Design and Evaluation of Modified-Release Bilayer Tablets of Linagliptin with Empagliflozin for Biphasic Release Profile

Pranjal Dhondiba Chavan[1], A. M. Patel[2] , Swapnil S. Harale[3], A. A. Alman[4], Arati R. Kapase [5], Mayuri A. Sutar [6]

[1-6]Sant Gajanan Maharaj College of Pharmacy, Mahagaon. Site- Chinchewadi Tal-Gadhinglaj Dist-Kolhapur, Maharashtra-416503

Corresponding Author:

Pranjal Dhondiba Chavan,

Address: Sant Gajanan Maharaj College of Pharmacy, Mahagaon. Site- Chinchewadi Tal-Gadhinglaj Dist-Kolhapur, Maharashtra-416503.

Email: chavanpranjal135@gmail.com

ABSTRACT

Objectives: The research aimed to design and evaluate modified-release bilayer tablets containing Linagliptin (LINA) and Empagliflozin (EMPA) to achieve a biphasic release profile. It sought to integrate both drugs into a single dosage form, optimizing drug release and evaluating the tablets' physical, chemical, and performance properties.

Materials and Methods: The study utilized 3^2 factorial designs to optimize the bilayer tablet formulation, with HPMC K 100 M and Ethyl Cellulose as independent variables. The % drug release after 8 hours and 12 hours were the dependent variables. Pre-compression and post-compression evaluations, including spectroscopic analysis, Differential Scanning Calorimetry (DSC), and FT-IR, were conducted to assess the tablets. The E7 batch was selected as optimized based on these evaluations.

Results and Discussion: The optimized bilayer tablets exhibited favorable characteristics: angle of response of 24.16°C, bulk density of 0.346 g/cm³, tapped density of 0.414 g/cm³, compressibility of 14.38%, and Hausner ratio of 1.178. The tablets showed low weight variation (288.16±2.0 mg), uniform thickness (3.90±0.2 mm), sufficient hardness (7.84±0.3 Kg/cm²), low friability (0.116%), and rapid disintegration (7.64 min). The drug release profile was optimal, with 84.763% release after 8 hours and 99.972% after 12 hours for the sustained release layer. In-vivo studies indicated that the bilayer tablets performed significantly better than marketed tablets.

Conclusion: The modified-release bilayer tablets of LINA and EMPA demonstrated effective drug delivery with optimized release characteristics. Stability testing over 30 days confirmed the tablets' consistency and suitability for therapeutic use, highlighting their potential to improve treatment outcomes in diabetes management.

KEYWORDS: Linagliptin, Empagliflozin, Immediate drug release, Sustain drug release.

INTRODUCTION

A drug is a single active chemical entity in medicinal products for diagnosing, preventing, treating, or curing diseases, excluding use in contraceptives or for general health improvement. The WHO defines a drug broadly as any substance or product for diagnosing, curing, mitigating, treating, or preventing disease in humans or animals and includes substances with addictive or abuse potential. Essential Drugs are those addressing priority healthcare needs, available in sufficient quantities and quality within healthcare systems.[1]

Diabetes Mellitus

Diabetes mellitus is a chronic metabolic disorder characterized by high blood glucose levels due to inadequate insulin secretion or insulin resistance. Diagnosis is confirmed by fasting plasma glucose ≥ 7.0 mmol/L or plasma glucose ≥ 11.1 mmol/L two hours post-meal.[2-3]

Global Challenge of Diabetes Mellitus

Diabetes mellitus, driven by genetic, epigenetic, and societal factors like dietary habits and environmental risks, is increasingly prevalent worldwide. In 2013, 382 million people had diabetes, projected to reach 592 million by 2035. Despite the challenges, progress includes identifying undiagnosed type 2diabetes and developing therapies for high-risk populations. Some developed nations show stabilization or reduction in obesity rates, potentially decreasing type 2 diabetes incidences. Addressing prenatal nutrition, genetic predisposition, and societal issues requires a collaborative effort from researchers, healthcare providers, policymakers, and the public.[4]

India faces challenges in diabetes management due to increasing prevalence, limited awareness, inadequate healthcare resources, high treatment costs, and poor glycemic control. Insulin therapy requires multiple daily injections, and long-term adherence is often hampered by patient compliance issues due to discomfort.[5]

Bilayer Tablets in Diabetes Treatment

Bilayer tablets offer an innovative approach to diabetes treatment by combining two distinct layers for immediate and sustained drug release. This design enhances therapeutic outcomes by providing both rapid and prolonged medication effects, ensuring more consistent blood glucose control. Tablets remain the preferred oral dosage form for anti-diabetic medications due to their convenience, precise dosing, and high patient acceptance. The incorporation of drugs like Linagliptin and Empagliflozin in bilayer tablets exemplifies advanced therapeutic strategies, optimizing glucose management while minimizing adverse effects, thus significantly improving patient outcomes.[6]

Conventional and Novel Oral Formulations

Traditionally, drugs are administered orally in forms such as solutions, suspensions, emulsions, tablets, and capsules. However, novel oral formulations are emerging, including floating, pulsatile, and mucoadhesive tablets, as well as solid dispersion nanoparticles, liposomes, microspheres, and proliposomes. These advanced formulations aim to enhance drug bioavailability, control release profiles, and improve patient adherence to treatment regimens.[7]

Sustained Release Dosage Forms

Sustained release dosage forms are designed to maintain constant drug concentrations over extended periods, thereby optimizing therapeutic effects and improving patient compliance. These formulations minimize side effects and reduce overall healthcare costs by maintaining steady plasma drug levels. Despite their advantages, sustained release forms can be more expensive, risk dose dumping, and have less predictable in vitro-in vivo correlations.[8]

Need and Advantages of Bi-Layer Tablets

Bi-layer tablets meet the need for dual-release fixed-dose combinations of different APIs, allowing for the modification of drug delivery rates and the combination of incompatible APIs in one dosage form. They offer precise dosing, cost-effectiveness, ease of packaging, and enhanced patient compliance. However, they may pose challenges such as being difficult to swallow for some patients and complex to formulate for drugs with poor wetting or dissolution properties.

Bi-Layer Tablet Types and Quality Requirements

Bi-layer tablets can be homogeneous, enabling dual release of drugs, or heterogeneous, separating incompatible chemicals or combining medications with different release profiles. Producing high-quality bi-layer tablets requires a press capable of achieving high yield, sufficient tablet hardness, prevention of cross-contamination between layers, clear visual separation and accurate weight control of each layer. These stringent quality requirements ensure the efficacy and safety of bi-layer tablets in therapeutic applications.[9]

Materials and Methods:

Materials

The active pharmaceutical ingredients (APIs) used were Linagliptin and Empagliflozin, both procured from UniChem, Kolhapur. Several excipients were utilized to enhance the formulation's properties. Super-disintegrants, including sodium starch glycolate, croscarmellose sodium, and crospovidone, were obtained from Research Lab, Mumbai. To achieve sustained release, HPMC K 100 M was employed. Ethyl cellulose and PVP K30 served as binders, while magnesium stearate acted as a glidant. Talc was included as a lubricant, and microcrystalline cellulose was used as a diluent, all sourced from Research Lab, Mumbai. This combination of materials facilitated the development of an optimized bilayer tablet for effective diabetes management.

