Original article:

Comparative *in-vitro* evaluation of ebastine tablets (10 mg) available in bangladesh

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Abstract:

Background: The symptoms of allergic responses, such as hay fever or urticaria, can be relieved with the use of ebastine, an efficient anti-allergic medication. ***Objective & Method:*** This study's primary objective was to assess and contrast various quality control parameters, such as weight variation, % friability, hardness, disintegration time, dissolution profile, and potency, of the twelve different brands of Ebastine tablets that were readily available in Bangladesh in accordance with compendia procedures. It's interesting to note that, according to various official requirements, all of the quality parameters indicated above were within acceptable bounds. ***Conclusion:*** Therefore, doctors can advise patients to use any of the Ebastine brands that are sold in Bangladesh.

Keywords: Ebastine; Friability; Disintegration time; Dissolution profile; Potency.

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Introduction:

Medicines must be safe, effective, and of great quality to have the best therapeutic effect. The efficacy of a medicine is a key component in ensuring the health and wellbeing of a patient. However, low-quality medications can raise a nation's mortality and morbidity rates[1]. Even though Bangladesh's pharmaceutical industries have improved the country's reputation on international markets by delivering 98 percent of the nation's yearly medication market, counterfeit, contaminated, and subpar drugs continue to pose a threat on the local market despite their low prevalence[2]. According to studies, some manufacturers make substandard medications and purposefully copy the cash cow items of some major drug manufacturers[3].

Ebastine is a second-generation histamine receptor antagonist that is long-acting, non-sedating, and exclusively binds to peripheral H1 receptors. It comes in 10mg and 20mg tablets, fast-dissolving tablets, and pediatric syrup (1mg/ml)[4]. The chemical name of Ebastine is 4-(4-benzhydryloxy-1- piperidyl)-1-(4-tert-butylphenyl) butan-1- one[5]. It prevents bronchoconstriction brought on by histamine and has antihistaminic and anti-allergic action. Additionally, Ebastine improves the prognosis of allergic disorders by increasing interferon gamma (IFN-) production in those with chronic allergic rhinitis. Additionally,

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decreased calcium ion concentration results in improved mast cell stability, which further reduces histamine release[6].

The growing popularity of Ebastine in the pharmaceutical market is attributed to its well tolerability and effectiveness in improving the symptoms of seasonal allergic rhinitis and idiopathic urticaria in comparison with the other antihistamine drugs available in the market. Furthermore, Ebastine ensures cardiac safety with no serious adverse cardiac effect and its overdose up to 100 times causes no toxicity[5]. Ebastine's therapeutic uses include the symptomatic treatment of allergic rhinitis, idiopathic chronic urticaria, and, in rare instances, Th-2 type autoimmune disease, allergic dermatitis, and occasionally asthma. It is also frequently used to treat atopic dermatitis and/or provide relief from mosquito bites[7].

Entire product quality must be assured in the pharmaceutical sector in order to eradicate products that do not meet the criteria and requirements outlined in the Pharmacopoeias. Around 18 different brands of Ebastine BP (British Pharmacopoeia) tablets are marketed in Bangladesh. Therefore, choosing the best option within a reasonable price range can be challenging. In order to correlate and compare the quality of twelve distinct brands of Ebastine BP tablets produced by both foreign and local pharmaceutical businesses and sold in Bangladesh's market, the study was designed to evaluate certain important in vitro quality control criteria. The study also looks at whether or not these tablets meet the declared physical requirements and compendial (Pharmacopeial) standards made by the manufacturing companies.

Materials and methods:

2.1. Materials

A total of twelve film-coated Ebastine BP tablets of local and international brands, containing 10 mg active pharmaceutical ingredients (API) per tablet were purchased from registered drug stores in Bangladesh. All the samples were properly checked for their price, manufacturing and expiry dates and these samples were assigned a brand code from E1-E12 randomly to hide their identity. Moreover, standard Ebastine drug having purity of 99.9% was used as a reference, a kind gift from one of the leading pharmaceutical companies of Bangladesh, Eskayef Pharmaceuticals Ltd. 37% hydrochloric acid and methanol were used in this study which were purchased from Sigma-Aldrich.

