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Bioequivalence Assessment of Two Fixed-Dose Formulations of Empagliflozin/Linagliptin (25 mg/5 mg) Coated Tablets in Healthy Subjects under Fasting and Fed Conditions

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Abstract

Objective: This study compared the pharmacokinetic and safety profiles of two fixed-dose combinations (FDCs) and the mean ratios of the test to reference formulations of empagliflozin/linagliptin (25 mg/5 mg) coated tablets, evaluating the bioequivalence within both formulations under two conditions. **Methods:** Two open-label, randomized, single-dose, two-period crossover studies were conducted under fasting (n = 32) and fed (n = 32) conditions. Eligible healthy subjects received a single oral dose of empagliflozin/linagliptin of test or reference formulations, followed by a 42-day washout interval between period I and period II. A total of 16 blood samples were collected up to 72 h after administration in each period. The primary pharmacokinetic (PK) parameters were calculated using the non-compartmental method. Bioequivalence (BE) was established for both studies, as the 90% confidence interval of the ratio of adjusted geometric mean (GMR) for C_{max} and $AUC_{0\text{-}72}$ was contained within the predefined BE criteria range of 80% - 125%. **Results:** The 90% confidence intervals (CIs) for the GMR of the test and reference formulations were: C_{max} (88.56% - 102.75%), $AUC_{0\text{-}72}$ (88.46% - 97.50%) for empagliflozin, and C_{max} (81.88% - 97.23%), $AUC_{0\text{-}72}$ (86.68% - 104.09%) for linagliptin under fasting conditions. The PK parameters of C_{max} (87.17% - 100.94%), $AUC_{0\text{-}72}$ (80.78% - 92.02%) were reported for empagliflozin, and C_{max} (90.83% - 105.94%), $AUC_{0\text{-}72}$ (86.12% - 109.51%) were reported for linagliptin under fed conditions. **Conclusion:** The BE of two FDC formulations, empagliflozin/linagliptin, 25 mg/5 mg, coated tablets as the test product and Jardianz® DPP, (empagliflozin/linagliptin) 25 mg/5 mg coated tablets as the reference prod-

uct, were demonstrated under fasting and fed conditions. No adverse events were reported.

Keywords

Bioequivalence, Fixed-Dose Combination, Empagliflozin, Linagliptin

1. Introduction

The pharmacology management of type 2 diabetes mellitus (T2DM) involves the use of new therapies for glycemic control and the prevention of cardiovascular complications of this pathology, as well as decreasing morbidity and mortality [1].

Among patients for whom a single anti-diabetic drug fails to adequately control blood glucose, the American Diabetes Association (ADA) and European Association for the Study of Diabetes (EASD) recommend initiation of combination therapy [2]. Fixed-dose combination (FDC) therapies with complementary mechanisms of action can improve medication adherence by reducing pill burden and may improve glycemic control in T2DM [1] [3]. The American Association of Clinical Endocrinologists' and the American College of Endocrinology's (AACE/ACE) recommends initial combined therapy when HbA1c is ≥7.5%, offering a rapid reduction of HbA1c and avoiding the harmful effects of glucotoxicity and the adverse events associated with high doses in monotherapy to achieve glycemic control [4].

The chemical name of empagliflozin is D-Glucitol, 1,5-anhydro-1-C-[4-chloro-3-[[4-[[(3S)-tetrahydro-3-furanyl] oxy] phenyl] methyl] phenyl], (1S)-. SGLT2 is mainly located in the apical brush border membrane of the S1 segment of the proximal convoluted tubules, which regulates 90% of glucose reabsorption from the glomerular filtrate [5]. The expression of SGLT2 is up-regulated, and the urinary glucose excretion threshold is also higher in patients with hyperglycemia compared with healthy humans [1] [5]. Inhibition of SGLT2 reduces glucose reabsorption, promotes urinary glucose excretion, and produces negative caloric balance, which leads to weight loss [1] [5] [6].

The empagliflozin pharmacokinetic (PK) profile, following oral administration, reaches peak plasma concentrations in approximately 1.5 hours (T_{max}). At steady-state, plasma AUC and C_{max} were 1870 nmol·h/L and 259 nmol/L, respectively, following therapy with 10 mg daily and 4740 nmol·h/L and 687 nmol/L, respectively, following therapy with 25 mg daily [6]. Administration with food does not significantly affect the absorption of empagliflozin [5] [6]. After oral administration of radiolabeled empagliflozin, approximately 41.2% of the administered dose was eliminated in the feces and 54.4% in the urine. The estimated apparent steady-state volume of distribution is 73.8 L. Oral clearance was found to be 10.6 L/h. SGLT2i can thus be used on top of other oral glucose-lowering drugs and insulin

to exert additive anti-hyperglycemic effects [5] [6]. Clinical trials have reported that empagliflozin improves cardiovascular outcomes in patients with heart failure, patients with T2DM who are at high cardiovascular risk, and patients with chronic kidney disease (EMPEROR-Reduced trial, EMPA-KIDNEY, EMPA-REG OUTCOME, CONFIDENCE, and PIONEER 2 Trial) [7]-[12].

The dipeptidyl peptidase-4 (DPP4) inhibitor, linagliptin has the chemical name 8-[(3R)-3-aminopiperidin-1-yl]-7-but-2-ynyl-3-methyl-1-[(4-methylquinazolin-2-yl)methyl]purine-2,6-dione. Linagliptin inhibits the proteolytic activity of the DPP4 enzyme, which results in decreased plasma glucose levels via enhanced insulin secretion from beta cells and decreased glucagon secretion from alpha cells in the pancreas [1]-[6] [13]. The PK profile after oral administration of a single 5-mg dose to healthy subjects reaches peak plasma concentrations at approximately 1.5 hours post-dose (T_{max}); the mean plasma area under the curve (AUC) was 139 nmol·h/L, and maximum concentration (C_{max}) was 8.9 nmol/L. The bioavailability of linagliptin is approximately 30%. A high-fat meal reduced C_{max} by 15% and increased AUC by 4%; this effect is not clinically relevant. Linagliptin may be administered with or without food [1] [13] [14]. Following administration of an oral (14C)-linagliptin dose to healthy subjects, approximately 85% was eliminated in the feces and 5.4% in the urine. Renal clearance at steady state was approximately 70 mL/min. A dose of 5 mg results in a volume of distribution of 1110 L. Linagliptin has a total clearance of 374 mL/min [1] [4]-[6] [13] [14]. The cardiovascular safety of linagliptin among patients with T2DM and elevated cardiovascular risk has been demonstrated in the CARMELINA and CAROLINA trials [1] [3] [15]-[17]. Linagliptin reduces HbA1c, is weight-neutral, has an excellent safety profile, and a low risk of hypoglycemia [1] [4]-[6].

