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Formulation and In-vitro Evaluation Of Sustained Release Tablets Of Bosentan

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ABSTRACT

Bosentan is a dual endothelin receptor antagonist important in the treatment of pulmonary artery hypertension (PAH). It is licensed in the United States, the European Union and other countries by Actelion Pharmaceuticals for the management of PAH under the trade name Tracleer®. Bosentan is used to treat pulmonary hypertension by blocking the action of endothelin molecules that would otherwise promote narrowing of the blood vessels and lead to high blood pressure. The aim of the present study was to develop sustained release formulation of Bosentan to maintain constant therapeutic levels of the drug for over 12 hrs. Various synthetic polymers were employed as polymers. Bosentan dose was fixed as 62.5 mg. Total weight of the tablet was considered as 200 mg. the tablets are prepared by employing direct compression method using 8mm punch. And the Polymers were used in the concentration of 31.5, 41.25, 62.5 mg concentration. All the formulations were passed various physicochemical evaluation parameters and they were found to be within limits. And the drug and excipient compatibility studies showed that there is no interaction between the drug and polymers employed in the formulation. Whereas from the dissolution studies it was evident that the formulation F3, F16 showed better and desired drug release pattern i.e., 98.15±0.06, 96.64±0.02 % respectively in 12 hours. the project work is successful in developing sustained release formulations of Bosentan.

Keywords: Bosentan, anti hypertension, sustained release, etc.

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INTRODUCTION

Hypertension [HTN or HT], also known as high blood pressure or arterial hypertension, is a chronic medical condition in which the blood pressure in the arteries is elevated. Blood pressure is expressed by two measurements, the systolic and diastolic pressures, which are the maximum and minimum pressures, respectively, in the arterial system. The systolic pressure occurs when the left ventricle is most contracted; the diastolic pressure occurs when the left ventricle is most relaxed prior to the next contraction. Normal blood pressure at rest is within the range of 100–140 mmHg systolic and 60–90 mmHg diastolic. Hypertension is present if the blood pressure is persistently at or above 140/90 millimeters mercury [mmHg] for most adults; different criteria apply to children[1].

Hypertension usually does not cause symptoms initially, but sustained hypertension over time is a major risk factor for hypertensive heart disease, coronary artery disease, stroke, aortic aneurysm, peripheral artery disease, and chronic kidney disease.

Hypertension is classified as either primary [essential] hypertension or secondary hypertension. About 90–95% of cases are categorized as primary hypertension, defined as high blood pressure with no obvious underlying cause. The remaining 5–10% of cases are categorized as secondary hypertension, defined as hypertension due to an identifiable cause, such as chronic kidney disease, narrowing of the aorta or kidney arteries, or an endocrine disorder such as excess aldosterone, cortisol, or catecholamine's[2].

Dietary and lifestyle changes can improve blood pressure control and decrease the risk of health complications, although treatment with medication is still often necessary in people for whom lifestyle changes are not enough or not effective. The treatment of moderately high arterial blood pressure [defined as >160/100 mmHg] with medications is associated with an improved life expectancy. The benefits of treatment of blood pressure that is between 140/90 mmHg and 160/100 mmHg are less clear, with some reviews finding no benefit and other reviews finding benefit[4].

Most conventional oral drug products, such as tablets and capsules, are formulated to release the active drug immediately after oral administration, to obtain rapid and complete systemic drug absorption. Such immediate-release products result in relatively rapid drug absorption and onset of accompanying pharmacodynamic effects. However, after absorption of the drug from the dosage form is complete, plasma drug concentrations decline according to the drug's pharmacokinetic profile. Eventually, plasma drug concentrations fall below the minimum effective plasma concentration [MEC], resulting in loss of therapeutic activity. Before this point is reached, another

dose is usually given if a sustained therapeutic effect is desired. An alternative to administering another dose is to use a dosage form that will provide sustained drug release, and therefore maintain plasma drug concentrations, beyond what is typically seen using immediate-release dosage forms[5].

Traditional drug delivery system has been characterized by immediate release and repeated dosing of the drug which might lead to the risk of dose fluctuation, this arises the need of a formulation with control release that maintain a near-constant or uniform blood level. The desire to maintain a near-constant or uniform blood level of a drug often translates into better patient compliance, as well as enhanced clinical efficacy of the drug for its intended use.

Sustained Release Concept:

Sustained release, sustained action, prolong action, controlled release, extended action, depot are terms used to identify drug delivery systems that are designed to achieve prolong therapeutic effect by continuously releasing medication over an extended period of time after administration of single dose. In the case of orally administer this period is measured in hours while in the case of injectable this period varies from days to months.[6]

Advantages of Sustained Release Dosage Forms[6]:-

1. Control of drug therapy is achieved.
2. Rate and extent of drug absorption can be is modified
3. Frequency of drug administration is reduced.
4. Patient compliance can be improved.
5. Drug administration can be made convenient
6. Maximizing the availability of drug with minimum dose.
7. The safety margin of high potency drug can be increased.

Disadvantages of Sustained Release Dosage Forms[6]: -

1. It not permits prompt termination of therapy.
2. Less flexibility in dose adjustment.
3. These dosage forms are designed on the basis of average biological half life.
4. They are costly.

Drawbacks of Conventional Dosage Forms[4,5]: -

1. Poor patient compliance, increased chances of missing the dose of a drug with short half-life for which frequent administration is necessary.
2. The unavoidable fluctuations of drug concentration may lead to under medication or over medication.

3. A typical peak-valley plasma concentration time profile is obtained which makes attainment of steady-state condition difficult.
4. The fluctuations in drug levels may lead to precipitation of adverse effects especially of a drug with small Therapeutic Index [TI] whenever over medication occur.

Aim of the present study is to formulate and to evaluate the Bosentan sustained release formulations. To perform compatibility studies for the prepared formulations and optimising the formulations with desired polymers. To perform in-vitro evaluation tests for the optimised formulations to calculate the in-vitro drug/dose release for the formulated Bosentan sustained release tablets and to evaluate the tablets for their stability characteristic features.

Bosentan is a dual endothelin receptor antagonist important in the treatment of pulmonary artery hypertension [PAH]. It is licensed in the United States, the European Union and other countries by Actelion Pharmaceuticals for the management of PAH under the trade name Tracleer®. Bosentan is used to treat pulmonary hypertension by blocking the action of endothelin molecules that would otherwise promote narrowing of the blood vessels and lead to high blood pressure. Patients with PAH have elevated levels of endothelin, a potent blood vessel constrictor, in their plasma and lung tissue. Bosentan blocks the binding of endothelin to its receptors, thereby negating endothelin's deleterious effects[7].

MATERIALS AND METHOD

Bosentan [API] Bought from Natco pharma pvt ltd., Eudragid RSPO, HPMC Chitosan, Magnesium Stearate, Aerosol, Micro Crystalline Cellulose are Bought from SD Fine-Chem. Pvt., Mumbai, India.

METHODOLOGY

Analytical method development[8]:

Preparation Of 0.1N HCl [pH 1.2]:

Take 8.5ml of Hcl in a measuring cylinder and transfer it into a beaker containing 1000ml of distilled water carefully and stir it thoroughly it gives us 0.1N HCl.

