

# Bioequivalence of Two Formulations of Metformin Hydrochloride 1000 mg XR Tablets, Fasting Condition in Healthy Adults Subjects

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## Abstract

**Background:** This study aimed to evaluate the bioequivalence of the two formulations of metformin 1000 mg XR tablets in healthy male volunteers under fasting conditions. **Methods:** This was a randomized, single-dose, open-label, two-period, two-sequence, crossover and single-dose study to compare the bioequivalence (BE) profile of Metformin (metformin hydrochloride) 1000 mg XR tablet of Laboratorios Leti S.A.V., with Glucophage® (metformin hydrochloride) 1000 mg XR tablet of Merck Serono GmbH in 26 adult healthy subjects. The pharmacokinetic (PK) parameters  $C_{max}$ ,  $AUC_{0-t}$  and  $AUC_{0-\infty}$  were calculated based on the plasma drug concentration-time profile measured by liquid chromatography-mass spectrometry (LC-MS/MS). The safety was assessed throughout the study. The two formulations test (T) and reference (R) were considered bioequivalent if 90% confidence interval (CI) were within BE acceptance range of 80.00% - 125.00% for  $C_{max}$ ,  $AUC_{0-t}$  and  $AUC_{0-\infty}$ . **Results:** A total of 25 subjects completed both study periods 90% confidence intervals (CIs) of the test/reference ratios were  $C_{max}$ : 92.30% (89.08% - 96.97%),  $AUC_{0-t}$ : 91.83% (87.11% - 94.11%) and  $AUC_{0-\infty}$ : 91.83% (87.23% - 94.85%) to metformin 1000 mg extended release. PK parameters were within the accepted bioequivalence criteria. The results show that no significant differences were observed between the pharmacokinetic profiles of the T and R formulations. No serious adverse events were reported in this study. **Conclusion:** The two formulations of metformin hydrochloride 1000 mg XR were bioequivalent in healthy subjects under fasting conditions.

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## Keywords

Bioequivalence, Metformin, Pharmacokinetic

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### 1. Introduction

Metformin hydrochloride, is a member of the biguanide class [1]. Metformin is a biguanide with a molecular formula,  $C_4H_{11}N_5$  and molecular weight of 129.16 with antihyperglycaemic effects, lowering both basal and postprandial plasma glucose [1] [2]. It does not stimulate insulin secretion and therefore does not produce hypoglycaemia [2]. Metformin may act via three mechanisms: 1) Reduction of hepatic glucose production inhibiting gluconeogenesis and glycogenolysis, 2) Modestly increasing insulin sensitivity, improving peripheral glucose uptake and 3) Utilization and delaying intestinal glucose absorption [3]. Metformin stimulates intracellular glycogen synthesis by acting on glycogen synthase. Metformin increases the transport capacity of specific types of membrane glucose transporters (GLUT-1 and GLUT-4) [4].

After an oral dose of metformin 500 mg, the  $T_{max}$  was reached in 2.5 h and the bioavailability was 50% - 60% [5]-[7]. The non-absorbed fraction recovered in faeces was 20% - 30%. The elimination half-life of metformin during multiple dosages in patients with good renal function is approximately 5 hours. The blood peak is lower than the plasma peak concentration and appears at approximately the same time. Metformin is excreted unchanged in the urine. No metabolites have been identified in humans. Renal clearance of metformin is  $>400$  mL/min, indicating that metformin is eliminated by glomerular filtration and tubular secretion. Following an oral dose, the apparent terminal elimination half-life is approximately 6.5 h. When renal function is impaired, renal clearance is decreased in proportion to that of creatinine and thus the elimination half-life is prolonged, leading to increased levels of metformin in plasma [1]-[6]. Gastrointestinal symptoms have been reported as adverse events in clinical and post marketing trials with metformin [6].

Metformin has been the gold standard for the monotherapy as first line in the treatment of T2DM to achieve the goals for effective glycaemic control [3]-[7].

