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A Bioequivalence Study of Empagliflozin/Metformin Fixed-Dose Combination in Healthy Subjects under Fasting Conditions

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Abstract

Background: This study evaluated the bioequivalence of empagliflozin 12.5 mg/metformin 1000 mg tablets compared to Synjardy® (Empagliflozin 12.5 mg/metformin 1000 mg) tablets in healthy male subjects under fasting conditions. **Methods:** This was a phase I, randomized, single-dose, two-period, two-sequence, crossover study to evaluate the bioequivalence (BE) profiles of two fixed-dose combinations (FDCs) of empagliflozin/metformin. C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ from test and reference formulations were evaluated to access BE. The plasma concentrations were measured using a validated liquid chromatography-mass spectrometry (LC-MS/MS) method. Of the 24 subjects enrolled, 23 completed both periods of the study. The two formulations test and reference were considered bioequivalent if 90% confidence interval (CI) fell within 80.00% - 125.00% for C_{max}, AUC_{0-t} and $AUC_{0-\infty}$. Tolerability and safety were assessed throughout the study. **Results:** The pharmacokinetic (PK) parameters were similar between the test product (T) and reference product (R) Synjardy®. The 90% CI of the test/reference ratios of log-transformed PK parameters point estimates was C_{max}: 89.87% (85.68% - 94.27%), AUC_{0-t}: 87.91% (83.65% - 92.39%) and $AUC_{0-\infty}$: 87.16% (82.80% - 91.75%) to empagliflozin and C_{max}: 92.19% (87.95% - 96.65%), AUC_{0-t}: 91.38% (84.42% - 98.91%) and $AUC_{0-\infty}$: 93.78% (83.82% - 104.93%) to metformin respectively (90% CI for all PK parameters fell within 80.00% - 125.00%). **Conclusion:** Our results demonstrated BE between the test and reference formulations of oral tablets of empagliflozin 12.5 mg/metformin 1000 mg (FDC) in healthy male subjects under fasting conditions.

Keywords

Bioequivalence, Fix Dose Combinations, Pharmacokinetic, Empagliflozin

1. Introduction

Global diabetes prevalence in 2017 indicated that 451 million people aged 18 - 99 years are affected with diabetes worldwide and 693 million people aged are projected to have diabetes by the year 2045 with most individuals suffering from type 2 diabetes mellitus (T2DM) [1]. Central American, South American, and other Hispanic, Latino or Spanish adults had prevalences ranging from 5.0% to 7.3% [2]. Uncontrolled patients with T2DM have a higher risk of cardiovascular (CV) complications, leading cause of death [2] [3]. The current management of this condition involves a patient-centered, comprehensive approach where by hyperglycemia and other risk factors for cardiovascular disease (CVD), such as hypertension, obesity, dyslipidemia, and lifestyle interventions as the use of effective pharmacological therapies [1]-[3].

Most patients with T2DM who received monotherapy are unable to maintain glucose levels with the progress of disease, and combined therapy with two or more anti-diabetic agents (ADAs) of different therapeutical classes has been indicated to achieve better glycemic control [3] [4]. Metformin is the most commonly used drug for patients with T2DM, benefiting from its efficacy and safety, however, several patients require dual or triple therapy to achieve glycemic control due to progressive deterioration of beta-cell function [3]. Empagliflozin is an excellent candidate as an add-on therapy to metformin based on it is different glucose-lowering mechanism inhibiting sodium-glucose cotransporter-2 (SGLT2) mediated renal glucose absorption [3] [4].

Metformin is a biguanide used to lower blood sugar by reducing hepatic glucose output and improving peripheral insulin resistance [4]-[7]. Metformin is recommended in the guidelines for the diagnosis and treatment of diabetes formulated by many countries and international organizations as the first-line drug for controlling hyperglycemia in T2DM patients as the basic drug in drug combination [2]-[7].

Metformin (3-(diaminomethylidene)-1,1-dimethylguanidine) ($C_{23}H_{27}ClO_7$) chemically is a hydrophilic base that exists at physiological pH as the cationic species (>99.9%). The mean ± SD fractional oral bioavailability of metformin is 55% ± 16%. It is absorbed predominately from the small intestine. Metformin is excreted unchanged in urine. The elimination half-life of metformin during multiple dosages in patients with good renal function is approximately 6.5 hours. The population mean renal clearance (CL(R)) and apparent total clearance after oral administration (CL/F) of metformin were estimated to be 510 ± 130 mL/min and 1140 ± 330 mL/min, respectively, in healthy subjects and diabetic patients with good renal function. Over a range of renal function, the population mean values

of CL(R) and CL/F of metformin are 4.3 ± 1.5 and 10.7 ± 3.5 times as great, respectively, as the clearance of creatinine (CL(CR)) [4] [7]. Metformin is excreted unchanged in the urine and does not undergo hepatic metabolism (no metabolites have been identified in humans) or biliary excretion. Approximately 90% of the drugs are eliminated in 24 hours in those with healthy renal function. Renal clearance of metformin is approximately 3.5 times and the tubular secretion is the primary mode [4]-[8]. The dosage of metformin should be reduced in patients with renal impairment in proportion to the reduced CL(CR) [4] [7]. The effect of food is minimal with combination tablets [4]-[8].