Preparation of 6.8 Phosphate Buffer:

Take 6.8 gms of KH_2PO_4 [potassium di hydrogen ortho phosphate] and add it to 1000 ml of distilled water and stir well until the solid content dissolves completely.

Take 0.896 gms of sodium hydroxide pellets and add them to 100ml of distilled water and stir well until the solid content completely dissolves add this solution to above solution drop by drop until the desired pH is obtained.

Determination of Absorption Maxima:

A solution containing the concentration 10 µg/ ml drug was prepared in 0.1N HCl and pH 6.8 Phosphate buffer UV spectrums was taken using Double beam UV/VIS spectrophotometer. The solution was scanned in the range of 200 – 400.

Preparation Calibration Curve:

Construction of Bosentan Calibration Curve with 0.1 N HCl pH 1.2:

100mg of Bosentan pure drug was dissolved in 100ml of Methanol [stock solution]10ml of above solution was taken and make up with100ml by using 0.1 N HCl [100µg/ml].From this 10ml was taken and make up with 100 ml of 0.1 N HCl [10µg/ml]. The above solution was subsequently diluted with 0.1N HCl to obtain series of dilutions Containing 0.5, 0.10, 0.15, 0.20, 0.25, 0.30, 0.35 and 0.40µg/ml of Bosentan per ml of solution. The absorbance of the above dilutions was measured at 242 nm by using UV-Spectrophotometer taking 0.1N HCl as blank. Then a graph was plotted by taking Concentration on X-Axis and Absorbance on Y-Axis which gives a straight line Linearity of standard curve was assessed from the square of correlation coefficient [R^2] which determined by least-square linear regression analysis.

Construction of Bosentan Calibration Curve with Phosphate Buffer pH 6.8:

100mg of Bosentan was dissolved in 100ml of phosphate buffer pH 6.8 to give a concentration of 1mg/ml [1000µgm/ml]. From the above standard solution [1000µgm/ml] 10 ml was taken and diluted to 100ml with phosphate buffer pH 6.8 to give a concentration of 100µgm/ml. From this stock solution aliquots of 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4 and 1.6 ml were pipette out in 10ml volumetric flask and the volume was made up to the mark with phosphate buffer PH 6.8 to produce concentration of 2,4,6,8 and 10 µgm/ml respectively. The absorbance [abs] of each conc. was measured at respective [λ_{max}] i.e., 244 nm.

Drug – Excipient Compatibility Studies[8,9]

Fourier Transform Infrared [FTIR] Spectroscopy:

The physical properties of the physical mixture were compared with those of plain drug. Samples was mixed thoroughly with 100mg potassium bromide IR powder and compacted under vacuum at a pressure of about 12 psi for 3 minutes. The resultant disc was mounted in a suitable holder in Perkin Elmer IR spectrophotometer and the IR spectrum was recorded from 3500 cm^{-1} to 500 cm^{-1}. The resultant spectrum was compared for any spectrum changes.

Pre-formulation Parameters

The quality of tablet, once formulated by rule, is generally dictated by the quality of physicochemical properties of blends. There are many formulations and process variables involved in mixing and all these can affect the characteristics of blends produced. The various characteristics

of blends tested as per Pharmacopoeia[9,10,11].

Angle of Repose:

The frictional force in a loose powder can be measured by the angle of repose. It is defined as, the maximum angle possible between the surface of the pile of the powder and the horizontal plane. If more powder is added to the pile, it slides down the sides of the pile until the mutual friction of the particles producing a surface angle, is in equilibrium with the gravitational force. The fixed funnel method was employed to measure the angle of repose. A funnel was secured with its tip at a given height [h], above a graph paper that is placed on a flat horizontal surface. The blend was carefully pored through the funnel until the apex of the conical pile just touches the tip of the funnel. The radius [r] of the base of the conical pile was measured. The angle of repose was calculated using the following formula:

$\tan \theta = h / r$

$\tan \theta$ = Angle of repose

h = Height of the cone, r = Radius of the cone base

Bulk Density:

Density is defined as weight per unit volume. Bulk density, is defined as the mass of the powder divided by the bulk volume and is expressed as gm/cm^3. The bulk density of a powder primarily depends on particle size distribution, particle shape and the tendency of particles to adhere together. Bulk density is very important in the size of containers needed for handling, shipping, and storage of raw material and blend. It is also important in size blending equipment. 10 gm powder blend was sieved and introduced into a dry 20 ml cylinder, without compacting. The powder was carefully leveled without compacting and the unsettled apparent volume, [Vo], was read.

The bulk density was calculated using the formula:

$$\text{Bulk Density} = M / V_o$$

Where, M = weight of sample

V_o = apparent volume of powder

Tapped Density:

After carrying out the procedure as given in the measurement of bulk density the cylinder containing the sample was tapped using a suitable mechanical tapped density tester that provides 100 drops per minute and this was repeated until difference between succeeding measurement is less than 2 % and then tapped volume, V measured, to the nearest graduated unit. The tapped density was calculated, in gm per L, using the formula:

$$\text{Tap} = M / V$$

Where, Tap= Tapped Density

M = Weight of sample

V= Tapped volume of powder

Measures Of Powder Compressibility:

The Compressibility Index [Carr's Index] is a measure of the propensity of a powder to be compressed. It is determined from the bulk and tapped densities. In theory, the less compressible a material the more flowable it is. As such, it is measures of the relative importance of interparticulate interactions. In a free- flowing powder, such interactions are generally less significant, and the bulk and tapped densities will be closer in value.

For poorer flowing materials, there are frequently greater interparticle interactions, and a greater difference between the bulk and tapped densities will be observed. These differences are reflected in the Compressibility Index which is calculated using the following formulas:

$$\text{Carr's Index} = [[\text{tap} - b] / \text{tap}] \times 100$$

Where, b = Bulk Density

Tap = Tapped Density

Formulation Development of Tablets:

All the formulations were prepared by direct compression. The compositions of different formulations are given in Table 3 &4. The tablets were prepared as per the procedure given below and aim is to prolong the release of Bosentan. Total weight of the tablet was considered as 200mg.

Table 1: Formulation composition for tablets F1-F9.

Drug &Excipients	F1	F2	F3	F4	F5	F6	F7	F8	F9
Bosentan [mg]	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5
Eudragid-RSPO[mg]	31.25	62.5	41.65	0	0	0	0	0	0
EudragidS100[mg]	0	0	0	31.25	62.5	41.65	0	0	0
EudragidL100[mg]	0	0	0	0	0	0	31.25	62.5	41.65
Mag.stearate[mg]	5	5	5	5	5	5	5	5	5
Aerosil [mg]	5	5	5	5	5	5	5	5	5
MCC[mg]	97.65	65	85.5	97.65	65	85.5	97.65	65	85.5

Table 2: Formulation composition for tablets F10-F16.

