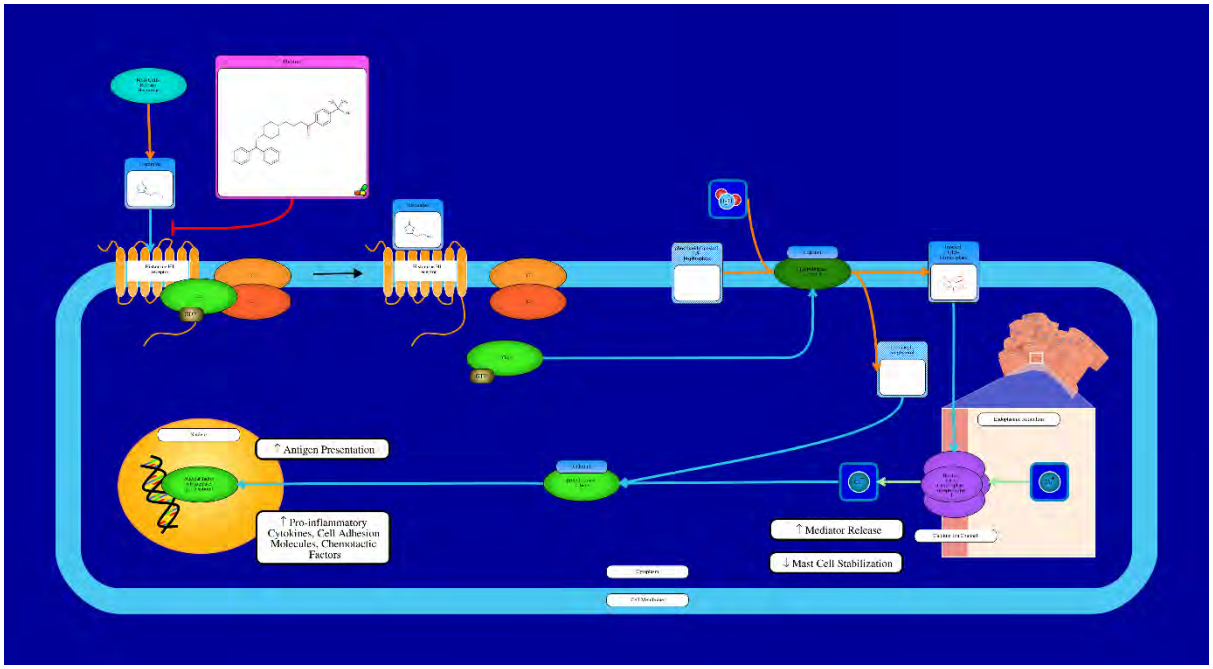

Figure 3: Mechanism of action of Ebastine (Simons & Simons, 2011).

1.5.3 Therapeutic indications

Ebastine 10 mg/20 mg tablet and syrup (1 mg/ml) are indicated for the symptomatic cure of:

- Allergic rhinitis (seasonal and perennial), whether or not linked with allergic conjunctivitis.
- Idiopathic chronic urticaria and in rare case Th-2 type autoimmune disease
- Allergic dermatitis and sometimes asthma
- In several countries for relief from mosquito bites or atopic dermatitis (Patel, 2018)

1.6 Pharmacokinetic properties

The preferential metabolization of ebastine occurs in the liver. The regular adult therapeutic quantity is 10-20 mg, with an onset of action ranging from 1 to 3 hours (Mittal et al., 2016). Pharmacokinetic investigations show that routine administration of 10 mg of ebastine results in a peak carebastine concentration within 2.6-5.7 hours and a half-life ranging from 10.3-19 hours. It binds heavily to plasma proteins (>95%), much of which is excreted through urine. When ebastine is taken with food, gastrointestinal absorption and pharmacokinetics are unaffected. Pharmacokinetic parameters in children and aged volunteers are usually similar to those seen in healthy adults, although C_{max} and AUC values are greater in children than in adults (Hurst & Spencer, 2000). Liver or serious kidney impairment patients (creatinine clearance <1.8 L/h/1.73m^2) had considerably increased for $t_{1/2}$ values compared to those acquired from healthy volunteers. In healthful volunteers getting multiple doses of either ketoconazole or erythromycin (medicines that inhibit CYP metabolism), the metabolism of single doses of ebastine 20 mg was considerably affected (Adwi, Ahmed, & Osama, 2017). Cimetidine, on the other hand, had no major influences on the metabolism of a single dose of 20 mg of ebastine (Frare & Singh, 2018).

1.7 Pharmacodynamic properties

Ebastine is known as inverse agonist of histamine instead of H_1-receptor antagonist. Its antagonistic effect repeatedly prevents histamine from working, particularly in instantaneous hypersensitivity (Ciprandi, 2010). It mainly functions on bronchi, capillaries, and several other smooth muscles and is used to inhibit or mitigate motion sickness, seasonal rhinitis, and allergic dermatitis (Adwi et al., 2017). In inhibiting histamine-induced wheal and flare, a single dose of ebastine (10 mg or more) is considerably superior to placebo. Single and multiple ebastine therapeutic doses do not interfere with psychomotor functioning or driving ability in volunteer. Thus far, the literature has not defined any crucial cardiovascular effects

of ebastine (Hurst & Spencer, 2000). Furthermore, ebastine therapy, 20 mg daily for 1 week, did not induce the psychomotor performance diminishing effects or the depressive implications of ethanol of diazepam (Frare & Singh, 2018).

1.8 Safety and tolerability

Ebastine tablet (10 mg/20 mg) is administered orally once daily. Generally, ebastine is well tolerated and effective at relieving the symptoms of allergic rhinitis and chronic idiopathic urticaria. Headache, drowsiness and dry mouth are the most prevalent adverse events. Ketoconazole or erythromycin co-administration is not clinically significant for adverse cardiac events. 10 mg and 20 mg of ebastine are not sedating and do not impair cognitive or psychomotor performance, including driving ability (Ciprandi, 2010). At doses up to 5 times the prescribed therapeutic dose, no relevant changes in the QTc interval were observed with ebastine. Thus, for the first-line treatment of allergic rhinitis and chronic idiopathic urticaria, once daily ebastine is an efficient solution to other second-generation antihistamines. No significant cardiac abnormalities appear to be associated with ebastine (Van Cauwenberge, De Belder, & Sys, 2004).

1.9 Dosage and administration

Ebastine tablets and syrup are for oral administration. For orodispersible (Fast dissolving tablet) tablet it should be placed on the tongue where the drug will release, no water or other fluid is needed for this. Ebastine may be administered with or without food as there is no effect of food on drug (Sastre, 2019).

Children between 2 and 5 years: 2.5 ml (half teaspoonful) once a day (in severe situations such as perennial allergic rhinitis up to 5 ml)

Children between 6 and 12 years: 5 ml (one teaspoonful) or 5 mg (half tablet) once a day (up to 10 ml in serious conditions such as perennial rhinitis)

Adults and older children more than 12 years old: Once daily 10 mg (1 tablet) or 10 ml (2 teaspoonful) (“Ebastine Drug Information - Indications, Dosage, Side Effects and Precautions,” 2019).

1.10 Drug interactions and precautions

Pharmacokinetic interaction with CYP3A4 metabolized drugs, for example with ketoconazole, itraconazole or erythromycin, leads to enhanced ebastine or carebastine plasma levels and trigger an extension of the QTc interval. Pharmacokinetic interaction with rifampin (rifampicin), which results in decreased carebastine plasma concentration. Theophylline, warfarin, cimetidine, diazepam or alcohol kinetics do not interact with ebastine. Alcohol and diazepam sedation effect may be improved (Sastre, 2019). Therefore, caution is to be advised while administering ebastine with other drugs mentioned.

1.11 Contraindications

Ebastine is contraindicated in patients with diarrhea, cardiac arrhythmias and recognized hypersensitivity to ebastine or to any of its excipients, in hepatic impairment, renal insufficiency, special caution is advised (Adwi et al., 2017).

1.12 Use of Ebastine during pregnancy and lactation

1.12.1 Pregnancy

Data from the use of ebastine in pregnant females are limited. Animal studies do not show detrimental effects on reproductive toxicity, either directly or indirectly. As a precautionary measure, the use of ebastine during pregnancy should be avoided (Ciprandi, 2010) .

1.12.2 Lactation

Whether ebastine is excreted in human milk is not known. High protein binding (>97%) of ebastine and its active metabolite, carebastine, does not suggest drug excretion and drug excretion into breast milk. It is preferable to avoid using ebastine during lactation as a precautionary measure ("Information on Ebastine and its use," 2018) .

1.13 Side effects

Headache, dry mouth and drowsiness are the most prevalent side effects. Pharyngitis, abdominal pain, dyspepsia, asthenia, epistaxis, rhinitis, sinusitis, nausea, and insomnia are less frequently reported side effects ("Information on Ebastine and its use," 2018).

1.14 Physicochemical properties

1.14.1 Molecular formula and molecular mass

$C_{32}H_{39}NO_2$ and 469.7 g/mol (Frare & Singh, 2018).

1.14.2 Appearance & physical state

non-hygroscopic, white to off-white crystals or crystalline powder (Hurst & Spencer, 2000)

1.14.3 Melting point

80 – 82ºC (Frare & Singh, 2018)

1.14.4 Boiling Point

596.3ºC (“Ebastine - Chemical Book,” 2017)

1.14.5 Solubility

It is easily soluble in acetic acid, in methanol it is also soluble, in water practically insoluble, partially soluble in ethanol (“Japanese Pharmacopoeia XVII,” 2017)

1.14.6 Storage Condition

25ºC (“Information on Ebastine and its use,” 2018)

1.14.7 λ_{max}

252 nm (wavelength of maximum absorption) (“Ebastine - JP XVII - Ultraviolet-visible Reference Spectra,” 2017)

1.15 Conventional oral dosage forms

The most common form of oral route administration that comes to mind are tablets that have the most major and meaningful place among all pharmaceutical formulations. In the pharmaceutical industries, the solid oral dosage forms are more important. Tablets are solid dosage forms generally obtained through single or multiple powder or granule compressions. In certain cases, molding or extrusion methods may be used to obtain tablets. They can be uncoated or coated and there can be one or more active ingredients in tablets (Ansel, 2015). Tablets may contain excipients such as diluents, binders, disintegrants, glidants, lubricants substances that can alter the behavior of the dosage forms and the active substance in the gastrointestinal tract, coloring materials and flavoring materials approved by the suitable national or regional authority. While using such excipients, the stability, dissolution rate, bioavailability, safety or effectiveness of the active ingredient must be ensured; any component of the dosage form must not be incompatible (Ansel, 2015). Tablets are

preparations for oral administration with a single dose. Some are meant to be swallowed whole, some after chewing and others after crushing, some are meant to be dispersed or dissolved in water afore being taken and a number of them should be kept in the oral cavity where the active drug substance is released ("The International Pharmacopoeia , Eighth Edition," 2018).

1.16 Advantages of conventional oral dosage forms

1) Tablets are unit dose form, offering the utmost abilities of all dosage forms for the highest precision dose and the latest variation in active substance.
2) The price is lowermost of all dosage forms.
3) They are the lightest and most solid of all forms of oral dosing.
4) Of all oral dosage forms, they are generally easiest and cheapest to package and ship.
5) Product recognition is possibly the easiest and low-priced, and no further operating actions are required when using an embossed or monogrammed punch face
6) They may possibly deliver the supreme ease of swallowing with the slightest possibility to remain above the stomach, especially when coated, as long as the disintegration of the tablet is not too fast.
7) Tablets offer themselves to specialized release profile medications such as enteric or delayed-release tablets.
8) Tablets are more appropriate for large-scale manufacturing than other oral forms of units.
9) They have the best overall characteristics of all oral forms of chemical, mechanical and microbiological stability (Aulton, 2005).

