



Durgappagari Rakesh Goud^{1*}, Dr. Kondi Vanitha²

1. Vishnu Institute of Pharmaceutical Education and Research, Department of Pharmaceutics, Vishnupur, Narsapur, Medak, India.
2. Vishnu Institute of Pharmaceutical Education and Research, Department of Pharmaceutics, Head of the department, Vishnupur, Narsapur, Medak, India.

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CORRESPONDING AUTHOR

Vishnu Institute of Pharmaceutical
Education & Research, Vishnupur,
Narsapur, Medak, Telangana, India
Pin code: 502313.

✉ rakeshgoud601@gmail.com

☎ +91-9441487784

ABSTRACT

The objective of present study was to formulate and evaluate bilayer tablet of Valsartan (VS) and Spirulina (SP) for treatment of hypertension and diabetes. The combination of Valsartan and Spirulina is used, as Valsartan establishes the immediate release layer (initial dose) and Spirulina as sustained release layer (maintenance dose) respectively. The bilayer tablets of VS and SP was particularly designed to minimize the risk of hypertension, diabetes and improve the therapeutic efficacy and to prolong the drug release and patient compliance. The formulation variables for immediate release layer include sodium starch glycolate and crospovidone as super disintegrants and micro crystalline cellulose as filler. HPMC K100, HPMC E15, was used as polymers for sustained release. The result of in-vitro release data showed that HPMC K100 can sustain the drug release up to 12hrs. From these studies HPMC K100 has been selected for further studies of bilayer tablet. Valsartan and Spirulina bilayer tablets were prepared by direct compression method. The hardness of the bilayer tablets was found to be $6.30 \pm 0.02 \text{ kg/cm}^2$. The thickness of the bilayer tablets was found to be $3.15 \pm 0.02 \text{ mm}$. The drug content of Valsartan and Spirulina was 98.10 ± 0.90 (SR) and 98.84 ± 0.09 (IR). The *in-vitro* drug release of bilayer tablets has Valsartan immediate release was within 45minutes and Spirulina from the tablets was found sustained over 12 hours with Zero order equation to analyze the release pattern of the drug from the polymeric system. The value of "n" was in the range of 0.1531, indicating the drug release followed fickian release. Fourier transform infrared spectroscopy (FT-IR) analysis indicated that the chemical interactions between the drugs and excipients were absent. The results of Accelerated stability studies showed that all parameters were within the expected specifications and there were no significant changes observed from initial to 3 months, indicating good stability.

INTRODUCTION

It has been experienced for decades that oral drug delivery as the most widely utilized route of administration among all the routes of administration that have been explored for the systemic delivery of drugs. The main goal of any drug delivery system is to provide a therapeutic amount of the drug at the site of action and effective throughout the entire duration of therapy by maintaining the desired drug concentration. Conventional dosage form produces an extensive range of fluctuations in drug concentration in the blood stream and tissues which leads to reduction or loss in drug effectiveness or increased frequency of side effects with subsequent undesirable toxicity and poor efficiency. However, sustained or controlled drug delivery systems can decrease the repetition of the dosing and also increases the potency of the drug by localization at the site of action, reducing the dose requirement and providing uniform drug delivery^[1]. Different types of advancements have been proposed to formulate sustained release tablets for reserve dosage form in stomach. These include bioadhesive or mucoadhesive systems^[2], swellable, expandable systems, floating systems and other delayed gastric emptying devices. In recent years, a growing interest has been developed in designing the drug delivery systems that includes an immediate release (IR) component to extended release (ER) dosages. The addition of an IR component allows one to design delivery systems having optimal pharmacokinetic profiles and enables the combination of different drugs thereby improving patient compliance^[3]. In certain conditions (migraine and sleeping disorders), drug treatment may be advantageous to be delivered in a biphasic manner rather than a single phase extended release preparation. In the first phase of drug release, the immediate release dose fraction (also called loading-dose) reaches a curative drug level in the blood plasma quickly after administration, while in second phase extended release of drug (called the maintenance-dose) provides the dose fraction, required to maintain an effective therapeutic level for a prolonged period. It is provided by bilayer tablets, drug layered matrices or combinations of immediate and extended release multi particulates. Mucoadhesive bilayer tablet is new concept for successful development of sustained release formulation along with various features to provide a way of successful drug delivery system that include an immediate release (IR) layer and a Sustained release (SR) layer. Immediate release layer provides therapeutically effective plasma drug concentration for a short period of time and Sustained release (SR) layer maintain uniform drug levels over a sustained period to reduce dosing intervals, side effects and increase the safety margin for highly-potent drugs and thus offer better patient compliance. It also includes bimodal drug delivery profile (fast release / slow release / fast release). This type of system is used primarily when maximum relief needs to be achieved quickly and then it is followed by a sustained release phase to avoid frequent administration. Suitable candidate drugs for this type of administration include nonsteroidal anti-inflammatory drugs (NSAIDs) and antihypertensive, antihistaminic, and ant allergic agents, anti-psychotics, hypnotics^[4, 5]. Generally, conventional extended dosage forms delay the drug release and do not provide a rapid onset of action. Whereas Immediate release DDS disintegrate rapidly and show instant drug release. They are associated with a fast increase and decrease drug release pattern and hence fluctuations in the plasma drug levels are seen which leads to reduction or loss of drug effectiveness or increased incidence of side effects. Administration of the DDS several times per day is therefore necessary to recognize the loss and decrease in plasma drug concentration due to metabolism and excretion. In many therapies, extended-release formulations are considered desirable but for many drugs, significant daily variations in pharmacokinetics and/or drug effects have been demonstrated on human beings. To maintain the drug concentration within

the therapeutic window a relatively constant plasma level of a drug is often preferred. However, it is difficult to achieve, especially in case of once a day dosage forms, partly because the environment for drug diffusion and absorption changes along the gastrointestinal (GI) tract. A constant plasma concentration may not be obtainable even though a dosage form with a zero-order *In-vitro* release is administered. It is conceivable that a delivery system that can provide a release profile with an increased release initially & followed by a relatively steady release or an accelerated release at a late stage may offer a better solution. Such a release profile, namely pseudo zero-order release with initial burst or bimodal release may compensate for the lower absorption rate in the stomach and the large intestine [6]. Moreover, for some drugs (such as antihypertensive, anti-allergic, NSAIDs, antihistaminic agents) a prompt disposition of a fraction of the dose should reach within in the shortest time possible to relieve the symptoms of the disease and then the continuation of the drug effect should be prolonged for some hours to optimize the therapy. For these types of drugs, extended release formulations generally lead to a delayed appearance of effective plasma levels and they cannot provide a prompt disposition of the dose immediately after administration. To fulfil the specific therapeutic requirements of the different diseases, new drug delivery devices are required for a more specific time-programmed administration of the active ingredients. On the basis of these considerations, a new oral delivery device was proposed, it is in the form of a double-component tablet, in this one portion is formulated to obtain a prompt release of the drug with the aim of reaching a high serum concentration in a short time period & the second portion is a prolonged-release layer which is designed to maintain an efficacious plasma level for a prolonged time period. The pharmacokinetic advantage relies on the fact that drug release from fast releasing component leads to a sudden rise in concentration of drug in blood. However, the blood level is kept maintained at steady state as the drug is released from the sustaining layer.

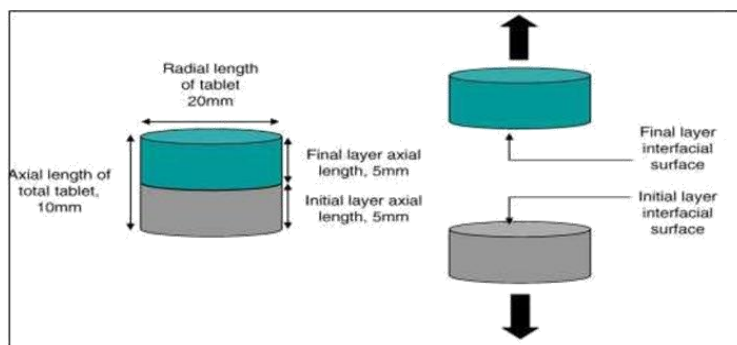


FIG-1. Diagram showing the definitions of the axial lengths, radial length and interfacial fracture surfaces.

