RESEARCH PAPER

Comprehensive in Vitro, in Vivo, and Stability Evaluation of Optimized Modified-Release Bilayer Tablets of Empagliflozin and Metformin

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ABSTRACT

The present study aimed to comprehensively evaluate the in vitro–in vivo performance, release kinetics, and stability of an optimized modified-release bilayer tablet containing empagliflozin and metformin hydrochloride for improved diabetes management. The bilayer system was designed to provide an immediate release of empagliflozin for rapid onset of action and a sustained release of metformin hydrochloride for prolonged glycemic control. In vitro dissolution studies demonstrated that the empagliflozin layer released more than 85% of the drug within 15 minutes, confirming its immediate-release behavior, while the metformin layer exhibited a controlled release profile with approximately 54-56% drug release at 2 hours, 83–87% at 6 hours, and more than 85% at 10 hours. Release kinetic modeling indicated that the optimized formulation best fitted the Korsmeyer–Peppas model (R^2 = 0.9935), suggesting a non-Fickian diffusion-controlled release mechanism. In vivo pharmacokinetic studies in male Wistar rats revealed rapid absorption of empagliflozin (T_{max} = 1.2 ± 0.3 h) and sustained plasma concentrations of metformin, with the test formulation showing slightly higher systemic exposure compared to the marketed reference product. Pharmacodynamic evaluation demonstrated a rapid reduction in blood glucose levels within 30 minutes, followed by prolonged glycemic control over 24 hours, with statistically significant improvement compared to both control and reference groups ($p < 0.05$). Stability studies conducted under accelerated and long-term storage conditions, in accordance with ICH guidelines, confirmed that the formulation maintained consistent disintegration time, dissolution behavior, and release characteristics, with similarity factor (f_2) values exceeding 65 for both drugs. Overall, the optimized bilayer tablet successfully integrates immediate and sustained drug release within a single oral dosage form, offering predictable performance, enhanced therapeutic efficacy, and good stability. This dual-release approach represents a promising strategy for improving patient compliance and long-term management of diabetes mellitus.

Keywords: Empagliflozin; Metformin hydrochloride; Bilayer tablet; Modified-release drug delivery; Diabetes mellitus.

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GRAPHICAL ABSTRACT

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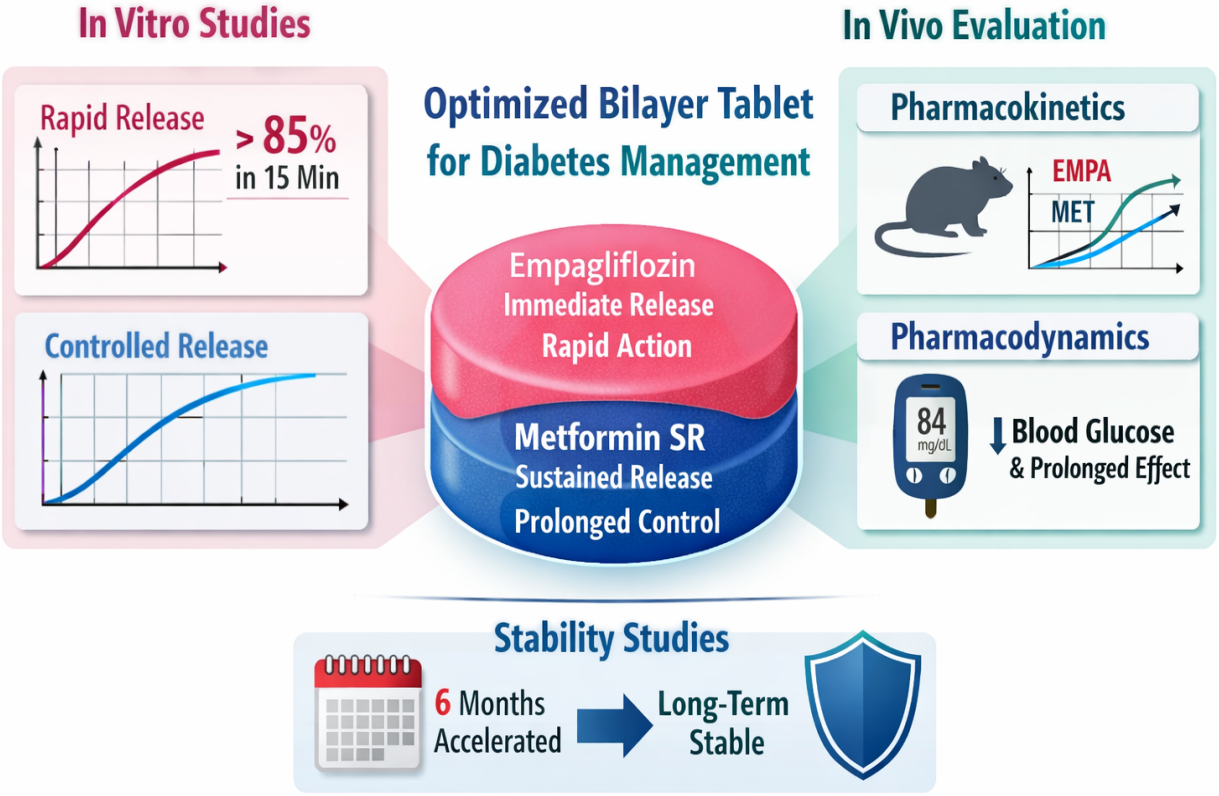

1. INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Long-term management of the disease requires sustained glycemic control to prevent or delay microvascular and macrovascular complications [1]. In clinical practice, the need for lifelong therapy often leads to challenges such as poor patient adherence, fluctuating plasma drug concentrations, and dose-related adverse effects. These issues have encouraged the development of therapeutic strategies that can provide effective glycemic control while improving patient convenience and safety.[2]

Metformin hydrochloride remains the first-line pharmacotherapy for type 2 diabetes mellitus due to its ability to improve insulin sensitivity and reduce hepatic glucose production. However, its short biological half-life and gastrointestinal intolerance at higher or frequent doses can limit patient compliance [3,4]. Empagliflozin, a sodium–glucose co-transporter-2 (SGLT2) inhibitor, offers a complementary mechanism of action by promoting urinary glucose excretion independent of insulin secretion. The combination of metformin and empagliflozin has therefore emerged as an effective therapeutic approach, providing additive glycemic control while reducing the risk of hypoglycemia and supporting weight management. Despite these advantages, conventional immediate-release dosage forms of such combinations may still require multiple daily dosing, which can compromise adherence in chronic therapy [5].

