

Bioequivalence of Two Brands of Metformin 850 mg Coated Tablets in 12 Healthy Algerian Volunteers: A Pilot Study

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Abstract: A randomized, two-way, crossover study was conducted in 12 fasting, healthy, Algerian volunteers to compare the bioavailability of two brands of metformin hydrochloride 850 mg coated tablets. The present study aimed to appreciate the bioequivalence of the generic product and to evaluate the intra-subject variability of this active substance in the Algerian population. The test brand was compared to Glucophage (Merck UK) as the reference product. The study was performed at the bioequivalence center of the national control laboratory for pharmaceuticals products from 03 to 04, 2011, in joint venture with specialized medical hospital center of El Hadi Flici, Algiers, Algeria. The drug was administered with 200 mL of water after a 10 h overnight fasting on two treatment days separated by one week washout period. After dosing, serial blood samples were collected for a period of 12 h. A reliable, simple, and robust liquid chromatography-tandem mass spectro-metric (LC-MS/MS) method has been developed and validated for estimation of metformin in human plasma using propranolol as internal standard. The analytes were extracted from plasma by using the protein precipitation extraction technique. The assay was found to be linear over the range of 50-3000 ng/mL with a lower limit of quantitation of 50 ng/mL. Various pharmacokinetic parameters including AUC_{0-t} , $AUC_{0-\infty}$, C_{max} , T_{max} , and $T_{1/2}$ were determined from plasma concentrations of both formulations and found to be in good agreement with reported values. The pharmacokinetic and statistical analysis was conducted with Kinetica 4.4.1. AUC_{0-t} , $AUC_{0-\infty}$ and C_{max} were tested for bioequivalence after log-transformation of data. No significant difference was found based on ANOVA; 90% confidence interval ([91.62 %, 115.66%] for AUC_{0-t} ; [92.07 %, 115.53 %] for $AUC_{0-\infty}$; [94.58%, 119.58 %] for C_{max}) of test/reference ratio for these parameters were found within bioequivalence acceptance range of 80-125%. Based on these statistical inferences, it was concluded that Metformin hydrochloride test is bioequivalent to Glucophage.

Key words: Metformin hydrochloride, bioequivalence, LC-MS/MS, pharmacokinetics.

1. Introduction

Bioequivalence of two formulations of the same drug includes equivalence with respect of the rate and extent of their absorption. The area under concentration time curve (AUC) generally serves as the characteristic of the extent of absorption while the peak concentration (C_{max}) and the time of its occurrence (T_{max}) reflect the rate of absorption [1].

Metformin is an oral hypoglycemic agent that belongs to the class known as biguanides, the ultimate goals of metformin are to lower blood sugar to a

normal level and maintain this level. Metformin improves peripheral glucose tolerance, decreases hepatic glucose output, and improves muscle sensitivity to insulin and glucose uptake non-insulin-dependent diabetes mellitus. In patients receiving metformin, a significant reduction in hepatic glucose output has been observed [2].

Metformin is the first-line medication for the treatment of type 2 diabetes, particularly in people who are overweight. It helps diabetics to respond normally to insulin. Like most diabetic drugs. Metformin can be used in conjunction with other diabetic drugs [3, 4]. Because of the low bioavailability and interindividual

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variability in the absorption of the different pharmaceutical forms of metformin, it is necessary to perform comparative bioavailability studies. Thus, regulatory authorities and medical prescribers would have the scientific support to expect a therapeutic equivalence if bioequivalence among the compared pharmaceutical forms is demonstrated. The aim of this study was to evaluate, in healthy Algerian volunteers, the bioequivalence of a generic of metformin and the reference product Glucophage® from Merck laboratories in order to evaluate the intrasubject variability of metformin (Coefficient of variation intrasubject: CV_{intra} of C_{max} and AUCs) and to validate the application of developed LC-MS/MS metformin hydrochloride quantification method.

