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In vitro quality assessment of ketotifen fumarate tablets commercially available in Bangladesh

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Abstract

Quality defines the standard of a product and every company strives to achieve it, although it is often challenging. Quality of pharmaceutical products is very important for achieving the therapeutically activity of the standard drugs. This research work was aimed to investigate the quality control parameters of five brands of ketotifen that are available in the market. The five ketotifen brands were collected from the Bangladeshi local market, after which they underwent various physical parameter assessments including weight variation, hardness, friability, potency, disintegration, and acid-base degradation assessments. The brands passed the tests as no tablets crossed the ±10 % weight variation. The percentage friability of the five brands was less than 1%, hence they satisfied the standards. The disintegration time of the tablets did not exceed 5 minutes; therefore, they met the standards. All the brands showed potency ranging from 90 to 110%. In the degradation studies, no products met the USP specified limit. The average hardness of all brands did not meet the standard criterion.

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1. Introduction

Ketotifen fumarate is the fumarate salt of ketotifen. As per the International Union of Pure and Applied Chemistry (IUPAC) it is known as (E)-but-2-enedioic acid, 10-(1- methylpiperidin-4-ylidene)-5H-benzo [1,2] cyclohepta [3,4-b] thiophen-4-one, a cycloheptathiophene derivative with antiallergenic properties. Ketotifen specifically inhibits histamine (H1) receptors, preventing the typical signs and symptoms associated with

release of histamine (Hanuskova & Plevkova, 2013). This drug also inhibits the production of inflammatory mediators by mast cells implicated in hypersensitive responses, hence lowers the chemotaxis and eosinophil activation. In Bangladesh, this medicine is available in both syrup and tablet forms. It has been used to treat many conditions such as rhinitis, asthma, anaphylaxis and skin allergies etc. (Kar, Krishnan & Mohankar, 2012).

Ketotifen is an insufficiently competitive, selective histamine antagonist (H1-receptor) that also stabilizes mast cells. Ketotifen blocks the release of mediators from mast cells that cause hypersensitivity responses. Decreased chemotaxis and eosinophil activation have also been observed. It also hinders cAMP phosphodiesterase. Ketotifen's features that may cause to its antiallergic activity and ability to affect the underlying pathology of asthma include inhibition of the development of airway hypersensitivity related with activation of platelets by PAF (Platelet Activating Factor), inhibition of PAF-induced accumulation of eosinophils and platelets in the airways, suppression of eosinophil priming by human regenerated cytokines, and antagonism of limiting of the due Ketotifen suppresses the production of allergic mediators including histamine, leukotrienes C4 and D4 (SRS-A), and PAF. Quality control (QC) testings ensure that the drugs are safe, efficacious, and have minimal side effects. It employs specific equipment to ensure the quality of assaying in accordance with the established guidelines. Friability, weight variation, disintegration, dissolution, and drug assay tests are some of the required procedures. Friability assesses content homogeneity and weight fluctuations. The tablet's affinity to divide, powder, or chip can influence its appearance and uniformity, whereas weight variation concerns drug distribution uniformity. The weight variation test is performed when the drug substance exceeds 50 mg, or 50% of the tablet weight. The bioavailability of drug is determined using dissolution test apparatus. It conveys the amount of medication which enters a solution per unit time under typical conditions. Prior to do this test the disintegration time (DT) measurement is required. DT is the time necessary to break down tablet ingredients into particles in simulated biological fluid. It employs a 10-mesh screen, and time is measured as dissolved particles pass through (Shenoy, Agrawal & Pandey, 2003). Potency, a very important requirement of a drug to exhibit the bioavailability, is determined by chemical and instrumental methods of testing. It uses a UV-visible spectrophotometer to find out the quantity and quality of the given analyte based on the intensity of light absorbed.

Overall, it is critical to maintain a product's quality by executing numerous quality control (QC) tests to ensure that it is effective and safe for general use. Simultaneously, it maintains the product's overall efficiency and quality. QC testing also ensures that the medicine meets the specifications listed on the label. It includes determining purity of drug and presence of any impurities, drug absorption by the body etc. *In-vitro* evaluation of the selected tablets as per guidelines of the United States Pharmacopoeia (USP) and the British Pharmacopoeia (BP) as allowed to assess the quality of the ketotifen fumarate tablets and we report the results of our preliminary findings.

2. Materials and methods

a. Machineries and apparatus

Friabilator, digital analytical balance, blender, UV-visible spectrometer, disintegration machine, digital pH meter, and dissolution machine were used in this study (Kar, Krishnan, & Mohankar, 2012)

b. Collection of samples

Five market preparations of ketotifen fumarate manufactured in the country were procured from some pharmacy outlets in Chattogram city's Oxygen area. The products were given codes ranging from KOT- 1 to KOT- 5 for ethical reasons; "KOT" stood for ketotifen oral tablets. Every brand's tablet was reported to contain (in mg) of the active ingredient (Table I).

