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In-vitro bioequivalence study of 8 brands of metformin tablets in Iran market

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ABSTRACT

Bioequivalence studies are the commonly accepted methods displaying therapeutic equivalence between two products. This study was conducted to evaluate the bioequivalence between different formulations of metformin 500 mg and 1000 mg tablets which were marketed in Iran, and innovator brand. Considering that only in vitro bioequivalence studies can predict the in vivo bioequivalence, and to save time and cost, three essential in vitro tests including assay, weight variation and a comparative in vitro dissolution study were performed. In order to compare formulations, dissolution profiles were taken and compared through two model independent methods, difference factor (f1) and similarity factor (f2). All the tested brands released more than 80% drug in 30 minutes and contained 95-96.3% of labeled amount except b and C. The acceptance value in all cases were below 15. Therefore it is evident that test products except brand C were bioequivalent to the reference product, and could be used as a generic substitute for the innovator product. Results emphasize to need for post marketing investigation for new formulations.

Keywords: Metformin tablets, Bioequivalence, In vitro, Dissolution profile.

INTRODUCTION

In recent years, in many countries generic copies of the reference medicinal products containing identical amounts of the same active ingredient in the formulation and same route of administration were made and generic drug products have become very popular. Evidences point to the fact that different products with the same amount of active pharmaceutical ingredient have shown distinct differences in their therapeutic effects (Esimone, Okoye *et al.*, 2008; Fujii, Yasui-Furukori *et al.*, 2009). This may be due to the differences in rate and extent of absorption, possibly by the reason of difference between the purity of active ingredients, type of excipients, proportion between them and the manufacturing variables such as the influence of mixing method and granulation procedure as well as coating parameters (Pillay and Fassihi 1998; Maggio, Castellano *et al.*, 2008). Therefore there are serious concerns that various generic substitutions may have different bioavailability and couldn't be used interchangeably.

Two pharmaceutical products are considered to be equivalent when their bioavailability factors are so similar that they could show clinically comparable therapeutic effects. Release of active pharmaceutical ingredient from the final product, its dissolution under physiological conditions and its permeability across the gastrointestinal tract are essential steps in drug absorption. Bioequivalence (BE) studies focus on the drug release from the formulation and subsequent absorption into the systemic blood circulation which consist of both in vivo and in vitro studies. Considering the first two step in absorption, in vitro dissolution may be applicable to the prediction of in vivo BE (Polli 2008). Until recent years, bioequivalence was determined only by in vivo tests. However, there are many reports that have been utilized in vitro bioequivalence studies instead of in vivo bioequivalence tests for immediate release solid oral dosage forms of highly soluble class I and III drugs. Therefore in vitro tests can be used solely to determine bioequivalence of products (Anderson, Bauer *et al.*, 1998; Pillay and Fassihi 1998; Yuksel, Kanik *et al.*, 2000; Yu, Amidon *et al.*, 2002; 2003; Gupta, Barends *et al.*, 2006; Maggio, Castellano *et al.*, 2008; Polli 2008). According to US Pharmacopeia, necessary in vitro tests are assay, content uniformity and dissolution studies. Three categories of dissolution test specification for immediate release products are described in the guidance provided by the Center for Drug Evaluation and Research at the Food and Drug Administration: (a) single point specifications, (b) two-point specifications, and (c) dissolution profile comparison. The dissolution profile comparison is more precise than others to characterize the drug product (Yuksel, Kanik *et al.*, 2000). To compare dissolution profiles, two model independent fit factors, the difference factor (f1) and the similarity factor (f2) introduced by Moore and Flanner (1996) as mathematical indices, were used in this study (Anderson, Bauer *et al.*, 1998; Cheng, Yu *et al.*, 2004; Maggio, Castellano *et al.*, 2008). Metformin is a biguanide which is used orally in hyperglycemic patients. Nowadays it is widely used in the management and control of non-insulin dependent diabetes mellitus (NIDDM). Absolute bioavailability of metformin when given orally is 50–60% and biological half-life of metformin is 1.5–1.6 h. Chemically it is N,N-dimethylimidodicarbonimidic diamide and freely soluble in water and has low permeability to cell membranes. Therefore, it can be classified as a BCS Class III drug (Stepensky, Friedman *et al.*, 2001; Adikwu, Yoshikawa *et al.*, 2004; 2006; Hu, Liu *et al.*, 2006; Ali, Arora *et al.*, 2007). Despite of widespread of NIDDM in Iran, and extensive use of metformin, there are no reports on the bioavailability and bioequivalence of the various brands of metformin tablets in our country. Hence the present study was carried out to investigate the bioequivalence of metformin tablets in Iran market and innovator product.

MATERIALS AND METHODS

Materials

Eight different brands of metformin tablets were used which are shown in table 1. Metformin and phosphate buffer were purchased from Merck –Germany.

Table 1: Characteristics of tested metformin tablets.

