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Pharmacokinetic Comparison and Bioequivalence Evaluation of Two Empagliflozin/Metformin Fixed-Dose Combination Tablets in Healthy Subjects under Fed Conditions

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Abstract

Background: Empagliflozin, an SGLT2 inhibitor, and Metformin, a biguanide, are commonly prescribed together for the management of type 2 diabetes. This study evaluates the bioequivalence of a fixed-dose combination of Empagliflozin and Metformin (12.5/1000 mg) in healthy subjects under fed conditions. The goal is to ensure that generic versions deliver the same therapeutic effect as the reference product. **Materials and Methods:** This study was a randomized, open-label, two-period, two-sequence, crossover trial aimed at evaluating the bioequivalence (BE) profiles of two fixed-dose combinations (FDCs) of Empagliflozin and Metformin. The assessment of bioequivalence focused on the maximum plasma concentration (C_{max}), area under the concentration-time curve from time zero to time t (AUC_{0-t}), and area under the concentration-time curve from time zero to infinity ($AUC_{0-\infty}$) for both the test and reference formulations. Out of 46 screened participants, 17 were enrolled, of whom 15 completed both treatment periods. In each period, serial blood samples were collected for a duration of up to 72 hours following the oral administration of the study medications. Plasma concentrations of Empagliflozin and Metformin were quantified using a liquid chromatography-tandem mass spectrometry (LC-MS/MS) methodology. The drug products were deemed bioequivalent if the 90% confidence interval (CI) for the test/reference ratios was within the range of 80.00% to 125.00% for the natural logarithm-transformed C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$. Tolerability and safety were continuously monitored throughout the study. **Results:** Based on the rate and extent of absorp-

tion, the pharmacokinetic (PK) parameters were similar between the test product (T) and reference product (R). The 90% CI of the test/reference ratios of log-transformed PK parameters point estimates for Empagliflozin were C_{max}: 97.39% (87.80% - 108.02%), AUC_{0-t}: 95.40% (90.67% - 100.37%), and $AUC_{0-\infty}$: 95.98% (90.93% - 101.32%) and C_{max}: 96.72% (84.39% - 110.84%), AUC_{0-t}: 98.30% (89.94% - 107.43%), and $AUC_{0-\infty}$: 97.69% (89.53% - 106.59%) for Metformin, respectively (90% CI for all PK parameters fell within 80.00% - 125.00%). **Conclusion:** Our findings confirmed in vivo bioequivalence between the test and reference formulations of Empagliflozin 12.5 mg/Metformin 1000 mg under fed conditions.

Keywords

Empagliflozin, Metformin, Bioequivalence, Pharmacokinetics

1. Introduction

Diabetes mellitus represents a significant global health crisis, particularly in South Asia, where approximately one-quarter of the world's population is at elevated risk for developing Type 2 diabetes (T2DM) [1]. Notably, over 90% of diabetes cases in this region pertain to Type 2 diabetes [2], with South Asians demonstrating a higher susceptibility compared to other ethnic groups [3] [4]. Projections indicate that cases will rise by 151% between the years 2000 and 2030, followed by an anticipated further increase of 40% [5]. The region faces considerable challenges in the management of diabetes, which includes an increased risk of both microvascular and macrovascular complications resulting from hyperglycemia and metabolic syndrome. Moreover, individuals within this population are at a greater risk of experiencing major adverse cardiac events, arrhythmias, and heart failure, as well as elevated rates of hospitalisation and mortality [6]. As the demand for effective treatment options escalates, it is imperative to ensure that South Asian communities have access to well-researched and bioequivalent therapeutic alternatives. Current pharmacological treatment objectives focus on controlling hyperglycemia and delaying or preventing both microvascular and potentially macrovascular complications by maintaining long-term euglycemia. The existing guidelines for glycemic control in Type 2 diabetes recommend aggressive targets, such as achieving an HbA1c level of less than 7% or even below 6.5%, necessitating a re-evaluation of treatment strategies by clinicians [7].

Metformin, classified as a biguanide, effectively lowers both basal and postprandial glucose levels through mechanisms that reduce hepatic glucose production, decrease intestinal glucose absorption, and enhance insulin sensitivity [8]. With an average bioavailability of 40% - 60%, Metformin is distributed rapidly throughout the body, achieving peak plasma concentrations approximately three hours post-administration [8] [9]. The liver does not significantly metabolise the drug and is excreted unchanged in urine, possessing a half-life of approximately 5 hours

[10].