The fixed-dose combination of empagliflozin and linagliptin has demonstrated good tolerability in phase III clinical trials [4]-[6]. The once daily oral combination of linagliptin plus empagliflozin does not increase the risk of hypoglycemia and has tolerability and discontinuation rates similar to those with each agent as monotherapy [4]-[6]. At HbA1c values below 8.5%, linagliptin/empagliflozin treatment produces an additive effect, whereas above 8.5%, there is a less than additive reduction with combination therapy compared with the effect of each agent alone [1] [4]-[6]. Linagliptin/empagliflozin addition is a logical combination in patients with T2DM, especially those with an HbA1c < 8.5%, as described in published studies [13] [16].

The purpose of the present study was to assess and compare the PK profile and safety of Jardianz® DPP (empagliflozin/linagliptin) 25 mg/5 mg, coated tablets (Boehringer Ingelheim Pharma GmbH & Co) as a reference product (R) vs empagliflozin/linagliptin 25 mg/5 mg coated tablets (Laboratorios Leti, S.A.V., República Bolivariana de Venezuela) as a test product (T) in healthy adult subjects under fasting and fed conditions. Both studies were required to demonstrate BE within the two formulations. This study was conducted in India by CRO ICBio Clinical Research Pvt, Ltd.

2. Materials and Methods

2.1. Ethical Considerations

The study was conducted ethically in accordance with the principles of the ICMR guidelines (2017) [18]. New Drugs & Clinical Trials Rules 2019, India [19], and adhered to the ethical principles of the Declaration of Helsinki [20] and the International Conference on Harmonization Good Clinical Practice Guidelines [21]. The study protocol was approved by an Independent Ethical Committee (ECR/141/indt/KA/2013/RR-19), Application No. EC/RENEW/IND2019/6255, and certified by CDSCO/DGHS to ICBio Clinical Research Pvt, Ltd., Study numbers: ICBio/010/0224 and ICBio/013/0224.

2.2. Study Drugs

The test formulation was an empagliflozin/linagliptin 25 mg/5 mg coated tablet provided by Laboratorios Leti, S.A.V., República Bolivariana de Venezuela, batch number: EP 0623553-6R; expiry date: February 2026. The reference formulation was Jardianz® DPP coated tablets from Boehringer Ingelheim Pharma GmbH & Co. (empagliflozin/linagliptin) 25 mg/5 mg coated tablets, batch number: 303018; expiry date: April 2026. Each test or reference formulation was administered during each treatment period.

2.3. Subjects

Healthy adult human subjects aged over 18 years (21 - 44), with body mass index (BMI) between 19.96 and 29.76 kg/m^2. A complete clinical history valid for the 6 months prior to the start of the study**;** normal laboratory values as determined by medical history and physical examination at the time of screening; normal vital signs (blood pressure, pulse rate, and axillary temperature) and physical examination; creatinine clearance (CrCl) value of more than 50 mL/min; negative tests for hepatic transaminases, hepatitis B and C, HIV, and VDRL; normal 12-lead EKG values, normal chest radiography, and negative result in urine drug tests. Subjects included non-smokers as well as smokers who had not smoked for at least 10 hours before the start of the study. All the subjects were informed about the possible risks and the benefits of their participation, such as blood lab tests, electrocardiograms, and travel and food expenses. They all signed the informed consent.

The exclusion criteria were as follows, a history of hypersensitivity to the study medication or to any other medication belonging to the study group, or any cardiovascular, renal, hepatic, metabolic, gastrointestinal, neurological, endocrine, hematopoietic, psychiatric, or other organic abnormalities; taking medications that interfere with the quantification and/or kinetics of the study medication, or potentially toxic medications within 30 days prior to the start of the study; having taken any medication, been hospitalized for any reason, or been seriously ill within the 90 days prior to the study; donated or lost 300 mL or more of blood within 90 days prior to the start of the study; recent history of drug abuse, including alcohol; consumed products such as cola drinks containing caffeine, theobro-

mine, or theophylline in the 48 h prior to the study; consumption of grapefruit juice within 72 hours prior to the study.

2.4. Study Design

This study comprised two independent clinical trials (fasting and fed study), each of which was a randomized, open-label, two-formulation, single-dose, two-sequence, two-period crossover bioequivalence study performed at the Phase I, screening, clinical study center, and bioanalytical facility by ICBio Clinical Research Private Limited, India.

All subjects in each trial were randomly assigned to either the T-R or R-T group (T was the test product and R was the reference product) at a 1:1 ratio according to a random number table generated by SAS statistical software (version 9.1.3). Group T-R subjects, who received the test product in the first treatment period, received the reference product in the second treatment period, whereas Group R-T received the opposite administration sequence. A 42-day washout period was used between the two treatment periods for both fed and fasting studies.

Under fasting conditions, each subject received a single dose of the test or reference tablet, administered orally with 240 mL of a 20% glucose solution in water, followed by 60 mL of the glucose solution administered every 15 minutes for up to 4 hours after dosing in a sitting position 2 h after at least a 10-hour overnight fast [22] in a sitting position, whereas the subjects under fed conditions received a standard high-fat (1394 kcal) breakfast (approximately 32.8 g of protein, 266 g of carbohydrate, and 22.5 g of fat) 30 min before each drug administration and followed the same scheme. Water intake was prohibited for 1 h before and after administration; outside of this restriction, it was provided ad libitum. Standardized lunches, snacks, and dinners were provided at 4, 8, and 12 hours after drug administration in each period.

A total of 16 × 6 mL blood samples of 6 mL each were collected through a cannula from each subject during each period at pre-dose and at 0.50, 1.00, 1.25, 1.50, 1.75, 2.00, 2.50, 3.00, 4.00, 6.00, 8.00, 12.00, 24.00, 48.00, and 72.00 hours after dosing. Samples at 48.00 and 72.00 hours were collected by direct venipuncture in both fasting and fed studies.

2.5. Analytical Procedure

The blood samples were collected in pre-labeled K_2 and ethylenediaminetetraacetic acid (EDTA) vacutainers and were centrifuged at 4000 rpm for 10 min at 2˚C - 8˚C. Plasma was separated, labeled, and stored at −70˚C ± 5˚C before analysis. Empagliflozin and linagliptin concentrations in K_2EDTA, Human plasma samples collected from study ICBio/010/0224, were determined using the Method SOP N˚ MV-020-00. Sample analysis was conducted from January 27, 2025 to February 09, 2025 for analytes by liquid-liquid extraction method using a validated high-performance liquid chromatographic method with mass spectrometric detection (LC-MS/MS). All processes are inspected by the Quality Assurance Unit. The

method demonstrated high accuracy and precision in measuring calibration standards for Empagliflozin (Analyte 10.030 to 1016.135 ng/ml, mean accuracy 100.06% and mean precision 3.15%) and Linagliptin (Analyte 0.213 to 13.585 ng/ml, mean accuracy 100.86% and mean precision 3.92%).