Methods

Melting Point

The melting points of Linagliptin (LINA) and Empagliflozin (EMPA) were determined using a digital melting point apparatus. Each drug was finely powdered and placed into separate capillary tubes. These tubes were gradually and uniformly heated by the apparatus, with a digital thermometer monitoring the temperature. The onset and complete melting temperatures were recorded to confirm drug purity and identity by comparing with standard references.[10]

Solubility

The solubility of LINA and EMPA was assessed using the saturation solubility method. Excess amounts of the drugs were dissolved in a solvent, shaken for 12 hours using a rotary shaker, and then allowed to stand for 24 hours. After reaching equilibrium, the samples were filtered, diluted with solvent, and examined using UV spectroscopy at their respective wavelengths (λ max).[2]

Pre-formulation Studies

Pre-formulation studies were conducted to standardize Linagliptin (LINA) and Empagliflozin (EMPA), and to assess potential drug-excipient interactions using FTIR and DSC. The investigation ensured the compatibility of the drugs with polymers used in the formulations.[11]

Spectroscopic Analysis

The absorbance maxima (λmax) of LINA were determined using UV spectrophotometry. Initially, 100 mg of LINA was dissolved in 0.1 N HCl to prepare a 1000 µg/ml Stock Solution I. This was further diluted to create Stock Solution II (100 µg/ml) and Stock Solution III (10 µg/ml). The final solution was scanned from 200-800 nm to identify the peak absorbance, crucial for further analysis and quantification. The same procedure was repeated in a pH 6.8 buffer.

To construct a calibration curve for LINA, a series of dilutions were prepared from Stock Solution II, resulting in final concentrations of 2, 4, 6, 8, and 10 µg/ml. Absorbance was measured at 296 nm using a UV spectrophotometer, providing precise calibration data for unknown sample analysis. This process was also performed in a pH 6.8 buffer.

Similarly, the λmax for EMPA was determined by dissolving 100 mg in 0.1 N HCl, following the same dilution steps to prepare Stock Solutions I, II, and III. The final solution was scanned to identify the highest absorbance peak. A calibration curve for EMPA was established by preparing dilutions from Stock Solution II, measuring absorbance at 224 nm. This method was also replicated in a pH 6.8 buffer for comprehensive analysis.[11]

Simultaneous Estimation of LINA and EMPA

To prepare standard stock solutions, 10 mg each of Linagliptin (LINA) and Empagliflozin (EMPA) were dissolved in separate 10 mL volumetric flasks with 0.1 N HCl, yielding concentrations of 1 mg/mL (1000 µg/mL). These were further diluted to 100 µg/mL for working solutions. For analysis, pure samples of LINA and EMPA were scanned between 200-400 nm, identifying 296 nm and 224 nm as the analytical wavelengths. Calibration curves were constructed using concentrations of 2, 4, 6, 8, and 10 µg/mL for both drugs. Absorptivity values at 296 nm and 224 nm were determined, and these values were used in Cramer's rule to calculate the concentrations of LINA and EMPA from the absorbance measurements.[12]

Differential Scanning Calorimetry (DSC):

A METTLER TOLEDO differential scanning calorimeter was used to analyze the pure drugs LINA and EMPA, along with excipients. Approximately 5 mg of the sample was precisely weighed and placed in a sealed aluminum container. The sample was heated at a rate of 10°C/min under a continuous nitrogen flow (45 CC/min), and energy changes were recorded throughout the temperature range.[13]

Fourier Transform Infrared Spectroscopy (FT-IR):

FT-IR analysis was performed to assess drug-excipient interactions. The spectra of pure LINA and EMPA, as well as their mixtures with various excipients, were recorded to ensure compatibility.[14]

Formulation of Bilayer Tablet of LINA and EMPA:

The bilayer tablet development involved two stages: an immediate-release (IR) layer of LINA and a sustained-release (SR) layer of EMPA. Preliminary trials were conducted to optimize the formulation of each layer separately. After optimization, the bilayer tablet was prepared using the finalized formulas, dividing the experimental work into three parts.[15]

Preparation of bilayer tablet

Bilayer tablets were prepared by combining of fast release layer and sustained release layer. After the compression upper punch was lifted and the blend of powder for immediate release layer was poured into the die, containing initially compressed matrix tablet on RIMEK multi station punching machine using flat punches, with the hardness of 6.5 kg/cm^2.

Table 1: Formulation table for immediate release layer of LINA

Formulation code	L1	L2	L3
LINA (mg)	5	5	5
Sodium Starch Glycolate	6	-	-
Croscarmellose sodium	-	6	-
Crospovidone	-	-	6
Microcrystalline Cellulose	Q. S	Q. S	Q. S
PVP K 30	3	3	3
Magnesium Stearate	4	4	4
Total	100	100	100

Table 2: Formulation table for sustain release layer of EMPA

Formulation code	EMPA (mg)	HPMC K 100 M (mg)	Ethyl Cellulose (mg)	Magnesium stearate (mg)	Talc (mg)	PVP K 30 (10%) solution
E1	25	90	16	10	5	q.s. to 200 mg
E2	25	110	16	10	5	q.s. to 200 mg
E3	25	110	12	10	5	q.s. to 200 mg
E4	25	70	16	10	5	q.s. to 200 mg
E5	25	70	20	10	5	q.s. to 200 mg
E6	25	90	20	10	5	q.s. to 200 mg
E7	25	90	12	10	5	q.s. to 200 mg
E8	25	110	20	10	5	q.s. to 200 mg
E9	25	70	12	10	5	q.s. to 200 mg

Evaluation parameters

The evaluation of Bilayer Tablet of LINA and EMPA includes pre-compression checks (angle of repose, bulk density, tapped density, % compressibility, Hausner ratio) for optimal powder flow. Post-compression parameters (weight variation, thickness, hardness, friability, disintegration time) ensure the final product meets performance standards.[16-17]

In-Vitro Dissolution Release for LINA:

To assess LINA's immediate release layer tablets, a dissolution apparatus type II (basket type) is used. Tablets are placed in a basket and the dissolution medium is maintained at pH 1.2 for 2 hours and pH 6.8 for 12 hours, simulating physiological conditions, with the basket rotating at 50 rpm at 37°C. Samples are taken hourly, 5 mL each, and replaced with fresh medium. The samples are diluted and measured for absorbance at 296 nm using a UV spectrophotometer. This process helps analyze the drug release profile and ensures consistent performance.[18]

In-Vitro Dissolution Release for EMPA:

For EMPA sustained-release (SR) layer tablets, a dissolution apparatus type II (basket type) is used. Tablets are placed in the basket, with the medium maintained at pH 1.2 for 2 hours and pH 6.8 for 12 hours, rotating at 50 rpm and held at 37°C. Sampling occurs hourly with 5 mL withdrawn and replaced with fresh medium. The samples are diluted and their absorbance measured at 224 nm using

a UV spectrophotometer. This method provides insights into the release profile and performance consistency of EMPA SR tablets.[18]

Release Kinetics Study:

To determine the drug release mechanism from the tablets, in-vitro data for the LINA and EMPA bilayer formulations were analyzed using several release models. The zero-order kinetics model was assessed by plotting cumulative % drug released against time, with zero-order kinetics indicating an ideal sustained release profile. The first-order kinetics were evaluated by plotting the log of % cumulative drug remaining versus time. Higuchi kinetics were analyzed by plotting % cumulative drug remaining against the square root of time, reflecting the inverse relationship between drug release rate and the square root of time. Finally, the Korsmeyer-Peppas model was used to plot log cumulative % drug released against log time, helping to evaluate the specific release mechanism.[19]

Stability Study:

Stability studies were performed to assess the product's quality, safety, and efficacy over time, focusing on how factors like temperature, humidity, and light affect the dosage form. Over one month, the formulation was tested at accelerated conditions of fewer than 40°C and 75% RH. Samples were taken at 0, 15, and 30 days and analyzed for in-vitro drug release.[10,20]

In-Vivo Bioavailability Study:

To evaluate the bioavailability of LINA and EMPA, rabbit plasma samples were prepared using a liquid-liquid extraction method after oral tablet administration. Blood samples (2 mL) were collected at 0.15, 0.30, 2, 4, 8, and 12 hours from fasted Wistar rabbits (n=2, 1-2 kg) and processed with perchloric acid, vortexed, and centrifuged at 3500 rpm before being transferred to vials. Standard stock solutions were made by dissolving 50 mg of each tablet in 80 mL of mobile phase, sonicating, and diluting to 100 µg/mL. The area under the curve (AUC) was calculated using the linear trapezoidal rule, and pharmacokinetic parameters such as Cmax and Tmax were analyzed. The study used a Jasco PU-2085 Plus HPLC system with a Hypersil ODS C18 column, with the mobile phase consisting of methanol and water (50:50, pH 4.5), and samples were analyzed at 247 nm. Rabbits were acclimatized for seven days and maintained in a controlled environment with a temperature of 22-23°C, single cage accommodation, and ad libitum access to pelleted feed and RO-filtered water.[21-22]

RESULTS AND DISCUSSION

Preformulation Study

Melting point and solubility

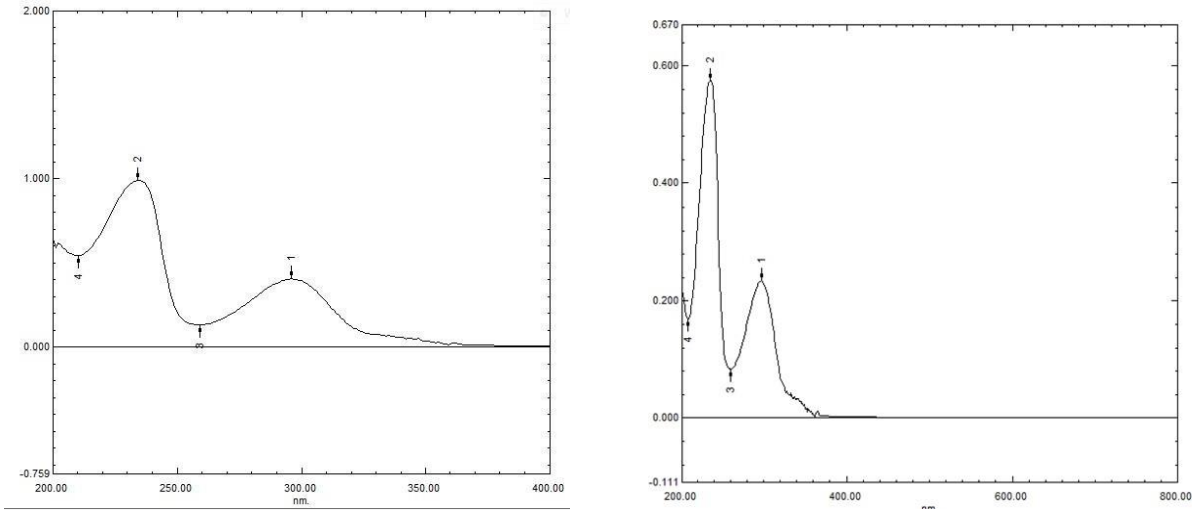
The melting point data in Table 10 shows the melting points of LINA (207°C ± 1.5°C) and EMPA (154°C ± 0.15°C), which are crucial for assessing the purity and stability of these compounds. LINA’s solubility ranges from 0.156 mg/ml in water to 0.384 mg/ml in a 6.8 pH buffer, while EMPA

ranges from 0.106 mg/ml in water to 46.318 mg/ml in methanol. This information helps in understanding how these compounds dissolve in different environments.

Spectroscopic Analysis

Determination of λmax of LINA

The λmax of LINA was found to be 296 nm in 0.1 HCl by UV spectrophotometer. The λmax of LINA was found to be 230 nm in pH 6.8 buffer by UV spectrophotometer.

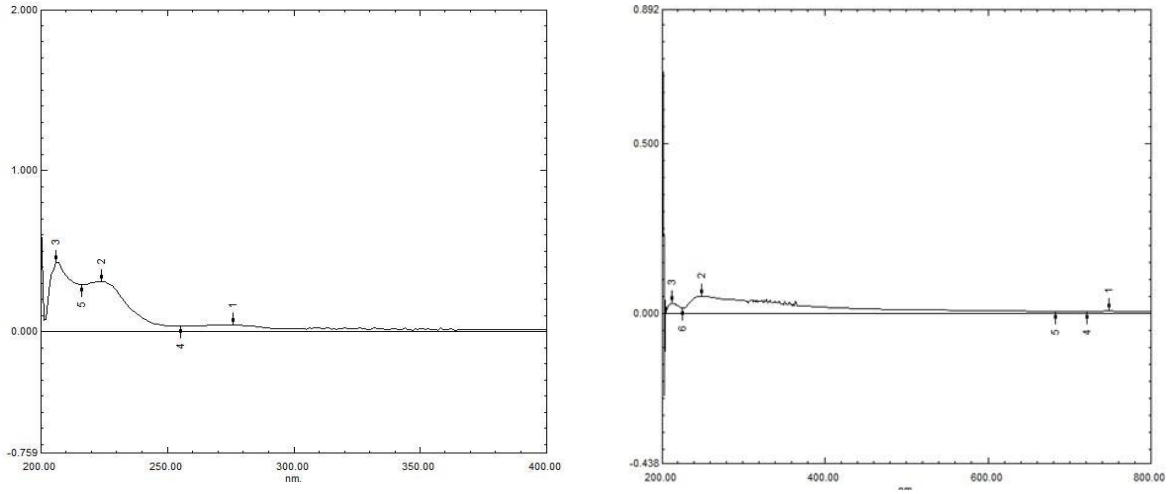


A **B**

Figure 1: UV absorption Spectrum of LINA A) at 296 in 0.1 HCl B) at 230 nm in pH 6.8 buffer

Determination of λmax of EMPA

The λmax of EMPA was found to be 224 nm in 0.1 HCl by UV spectrophotometer. The λmax of EMPA was found to be 248 nm in 6.8 buffers by UV spectrophotometer.

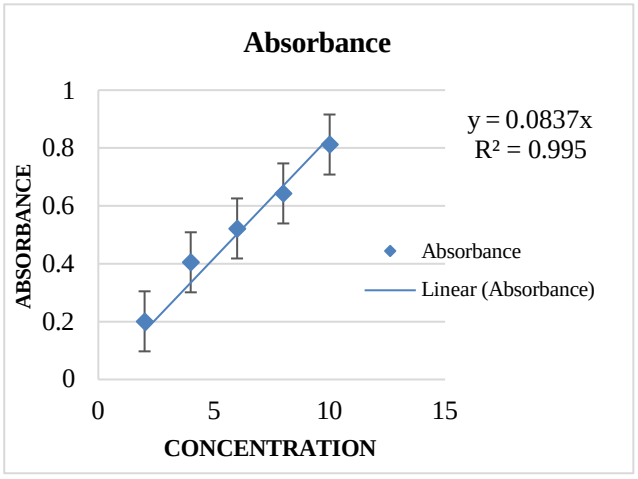


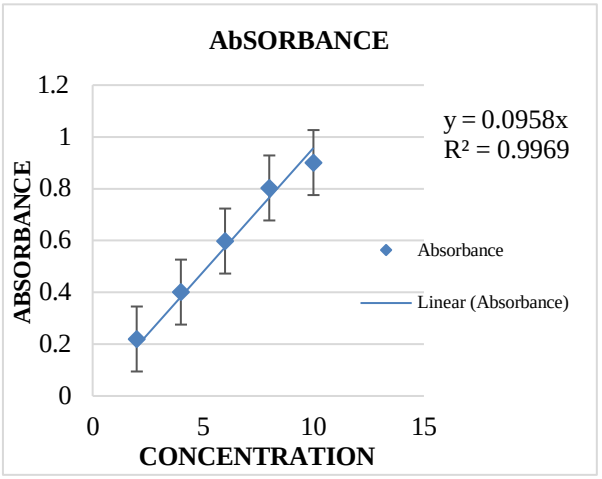
A **B**

Figure 2: UV absorption Spectrum of EMPA A) at 224 in 0.1 HCl B) at 248 nm in pH 6.8 buffers

Calibration curve of LINA in 0.1 N HCl and 6.8 buffer

The maximum wavelength (λmax) for LINA is 296 nm. The standard calibration curve for LINA 0.1 N HCl, plotted at this wavelength, shows a linear relationship with a correlation coefficient of 0.995. The equation of the line is y = 0.0837x, and absorbance values for various concentrations (2, 4, 6, 8, and 10 µg/ml) were measured. The UV absorption peak for LINA was confirmed at 296 nm. The maximum wavelength (λmax) for LINA was 230 nm. The standard calibration curve of LINA in 6.8 buffer, plotting absorbance against concentration at this wavelength, was linear with a correlation coefficient of 0.9969, following the equation y = 0.0958x. Absorbance data for LINA showed a linear response in the 2-10 µg/ml concentration range.