Bilayer tablet releases two drugs in combination sequentially, it is suitable to separate two incompatible drugs and also for sustained release tablet in which one layer is immediate release (IR) as initial dose and second layer is sustained release layer as maintenance dose. Bilayer tablets have been developed to attain controlled delivery of various drugs with pre-determined release profiles. In the last ten years, interest in developing a combination of two or more API's in a single dosage form has been enlarged in the pharmaceutical industry, promoting patient convenience and compliance. This article express about different kinds of techniques of bi-layer tablet and why the evaluation and production of quality bi-layer tablets need to be carried out on purpose built tablet presses and also explains about how to defeat the common bilayer tablet problems, such as layer separation, inaccurate individual layer weight control, inadequate hardness, cross contamination between the layers, reduced yield etc. bi-layer tablet consists of monolithic partially coated or multilayered matrices have many applications [7].

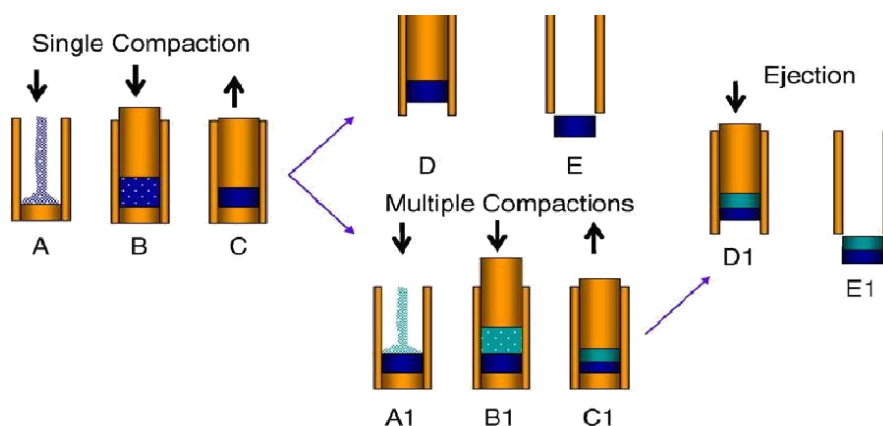


FIG-2. Schematic presentation for compression of bi-layer tablet.

Conventional dosage form produces several alterations in drug concentration in the blood plasma and tissues with undesirable toxicity and poor efficiency. These dynamics such as dosing and unreliable absorption led to the idea of controlled drug delivery systems. The aim in designing sustained or controlled delivery systems is to increase efficacy of the drug by localization at the site of action, to decrease the frequency of the dosing or, reducing the dose required or provides uniform drug delivery. The main objective of sustained release drug delivery is to make sure about safety and to improve efficacy of drugs as well as patient compliance. Many pharmaceutical companies are presently developing bi-layered tablets for different reasons like patent extension, therapeutic and marketing purpose [8]. Formulation of layers are done by using more than one rate controlling polymer, thus enabling different types of drug delivery of one or more drugs where the drug may liberate with a bolus and then at a controlled rate or by targeted drug delivery in the GI tract.

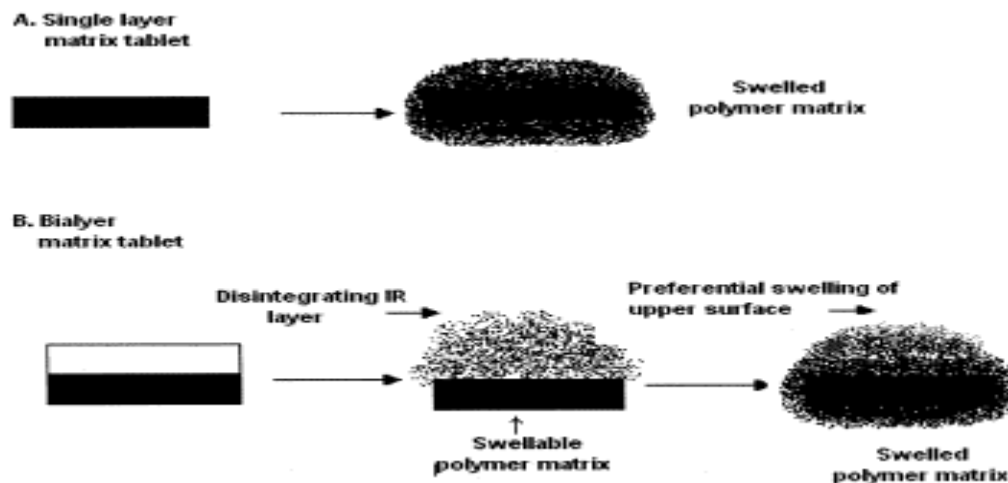


FIG-3. Drug release mechanism.

Need of developing bi-layer tablets ^[9]

For the supervision of fixed dose combinations of drugs, prolong the drug product life cycle, manufacture novel drug delivery systems such as floating or mucoadhesive bilayer tablets for gastro - retentive drug delivery systems.

1. Controlling the delivery rate of either single or two different active pharmaceutical ingredients (API'S).
2. To adapt the total surface area available for API layer either by sandwiching with one or two inactive layers in order to achieve swellable / erodible barriers for controlled release.
3. To divide the API's which are incompatible with each other, & to control the release of one layer by using the functional property of the other layer (such as osmotic property).

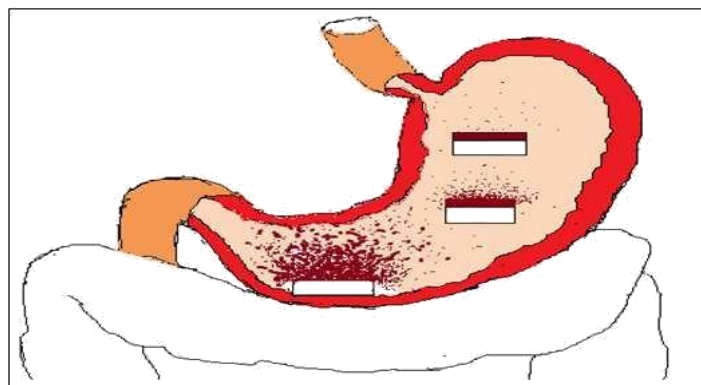


FIG-4. Figure showing immediate drug release from IR layer in stomach.

Valsartan:

Valsartan is an angiotensin-receptor blocker (ARB) that may be used to treat a variety of cardiac conditions including hypertension, diabetic nephropathy and heart failure. Valsartan lowers blood pressure by antagonizing the renin-angiotensin-aldosterone system (RAAS) [10].

Spirulina: Scientific name: *Arthrospira plantensis*, Family: Spirulinaceae, Kingdom: Eubacteria, Phylum: Cyanobacteria, Empire: Prokaryote, Class: Cyanophyceae, Subclass: Oscillatorioophycidae, Order: Spirulinales, Genus: *Spirulina* [11]. Benefits of Spirulina: Spirulina is considered as super food because it contains many nutrients, proteins. Some amounts of magnesium, potassium and manganese & carbohydrates etc. It contains several properties like anti-oxidant, anti-inflammation and prevents the cell damage, It is Effective Against Anemia, hypertension & Help in maintaining Blood Sugar level, Spirulina Appears to Have Anti-Cancer Properties, Especially Against Oral Cancer, It improves the muscle strength hence it is used as good diet supplement food [12].

Hypertension [13]:

Hypertension means high blood pressure, sometimes it is also called as arterial hypertension, and it is a chronic condition in which the blood pressure in the arteries gets elevated. Blood pressure is measured by systolic and diastolic pressures, which depend on the heart muscle is contracting (systole) or relaxed between beats (diastole). Generally Normal blood pressure is within the range of 100–140mmHg systolic and 60–90mmHg diastolic. If the blood pressure is present often at or above 140/90 mmHg it indicates high blood pressure. Hypertension puts strain on the heart, leading to hypertensive heart disease and coronary artery disease if it is not treated. Hypertension is also a serious cause for occurrence of stroke, aneurysms of the arteries (e.g. aortic aneurysm), and peripheral arterial disease and also cause chronic kidney disease. By Dietary intake and lifestyle changes can improve blood pressure control and can diminish the risk of health complications, although drug treatment is still often necessary in people for whom lifestyle changes are not enough or not effective.

Diabetes [14]:

Diabetes is a metabolic disorder which is categorized by a chronic hyperglycemic condition resulting from defects in insulin secretion, insulin action or both. Type 1 diabetes is occurred due to the result of an autoimmune reaction caused to proteins of the islets cells of the pancreas while type 2-diabetes is caused by the collaboration of genetic factors related to impaired insulin secretion, insulin resistance and environmental factors such as obesity, overeating, lack of exercise and stress, as well as aging. The pathogenesis of selective β -cell destruction within the islet in type 1 diabetes mellitus is difficult to follow due to marked heterogeneity of the pancreatic lesions. At the onset of overt hyperglycemia, a mixture of pseudo atrophic islets with cells producing glycogen, somatostatin and pancreatic polypeptide, normal islets and islets containing both β -cells and infiltrating lymphocytes and monocytes may be seen. Of when in pancreatic β -cells the autoimmune destruction occurs, it leads to a deficiency of insulin secretion that leads to the metabolic derangements associated with type-1 diabetes.