Empagliflozin: Subsequently, the plasma samples were processed. The calibration curve of internal standards (IS), Empagliflozin D4 (Vivian Life Sciences, Bangalore, India), and quality control (QC) samples were thawed and vortexed for preparation and analysis. Empagliflozin was selectively isolated from 300 µL plasma by a liquid-liquid extraction method. Aliquots of 0.300 µL were mixed with 0.300 µL of extraction buffer (50 mM sodium carbonate in water) and vortexed. After adding 2.500 mL of TBME (methyl tert-butyl ether) and shaking for 10 min, the samples were centrifuged at 4000 rpm for 5 min at 4˚C. The supernatant layer (2.000 mL) was transferred and dried in a nitrogen evaporator at 40˚C ± 2˚C. The samples were reconstituted with 0.50 mL of reconstitution solution and spiked with IS over the concentration range of 10.030 - 1016.135 ng/mL. Analytes, IS, and QC samples were transferred to pre-labeled vials in the autosampler at 5˚C ± 3˚C and injected into a liquid chromatography electrospray ionization tandem mass spectrometry (LC-ESI-MS-MS) instrument (ICBio II/BA/LCMS/0003, Bangalore, India). The chromatographic separation was performed by a BDS Hypersil C18, 4.6 × 50 mm, 5 µm column (Exion LC, Bangalore, India). The mass spectrometer was operated in positive electrospray mode. Identifications were based on multiple reactions monitoring transitions; m/z 355.200 - 468.300 for empagliflozin and m/z 359.100-472.100 for IS. The inter-batch calibration standard accuracy was 96.70% - 102.68% with precision values of 2.34% - 5.50% and a linearity range of 10.030 - 1016.135 ng/mL.

Linagliptin: The plasma samples were processed. The calibration curve of internal standards (IS) Linagliptin D4 (Vivian Life Sciences Pvt. Ltd., Bangalore, India) and quality control (QC) samples were thawed and vortexed for preparation and analysis. Linagliptin was selectively isolated from 300 µL of plasma by a liquid-liquid extraction method using a validated high-performance liquid chromatographic method with mass spectrometric detection. Aliquots of 0.300 mL were mixed with 50 µL IS dilution, 300 µL of extraction buffer (10% ammonia solution in water), and 2.5 mL of ethyl acetate, then vortexed. They were then centrifuged at 4500 rpm for 5 min at 4˚C. Was transferred 2.0 mL of the supernatant layer and dried in a nitrogen evaporator at 40˚C ± 2˚C. The samples were reconstituted with 0.400 mL of reconstitution solution and spiked with IS over a concentration range of 0.213 ng/mL to 13.584 ng/mL. Analytes, IS, and QC samples were transferred to pre-labeled vials in the autosampler at 5˚C ± 3˚C and injected into a liquid chromatography electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) instrument (Vivian Life Sciences, VL/S-LG-052/c, Bangalore, India). The chromatographic separation was performed by a BDS Hypersil C18, 4.6 × 50 mm, 5 µm column (Exion LC, Bangalore, India). Identifications were based on multiple reaction monitoring transitions; m/z

420.000 - 473.300 for linagliptin and m/z 424.000 - 477.200 for IS. The inter-batch calibration standard accuracy was 97.53 - 104.10%, with precision values of 1.11% - 4.63%, and a linearity range of 0.213 - 13.584 ng/mL.

2.6. Pharmacokinetic Analysis

PK parameters were evaluated and adhered to FDA regulations [22]. The PK parameters calculated were maximum peak concentration (C_{max}), area under the curve (AUC) from time 0 h to the last measurable concentration ($AUC_{0\text{-}72}$). Other secondary PK parameters evaluated were: time to reach C_{max} (T_{max}), $T_{1/2}$, and Kel in plasma concentration for empagliflozin and linagliptin. PK analysis was performed using the non-compartmental model of SAS® Statistical Analysis System (software version 9.1.3, SAS Institute Inc., CARY, USA).

2.7. Statistical Analysis

The sample size was based on the intra-subject coefficient of variation (CV%) for empagliflozin and linagliptin. With an expected coefficient of variation for both C_{max} and AUC not exceeding 20% and 26% respectively and assuming the true ratio of the test and reference formulations would fall between 92% -108%, the study required at least 32 evaluable subjects to demonstrate bioequivalence with a power greater than 90% at a 5% level of significance [5] [6] [23] [24].

The Ln-transformed pharmacokinetic parameters C_{max} and $AUC_{0\text{-}72}$ of empagliflozin and linagliptin, under fasting and fed conditions, will be subjected to analysis of variance (ANOVA) for bioequivalence assessment. The ANOVA model will include the main effects of period and treatment as fixed effects and subject nested within sequence as a random effect. A separate ANOVA model will be used to analyze each of the parameters. The period, treatment, and sequence effects will be considered statistically significant if the probability values of the respective effects (p-values) are ≤ 0.05. The significance of the sequence effect will be tested using the mean square of subjects nested within sequences as the error term and reported.

Bioequivalence assessment was performed on the basis of the 90% confidence intervals of the differences of the least squares treatment means for Ln-transformed pharmacokinetic parameters C_{max} and $AUC_{0\text{-}72}$ of empagliflozin and linagliptin obtained after single-dose administration under fed and fasting conditions.

2.8. Safety Analysis

Safety evaluation was performed before, during, and at the end of the study. Vital signs, blood pressure, pulse rate, respiratory rate, and ancillary temperature were measured and recorded at check-in, before dosing (within 2 hours), 3.00 hours, 7.00 hours, and 11.00 hours post-dosing, during check-out, and at ambulatory visits of each period. A twelve-lead electrocardiogram and glycated hemoglobin (HbA1c) were recorded during the screening. Blood glucose level monitoring was done pre-dose (within 2 hours of dosing) at 1.0 hours, 3.0 hours, and 9.0 hours

post-dose of each period for fed and fasting condition studies. Subjects were monitored for adverse events or serious adverse events throughout the study.

3. Results

3.1. Demographic Data

The demographic profile of the subjects completing the bioequivalence study under fasting and fed conditions is summarized in Table 1.

Table 1. Demographic data.

BE Study	Fasting Condition N = 28	Fed Condition N = 28
Age (years)		
Mean ± SD	**34.57 ± 6.84**	**35.39 ± 5.02**
Range (%)		
(21 - 40)	**25 (89.28%)**	**24 (85.72%)**
(41 - 43)	**3 (10.72%)**	**4 (14.28%)**
Weight (kg)		
Mean ± SD	**68.71 ± 7.41**	**71.42 ± 11.36**
Range	**(54 - 80)**	**(56 - 98)**
Height (m)		
Mean ± SD	**1.67 ± 0.06**	**1.69 ± 0.07**
Range	**(1.57 - 1.79)**	**(1.62 - 1.84)**
BMI (Kg*m²SC)		
Mean ± SD	**24.42 ± 2.38**	**24.81 ± 2.61**
Range	**(19.96 - 29.76)**	**(20.31 - 29.41)**
Race		
Asian (%)	**100%**	**100%**
Sex (M/F)		
Male (%)	**100%**	**100%**

Fasting and Fed Study

A total of 32 subjects were enrolled in the fasting BE study in period I, and 28 subjects in period II. 4 participants were considered dropouts because they did not return to period II. In the fed condition study, 28 subjects completed the study and were included in the PK and statistical evaluation. The demographic characteristics of the individual sequence groups did not differ significantly. The washout period of 42 days was maintained between IP administrations in each treatment period in both studies, in fasting and fed conditions.

