Research Article

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American Journal of Pharmacology and Pharmacotherapeutics
ISSN : 2393-8862

2024
Vol. 11 No. S1: 02

Bioequivalence Comparison of Two Formulations of Fixed Dose Combination Sitagliptin/Metformin (50/1000 mg) Immediate Release (IR) Tablets in Fed Condition

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Citation: Pena E, Inatti A, Chacon J, Taly A, Marti SX (2024) Bioequivalence Comparison of Two Formulations of Fixed Dose Combination Sitagliptin/Metformin (50/1000 mg) Immediate Release (IR) Tablets in Fed Condition. Am J Pharmacol Pharmacother Vol.11 No. S1: 02.

Abstract

Background: This study aimed to evaluate the bioequivalence of the Fixed-Dose Combination (FDC) sitagliptin 50 mg/metformin 1000 mg tablets compared to Janumet® (sitagliptin 50 mg/metformin 1000 mg tablets) in healthy male volunteers under fed 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 two FDC of sitagliptin 50 mg/metformin 1000 mg Immediate Release (IR) in 26 adult healthy subjects. The Pharmacokinetic (PK) parameters C_{max} and AUC_{0-t} 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. Bioequivalence was evaluated using 90% Confidence Intervals (CIs) for the ratio test/reference of log Area Under the Concentration-Time Curve (AUC) from time 0 to the last quantifiable concentration and log peak concentration. 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} and AUC_{0-t}.

Results: All 26 subjects completed both study periods. The 90% Confidence Intervals (CIs) of the test/reference ratio were C_{max}: 110.46% (103.26%-118.15%) and AUC_{0-t} 104.82% (99.81%-110.08%) of sitagliptin and C_{max}: 99.85% (93.61%-106.52%) AUC_{0-t}: 102.51 (96.57%-108.82%) to metformin. 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 sitagliptin 50 mg/metformin 1000 mg IR (FDC) were bioequivalent in healthy subjects under fed conditions. The geometric mean ratio and 90% CI for primary PK parameters, C_{max} and AUC_{0-t}, of T and R formulation were within the range 80% to 125%.

Keywords: Bioequivalence; Fixed dose combinations; Pharmacokinetic; Sitagliptin

Received date: October 22, 2024, Manuscript No. IPAPP-24-19825; **Editor assigned date:** October 25, 2024, PreQC No. IPAPP-24-19825 (PQ); **Reviewed date:** November 8, 2024, QC No. IPAPP-24-19825; **Revised date:** November 15, 2024, Manuscript No. IPAPP-24-19825 (R); **Published date:** November 22, 2024, DOI: 10.36648/2393-8862.11. S1.02

Introduction

Sitagliptin and metformin HCl tablets contain two oral antihyperglycemic drugs used in the management of Type 2 Diabetes Mellitus (T2DM). Sitagliptin phosphate is a Dipeptidyl Peptidase-4 (DPP-4) inhibitor and metformin hydrochloride, is member of the biguanide class [1]. Sitagliptin phosphate with a molecular formula: $C_{16}H_{15}F_6N_5O$ and weight of 523.32 g/mol,

is an orally-active, potent and highly selective inhibitor of the Dipeptidyl Peptidase-4 (DPP-4) enzyme for the treatment of Type 2 Diabetes Mellitus (T2DM) [2]. The DPP-4 inhibitors are a class of agents which are incretin enhancers secretion from pancreatic alpha cells, leading to reduced hepatic glucose production. When blood glucose levels are low, insulin release is not enhanced and glucagon secretion is not suppressed. Sitagliptin is a potent and highly selective inhibitor of the enzyme DPP-4 and does not inhibit the closely-related enzymes DPP-8 or DPP-9 at therapeutic concentrations. By inhibiting the DPP-4 enzyme, sitagliptin increases the levels of two known active incretin hormones, Glucagon-Like Peptide-1 (GLP-1) and Glucose-dependent Insulinotropic Polypeptide (GIP). The incretins are part of an endogenous system involved in the physiologic regulation of glucose homeostasis. When blood glucose concentrations are normal or elevated, GLP-1 and GIP increase insulin synthesis and release from pancreatic beta cells. GLP-1 also lowers glucagon [2-4]. Following oral administration of a 100 mg dose in healthy subjects, sitagliptin was rapidly absorbed, with peak plasma concentrations (median T_{max}) occurring 1 h to 4 h post-dose, the mean plasma AUC was 8.52 µM.h, C_{max} was 950 nm. The absolute bioavailability of sitagliptin is approximately 87%.