Drug &Excipients	F10	F11	F12	F13	F14	F15	F16
Bosentan[mg]	62.5	62.5	62.5	62.5	62.5	62.5	62.5
HPMC-K4M[mg]	31.25	62.5	0	0	0	0	0
HPMC-K15[mg]	0	0	31.25	62.5	0	0	0
HPMC-K100[mg]	0	0	0	0	31.25	62.5	0
CHITOSAN[mg]	0	0	0	0	0	0	62.5
Mg. stearate[mg]	5	5	5	5	5	5	5
Aerosil [mg]	5	5	5	5	5	5	5
MCC[mg]	97.65	65	97.65	65	97.65	65	65

All the quantities were in mg for a total weight of 200mg.

Procedure:

1. Bosentan and all other ingredients were individually passed through sieve no ≠ 60.
2. All the ingredients were mixed thoroughly by triturating up to 15 min.
3. The powder mixture was lubricated with aerosol.
4. The tablets were prepared by using direct compression method.

Evaluation of Post Compression Parameters for Prepared Tablets[8]

The designed formulation tablets were studied for their physicochemical properties like weight variation, hardness, thickness, friability and drug content.

Weight Variation Test:

To study the weight variation, twenty tablets were taken and their weight was determined individually and collectively on a digital weighing balance. The average weight of one tablet was determined from the collective weight. The weight variation test would be a satisfactory method of deter mining the drug content uniformity. Not more than two of the individual weights deviate from the average weight by more than the percentage shown in the following table and none deviate by more than twice the percentage. The mean and deviation were determined. The percent deviation was calculated using the following formula.

$$\% \text{ Deviation} = [\text{Individual weight} - \text{Average weight} / \text{Average weight}] \times 100$$

Hardness:

Hardness of tablet is defined as the force applied across the diameter of the tablet in order to break the tablet. The resistance of the tablet to chipping, abrasion or breakage under condition of storage transformation and handling before usage depends on its hardness. For each formulation, the hardness of three tablets was determined using Monsanto hardness tester and the average is calculated and presented with deviation.

Thickness:

Tablet thickness is an important characteristic in reproducing appearance. Tablet thickness is an important characteristic in reproducing appearance. Average thickness for core and coated tablets is calculated and presented with deviation.

Friability:

It is measured of mechanical strength of tablets. Roche friabilator was used to determine the friability by following procedure. Preweighed tablets were placed in the friabilator. The tablets were rotated at 25 rpm for 4 minutes [100 rotations]. At the end of test, the tablets were re weighed, loss in the weight of tablet is the measure of friability and is expressed in percentage as

$$\% \text{ Friability} = [[W1-W2] / W] \times 100$$

Where, W1 = Initial weight of three tablets

W2 = Weight of the three tablets after testing

Determination of Drug Content:

Tablets were tested for their drug content. Ten tablets were finely powdered quantities of the powder equivalent to one tablet weight of Bosentan were accurately weighed, transferred to a 100 ml volumetric flask containing 50 ml water and were allowed to stand to ensure complete solubility of the drug. The mixture was made up to volume with water. The solution was suitably diluted and the absorption was determined by UV –Visible spectrophotometer. The drug concentration was calculated from the calibration curve.

Note: the regression value for Bosentan from standard graph is.

$$\text{Concentration [mcg/ ml]} = \frac{\text{Absorbance} - \text{Intercept}}{\text{Slope}}$$

Drug content [mg]= concentration x dilution factor

$$\%\text{Drug content} = \frac{\text{Drug content [mg]}}{\text{Lable claim [mg]}}$$

***In vitro* drug release studies**

Dissolution Parameters:

Apparatus	--	USP-II, Paddle Method
Dissolution Medium	--	0.1 N HCl , p H 6.8 Phosphate buffer
RPM	--	50
Sampling intervals [hrs]	--	0.5,1,2,3,4,5,6,7,8.
Temperature	--	37°C+ 0.5°C.

Procedure:

900ml 0f 0.1 HCl was placed in vessel and the USP apparatus –II [Paddle Method] was assembled. The medium was allowed to equilibrate to temp of 37°C+ 0.5°C. Tablet was placed in the vessel and the vessel was covered the apparatus was operated for 2 hours and then the medium 0.1 N HCl was removed and pH 6.8 phosphate buffer was added process was continued from upto 12 hrs at 50 rpm. At definite time intervals of 5 ml of the receptors fluid was withdrawn, filtered and again 5ml receptor fluid was replaced. Suitable dilutions were done with receptor fluid and analyzed by spectrophotometrically at 242 and 244 nm using UV-spectrophotometer.

Application of Release Rate Kinetics to Dissolution Data[12]:

Various models were tested for explaining the kinetics of drug release. To analyze the mechanism of the drug release rate kinetics of the dosage form, the obtained data were fitted into zero-order, first order, Higuchi, and Korsmeyer-Peppas release model.

RESULTS AND DISCUSSION

The present study was aimed to developing sustained release tablets of Bosentan using various polymers. All the formulations were evaluated for physicochemical properties and in vitro drug release studies.

Analytical Method

Analytical UV Spectrums of Bosentan in 0.1N HCl and 6.8pH Phosphate Buffers:

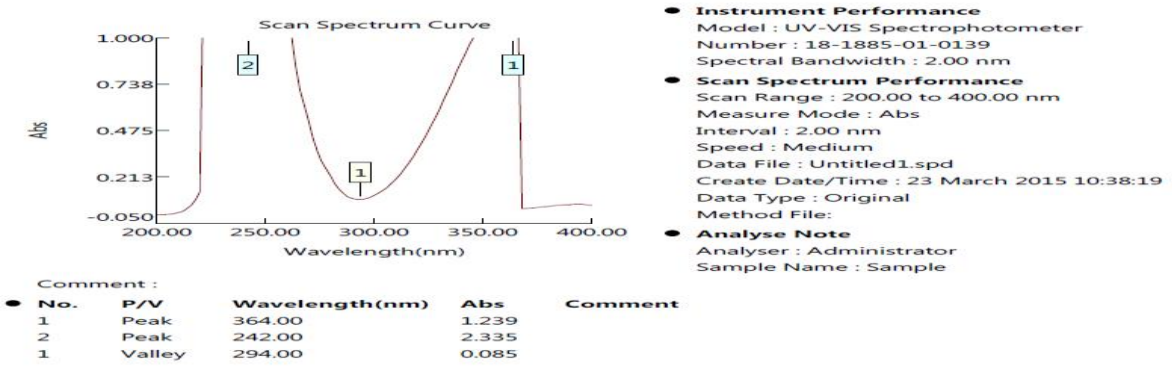


Figure 1: UV absorbance spectrum of Bosentan in 0.1N HCl.