In type-2 diabetes the main pathophysiological features are impaired insulin secretion and increased insulin resistance. The impairment of pancreatic β cell function specially shows progression overtime in type 2 diabetes although aging, obesity, insufficient energy intake, alcohol drinking, smoking, etc. are independent risk factors of pathogenesis of type 2 diabetes mellitus.

MATERIALS

Valsartan was obtained from Startech laboratories, Hyderabad, Spirulina was obtained from India mart, Hydroxy propylmethyl cellulose E15, K100, Microcrystalline cellulose was obtained from Dr. Reddy's laboratories, Hyderabad, PVP K 30 obtained from BMR PHARMA, Hyderabad, Sodium Starchglycolate, Crospovidone obtained from MSN Laboratories, Hyderabad, Magnesium stearate Talc, Sodium hydroxide, Potassium phosphate, 0.1N Hydrochloric acid obtained from S.D fine chem. Ltd, Mumbai.

METHODS

Drug-excipient compatibility studies ^[15]: Sample were reduced to powder and analyzed by using a Fourier transform infrared (FT-IR) spectroscope (Bruker). The sample was placed in the sample holder and spectral scanning was taken in the wavelength region Between 400 - 4000 cm^{-1} at a resolution of 4 cm^{-1} with scan speed of 1 cm/sec .

Analytical Method Development: Construction of Calibration Curve of Valsartan and Spirulina. Calibration curves were constructed by preparing stock solution of 1000 $\mu\text{g}/\text{ml}$ from this stock, concentrations of 10 μg to 50 μg per ml were prepared and the above obtained concentrations are observed for absorbance in UV spectrophotometer at a λ max of 214 nm for Valsartan by using pH 1.2 (0.1 N hydrochloric acid buffer) and 202nm for Spirulina by using pH 6.8 phosphate buffer. The calibration curves were be plotted by taking concentration on x-axis and absorbance on y-axis.

Pre-Compression Properties: Sample were taken and the angle of repose was measured by fixed funnel method. Bulk and tapped densities were determined by tapped density apparatus from which compressibility index and Hausner's ratio values were calculated and from the values obtained flow property of powder was determined from (Table 1) ^[16].

Flow Character	Angle of Repose (degrees)	Hausner's Ratio	Compressibility Index (%)
Excellent	25–30	1.00-1.11	≤ 10
Good	31–35	1.12-1.18	11-15
Fair	36–40	1.19-1.25	16-20
Passable	41–45	1.26-1.34	21-25
Poor	46–55	1.35-1.45	26-31
Very Poor	56–65	1.46-1.59	32-37
Very, very Poor	>66	> 1.60	< 38

Table 1. Scale of flowability (USP).

Post-Compression Properties: Thickness of tablets was measured using digital Vernier calipers and recorded. Weight variation was performed on a digital weighing balance, Hardness test was determined using Monsanto hardness tester, Friability by using Roche Friabilator and calculated % friability.

Drug Content Uniformity: One tablet was weighed and crushed in a mortar and then weighed powder containing 100mg equivalent of drug transferred in 100ml of 0.1N hydrochloric acid for valsartan and 100ml of pH 6.8 phosphate buffer and 100ml pH1.2 (0.1N HCL) for Spirulina. Its concentration is 1000 µg/ml then 10ml from this stock solution was taken and diluted to 100ml by using 0.1N hydrochloric acid for valsartan, diluted to 100ml pH 6.8 phosphate buffer and 100ml 0.1N HCL for Spirulina and it makes 100µg/ml. Then 1ml from this stock solution was taken and diluted to 10ml 0.1N hydrochloric acid for Valsartan and 10ml of pH 6.8 phosphate buffer and 100ml pH1.2(0.1N HCL) for Spirulina, it make 10µg/ml was prepared and the absorbance measured at 214 nm for valsartan and 202 nm for Spirulina.

Disintegration test: Disintegration test was performed to determine rate of disintegration of tablets. By Disintegrating apparatus. Take 6 tablets and place 1 tablet in each of the 6 tubes and added a disc to each tube. Maintain the temperature of the disintegration media at $37\pm 5^{\circ}\text{C}$ as specified in the monographs. At the end of time limit specified, lift the basket from fluid and observe the tablets. If 1 or 2 tablets fail to disintegrate completely repeat the test on 12 additional tablets. Not less than 16 out of 18 tablets tested disintegrate completely.

In-Vitro dissolution studies: The *in vitro* dissolution was carried out using USP Dissolution testing apparatus type-II (Paddle method; DBK instrument). The tablets were placed in the buffer solutions then the apparatus was run at $37^{\circ}\text{C}\pm 0.5^{\circ}\text{C}$ and a rotating speed of 50 rpm in a 900 ml dissolution medium. The 10 ml aliquots were withdrawn at intervals of 5 minutes, 10 minutes, 15 minutes, 20 minutes, 30 minutes, 45 minutes, 60 minutes, 75 minutes, 90 minutes, 120 minutes and replacement was done each time with equal amounts of fresh dissolution medium maintained at same temperature. Each 10 ml aliquot was filtered through Whatmann filter paper (No.41). 1 ml of sample was diluted to 9 ml 0.1N HCL and absorbance was measured at 214 nm for valsartan, same procedure was repeated for spirulina but the dissolution medium used for first 2 hours was 0.1N HCl buffer solution and then the dissolution medium was changed by replacing with pH 6.8 phosphate buffer solutions for further 10 hours and absorbance was measured at 202 nm. For absorbance was determined by using UV/VIS spectrophotometer (TG-60, PG instruments). Drug concentrations in the samples were determined from respective standard calibration curves.

Accelerated Stability study of the optimized batch ^[17]:

The purpose of stability testing is to provide evidence on how the quality of drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity, and light. The optimized bilayer tablets were subjected to stability studies at accelerated storage condition at temperature $25^{\circ}\text{C}\pm 2^{\circ}\text{C}/60\%\text{RH}$, $40^{\circ}\pm 2^{\circ}\text{C}/75\%\pm 5\%\text{RH}$ in a humidity chamber for 2 months. Sample were withdrawn after 30 days interval and evaluated for changes in the drug release.

Preparation and evaluation of individual layers:

Preparation of Immediate Release layer of Valsartan: Valsartan and microcrystalline cellulose were mixed with disintegrant for 15 min in porcelain mortar, passed through 80# sieve. This blend was mixed with talc and magnesium stearate for 5min and processed for direct compression by using 8 mm round concave-faced punch at 10 station tablet press. Compression force was maintained at constant level and magnesium stearate as lubricant was fixed at 2% w/w for all formulations. Disintegrants are used at 2, 4 and 6% in tablets. Compositions of all batches are represented in (Table 8).

Preparation of Sustained Release layer of Spirulina: Sustained release tablet layer was prepared by direct compression method according to the formula given in table. All the ingredients including drug were weighed accurately and passed through 80# sieve separately. The drug and polymer were mixed by small portion of both each time and blend it to get a uniform mixture and kept aside. Then all the ingredients weighed are mixed in geometrical order excluding magnesium stearate to get a uniform blend. Finally, mixture is blended with magnesium stearate and tablets were compressed of 10mm sizes concave round punch to get tablet using shiv pharma Compression Machine. Composition of all batches are represented in (Table 9).

Evaluation of individual layers ^[18]: All the batches of tablets were evaluated for various physical parameters like thickness, weight variation, friability, hardness, disintegration, drug content and dissolution as per pharmacopoeia standards.

Preparation of bilayer tablet: Optimized batch of Valsartan (IR4) and Spirulina (SR7) layers were selected for preparation of bilayer tablet. The quantity of powder blend for the sustained release layer was compressed lightly at 10 station shiv pharma tablet press using 10mm round concave punches. Over this compressed layer, required quantity of powder blend for immediate release layer was placed and compressed with the hardness in the range of 3-5kg/Cm² to form a bilayer matrix tablet prepared bilayer tablets are shown below in (FIG-5).



FIG-5. Bilayer tablet of Valsartan and Spirulina.

Evaluation of Bilayer layer tablet: Hardness, thickness, weight variation and friability, in vitro dissolution, stability studies was performed using same method as describe above.

