FORMULATION AND EVALUATION OF BILAYER TABLET OF METFORMIN HCL AND ENALAPRIL MALEATE

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Abstract: *The present research work was to establish the bilayer tablet of Metformin HCl and Enalapril Maleate for patients having Diabetes Mellitus and Hypertension. The bilayer tablet of Metformin HCl and Enalapril Maleate was developed by direct compression method. The bilayer tablet contained the Sustained release layer of Metformin HCl and Immediate release layer of Enalapril Maleate. The SR layer of Metformin HCl developed by using the HPMC K4M and HPMC K100M for controlled release of drug. While, the IR layer of Enalapril Maleate was developed by using Croscarmellose Sodium (CCS) and Sodium Starch Glycolate (SSG). The pre-compression study was performed and the results were reported. In pre-compression study the angle of repose, bulk density, tapped density, Hausner's ratio and Carr's index was calculated and results shown the good compliance as per IP standards. The tablets parameters were evaluated by testing thickness, hardness, weight variation, friability test etc. The % drug content was found to be in the range of 97.7±0.33 to 99.12±0.56 for SR layer of Metformin HCl and for IR layer Enalapril Maleate was found to be 97.03±0.62 to 99.53±0.35. The disintegration time for IR layer was found to be 45.31±0.39 to 78.27±0.55 sec. The formulation F3 shown the best results of dissolution study containing IR3 and SR3 layer of Croscarmellose and HPMC K4M respectively. The IR3 layer of F3 formulation shown 98.93±0.37 % drug release at the end of 40 min. and which was the best drug release than other formulations. The SR3 layer of F3 formulation shown the controlled drug release for 12 hrs. with maximum drug release than other formulations and was found to be 97.99±0.46 % drug release. Further, the stability study of the bilayer tablet was checked as per ICH guidelines and the results were complies to the IP standards. From the drug release study it is possible to reduce the frequency of dose.*

Keywords: *Diabetes Mellitus, Hypertension, Metformin HCl, Enalapril Maleate, CCS, SSG, HPMC K4M, HPMC K100M*

INTRODUCTION

Diabetes mellitus (DM) is a clinical syndrome characterized by varying degrees of insulin hyposecretion and/or insulin insensitivity leading to hyperglycaemia. Lack of insulin affects the metabolism of carbohydrates, protein and fat, which lead to metabolic alterations, and it causes a significant disturbance of water and electrolyte homeostasis [1]. According to the International Diabetes Federation, in 2019, approximately 463 million adults (20-79 years) were living with diabetes worldwide, and this number is projected to increase to 700 million by 2045 [2]. Diabetes is classified into several types, including type 1

diabetes [Insulin-dependent DM (IDDM)], type 2 diabetes [Noninsulin-dependent DM (NIDDM)], gestational diabetes, and other specific types [3].

Type 1 diabetes (T1D)

Type 1 diabetes, also known as insulin-dependent diabetes. T1D is less common in India than type 2 diabetes and is characterized by an absolute deficiency of insulin due to the destruction of pancreatic beta cells. The prevalence of T1D in India is estimated to be around 0.5% of all diabetes cases [4].

Type 2 diabetes (T2D)

Type 2 diabetes, on the other hand, is characterized by insulin resistance, which leads to a relative deficiency of insulin. T2D is the most common form of diabetes in India, accounting for around 90% of all cases. Risk factors for T2D in India include age, obesity, physical inactivity, and a family history of diabetes. According to the International Diabetes Federation (IDF), the global prevalence of diabetes in adults was estimated to be 9.3% in 2019, with type 2 diabetes accounting for the majority of cases [5].

Approximately, 30-60% of diabetics have systemic arterial hypertension (SAH), which shows the close relationship between such diseases [6]. SAH, in turn substantially contributes to morbidity in patients with diabetes [7], with oxidative stress (OS) configuring an important mechanism in the pathophysiology of DM and SAH [8-10]. Increased oxygen free radical activity, coupled with reduced protection against OS, could play a role in the etiology of neurovascular abnormalities in experimental DM [11]. Production of reactive oxygen species (ROS) is increased in diabetic patients, especially in those with poor glycaemic control. Diabetes and hypertension (high blood pressure) are two common chronic conditions that often coexist and can have a significant impact on a person's health. The exact mechanisms underlying the relationship between diabetes and hypertension are complex and not fully understood. However, several physiological processes contribute to the interaction between the two conditions. These include insulin resistance, endothelial dysfunction, sympathetic nervous system overactivity, and abnormal renal sodium handling [12].

Anti-hyperglycaemic medication metformin HCL helps type II diabetics' ability to tolerate glucose. According to reports, 50–60% of metformin HCL administered orally has a 100% bioavailability. The proximal small intestine is the primary location of metformin HCL's absorption, and its biological half-life is 1.5–1.6 hours [13]. It belongs to the class of drugs known as biguanides and is considered a first-line treatment for diabetes. Metformin HCl works by reducing glucose production in the liver and increasing the body's sensitivity to insulin. It does not stimulate the pancreas to produce insulin [14].

Enalapril maleate is a medication commonly prescribed for the treatment of hypertension (high blood pressure) and heart failure. It belongs to the class of drugs known as angiotensin-converting enzyme (ACE) inhibitors. Enalapril maleate works by inhibiting the action of the ACE enzyme in the body. ACE is responsible for the conversion of angiotensin I to angiotensin II, a potent vasoconstrictor. By blocking ACE, enalapril reduces the production of angiotensin II, leading to vasodilation (widening of blood vessels) and a decrease in blood pressure [15,16].

Metformin HCl and Enalapril Maleate, these two medications are used to treat type 2 diabetes and high blood pressure, respectively. Combination medication treatment minimizes dosage burden because 40–80% of persons with diabetes also have hypertension. When anti-diabetic and anti-hypertensive medications are taken, they lead to synergistic pharmacodynamic effectiveness. While Metformin HCl and Enalapril Maleate have different mechanisms of action and primary indications, they may have some potential pharmacodynamic synergistic effects when used together. This means that their combined action may provide additional benefits beyond what each medication can achieve on its own.

Both Metformin HCl and Enalapril Maleate have been shown to have positive effects on cardiovascular health. Metformin has been associated with reduced cardiovascular events and improved outcomes in patients with diabetes, while enalapril has been shown to reduce blood pressure and improve heart function. When used together, these medications may have a complementary effect in managing conditions such as diabetes and hypertension.

Additionally, the combination of enalapril maleate and metformin may also have potential benefits for patients with kidney disease. Both medications have been shown to have Reno protective effects, meaning they can help protect the kidneys from damage and slow down the progression of kidney disease. Studies have indicated that the combined use of enalapril maleate and metformin in patients with diabetic nephropathy (kidney disease caused by diabetes) may have synergistic effects on renal function and reduce proteinuria (excessive protein in the urine).
Bilayer tablet of Immediate released Enalapril Maleate and Sustained released Metformin HCl may help to reduce the dosage burden of patients having Diabetes and Hypertension due to which the patient compliance will be improved. The sequential release of Metformin HCl and Enalapril Maleate shows the synergistic effect by reducing the Oxidative Stress. Bilayer tablet is an advanced technology that helps in overcoming the limitations of a single-layered tablet.