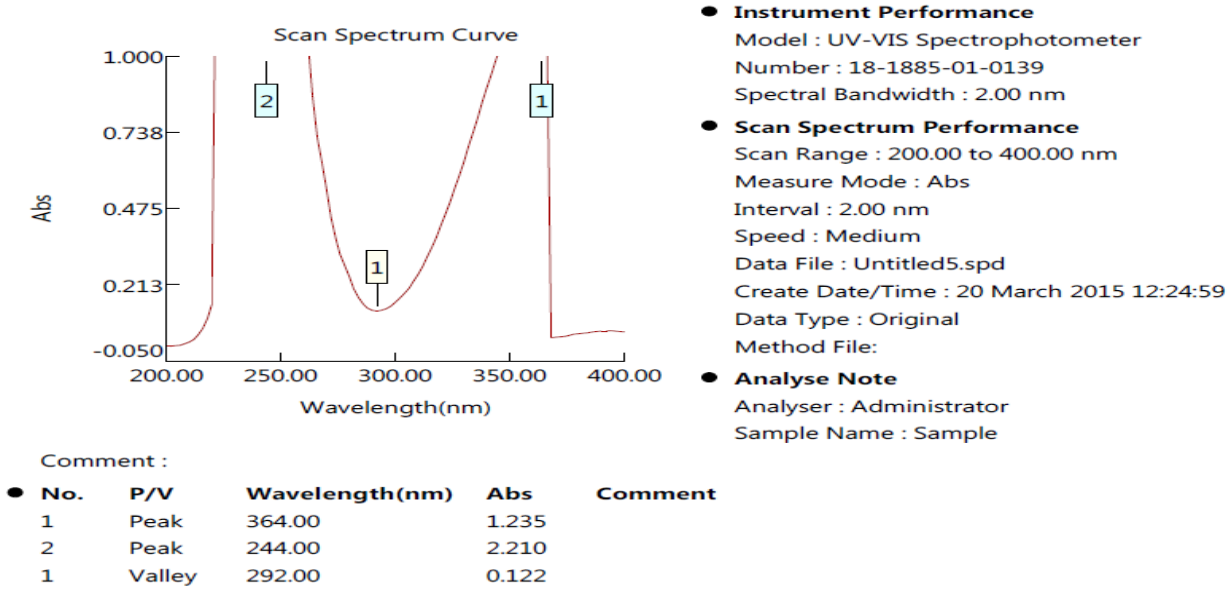


Figure 2: UV absorbance spectrum of Bosentan in 6.8pH buffer.

Graphs of Bosentan was taken in Simulated Gastric fluid [pH 1.2] and in p H 6.8 phosphate buffer at 242 nm and 244 nm respectively.

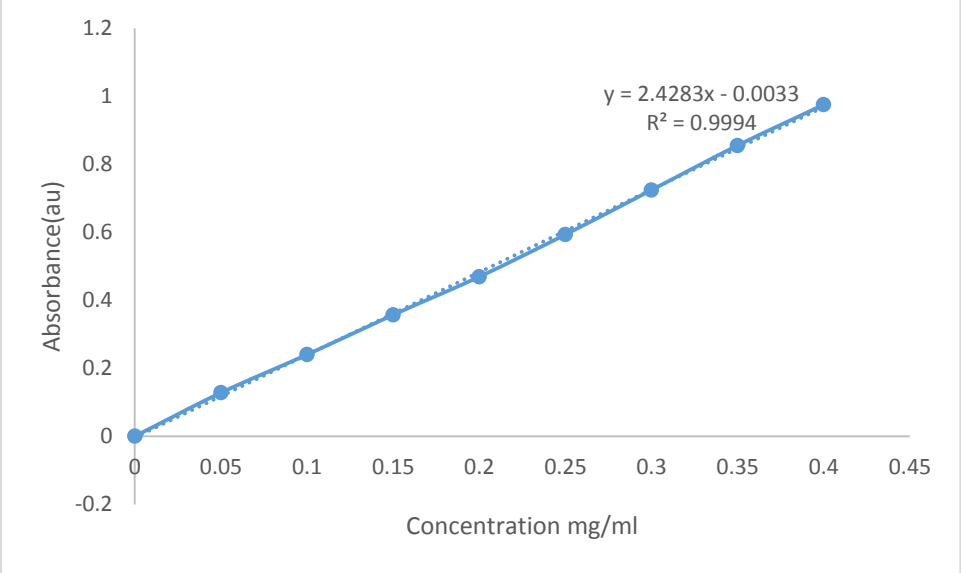


Figure 3: Standard graph of Bosentan in 0.1N HCl

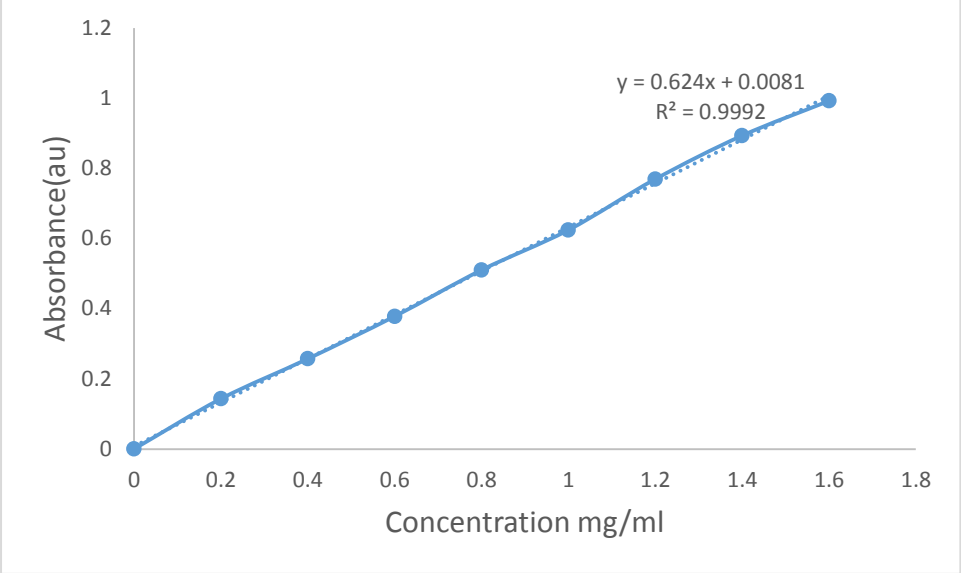
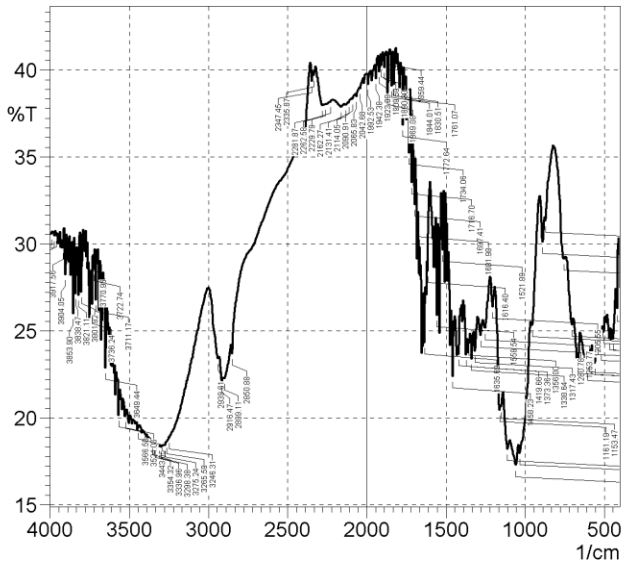


Figure 4: Standard graph of Bosentan in pH 6.8 phosphate buffer [244nm]

Drug and Excipient Compatibility Studies:

FTIR Spectrum of Pure Drug:

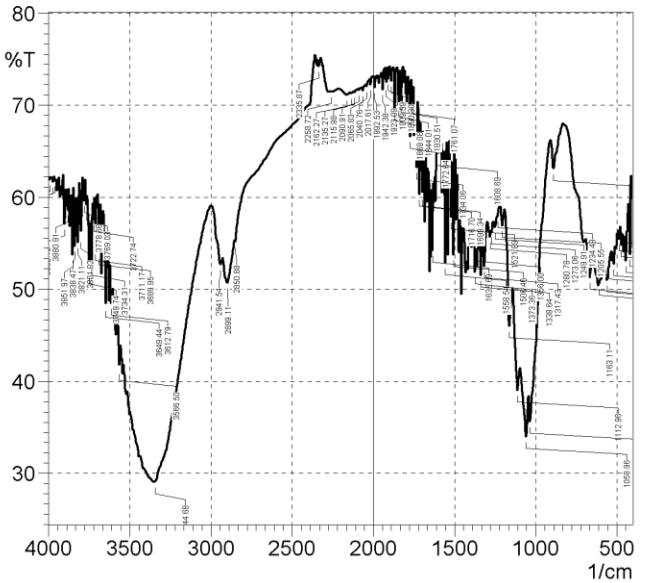


SHIMADZU

	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	420.5	26.203	1.364	422.42	412.78	5.304	0.099
2	443.64	24.556	1.378	453.29	430.14	13.764	0.291
3	466.79	24.581	0.863	480.29	462.93	10.415	0.137
4	503.44	24.943	0.601	509.22	497.65	6.92	0.069
5	518.87	23.973	0.569	520.8	511.15	5.874	0.05
6	540.09	24.286	0.096	542.02	534.3	4.715	0.006
7	559.38	23.157	0.492	563.23	551.66	7.275	0.058
8	592.17	23.316	0.379	596.02	580.59	9.643	0.031
9	605.67	22.834	0.729	623.03	597.95	15.946	0.21
10	665.46	23.222	0.503	667.39	651.96	9.525	0.052
11	704.04	25.382	0.834	740.69	698.25	24.181	0.317
12	756.12	29.155	0.668	813.99	750.33	31.387	0.268
13	871.85	31.341	0.442	875.71	823.63	24.529	0.042
14	889.21	30.126	1.906	906.57	877.64	14.658	0.383
15	954.8	25.325	1.241	962.51	908.5	29.143	0.322
16	1033.88	17.988	1.172	1041.6	964.44	53.076	2.061
17	1060.88	17.281	1.26	1095.6	1043.52	38.847	0.78
18	1112.96	18.203	1.525	1139.97	1097.53	30.451	0.784
19	1153.47	20.63	0.151	1155.4	1141.9	9.154	0.034
20	1161.19	20.47	0.974	1192.05	1157.33	21.761	0.234
21	1205.55	26.384	1.383	1220.98	1193.98	15.293	0.275
22	1253.77	25.167	1.11	1265.35	1222.91	24.636	0.414
23	1280.78	24.766	0.937	1298.14	1267.27	18.434	0.227
24	1317.43	23.865	1.36	1325.14	1300.07	15.19	0.276
25	1338.64	23.398	1.523	1346.36	1327.07	11.851	0.219
26	1356	24.287	0.222	1357.93	1348.29	5.887	0.023
27	1373.36	23.65	1.498	1383.01	1367.58	9.439	0.215
28	1419.66	23.657	0.852	1421.58	1410.01	6.934	0.05
29	1458.23	22.4	3.054	1462.09	1452.45	5.992	0.257
30	1521.89	29.686	3.324	1529.6	1514.17	7.746	0.32
31	1558.54	25.901	5.369	1562.39	1552.75	5.186	0.318
32	1616.4	28.457	1.433	1620.26	1600.97	9.671	0.066
33	1635.69	24.352	3.078	1641.48	1631.83	5.631	0.213
34	1681.98	31.097	1.078	1691.63	1680.05	5.79	0.207
35	1697.41	32.286	3.122	1710.92	1693.56	8.105	0.378
36	1716.7	33.541	3.831	1726.35	1712.85	5.947	0.221
37	1734.06	35.233	3.158	1737.92	1728.28	4.161	0.157
38	1761.07	39.002	1.039	1764.93	1755.28	3.906	0.066
39	1772.64	36.859	2.92	1776.5	1766.85	4.009	0.15
40	1830.51	39.188	1.93	1834.36	1820.86	5.339	0.133
41	1844.01	39.1	1.994	1851.72	1840.15	4.58	0.112

Figure 5: FTIR spectrum of pure drug

FTIR Spectrum of Optimized Formulation [F3]:

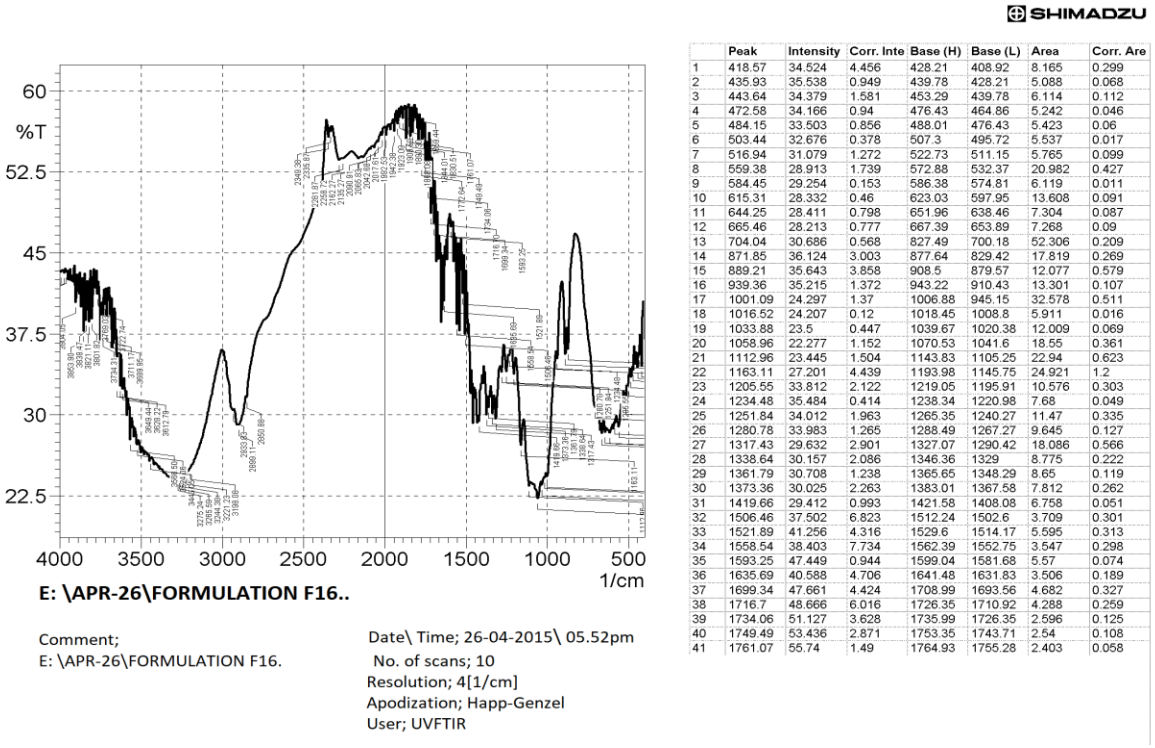


SHIMADZU

	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	418.57	53.82	6.978	428.21	414.71	3.237	0.289
2	435.93	56.27	0.713	437.86	430.14	1.84	0.017
3	443.64	53.129	2.866	449.43	439.78	2.544	0.115
4	472.58	54.807	1.787	476.43	462.93	3.43	0.068
5	484.15	55.467	1.125	488.01	478.36	2.417	0.038
6	518.87	52.745	1.014	520.8	511.15	2.607	0.041
7	547.8	52.392	0.295	549.73	540.09	2.65	0.013
8	559.38	51.024	0.731	563.23	551.66	3.331	0.043
9	607.6	50.669	0.293	611.45	597.95	3.948	0.024
10	665.46	51.333	0.851	667.39	651.96	4.28	0.041
11	891.14	63.083	3.55	908.5	833.28	13.766	0.748
12	1033.88	35.667	4.17	1041.6	910.43	38.711	1.312
13	1058.96	34.012	5.321	1095.6	1043.52	22.335	1.535
14	1112.96	39.045	5.426	1141.9	1097.53	16.402	1.188
15	1163.11	46.024	7.595	1190.12	1143.83	13.584	1.196
16	1205.55	56.948	1.939	1217.12	1192.05	5.947	0.176
17	1234.48	57.919	0.143	1236.41	1220.98	3.572	0.001
18	1249.91	56.778	0.713	1255.7	1240.27	3.743	0.048
19	1273.06	56.222	0.288	1274.99	1263.42	2.848	0.018
20	1280.78	55.722	0.761	1298.14	1276.92	5.274	0.057
21	1317.43	52.564	3.354	1327.07	1300.07	7.063	0.324
22	1338.64	51.533	3.627	1346.36	1329	4.696	0.206
23	1356	54.339	0.291	1357.93	1348.29	2.515	0.01
24	1373.36	51.88	3.107	1383.01	1367.58	4.167	0.179
25	1506.46	53.795	10.92	1512.24	1502.6	2.157	0.334
26	1521.89	57.851	6.888	1529.6	1514.17	3.295	0.361
27	1558.54	52.893	12.051	1562.39	1548.89	2.861	0.35
28	1608.69	64.742	0.667	1610.61	1599.04	2.084	0.017
29	1635.69	54.235	6.906	1641.48	1631.83	2.268	0.207
30	1699.34	60.349	6.443	1708.99	1693.56	3.074	0.378
31	1716.7	60.322	8.642	1726.35	1710.92	2.778	0.301
32	1734.06	63.289	4.943	1735.99	1726.35	1.676	0.14
33	1761.07	70.425	2.005	1764.93	1755.28	1.421	0.061
34	1772.64	66.63	5.27	1776.5	1766.85	1.538	0.154
35	1830.51	70.542	2.798	1832.44	1820.86	1.646	0.114
36	1844.01	70.033	3.792	1851.72	1840.15	1.639	0.116
37	1869.08	69.651	4.211	1872.94	1863.3	1.39	0.121
38	1890.3	72.665	1.069	1892.23	1880.66	1.547	0.028
39	1909.59	72.902	1.268	1913.45	1899.95	1.786	0.037
40	1923.09	72.269	1.651	1926.95	1915.38	1.588	0.063
41	1942.38	71.733	1.932	1950.1	1938.52	1.593	0.056

Figure 6: FTIR spectrum of optimized formulation [F3]

FTIR Spectrum of Optimized Formulation [F16]:



	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	418.57	34.524	4.456	428.21	408.92	8.165	0.299
2	435.93	35.538	0.949	439.78	428.21	5.088	0.068
3	443.64	34.379	1.581	453.29	439.78	6.114	0.112
4	472.58	34.166	0.94	476.43	464.86	5.242	0.046
5	484.15	33.503	0.856	488.01	476.43	5.423	0.06
6	503.44	32.676	0.378	507.3	495.72	5.537	0.017
7	516.94	31.079	1.272	522.73	511.15	5.765	0.099
8	559.38	28.913	1.739	572.88	532.37	20.982	0.427
9	584.45	29.254	0.153	586.38	574.81	6.119	0.011
10	615.31	28.332	0.46	623.03	597.95	13.608	0.091
11	644.25	28.411	0.798	651.96	638.46	7.304	0.087
12	665.46	28.213	0.777	667.39	653.89	7.268	0.09
13	704.04	30.686	0.568	827.49	700.18	52.306	0.209
14	871.85	36.124	3.003	877.64	829.42	17.819	0.269
15	889.21	35.643	3.858	908.5	879.57	12.077	0.579
16	939.36	35.215	1.372	943.22	910.43	13.301	0.107
17	1001.09	24.297	1.37	1006.88	945.15	32.578	0.511
18	1016.52	24.207	0.12	1018.45	1008.8	5.911	0.016
19	1033.88	23.5	0.447	1039.67	1020.38	12.009	0.069
20	1058.96	22.277	1.152	1070.53	1041.6	18.55	0.361
21	1112.96	23.445	1.504	1143.83	1105.25	22.94	0.623
22	1163.11	27.201	4.439	1193.98	1145.75	24.921	1.2
23	1205.55	33.812	2.122	1219.05	1195.91	10.576	0.303
24	1234.48	35.484	0.414	1238.34	1220.98	7.68	0.049
25	1251.84	34.012	1.963	1265.35	1240.27	11.47	0.335
26	1280.78	33.983	1.265	1288.49	1267.27	9.645	0.127
27	1317.43	29.632	2.901	1327.07	1290.42	18.086	0.566
28	1338.64	30.157	2.086	1346.36	1329	8.775	0.222
29	1361.79	30.708	1.238	1365.65	1348.29	8.65	0.119
30	1373.36	30.025	2.263	1383.01	1367.58	7.812	0.262
31	1419.66	29.412	0.993	1421.58	1408.08	6.758	0.051
32	1506.46	37.502	6.823	1512.24	1502.6	3.709	0.301
33	1521.89	41.256	4.316	1529.6	1514.17	5.595	0.313
34	1558.54	38.403	7.734	1562.39	1552.75	3.547	0.298
35	1593.25	47.449	0.944	1599.04	1581.68	5.57	0.074
36	1635.69	40.588	4.706	1641.48	1631.83	3.506	0.189
37	1699.34	47.661	4.424	1708.99	1693.56	4.682	0.327
38	1716.7	48.666	6.016	1726.35	1710.92	4.288	0.259
39	1734.06	51.127	3.628	1735.99	1726.35	2.596	0.125
40	1749.49	53.436	2.871	1753.35	1743.71	2.54	0.108
41	1761.07	55.74	1.49	1764.93	1755.28	2.403	0.058

Figure 7: **FTIR spectrum of optimized formulation [F16]**

Following the above FTIR spectrum, it was confirmed that there was no change in the functional group peaks of the pure in the optimized formulas, hence considered no interactions and compatible.