3.2. Pharmacokinetics and Statistics Results

A non-compartmental PK analysis was applied to estimate the plasma concentration vs. time of empagliflozin and linagliptin. The oral dosing of empagliflozin/linagliptin for 72 h post-dose is represented on linear and logarithmic scale plots for the mean ± SD plasma concentration vs time curves for both test and reference products, Figures 1-4 for fasting conditions and Figures 5-8 for fed conditions. Descriptive statistics for all PK parameters are presented in Tables 2-7 for test and reference products, in fasting and fed conditions.

3.2.1. PK in Fasting Conditions

As shown in Figure 1 and Figure 2 for the empagliflozin test (T) and reference (R)

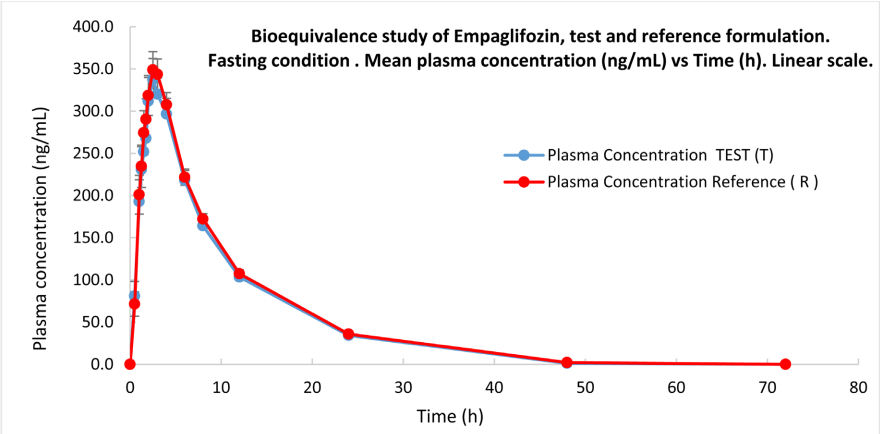

Figure 1. Empagliflozin 25 mg mean plasma concentration vs. time (h) profile for each formulation is presented in a linear scale, following a single oral dose in fasting conditions. Blue line indicates Empaliflozin (Empagliflozin/Linagliptin 25 mg/5 mg coated tablets, test product of Laboratorios Leti S.A.V) and red line indicates Empagliflozin Jardianz® DPP (Empagliflozin/Linagliptin 25 mg/5 mg coated tablets, reference product of Boehringer Ingelheim).

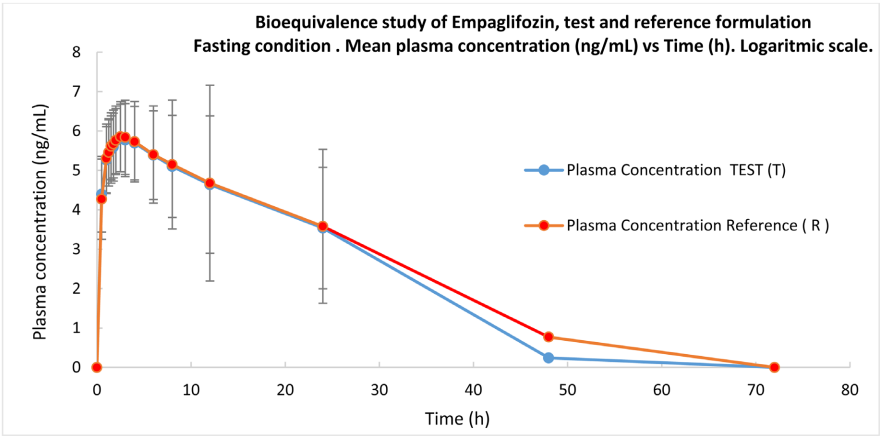

Figure 2. Empagliflozin 25 mg mean plasma concentration vs time (h) profile for each formulation is presented on a logarithmic scale, following a single oral dose in fasting conditions. Blue line indicates Empaliflozin (Empagliflozin/Linagliptin 25 mg/5 mg coated tablets, test product of Laboratorios Leti S.A.V) and red line indicates Empagliflozin Jardianz® DPP (Empagliflozin/Linagliptin 25 mg/5 mg coated tablets, reference product of Boehringer Ingelheim).

products and Figure 3 and Figure 4 for linagliptin, T and R products were comparable across treatments. Mean values for empagliflozin PK parameters were: C_{max} 391.03 ng/mL and T_{max} 2.43 h for T product, and C_{max} 398.68 ng/mL and T_{max} 2.30 h for R product, respectively.

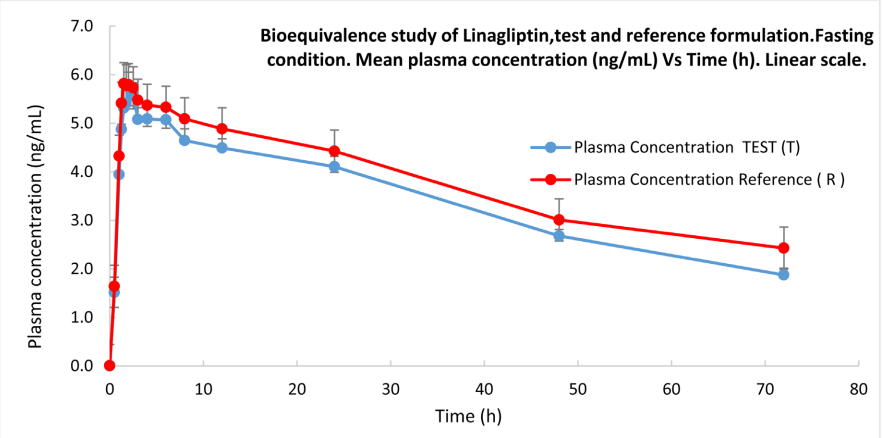

Figure 3. Linagliptin 5 mg mean plasma concentration vs time (h) profile for each formulation is presented in a linear scale, following a single oral dose in fasting conditions. Blue line indicates Linagliptin (Empagliflozin/Linagliptin 25 mg/5 mg, coated tablets, test product of Laboratorios Leti S.A.V) and red line indicates Linagliptin, Jardianz® DPP (Empagliflozin/Linagliptin 25 mg/5 mg coated tablets, reference product of Boehringer Ingelheim).