MATERIALS AND METHOD

Materials

Metformin HCl by Aarti Chemicals and Distributors Mumbai, Enalapril Maleate from Yarrow Chemicals Mumbai. HPMC K4M, HPMC K100M, Croscarmellose, Sodium Starch Glycolate (SSG) are received from Research lab Fine Chem. Industries, Mumbai. Microcrystalline Cellulose from Prachin Chemical, Ahmedabad and Magnesium stearate and Talc is received from Signet Excipients Pvt. Ltd. Mumbai.

Method

Identification of pure drug [17]

Identification of pure drug was carried out by UV Visible spectroscopy and Fourier Transform Infra-Red Spectrophotometry scanned in the range of 200-400nm and shown in Fig.7 to Fig.14.

Drug-excipient compatibility study [18]

Studies of drug-excipient compatibility are important to ascertain drug and excipients are compatible with each other. Fourier Transform Infra-Red Spectrophotometry (FTIR) are used to study drug excipient compatibility.

Calibration curve of Metformin HCl Enalapril Maleate in 0.1N HCl and Metformin HCl and Enalapril Maleate in pH 6.8 Phosphate buffer [19]

100 mg of pure drug was dissolved in 100 mL of 0.1N HCl / pH 6.8 Phosphate buffer (stock solution-I; 1000 µg/mL) and then placed in a Sonicator for 10 min, from this 10 mL of solution was taken and the volume was adjusted to 100 mL with 0.1N HCl / pH 6.8 Phosphate buffer (stock solution-II; 100 µg/mL). The stock solution-II; was suitably diluted with 0.1N HCl/ pH 6.8 Phosphate buffer to obtain the series of working dilutions: 2, 4, 6, 8, 10 µg/mL of drug solution. The median concentration was scanned for λ_{max} and at the respective λ_{max} working dilutions were analyzed by using a double beam UV-Vis. spectrophotometer (Shimadzu, Japan UV-1800 double beam Spectrophotometer). The λ_{max} of pure drug in 0.1N HCl and 6.8 phosphate buffer shown in Fig.1,3,5. The standard calibration curve was plotted by taking concentration on X-axis and absorbance on Y-axis was shown in Fig.2,4,6.