Preformulation Parameters of Powder Blend

Table 3: Pre-formulation parameters of Core blend

Formulation Code	Angle of Repose(N^0)	Bulk density [gm/ml]	Tapped density [gm/ml]	Carr's index [%]	Hausner's Ratio
F1	25.11±0.05	0.49±0.04	0.54±0.04	16.21±0.06	0.86±0.06
F2	25.67±0.02	0.52±0.09	0.52±0.04	16.87±0.05	0.98±0.05
F3	25.54±0.09	0.50±0.05	0.58±0.05	17.11±0.01	0.64±0.03
F4	25.43±0.04	0.51±0.06	0.54±0.07	17.67±0.08	1.12±0.04
F5	25.34±0.05	0.52±0.03	0.57±0.03	16.92±0.04	1.2±0.08
F6	24.22±0.04	0.53±0.04	0.56±0.06	17.65±0.09	1.06±0.09
F7	25.18±0.03	0.54±0.06	0.59±0.04	16.43±0.05	0.76±0.03
F8	24.22±0.08	0.58±0.04	0.67±0.02	17.97±0.02	1.15±0.09
F9	25.05±0.07	0.55±0.08	0.52±0.03	17.54±0.09	1.17±0.02
F10	25.12±0.02	0.49±0.04	0.54±0.04	16.23±0.06	0.67±0.04
F11	25.54±0.07	0.51±0.09	0.52±0.04	16.65±0.05	0.87±0.05
F12	25.34±0.05	0.47±0.05	0.58±0.05	17.16±0.01	0.54±0.03
F13	25.12±0.05	0.51±0.06	0.54±0.07	17.54±0.08	1.11±0.02
F14	25.23±0.04	0.53±0.03	0.57±0.03	16.16±0.04	1.24±0.05
F15	24.47±0.02	0.52±0.04	0.56±0.06	17.63±0.09	1.02±0.09
F16	25.63±0.03	0.53±0.06	0.59±0.04	16.84±0.05	0.65±0.03

N=3, ± SD standard deviation.

Tablet powder blend was subjected to various pre-formulation parameters. The angle of repose values indicates that the powder blend has good flow properties. The bulk density of all the formulations was found to be in the range of 0.43±0.07 to 0.58±0.06 [gm/cm3] showing that the powder has good flow properties. The tapped density of all the formulations was found to be in the range of 0.52±0.03 to 0.67±0.02 gm/ml. showing the powder has good flow properties. The compressibility index of all the formulations was found to be ranging from 16.16±0.04 to 17.97±0.02% which shows that the powder has good flow properties. All the formulations has shown the hausner ratio ranging between 0.64±0.03 to 1.24±0.05 indicating the powder has good flow properties.

Quality Control Parameters for Tablets:

Tablet quality control tests such as weight variation, hardness, and friability, thickness, and drug release studies in different media were performed on the compression coated tablet.

Table 4: Post compression parameters of all formulations.

Formulation codes	Weight variation [mg]	Hardness [kg/cm2]	Friability [%loss]	Thickness [mm]	Drug content [%]
F1	201.5±0.03	4.5±0.03	0.50±0.02	1.8±0.01	99.76±0.058
F2	200.4±0.05	4.5±0.02	0.51±0.05	1.9±0.02	99.45±0.02
F3	199.6±0.03	4.4±0.03	0.51±0.06	1.9±0.01	99.34±0.03
F4	200 .6±0.08	4.5±0.04	0.55±0.04	1.9±0.03	99.87±0.04
F5	199.4±0.04	4.4±0.05	0.56±0.08	1.7±0.03	99.14±0.05
F6	200.7±0.02	4.5±0.04	0.45±0.06	1.5±0.02	98.56±0.02
F7	202.3±0.09	4.1±0.05	0.51±0.07	1.4±0.04	98.42±0.08
F8	201.2±0.05	4.3±0.04	0.49±0.06	1.7±0.03	99.65±0.04
F9	199.3±0.06	4.5±0.02	0.55±0.04	1.6±0.01	99.12±0.06
F10	203.4±0.05	4.5±0.03	0.51±0.03	1.8±0.03	99.24±0.06
F11	198.6±0.07	4.4±0.03	0.52±0.04	1.4±0.04	99.34±0.04
F12	200.1±0.06	4.5±0.02	0.53±0.02	1.7±0.02	99.51±0.03
F13	199.2±0.08	4.4±0.01	0.57±0.08	1.8±0.02	99.26±0.04
F14	200.4±0.06	4.5±0.03	0.45±0.04	1.9±0.01	98.38±0.01
F15	202.3±0.03	4.1±0.04	0.54±0.05	1.7±0.03	98.42±0.03
F16	201.2±0.07	4.3±0.02	0.46±0.02	1.6±0.04	98.13±0.01

N=3, ±SD standard deviation.

In-vitro Quality Control Parameters for Tablets

All the parameters such as weight variation, friability, hardness, thickness and drug content were found to be within limits.

***In-Vitro* Drug Release Studies**

Table 5: Dissolution profile of Bosentan F1-F9

Time[hr]	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
0.5	25.54±0.06	20.13±0.05	16.43±0.04	17.25±0.04	16.42±0.08	14.62±0.05	10.4±0.03	9.4±0.06	8.5±0.08
1	46.73±0.03	39.46±0.04	26.77±0.07	38.26±0.06	25.73±0.02	19.86±0.08	16.5±0.08	15.6±0.03	14.5±0.06
2	76.57±0.08	55.38±0.02	34.62±0.04	54.16±0.04	36.63±0.06	22.35±0.07	28.6±0.04	21.4±0.08	18.4±0.04
3	98.41±0.05	75.34±0.02	42.45±0.07	72.01±0.07	45.04±0.01	31.45±0.05	39.5±0.05	36.7±0.06	23.4±0.08
4		87.36±0.06	55.42±0.03	88.26±0.08	58.25±0.08	39.80±0.03	48.5±0.08	42.4±0.04	28.2±0.06
5		99.43±0.04	67.40±0.04	97.10±0.01	65.33±0.05	45.25±0.05	59.4±0.01	49.6±0.06	34.8±0.04
6			85.45±0.01		76.41±0.01	58.24±0.04	69.2±0.05	55.3±0.04	40.2±0.07
8			91.54±0.08		84.84±0.08	66.73±0.02	74.5±0.08	60.3±0.05	44.8±0.01
12			98.15±0.06		92.80±0.04	71.34±0.01	82.3±0.01	72.8±0.05	50.4±0.05

Table 6: Dissolution Data of Bosentan Tablets F10-F16

Time [hrs]	F10	F11	F12	F13	F14	F15	F16
0	0	0	0	0	0	0	0
1	10.45±0.04	10.45±0.04	14.56±0.05	10.54±0.08	11.56±0.05	13.65±0.05	15.67±0.06
2	20.46±0.06	21.78±0.08	21.67±0.07	21.56±0.04	25.75±0.07	19.78±0.07	22.78±0.07
3	32.65±0.05	27.76±0.06	34.62±0.02	29.87±0.01	37.74±0.04	28.18±0.01	34.76±0.03
4	48.71±0.01	38.76±0.07	48.43±0.04	39.11±0.02	49.54±0.04	38.89±0.08	43.78±0.07
5	56.62±0.06	45.87±0.08	58.92±0.02	44.98±0.08	56.27±0.02	48.67±0.06	62.18±0.08
6	69.35±0.03	55.63±0.06	63.43±0.04	56.92±0.02	66.75±0.05	59.91±0.01	79.15±0.07
8	77.51±0.01	69.43±0.03	77.13±0.01	68.77±0.07	79.63±0.06	69.41±0.04	85.26±0.04
12	81.54±0.04	79.56±0.05	85.34±0.03	77.65±0.05	85.75±0.05	76.98±0.08	96.64±0.02

N=3,±SD standard deviation.