Empagliflozin is a selective inhibitor of SGLT2, (D-Glucitol,1,5-anhydro-1-C-[4-chloro-3-[[4-[[(3S)-tetrahydro-3furanyl]oxy]phenyl]methyl]phenyl]-(1S)) increase glucose excretion in urine, acting independently of insulin with an excellent glycemic effect, in addition to its there is much evidence of the extra-glycemic benefits [4] [9]. The pharmacokinetic profile after oral administration, peak plasma concentrations (C_{max}) of empagliflozin were reached at 1.33 - 3.0 h post-dose [4] [9]-[11]. The steady-state mean plasma in the area under curve (AUC) and C_{max} were 1870 nmol·h/L and 259 nmol/L, respectively, with 10 mg empagliflozin once-daily treatment, and 4740 nmol·h/L and 687 nmol/L, respectively, with 25 mg empagliflozin once daily treatment [9]-[11]. The single-dose and steady-state pharmacokinetic parameters of empagliflozin were similar, suggesting linear pharmacokinetics with respect to time [4] [9]-[11]. The apparent terminal elimination half-life of empagliflozin was estimated to be 12.4 h and apparent oral clearance was 10.6 L/h (41.2%), based on the population pharmacokinetic analysis [4] [9]-[11]. No major metabolites of empagliflozin were detected in human plasma and the most abundant metabolites were three glucuronide conjugates (2-O-, 3-O-, and 6-O-glucuronide) [9]-[11]. Following once-daily dosing, up to 22% accumulation, with respect to plasma AUC, was observed at steady-state, which was consistent with empagliflozin half-life ($V_{1/2)}$ [4] [9]-[11]. The apparent steady-state volume of distribution (Vd) was estimated to be 73.8 L based on a population pharmacokinetic analysis and plasma protein binding was 86.2%) [6]. Approximately 95.6% of the drug-related radioactivity was eliminated in feces (41.2%) or urine (54.4%) [4] [11]-[13].

Empagliflozin and metformin have complementary mechanism of action, empagliflozin inhibits SGLT2, which is responsible for glucose reabsorption and increases urinary glucose excretion and metformin lowers hepatic glucose production through stimulating AMP-activated protein kinase, increasing insulin sensitivity. Combination therapy, empagliflozin and metformin provided synergetic glucose-lowering activity in patients with T2DM. Fixed-dose tablets could simplify the dosification schedule, improving patient adherence and treatment compliance [3] [4] [9]. This combined therapy with two drugs that have complementary mechanism of action, metformin and empagliflozin, ensures appropriate glycemic control, with a low risk of hypoglycemia and acceptable tolerability without overlapping adverse events [4].

The purpose of the present study was to assess and compare the PK profile and safety of two fixed-dose combination tablets of empagliflozin 12.5 mg/metformin 1000 mg, Synjardy® (Boehringer Ingelheim Pharmaceuticals, Inc.) as reference (R) formulation vs empagliflozin/metformin 12.5 mg/1000 mg (Laboratorios Leti, S.A.V.) as a test (T) formulation, in healthy adult subjects under fasted conditions. This study was conducted in India, by CRO ICBio Clinical Research Pvt. Ltd.

2. Materials and Methods

The study was conducted ethically in accordance with the principles of the ICMR guidelines (2017) [14], New Drugs & Clinical Trials Rules 2019 India [15], and adhered to the ethical principles of the Declaration of Helsinki [16], the International Conference on Harmonization Good Clinical Practice Guidelines [17]. The study protocol was approved by an Independent Ethical Committee (ECR/141/indt/KA/2013/RR-19), Application N˚ EC/RENEW/IND2019/6255 and certified by CDSCO/DGHS to ICBio Clinical Research Pvt. Ltd., Study number: ICBio/028/0722 (date 4 March 2023).

