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pH-dependent pulsatile delivery of Ambrisentan for the treatment of hypertension

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ABSTRACT: The greatest number of mortality in the world occurred due to hypertension. Several bodily functions are based on circadian rhythm including hypertension. The stimulation of the sympathetic nervous system in the early morning hours resulted in accelerating blood pressure which is a highly dangerous condition. This lead to cardiac failure and damage to the vital organs of the body. The current research focuses on pulsatile delivery of Ambrisentan for the treatment of high blood pressure which released the medicament at the right time and the right place. Primarily, core tablets were prepared with Ambrisentan. The compatibility of Ambrisentan with excipients was confirmed by FTIR analysis. The core tablets were evaluated for flowing characteristics which showed better flowing behavior and optimized core batch CT6 showed consolidation index 16.07±0.11 and angle of repose 27.02±0.14. Moreover, due to the least disintegration time (2.23±0.10) and greater dissolution release of 99.70 % ±0.49, CT6 was selected as an optimized core tablet for pulsatile delivery. Further, the combination of Eudragit L100 and ethyl cellulose were utilized for coating purposes in different proportion were utilized for achieving the desired lag time of 6 hrs. The optimized batch PC6 (Eudragit L100: EC 10) which comprised of 25:75 proportion showed a lag time of 6 hours, cumulative release of 99.25 % and content uniformity of 99.29±0.82. The dissolution release profile predicted zero-order release for Ambrisentan pulsatile tablets. The accelerated stability testing showed minimal loss of drug content when exposed at 40 ^{0}C and 75 % relative humidity without altering lag time.

KEYWORDS: Ambrisentan; Pulmonary hypertension; Pulsatile delivery; Eudragit L100; Ethylcellulose.

1. INTRODUCTION

Hypertension is a chronic critical condition that arises when the blood pressure of the body goes beyond the normal range (> 130/80 mmHg) [1]. High blood pressure is an extreme risk factor in the progressive development of cardiovascular diseases (CVD) and is also associated with damage to other essential organs of the body. A large number of the population in the world suffer from kidney failure, heart attack and stroke due to hypertension [2]. The prime reason for mortality in the world is mainly due to CVD which accounts for > 17.9 million deaths annually out of 31 % of total deaths. The reported data estimated that > 874 million people suffered due to hypertension and had an annual mortality rate of 9.4 % [3].

Hypertension increases with the age and is also observed during modern lifestyle changes including intake of fast foods or processed food containing high amounts of sodium, low potassium intake in the diet, alcohol consumption as a regular habit or way of enjoyment, obesity, smoking and lack of exercises [4]. The several dysfunctioning reported in the body such as sodium homeostasis, renin-angiotensin-aldosterone system (RAAS), sympathetic nervous system and swelling leads to the development of hypertension [5] [6]. Moreover, pulmonary arterial hypertension (PAH) is defined as when the blood pressure in the arteries exceeds normal (>20-30 mmHg) in the lungs and heart. During the Covid-19 pandemic, a large population experiences a rise in the blood pressure due to the direct attack on the lungs and rapid release of a cytokine such as interleukins (IL-1, 2, 6, and 10). These are inflammatory and create excessive stress, thereby resulting in the dysfunctioning of the system responsible for inducing hypertension [7].

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The oscillations in our body which are completed in 24 hours are known as Circadian rhythms [8]. Various essential functions of the body are regulated via circadian rhythm which includes the release of hormones, blood pressure, etc. During circadian rhythm, it is noticed that blood pressure goes down in the night cycle and increases rapidly in the early morning (early morning surge) and also in the afternoon. The mechanistic approach for early morning surge is attributed due to the stimulation of the sympathetic nervous system [9]. The temporal rhythm of the body strongly impacted the number of diseases along with the pharmacokinetics and pharmacodynamics of maximum molecules in use [10]. Chronopharmaceutics include pharmaceutical applications of chronobiology in drug delivery. The main objective of chronotherapy in the therapy of numerous diseases is to convey the drug in greater quantity during the time of extreme requirement consistent with the circadian onset of the disease or syndrome [11].

The zero order or first order release can be achieved through oral controlled release dosage forms in which the active ingredient is released at a markedly fixed amount per unit time. The time-release products are a more effective tool in chronopharmacotherapy, because of their unique drug release characteristics following circadian rhythms in physiologic and pathophysiologic functions [12] [13]. A time-release formulation could permit drug release at a greater plasma drug concentration specifically when clinical signs are developed. Disease conditions such as early morning symptoms of blood pressure, asthma, arthritis, and ischemic heart disease, a time-controlled pulsatile drug delivery system offer more benefits [14].