2. Materiel and Methods

2.1 Study Products

Two oral formulations of metformin hydrochloride 850 mg were evaluated:

Reference formulation: GLUCOPHAGE® 850 g coated tablet (batch number MC3, expiry date 09/2011 manufactured by Merck UK).

Test formulation: metformin hydrochloride 850 mg coated tablet (batch number 500011, expiry date 12/2013).

2.2 Study Subjects

12 healthy algerian subjects (04 male and 08 female), suitable for a pilot study, were enrolled into the study with mean (SD) age, 27.08 (2.87) years (range 23-35); mean (SD) body weight, 62.58 (10.13) kg (range 48-85 kg); mean (SD) height, 1.6583(0.01027) m (range 1.53-1.85 m) and mean(SD) body mass index (BMI), 22.75 (3.05) kg/m^2 (range 18.5-26.7 kg/m^2).

The volunteers were screened by a complete clinical examination and laboratory tests (hematological, biochemical and urinary analysis and serological test) and were requested to be abstained from taking any medication for 2 weeks before and during the study, from taking vitamins 2 days prior the study, from taking grapefruit 7 days before the study and from smoking, as well as consuming caffeine or drinks or foods containing xanthines related for 48 h prior to the study drug administration.

2.3 Ethical Consideration

This research was carried according to the Declaration of Helsinki (Seoul, 2008) and GCP (good clinical practice) Guidelines.

The study was conducted at National Control Laboratory for Pharmaceuticals Products (Algiers, Algeria) according to a protocol approved by Research

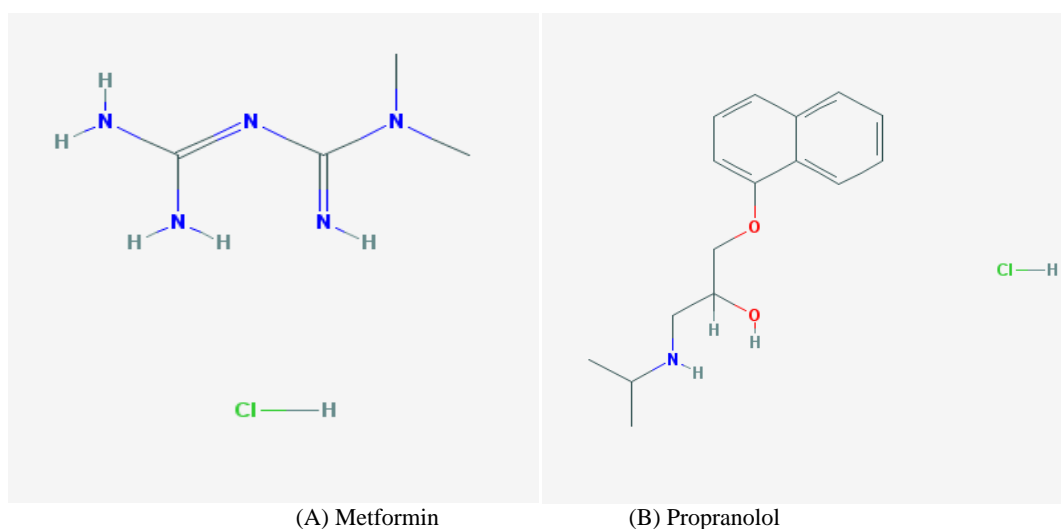


Fig. 1 Chemical structures of metformin hydrochloride (3-(diaminomethylidene)-1,1-dimethylguanidine;hydrochloride) and propranolol hydrochloride (1-naphthalen-1-yloxy-3-(propan-2-ylamino)propan-2-ol;hydrochloride) [5, 6].

Ethics Committee of the Specialized Medical Hospital center of El kettar and by ministry of health.

All the subjects provided written informed consent before entering the study.

2.4 Study Design

The study was based on a randomized, single dose, two way crossover designs under fasting condition with a washout period of one week.

The first period was in 03/03/2011 and the second period was in 10/03/2011.