Results and Discussion:

Drug-exipient compatibility studies: FT-IR spectra of pure Valsartan and Spirulina were presented in (FIG- 6, 7, and 8) (Table-2,3 and 4) respectively. The characteristic peaks of pure Valsartan and Spirulina (1371.33cm^{-1} , 1470.07cm^{-1} , 1500.03cm^{-1} , 1605.20cm^{-1} , 13421.15cm^{-1} , 2962.99cm^{-1} , 2852.48cm^{-1} , 1654.00cm^{-1} , 1555.86cm^{-1} , 1404.29cm^{-1}) appeared in both the spectra of Valsartan and Spirulina pure drugs, the physical mixture of Valsartan and Spirulina HPMC K 100, Crospovidone, MCC, PVP K30, and the pure drugs without or with very minute shifting. The characteristic peaks 1371.33cm^{-1} due to C=O, 1470.07cm^{-1} due to CH₂, 1500.03cm^{-1} due to N-H, 1605.20cm^{-1} C=C and peaks, 3421.15cm^{-1} is due to O-H group, 2962.99cm^{-1} due to -CH₃, 2852.48cm^{-1} due to O-H, 1654.00cm^{-1} due to N-H, 1555.86cm^{-1} due to =N-H and 1404.29cm^{-1} due to α-CH₂ In optimized formulation. C=O is observed at 1356.90cm^{-1} , CH₂ is observed at 1432.66cm^{-1} , N-H is observed at 1549.03cm^{-1} , C=C is observed at 1669.23cm^{-1} , O-H is observed at 3536.63cm^{-1} , -CH₃ is observed at 2962.82 , O-H is observed at 2852.49cm^{-1} , N-H is observed at 1679.58cm^{-1} , =N-H is observed at 1545.63cm^{-1} and 1403.86cm^{-1} This phenomenon indicates that chemical interaction between the drug and the excipients was absent.

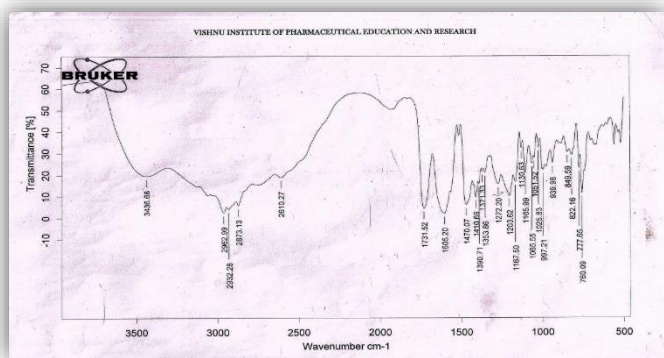


FIG-6. FT-IR spectra of Valsartan pure drug.

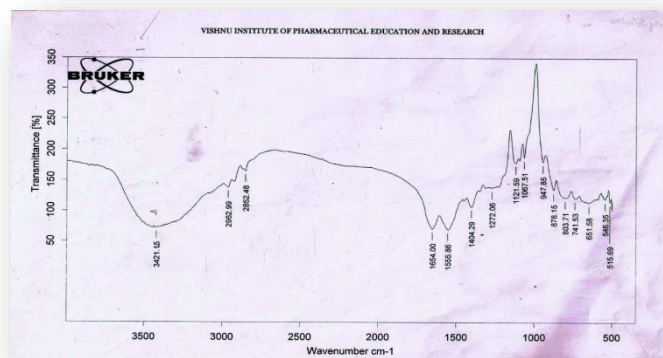


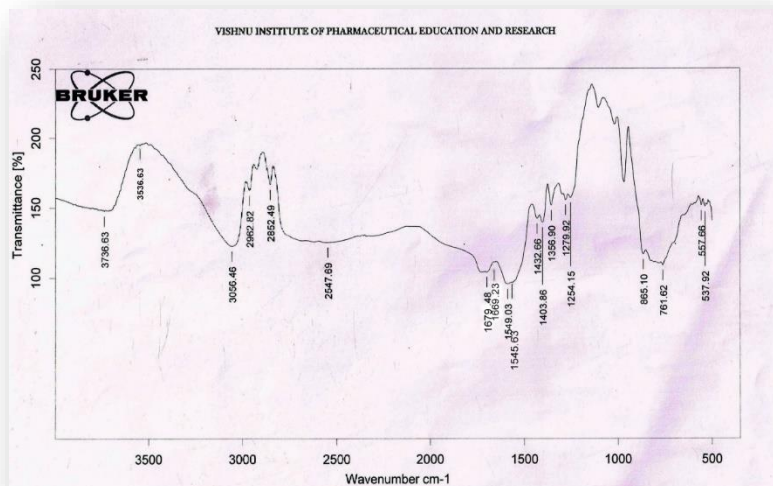
FIG-7. FT-IR spectra of Spirulina pure drug.

Functional group	Wavelength cm ⁻¹	Range (cm ⁻¹)
C=O bending	1371.33	144-1390
CH ₂ bending	1470.07	1430-1470
N-H bending	1500.03	1500-1560
C=C stretching	1605.20	1600-1680

Table 2. Characteristic peak of Valsartan pure drug.

Functional group	Wavelength cm ⁻¹	Range (cm ⁻¹)
O-H stretching	3421.15	3200-3570
-CH ₃ stretching	2962.99	2950-2970
O-H stretching	2852.48	2500-3300
N-H bending	1654.00	1590-1650
=N-H bending	1555.86	1550-1650
α-CH ₂ bending	1404.29	1400-1450

Table 3. Characteristic peak of Spirulina pure drug.



Functional group	Wavelength cm^{-1}	Range(cm^{-1})
C=O bending	1356.90	144-1390
CH ₂ bending	1432.66	1430-1470
N-H bending	1549.03	1500-1560
C=C stretching	1669.48	1600-1680
O-H stretching	3536.63	3200-3570
-CH ₃ stretching	2962.82	2950-2970
O-H stretching	2852.49	2500-3300
N-H bending	1679.48	1590-1650
=N-H bending	1545.63	1550-1650
α-CH ₂ bending	1403.86	1400-1450

FIG-8. FT-IR spectra of pure Valsartan and Spirulina mixture and its blend with other excipients.

Table 4. Characteristic peak of pure Valsartan and Spirulina mixture and its blend with other excipients.

Analytical Method Development:

The Valsartan was estimated using 0.1N HCl solution and the calibration curve was constructed at 214 nm (Table 5 and FIG-9). It obeys Beer-Lambert’s law in the studied range of 10-50 µg/ml with high R² value of 0.9955.

The Spirulina was estimated using 0.1N HCl solution and the calibration curve was constructed at 202 nm (Table 6 and FIG-10). It obeys Beer-Lambert’s law in the studied range of 10-50 µg/ml with high R² value of 0.9973.

The calibration curve for Spirulina was constructed in phosphate buffer pH 6.8 at 202nm as shown in (Table 7 and FIG-11). The method obeyed Beer-Lambert’s law in the studied range of 10-50µg/ml with a HighR² in 0.9988.

Concentration (µg/ml)	Absorbance
0	0
10	0.13
20	0.23
30	0.32
40	0.41
50	0.54

Table 5. Calibration curve absorbances of Valsartan in 0.1 N HCl solution.

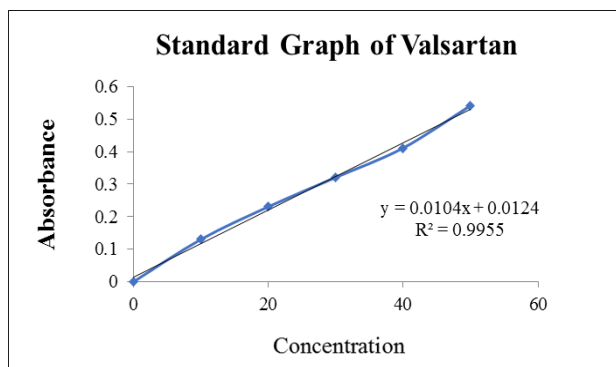


FIG-9. Calibration curve absorbances of Valsartan in 0.1 N HCl solution.

Concentration (µg/ml)	Absorbance
0	0
10	0.10
20	0.204
30	0.324
40	0.414
50	0.553

Table 6. Calibration curve absorbances of Spirulina in 0.1 N HCl solution.