Following a single oral dose of metformin extended release (XR),  $C_{max}$  is achieved with a median value of 7 hours and a range of 4 to 8 hours. Peak plasma levels are approximately 20% lower compared to the same dose of metformin IR [3]-[7] however, the extent of absorption (as measured by AUC) is similar to metformin IR. At steady state, the AUC and  $C_{max}$  are less than dose proportional for metformin XR within the range of 500 to 2000 mg administered once daily. Peak plasma levels are approximately 0.6, 1.1, 1.4, and 1.8  $\mu\text{g/mL}$  for 500, 1000, 1500, and 2000 mg once-daily doses, respectively. The extent of metformin absorption (as measured by AUC) from metformin XR at a 2000 mg once-daily dose is similar to the same total daily dose administered as metformin immediate release (IR) tablets 1000 mg twice daily. After repeated administration of metformin XR, metformin

did not accumulate in plasma. Within-subject variability in  $C_{\max}$  and AUC of metformin from metformin XR is comparable to that with metformin IR, there was no effect of food on  $C_{\max}$  and  $T_{\max}$  of metformin. Both high and low fat meals had the same effect on the pharmacokinetics of metformin XR [6].

Metformin pharmacokinetic parameters did not differ significantly between normal subjects and patients with type 2 diabetes when analyzed according to gender (males = 19, females = 16). Similarly, in controlled clinical studies in patients with type 2 diabetes, the antihyperglycemic effect of metformin IR was comparable in males and females. A 24-week, double-blind, placebo-controlled study of metformin XR, taken once daily with the evening meal, was conducted in patients with type 2 diabetes who had failed to achieve glycemic control with diet and exercise (HbA1c: 7.0% - 10.0%, FPG: 126 - 270 mg/dL). Patients entering the study had a mean baseline HbA1c of 8.0% and a mean baseline FPG of 176 mg/dL. After 12 weeks treatment, mean HbA1c had increased from baseline by 0.1% and mean FPG decreased from baseline by 2 mg/dL in the placebo group, compared with a decrease in mean HbA1c of 0.6% and a decrease in mean FPG of 23 mg/dL in patients treated with metformin XR 1000 mg once daily [6] [7].

The purpose of this bioequivalence study was to assess and compare the PK profile and safety of a single-tablet, of Metformin (Metformin Hydrochloride 1000 mg) 1000 mg, Extended Release (XR) of Laboratorios Leti S.A.V., as test (T) formulation vs Glucophage® 1000 mg XR tablet (Metformin Hydrochloride 1000 mg) of Merck Serono GmbH in healthy adult human subjects under fasting condition [8]-[10].

This study was conducted by a clinical research operator (CRO) ICBio Clinical Research Private Limited, Bangalore, India.

## 2. Materials and Methods

### 2.1. Ethical Considerations

The study was conducted ethically in accordance with the principles of the ICMR guidelines (2017) [11], New Drugs and Clinical Trials Rules 2019 India [12] and adhered to the ethical principles of the Declaration of Helsinki [13] the International Conference on Harmonization Good Clinical Practice Guidelines [14]. The study protocol (N°096-22) was approved by Aavishkar Ethics Committee, on March 06, 2023 (Version: 00, Dated 06 Dec 2022) and certified by CDSCO/DGHS to ICBio Clinical Research Pvt, Ltd.

### 2.2. Study Design

This was an open-label, randomized, two-treatment, two-period, two-sequence, single oral dose, and crossover bioequivalence (BE) study under fasting conditions comparing two formulations of Metformin (metformin hydrochloride) 1000 mg tablets extended release (XR), batch N°18E, date of expiry 04/2023, of Laboratorios Leti S.A.V., as test formulation (T) vs Glucophage® (metformin hydrochloride) 1000 mg tablets XR, batch N°BR131581, date of expiry 03/2024, of Merck Serono, GmbH, Germany as reference formulation (R).

The subjects were randomized, to one of the two sequences (T - R) or (R - T). The randomization schedule was generated using Statistical Analysis Software (SAS®, version 9.4, Institute. Inc., CARY, USA). One single dose was administered in each period. Subjects who received T product in period I were administered R product in period II and *vice versa*. Pre-screening period was 21 days. The total duration of the clinical phase of this study was of 12 days from the day of check-in of period I to last blood sample collection of period II (April 27, 2022-May 08, 2022) separated by a washout period of 07 days, considering the terminal half-life for metformin 6.5 h [6]. This BE study met the principles described in bioequivalence EU BE guideline [9] [10].