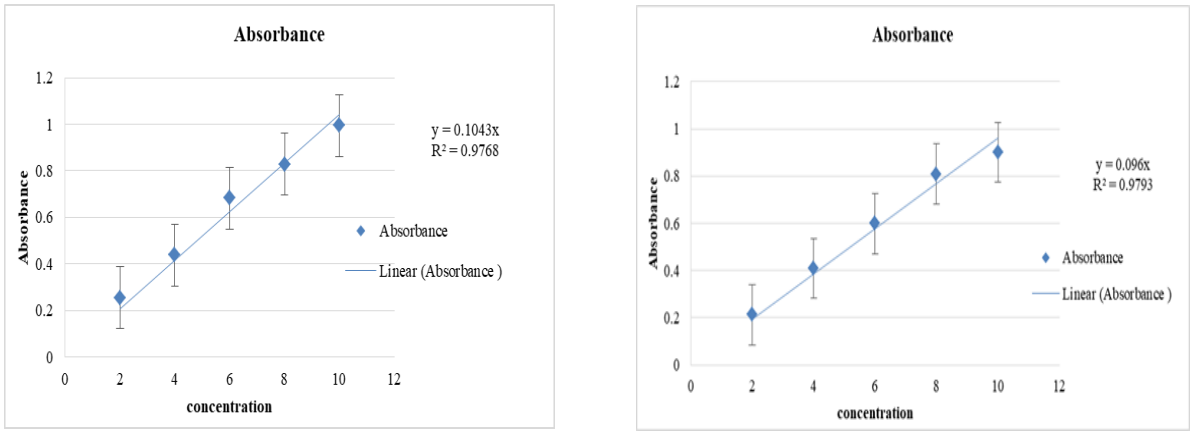


A **B**

Figure 3: Calibration curve for LINA drug in A) in 0.1 N HCl B) in 6.8 buffer

Calibration curve of EMPA in 0.1 N HCl and 6.8 buffer

For EMPA in 0.1 N HCl, the maximum wavelength (λmax) was 224 nm. The calibration curve of EMPA in 0.1 N HCl, plotting absorbance against concentration at this wavelength, was linear with a correlation coefficient of 0.9966, following the equation y = 0.1043x. Absorbance was linear in the 2-10 µg/ml concentration range. The maximum wavelength (λmax) for EMPA was 248 nm. The standard calibration curve of EMPA in 6.8 buffer at this wavelength was linear, with a correlation coefficient of 0.9967 and the equation y = 0.096x. Absorbance vs. concentration was linear in the 2-10 µg/ml range.



A **B**

Figure 4: Calibration curve for EMPA drug in A) in 0.1 N HCl B) in 6.8 buffer

Simultaneous estimation of LINA and EMPA

For LINA, absorbances at λ1 and λ2 were 0.280 and 0.801, with absorptivities of 0.14 and 0.1001, respectively. For EMPA, absorbances were 0.545 and 0.799, with absorptivities of 0.272 and 0.099. The absorbance values A1 and A2 were 1.055 and 1.57, respectively.

The % recovery for LINA and EMPA in simultaneous estimation. LINA, with a concentration of 4.38 units, had a recovery rate of 87.6%, while EMPA, with a concentration of 20.48 units, had a recovery rate of 81.92%. These recovery percentages reflect the accuracy and efficiency of the analytical methods used for assessing these compounds.

FTIR result analysis of Physical mixture

(LINA, EMPA, sodium starch glycolate, crosspovidone, croscarmellose sodium, HPMC K 100 M, and ethyl cellulose)

This graph shows the IR spectra of pure drug and excipients. Drug excipients compatibility study was done by FT-IR spectra. According to a study on compatibility on drug and excipients it was proved that there are slightly changes in peak of pure drug and physical mixture. So, all the ingredients were compatible with each other. So, there was no any incompatibility seen between drug and physical mixture.

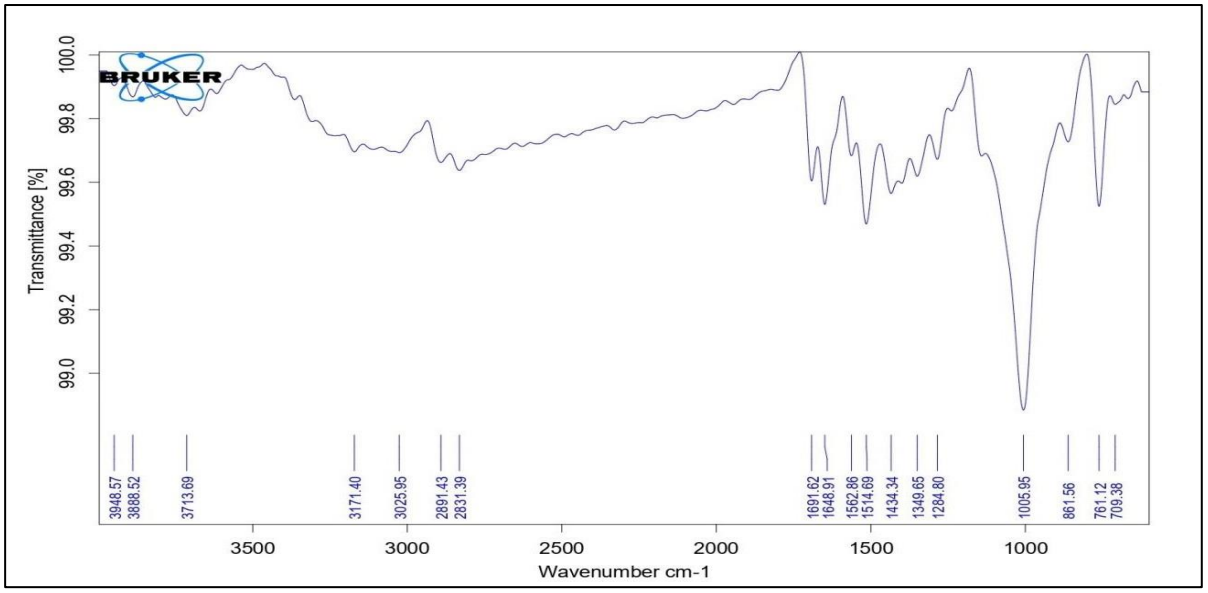


Figure 5: FTIR Spectra of Physical mixture

Differential Scanning Colorimetry

The DSC curve for a physical mixture of LINA, EMPA, and various excipients shows a melting point of 155.88°C, with the melting process starting at 152.70°C. The absorbed energy during melting is -25.03 mJ (-12.52 J/g). These thermal properties are important for characterizing the mixture and its behavior in pharmaceutical formulations.