A pulsatile drug delivery system (PDDS) is defined as the ''system which releases the drug rapidly and completely after a lag time, thus providing spatial and temporal delivery and increasing patient compliance'' [15]. PDDS is differentiated from conventional for offering improved bioavailability, reduced adverse effects and tolerability, no risk of dose dumping, improved stability, patient comfort and compliance and achieved a unique release pattern [16].

Ambrisentan is categorized as an endothelin receptor antagonist and utilized for the therapy of PAH. Ambrisentan avoids the constriction of muscles in the blood vessels and thereby relaxes them which resulted in the reduction of blood pressure. It is suitable for both idiopathic and connective tissue diseases. Ambrisentan is administered orally and showed higher bioavailability at raised pH [17]. The current research focused on the development of pulsatile tablets of Ambrisentan for the treatment of early morning surges in blood pressure and thereby providing the right drug at the right time and right place.

2. RESULT AND DISCUSSION

2.1. Preformulation

Ambrisentan existed as white to colorless powder. The melting point was determined by digital melting point apparatus which showed in the range of 165-167 ^{0}C. The loss on drying (LOD) of Ambrisentan powder was found to be 0.36% ±0.29.

2.2. Estimation of solubility

Ambrisentan is practically insoluble in water (0.005mg/ml), hence needs to improve its solubility. The solubility was checked in several polar and organic solvents and indicated soluble in most of the organic solvents like ethanol, and acetone. Thereafter, solubility was checked in various non-volatile solvents and found the best solubility in PEG 400. As PEG 400 is liquid in nature addition of it, converting solid powder into liquid medicament and again transforming into the solid increases the weight of the tablets. Hence, a solid form of PEG 6000 was blended with Ambrisentan by solid dispersion method in various ratios (1:1, 1:2, 1:3 and 1:4). The desired improvement of Ambrisentan was observed with a 1:4 ratio, hence selected for further processing.

2.3. FTIR analysis

Identification of received sample of Ambrisentan was subjected towards FTIR (Affinity, 1s, Shimadzu, Japan) and conclude that pure sample of Ambrisentan from the below peaks. The C-O-C stretching was observed at 1172 and 1192 cm^{-1}, aromatic carbon ring at 1567, 1751 and 1975 cm^{-1}, methylene C-H bending at 1446 cm^{-1}, C=C at 1558, 1597 cm^{-1}, Aromatic C-H at 702, 875 cm^{-1}, and C=N at 1558, 1597 cm^{-1}. The physical interaction of Ambrisentan with other selected excipients was a check for any possible interactions and indicated no such interaction. The Ambrisentan was found compatible with all excipients. The FTIR spectra of pure Ambrisentan were depicted in Fig. 1 and with other excipients in Fig. 2 to 3.