The sample size calculation for the study was based on intra-subject coefficient of variation (CV%) obtained of published data for metformin  $C_{max}$ : 11.4%, and  $AUC_{0-t}$  13.63% [3] [6]. Based on referred literature using the reported 90% confidence intervals, intrasubject CV% was estimated for pharmacokinetics parameters of metformin hydrochloride and the maximum intrasubject CV% with the expected % CV not exceeding 20% and the ratio within 80 and 125% [3] [6]. The study required 22 evaluable subjects to demonstrate BE with a power of  $\geq 90\%$  at 5% level of significance. Thus, with the expected coefficients of variation for  $C_{max}$  and AUC not exceeding 24% and assuming the true ratio falling within 95% to 105% (*i.e.* a true treatment difference of 5%), the study had at least 22 evaluable subjects to show the bioequivalence with a power of greater than 80% at 5% level of significance. Further, expecting certain dropouts and/ or withdrawals in the study, 04 additional subjects were included [15]-[17].

### 2.3. Subjects

All volunteers underwent a screening procedure. A total of 26 healthy, adult male volunteers who met the inclusion and exclusion criteria were enrolled, with a mean age of 33.96 years, mean weight 70.88 Kg, mean height 1.73 cm, and body mass index (BMI) of 23.66 kg/m<sup>2</sup> (Table 1).

**Table 1.** Demographic profile of subjects.

bioequivalence study (n = 26)				
Age	Mean ± SD	33± 5.8		
(years)	Range	23 - 45		
Age group		Male	%	Total
	23 - 40	22	84.8%	22/84.8%
	41 - 43	4	15.2%	4/15.2%
Total	26	26	100%	26/100%
BMI (kg/m <sup>2</sup> )	Mean ± SD	23.66 ± 2.62		
	Range	(19.71 - 28.65)		
Race	Asian	26	100%	

A complete clinical history valid for 6 months before the start of the study; normal laboratory values as determined by medical history and physical examination at the time of screening; normal vital signs and physical examination; creatinine clearance of more than 50 mL/min; negative tests for hepatic transaminases, hepatitis B and C, human immunodeficiency virus, and venereal diseases research laboratory; and normal 12-lead EKG values, normal chest radiography, and negative result in urine drug tests. Urine for drugs of abuse and urine test for alcohol consumption were performed on day of check in of each period. Random blood glucose test was performed in screening. Fasting blood glucose test was conducted prior to check-in of period I. In each period, subject's blood glucose monitoring was performed before dosing and at 02.00, 04.00, 06.00 and 11.00 h  $\pm$  (30 minutes) after dosing. Other key inclusion criterion was that subjects must be non-smokers or smokers who had not smoked at least 10 h before the start of the study. They all signed the informed consent.

The exclusion criteria included volunteers incapable of understanding the informed consent, history of diabetes, tuberculosis and systemic hypertension. A history of hypersensitivity to the study medication or to any other medication belonging to the study group or cardiovascular, renal, hepatic, metabolic, gastrointestinal, neurological, endocrine, hematopoietic, psychiatric, or other organic abnormalities; under medication that interferes with the quantification, drugs that can potentially affect the hepatic metabolism of other drugs.

#### 2.4. Drug Administration

The subjects were admitted to the facility one night before study. All the subjects were fasted for at least 10 hours pre-dose and 4 hours post-dose. The subjects received standardized meals at 04.00, 08.00, 12.00 and 24.00 hours after dosing in each Period. During housing the meal menu was same in both the periods and drinking water was provided *ad libitum* (As desired).

The subjects received standard meals at 04.00 (lunch), 08.00 (snacks) and 12.00 (dinner) hours after dosing in each period. All meal plans were identical for each period of study.

A single oral dose (metformin 1000 mg tablets XR) either one T or R, following randomization schedule and was administered with 240 mL  $\pm$  2 mL of glucose solution in water, followed by 60 mL of 20% glucose solution in water administered every 15 minutes for up to 04 hours after dosing at ambient temperature to each subject in sitting position [8]-[10].