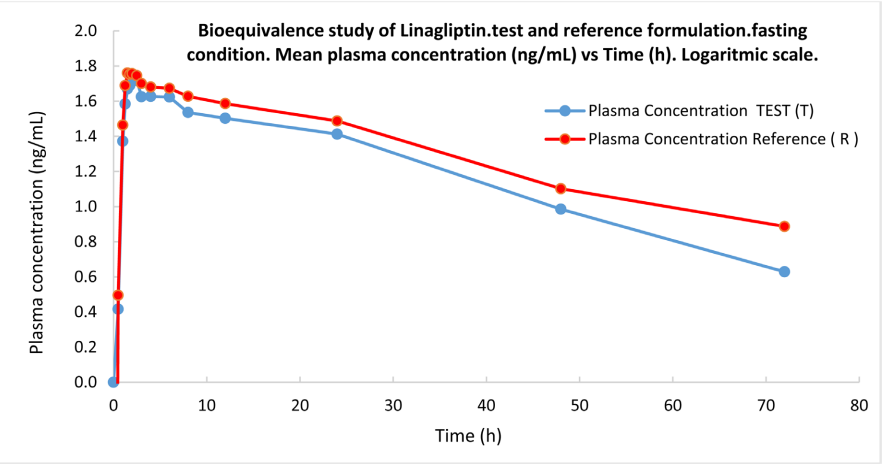

Figure 4. Linagliptin 5 mg mean plasma concentration vs time (h) profile for each formulation is presented in a logarithmic scale, following a single oral dose in fasting conditions. Blue line indicates Linagliptin (Empagliflozin/Linagliptin 25 mg/5 mg coated tablets, test product of Laboratorios Leti S.A.V) and red line indicates Linagliptin, Jardianz® DPP (Empagliflozin/Linagliptin 25 mg/5 mg coated tablets, reference product of Boehringer Ingelheim).

The $AUC_{0\text{-}72}$ was 3277.27 ng·h/mL for T and 3452.26 ng·h/mL for R product. (Table 2)

Mean peak linagliptin plasma concentrations were comparable across treatments and PK parameters: C_{max} 6.35 ng/mL and T_{max} 4.17 h for the T product and C_{max} 7.04

ng/mL and T_{max} 3.39 h for the R product. The AUC_{0-72} was 243.85 ng·h/mL for T and 261.94 ng·h/mL for the R product. (Figure 3 and Figure 4)

The PK parameters C_{max}, AUC_{0-t}, T_{max}, Kel (h^{-1}), and $T_{1/2}$ of empagliflozin and linagliptin plasma concentrations are presented in Table 3.

Table 2. Pharmacokinetic parameters of Empagliflozin/Linagliptin (25 mg/5 mg) coated tablets for Empagliflozin test product (T) and reference product (R), fasting condition N = 28.

Empagliflozin 25 mg Test Product (T)					
Parameter	**C_{max} (ng/mL)**	**AUC_{0-72} (ng·h/mL)**	**T_{max} (h)**	**K_{el} (1/h)**	**$T_{1/2}$ (h)**
N	28	28	28	28	28
Mean	391.03	3277.27	2.43	0.09	7.19
Standard Deviation	141.51	1091.22	0.90	0.01	1.08
Empagliflozin 25 mg Reference Product (R)					
Parameter	**C_{max} (ng/mL)**	**AUC_{0-72} (ng·h/mL)**	**T_{max} (h)**	**K_{el} (1/h)**	**$T_{1/2}$ (h)**
N	28	28	28	28	28
Mean	398.68	3452.26	2.30	0.09	7.28
Standard Deviation	108.10	836.38	0.74	0.017	1.48

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_{0-72}, area under the plasma concentration-time curve from time 0 to 72 hours, T_{max} time to reach C_{max}, Kel (1/h) is the fraction of drug that is eliminated per unit of time, $T_{1/2}$ time required for plasma concentration to decrease by 50%.

Table 3. Pharmacokinetic parameters of Empagliflozin/Linagliptin (25 mg/5 mg) coated tablets for Linagliptin, fasting condition N = 28.

Linagliptin 5 mg Test Product (T)					
Parameter	**C_{max} (ng/mL)**	**AUC_{0-72} (ng·h/mL)**	**T_{max} (h)**	**K_{el} (1/h)**	**$T_{1/2}$ (h)**
N	28	28	28	28	28
Mean	6.35	243.85	4.17	0.016	45.05
Standard Deviation	2.27	51.29	4.56	0.005	13.03
Linagliptin 5 mg Reference Product (R)					
Parameter	**C_{max} (ng/mL)**	**AUC_{0-72} (ng·h/mL)**	**T_{max} (h)**	**K_{el} (1/h)**	**$T_{1/2}$ (h)**
N	28	28	28	28	28

Continued

Mean	7.04	261.94	3.39	0.014	56.77
Standard Deviation	2.17	72.10	4.70	0.005	25.26

Data presented as a mean ± SE. C_{max}, maximum concentration, $AUC_{0\text{-}t}$, area under the plasma concentration-time curve from time 0 to the last measurable concentration; $AUC_{0\text{-}72}$, area under the plasma concentration-time curve from time 0 to 72 hours, T_{max} time to reach C_{max}, Kel (1/h) is the fraction of drug that is eliminated per unit of time, $T_{1/2}$ time required for plasma concentration to decrease by 50%.

The geometric mean ratios (GMR), 90% CI, and power of variation of both products for Ln-transformed pharmacokinetic parameters C_{max} and $AUC_{0\text{-}72}$ for empagliflozin and linagliptin are presented in Table 4.

Table 4. Geometric mean for C_{max} and $AUC_{0\text{-}72}$ test and reference products, fasting conditions N = 28.

Empagliflozin 25 mg						
Parameter	**Geometric Least Square Mean (GLSM)**				**LSM Ratio**	**90% CI**
	N	**Test Product (T)**	**N**	**Reference Product (R)**		
C_{max} (ng/mL)	28	364.18	28	381.78	95.39	(88.56% - 102.75%)
$AUC_{0\text{-}72}$ (ng·hr/mL)	28	3107.21	28	3345.79	92.87	(88.46% - 97.50%)
Linagliptin 5 mg						
Parameter	**Geometric Least Square Mean (GLSM)**				**LSM Ratio**	**90% CI**
	N	**Test Product (T)**	**N**	**Reference Product (R)**		
C_{max} (ng/mL)	28	6.03	28	6.76	89.23	(81.88% - 97.23%)
$AUC_{0\text{-}72}$ (ng·hr/mL)	28	238.61	28	251.20	94.99	(86.68% - 104.09%)

Geometric Least Square Mean (GLSM) for C_{max} and $AUC_{0\text{-}72}$, and their respective 90% CI ratios, fell within the accepted bioequivalence range.

For empagliflozin, the GLSM was: C_{max} 95.39% (CI: 88.56% - 102.75%) and $AUC_{0\text{-}72}$: 92.87% (88.46% - 97.50%), and for linagliptin, the GLSM was: C_{max} 89.23% (81.88% - 97.23%) and $AUC_{0\text{-}72}$ 94.99% (86.68% - 104.09%) under fasting conditions.

