Formulation and Evaluation of Bilayer tablet of Selexipag as a Sustained release and Ambrisentan as an Immediate release for Pulmonary Arterial Hypertension treatment.

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Abstract:

The present study aimed to develop a bilayer tablet of Selexipag (a selective non-prostanoid prostacyclin receptor agonist) and Ambrisentan (selectively blocking endothelin type A receptors) to treat pulmonary arterial hypertension. The bilayer tablet was formulated to reduce the frequency of administration. The tablets were formulated using super disintegrants such as Sodium Starch Glycolate in immediate release and polymers such as ethyl cellulose and methyl cellulose in sustained release. Bilayer tablets were prepared by wet granulation.

Selexipag is a selective non-prostanoid prostacyclin receptor agonist, and Ambrisentan is an endothelin receptor antagonist widely used for the treatment of Pulmonary Arterial Hypertension. (PHA). The present work involves the bi-layer tablet for the sustained release of

Selexipag using Methylcellulose and ethyl cellulose as a retardant polymer and for the Immediate release of Ambrisentan using sodium starch glycolate as a super disintegrant.

Keywords: Ambrisentan, Selexipag, Pulmonary Arterial Hypertension, Bi-layer tablet, Sustained release tablet, Immediate release tablet

Introduction

Tablet dosage form

Tablets are solid preparations that are mainly intended for administration orally. Tablets are made by compressing in accordance with the amounts of particles and contain a single dose of one or more active ingredients. Tablets are the most often recommended dosage form because they are easy to use, offer dosage homogeneity from tablet to tablet, are stable over long periods and under various storage circumstances, and can be made using high-speed compression, labelling, and packing equipment. (1) The goal of tablets is to produce the correct pharmacological reaction. Tablets are solid dosage forms made by dry granulation, moist granulation, or direct compression. According to the dosage form and delivery route, many varieties of tablets are produced. (2) Depending on the quantity of therapeutic ingredients and the intended manner of administration, they vary in size and weight. It is the most widely used dose form, and tablets make up about 70% of all medications prescribed. (3)

Immediate Release Drug Delivery System (4)

The term "immediate release" refers to pharmaceutical formulations in which the drug is released and absorbed without any intentional delay or modification through formulation techniques. In such formulations, commonly used diluents or carriers do not significantly slow down the drug's release or absorption.

Sustained Release Drug Delivery System

The primary objective of therapy is to maintain a consistent blood concentration that remains both effective and safe over an extended duration.. Various drug delivery strategies, such as sustained release, controlled release, extended action, and timed-release systems, have been developed to support this goal. (5) The term-controlled release has become associated with those systems from which therapeutic agents may be automatically delivered at predetermined rates over a long period. (6)

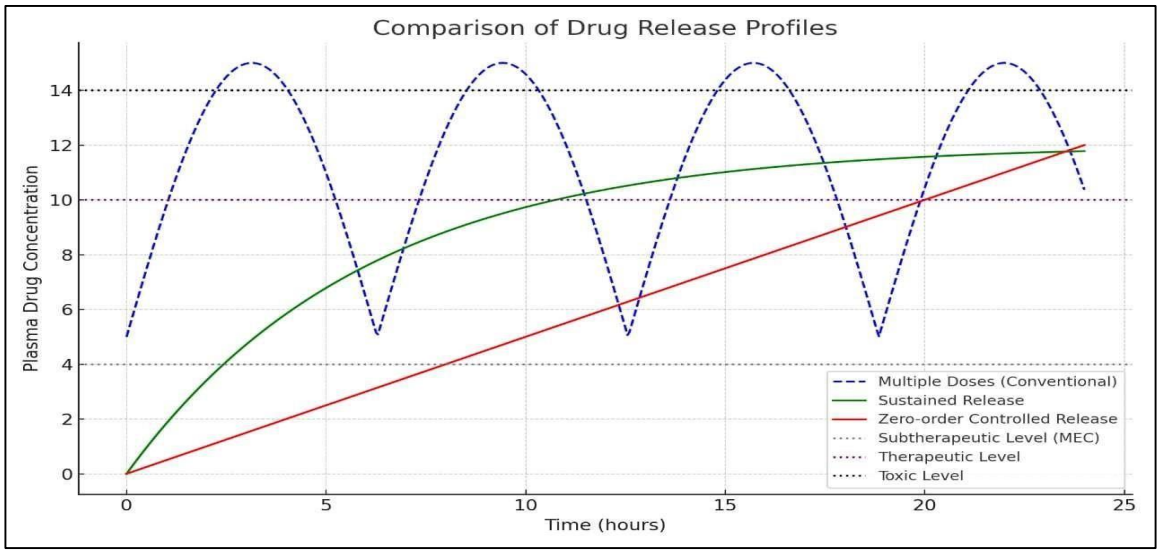


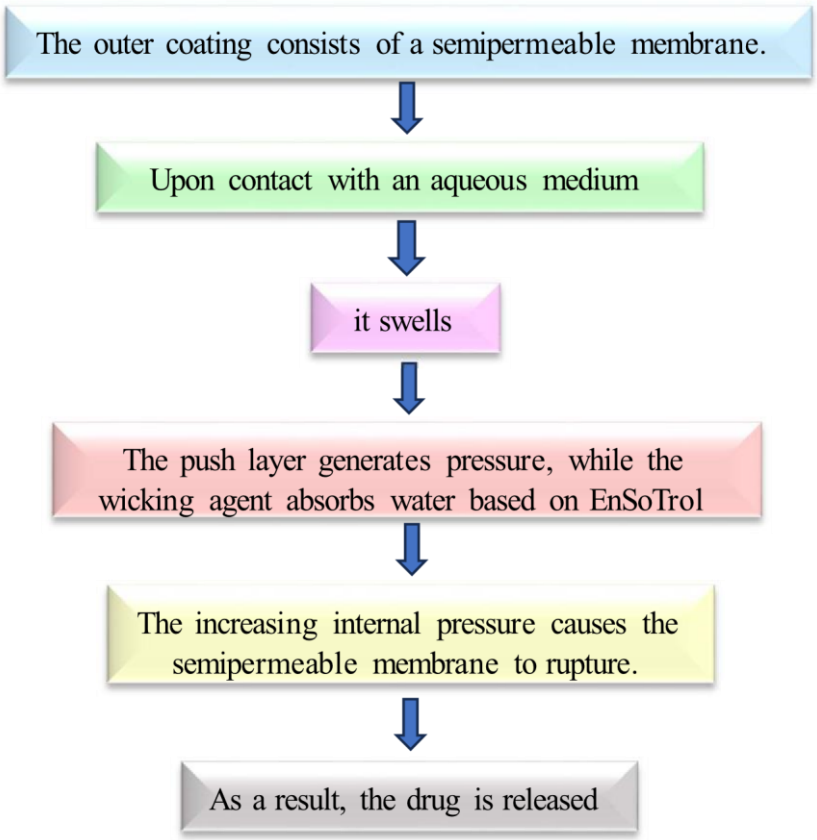
fig no. 1: A Hypothetical Plasma Concentration Vs. Time Profile.

Bilayer tablet (7)

Bilayer tablets represent a novel advancement in the development of controlled-release formulations, offering several advantages over traditional dosage forms. This bilayer tablet technology addresses the limitations associated with conventional single-layer tablets. Typically, the immediate-release layer, containing Superdisintegrants, facilitates a rapid onset of action (loading dose), while the sustained-release layer ensures a prolonged therapeutic effect (maintenance dose).