A total of 19  $\times$  5 ml of venous blood samples were collected through cannula from each subject during the two periods of the study, withdrawn at pre-dose (00.00 h) and 00.50, 01.00, 01.50, 02.00, 02.50, 03.00, 03.50, 04.00, 05.00, 06.00, 07.00, 08.00, 10.00, 12.00, 16.00, 24.00, 48.00 and 72.00 hours after dosing in each period. While 24.00, 48.00 and 72.00 hours, post dose, blood samples were collected by direct venipuncture. The subjects received standardized meals at 04.00, 08.00, 12.00 and 24.00 hours after dose in each period (800 - 1000 Kcal) and drinking water was provided *ad libitum*.

## 2.5. Analytical Procedure

Venous blood samples were collected in pre-labelled K<sub>2</sub>EDTA (ethylenediaminetetraacetic acid) vacutainers and were centrifuged at 4000 rpm for 10 min at 2°C - 8°C within 45 minutes of sample collection. Plasma was separated, labeled and stored at -70°C ± 15°C before analysis. Subsequently, the plasma samples were processed, calibration curve of internal standards (ISTD) metformin D6, (Vivian Life Sciences Private Limited, Mumbai, India) and quality control (QC) samples were thawed and vortexed for preparation and analysis. Method of validation of metformin was conducted with calibration 14.040 ng/mL to 4004.046 ng/mL respectively and validation results were reported in Method Validation Report MVR-SOP MV-073-00. The biostudy was performed on Protein Precipitation Method coupled with LC using metformin D6 as internal standard. The interface used was turbo iron spray and positive ions were measured in MRM mode. The samples were extracted by a solid phase extraction chromatography column coupled to a BDS Hypersil C18, 4.6 × 50 mm, 5 µm column. Elution was performed at 25°C temperature, with a mobile phase of acetonitrile and HPLC water mixture (50:50% v/v). The lower limit of quantification was 10.495 ng/mL whereas the upper limit of quantification was 3053.826 ng/mL. These data were acquired, integrated and quantified on ABSCIEX systems Shimadzu, analyst version 1.7.3 software.

All solutions were prepared using diluent (metanol: water, 50:50 v/v) and stored at 2.0°C - 8.0°C. The supernatant identifications were based on multiple reactions monitoring transitions; m/z 60.200 - 130.050 for metformin and m/z 60.200 - 136.100 for the IS-metformin D6. The inter-batch calibration standard (IBCS) for metformin was 3.68% to 5.04%, accuracy 97.61% to 104.50%.

## 2.6. Statistical and Pharmacokinetic Analysis

Statistical analysis was conducted on all of the subjects who complete both periods of the study as per protocol, using SAS® (software version 9.4, Institute. Inc., CARY, USA).

The blood concentration-time curve (C-T curve) was obtained by mapping the plasma metformin concentration at each time point. The primary PK variables evaluated were maximum peak concentration ( $C_{max}$ ), area under curve from time 0 to last measurable concentration ( $AUC_{0-t}$ ) and area under the plasma concentration-time curve from time 0 to infinity ( $AUC_{0-\infty}$ ) The peak concentration ( $C_{max}$ ) was obtained from this graph. The area under the curve (AUC) from time 0 to the time of the last measurable concentration ( $AUC_{0-t}$ ) was calculated using the linear trapezoidal rule. The AUC from time 0 to infinity ( $AUC_{0-\infty}$ ) was obtained using the formula  $AUC_{0-\infty} = AUC_{0-t} + C_t / \lambda_z$ , where  $C_t$  is the final measurable concentration and  $\lambda_z$  is the elimination rate constant, which is the slope of the terminal segment of the linear regression curve of the logarithmically transformed drug concentration values vs. Time. Others secondary PK parameters evaluated were:  $T_{max}$  (time to reach  $C_{max}$ ) and was obtained directly from the C-T