Modified-release oral drug delivery systems have gained importance in the management of chronic diseases as they can maintain drug concentrations within the therapeutic window for extended periods [6]. By controlling the rate and site of drug release, these systems can minimize peak-related side effects, reduce dosing frequency, and enhance overall therapeutic outcomes. Bilayer tablet technology represents a practical and flexible approach within this context, allowing the incorporation of drugs with different release requirements into a single dosage form. An immediate-

release layer can provide rapid onset of action, while a sustained-release layer can maintain prolonged therapeutic levels, thereby aligning drug release profiles with pharmacokinetic and pharmacodynamic needs [6,7].

While formulation development and optimization are essential initial steps, the clinical and pharmaceutical relevance of a modified-release dosage form ultimately depends on its *in vitro* and *in vivo* performance. In vitro dissolution studies provide critical information on drug release behavior under simulated physiological conditions and serve as a predictive tool for in vivo performance. Furthermore, kinetic modeling of dissolution data helps elucidate the underlying mechanisms of drug release, such as diffusion, erosion, or a combination of processes, which is essential for understanding and controlling formulation behavior. However, in vitro data alone are insufficient to confirm therapeutic effectiveness, making in vivo evaluation necessary to assess pharmacokinetic profiles, onset and duration of action, and overall efficacy in a biological system [8,9].

In addition to performance evaluation, stability assessment is a key requirement for the successful translation of a pharmaceutical formulation from laboratory to clinical and commercial use. Stability studies conducted under accelerated and long-term conditions provide insight into the robustness of the dosage form, its ability to maintain release characteristics over time, and its compliance with regulatory expectations. For modified-release bilayer tablets, maintaining consistent disintegration and dissolution behavior during storage is particularly important to ensure dose uniformity and therapeutic reliability throughout the product's shelf life [10,11].

In this context, the present study focuses on the comprehensive evaluation of an optimized modified-release bilayer tablet containing empagliflozin and metformin hydrochloride. Building upon prior formulation development and optimization, the work aims to systematically investigate the *in vitro* drug release characteristics, analyze release kinetics, evaluate *in vivo* pharmacokinetic and pharmacodynamic performance, and assess the stability of the optimized formulation under ICH-recommended conditions. This integrated evaluation is intended to establish the reliability, predictability, and therapeutic suitability of the bilayer tablet system for effective diabetes management [12,13].

2. MATERIAL AND METHOD

2.1 Material

Empagliflozin (EMP) was obtained from USV Pvt. Ltd., India. Metformin hydrochloride (Met HCl) was procured from Alkem Laboratories Pvt. Ltd., India. Microcrystalline cellulose (Avicel PH-112), spray-dried lactose (Supertab® 11SD), hydroxypropyl cellulose, croscarmellose sodium (Ac-Di-Sol), and talc were used as pharmaceutical excipients. All chemicals and reagents employed in the study were of analytical grade.

2.3 *In Vitro* Drug Release Study and Release Kinetic Analysis

2.3.1 In Vitro Drug Release Study

The *in vitro* drug release behavior of the optimized bilayer tablets was evaluated using a USP Type II (paddle) dissolution apparatus (Electrolab, India) operated at a paddle rotation speed of 100 rpm. The dissolution studies were carried out using 900 mL of dissolution medium, with the temperature maintained at 37 ± 0.5 °C throughout the experiment [14,15].

For the empagliflozin immediate-release layer, dissolution testing was performed in simulated gastric fluid consisting of 0.1 N hydrochloric acid (pH 1.2). Samples (5 mL) were withdrawn at 5, 10, 15, 30, 45, and 60 minutes. Each withdrawn sample was filtered through Whatman filter paper, suitably diluted, and analyzed. An equal volume of fresh dissolution medium was immediately replaced to maintain constant volume and sink conditions. For the metformin hydrochloride sustained-release layer, dissolution studies were conducted using a pH-shift method to simulate gastrointestinal conditions. Initially, tablets were subjected to 0.1 N hydrochloric acid (pH 1.2) for the first 2 hours. Subsequently, the dissolution medium was replaced with simulated intestinal fluid (phosphate buffer, pH 6.8), and the study was continued for up to 12 hours. Aliquots of 5 mL were withdrawn at 0.5, 1, 2, 4, 6, 8, and 10 hours, filtered, and suitably diluted prior to analysis. After each sampling, an equal volume of fresh dissolution medium was added to maintain sink conditions [16,17].

2.3.2 Release Kinetic Analysis

To elucidate the drug release mechanism from the optimized bilayer tablets, the in vitro dissolution data were subjected to mathematical modeling using different kinetic equations. The release data were fitted to zero-order, first-order, Higuchi, Korsmeyer–Peppas, and Hixson–Crowell models to identify the pattern and mechanism of drug release [18,19].

Zero-Order Kinetic Model

The zero-order model describes systems in which the drug release rate is independent of its concentration. The release data were analyzed by plotting cumulative percentage drug released versus time according to the following equation (1) [20]:

$Qt = K_0t$……(1)

where Qt is the amount of drug released at time t and K_0 is the zero-order release rate constant. A linear plot indicates adherence to zero-order kinetics.

First-Order Kinetic Model

The first-order model assumes that the drug release rate is concentration dependent. The data were analyzed by plotting the logarithm of cumulative percentage drug remaining versus time using the equation (2) [21]:

$Log\ Q = Log\ Q_o - K_1t/2.303$……(2)

where Q is the amount of drug remaining at time t, Q_0 is the initial amount of drug, and K1 is the first-order rate constant.

