

Metformin IR tablets: partial in vitro dissolution profiles differences do not preclude in vivo bioequivalence

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Abstract— Objectives: To investigate whether Metformine Profarma 850 mg tablets (test product) are bioequivalent to Glucophage® 850 mg tablets (Merck Santé laboratories - reference product) despite partial in vitro dissolution profiles differences observed.

Methods: A randomized, open-label, single-dose, two-period, one-week wash out, crossover study was performed in 20 healthy male and female volunteers at "Mother Theresa" University Hospital Centre, Tirana, Albania, after obtaining the approval by National Ethics Committee. A single 850mg dose of metformin was administrated with 200 ml of water after overnight fasting and blood samples were collected at 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, 12 and 14 h after dosing. Plasma concentrations were measured by using a validated ion-pair HPLC method with UV-DAD. Non-compartmental pharmacokinetic parameters such as C_{max} , $AUC_{0-14\ h}$, AUC_{0-inf} and T_{max} were determined using PKSolver Version 2.

Results: Administration of single Metformin Profarma 850 mg and Glucophage® 850 mg tablets resulted in comparable systemic exposures to metformin, as determined by C_{max} , AUC_{0-14} and AUC_{0-inf} . ANOVA analysis of the ln-transformed C_{max} , AUC_{0-14} and AUC_{0-inf} values indicated that none of the effects examined (formulation, period, sequence and carry over) was statistically significant. The geometric mean ratios of C_{max} , AUC_{0-14} and AUC_{0-inf} were 103.0%, 99.3% and 98.8%, respectively, and 90% confidence intervals of C_{max} , AUC_{0-14} and AUC_{0-inf} were contained within the bioequivalence acceptance limits of 80% to 125%.

Conclusions: Metformin Profarma 850 mg and Glucophage® 850 mg tablets were shown to be bioequivalent despite the in vitro dissolution profiles indicate a faster dissolution rate for Metformine Profarma 850 mg tablets, at least in one dissolution medium.

Keywords— metformin; dissolution profile; pharmacokinetics; bioequivalence

I. INTRODUCTION

Metformin, an oral biguanide, is actually considered the first-line drug of choice for the treatment of type 2 diabetes [1]. Although the mechanisms of action are not fully elucidated, the suppression of hepatic glucose production is clearly the major one. Also, some evidence exists, which suggests metformin may have a role in the prevention of cardiovascular events [2] and cancer [3] in diabetic patients.

In Albanian pharmaceutical market, apart the innovator Glucophage, several generics are available, of which the most prescribed are Metformin Profarma and Siofor. Generic drugs, defined as medicinal products containing identical amounts of the same active substance as the reference formulation (innovator), have become very popular in recent years. Lower medication cost is their major advantage. However, there are still concerns that substitution of an innovator medicinal product with the respective generic may lead to a different bioavailability and, for this reason, they couldn't be used interchangeably. In practice, but also from the regulatory point of view, this problem generally is overcome by in vivo bioequivalence studies. Despite this, increasing evidence has shown that in vitro bioequivalence studies may accurately predict in vivo bioequivalence for immediate release solid oral dosage forms of highly soluble class I and III drugs. Furthermore, it appears that in vitro studies are sometimes better than in vivo studies in assessing bioequivalence of immediate release solid oral dosage forms [4].

This study was carried out to investigate, by means of dissolution profile comparison, the in vitro bioequivalence of metformin generic tablets in Albanian market and innovator product and to compare the results with those obtained from respective in vivo bioequivalence test.

II. METHODS

A. Dissolution profile comparison (in vitro bioequivalence)

Product selection

Three most prescribed brands of metformin tablets were used (Table I).

TABLE I. CHARACTERISTICS OF METFORMIN IMMEDIATE RELEASE TABLETS INCLUDED IN THE STUDY

Product name	Dosage	Content %	Batch number	Producer
Glucophage	850 mg	102.2	500911	Merck Sante
Siofor	850 mg	101.1	48273	Berlin Chemie
Metformine	850 mg	97.6	1310	Profarma Sh.a

Dissolution test

An USP apparatus II (model TDT – 08L, Pharma Alliance Group) was employed for the dissolution testing. For each brand 12 tablets were used. Samples (10 millilitres) were taken at 0, 10, 15, 20, 30, 45 and 60 minutes. Dissolution medium was replaced after each sampling to maintain the sink conditions. Two dissolution media were used:

- 900 ml of pH 6.8 phosphate buffer (0.68% w/v of potassium dihydrogen orthophosphate adjusted to pH 6.8 by the addition of 1M sodium hydroxide) at 37°C, with 100 rpm (compendial test)..
- 900 ml of either pH 4.5 acetate buffer or pH 1.2 HCl buffer, both containing 0.01% sodium lauryl sulphate at 37°C, with 150 rpm (non-compendial test).

