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Bioequivalence Assessment of the Fixed Dose Combination of Linagliptin/Metformin (2.5mg/850mg) Film Coated Tablets in Healthy Human Subjects: A Randomized, Open-Label, Two-Period Crossover Study

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Abstract

Type 2 diabetes mellitus (T2DM) is a chronic, progressive disease with substantial global prevalence and far-reaching health consequences. Combination oral anti-diabetic therapies often provide greater glycemic control compared to monotherapy. Among these, the fixed-dose combination (FDC) of linagliptin and metformin is widely used due to complementary mechanisms of action that enhance glycemic management: metformin acts predominantly via hepatic and peripheral insulin sensitization, while linagliptin, a dipeptidyl peptidase-4 (DPP-4) inhibitor, amplifies incretin activity to promote insulin secretion and inhibit glucagon release. The present study investigates the bioequivalence of two formulations, each containing 2.5mg linagliptin and 850mg metformin, under fasting conditions in healthy Indian males. A single-center, open-label, randomized, two-period crossover trial was conducted. The study enrolled 28 healthy male volunteers, each receiving single doses of both the test and reference formulations with a 36-day washout. Pharmacokinetic (PK) parameters (C_{max}, AUC_{0-t}, $AUC_{0-\infty}$, T_{max}, $t_{1/2}$) were assessed using validated LC-MS/MS. Bioequivalence was determined by the 90% confidence intervals (CIs) for the geometric mean ratio (GMR) of ln-transformed C_{max} and AUC remaining within 80–125%. The results confirmed bioequivalence, as all CIs fell within the regulatory limits. No significant adverse events were observed, and both drugs were well tolerated, supporting their interchangeable use in clinical practice in India.

Keywords: *Bioequivalence, Crossover Study, LC-MS/MS, Linagliptin, Metformin, Pharmacokinetics.*

Introduction

Public Health Impact and Need for Fixed-Dose Combinations

Type 2 diabetes mellitus (T2DM) is characterized by both insulin resistance and beta-cell dysfunction, leading to chronic hyperglycemia and its associated complications, which increasingly strain public health systems worldwide. Optimal glycemic control is critical for reducing microvascular and macrovascular risks [8, 17]. While lifestyle modification remains foundational, pharmacological intervention is typically required at diagnosis or shortly thereafter.

Metformin, a biguanide, is widely regarded as the cornerstone of first-line oral therapy due to its efficacy, cardiovascular neutrality, and long safety record [5, 13]. Its hepatic and peripheral actions reduce glucose output and improve insulin sensitivity. However, as T2DM progresses, monotherapy may be inadequate.

Dipeptidyl peptidase-4 (DPP-4) inhibitors such as linagliptin offer an insulinotropic

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approach that enhances endogenous incretin hormone activity [6, 7, 16], heightening glucose-dependent insulin secretion while suppressing inappropriate glucagon secretion. This drug class is associated with minimal hypoglycemia and weight neutrality, making it attractive for combination regimens.

FDCs, such as Isentin-M and Trayenta Duo, amalgamate efficacy, reduce pill burden, and improve adherence, all of which are crucial for real-world glycemic control and long-term outcomes [18, 20].

Regulatory Context for Bioequivalence

Bioequivalence forms the regulatory and scientific bedrock that permits generic drugs [1, 2, 10]—and especially fixed-dose combinations (FDCs)—to be approved as alternatives to established, branded medicines [1, 2, 4, 11]. Rather than requiring full-scale clinical trials for each new generic product, regulatory agencies accept robust bioequivalence studies [1, 2, 19, 21] as evidence that a generic will deliver the same clinical effect as the original branded product. This facilitates timely access to affordable medicines without compromising on efficacy or safety.

Global Regulatory Bodies and Harmonization

United States (FDA), European Union (EMA), India (CDSCO), and International Council for Harmonization (ICH) all require that for an FDC to be approved as a generic or similar alternative, there must be clear demonstration of [1-4]:

Therapeutic equivalence: Both drug products must produce the same therapeutic effects.

Physicochemical equivalence: The products must have similar physical and chemical characteristics.