1.17 Disadvantages of conventional oral dosage forms

1) Due to their amorphous nature or flocculent, low-density feature, some drugs block compression into dense compacts.
2) Drug substances with limited wetting, slow dissolution characteristics, moderate to bulky doses, optimal elevated absorption in the gastrointestinal tract, or any compilation of these characteristics can be hard or even unfeasible to formulate and develop as a tablet that will nevertheless impart sufficient or complete bioavailability of drugs.
3) Drugs may need encapsulation or entrapment prior to compression or the tablets may entail coating if drugs are bitter tasting, have unpleasant odor or susceptible to oxygen or atmospheric humidity (Ansel, 2015).

1.18 *In vitro* study vs. *in vivo* study

1.18.1 *In vitro* study

In vitro (Latin for inside the glass) relates to the method of performing a particular procedure outside a living organism in a controlled setting. Many studies are performed outside of organisms or cells in cell biology. One of the repeated weaknesses of *in vitro* studies is that they do not mimic an organism's accurate cellular circumstances, especially a microbe. For example, According to one estimate, 99.6% of human microbiota species have not or cannot be identified by *in vitro* methodologies. But still in vitro studies are very important in estimating and evaluating many procedures, processes, methods and quality standards before performing them on living organisms to get the practical results in a cost-effective way (Murray, Arias, Li, Bhoopathy, & Hidalgo, 2016).

1.18.2 *In vivo* study

In vivo ("within the living" in Latin) relates to testing using an entire living organism as opposed to a partial or dead organism. Two types of *in vivo* study are animal studies and clinical trials. *In vivo* experimentation is often used over *in vitro*, as it is more convenient to observe an experiment's overall effects on a living subject. Although there are many reasons for believing that *in vivo* studies have the ability to provide decisive insights about the nature of medicine and disease, there are various ways in which these findings can be misleading. A therapy, for instance, can provide a short-term benefit, but a long-term damage (Etman, Shekedef, Nada, & Ismail, 2017).

1.19 *In vitro* quality control parameters and tests for tablets

For a drug to be called as a finished product and launched in market it needs to meet the physical specification, compendial requirements and quality standards prescribed and described in different pharmacopeial monographs during production and some crucial factors should remain controlled throughout the manufacturing process. They are called in-process quality control parameters as well as quality control tests. There is a chance of errors occurring when the production process is running, so it is important to control the flaws that may occur during the production process, and to assess the quality of the product, strict quality control tests must be carried out. Furthermore, the finished products also need to go through some rigorous quality measurements and standards to satisfy the requirements and pass or qualify the finalized product quality control tests to assure the quality of the products so that they cannot be called as misbranded or sub-standard drugs or medicines. Therefore, both in process and finished product quality control tests are vital requirements to ensure the overall quality of the product (Shabana, 2016). Below some crucial (official and non-official) quality control tests for tablets are described in brief to know the importance of them.

1.19.1 Weight variation test

This test is an official test for tablets (United states pharmacopoeial commision, 2016). The weight uniformity test is used to verify that each tablet contains the specified quantity of drug substance with little deviation among the tablets within a batch. The purpose of this test is to evaluate uniformity of tablet weights with respect to the dose. Generally 20 tablets are taken to perform the test, average weight of 20 tablets is recorded and % variation or % deviation of each tablet from the mean or average weight is measured and the % weight variation should be within the percentage limit range listed in USP, BP and IP (Kumar, 2013). Only apparatus that is needed for this procedure is an analytical balance.

1.19.2 Tablet thickness test

For very few tablets, thickness testing is performed as it is not an official test to conduct. Venire caliper or screw gauge is used to measure tablet thickness (Ansel, 2015).

1.19.3 Content uniformity test

It is an official quality control test (United states pharmacopoeial commision, 2016) . Content uniformity test was established to guarantee the consistency of active drug ingredients in dosage units within a limited range around the label claim. This test is essential for tablets with a drug substance below 25 mg or where the active drug is less than 25% of the entire tablet weight. 30 tablets are used to execute the experiment, 10 tablets are assayed individually as prescribed in the individual monograph and if it is found that quantity of the active drug in every dosage unit remains within the limit of 85% to 115% of the claimed value, the procedure is considered done and the tablets pass. Remaining 20 tablets are used when this experiment fails to comply (Kongsuk, 2011).

1.19.4 Content of active ingredient (Assay)

This is also an official test described in individual drug monograph of pharmacopeia. The objective of this experiment is quantitative and qualitative analysis of active contents in tablets. The requirement of this test is to confirm and check whether labeled amount of active drug is present in the given dosage form. This is calculated from a sample of 20 tablets that should be chosen casually from a tablet batch (Savale, Laboratories, Nasik, & Estimation, 2018). The tablets are weighed and crushed with just a pestle in a mortar. In an analytical balance, a quantity equivalent to the theoretical content of each tablet or the average crushed tablet is weighed out in the balance. The weighted sample is dissolved either in a solvent where the active drug is readily soluble or in a solvent prescribed in the individual compendial monograph then the resultant solution is filtered and the stipulated assay procedures are subjected to an aliquot of the resulting supernatant (Gupta, 2017). Spectrophotometry or High-Performance Liquid Chromatography (HPLC) is generally used to analyze the active drug (Allen, Bassani, Elder, & Parr, 2014).

1.19.5 Hardness test

The purpose of this test is to check whether or not the tablets can withstand extreme handling and pressure by measuring the crushing strength property which is defined as the compressional force applied to a tablet along the diameter (Savale et al., 2018). Most of the time, the test is carried out to satisfy the need for pressure adjustments on the tablet machine. Hardness influences the test for disintegration. The newton, as accepted by the SI system, is the recommended unit of force. However, the kilogram may also be used as the measuring unit for hardness test. But newton will be the unit that should be used to measure the tablet crushing strength, 1 kilogram = 9.807 newton (Gupta, 2017). Hardness tester is used to measure the degree of force for crushing the tablets (Shabana, 2016).

1.19.6 Friability test

The purpose of this test is to check tablets' durability (physical strength) and how well tablets stand up to abrasion during handling, coating, packaging, shipping and other processing conditions (Shabana, 2016). To perform the test friabilator or friability tester is used with the help of tumble motion. Loss of weight of 10 tablets is the measuring point of this test after a specific period of time, friabilator has a rotating drum which rotates for 4 minutes at 25 rpm means that 100 revolutions (S. Interim, Announcement, & Friability, 2016). Initial weight of 10 tablets is calculated and after the rotation 10 tablets are weigh again for final weight and from that % weight loss is measured (Ansel, 2015).

1.19.7 Disintegration test

The purpose of this test is to determine whether tablets break down into small pieces or not under experimental conditions within the recommended duration of time while putting in a liquid medium ("The British pharmacopoeia," 2013). This is the first significant step towards drug dissolution (T. I. Pharmacopoeia, 2011). This is an official test that illustrates how long it takes to break the tablet into fine particles and tiny fragments improving its solvent solubility ("The International Pharmacopoeia, Eighth Edition," 2018). The disintegration process can be integrated into the dissolution process where a solid ingredient breaks down into small pieces while dispersing in a liquid until a homogeneous solution of the solute and the solvent is formed. Disintegration affects dissolution test. All tablets must complete a disintegration test that is performed *in vitro* using a test device for disintegration (T. I. Pharmacopoeia, 2016). The USP disintegration device consists of a basket-rack setup comprising 6 USP-specified dimensional open-ended transparent tubes placed vertically on a 10-mesh size (2 mm) stainless steel wire mesh (Pharmacopeia, 2008). For the duration of experimentation, a tablet is put in every single of the basket's 6 chambers and the basket is lifted and dropped in a fluid reservoir for example, water (most of the time) or any other

solvent recommended in the individual monograph at 29 to 32 cycles per minute by using a mechanical device (T. I. Pharmacopoeia, 2016). The wire screen should always be below the level of the fluid. The temperature of the immersion medium should be maintained at 37º ± 2ºC and media volume should be 600 ml in each 1000 ml beaker (Pharmacopeia, 2008). Disintegration is considered to be achieved if there are no residue or fragments (other than coating fragments adhered to the lower surface of the disk) on the screen or if there are particles left, they are soft mass without any palpably firm, un-moistened core (T. I. Pharmacopoeia, 2016). Disintegration procedure helps to understand the solubility of the API (active pharmaceutical ingredient) in the digestive system's gastric fluid or in the intestinal fluid (Assembly, 2019).

1.19.8 Dissolution test

Dissolution test is considered as an official test and it is performed to determine the percentage release of drug substance from the dosage forms determining amount of active substance dissolved in the medium at stated period. Normal dissolution is the mechanism by which solid, gaseous or liquid materials are dissolved to create a solution in a solvent. However, the solvent must be compatible with the materials to make a solution; the solution produced by the mechanism of dissolution is often homogeneous. Dissolution is a kinetic process, so that kinetic energy emerging from high temperature will accelerate the mechanism of dissolving a solute in a liquid. Dissolution testing is an *in vitro* procedure which defines how an API is extracted out of a solid dosage unit into a solution within the gastrointestinal tract ("*In Vitro* Dissolution Testing for Solid Oral Dosage Forms," 2010). The FDA instruction on dissolution testing for types of immediate release solid oral dosage involves the execution of the Biopharmaceutical Classification System (BCS) guidelines and criteria for bio-relevant dissolution studies and that is based on the solubility and permeability of the API ("Biopharmaceutical Classification System and Formulation

Development," 2011). *In vitro* dissolution experimentation may be an effective tool for estimating *in vivo* drug content efficiency and potentially reducing the number of required bioavailability or bioequivalence studies. Selection of appropriate dissolution medium and temperature of the medium are the 2 most important factors, *in vitro* conditions must mimic the *in vivo* conditions to get better *in vivo* drug dissolution (United States Pharmacopeial Convention, 2011). Therefore, the tablet dissolution rate is a very important factor for drug absorption inside the body as increased the dissolution rate → increased the absorption rate→ increased bioavailability of drug (Shargel, Leon & B.C. Yu, 2017). USP apparatus I (basket) and USP apparatus II (paddle) remain the most widely operated dissolution-testing instrument. 37º ± 0.5ºC is the optimum temperature condition that is maintained mostly and paddle rotation may vary from 25, 50, 75 or 100 rpm. 1000 ml beaker is used in the apparatus and in each 1000 ml of beaker 900 ml of media volume is generally placed to perform the method (F. Interim & Announcement, 2016).

1.20 Aim of the study

This study aimed at evaluating some relevant *in vitro* quality control parameters to correlate and compare the quality of twelve different brands of ebastine BP tablets of both international and local pharmaceutical companies available in the Bangladesh market. The study also focuses on determining whether these tablets comply with the physical specifications and compendial (pharmacopeial) requirements as claimed by the manufacturing companies or not.

1.21 Objectives of the study

In the pharmaceutical industry, total product quality must be assured to remove the product that does not fulfill the standards and specifications specified in the Pharmacopoeias. There are many brands (approximately 18 brands) of ebastine BP tablets available all around the Bangladesh. Therefore, it is quite difficult to select the most effective and the safest one within the affordable price range. Objectives of this study are as follows:

1) To determine safety and efficacy of the tablets.
2) To find out the tablets with flaws, defects, errors, and the tablets which will fail to pass the quality standards and measurements.
3) To find out the dosage forms that will not meet the compendial requirements of tablets among these twelve brands.
4) To find out the misbranded (mislabeled), sub-standard and counterfeit (falsified) tablets.
5) To compare and correlate the quality of the twelve brands of ebastine tablets.
6) To find out the safest and the most effective brand within an affordable price.
7) To establish possible equivalence among the brands of ebastine tablets.

Chapter 2

Materials and methods

2.1 Materials

2.1.1 Study samples

Samples of this study were film-coated ebastine BP tablets (containing 10 mg API in each tablet) of 12 different brands and all of them are immediate release solid oral dosage forms.