In the morning of period I and II, after an overnight fast (10 h) volunteers were given a single dose of either formulation (reference or test) of metformin 850 mg with 200 mL of water. No food was allowed until 4 h after dose administration. The volunteers take 100 mL of glucose 10% solution after 2 h and 100 mL of glucose 5% after 03 h of drug administration, lunch and snack were given to all volunteers according to a time schedule. The volunteers were continuously monitored by Specialized Medical Center Hospital of El kettar staff throughout the confinement period of the study.

2.5 Blood Sampling

Approximately, 04 mL of blood samples for metformin assay was obtained through a heparin-locked catheter before (0 h) and at 0.33, 0.66, 1.0, 1.33, 1.66, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0, 8.0, 10.0, 12.0 after dosing. The blood samples were collected in glass tubes containing heparin, and centrifuged at 3500 rpm for 10 min; plasma was separated and kept frozen at -80 °C in properly labelled tubes. After a period of 7 days, the study was repeated in the same manner to complete the crossover design.

2.6 Optimization of MS Parameters and Chromatographic Conditions

An LC-MS/MS method was developed and validated, for metformin analysis in plasma samples. All solvents were HPLC grade, other chemicals and reagents were analytical grade. Metformin

hydrochloride and propranolol hydrochloride (internal standard) were used as reference standards.

The LC-MS/MS system consisted of HPLC Perkin Elmer SER 200 witch containing an autosampler SER 200 and a binary pump (LC-200Q/410).

Masse spectrometer AB Sciex Instruments, 3200 Q Trap triple quadrupole instrument was equipped with an ESI source. Analyste 1.5.1 software was used for data interpretation.

The method was developed in positive mode with turbospray source (ESI) by infusion of 0.1 µg/mL aqueous solutions of metformin and propranolol reference standards. The ion transitions m/z 130.1→71.1, 130.1→60.1 and 260.2→116.3, 260.2→183.2 were selected for the MRM of metformin and propranolol respectively. The compound parameters were optimized as follows: Declustering potential: 30 V, entrance potential: 5 V, collision cell entrance potential: 12.97 V, 18.18 V collision cell exit potential: 2.90 V, 3.74 V, and collision energy: 32 V, 23.84 V for metformin and propranolol respectively. The source/gas parameters were optimized as follows: Curtain gas: 20, CAD: Medium, ion source gas-1: 50, ion source gas-2: 60, ion spray voltage: 4,000 V and temperature: 550 °C.

Chromatographic separation was performed using SUPELCO Ascentis™ Phenyl (250 × 4.6 mm, 5 µm) column. The mobile phase consisted of 80% acetonitrile grade HPLC and 20% 50 mM ammonium acetat with 0.5% acetic acid buffer. The mobile phase was eluted at a flow rate of 1.0 mL/min in isocratic mode, each analysis required 6 min. The retention time was 3.60 min and 4.65 min for metformin and propranolol respectively [7]. Quantitation was achieved by measurement of the peak area ratio of the drug to the internal standard, using Analyst 1.5.1 software.

The method was validated according to FDA guidelines [8]. The calibration curves were validated over the concentration range of 50-3,000 ng/mL for metformin in human plasma in the low limit of

quantification LLOQ of 50 ng/mL.

2.7 Sample Preparation

A 50 µl internal standard (propranolol, 500 µg/mL) was added to 250 µL plasma sample and vortexed for 30 seconds then 10 µL of perchloric acid 70% was added and vortexed for 30 seconds and then centrifuged for 5 min at 19,000 g. 1 mL of dilution solvent (20 mL acetonitrile, 180 mL of pure water and 200 µL of NaOH) was added to 20 µL of the supernatant and vortexed for a few seconds then transferred to a vial. 10 µL of the aliquot was injected to the column.

The procedure described here was applied not only to subject's samples, but also to the extraction of samples for calibration curve and QC (quality control) process.