Bioequivalence: The rate and extent to which the active pharmaceutical ingredients (APIs) reach the systemic circulation must be statistically equivalent between the test (generic) and reference (brand) formulations.

International guidelines—including the updated ICH M13A [4, 25]—are moving toward global harmonization [4], ensuring that the same standards and evaluation criteria apply in all major markets.

Key Endpoints: What Must Be Measured

Bioequivalence focuses on whether two products deliver the same amount of active agent into the bloodstream at the same rate. The international gold standards for measuring and comparing this are:

Cmax (Maximum Observed Plasma Concentration): The highest concentration the drug achieves in blood plasma. It reflects both the rate and extent of absorption.

AUC_{0-t} (Area Under the Plasma Concentration-Time Curve, until last measurable sample): This represents the total amount of drug absorbed over a defined sampling period.

$AUC_{0-\infty}$ (AUC extrapolated to infinity): This extends the AUC calculation to account for remaining (unmeasured) absorption, giving a theoretical total exposure from a single dose.

These endpoints are typically measured in single-dose, cross-over study designs under controlled (fasting and/or fed) conditions, which help isolate differences due to the formulation rather than external variables.

Study Design and Statistical Analysis

Cross-Over Design: Most bioequivalence studies use a randomized, two-period, two-sequence cross-over design, where each healthy volunteer receives both the test and reference products, separated by a sufficient washout period.

Log Transformation: Due to the skewed distribution of pharmacokinetic parameters (especially Cmax and AUC), their data are natural log-transformed prior to statistical analysis to stabilize variance and approach

normality, which is essential for valid parametric inference.

Statistical Testing: The geometric mean ratio (GMR) of the test to reference values for Cmax, AUC_{0}–t, and AUC_{0}–∞ is calculated. To account for study variability, the 90% confidence interval (CI) for the GMR is determined using an analysis of variance (ANOVA). This approach accounts for intra- and inter-subject variability and ensures robust, unbiased results.

Acceptance Criteria: The 80–125% Rule

Bioequivalence is considered demonstrated if the entire 90% confidence interval for the GMR (test/reference) for both Cmax and AUC parameters falls within the acceptance range of 80–125%.

These limits are not arbitrary; they stem from a consensus that up to ±20% variability is not expected to have significant clinical impact for most drugs (except those with a narrow therapeutic index, which may require tighter criteria).

Meeting these criteria assures regulatory authorities that the test product will match the original in clinical efficacy and safety if substituted in therapy.

Special Considerations for FDCs

For FDCs, each individual component must independently meet bioequivalence criteria when compared against the reference co-administered as single ingredients or as a branded FDC, as applicable.

Regulatory authorities may waive some in vivo BE studies for additional strengths of the same FDC if in vitro data demonstrate uniformity (biowaivers), especially if pharmacokinetics are dose-proportional.

Country-Specific Regulations

US FDA: Adheres closely to the above, requiring demonstration of similar rate and extent of absorption (rate = Cmax; extent = AUC), detailed in 21 CFR 320 and relevant FDA guidance.

EMA (Europe): Requires BE for both rate and extent, with added requirements around study population, design, and data reporting. Adopts ICH international standards.

CDSCO (India): Mandates BE for generics to ensure safety and interchangeability, aligning with Schedule Y of the Indian Drugs and Cosmetics Act and GCP guidelines.

In summary, Regulatory assessment of bioequivalence for FDCs relies on sophisticated study designs and strict acceptance criteria for key pharmacokinetic endpoints. Products are only deemed interchangeable if rigorous statistical analysis proves that their rate and extent of absorption are so similar that switching between them will neither compromise nor alter therapeutic effect. This harmonized approach maintains clinical standards while supporting the introduction of safe, effective, and more accessible generics into the marketplace.

Materials and Methods

Study Site and Ethical Compliance

The study was conducted at Quest Life Sciences Pvt Ltd, a certified clinical research facility in Chennai, India. Protocol approval was obtained from an Independent Ethics Committee, with procedures executed per the Declaration of Helsinki, ICH-GCP, ICMR guidelines, and regional regulatory standards. All subjects provided written informed consent.