2.2. Methods

2.2.1. Weight Variation test

Firstly, twenty randomly selected tablets from each brand were weighed accurately using weighing balance (Electronic balance 3-digit, PA-213, Ohaus Corporation-USA). Then the average weight of all the 20 tablets from each brand was noted with standard deviation (SD). Percentage (%) weight variation of individual tablet was determined by using the following equation:

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

2.2.2. Hardness test

A total 10 tablets (randomly selected) from each brand were crushed individually by putting it in between the upper and lower punch of the hardness tester (Hardness tester USP, EH-01, Electrolab-India). The pressure required to crush the tablets was unique to each tablet from all the 12 brands. Finally, the average hardness was calculated with SD.

2.2.3. Friability test

Initial weight of random ten tablets was recorded and then those tablets were placed inside the transparent drum of the United States pharmacopeia (USP) friability tester (Tablet friability tester, EF2-USP, Electrolab-India). Then the final weight was recorded of the same ten tablets after the tumbling or rotation of the drum for 4 minutes at 25±1 rotation per minute (rpm). The % weight loss of these tablets was calculated by using the following equation:

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

2.2.4. Disintegration test

600 mL of 0.1M HCl was used for six randomly chosen tablets from each brand and temperature was maintained at 37º±2ºC as per the direction by USP, BP, JP, Indian Pharmacopeia (IP), European Pharmacopeia (Ph. Eur.)[1, 8]. After placing the tablets into six transparent tubes of the basket and reassembling it with the rack of the device containing the immersion fluid, the machine (Tablet disintegration tester USP, ED-2L, Electrolab-India)

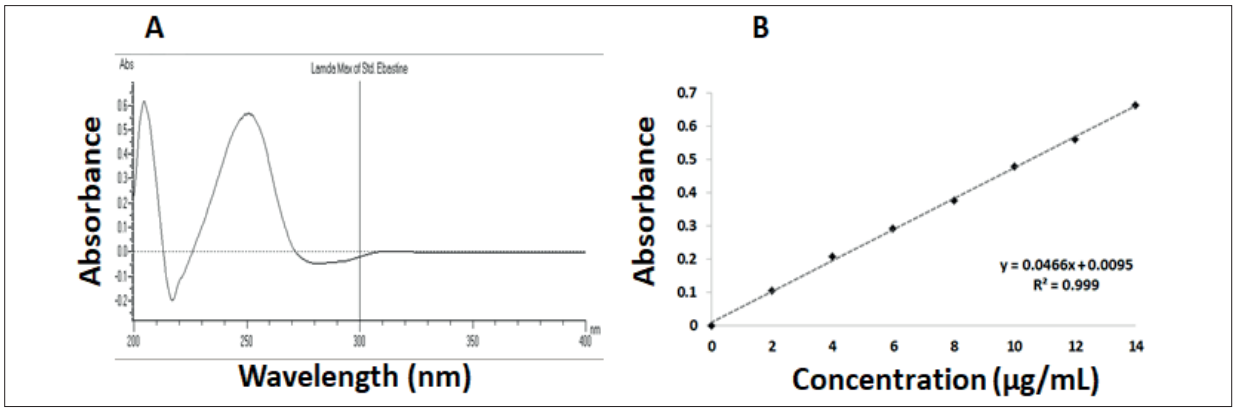

Figure 1: *(A) λmax determination. (B) Standard curve preparation.*

was turned on with timer until all the 6 tablets were broken down completely into small fragments and no residue was left on the screen (mesh) of the tube[9]. Then, the time was noted and the average time was calculated with SD. The test was carried out for tablets from all the 12 brands.

2.2.5. λ_{max} determination and standard curve preparation in different media

For the determination of λ_{max} of Ebastine, solution having concentration 20 µg/mL of Ebastine in methanol was scanned using UV-VIS spectroscopy (Shimadzu Corporation, Japan) in the range within 200–400 nm and maximum absorbance was found at 252 nm (Figure 1A). 50 mg of pure drug was dissolved in 50 mL HCl medium (pH 2) and 50 mL methanol separately. Solutions of concentration ranging from 2 – 14 µg/mL were prepared and using the absorbance of each of the solutions at 252 nm, standard curve (absorbance vs. concentration graph) of Ebastine was constructed (Figure 1B).