Empagliflozin, recognised as the first approved SGLT2 inhibitor, serves as a promising adjunct therapy to Metformin, contributing to improved glycemic control and cardiovascular outcomes for individuals with T2DM [11]. Its mechanism of action involves increasing urine glucose excretion and mitigating hyperglycemia through the inhibition of the SGLT2 transporter, which is responsible for renal glucose reabsorption [12]. Empagliflozin's pharmacokinetics have been extensively studied in both type 2 diabetic patients and healthy individuals. Following oral administration, Empagliflozin is rapidly absorbed, reaching peak plasma concentrations at a median time of 1.5 hours post-dose [13]. The systemic exposure to Empagliflozin increases in a dose-proportional manner within the therapeutic range [14] [15]. Subsequent plasma concentrations decline in a biphasic manner, characterised by a notably slow terminal phase and a rapid distribution phase, ultimately yielding a terminal half-life of 13 hours [16].

Although Metformin remains the preferred first-line monotherapy for managing T2DM, the most recent treatment algorithms from the American Diabetes Association (ADA) and the American Association of Clinical Endocrinology (AACE) recommend the inclusion of sodium-glucose cotransporter-2 (SGLT2) inhibitors as part of dual combination therapy [17]. This trend towards combination therapy is increasingly recognised due to the progressive nature of T2DM and the limited efficacy of Metformin when used as monotherapy [18] [19]. The complementary mechanisms of Empagliflozin and Metformin allow for more effective management of type 2 diabetes, as Empagliflozin reduces hyperglycemia through the inhibition of the SGLT2 transporter, while Metformin reduces hepatic glucose production and enhances insulin sensitivity and peripheral glucose uptake [20]-[23]. The utilisation of both medications results in synergistic glucose-lowering effects, providing superior glucose control compared to either agent used independently [23] [24].

The FDA has approved the fixed-dose combination medication of empagliflozin and metformin, predicated on pharmacokinetic studies that compare it to the separate administration of individual tablets of both agents in healthy subjects [25]. The formulation of a single-pill, fixed-dose combination simplifies the dosing regimen, thereby potentially improving patient adherence and compliance with treatment [26]. For individuals with type 2 diabetes, this combinatory therapy, which offers various dosage options, facilitates individualised treatment [27]. Unlike "loose-dose combinations," single-pill formulations significantly reduce the pill burden and streamline the dosage schedule. Such an approach has been correlated with increased patient satisfaction, enhanced adherence to therapeutic regimens, and a decrease in healthcare costs [28]. Improved adherence to therapy is likely to correlate with better glycemic control among patients with T2DM [28].

Given the substantial burden of T2DM within South Asia and the imperative for accessible treatment options, this study aims to evaluate and compare the pharmacokinetic profile and safety of two fixed-dose combination tablets of Em-

pagliflozin 12.5 mg/Metformin 1000 mg: Synjardy® (Boehringer Ingelheim International GmbH, Germany) as the reference formulation and Empagliflozin 12.5 mg/Metformin 1000 mg (Beximco Pharmaceuticals Limited, Bangladesh) as the test formulation, administered to healthy adult subjects under fed conditions.

2. Materials and Methods

2.1. Study Design

This was a single-centre, randomised, open-label, balanced, laboratory-blind, single oral dose, two-treatment, two-sequence, two-period, two-way crossover oral bioequivalence study of Empagliflozin and Metformin hydrochloride tablets, 12.5/1000 mg tablet manufactured by Beximco Pharmaceuticals Limited, Bangladesh (T), and Synjardy 12.5/1000 mg tablet (Empagliflozin and Metformin hydrochloride tablets, 12.5/1000 mg) of Boehringer Ingelheim International GmbH, Germany, in healthy adult male human subjects under fed conditions. The study was conducted in Bangladesh by the Contract Research Organization, Novus Clinical Research Services Limited. The clinical phase spanned 14 days, from 20 February 2024 to 4 March 2024, and included a washout period of 7 days. This duration was determined based on the terminal elimination half-lives of Empagliflozin (12.4 hours) and Metformin (6.2 hours), which exceeds the seven half-lives recommended by the Food and Drug Administration (FDA). Treatment allocation and randomisation were executed using Statistical Analysis Software (SAS). Notably, both participants and study personnel were not blinded to the treatment assignments throughout the study. However, during the analytical processes, the analyst remained blinded to the sequence of treatment allocation.

2.2. Ethical Approval

The study was conducted in compliance with the ethical standards established by the Bangladesh Medical Research Council (BMRC) guidelines (2017) [29], the Good Clinical Practice (GCP) guidelines of the Directorate General of Drug Administration (DGDA) [30], as well as the ethical principles set forth in the Declaration of Helsinki [31] and the International Council for Harmonization's Good Clinical Practice Guidelines [32]. In August 2023, the National Research Ethics Committee (NREC) of the BMRC reviewed and approved the study documentation, which included the protocol, informed consent form, and case report form (Reference No.: BMRC/NREC/2022-2025/210). Furthermore, in October 2023, the Directorate General of Drug Administration (DGDA) granted authorisation for the study (Reference No.: DGDA/CTP-04/2016/19424).