Study Design

This was a randomized, open-label, two-period, two-sequence, crossover study enrolling 28 healthy adult Indian males (age: 22–42 years; BMI: 18.5–30.0kg/m^2), meeting comprehensive inclusion/exclusion criteria. Screening included medical history, physical exam, laboratory investigations, and ECG to exclude underlying pathology.

Study Demographic characteristics of subjects are shown in Table 1.

Table 1. Study Demographic Characteristics of Subjects

Parameter	Mean ± SD	Range
Age (years)	33.2 ± 6.0	22–42
Height (cm)	168.6 ± 6.6	157–181
Weight (kg)	69.4 ± 6.8	58.2–85.7
BMI (kg/m²)	24.4 ± 1.7	19.7–27.2

Treatment and Randomization

Each subject received a single oral dose of either the test (linagliptin/metformin 2.5mg/850mg) or reference (Trayenta Duo, linagliptin/metformin 2.5mg/850mg) formulation in each period. Treatments were separated by a 36-day washout, exceeding at least five elimination half-lives to nullify carryover.

Procedure and Sampling

Subjects were admitted under standardized dietary and environmental conditions 11h prior to dosing and remained for 12h post-dose. Fluid and food access were controlled to reduce PK variability. Nineteen venous blood samples per period were collected: pre-dose and at 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 12, 24, and 48h post-dose.

Description of the Laboratory Methods

Plasma concentrations of both linagliptin and metformin were measured using Liquid Chromatography coupled with Tandem Mass Spectrometry (LC-MS/MS). This is a gold-standard, highly sensitive, and selective analytical technique. It separates components in complex biological matrices (like plasma) and then detects and quantifies each component based on its mass-to-charge ratio. LC-MS/MS is routinely used for bioequivalence and pharmacokinetic studies due to its robust ability to detect very low levels of drugs in biological samples.

All LC-MS/MS procedures were developed and validated strictly according to guidance documents from the US FDA and EMA [1, 2]. These agencies require demonstrated reliability of analytical methods in quantitative drug assessments for regulatory acceptance. Linearity range is shown in Table-2.

Table-2. Validated Linearity Range

Linagliptin quantitation:	98.73–7,521.56 pg/mL
Metformin quantitation:	102.39–15,200.35 ng/mL

This method could reliably detect and accurately quantify plasma concentrations as low as about 99pg/mL for linagliptin and 102ng/mL for metformin, up to 7,500pg/mL and 15,200ng/mL, respectively. These wide calibration ranges ensure that all expected drug concentrations post-dose are measurable throughout the pharmacokinetic sampling window, capturing both peak and elimination phases.

Analytical Method Validation

Validation is essential to ensure bioanalytical data are accurate, reliable, and reproducible. The following parameters were specifically assessed:

This analytical method was developed as per EMA and ICH standards to ensure regulatory compliance [2, 12, 24].

Accuracy: The method's ability to measure the true concentration of the analyte. Measured (experimental) concentrations are compared to known reference standards across the calibration range.

Precision: The reproducibility of the method under the same conditions, including intra- and inter-batch consistency. It is typically expressed as the coefficient of variation (CV%).

Linearity: Demonstrates that the assay's measured response is directly proportional to the analyte concentration over the calibration range (i.e., a straight line is obtained when plotting concentration vs. instrument response).

Specificity: Confirms the method measures the target drugs (linagliptin and metformin) without interference from endogenous substances or other drugs that might be present in plasma.

Recovery: The efficiency with which the analytes (linagliptin and metformin) are extracted from plasma during sample processing.

Stability: Demonstrates that analyte concentrations do not degrade under sample handling and storage conditions studied. This typically includes bench-top stability (room temperature), freeze-thaw stability, autosampler stability, and long-term storage stability.

Pharmacokinetic Data Analysis

Pharmacokinetic Data Analysis Using Phoenix WinNonlin

Phoenix WinNonlin (v6.3; Certara) is a specialized software widely used in pharmacokinetics (PK) for analyzing drug concentration versus time data. The analysis performed is non-compartmental analysis (NCA), which is a model-independent method that derives pharmacokinetic parameters directly from observed plasma drug concentration-time data without assuming any specific physiological compartments.

