EVALUATION OF PHARMACEUTICAL EQUIVALENCE OF VARIOUS LOCAL BRANDS OF METFORMIN HCL TABLETS IN PAKISTAN

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ABSTRACT

Present study aimed to assess pharmaceutical equivalence of five different local brands of Metformin tablets in Pakistan. The brands were treated for different quality attributes according to the BP and USP pharmacopeial and non-pharmacopeial methods. Shelf life was determined by R-Gui software while model independent and dependent approaches were used for in-vitro dissolution profile study. Among all brands, A (2.83) and D (3.5) passed claimed shelf life of 3 years. Moreover, only one out of five brands that is brand A (f2= 53.58 and f1= 5.98) showed equivalence release to innovator (brand C) at each time point in BP mentioned pH 6.8 media, falling in standard range of f2=50-100 and f1=0-15 therefore they are interchangeable (P<0.01). However, the other 3 brands showed insignificant similarity. Present findings supports the need to conduct post-quality evaluation by regulatory authority for locally available pharmaceutical products.

Keywords: *In-Vitro* dissolution profile, Metformin, pharmaceutical equivalence

INTRODUCTION

Metformin HCl belongs to a biguanide class, mainly indicated for type 2 diabetic mellitus. It is an oral anti diabetic drug [1]. Mechanism of action of Metformin is basically to enhance the body sensitivity towards insulin and helps it to consume glucose in a usual way [2]. Moreover, Metformin HCl is a drug of choice for diabetic patients who are overweight or obese, but their renal function also works properly. From research evidence, its use has also been repurposed that Metformin has also been used increasingly for polycystic ovary syndrome [3], a syndrome manifesting hyperandrogenism and ovarian dysfunction [4]. Evidence recommends that Metformin not only acts as insulin sensitizer, but it also recovers hyperandrogenism and shows progress in ovulation and pregnancy rates in patients with PCOS, premature puberty and non-alcoholic fatty liver disease [3].

Those drug products which are equivalent chemically and bio-pharmaceutically must be similar in their purity, strength, quality, and active ingredient release profile so their route

of administration and dosage form must be same too. To monitor batch to batch consistency of drug release, dissolution study of drug product is performed for this is the essential measure to assess it [5]. For in vivo studies, dissolution test is an economical and practical approach in developing countries with limited resources and technology [6]. In this study, the release of active pharmaceutical ingredients from the drug product for dissolution medium is determined in comparison with gastrointestinal tract fluid. It can be considered for drug absorption that in vitro dissolution is helpful in assessing in vivo performance [7]. Other ingredients besides active pharmaceutical ingredient (API) including binders, thickening agents, disintegrants, glidants, colorants, sweetening agents may play role in quality and dissolution of drug product. To keep the proper amount of drug content the percentage content of active ingredient must be monitored regularly [8].

The aim of this study was to evaluate the quality of locally manufactured generics of different brands of Metformin HCl available locally in Pakistan. Metformin has been chosen as model drug because it is a first line drug for the treatment of type 2 diabetes mellitus and highly consumed by local people [9].

MATERIALS AND METHODS

Collection of Sample

With label claim of 500mg each Metformin HCl tablets of five different brands were purchased from local licensed pharmacy in Pakistan to evaluate the Quality Control tests and stability studies. These all-available brands were label claimed 500mg and these brands have been assigned codes as brand A, brand B, brand C (innovator), brand D and brand E.

Instruments

For carrying out dissolution and stability testing, following equipment has been used during quality assessment of different samples of Metformin tablets. Analytical Weighing Balance (Sartorius CP224S, Germany range 0-60g, readability: 0.0001g), Hardness Tester (Fujiwara, Japan, Range 1-20 Kp), Disintegration Apparatus (Sotaxdt 3 ch- 4123Allschwill/ Basal, Switzerland, No. of baskets 2), Dissolution Tester (ErweaDt 600, Japan, GmbH Apparatus), UV-Visible Spectrophotometer (UV 1800 Shimadzu, Japan, Double beam, Diode Array detector, Tungsten Lamp, ±0.3 accuracy, Wavelength range: 190-900 nm), Stability Chamber (LD-15 U, India, Shelves: 4, Power supply 230V, 50Hz, Temperature range: 20-60 ±0.2˚C, Humidity Range (R.H) 40-98 ±0.2%), and Friability Tester (Ft-400, India, No. of drums: 2, Drum Rotation: 20-60 Rpm).

