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IN VITRO EQUIVALENCE STUDY OF GENERIC METFORMIN HYDROCHLORIDE TABLETS UNDER BIOWAIVER CONDITIONS

Ruaa H. AL Arwadi
Pharm D, Faculty of Pharmacy, Beirut Arab University, Beirut, Lebanon, ruaa.arwadi@gmail.com

Azza A. K. Gazy
Professor of Pharmaceutical Analytical Chemistry and Drug Quality Control, Faculty of Pharmacy, Beirut Arab University, Beirut, Lebanon, a.ghazi@bau.edu.lb

Lama Soubra
Associate Professor of Toxicology and Risk Assessment, Faculty of Pharmacy, Beirut Arab University, Beirut, Lebanon, l.soubra02@bau.edu.lb

Rola D. Ajami
Pharm D, Faculty of Pharmacy, Beirut Arab University, Beirut, Lebanon, rola.dbouk@opl.org.lb

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Abstract

Background: Generic drugs are smarter alternative to expensive brands, it is bio- equivalent formula of any branded drug. FDA approved that generic drugs are the safest to consume, the medicines meet the similar manufacturing standards followed while producing an innovator drug, however, the color, shape, taste and packaging of generics is different from the innovator product. In short, a generic drug should be bioequivalent to its brand counterpart. Metformin was initially marketed under the name of Glucophage®, and now the market is loaded by generics of different origin, and price variability. Method: Our study was conducted to determine whether metformin generics are bioequivalent to the innovator drug Glucophage®. In-vitro bioequivalence testing under Biowaiver conditions can predict bioequivalence in a safe, fast, and less expensive method. Thus, study was performed on Metformin tablets to assess whether generics are bioequivalent to the innovator and hence be interchangeable. Results: The quality control results of the thickness, hardness, friability, disintegration, weight uniformity, content uniformity, and assay showed that most metformin tablets complied with the USP 34 NF29 2011 specifications. Dissolution testing under biowaiver conditions showed different results. All tablets of the generics and innovator Glucophage® were able to dissolve by more than 85% within 15 min. Two generics were bioequivalent to the innovator Glucophage® having $f2 \geq 50$ in the three dissolution media. The rest of generics showed variable results. Conclusion: Generics of metformin varied in their bioequivalency to the innovator Gluocophage®. This variation could be explained by different excipients, and manufacturing conditions. In-vivo bioequivalence testing should be conducted to confirm that the innovator could be safely interchangeable with the brand and this variation won't affect the safety and efficacy of the drug.

Keywords

1. INTRODUCTION

Diabetes is a chronic complex metabolic disorder that usually develops when the endogenous insulin is insufficient to control the plasma glucose blood level because of insufficient insulin , this condition requires continuous treatment and patient self-monitoring to prevent its complications. Insulin, a hormone naturally secreted by the pancreas in response to high glucose concentration, controls the body metabolism via regulating the body's ability to utilize and store glucose and fat. Among the different types of diabetes, type 2 is the most common form making more than 90% of the cases. It is more common in patients who are obese, having family history of diabetes, or gestational diabetes. They usually suffers from insulin resistance, high triglyceride, and low high density lipoprotein (Yehuda, H. Z., et al. 2015). Metformin is the most common used oral anti-hyperglycemic drug used for treating type 2 diabetes. It is considered as the first line drug of choice for the treatment of type 2 diabetes, particularly in overweight or obese people and those with normal kidney function. Metformin can lower basal and post-prandial glucose levels. It produces its effect by decreasing hepatic glucose production, decreasing intestinal absorption, and improving insulin sensitivity by increasing glucose uptake and utilization. Metformin unlike other anti-diabetic doesn't cause hypoglycemia, and hyperinsulinemia. In addition to its anti-diabetic profile, metformin nowadays is used to treat polycystic ovary syndrome (PCOS) which is a syndrome of ovarian dysfunction and hyperandrogenism. Metformin, an insulin sensitizer, improves not only hyperandrogenism but also ovulation and increases pregnancy rates in patients with PCOS. Recently, studies showed that it may be beneficial in reducing the incidence of cancer.Bristol-Myers Squibb Company was the first to introduce Metformin hydrochloride tablets in the market under the name of Glucophage®. It was approved in 1994 in the USA. And now the market is full of different generics of metformin hydrochloride. The main side effect of metformin is gastrointestinal disturbances. Metformin is contraindicated in patients having their creatinine clearance less than30 ml/min (Drugs.com. 2019). Metformin hydrochloride is found in different dosage forms either immediate release (500, 850, 1000), or extended release dosage forms (500 XR, 750 XR, 1000 XR)
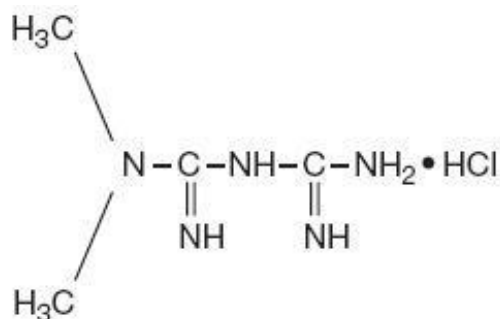
Metformin