2.3. Study Population

The study included 46 healthy volunteers aged 18 - 55 years with a BMI between 18 and 30 kg/m^2 who underwent a screening procedure. Thirty were found eligible, 17 were enrolled in the study, and 15 subjects completed the clinical phase.

The volunteers were assessed as healthy after medical examinations, physical examinations, 12-lead electrocardiography, and clinical laboratory tests. Clinical laboratory tests included hematology, biochemistry, serology and urinalysis. They also underwent chest radiography, tests for alcohol and drug abuse, and a COVID antigen test. Participants with dietary or medical conditions that could affect drug distribution, metabolism, excretion, or absorption were not allowed to participate. Exclusions included those with a history of hypersensitivity to study medication, drug or alcohol abuse, participation in a clinical investigation requiring repeated blood sampling, blood donation, or blood loss within 90 days. Other exclusions were the use of prescribed drugs within 14 days of first dosing and throughout the study periods, the use of over-the-counter (OTC) products within 7 days of first dosing and throughout sampling time points, and the use of medication that could affect drug metabolism within one month and throughout the study periods. All participants received a full explanation of the study, including possible risks and benefits, from the researchers and voluntarily signed an informed consent form.

2.4. Drug Products

The pharmaceutical products utilised in this study underwent a prior assessment to evaluate the drug content of each item. These products were classified as pharmaceutical equivalents, as drug content of the test product did not differ from the reference product by more than 5%. A comprehensive summary of each investigational product employed during the study procedures is presented in Table 1.

Table 1. Identity of Investigational Product(s) [Test (T) and Reference (R)].

Product details	Test product (T)	Reference product (R)
Trade Name	Jardimet® 12.5/1000 mg tablet	Synjardy® 12.5/1000 mg tablet
Generic Name	Empagliflozin & Metformin Hydrochloride 12.5/1000 mg tablet	Empagliflozin & Metformin Hydrochloride 12.5/1000 mg tablet
Batch/Lot No.	LTN (046/23) 077D	202707A
Manufacturing Date	02/2024	N/A
Expiry Date	01/2026	03/2025
Name and Address of the manufacturer	Beximco Pharmaceuticals Ltd., Bangladesh	Boehringer Ingelheim International GmbH., Germany

2.5. Drug Administration

Participants were required to remain within the facility for a minimum of 12 hours prior to the administration of the study medication during each study period. A

single oral dose of the investigational drug was administered under fed conditions, accompanied by 240 mL of a 20% glucose solution at ambient temperature. Additionally, 60 mL of the same 20% glucose solution was provided every 15 minutes for a duration of up to 4 hours post-dosing, all under the supervision of the Principal Investigator. Participants were randomised to receive a single oral dose (1 × 12.5/1000 mg tablet) of either the test product or the reference product. To mitigate bias, a randomisation schedule was generated utilising SAS® software. A washout period of seven days was implemented between the two treatment regimens.

2.6. Standard Meal and Fluid

The study participants were required to fast for 10 hours prior to and 4 hours following the administration of the drug. They consumed a high-fat, high-calorie breakfast, consisting of approximately 800 to 1000 kcal, 30 minutes before receiving the drug. Standardised meals were provided at 04:00, 08:00, 12:00, and 25:00 hours subsequent to dosing within each study period. Throughout the housing period, the meal plan remained consistent across both study periods. With the exception of one hour prior to and one hour following drug administration, as well as during the 15-minute interval for glucose solution administration, participants were permitted to drink an unlimited amount of water.

2.7. Blood Sample Collection

Five millilitres of 23 blood samples were taken through an indwelling cannula from each subject during each period, while 48 and 72 h samples were collected by direct venipuncture. The venous blood samples were withdrawn at 00.00 (predose), 00.33, 00.67, 01.00, 01.33, 01.67, 02.00, 02.33, 02.67, 03.00, 03.33, 03.67, 04.00, 04.50, 05.00, 06.00, 08.00, 10.00, 12.00, 16.00, 24.00 (check-out), 48.00 (ambulatory) and 72.00 (ambulatory) hours post-dose.

2.8. Analytical Methodology

Empagliflozin and Metformin plasma concentrations were determined using a validated liquid chromatography-tandem mass spectrometry method. Blood samples were collected in K_2EDTA tubes, and immediately after sampling, they were centrifuged at 3500 RPM for 10 minutes at 5˚C ± 3˚C. Protein precipitation was used to pretreat plasma samples. Following sample separation, the supernatants were stored below 70˚C until analysed further. The chromatographic separation was performed by a Symmetry (C18, 4.6 × 100 mm, 5.0 µm) column (for Empagliflozin) and Zorbax Eclipse (XDB-C18, 4.6 × 150 mm, 5.0 µm) column (for Metformin). In the positive electrospray mode, the mass spectrometer was used. As the internal standard, Olmesartan was used. The validated analytical method was linear throughout the range of 10 - 800 ng/mL for Empagliflozin, and the method for Metformin was linear over the range of 10 - 3000 ng/mL ($r^2 > 0.99$). For both analytes, the lower limit of quantification was set at 10 ng/mL, and the method

validation complied with regulatory requirements and showed no significant interference. The method's precision and accuracy were evaluated using quality control samples at four concentrations (Empagliflozin: 30.00, 100.00, 300.00, 600.00 ng/mL; Metformin: 25.00, 250.00, 1000.00, 2500.00 ng/mL), which were evenly distributed among the plasma samples of participants. This validated method ensured accurate and reliable pharmacokinetic assessments of Empagliflozin and Metformin in plasma samples.