Formulation code	Producer	Dosage	Country	Batch number
A	Exir	500 mg	Iran	082012.843
B	Kimi Daru	500 mg	Iran	6012016
C	Aria	500 mg	Iran	901237
D	Apo	500 mg	Canada	248939
E	Tehran Shimi	500 mg	Iran	0491-278
F	Soha	1000 mg	Iran	S004
G	Hexal	1000 mg	Germany	Ay 4247
H	Merck	500 mg	Germany	102662

Content uniformity

10 tablets from each of the 8 brands were selected by chance, weighted individually with an analytical weighting balance (GR-200, Max 219g, min 10mg, e=1mg, d=0.1mg). The average weight, SD, RSD and acceptance value for each brand were calculated. Then the drug substance content, expressed as % of label claim, of each tablet from the weight of the individual tablet and the result of the assay was calculated. For calculation of acceptance value (AV) the following formula was used (2007).

$$\text{Acceptance value} = |M - \bar{X}| + ks$$

In which M is the reference value, \bar{X} is the mean of individual contents expressed as a percentage of the label claim, k is the acceptability constant (If the number of individuals is 10 then k=2.4, and if the number of individuals is 30, then k=2.0), and s is the sample standard deviation.

Assay

Standard solution was prepared by dissolving pure metformin in distilled water (1mg/ml). To prepare the test solutions, 10 tablets of each brand were crushed, finely powdered, then equivalent to 100 mg of the metformin were weighted and dissolved in 100 ml distilled water. The active pharmaceutical ingredient content of standard and sample solutions were determined by measuring their absorbance after dilution versus the blank at 232 nm using an ultraviolet spectrophotometer (shimadzu UV -1800, Japan). The ratio of drug content in samples compared to standard were calculated and reported in percentage for each brand (2007).

Dissolution test

Before performing dissolution test, to calculate the concentration, 6 serially diluted solutions of pure metformin with the concentration of 0.3125 ($\mu\text{g/ml}$) to 10 ($\mu\text{g/ml}$) were prepared from a stock solution, and standard curve was drawn. The curve was linear between 0.3125 $\mu\text{g/ml}$ and 10 $\mu\text{g/ml}$ with a correlation coefficient of 0.9995. The dissolution test was undertaken using USP apparatus II (Erweka DT6R) with the rate of 100 rpm at 37°C on 6 tablets of each brand (2007). The dissolution medium was 900 ml phosphate buffer (pH=6.8). To draw dissolution profile, 5 ml of dissolution samples were withdrawn at different time intervals up to 60 min and replaced with the same volume of prewarmed dissolution medium. Subsequently samples after 100 fold dilution were assayed by ultraviolet spectrophotometer at an absorbance wavelength of 232 nm. The concentration of each sample was

determined from a calibration curve. The main purpose of performing dissolution study for test and reference product was to compare product's dissolution profiles. All drug products has dissolution specification, Q, stated in the USP, and for passing the test, all metformin immediate release tablets must release 80% of drug within 30 minutes (2007). The dissolution profiles were compared using two model independent parameters: the difference factor (f1) and the similarity factor (f2) derived from the dissolution profiles. The f1 factor measures the percent difference between two concentration curves and the f2 factor shows similarity between them over all time points. f1 is zero and f2 is 100 when the test and reference drug profiles are identical. f1 increases and f2 decreases proportionally as the dissimilarity increases. Two dissolution profiles are verified similar if f1 is between 0 and 15 and if f2 is between 50 and 100. f1 and f2 can be calculated from following equations:

$$F1 = \left\{ \frac{\sum_{t=1}^n wt |R_t - T_t|}{\sum_{t=1}^n wt R_t} \right\} \times 100\%$$

$$F2 = 50 \log \left\{ \left[1 + \frac{1}{n} \sum w_i (R_i - T_i)^2 \right]^{-0.5} \times 100 \right\}$$

Where R_t and T_t are the cumulative percentage of dissolved drug for the reference and test formulation at time t , respectively, n is the number of time points and w_i is an optional weight factor (Anderson, Bauer *et al.*, 1998; Pillay and Fassihi 1998; Su, Chou *et al.*, 2003; Menegola, Steppe *et al.*, 2007). The dissolution results have served as a means to evaluate different formulations and determine final dissolution qualifications for pharmaceutical dosage form.

RESULTS AND DISCUSSION

Weight variation

Uniformity of Dosage Form can be demonstrated by two methods, content uniformity and weight variation. If uncoated or film coated tablets contain 25 mg or more drug substance that comprise 25 % of each tablet weight, weight variation is applicable for the test of Uniformity of Dosage Form. Therefore considering the high amount of active ingredient (500 mg) in each dosage form in the present study, weight variation method was performed. 10 tablets of each brand weighted and the mean weight, SD, RSD and acceptance value were calculated, and illustrated in Table 2. The requirement for dosage uniformity was met, since the calculated acceptance values of the first 10 dosage units for each brand is less than L1% or 15. (2007). Thus all brands used in Iran market and

innovator products were suitable in the case of weight variation and all were in the acceptable value range.