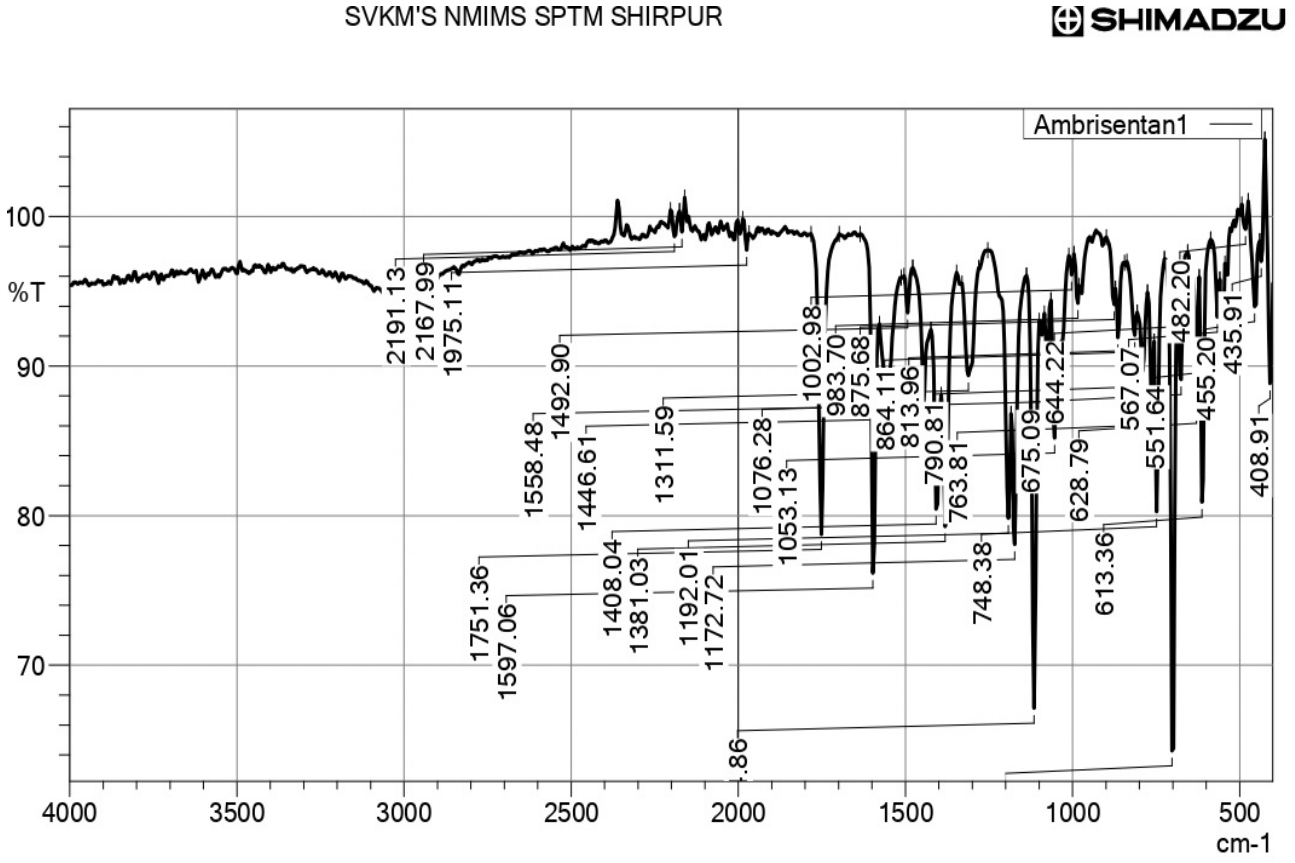

Fig. 1. FTIR Spectra of Pure Ambrisentan

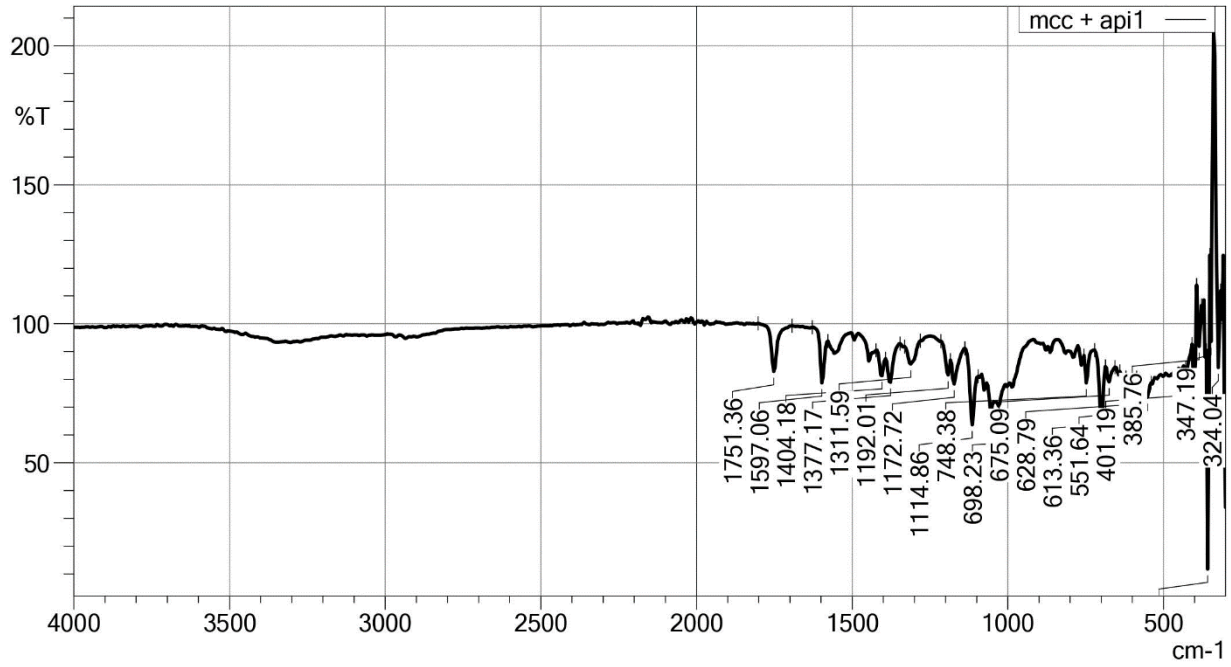

Fig. 2. FTIR Spectra of Ambrisentan.jpg and MCC

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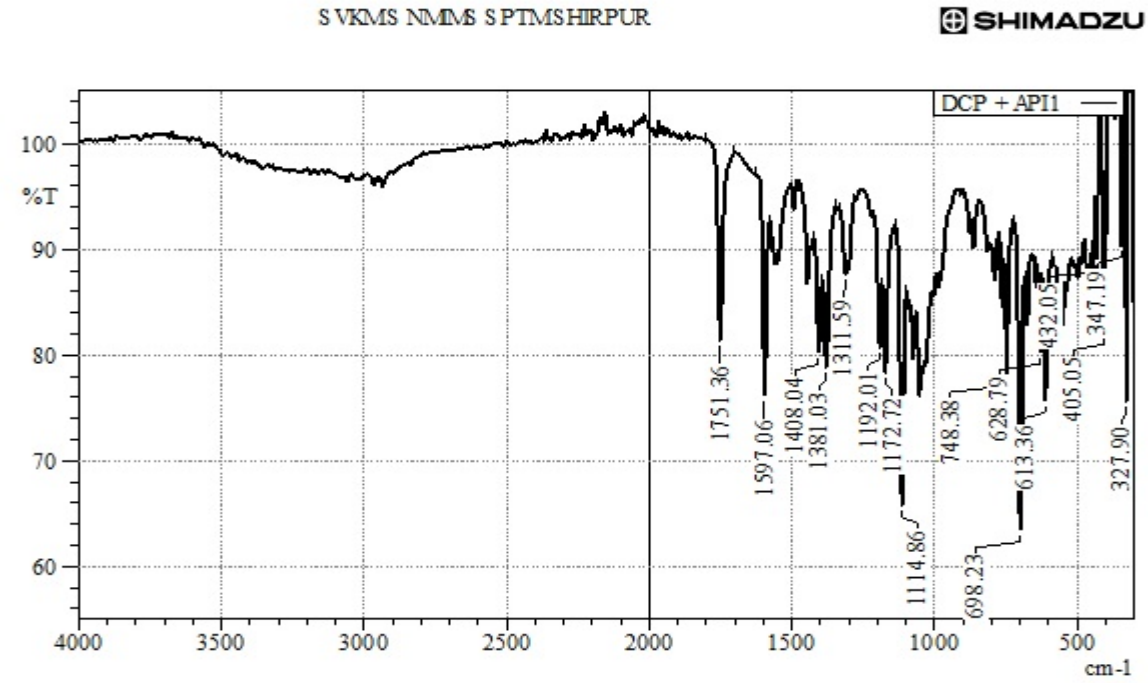

Fig. 3. FTIR Spectra of Ambrisentan.jpg and DCP

2.4. Analysis of flowing characteristics

The powder blends were assessed for their flowing behavior which comprises bulk density, tapped density, Carr's index, angle of repose and Hausner's ratio. The ingredients opted for direct compression method were compressible and hence showed good flowability. The distinctions in the series of consolidation index and angle of repose were recognized due to the slight variation in the volume of directly compressible agents. The bulk density of all batches was recorded in the range of 0.46±0.04 to 0.47±0.12. After operating the bulk density apparatus with 100 tapings, densities of all batches were observed between 0.57±0.06 to 0.59±0.10. The