2.9. Safety Assessment

Safety evaluation was carried out through the assessment of adverse events (AEs) monitoring for all the participants who received the drugs at least once throughout the study. Data from clinical laboratory testing, physical examinations, subject interviews, and vital signs were integrated to identify any adverse events (AEs). All adverse events were documented using the Medical Dictionary for Regulatory Activities. AE severity was assessed using the standard terminology criteria. Vital signs (temperature, pulse rate, blood pressure, and respiratory rate), along with random blood sugar monitoring, were measured at baseline, 1.50, 3.50, 5.50, 7.00, 9.00, 11.00, 13.00, 24.00 (check-out), 48.00 and 72.00 (ambulatory) hours post-dose. Throughout the studies, adverse events were documented, assessed for severity and drug relationship, and monitored using clinical laboratory results after the study.

2.10. Statistical Analysis of PK Parameters for BE Determination

The non-compartmental model method (NCA module) was used to determine pharmacokinetic parameters. The PK parameters calculated were maximum peak concentration (C_{max}), AUC from time 0 h to the last measurable concentration ($AUC_{0\text{-}t}$), and AUC from time 0 to infinity ($AUC_{0\text{-}\infty}$) as primary parameters. Other secondary PK parameters evaluated were as follows: AUC_% Extrap_obs, T_{max}, $T_{1/2}$, and λ_z/K_{el} of Empagliflozin & Metformin in plasma. SAS software, version 9.4, SAS Institute Ind., USA, was used to conduct statistical analyses. Using log-transformed data for these parameters, ANOVA for a 2 × 2 crossover design was carried out at the 5% significance level ($\alpha = 0.05$), where period, sequence and treatment were considered as fixed effects. When 90% CIs of the test (T) and reference (R) ratio of these parameters fell between 80% and 125%, the formulations were considered bioequivalent.

3. Results

3.1. Subject Characteristics

A total of 18 subjects were randomised, and 17 were enrolled in the study. Sixteen subjects completed the clinical phase of period I, and 15 subjects successfully completed both periods of the study. Plasma samples of 15 subjects were analysed. The data from 15 subjects were considered for safety, pharmacokinetic, and statistical

analysis. The baseline demographics of subjects who completed the study are summarised in Table 2.

Table 2. Demographic details for all evaluable subjects in the study (n = 15).

	Age (year)	Height (cm)	Weight (kg)	BMI (kg/m^2)
Minimum	21	160.0	50.10	18.60
Maximum	35	177.0	78.50	28.30
Mean	26	166.1	63.39	23.01
SD	3.3778	4.8912	8.0257	3.0308
Median	25	165.0	61.20	22.60

3.2. Safety Analysis

All subjects who took at least one dose of the study drug were included in the tolerability assessment. Four subjects reported individual events of diarrhoea, vomiting and vertigo, which adverse events (AEs)were mild. The test product and the reference product indicated good tolerance in all volunteers, and there were no serious AEs or drug reactions. No serious adverse events were reported, and no significant clinical findings were found in hematology tests, blood chemistry examinations, urine tests, ECG, blood pressure, pulse rate, body temperature, or physical examination results.

3.3. Pharmacokinetic Properties

Table 3(a) and Table 3(b) summarise the pharmacokinetic results of Empagliflozin and Metformin Hydrochloride for test product (T) and reference product (R).

Table 3. (a): Summary of Pharmacokinetic Parameters (Empagliflozin); (b): Summary Results of Pharmacokinetic Parameters (Metformin).