Since co-administration of a high-fat meal with sitagliptin had no effect on the pharmacokinetic, sitagliptin may be administered with or without food. The mean volume of distribution at steady state is approximately 198 L and the fraction of sitagliptin reversibly bound to plasma proteins is low (38%) [2,3]. Sitagliptin is primarily eliminated unchanged in urine and metabolism is a minor pathway. Approximately 79% is excreted unchanged in the urine. Sitagliptin was eliminated in faeces (13%) or urine (87%) within one week of dosing. The apparent terminal $t_{½}$ following a 100 mg oral dose of sitagliptin was approximately 12.4 h [2-6]. In monotherapy, adverse events more frequently reported were headache, hypoglycaemia, constipation and dizziness [3,6]. Metformin is a biguanide with a molecular formula, C4H11N5 and molecular weight of 129.16 with anti-hyperglycaemic effects, lowering both basal and postprandial plasma glucose [7]. It does not stimulate insulin secretion and therefore does not produce hypoglycaemia [8]. 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 [9].

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). After an oral dose of metformin 500 mg, the T_{max} was reached in 2.5 h and the bioavailability was 50%-60% [8-10]. 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 h. 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 [8-11]. Gastrointestinal symptoms have been reported as adverse events in clinical and post marketing trials with metformin [12]. Metformin has been the gold standard for the monotherapy treatment of T2DM and DPP-4 inhibitor such as sitagliptin added to metformin are a good combination to achieve the goals of effective glycaemic control [13,14]. Using monotherapy to target a single defect is often inadequate to achieve glycemic goals and the result is prolonged exposure to hyperglycaemia and an increased risk of diabetic complications [13,15]. Combined therapy using agents with complementary mechanisms of action has become a fundamental of T2DM management. The algorithm created for the American Diabetes Association and European Association for the study of diabetes recommends combination therapy for all patients with glycated haemoglobin (A1C) >7% after 2-3 months of metformin monotherapy [4,10]. An algorithm created for the American Association of Clinical Endocrinologists and the American College of Endocrinology advises combination therapy when oral monotherapy fails to achieve or maintain A1C ≤ 6.5% after 2-3 months and consideration of combination therapy at the time of diagnosis for patients with A1C >7.5% [6-7]. Some adverse events were observed more frequently in studies with this combination, sitagliptin/metformin as hypoglycaemia, constipation and headache [8,13].

Combination therapies are often essential for the majority of patients with T2DM, as these individuals frequently have hypertension, dyslipidaemia and other comorbidities that require pharmacotherapy. Consequently, they may need to take multiple medications, leading to polypharmacy, which is a common challenge in this population, and the use of Fixed-Dose Combinations (FDCs) is a rational leading to polypharmacy which is a common challenge in this population and the use of FDCs is a rational approach to achieving and maintaining glycaemic control, guaranteeing compliance and adherence to treatment [1,8,9]. A Hungarian study demonstrated a significant difference between the persistence of one year of treatment with metformin monotherapy and the fixed-dose combination of sitagliptin/metformin in Type 2 diabetic patients, with favourable adherence in the sitagliptin/metformin group compared to metformin monotherapy [10,11]. The purpose of this bioequivalence study was to assess and compare the PK profile and safety of a single-tablet, fixed-dose combination of sitagliptin 50 mg and metformin 1000 mg, Immediate Release (IR) of Laboratorios Leti S.A.V, as Test (T) formulation *vs.* Janumet® (sitagliptin 50 mg-metformin 1000 mg) of Merck Sharp and Dohme Corp, as Reference (R) formulation in healthy adult subjects in randomized cross over study under fed conditions. This study was conducted by a Clinical Research Operator (CRO) VerGo Pharma Research Pvt. Ltd., India.

Materials and Methods

The study was conducted ethically in accordance with the principles of the ICMR guidelines (2017) [16], New Drugs and Clinical Trials Rules 2019 India [17] and adhered to the ethical principles of the Declaration of Helsinki [18] the International Conference on Harmonization Good Clinical Practice Guidelines [19]. 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 VerGo Clinical Research Pvt, Ltd.

Study design

This was an open-label, randomized, two-treatment, two-period, two-sequence, single oral dose and crossover Bioequivalence (BE) study under fed conditions comparing, two formulations of sitagliptin 50 mg/metformin 1000 mg tablets Immediate Release (IR), batch N°004, date of expiry 06/2024, of Laboratorios Leti S.A.V., as test formulation *vs.* Janumet® (sitagliptin 50 mg/metformin 1000 mg) IR, batch N°W006655, date of expiry 11/2023, of Merck Sharp & Dohme Corp, as reference formulation [16-19].