The primary pharmacokinetic parameters calculated include:

C_{max} (Maximum Observed Plasma Concentration): This is the highest drug concentration measured in plasma after administration. It indicates the peak level of drug exposure in systemic circulation.

T_{max} (Time to Maximum Concentration): The time point at which Cmax occurs, representing how quickly the drug reaches its peak concentration after dosing.

AUC_{0-t} (Area Under the Concentration-Time Curve from Time Zero to Last Measurable Concentration): This reflects the total drug exposure over the time period from dosing until the last quantifiable plasma concentration. The AUC is calculated using the trapezoidal rule integrating the plasma concentration over time.

$AUC_{0-\infty}$ (Area Under the Curve from Zero to Infinity): This extends AUC0-t by estimating the additional drug exposure beyond the last measurable time point to infinite time, usually by extrapolating based on the elimination rate. It provides a measure of total drug exposure.

$t_{½}$ (Elimination Half-Life): The time it takes for the plasma drug concentration to decrease by 50% during the elimination phase. It's calculated from the slope of the terminal elimination phase on a log concentration-time plot.

K^{el} (Elimination Rate Constant): The rate at which the drug is eliminated from the plasma, calculated as the slope of the terminal phase in the log-linear concentration-time graph.

These parameters comprehensively summarize the rate and extent of drug absorption, distribution, and elimination — critical for bioequivalence assessment.

Bioequivalence Assessment

Bioequivalence between the test and reference formulations was assessed according to internationally accepted guidelines by calculating the geometric mean ratios (GMRs) for the log-transformed pharmacokinetic parameters, along with their corresponding 90% confidence intervals (CIs) using Schuirmann's two one-sided tests procedure. The protocol defined bioequivalence as achieving 90% CIs within the regulatory acceptance interval of 80% to 125% for both C_{max} and AUC parameters. Additional summaries included descriptive statistics for all PK parameters (arithmetic mean, geometric mean, standard deviation, range, coefficient of variation) and computation of power estimates and intra-subject coefficients of variation (CV). Sample size determination was based on literature-reported variabilities for linagliptin and metformin; with an intra-subject CV estimated at 21%, and assuming a test-to-reference ratio close to 100%, recruiting 28 subjects was calculated to provide at least 80% power to detect potential differences while minimizing the risk of type II error. This comprehensive strategy ensured statistical robustness and regulatory compliance for all analytical comparisons undertaken in the study.

Results

Subject Disposition

In total, 26 subjects had completed both the periods of the study and were included in

pharmacokinetic analysis and statistical analysis.

Pharmacokinetic Parameters

Descriptive statistic includes Mean, Standard Deviation (SD), Geometric mean, Coefficient of Variation (CV %) and Range (Minimum and Maximum) were calculated for C_{max}, T_{max}, K^{el}, $T_{1/2}$, AUC_{0-t} and $AUC_{0-\infty}$, $AUC_{\%extra\_Obs}$ for test and reference product of Linagliptin and Metformin using Phoenix® WinNonlin® version 6.3 and were given in the Table 3. Linear and Semilog Plot of Mean Plasma Concentration vs. Time (N=26) of Metformin are shown in Figure 1 and 2. Similarly Figure 3 and 4 shows the Linear and Semilog Plot of Mean Plasma Concentration vs. Time (N=26) of Linagliptin.

Table 3. Pharmacokinetic Results for Metformin and Linagliptin (N=26)

Parameter	Metformin Test (Mean ± SD)	Metformin Reference (Mean ± SD)	Linagliptin Test (Mean ± SD)	Linagliptin Reference (Mean ± SD)
Cmax	10,470.9 ± 3,724.1 ng/mL	10,721.4 ± 3,491.4 ng/mL	2,483.3 ± 3,156.2 pg/mL	2,202.4 ± 2,635.5 pg/mL
Tmax	2.54 ± 1.01 h	2.41 ± 0.93 h	1.65 ± 0.47 h	0.67 ± 0.29 h
AUC0–t	35,411.3 ± 11,692.7 ng*h/mL	36,895.6 ± 12,147.9 ng*h/mL	4,814.8 ± 7,046.1 pg*h/mL	4,376.5 ± 5,220.1 pg*h/mL
AUC0–∞	35,801.1 ± 11,710.3 ng*h/mL	37,273.5 ± 12,185.8 ng*h/mL	5,210.6 ± 7,041.5 pg*h/mL	4,695.1 ± 5,240.6 pg*h/mL
t½	1.36 ± 0.27 h	1.33 ± 0.22 h	1.92 ± 3.70 h	1.52 ± 1.78 h