Higuchi Model

The Higuchi model describes drug release from a matrix system primarily governed by diffusion. The data were fitted to the Higuchi equation (3) [22]:

$Qt = KH\ t^{1/2}$……(3)

where Qt is the amount of drug released at time t and KH is the Higuchi dissolution constant.

Korsmeyer–Peppas Model

The Korsmeyer–Peppas model was applied to analyze drug release when the mechanism is not well defined or involves more than one process. The release data were fitted to the following equation (4):

$Mt/M_\infty = KKPt^n$ ……(4)

where Mt/M_∞ is the fraction of drug released at time t, KKP is the kinetic constant, and n is the release exponent.

Hixson–Crowell Model

The Hixson–Crowell model accounts for changes in surface area and particle diameter during dissolution. The release data were analyzed using the equation (5) [23]:

$Q_0^{1/3} - Q_t^{1/3} = K_{HC}*t$ ……(5)

where Q_0 is the initial amount of drug, Qt is the amount of drug remaining at time t, and KHC is the Hixson–Crowell rate constant.

Model Selection Criteria

The suitability of each kinetic model was evaluated based on the correlation coefficient (R^2), residual sum of squares (SSR), and Akaike information criterion (AIC). The model exhibiting the highest R^2 value and the lowest SSR and AIC values was considered to best describe the drug release behavior of the optimized bilayer tablet formulation [14,24,25].

2.4 *In Vivo* Evaluation of Optimized Bilayer Tablets

2.4.1 Animals and Ethical Approval

Male Wistar rats weighing 200–250 g were used for the in vivo evaluation of the optimized bilayer tablet formulation. The animals were housed under standard laboratory conditions with a controlled temperature of 22–25 °C, relative humidity of 45–55%, and a 12 h light–dark cycle, with free access to standard rodent feed and water. The experimental protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC) under approval number IAEC/1865/22-23/P-22, and all procedures were carried out in accordance with the guidelines of the Committee for Control and Supervision of Experiments on Animals (CPCSEA). Ethical considerations were strictly followed to minimize animal discomfort and ensure humane handling throughout the study [26,27].

2.4.2 Study Design and Dosing

A total of 18 rats were randomly divided into three groups (n = 6 per group). The control group received a suspension of drug-free bilayer tablets, while the test group was administered the optimized bilayer tablet formulation containing empagliflozin as the immediate-release layer and metformin hydrochloride as the sustained-release layer. The reference group received a marketed formulation containing the same active pharmaceutical ingredients for comparative evaluation. Animals were fasted overnight for 12 h prior to dosing, with free access to water [28,29].

The therapeutic human doses of empagliflozin (10 mg) and metformin hydrochloride (500 mg) were converted to rat-equivalent doses using a body surface area (BSA) scaling method, corresponding to approximately 0.9 mg/kg and 45 mg/kg, respectively. Tablets were finely powdered and suspended in 0.5% carboxymethylcellulose (CMC) solution, and the prepared suspensions were administered orally by gavage. Individual doses were adjusted according to body weight to ensure accurate and consistent dosing [30,31].

2.4.3 Sample Collection and Analysis

Blood samples (approximately 0.5 mL) were collected from the retro-orbital sinus at 0, 0.5, 1, 2, 4, 6, 8, 12, and 24 h following oral administration. Samples were collected into EDTA-coated tubes and centrifuged at 3,000 rpm for 10 min to separate plasma. The plasma samples were stored at −20 °C until further analysis. At the completion of the study, animals were humanely

euthanized, and liver and kidney tissues were collected for additional evaluation [32,33].

Plasma concentrations of empagliflozin and metformin hydrochloride were determined, and pharmacokinetic parameters including maximum plasma concentration (C_max), time to reach maximum concentration (T_max), area under the plasma concentration–time curve (AUC_0–t), and elimination half-life (t_1/2) were calculated using non-compartmental analysis. Pharmacodynamic evaluation was performed by monitoring blood glucose levels using a glucometer at baseline and at regular intervals post-administration to assess the hypoglycemic effect and duration of glycemic control [34,35].

2.4.4 Statistical Analysis

All experimental data were expressed as mean ± standard deviation (SD). Statistical comparisons among the control, test, and reference groups were performed using one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test. A p-value of less than 0.05 was considered statistically significant [36,37].

2.5 Stability Studies

Stability studies were conducted to evaluate the effect of storage conditions on the performance and drug release characteristics of the optimized bilayer tablets containing empagliflozin and metformin hydrochloride. The studies were performed in accordance with ICH guideline Q1A(R2) under accelerated (40 ± 2 °C / 75 ± 5% RH) and long-term (25 ± 2 °C / 60 ± 5% RH) conditions. Tablets were stored in high-density polyethylene (HDPE) containers and withdrawn for evaluation at 0, 1, 3, and 6 months under accelerated conditions and at 0, 3, 6, and 12 months under long-term conditions [38,39].

2.5.1 Disintegration Study

Disintegration testing was carried out using a USP disintegration apparatus (Type A). Six tablets from each sampling point were placed individually in the apparatus tubes containing 900 mL of distilled water maintained at 37 ± 0.5 °C. The time required for complete disintegration of the empagliflozin immediate-release layer was recorded. As metformin hydrochloride was incorporated in the sustained-release layer, complete disintegration of this layer was not expected and was therefore not evaluated using conventional disintegration criteria. All measurements were performed in triplicate and mean values were reported [40,41].

2.5.2 Dissolution Study

In vitro dissolution studies during stability testing were performed separately for both layers. Dissolution of the empagliflozin immediate-release layer was evaluated using a USP Type II (paddle) dissolution apparatus operated at 50 rpm in 900 mL of phosphate buffer (pH 6.8) maintained at 37 ± 0.5 °C. Samples (5 mL) were withdrawn at 5, 10, 15, 20, and 30 minutes, filtered, and analyzed spectrophotometrically at the λ_max of empagliflozin [42,43].