Various techniques for bilayer tablets (8)(9)

General mechanism of release of drug of all techniques



Pulmonary Arterial Hypertension

Pulmonary arterial hypertension (PAH) is a progressive disease marked by increased pressure in the pulmonary arteries, eventually leading to failure of the right ventricle. Over the past decade, treatment options have significantly expanded, with six medications now approved by the U.S. Food and Drug Administration, leading to improved patient outcomes. Recent advancements in understanding its development and prognosis, the latest treatment strategies, and emerging therapeutic targets for the future. (10)

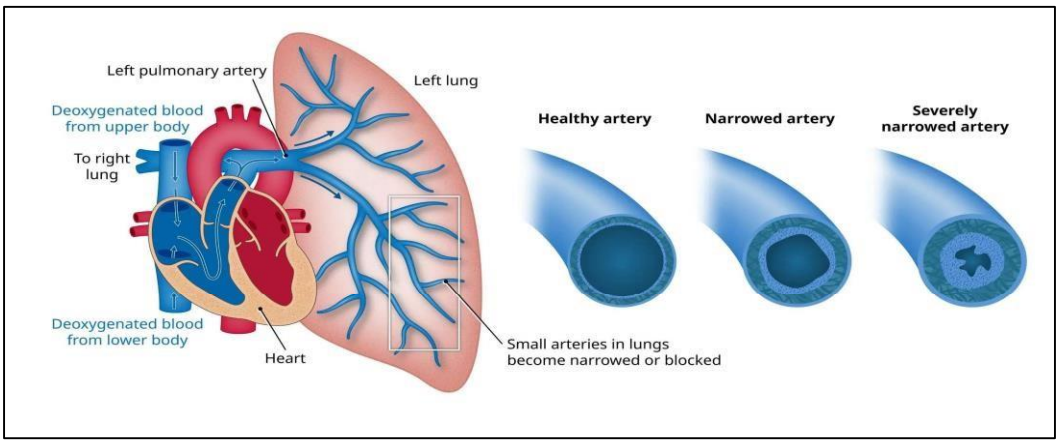


Fig no 2: Pulmonary Arterial Hypertension

MATERIALS AND EQUIPMENTS

6.1 Materials and their suppliers

Table No.1: Materials and their suppliers

Sr.no	Ingredients	Company name
1	Selexipag	Alembic Pharmaceutical Limited, Kharakhadi.
2	Ambrisentan	Mylan lab. Limited Vizianagaram
3	Strach	s d Fine-Chem limited, Mumbai
4	Sodium starch glycolate	Dolphin Chemicals, Mumbai
5	Gelatin	Balaji drugs, Surat
6	Propyl paraben	Modern Industries, Nashik
7	Methyl paraben	Modern Industries, Nashik
8	Talc	Loba Chemie, Palghar
9	Magnesium stearate	s d Fine-Chem limited, Mumbai
10	Lactose	Dolphin Chemicals, Mumbai
11	Aerosil	s d Fine-Chem limited, Mumbai
12	Methylcellulose	Dolphin Chemicals, Mumbai
13	Ehylcellulose	Dolphin Chemicals, Mumbai
14	PVP K30	Modern Industries, Nashik

EXPERIMENTAL WORKS

9.1 PREFORMULATION STUDY

Organoleptic Properties

Medications typically have distinct smells and preferences, and unpleasant ones are masked later in the formulation process. This includes using descriptive terminology to note the new drug's colour, odour, and taste. Early batch colour records are extremely useful in establishing appropriate specifications for later production.

Determination of melting point:

The drug was administered through a capillary with one end sealed with a flame. The vein containing the drug was dipped in liquid paraffin inside the melting point apparatus. The melting point was the first indicator of sample purity because a relatively small amount of impurity can be detected by a decrease and an increase in the melting point range. melting point of the drug can also be determined using the melting point apparatus.

Solubility studies

The solubility of drugs was determined by using various solvents.

The solubility of Selexipag was determined in water and DMSO.

The solubility of Ambrisentan was determined in 0.1 N NaOH.

UV-Spectroscopic scanning -spectral analysis:

Determination of λmax of Selexipag:

Selexipag 10 mg was accurately weighed, and stock solution (100µg/ml) was prepared. The stock solution was further diluted using DMSO to get serial dilutions. A dilution was kept in a cuvette. The UV spectrum was recorded using a double-beam UV-visible spectrophotometer in the range of 200nm-400nm wavelength.

Preparation of standard curve:

A stock solution of Selexipag (100µg/ml) was prepared by dissolving 10mg of the drug in DMSO, and the final volume was made to 100 ml. The solutions in the concentration range of 5-25µg/ml were prepared by appropriate dilutions of stock solutions. The UV absorbance of these solutions was determined spectrophotometrically at λmax 306 nm.

Determination of λmax of Ambrisentan

Ambrisentan (10mg) was accurately weighed, and 100µg/ml stock solution was prepared. The stock solution was further diluted using 0.1N NaOH to get dilutions. The UV spectrum was recorded using a double-beam UV-visible spectrophotometer in the range of 200-400nm wavelength.

Preparation of standard curve

A stock solution of Ambrisentan was prepared by dissolving 10mg of Ambrisentan in 0.1N

NaOH, and the final volume was made to 100 ml. The solutions in the concentration range of 5-25µg/ml were prepared by appropriate dilutions of stock solution. The UV absorbance of these solutions was determined spectrophotometrically at λmax 264 nm.

9.2 DRUG-EXCIPIENTS COMPATIBILITY STUDY

Differential Scanning Calorimetry

Thermo gram was recorded on Ta instruments/Q 20 models. DSC thermograms of Selexipag, Ambrisentan, a mixture of Selexipag, Ambrisentan, and excipients (1:1:1). The drug exhibited a sharp melting endotherm at 134°C, 165°C respectively. No change in the endotherm of the drugs was observed in the mixture, which indicates no interaction between the excipients and drugs.

FORMULATION OF BILAYER TABLET

Table no. 2 Formulation table of the sustained release layer

Sr.no	Ingredients	SR1	SR2	SR3	SR4	SR5	SR6	SR7	SR8	SR9
1	Selexipag	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
2	Ethyl cellulose	25	37.5	50	-	-	-	12.5	18.75	25
3	Methyl cellulose	-	-	-	25	37.5	50	12.5	18.75	25
4	Starch	177.1	164.1	152.1	177.1	164.1	152.1	177.1	164.1	152.1
5	PVP k-30	37.5	37.5	37.5	37.5	37.5	37.5	37.5	37.5	37.5
6	Methyl paraben	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125
7	Propyl paraben	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125
8	Magnesium stearate	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
9	Talc	5	5	5	5	5	5	5	5	5
10	Tartrazine	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
11	Total weight (mg)	250	250	250	250	250	250	250	250	250

All quantities are expressed in mg.

Table no. 3: Formulation table of Immediate Release Layer

Sr.no	Ingredients	IR1	IR2	IR3	IR4	IR5	IR6	IR7	IR8	IR9
1	Ambrisentan	37.5	37.5	37.5	37.5	37.5	37.5	37.5	37.5	37.5
2	Starch	30	45	60	-	-	-	15	22.5	30
3	Sodium Starch glycolate	-	-	-	30	45	60	15	22.5	30
4	Lactose	178.2	163.2	148.2	178.2	163.2	148.2	178.2	163.2	148.2
5	Gelatin	45	45	45	45	45	45	45	45	45
6	Methyl paraben	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
7	Propyl paraben	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
8	Aerosil	3	3	3	3	3	3	3	3	3
9	Talc	6	6	6	6	6	6	6	6	6
10	Total weight (mg)	300	300	300	300	300	300	300	300	300

PREPARATION OF GRANULES

Preparation of sustained-release granules of Selexipag.