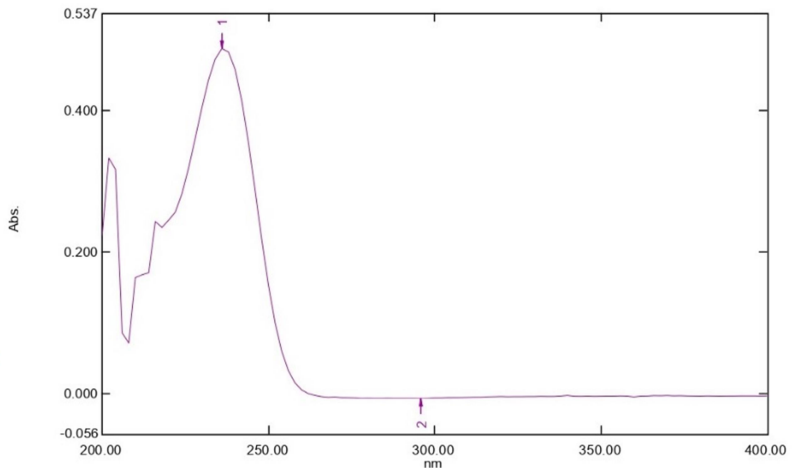


Fig 1: UV Spectrum of Metformin HCl in 0.1N HCl

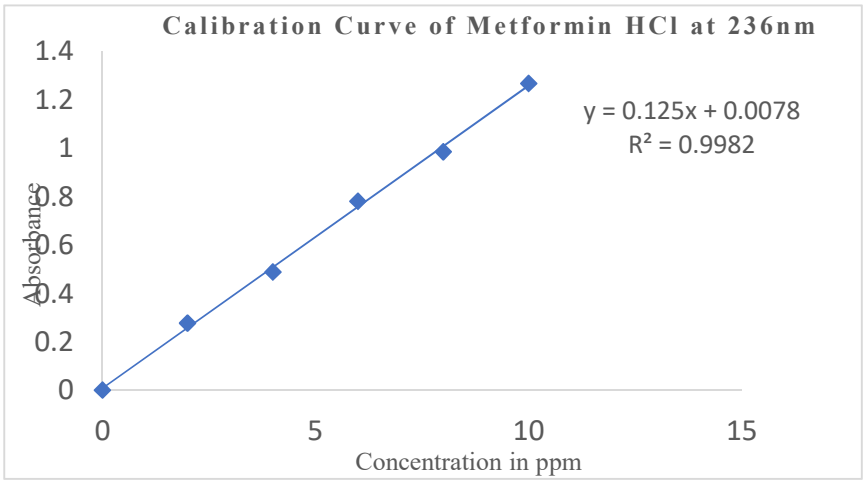


Fig 2: Calibration curve of Metformin HCl in 0.1 N HCl

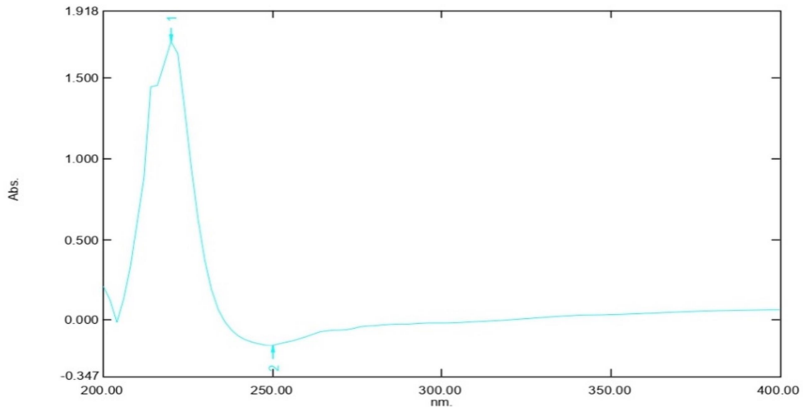


Fig 3: UV Spectrum of Metformin HCl in 6.8 Phosphate Buffer

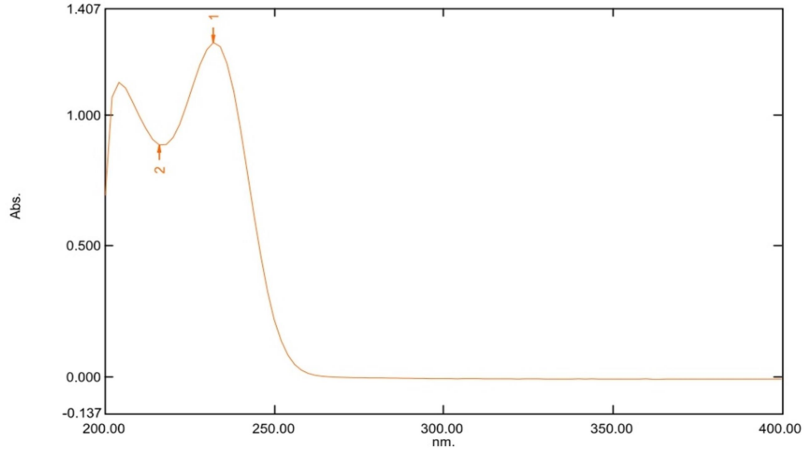


Fig 4: Calibration Curve of Metformin HCl in 6.8 pH Phosphate Buffer

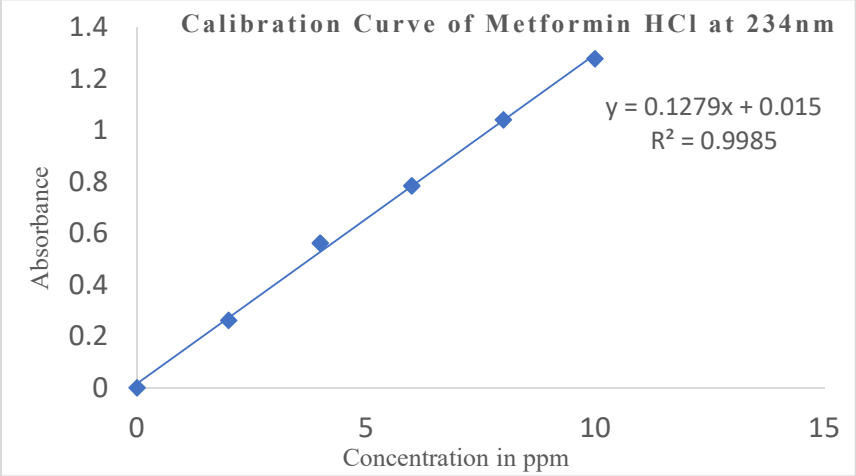
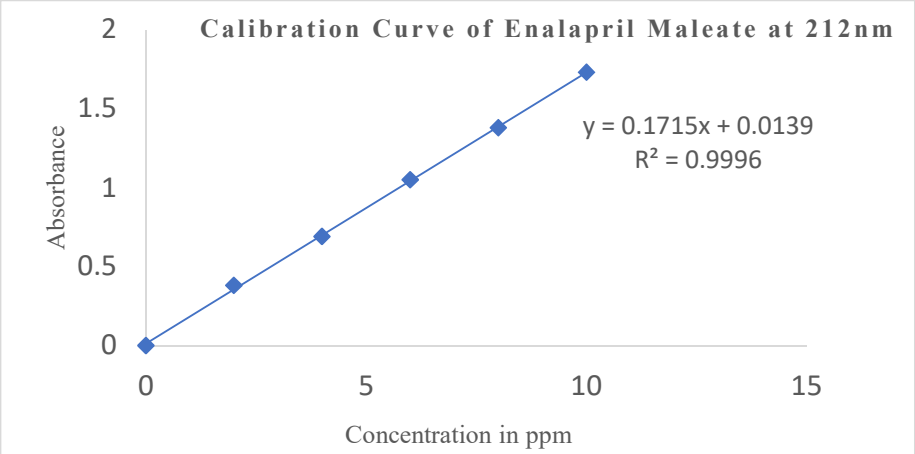


Fig 5: UV Spectrum of Enalapril Maleate in 0.1N HCl

Fig 6:



Calibration Curve of Enalapril Maleate in 0.1 N HCl

Drug – Excipient Compatibility Study

Fourier Transform Infrared Spectroscopy (FTIR) [20]

FT-IR spectrum of Metformin HCl and Enalapril Maleate was recorded to confirm its purity on FTIR spectrophotometer (FTIR 8400S, Shimadzu) by using KBr powder press technique. The base line correction was done using dried potassium bromide. The instrument was operated under dry air purge with resolution of cm^{-1} over the region 4000-400 cm-1. The scans were evaluated for presence of principle peaks of drug. The identified peaks were compared with peaks of reported IR spectrum.