2.2.6. In-vitro dissolution test procedure

According to the JP's current monographs, the dissolve must be carried out using the paddle method, at a speed of 50 rpm, using 900 mL of a 0.2% sodium chloride solution. The pH must also be adjusted to 1.2 with HCl, simulating the gastric fluid of a fasting human stomach. Here, a single-point dissolution study was carried out, in which 10 ml of sample was removed after 30 minutes, filtered using Whatman (grade 41) filter paper, and the filtrate was five times diluted with fresh acidic solvent (0.01M HCl). The concentration of the drug in the dosage form was determined by utilizing the linear equation of the standard calibration curve of Ebastine in an acidic medium (pH 2). Each tablet from each of the 12 brands was placed in a separate beaker of the dissolution tester device (USP dissolution apparatus II, UDT-804, Logan instruments corporation-USA) throughout this process, which was carried out for all 12 brands[10]. Amount of released drug was calculated by the following equation:

$$\text{Amount of released drug (mg/tablet)} = \frac{x(\mu g/ml) \times 5 \times 900ml}{1000}$$

Where, dilution factor = 5 and 1000 is used to convert µg to mg.

Equation for % drug release is as follow:

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

2.2.7. Assay of Ebastine tablet by UV-spectroscopy

Average weight of 20 Ebastine tablets of different brands were taken and crushed with mortar and pestle. Powder equivalent to 50 mg of pure Ebastine was taken and dissolved in 50 mL methanol and filtered by using Whatman (grade 41) filter paper. The filtrate was diluted 100 times with the same fresh solvent of methanol to make it 10 µg/mL. The absorbance was measured at 252 nm and concentration of drug was calculated using standard curve and it was triplicated. Amount of drug content per unit dosage form was calculated by using the following 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 and 1000 is used to convert µg to mg.

Following equation is used to calculate the % drug

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

Data processing and analysis

Data for each individual tablet was recorded and organized as instructed by the manufacturer on a separate page once all test procedures had been performed. Using the above mathematical equations and MS-Excel ®, 2013, data were finally analyzed.

Results:

3.1. Weight variation and potency determination

Individual weight (mg) and average weight (mg) with standard deviation (SD) of 20 tablets from each brand was calculated and plotted in a graph (Figure 2A). Each tablet of 12 different brands was within the accepted limit according to BP guidelines. The average of drug content percentage was calculated and outlined in a graph (Figure 2B) which complies with different official guidelines. Maximum drug content was found in case of the tablets of E2 brand and the minimum was found with the tablets of E9 and E12 brands.

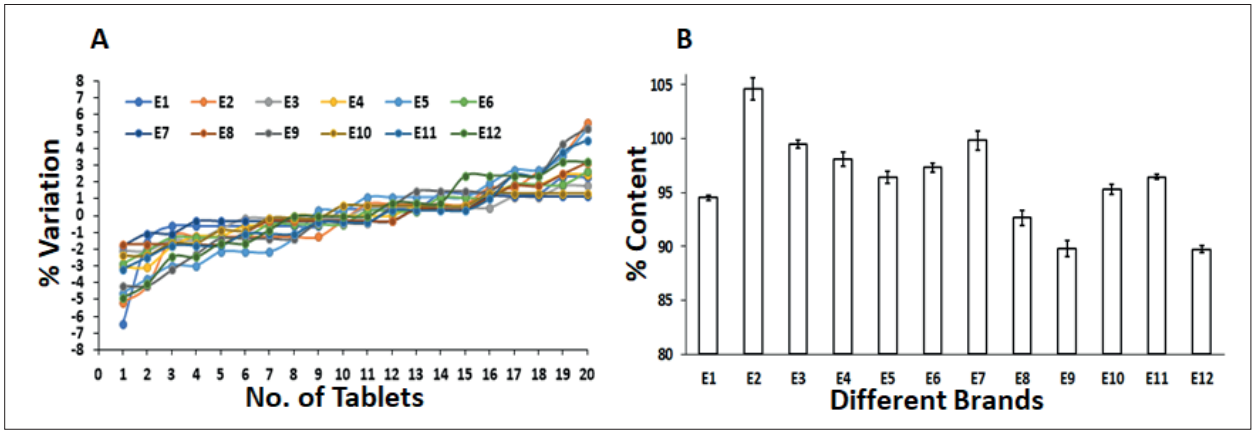

Figure 2: (A) % Weight variation of 20 tablets of twelve different brands. (B) % Content (Potency) of each brand.