- Granulation done by wet granulation method.
- Weigh all the ingredients and pass through #60 mesh.
- Mixed all ingredients except lubricant and added binder solution of PVP K30 and formed uniform dough mass and pass through # 8 mesh.
- Dried granules at 60ºC in a hot air oven for 15 minutes.
- The dried granules were then sieved with #16 mesh.
- Then Added magnesium stearate and Talc and mixed for 2 min.

- Then compressed into a tablet.

Preparation of immediate-release granules of Ambrisentan.

- Granulation done by wet granulation method.
- Weigh all the ingredients and pass through #60 mesh.
- Mixed all ingredients except lubricant and added binder solution of gelatin and formed uniform dough mass and passed through #8 mesh.
- Dried granules at 60ºC in a hot air oven for 15 minutes.
- The dried granules were then sieved with #16 mesh.
- Added Talc and Aerosil and mixed for 2 min.
- Then compressed into a tablet.

Pre-compression parameters for granules.

Bulk density:

The granules' bulk density was measured using bulk density equipment. The weight of granules was taken. Granules were taken into cylinders, and volume was measured. It can be measured by using the formula.

$$\text{Bulk Density} = \text{Mass/Volume}$$

Tapped Density:

Granules were placed in the tapped density tester and tapped 100 times. After 100 taps final volume was measured. Measured by using the formula.

$$\text{Tapped Density} = \text{Mass/Tapped volume}$$

Compressibility index:

The compressibility index gauges the ow characteristics of the powder being compressed. It is expressed in % and was determined using Carr's compressibility index formula Carr's compressibility index (%) = [(Tap Density-Bulk density)/Tap density] × 100

Hausner ratio:

The flowability of the powder or granules is associated with the Hausner's ratio or this indirectly measures the flowability of powder.

Hausner ratio = Tap density / Bulk density

The angle of repose:

The funnel method was used to calculate the angle of repose, which represents the granules' ow characteristics. The carefully measured powder was put into a funnel. The funnel's height was modified such that its tip just touched the highest point of the powder mound. Onto a clean surface, the powders were allowed to ow freely through the funnel. The powder cone's diameter was measured. It was calculated using the following formula.

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

h = height of the pile, r

= radius of the pile

EVALUATION OF BILAYER TABLET (Post compression parameters) Uniformity of

weight:

The weight variation test was taken out to ensure that the weight was uniform of the tablets in a batch. The total weight of 20 tablets from formulation was determined, and the average was calculated.

Hardness:

The Tablet was held along its oblong axis in between the two jaws of the tester. At this point, the reading should be zero kg/cm² .The value at this point was noted in kg. The constant force was applied by rotating the knob until the Tablet fractured. For each formulation, the hardness of the Tablet was determined using the Monsanto hardness tester. Tablet hardness and strength are essential to see that the Tablet can with shock and stress during manufacturing, packaging, and transportation and while handled by the patient.

Thickness:

The shape and size of a tablet would vary based on the tooling used in tablet manufacturing. The crown size is measured by using a micrometre, and a sliding calliper scale is used to measure the length of 5 to 10 at a time on a laboratory scale; the Vernier calliper measures tablet size.

Friability:

Twenty tablets were rotated in a friability (Roche) at 25 rpm for 4 min. The tablets were then deducted, and the loss in weight due to fracture or abrasion was recorded as percentage weight loss (% friability)

$$\text{Friability (\%)} = \text{Initial Weight} - \text{Final Weight}$$

Drug Content:

Preparation of standard stock solution of Selexipag:

Selexipag, equivalent to 30 mg, was accurately weighed. DMSO was added and sonicated for 10 min. The volume was made up to 100 ml with DMSO. 2 ml of the solution was diluted with DMSO up to 10 ml. The absorbance of the solution was measured at 306 nm.

Preparation of standard stock solution of Ambrisentan:

Ambrisentan, equivalent to 25 mg, was accurately weighed. 0.1N NaOH was added and sonicated for 10 min. The volume was made up to 100 ml with 0.1N NaOH. 2 ml of the solution was diluted with 0.1N NaOH up to 10 ml. The absorbance of the solution was measured at 264 nm.

Preparation of sample solution

Twenty tablets were accurately weighed, and the average weight was calculated. Powdered the tablets. Powder equivalent to 550 mg was weighed and transferred into a 100 ml standard ask. After that, the powder was then dissolved in DMSO and sonicated. The volume was prepared up to 100 ml with DMSO. 2 ml of the solution was diluted with DMSO up to 10 ml. The absorbance of the resulting solution was measured at 306 nm and 264 nm, respectively. The amount of both drugs is determined.

In vitro dissolution studies of SR tablets:

The release rate of Selexipag from Bilayer tablets was determined up to 12 hrs. using a USPtype II dissolution testing paddle apparatus. The dissolution medium of 900 ml containing phosphate buffer 6.5 was maintained at 37.0 ± 0.5°C. A 5 ml sample was withdrawn at specific time intervals, and the same volume of the fresh medium was replaced. The withdrawn samples were diluted with pH 6.5, filtered, and analyzed on a UV at 306 nm. Percentage cumulative drug release was calculated.

In vitro dissolution studies of IR tablets:

The release rate of Ambrisentan from Bilayer tablets was determined up to 60 minutes using a USP-type II dissolution testing paddle apparatus. The dissolution medium of 900 ml containing phosphate buffer 6.5 was maintained at 37.0 ± 0.5°C. The withdrawn samples were diluted with pH 6.8, filtered, and analyzed on a UV spectrophotometer at 264nm. Percentage cumulative drug release was calculated.

In-Vitro Disintegration Time: (Immediate release layer)

In vitro disintegration time was performed by apparatus specified in USP at 50 rpm. Phosphate buffer pH-6.8, 900 ml was used as the disintegration medium, the temperature of which was maintained at 37±2°C, and the time in seconds taken for complete disintegration of the Tablet with no palpable mass remaining in the apparatus was measured in seconds.

Kinetic modelling of drug release

To find out the mechanism of drug release. Optimized formulations of bilayer tablets of Selexipag and Ambrisentan were prepared and subjected to in vitro drug release studies. The results obtained from in vitro release studies were plotted in different kinetic models of release data are given as follows: 1. Cumulative % of drug release vs. time (zero order rate kinetics) 2. Log cumulative % drug retained vs. time (first-order rate kinetics) 3. Log cumulative % drug release vs. square root of time (Higuchi's classical diffusion equation) 4. Log cumulative % drug release vs. log time (Pappas exponential equation).

Stability Studies:

Stability studies were conducted according to the ICH guidelines. The prepared Bilayer tablet was kept at three different temperatures. The first formulation was kept at room temperature, the second was held at a cold temperature, and the third was kept in the stability chamber at a temperature of 40°C/ 75%RH for three months. At the end of 3 months, samples were withdrawn, observed for physical appearance, and investigated for % drug release.