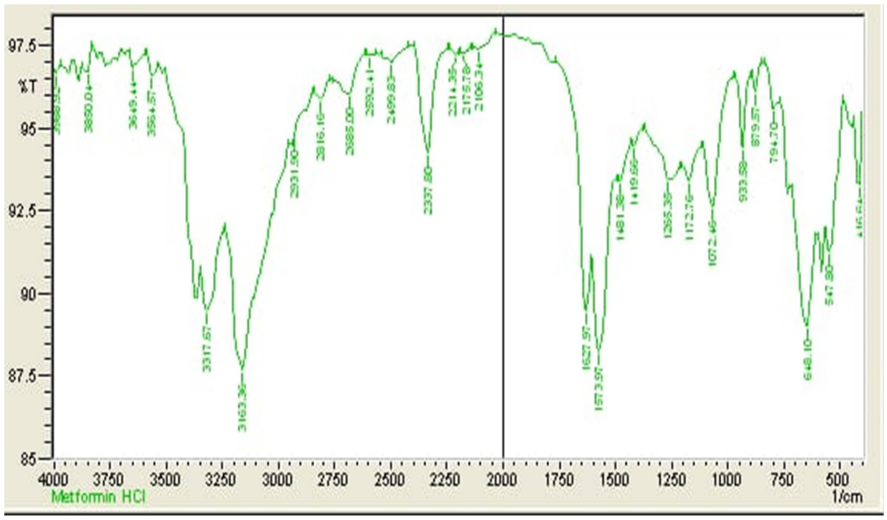


Fig 7: FT-IR Spectra of Metformin HCl

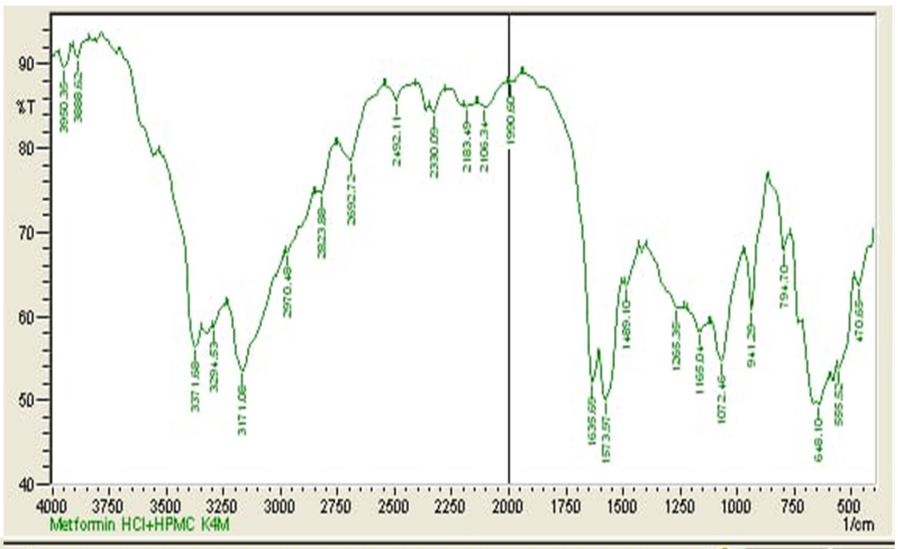


Fig 8: FT-IR Spectrum of Physical Mixture of Metformin HCl +HPMC K4M

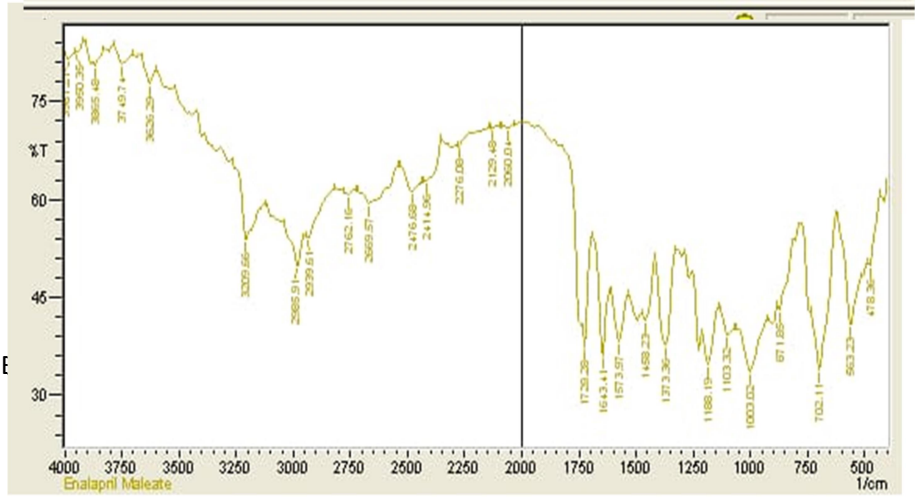


Fig 9: FT-IR Spectrum of Physical mixture of Metformin HCl + HPMC K100M

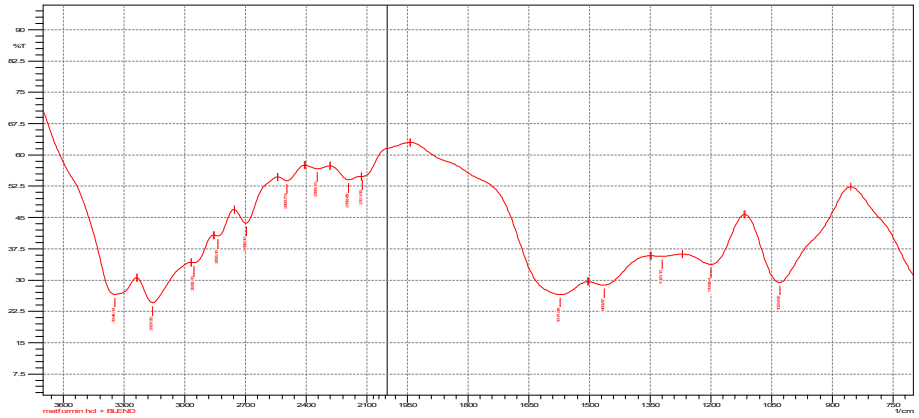


Fig 10: FT-IR Spectrum of Physical mixture of Metformin HCl + other Excipients

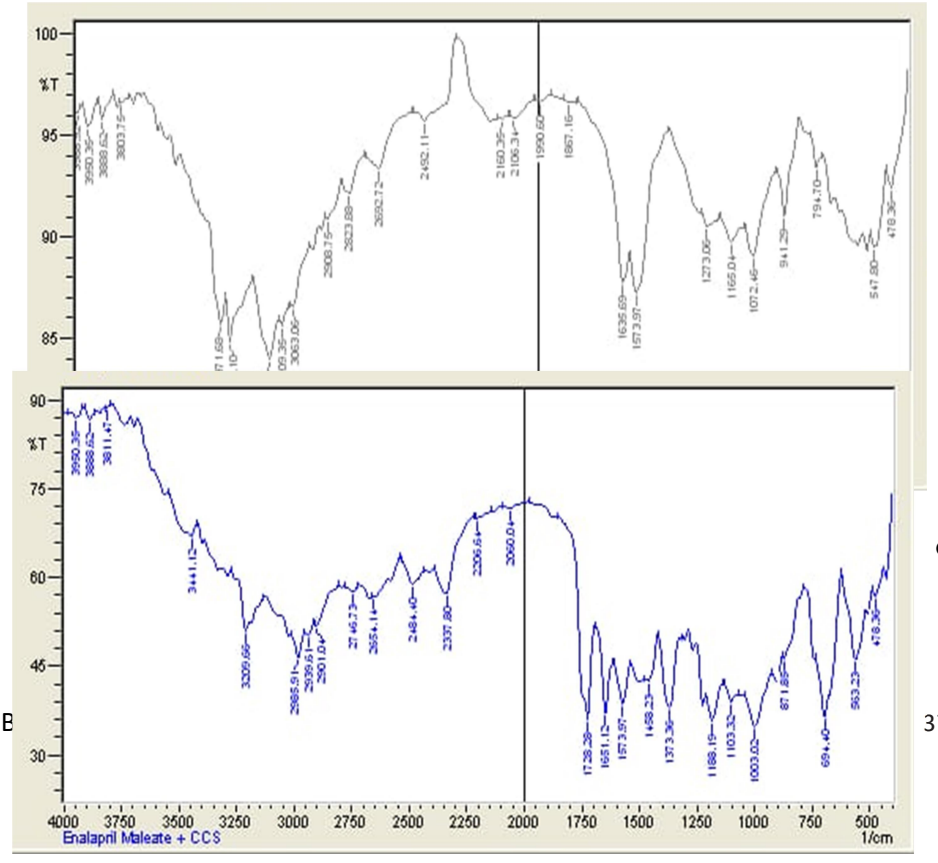


Fig 11: FT-IR Spectrum of Enalapril Maleate

Fig 12: FT-IR Spectrum of Physical mixture of Enalapril Maleate + Croscarmellose

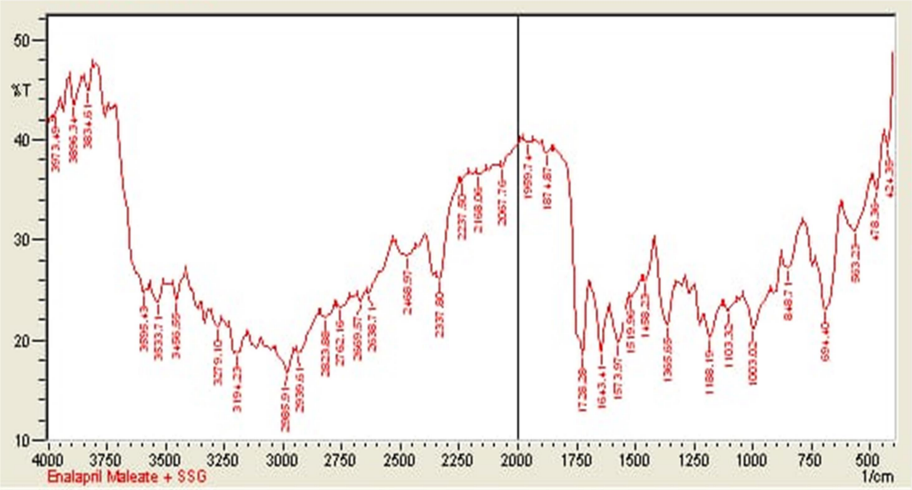


Fig 13: FT-IR Spectrum of Physical mixture of Enalapril Maleate + Sodium Starch Glycolate