2.1. Study Design

An open-label study, randomized, two-treatment, two-period, two-sequence, single oral dose, crossover in fasted condition, designed to evaluate bioequivalence of two FDC tablets. Empagliflozin 12.5 mg/metformin 1000 mg tablets were provided as test formulation (T) by Laboratorios Leti S.A.V., República Bolivariana de Venezuela, batch N˚ EP-0322529-17 (Expiry date Sep. 2024) and reference formulation (R) was branded Synjardy® (Empagliflozin 12.5 mg/metformin 1000 mg) tablets of Boehringer Ingelheim Pharmaceuticals, Inc., Germany, batch N˚E0201453A (Expiry date: Feb. 2025). According to randomization schedule, a single dose of the study drug (T or R) 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 study lasted for 15 days (17/02/23 to 03/03/23) including 12 days of washout considering the terminal half-life of 6.6 and 12.5 hours for metformin and empagliflozin respectively [3] [4] [7] [11]. The randomization schedule was generated using Statistical Analysis Software (SAS® version 9.1.3).

2.2. Subjects

All volunteers underwent a screening procedure. Twenty-four healthy male volunteers who met the inclusion and exclusion criteria were enrolled. They had a mean age of 34.42 years, mean weight of 70.46 Kg, mean height of 169 cm, and body mass index (BMI) of 24.82 kg/m^2 (**Table 1**).

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 drug tests for drugs of abuse and alcohol consumption were performed on day of check-in of each period). Another 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.

Table 1. Demographic profile of subjects completing the bioequivalence study (n = 23).

Age	Mean ± SD		34 ± 6.45	
(years)	Range		20 - 43	
Age group		**Male**	**%**	**Total**
	18 - 40	19	82.60%	19/82.60%
	41 - 44	4	17.39%	4/17.39%
Total	23 - 44	23	100%	23/100%
BMI (kg/m²)	Mean ± SD		24.82 ± 2.8	
	Range		(19.84 - 28.73)	
Race	Asian		23	100%

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.

2.3. Drug Administration

The subjects were admitted to the facility overnight before study. In each period, after an overnight fasting of 10 hours, each subject received a single oral dose (1 X empagliflozin 12.5 mg/metformin 1000 mg) FDC tablets of either one T or R, following randomization schedule with 240 mL ± 5 mL of 20% of glucose water at ambient temperature in sitting position followed by the dosing 60 ml of the 20% glucose water at ambient temperature in each period. A total of 21 × 6 ml of venous blood samples were collected through cannula from each subject during each period, withdrawn at pre-dose (0.00 h) and 0.125, 0.50, 0.75, 1.00, 1.25, 1.50, 1.750, 2.00, 2.50, 3.00, 4.00, 5.00, 5.50, 6.00, 7.00, 8.00, 10.00, 12,00, 24.00, 48.00 and 72.00 h post dose, following drug administration in each administration.

All the subjects received standardized meals at 4.00, 8.00, 12.00 and 24.00 hours

after dosing in each period (2500 Kcal) and drinking water was provided *ad libitum*.

2.4. Analytical Procedure

ICBio has determined empagliflozin and metformin concentrations in K_2EDTA (ethylenediaminetetraacetic acid) venous plasma samples by solid phase extraction method using a validated high-performance liquid chromatographic method with mass spectrometric detection. Venous blood samples were centrifuged at 3800 rpm for 10 min at 10˚C within 45 minutes of sample collection. Plasma was separated, labelled and stored at –70˚C ± 15˚C before analysis. Data performance of assay method using SOP N˚ MV 003 and analyte across for this study to empagliflozin, linearity range was 5.087 to 807.490 ng/ml, (% CV 2.65 - 6.26) to metformin the linearity range was 31.210 to 5.004.012 ng/ml (% CV 2.03 - 5.73), with calibration standards, mean accuracy (99.82 - 100.00) mean precision and quality control samples (Vivian Life Sciences Private Limited, Mumbai, India). All samples were thawed and vortexed for preparation and analysis. LC-MS/MS and mass spectrometric were used as internal standard (IS) (Vivian Life Sciences) for empagliflozin D4 HCL and metformin D6 HCL. The major instruments used for determination of empagliflozin and metformin in plasma were: ICBio-II/BA/LCMS/001 quaternary pump, autosampler and column Exion LC, detector; Sciex triple quad 4500. Column (BDS Hypersil C_{18}, 4.6 × 100 mm, 5 µm). The mass spectrometer was operated in positive electrospray mode. Identifications were based on multiple reactions monitoring transitions to each formulation: m/z 359.00 - 472.05 for empagliflozin and m/z 355.10 - 468.15 for the empagliflozin IS and m/z 400.10 - 722.00 to metformin and m/z 413.10 - 730.00 for metformin IS.