Each of the withdrawn samples was filtered with a 0.45µm syringe filter, further diluted, and the absorbance was measured at 233 nm. At each run a calibration curve was constructed to calculate the concentrations.

Dissolution profiles

For each pharmaceutical product a dissolution profile was constructed by plotting the mean of cumulative percentage released in a specific dissolution media against the sampling time.

The dissolution profiles were compared using two model independent parameters: the difference factor (f1) and the similarity factor (f2) [5]. Two dissolution profiles are considered similar if f1 is between 0 and 15 and if f2 is between 50 and 100.

B. Bioequivalence test

Subjects

20 healthy male and female adult volunteers, of age between 18 to 50 years, were enrolled in the study. Pre-study baseline health status assessments for each individual included medical history, physical examinations, vital signs and clinical laboratory tests.

Subjects with a positive history or any evidence of hepatic, renal, gastrointestinal, hematologic or allergic disorders, any acute or chronic diseases, drug allergy or receiving any kind of treatment were not permitted to participate. Volunteers were asked to abstain from taking alcohol or non prescription medical products at least 1 week prior to and during the study period.

The study protocol was approved by National Ethics Committee. All participants signed the Informed Consent after explaining the possible risks and benefits, and the purpose of the study.

The study was conducted in accordance with local regulatory requirements and with ethical standards for human clinical trials established by the Declaration of Helsinki.

Study design

A randomised, open-label, single dose, two-period cross-over design was used to investigate the bioequivalence of Glucophage and Metformine 850 mg immediate release tablets. A wash-out period of at least 5 days was allowed between treatment periods. After an overnight fasting, the subjects were given single oral doses of study medication with approximately 200 ml water. Water was allowed *ad libitum* 2 hours after drug administration, while food after 4 hours. Volunteers were ambulatory during the study.

Blood sampling

Approximately, 5 ml of blood were drawn through a forearm vein indwelling cannula before and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, 12 and 14 hours after drug administration in heparin lithium containing tubes and were centrifuged at 3500 x rpm for 10 minutes at room temperature within 30 minutes. Plasma was collected in two aliquots and kept frozen at -20°C until analysis.

C. Bioanalytical method

Plasma concentrations of metformin were determined using a validated ion-paired HPLC method described in details elsewhere [6]. Briefly, to 500 µl plasma were added acetonitrile (1:1) and the mixture was vortexed for 30 seconds and centrifuged at 10000 rpm for 10 minutes. The upper layer (about 0.75 mL) was collected into a clean glass tube and 1.5 ml dichloromethane were added. After mixing for 30 seconds, the sample was centrifuged at 5000 rpm for 10 minutes. Then 20 µL of supernatant was injected into an Agilent 1200 chromatograph. The separation was performed on an LiChroCart® 100 RP 18 (125/× 4.0 mm i.d. 5 µm, particle size) column. The mobile phase was prepared by mixing 0.01 M of sodium phosphate buffer (pH=6.0), 0.3% sodium dodecyl sulphate, and acetonitrile in a ratio of 67.5:32.5, adjusting with H₃PO₄ to 6.0 as necessary. The flow rate and the column temperature were 1.25 ml/minute and 50°C, respectively. The detection of metformin was carried out at 236 nm.

Assay performance during the study was assessed by measurement of quality control samples and back-calculation of calibration standards.

Pharmacokinetic analysis

Pharmacokinetic analyses were carried out using non-compartmental analysis methods [7].

Maximum concentration (C_{max}) and time to maximum concentration (T_{max}) values were directly obtained from the plasma concentration profiles.

Areas under the plasma concentration-time curve (AUC₀₋₁₄, AUC_{0-inf}) and other standard pharmacokinetic parameters, such as apparent clearance (CL/F), terminal half-life (t_{1/2}) and apparent volume of distribution (V_z/F), were calculated using PKSolver add-in program for Microsoft Office.

Statistical analysis

For the purpose of bioequivalence analysis, AUC_{0-14} , AUC_{0-inf} and C_{max} were considered as primary pharmacokinetic endpoints. After log transformation (natural logarithm) of the data, the analysis of variance for crossover design was used to assess the effect of formulations, periods, sequences and subjects on these parameters. Parametric 90% confidence intervals based on the ANOVA of the mean test/reference (T/R) ratios of AUCs and C_{max} were computed. Difference between two related parameters was considered statistically significant if $p < 0.05$.