2.1.2 Sample collection and identification

All the tablet samples were purchased from different local registered drug stores of dhanmondi, kalabagan, azimpur and panthapath of Dhaka city. Before purchasing, all the samples were accurately verified for their price, manufacturing and expiry dates. All the sample brands were given a brand code assigned to each one of them from E1-E12 randomly to hide their identity. Label information of the purchased 12 different brands of ebastine tablets is given below.

Table 1: Label information of randomly selected twelve different brands of Ebastine tablets (10 mg)

Brand Code	Strength	Dosage form	Mfg. Date	Exp. Date	Pack size found	Price of pack found (BDT)	Price / 10 units (BDT)
E1	10 mg	Tablet	Jan-19	Dec-22	30	240	80
E2	10 mg	Tablet	Nov-18	Nov-20	30	180	60
E3	10 mg	Tablet	Apr-19	Apr-21	50	400	80
E4	10 mg	Tablet	Apr-19	Apr-21	50	250	50
E5	10 mg	Tablet	Mar-19	Feb-21	60	480	80
E6	10 mg	Tablet	Oct-18	Oct-20	50	400	80
E7	10 mg	Tablet	Oct-19	Oct-21	30	180	60
E8	10 mg	Tablet	Sep-19	Aug-20	30	180	60
E9	10 mg	Tablet	Sep-18	Sep-20	30	180	60
E10	10 mg	Tablet	Nov-18	Nov-20	50	300	60
E11	10 mg	Tablet	Aug-18	Aug-21	30	180	60
E12	10 mg	Tablet	Dec-18	Dec-20	30	180	60

2.1.3 Reference standard drug

Standard ebastine drug (Purity 99.9%) was a kind gift from one of the leading pharmaceutical companies of Bangladesh, Eskayef Pharmaceuticals Ltd.

2.1.4 Glassware and paper materials

Table 2: Glassware and paper materials used in the experiment

Serial No.	Name	Specification
1	Volumetric flask	10ml, 50ml, 100ml
2	Pipette	2ml, 5ml, 10ml
3	Beakers	50ml, 100ml, 500ml
4	Funnel	Medium
5	Measuring cylinder	50ml, 100ml
6	Pipette filler	Medium
7	Filter paper	Whatman Grade 41
8	Mortar and pestle	Large
9	Glass rod	Small, medium
10	Spatula	Small, medium
11	Weighing paper	Small, medium
12	Test tube	Small
13	Aluminum foil paper	Medium

2.1.5 Solvents and reagents

Table 3: Solvents and reagents used in the experiment

Serial No.	Name	Purpose
2	37% Hydrochloric acid	Dissolution test and Disintegration test
4	Methanol	Potency test (Assay)
5	Distilled Water	Dissolution test and Disintegration test

2.1.6 Equipment and instruments

Table 4: Equipment and instruments used in the experiment

Serial no.	Equipment Name	Model No.	Manufacturer	Origin
1	Electronic Balance 3-Digit	PA-213	Ohaus Corp.	USA
2	Tablet Friability Tester (USP)	EF2-USP	Electrolab	India
3	Tablet Disintegration Tester (USP)	ED-2L	Electrolab	India
4	Hardness Tester (USP)	EH-01	Electrolab	India
5	UV Spectrophotometer	UV-1800	Shimadzu	Japan
6	USP Dissolution Apparatus II	UDT-804	Logan Instruments Corp.	USA
7	Ultra-Sonic Sound Bath (Sonicator)	KSU-500	Labtech	Korea
8	Vortex Mixer	VM-2000	Digisystem	Taiwan
9	Hot Plate with Stirrer	LMS3006	Labtech	Korea

2.2 Methods

2.2.1 Weight variation test

With the help of electronic analytical balance 20 tablets from each brand were weighed accurately which were selected randomly and then average weight was calculated. Percentage deviation of individual tablet weight from the average weight was determined by using the following equation:

$$\% \text{ Weight variation} = \frac{\text{Individual weight} - \text{Average weight}}{\text{Average weight}} \times 100$$

Not more than two of the individual tablet weights would deviate from the mean weight more than the percentage limit range recommended by USP, IP and BP that is listed below and none should deviate from the mean weight more than twice the percentage limit range

(Kumar, 2013). Furthermore, average weight of 20 tablets from each brand was noted with standard deviation.

Table 5: USP, BP and IP acceptance limits of maximum weight variation (%) (Kumar, 2013).

BP/IP standards of average weight	Maximum allowed % variation limit	USP standards of average weight
80 mg or less	± 10 %	130 mg or less
80 mg – 250 mg	± 7.5 %	130 mg – 324 mg
More than 250 mg	± 5 %	More than 324 mg

2.2.2 Hardness test

Hardness tester (Electrolab) was operated to measure the crushing strength of the 10 tablets from each brand that were selected randomly. The unit to measure the degree of force was newton (N) and by putting each tablet once between the upper punch and lower punch of the tester and then each tablet were crushed between them and the pressure required to crush them was determined for 10 tablets individually from each of the 12 brands and average hardness was noted with standard deviation. The standard range of hardness or crushing strength is 39.24 – 78.48 N (Karmoker J, Joydhar P, Sarkar S, 2016), (Kumar, 2013).

2.2.3 Friability test

10 tablets were picked randomly from each brand for this test and the test was run in the device known as Roche friabilator. At first, 10 tablets were weighed together before placing them inside the transparent drum of the USP friability tester and the initial weight of 10 tablets were recorded. Then after the tumbling of the drum or rotation of the drum for 4 minutes at 25 ± 1 rpm final weight was recoded of the same 10 tablets that were in the drum to determine any % loss of weight of these 10 tablets after 4 minutes. USP recommends that

% loss of weight of the tablets should not be more than 1% and below 0.5% is most accepted (S. Interim et al., 2016). The % weight loss is calculated by the following equation:

$$\% \text{ Weight loss} = \frac{(\text{Initial weight} - \text{Final weight})}{\text{Initial weight}} \times 100$$

2.2.4 Disintegration test

For this test 6 tablets from each brand were chosen at random and were placed inside the six open-ended transparent tubes of the disintegration device's basket and then the basket was reassembled with the rack of the device as this tester is known as basket-rack assembly (Assembly, 2019). Finally, discs were put on top of each six tablets as it was specified in individual monograph of USP, BP and JP for disintegration of film-coated tablet (Al-Gousous & Langguth, 2015). IP, JP, Ph. Eur. and BP prescribed in their individual monograph about the conditions for plain-coated or film-coated tablet disintegration:

Table 6: Disintegration test conditions of film coated tablets according to IP, JP, BP and Ph. Eur. (Al-Gousous & Langguth, 2015), (Savale et al., 2018).

Immersion medium	Temperature	Time Limit
Water with discs	37º ± 2º C	Not more than 30 minutes

However, JP suggested a different media condition for the disintegration of ebastine film coated tablet and that is using of 0.1 M HCl (pH 1.2) as the immersion fluid or medium with discs, rest of the parameters are the same as BP, IP and Ph. Eur. recommended (Al-Gousous & Langguth, 2015), (Kumar, 2013). So, for this test USP GSF (gastric simulated fluid) was used as medium as it was recommended by JP ("Japanese Pharmacopoeia XVII," 2017). 600 ml of immersion medium was prepared using 0.1 M HCl for 6 tablets from each brand and the medium was transferred into the 1000 ml beaker of the device and the temperature was maintained at 37º ± 2ºC using the device's thermostat. After placing the tablets into the 6

tubes of the basket and reassembling it with the rack of the device which was having 1000 ml of beaker containing 600 ml of immersion fluid, the machine was turned on with timer and the basket was continuously raised and lowered from and into the medium until all the six tablets were broken down completely into small fragments and there was no residue left on the stainless steel screen (mesh) of the tube (Pharmacopeia, 2008). When all the 6 tablets were broken into small particles and all the particles passed from tube mesh to outer beaker that time was recoded and average time was noted with standard deviation for future analysis and it was carried out for each of the 12 brands. It was made sure before closing the test that all the ebastine tablets were disintegrated within the specified time.

2.2.5 *In vitro* dissolution test

According to BCS (Biopharmaceutical classification system) ebastine is class II drug meaning that it has high permeability but low solubility (Kamisetti & Gupta, 2017). Ebastine (active ingredient) is not freely soluble in water. Therefore, considering this situation dissolution test of this type of active drug needs special technique and method. For conventional release tablet formulations comprising poorly water-soluble active components (BCS Class II and IV), choices are made on a case-by-case groundwork on the suitable experiment conditions. Normally, a single-point dissolution test is used. Due to the low aqueous solubility, it may be necessary to dissolve the drug in dissolution volume of 900 ml and add a surfactant to increase its solubility in water (I. Pharmacopoeia, 2015).

BCS Class	Solubility	Permeability	Oral Dosage Form Approach
1	High	High	Simple solid oral dosage form
2	Low	High	• Techniques to increase surface area like particle size reduction, solid solution, solid dispersion • Solutions using solvents and/ or surfactants
3	High	Low	Incorporate permeability enhancers, maximize local lumenal concentration
4	Low	Low	Combine 2 and 3

Figure 4: Approaches to enhance solubility and permeability for BCS class I – IV drugs ("Biopharmaceutical Classification System and Formulation Development," 2011).

The duration of the dissolution is generally 30 to 60 minutes for immediate release dosage forms and if apparatus II (paddle) is used, 50 and 75 rpm are the frequently used rotational speeds. In Japanese Pharmacopeia, ebastine tablet dissolution procedure is described. As per the existing monographs, the dissolution have to be conducted with a paddle technique, at 50 rpm speed 900 ml of 0.2 % sodium chloride (NaCl) solution, with pH fixed to 1.2 with HCl mimicking gastric fluid of the human stomach at fasted state, this prepared fluid is known as simulated gastric fluid (SGF). The dissolution rate for ebastine tablets is no less than 75% in 30 minutes ("Japanese Pharmacopoeia XVII," 2017). An alternative approach is described for ebastine dissolution profile analysis. The writers researched various dissolution media at varying pH, with or without surfactant. Based on the findings, the writers recommended a procedure of dissolution using apparatus II with a 900 ml (acidic) medium at 75 rpm (Arend, 2010). BP and USP do not have any official monograph for ebastine tablet dissolution. So, methods prescribed and described in individual monograph of Japanese pharmacopeia about ebastine dissolution was followed during this experiment. Here, the alternative method of ebastine tablet dissolution was implemented using 0.01M HCl as the dissolution media as 0.01M at 75 rpm showed highest amount of active drug getting dissolved and giving highest

percentage of drug release and pH was maintained between 2 – 2.5 using HCl (Arend, 2010). The conditions of this dissolution experiment were as follows:

Table 7: Dissolution test conditions of Ebastine tablets

Equipment	**USP II (paddle)**
Dissolution media	900 ml, 0.01M Hydrochloric acid
Temperature	37º ± 0.5ºC
pH	2
Rotation	75 rpm
Time	30 minutes

Preparation of stock solution and standard curve: Accurately weighed 5 mg of pure drug was taken in a 50 ml of volumetric flask and dissolved in acidic medium (pH 2) up to the mark. For better dissolution of the pure drug in the acidic medium, the solution was taken in a beaker with a magnet and it was put on the hot plate with magnetic stirrer and it was kept there for 30 minutes at 40ºC. Acidic medium or fluid was made by diluting 37 % HCl (12M) to 0.01M HCl by using $V_1 \times C_1 = V_2 \times C_2$ equation and the pH was adjusted to 2 using further HCl if needed. So, the concentration of the solution was 0.1 mg/ml or 100 μg/ml and from this 100 μg/ml solution, 20 μg/ml of solution of 100 ml was prepared by diluting it 5 times and this was used as stock solution. Using this stock solution, solutions of concentration ranging from 2 – 14 μg/ml were prepared as working standard solutions by using this $V_1 \times C_1 = V_2 \times C_2$ equation. After that absorbance of each of the solutions of pure drug was measured and noted at 252 nm (wavelength of maximum absorption of ebastine) using UV-spectrometer against a suitable blank solution of acidic fluid then a calibration curve or standard curve (absorbance vs. concentration graph) of ebastine was constructed according to Beer-Lambert's law.