2.8 Pharmacokinetic Analysis

Pharmacokinetic analysis was performed by means of a model independent method using a Kinetica 4.4.1 computer program [9]. The elimination rate constant (k_z) was obtained as the slope of the linear regression of the log-transformed concentration values versus time data in the terminal phase. The elimination half-life ($T_{1/2}$) was calculated as $0.693/k_z$. The area under the curve to the last measurable concentration AUC_{0-t} was calculated by the linear trapezoidal rule. The area under the curve extrapolated to infinity $AUC_{0-\infty}$ was calculated as $AUC_{0-t} + Ct/k_z$, where Ct is the last measurable concentration.

2.9 Statistical Analysis

For the purpose of bioequivalence analysis AUC_{0-t} , $AUC_{0-\infty}$ and C_{max} were considered as primary variables. The bioequivalence of the two products was assessed by means of an analysis of variance (ANOVA GLM procedure; Kinetica 4.4.1 Computer program [9] for crossover design and calculating standard 90% confidence intervals of the ratio test/reference (T/R) using log-transformed data. The products were considered bioequivalent if the difference between the two compared parameters was found statistically insignificant ($p \geq 0.05$) and 90% confidence intervals for these parameters fell within 80%-125% [10, 11].

3. Results and Discussion

Total of twelve volunteers were enrolled, all of whom completed both treatment periods of the study with no protocol violations. Metformin was well tolerated by all volunteers.

The relationship between concentration and peak area ratio was found to be linear within the range 50-3,000 ng/mL with LLOQ of 50 ng/mL. As shown in Table 1, the intraday accuracy of the method ranged from 94.9% to 102.61% while the intraday precision ranged from 3.56% to 4.16%. The inter-day accuracy ranged from 95.61% to 106.71% while the inter-day precision ranged from 6.82% to 10.99%.

This reproducibility of metformin was able to increase assay senility. Therefore, simple serum deproteinization procedure using perchloric acid 70%

Table 1 Precision and accuracy of metformin in human plasma.

Concentration ng/mL	Precision (CV %)		Accuracy (%)	
	Intra-day	Inter-day	Intra-day	Inter-day
50 (LLOQ)	3.71	10.99	99.95	99.55
150	3.56	6.82	102.61	106.71
1,500	3.67	8.72	94.9	95.61
2,500	4.16	9.28	95.63	101.29
Mean ± SD	3.77 ± 0.26	8.95 ± 1.71	98.27 ± 3.65	100.79 ± 4.60

LLOQ = lower limit of quantification. CV = coefficient of variation = (SD/mean)*100. All the data were presented as arithmetic means.

and dilution solvent has been successfully applied to the extraction of metformin from human plasma.

Stability studies showed that metformin was stable in plasma for 4 weeks when stored at -20°C . Metformin was well tolerated, and all the subjects carried the study for the end.

Both formulations were rapidly absorbed from the gastrointestinal tract and metformin was measurable at the first sampling time (0.33 h) in all the volunteers. The peak concentration of 2494.2 ng/mL and 2667.5

ng/mL for metformin were attained at 2.27 h and 2.38 h after administration of reference and test products, respectively and then declined rapidly and remained detectable up until 12 h. Table 2 shows the pharmacokinetic parameters of metformin for the two brands.

The relative bioavailability of metformin test was 100.31% for AUC_{0-t} , 100.32% for $\text{AUC}_{0-\infty}$, and 100.78% for C_{max} .

The 90 % confidence limits for AUC_{0-t} , $\text{AUC}_{0-\infty}$, and

Table 2 Pharmacokinetic parameters of metformin hydrochloride coated tablets (arithmetic mean \pm standard deviation, $n = 12$).