Table I

Label information of collected sample.

Sl. No	Market preparation	Date of Mfg	Date of Exp	Manufacturing Company
1.	KOT-1	Jul, 2015	July, 2018	Manufacturer-A
2.	KOT-2	Nov, 2014	Nov, 2016	Manufacturer-B
3.	KOT-3	Jan, 2013	Jan, 2016	Manufacturer-C
4.	KOT-4	Mar, 2015	Mar, 2018	Manufacturer-D
5.	KOT-5	Dec,15	Oct, 2016	Manufacturer-E

c. General appearance

The comprehensive assessment of tablets, encompassing their visual identity and general appeal, consistency across different lots, and uniformity among individual tablets, is essential for gaining customer approval. The overall appearance is influenced by various elements, such as size, shape, and thickness. These characteristics are vital for determining suitable packaging and selecting the right equipment for tablet packaging. All tablets exhibited a favorable appearance regarding uniformity in size and shape among other attributes.

d. Organoleptic properties

These characteristics are essential as they include the tablet's color and odor. The color of numerous pharmaceutical tablets is vital for enabling rapid recognition and improving customer acceptance. Furthermore, the flavor of chewable tablets greatly influences consumer acceptance. The organoleptic characteristics of the tablets, encompassing color, odor, and taste (specifically for chewable variants), were evaluated to confirm adherence to established specifications. Color assessment was conducted visually under controlled lighting conditions, while odor evaluation involved direct olfactory inspection. In the case of chewable tablets, taste was subjectively evaluated by a trained panel to ascertain consumer acceptability.

e. Weight variation test

The impact of weight variation ensures good manufacturing practices (GMP) parameter, an appropriate tablet size, and formulation content homogeneity (Yoshida & Sakai, 1999). The British Pharmacopoeia (BP) stipulates guidelines for tablet weight variation among intact dose units like tablet. A random sample of 20 tablets was selected and individually weighed using an analytical balance (Model: ATY-224R Shimadzu Japan). The average weight was calculated.

$$\text{Average weight, } X = \frac{X1+X2+X3+\cdots+Xz}{20}$$

Then each tablet, such as X1, X2, X3,... Xz, was weighed independently to observe if it remains within the range. Individual weights were compared to the average weights in accordance with the BP weight variation test protocol criteria. If no more than two tablets exceed the percentage limit and no tablet deviates by more than twice the percentage restriction, the tablets meet the BP requirements. The formula for calculating the % weight variation is:

$$\textit{Percentage weight variation} = \frac{\textit{average weight} - \textit{individual weight}}{\textit{individual weight}} x100\ \%$$

f. Hardness test

When a tablet is placed on its edge, the force required to break it can be employed to determine its hardness. Hardness is thus sometimes characterized as tablet's crushing strength. It usually affects the drug dissolution and release, and it may affect bioavailability. The crushing strength was determined using a Monsanto tablet hardness tester (Intech, Korea). For this test, four tablets were randomly picked from each brand (Karmakar & Kibria, 2012). The average crushing strengths (hardness values) were calculated.

g. Friability test

The test was carried out by taking weights of 10 tablets in total, which as referred to as the initial weight, W_1. All of the tablets were placed in the chamber of the friability tester and rotated for four minutes at a speed of 100 rpm (or 25 rpm for one minute). The tablets were then removed, counted, and weighed again, with only the tablets that were still whole being examined. This is referred to as the final weight, W_2 (Chandrasekaran, Han, Chung, Cheang & Ping, 2011). The percentage friability (weight loss) of the tablets was then calculated using the following equation.

$$Percentage\ friability = \frac{W1 - W2}{W1} x\ 100\%$$

h. Disintegration test

The disintegration test measures the time it takes for a group of tablets to disintegrate into particles small enough to pass through a 10-mesh screen. The tablet disintegration tester was used to determine disintegration time of tablet. Six (6) tablets from each market preparation were studied in simulated gastric fluid at 37°C with a disintegration apparatus. The disintegration time, the time when no particle remained on the basket of the system was noted down (Karmakar & Kibria, 2012).