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 11 days from the day of check-in of period I to last blood sample collection of period II (April 15, 2023-April 22, 2023) separated by a washout period of 7 days, considering the terminal half-life for sitagliptin is 12.4 h. [2,6] and metformin 6.5 h. [8,10,13]. This BE study in FDC met the principles described in bioequivalence European Union (EU) guideline [20] and the in fed condition selection was based on metformin immediate release and Summary of Product Characteristics (SmPC) recommendations [20,21]. Sitagliptin EU guidelines recommend to do BE with highest strength, is possible to use the lower strength with a linear PK and high solubility [22].

All volunteers underwent a screening procedure. A total of 26+2 (stand by) healthy, adults male volunteers who met the inclusion and exclusion criteria were enrolled, with a mean age of 32.30 years, mean weight 71.02 kg, mean height 1.67 cm and Body Mass Index (BMI) of 25.18 kg/m² **(Table 1)**.

Table 1: Demographic profile of subjects completing the bioequivalence study (n=26).

Age	**Mean ± SD**	**32 ± 4.87**		
Years	Range	25-43		
Age group		**Male**	%	**Total**
	25-40	24	92.30%	24/92.30%
	41-43	2	7.70%	2/7.70%
Total	26	26	100%	26/ 100%
BMI (kg/m²)	Mean ± SD	25.18 ± 2.7		
	Range	(18.90-29.39)		
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 Electrocardiogram (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 ± (30 min) 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.

Drug administration

The subjects were admitted to the facility one night before study. Each subject received standard dinner on the day of check in after which they fasted for 10 h prior to consuming standard high-fat, high-calorie, non veg breakfast (800-1000 k_{cal}) which was served 30 min before scheduled time of dosing. Being a fed study, subjects were served high-fat, high-calorie breakfast on the dosing day. Subjects were fasted for 4 h after dosing in each period. The subjects received standard meals at 04.00 (lunch), 08.00 (snacks) and 12.00 h (dinner) after dosing in each period. All meal plans were identical for each period of study.

A single oral dose (sitagliptin 50 mg/metformin 1000 mg tablets IR) either one T or R, following randomization schedule and was administered with 240 mL ± 2 mL of glucose solution in water, followed by 60 mL of 20% glucose solution in water administered every 15 min for up to 4 h after dosing at ambient temperature to each subject in sitting position [23,24].

A total of 24 × 5 ml of venous blood samples were collected through cannula from each subject during the two periods of the study, withdrawn at pre-dose (-02.00-00.00 h) and 00.50, 01.00, 01.33, 01.67, 02.00, 02.33, 02.67, 03.00, 03.33, 03.67, 04.00, 04.50, 05.00, 05.50, 06.00, 07.00, 08.00, 10.00, 12.00,16.00,

24.00, 36.00 and 48 h after dosing in each period. While 24.00, 36.00 and 48.00 h, post dose, blood samples were collected by direct venepuncture. The subjects received standard high-fat, high-calorie, non-veg breakfast (800-1000 k_{cal}) 30 min before scheduled time of dosing and drinking water was provided ad libitum.

Analytical procedure

Venous samples were collected in pre-labelled K2 EDTA (ethylenediaminetetraacetic acid) vacutainers and were centrifuged at 3800 rpm for 10 min at 10°C within 45 min 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) sitagliptin D4 and 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 sitagliptin and metformin was conducted with calibration 2.101 ng/mL to 599.208 ng/mL and 14.040 ng/mL to 4004.046 ng/mL respectively and validation results were reported in Method Validation Report (MVR) 191-20 version 00. The bio study was performed on API 4000 system coupled with LC using sitagliptin D4 and 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 (StrataTM) × 33 µm coupled to a ZORBAX SB-C18, 46 × 75 mm, 3.5 µm column. Elution was performed at 25°C, with a mobile phase of methanol: 10 mM ammonium formate buffer (70:30/v/v). The lower limit of quantification was 2.102 ng/mL to sitagliptin and 14.083 ng/mL for metformin whereas the upper limit of quantification was 597.549 ng/mL for sitagliptin and 4003.999 ng/mL for metformin. These data were acquired, integrated and quantified on AB Sciex systems Shimadzu, analyst version 1.7.3 software.

A Stock Solution (SS) for sitagliptin was prepared with 2.4483 mg to Calibration Curve (CC) standards and 2.3767 and for QC, SS was done by two different analysts (CC-ISTD 951511.373 ng/mL), (QC-ISTD 923684.630 ng/mL) to sitagliptin D4, the CC standard was 114820.061 ng/mL.