***In vitro* dissolution test procedure:** The dissolution test was performed by using USP II apparatus (paddle) dissolution tester. To determine drug release from the dosage form 900 ml of acidic fluid prepared by using 0.01M HCl (pH 2) was used as the dissolution medium. A single-point dissolution study was performed as only after 30 minutes, sampling was done meaning that 30 minutes was set as the sampling time. As the apparatus has no auto-sampler mechanical device, sampling was done manually. The temperature was maintained at 37º ± 0.5ºC by an auto heater and rotation of the paddle was set at a speed of 75 rpm. After 30 minutes, 10 ml of sample was withdrawn and then it was filtered with whatman (grade 41) filter paper of pore size 20 µm and the filtrate was diluted 5 times with fresh acidic solvent (0.01M HCl). 2 ml from that 10 mL of sample was taken in a separate volumetric flask of 10 ml and fresh acidic fluid was added up to the mark to dilute 5 times. Amount of dissolved ebastine was determined by using UV-spectrometer by taking absorbance values at about 252 nm (λ_{max}) against the blank solvent of dissolution medium. After that by using the linear equation of the standard calibration curve of ebastine in acidic medium (pH 2), concentration of drug from the dosage form was calculated by using the absorbance values. This process was done for all 12 brands by using 6 tablets from each brand and putting each tablet in one beaker thus using 6 beakers at a time of the dissolution tester device (United States Pharmacopeial Convention, 2011). Average percentage drug release was calculated along with the standard deviation for using 6 tablets from each brand. Amount of released drug was calculated by the following equation:

$$\text{Amount of released drug (mg / tablet)} = \frac{\text{x (µg/ml)} \times 5 \times 900\text{ml}}{1000};$$ where, dilution factor = 5 and 1000 is used to convert µg to mg. Equation for % drug release is as follow:

$$\%\ \text{Drug release} = \frac{\text{Amount of released drug}}{\text{Labeled Amount}} \times 100$$

Table 8: Acceptance criteria for in vitro dissolution test of immediate release tablet (WHO Department of Essential Medicines and Health, 2018).

Level	Samples tested	Acceptance criteria
S_1	6	Each magnitude is not less than Q + 5%
S_2	6	The mean magnitude of the twelve units of dosage (S1 + S2) is equal to or higher than Q and no unit is less than Q-15%
S_3	12	The mean magnitude of twenty four units of dosage (S1 + S2 + S3) is equal to or higher than Q; not more than two units are less than Q-15% ; not less than Q-25%

Q (dissolution limit) is the stated amount of dissolved active ingredient expressed as a percentage of the labeled amount. Here for Ebastine 10 mg tablet, Q value is not less than 75% according to JP (Frare & Singh, 2018). 5%, 15% and 25%; these percentages in the acceptance criteria are percentages of the labeled amount so as to these magnitudes and Q are in the similar forms (WHO Department of Essential Medicines and Health, 2018).

2.2.6 Assay of Ebastine tablet by UV-spectroscopy

Preparation of stock solution and standard curve: Accurately weighed 50 mg of pure drug was taken in a 50 ml of volumetric flask and dissolved in a suitable solvent methanol. For better solubility of the pure drug in methanol, the solution was taken in a beaker and covered with aluminum foil as methanol is volatile and there is chance of methanol evaporation. Then the beaker was put inside the ultra-sonic sound bath (sonicator) as it was subjected to sonication to dissolve the powder properly in the methanol. Therefore, the concentration of the solution was 1 mg/ml or 1000 μg/ml and from this 1000 μg/ml solution 100 μg/ml solution was made by diluting it 10 times. Further, from that solution, 20 μg/ml of solution of 100 mL was prepared by diluting it 5 times and this was used as stock solution. Using this stock solution, solutions of concentration ranging from 2 – 14 μg/ml were prepared as

working standard solutions by using this $V_1 \times C_1 = V_2 \times C_2$ equation. After that absorbance of each of the solutions of pure drug was measured and noted at 252 nm (wavelength of maximum absorption of ebastine) using UV-spectrometer against a suitable blank solvent methanol. Then a calibration curve or standard curve (absorbance vs. concentration graph) of ebastine was constructed according to Beer-Lambert's law. Maximum wavelength was obtained by scanning 20 μg/ml concentration of solution of pure drug from 400 nm – 200 nm in UV-spectrometer against methanol as blank after the correction of baseline (Dahivadkar, Jain, & Gujar, 2013).

Assay procedure: To perform this test 20 ebastine tablets from each brand selected randomly were accurately weighed together and then average weight of those 20 tablets were noted. After that, those 20 tablets from each brand were crushed by using mortar and pestle and fine powder was made, proper trituration is a very important consideration over here. Then powder equivalent to 50 mg of pure ebastine was weighed as well as taken in a beaker of 250 ml and then it was dissolved in a suitable solvent in which it is completely soluble. That solvent is methanol and 50 ml of it was used to dissolve the powder material. After that, beakers containing powder materials dissolved in methanol were placed inside the ultra-sonic sound bath (sonicator) as they were subjected to sonication to dissolve the powder properly in the methanol. As methanol is volatile aluminum foil was used to cover the beakers containing tablet powder dissolved in methanol so that specified amount of methanol did not get lost. Next, when the powder was completely soluble in methanol the solution was purified by using whatman (grade 41) filter paper of pore size 20 μm. The filtrate was diluted 100 times with the same fresh solvent of methanol to make it 10 μg/ml. 0.1 ml of the filtrate was taken in a separate volumetric flask of 10 mL and methanol was added up the mark to dilute 100 times. After that absorbance value was measured at 252 nm (λ_{max}) of that concentration of solution against the blank solvent of methanol using UV-spectrometer (Savsani, Goti, &

Patel, 2013). Furthermore, by using the linear equation of the standard calibration curve of ebastine in methanol, concentration of drug of the dosage form was calculated by using the absorbance values and available amount of drug was also determined and % drug content or potency was calculated. For accuracy, the process was done 3 times for all the brands and average % drug content was determined along with standard deviation. Amount of drug (active ingredient) content per unit dosage form was computed by utilizing the subsequent equation:

$$\text{Drug content (mg / tablet)} = \frac{x\left(\frac{\mu g}{mL}\right)\times 100\times 50ml\times \text{Avg. Wt. of Each Dosage Unit (mg)}}{\text{Amount of Sample(mg)}\times 1000};$$ where,

100 is dilution factor; amount of sample = equivalent weight to 50 mg of ebastine API of powdered 20 tablets and 1000 is used to convert µg to mg. After obtaining ebastine content (mg / tablet) for all the brands, an equation was used to determine potency to check percentage drug content of claimed amount in each tablet unit as an estimation. Moreover, this assay test was carried out to make sure manufacturers are providing what they are actually claiming. Equation for % drug content (potency) is as follow:

$$\text{Potency (\%)} = \frac{\text{Drug Content present per unit (mg)}}{\text{Labeled content (mg)}} \times 100$$

2.2.7 Data processing and analysis

Once all test processes had been completed, values of each individual tablet was noted and separated as per the manufacturer on a separate sheet. Utilizing the above-mentioned mathematical equations and MS-Excel ®, 2013, data were finally analyzed.

Chapter 3

Results

3.1 Average weight and weight variation test

Individual weight (mg) and average weight (mg) with standard deviation of 20 tablets from each brand had been tabulated and the table has been given below:

Table 9: Measured weight and average weight of Ebastine 10 mg tablets of twelve different brands (a)

Tablet No.	E1	E2	E3	E4	E5	E6
1	102	97	155	163	120	124
2	102	104	154	164	123	126
3	104	104	159	160	129	128
4	101	100	156	169	121	129
5	101	102	158	164	126	128
6	103	101	156	167	120	126
7	102	103	156	166	127	130
8	104	108	155	169	122	128
9	104	104	155	164	126	128
10	102	99	156	165	117	129
11	103	104	153	166	122	127
12	103	107	158	164	122	125
13	105	101	154	162	128	131
14	110	104	153	164	122	129
15	104	102	155	165	123	129
16	104	104	155	160	126	125
17	104	102	155	161	122	127
18	103	102	156	161	125	127
19	102	104	157	163	119	125
20	104	102	159	163	127	126
Average ± SD	103.35 ± 1.97	102.70 ± 2.52	155.75 ± 1.74	164.00 ± 2.58	123.35 ± 3.25	127.35 ± 1.87

Table 10: Measured weight and average weight of Ebastine 10 mg tablets of twelve different brands (b)

Tablet No.	E7	E8	E9	E10	E11	E12
1	135	144	109	136	143	123
2	135	141	111	134	143	120
3	137	146	108	133	146	126
4	137	144	107	133	145	121
5	137	144	110	135	138	123
6	137	143	108	135	146	121
7	139	143	111	137	144	124
8	135	142	107	138	142	129
9	137	146	108	136	137	130
10	136	139	101	133	143	124
11	137	144	106	133	145	124
12	135	146	105	133	144	123
13	137	141	105	134	148	127
14	136	140	102	134	144	120
15	137	145	104	134	147	121
16	135	143	105	134	145	126
17	138	144	104	137	140	125
18	138	144	108	138	143	124
19	136	146	107	135	140	121
20	137	146	105	134	146	127
Average ± SD	136.55 ± 1.16	143.55 ± 2.09	106.55 ± 2.72	134.80 ± 1.69	143.45 ± 2.89	123.95 ± 2.89

Note: SD = Standard Deviation

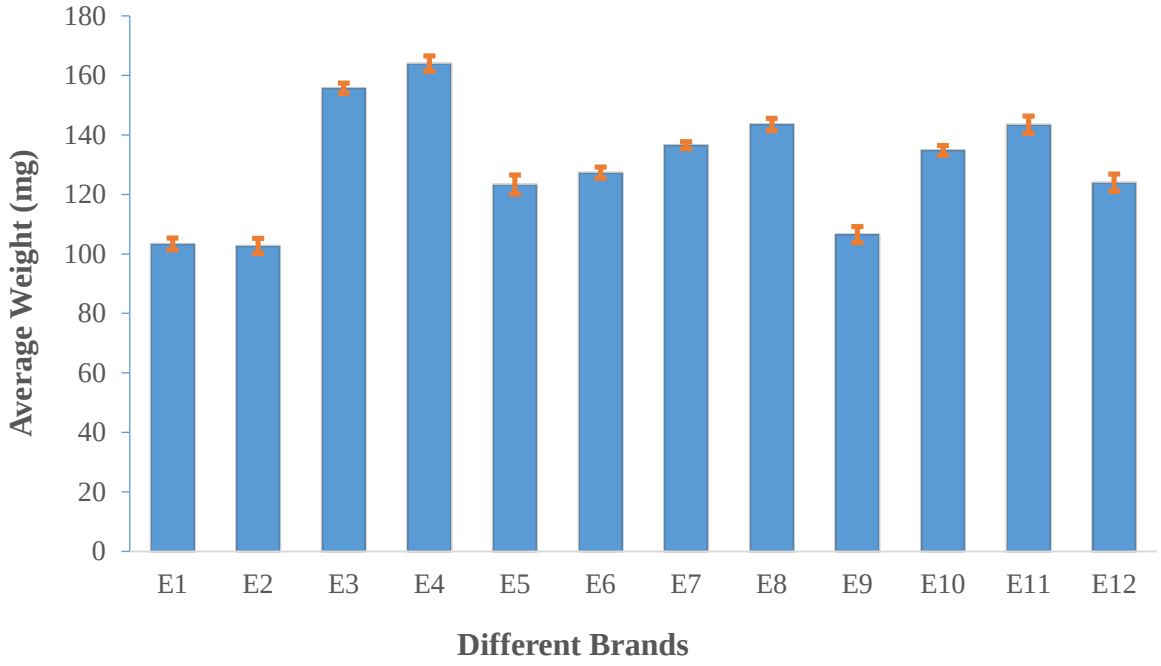

Figure 5: Average weight of different brands of Ebastine tablets with error bars indicating standard deviation