(a)							
Empagliflozin (Reference Product)							
Variable	**N**	**Arithmetic Mean**	**SD**	**CV%**	**Min**	**Median**	**Max**
T_{max} (hr)	15	3.67	1.39	37.8	2.00	3.33	6.00
C_{max} (ng/mL)	15	154.177	33.862	22.0	71.847	161.050	192.754
AUC_{0-t} (hr*ng/mL)	15	1308.675	291.083	22.2	573.928	1378.517	1692.102
$AUC_{0-\infty}$ (hr*ng/mL)	15	1467.887	317.964	21.7	652.897	1524.063	1847.131
$AUC_{\_\%Extrap\_obs}$ (%)	15	10.90	3.27	30.0	6.17	10.49	18.38
$T_{1/2}$ (hr)	15	6.459	1.400	21.8	4.105	6.434	9.279
K_{el} (hr^{-1})	15	0.112	0.026	23.3	0.075	0.108	0.169

Continued

Empagliflozin (Test Product)							
	N	Arithmetic Mean	SD	CV%	Min	Median	Max
T_{max} (hr)	15	3.18	2.15	67.6	1.00	2.33	8.00
C_{max} (ng/mL)	15	154.172	44.232	28.7	67.043	145.911	248.400
AUC_{0-t} (hr*ng/mL)	15	1258.879	307.970	24.5	508.256	1307.779	1633.321
$AUC_{0-\infty}$ (hr*ng/mL)	15	1418.275	347.016	24.5	586.532	1470.937	1876.943
$AUC_{\_\%\ Extrap\_obs}$ (%)	15	11.22	4.56	40.6	6.06	10.24	24.18
$T_{1/2}$ (hr)	15	6.143	1.530	24.9	3.915	6.167	9.596
K_{el} (hr^{-1})	15	0.119	0.029	24.6	0.072	0.112	0.177

(b)

Metformin (Reference Product)							
Variable	N	Arithmetic Mean	SD	CV%	Min	Median	Max
T_{max} (hr)	15	3.45	1.66	48.2	1.33	3.33	8.00
C_{max} (ng/mL)	15	1600.807	371.265	23.2	939.174	1599.834	2285.761
AUC_{0-t} (hr*ng/mL)	15	12381.282	2419.911	19.5	8046.840	12320.824	16435.209
$AUC_{0-\infty}$ (hr*ng/mL)	15	12665.123	2409.130	19.0	8542.896	12925.466	16687.474
$AUC_{\_\%\ Extrap\_obs}$ (%)	15	2.32	1.81	77.7	0.67	1.75	6.67
$T_{1/2}$ (hr)	15	5.181	3.070	59.3	3.135	3.914	15.409
K_{el} (hr^{-1})	15	0.157	0.048	30.7	0.045	0.177	0.221
Metformin (Test Product)							
	N	Arithmetic Mean	SD	CV%	Min	Median	Max
T_{max} (hr)	15	2.98	1.75	58.7	1.00	2.67	8.00
C_{max} (ng/mL)	15	1610.065	537.046	33.4	863.675	1577.452	2646.548
AUC_{0-t} (hr*ng/mL)	15	12151.223	2176.004	17.9	8979.629	11526.530	17786.623
$AUC_{0-\infty}$ (hr*ng/mL)	15	12355.745	2178.457	17.6	9142.771	11866.675	17971.397
$AUC_{\_\%\ Extrap\_obs}$ (%)	15	1.69	0.85	50.5	0.52	1.59	3.43
$T_{1/2}$ (hr)	15	4.169	1.340	32.2	2.900	3.700	7.922
K_{el} (hr^{-1})	15	0.178	0.041	23.0	0.088	0.187	0.239

C_{max}: maximum plasma concentration of the drug, AUC_{0-t}: area under the plasma concentration-time curve from time zero to the time of the last measurable concentration, $AUC_{0-\infty}$: area under the plasma concentration-time curve from time zero to infinity, T_{max}: time to reach maximum plasma Concentration, $T_{1/2}$: half-life of the drug, K_{el}: elimination rate constant.

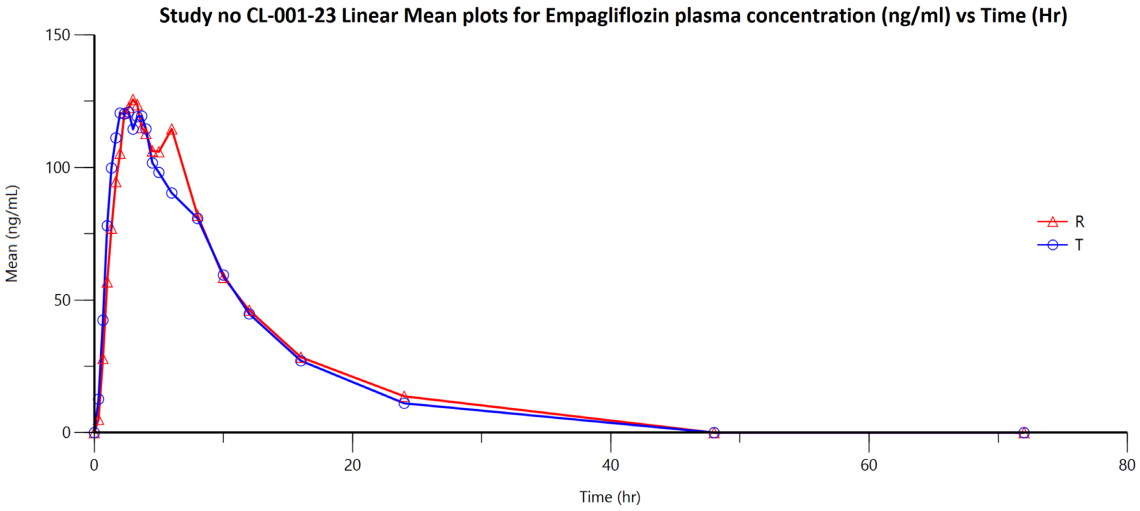

Figure 1. Linear plot of mean plasma concentration versus time for test and reference product (Empagliflozin).