III. RESULTS

Dissolution profile comparison (in vitro bioequivalence)

As shown in Table I, the content of active ingredient of three metformin tablets included in the study were within the pharmacopoeial specification (95% to 105% of stated amount).

The dissolution profiles in 900 ml of pH 6.8 phosphate buffer at 37°C, with 100 rpm, for Glucophage (innovator), Siofor and Metformine (generics) are given in Fig. 1.

The dissolution profiles in 900 ml of pH 1.2 HCl buffer, containing 0.01% sodium lauryl sulphate (SLS), at 37°C, with 150 rpm, for Glucophage (innovator), Siofor and Metformine (generics) are given in Fig. 2.

The similarity factor f_2 and the difference factor f_1 method are used to compare two dissolution profiles. The results of f_2 and f_1 are shown in Table II and Table III comparing the dissolution curves of Metformine and Siofor with the innovator Glucophage. For Glucophage, being the reference product, f_1 and f_2 values are by definition 0 and 100, respectively.

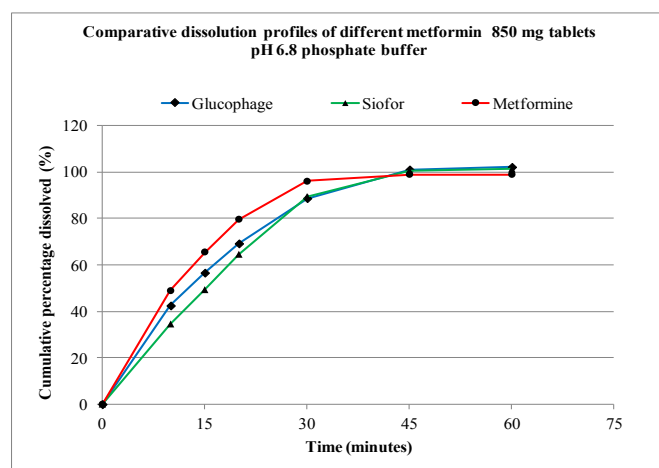


Fig. 1. Comparative dissolution profiles of metformin tablets (12 tablets for each product) in pH 6.8 phosphate buffer medium.

TABLE II. THE CALCULATED SIMILARITY AND DIFFERENCE FACTORS FOR TESTED PRODUCTS VS. GLUCOPHAGE AND PERCENTAGE OF ACTIVE INGREDIENT DISSOLVED AT SPECIFIC TIMES (PH 6.8).

Product name	f1	f2	% dissolved at 15 min	% dissolved at 30 min
Glucophage			56.6%	88.6%
Siofor	8	61	49.3%	89.3%
Metformine	13	54	65.5%	95.9%

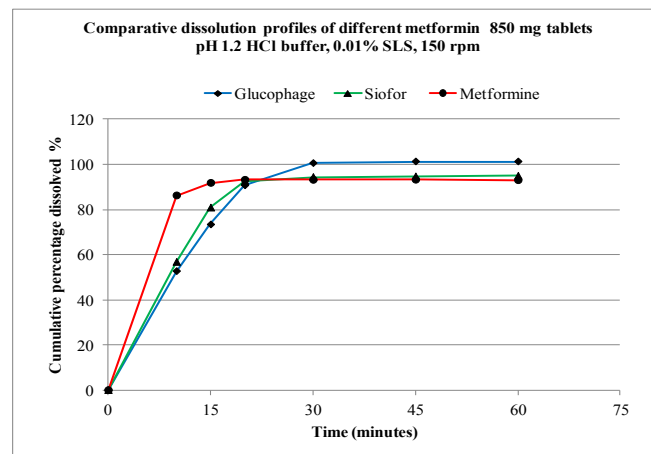


Fig. 2. Comparative dissolution profiles of metformin tablets (12 tablets for each product) in pH 1.2 HCl buffer medium, 0.01% SLS, 150 rpm.

TABLE III. THE CALCULATED SIMILARITY AND DIFFERENCE FACTORS FOR TESTED PRODUCTS VS. GLUCOPHAGE AND PERCENTAGE OF ACTIVE INGREDIENT DISSOLVED AT SPECIFIC TIMES (PH 1.2).

Product name	f1	f2	% dissolved at 15 min	% dissolved at 30 min
Glucophage			73.4%	100.6%
Siofor	6	65	80.6%	94.4%
Metformine	25	33	91.8%	93.2%

Bioequivalence test

All volunteers participating the clinical part of the study showed a good tolerance to both tablets of metformin. No unexpected side effects or other complications that could have influenced the outcome did occur during the study. No alterations in blood and urine biochemistry analyses were reported at the end of the study. There were no reports of deviations from the scheduled blood collection times which by protocol are considered significant (more than 5 minutes). Mean plasma concentration of metformin versus time, after single oral administration of Metformine 850 mg tablets and Glucophage 850 mg tablets, are shown in Fig. 3.