Results of weight variation test of 12 different brands of ebastine tablets using weight variation equation have been tabulated and table is given below:

Table 11: Results of weight variation test (a)

% Weight variation						
Tablet No.	**E1**	**E2**	**E3**	**E4**	**E5**	**E6**
1	-1.31	-5.55	-0.48	-0.61	-2.72	-2.63
2	-1.31	1.27	-1.12	0	-0.28	-1.06
3	0.63	1.27	2.09	-2.44	4.58	0.51
4	-2.27	-2.63	0.16	3.05	-1.91	1.3
5	-2.27	-0.68	1.44	0	2.15	0.51
6	-0.34	-1.66	0.16	1.83	-2.72	-1.06
7	-1.31	0.29	0.16	1.22	2.96	2.08
8	0.63	5.16	-0.48	3.05	-1.09	0.51
9	0.63	1.27	-0.48	0	2.15	0.51
10	-1.31	-3.6	0.16	0.61	-5.15	1.3
11	-0.34	1.27	-1.77	1.22	-1.09	-0.27
12	-0.34	4.19	1.44	0	-1.09	-1.85
13	1.6	-1.66	-1.12	-1.22	3.77	2.87
14	6.43	1.27	-1.77	0	-1.09	1.3
15	0.63	-0.68	-0.48	0.61	-0.28	1.3
16	0.63	1.27	-0.48	-2.44	2.15	-1.85
17	0.63	-0.68	-0.48	-1.83	-1.09	-0.27
18	-0.34	-0.68	0.16	-1.83	1.34	-0.27
19	-1.31	1.27	0.8	-0.61	-3.53	-1.85
20	0.63	-0.68	2.09	-0.61	2.96	-1.06

Table 12: Results of weight variation test (b)

% Weight variation						
Tablet No.	**E7**	**E8**	**E9**	**E10**	**E11**	**E12**
1	-1.14	0.31	2.3	0.89	-0.31	-0.77
2	-1.14	-1.78	4.18	-0.59	-0.31	-3.19
3	0.33	1.71	1.36	-1.34	1.78	1.65
4	0.33	0.31	0.42	-1.34	1.08	-2.38
5	0.33	0.31	3.24	0.15	-3.8	-0.77
6	0.33	-0.38	1.36	0.15	1.78	-2.38
7	1.79	-0.38	4.18	1.63	0.38	0.04
8	-1.14	-1.08	0.42	2.37	-1.01	4.07
9	0.33	1.71	1.36	0.89	-4.5	4.88
10	-0.4	-3.17	-5.21	-1.34	-0.31	0.04
11	0.33	0.31	-0.52	-1.34	1.08	0.04
12	-1.14	1.71	-1.45	-1.34	0.38	-0.77
13	0.33	-1.78	-1.45	-0.59	3.17	2.46
14	-0.4	-2.47	-4.27	-0.59	0.38	-3.19
15	0.33	1.01	-2.39	-0.59	2.47	-2.38
16	-1.14	-0.38	-1.45	-0.59	1.08	1.65
17	1.06	0.31	-2.39	1.63	-2.41	0.85
18	1.06	0.31	1.36	2.37	-0.31	0.04
19	-0.4	1.71	0.42	0.15	-2.41	-2.38
20	0.33	1.71	-1.45	-0.59	1.78	2.46

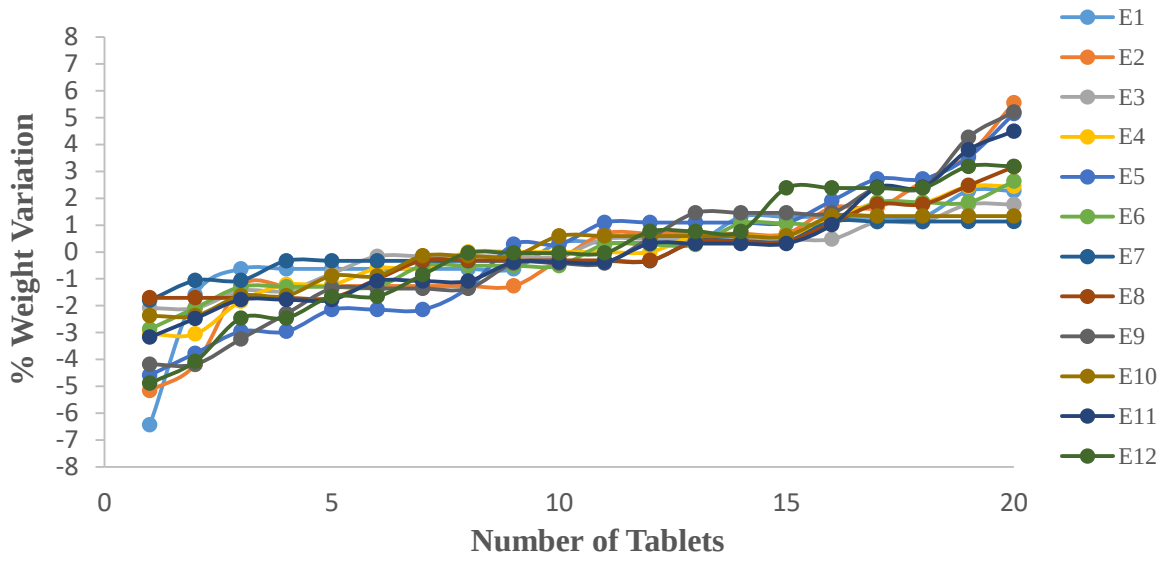

Figure 6: Comparison of weight variation test of twelve different brands of Ebastine tablets. No value lies beyond the acceptable limit

Note: To avoid complexity the values of weight variation had been sorted from smallest to largest.

3.2 Hardness test

Results of hardness test (measuring unit was Newton – N) of 10 tablets from each brand had been tabulated and the table has been given below:

Table 13: Results of hardness test

Tablet no.	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12
1	66.6	59.5	67.8	69.4	43.2	65.4	54.3	32.5	28.5	112.2	81.3	69.8
2	69.4	60.7	78.1	67.8	56.3	58.3	65	31.3	30.1	104.7	77.7	53.9
3	67.8	71	69.8	59.9	46	59.9	69.4	42.4	19.4	97.1	83.3	68.6
4	57.1	61.5	73.4	61.1	54.2	68.6	61.3	33.3	25.4	104.3	81.3	66.2
5	59.5	58.7	72.6	61.9	42.4	60.3	59.1	34.1	23.8	96.7	83.3	65.4
6	60.3	66.6	63.8	65.8	39.3	62.3	61.1	32.5	25.8	107.9	71.4	72.6
7	65	53.9	73	79.3	39.7	59.9	59.1	34.1	22.2	101.1	66.6	60.3
8	53.5	67	67.4	73.8	46.4	62.3	69.4	38.1	19.8	99.9	70.2	53.9
9	64.2	67.8	73.8	70.2	48	58.7	58.3	38.9	24.5	115	74.9	61.1
10	61.1	70.6	66.6	65.8	47.6	57.4	63.8	33.3	19.4	99.9	76.5	65.4
Average ± SD	62.45 ± 5.03	63.73 ± 5.67	70.63 ± 4.28	67.50 ± 6.02	46.31 ± 5.62	61.31 ± 3.46	62.08 ± 4.86	35.05 ± 3.55	23.89 ± 3.74	103.88 ± 6.21	76.65 ± 5.83	63.72 ± 6.35

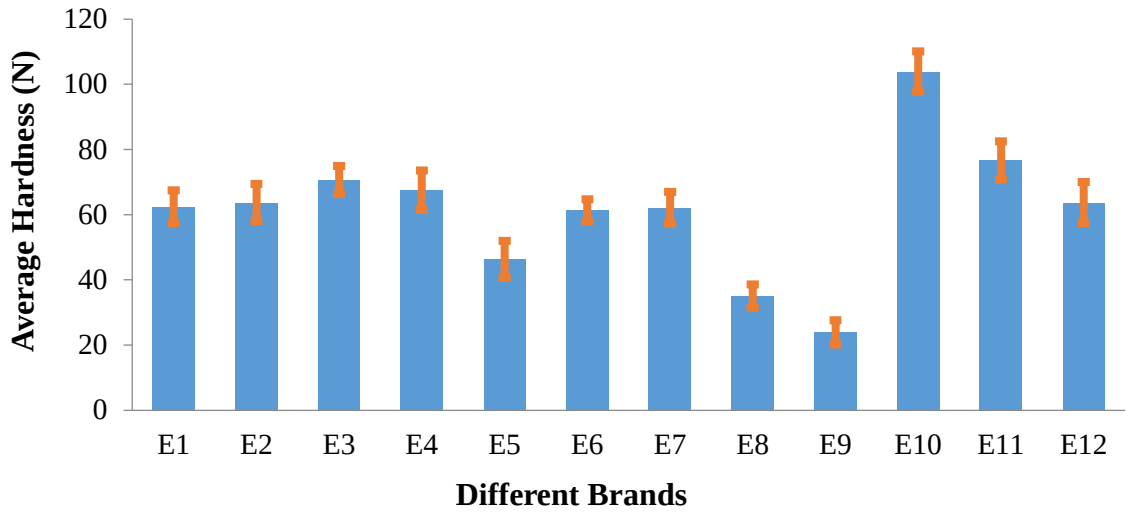

Figure 7: Comparison of hardness test of different brands of Ebastine tablets with error bars indicating standard deviation

3.3 Friability test

Results of friability test of 10 tablets from each brand had been tabulated and the table has been given below:

Table 14: Results of friability test

Brands	Initial. Wt. (mg) (10 tablets)	Final. Wt. (mg) (10 tablets)	% Weight loss
E1	1030	1027	0.29
E2	1022	1021	0.09
E3	1564	1562	0.13
E4	1631	1631	0
E5	1234	1233	0.08
E6	1286	1284	0.16
E7	1369	1367	0.14
E8	1443	1440	0.21
E9	1075	1075	0
E10	1346	1343	0.22
E11	1429	1429	0
E12	1242	1242	0

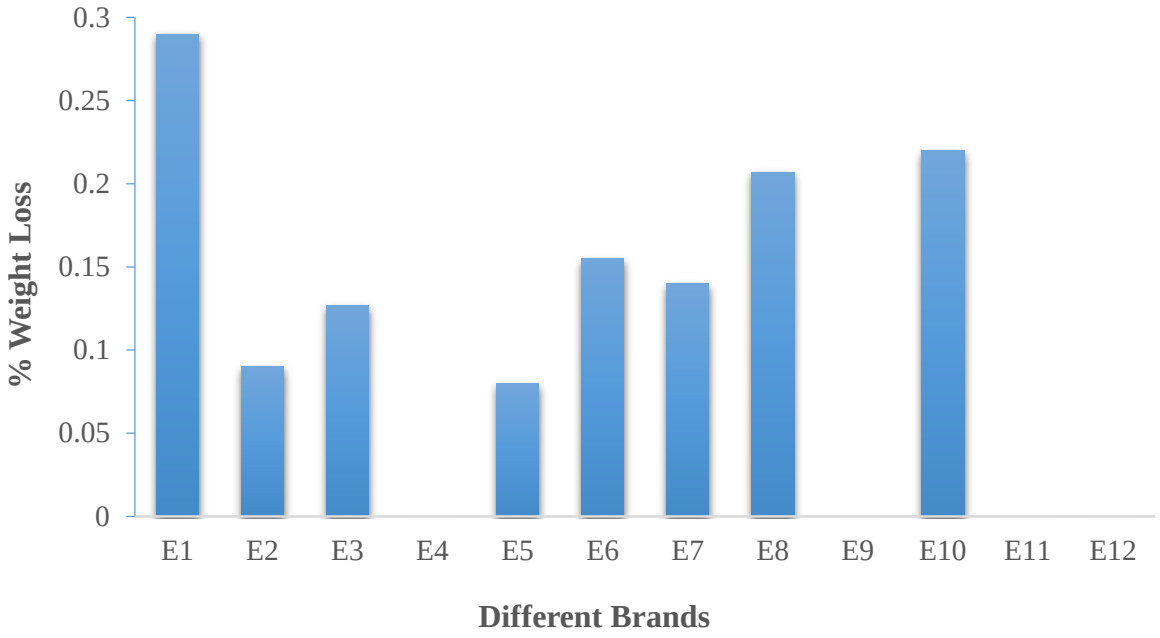

Figure 8: Comparison of friability test of different brands of Ebastine tablets

3.4 Disintegration test

Results of disintegration test (measuring unit of time was minute) of 6 tablets from each brand had been tabulated and the table has been given below:

Table 15: Results of disintegration test

Tablet No.	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12
1	1.17	1.4	1.03	0.75	2.02	0.38	0.75	1.62	0.28	1.58	1.68	9.02
2	1.1	1.37	0.87	0.91	1.68	0.41	0.9	1.48	0.38	1.45	1.58	8.12
3	1.03	1.33	0.9	0.88	1.87	0.51	0.8	1.53	0.33	1.48	1.63	8.5
4	1.27	1.25	1.08	0.78	1.63	0.45	0.65	1.45	0.3	1.52	1.91	8.3
5	1.01	1.28	0.97	0.97	1.58	0.7	0.95	1.32	0.48	1.67	1.45	8.71
6	1.2	1.26	1.02	0.72	1.8	0.65	0.98	1.41	0.37	1.72	1.8	8.61
Average ± SD	1.13 ± 0.10	1.32 ± 0.06	0.98 ± 0.08	0.84 ± 0.10	1.76 ± 0.17	0.52 ± 0.13	0.84 ± 0.13	1.47 ± 0.10	0.36 ± 0.07	1.57 ± 0.11	1.68 ± 0.16	8.54 ± 0.32

Note: SD = Standard Deviation

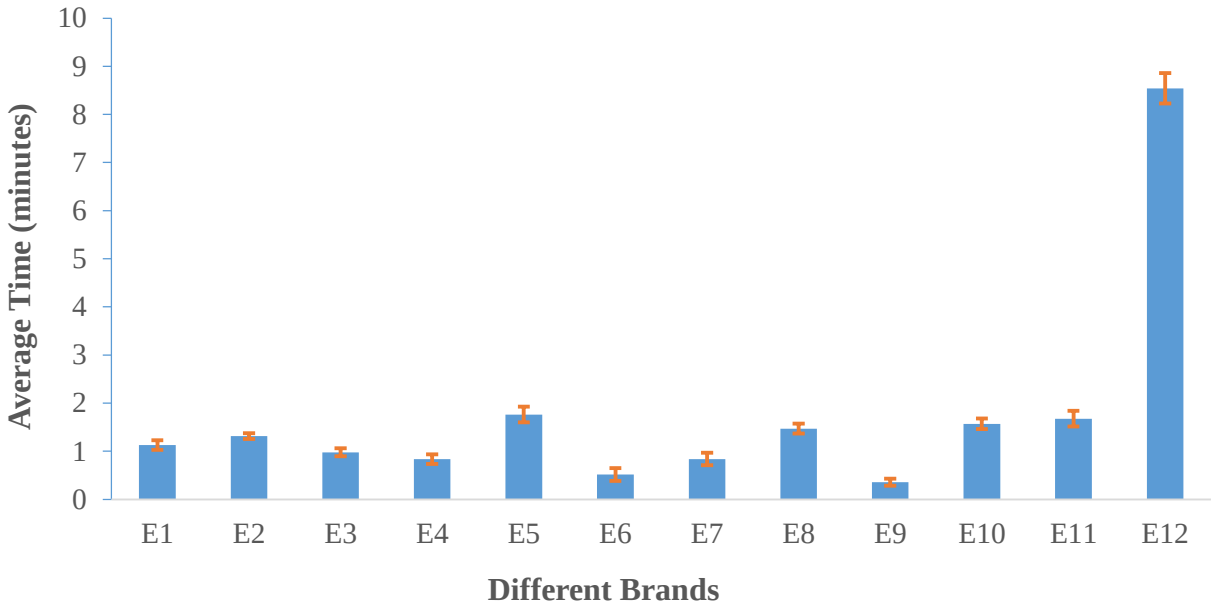

Figure 9: Comparison of disintegration test of different brands of Ebastine tablets with error bars indicating standard deviation

3.5 Assay of Ebastine tablet by UV-spectroscopy

3.5.1 Determination of λ_{max} of Ebastine for analysis

For the determination of wavelength of maximum absorption of ebastine, solution having concentration (20 µg/ml) of ebastine in suitable solvent methanol was scanned using UV-spectrometer in 'spectrum mode' in the range within 400 – 200 nm to obtain the λ_{max} of ebastine using methanol as blank after baseline correction and it was found at 252 nm.

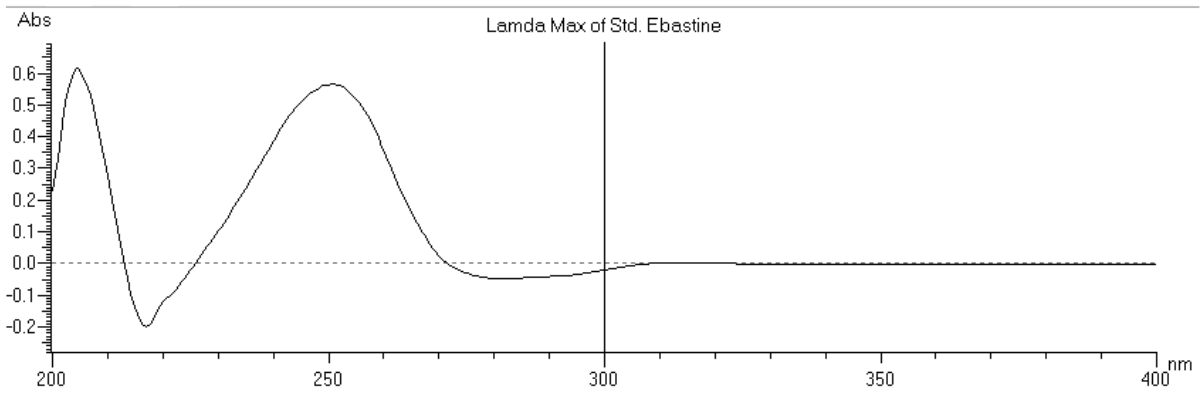

Figure 10: Spectrum of Ebastine (20µg/ml) determined during the laboratory experiment

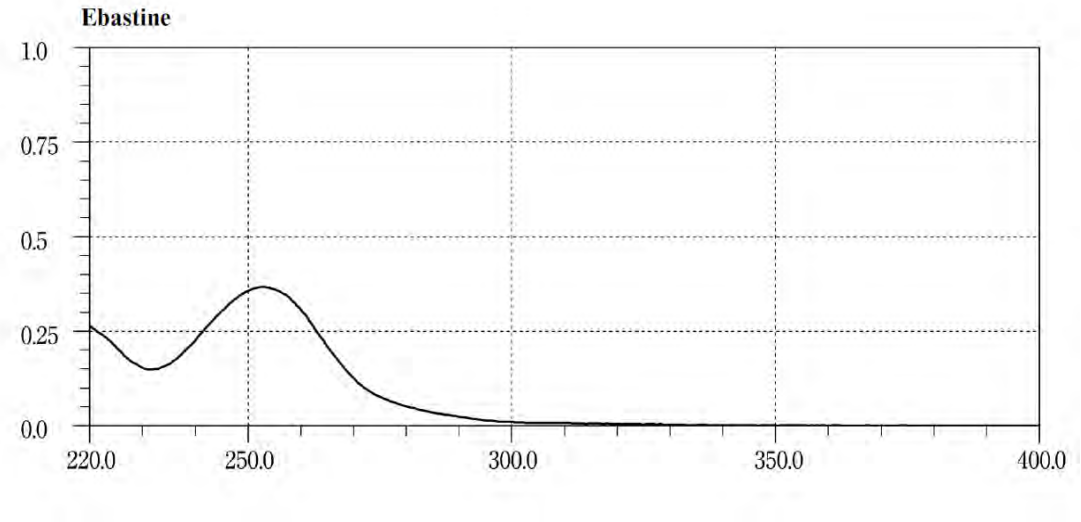

Figure 11: Spectrum of Ebastine referred in Japanese Pharmacopeia ("Ebastine - JP XVII - Ultraviolet-visible Reference Spectra," 2017).

3.5.2 Standard curve of Ebastine in methanol

Table 16: Absorbance of Ebastine standard solutions (in methanol) of various concentrations at 252 nm

Concentration	Absorbance at 252 nm
0	0
2	0.105
4	0.208
6	0.292
8	0.376
10	0.479
12	0.562
14	0.664

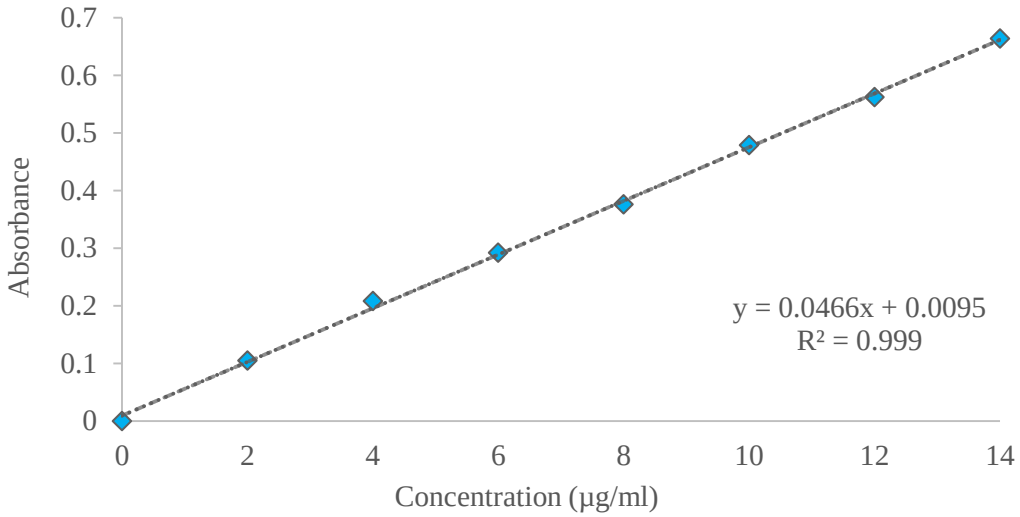

Figure 12: Standard curve of Ebastine in methanol

3.5.3 Assay results

Results of assay of ebastine tablets are obtained by using linear equation of the standard calibration curve of ebastine in methanol. With the help of absorbance values acquired from the test, the concentration of ebastine is determined from the standard curve, thus ebastine content per unit and percentage drug content of claimed amount is determined using the equations mentioned earlier. As the coefficient of determination of this curve (R^2 value) is close to 1, all the values obtained from this graph would be more accurate and more valid and close to values of pure ebastine in methanol. Results had been tabulated and that table is given below:

Table 17: Results of assay of Ebastine tablets of different brands

	% Drug content											
Trial No.	**E1**	**E2**	**E3**	**E4**	**E5**	**E6**	**E7**	**E8**	**E9**	**E10**	**E11**	**E12**
1	94.74	105.9	99.25	97.53	96.46	97.75	99.03	93.45	89.36	94.96	96.46	90.02
2	94.31	107.19	99.25	97.96	95.82	96.89	100.75	92.38	90.67	95.82	96.67	89.81
3	94.53	107.83	99.89	98.82	96.89	97.32	99.68	92.17	89.38	95.17	96.24	89.38
Average ± SD	94.53 ± 0.22	106.97 ± 0.98	99.46 ± 0.37	98.1 ± 0.66	96.39 ± 0.54	97.32 ± 0.43	99.82 ± 0.87	92.67 ± 0.69	89.81 ± 0.74	95.31 ± 0.45	96.46 ± 0.22	89.73 ± 0.33