From the dissolution data it was evident that the formulations prepared with guar gum as polymer were unable to retard the drug release up to desired time period i.e., 12 hours.

Whereas the formulations prepared with Eudragit RSPO, chitosan retarded the drug release in the concentration of 41.25 mg showed required release pattern i.e., retarded the drug release up to 12 hours and showed maximum of 98.15±0.06% in 12 hours with good retardation. Also F16 formulation shows 96.64±0.02% as it is prepared with chitosan. Both F3, F16 are the best formulations among the all 16 formulations.

Release Kinetics:

Kinetic Studies for F3 Formulation

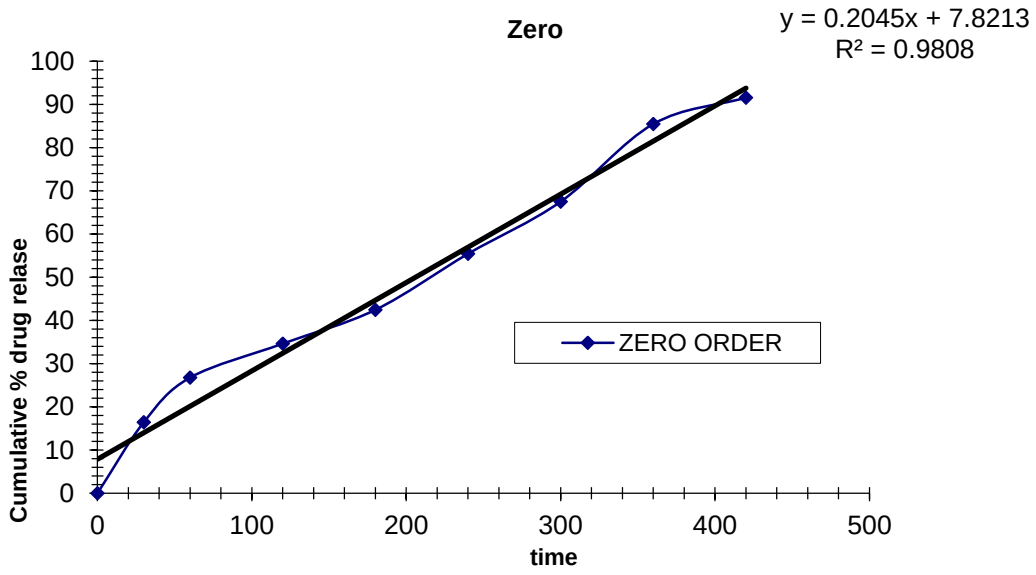


Figure 8: Zero order kinetics for F3 formulation.

To analyse the mechanism of the drug release rate kinetics of the dosage form, the data obtained were graphed as:

- The graph plotted against Percentage drug released and square root of time is the Higuchi's plot and the R^2=0.951
- The graph plotted against Log percentage drug remaining and time is the First order kinetics and the R^2=0.906
- The graph plotted against Log drug released and log time Peppas plot and the R^2=0.974,n value found to be 0.633.
- The graph plotted against cumulative Percentage drug released and time is the zero order kinetics and the R^2=0.980.

From observing the above values it is evident that the optimised formulation [F3] follows the zero

order release kinetics.

Kinetic Studies For F16 Formulation

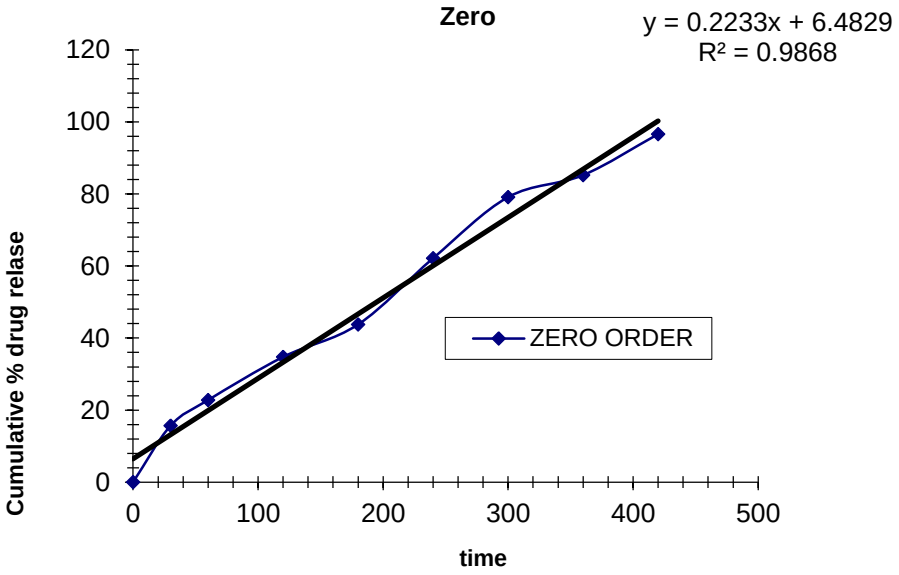


Figure 9: Zero order release kinetics for F16 formulation

To analyze the mechanism of the drug release rate kinetics of the dosage form, the data obtained were graphed as:

- The graph plotted against Percentage drug released and square root of time is the Higuchi's plot and the R^2=0.954
- The graph plotted against Log percentage drug remaining and time is the First order kinetics and the R^2=0.874
- The graph plotted against Log drug released and log time Peppas plot and the R^2=0.874,n value found to be 0.706.
- The graph plotted against cumulative Percentage drug released and time is the zero order kinetics and the R^2=0.986

From observing the above values it is evident that the optimised formulation [F16] follows the zero order release kinetics.

Stability studies for the optimised formulations:

Table 7: Stability studies for optimised formulation [F3]

S.No	Duration	25^0C[75%RH]	37^0C[75%RH]
1	1 MONTH	97.85%	97.92%
2	2 MONTH	97.35%	97.80%
3	3MONTH	97.10%	97.75%

N=1.

By observing the stability studies it is concluded that the optimised formulation is stable through

the entire period of 3 months and the drug release profile is also intact throughout the time being.

CONCLUSION

In the present research work sustained release tablets of Bosentan are prepared by direct compression method using different grades of HPMC and Eudragit and chitosan as polymers. The formulations are checked for pre compression parameters and compatibility. The prepared tablets were evaluated for various physicochemical parameters which are shown in results chapter above and found to be within the limits. The best formulation is selected using dissolution data both F3, F16 showed 98.15±0.06, 96.64±0.02% respectively in 12 hours. And considered best among the prepared formulations, both F3, F16 formulations followed zero order release kinetics. These formulations subjected to stability testing and it is confirmed that the deterioration is minimum and acceptable.

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