consolidation index was calculated from both these densities and observed as 16.071±0.14 to 17.857±0.20 %. The angle of repose was observed from 26.82±0.20 to 29.38±0.16 and Hausner's ratio from 1.19±0.06 to 1.21±0.09. The results of these powder blends for flowing behavior showed that materials have the potential of flowing from the hopper to the dies during compression and don't require further addition or modification in the formula. The results of flow behavior were depicted in **Table 1**.

Table 1: The evaluation of powder blends of Ambrisentan

Batch	Bulk density	Tapped density	Carr's index (%)	Angle of repose (θ)	Hausner's index
CT1	0.46±0.10	0.58±0.14	16.364±0.09	27.25±0.18	1.19±0.06
CT2	0.47±0.12	0.58±0.09	17.544±0.16	28.71±0.14	1.21±0.07
CT3	0.46±0.10	0.57±0.08	17.857±0.20	29.38±0.16	1.21±0.09
CT4	0.47±0.08	0.57±0.06	16.071±0.14	26.82±0.20	1.19±0.02
CT5	0.46±0.04	0.59±0.10	16.364±0.08	27.30±0.12	1.19±0.06
CT6	0.47±0.08	0.57±0.08	16.07±0.11	27.02±0.14	1.19±0.8

All values are n =3 ±SD.