3.2. Hardness and friability assessment

Hardness of 10 tablets from each brand was noted in Newton-N whereas percentage (%) of weight loss (% friability) of 10 tablets from each brand was calculated and plotted in a graph (Figure 3A). Here, brand E1 showed the highest percentage weight loss which was 0.29% whereas E4, E9, E11 and E12 showed the lowest 0% weight loss (Figure 3A). Ebastine tablets of every brand complied with the USP guidelines.

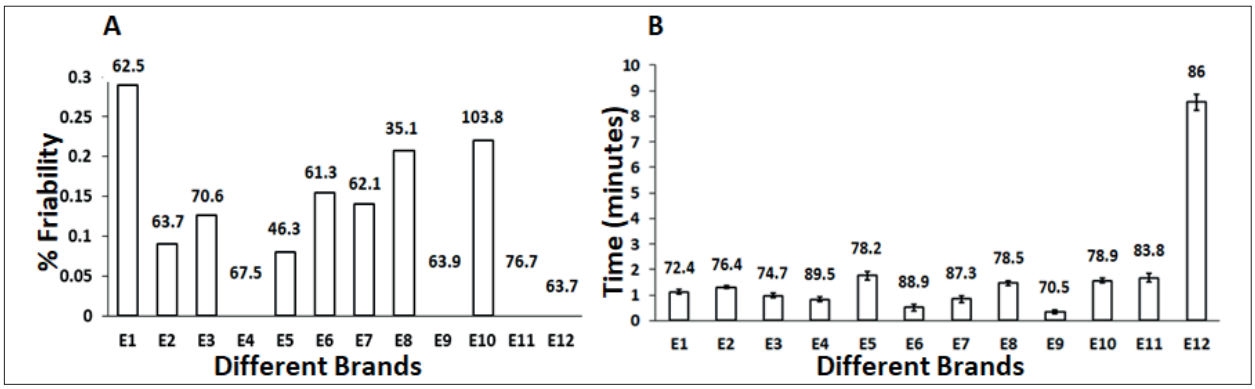

Figure 2: *(A) Bar diagram of % friability of different brands where average hardness value of each brand is placed at the top of respective brand. (B) Bar diagram of disintegration value where % drug release after 30 minutes has been placed at the top of respective brand.*

3.3. Disintegration and dissolution test

Outcomes of disintegration test (measuring unit of time was minute) of 6 tablets from each brand was noted and not a single tablet was out range of accepted limit according to BP (Figure 2B). Maximum disintegration time 8.54 minutes was observed in case of brand E12 whereas brand E9 showed the lowest disintegration time 0.36 minutes. % Release of Ebastine tablets after 30 minutes were calculated and integrated in the (Figure 3B). Here, tablets of E (4, 6, 7, and 12) brands meet the 1st stage dissolution test criteria (Figure 3B) where tablets of E (1, 2, 3, 5, 8, 9, 10, and 11) meet the 2nd stage dissolution test criteria according to JP (data not shown here).

Discussion:

One of the most important tests to verify dosage unit homogeneity is the weight variation test. Each brand's tablets typically weigh between (80-250) mg in weight. The greatest weight fluctuation permitted for the tablets within this range, according to BP, is 7.5%[11]. This analysis revealed that no tablet had ever gone over this range's upper bound. As a result, it can be presumed that the drug content is evenly dispersed throughout each brand's tablet.

Even though the hardness test is regarded as an unofficial test, it is still widely used to assess the quality of tablets. All 12 brands' tablets had an average hardness that fell between 23.89 N and 103.88 N. For a satisfactory tablet, the permissible range of crushing strength is 39.24 N–78.48 N[12, 13]. Although the tablets of the E8 brand fell beyond of the acceptable range, their% friability was still within the USP-accepted range. However, despite having a hardness of 103.88N, tablets of the E10 brand disintegrated on average in just 1.57 minutes.