i. Potency test/Assay

Ten (10) tablets' weight from each market preparation was obtained separately and the average weight was calculated. Each sample was crumpled with a mortar-pestle and the proper quantity was weighed. Water was put into a 500 mL volumetric flask. The individual samples were placed in 50 ml volumetric flasks, which were properly labelled. A little quantity of prepared methanol was then added to each flask until it reached 50 ml. The solutions were sonicated for 15 minutes with a sonicator before filtering to produce a clear solution. Using a 5 ml pipette, the filtrate (2.5 ml) was taken from every volumetric flask and placed into a fresh 50 ml sized volumetric flask, filling it up to the mark. A small quantity was transferred to a UV cell. In case of standard ketotifen fumarate, the same protocol was applied. The absorbance was measured at 298 nm using a UV spectrophotometer (Singhvi & Sachdeva, 2009). The percentage and milligram content of each sample were then calculated and compared to the standard. The percentage of potency evaluation is computed using the formula mentioned below:

$$Percentage\ of\ the\ assay = \frac{Absorbance\ of\ Brand}{Absorbance\ of\ Standard}\ x\ 100\%$$

j. Acid base degradation study

For acid

Each sample's 5 ml of 20 ppm solution was put into four different test tubes, and then 5 ml of 0.1N HCl was added to each tube to evaluate the effect of the acid. After that, the combinations were let to stand for half an hour. The absorbance at a wavelength of 298 nm was then measured after each solution was moved separately to a UV cell.

For base

To evaluate the impact of base, the procedure is almost like use for acid. Instead of HCl (0.1N), NaOH (0.1 N) solution was used as reagent (Naveed, Shafiq, Khan, Jamal, Zafar, Hashim & Uroo, 2014).

3. Results and discussion

a. Physical appearance

The physical appearance and the shapes of different brands of ketotifen were found to be nearly identical, but they had different colors, as shown in Table II.

Table II

Physical characteristics of different market preparation.

Sl. No	Market preparation	Color	Tablet type and Shape
1.	KOT-1	Yellow	Uncoated and round
2.	KOT-2	White	Uncoated and round
3.	KOT-3	Yellow	Uncoated and round
4.	KOT-4	Ash	Uncoated and round
5.	KOT-5	Slight Yellow	Uncoated and round

b. Weight variation test

If the weight of the tablets exceeds the recommended range, the assay or content uniformity may be high. If tablet weight falls below the recommended range, assay or content uniformity may suffer. As a result, the weight of tablets should be carefully monitored during the compression process. The United States Pharmacopoeia (USP) specifies weight variation limits for tablet weights. These limits are as follows: 10% for tablets weighing less than 130 mg, 7.5% for tablets weighing between 130 mg and 324 mg, and 5% for tablets weighing more than 324 mg. The specified limit was not exceeded by any brand (Table III).

Table III
The weight variation results of various brands.

SL. No	Market preparation	Average weight (mg)	Weight variation limit
1.	KOT-1	164.9	-1.15 to +0.06%
2.	KOT-2	188.2	-1.64 to +2.44%
3.	KOT-3	63.9	-4.22 to +2.97%
4.	KOT-4	184.0	-1.73 to +2.28%
5.	KOT-5	168.4	-0.59 to +2.07%

c. Hardness test

The aptness of the tablet regarding mechanical stability during packaging and shipment can usually be expected based on hardness. Tablet hardness, in turn, affects tablet density and porosity. It may affect tablet friability and disintegration time.

Hardness indicates a tablet's ability to withstand the mechanical shocks during manufacturing, packaging, and shipping. The acceptable range of hardness or crushing strength of tablet is 4 to 7 kgf (kilogram of force). Five tablets from each brand were tested to determine their hardness. Most likely, the Monsanto hardness tester was not suitable for small size tablet. All tablets were too small, and no brand complied with the specified limit as shown in Table IV.

Table IV
Average hardness of various brands.

Sl. No.	Market preparation	Average hardness (kg/f)
1.	KOT-1	1.5 kg/f
2.	KOT-2	1.3 kg/f
3.	KOT-3	1.25 kg/f
4.	KOT-4	1.25 kg/f
5.	KOT-5	3.00 kg/f

d. Friability test

Friability refers to the tablet's tendency to crumble. It is critical for the tablet to withstand wear to its edges. Tablets are susceptible to pressures during manufacture and handling due to collision and sliding against one another and other solid surfaces, which can cause minute shards and particles to be removed from the tablet surface. The tablet friability test evaluates the ability to tolerate abrasion during handling, packing and transportation. During the test, all market preparations had acceptable friability values. The friability assessments for ketotifen tablets ranged from 0 to 0.603%. All brands had a percent (%) friability of less than 1%, indicating that all tablets were intuitively steady (Table V).

Table V.
The average percentage of friability among different brands.