Table No.4: ICH Q1A (R2) Accelerated Stability Guideline

Sr.no	Stability Study Storage conditions general case study	Storage Conditions	The maximum Period covered by data at submission

1	Long term	1)25°C,60%RH±5%RH 2)30°C±2°C,65%RH±5%RH	3 Months
2	Intermediate	30°C±2°C,65%RH±5%RH	2 Months
3	Accelerated	40°C±2°C.75%RH±5%RH	1 Months

RESULT & DISCUSSION

Characterization of drug:

Table no.5: Organoleptic properties of drug

Sr. no	Sample	Colour	Odour	Taste	Melting point	Solubility
1	Selexipag	Off White Powder	Odorless	Bitter	134 c □	DMSO
2	Ambrisentan	White Powder	Odorless	Bitter	150 c □	0.1N NaOH

UV-Visible Spectroscopic scanning-spectral analysis:

Determination of UV Absorbance Maxima of Selexipag

A suitable analytical method was developed for Selexipag using UV spectroscopy, and an analytical wavelength of λmax 306 nm was identified in DMSO. Calibration curves were constructed in these media. The R2 value was 0.9994 for the DMSO solution. Beer-Lambert law obeyed in the range of 5 25µg/ml.

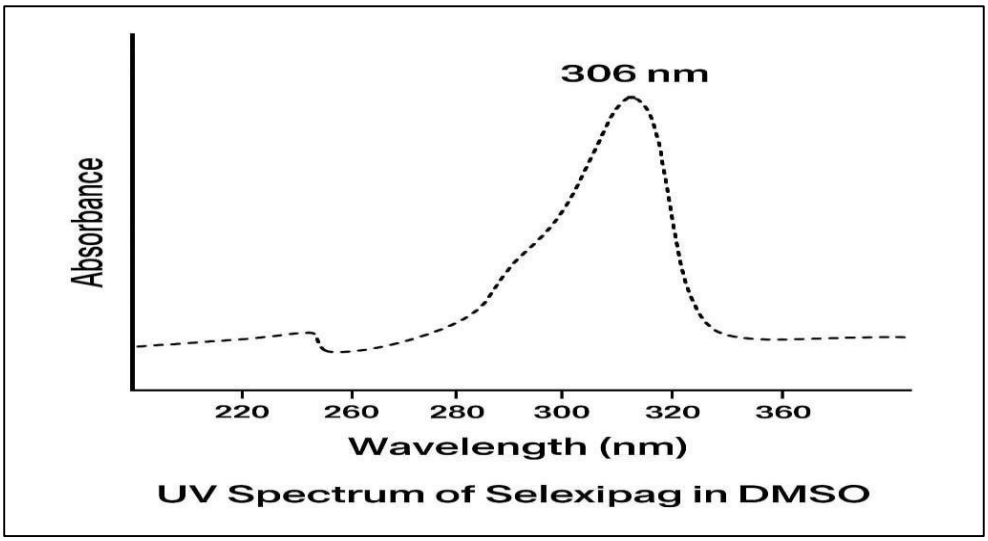


Fig no.3: Wavelength maxima of Selexipag in DMSO

Standard Calibration curve of Selexipag

The standard calibration curve was plotted as an absorbance vs. concentration in a DMSO solution. The absorbance value is plotted in Table. The beer range of the Selexipag obeyed was 5-25 µg/ml.

Table No. 6: Standard calibration curve data for Selexipag in DMSO.

Sr.no	Conc. (µg/ml)	Abs. at (306nm)
1	0	0
2	5	0.188497
3	10	0.375249
4	15	0.519478
5	20	0.696869
6	25	0.855639

Fig no.29: Calibration curve of Selexipag in DMSO (Linearity of Selexipag)

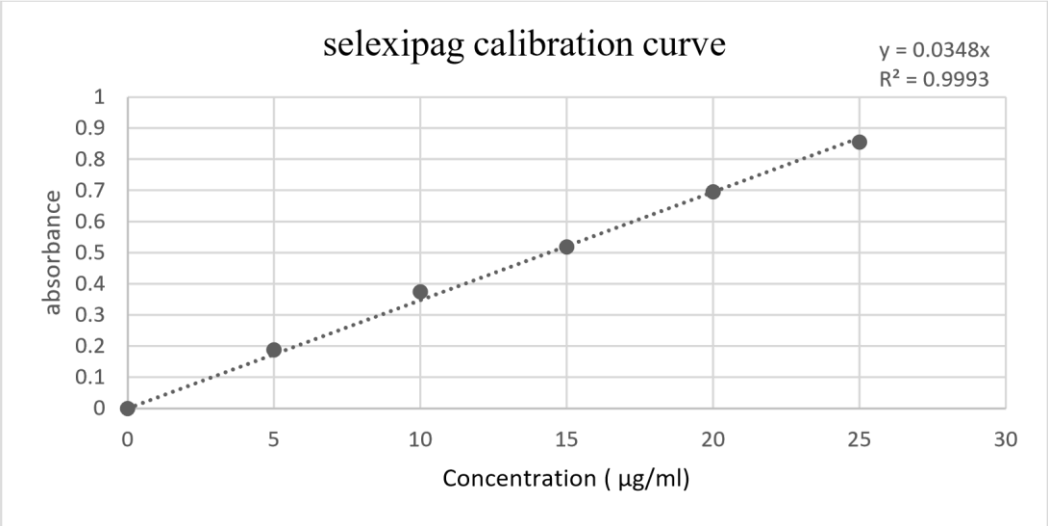


Fig no.3: Selexipag calibration curve

Determination of UV Absorbance Maxima of Ambrisentan

A suitable analytical method was developed for Ambrisentan using UV spectroscopy, and an analytical wavelength of λmax 264 nm was identified in 0.1N NaOH. Calibration curves were constructed in these media. The R2 value was 0.9994. Beer-Lambert law obeyed in the range of 5-25µg/ml.

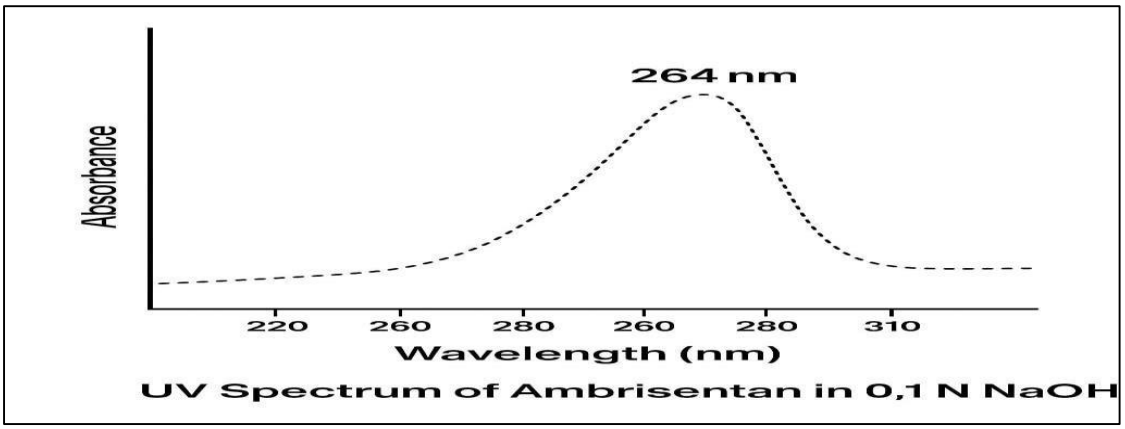


Fig no.4: Wavelength maxima of Ambrisentan in 0.1N NaOH.

Standard Calibration curve Ambrisentan

The standard calibration curve was plotted as absorbance vs concentration in 0.1 N NaOH. Standard calibration curve data for Ambrisentan in 0.1 N NaOH.