Note: SD = Standard Deviation

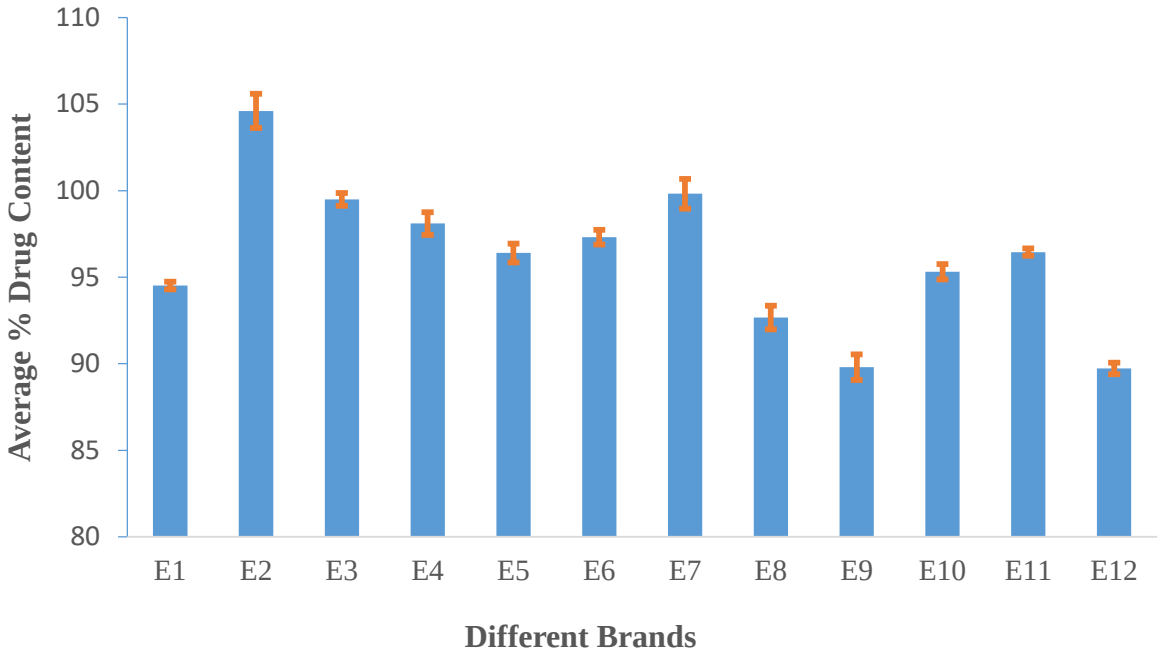

Figure 13: Comparison of potency (%) of different brands of Ebastine tablets with error bars indicating standard deviation

3.6 *In vitro* dissolution test

3.6.1 Standard curve of Ebastine in 0.01M HCl (acidic medium)

Table 18: Absorbance of Ebastine standard solutions (in 0.01M HCl) of various concentrations at 252 nm

Concentration	Absorbance at 252 nm
0	0
2	0.061
4	0.11
6	0.162
8	0.209
10	0.268
12	0.318
14	0.368

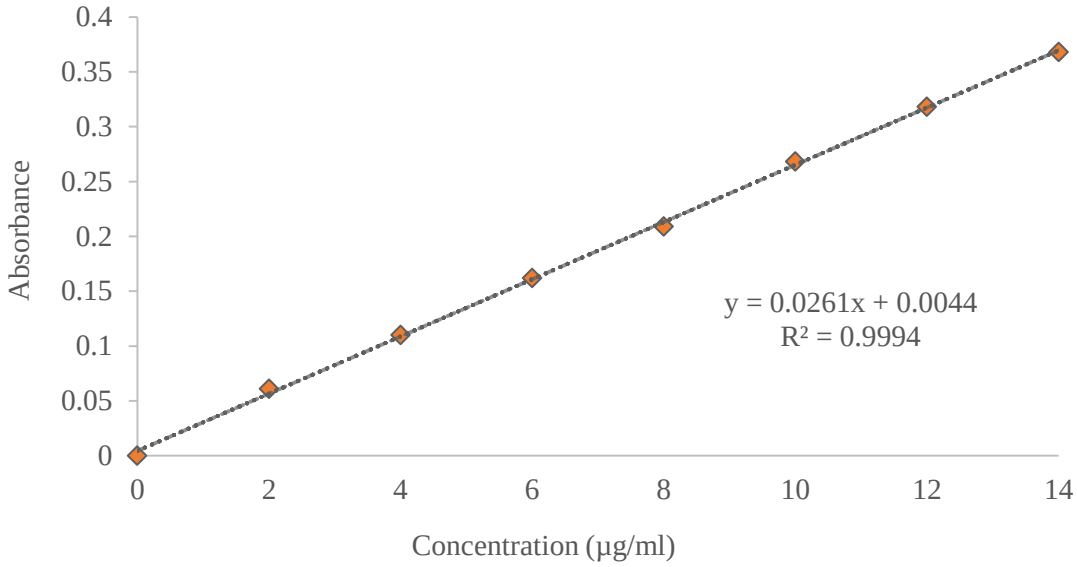

Figure 14: Standard curve of Ebastine in 0.01M HCl (acidic medium)

3.6.2 Results of dissolution test

Results of dissolution study of ebastine tablets are obtained by using linear equation of the standard calibration curve of ebastine in acidic medium (pH 2 – 2.5). With the help of absorbance values acquired from the test, the concentration of ebastine is determined from the standard curve, thus amount of released drug or dissolved drug and percentage drug release is determined using the equations mentioned earlier. As the coefficient of determination of this curve (R^2 value) is close to 1, all the values obtained from this graph would be more accurate and more valid and close to the values of pure ebastine in acidic medium. Results had been tabulated and the table is given below:

Table 19: Results of dissolution test

% Drug Release (After 30 minutes)												
Tablet No.	**E1**	**E2**	**E3**	**E4**	**E5**	**E6**	**E7**	**E8**	**E9**	**E10**	**E11**	**E12**
1	68.27	76.89	70	92.41	83.79	87.24	88.97	73.45	73.45	78.62	76.89	94.14
2	73.45	70	73.45	91.73	81.45	87.97	89.51	76.26	68.77	76.81	79.87	90.78
3	70.65	74.84	73.45	90.69	76.89	87.24	83.79	83.79	66.55	75.17	83.79	82.07
4	74.27	78.49	76.30	85.51	75.20	88.64	85.10	80.21	72.34	81.84	83.51	85.66
5	75.17	82.07	80.34	88.66	73.45	92.41	88.97	78.62	71.72	82.07	83.79	80.34
6	72.17	76.27	75.88	87.90	78.29	90.10	87.69	78.52	70.10	78.61	81.27	82.76
Average ± SD	72.37 ± 2.55	76.43 ± 4.00	74.70 ± 3.62	89.48 ± 2.61	78.18 ± 3.88	88.93 ± 2.01	87.34 ± 2.36	78.48 ± 3.51	70.49 ± 2.54	78.85 ± 2.72	81.52 ± 2.77	85.96 ± 5.42

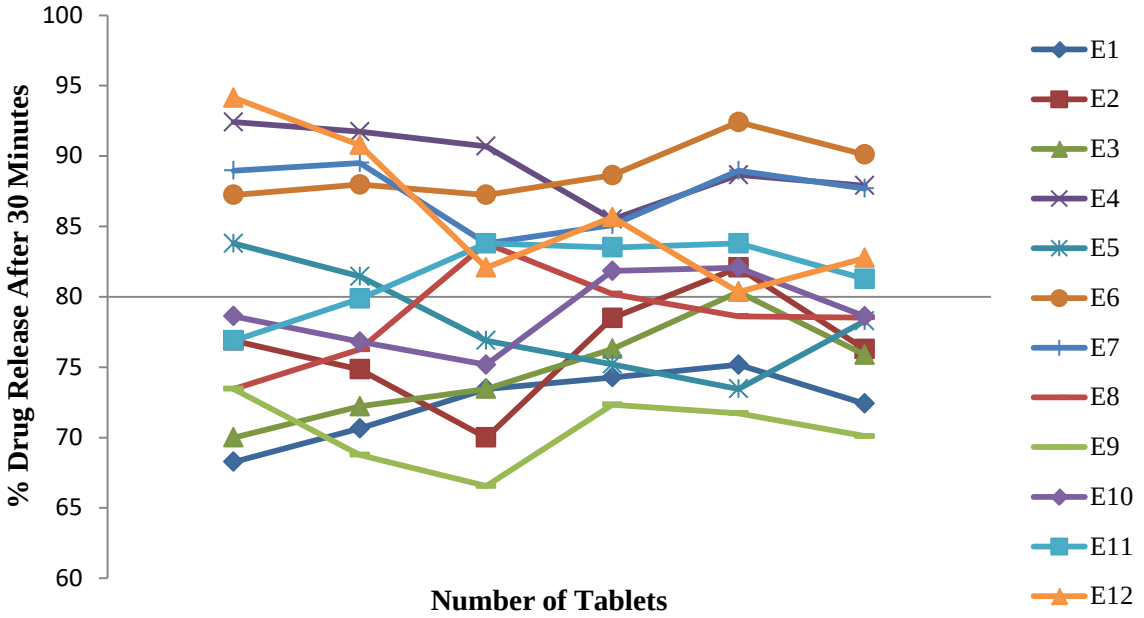

Figure 15: Individual % drug release of different tablets of different brands. Tablets of E4, E6, E7 and E12 brands showed % drug release which meet the criteria of 1st stage dissolution test according to JP.

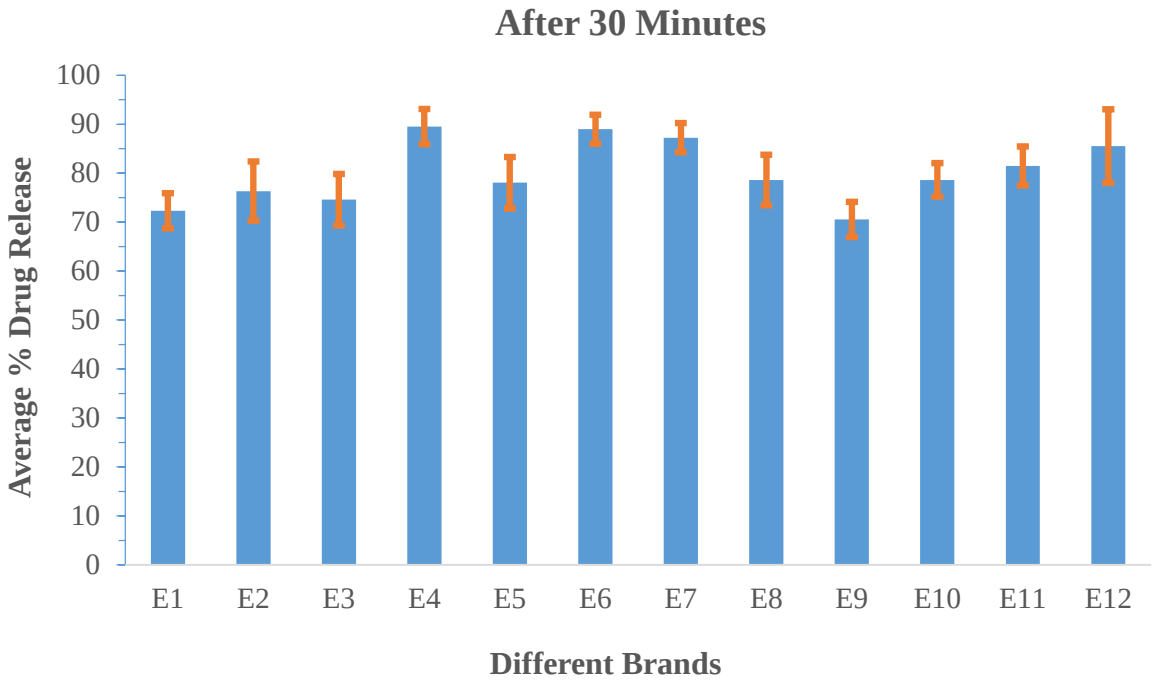

Figure 16: Comparison of drug release profile of twelve different brands of Ebastine tablets at pH 2 with error bars indicating standard deviation