curve, time required for plasma concentration to decrease by 50% ( $T_{1/2}$ )  $T_{1/2}$  is the elimination half-life, calculated as  $0.693/\lambda_z$ . Log-transformed,  $AUC_{\%Extrap}$ , constant of elimination ( $K_{el}$ ) and the number of points ( $N_{points}$ ) of the terminal log-linear phase used to estimate the terminal rate constant. The natural log transformed (*i.e.*, Ln-transformed) values for the pharmacokinetic parameters  $C_{max}$ ,  $AUC_{0-t}$  and  $AUC_{0-\infty}$  was analyzed for statistical difference between test and reference formulations (metformin T & R) with ANOVA by a Generalized Linear Model (GLM) ANOVA using SAS®. Based on these parameters, the 90% confidence intervals (CIs) were constructed for the least square mean differences of log-transformed PK parameters  $C_{max}$ ,  $AUC_{0-t}$  and  $AUC_{0-\infty}$ . The metformin formulations regarded as bioequivalent when the 90% (CIs) of the T and R ratio of  $C_{max}$ ,  $AUC_{0-t}$ , and  $AUC_{0-\infty}$  ranged from 80% to 125% [8] [9].

## 2.7. Safety Assessments

Safety assessments were performed during screening, during the study and the end of the study and the adverse events (AEs) were monitored throughout the study. Vital signs were measured during baseline screening, and at the conclusion of the study. Twelve-lead electrocardiogram was recorded during screening. Random blood glucose test was performed in screening. Fasting blood glucose test (FBGT) was conducted prior to check in of period I. In each period, blood glucose monitoring was performed before dosing and at 03.00 and 06.00 h ( $\pm$  15 minutes) after dosing. Post study safety evaluation was performed on all the subjects who came for period II of clinical study. Total 25 subject's post safety samples were collected on last ambulatory visit (72.000 hr) and subject N° 19 reported as drop-out in period II.

## 3. Results

### 3.1. PK Evaluation

A total of 25 subjects completed the study and were included in the PK and statistics evaluation. A non-compartmental analysis was applied for the estimation of PK parameters  $C_{max}$ ,  $AUC_{0-t}$ ,  $AUC_{0-\infty}$ ,  $T_{max}$ ,  $K_{el}$  (h<sup>-1</sup>), and  $T_{1/2}$ , of metformin in plasma concentration which are presented in **Table 2**.

**Table 2.** Pharmacokinetic parameters for metformin XR after administration of test product (T) and reference product (R) N = 25.

PK Parameters	Values $\pm$ SD	
	Test (T)	Reference (R)
$C_{max}$ (ng/mL)	1073.8982 $\pm$ 293.35014	1148.9839 $\pm$ 269.50783
$AUC_{0-t}$ (ng*hr/mL)	10201.9170 $\pm$ 5391.58104	11117.0503 $\pm$ 5567.26647
$AUC_{0-\infty}$ (ng*hr/mL)	10396.8852 $\pm$ 5384.89716	11321.4541 $\pm$ 5559.82627
$T_{max}$ (h)	4.000 (3.000 - 10.000)	4.000 (3.000 - 12.000)

## Continued

$K_{el}$ (hrs <sup>-1</sup> )	0.15971 ± 0.032701	0.15897 ± 0.035179
$T_{1/2}$ (hrs)	4.5478 ± 1.07553	4.6018 ± 1.16175

Data presented as a mean ± standard deviation  $C_{max}$ , maximum concentration,  $AUC_{0-t}$ , area under the plasma concentration-time curve from time 0 to the last measurable concentration;  $AUC_{0-\infty}$ , area under the plasma concentration-time curve from time 0 to infinity,  $T_{max}$  time to reach  $C_{max}$ ,  $K_{el}$  elimination constant.  $T_{1/2}$  time required for plasma concentration to decrease by 50%. Median (range).

The AUC from 0 to last time point with measurable plasma concentrations was computed using linear trapezoidal-rule.

### 3.2. Bioequivalence Evaluation

Analysis of variance analysis from Ln  $C_{max}$ ,  $AUC_{0-t}$  and  $AUC_{0-\infty}$ , there were no statistically significant differences between the PK parameters of the metformin formulations ( $P > 0.05$ ) (Table 2 and Table 3).