Innovator drugs, also known as brands, are the first discovered and introduced drugs to the market, its patency up to 10 years. After patent expiration, it can be copied under different generic names. Generic must be bioequivalent to the original innovator having the same rate and extent, dose, route of administration, use, side-effect, safety, and strength. They must be therapeutically and pharmaceutically equivalent. They can only differ in their excipients, color, shape, and cost (Olubukola O., et al 2012).

To verify that the brand is bioequivalent to the generic, in vivo and in vitro bioequivalence tests must be done to assess compatibility of the products. The main limitation of the In-vivo tests that make them unpractical is that generally their cost, time consuming, and invasiveness. In order to reduce its use, the biopharmaceutics classification system must be used.

Biopharmaceutical classification system (BCS), is a system that is used to classify drugs according to their dose, solubility ratio, and intestinal permeability. For any drug to determine its bioequivalency BCS should be done (Rohilla S., et al 2012).

Table 1: Biopharmaceutics classification system (BCS)

Class	Description
I	High solubility, High permeability
II	Low solubility, High permeability
III	High solubility, low permeability
IV	Low solubility, low permeability

Drugs classified as BCS class I , II, or III can be tested for their bioequivalence of brand and generics using biowaiver dissolution testing according to the FDA, and WHO (Drugs.com. 2019, Rohilla S.,et al , 2012) Table 1. According to the WHO, and FDA (Rohilla S., et al 2012), Biowaiver dissolution testing is a clinical bioequivalence testing for drugs having BCS I , II, or III. The drugs tested must dissolve rapidly ($\geq$85% dissolved within 15 min) in 3 different pH media ranging from 1.2 to 6.8 having f2$\geq$50 in the 3 medias. Biowaiver helps in predicting bioequivalence in a safer, inexpensive, faster, and effective way than in–vivo testing. In addition, it could be used for drugs of narrow therapeutic index. Certain criteria must be present to validate this method. Where f2 is the similarity factor and f1 is the difference factor and calculated according to the following formulas:

F1= {[Σnt=1|Rt-Tt|]/[Σt=1nRt]}×100

F2 = 50×log {[1 + (1/n)Σn=1(Rt – Tt)2]-0.5 x 100}

Where n is the number of dissolution sample times, Rt and Tt are the mean percent dissolved at each time point, t, for the reference and test dissolution profiles, respectively. Criteria for biowaiver:

To meet the biowaiver criteria, the tested drug must be highly soluble (highest dose is soluble in 250 ml at pH 1.2-6.8), highly permeable (extent of absorption is greater than 85%), and rapidly dissolving (85% or greater dissolves within 15 min). The dissolution must be performed in 3 different pH media 1.2, 4.5, and 6.8 mimicking the gastric and intestinal fluids of the body without enzymes. Twelve or six samples should be placed in 900ml buffer solution using either the paddle type at 50 rpm or basket type at 100 rpm. 5ml samples to be withdrawn at different time interval 10, 15,20,30,45, and 60min. The profiles of the test and reference products must be similar in all 3 media having similarity factor f2$\geq$50 (Rohilla S.,et al ., 2012).

Our studied drug metformin hydrochloride is BCS class III, and hence it is highly soluble with low permeability and candidate for a biowaiver in vitro dissolution testing based on the WHO classification system for class III drugs (Drugs.com. 2019, Rohilla S., et al, 2012).

This study sought to apply the BCS biowaiver requirements in order to assess the equivalence of generic metformin tablets obtained from Lebanese, Egyptian, European, and United Arab Emirate markets with the innovator GLUCOPHAGE® that is a registered trademark of Merck Santé S.A.S., an associate of Merck KGaA of Darmstadt, Germany. Our study also aimed to compare and evaluate the tested brand and generics by applying quality control tests following the USP pharmacopeia.

2. MATERIALS AND METHODS
2.1. Materials:

The drug samples investigated in our study were all immediate release solid dosage forms, purchased from registered pharmacies in Lebanon and Egypt, and all were within product expiration dates. The selection of these brands was done according to their availability in the Egyptian and Lebanese markets.