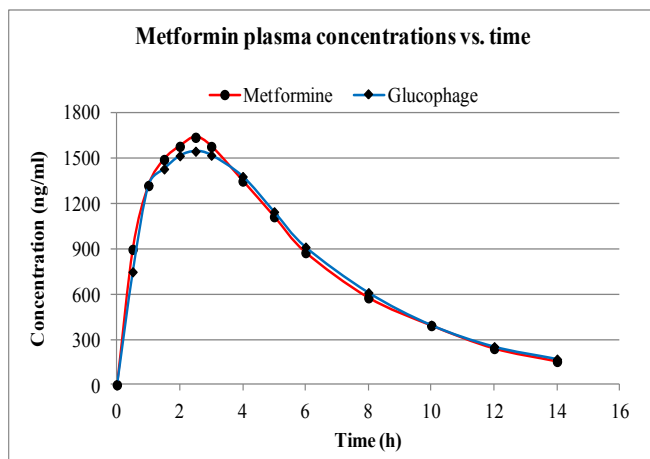


Fig. 3. Plasma concentration-time profiles of metformin after single oral administration of Metformine 850 mg tablets and Glucophage 850 mg tablets.

Systemic exposures to metformin, as demonstrated by mean plasma drug concentration profiles of the two products were closely similar.

Using non-compartmental analysis of plasma concentrations after extra vascular input feature of PKSolver, main pharmacokinetic parameters were calculated for both products (Table IV).

Analysis of variance (ANOVA) for AUC_{0-14} , AUC_{0-inf} and C_{max} , after log-transformation of the data, showed no statistically significant difference between Metformine 850 mg tablets and Glucophage 850 mg tablets either in periods, formulations or sequence. 90% confidence intervals also demonstrate that the ratios of AUC_{0-14} , AUC_{0-inf} and C_{max} of the study products lie within the regulatory acceptable range of 80–125% (Table V).

TABLE IV. MAIN PHARMACOKINETIC PARAMETERS, FOR METFORMINE 850 MG TABLETS AND GLUCOPHAGE 850 MG TABLETS.

Parameter	Unit	Metformine	Glucophage	p- value
λ_z	1/h	0.216	0.212	
$t_{1/2}$	h	3.204	3.274	
Tmax	h	2.5	2.5	NS
Cmax	ng/ml	1635.2	1543.1	NS
AUC 0-14	ng/ml*h	10966.9	10970.8	NS
AUC 0-inf	ng/ml*h	11679.2	11770.6	NS
Vz/F	(mg)/(ng/ml)	0.327	0.331	
Cl/F	(mg)/(ng/ml)/h	0.071	0.070	

NS = not significant

TABLE V. POINT ESTIMATE AND TWO-SIDED 90% CONFIDENCE INTERVAL LIMITS FOR PRIMARY PHARMACOKINETIC ENDPOINTS.

PK endpoint	Geometric mean ratio (T/R) %	90% confidence interval		CV %
		Lower limit %	Upper limit %	
Cmax	103.0	91.8	115.5	21.2
AUC0-14	99.3	90.6	108.9	16.9
AUC0-inf	98.8	90.3	108	16.5

Reference= Glucophage 850 mg tablet

Test= Metformine 850 mg tablet

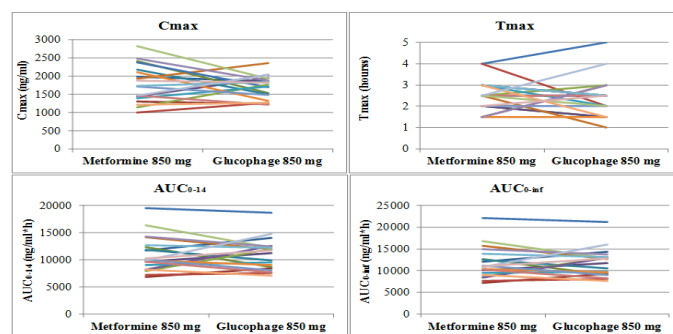


Fig. 4. Intraindividual comparison of main pharmacokinetic parameters after single oral administration of Metformine 850 mg tablets or Glucophage 850 mg tablets.