2.5. Evaluation of core tablets of Ambrisentan

The direct compression method was utilized for the manufacturing of core tablets of Ambrisentan by incorporating MCC and DCP as directly compressible agents. Several proportions of cross carmellose sodium were added to achieve desired release pattern. The formulated tablets passed from the weight variation test according to the specifications of pharmacopeia. The weight variation of tablets was observed from 174±0.60

to 177±0.32. The hardness of all tablets was measured with a Monsanto hardness tester and found to be 4.2±0.15 to 4.4±0.18. The hardness of tablets varies slightly due to the different proportions of ingredients. Subjected to all the batches in Roche friabilator, the friability was noted from 0.48±0.10 to 0.53±0.16. The disintegration time was observed as 2.23±0.10 to 2.27±0.07. The variation among the batches in terms of disintegration time was due to different concentrations of superdisintegrants. As the concentration increases the disintegration time is found to be decreasing due to rapid swelling characteristics on exposure to the solvent. The prepared batches showed high content uniformity and ranged from 98.94±0.82 to 99.67±0.80. The results were depicted in **Table 2**.

Table 2: Evaluation of core tablets of Ambrisentan

Batch	Weight variation (mg)	Hardness (kg/cm²)	Friability (%)	Disintegration (min)	Content uniformity (%)
CT1	176±0.28	4.3±0.10	0.49±0.16	2.26 ±0.04	99.34±0.46
CT2	177±0.32	4.3±0.14	0.50±0.14	2.25±0.08	99.27±0.52
CT3	175±0.26	4.2±0.15	0.53±0.16	2.24±0.06	99.47±0.64
CT4	174±0.60	4.4±0.18	0.48±0.10	2.27±0.07	98.94±0.82
CT5	175±0.56	4.4±0.10	0.48±0.11	2.25±0.09	99.53±0.78
CT 6	176±0.36	4.3±0.11	0.50±0.20	2.23±0.10	99.67±0.80

All values are n =3 ±SD.

2.6. In-vitro dissolution studies of core tablets of Ambrisentan

Dissolution is the prime parameter for the selection of optimized core tablets. Hence, the CT 1 to CT 6 batches were subjected to in-vitro dissolution testing. The discharge of ambrisentan was speedy due to the creation of matrix with PEG 6000. The noticeable enhancement in wetting tendency followed by quick dissolution was noticed after the incorporation of PEG 6000 which diminish the interfacial tension between ambrisentan and solvent molecules. The drug release from the CT1 batch was found to be 99.21 % after 45 min. whereas, the cumulative amount of 99.27 % of Ambrisentan was recoreded in the CT2 batch. The release rate was slightly more in the CT3 batch was 99.58 % compared with the CT1 and CT2. The rapid release of Ambrisentan was attributed due to the greater concentration of superdisintegrants. İn batch CT3. Similarly, the cumulative percentage of drugs released from the batches CT4 to CT6 were 99.30 %, 99.59 %, and 99.70 % respectively. The batches comprised of DCP also showed a similar release profile as that of MCC containing CT1 to CT3 batches and the slight variations in the drug release were due to the composition and hardness of the tablets. The in-vitro dissolution release from core tablets was showed in Fig. 4.