For the metformin hydrochloride sustained-release layer, dissolution was carried out using the same apparatus in 900 mL of 0.1 N hydrochloric acid for the first 2 hours, followed by phosphate buffer (pH 6.8) for up to 12 hours to simulate gastrointestinal pH conditions. Samples were withdrawn at 1, 2, 4, 6, 8, and 12 hours, filtered, and analyzed at the λ_max of metformin hydrochloride. After each sampling, an equal volume of fresh dissolution medium was added to maintain sink conditions [44,45].

3. RESULTS AND DISCUSSION

3.1 *In Vitro* Drug Release Study of Bilayer Tablets

The *in vitro* drug release behavior of the bilayer tablets was evaluated to confirm the immediate release of empagliflozin (EMPA) from the upper layer and the sustained release of metformin hydrochloride (Met HCl) from the lower layer. The cumulative drug release profiles obtained for different batches clearly demonstrate the ability of the bilayer system to achieve distinct and controlled release patterns for both drugs.

3.1.1 Immediate-Release Layer of Empagliflozin

The cumulative percentage drug release (% CDR) of empagliflozin from the immediate-release layer is summarized in **Table 1**, and the corresponding release profiles are illustrated in **Figure 1**. All batches exhibited rapid drug release within the initial 30 minutes, indicating effective disintegration and dissolution behavior suitable for immediate-release delivery.

Among the tested batches, batch B7 showed the highest % CDR, reaching 96.7 ± 1.86% at 30 minutes, indicating superior immediate-release performance. Other batches, such as B6 and B9, also demonstrated satisfactory release, achieving 90.6 ± 1.77% and 91.0 ± 1.39%, respectively, within the same time period. However, comparatively lower release was observed for certain batches, suggesting batch-to-batch variation attributable to formulation and processing factors.

The rapid and consistent release observed for batch B7 confirms its suitability for achieving prompt onset of action, which is critical for empagliflozin to exert its therapeutic effect. Overall, the results indicate that the immediate-release layer of the bilayer tablet effectively fulfills its intended function.

Table 1: % CDR of immediate release batches.

Time (Min)	BI	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12	B13
0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	60.7 ± 1.57	61.4 ± 2.31	62.2 ± 1.43	61.7 ± 1.77	63.1 ± 1.73	68.4 ± 1.82	69.4 ± 2.21	71.7 ± 1.12	70.9 ± 1.68	61.9 ± 1.68	60.9 ± 1.68	63.9 ± 1.68	65.9 ± 1.68
10	71.2 ± 1.25	70.7 ± 1.91	73.8 ± 1.75	71.9 ± 2.36	68.6 ± 1.92	80.3± 2.09	78.5 ± 1.85	72.4 ± 1.29	72.7 ± 1.53	73.7 ± 1.53	70.7 ± 1.53	73.7 ± 1.53	75.7 ± 1.53
15	80.4 ± 1.43	81.9 ± 2.33	82.3 ± 1.55	78.5 ± 1.89	79.3 ± 1.67	81.3 ± 2.57	89.4 ± 2.89	85.3 ± 2.40	85.9 ± 1.97	84.9 ± 1.97	81.9 ± 1.97	78.9 ± 1.97	80.9 ± 1.97
20	82.3 ± 1.23	81.1 ± 1.36	83.5 ± 1.14	81.1 ± 1.41	88.2 ± 1.39	86.7 ± 1.81	93.9 ± 1.18	86.3 ± 2.22	87.9 ± 1.38	85.9 ± 1.38	86.9 ± 1.38	90.9 ± 1.38	90.9 ± 1.38
30	84.1 ± 1.80	85.5 ± 1.32	84.9 ± 1.26	86.1 ± 1.71	86.4 ± 1.47	90.6 ± 1.77	96.7 ± 1.86	88.8 ± 2.36	91.0 ± 1.39	94.0 ± 1.39	91.0 ± 1.39	93.0 ± 1.39	91.0 ± 1.39

Values are mean ± SD (n = 3); CDR = cumulative drug release

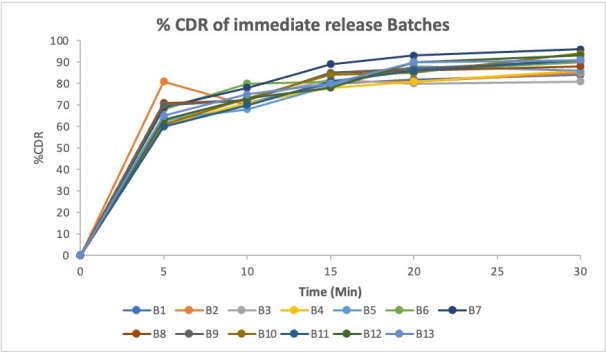

Figure 1: CDR of immediate-release batches.

3.1.2 Sustained-Release Layer of Metformin Hydrochloride

The *in vitro* drug release profile of metformin hydrochloride from the sustained-release layer was evaluated over a period of 10 hours. The % CDR values are presented in **Table 2**, and the corresponding dissolution profiles are depicted in **Figure 2**. All batches exhibited a controlled and gradual release pattern, confirming the effectiveness of the sustained-release matrix system.

Batch B7 demonstrated the most desirable release profile, achieving 95.2 ± 1.57% CDR at 10 hours, indicating efficient and prolonged drug release. Other batches, including B10 and B13, also showed satisfactory sustained-release behavior with % CDR values of 91.7 ± 1.15% and 92.9 ± 1.25%, respectively. The gradual increase in cumulative drug release over time reflects effective control of drug diffusion from the matrix, minimizing dose dumping and ensuring prolonged therapeutic action.

The combined dissolution results for empagliflozin and metformin hydrochloride clearly demonstrate that the bilayer tablet system successfully provides a rapid release of empagliflozin followed by a sustained release of metformin hydrochloride, which is desirable for effective glycemic control in diabetes management.

Table 2: % CDR of extended-release batches.