Pharmacokinetic parameter	test	reference
C_{max} (ng/mL)	2,667.5 \pm 748.48	2,494.2 \pm 631.89
SSC_{0-t} (ng. h/mL)	13,783 \pm 3,310.8	13,448 \pm 3,330.3
$\text{SSC}_{0-\infty}$ (ng. h/mL)	14,972 \pm 3,350.4	14,653 \pm 3616.7
t_{max} (h)	2,385 \pm 0.90818	22,742 \pm 0.72268
$t_{1/2}$ (h)	32,522 \pm 0.87673	30,124 \pm 0.486

Table 3 The statistical evaluation of bioequivalence after oral dosage of 850 mg metformin hydrochloride of each formulation.

	Geometric Mean \pm SD		CI	CV_{intra}	t -test	
	Test	Reference			Lower	Upper
AUC_{0-t} (ng/mL·h)	9.50 \pm 0.27	9.47 \pm 0.22	[91.62%, 115.66%]	15.83%	3.68	2.84
$\text{AUC}_{0-\infty}$ (ng/MI·h)	9.58 \pm 0.22	9.55 \pm 0.27	[92.07%, 115.53%]	15.28%	4.58	2.67
C_{max} (ng/mL)	7.84 \pm 0.28	7.78 \pm 0.26	[94.58%, 119.58%]	15.94%	3.35	2.07

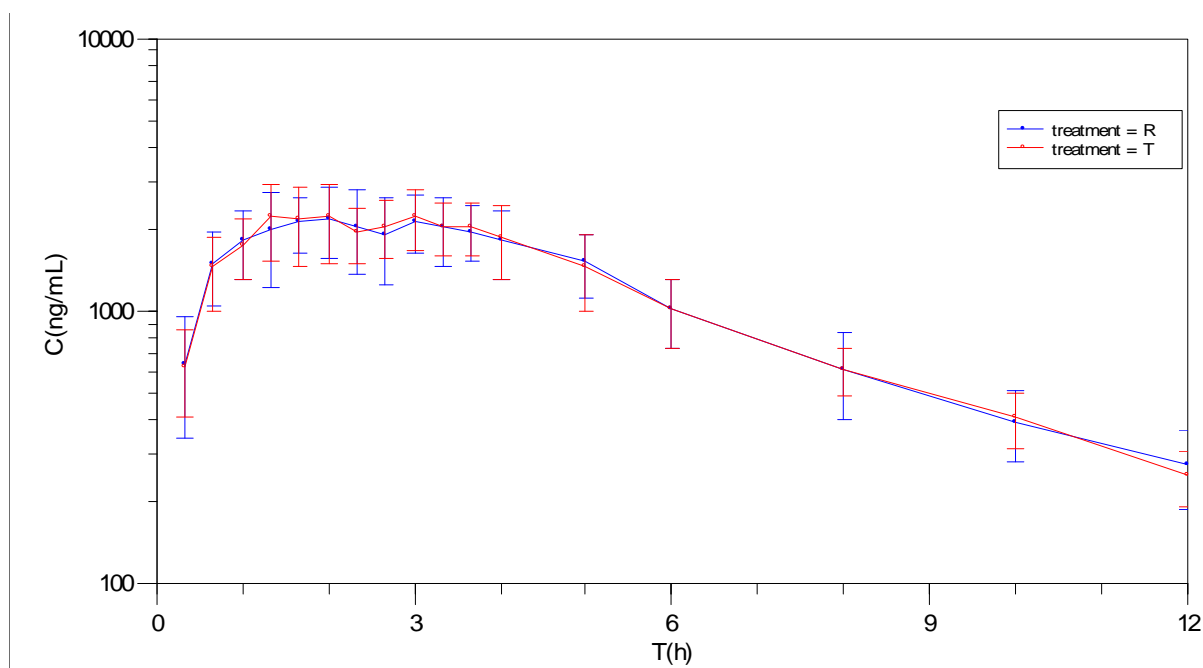


Fig. 3 Mean drug plasma concentration-time profiles of metformin test and reference (In transformation).

C_{\max} as well as the results of the Schuirmann's two one-sided t -tests are also shown in Table 3.

Mean drug plasma concentration-time profiles of metformin (Fig. 3) were nearly identical, suggesting an equal *in vivo* performance of the two products.