2.5. Statistical Analysis

The sample size calculation for the study was based on intra-subject coefficient of variation (CV%) for empagliflozin and metformin obtained from published literature. Empagliflozin as obtained (C_{max} 14% - 25%, $AUC_{0-\infty}$ 16% - 24%) with the expected % CV for C_{max} and AUC not exceeding 20% and the ratio between within 95% to 105% (*i.e.* a true treatment difference of 5%), twenty evaluable subjects would be enough to demonstrate BE [4] [11] [18]-[23]. For metformin, the C_{max} and AUC were calculated in 20%, with the expected % CV not exceeding 22% and the ratio within 95 and 105%, requiring 18 evaluable subjects to demonstrate BE with a power of ≥80% at 5% level of significance [4] [7] [18]-[21]. Based on these data to a sample size, 20 subjects were sufficient to demonstrate BE between the two FDC tablets. Additional subjects (4) were included in the study for possible dropouts/withdrawals. Statistical analysis was conducted on all of the subjects who completed both periods of the study as per protocol, using SAS® software version 9.1.3 (Institute. Inc., CARY, USA).

The PK parameters calculated were maximum peak concentration (C_{max}), area-under-curve (AUC) from time 0 h to the last measurable concentration (AUC_{0-t}),

AUC from time 0 to infinity ($AUC_{0-\infty}$), time to reach C_{max} (T_{max}) as primary parameters to PK analysis. Other secondary PK parameters evaluated were: AUC _% Extrap_obs, T_{max}, $T_{1/2}$ and λ_z/K_{el} of empagliflozin and metformin in plasma. The log-transformed pharmacokinetic parameters were analysed using a general linear model (Proc GLM of SAS® Mumbai, India). The formulations were regarded as bioequivalent when the 90% confidence intervals (CIs) of the T and R ratio of AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} ranged from 80% - 125% [18]-[22]. This is the standard accepted by the United States Food and Drug Administration [18].

2.6. Safety Assessments

The safety of two formulations (T and R) was evaluated through the assessment of adverse events monitoring throughout the study. According to FDA rules, subjects were a test or parent reference formula in 240 mL of 20 percent glucose solution and 60 mL of 20 percent glucose solution every 15 minutes for 4 hours to reduce the risk of hypoglycaemia [18]. All adverse events (AEs) were recorded based on the Medical Dictionary for Regulatory Activities (MEDRA). The Common Terminology Criteria for Adverse Events were used for assessment of severity. Vital signs were measured during baseline screening, and at the conclusion of the study. Twelve-lead electrocardiogram and clinical laboratory such as urine analysis, haematology, blood biochemistry including glycaemia evaluation were conducted during screening and again 72 hours after the study.

3. Results

A total of twenty-three subjects completed the study and were included in the PK and statistics evaluation. Plasma concentration data for empaglifozin and metformin were evaluated and presented in arithmetic and logarithmic scales in Table 2 and Table 3. A non-compartmental analysis was applied for the estimation of PK parameters C_{max}, AUC_{0-t}, $AUC_{0-\infty}$, T_{max}, K_{el} ($^{h-1}$), and $T_{1/2}$, of empagliflozin and metformin in plasma concentration, which are presented in Table 4 and

Table 2. Empagliflozin plasma concentations per time in arithmetic and logarithmic scale. Mean values per hour (n = 23).

Plasma concentrations: arithmetic values and standard errors (SE)					Plasma concentrations: logarithmic values (Ln) and standard errors (SE)			
Time (h)	Plasma concentration TEST (T)	Plasma concentration Reference (R)	SE (T)	SE (R)	Plasma concentration Ln TEST (T)	Plasma concentration Ln Reference (R)	SE (T)	SE (R)
0	0.00	0.00	0.000	0.000	0	0	0	0
0.125	0.28	0.00	3.533	0.000	0	0	0	0
0.5	27.67	30.16	0.207	0.178	3.32	3.40	0.833	0.815
0.75	60.26	65.24	0.172	0.141	4.09	4.17	0.708	0.699
1	90.47	97.83	0.165	0.115	4.50	4.58	0.664	0.656