Time hrs	BI	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12	B13
0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.5	16.7 ± 1.15	14.5 ± 1.24	10.2 ± 1.17	21.2 ± 2.36	16.9 ± 1.72	13.7 ± 2.67	25.1 ± 1.63	17.3 ± 2.55	16.2 ± 2.40	18.3 ± 2.55	19.2 ± 2.40	18.3 ± 2.55	20.2 ± 2.40
1	32.70 ± 2.67	31.33 ± 0.57	28.62 ± 0.87	24.66 ± 0.87	21.34 ± 0.59	22.66 ± 0.32	35.33 ± 0.25	28.63 ± 0.78	24.16 ± 0.85	21.63 ± 0.78	20.16 ± 0.85	18.63 ± 0.78	21.16 ± 0.85
2	40.08 ± 1.41	42.20 ± 0.29	41.23 ± 014	39.34 ± 0.27	38.54 ± 0.24	41.34 ± 0.17	54.54 ± 0.14	42.43 ± 0..27	39.46 ± 0.89	38.43 ± 0..27	45.46 ± 0.89	46.43 ± 0..27	49.46 ± 0.89
4	51.07 ± 1.81	56.9 ± 2.33	57.3 ±1.55	54.5 ± 1.89	59.3 ± 1.67	61.3 ± 2.57	76.4 ± 2.89	63.3 ± 2.40	64.9 ± 1.97	58.3 ± 2.40	53.9 ± 1.97	61.3 ± 2.40	58.9 ± 1.97
6	70.83 ± 1.34	72.11 ± 1.34	69.12 ± 1.34	70.18 ± 1.34	74.68 ± 1.34	73.43 ± 1.34	82.78 ± 1.34	78.38 ± 1.34	74.58 ± 1.34	68.38 ± 1.34	74.58 ± 1.34	79.38 ± 1.34	80.58 ± 1.34
8	75.92 ± 2.37	74.40 ± 1.86	70.30 ± 1.78	75.22 ± 1.23	79.5 ± 1.85	78.2 ± 2.56	85.5 ± 1.64	80.1 ± 2.17	78.6 ± 1.35	76.1 ± 2.17	75.6 ± 1.35	81.1 ± 2.17	81.6 ± 1.35
10	85.0 ± 1.55	79.87 ± 1.36	81.48 ± 2.34	83.1 ± 2.54	80.5 ± 1.52	83.6 ± 1.20	95.2 ± 1.57	83.7 ± 1.15	81.9 ± 1.25	91.7 ± 1.15	82.9 ± 1.25	90.7 ± 1.15	92.9 ± 1.25

Values are mean ± SD (n = 3); CDR = cumulative drug release.

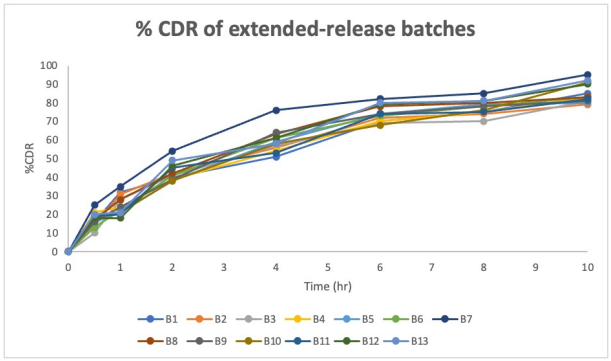

Figure 2: % CDR of extended-release batchs.

3.2 Drug Release Kinetic Analysis

To elucidate the mechanism of drug release from the optimized bilayer tablet formulation, dissolution data of the optimized batch (B7) were fitted to various kinetic models, including zero-order, first-order, Higuchi, Korsmeyer–Peppas, and Hixson–Crowell models. The kinetic parameters, correlation coefficients (R^2), residual sum of squares (SSR), and Akaike information criterion (AIC) values are summarized in **Table 3.**

Among the evaluated models, the Korsmeyer–Peppas model exhibited the highest correlation coefficient (R^2 = 0.9935), along with comparatively lower SSR and AIC values, indicating the best fit for the dissolution data. The release exponent (n) value obtained from the Korsmeyer–Peppas equation was 0.472, which falls within the range of $0.45 < n < 0.89$, suggesting a non-Fickian (anomalous) diffusion mechanism.

These findings indicate that drug release from the optimized bilayer tablet is governed by a combination of diffusion and matrix relaxation mechanisms rather than a purely diffusion-controlled or erosion-controlled process. The Higuchi model also showed a high R^2 value (0.9922), further supporting diffusion-based release behavior, whereas comparatively lower R^2 values were observed for the zero-order and Hixson–Crowell models. Overall, the kinetic analysis confirms that the optimized bilayer formulation (batch B7) exhibits a controlled and predictable release profile, aligning with the intended design objectives of immediate release for empagliflozin and sustained release for metformin hydrochloride.

release follows the diffusion-controlled mechanism.

Table 3: Fitting of release profile of optimized formulation to kinetic models.

Batch	Model	Parameters Used				
		R2	R	K	Residual sum of squares (SSR)	Akaike Information Criteria (AIC)
B7	Zero-order	0.7429	0.9494	12.177	2381.0501	64.2024
	First-order	0.9773	0.9902	0.3422	210.0399	44.7784
	Higuchi	0.9922	0.9963	33.3888	72.0347	36.2172
	Korsmeyer–Peppas	0.9935	0.9968	35.1104 n=0.472	60.4890	36.8197
	Hixson Crowell	0.9616	0.9882	0.0900	355.4174	48.9863

R^2 = coefficient of determination; K = release rate constant; n = release exponent; SSR = residual sum of squares; AIC = Akaike information criterion.

3.3 *In Vivo* Evaluation of Optimized Bilayer Tablets

3.3.1 Pharmacokinetic Analysis

The in vivo pharmacokinetic evaluation of empagliflozin (EMPA) and metformin hydrochloride from the optimized bilayer tablet formulation demonstrated favorable absorption, bioavailability, and elimination characteristics. The pharmacokinetic performance of the test formulation was comparable to, and in certain parameters marginally superior to, the marketed reference formulation, confirming the effectiveness of the bilayer design.