Table no.7: Standard calibration curve data for Ambrisentan in 0.1N NaOH

Sr.no	Conc. (µg/ml)	Abs. at (306nm)
1	0	0
2	5	0.207892
3	10	0.399456
4	15	0.657234
5	20	0.865123
6	25	1.052346

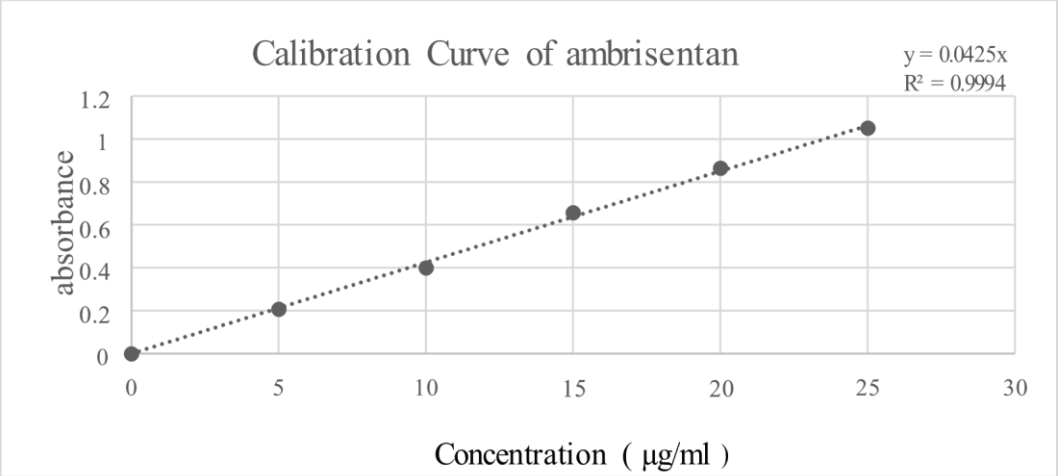


Fig no.5: Calibration Curve of Ambrisentan

DRUG-EXCIPIENT COMPATIBILITY STUDIES

Differential Scanning Calorimetry (DSC) study:

DSC thermograms of Selexipag, Ambrisentan and a mixture of Selexipag + Ambrisentan+ Excipients are depicted in Fig. The drug exhibited a sharp melting endotherm at 134°C and 165°C, respectively. No change in the endotherm of the drug was observed in the mixture, which indicates the absence of any interaction between the drug and the excipient.