Time Vs Mean,
Linagliptin/Metformin HCl 2.5/850 mg Film Coated Tablets
Analyte : Metformin

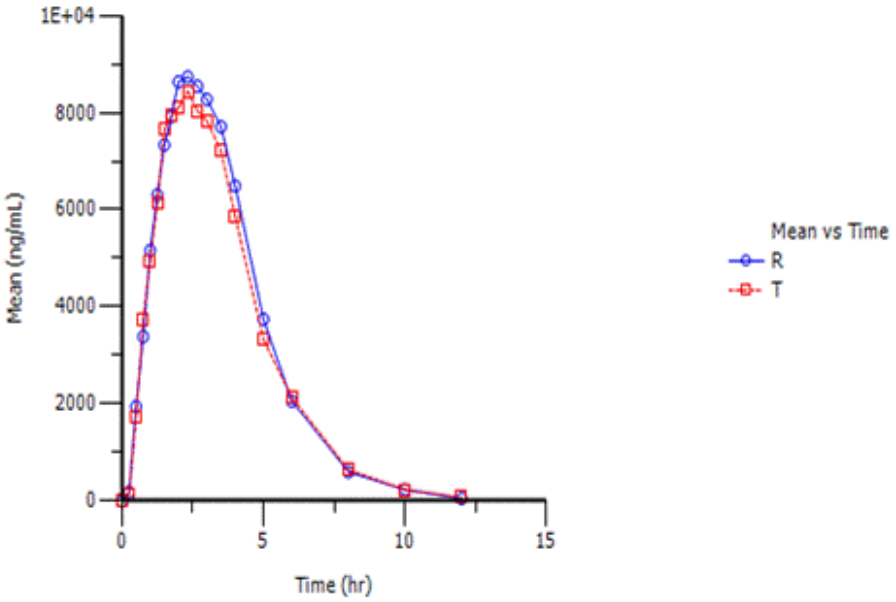

Figure 1. Linear Plot of Mean Plasma Metformin Concentration vs. Time (N=26)

Semilog, Time Vs Mean,
Linagliptin/Metformin HCl 2.5/850 mg Film Coated Tablets
Analyte : Metformin

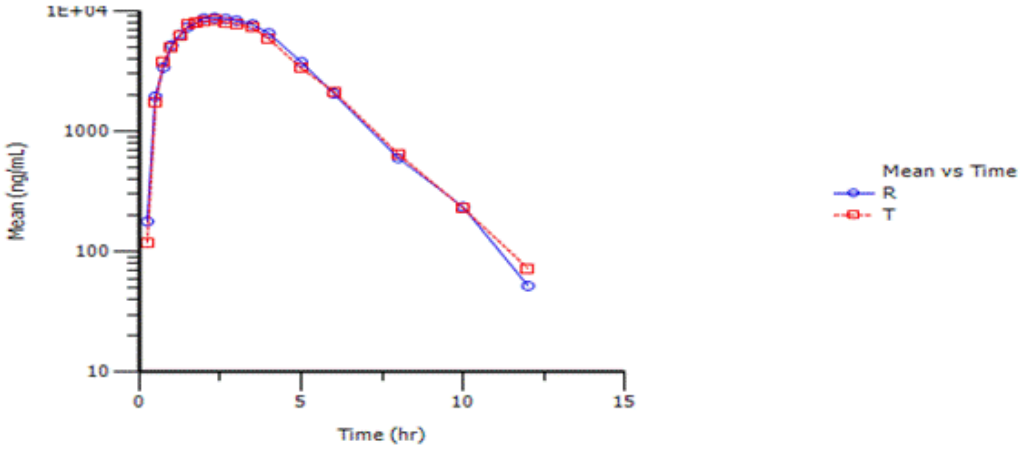

Figure 2. Semi-log Plot of Mean Plasma Metformin Concentration vs. Time (N=26)