Table 2: Coding Of Metformin Hydrochloride Tablets

Manufactured country	CODE	Batch number	Expiry Date
Germany	Innovator- Glucophage® 1000 mg	ELE3901	NOV 2017
European	GM001 1000 mg	41071	FEB 2017
Egyptian	GM002 1000 mg	1573011	MAR 2017
Egyptian	GM003 1000 mg	01140533	AUG 2017
Emirate (U.A.E)	GM004 1000 mg	0040	OCT 2019
Lebanese	GM005 850 mg	LT9184	JAN 2017

All reagents used to prepare the buffer solutions were of analytical grade and provided by Fluka. These were potassium chloride, sodium chloride, concentrated hydrochloric acid, potassium dihydrogen phosphate, and Dihydrogen phosphate. Distilled water was used throughout the work. Metformin hydrochloride authentic powder was a gift from CID co. pharmaceuticals, Cairo, Egypt.

2.2. Method

2.2.1. Buffer solutions

The buffer solutions were prepared according to the EUROPEAN PHARMACOPOEIA 5.0, and the pH was confirmed using a pH meter. Buffer solution of pH 1.2 was prepared by dissolving 6.57 g of potassium chloride in distilled water. 50ml of the solution was added to 85 ml of 0.2 M hydrochloric acid solution. The solution was then diluted with distilled water till 200 ml volumetric flask. The phosphate buffer solution of pH 4.5 was prepared by dissolving 6.8 g of potassium dihydrogen phosphate in 1000 ml distilled water. The phosphate buffer solution of pH 6.8, was prepared from 2 solutions A (50ml), and B (22.4 ml) and completed to 200 ml by distilled water. Solution A was prepared by dissolving 9.08 g of potassium dihydrogen phosphate in 1L of distilled water, while solution B consisted of 0.2 M sodium hydroxide (NaOH) (2g/L).

2.2.2. Standard metformin hydrochloride stock solution

In order to prepare the standard metformin hydrochloride stock solution, 100mg metformin hydrochloride reference standard was accurately weighted and transferred into 100 ml volumetric flask, then completed to the line marked using distilled water.

The wavelength was measured using Jasco® UV-vis spectrophotometer (Model No. V-530). Absorption was measured at a range of 200-300 nm. The maximum wavelength of standard Metformin Hydrochloride was found at 232 nm.

The Calibration curve was obtained by measuring the absorbance's against blank of different serial dilutions of metformin hydrochloride ranging from 0.4mg/ml to 1.4mg/ml. The calibration curve is shown in figure 1.
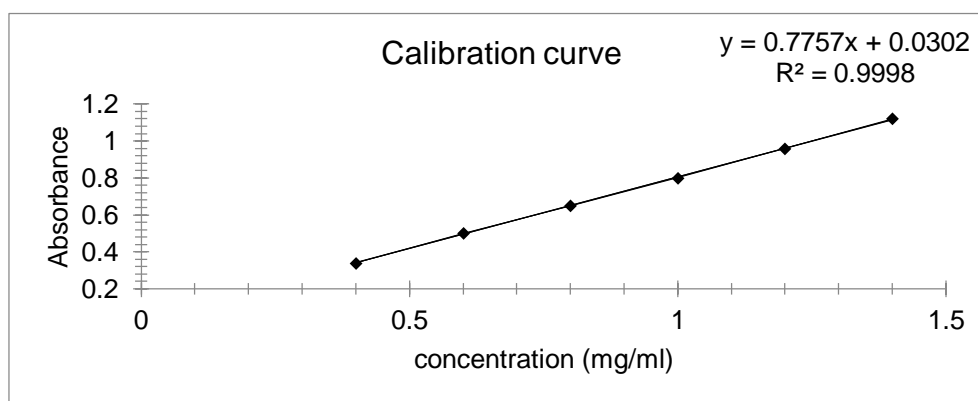
Fig.1: Calibration Curve

2.2.3. Dissolution Rate Determination under Biowaiver conditions

The dissolution test carried in 3 pH media 1.2, 4.5, 6.8 was performed using the Dissolution rate apparatus ERWEKA DT6R (Heusenstamm, GERMANY). 900 ml buffer solution was placed in each compartment of the apparatus with one tablet placed in each basket. The dissolution was performed at 100 rpm at a temperature of 37 ± 0.5 C°. Six units from each brand were evaluated in the three pH media. Sample aliquots of 5 ml were withdrawn from a zone midway between the surfaces of the dissolution medium to the top of the basket at specified time intervals (5,10,15,30,45,60 min) and replaced with 5 ml of the appropriate medium to maintain sink conditions. Samples were filtered using 0.45µm milipore filter paper, and diluted 100 folds with distilled water. The diluted samples were then analyzed spectrophotometrically at 232 nm to determine the dissolved amount of metformin hydrochloride. The dissolution system met the USP, BP, and the FDA performance verification test requirements for dissolution testing under biowaiver conditions. The percentage of drug dissolved was calculated using the following formula:

% Drug dissolved = [amount of drug dissolved (mg) / drug content per tablet (mg)] $\times100$.