Tablet friability is frequently studied to reassure and reevaluate the durability of tablets because tablet hardness is not a full strength indicator[11]. The standard requirement for the friability test is that the percentage of weight loss (friability) must not exceed 1%; ideally, it should be less than 0.5%[14]. According to the findings of our investigation, all of the friability values for the 12 brands tested were below 0.5% and ranged from 0% to 0.29% (Figure 3A), which is in compliance with USP specifications.

During disintegration time drug becomes isolated from the dosage form[10, 15]. It should be highlighted that since the tests for disintegration serve as a component in the overall quality control of tablet production, a product that doesn't disintegrate would likely fail the criterion for dissolution. Film coated tablets must dissolve within 30 minutes in accordance with the specifications of BP, JP, Ph. Eur., and Ph. International[1, 9, 11, 16]. In this study, the disintegration time observed for all tablets of twelve different brands was satisfactory.

Regarding the in vitro dissolution test, the percentage of drug release was determined immediately after 30 minutes without any intervals or the replacement of the dissolving medium. According to JP, the Q value for the Ebastine pill is 75%, hence JP only allowed the dissolution study to run for 30 minutes[17]. Therefore, all 6 tablets used for the dissolution research should release at least 80% of the drug after 30 minutes for the first stage dissolution test[10]. According to the findings of the dissolution investigation, each tablet from the brands E4, E6, E7, E11, and E12 demonstrated drug release of more than 80% after 30 minutes, indicating that these tablets comply with the regulatory criteria[10]. Others quest for the further dissolution study to meet the official requirement (data not shown here).

Potency test is one of the most crucial quality control parameters that must be assessed to ensure the presence of exact amount of active drug substance in each of the dosage units to produce the desired pharmacological action comparing with the amount stated or claimed in the label[9]. In the study, potency (%) was found within the range of 89.73% – 106.97% for all the brands (Figure 1B). Here, brand E3 (99.46%), E4 (98.10%) and E7 (99.82%) had the available drug content close to 100% (Figure 1B). Additionally, brand E2 demonstrated the lowest potency (89.73%) and brand E12 the highest potency (106.97%) among these 12 brands, respectively. For very potent, low-dose medicines, the acceptable range is often 90% to 110% of the stated quantity[18]. Based on this assay limit range, every brand showed optimum potency.

Conclusion:

Although there is now a broad variety of antihistamine drugs, Ebastine still remains as one of the most common option in anti-allergic treatment comparing to other most popular antihistamine drugs marketed

in Bangladesh, particularly for oral administration[18]. Pharmaceutical quality assurance and quality control depends on inspecting the composition and consistency of the processing of the drug substance and in the final product. In-vitro testing are crucial in the current industrial practice for comparing with multi-brand generic molecules and determining the dosage form's adequate therapeutic activity, which may finally translate to the drug's in-vivo performance. The physical and chemical analysis of certain commercial brands of Ebastine tablets sold in Bangladesh revealed that they meet both BP and JP specifications and are of a high enough quality and effectiveness to be used. There is often quantitative variation among different manufacturers' drugs. Nevertheless, most drug products are within the official limit, despite the variation. Considering these evaluation parameters, E4 may be regarded as the best brand among these twelve brands in Bangladesh. In spite of that, patients can safely move among different other brands as very little variation exists there.

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Author contribution

Syeda Fahria Hoque Mimmi: Investigation, Methodologies, Statistical analysis. **Saqib Rahman:** Investigation, Methodologies. **Prethula Areefin**: Investigation. **Md. Shaki Mostaid:** Statistical analysis. **Md. Ayman Siddique:** Investigation. **Md. Aminul Haque:** Concept and design, Critical revision of manuscript, Supervision.

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Conflict of interest

"None to declare"

Ethics approval

Not Applicable

Availability of Data and Materials

All the datasets used and/or analyzed during the present study are with the corresponding author [Md. Aminul Haque], and can be made available upon reasonable request.

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