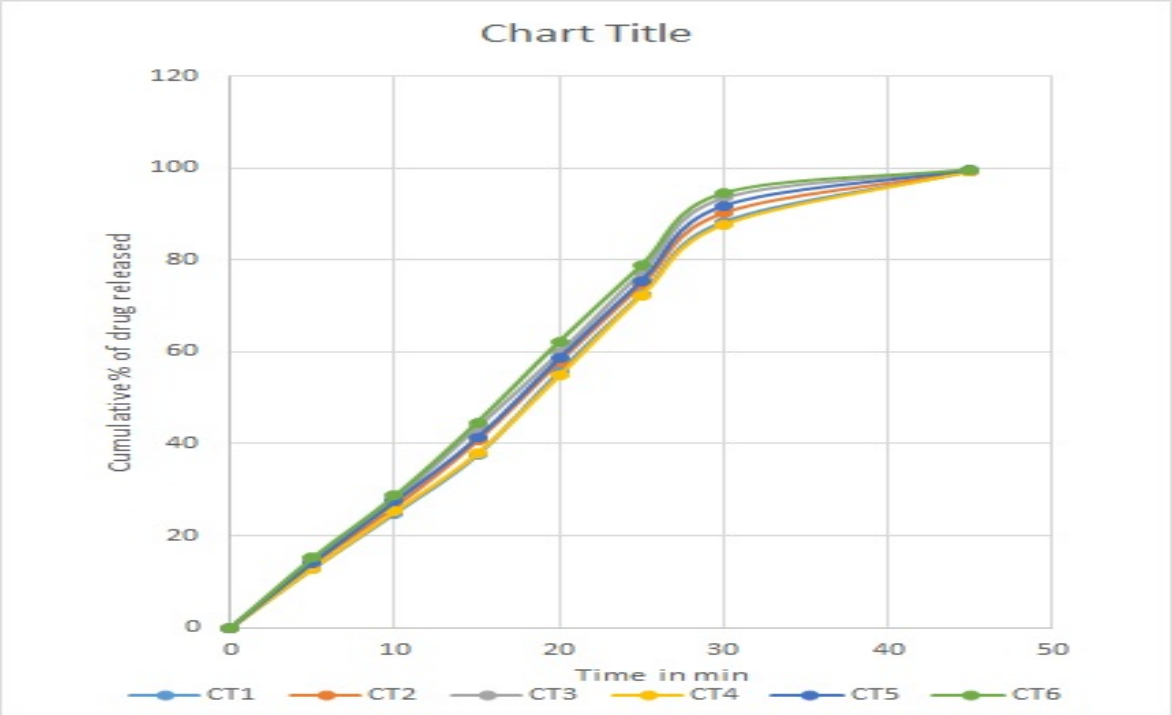

Fig. 4. Dissolution profile of Core tablets. All values in n=3, ±SD

2.7. Optimization of core tablets

The core tablet was picked which showed the least disintegration time, higher dissolution release and good content uniformity. From the several batches, CT6 was chosen as the optimized batch for pressing coating.

2.8. Evaluation of pulsatile coated tablets

The compression-coated tablets were prepared using a combination of Eudragit L100 and erodible material ethyl cellulose as coating agents. The pulsatile tablets were evaluated for their post-compressional characteristics similar to the core tablets. The results of pulsatile-coated tablets were depicted in **Table 3**. The prepared tablets passed from weight variation test according to the specifications of pharmacopeia. The weight variation of tablets was observed from 428±0.46 to 432±0.82. The hardness of all tablets was measured with a Monsanto hardness tester and found to be 8.5±0.04 to 8.7±0.10. The hardness of tablets varies slightly due to the different proportions of ingredients. Subjected to all the batches in Roche friabilator, the friability was noted from 0.20±0.018 to 0.26±0.13. The prepared batches showed high content uniformity and ranged from 98.09±0.64 to 99.29±0.82.

Table 3: The post-compression evaluations of Ambrisentan Pulsatile Tablets

Batch	Weight variation (mg)	Hardness (kg/cm²)	Friability (%)	Content uniformity (%)
PC1	429±0.77	8.7±0.10	0.20±0.018	98.45±0.67
PC2	431±0.65	8.6±0.14	0.22±0.014	98.57±0.54
PC3	430±0.48	8.6±0.08	0.23±0.010	99.13±0.46
PC4	431±0.71	8.5±0.04	0.25±0.15	98.74±0.74
PC5	432±0.82	8.6±0.09	0.23±0.19	98.09±0.64
PC6	430±0.60	8.7±0.07	0.21±0.21	99.29±0.82
PC7	429±0.52	8.7±0.09	0.22±0.12	99.15±0.70
PC8	428±0.46	8.6±0.08	0.22±0.17	99.06±0.56
PC9	430±0.58	8.5±0.11	0.26±0.13	98.81±0.51

All values in n=3 ±SD

2.9. In-vitro dissolution studies of pulsatile tablets of Ambrisentan

The in-vitro dissolution study of Ambrisentan pulsatile tablets was performed with USP Dissolution apparatus II (Paddle) using 0.1 N HCl initially for the first 2 hours and later on replaced with pH 7.4 phosphate buffer. The paddle was allowed to rotate at a speed of 50 rpm, at 37±0.5° C. The samples were withdrawn at an interval of 1 hour, diluted, filter through a 0.45 µm membrane filtered and analyzed spectrophotometrically at 264 nm.

The pH-dependent coating material Eudragit L100 which is a derivative of polymethacrylate and ethyl cellulose (7 and 10 cps) was utilized for getting the preferred lag time for pulsatile tablets. These coating agents were utilized in different proportions (100:0, 25:75, 50:50, 75:20 and 0:100) containing Eudragit L100 and EC respectively. Eudragit L100 is dissolved above the pH 5.5, hence the drug was not released for about 3 hours and after dissolving the outer coat, the drug was released quickly from the PC1 batch. The batches PC5 and PC9 are comprised of fully ethyl cellulose which is impermeable to the water and due to this reason takes a long time to rupture the outer coat. The batch comprised of EC7 and EC10 showed the lag time of 4 and 5 hours respectively. The batches which comprised of single coating material were unable to produce the desired lag time of 6 hours.