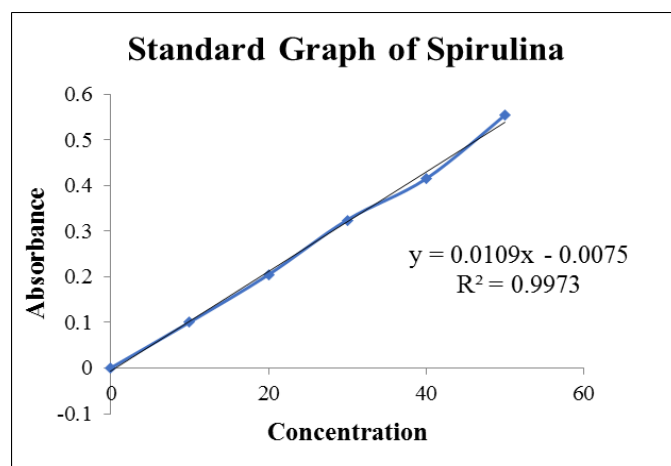


FIG-10. Calibration curve of Spirulina in 0.1 N HCl solutions.

Concentration (µg/ml)	Absorbance
0	0
10	0.14
20	0.31
30	0.45
40	0.60
50	0.73

Table 7. Calibration curve absorbances of Spirulina in pH6.8 phosphate buffer solution.

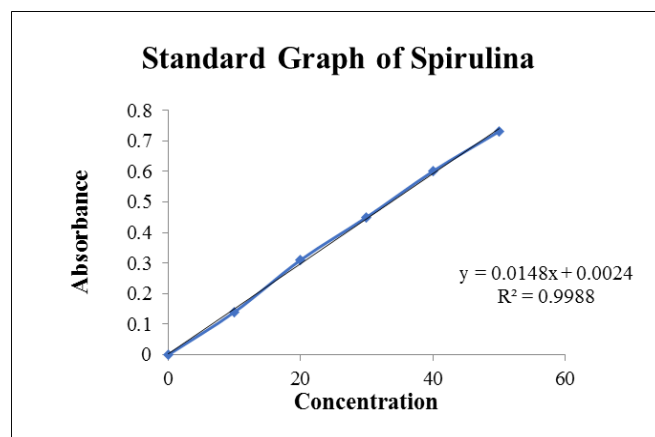


FIG-11. Calibration curve of Spirulina in 0.1 N HCl solutions.

Ingredients	IR1	IR2	IR3	IR4	IR5	IR6
Valsartan	40*	40*	40*	40*	40*	40*
Sodium starch glycolate	2*	4*	6*	–	–	–
Crospovidone	–	–	–	2*	4*	6*
Microcrystalline cellulose	54*	52*	50*	54*	52*	50*
Magnesium Stearate	2*	2*	2*	2*	2*	2*
Talc	2*	2*	2*	2*	2*	2*
Total weight of tablets	100*	100*	100*	100*	100*	100*

Ingredients	SR1	SR2	SR3	SR4	SR5	SR6
Spirulina	500*	500*	500*	500*	500*	500*
HPMC K100	30*	20*	10*	–	–	–
HPMC E15	–	–	–	30*	20*	10*
Microcrystalline cellulose	10*	20*	30*	10*	20*	30*
PVP K30	6*	6*	6*	6*	6*	6*
Magnesium stearate	2*	2*	2*	2*	2*	2*
Talc	2*	2*	2*	2*	2*	2*
Total Weight of tablets	550*	550*	550*	550*	550*	550*

Table 8. Composition of Valsartan immediate release layer.

Table 9. Composition of Spirulina sustained release layer.

*ALL Quantities in mg per tablet IR = formulation codes

*ALL Quantities in mg per tablet SR = formulation

Batch code	Bulk density (gm/cm ³)	Tapped density (gm/cm ³)	Carr's index (%)	Hausner ration (HR)	Angle of repose (θ)
IR1	0.419±0.07	0.55±0.05	23.8±0.10	1.31±0.07	18.26±0.05
IR2	0.455±0.01	0.56±0.01	18.75±0.01	1.23±0.01	16.17±0.01
IR3	0.50±0.05	0.65±0.05	23.07±0.10	1.3±0.04	27.92±0.05
IR4	0.452±0.01	0.55±0.01	17.81±0.05	1.21±0.02	17.22±0.05
IR5	0.40±0.07	0.47±0.01	14.89±0.05	1.17±0.07	29.68±0.01
IR6	0.59±0.05	0.75±0.01	21.33±0.05	1.27±0.07	31.79±0.05

Table 10. Precompression parameters of Valsartan Immediate release layer.

Batch code	Bulk density (gm/cm ³)	Tapped density (gm/cm ³)	Carr's index (%)	Hausner ration (HR)	Angle of repose (θ)
SR1	0.53±0.04	0.66±0.05	19.6±0.09	1.24±0.07	23.7±0.06
SR2	0.54±0.02	0.67±0.04	19.40±0.06	1.24±0.03	30.1±0.01
SR3	0.60±0.01	0.65±0.01	7.60±0.07	1.08±0.02	20.3±0.01
SR4	0.63±0.04	0.76±0.05	17.70±0.11	1.20±0.04	18.26±0.05
SR5	0.71±0.01	0.92±0.02	22.8±0.09	1.29±0.08	39.69±0.00
SR6	0.57±0.07	0.71±0.03	19.7±0.11	1.24±0.03	37.23±0.10

Table 11. Precompression parameters for Spirulina sustained release layer.

Pre-Compression Properties

The precompression parameters for Valsartan immediate release layer are shown in (Table 10). The bulk density was found in range of 0.40 to 0.59 gm/cm³. This value of bulk density indicated good to passable packing characteristic. The tapped density was found between 0.47 to 0.75 gm/cm³. The bulk and tapped density was used to calculate compressibility index and Hauser ratio. The Carr's compressibility index was in the range of 14.89 to 23.81% suggested good to passable compressibility of blend. The values of Hauser ratio were found in the range of 1.17 to 1.31 suggested good to passable flowability of powder blend. The angle of repose of the entire blend was within range of 16.17 to 31.79 indicated excellent to passable flow property of powder blend.

The precompression parameters of powder blend for Spirulina sustained release layer are shown in (Table 11). The bulk density was within range of 0.53 to 0.71 gm/cm³ with tapped density in range of 0.65 to 0.92 gm/cm³ indicated good to passable packaging capacity of blend. The Carr's compressibility index was within 7.60 to 22.8 value suggested good to passable compressibility of powder blend. The angle of repose was 18.26 to 39.69 with Hausner ratio of 1.08 to 1.29 suggested good to passable flowability of powder blend.

Hence all the precompression parameters obtained for the powder blends to be compressed as Valsartan immediate release layer and Spirulina sustained release layer were within the acceptable limits of pharmacopeia specification.

Batch code	Hardness (Kg/cm ²)	Thickness (mm)	% Friability	Weight variation (mg)	Drug content %	Disintegration time(sec)
IR1	3.43±0.02	2.23±0.01	0.36%	92±0.005	95.78±0.44	40.59±8.9
IR2	3.83±0.06	2.23±0.007	1.75%	87±0.003	98.84±0.50	27.60±3.3
IR3	3.50±0.07	2.17±0.009	1.07%	94±0.007	97.26±0.15	38.56±0.88
IR4	3.76±0.05	2.27±0.21	0.53%	94±0.009	96.89±0.53	31.42±6.6
IR5	3.70±0.06	2.23±0.01	0%	97±0.00	98.87±0.04	22.56±0.7
IR6	3.56±0.06	2.25±0.01	0.57%	87±0.007	93.89±0.47	35.33±6.8

Table 12. Post compression parameters of Valsartan Immediate release layer.

Batch code	Hardness (Kg/cm ²)	Thickness (mm)	% Friability	Weight variation (mg)	Drug content %
SR1	6.27±0.03	2.73±0.01	2.6%	542±0.006	85.02±0.04
SR2	6.00±0.04	2.21±0.03	3.5%	542±0.002	97.16±0.12
SR3	6.37±0.01	2.81±0.009	0.6%	548±0.002	99.66±0.07
SR4	6.97±0.07	2.33±0.21	3.4%	537±0.006	96.32±0.26
SR5	6.64±0.1	2.83±0.01	3.4%	531±0.004	89.23±0.18
SR6	6.74±0.1	2.16±0.01	1.5%	540±0.01	96.02±0.30

Table 13. Post compression parameters of Spirulina sustained release layer.