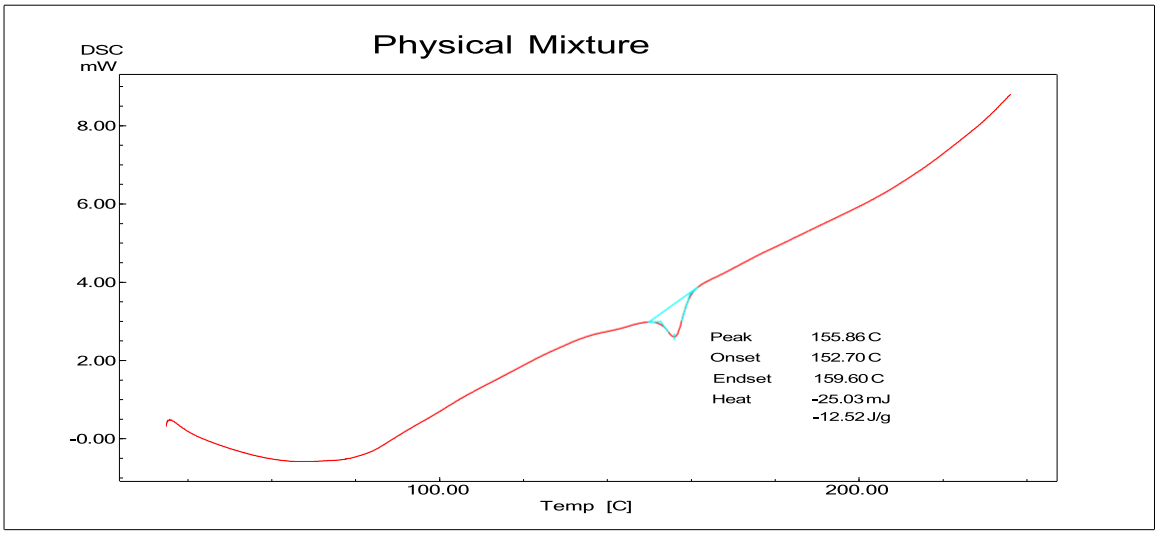


Figure 6: DSC Thermogram of Physical Mixture

Formulation development of bilayer of LINA and EMPA

The details of batches were given in following table 3.

Table 3: Experimental runs based on the factorial design of bilayer of LINA and EMPA

Immediate release layer (100 mg) *	
Formulation code	**L3**
LINA (mg)	5
Crospovidone	6

Microcrystalline Cellulose	Q. S								
PVP K 30	3								
Magnesium Stearate	4								
Total	100								
Sustained release layer (200 mg) *									
Formulation code	E1	E2	E3	E4	E5	E6	E7	E8	E9
EMPA (mg)	25	25	25	25	25	25	25	25	25
HPMC K 100 M (mg)	90	110	110	70	70	90	90	110	70
Ethyl Cellulose (mg)	16	16	12	16	20	20	12	20	12
Magnesium stearate (mg)	10	10	10	10	10	10	10	10	10
Talc (mg)	5	5	5	5	5	5	5	5	5
PVP K 30 (10%) solution	Q. S.	Q. S.	Q. S.	Q. S.	Q. S.	Q. S.	Q. S.	Q. S.	Q. S.
Total wt.	300	300	300	300	300	300	300	300	300

Figure 7: Prepared EMPA sustain release layer tablets

Pre-compression parameter

Pre-compression parameters assess the properties of powder blends or granules before tablet compression. Evaluating factors like flowability, moisture content, and compressibility is essential for ensuring efficient and consistent tablet production, impacting the medication's quality and safety.

Table 4: Pre-compression parameters of granules

Batches	Bulk density	Tapped density	% compressibility	Hausner ratio	Angle of repose (θ)

E1	0.388±0.50	0.480±0.23	19.16	1.201	34.94±1.34
E2	0.384±0.38	0.476±1.59	19.30	1.216	34.83±1.59
E3	0.371±0.43	0.425±1.42	12.70	1.142	29.42±0.83
E4	0.384±0.32	0.434±1.59	11.52	1.176	33.02±1.23
E5	0.384±0.59	0.476±1.45	19.32	1.214	34.37±0.49
E6	0.407±1.32	0.460±0.35	11.52	1.147	38.12±0.86
E7	0.404±0.18	0.459±0.36	11.98	1.184	28.36±0.38
E8	0.425±1.76	0.485±0.49	12.37	1.216	34.37±0.29
E9	0.412±1.16	0.467±1.23	12.27	1.117	35.24±0.23

Flow properties are crucial for evaluating the compression of EMPA sustained-release tablets. A smaller angle of repose (<30°) indicates better flow due to less internal friction. The pre-compression powder blend showed an angle of repose between 28.36° and 38.12°, suggesting good to passable flow. Carr's index ranged from 11.52 to 19.32 and Hausner's ratio from 1.132 to 1.146, both indicating good flow properties.

Post -Compression parameters

Post-compression parameters play a crucial role in evaluating the quality of manufactured tablets. These parameters are essential to guarantee that the tablets adhere to specified standards concerning mechanical strength, dissolution rate, and overall performance characteristics. They serve as critical checkpoints to ensure the tablets meet the necessary criteria for efficacy and stability throughout their shelf life.

Table 5: Post Compression parameters of bilayer tablet of LINA and EMPA

Batches	**Weight variation (mg) SD (n=3)**	**Thickness (mm)**	**Hardness (Kg/cm^2) SD (n=3)**	**% Friability**	**Disintegration time (min)**
E1	299.85± 4.0	3.93±0.2	8.06 ± 0.4	0.106±0.78	8.16±0.76
E2	297.80± 5.0	3.94±0.4	7.15 ± 0.4	0.151±0.40	9.36±1.93
E3	298.25± 3.0	3.91±0.3	7.64 ± 0.3	0.162±1.48	7.67±1.34
E4	297.00± 5.0	3.87±0.3	8.21 ± 0.3	0.214±1.73	5.39±0.89
E5	297.14± 3.0	3.86±0.3	8.00 ± 0.2	0.381±1.33	8.67±0.84
E6	296.23± 4.0	3.91±0.4	8.12 ± 0.4	0.368±1.43	6.94±0.53

E7	299.08± 3.0	3.90±0.3	7.63± 0.2	0.158±0.43	9.38±0.83
E8	297.53± 2.0	3.86±0.2	8.17± 0.4	0.147±0.28	8.37±0.58
E9	298.25± 3.0	3.91±0.3	7.64 ± 0.3	0.162±0.49	7.67±0.38

The table outlines post-compression parameters for bilayer tablets containing LINA and EMPA across batches B1 to B9. Weight variation ranges from 296.23 mg to 299.85 mg, while tablet thickness is between 3.86 mm and 3.94 mm. Hardness varies from 7.15 Kg/cm² to 8.21 Kg/cm², and friability ranges from 0.106% to 0.381%, with acceptable durability across batches. Disintegration time ranges from 5.39 to 9.38 minutes, ensuring proper dissolution. This assessment confirms that the tablets meet quality standards for effective and safe use.

Table 6: Specification of bilayer tablets of LINA with EMPA

Average weight of 1 Tablet	297.90±4.17 mg
Appearance	White color, round shape with both side plane
Thickness of 1 tablet	3.870±0.2%
Hardness of 1 tablet	7.95 ± 0.2kg

***In-vitro* drug release study**

The in vitro drug release study evaluated the release profiles of LINA and EMPA from a bilayer tablet, focusing on achieving both immediate and sustained release. The cumulative release percentages were monitored at set intervals to analyze the kinetics and mechanisms of drug release. The goal was to assess if the bilayer tablet effectively delivered LINA and EMPA in a controlled, sequential manner, aligning with therapeutic needs.