Furthermore, combinations of Eudragit L100 with different viscosity grades of ethyl cellulose were tried for desired lag time. The batch PC2 was composed of 25:75 proportions of Eudragit L100 and EC 7 was just short of destination and showed a lag time of 5 hours with a cumulative release of 98.86 % after 9 hours. Whereas, PC6 containing the same ratio with a higher viscosity grade of ethyl cellulose EC10 was capable of producing desired lag time of 6 hours and cumulative release of 99.25 % after 10 hours. The batches PC3 and PC7 comprised of 50:50 proportions and later with a high viscosity grade of ethyl cellulose showed the lag times of 3 and 5 hours respectively with the cumulative release of 98.90 % and 99.07 % respectively after 9 hours. Finally, batches PC4 and PC8 comprised 75: 25 proportions of Eudragit L100 and ethyl cellulose showed the lag time of 3 and 4 hours only. This indicated that as the concentration of Eudragit L100 increases, the lag time decreases and increasing ethyl cellulose concentration and viscosity lag time increase.

The perfect blend of the enteric and hydrophobic combination was required to elicit a pulsatile response which showed from the batch PC6. Due to its highest lag time, zero order release kinetics was predicted from this batch. The cumulative release of all batches was showed in Fig. 5 and 6 respectively.

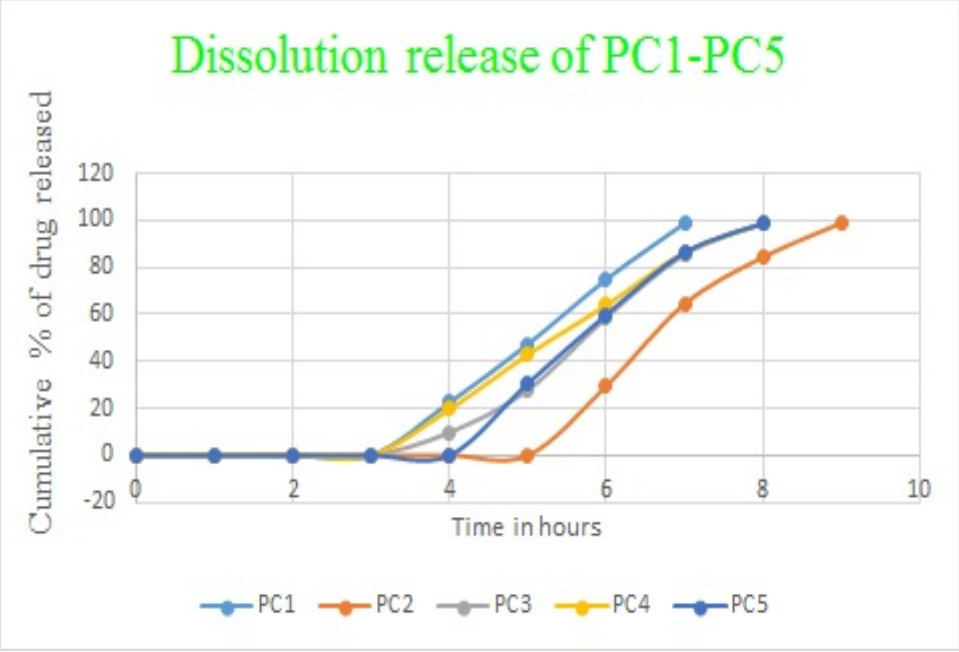

Fig. 5. Dissolution release of Pulsatile tablets PC1-PC5. All values in n=3, ±SD

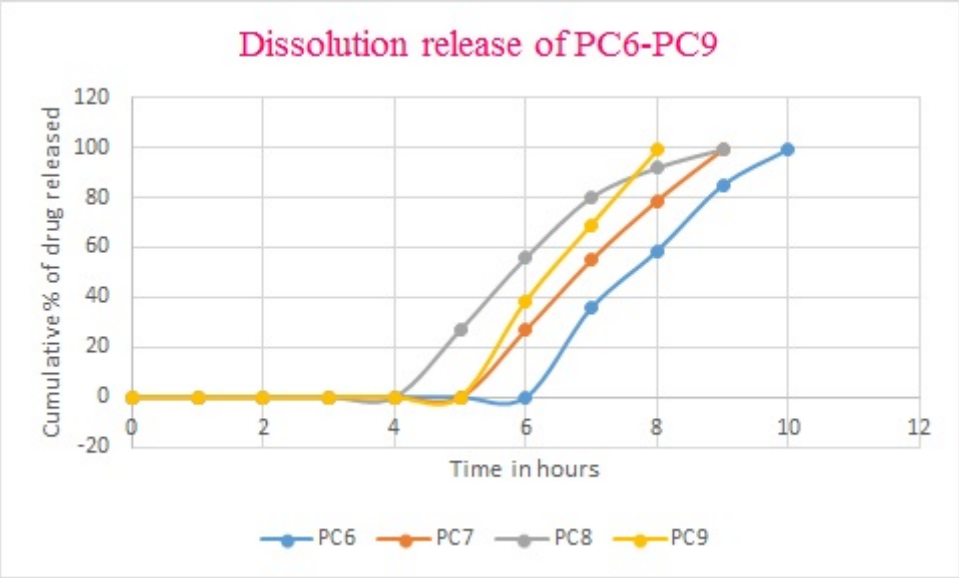

Fig. 6. Dissolution release of Pulsatile tablets PC6-PC9. All values in n=3, ±SD

2.10. Preliminary study

The optimized batch PC6 was assessed for accelerated stability testing at 40 ^{0}C and 75 % relative humidity. The optimized batch showed highly acceptable results and passed stability testing. The prepared tablets were packed in aluminum-aluminum foil and further dispatched to the market for sale. The results of stability testing were depicted in **Table 4**.

Table 4: Preliminary study of an optimized batch PC6

Parameters	Initial	After 1 month	After 2 months	After 3 months
Appearance	Good	Good	Good	Good
Hardness (kg/cm^2)	8.7±0.09	8.6±0.08	8.4±0.12	8.3±0.10
Friability (%)	0.21±0.21	0.24±0.14	0.27±0.12	0.28±0.16
Lag time (Hour)	6	6	6	6
Content uniformity (%)	99.29±0.82	99.16±0.47	99.03±0.38	98.72±0.32

n =±3, SD

3. CONCLUSION