Post-Compression Properties:

The Valsartan immediate release layer was evaluated for hardness, thickness, friability, weight variation, drug content uniformity and *in vitro* disintegration time in (Table 12). The hardness was in the range of 3.43 to 3.83 kg/cm² which was in accordance with The Immediate release tablet. The thickness was from 2.17 to 2.27mm suggested uniformity in thickness for immediate release layer. The friability was from 0-1.75% indicated Excellent to fair handling of the layer. The weight variation results suggested uniformity in weight of layers. The content uniformity was in range of 93.89% to 98.87% indicated uniform dispersion of Valsartan in the layer. The *in vitro* disintegration time for the layer containing sodium starch glycolate was 27.60 to 40.59 sec, for the layer containing crospovidone 22.56 to 35.33 sec the disintegration time follow the order according to superdisintegrants as sodium starch glycolate >crospovidone. As the concentration of superdisintegrants was increased there was decreased in the disintegration time, which was due to the fact that higher level of disintegrants probably made the large pores with continuous network of skeleton providing enough pressure within matrix for faster disintegration. Hence the disintegration time for all the prepared layer was less than 1 minutes indicated that the prepared layer was immediate release layer tablet.

The dissolution study of Valsartan immediate release layer was conducted using 0.1N HCl as dissolution media. The *in vitro* release data of Valsartan is show in (Table 14). The *in vitro* release of Valsartan was plotted as percent drug release versus time as depicted in (FIG-12).

The *in vitro* release of Valsartan was rapid from all the layers. The layer prepared by using sodium starch glycolate showed 48.525 to 51.02% within 45 mins which was due to enormous swelling followed by rapid disintegration. The *In vitro* release of Valsartan was 71.70 to 99.60% within 60 min from immediate release layer containing crospovidone as super disintegrating agent which was attributed to high capillary activity with pronounced hydration capacity of the super disintegrants. The *in vitro* release of Valsartan followed the rank order of sodium starch glycolate<crospovidone as the concentration of super disintegrants increased in all the formulated layers, the release was more and rapid which was due to rapid disintegration in shortest time.

Hence based on the disintegration time and in vitro release study IR5 (CP 4mg) layer was selected as Valsartan immediate release layer of Valsartan for further preparation of bilayer tablet.

Time in minutes	In vitro drug release data of Valsartan immediate release layer % Drug release					
	IR1	IR2	IR3	IR4	IR5	IR6
0	0	0	0	0	0	0
5	28.12	31.05	26.62	20.40	39.90	28.27
10	35.55	35.40	28.35	27.30	50.02	36.07
15	39.30	41.32	40.725	33.07	53.62	42.67
20	42.37	45.30	43.27	48.07	64.65	49.65
30	47.70	48.73	45.45	49.27	73.27	55.20
45	48.52	53.77	51.02	63.07	99.57	65.92
60	56.02	74.85	82.35	76.27	99.60	71.70
75	75.45	91.42	75.60	87.37	-	88.50
90	96.52	-	81.37	91.57	-	-
120	98.02	-	-	98.77	-	-

Table 14. In vitro drug release data of Valsartan immediate release layer (% drug release).

Time in minutes	In vitro release data of Spirulina from sustained release layer % Drug release					
	SR1	SR2	SR3	SR4	SR5	SR6
0	0	0	0	0	0	0
15	5.47	12.97	9.97	7.72	3.97	2.47
30	8.47	17.47	15.97	14.47	13.72	4.72
45	12.97	21.97	21.97	18.97	20.47	9.22
60	15.22	25.72	24.97	25.72	22.72	12.97
2hr	18.97	27.97	30.22	29.47	32.47	17.47
3hr	21.22	30.22	39.22	38.47	37.72	27.97
4hr	24.22	36.97	44.47	52.72	45.97	32.47
5hr	27.97	47.47	52.47	62.47	50.47	38.47
6hr	35.47	51.97	69.22	66.97	53.47	45.22
7hr	38.47	60.97	93.22	82.72	61.72	51.22
8hr	46.75	72.97	95.47	-	66.97	63.22
9hr	50.47	88.72	97.72	-	72.22	68.47
10hr	53.47	-	99.97	-	83.47	87.97
11hr	65.47	-	-	-	93.97	92.47
12hr	71.47	-	-	-	97.72	-

Table 15. In vitro drug release data of Spirulina from sustained release layer (% drug release).

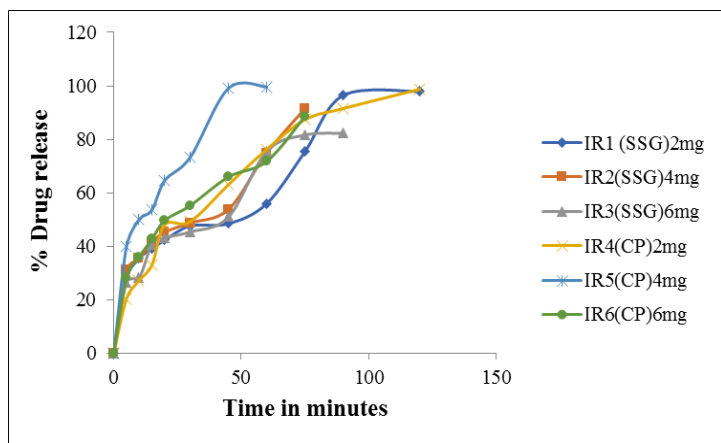


FIG-12. In- vitro release of Valsartan from immediate release layer containing sodium starch glycolate and Crospovidone.

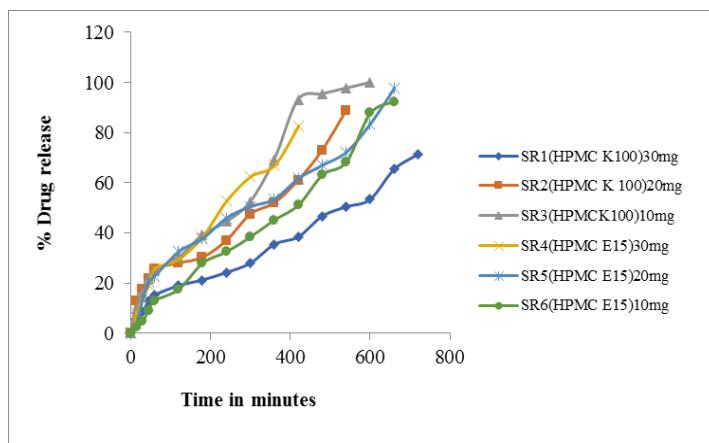


FIG-13. In vitro release of Spirulina from sustained release layer containing HPMC K100, HPMC E15.

The prepared sustained release layer of Spirulina was evaluated for post compression parameters and drug content and results in (Table 13). The hardness of prepared Spirulina layer was in the range of 6.00 to 6.97 kg/cm² which was in acceptable range of sustained release formulation. The thickness of the entire formulated sustained release layer was in range of 2.16 to 2.83mm due to the constant tablet press setting across all the batches irrespective of weight variation. The average weight of formulated layer was found to be uniform in the range of 531 to 548 and the percent deviation in weight variation for all the formulated layer was within the acceptable range of pharmacopeia specification. The percent friability value for all formulated layer was in range 0.6% to 3.5% indicated good to fair handling properties of formulated layers. The drug content was in the range of 85.02 to 99.66% for the entire formulated layer suggested uniform dispersion of Spirulina in formulated sustained release layer.

The *in vitro* release study of Spirulina from sustained release layer was conducted for first 2 hours in 0.1N HCl and then the dissolution study was continued replacing with pH 6.8 phosphate buffer for next 10 hour. The *in vitro* release data of Spirulina from sustained layer is in (Table 15) and illustrated in (FIG-13). The *in vitro* release of Spirulina was slow in 0.1N HCl due to the slow swelling of polymer matrix used in the preparation of sustained release layer. After two hours the formulation SR1, SR2, SR3 have 18.97, 27.97 & 30.22 % from HPMC K100 and formulation SR4, SR5, SR6 have 29.47, 32.47 & 17.47% from HPMC E15 polymer matrix of sustained release layer was released.

The *in vitro* release was rapid in pH 6.8 phosphate buffer due to the more swelling of polymer matrix in alkaline medium. A maximum of 71.47%, 88.72%, 99.97% from HPMC K100, 82.72%, 97.72%, 92.47% from HPMCE15, polymer matrix of sustained release layer was released within 12hr. The *in vitro* release is depending upon nature of drug, nature of polymer, drug to polymer ratio and the medium used. In the present work HPMC K100, HPMC E15 were used as hydrophobic polymer as a matrix in the preparation of sustained release layer. The *in vitro* release followed the rank order according to polymer matrix as HPMC K100 > HPMC E15. The highest release was observed with HPMC K100 which is commonly used hydrophobic matrix, gets swelled and dissolved in aqueous media forming viscous gel thereby rapidly releasing the drug.