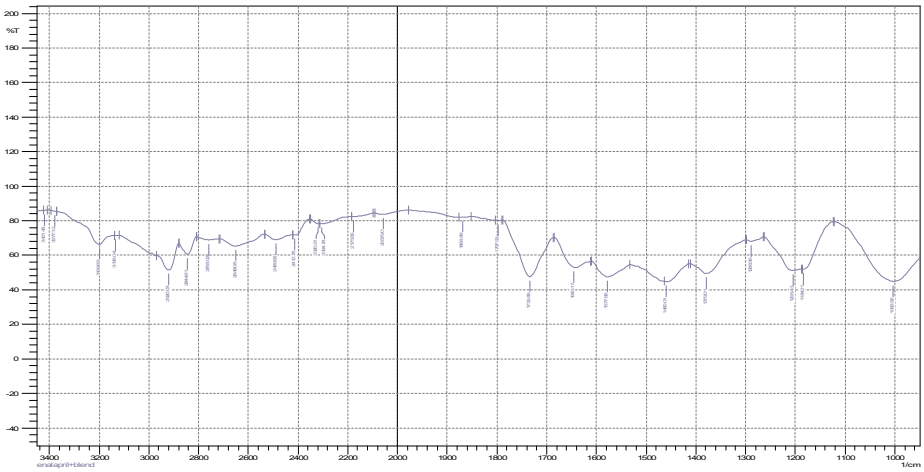


Fig 14: FT-IR Spectrum of Physical mixture of Enalapril Maleate + other Excipients

Preparation Of Bilayer Tablet

Preparation of Immediate Release Layer

The Immediate release layer were prepared by Direct Compression technique by blending Enalapril Maleate uniformly with Sodium Starch Glycolate and Croscarmellose sodium as a superdisintegrants, Microcrystalline cellulose as a direct compressible agent, Magnesium Stearate as a diluent as per the formula. The blend obtained was passed through a 40# sieve. The powder blend was mixed with talc.

Preparation of Sustained Release Layer

The sustained release layer was prepared by direct compression method by blending Metformin hydrochloride uniformly with HPMC K4M and HPMC K100M as a sustained release polymer, Microcrystalline Cellulose as a compressible agent. The blend powder was mixed with talc and magnesium stearate.

Table 1: Formulation of Sustained Release Metformin HCl Layer

Immediate Release Layer	IR1	IR2	IR3	IR4	IR5	IR6
Enalapril Maleate	5	5	5	5	5	5
Croscarmellose Sodium	5	10	15	---	---	---
Sodium starch Glycolate	---	---	---	5	10	15
Microcrystalline cellulose	88	83	78	88	83	78
Magnesium stearate	1	1	1	1	1	1
Talc	1	1	1	1	1	1
Ferric oxide	Q. S	Q. S	Q. S	Q. S	Q. S	Q. S
Total(mg)	100	100	100	100	100	100

Table 2: Formulation of Immediate Released Enalapril Maleate Layer

Formulation in mg	F1	F2	F3	F4	F5	F6
Sustained Release Layer	**SR1**	**SR2**	**SR3**	**SR4**	**SR5**	SR6
Metformin HCl	500	500	500	500	500	500
HPMC K100M	150	170	190	---	---	---
HPMC K4M	---	---	---	150	170	190
Microcrystalline Cellulose	84	64	44	84	64	44
Magnesium Stearate	8	8	8	8	8	8
Talc	8	8	8	8	8	8
Total (mg)	750	750	750	750	750	750

Pre-Compression Studies [21]

Directly compressible tablet blends of Enalapril Maleate-IR layer and Metformin HCl-SR layer were evaluated for angle of repose (θ), bulk density (BD), tapped density (TD), Carr's Index (CI) & Hausner's Ratio (HR). The obtained results of pre-compression studies of IR and SR layers were reported in **Table 3** and **Table 4.**

Angle of Repose [22]

This is the maximum angle possible between the height of pile of blend powder and horizontal plane. The frictional forces in the lose powder can be measured by angle of repose. The tangent of angle of repose is equal to the coefficient friction (θ) between the particles. Hence the rougher and more irregular the surface of particles the greater will beangle of repose.

$\theta = \tan^{-1} (h/r)$

Where, h = height of the pile r = radius of the pile

Bulk Density [23]

Bulk density is the ratio of the mass by the volume of an untapped powder sample. The bulk density is measured in g/ml. The bulk density depends on both the density of the powder particles and the arrangement of the powder particles. The bulk density influences preparation, storage of the sample. The mathematical re-presentation is given below.
Bulk density = Weight of the drug / Bulk volume

Tapped Density [24]
In tapped density, the bulk powder is mechanically tapped in a graduated cylinder until the volume is observed. Here the tapped density is calculated as mass divided by the final volume of the powder
Tapped density = Weight of the granules / tapped volume

Carr's Index [25]
It is one of the most important parameters to characterize the nature of granules.
Carr's index (%) = (Tapped density – Bulk density/ Tapped density) × 100

Hausner's ratio [25]
It is an important character to determine the flow property of granules in the presence of different compositions of polymers. The following formula can calculate this.
Hausner's ratio = Tapped density / Bulk density
Values less than 1.18 indicate good flow, and greater than 1.18 indicate poor flow.

Post-Compression Studies Of Bi-Layered Tablets [21,22,23,24,25]
Average weight of tablets 20 tablets was randomly selected from each batch and their weight was determined by an electronic balance (Shimadzu, Japan) the results shown in **Table 5.**

Thickness
For the thickness, tablets were randomly selected from each batch and their thickness was measured using a vernier calipers (Mitutoyo Corporation, Japan.).

Hardness
To check the hardness of the tablet, tablets were randomly selected from each batch and their hardness was measured using a Monsanto hardness tester (Pfizer mLabs-SE-276).

Friability
The friability of the 20 tablets from each batch was tested by a Friabilator (Roche-Friabilator, Germany) at a speed of 25 RPM for 4 min. The tablets were then de-dusted, re-weighed, and percentage weight loss was calculated by the equation below,
% Friability = (Initial Wt.− Wt. after friability) / Initial Wt. × 100

Disintegration test
The disintegration time for the tablet was determined using the disintegration apparatus. One tablet was placed in each of six tubes placed in a beaker containing 1000 ml of purified water maintained at 37±50ºC and the apparatus was operated. The time taken for the tablets to disintegrate and pass through the mesh was noted.

Drug Content Uniformity
Twenty tablets were finely powdered and an amount equivalent to 500 mg of Metformin hydrochloride and 5mg of Enalapril Maleate was accurately weighed and transferred to a 100 mL volumetric flask and was shaken for 10 min. with 70 mL of methanol. Finally, the volume was made up to mark with methanol. This was filtered through Whatman filter paper No.41 and suitably diluted. Drug content was determined using U.V spectrophotometer at 232nm and 208nm respectively

In vitro Dissolution study of IR layer

Dissolution test was carried out using dissolution apparatus USP Type-II using 0.1N HCl as the dissolution medium, maintained at a temperature of 37±0.5ºC. Randomly selected three tablets from each batch were taken for the evaluation undergone dissolution in the USP-II (paddle) dissolution apparatus (Electro lab, Mumbai (Model TDT-08L)), each flask was filled with 900 mL of 0.1N HCl; speed of paddle was maintained at 50 rpm, the temperature was kept constant at 37°C ± 0.5°C. At time points 0, 5, 10, 15, 20, 25, 30, 35, 40 min. 5 mL of dissolution media was withdrawn, filtered through 0.45µm membrane filter, suitably diluted and analyzed at respective λ_{max} of Enalapril Maleate using a double beam UV-Vis. spectrophotometer (Shimadzu-1800, Japan). Each sample withdrawn was replaced with an equal amount of fresh 0.1 N HCl, to keep the volume constant. In vitro dissolution profiles of Enalapril Maleate-IR tablets were shown in **Table 6** and **Fig.15**.