**Table 3.** Bioequivalence assessment of a single dose of metformin 1000 mg XR test and reference formulations. Log transformed  $C_{max}$ ,  $AUC_{0-t}$  and  $AUC_{0-\infty}$  (N = 25).

Parameters (Units)	Least Square Means		Geometric Least Square Means		Ratio (%) (T Vs R)	90% Confidence Intervals (%)	Intra Subject CV (%)	Power (T Vs R) (%)
	T	R	T	R				
Ln ( $C_{max}$ ) (ng/mL)	1037.93	1115.59	1039.93	1118.91	92.94	89.08 - 96.97	8.76	100.00
Ln ( $AUC_{0-t}$ ) (hr*ng/mL)	9030.82	9930.03	9066.95	1512.792	92.30	87.11 - 94.77	8.70	100.00
Ln ( $AUC_{0-\infty}$ ) (hr*ng/mL)	9237.71	10147.10	9274.94	10196.79	91.96	87.23 - 94.85	8.65	100.00

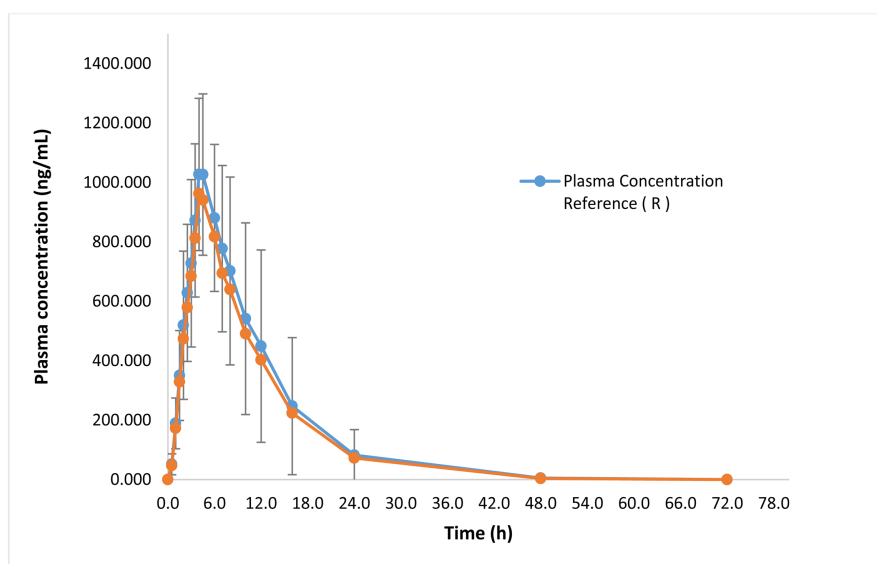
Least Square Mean (LSM), Geometric Least Square Mean (GLSM), Ratio, 90% confidence intervals (CI), Intra subject Variability (ISCV) and Power for the Log transformed  $C_{max}$  and  $AUC_{0-t}$ . Metformin 1000 mg extended release (ER) of Laboratorios Leti S.A.V. as Test formulation and Glugofage® (metformin 1000 mg ER) of Merck Serono as Reference formulation.

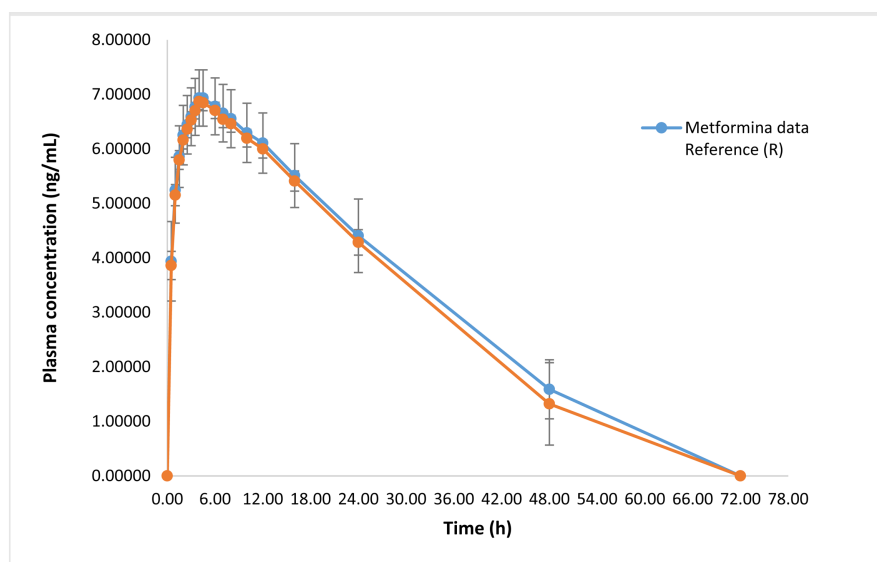
The geometric mean ratios for primary PK parameters were: Ln  $C_{max}$  92.94 (90% IC: 89.08 - 96.97), Ln  $AUC_{0-t}$  92.30 (90% IC: 96.57 - 108.82) and Ln  $AUC_{0-\infty}$  92.30 (90% IC: 96.57 - 108.82 (Table 3), intervals for test and reference formulations were within the acceptance limits (80.00% - 125.00%) to establish bioequivalence.