Chemicals

Various brands of Metformin HCl of strength 500mg (label claim) has been collected from licensed local pharmacy in Pakistan and quality assessment accomplished within expiry date of the product. The standard Metformin HCl, for reference was obtained from Mass Pharma (Pvt) Ltd. Reagents used were Sodium Hydroxide (NaOH) pellets (Merck, Darmstadt, Germany), Potassium Dehydrogenate

Orthophosphate (KH_2PO_4) (Merck, Darmstadt, Germany). The above reagents spent for testing was of analytical grade. Distilled water was being utilized throughout the performance of testing.

Dissolution Test

ERWEKA DT 600 dissolution apparatus (Heusenstamm, Germany) has been used for performing dissolution studies taking simulated intestinal fluid pH 6.8. At temperature of $37 \pm 0.5°C$, in each compartment of the apparatus a tablet was placed containing 1000mL of medium, rotating at 100 rpm. Then at different intervals of 10, 20, 30,45 and 60 min; 10 ml of sample was drawn with the help of pipette. Also, a fresh dissolution medium with the same volume was added to replace the withdrawn sample volume for sustaining the sink condition. The withdrawn sample was then filtered with a syringe filter 0.45μm and diluted the filtrate. Using UV visible spectrophotometer, the absorbance was measured at 233nm, and concentration was determined against standard solution of known concentration of Metformin HCl. The percentage of drug release is determined by equation **[1]**.

$$\text{Drug release}\,(\%) = \frac{\text{Amount of drug released}\left(\frac{mg}{ml}\right)}{\text{Drug content in a tablet}\,(500)} \times 100$$

Disintegration Test

The randomly selected six tablets of different brands were placed in disintegration apparatus in its mesh of hole at a particular temperature of $37°C \pm 2°C$. Disintegration time of different brands tablets was assessed in distilled water at30 rpm. At the time when

no granule of any tablet was left on the mesh considered to be the disintegration time of that tablet **[10]**.

Friability Test

From each brand, twenty tablets were selected randomly and weighed on analytical balance before carrying out friability test. After de- dusting the tablet, it was then put on friability drum at 100 revolutions per 4 min. Tablets were de-dusted once again and re-weighed. The percent loss was measured from following formula and this weight loss should not be greater than 1% according to BP **[11]**. Percent Friability $= \frac{Wo - W1}{Wo} \times 100$

Where,

w_o denotes initial weight.

w_1 denotes final weight.

Thickness Test

For thickness measurement for both round and oblong shaped tablets, ten tablets were randomly selected from each brand. Vernier caliper was used for thickness measurement test. Afterwards, their mean and standard deviation were calculated.

Hardness Test

With the help of Hardness tester (Fujiwara, Japan), hardness test was performed on different brands of metformin HCl via selecting ten tablets randomly. These tablets were kept under pressure and temperature. Hardness was measured by measuring their crushing strength. The values were noted in Unit of Newton **[11]**.

Weight Variation

A random selection of 20 tablets from each brand has been made separately then each

tablet weighed individually on an analytical balance (Sartorius, CP224S, Germany). Average weight was calculated then corresponding percentage deviation from average weight has been determined for each tablet using equation **[11]**.

Percentage Deviation =

$$\frac{Individual\ Weight - Average\ Weight}{Average\ Weight} \times 100$$

Assay of Active Ingredient/ Metformin Tablets (500mg) using **UV- Visible Spectrophotometry**

To determine the claimed amount of the active ingredient in each Metformin tablets as label mentioned were evaluated by a validated and developed method of Assay with UV- visible Spectrophotometry with 0, 1, 3 and 6-month intervals and evaluated its stability.