3.2.2. PK in Fed Condition

As shown in Figure 5 and Figure 6 for empagliflozin and Figure 7 and Figure 8 for linagliptin, across test and reference treatments, plasma concentration-time profiles were similar.

Mean values for empagliflozin PK parameters were comparable: C_{max} 188.59 ng/mL with a T_{max} of 3.36 h for the T product and C_{max} 202.78 ng/mL with a T_{max} of 3.5 h for the R product, respectively. The $AUC_{0\text{-}72}$ was 175.35 ng·h/mL for the T and 179.10 ng·h/mL for the R product (Table 5).

Mean linagliptin 5 mg PK parameters were comparable across treatments: C_{max} 4.18 ng/mL with a T_{max} of 4.55 h for the T product and C_{max} 4.21 ng/mL with a T_{max} of 5.78 h for the R product.

The $AUC_{0\text{-}72}$ was 243.85 ng·h/mL for the T and 261.94 ng·h/mL for the R product (Table 6).

The PK parameters C_{max}, $AUC_{0\text{-}t}$, T_{max}, Kel (h^{-1}), and $T_{1/2}$ of linagliptin plasma concentrations are presented in Table 6.

Geometric least square mean (GLSM) and the ratios of the GLSM for C_{max} and $AUC_{0\text{-}72}$ are summarized in Table 7.

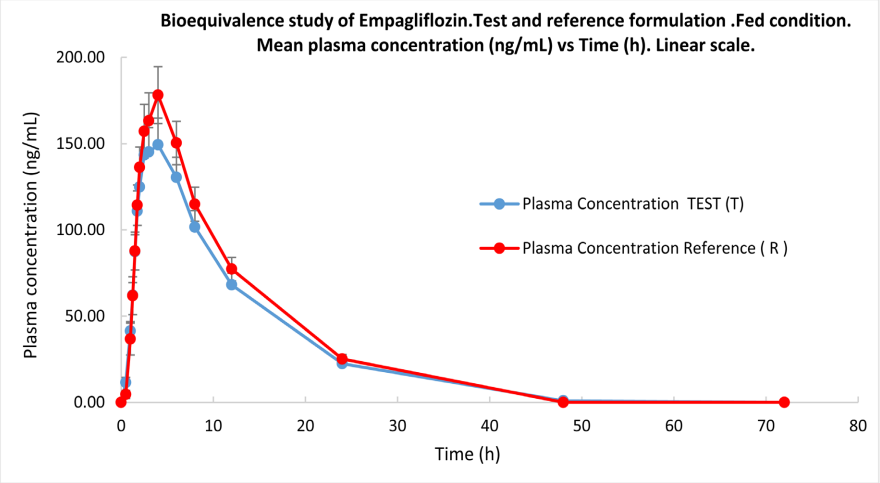

Figure 5. Empagliflozin 25 mg mean plasma concentration vs time (h) profile for each formulation is presented in a linear scale, following a single oral dose in fed conditions. Blue line indicates Empaliflozin (Empagliflozin/Linagliptin 25 mg/5 mg coated tablets, test product of Laboratorios Leti S.A.V) and red line indicates Empagliflozin Jardianz® DPP (Empagliflozin/Linagliptin 25 mg/5 mg, coated tablets, reference product of Boehringer Ingelheim).

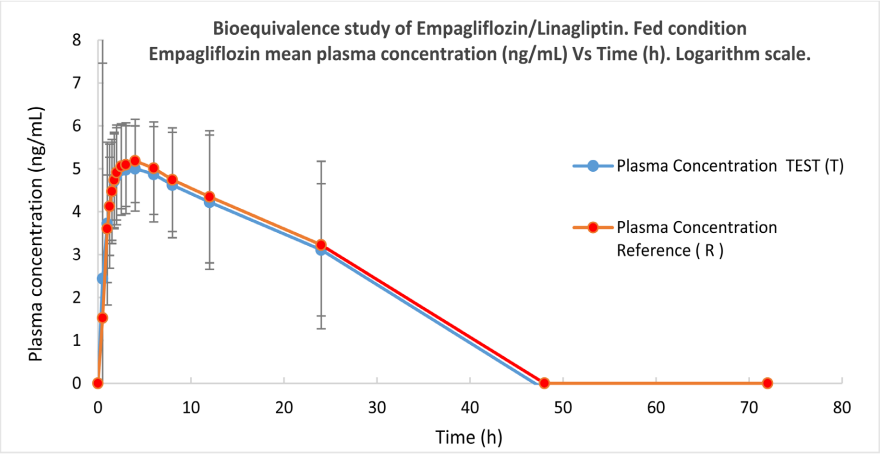

Figure 6. Empagliflozin 25 mg mean plasma concentration vs time (h) profile for each formulation is presented on a logarithmic scale, following a single oral dose in fed conditions. Blue line indicates Empaliflozin (Empagliflozin/Linagliptin 25 mg/5 mg, test product of Laboratorios Leti S.A.V) and red line indicates Empagliflozin Jardianz® DPP (Empagliflozin/Linagliptin 25 mg/5 mg, reference product of Boehringer Ingelheim).

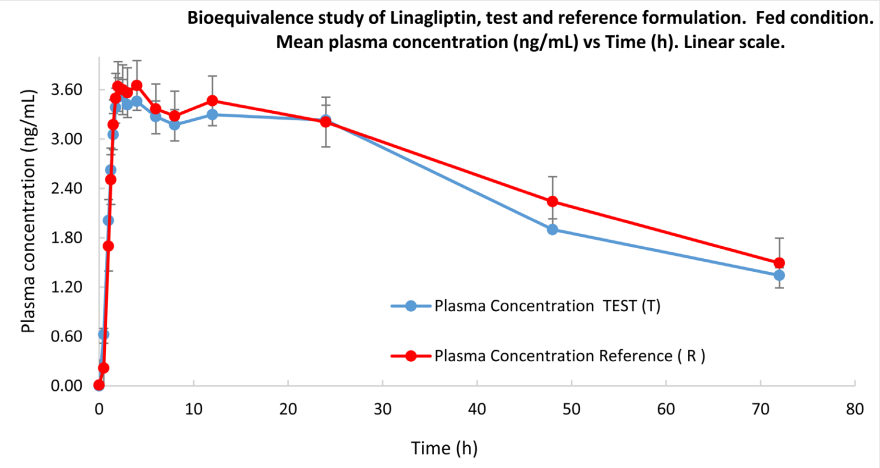

Figure 7. Linagliptin 5 mg mean plasma concentration vs time (h) profile for each formulation is presented in a linear scale, following a single oral dose in fed conditions. Blue line indicates Linagliptin (Empagliflozin/Linagliptin 25 mg/5 mg coated tablets, test product of Laboratorios Leti S.A.V) and red line indicates Linagliptin Jardianz® DPP (Empagliflozin/Linagliptin 25 mg/5 mg coated tablets, reference product of Boehringer Ingelheim).