For metformin SS were prepared with 2.5319 mg for CC and 2.4417 mg for QC, the concentration of SS was 973735.138 ng/mL (CC) and 939045.415 ng/mL (QC). Metformin D6 (SS) was prepared with 1.0869 mg stock concentration of ISTD was 855789.324 ng/mL were made using diluent (Methanol: Water in ratio 50:50 v/v) and stored at 2.0-8.0.

Metformin was prepared with 1.0869 mg (ISTD 855789.324 ng/mL) and dissolved in 1000 mL of methanol. All solutions were prepared using diluent (Methanol: 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 235.100-408.200 for sitagliptin and m/z 239.000-412.300 for the IS-sitagliptin D4. And m/z 71.200-130.100 for metformin and m/z 77.200-136.200 for the IS-metformin D6. The Inter-Batch Calibration Standard (IBCS) was 1.19% to 3.33%, accuracy 93.20% to 103.21% for sitagliptin and IBCS was 1.01% to 3.28%, accuracy 97.61% to 104.50% for metformin.

Statistical analysis

The sample size calculation for the study was based on intra-subject Coefficient of Variation (CV%) obtained of published data for sitagliptin (C_{max}: 16.61% and AUC_{0-t} 5.57%) [2,4,6,13] and metformin obtained from published literature (C_{max}: 11,4% and AUC_{0-t} 13.63%), with the expected CV% not exceeding 20% and the ratio within 80 and 125% [10,13,14].

The study required 26 evaluable subjects to demonstrate BE with a power of ≥ 90% at 5% level of significance. Based on a sample size, 26 subjects were sufficient to demonstrate BE between the two S/M formulations. 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 BE was determined using a PK sampling scheme suitable for the determination of the PK parameters of each individual component (sitagliptin/metformin), adhered to European Medicines Agency (EMA)-specific bioequivalence guide [21,22] and International Council for Harmonisation (ICH) M13A, guideline on bioequivalence for immediate release solid oral dosage forms [20].

The primary PK variables evaluated were maximum peak concentration (C_{max}) and area under curve from time 0 to last measurable concentration (AUC_{0-t}). Others secondary PK parameters evaluated were: T_{max} (time to reach C_{max}), time required for plasma concentration to decrease by 50% ($T_{1/2}$), area under the plasma concentration-time curve from time 0 to infinity (AUC_{0-inf}), $AUC_{-\%Extrap}$, Constant of Elimination (K_{el}) and the Number of points (Npoints) 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} and AUC_{0-t} was analysed for statistical difference between test and reference formulations to each compound, sitagliptin and metformin Test and Reference (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} and AUC_{0-t}. The formulations, sitagliptin and metformin were regarded as bioequivalent when the 90% (CIs) of the T and R ratio of C_{max} and AUC_{0-t}, ranged from 80% to 125%. This is the BE standard accepted by the EMA guide for each individual compound [21,22].

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 02.00, 04.00, 06.00 and 11.00 h (± 30 min) after dosing.

Results

All subjects (26) 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}, T_{max}, K_{el} (h-1) and $T_{½}$, of sitagliptin/metformin (S/M) in plasma concentration which are presented in **Table 2**, the AUC from 0 to last time point with measurable plasma concentrations was computed using linear trapezoidal-rule.

Analysis of variance analysis from Ln C_{max} and AUC_{0-t}, there were no statistically significant differences between the PK parameters of the two (S/M) formulations (p>0.05) **(Tables 2 and 3)**.

The geometric mean ratios of the test and reference formulations for primary PK parameters C_{max} and AUC_{0-t} for sitagliptin, C_{max} was 110.46 (90% IC 103.24-118.15) and AUC_{0-t} was 104.82 (90% IC 99.81-110.08). For metformin, C_{max} was 99.85 (90% IC 93.61-106.52) and AUC_{0-t} was 102.51 (90% IC96.57-108.82) **(Table 3)**. C_{max} and AUC_{0-t} intervals for test and reference formulations were within the acceptance limits (80.00%-125.00%) to establish bioequivalence [21,22].

The oral dosing of S/M for 48 h post-dose is represented on arithmetic and logarithm scales, test and reference formulations for sitagliptin 50 mg and or metformin 1000 mg Ir as shown in **Figures 1 and 2, Tables 4 and 5**.

Tolerability and safety

All subjects (26) were included in the safety evaluation. A total of 3 adverse events were reported during the study, in the subjects dosed with test product. Two subjects (N14 and N26) experienced diarrhoea as an adverse event, however, both cases were not serious and resolved in <24 h. The subject N01 reported an AE of laboratory, increased potassium value, but it no was clinically significant. No clinically significant values were observed during the vital signs examination. There were no reports of death, serious or unexpected adverse events during the course of the study **(Table 6)**. Hence the test product and reference product were found to be safe and well tolerated upon single dose administration in healthy male adults under fed conditions.