Hence based on the *in vitro* release study, formulation SR3 (HPMC K100) layer was selected as Spirulina sustained release layer of Spirulina for further preparation of bilayer tablet.

Formulation of bilayer tablet of Spirulina and Valsartan

Preparation and evaluation of Bilayer tablets:

The bilayer tablet were prepared by double compression of optimized Spirulina sustained release layer (SR3) and Valsartan immediate release layer (IR5) as shown in (Table 16) using 10 mm round punches on a shiv pharma tablet press. The bilayer tablets were evaluated for different physical parameter like hardness, thickness, friability, weight variation and *in vitro* disintegration time. The results of parameters are represented in (Table 17). The hardness of bilayer tablet was found to be $6.30 \pm 0.02 \text{ kg/cm}^2$ which was more as compare to individual layer because of double compression. The thickness of the bilayer tablet was found to be 3.15 ± 0.02 mm which was increased as compare to individual layer because of increase in amount of excipients. The friability was 0.6% for the entire bilayer tablet which was less than 1% indicating good handling of tablet. The weight variation of the bilayer tablet $653 \pm 0.007 \text{ mg}$ was found to be within the limits ($10 \pm 5\%$). content uniformity of Valsartan and Spirulina in the bilayer tablet was found to be 98.10 ± 0.90 and $98.84 \pm 0.09\%$ respectively. The *in vitro* disintegration time was $28.56 \pm 2.15 \text{ sec}$ for all the tablets suggested rapid disintegration of only Valsartan layer whereas the Spirulina layer was not disintegrated but swells. Hence the physical parameter evaluated for all the bilayer tablet were within acceptable range of pharmacopeia norms with good physical properties.

Ingredients	BF1
Spirulina	500mg
HPMC K100	10mg
Micro crystalline cellulose	30mg
PVP K30	6mg
Magnesium stearate	2mg
Talc	2mg
Valsartan	40mg
Crospovidone	4mg
Micro crystalline cellulose	52mg
Magnesium stearate	2mg
Talc	2mg
Total weight of the tablet	650mg

Table 16. Composition of bilayer tablet of Spirulina and Valsartan.

Batch code	Hardness (Kg/cm ²)	Thickness (mm)	%Friability	Weight variation(mg)	Drug content%	Disintegration time(sec)
BF1	6.30 ± 0.02	3.15 ± 0.02	0.6%	650 ± 0.007	98.10 ± 0.90 (SR)	28.56 ± 2.15

Table 17. Evaluation parameters of Bilayer tablet formulation (BF1).

Time	% drug release	
	Immediate release layer	Sustained release layer
0.1N HCL buffer solution		
10	20.02	1.05
15	45.6	2.17
20	64.57	3.60
30	71.92	4.955
45	99.37	5.40
pH6.8 phosphate buffer solution		
1hr	-	9.97
2hr	-	15.97
3hr	-	27.97
4hr	-	34.72
5hr	-	48.97
6hr	-	53.47
7hr	-	60.22
8hr	-	65.47
9hr	-	71.47
10hr	-	84.97
11hr	-	91.72
12hr	-	98.47

Table 18. *In vitro* release data of Valsartan (IR5) and Spirulina (SR3) Bilayer formulation (BF1).

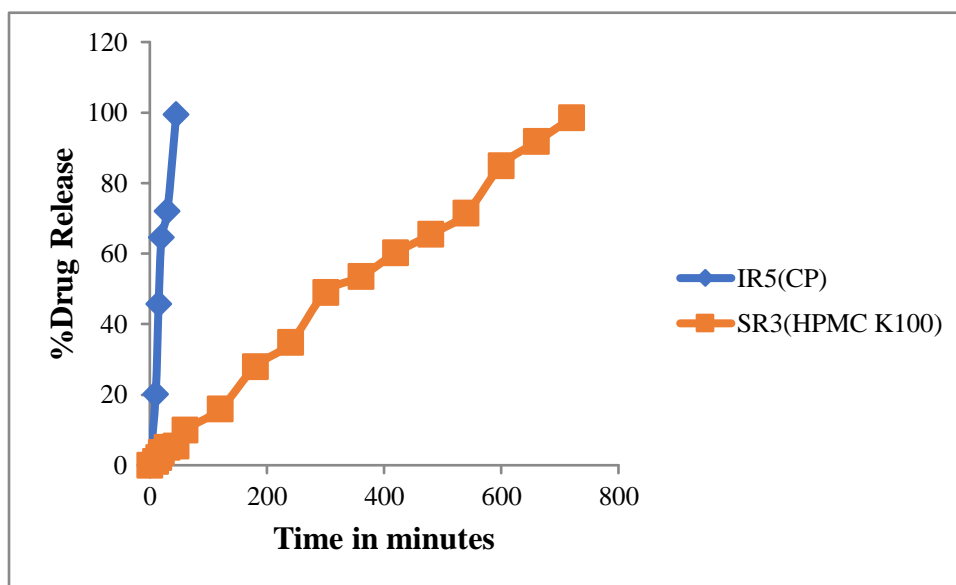


FIG-14. *In vitro* release data of Valsartan (IR5) and Spirulina (SR3) Bilayer formulation (BF1).

The *in vitro* release study of Bi-layer tablets of Valsartan immediate release layer and Spirulina sustained release layer was conducted for first 2 hours in 0.1N HCl and then the dissolution study was continued replacing with pH 6.8 phosphate buffer for next 10 hour. The dissolution study suggested that Valsartan was released within 45min in simulated gastric fluid, while Spirulina was released in much smaller amount (1.05 to 5.4%) within 45minutes. Subsequent to replacing media with phosphate buffer (pH6.8), Spirulina dissolution was found to be increased. The graphical representations of cumulative percent drug release vs time plot for Valsartan and Spirulina in bilayer tablet are represented in (FIG-14).

Kinetic modelling of drug release

The *In vitro* drug release from various Bi-layer tablets was evaluated by using various release kinetic models. The drug release kinetics was evaluated by using the linear regression method (Table 19). For Zero order, R² value were in 0.9934, for first order, R² value were in 0.0092, for Higuchi, R² values were in 0.9202 and for Hixson Crowell, R² value were in 0.9881 and for Korse Meyer, R² values were in 0.9868, The best fit with the highest determination R² coefficients was shown by both the Zero order equations (FIG-15).

Formulation code	Zero order		First order R ²	Higuchi R ²	Korsemeyer-peppas R ²	Hixson Crowell R ²	Best fitting model
	R ²	N					
BF1	0.9934	0.1531	0.0092	0.9202	0.9868	0.9881	Zero order

Table 19. Drug release kinetics of bilayer tablet of Valsartan and Spirulina.

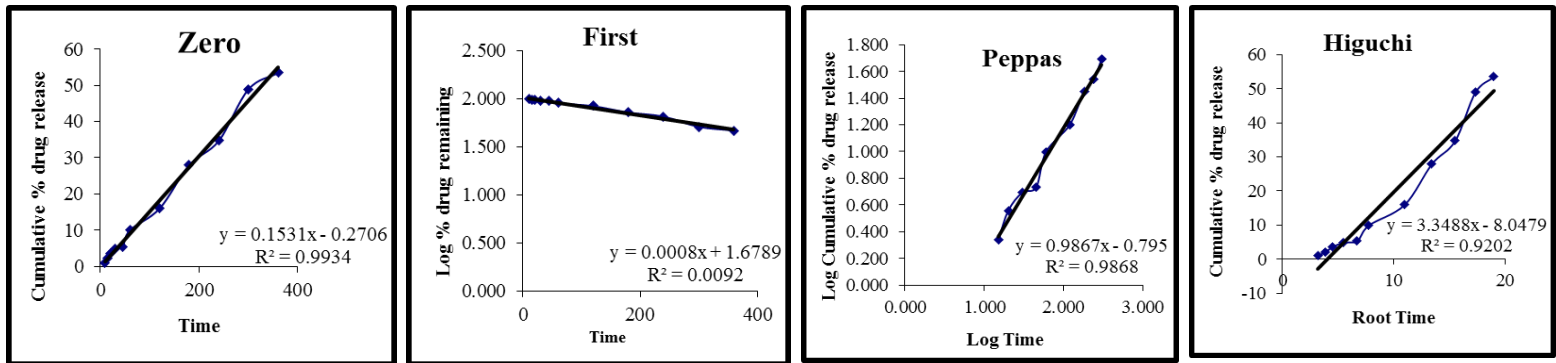


FIG-15. Drug release kinetics of Bilayer tablet formulation (BF1).