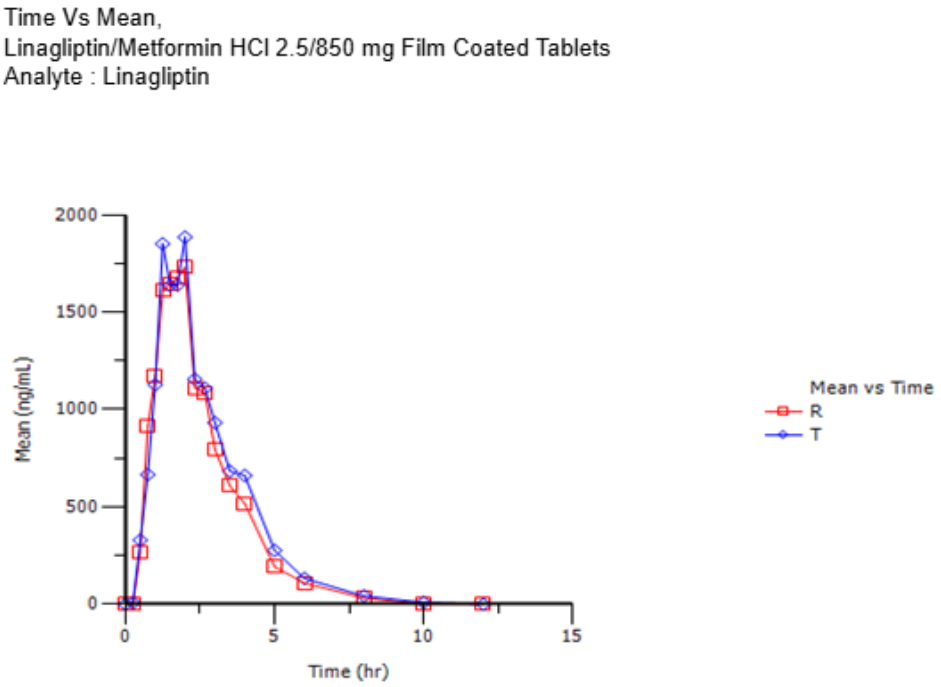

Figure 3. Linear Plot of Mean Plasma Linagliptin Concentration vs. Time (N=26)