Table 2: Pharmacokinetic parameters for sitagliptin after administration of Test product (T) and Reference product (R) N=26.

PK parameters (Units)	Sitagliptin (Mean ± SD)	
	Test (T)	Reference (R)
C_{max} (ng /mL)	146.8720 ± 34.23664	134.7474 ± 36.22948
AUC_{0-t} (h*ng/mL)	1601.8737 ± 227.43847	1534.1097 ± 246.13779
AUC_{0-inf} (h*ng /mL)	1657.7560 ± 241.90543	1587.2834 ± 263.00806
$AUC_{\%Extrap}$ (%)	3.317 ± 1.2643	3.285 ± 1.5428
$T_{1/2}$ (h)	9.412 ± 1.3557	9.373 ± 1.2096
K_{el} (1/h)	0.07521 ± 0.011554	0.07505 ± 0.008946
$T_{1/2}$ (h)	9.258 (6.25-12.00)	8.982 (7.36-12.56)
PK parameters (Units)	**Metformin (Mean ± SD)**	
	Test (T)	**Reference (R)**
C_{max} (ng/mL)	1575.8543 ± 304.13060	1584.5823 ± 329.17890
AUC_{0-t} (hr*ng/mL)	14422.9986 ± 2895.62014	14036.4871 ± 2560.61132
AUC_{0-inf} (hr*ng /mL)	14643.7088 ± 2877.18270	14313.2584 ± 2538.94493
$AUC_{\%Extrap}$ (%)	1.581 ± 0.8877	2.019 ± 1.0266
$T_{1/2}$ (hr)	4.373 ± 0.7524	4.473 ± 1.0758
K_{el} (1/hr)	0.16277 ± 0.026117	0.16207 ± 0.031401
$T_{1/2}$ (hr)	4.313 (3.38-6.04)	3.999 (3.29-7.41)

Note: 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; AUC0-∞: Area under the plasma concentration-time curve from time 0 to infinity, T_{max}: Time to reach; K_{el}: Elimination constant. $T_{1/2}$ time required for plasma concentration to decrease by 50%; Median (range).

Table 3: Bioequivalence assessment of a single dose of sitagliptin 50 mg test and reference products. Log transformed C_{max} and AUC_{0-t} (N=26).

Sitagliptin								
Parameters (units)	Least square means		Geometric least square means		Ratio (%) (T *vs.* R)	90% confidence intervals (%)	Intra subject	Power (T *vs.* R)
	T	R	T	R			CV (%)	(%)
Ln (C_{max}) (ng/mL)	4.965	4.865	143.275	129.712	110.46	103.26-118.15	14.27	99.97
Ln (AUC_{0-t}) (hr*ng/mL)	7.369	7.322	1585.748	1512.792	104.82	99.81-110.08	10.34	100
Metformin								
Parameters (units)	Least square means		Geometric least square means		Ratio (%) (T *vs.* R)	90% confidence intervals (%)	Intra subject	Power (T *vs.* R)
	T	R	T	R			CV (%)	(%)
Ln (C_{max}) (ng/mL)	7.3446	7.346	1547.783	1550.076	99.85	93.61-106.52	13.68	99.98
Ln (AUC_{0-t}) (hr*ng/mL)	9.5572	9.532	14146.37	13799.83	102.51	96.57-108.82	12.63	100

Note: LSM: Least Square Mean; GLSM: Geometric Least Square Mean; Ratio; 90% Confidence Intervals (CI); ISCV: Intra-Subject Coefficient of Variation and power for the log transformed C_{max} and AUC_{0-t}. Sitagliptin 50 mg-metformin 1000 mg Immediate Release (IR) of Laboratorios Leti S.A.V. as test formulation and Janumet® (sitagliptin 50 mg-metformin 1000 mg IR) of Merck Sharp & Dohme Corp, as reference formulation.