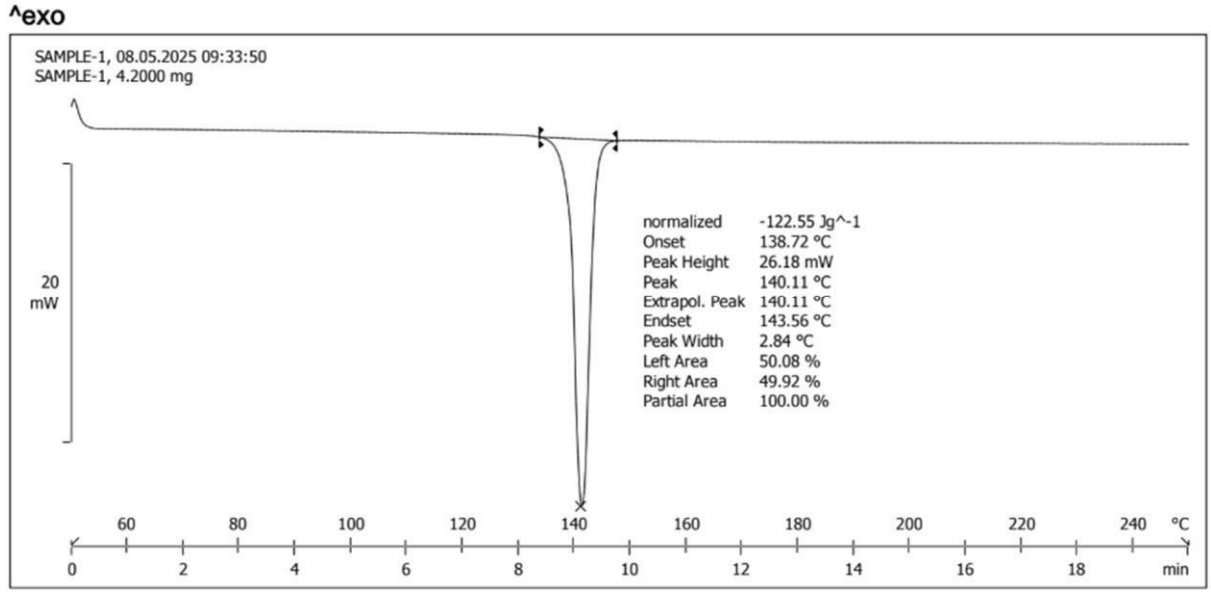


Fig no. 6: - DSC Thermogram of API Selexipag

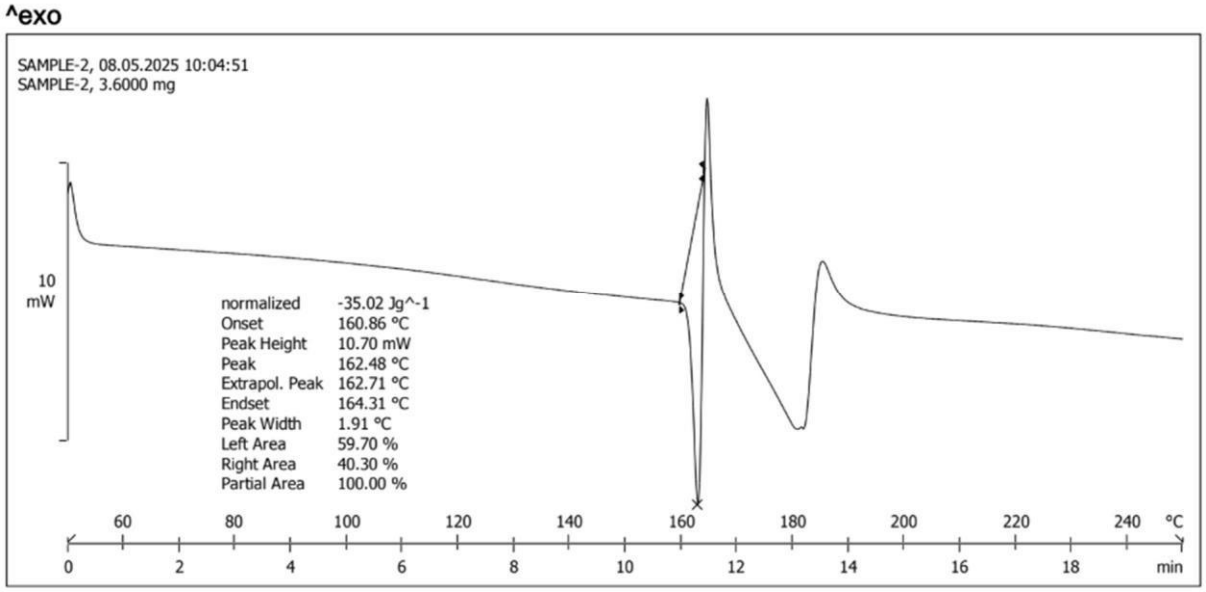


Fig no. 7: - DSC Thermogram of API Ambrisentan

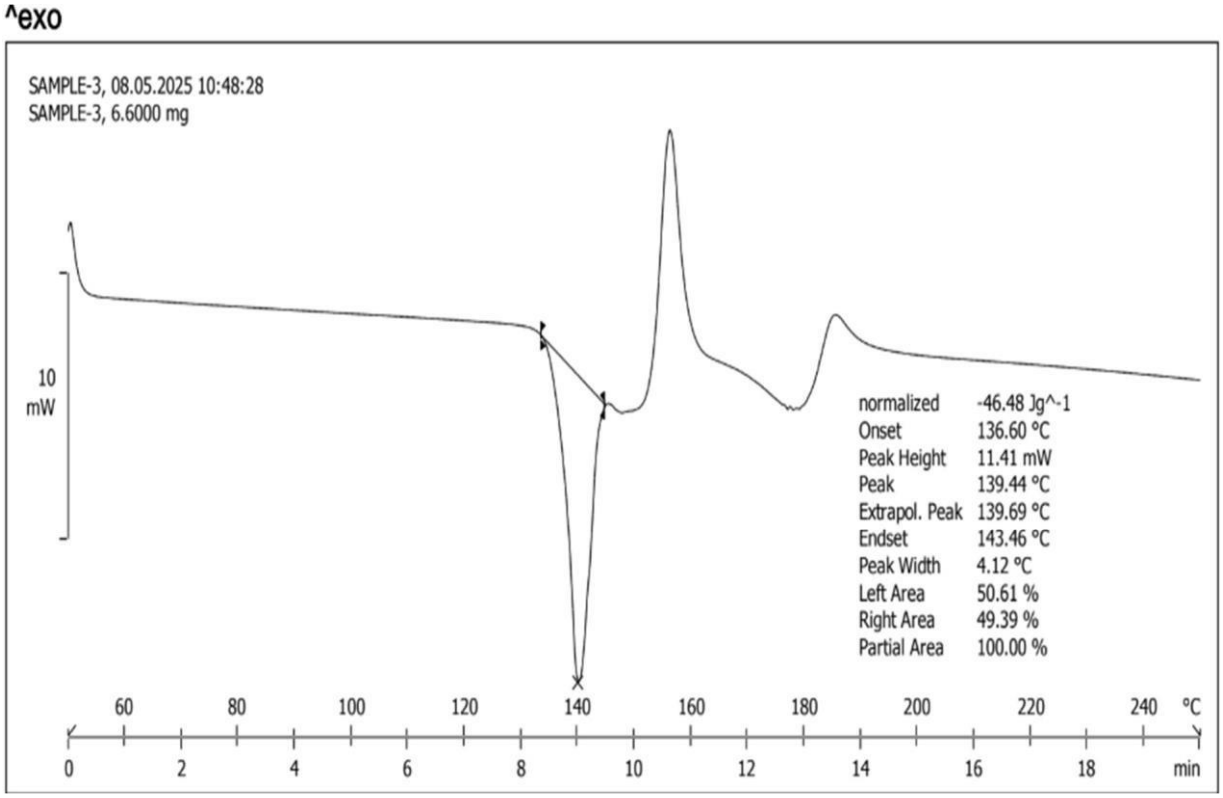


Fig no. 8: DSC Thermogram of API Selexipag + API Ambrisentan

10.3 PRE-COMPRESSION PARAMETERS FOR GRANULES (SUSTAINED RELEASE)

Pre compression parameters of immediate release layer and Sustained release layer granules shows Angle of repose, Carr's index, Hausner's ratio are in the range given in official standards.

Table 8: Pre compression parameters for Sustained release layer granules (Selexipag)

Formulation	Bulk density (g/ml)	Tapped density (g/ml)	Carr's index (%)	Hausners ratio	Angle of repose (θ)
SR1	0.48±0.005	0.54±0.004	11.11±0.22	1.13±0.073	29.45±0.18
SR2	0.54±0.004	0.62±0.003	12.90±0.10	1.15±0.026	29.41±0.15
SR3	0.43±0.002	0.50±0.005	14.00±0.21	1.16±0.034	30.85±0.24

SR4	0.58±0.005	0.67±0.003	13.43±0.45	1.16±0.026	28.52±0.19
SR5	0.51±0.003	0.58±0.003	12.06±0.22	1.14±0.029	31.23±0.31
SR6	0.54±0.010	0.60±0.007	10.00±0.32	1.11±0.045	28.52±0.35
SR7	0.53±0.004	0.60±0.006	11.66±0.43	1.13±0.061	29.98±0.17
SR8	0.47±0.004	0.54±0.002	12.96±0.42	1.16±0.029	33.47±0.24
SR9	0.50±0.008	0.56±0.003	10.71±0.23	1.12±0.034	23.49±0.26

PRE-COMPRESSION PARAMETERS FOR GRANULES (IMMEDIATE RELEASE)

Table no 9: Pre compression parameters for Immediate release layer granules (Ambrisentan)

Formulation	Bulk density (g/ml)	Tapped density (g/ml)	Carr's index (%)	Hausners ratio	Angle of repose (θ)
IR1	0.32±0.005	0.38±0.003	15.78±0.10	1.18±0.029	28.85±0.24
IR2	0.34±0.008	0.40±0.002	15.00±0.10	1.17±0.045	30.12±0.19
IR3	0.38±0.005	0.44±0.003	13.63±0.41	1.16±0.025	29.25±0.26
IR4	0.33±0.004	0.38±0.007	13.15±0.22	1.16±0.071	25.45±0.35
IR5	0.37±0.002	0.42±0.004	11.9±0.31	1.15±0.034	29.22±0.32
IR6	0.33±0.002	0.37±0.002	10.81±0.22	1.12±0.029	26.58±0.19
IR7	0.34±0.005	0.38±0.005	10.52±0.21	1.13±0.024	29.25±0.24
IR8	0.37±0.004	0.42±0.003	11.90±0.45	1.15±0.022	28.45±0.15
IR9	0.36±0.010	0.40±0.006	10.00±0.19	1.12±0.026	22.38±0.17

POST COMPRESSION PARAMETERS FOR BILAYER TABLET

Table shows post compressional parameters i.e. Weight variation, Thickness, Hardness, Friability, Drug content, Disintegration time within the acceptable official limits.

Table no.10: Post compression parameters of bilayer tablet

Batch	Uniformity of weight (mg)	Thickness (mm)	Hardness (kg/cm^{2})	Friability (%)
F1	550.00±1.4	4.81±0.04	5.1±0.05	0.22±0.04
F2	549.60±1.6	4.30±0.07	5.3±0.03	0.24±0.06
F3	549.97±1.6	4.28±0.14	5.3±0.07	0.28±0.02
F4	550.30±1.5	4.31±0.02	5.9±0.05	0.24±0.02
F5	551.27±1.4	4.34±0.07	5.2±0.03	0.24±0.06
F6	550.30±1.5	4.31±0.02	5.9±0.05	0.22±0.02
F7	549.10±1.5	4.50±0.02	5.5±0.02	0.26±0.04
F8	548.23±1.6	4.33±0.08	5.8±0.03	0.24±0.03
F9	550.03±1.6	4.30±0.06	4.1±0.03	0.28±0.06

Batch	Drug Content (%)		In Vitro Disintegration time (min) IRL
	SR	IR	
F1	90.09±0.32	90.44±0.78	3.0±1.00
F2	92.07±0.57	94.30±0.53	2.5±1.01
F3	96.27±0.52	97.29±0.59	2.9±1.00
F4	97.01±0.49	98.30±0.44	4.8±1.03
F5	96.31±0.58	97.23±0.58	6.0±1.00
F6	96.30±0.40	96.24±0.56	3.0±1.22
F7	97.43±0.56	96.28±0.58	2.9±1.03