The difference factor f1 and the similarity factor f2 was calculated for each generic with respect to the innovator using the following formulas.

F1= {[$\Sigma nt=1$|Rt-Tt|]/[$\Sigma t=1nRt$]}$\times100$
F2 = $50\times\log$ {[1 + (1/n)$\Sigma n=1$(Rt – Tt)2]-0.5 x 100}

Where n is the number of dissolution sample times, Rt and Tt are the mean percent dissolved at each time point, t, for the reference and test dissolution profiles, respectively.

2.2.4 Quality control tests

- Tablet Thickness (mm): The thickness of metformin innovator and generic drugs was measured using a micrometer. Ten tablets from each brand were examined. The Average thickness was calculated and expressed in mm. The limit required by the pharmacopeia should be $\pm$ 5% from the average weight calculated.
- Tablet hardness Test: The hardness of metformin innovator and generics was measured using ERWEKA Monsanto hardness tester. Ten tablets from each smaple were taken, and then each tablet was placed between the spindle of the machine until the tablet breaks. The pressure required to break the tablets was recorded, and the average hardness of the ten tablets was calculated and expressed in kg/cm2.

- Friability Test: To determine the weight loss of the tablets after abrasion, ten intact tablets from each sample were weighted and placed in the Roche Friabilator at 25 revolutions per minute (rpm) for 4 minutes. Dust was removed from the tablets before re- weighting them. The Friability of the tablets were then calculated using the following formula:

% friability = [initial wt –final wt]/initial weight ×100

- Uniformity of weight (mg): Twenty intact tablets from each tested sample were weighed individually using a digital balance, then their average weight was calculated. The uniformity of weight was calculated by the percentage deviation between the weight of each tablet to the average weight of the 20 tablets.

- Disintegration: To determine the disintegration time required by each tablet, 6 tablets from each tested sample was placed in the ERWEKA (Heusenstamm, Germany) disintegration apparatus at 37.2 ± 0.5 C° in distilled water. The time taken by each tablet to disintegrate completely was recorded. Complete disintegration is defined when there was no more tablet residues left in the mesh.

- Assay: The assay was done to ensure that the actual amount of the active ingredient present in the tablet is the same as the labeled amount. Twenty tablets from each sample were weighted and powdered. From the powder, an amount equivalent to 0.1g of Metformin hydrochloride was transferred to a 100ml volumetric flask. The powder was dissolved in 70ml distilled water and shacked for 15 min, then diluted to 100ml distilled water and filtered using filter paper. 10ml of the filtrate was then diluted to 100ml. This procedure was repeated one more time. The absorbance of the resulting solution was measured at a wavelength of 232nm against blank. The amount in mg of metformin hydrochloride present per portion was calculated using the following formula:

10C (AU/AS)

Where C is the concentration of Metformin Hydrochloride reference standard in µg/ml, and AU and AS are the absorbance's obtained from assay preparation and standard preparation respectively.

- Content uniformity: Ten tablets from each batch of the studied generics and innovator were used to perform this test. Each tablet was firstly placed in 100ml volumetric flask. 50ml distilled water was added into the flask. Then the flask was shaken to ensure complete dissolution of metformin, and completed till the line mark by distilled water. 0.1 ml was withdrawn from the prepared solution using a pipette and diluted in 100ml distilled water. The absorbance of the following solution was measured at 232nm against blank. The content uniformity was calculated by using the same formula of that for the assay.

3. RESULTS

3.1 Dissolution Rate Determination

Figures 2, 3, and 4 illustrate the dissolution profile of the innovator and the five tested metformin hydrochloride generics. The dissolution curves for each product reflects the average dissolution time of six tablets. Table 3 showed the percentage dissolved of metformin hydrochloride at each time interval. The results of pH 2 showed that, the dissolution of Glucophage® and its generic ranged from 85.55% to 101.17% within 30 min. At pH 4.5, Glucophage® recorded 87.77%, while its generics recorded higher readings from 90% till 96.67%. At pH 6.8, similar results were shown Glucophage® dissolved by 97.82% at 30 min, and other generics dissolved between 97.82% and 105.43%. This result complied with the USP metformin monograph, and the WHO specifications for dissolution rate determination under biowaiver conditions. Dissolution profile of metformin generics was compared to the innovator Glucophage® using the similarity factor f2, and the difference factor f1. For GM001 and GM003, their similarity factor f2 had a value greater than 50 in the 3 dissolution media and a low difference factor f1<15 indicating their bioequivalence to the innovator.