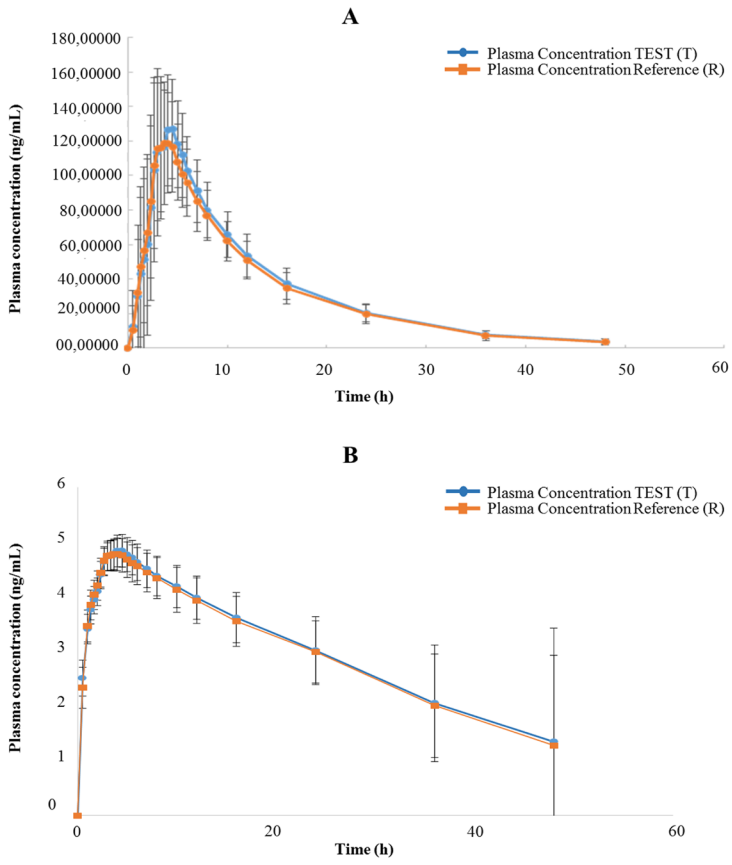

Figure 1 Bioequivalence study of Sitagliptin. Mean plasma concentration (ng/mL) *vs.* Time (h). **Note:** A) Linear scale; B) Logarithmic scale.

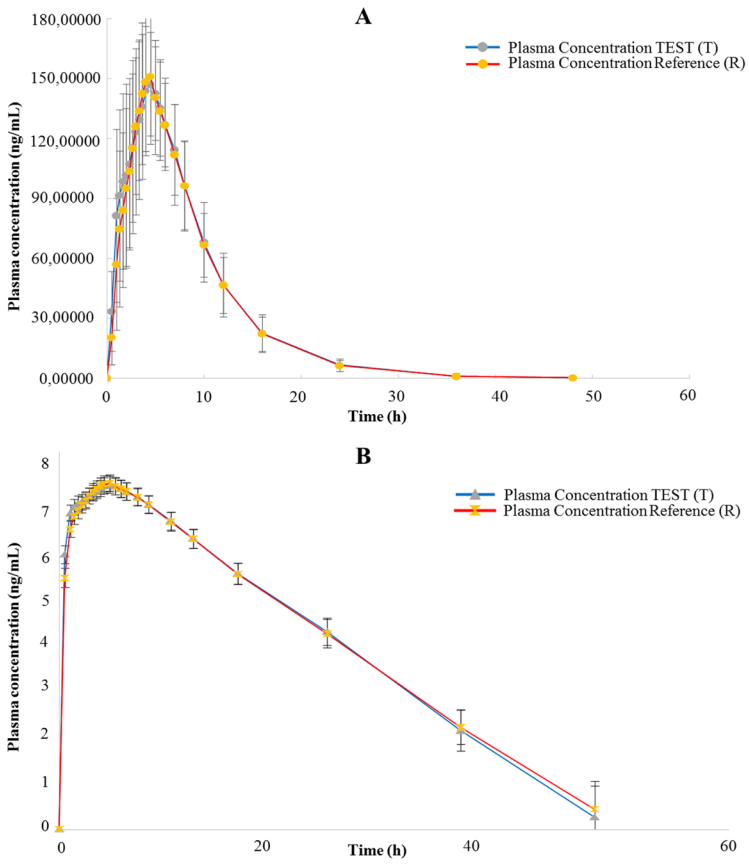

Figure 2 Bioequivalence study of metformin. Mean plasma concentration (ng/mL) *vs.* Time (h). **Note:** A) Linear scale; B) Logarithmic scale.

Table 4: Sitagliptin plasma concentration over 48 h following a single dose of 50 mg oral tablet under fed condition. Test formulation (T) of laboratorios Leti S.A.V. and Reference formulation (R) of Merck Sharp & Dohme Corp.