Plasma Concentration–Time Profiles

The plasma concentration–time profiles of EMPA and metformin are illustrated in **Figure 3** and **Figure4**, respectively. EMPA exhibited rapid absorption with an early peak plasma concentration, consistent with its immediate-release design. In contrast, metformin showed a gradual and sustained plasma concentration profile over 24 hours, confirming controlled drug release from the sustained-release layer of the bilayer tablet.

These distinct profiles clearly demonstrate the ability of the bilayer system to achieve immediate release for EMPA and sustained release for metformin within a single oral dosage form.

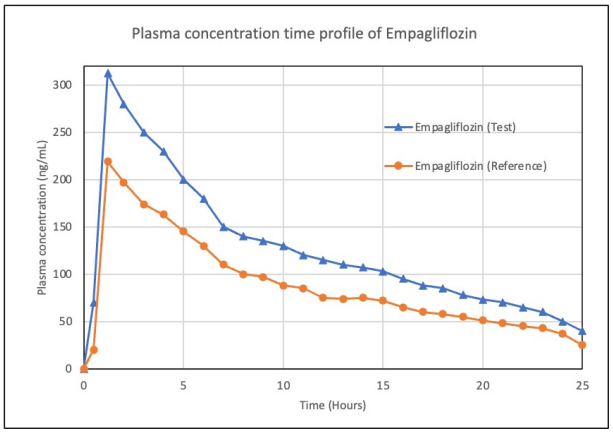

Figure 3: Plasma concentration-time profile of EMPA for test and reference groups.

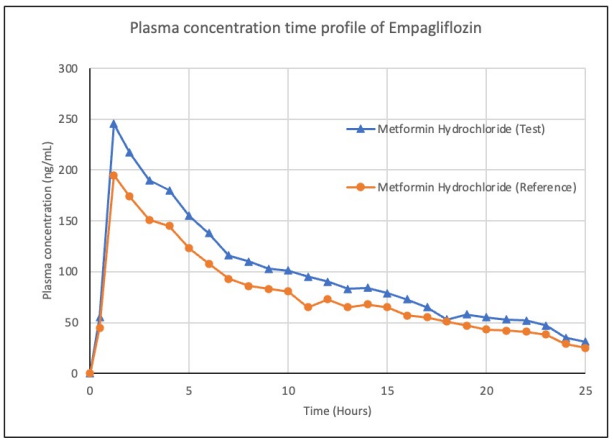

Figure 4: Plasma concentration-time profile of metformin for test and reference groups.

Pharmacokinetic Parameters

The key pharmacokinetic parameters, including maximum plasma concentration (C_max), time to reach maximum concentration (T_max), area under the plasma concentration–time curve (AUC_0–t), and elimination half-life (t_1/2), are summarized in **Table 4.**

For EMPA, the optimized bilayer tablet achieved a C_max of 312.5 ± 12.3 ng/mL, which was higher than that observed for the reference formulation (219.7 ± 10.8 ng/mL). The T_max value for the test formulation (1.2 ± 0.3 h) indicated rapid absorption, aligning well with the immediate-release objective of EMPA. Furthermore, the AUC_0–t of the test formulation (1450.2 ± 75.4 ng·h/mL) was marginally higher than that of the reference formulation (1020.8 ± 70.3 ng·h/mL), suggesting improved systemic exposure.

For metformin hydrochloride, the sustained-release behavior of the test formulation was evident from the prolonged plasma concentration profile and comparable elimination half-life values (11.2 ± 0.8 h for the test formulation versus 11.0 ± 0.7 h for the reference formulation). The AUC_0–t for the test formulation (2320.5 ± 110.7 ng·h/mL) was slightly higher than that of the reference formulation (1900.8 ± 105.6 ng·h/mL), indicating prolonged drug exposure and effective sustained release.

Statistical analysis using one-way ANOVA revealed that differences in C_max and AUC values between the test and reference groups were statistically significant ($p < 0.05$), confirming the pharmacokinetic advantage of the optimized bilayer tablet.

Table 4: Pharmacokinetic parameters of EMPA and Met-HCL

Parameter	Empagliflozin (Test)	Empagliflozin (Reference)	Metformin (Test)	Metformin (Reference)
Cmax (ng/mL)	312.5 ± 12.3	219.7 ± 10.8	245.4 ± 15.6	195.6 ± 14.2
Tmax (h)	1.2 ± 0.3	1.5 ± 0.2	3.5 ± 0.4	3.9 ± 0.3
AUC0–t (ng·h/mL)	1450.2 ± 75.4	1020.8 ± 70.3	2320.5 ± 110.7	1900.8 ± 105.6

Values are mean ± SD (n = 6).

3.3.2 Pharmacodynamic Analysis

The pharmacodynamic evaluation was performed to assess the hypoglycemic efficacy of the optimized bilayer tablet containing EMPA as the immediate-release component and metformin hydrochloride as the sustained-release component. Blood glucose levels were measured over a 24-hour period in the control, test, and reference groups.

Blood Glucose Reduction

The blood glucose values recorded at different time intervals are presented in **Table 5,** and the corresponding glucose reduction profiles are depicted in **Figure 7.27**. The test group exhibited a rapid reduction in blood glucose levels within 30 minutes of administration, with

the maximum hypoglycemic effect observed at 2 hours. This rapid onset of action is attributed to the immediate-release layer of EMPA, which facilitates prompt glucose lowering.

In addition to the rapid initial response, the test formulation maintained significantly reduced blood glucose levels over the 24-hour study period. This sustained glycemic control reflects the controlled release of metformin from the bilayer tablet, minimizing fluctuations in blood glucose levels and providing prolonged therapeutic coverage. Compared to the reference group, the test formulation consistently demonstrated lower glucose levels across all time points. Statistical analysis using one-way ANOVA followed by Tukey's post-hoc test showed that the reductions in blood glucose levels achieved by the test formulation were statistically significant ($p < 0.05$) when compared with both the control and reference groups.

Comparative Efficacy and Therapeutic Significance

The optimized bilayer tablet formulation demonstrated superior hypoglycemic efficacy in terms of both onset and duration of action compared to the marketed formulation. The immediate glucose-lowering effect of EMPA effectively addressed early post-dose glucose levels, while the sustained release of metformin ensured prolonged glycemic control throughout the day.