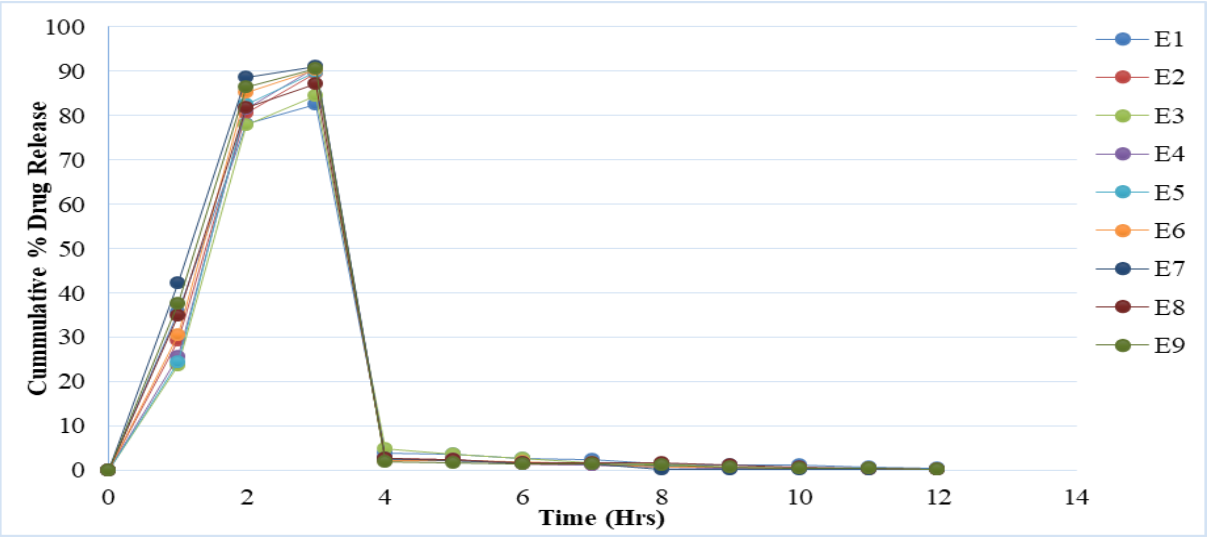


Figure 8: Cumulative percent drug release versus time plots of LINA immediate release layer.

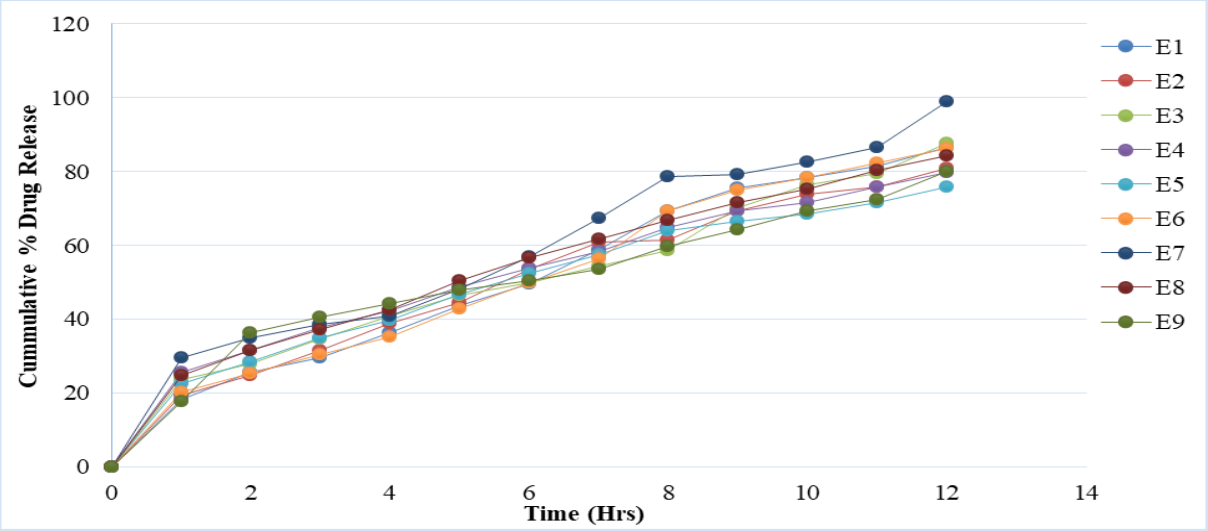


Figure 9: Cumulative percent drug release versus time plots of EMPA sustain release layer.

***In-vitro* release study of Optimized batch (OB)**

The figure 10 displays the drug release percentages from a bilayer tablet with LINA and EMPA over 12 hours for the optimized batch (OB). By 8 hours, 84.76% ± 2.3 of EMPA and 1.05% ± 0.50 of LINA were released. By 12 hours, nearly complete release was achieved, with 99.97% ± 3.7 of EMPA and 0.15% ± 0.11 of LINA released. This indicates a controlled release profile, with the immediate-release layer providing a quick effect and the sustained-release layer ensuring prolonged delivery. The optimized formulation, with Hydroxy Propyl Methyl Cellulose (HPMC) K100M and Ethyl Cellulose, showed superior performance, validating the optimization process. The release study indicated that the optimized formulation is stable and robust under the test conditions. The batch maintained its integrity and performance, suggesting good formulation stability and manufacturing reproducibility.

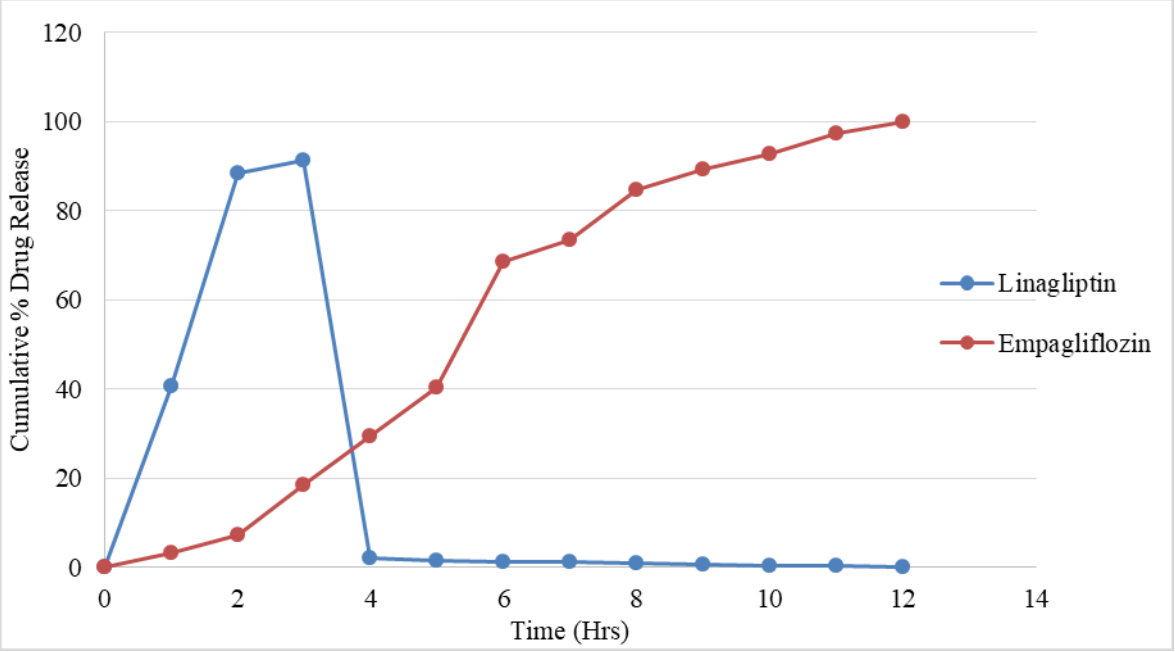


Figure 10: *In vitro* profile of Optimized Batch

Release kinetics study

The dissolution data for sustained-release EMPA were analyzed using zero-order, first-order, Higuchi, and Korsmeyer-Peppas models to study drug release kinetics. The Higuchi model fit the dissolution profiles best, with R^2 values between 0.97 and 1, indicating drug release was proportional to the square root of time. The Korsmeyer-Peppas model showed "n" values between 0.48 and 0.68, suggesting the release mechanism was anomalous transport.