IV. DISCUSSION

A generic drug is a pharmaceutical product, usually intended to be interchangeable with an innovator product, that is marketed after the expiry date of the patent. Generic drugs are frequently as effective as, but much cheaper than, respective innovators. Because of their low price, they are often the only medicines that the poorest social groups can access. In most cases the lower prices of generic drugs are related to the fact that generic manufacturers do not incur the costs of drug discovery and clinical trials, used to demonstrate their safety and efficacy. However, most regulatory agencies require generic drug manufacturers to prove their products are bioequivalent to the innovator product and therefore therapeutically interchangeable. As a gold standard assessment of “interchangeability” between the generic and the innovator product is carried out by a study of in vivo equivalence or bioequivalence [8].

The development of the biopharmaceutical classification system (BCS) [9] as a framework for classifying a drug substance based on its aqueous solubility and intestinal permeability has made possible to predict the intestinal absorption of orally administered drugs using such parameters. Based on the BCS, most regulatory agencies (i.e. US FDA, EMA) have recommended that generic manufacturers may replace the bioequivalence studies for immediate-release solid oral dosage forms of highly soluble and highly permeable drugs (Class I) with in vitro dissolution profiles studies, when appropriate. The same is expected for rapid dissolving dosage

forms of Class III high solubility-low permeability drugs, although the criteria will be more restrictive.

Dissolution of drug from oral solid dosage forms is an important aspect for drug bioavailability (i.e., the drug must be solubilised in the aqueous environment of the gastrointestinal tract to be absorbed). Accordingly, dissolution testing of solid oral drug products has become one of the most important tests not only for assuring product uniformity and batch-to-batch equivalence, but also for demonstrating bioequivalence [10,11]. Dissolution test is currently used as an in vitro bioequivalence test, generally for figuring out dissolution profile and profile comparison, establishing the similarity of pharmaceutical dosage forms [9, 12].

Metformin is a typical BCS Class III drug as it shows high solubility in water and low permeability to cell membranes [12].

In the present study, it was observed that in pH 6.8 phosphate buffer medium the dissolution of metformin from all tablets was more than 85% in 30 min and the similarity factor f_2 for Metformine and Siofor, compared with the innovator Glucophage, was greater than 50 (Fig. 1 and Table II). In such a situation, evidence supports the conclusion that Metformine and Siofor 850 mg tablets are bioequivalent with the innovator Glucophage. If different dissolution conditions are applied (e.g. pH 1.2 HCl buffer, containing 0.01% SLS, and with 150 rpm), it becomes evident that Metformine tablets, but not Siofor, dissolve more rapidly than Glucophage tablets, with similarity factor f_2 being smaller than 50 (Fig. 2 and Table III). In such a situation, evidence does not support the conclusion that Metformine 850 mg tablets are bioequivalent with the innovator Glucophage or other generic Siofor.

To overcome this ambiguity, we performed a bioequivalence test. Twenty healthy volunteers participated in a randomised, open-label, single dose, two-period, cross-over clinical trial, aiming to investigate the bioequivalence of Glucophage and Metformine 850 mg immediate release tablets. Statistical comparison of the AUC₀₋₁₄, AUC_{0-inf} and C_{max} clearly indicated no significant difference between Metformine and Glucophage tablets (Fig. 3 and Table IV). The 90% confidence intervals for the ratios of mean AUC₀₋₁₄, AUC_{0-inf} and C_{max} were entirely within the bioequivalence acceptance range of 80–125% (Table V).

The demonstration of in vivo bioequivalence between Metformine and Glucophage tablets confirms the results obtained with compendial in vitro dissolution test (pH 6.8 phosphate buffer medium), that is in vitro bioequivalence.

However, the reason why more rapidly dissolving Metformine tablets, also showing different dissolution profile in non-compendial dissolution test, are in vivo bioequivalent with Glucophage tablets, still needs an explanation. In the case of metformin, it has been demonstrated that the solution dosage form is bioequivalent to an immediate-release tablet that dissolved completely within 1 h [13]. The examination of dissolution profiles clearly demonstrates that Metformine, Siofor and Glucophage tablets are dissolved completely within 1 h. On the other side, it seems that for a BCS Class III drug,

the low permeability to cell membranes blunts the temporary difference in the solubilisation of active ingredient created when a more rapidly than innovator generic tablet is administered orally. The contrary may be not true.

V. CONCLUSIONS

Metformin 850 mg and Glucophage 850 mg tablets were shown to be bioequivalent despite the in vitro dissolution profiles indicate a faster dissolution rate for Metformine 850 mg tablets, at least in one non-compendial dissolution medium. However, the compendial in vitro dissolution test (pH 6.8 phosphate buffer medium, 100 rpm) was capable to predict in vivo bioequivalence between Metformine and Glucophage tablets.

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