SL. No	Market Preparation	Average friability percentage
1.	KOT-1	0.53%
2.	KOT-2	0.36%
3.	KOT-3	0.603%
4.	KOT-4	0.167%
5.	KOT-5	0%

e. Disintegration test

Drugs must first be in solution in order to be absorbed from solid dosage forms following oral administration. Disintegration, or the breaking up of the tablet, is typically the first significant step toward this condition. The disintegration test determines the time it takes for a batch of tablets to break up into particles that can fit through woven wire 10 mesh screens under specific circumstances. For standard dose forms, the test is often helpful as a quality assurance tool. The duration of a drug's disintegration affects both its therapeutic efficacy and its rate of absorption. It is hard to say that the drug is effective enough if the disintegration time is less than ideal. The optimal disintegration time for an uncoated tablet is five minutes, according USP guidelines, but most coated tablets disintegrate in 30 minutes (USP, 2020). All brands stand by this limit (Table VI).

Table VI
Disintegration time for ketotifen tablets.

Sl. No.	Market Preparation	Average disintegration time (DT)
1.	KOT-1	1 min 14 Sec
2.	KOT-2	1 min 25 sec
3.	KOT-3	3 min 5 sec
4.	KOT-4	1 min 24 Sec
5.	KOT-5	1 min 3 sec

f. Potency

The study was conducted to evaluate the different market preparations of ketotifen tablets available in Bangladesh and determine whether they meet the standards established in the official publications. As per BP, a tablet is deemed compliant if every single content level is between 85% and 115% and none surpasses the range of 75% to 125% of the average content. An extra twenty tablets' individual contents must be evaluated if an individual content measurement falls between 75% and 125% but is above 115% or below 85%. If none of the 30 tablets' individual contents fall outside of the 85% to 115% range of the average content and none of them beyond the 75% to 125% limitations of the average content, the tablet satisfies compliance standards (British Pharmacopoeia Commission, 2020). Hence,

all brands of ketotifen were found found to comply the specified limit (Table VII).

Table VII
Percentage of brand potency tested against standard ketotifen.

Sl. No.	Market Preparation	Potency result
1.	KOT-1	98.2%
2.	KOT-2	98%
3.	KOT-3	100%
4.	KOT-4	95%
5.	KOT-5	92.4%

g. Acid-base degradation

Degradation studies aim to analyze product changes, determine shelf life, and establish storage guidelines for future batches produced under identical conditions. In this investigation, UV spectrometry was used to analyze ketotifen degradation, and the USP limit of the test indicated that the level might be between 95% and 105% of the labelled quantity. It was observed that none of ketotifen complied with the USP specified limit (Table VIII and IX).

Table VIII
Impact of acidic pH.

Sl. No.	Market Preparation	% Assay
1.	KOT-1	61%
2.	KOT-2	56%
3.	KOT-3	53%
4.	KOT-4	61%
5.	KOT-5	58%

Table IX
Impact of basic pH.

Sl. No.	Market Preparation	% Assay
1.	KOT-1	58%
2.	KOT-2	54%
3.	KOT-3	56%
4.	KOT-4	58%
5.	KOT-5	61%

Quality product is the prime goal of developing intention for any pharmaceutical product manufactured by a pharmaceutical company. The pharmaceutical manufacturers are currently interested in a variety of approaches to achieving such quality (Nandhakumar, Dharmamoorthy, Rameshkumar & Chandrasekaran, 2011). Antihistamines are a diverse class of drugs that can prevent a number of symptoms associated with histamine. Usually structurally identical to histamine, these medications work by competing with histamine for binding to its receptors, which stops the activation of the receptor. Therefore, their therapeutic value is

not in correcting the effects of histamine but in preventing them. Various antihistamines work by inhibiting particular histamine receptors, such as H_1 or H_2 receptors (Pearlman, 1976). The data above, showed the various quality parameters of the different ketotifen brands available in Bangladesh.

Conclusion

In current manufacturing practice, *in vitro* quality control factors play important roles in comparing various generic branded products and providing adequate therapeutic activity of the tablet dosage form. The drug ketotifen is a noncompetitive antagonist of histamine that also stabilizes mast cells. Current pharmaceutical market of Bangladesh provides a wide range of products. Quality characteristics affect a formulation's therapeutic response. The current study found that most brands met the specifications in many aspects. The disintegration time (DT) of the tablets fell within the acceptable parameters, and the compliance rates for all brands were notably high. In the acid-base degradation analysis, all brands typically demonstrated degradation levels that remained within the acceptable limits. Nevertheless, none of the ketotifen brands fulfilled the required standards in the hardness test evaluation. This may have occurred due to the improper use of a hardness tester. The available hardness testers are typically designed for larger tablets, while the tablets examined in this study were smaller in size. Finally, because quality control characteristics are linked from the first step to the drug's pharmacological effect, a superior tablet, whether alone as well as combination form, would fulfil all quality parameters to achieve the required therapeutic response. Further research should be undertaken to examine the potency and dissolving profile of the brands to prove their pharmacological equivalency.

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