Table 7: Mathematics models kinetics of EMPA

Sr. No.	Formulation	Zero order	First order	Higuchi Model	Korsmeyer Peppas Model		Hixson Crowell Model
		R^2	R^2	R^2	n	R^2	R^2
1	E1	0.9891	0.9703	0.9871	0.6682	0.9752	0.9865
2	E2	0.9813	0.9902	0.9946	0.6157	0.9855	0.9932
3	E3	0.9806	0.9278	0.9925	0.544	0.9599	0.9625
4	E4	0.9649	0.9893	0.9992	0.4801	0.9845	0.9843
5	E5	0.9673	0.9918	0.999	0.5174	0.9891	0.9825
6	E6	0.9886	0.9649	0.9869	0.6399	0.961	0.9828
7	E7	0.9764	0.7281	0.9918	0.5162	0.9203	0.8967
8	E8	0.9709	0.9859	0.9987	0.5161	0.9856	0.9904
9	E9	0.9604	0.9594	0.9971	0.5237	0.9513	0.9600

Stability study of optimized Batch

Stability studies are essential to ensure that the pharmaceutical product maintains its intended quality, safety, and efficacy throughout its shelf life. The optimized batch of LINA with EMPA bilayer tablets was subjected to stability testing under specified conditions to evaluate its physical, chemical, and performance characteristics over time. These results indicate that the tablets maintain their physical integrity and quality over three months.

Table 8: Stability study of optimized Batch

Optimized Batch	Physical Appearance	% Drug Release after 8 hours	% Drug Release after 12 hours
0 Days	No significant changes in color & texture,	84.763± 2.3	99.972± 3.7

15 Days	No significant changes in color& texture	81.125±1.23	96.763±0.62
30 Days	No significant changes in color & texture	80.26±0.56	95.26±1.24

***In-vivo* Bioavailability Study:**

Pharmacokinetic Parameters for LINA & EMPA:

The maximum concentration (Cmax) of 65.29 µg/ml is achieved at 480 minutes (Tmax). The time lag (Tlag) before the drug's effect begins is 0 minutes. The ratio of the last observed concentration to the maximum concentration (Clast-obs/Cmax) is 0.2074. The area under the concentration-time curve from time 0 to the last measurable concentration (AUC 0-t) is 30,339.075 µg/ml*min, representing the drug's overall exposure in the body over time.

The maximum concentration (Cmax) of 1.68 µg/ml was reached at 480 minutes (Tmax). The time lag (Tlag) before the drug's effect begins is 0 minutes. The ratio of the last observed concentration to the maximum concentration (Clast-obs/Cmax) is 0.9465. The area under the concentration-time curve from time 0 to the last measurable concentration (AUC 0-t) is 1123.6889 µg/ml*min, indicating the drug's overall exposure in the body over time.

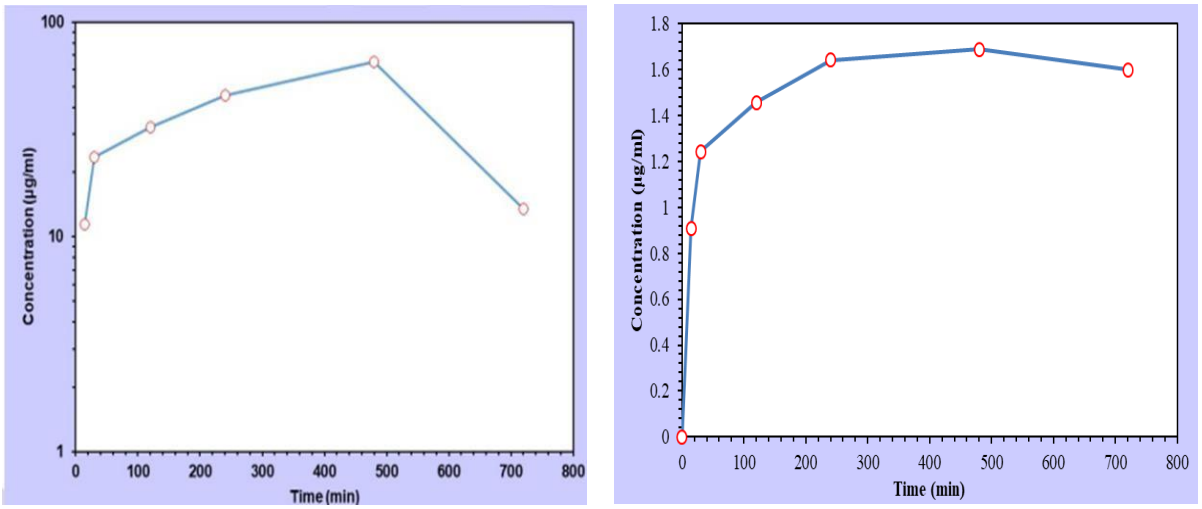


Figure 11: Time in (min)Vs Concentration (µg/ml)- LINA & EMPA

***In-vivo* bioavailability results for LINA & EMPA (Marketed Tablet):**

The in vivo bioavailability results for LINA show that Tmax is 720 minutes, indicating peak plasma concentration is reached at this time. Cmax is 74.11 µg/ml, the highest drug level observed. Tlag is 0 minutes, showing rapid absorption. The Clast-obs/Cmax ratio of 1 means the last observed concentration equals the maximum, indicating peak levels were maintained. The AUC_{0}-t is 39,815.7 µg/ml min, representing total drug exposure over 12 hours.

The in vivo bioavailability results for EMPA show a Tmax of 720 minutes, meaning EMPA peaks in plasma at this time. The Cmax is 76.24 µg/ml, the highest concentration observed. With a Tlag of 0 minutes, EMPA is rapidly absorbed. The Clast-obs/Cmax ratio of 1 indicates the concentration stayed at its peak during the last measurement. The AUC_0-t is 40,932.825 µg/ml*min, representing total drug exposure over 12 hours.

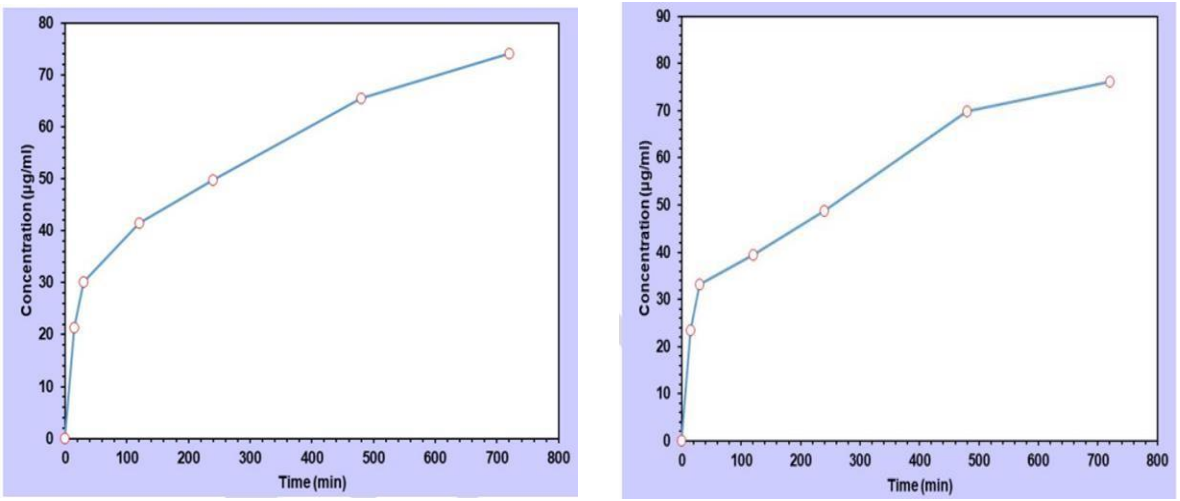


Figure 12: Time in (min) Vs Concentration (µg/ml)- LINA & EMPA

The method successfully quantified LINA and EMPA concentrations in combination and marketed tablets in rabbits. Key pharmacokinetic parameters, including Cmax, AUC_0 to t, and Tmax, were evaluated. The results, detailed in the profile, show the drugs' absorption, distribution, metabolism, and excretion, demonstrating sustained release and effective plasma level maintenance for optimal therapeutic outcomes.