Sample Preparation

For each brand, an average weight of twenty tablets has been weighed with the help of an analytical balance. Then these tablets were crushed into fine powder by mortar and pestle. The powder containing active equivalent to 0.1g of Metformin HCl was mixed with 70ml of distilled 1800 Shimadzu at 232 nm. By using equation, **[4]** percent of active content (Metformin HCl) in the tablets was calculated, while taking A1% 1cm as 798.

$$\% \text{ Metformin hydrochloride} = \frac{Absorbance\ of\ sample}{A\ 1\%\ 1cm\ (798) \times b(1)} \times$$

1000 (dilution factor) × 100

Data Analysis

SPSS version 20 was employed to evaluate experimental data graphically and statistically for present investigation while R-Gui software was used to determine the shelf life of all the brands of tablets. At 0.05 level of significance, the statistically significant difference was assessed applying one way ANOVA for % assay. In addition, the dissolution profiles for different selected brands of Metformin HCl were compared using one way ANOVA that is considered as model independent method. Difference factor (f1) and similarity factor (f2) was another model independent method used for comparing the dissolution curves. These factors help to understand the difference and similarity in percentage release of drug from tablet between test and reference samples respectively at each time point. Formulae to obtain f1 and f2:

$$f1 = \left\{ \frac{[\sum_{t=1}^{n} |Rt - Tt|]}{[\sum_{t=1}^{n} Rt]} \right\} \times 100$$

$$f2 = 50 \times \log \left\{ \left[1 + (1 \div n) \sum_{t=1}^{n} (R_t - T_t)^2 \right]^{-0.5} \times 100 \right\}$$

Where, t represents the time point, Tt and Rt denote the mean of drug released at each point of time for respective test and reference profiles of dissolution. The standard value for f1 and f2 should be in the range of 0-15 and 50-100, respectively for two comparative dissolution profiles when they show similar and identical pattern of drug release.

RESULTS AND DISCUSSION

All the brands of Metformin HCl used in the present study were purchased from local manufacturers. Moreover, the dissolution tests and other quality parameters such as assay, friability, hardness, and weight variation were carried out as part of essential quality control tests.

Assay and Kinetic Degradation

% Assay test was performed to evaluate the change in concentration of active ingredient in selected five brands of metformin HCl. A minimal difference was observed respecting the percent content of all five brands. Brand C had the lowest % content (98.42%) while brand B possessed the highest content (99.48) among all brands. Statistical analysis using one-way ANOVA test performed for average difference in percent content of drug also showed that with 95% confidence interval, no significant difference ($P>0.05$) was observed for five brands of metformin HCl.

R-Gui software was used to evaluate the first order kinetics and shelf life. The claimed shelf life for all brands was three years, followed by only two brands C (innovator) and D (Table 1). Brand D was shown to be the most stable formulation among all with less deviation in % assay values and maximum shelf life (3.5 years). Hydrolysis, packaging material, and incompatibility of excipients with active are some of the prime factors affecting the stability of tablets [12].

Disintegration test

Disintegration of tablets into primary particles is significant for assuring the formation of large surface area of a drug to facilitate subsequent dissolution and ultimate absorption of drug [13]. Disintegration test is an official requirement of pharmaceutical products like tablets to be accepted as quality formulation. According to USP 34 NF 29 (2011), an acceptable formulated tablet disintegrates within 15 minutes. Disintegration test showed tablets of all the brands of Metformin HCl were disintegrated within 15 minutes under the specified storage conditions over six months (Table 1). Minimum disintegration time recorded was 4.91 minute for brand B while maximum time observed was 6.34 minute for brand E. moreover, a minimal change was noticed for disintegration time of all the tablets that might be due to absorption of moisture that cause swelling of disintegrates [14].

Thickness test

Change in thickness of tablet may alter dose distribution and associated therapeutic effect. Therefore, this parameter must be considered and checked during formulating a finished batch of tablets. Table 1 depicts no variation in thickness of tablets of all the selected brands over a period of six months with SD of 0.01 for brand B to 0.04 for brand C. In this way, thickness of tablets was found controlled within 5% of limit by standard. These results indicate even filling of die and uniform speed of compression machine manufacturing of tablets [14].