Continued

1.25	115.99	130.85	0.136	0.103	4.75	4.87	0.641	0.631
1.5	127.31	141.12	0.139	0.120	4.84	4.94	0.633	0.625
1.75	136.01	155.63	0.139	0.116	4.91	5.04	0.628	0.617
2	140.68	160.74	0.131	0.112	4.94	5.07	0.625	0.615
2.5	134.81	156.92	0.174	0.122	4.90	5.05	0.628	0.617
3	130.86	148.13	0.175	0.156	4.87	4.99	0.631	0.621
4	112.65	126.44	0.270	0.189	4.72	4.83	0.644	0.634
5	93.30	101.68	0.283	0.261	4.53	4.62	0.661	0.653
6	79.65	86.83	0.278	0.277	4.37	4.46	0.677	0.668
7	64.44	72.93	0.400	0.298	4.16	4.289	0.700	0,686
8	57.53	64.52	0.432	0.425	4.05	4.16	0.714	0.700
10	45.57	52.00	0.510	0.490	3.81	3.95	0.746	0.727
12	34.76	41.55	0.008	0.512	3.54	3.72	0.789	0.760
24	10.51	12.65	0.012	1.204	2.35	2.53	1.169	1.073
48	0.00	0.00	0.000	0.000	0.000	0.00	0.00	0.00
72	0.00	0.00	0.000	0.000	0.000	0.00	0.00	0.00

Table 3. Metformin plasma concentrations per time, arithmetic and logarithmic values. Mean values per hour (n = 23).

	Plasma concentrations: arithmetic values and standard errors (SE)				Plasma concentrations: logarithmic values (Ln) and standard errors (SE)			
Time (h)	Metformin Plasma Concentration Test (T)	Metformin Plasma Concentration Reference (R)	SE Test (T)	SE Reference (R)	Metformin Ln Test (T)	Metformin Ln Reference (R)	SE Test (T)	SE Reference (R)
0	-	0.00	-	-	0	0	0	0
0.125	96.53	40.10	0.010	0.025	4.56	3.69	0.029	0.081
0.5	691.19	527.47	0.001	0.002	6.54	6.27	0.008	0.018
0.75	1055.95	939.85	0.001	0.001	6.96	6.85	0.008	0.015
1	1254.44	1223.20	0.001	0.001	7.13	7.11	0.009	0.012
1.25	1373.37	1431.30	0.001	0.001	7.23	7.27	0.010	0.012
1.5	1453.23	1523.48	0.001	0.001	7.28	7.33	0.010	0.014
1.75	1494.08	1617.86	0.001	0.001	7.31	7.39	0.011	0.012
2	1575.24	1737.15	0.001	0.001	7.36	7.46	0.011	0.011
2.5	1617.85	1766.39	0.001	0.001	7.37	7.48	0.013	0.010
3	1592.44	1734.75	0.001	0.001	7.39	7.46	0.013	0.011
4	1493.24	1669.15	0.001	0.001	7.31	7.42	0.016	0.011
5	1242.84	1370.04	0.001	0.001	7.13	7.22	0.017	0.014
6	960.54	1099.27	0.001	0.001	6.87	7.00	0.018	0.015

Continued

7	694.60	820.79	0.001	0.001	6.54	6.71	0.027	0.020
8	580.14	624.76	0.002	0.002	6.36	6.44	0.038	0.028
10	408.19	430.90	0.002	0.002	6.01	6.07	0.022	0.040
12	246.31	291.02	0.004	0.003	5.51	5.67	0.072	0.040
24	93.37	54.29	0.011	0.018	4.54	3.99	0.106	0.090
48	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
72	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table 4. Pharmacokinetics parameters after a single oral dose of test (T) and reference (R) formulations of empagliflozin 12.5 mg (n = 23).

PK parameters (units)	Media ± SD	
	Test (T)	Reference (R)
C_{max} (ng/mL)	140.68 ± 33.68	160.64 ± 74.78
AUC_{0-t} (ng·h/mL)	1198.9547 ± 218.36.08571	1362.3670 ± 242.7969
$AUC_{0-\infty}$(ng·h/mL)	1306.5089 ± 245.35724	1496.4944 ± 267.16367
T_{max} (hrs)	2.000 (1.750 - 4.000)	2.000 (1.750 - 4.000)
K_{el} (hrs^{-1})	0.1035 ± 0.01440	0.1034 ± 0.01968
$T_{1/2}$ (hrs)	6.8307 ± 1.02535	6.9733 ± 1.52330

Data presented as a mean ± SE. 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; K_{el}: Elimination rate constant; T_{max}: Time to reach C_{max}; $T_{1/2}$: Time required for plasma concentration to decrease by 50%; h: Hour; hrs: Hours.

Table 5, analysis of variance from Ln C_{max}, Ln AUC_{0-t} and Ln $AUC_{0-\infty}$ in Table 6 and Table 7. The oral dosing of empagliflozin 12.5 mg/metformin 1000 mg FDC tablets, for 48 h post-dose is represented on arithmetic and logarithm scales, as shown in Figure 1 and Figure 2 to empagliflozin (T and R) and Figure 3 and Figure 4 to metformin (T and R).