Generics GM002, GM004, and GM005 failed to show their bioequivalence to Glucophage® as they had a similarity factor less than 50 and a higher difference factor f1 ranging from 11 to 24 in the 3 dissolution media.
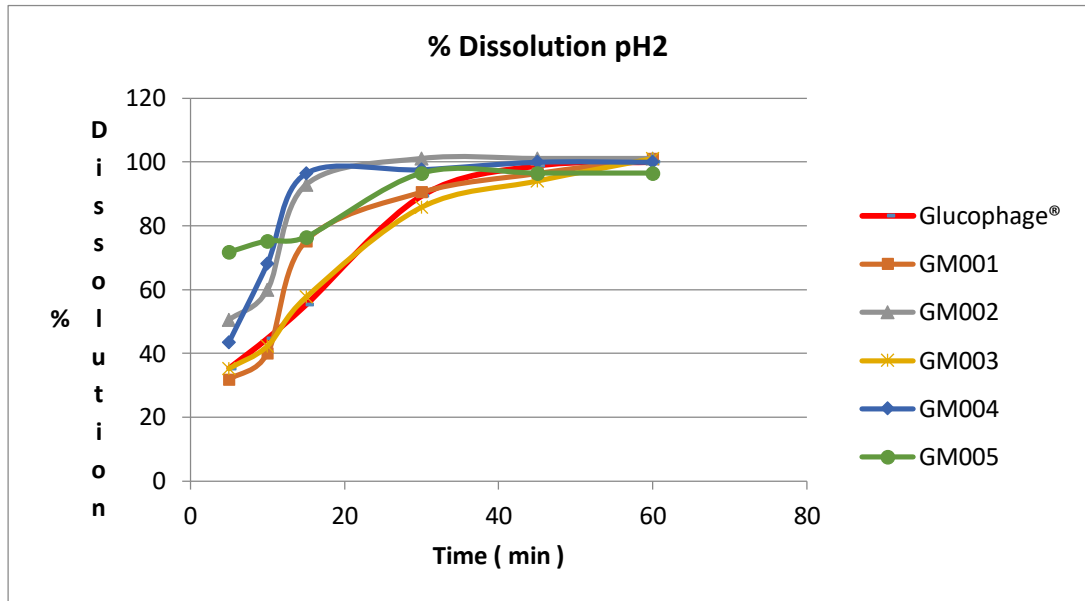

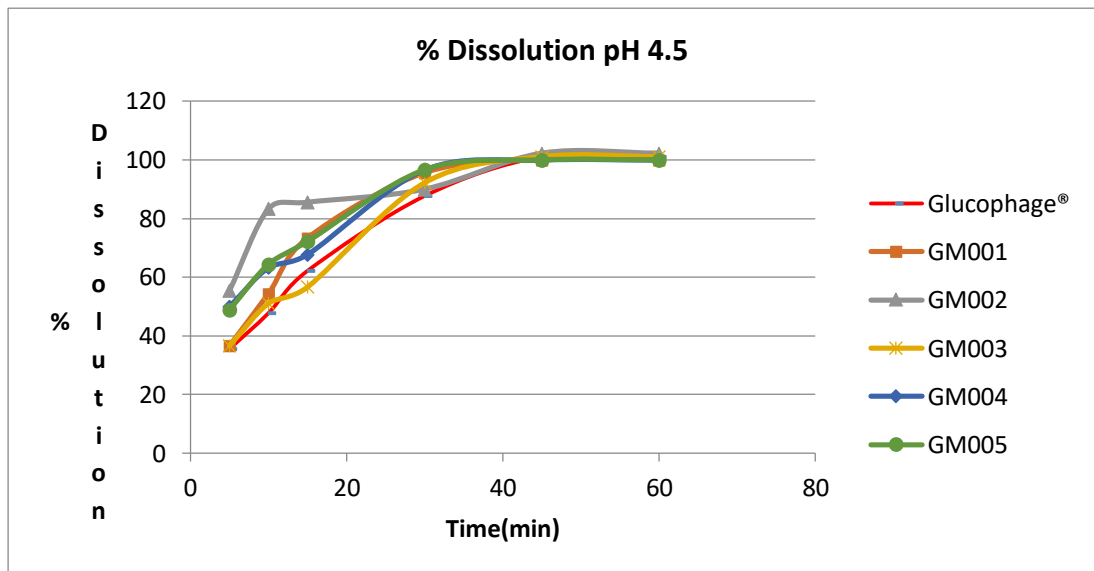Fig.2: Dissolution rate profile of Glucophage® innovator and generics in pH 2