Accelerated Stability studies of Bi-layer tablet of Valsartan and Spirulina.

A drug product may undergo changes in its physicochemical characteristics during storage and these changes can affect the bioavailability of drug from the dosage forms. Bilayer tablet have to meet the pharmacopeia specifications, such as weight variation, friability, hardness, thickness, and the drug release during its shelf life [19]. Accordingly, the effect of storage at room temperature, 25°C /60%RH and 40°C/75% RH for 3 months on the *in vitro* characterization and *in vitro* release of formulation BF1 was investigated [ICH Q1A (R2), 2003]. All the stored Bilayer tablets didn't show any change in their colour or appearance throughout the storage period. The physical characteristics of the stored Bilayer tablets in comparison to the fresh ones are compiled as the stability data and presented in Table 17,18 and 19 it is evident that the drug content of formulation BF1 remained within the acceptable limits. A slight decrease in hardness (kg/cm²) was observed compared to the fresh ones. Evidently, a slight increase in drug release was observed on comparing the fresh Bilayer tablets to the stored Bilayer tablets. *In-vitro* release profiles of formulation BF1 at room temperature, 25°C /60%RH and 40°C/75% for 3 months are represented in (Table 20,21,22) and (FIG-16, 17, 18) respectively. However, even with this increment, the stored Bilayer tablets complied with the reported specifications of sustained-release products. This indicates that the formulation BF1 is fairly stable at accelerated storage condition.

Time (months)	Hardness (kg/cm ²)	Thickness (mm)	% friability	Weight variation(mg)	Drug content %	Disintegration (Sec)
0 month	6.30±0.02	3.15±0.02	0.60%	650±0.007	98.10±0.90 (SR)	28.56±2.15
					98.84±0.09 (IR)	
1 month	6.10±0.15	3.15±0.12	0.61%	647.±0.04	98.01±0.70 (SR)	29.19±2.17
					98.60±0.08 (IR)	
2 month	5.93±0.15	3.15±0.14	0.63%	646±0.03	97.97±0.60 (SR)	29.26±2.19
					98.00±0.06 (IR)	
3 month	5.90±0.5	3.15±0.14	0.67%	646±0.23	97.57±0.60 (SR)	30.37±1.20
					97.90±0.06 (IR)	

Table 20. *In vitro* characterization of formulation BF1 at room temperature.

Time (months)	Hardness (kg/cm ²)	Thick ness (mm)	% friability	Weight variation(mg)	Drug content %	Disintegration (Sec)
0 month	6.30±0.02	3.15±0.02	0.60%	650±0.007	98.10±0.90 (SR)	28.56±2.15
					98.84±0.09 (IR)	
1 month	6.20±0.17	3.15±0.14	0.62%	649±0.02	98.04±0.60 (SR)	22.98±2.16
					98.40±0.02 (IR)	
2 month	5.96±0.15	3.15±0.16	0.63%	649±0.04	97.99±0.90 (SR)	23.10±2.19
					98.20±0.06 (IR)	
3 month	5.96±0.15	3.15±0.10	0.63%	647±0.03	97.99±0.90 (SR)	23.12±2.03
					98.10±0.03 (IR)	

Table 21. *In vitro* characterization of formulation BF1 at 25°C±2°C/60%RH.

Time (months)	Hardness (kg/cm ²)	Thick ness (mm)	% friability	Weight variation(mg)	Drug content %	Disintegration (Sec)
0 month	6.30±0.02	3.15±0.02	0.60%	650±0.007	98.10±0.90 (SR)	28.56±2.15
					98.84±0.09 (IR)	
1 month	6.15±0.13	3.15±0.11	0.63%	647±0.05	98.00±0.70 (SR)	22.24±2.05
					98.40±0.05 (IR)	
2 month	5.99±0.16	3.15±0.13	0.61%	645±0.03	97.98±0.60 (SR)	23.16±2.01
					97.89±0.03 (IR)	
3 month	5.99±0.16	3.15±0.05	0.63%	645±0.08	97.78±0.06 (SR)	23.19±2.1
					97.69±0.30 (IR)	

Table 22. *In vitro* characterization of formulation BF1 at 40°C ±2°C/75%RH.

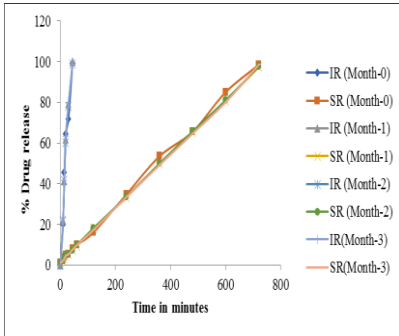


FIG-16. *In vitro* dissolution profiles of formulation BF1 at room temperature.

Time (hrs)	0	10 (IR)	15 (IR)	20 (IR)	30 (IR)	45 (IR)	60 (SR)	2 (SR)	4 (SR)	6 (SR)	8 (SR)	10 (SR)	12 (SR)
Time 0(mns)	0	20.025	45.60	64.57	71.92	99.37	9.97	15.97	34.72	53.47	65.47	84.97	98.47
1	0	22.90	41.30	60.75	78.82	99.92	9.34	17.93	33.31	49.90	65.35	80.73	97.36
2	0	23.03	41.89	61.01	79.00	100	9.83	18.06	33.72	50.00	65.70	81.12	97.80
3	0	22.04	41.78	60.07	78.09	99.05	9.74	17.19	33.46	49.20	64.64	80.10	98.53

Table 23. *In vitro* dissolution profiles of formulation BF1 at room temperature.

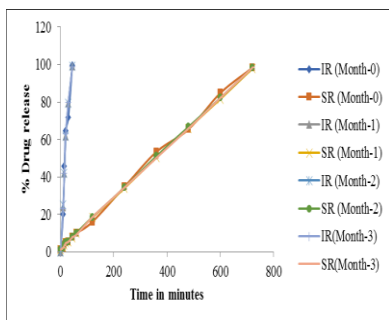


FIG-17. *In vitro* dissolution profiles of formulation BF1 at 25°C ± 2°C / 60% RH.

Time (hrs)	0	10 (IR)	15 (IR)	20 (IR)	30 (IR)	45 (IR)	60 (SR)	2 (SR)	4 (SR)	6 (SR)	8 (SR)	10 (SR)	12 (SR)
Time 0(mns)	0	20.025	45.60	64.57	71.92	99.37	9.97	15.97	34.72	53.47	65.47	84.97	98.47
1	0	23.98	42.12	61.82	79.39	98.95	9.91	18.23	33.89	50.31	65.80	81.53	97.75
2	0	24.21	42.99	63.27	80.00	99.79	10.12	18.62	34.41	51.11	66.71	82.22	98.32
3	0	23.12	41.87	63.16	79.89	98.87	9.13	18.51	34.30	50.15	65.82	82.13	98.21

Table 24. *In vitro* dissolution profiles of formulation BF1 at 25°C ± 2°C / 60% RH.

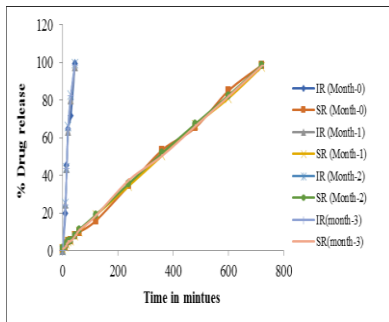


FIG-18. *In vitro* dissolution profiles of formulation BF1 at 40°C ± 2°C / 75% RH.

Time (hrs)	0	10 (IR)	15 (IR)	20 (IR)	30 (IR)	45 (IR)	60 (SR)	2 (SR)	4 (SR)	6 (SR)	8 (SR)	10 (SR)	12 (SR)
Time 0(mns)	0	20.025	45.60	64.57	71.92	99.37	9.97	15.97	34.72	53.47	65.47	84.97	98.47
1	0	24.89	43.63	63.52	80.11	97.85	10.45	18.93	34.62	50.71	66.86	81.21	97.87
2	0	25.12	44.21	65.93	82.77	99.59	11.18	19.00	35.67	51.82	67.32	82.48	98.50
3	0	24.18	43.78	64.98	82.57	98.84	11.10	18.84	37.78	50.71	66.43	82.24	98.36

Table 25. *In vitro* dissolution profiles of formulation BF1 at 40°C ± 2°C / 75% RH.

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