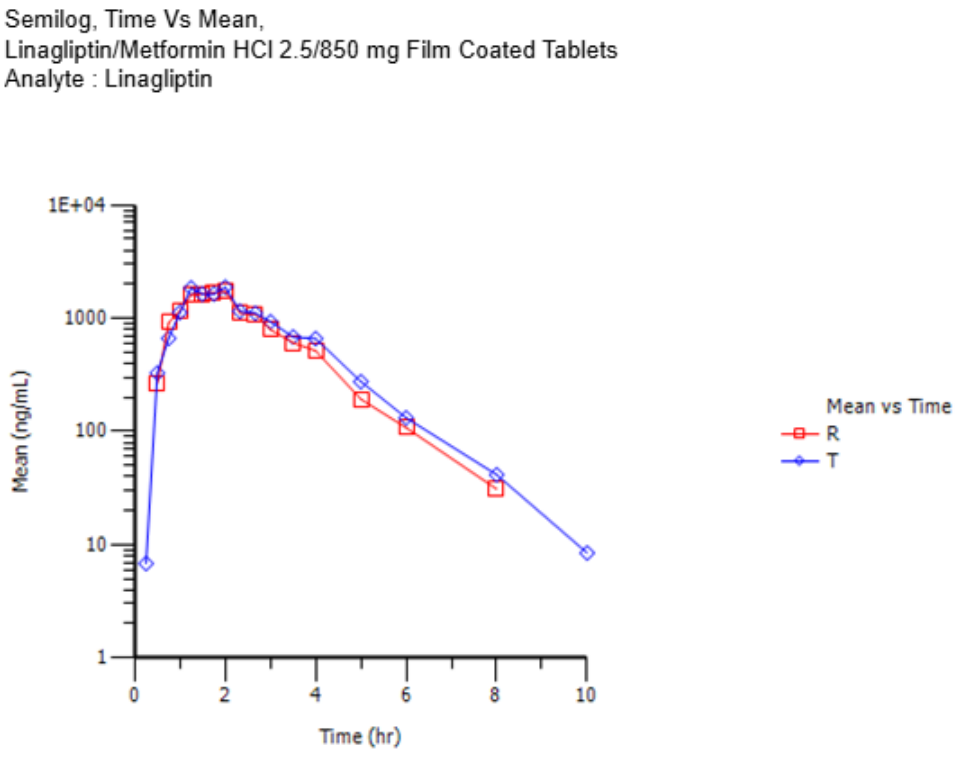

Figure 4. Semi-log Plot of Mean Plasma Linagliptin Concentration vs. Time (N=26)

Bioequivalence Statistics

The 90% confidence interval of geometric least square mean of Tests to Reference falls within the acceptance range of 80.00% – 125.00% for Ln-transformed pharmacokinetic parameter of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ for both Metformin and Linagliptin are given in Table 4. All 90% CIs were within the regulatory range (80–125%), confirming bioequivalence. Hence bioequivalence of the test product with that of the reference product was concluded.

Table 4. Geometric Mean Ratios (GMR) and 90% CI for Bioequivalence

Analyte	Parameter	GMR (%)	90% CI (%)	Intra-CV (%)
Metformin	Cmax	96.8	87.6–107.0	21.3
	AUC0–t	96.0	86.8–106.2	21.4
Linagliptin	Cmax	105.5	96.3–115.6	19.4
	AUC0–t	100.9	93.8–108.7	15.6

Safety and Tolerability

No deaths or serious adverse events occurred. No clinically significant changes were observed in laboratory or vital sign parameters. Minor non-serious deviations (e.g., cannula block) did not affect outcomes.

Discussion

This investigation unequivocally established the bioequivalence of two formulations of fixed dose combination of linagliptin/metformin (2.5mg/850mg), in Indian healthy males under fasting conditions, utilizing a robust crossover methodology compliant with global regulatory guidance. The PK profiles obtained—C_{max}, AUC, and associated intervals—were consistent with previously published studies [5, 6, 13, 14] for both molecules in similar populations and settings, reinforcing the reliability of the data.

No significant period or sequence effects were apparent within the crossover ANOVA, reflecting optimal randomization and washout efficacy. Analytically, validated LC-MS/MS ensured precision and sensitivity across the relevant PK range. The comprehensive safety analysis concurred with the long-established benign profile [6, 7, 15] of both agents when used as monotherapy or in combination.

These data directly address Indian regulatory requirements for substitution and contribute valuable real-world evidence to global generic drug policy. The main study limitations included the all-male, healthy volunteer cohort, which may not generalize directly to target patient populations (women, elderly, those with comorbidities or organ dysfunction). Future studies could expand to longer-term use in T2DM patients to reinforce external validity.

Conclusion

A single oral dose of the test formulation of the fixed dose combination of Linagliptin and Metformin HCl (2.5mg/850mg) is bioequivalent to the reference product Trayenta Duo (Linagliptin and Metformin HCl (2.5mg/850mg)) regarding systemic exposure (C_{max}, AUC) under fasting conditions in healthy adult males, with excellent safety and tolerability. These findings support the therapeutic interchangeability of the test product in diabetes care and will inform regulatory and clinical policy in South Asia [1, 9, 10].

Conflict of Interest

The authors declare no conflict of interest.

Data Availability

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to the restrictions of the information that could compromise the privacy of research participants.

Author Contribution

All the authors have equally contributed in this research.

Ethical Approval

The protocol was approved by the Independent Ethics Committee and all procedures conformed to international ICH-GCP and local regulatory requirements. Ethical compliance was ensured according to the Declaration of Helsinki and ICMR guidelines [3, 22].

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