Empagliflozin PK mean values of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ were respectively 140.68 ng/mL, 1198.95 ng·h/mL and 1306.50 ng·h/mL to test formulation and 160.64 ng/mL, 1362.36 ng·h/mL and 1496.49 ng·h/mL to reference formulation (Table 4).

Metformin PK mean values of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ were respectively 1617.85 ng/mL, 12563.48 ng·h/mL and 13961.79 ng·h/mL to test formulation and 1766.64 ng/mL, 13610.53 ng·h/mL and 14329.85 ng·h/mL to reference formulation.

The test/reference geometric mean ratios and 90% CIs for the logarithm of C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ are presented in Table 6 and Table 7. The BE results were as follows: Ln C_{max}: 89.87% (85.68% - 94.27%), AUC_{0-t}: 87.91% (83.65% - 92.39%) and $AUC_{0-\infty}$: 87.16% (82.80% - 91.75%) to empagliflozin and C_{max}: 92.19% (87.95% - 96.65%), AUC_{0-t}: 91.38% (84.42% - 98.91%) and $AUC_{0-\infty}$: 93.78% (83.82% - 104.93%)

to metformin, these values are within the 90% CIs and acceptance criteria of 80% - 125%. There were no significant differences between the PK parameters of two formulations of empagliflozin 12.5 mg/metformin 1000 mg fix dose tablets ($P > 0.05$). These results met the predefined bioequivalence requirements.

Table 5. Pharmacokinetics parameters after a single oral dose of test (T) and reference (R) formulations of metformin 1000 mg (n = 23).

PK parameters (units)	Media ± SD	
	Test (T)	Reference (R)
C_{max} (ng/mL)	1617.85 ± 33.68	1766.64 ± 39.78
AUC_{0-t} (ng·h/mL)	12563.4882 ± 3616.37447	13610.5364 ± 3278.69738
$AUC_{0-\infty}$ (ng·hr/mL)	13961.7976 ± 6227.38257	14329.8573 ± 3559.79946
T_{max} (hrs)	2.500 (0.500 - 4.000)	2.500 (1.000 - 6.000)
K_{el} (hrs^{-1})	0.1597 ± 0.05339	0.1550 ± 0.04178
$T_{1/2}$ (hrs)	4.8930 ± 2.06590	5.0049 ± 2.34401

Data presented as a mean ± SE. 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; K_{el}: Elimination rate constant; T_{max}: Time to reach C_{max}; $T_{1/2}$: Time required for plasma concentration to decrease by 50%; h: Hour; hrs: Hours.

Table 6. Correlation of 90% CIs of Ln-transformed of main PK variables for empagliflozin 12.5 mg after administration of two formulations (test and reference).

Parameters (units)	Geometric mean ratio GMR		Ratio (%) (T/R)	90% confidence intervals (%)	Power (T vs R) (%)
	T	R			
Ln (C_{max}) (ng/mL)	150.569	167.278	89.7	85.68 - 94.27	100.0
Ln (AUC_{0-t}) (h·ng/mL)	1180.294	1341.268	87.91	83.65 - 92.39	100.0
Ln ($AUC_{0-\infty}$) (h·ng/mL)	1285.414	1473.305	87.16	82.90 - 91.75	100.0

Data presented as a % mean Ln transformed. C_{max}: Maximum concentration; AUC_{0-t}: Area under the plasma concentration-time curve from time 0 to the last measurable concentration, BE acceptance criteria of 80% - 125%; GMR: Geometric mean ratios; CI: Confidence interval; PK: Pharmacokinetic.

Table 7. Correlation of 90% CIs of Ln-transformed of main PK variables for metformin 1000 mg after administration of two formulations (test and reference).

Parameters (units)	Geometric mean ratio GMR		Ratio (%) (T/R)	90% confidence Intervals (%)	Power (T vs R) (%)
	T	R			
Ln (C_{max}) (ng/mL)	1738.406	1886.854	92.19	87.95 - 96.65	100.0
Ln (AUC_{0-t}) (h·ng/mL)	12111.703	13242.269	91.38	84.42 - 98.91	99.42
Ln ($AUC_{0-\infty}$) (h·ng/mL)	13100.783	13946.410	93.78	83.82 - 104.93	90.27

Data presented as a % mean Ln transformed. C_{max}: Maximum concentration; AUC_{0-t}: Area under the plasma concentration-time curve from time 0 to the last measurable concentration, BE acceptance criteria of 80% - 125%; GMR: Geometric mean ratios; CI: Confidence interval; PK: Pharmacokinetic.