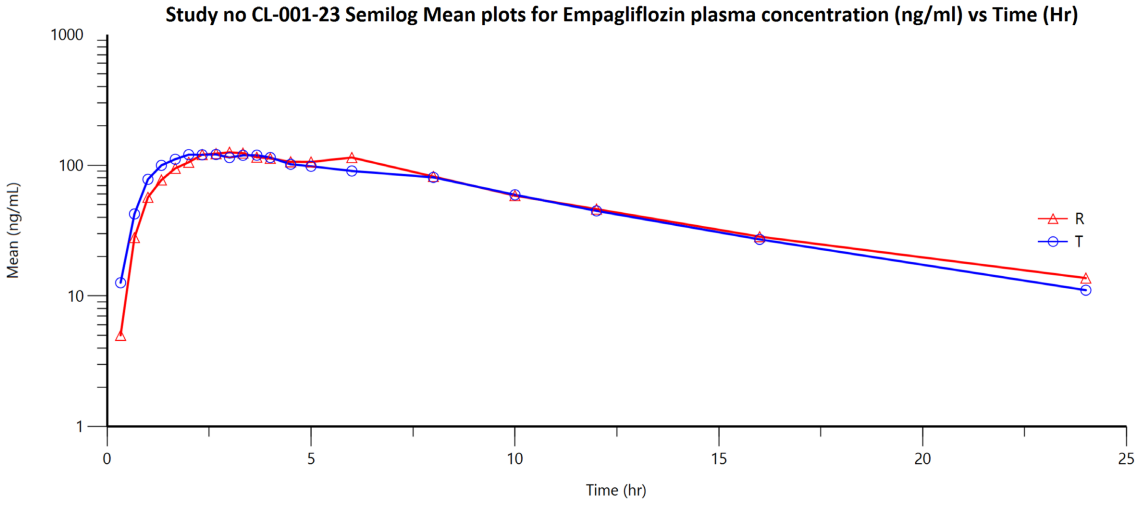

Figure 2. Semilog plot of mean plasma concentration versus time for test and reference product (Empagliflozin).

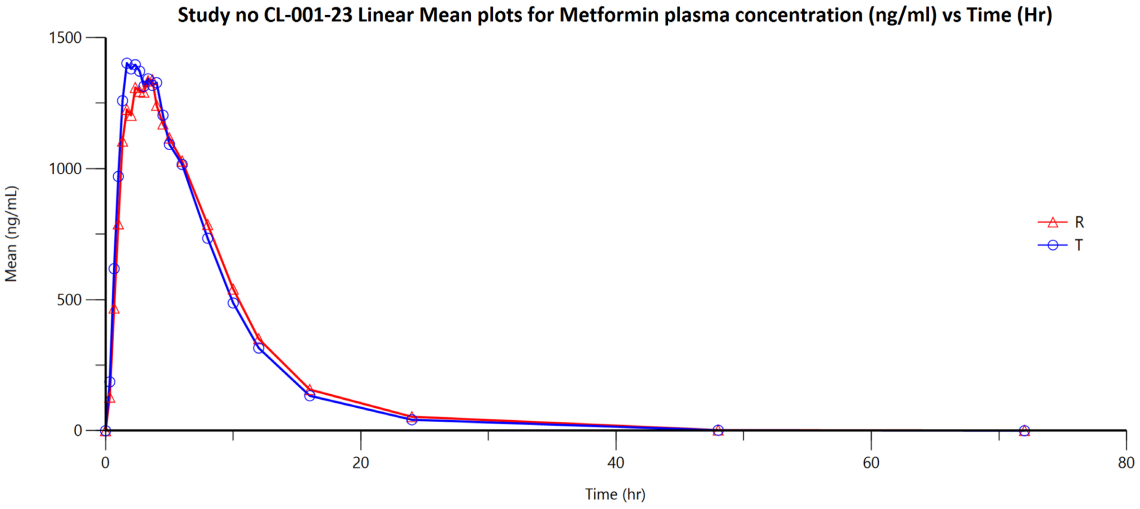

Figure 3. Linear plot of mean plasma concentration versus time for test and reference product (Metformin).