In vitro Dissolution study of SR Layer

Dissolution test was carried out using dissolution apparatus USP Type-II using 0.1N HCl as the dissolution medium, maintained at a temperature of 37±0.5ºC. Randomly selected three tablets from each batch were taken for the evaluation undergone dissolution in the USP-II (paddle) dissolution apparatus (Electro lab, Mumbai (Model TDT-08L)), each flask was filled with in 900 mL of 0.1N HCl for first 2 hr. and in 900 mL of pH 6.8 Phosphate buffer up to 12 hr. Speed of paddle was maintained at 50 RPM, the temperature was kept constant at 37°C ± 0.5°C. Samples were collected at time points 1,2,4,6,8,10,12hr, 5 mL of dissolution media was withdrawn, filtered through 0.45µm membrane filter, suitably diluted and analyzed at respective λ_{max} of Metformin HCl other time points using a double beam UV is spectrophotometer (Shimadzu-1800, Japan). Each sample withdrawn was replaced with an equal amount of fresh 0.1N HCl, to keep the volume constant. In vitro dissolution profiles of SR bi-layered tablets were shown in **Table 7** and **Fig.16**.

Stability testing of formulated bilayer tablet of optimized batch

The formulated bilayer tablets were kept at different storage conditions. The test samples were kept at was kept at 25±2^{o} C, 60% RH and at 40±2^{o} C, 75% RH according to ICH guidelines. The hardness, friability and drug content of the optimized formulation after 3 months were reported in **Table 10**.

RESULT AND DISCUSSION

Calibration curve of Glyburide and Metformin HCl

λ_{max} of Metformin HCl in 0.1N HCl; Enalapril Maleate in 0.1N HCl and Metformin HCl in pH 6.8 Phosphate buffer are 236 nm, 212 nm and 232 nm respectively. The standard curves are following linearity with a regression coefficient of (r^2=0.999). They are obeying the Beer's law in the conc. range of 0-10 µg/ml. As the excipients used in the study were not interfering and good % recovery of drug(s) indicates this spectrophotometric method was suitable for the estimation of drug(s) in dissolution studies and % assay of formulations.

Drug-excipient compatibility studies by FT-IR

An interpretation of FT-IR spectrum of Metformin HCl and Enalapril Maleate (pure drugs) reveals that the IR bands of pure drug and drug(s) + excipients show no significant shifts or reduction in intensity of the FT-IR bands. Hence there was no incompatibility problem between the drug and excipients used in the study.

Pre-Compression Studies

Table 3: Evaluation of prepared tablet blends for pre-compression study of Sustained Release Layer

Formulations	Angle of Repose(θ˚)	Bulk Density (gm/ml)	Tapped Density (gm/ml)	Hauser's Ratio (HR)	Carr's Index (%)
F1	30.46±0.73	0.27±0.02	0.32±0.04	1.18±0.02	15.62±0.23
F2	29.05±0.83	0.28±0.06	0.33±0.05	1.17±0.03	15.15±0.26
F3	31.60±0.67	0.27±0.03	0.31±0.06	1.14±0.04	12.9±0.17
F4	29.74±0.69	0.27±0.05	0.31±0.04	1.14±0.06	12.9±0.34
F5	30.83±0.79	0.29±0.03	0.32±0.02	1.10±0.03	9.3±0.28
F6	28.39±0.8	0.28±0.05	0.32±0.03	1.14±0.05	12.5±0.15

Table 4: Evaluation of prepared tablet blends for pre-compression study of Immediate Release Layer

Formulations	Angle of Repose(θ˚)	Bulk Density (gm/ml)	Tapped Density (gm/ml)	Hauser'sRatio (HR)	Carr's Index(%)
F1	27.55±0.61	0.33±0.02	0.37±0.06	1.15±0.0.21	12±0.3
F2	28.50±0.49	0.31±0.05	0.33±0.04	1.06±0.015	6.06±0.024
F3	27.24±0.45	0.31±0.04	0.35±0.02	1.12±0.023	11.4±0.018
F4	28.08±0.63	0.33±0.03	0.35±0.05	1.06±0.012	6.06±0.034
F5	26.56±0.028	0.31±0.02	0.37±0.03	1.19±0.013	16.21±0.027
F6	28.95±0.83	0.3±0.03	0.33±0.01	1.1±0.025	9.09±0.023

Post-Compression Parameters

Table 5: Post-Compression Parameter of Bilayer Tablet

Formulations	Weight variation (mg)	Thickness (mm)	Hardness (Kg/cm^2)	Friability (%)	Drug Content		Disintegration time(sec)
F1	849.7±0.38	5.7±0.034	6.4±0.15	0.87±0.03	97.82±0.73	97.96±0.32	68.45±0.47
F2	848.3±0.55	5.6±0.087	6.5±0.23	0.83±0.06	98.2±0.53	99.53±0.21	54.82±0.33
F3	850.1±0.32	5.5±0.064	6.9±0.37	0.8±0.02	98.6±0.62	98.72±0.46	45.31±0.29
F4	848.5±0.23	5.7±0.043	6.7±0.28	0.84±0.05	98.20±0.33	97.55±0.35	78.27±0.55
F5	852.2±0.62	5.5±0.053	6.9±0.10	0.91±0.02	97.7±0.37	99.01±0.57	67.59±0.25
F6	850.8±0.46	5.6±0.027	6.3±0.2	0.85±0.04	99.12±0.29	97.03±0.60	52.87±0.39

The bilayer tablets of Metformin HCl and Enalapril Maleate were evaluated for post compression parameter like weight variation test, friability, thickness, hardness, and drug content as shown in the **Table 5**. The percent variation was under the pharmacopoeia limit of 5%, thus all the tablets passed the weight variation test. It ranged from 848.5±0.23 to 852.2±0.62 mg. Thickness was found in the range from 5.5±0.053 to 5.7±0.043 mm. Hardness test was performed by Monsanto hardness tester. Hardness was maintained to be within 6.3±0.2 to 6.9±0.10 kg/cm^2. The friability was found well within the approved range of 0.8±0.02 to 0.91±0.02 % i.e., less than1%. The % drug content was found to be in the range of 97.7±0.33 to 99.12±0.56 for SR layer of Metformin HCl and for IR layer Enalapril Maleate was

found to be 97.03±0.62 to 99.53±0.35. The disintegration time for IR layer was found to be 52.87±0.39 to 78.27±0.55 seconds.