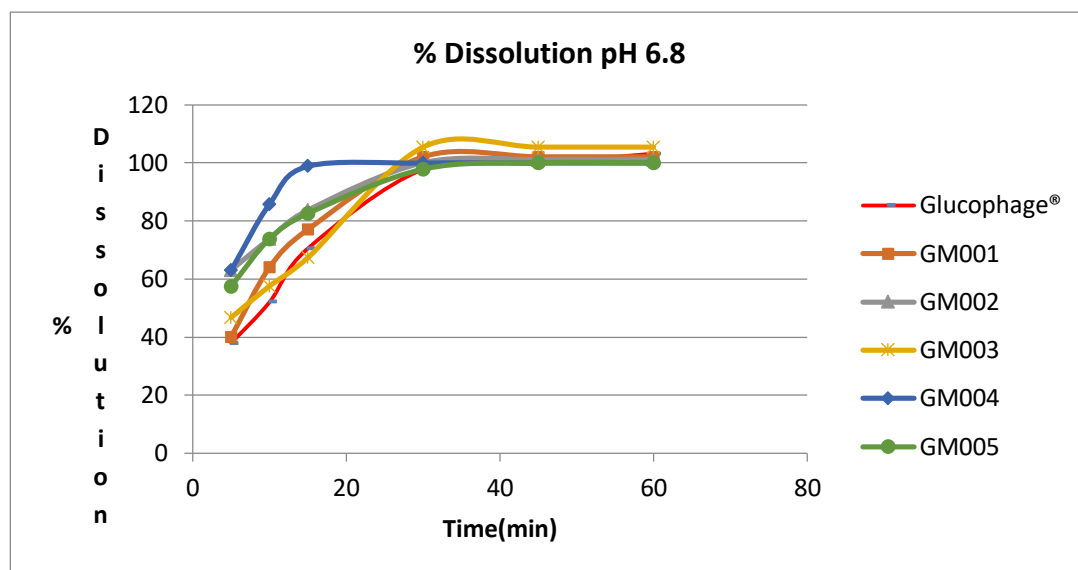Fig.3: Dissolution rate profile of Glucophage® innovator and generics in pH 4.5

Fig.4: Dissolution rate profile of Glucophage® innovator and generics in pH 6.8

Table 3: Results of f2, and f1 calculations for Glucophage® innovator and generics in different pH media

Time (min)	Glucophage® % Dissolved	GM001 % Dissolved	GM002 % Dissolved	GM003 % Dissolved	GM004 % Dissolved	GM005 % Dissolved
pH 2						
5	35.29	31.76	50.59	35.29	43.52	71.76
10	44.70	40	60	42.35	68.23	75.29
15	55.29	75.29	92.94	57.64	96.47	76.47
30	89.41	90.59	101.17	85.88	97.64	96.47
45	98.82	96.47	101.17	94.11	100	96.47
60	100	101.17	101.17	101.17	100	96.47
		f1=8	**f1=20**	**f1=3**	**f1=19**	**f1=24**
		f2=53	**f2=37**	**f2=76**	**f2=35**	**f2=33**
pH 4.5						
5	35.55	36.67	55.55	36.67	50	48.89
10	47.77	54.44	83.33	51.11	63.33	64.44
15	62.22	73.33	85.55	56.67	67.78	72.22
30	87.77	95.55	90	92.22	96.67	96.67
45	101.11	100	102.22	101.11	100	100
60	101.11	100	102.22	101.11	100	100
		f1=7	**f1=19**	**f1=3**	**f1=11**	**f1=12**
		f2=60	**f2=36**	**f2=73**	**f2=51**	**f2=49**
pH 6.8						
5	38.04	40.22	63.043	46.74	63.04	57.60
10	52.17	64.130	73.91	57.61	85.87	73.91
15	70.65	77.17	83.69	67.39	98.91	82.60
30	97.82	102.17	100	105.43	100	97.82
45	100	102.17	101.087	105.43	100	100
60	103.26	102.17	101.087	105.43	100	100
		f1=6	**f1=14**	**f1=7**	**f1=20**	**f1=12**
		f2=61	**f2=42**	**f2=61**	**f2=34**	**f2=44**

3.2 Quality Control Tests:

Thickness, hardness, friability, uniformity of weight, disintegration, content uniformity, and drug content per tablet (assay) were assessed and are shown in Table 4.