F8	97.58±0.54	97.28±0.59	3.0±1.15
F9	99.14±0.58	99.23±0.49	2.0±1.00

10.5 IN-VITRO DRUG RELEASE STUDY

The in vitro dissolution study of the formulated Sustained release tablets is given in table no.18 and fig no.38 and immediate release tablets is given in table no. 19 and fig no. 39

Table no: 11: In-vitro Drug Release studies formulations (SR1-SR9) Selexipag (SRL)

Time (hours)	Percentage Cumulative Drug Release profile (%)								
-	SR1	SR2	SR3	SR4	SR5	SR6	SR7	SR8	SR9
0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
1	15.616	10.714	13.607	14.435	22.960	11.931	12.009	20.774	23.109
2	26.602	20.159	16.828	18.097	32.125	14.337	16.855	28.510	33.821
3	35.460	28.981	21.167	21.071	36.451	17.040	25.779	39.556	38.153
4	42.265	38.941	28.034	28.166	43.500	21.043	41.143	51.643	45.222
5	47.004	43.597	32.325	32.337	47.778	26.069	45.746	56.772	57.975
6	54.862	47.135	41.506	42.325	58.928	29.260	57.964	62.145	63.840
7	62.805	53.979	47.972	50.504	66.110	42.650	68.081	70.585	71.951
8	68.591	58.552	57.390	65.589	72.904	50.342	73.663	72.012	74.629
9	70.641	65.599	63.680	71.824	80.711	65.523	75.159	73.022	81.720
10	74.538	72.515	73.063	78.191	-	76.181	76.141	76.577	85.767
11	76.255	74.896	75.700	84.364	-	78.995	78.535	78.681	93.514
12	78.404	77.833	78.920	-	-	80.327	82.071	86.422	99.690

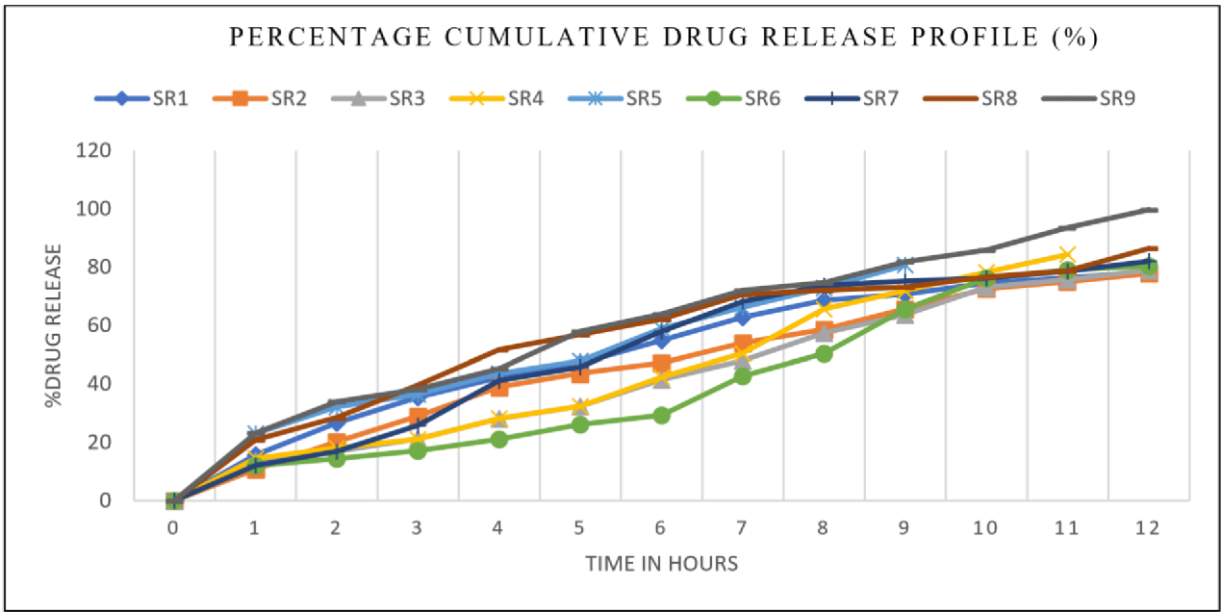


Fig no. 9: In-Vitro Release of Selexipag for batches

Table no.12: In-vitro Drug Release studies formulations (IR1-IR9) Ambrisentan (IRL)

Time (Min)	Percentage Cumulative Drug Release profile (%)								
-	IR1	IR2	IR3	IR4	IR5	IR6	IR7	IR8	IR9
0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
10	35.802	35.802	47.554	27.158	29.277	35.351	31.275	35.802	48.066
20	49.974	58.501	62.232	49.201	40.572	42.986	44.538	58.500	63.275
30	62.204	73.330	71.880	69.193	52.710	60.681	55.451	73.331	74.830
40	74.274	75.699	73.648	71.753	70.484	73.752	77.252	85.699	87.283
50	75.271	78.318	76.935	74.257	77.444	75.585	84.440	87.318	94.636
60	78.533	80.025	78.835	76.661	80.886	79.303	85.537	90.025	98.993

Percent cumulative drug release from batch (IR1-IR9) immediate reales Ambrisentan

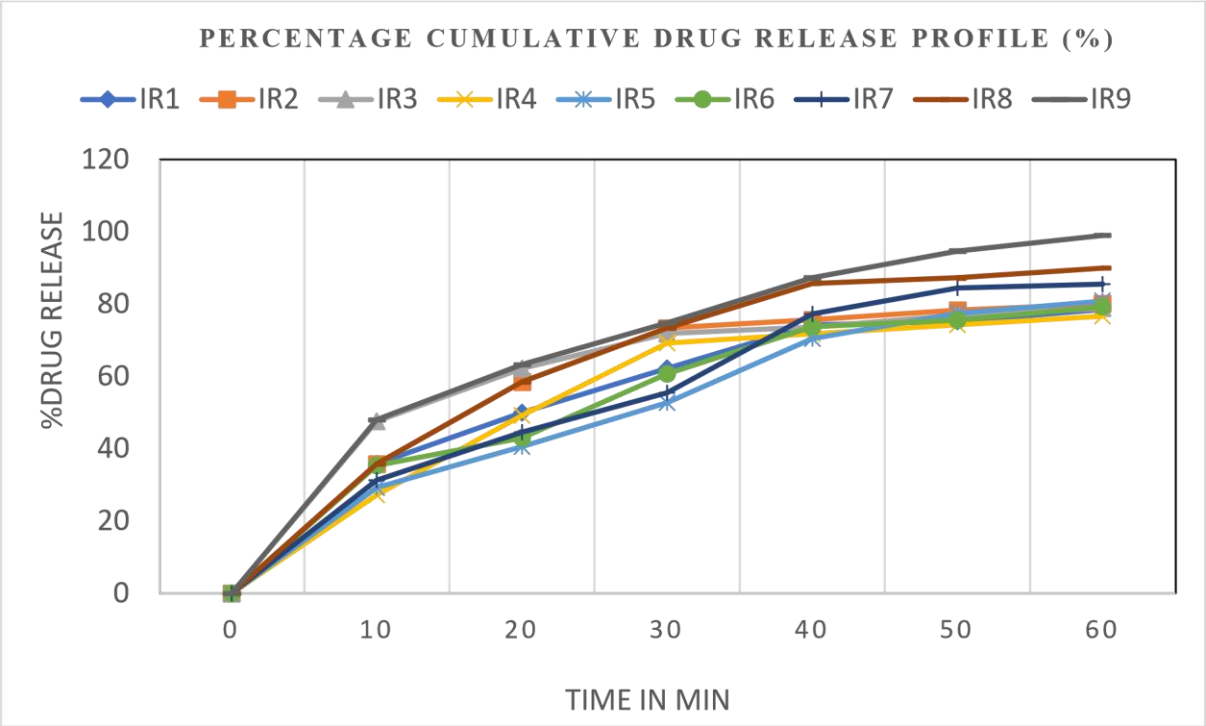


Fig no. 10: In-Vitro Release of Ambrisentan for batches (IR1-IR9)

RELEASE KINETICS

Release kinetics for sustained release layer:

Dissolution data of the optimized batch SR9 was fitted to various mathematical models like zero-order, First-order, Higuchi, Korsmeyer-Peppas and Hixson Crowell models in order to describe the kinetics of drug release. The smallest value of the sum of squared residuals (SSR), PCP dissolution software and best goodness-of-t test (R2) were taken as criteria for selecting the most appropriate mode. The Korsmeyer-Peppas model was the best-t model for batch SR9.