Chapter 4

Discussion

4.1 Weight variation test

Weight variation test is one the most crucial test to ensure uniformity of dosage unit. It determines whether the flow property of powder or granules of the desired tablet is good or not. Fluidity or flow property is required for manufacturing of tablets of consistent weight and uniform hardness (Roy, Ahmed, & Nahid, 2017). The weight variation procedure would be a satisfying way of evaluating uniformity of the drug substance in tablet if uniformity of the drug distribution was perfect in the granulation or powder form from which the tablets were made (Savale et al., 2018). The average weight of tablets from each brand fall into the range of 80 mg – 250 mg of BP standards of average weight. According to BP, tablets weighing from 80 mg to 250 mg have a maximum allowed percentage weight variation limit range of ±7.5% (Uddin, Mamun, Rashid, & Asaduzzaman, 2015). From the results, it can be concluded depending upon the BP specification that, percentage weight variation of all twelve different brands of ebastine 10 mg tablets were within the range of ±7.5%. No tablet had crossed the limit of weight variation range. Therefore, all the brands comply with BP specification of weight variation and it can be assumed that drug content is uniformly distributed throughout the tablets of each brand. Positive highest % weight variation was seen in a tablet of E2 brand (+5.56%) and negative highest % weight variation was seen in a tablet of E1 brand (–6.43%). Weight variation is the key to maintaining tablet crushing strength and friability.

4.2 Hardness test

Hardness test is referred to as a non-official test; still hardness is the second most significant physical factor for evaluating tablet quality. The hardness of the tablet relies on the materials used, the quantity and quality of binder, the gap between the upper and lower punches and the pressure implemented during the compression phase (Kumar, 2013). Testing tablet hardness plays a crucial part in both product development and overall quality control, as elevated hardness levels can lead to elevated disintegration period and increased dissolution time thus reducing dissolution rate ("Assessment of physicochemical properties of metronidazole tablets marketed in Zaria, Nigeria," 2011). Measuring a tablet's hardness is not a precise pointer of tablet strength because various formulations compressed into very rigid tablets tend to cap or miss their portions of the crown on abrasion (Raka, Rahman, & Bodiuzzaman, 2017). Average hardness of the tablets of all 12 brands was found within the range of 23.89 N – 103.88 N. Significant standard deviation can be seen among the tablets within a brand regarding hardness. A force of 40 N is regarded as the lowest prerequisite for a satisfying tablet (Ansel, 2015). The acceptable range of crushing strength is 39.24 – 78.48 N (Ali, Faizah Ali, Akhter Rita, & Ahmed Bhuiyan, 2018). However, 2 brands were below the limit range and they were E8 and E9, having hardness of 35.05 N and 23.89 N respectively. Therefore, brand E9 had the least hard tablets. On the other hand, one brand was above the limit range and it brand E10, having hardness of 103.88 N. Remaining 8, brands were within the acceptable range of hardness.

4.3 Friability test

It is the inclination of tablets to powder, chip, or fragment, which can alter the tablet's elegance, customer satisfaction, and also add weight variation or content uniformity issues of tablet (Ali et al., 2018). As tablet hardness is not a complete strength indicator so another measure of the strength of a tablet friability is often measured to reassure and reevaluate the

durability of tablet (Uddin et al., 2015). Friability test is now regarded as official test in USP (S. Interim et al., 2016). The compendial specification for friability test is that, percentage of weight loss (friability) must not be more than 1% and below 0.5% is most desired (S. Interim et al., 2016). All brands displayed remarkable values of friability in the friability test as from the results it can be seen that all the friability values of 12 brands were below 0.5% and the values range from 0% to 0.29% which made sure all the tablets from each brand were mechanically durable and compact and it can be concluded that all 12 different brands of ebastine tablets comply with USP specification of friability test of tablet. Brand E1 showed the highest percentage weight loss or friability (%) which was 0.29% and E4, E9, E11 and E12 these 4 brands showed the lowest percentage friability and that was 0% meaning that they were completely intact and stable after the test. Interestingly, tablets from E9 brand showed 0% friability with 23.89 N hardness (lowest). This could have been a good formulation if it had complied with other *in vitro* quality test criteria.

4.4 Disintegration test

The time of disintegration relies on the product, the velocity of stirring, immersion medium etc. (Oyetunde, Tayo, Akinleye, & Aina, 2012). Time of disintegration influences extraction of the drug material from its dosage form ("In Vitro Dissolution Testing for Solid Oral Dosage Forms," 2010). It must be observed that a product that fails to disintegrate will probably fail the criteria for dissolution because the tests for disintegration deliver as an element in the overall quality control of tablet production (Kumar, 2013). Tablets ' hardness or crushing strength is closely related to the time of disintegration, harder the tablet more will be the disintegration time of that tablet (Karmoker, Sarkar, Joydhar, & Chowdhury, 2016). Fortunately, in this study hardness of the tablets had a very little influence on the disintegration time and this can be seen from the result section, only influence of hardness on disintegration can be seen in the case of brand E9 as the tablets of this brand were least hard

so this brand showed the minimum disintegration time among the twelve brands. The disintegration time of each tablet brands of ebastine (10 mg) was acceptable, as according to BP, JP, Ph. Eur. and Ph. Int. specifications film coated tablet should disintegrate within 30 minutes time limit (Al-gousous & Langguth, 2015), (T. I. Pharmacopoeia, 2011), (Uddin et al., 2015). Here, all brands of ebastine tablets were film coated and they all disintegrated within the prescribed time with maximum disintegration time 8.54 minutes that was observed in case of brand E12 and minimum disintegration time 0.36 minutes which was observed in case of brand E9.

4.5 *In vitro* dissolution test

All the tablets were studied as stated by Japanese pharmacopeia for *in vitro* dissolution of ebastine tablets ("Japanese Pharmacopoeia XVII," 2017). Percentage of released drug in the dissolution medium was calculated following the *in vitro* dissolution method of ebastine (10mg) tablet developed by Marcela Zart (Arend, 2010). In the result section that has been illustrated and the outcome was satisfactory. Dissolution was another study of important parameters of quality control closely connected to drug absorption and bioavailability (Karmoker J, Joydhar P, Sarkar S, 2016). A single-point dissolution study was developed in which percent release of drug was only measured and calculated after a certain period of time (after 30 minutes) (Popy, Dewan, & Islam, 2012). This was done because for low or poor water soluble drugs, cumulative drug release procedure is not followed in which over a long range of time drug release rate is checked, calculated and measured at different specific intervals which is known as multi-point study (I. Pharmacopoeia, 2015). Amount of drug release and percent drug release was calculated directly after 30 minutes with no interval points and no replacement of the dissolution medium. The running time of this dissolution study was only for 30 minutes as prescribed by JP and according to JP, the Q value for ebastine tablet is 75% ("Japanese Pharmacopoeia XVII," 2017). So, for 1st stage dissolution

test, all 6 tablets taken for the dissolution study should release not less than 80% drug after 30 minutes (WHO Department of Essential Medicines and Health, 2018). From the results of dissolution study, it was found that each tablet of E4, E6, E7 and E12 brands showed drug release more than 80% after 30 minutes which indicates that tablets from these brands complied with the official requirements (WHO Department of Essential Medicines and Health, 2018).

Average drug release was more than 75% for the tablets of E2, E3, E5, E8, E10 and E11 brands which quest for the 2^{nd} stage dissolution study to meet the official requirement. However, in this study, we did not perform the 2^{nd} stage dissolution study, as this one is a comparative study.

Tablets from E1 and E9 brands showed very poor % drug release. Average % drug release was less than 75% after 30 minutes which reflects their poor product quality.

Furthermore, disintegration time of the tablets from each brand had little influence or effects on the dissolution profile because brand E9 had the minimum disintegration time but it had the lowest drug release percentage out of all the 12 brands after the specified dissolution time. Moreover, E12 brand had the maximum disintegration time out of all the brands but it showed release of drug 85.96% within the specific dissolution time limit.

4.6 Assay of Ebastine tablets by UV-spectroscopy

Drug assay or drug potency test is one of the most important quality control parameters or quality standards that must be assessed and maintained to ensure the presence of exact quantity of active drug ingredient in each of the dosage units to produce the desired pharmacological action comparing with the amount stated or claimed in the label (Kumar, 2013). In this study, percent drug content of the claimed value in each ebastine film-coated tablet of 12 different brands was estimated using specific UV-spectrometric method and from

the results of the drug assay, it is evident that all the brands of ebastine tablets showed satisfactory percentage drug content available in each of these tablets of 12 different brands. Potency for all the brands (%) was found within the limit of 89.73% – 106.97% among them brand E3 (99.46%), E4 (98.10%) and E7 (99.82%) had the available drug content close to 100%. Brand E2 showed the highest potency (106.97%) having drug content available more than 100% and brand E12 showed the lowest potency (89.73%) among these 12 brands. There is currently no official specification for the potency of the drug. The range is generally not less than 90% and not more than 110% of the labeled content for highly potent, low-dose medicines (Roy et al., 2017). Since this test was performed with low dose of ebastine tablets (10 mg) and by comparing with USP specification of another drug of the same class (second generation antihistamine), cetirizine 10 mg tablet (potency range: 90 % -110 %) we can say the percentage of potency was within 90% -110% (Bulletin, 2013). Based on this assay limit range, every brand showed optimum potency.

Chapter 5

Conclusion

In the existing industrial practice, *in-vitro* tests play a vital role in comparing with multi-brand generic molecules and providing adequate therapeutic activity of the dosage form which may eventually reflect in vivo functioning of the drug. Although in the field of most of the cases, i*n vitro-in vivo* relation of a specific drug is hard to establish. The physical and chemical study of chosen commercial brands of ebastine tablet marketed in Bangladesh has displayed quality and effectiveness in accordance with the compendial standards and requirements. Since the parameters of quality control are associated to each other from the early step to the drug's pharmacological action, a high-quality tablet must fulfill all established quality parameters to achieve its required therapeutic action. Since all the formulations except three satisfy all the compendial specifications, we can say they will produce the desired antihistaminic effect. However, brand E1 and E9 showed poor percentage drug release among all the twelve brands. Then again, among all the brands, E4 exhibited decent % weight variation, excellent disintegration, 90% drug release after 30 minutes and 98.1% potency. Moreover, its unit price is also minimum. Considering these points, E4 may be regarded as the best brand among these twelve brands in Bangladesh. In spite of that, patients can safely move among E4, E6, E7 and E12 brands as very little variation exists from brand to brand among these brands.

Chapter 6

Future work

Future work of this project includes:

1) Content uniformity test of all the available brands of ebastine 10 mg tablet in Bangladesh.
2) Construction of plasma level-time curve and measurement of plasma drug concentration of ebastine by using both UV-spectrometric and HPLC method. This will be done by utilizing the human blood samples of multiple volunteers who will be orally administered ebastine tablets of all available brands. In other words, bioequivalence studies and analysis will be conducted using marketed ebastine tablets (10 mg) of different brands and *in vivo* performance of this drug will be checked and evaluated as well to correlate with the results obtained from *in vitro* study.
3) HPLC assay of ebastine tablets (10 mg) of various brands.

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