Assay

Results achieved from analysis of active ingredient in brands and innovator products exhibit in table 3. As USP specified, the content should not be less than 95% and not more than 105% of labeled amount (2007). Results in table 3 indicate that all products except brand C stayed on the acceptable limits. Table 3

Dissolution test

Oral dosage forms only become available for absorption following the process of disintegration and dissolution. Dissolution testing is employed to distinguish the influence of manufacturing variables such as binder effect, mixing effect, granulation procedure, excipients type and can be used as a tool to predict product behavior in vivo (Papadopoulou, Valsami *et al.* 2008). Consequently dissolution test is currently used as an in vitro bioequivalence (BE) test, generally for figuring out dissolution profile and profile comparison, establishing the similarity of pharmaceutical dosage forms (Amidon, Lennernas *et al.*, 1995; Cheng, Yu *et al.*, 2004; Esimone, Okoye *et al.*, 2008). Eight different brands of metformin tablets were studied, with brand H being the innovator. To compare the dissolution profiles, dissolution curve (based on mean percentages of drug released) of test and reference products were combined and depicted in figure 1. In this study, as expected for highly soluble compound, metformin, it was observed that for all products, at least 80% release in 30 min took place except brand C. Therefore all formulations excluding formulation C passed this acceptance pharmacopeia criterion (2007).

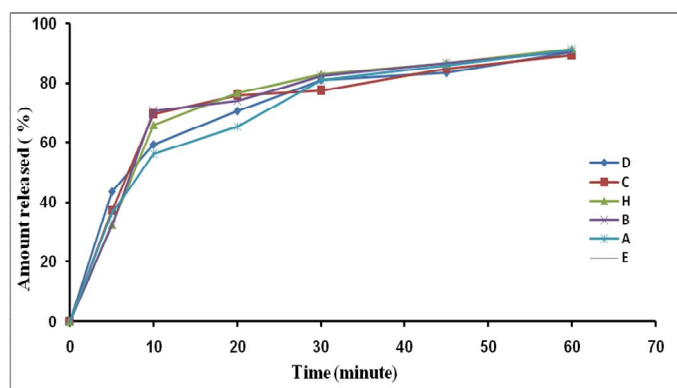


Fig. 1: Comparative dissolution profiles of metformin tablets.

Table 2: Mean weight, acceptance value, SD and RSD for uniformity of weight test for different metformin tablets.

Formulation code	A	B	C	D	E	F	G	H
Mean weight	552.1	683.7	566.4	623.3	575.6	1052.3	1052.3	531.8
Acceptance value	3.8	7.5	13.3	5.2	6.3	4.9	4.0	4.2
SD	3.2	13.6	6.4	6.8	7.1	7.0	8.6	4.8
RSD	0.6	1.9	1.1	1.1	1.2	0.7	0.6	0.9

Table 3: Amount (% of labeled) of metformin in tests and innovator products.

Formulation Code	A	B	C	D	E	F	G	H
Mean content (%)	96.3	95.5	87.5	95.7	95.0	95.1	95.9	96.5
SD	0.5	1.1	8.8	1.2	1.8	1.8	3.8	0.3
RSD	0.5	1.2	10.1	1.2	1.9	1.9	3.9	0.3

The use of fit factors was also recommended for dissolution profile comparison in the FDA's guides for industry. According to these guides, generally, f1 values up to 15 (0–15) and f2 values greater than 50 (50–100) ensure similarity or equivalence of the two curves (Yuksel, Kanik *et al.*, 2000). F1 and f2 values were calculated between the test products and reference product and illustrated in table 3. Significant differences were not observed in both parameters and this confirmed similarity between all brands formulations compared with innovator product and indicated that the release of metformin from all formulations were similar to reference. However comparison of the two dissolution curves shows that brand C couldn't release 80% of drug during 30 minutes. Therefore taking all results into account, all formulations are comparable with reference and there is essential similarity between all formulations with reference product except brand C.

Table 4: The calculated similarity and difference factors for tested products.

Formulation code	A	B	C	D	E
F1	6.53	2.09	4.3	6.95	2.65
F2	59.29	80.43	71.79	60.42	79.46

CONCLUSION

Compared to the *in vivo* BE tests, conventional *in vitro* studies are less complicated, fast, economic and useful quality control tool and evaluate more directly drug absorption than *in vivo* bioequivalence studies. For application and interpretation of dissolution profile, fit factors are easier to use; only one value is obtained to describe the similarity of the two dissolution profiles in order to demonstrate bioequivalence. However, the analyses must be made with the same time points and sufficient pairs of batches should be compared to obtain a statistically significant result (Anderson, Bauer *et al.*, 1998; Yuksel, Kanik *et al.*, 2000; Cheng, Yu *et al.*, 2004; Maggio, Castellano *et al.*, 2008). In the current study, the f1 and f2 values were in the range of 2–7 and 59–80 respectively. This suggests that the release of metformin from all formulations were similar with reference. On the other hand, for all products except formulation C the drug delivery was satisfactory since at least 80% was dissolved in 30 min. Therefore results confirm the presence of bioequivalence between the analyzed brands and reference product with the exception of brand C. This study verifies serious need for constant post marketing monitoring of the marketed products with the view to bioequivalence and agreement with pharmacopoeia standards.

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CONFLICT OF INTEREST

The authors indicate no conflicts of interest regarding the content of this article.

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