The integration of immediate-release and sustained-release components within a single bilayer tablet provides a synergistic therapeutic advantage by addressing both short-term and long-term glycemic management needs. This dual-release strategy not only enhances overall therapeutic efficacy but also has the potential to improve patient compliance by reducing dosing frequency and maintaining stable blood glucose levels over an extended period.

Table 5. Blood Glucose Levels (mg/dL) at Different Time Intervals.

Time (h)	Control Group	Test Group (Bilayer Tablet)	Reference Group (Marketed)
0	152.3	150.8	151.2
0.5	150.5	120.3	140.7
1	149.8	105.6	128.2
2	148.5	95.2	120.7
6	147.9	85.6	110.5
12	147.5	89.2	105.3
24	147.2	110.5	120.6

Values are mean ± SD (n = 6).

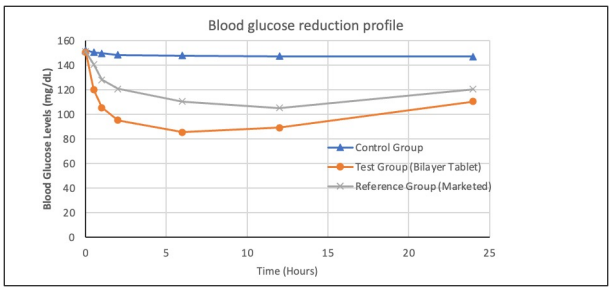

Figure 2: Blood glucose reduction profiles for control, test, and reference groups over 24 hours

3.4 Stability Studies

The stability of the optimized bilayer tablets containing empagliflozin and metformin hydrochloride was evaluated under accelerated and long-term storage conditions to assess the robustness of the formulation and the consistency of its release performance over time.

3.4.1 Disintegration Behavior

The disintegration time of the empagliflozin immediate-release layer remained within pharmacopeial limits (< 15 min) throughout the stability study. At initial testing (0 month), the disintegration time was 3.2 ± 0.5 min. After 6 months of accelerated storage and 12 months of long-term storage, the disintegration times were 3.6 ± 0.4 min and 3.5 ± 0.6 min, respectively. Statistical analysis indicated no significant difference in disintegration time during the study period ($p > 0.05$).

These results confirm that storage under both stressed and real-time conditions did not adversely affect the integrity or performance of the immediate-release layer. The disintegration profiles are illustrated in **Figure 6**, demonstrating consistent and reproducible behavior over the evaluated duration.

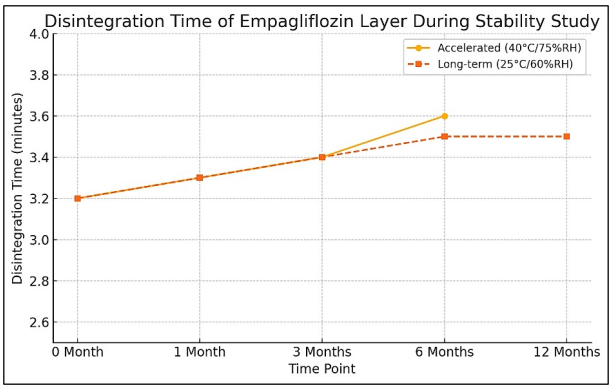

Figure 6: Disintegration study of EMPG.

3.4.2 Dissolution Behavior

The dissolution profiles of both empagliflozin and metformin hydrochloride remained stable throughout the

storage period. The empagliflozin layer consistently released more than 85% of the drug within 15 minutes at all sampling points, with no observable shift in the release pattern. This indicates preservation of immediate-release characteristics during storage.

For the metformin hydrochloride sustained-release layer, a controlled and gradual release profile was maintained across all stability time points. Approximately 30–35% drug release was observed at 2 hours, 60–70% at 6 hours, and more than 85% at 12 hours, closely matching the initial dissolution profile. These findings demonstrate that the sustained-release matrix retained its functionality under both accelerated and long-term storage conditions. The similarity factor (f_2) values between the initial and 6-month accelerated dissolution profiles were greater than 65 for both drugs, indicating no significant variation in dissolution behavior. The summarized dissolution data are presented in **Table 6**, while the comparative dissolution profiles of empagliflozin and metformin hydrochloride are shown in **Figure 7 and Figure 8**, respectively.

3.4.3 Stability Implications

The stability results confirm that the optimized bilayer tablet formulation is physically and chemically stable under both accelerated and long-term storage conditions. The absence of significant changes in disintegration time and dissolution profiles demonstrates the robustness of the bilayer design and supports its suitability for long-term storage and clinical use. The maintained immediate-release behavior of empagliflozin and sustained-release performance of metformin hydrochloride further validate the reliability of the formulation in delivering consistent therapeutic performance over time.

3.4 Stability Studies

The stability of the optimized bilayer tablets containing empagliflozin and metformin hydrochloride was evaluated under accelerated and long-term storage conditions to assess the consistency of disintegration behavior and dissolution performance over time.

3.4.1 Disintegration Behavior

The disintegration time of the empagliflozin immediate-release layer remained within pharmacopeial limits (< 15 min) throughout the study period. At initial testing (0 month), the disintegration time was 3.2 ± 0.5 min. After 6 months of accelerated storage and 12 months of long-term storage, the disintegration times were 3.6 ± 0.4 min and 3.5 ± 0.6 min, respectively. Statistical analysis indicated no significant difference in disintegration time during storage ($p > 0.05$), confirming the stability and integrity of the immediate-release layer under both stressed and real-time conditions.

The disintegration performance at different time points is illustrated in **Figure 6**, demonstrating consistent behavior throughout the storage period.