Table 4: Physiochemical Properties of Metformin Hydrochloride Tablets

	Thickness (mm)	Hardness (kg/cm^2)	Friability %	Uniformity of weight(g)	Disintegration time (min)	Content uniformity (w/v%)	Assay (w/v%)
Glucophage®	6.457 ±0.035	24.04 ± 1.63	0.043	1.071 ± 0.015	15 :55 ± 0:23	97.8 ±0.55	101.00 ± 1.26
GM001	6.33 ±0.035	23.56 ±1.97	0.019	1.101* ±0.024	10:41 ±0:38	98.0 ±1.03	103.00 ± 0.99
GM002	5.813 ± 0.025	19.14 ±1.622	0.022	1.165 ±0.019	7:48 ±0:10	105.5 ±1.55	105.39 ±1.88
GM003	6.412 ± 0.058	12.21 ± 2.25	0.007	1.068* ± 0.015	13:26 ±1:58	95.2 ±0.96	98.50 ± 1.5
GM004	6.706 ±0.065	26.93 ±3.49	0.021	1.14 ±0.019	8:57 ±0:05	102.0 ±0.61	104.50 ± 1.55
GM005	6.85 ± 0.042	15.58 ±1.67	0.027	0.91* ±0.0093	7:24 ±0:20	94.4 ±1.25	99.75 ±1.47

-Uniformity of weight USP limit ± 5% weight of tablet

-Friability % limit < 1% Disintegration time within 15 min

USP range for Content Uniformity, and Assay 95%-105%

* One way analysis of variance ANOVA followed by post-hoc test indicating that the results were statistically significant (p<0.01) than the innovator glucophag

4. Discussion:

Glucophage® and five available generics of Metformin Hydrochloride were examined for their in-vitro equivalency under biowaiver conditions (Drugs.com. 2019, Rohilla S., et al, 2012). The study also evaluated quality control tests (thickness, hardness, friability, disintegration, uniformity of weight, content uniformity, assay, dissolution) according to the USP pharmacopeia (USP 34 NF 29, 2011).

Dissolution:

Dissolution rate determination is considered among the most important in-vitro tests to assess the quality of oral pharmaceutical solid dosage forms as tablets (Anand, O., et al, 2011). Solubility, hydrophobicity of the formulation, amount of disintegrant, binder, and the manufacturing method (compression force of the tablets) are considered among the main factors influencing the dissolution rate of a drug. According to the USP 34 NF29 2011(USP 34 NF 29, 2011) metformin monograph, the accepted criteria for the dissolution of metformin tablets should not be less than 75% of the labeled amount dissolved within 30 min.

Dissolution of metformin is an important aspect for drug bioavailability since it belongs to a class III drug (Crison, J., et al, 2012) it must be solubilized in the aqueous environment of the gastrointestinal tract to be absorbed. The dissolution profile of Glucophage® and its generics did not show a wide variety in the results in the chosen pH media. In order to judge the bioequivalence of the tested samples under biowaiver conditions, all dissolution profiles were compared to that of the brand Glucophage® using the similarity factor f2 and the difference factor f1.

The higher the similarity factor f2, and the lower the difference factor f1, the more similar the dissolution profile of the generic to the innovator will be. According to the WHO, a generic is said to be bioequivalent to the innovator if the similarity factor f2 > 50 in 3 dissolution media (Rohilla S., et al, 2012). GM001 and GM003 were bioequivalent to Glucophage® as they showed f2 > 50 in 3 dissolution media. Regarding GM002, GM004, and GM005 their similarity factor was not significant to show their equivalency to the innovator. This difference could be attributed to various binders and disintegrates used by different companies. Based on this assessment, only generic GM001, and GM003 can be interchanged with the innovator. Physical inspection of Metformin tablets showed that almost all samples complied with the USP specifications.

Thickness:

Thickness of tablets depends on the shape of the tablet which differs from one company to another. According to the USP the allowable percentage difference between each tablet and the average thickness of 10 tablets is ± 10%. The average thickness of Glucophage® innovator was 6.457±0.035mm, and those of the generics ranged from 6.33±0.035 mm to 6.85±0.042 mm. All the tablets of the generic and the innovator was within the specified range, thus the results complied with the USP requirements. Tablet thickness may play a role in the patient compliance (U.S. Department of Health and Human Services, CDER, (2015).

Hardness:

Hardness reflects the ability of tablets to withstand shipping, abrasion, transportation, handling, and other breakage storage conditions. It is an important criterion as it affects both disintegration, and dissolution. Tablets must be hard enough to withstand packaging and shipping, and yet soft enough to disintegrate properly after swallowing. Compression pressure applied during manufacturing, types of excipients as binder, and whether the tablets coated or not can reflect the extent of tablet hardness (U.S. Department of Health and Human Services, CDER, (2015). The hardness of Glucophage®, and its generic were tested and found to be very hard compared to the USP34 NF29 2011(USP 34 NF 29, 2011) limit (5-8 kg). The average hardness of the six batches ranged from 15.58 kg/cm2 - 24.04 kg/cm2 with Glucophage® recording the highest results among its tested generics. All the tested tablets failed to pass the hardness test. This result could be explained by the fact that the tablets are film coated to decrease their main side effect which is gastrointestinal side effect. Also, different excipients presented in each generic may explain the variety of hardness among the batches. The hardness of Metformin tablets didn't affect their solubility.