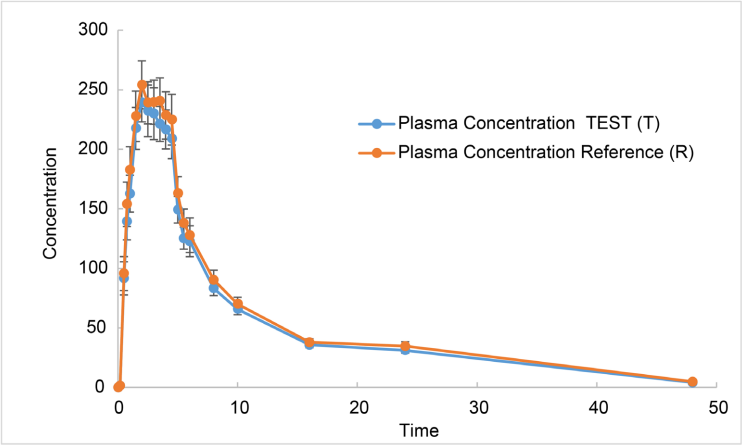

Figure 1. Empagliflozin plasma concentration (ng/mL) vs time (h). Arithmetic scale.

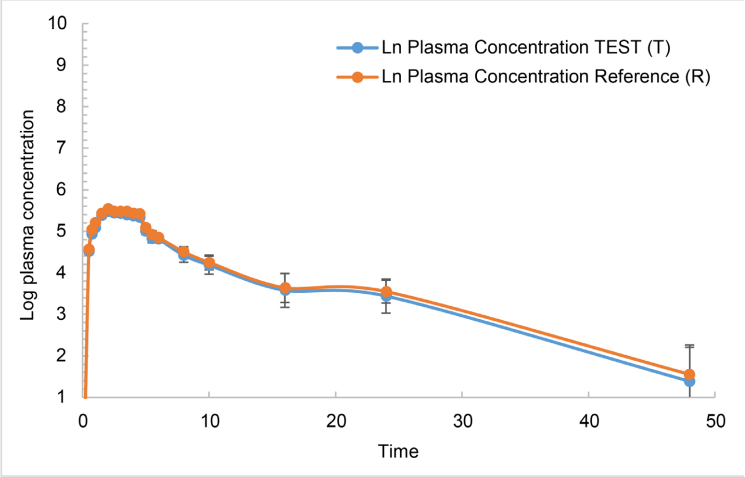

Figure 2. Empagliflozin plasma concentration (ng/mL) vs time (h). Logarithmic scale.

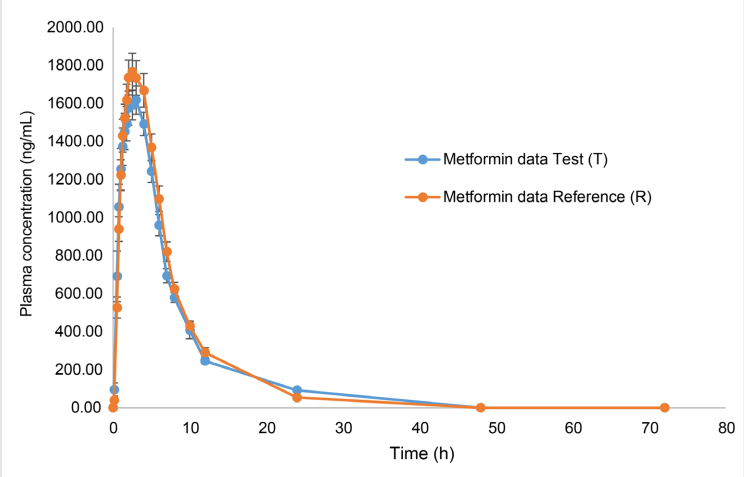

Figure 3. Bioequivalence study of metformin. Mean plasma concentration (ng/mL) vs time (h). Arithmetic scale.