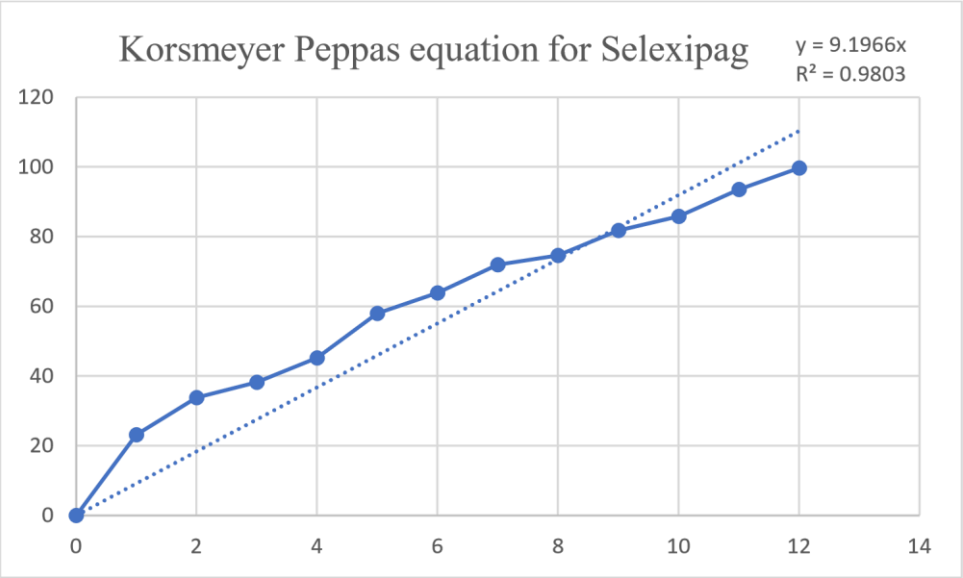


Fig no. 11: Korsmeyer Peppas equation for Selexipag

Table 13: Kinetics Release of SR9 optimize formulation

Models	Zero order	1st order	Higuchi	Korsmeyer-Peppas	Hixon-Crowel
R^2 Value	0.8953	0.9718	0.9764	0.9950(best fit model)	0.9806

Release kinetics for immediate release layer:

Dissolution data of the optimized batch IR9 was fitted to various mathematical models like zero-order, First-order, Higuchi, Korsmeyer-Peppas and Hixson Crowell models in order to describe the kinetics of drug release. The smallest value of the sum of squared residuals (SSR), PCP dissolution software and best goodness-of-t test (R2) were taken as criteria for selecting the most appropriate mode. A Higuchi kinetic model was the best-t model for batch IR9.

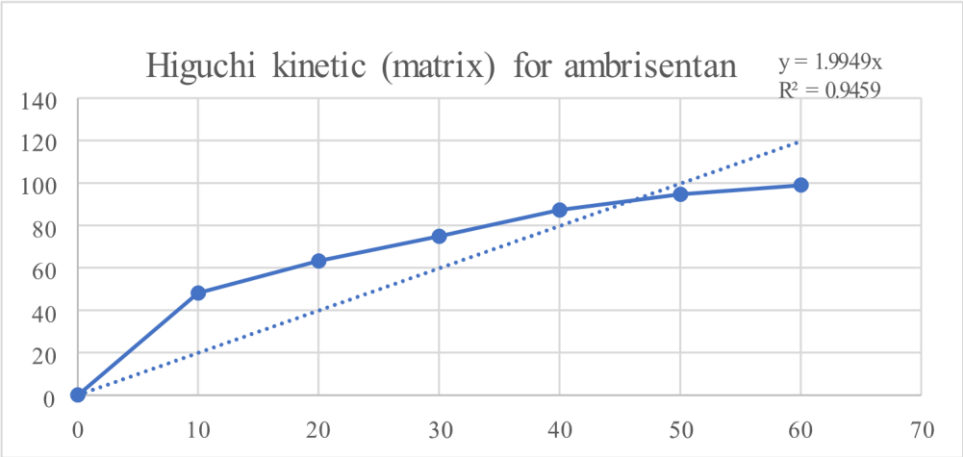


Fig. no. 12: Higuchi kinetic equation for Ambrisentan

Table 14: Kinetics Release of IR9 optimize formulation

Models	Zero order	1st order	Higuchi	Korsmeyer-Peppas	Hixon-Crowel
R^2 Value	0.7090	0.9876	0.9983 (Best fit model)	0.9897	0.9728

10.7 Stability study of optimized batch

A stability study was carried out at 40 ± 2°C and 75 ± 5% RH for up to 3 months in a stability chamber. At the end of one month, two-month, three-month tablets were evaluated for drug content. There were no observable significant modifications in any of the studied parameters during the study period.

Table no.15: Stability study of optimized formulation(f9)

Time Interval	Drug content (%)	
	Selexipag	Ambrisentan
0 day	99.14±0.58	99.23±0.49
30th day	98.06±0.34	98.17±0.31
60th day	97.56±0.23	97.89±0.40

90th day	97.01±0.14	97.31±0.19

Conclusion :

All the formulations were evaluated for physical characteristics, drug content, dissolution and release kinetics and stability study. The tablets were formulated using by wet granulation technique. The formulated tablets were found within the limits with respect to uniformity of weight, hardness, thickness and friability. The drug content of the bilayer tablet was estimated by simultaneous estimation, and it was within limits. Based on in vitro dissolution studies, Immediate Release formulation F9 was optimized and selected, which contains Sodium Starch Glycolate (25%) as a super disintegrant; drug release was found to be 99.83% drug release within 60 minutes.Based on in vitro dissolution studies, Sustained Release formulation F9 was optimized and selected, which contains Ethyl cellulose and Methylcellulose (12.5%: 12.5%) in the 1:1 ratio and drug release was found to be 99.69 % in 12 hours. To reduce the frequency of administration and to improve patient compliance, a bilayer tablet was prepared successfully.

So, from immediate release study formulation IR9 and sustained release study formulation SR9 were selected as the best formulation of each layer. Selexipag is a selective prostacyclin IP receptor agonist used for the long-term treatment of pulmonary arterial hypertension (PAH). It helps to dilate the pulmonary arteries and reduce vascular resistance. Ambrisentan is an endothelin receptor antagonist that works by blocking endothelin-1, a potent vasoconstrictor, thus reducing pulmonary blood pressure. It is effective for immediate symptom relief in PAH.

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