Table 1: Physical measurements of five brands of Metformin HCL tablets (500mg)

Brand Code	Assay (%) ± SD	Disintegration time (min) ± SD	Mean Thickness (mm) ± SD	Mean Hardness (N) ± SD	Mean Weight (mg) ± SD	Friability (%) ± SD	t_{90}** (years)
A	99.45±0.69	5.96 ± 0.58	5.21 ± 0.03	108.85 ± 7.84	546.05 ± 1.77	0.21 ± 0.07	2.83
B	99.46 ± 0.83	4.91 ± 0.54	5.02 ± 0.01	115.52 ± 10.29	529.77 ± 1.94	0.38 ± 0.09	2.41
C	98.42±0.65	5.05 ± 0.79	5.51 ± 0.04	109.24 ± 6.57	520.78 ± 3.27	0.32 ± 0.04	3.08
D	98.67± 0.58	6.50 ± 0.62	5.65 ± 0.02	101.79± 4.51	534.04 ± 2.36	0.21 ± 0.10	3.5
E	99.34 ±0.82	6.34 ± 0.47	5.63 ± 0.02	118.36 ± 6.77	593.89 ± 3.22	0.24 ± 0.07	2.41

** originated from R-Gui software

Hardness test

The hardness test basically helps to measure the capacity of a drug to withstand stress or pressure during packaging, handling, and transportation. Table 1 exhibits results of hardness test that indicates all the brands of metformin possessed a value of hardness more than 50. Thus, concluding that all products have assured the requirement criteria. In addition to this, all the brands possessed varied average values of hardness. Hardness of brand A and brand C is found to be close to each other with little difference. Hardness ranged from 101.79 N for brand D to 118.36 N for brand E. various factors are allied to such alteration concerning the hardness of tablets. These factors might include certain formulation conditions during

pharmaceutical manufacturing process like type and quantity of lubricants used, methods of granulation and different speed of machine **[15]**.

Weight variation test

Weight variation should be minimum respecting the dose of drug in a tablet as it is the measurement of concentration of active ingredient during tablet compression **[14]**. Variation of weight depends on various parameters such as powder density, distribution of particle size, flow properties, die, punches, and speed of compression machine. Result showed that brand E had a higher average weight (593.89 mg) while brand C had a smaller mean weight (520.78 mg). However, all the brands of metformin tablets in current work depicted the

negligible difference in weight variation (Table 1). All the brands did not show deviation in weight more than 5% and hence exhibited uniformity of content in acceptable range as specified by USP [10].

Friability

We cannot rely just on hardness test as best measure. The friability test must also be considered as drug handling and packaging may cause loss due to abrasion. Therefore, friability becomes a more relevant factor. The following study showed that the friability values of all metformin brands ranging from 0.21% to 0.38% (Table 1). These all brands are found to be up to the mark and passed pharmacopeial specification of friability, according to which there should not be maximum weight loss of more than 1% of the tablet [10] and this study conducted in Pakistan on metformin hydrochloride brands found within range like that conducted by Arwadi and others in Egypt (2020).

Dissolution Test

Dissolution is defined as the extent and rate in which a solid therapeutic agent is released from solid dosage form into solution or liquid state [14]. One of the important parameters of tablets is its bioavailability in gastrointestinal tract for better absorption and achieving desired therapeutic effect. It is one of the prime control tests required to assure batch to batch equivalency and uniformity of drug content [5]. Moreover, the dissolution studies predict the performance of solid oral drug products such as capsules and tablets at in-vivo scale. Figure 1 shows that in current investigation, the release of active from all