Arithmetic mean data					Logarithmic mean data			
TIME (h)	Plasma concentration Test (T) (ng/mL)	Plasma concentration Reference (R) (ng/mL)	Standard Error (SE) (T)	Standard Error SE®	Log plasma concentration Test (T)	Log plasma concentration Reference (R)	1/Ln SE (T)	1/Ln SE (R)
0	0	0	0	0	0	0	0	0
0.5	12.338	10.311	21.078	14.512	2.675	0.984	0.374	1.016
1	30.235	31.956	40.903	31.149	3.439	1.235	0.291	0.81
1.33	42.9	47.029	50.422	40.322	3.697	1.307	0.27	0.765
1.67	51.364	56.569	53.365	41.837	3.734	1.317	0.268	0.759
2	59.935	67.046	52.189	42.539	3.75	1.322	0.267	0.757
2.32	81.252	84.759	53.779	44.148	3.788	1.332	0.264	0.751
2.67	103.346	105.637	53.166	47.91	3.869	1.353	0.258	0.739
3	113.517	115.725	48.628	41.776	3.732	1.317	0.268	0.759

3.33	116.14	116.169	41.341	37.377	3.621	1.287	0.276	0.777
3.67	116.455	118.793	33.227	35.48	3.569	1.272	0.28	0.786
4	126.199	118.726	31.978	28.961	3.366	1.214	0.297	0.824
4.5	126.717	116.584	28.759	26.135	3.263	1.183	0.306	0.846
5	118.2	107.691	24.964	22.028	3.092	1.129	0.323	0.886
5.5	111.693	100.663	24.203	19.041	2.947	1.081	0.339	0.925
6	102.664	95.782	19.906	19.312	2.961	1.085	0.338	0.921
7	91.107	84.989	18.148	17.121	2.84	1.044	0.352	0.958
8	79.923	76.92	15.994	14.444	2.67	0.982	0.374	1.018
10	65.926	62.091	13.259	11.384	2.432	0.889	0.411	1.125
12	53.654	50.947	12.486	10.927	2.391	0.872	0.418	1.147
16	37.263	34.813	8.895	8.881	2.184	0.781	0.458	1.28
24	20.39	19.867	4.99	5.788	1.756	0.563	0.57	1.776
36	7.77	7.436	2.55	2.876	1.057	0.055	0.947	18.189
48	3.856	3.595	1.618	1.832	0.605	0.01	1.078	0.089

Table 5: Metformin Plasma Concentration over 48 h following a single dose of 1000 mg IR oral tablet under fed condition. Test formulation of laboratorios Leti S.A.V. and Reference (R) formulation of Merck Sharp & Dohme Corp.

Arithmetic mean data					Logarithmic mean data			
TIME (h)	Plasma concentration Test (T) (ng/mL)	Plasma concentration Reference (R) (ng/mL)	Standard Error (T) SE	Standard Error (R) SE	Log plasma concentration Test(T)	Log plasma concentration Reference (R)	1/Ln SE (T)	1/Ln SE (R)
0	0	0	0	0	0	0	0	0
0.5	334.45	203.329	199.496	137.241	4.922	1.594	0.203	0.627
1	812.513	569.003	432.465	329.984	5.799	1.758	0.172	0.569
1.33	914.384	745.679	428.923	390.2	5.967	1.786	0.168	0.56
1.67	984.099	840.5	439.012	387.091	5.959	1.785	0.168	0.56
2	1015.566	949.811	455.011	398.96	5.989	1.79	0.167	0.559
2.33	1070.384	1036.328	428.335	383.911	5.95	1.783	0.168	0.561
2.67	1156.16	1150.593	433.177	371.123	5.917	1.778	0.169	0.563
3	1232.704	1260.349	415.654	342.163	5.835	1.764	0.171	0.567
3.33	1293.315	1336.126	398.758	346.712	5.848	1.766	0.171	0.566
3.37	1357.507	1422.613	361.678	354.279	5.87	1.77	0.17	0.565
4	1436.457	1483.82	322.629	348.686	5.854	1.767	0.171	0.566

4.5	1470.615	1509.613	258.759	337.73	5.822	1.762	0.172	0.568
5	1424.16	1404.006	234.199	284.91	5.652	1.732	0.177	0.577
5.5	1352.682	1335.558	241.12	245.699	5.504	1.705	0.182	0.586
6	1270.261	1266.572	230.868	208.836	5.342	1.676	0.187	0.597
7	1141.93	1118.369	226.522	252.216	5.53	1.71	0.181	0.585
8	963.322	961.889	220.575	225.002	5.416	1.689	0.185	0.592
10	680.591	666.038	199.469	158.775	5.067	1.623	0.197	0.616
12	465.941	465.129	159.421	140.214	4.943	1.598	0.202	0.626
16	223.633	220.921	94.389	85.573	4.449	1.493	0.225	0.67
24	65.37	62.579	31.577	27.458	3.313	1.198	0.302	0.835
36	8.077	8.645	10.102	15.187	2.72	1.001	0.368	0.999
48	1.291	1.523	4.591	5.4002	1.686	0.523	0.593	0.342

Table 6: Intensity and causality of the adverse event for test product.