The oral dosing for 48 h post-dose is represented on arithmetic and logarithm scales, test and reference formulations as shown in Table 4 (Figure 1 and Figure 2) for metformin 1000 mg XR.

**Table 4.** Plasma concentrations of metformin 1000 mg XR, Test and Reference formulations (N = 25).

Time (h)	Arithmetic Data				Logarithmic Data			
	Plasma Concentration Test (T)	Plasma Concentration Reference (R)	Metformina data Test (T) SD	Metformina data Reference (R) SD	Metformina data Test (T)	Metformina data Test (T) SD	Metformina data Reference (R)	Metformina data Reference (R) SD
0.0	0.000	0.000	0.00000	0.00000	0.00000	0.00000	0	0
0.5	47.495	51.328	0.02105	0.01948	-3.93823	-0.25392	3.93823	0.72954
1.0	172.745	189.084	0.00579	0.00529	-5.24219	-0.19076	5.24219	0.6036
1.5	328.933	350.265	0.00304	0.00285	-5.85869	-0.17069	5.85869	0.56563
2.0	473.861	519.448	0.00211	0.00193	-6.25277	-0.15993	6.25277	0.54555
2.5	578.961	628.394	0.00173	0.00159	-6.44317	-0.15520	6.44317	0.53676
3.0	684.655	727.964	0.00146	0.00137	-6.59025	-0.15174	6.59025	0.53034
3.5	812.477	872.203	0.00123	0.00115	-6.77102	-0.14769	6.77102	0.52283
4.0	962.114	1027.058	0.00104	0.00097	-6.93445	-0.14421	6.93445	0.51639
4.5	940.649	1026.503	0.00106	0.00097	-6.93391	-0.14422	6.93391	0.51642
6.0	817.248	880.618	0.00122	0.00114	-6.78062	-0.14748	6.78062	0.52245
7.0	694.404	777.213	0.00144	0.00129	-6.65571	-0.15025	6.65571	0.52757
8.0	639.432	702.160	0.00156	0.00142	-6.55416	-0.15257	6.55416	0.53189
10.0	490.530	541.539	0.00204	0.00185	-6.29441	-0.15887	6.29441	0.54358
12.0	403.070	449.118	0.00248	0.00223	-6.10729	-0.16374	6.10729	0.55264
16.0	223.670	247.186	0.00447	0.00405	-5.51014	-0.18148	5.51014	0.58596
24.0	72.568	81.972	0.01378	0.01220	-4.40638	-0.22694	4.40638	0.67428
48.0	3.751	4.899	0.26662	0.20412	-1.58903	-0.62931	1.58903	0.5414
72.0	0.000	0.000	0.00000	0.00000	0.00000	0.00000	0	0

**Figure 1.** Bioequivalence study of Metformin XR 1000 Mg. Mean plasma concentration (ng/mL) Vs Time (h). Arithmetic scale.



**Figure 2.** Bioequivalence study of Metformin XR 1000 Mg. Mean plasma concentration (ng/mL) Vs Time (h). Logarithmic scale.