Friability:

Friability test is usually related to the hardness test. It is usually designed to assess the ability of the tablets to resist corrosion. During manufacturing and handling process, tablets are subjected to stressful conditions which can result in the removal of small fragments from the tablet surfaces (U.S. Department of Health and Human Services, CDER, (2015). According to the USP 34 NF29 2011(USP 34 NF 29, 2011), the maximum weight loss accepted should not exceeds 1%. Glucophage® friability was measured and found to have high percentage of friability compared to other generics recording 0.043%. The rest of generics had lower friability % ranging from 0.007% to 0.027%. Our tested samples passed this test with % friability <1%.

In general dose variation between tablets is measured by two tests, the weight uniformity and content uniformity test (USP 34 NF 29, 2011). Variation in weight may be attributed to either poor flow properties of the powder or mechanical problems of the tablet machine (USP 34 NF 29, 2011).

Content uniformity:

Content uniformity is done to ensure a constant dose of drug between individual tablets. The USP 34 NF29 2011(USP 34 NF 29, 2011) metformin monograph stated that the average content of metformin hydrochloride tablets ranged from 95% to 105. The content uniformity of the innovator, GM001, GM003, and GM004 were within the USP limit (95-105%) while GM002, and GM005 were out of the specified limit.

Uniformity of weight:

According to the USP 2010 (USP 33 NF 28. 2010), the allowed percentage deviation for tablets weighing more than 250 mg is ± 5% and not more than 2 tablets deviated outside the range, and none deviates by more than twice the allowed percentage.

The weight of Metformin hydrochloride tablets ranged from 1.068 g for GM003 to 1.165 g for generic GM002 except for generic GM005 0.91 g being lower in dose than the rest of the generics. GM002 deviated out of the specified range, while the rest of the generics had an accepted range and complied with the USP specifications except for GM002.

Disintegration:

Disintegration test aimed to test the ability of tablets to disintegrate within a prescribed time when present in a liquid media (USP 34 NF 29, 2011). The average disintegration time of the innovator Glucophage® was about 15:55 ± 0:23 sec and those of the generics ranging from 7:24 ±0:20 sec to 13:26 ±1:58 sec. The results shown complied with the USP specification (USP 34 NF 29, 2011), and the BCS class of metformin which stated that the disintegration time for class III BCS must not exceeds 15 min (Drugs.com. 2019, Rohilla S., et al , 2012).

Assay:

The assay method was used to prove that the amount of active ingredient of the product batch is close to its label claim. The results obtained from the assessment of the % content of active ingredients of the innovator and five tested generics of metformin hydrochloride showed values within the monograph specifications (95%-105%).

Main limitations of the study:

According to the FDA and WHO, In-vitro bioequivalence under biowaiver conditions could reflects the in-vivo bioequivalence for drugs classified as BCS 1,2,3 (Drugs.com. 2019, Rohilla S., et al ,2012). Even though, the real bioavailability and bioequivalence of the drugs can be confirmed and concluded by in-vivo studies. Factors such as human gastrointestinal tract with its own nature, excipients used, and different manufacturing conditions could affect the dissolution profile of the tested products. The study was conducted on only five generics of metformin hydrochloride.

5. CONCLUSION:

The debate whether generics could be interchangeable with brand remains a controversial issue. For that reason bioequivalence testing must be done to make a decision of which generic could be substituted by the innovator. The quality of the marketed products of metformin, brand and generics, was evaluated through quality control parameters done on the tablets.

They include tablet thickness, hardness, friability, disintegration, weight uniformity, content uniformity, and assay. According to the USP 34 NF 29 2011 (USP 34 NF 29, 2011), any change in these characteristics can significantly affect the safety and efficacy of the product. Glucophage® and generics showed compliance with the USP specifications and limits.

In-vitro bioequivalence testing was conducted under the WHO biowaiver testing procedure to determine whether generics could be interchangeable with the innovator Glucophage®. Dissolution profiles revealed differences between GM002, GM004, and GM005 with respect to the innovator Glucophage®. While GM001, and GM003 showed their bio-equivalency to the innovator Glucophage®.

Now and along with our study's result, we do recommend that Glucophage® could be substituted firstly by GM001, and GM003. Even though, we cannot judge that the reset of generics were not bio-equivalent to the innovator. In order to confirm the in-vitro results, in-vivo studies should be implemented. The difference among generics could be referred to different excipients used, various manufacturing conditions, and metformin's low permeability may affects its dissolution profile.

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