Adverse Event (AE)	Mild		Moderate		Severe		Total
	N=26						
	R	NR	R	NR	R	NR	
	n (%)		n (%)		n (%)		
Diarrhoea	2 (7.69)%	-	-	-	-	-	2 (7.69)%
Increase potassium value	-	1 (3.87%)	-	-	-	-	1 (3.87%)
Total	2 (7.69)%	1 (3.87%)	-	-	-	-	3 (11.54%)

Note: N: Total N° de subjects dosed with product T who had the Adverse Event; 1 and R: Probable/likely, possible/certain; 2 and NR: No relation, unlike relation, conditional/unclassified, unassessable/unclassifiable.

Discussion

This study was designed to evaluate the bioequivalence of two FDC formulations in a single-dose, two-period, crossover design involving healthy male subjects under fed conditions. The BE evaluation was based on 90% CIs ratios for sitagliptin and metformin and was assessed against bioequivalence standards of 80%-125% for C_{max} and AUC_{0-t}, as primary PK parameters, adhered to the EU guidelines [20-22]. 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 [23,24].

Due to the metformin component and SmPC recommendations to this fixed dose combination of sitagliptin, 50 mg/metformin 1000 mg IR, this study was done under fed condition [21]. 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} of the T and R formulations, no significant differences were demonstrated between the two formulations.

The 90% confidence intervals for the ratios of sitagliptin and metformin, met the acceptance range of 80.00%-125.00% for primary PK parameter AUC_{0-t} and C_{max} [20-22]. The Geometric Mean Ratio ((GMR) T/R) for the primary PK parameters to sitagliptin was C_{max} 110.46% (103.26%-118.15%) and AUC_{0-t} 104.82% (99.81%-110.08%). The PK parameters for metformin were C_{max} 99.85% (93.61%-106.52%) and AUC_{0-t} 102.51 (96.57%-108.82%). The mean plasma concentration-time curves for the sitagliptin 50 mg and metformin 1000 mg, IR tablets, were similar for both T and R formulations **(Figure 1)**.

The Analysis of Variance (ANOVA) was used for crossover design in bioavailability testing. Was reported 3 adverse events no serious during test administration, clinically no significant, hence the T and R formulations were found to be safe and well tolerated in this study. 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. Many patients with T2DM, who receive monotherapy, are unable to maintain glucose levels with the progress of disease and combined therapy [25] have been indicated to achieve the better glycaemic control [1,13,15,25].

Metformin is the gold standard drug used for patients with T2DM, however several patients requires dual or triple therapy to achieve a glycaemic control due progressive deterioration of beta-cell function. Sitagliptin is an excellent option for combined with metformin because it has glucose-dependent action with lower risk of hypoglycaemia, as well as beneficial effects on beta-cell function and eventual protective action on beta-cell mass [1,3,5,7,14,25].

These components sitagliptin and metformin in FDC can enhance adherence to therapy resulting in improved glycaemic control and reduction of disease management costs [26]. This BE study of sitagliptin/metformin, FDC a generic product, demonstrated to be bioequivalent to reference product, Janumet®, in this dose-strength formulation (sitagliptin 50 mg/metformin 1000 mg IR) that permit dosing flexibility. A generic FDC formulation that is bioequivalent to the original product and available for patients with T2DM, could reduce pill burden, improve patients adherence to treatment, increase compliance and optimize cost-effectiveness [26-29]. This combination provides synergetic glucose control in patients with T2DM, in addition to providing patient adherence and offers potential cost advantages.

Conclusion

Two FDC formulations containing 50 mg of sitagliptin and 1000 mg of metformin IR were evaluated and found to be bioequivalent in healthy subjects under fed condition. The pharmacokinetic profiles of the test and reference formulations were similar, as demonstrated by the 90% Confidence Intervals (CIs) of C_{max} and AUC_{0-t} within the accepted EU-BE criteria of 80%-125%.

Limitations of the Study

This study analyzed PK parameters of sitagliptin/metformin under fed conditions, followed EMA BE guidelines. This study included healthy males only.

Acknowledgements

This study was conducted at the third party Pharmadesk Solutions Pvt., Ltd and VerGo Pharma Research Pvt. Ltd, (Division-VerGo Clinicals) India.

Conflict of Interest

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

Author's Contributions

Evelyn Pena, Alfredo Inatti, Jose Chacon, Anyoli Taly and Xenon Serrano Marti performed the statistical analysis, interpretation, writing, review of the manuscript.

Declaration of Patient Consent

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

Financial Support and Sponsorship

This study was funded by Laboratorios Leti S.A.V.

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