### 3.3. Safety Evaluation

Post study safety evaluation was performed on all subjects who came for period II of the study. Total 25 subjects post safety samples were collected on last ambulatory visit (72.000 hr) in period II and subject N° 19 reported as dropout in period II. On evaluation all the safety parameters were found to be normal.

There were no serious adverse events (SAE) and other significant adverse events observed during the study. Hence the test product and reference product were found to be safe and well tolerated upon single dose administration in healthy male adults under fasting conditions.

## 4. Discussion

This study was designed to evaluate the bioequivalence of two metformin formulations extended release in a single-dose, two-period, crossover design involving healthy male subjects under fasting conditions. The BE evaluation was based on 90% CIs ratios for metformin, and was assessed against bioequivalence standards of 80% - 125% for  $C_{max}$ ,  $AUC_{0-t}$  and  $AUC_{0-\infty}$  as primary PK parameters, adhered to the EMA BE guidelines [9] [10].

The study included 26 male subjects covering the variability observed in others studies with a sufficient number of subjects to ensure statistical power to demonstrate the bioequivalence for both formulations [10].

Comparative bioavailability of T and R formulations was demonstrated. The total amount of drug reaching the systemic circulation is proportional to the area under curve and fraction of drug absorbed is determined by comparing  $AUC_{0-t}$  and  $AUC_{0-\infty}$  of the T and R formulations, no significant differences were demonstrated between the two formulations. The 90% confidence intervals for the ratios of metformin, met the acceptance range of 80.00% - 125.00% for primary PK

parameter  $C_{\max}$ ,  $AUC_{0-t}$  and  $AUC_{0-\infty}$  [10] [15]-[17]. The geometric mean ratio ((GMR) T/R) for the primary PK parameters for metformin were  $C_{\max}$  92.94 (90% IC: 89.08 - 96.97),  $AUC_{0-t}$  92.30 (90% IC : 96.57 - 108.82) and  $AUC_{0-\infty}$  92.30 (90% IC: 96.57 - 108.82. The mean plasma concentration-time curves for the metformin 1000 mg XR tablets, were similar for both T and R formulations (**Figure 1** & **Figure 2**). The analysis of variance (ANOVA) was used for crossover design in bioavailability testing [10] [15].

Type 2 Diabetes Mellitus (T2DM) is a complex, chronic illness characterized by persistent high blood glucose levels, with severe cardiovascular complications in patients uncontrolled, Metformin is the gold standard drug used for patients with T2DM, to initiate an effective glycemic control due progressive deterioration of beta-cell function with lower risk of hypoglycemia, as well as beneficial effects on beta-cell function and eventual protective action on beta-cell mass [6] [18]. The test product Metformin 1000 mg XR was developed by Laboratorios Leti S.A.V and this product will be available in some Latin American countries where Leti group is present following the regulations of each country [19] [20]. Metformin 1000 mg XR Tablet from Laboratorios Leti S.A.V., compared to Glucofage® (metformin Hydrochloride) 1000 mg XR of Merck, Serono GmbH demonstrated to be bioequivalent and safe in male subjects under fasting condition.

## 5. Limitations

This study analyzed PK parameters of metformin 1000 mg XR, under fasting conditions. This study was open to both male and female healthy subjects, but only male attended the screening.

## 6. Conclusion

Two XR formulations containing 1000 mg of metformin were evaluated and found to be bioequivalent in healthy subjects under fasting condition. The pharmacokinetic profiles of the test and reference formulations were similar, as demonstrated by the 90% confidence intervals (CIs) of  $C_{\max}$ ,  $AUC_{0-t}$  and  $AUC_{0-\infty}$  within the accepted BE criteria of 80% - 125%.

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## Author Contribution

E.P., A.I., A.T., X.S., and J.CH., performed the statistical analysis, interpretation, writing, and review of the manuscript.

## Declaration of Patient Consent

All volunteers provided written informed consent after being well informed about the study before screening.

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## Use of Artificial Intelligence (AI)-Assisted Technology for Manuscript Preparation

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

## Conflicts of Interest

All authors are employees of Industrias Biocontrolled C.A., (Leti Group), and may hold share and/or stock options in the company. The authors have no other potential conflicts of interest relevant to this study.

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