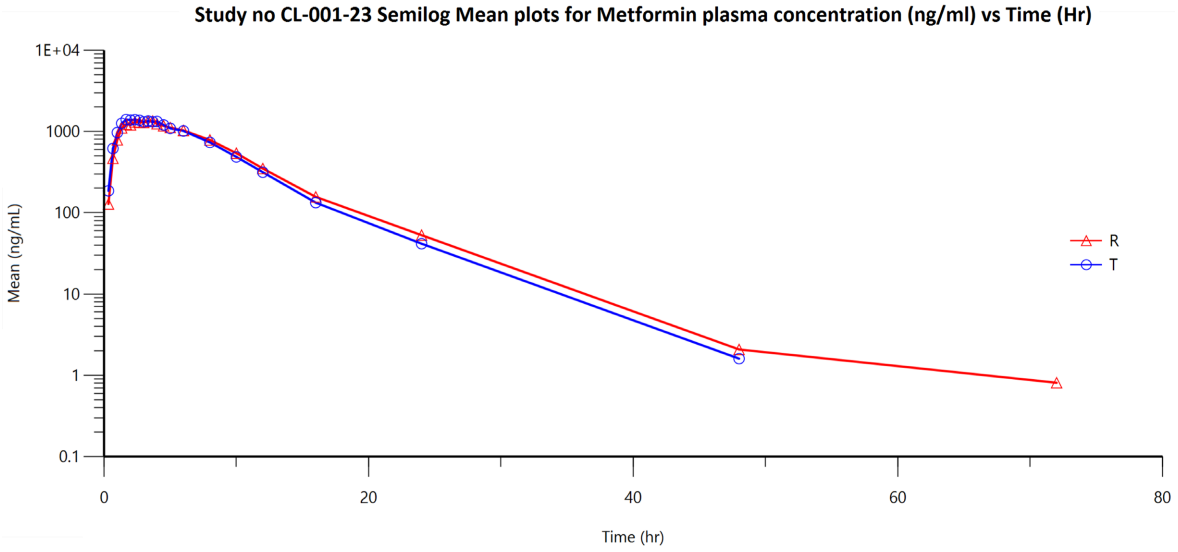

Figure 4. Semilog Plot of Mean Plasma Concentration versus time for Test and Reference Product (Metformin).

The mean plasma concentration-time curves of Untransformed and log-transformed data for the two formulations (test & reference) are shown in **Figures 1-4**.

3.4. Statistical Analysis

The geometric least squares mean intra-subject CV, power, the ratio of Test Product-T and Reference Product-R (T/R), and the 90% confidence interval for the ln-transformed pharmacokinetic parameters (C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$) for Empagliflozin and Metformin are summarized in **Table 4(a)** and **Table 4(b)**.

The 90% CI (**Table 4(a)** and **Table 4(b)**) for C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ were 87.80% - 108.02%, 90.67% - 100.37%, and 90.93% - 101.32% for Empagliflozin and 84.39% - 110.84%, 89.94% - 107.43%, and 89.53% - 106.59% for Metformin, respectively. The results indicate that the ratios of the test Product and the reference product for C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ fell within the range of 80.00% - 125.00%.

The coefficient of variation (CV%) values associated with C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ offer significant insights into the degree of variability within and between subjects. For Empagliflozin, the intra-subject CV ranged from 7.85% to 16.10% (as detailed in **Table 4(a)**), whereas for Metformin, the observed variation ranged from 13.52% to 21.26% (refer to **Table 4(b)**). The Analysis of Variance (ANOVA) conducted for both Empagliflozin and Metformin assessed the impacts of treatment, period, and sequence variability on pharmacokinetic parameters, as presented in **Table 5(a)** and **Table 5(b)**. The ANOVA results indicated no significant formulation or sequence effects ($p > 0.05$) for AUC_{0-t} and $AUC_{0-\infty}$. However, a notable period effect was identified for the C_{max} of Empagliflozin. In contrast, the ANOVA for Metformin revealed no significant impact ($p > 0.05$) attributable to period, formulation, or sequence on C_{max}, AUC_{0-t}, or $AUC_{0-\infty}$ parameters.

Table 4. (a): Summary results—Empagliflozin; (b): Summary results—Metformin.

(a)							
Parameter	Geometric Least Squares Means (GEOLSM)		T/R Ratio (%)	90% Confidence Interval		Intra Subject CV (%)	Power (%)
	Test Product	Reference Product		Lower Limit (%)	Upper Limit (%)		
C_{max} (ng/mL)	146.301	150.227	97.39	87.80	108.02	16.10	93.88
AUC_{0-t} (hr*ng/mL)	1211.635	1270.077	95.40	90.67	100.37	7.85	100.00
$AUC_{0-\infty}$ (hr*ng/mL)	1368.265	1425.508	95.98	90.93	101.32	8.36	99.99
(b)							
Parameter	Geometric Least Squares Means (GEOLSM)		T/R Ratio (%)	90% Confidence Interval		Intra Subject CV (%)	Power (%)
	Test Product	Reference Product		Lower Limit (%)	Upper Limit (%)		
C_{max} (ng/mL)	1512.180	1563.534	96.72	84.39	110.84	21.26	76.35
AUC_{0-t} (hr*ng/mL)	11947.117	12154.051	98.30	89.94	107.43	13.77	98.03
$AUC_{0-\infty}$ (hr*ng/mL)	12156.890	12444.677	97.69	89.53	106.59	13.52	98.30

Table 5. (a): Analysis of Variance (ANOVA)—Empagliflozin; (b): Analysis of Variance (ANOVA)—Metformin.