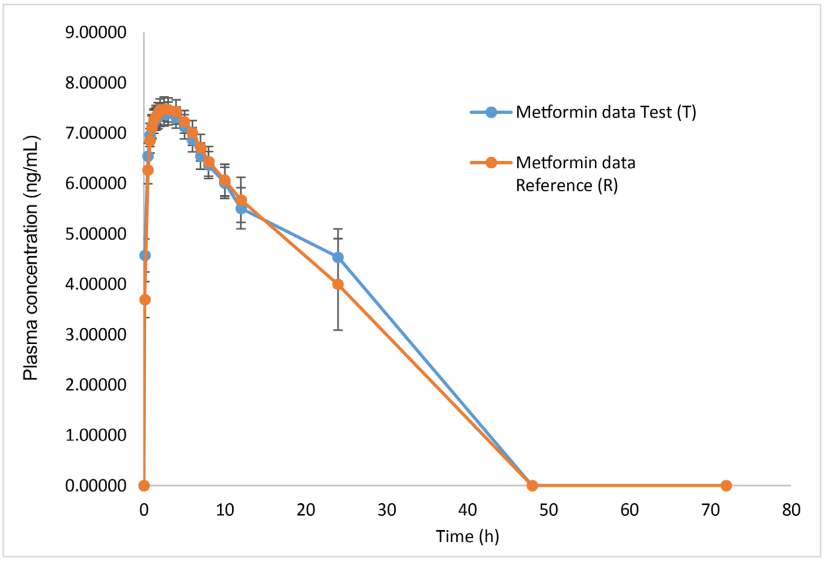

Figure 4. Bioequivalence study of metformin. Mean plasma concentration (ng/mL) vs time (h). Logarithmic scale.

Tolerability and Safety

A total of 23 subjects post-safety samples were collected, the subject 18 was reported as dropout in period II. No adverse events were reported during the course of the study. Hence, the T and R formulations were found to be safe and well tolerated.

4. Discussion

The present study was designed to evaluate the bioequivalence in one single-dose study of two treatments, two periods, and crossover in healthy subject's volunteers under fasting conditions [18]-[20] [22] [23]. The 90% CI to empagliflozin/metformin fix dose tablets was assessed by determined bioequivalence standards of 80% - 125% for C_{max}, AUC $_{0\text{-}t}$ and $AUC_{0\text{-}\infty}$ [18]-[20]. The study included 24 male subjects covering the variability observed in other studies with a sufficient number of subjects to ensure statistical power to demonstrate the bioequivalence of both formulations [18]-[20] [22].

Empagliflozin and metformin PK parameters (T and R formulations) were analyzed for the T/R ratios, all of them were within the 90% CI BE limits of 80% - 125% [18]-[20] [22].

BE was demonstrated between the T product and R product, oral tablets at dose of empagliflozin 12.5 mg/metformin 1000 mg, under fasting conditions. These similarities in values and shape of the plasma concentration-time curves can be seen in **Figures 1-4**, to test and reference formulations of empagliflozin and metformin.

Type 2 diabetes mellitus (T2DM) is a complex, chronic illness characterized by persistent high blood glucose levels, with severe cardiovascular complications in patients uncontrolled, some patients can receive a simple drug or a combination of

drugs, depending on glucose control levels. The combination of metformin and empagliflozin has been prescribed in accordance with health research association guidelines such as the National Institute of Health Care Excellence (NICE), American Diabetic Association (ADA) and American Association of Clinical Endocrinologist [2] [7] [11]. Combination therapy could significantly improve glycemic control in patients with T2DM [2]-[4] [9] [11] [15]. This bioequivalence study of empagliflozin/metformin fixed-dose tablets in generic presentations could reduce pill burden, improve patient adherence and compliance and optimize cost-effectiveness. This combination provides synergetic glucose control in patients with T2DM, in addition to providing patient adherence and offers potential cost advantages [24].

This therapy combined provides an alternative across a range of patients with T2DM with other associated pathologies and cardiovascular complications with a simplified treatment that improves adherence when multiple medications are required due to their condition.

Currently, regulatory agencies in Latin America are requiring bioequivalence studies to support the registration of generic drugs in these countries, demanding more attention in the process of conducting BE studies to make decisions, ensuring greater access and adherence to these treatments, especially in pathologies as diabetes with high risk of cardiovascular complications [24]-[27].

5. Limitations of the Study

This study analyzed PK parameters of metformin/empagliflozin under fasting conditions, and the results showed that pharmacokinetic behaviors of empagliflozin and metformin were clinically irrelevant to the effects of food and followed general BE guidelines [3] [4] [22]. This study included healthy males only.

6. Conclusion

The fixed-dose combination of two formulations containing 12.5 mg of empagliflozin and 1000 mg of metformin was evaluated, resulting in bioequivalent in healthy subjects under fasting conditions. The PK profile of test and reference formulations was similar, demonstrated by the 90% CIs of C_{max}, $AUC_{0\text{-}t}$ and $AUC_{0\text{-}\infty}$ within the accepted BE criteria of 80% - 125%.

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This study was conducted at the third-party ICBio Clinical Research Pvt. Ltd.

Authors' Contributions

E.P., AI., A.T., X.S., and J.C. 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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Conflict of Interest

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

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