CONCLUSION

The research successfully developed and evaluated bilayer tablets of Linagliptin and Empagliflozin, achieving an optimal biphasic release profile. The formulation demonstrated effective immediate release of Linagliptin and sustained release of Empagliflozin, meeting therapeutic needs for diabetes management. Preformulation studies using FTIR, DSC, melting point, and saturation solubility confirmed the compatibility and quality of the ingredients. The factorial design method enabled the successful development of sustained-release tablets, with the E7 batch showing excellent drug release characteristics (84.76% after 8 hours and 99.97% after 12 hours for Empagliflozin) and L3 batch optimized for immediate release. Stability testing over 30 days confirmed the formulation’s consistency and maintained drug release rates, while in-vivo studies showed significant improvement compared to marketed formulations.

AUTHORS CONTRIBUTIONS:

All authors have contributed equally.

CONFLICTS OF INTERESTS:

All authors have declared no conflict of interest.

REFERENCES:

1. Hammad RW, Sanad RA, Abdelmalak NS, Latif R. Cubosomal functionalized block copolymer platform for dual delivery of linagliptin and empagliflozin: Recent advances in synergistic strategies for maximizing control of high-risk type II diabetes. Drug Delivery and Translational Research. 2023 Oct 8:1-8.
2. Ch S, Raviteja R. Formulation development and stability studies of bilayer tablets of linagliptin and metformin. Journal of Innovations in Applied Pharmaceutical Science (JIAPS). 2021 Apr 30:08-14.
3. Unnikrishnan AG, Kalra S, Purandare V, Vasnawala H. Genital Infections with Sodium Glucose Cotransporter-2 Inhibitors: Occurrence and Management in Patients with Type 2 Diabetes Mellitus. Indian J Endocrinol Metab. 2018;22(6):837-842.
4. Katsiki N, Perakakis N, Mantzoros C. Effects of sodium-glucose co-transporter-2 (SGLT2) inhibitors on non-alcoholic fatty liver disease/non-alcoholic steatohepatitis: Ex quo et quo vadimus? Metabolism. 2019;98:iii-ix.
5. Kumar R, Saha P, Kumar Y, Sahana S, Dubey A, Prakash O. A review on diabetes mellitus: type1 & Type2. World Journal of Pharmacy and Pharmaceutical Sciences. 2020 Aug 2;9(10):838-50.
6. Gupta D, Pandey M, Maiti A, Pujari NM. Bilayer Tablet Technology: A Concept Of Immediate And Controlled Drug Delivery. Journal of Pharmaceutical Negative Results. 2023 Jan 14:503-12.
7. Akhtar M, Jamshaid M, Zaman M, Mirza AZ. Bilayer tablets: A developing novel drug delivery system. Journal of Drug Delivery Science and Technology. 2020 Dec 1;60:102079.
8. Abebe A, Akseli I, Sprockel O, Kottala N, Cuitiño AM. Review of bilayer tablet technology. International journal of pharmaceutics. 2014 Jan 30;461(1-2):549-58.
9. Payghan SA, Disuza JI. Formulation, evaluation and development of bilayer tablet. Int. J. Pharmaceut. Res. Dev. 2011;3:80-7.
10. Babu CS. Formulation and evaluation and stability studies of bilayer tablets of metformin and linagliptin. World Journal of Pharmaceutical Research (WJPR), Volume 8, Issue 13, 868-880.
11. Kale SS, Saste VS, Ughade PL, Baviskar DT. Bilayer tablet. International Journal of Pharmaceutical Sciences Review and Research. 2011 Jul;9(1):25-30.
12. Sushil D Patil, Swapnapurti V Bharambe, Sanjay Kshirsagar. Development and Validation of RP- HPLC Method for Estimation of Empagliflozin and its Stability Studies In vitro Simulated Gastric and Intestinal Fluids. Asian J. Pharm. Ana. 2019; 9(3):156- 160.

13. Patil SD, Chaure SK and Kshirsagar S: Development and validation of UV- spectrophotometer method for simultaneous estimation of Empagliflozin andMetformin HCl in bulk drugs. Asi J of Pharm Anal 2017; 7(2): 2231-67.
14. Kumar R, Saha P, Kumar Y, Sahana S, Dubey A, Prakash O. A review on diabetes mellitus: type1 & Type2. World Journal of Pharmacy and Pharmaceutical Sciences. 2020 Aug 2;9(10):838-50.
15. Nguyen NN, Pham DT, Nguyen DT, Trinh TT. Bilayer tablets with sustained-release metformin and immediate-release sitagliptin: preparation and in vitro/in vivo evaluation. Journal of Pharmaceutical Investigation. 2021 Sep;51(5):579-86.
16. Won DH, Park H, Ha ES, Kim HH, Jang SW, Kim MS. Optimization of bilayer tablet manufacturing process for fixed dose combination of sustained release high-dose drug and immediate release low-dose drug based on quality by design (QbD). International Journal of Pharmaceutics. 2021 Aug 10;605:120838.
17. Singh, S. Das, S. Gupta, and S. Ghosh, "The Challenges of Producing Bilayer Tablet: A Review," Journal of Drug Delivery and Therapeutics, vol. 11, no. 4-S, pp. 171–175, Aug. 2021, doi: 10.22270/jddt.v11i4-s.4922.
18. Kadam AT, Zameeruddin M and Arsul V: Development and validation of analytical methods for the simultaneous estimation of metformin hydrochloride and empagliflozine. J of Med and Pharm Innovation 2017; 2347-8136.
19. Kaku K, Haneda M, Tanaka Y, et al. Linagliptin as add-on to empagliflozin in a fixed-dose combination in Japanese patients with type 2 diabetes: Glycaemic efficacy and safety profile in a two-part, randomized, placebo-controlled trial. Diabetes Obes Metab. 2019;21: 136-145.
20. Katsiki N, Ofori-Asenso R, Ferrannini E, Mazidi M. Fixed-dose combination of empagliflozin and linagliptin for the treatment of patients with type 2 diabetes mellitus: A systematic review and meta-analysis. Diabetes, Obesity and Metabolism. 2020 Jun;22(6):1001-5.
21. Jyothirmai N, Begum KM, Supriya P. Novel stability indicating RP-HPLC method for the simultaneous estimation of Empagliflozin and Linagliptin in bulk and pharmaceutical formulations. Journal of Atoms and Molecules. 2016 May 1:6(3):977.
22. Sushil D. Patil, Sunil V. Amurutkar, Chatapalliwar V.A, Chandrashekhar D. Upasani. (2017). Development and validation of RP-HPLC method for empagliflozin and metformin HCL. Journal of Innovation in Pharmaceutical and Biological Science, 4(4), 185-189.