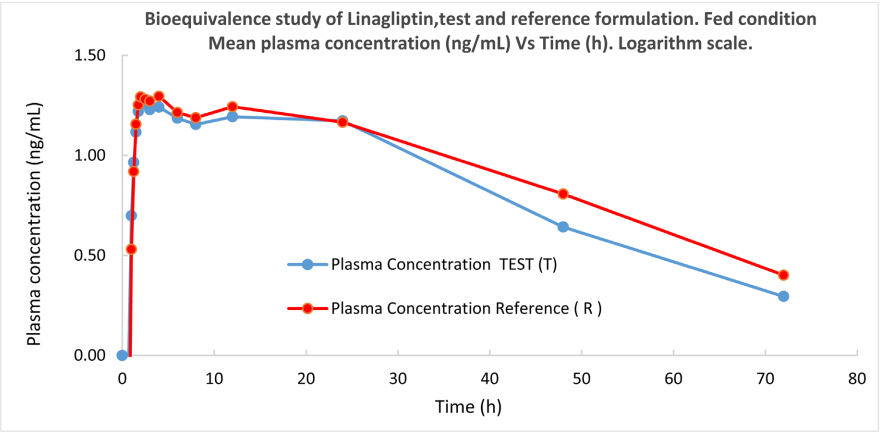

Figure 8. Linagliptin 5 mg mean plasma concentration vs time (h) profile for each formulation is presented in a logarithmic scale, following a single oral dose in fed conditions. Blue line indicates Linagliptin (Empagliflozin/Linagliptin 25 mg/5 mg, coated tablets, test product of Laboratorios Leti S.A.V) and red line indicates Linagliptin Jardianz® DPP (Empagliflozin/Linagliptin 25 mg/5 mg, coated tablets, Reference product of Boehringer Ingelheim).

Table 5. Pharmacokinetic parameters of Empagliflozin/Linagliptin (25 mg/5 mg) coated tablets for the Empagliflozin, fed condition N = 28.

Empagliflozin Test Product (T)					
Parameter	**C_{max} (ng/mL)**	**AUC_{0-72} (ng·h/mL)**	**T_{max} (h)**	**K_{el} (1/h)**	**$T_{1/2}$ (h)**
N	28	28	28	28	28
Mean	188.59	1841.86	3.36	0.10	7.25
Standard Deviation	73.87	1021.34	1.35	0.02	1.57

Continued

Empagliflozin Reference Product (R)					
Parameter	**C_{max} (ng/mL)**	**AUC_{0-72} (ng·h/mL)**	**T_{max} (h)**	**K_{el} (1/h)**	**$T_{1/2}$ (h)**
N	28	28	28	28	28
Mean	202.78	2037.91	3.50	0.096	7.31
Standard Deviation	84.12	899.25	1.44	0.015	1.07

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_{0-72}, area under the plasma concentration-time curve from time 0 to 72 hours, T_{max} time to reach C_{max}, Kel (1/h) is the fraction of drug that is eliminated per unit of time, $T_{1/2}$ time required for plasma concentration to decrease by 50%.

Table 6. Pharmacokinetic parameters of Empagliflozin/Linagliptin (25 mg/5 mg) coated tablets for Linagliptin, fed condition N = 28.

Linagliptin Test Product (T)					
Parameter	**C_{max} (ng/mL)**	**AUC_{0-72} (ng·h/mL)**	**T_{max} (h)**	**K_{el} (1/h)**	**$T_{1/2}$ (h)**
N	28	28	28	28	28
Mean	4.18	175.35	4.55	0.017	43.83
Standard Deviation	1.43	71.12	4.98	0.004	17.89
Linagliptin Reference Product (R)					
Parameter	**C_{max} (ng/mL)**	**AUC_{0-72} (ng·h/mL)**	**T_{max} (h)**	**K_{el} (1/h)**	**$T_{1/2}$ (h)**
N	28	28	28	27	27
Mean	4.21	179.10	5.78	0.014	64.40
Standard Deviation	1.24	65.31	9.31	0.005	68.89

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_{0-72}, area under the plasma concentration-time curve from time 0 to 72 hours, T_{max} time to reach C_{max}, Kel (1/h) is the fraction of drug that is eliminated per unit of time, $T_{1/2}$ time required for plasma concentration to decrease by 50%.%. Note: Kel and $T_{1/2}$ were calculated only for the 27 subjects in these 2 samples.

For empagliflozin and linagliptin, their respective 90% CIs for C_{max} and AUC_{0-72} (87.17% - 100.94%) and (80.78% - 92.02%) for empagliflozin, and (90.83% - 105.94%) and (86.12% - 109.51%) for linagliptin, respectively, fell within the acceptable bioequivalence range (80% - 125%).

Table 7. Geometric mean for C_{max} and AUC test and reference products, fed condition N = 28.

Empagliflozin 25 mg						
Parameter	Geometric Least Square Mean (GLSM)				LSM Ratio	90% CI
	N	Test Product (T)	N	Reference Product (R)		
C_{max} (ng/mL)	28	177.07	28	188.77	93.80	(87.17% - 100.94%)
AUC_{0-72} (ng·hr/mL)	28	1613.95	28	1871.99	86.22	(80.78% - 92.02%)
Linagliptin 5 mg						
Parameter	Geometric Least Square Mean (GLSM)				LSM Ratio	90% CI
	N	Test Product (T)	N	Reference Product (R)		
C_{max} (ng/mL)	28	3.94	28	4.02	98.09	(90.83% - 105.94%)
AUC_{0-72} (ng·hr/mL)	28	160.6343	28	165.4103	97.11	(86.12% - 109.51%)

The ANOVA results (Table 8) for log-transformed C_{max} data of empagliflozin showed no statistically significant variation for period, treatment, or sequence values between the test and reference formulations in fasting and fed conditions. However, the log-transformed AUC_{0-72} data showed significant variations for treatment values in fed conditions only, with no significant variation for period or sequence in the fasting and fed condition study. Linagliptin log-transformed Cmax data showed statistically significant variation for treatment in fasting condition study only, and the other parameters in both conditions; the ANOVA results for C_{max} and AUC_{0-72} indicated no significant variation for treatment, period, or sequence values.

Table 8. Pharmacokinetic parameters of Empagliflozin/Linagliptin (25 mg/5 mg) ANOVA.