Dissolution Study

Table 6: In Vitro Drug Release Profile of Immediate Release Layer

Time in minute	% Cumulative Drug Release					
0	0	0	0	0	0	0
5	9.21±0.47	12.15±0.37	14.04±0.50	7.60±0.38	10.02±0.21	12.08±0.37
10	22.44±0.42	26.18±0.48	27.65±0.42	20.72±0.35	24.61±0.11	25.66±0.21
15	30.99±0.37	37.30±0.42	41.77±0.37	29.80±0.47	33.03±0.28	38.41±0.47
20	47.25±0.63	51.32±0.59	56.51±0.58	45.22±0.38	49.29±0.37	53.98±0.43
25	60.77±0.48	66.03±0.32	71.64±0.32	59.36±0.53	63.40±0.58	67.64±0.21
30	70.17±0.69	74.07±0.37	78.81±0.39	68.45±0.34	71.47±0.11	76.07±0.42
35	82.17±0.43	84.02±0.48	86.28±0.48	79.40±0.38	83.02±0.43	85.10±0.31
40	93.59±0.64	96.35±0.54	98.93±0.37	92.53±0.37	94.41±0.53	96.67±0.57

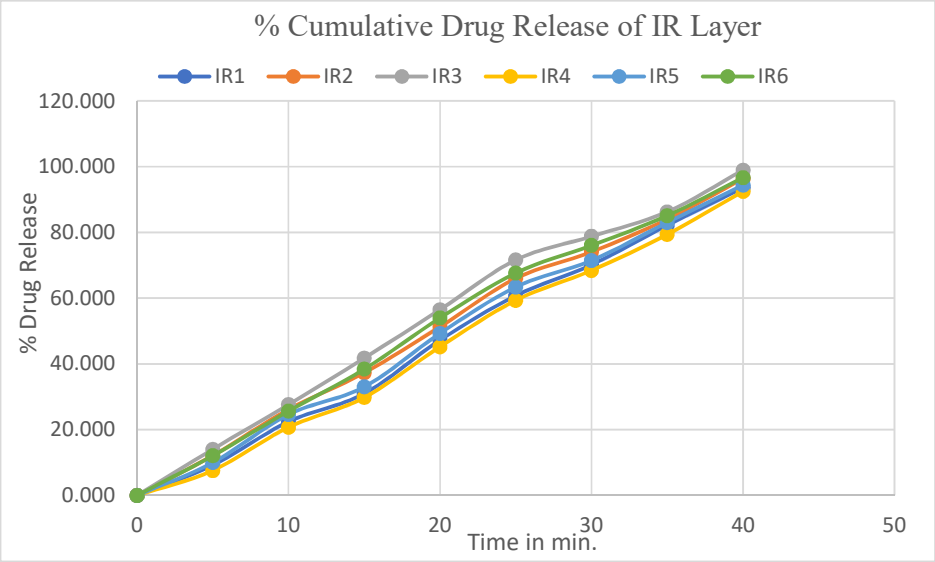


Fig 15: % Cumulative Drug Release of Immediate Release Layer

Immediate released tablets of Enalapril Maleate were prepared by using Croscarmellose and Sodium Starch Glycolate super-disintegrants. The release profiles of Enalapril Maleate Immediate Released tablet were plotted as in **Fig.15**. The effect of Super-disintegrants concentration on drug release could be clearly seen from the variation of dissolution profiles. It was found that drug release from IR3 composed of Croscarmellose in high concentration was 98.93±0.37% drug release up to 40 min. and also shows significantly higher drug release rate than other formulations. Formulation IR3 containing 15 mg of

Croscarmellose Sodium of Cumulative drug release which comparatively greater than other formulation batches so IR3 was selected for further formulation of bilayer tablet of Enalapril Maleate.

Table 7: In Vitro Drug Release Profile of Formulations of Sustained Released Tablet

Time in hour	% Cumulative Drug release					
0	0	0	0	0	0	0
1	12.82±0.44	10.34±0.65	7.64±0.53	10.26±0.53	8.50±0.45	6.10±0.34
2	23.43±0.50	18.63±0.33	17.31±0.51	20.36±0.42	18.05±0.60	16.14±0.36
4	44.20±0.91	40.47±0.50	29.34±0.55	38.55±0.49	34.61±0.46	27.44±0.44
6	64.35±0.71	57.75±0.48	50.77±0.40	57.86±0.58	55.79±0.55	47.06±0.37
8	79.45±0.55	76.69±0.71	69.52±0.47	75.05±0.47	73.81±0.40	66.93±0.4
10	97.44±0.71	95.18±0.73	83.39±0.58	96.24±0.46	93.85±0.55	81.13±0.35
12	-----	-----	97.99±0.46	------	-------	93.10±0.38

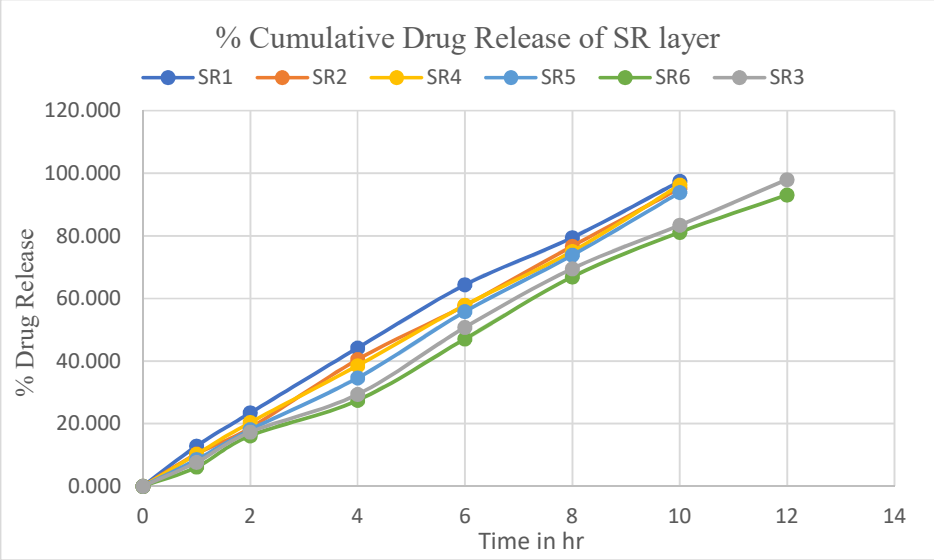


Fig 16: % Cumulative Drug Release of Sustained Release Layer

Sustained release tablets of Metformin HCl were prepared by using HPMC K4M and HPMC K100M polymers. The release profiles of Metformin HCl sustained Released tablet were plotted as in **Fig.16**. The release rate of Metformin HCl mainly controlled by the hydration and swelling properties of polymers. The formulation F1, F2, F4, and F5 shows the drug release up to 10 hrs. which shows the maximum drug release up to 10 hrs. after 10 hrs. the drug release was constant. The effect of polymer concentration on drug release could be clearly seen from the variation of dissolution profiles. It was found that drug release from SR3 composed of HPMC K4M in high concentration was 97.99±0.46 at the end of 12 hrs. and also shows significantly higher drug release rate than other formulations. Formulation SR3 containing 190 mg of HPMC K4M of Cumulative drug release which comparatively greater than other formulation batches so SR3 was selected for further formulation of bilayer tablet of Metformin HCl.

Stability Studies of Bilayer Tablet

Stability study for the developed formulation F3 were carried out as per ICH guideline by storing at

40°C/75% RH up to three months. The formulation F3 was selected on the basis of their cumulative percentage drug release. **Fig.17** and **Fig.18** shows cumulative percent release of F3 Formulation after three months which shows stability of formulation.