brands of Metformin tablet was found to be 80% and more in 45 minutes. In this way, all the tablets of selected brands passed the general specifications given in USP 34 as standard for dissolution test results for conventional release oral dosage forms. Brand B showed the highest release of drug, 88.25% at 45 minutes which is greater compared to innovator. In addition, tablets for all the brands A, B, C, D and E released more than 50% of Metformin HCl in 10 minutes that might be associated with the use of nature and number of excipients utilized, as well as formulation and processing factors. This result is supported by the findings obtained by [14] and his colleagues (2020) where > 50% Metformin was released from tablets of all the brands in pH 6.8 media. The present finding is also as same as found in the quality controls tests conducted by [16]. Moreover, figure 1 indicates difference in dissolution profiles of various brands at each time point. At 95% confidence interval, no significance difference was determined in dissolution profiles of Metformin HCl brands when one way ANOVA analysis was performed for specified time of 45 minutes given in pharmacopeia ($P > 0.05$). Thus, this indicates the Metformin HCl dosage forms are statistically equivalent respecting to in vitro pattern of dissolution profiles at 45 minutes. FDA recommended f2 and f1 tests [5], in this regard, were applied to assess the similarity and difference at each time point among all the dissolution profile via comparing them with the release profile of innovator (Brand C).
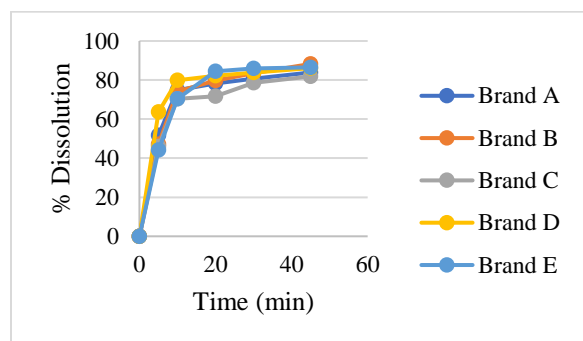
Figure 1. Comparison of in-vitro dissolution patterns of various selected brands of metformin HCl.

Similarity and difference factors show that all brands possessed the significance difference in release profiles at each time point when compared to dissolution pattern of originator brand C, except brand A that showed no significance dissimilarity to innovator (Table 2). F2 and F1 analysis between four selected brands of marketed tablets of Metformin HCl and originator Brand C exhibited significant similar release profile at each time point only in case of brand A and innovator where f2 > 50 indicating the more the value of f2, the higher will be similarity between two profiles of dissolution [14]. However, other three brands with f2 < 50 and f1 > 15 represented non-equivalent dissolution patterns to the originator brand C. Hence, brand A was found to be the most equivalent and similar marketed product in the locality to brand C (Table 2). There can be various factors allied to difference in marketed products for same active such as use of different disintegrates and binders by manufacturers. However, such findings proposed the requirement of post- marketing assessment of

pharmaceutical formulations by the regulatory bodies.

Table 2. Similarity and difference factors value for all five brands of Metformin HCl.

Marketed Brand of Metformin HCl	f1	f2
Brand A	5.98	53.58
Brand B	18.97	36.24
Brand C (Innovator)	0	100
Brand D	20	35.28
Brand E	16.24	41.95

One of the previous studies also performed the dissolution analysis of six available local brands of metformin HCl in Egypt and obtained the results [14]. Result revealed similar release of metformin HCl from two marketed brands with f2 > 50 and non-equivalent release profiles from three marketed brand (f2 < 50) when compared to innovator.

Dissolution testing imparts an imperative role in designing and manufacturing a quality, efficient and safe pharmaceutical product. In this regard, use of enhanced computational software and tools along with improved comprehension of drug release mechanisms from pharmaceutical dosage form, absorption processes, as well as the physiological environment required for drug release has contributed to develop in vitro dissolution methods for assessing the potential of *in-vivo* performance of finished solid oral medicinal products. In this way, the three brands with non-bioequivalent profiles may not be consumed inter-changeably to innovator

(Brand C) and required further evaluation [17]. The quality control tests are helpful to determine the accurate behavior and performance of pharmaceutical dosage forms and must be a part of evaluation for post marketed products to avoid the preventable factors deteriorating the quality and efficacy at any point.

CONCLUSION

Present study attempted to assess the certain physicochemical equivalence along with other quality parameters of locally manufactured and available five brands of metformin HCl in Pakistan. Evaluation at physicochemical level exhibited that all the tablets passed the specifications of quality encompassing assay, friability, weight variation, thickness, hardness, and disintegration time. Considering the shelf life and first kinetic study, all the brands; except comparator (brand C) and brand D; were failed to fulfil the claimed duration of shelf life. Regarding the comparative study via dissolution test, only one brand out of four proved the equivalence behavior in release pattern of active with comparator brand C. Current findings of this research emphasize on the requirement to capacitate and enforce the rules and mechanisms of regulatory authorities via focusing on the post-quality evaluation of pharmaceutical products manufactured by various originators and circulating in local markets.

CONFLICT OF INTERESTS

Authors declared no conflict of interest.

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