(a)			
ANOVA p Values			
Parameters	LC_{max}	$LAUC_{0-t}$	$LAUC_{0-\infty}$
Formulation	0.6584	0.1245	0.2028
Period	0.0035	0.7718	0.5314
Sequence	0.3005	0.9796	0.9274
(b)			
ANOVA p Values			
Parameters	LC_{max}	$LAUC_{0-t}$	$LAUC_{0-\infty}$
Formulation	0.6714	0.7375	0.6428
Period	0.0516	0.3656	0.4155
Sequence	0.4570	0.5936	0.6222

4. Discussion

The present study evaluated the bioequivalence of fixed-dose combination tablets containing Empagliflozin and Metformin among healthy volunteers under fed

conditions, utilising a single-dose, crossover design. The results demonstrated that the test/reference ratios for both analytes fell within the bioequivalence range of 80% to 125% as accepted by the Food and Drug Administration (FDA), thereby substantiating the bioequivalence of the test and reference products. This combination therapy has the potential to significantly enhance glycemic control in patients with Type 2 diabetes mellitus (T2DM), reducing the pill burden and improving patient adherence. It offers a streamlined treatment option that is particularly advantageous for T2DM patients with concurrent medical conditions, including cardiovascular complications, ultimately supporting better adherence in the context of polypharmacy.

The study observed that the intra-subject coefficient of variation (CV) for Empagliflozin ranged from 7.85% to 16.10%, while Metformin exhibited a variation between 13.52% and 21.26%. These findings highlight the consistent pharmacokinetic performance of both formulations across individuals, indicating relatively low intra-subject variability. Conversely, inter-subject variability was markedly more pronounced than intra-subject variability, suggesting that individual differences in drug absorption and metabolism significantly contributed to variations among subjects. Factors influencing inter-subject variability may include physiological elements such as gastric emptying rates, gastrointestinal transit times, and variations in metabolic enzyme activity. Intra-subject variability was minimised through the implementation of a standardised study design, a controlled diet, and a uniform sampling schedule. The results indicate that while physiological variations exist, intra-subject variability remains minimal, thereby enhancing the scientific rigour of bioequivalence assessments conducted under fed conditions.

Bioequivalence studies are essential components of the pharmaceutical industry, playing a critical role in ensuring patient safety, regulatory compliance, and cost-effectiveness. These studies validate that a generic drug performs consistently in comparison to its reference product, thereby mitigating adverse effects and adhering to regulations established by authorities such as the FDA and the European Medicines Agency (EMA). By promoting the development of affordable generic medications, bioequivalence studies significantly contribute to the economic efficiency of healthcare systems. The availability of generic bioequivalent products aids in reducing healthcare expenditure without compromising therapeutic efficacy, thus enhancing access to essential medications for a broader demographic, particularly among older adults, who may encounter challenges in adherence to treatment due to high prescription costs. While this study focused on pharmacokinetics, an exploration of the pharmacodynamics of Empagliflozin and Metformin could yield additional insights. Future investigations incorporating pharmacodynamic assessments could further elucidate these potential therapeutic advantages.

5. Limitations

The study has a few limitations. First, it enrolled only healthy male subjects to

minimise potential bias, but pharmacokinetics (PK) may differ in real-world populations, such as elderly patients or those on varying dosage regimens. The sample size was relatively small, which may limit the generalizability of the findings to broader patient populations. Larger sample size studies are needed to confirm and extend these results. Additionally, the study used a single-dose, crossover design, which may not capture the full variability in Empagliflozin-Metformin pharmacokinetics over time or with different dosing regimens. Future research with alternative study designs or multiple dosing regimens could offer a more comprehensive understanding of bioequivalence.

6. Conclusion

This study has demonstrated the bioequivalence of the test product, Empagliflozin and Metformin hydrochloride tablets, 12.5/1000 mg, developed by Beximco Pharmaceuticals Limited in Bangladesh, in comparison to the reference product, Synjardy 12.5/1000 mg tablet (Empagliflozin and Metformin hydrochloride tablets, 12.5/1000 mg), which is manufactured by Boehringer Ingelheim International GmbH in Germany. The evaluation was conducted on healthy adult human subjects under fed conditions.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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