Table 8: Comparative drug % of Sustained release layer of SR3 batch

Time in hr.	% Cumulative drug release	
0	0	0
1	7.64±0.53	6.13±0.034
2	17.31±0.51	16.10±0.36
4	29.34±0.55	28.31±0.49
6	50.77±0.40	47.13±0.37
8	69.52±0.47	67.13±0.52
10	83.39±0.58	79.72±0.35
12	97.99±0.46	93.25±0.34

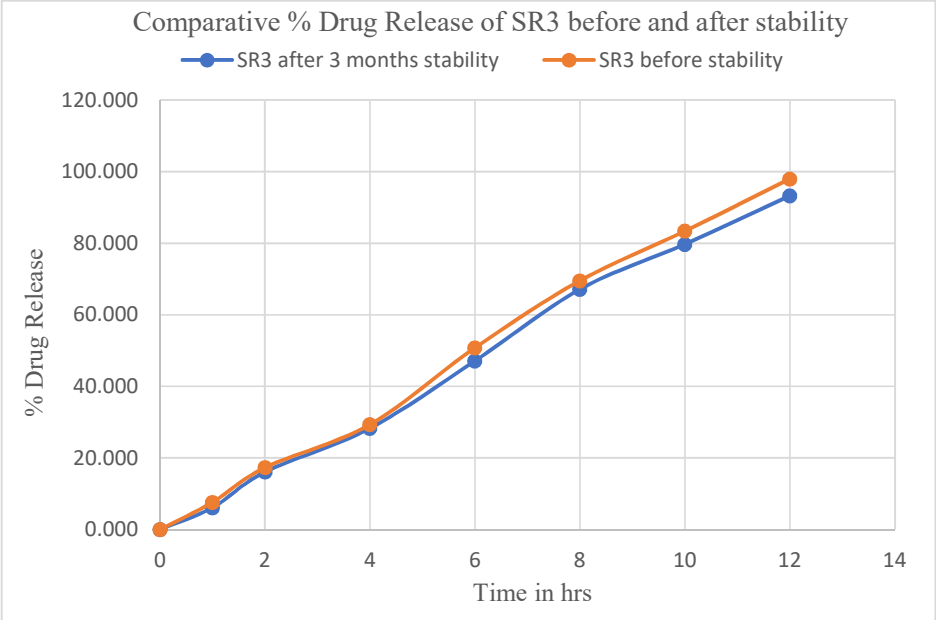


Fig 17: Comparative drug % release of Sustained Release layer of SR3 layer before and after 3 months stability

Table 9: Comparative % drug release of Immediate release layer IR3 Batch

Time in Minutes	% Cumulative drug release	
0	0	0
5	14.04±0.50	13.06±0.42

10	27.65±0.42	25.83±0.58
15	41.77±0.37	39.04±0.23
20	56.51±0.58	51.22±0.42
25	71.64±0.32	65.47±0.43
30	78.81±0.39	73.94±0.69
35	86.28±0.48	84.40±0.65
40	98.93±0.37	95.16±0.80

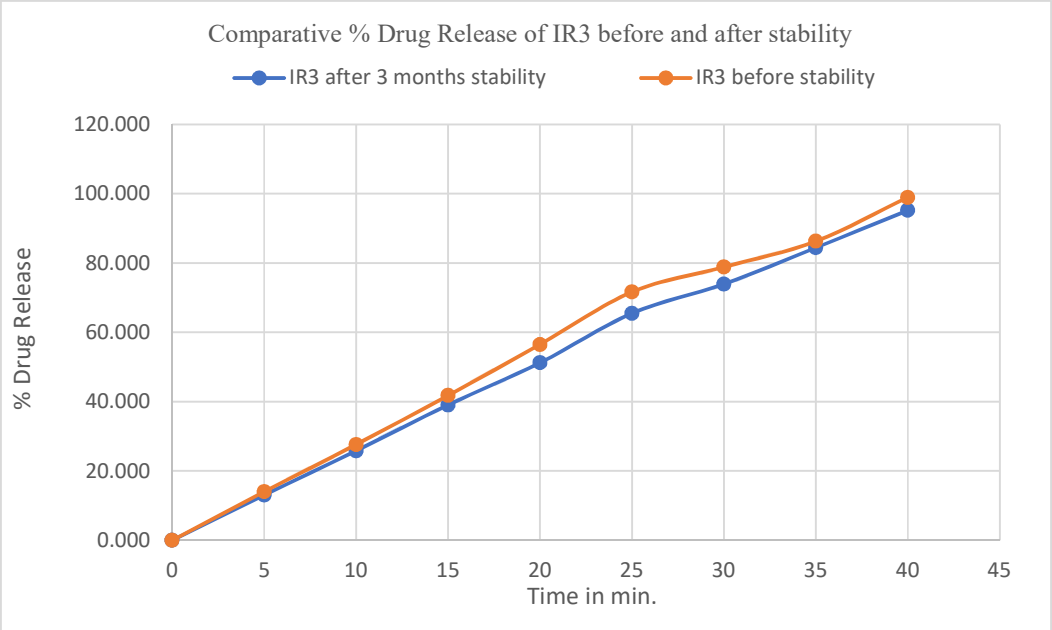


Fig 18: Comparative drug % of release Immediate Release layer of IR3 batch before and after 3 months stability

As there were no significant differences in post compression studies (weight variation, thickness, hardness, friability and in vitro dissolution studies) of initial and accelerated stability samples of optimized formulation IR3 and SR3 of batch F3 in the final up to three months, it passes the test for stability as per ICH guidelines. Comparative FT-IR spectra of optimized F3-Initial and 40°C / 75% RH-2M, reveals there is no significant change in the functional group's peaks of the Enalapril Maleate and Metformin HCl due to interaction with polymers and other excipients in the accelerated stability studies.

Table 10: Comparative stability study of Bilayer Tablet

Sr.No.	Parameter	Initial	After three months
1	Hardness (kg/cm$^{2)}$	6.9±0.37	6.5±0.24
2	Thickness(mm)	5.5±0.064	5.2±0.05
3	Friability (%)	0.8±0.02	0.85±0.06
4	Weight Variation(mg)	850.1±0.32	848±0.96

5	Disintegration Time (Sec)	45.31±0.29	54.63±0.34
6	Drug content (%)	98.6±0.62 (SR3) 98.72±0.46 (IR3)	97.96±0.32 (SR3) 97.42±0.57 (IR3)
7	% Drug Release	97.99±0.46 (SR3) 98.93±0.37 (IR3)	93.25±0.34 (SR3) 95.16±0.80 (IR3)

CONCLUSION

The present research work was carried out to develop antidiabetic bilayer tablet of Metformin HCl and Enalapril Maleate using polymer HPMC K4M and HPMC K100M at high concentration for Sustained Release layer and super disintegrant Croscarmellose Sodium and Sodium Starch Glycolate for Immediate Release layer.

The pre-compression study was performed and the results were reported. In pre-compression study the angle of repose, bulk density, tapped density, Hausner's ratio and Carr's index was calculated and results shown the good compliance as per IP standards. The tablets parameters were evaluated by testing thickness, hardness, weight variation, friability test etc. The % drug content was also calculated and the disintegration time study was performed by using disintegrating apparatus.

The formulation F3 shown the best results of dissolution study containing IR3 and SR3 layer of Croscarmellose Sodium and HPMC K4M respectively. The IR3 layer of F3 formulation shown 98.93% drug release at the end of 40 min. and which was the best drug release than other formulations. The SR3 layer of F3 formulation shown the controlled drug release for 12 hrs. with maximum 97.99 % drug release than other formulations. Further, the stability study of the bilayer tablet was checked as per ICH guidelines and the results were complies to the IP standards. The dissolution study shows the sustain release of the Metformin HCl for 12 hrs. which reduces the dose frequency and increases the patient compliances. The Metformin HCl and Enalapril Maleate show the combining effect that reduces Oxidative Stress.

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