The mean and standard deviation of AUC_{0-t} , $AUC_{0-\infty}$ and C_{\max} of the two products did not differ significantly, suggesting that the blood profiles generated by metformin test are comparable to those produced by Glucophage. ANOVA (Analysis of variance) for these parameters, after log-transformation of the data, showed no statistically significant difference between the two formulations either in periods, formulations or sequence, having p value greater than 0.05.

The intra subject variability was low (approximately 16%) and homogenous between the three parameters AUC_{0-t} , $AUC_{0-\infty}$, and C_{\max} : number of subject should be sufficient to demonstrate bioequivalence.

The 90 % confidence interval of AUC_{0-t} , $AUC_{0-\infty}$, and C_{\max} was within the acceptable bioequivalence range of 80 % to 125 %, and that the lower and upper limits of the calculated Schuirmann's t -tests were greater than the critical t -value.

4. Conclusions

The results of PK analysis suggested that the reference and test formulations of metformin 850 mg coated tablets were bioequivalent during fasting state in these healthy algerian volunteers. Because of the low intra-subject variability of metformin in this study, we conclude that this pilot study conducted with 12 volunteers was sufficient and doesn't need a pivot study to demonstrate the bioequivalence of this active substance.

In conclusion, of the two metformin, formulations are equivalent with respect to the rate and extent of absorption and it can be assumed to be therapeutically equivalent and exchangeable in clinical practice.

References

- [1] Food and Drug Administration. 2003. Guidance for Industry: Bioavailability and Bioequivalence Studies for Orally Administered Drug Products Center for Drug Evaluation and Research (CDER).
- [2] Dunn, J. C., and Peters, D. H. 1995. "Metformin: A Review of Its Pharmacological Properties and Therapeutic Use in Noninsulin-Dependent Diabetes Mellitus." *Drug* 49 (5): 721-49.
- [3] Goo, A. K. Y., Carson, D. S., and Bjelajac, A. 1996. "Metformin: A New Treatment Option for Non-Insulin-Dependent Diabetes Mellitus." *J Fam Pract* 42: 612-8.
- [4] Product Information: Glucophage (R), metformin hydrochloride. 1999. Bristol-Myers Squibb Company: Princeton, NJ.
- [5] Pubchem. 2005. Metformin hydrochloride[C4H12ClN5-PubChem [Internet]. [cit e 30 mars 2017]. Disponible sur: https://pubchem.ncbi.nlm.nih.gov/compound/Metformin_hydrochloride.
- [6] Pubchem. 2005. Propranolol hydrochloride[C16H22ClNO2-PubChem [Internet]. [cit e 30 mars 2017]. Disponible sur: https://pubchem.ncbi.nlm.nih.gov/compound/Propranolol_hydrochloride.
- [7] Sengupta, P., Bhaumik, U., Ghosh, A., Sarkar, A. K., Chatterjee, B., Bose, A., et al. 2009. "LC-MS-MS Development and Validation for Simultaneous Quantitation of Metformin, Glimepiride and Pioglitazone in Human Plasma and Its Application to a Bioequivalence Study." *Chromatographia* 69 (11-12): 1243.
- [8] Food and Drug Administration of the United States. 2001. Guidance for Industry-Bioanalytical Method Validation, US Department of Health and Human Services, Center for Drug Evaluation and Research (CDER), Center for Veterinary Medicine (CVM).
- [9] Kinetica, T. M. 2005. Version 4.4.1, Innaphase, User Manual.
- [10] Schuirmann, D. J. 1987. "Pharmacometrics: A Comparison of the Two One-sided Tests Procedure and the Power Approach for Assessing the Equivalence of Average Bioavailability." *Journal of Pharmacokinetics and Biopharmaceutics* 15 (6): 657- 81.
- [11] Food and Drug Administration. 2001. Statistical Approaches to Establishing Bioequivalence, Center for Drug Evaluation and Research (CDER), Rockville, Maryland.