Empagliflozin p Values						
Parameter	Sequence		Period		Treatment	
Study Condition	Fasting	Fed	Fasting	Fed	Fasting	Fed
C_{max} (ng/mL)	0.670	0.520	2.327	0.211	0.288	0.148
AUC_{0-72} (ng·hr/mL)	0.282	0.910	0.746	0.528	0.0155	0.000
Linagliptin p Values						
Parameter	Sequence		Period		Treatment	
Study Condition	Fasting	Fed	Fasting	Fed	Fasting	Fed
C_{max} (ng/mL)	0.61	0.909	0.329	0.441	0.032	0.673
AUC_{0-72} (ng·hr/mL)	0.053	0.884	0.098	0.050	0.346	0.680

3.3. Safety Results

3.3.1. Fasting Condition

All subjects were monitored for adverse events throughout the study. At the end

of the study, a post-study safety evaluation was done, which included hematology and clinical biochemistry tests. Post-study safety evaluation was performed on all the subjects (except subjects S11, S14, S17, and S21) who came for period II of the clinical study.

Twenty-eight subjects' post-study safety samples were collected, and upon evaluation, all post-study safety laboratory parameters were found to be normal.

3.3.2. Fed Condition

Post-study safety evaluation was performed on all the subjects (except subjects S05, S10, S26, and S32) who came for period II of the clinical study. Post-study safety samples were collected from 28 subjects, and upon evaluation, all post-study safety laboratory parameters were found to be normal.

In these studies (fasting and fed), no adverse events or serious adverse events were reported.

4. Discussion

This BE study compared the relative bioavailability of a combination of empagliflozin 25 mg and linagliptin 5 mg coated tablets of Laboratorios Leti S.A.V. with Jardianz® DPP (25 mg/5 mg) coated tablets of Boehringer Ingelheim Pharma GmbH & Co., in healthy adult subjects under fasting and fed conditions.

Empagliflozin/linagliptin, Jardianz® DPP, was the first fixed-dose combination (FDC) of an SGLT2 inhibitor and DPP-4 inhibitor approved by the FDA for the treatment of adult patients with type 2 diabetes mellitus (T2DM) as an adjunct to diet and exercise to improve glycemic control [4]-[6].

T2DM is a progressive disease that requires combined therapy in some cases to achieve effective control of hyperglycemia [1] [2] [13] [14]. Combined therapies of sodium-glucose cotransporter type 2 inhibitor (SGLT2), empagliflozin, and a dipeptidyl peptidase-4 inhibitor (DPP-4I), linagliptin, have been demonstrated to be effective with complementary mechanisms of action as a strategy to provide optimal control in these patients [1]-[4] [13]-[15] [23].

The use of this FDC empagliflozin/linagliptin in the treatment of T2DM can simplify drug dosing, reducing pill burden and improving treatment adherence and compliance, with additional benefits in body weight loss, blood pressure reduction, and low risk of hypoglycemia [23] [24].

This bioequivalence (BE) study, was found no significant differences in the main PK parameters C_{max} and $AUC_{0\text{-}72}$ for a FDC of empagliflozin/linagliptin (25 mg/5 mg)coated tablets as the T formulation of Laboratorios Leti S.A.V., and the R formulation, Jardianz® DPP (empagliflozin/linagliptin 25 mg/5 mg, coated tablets) of Boehringer Ingelheim Pharma GmbH & Co. after a single oral dose, under fasting and fed conditions, following the FDA's specific guidance on bioequivalence studies [22]. The geometric mean ratios (GMR) 90% CI to empagliflozin, were to C_{max} 95.39% (364.18 T/381.78 R) CI (88.56% - 102.75%) and $AUC_{0\text{-}72}$ 92.87% (3107.21 T 3345.79 R) CI (88.46% - 97.50%) for fasting conditions and C_{max} 93.80% (177.07 T/188.77 R) CI (87.17% - 100.94%) and $AUC_{0\text{-}72}$ 86.22%

(1613.95 T/1871.99 R) CI (80.78% - 92.02%) for fed conditions (Table 4 and Table 7). All the values applying GLSM were within the allowed range of BE established. LGMR was evaluated for linagliptin in fasting and fed conditions and was found within the BE range. The two formulations FDC empagliflozin/linagliptin (25 mg/5 mg coated tablets) were considered BE when the main PK parameters (C_{max} and $AUC_{0\text{-}72}$) were analyzed for T/R ratio and all of them were within 90% CI BE limits of 80% - 125% in both studies, under fasting and fed conditions.

Other PK parameters were evaluated as T_{max} and $T_{1/2}$ for both compounds. Empagliflozin reported T_{max}: 2.43 h for T and 2.30 h for R in fasting conditions and 3.36 h for T and 3.50 h for R in fed conditions, respectively. The $T_{1/2}$ was similar in both conditions for the T and R formulations (Table 2 and Table 5). Linagliptin reported a T_{max}: 4.17 h for T and 3.39 h for R; and 4.45 h for T and 5.78 h for R in fasting and fed conditions, respectively. (Table 3 and Table 6). The $T_{1/2}$ was similar in both conditions for the T and R formulations.

A decrease of C_{max} and $AUC_{0\text{-}72}$ for empagliflozin and linagliptin in the fed condition was observed, and the T_{max} was observed in empagliflozin only. The variability observed of empagliflozin test formulation in the fed condition compared to the fasting condition, resulting in $AUC_{0\text{-}72}$ decreased approximately 43% and C_{max} decreased approximately 48% compared to the fasting condition. For linagliptin, the $AUC_{0\text{-}72\ and}$ C_{max} decreased by 34% and 28%, respectively. These findings correspond to similar PK values reported in other studies for each compound of empagliflozin and linagliptin, separately in healthy adult subjects under fasting or fed conditions [5] [6] [23]-[28]. This variability in C_{max} or AUC in fasting or fed conditions for empagliflozin and linagliptin does not appear to affect the bioavailability of these products or their effect on glycemic control levels [1] [4] [5] [23]-[28]. Although the FDA suggests conducting a BE study for this FDC under fasting conditions, we decided to perform it under both conditions [5].

This evaluation included 28 male adult subjects in both studies, covering the variability and assuring statistical power to demonstrate the BE within test and reference formulations.

The generic formulation in FDC represents considerable advantages for diabetic patients, such as a reduction in the number of tablets to be consumed per day, reducing the risk of adverse events when increasing the dose in monotherapy, as well as improving treatment compliance, in addition to providing patient adherence, offering advantages in treatment compliance, especially in chronic pathologies such as T2DM [1] [2] [4] [12] [28].

5. Limitations

We could not access PK parameters in female volunteers, although the study was open to both males and females.

6. Conclusion

Two FDC containing 25 mg of empagliflozin and 5 mg of linagliptin were evalu-

ated, resulting in bioequivalence in healthy subjects under fasting and fed conditions. The PK profile of the test and reference formulations was similar, as demonstrated by the 90% CI of C_{max} and $AUC_{0\text{-}72}$ within the accepted BE criteria of 80% - 125%.

Author's Contributions

EP, AI, JGC, AT, and XS performed the statistical analysis, interpretation, writing, and review of the manuscript.

Declaration of Patient Consent

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

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.

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Conflicts of Interest

All authors are Laboratorios Leti S.A.V. employees 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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