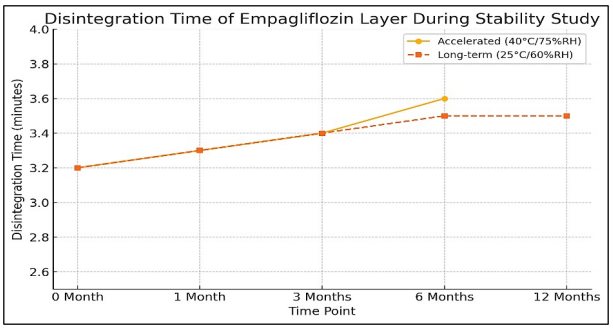

Figure 6: Disintegration study of EMPG

3.4.2 Dissolution Behavior

The dissolution profiles of both empagliflozin and metformin hydrochloride remained consistent during the stability study. The empagliflozin immediate-release layer released more than 85% of the drug within 15 minutes at all evaluated time points, with no observable shift in the release pattern, indicating preservation of immediate-release characteristics during storage. The dissolution profiles of empagliflozin at different stability intervals are shown in **Figure 7**.

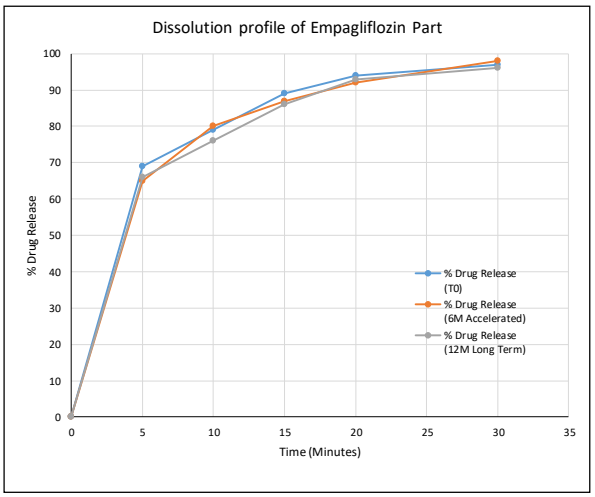

Figure7: Dissolution profile of EMPG.

For the metformin hydrochloride sustained-release layer, a controlled and gradual release profile was maintained throughout the study. Approximately 54–56% drug release was observed at 2 hours, 83–87% at 6 hours, and more than 85% at 10 hours, closely matching the initial dissolution profile. The comparative dissolution profiles

of metformin hydrochloride at different storage intervals are presented in **Figure 8**.

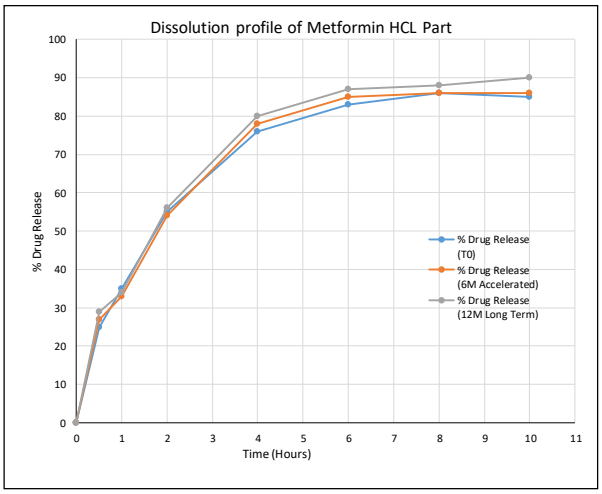

Figure 8: Dissolution study of Met-HCL.

The similarity factor (f_2) between the initial and 6-month accelerated dissolution profiles was greater than 75 for both drugs, indicating no significant variation in dissolution behavior during storage. The summarized dissolution data for both drugs under accelerated and long-term conditions are presented in **Table 6**.

Table 6: Summary of stability study.

Time	Empagliflozin - 0M	Empagliflozin - 6M Acc	Empagliflozin - 12M LT	Metformin - 0M	Metformin - 6M Acc	Metformin - 12M LT
5 min	69%	65%	66%	—	—	—
10 min	79%	80%	76%	—	—	—
15 min	89%	87%	86%	—	—	—
20 min	94%	92%	93%	—	—	—
30 min	97%	98%	96%	—	—	—
1 hr	—	—	—	25%	27%	29%
2 hr	—	—	—	35 %	33%	34%
4 hr	—	—	—	55%	54%	56%
6 hr	—	—	—	76%	78%	80%
8 hr	—	—	—	83%	85%	87%
12 hr	—	—	—	86%	86%	88%

Values represent cumulative drug release (%). Acc = accelerated storage; LT = long-term storage.

3.4.3 Stability Implications

The absence of significant changes in disintegration time and dissolution profiles confirms that the optimized bilayer tablet formulation retained its release characteristics and performance throughout the stability study. The formulation demonstrated physical and chemical stability under both accelerated and long-term storage conditions, supporting its suitability for long-term storage and consistent therapeutic performance.

CONCLUSION

In conclusion, the optimized modified-release bilayer tablet of empagliflozin and metformin hydrochloride successfully achieved rapid drug release from the immediate-release layer and sustained release from the extended-release layer. In vitro studies confirmed the desired dissolution behavior, while release kinetic analysis indicated a non-Fickian diffusion-controlled mechanism. In vivo pharmacokinetic and pharmacodynamic evaluations demonstrated rapid onset of action, prolonged glycemic control, and comparable or improved bioavailability relative to the marketed formulation. Stability studies further confirmed that the bilayer tablets maintained their disintegration and dissolution characteristics under accelerated and long-term storage conditions. Overall, the developed bilayer system represents a reliable and effective oral drug delivery approach for improved diabetes management.

ABBREVIATIONS

EMPA, empagliflozin; Met HCl, metformin hydrochloride; IR, immediate release; SR, sustained release; CDR, cumulative drug release; PK, pharmacokinetic; PD, pharmacodynamic; C_max, maximum plasma concentration; T_max, time to reach

maximum plasma concentration; AUC_0–t, area under the plasma concentration–time curve; t_1/2, elimination half-life; R^2, coefficient of determination; SSR, residual sum of squares; AIC, Akaike information criterion; Acc, accelerated storage; LT, long-term storage.

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CONFLICT OF INTEREST

No conflict of interest.

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