Cardiovascular diseases are a big health problem currently and are largely associated with the mortality in the world. Hypertension is one the critical condition and affects other vital organs of the body resulting in damage such as renal failure. The blood pressure followed the circadian rhythm and hence, it rises promptly in the early morning hours. At that time many peoples are in sleep or wake-up condition. Generally, any medicine is to be administered after breakfast to post lunch time. Pulsatile delivery is the only delivery that provides relief from high blood pressure in the early morning and hence, is considered the most promising drug delivery for hypertension and CVS. The optimized batch PC6 provided 6-hour lag time and patients have to administer is at bedtime around 10 pm which releases the drug after 4 pm when the body requires it.

4. MATERIALS AND METHODS

4.1. Materials

Ambrisentan was gifted by Cipla Pharmaceuticals, Mumbai. Dibasic calcium phosphate and magnesium stearate was gifted by Nitika Pharmaceutical, Nagpur. Eudragit L100 was supplied from Evonic Deggusa Pvt. Ltd, Mumbai. Ethyl cellulose (EC 10) were supplied by Ajanta Pharmaceutical, Aurangabad. Cross povidone and PEG 6000 were purchased from Loba chemicals, Mumbai. All other ingredients and chemicals were of analytical grade only.

4.2. Preformulation parameters

The pure sample of Ambrisentan was evaluated for color, melting point and loss on drying (LOD).

4.3. Estimation of solubility of Ambrisentan

An accurately weighed amount of 10mg of Ambrisentan powder was transferred into the 1ml of a test tube containing several non-volatile solvents including distilled water, Tween 80, Span 20, polyethylene glycol 400 and propylene glycol respectively. The sample was checked visually for solubility and further subjected to absorbance scanning under a UV-visible spectrophotometer at 241 nm [18] [19].

4.4. FTIR analysis

The pure sample of Ambrisentan was subjected to FTIR spectral analysis by scanning in the range of 400-5000 cm^2. Afterward, the Ambrisentan was mixed with dibasic calcium phosphate, and microcrystalline cellulose, to check for any possible interactions among the excipient [20].

4.5. Development of core tablets of Ambrisentan

Ambrisentan powder was accurately weighed and further blended with polyethylene glycol 6000(1:4). This solid dispersion was further mixed with remaining ingredients such as microcrystalline cellulose, dibasic calcium phosphate and cross povidone which passed through a sieve no. 60 to ensure uniformity among the powder samples. The powder blends were compressed on multi-tooling tablet compression machine using Rimek mini press II (Karnavati Engineering, Ahmadabad, India) [21] [22]. The formulation components of core tablets were depicted in **Table 5.**

Table 5: Formulation ingredients of core tablets of Ambrisentan

Sr. No	Ingredients	CT1	CT2	CT3	CT4	CT5	CT6
1	Ambrisentan	5	5	5	5	5	5
2	PEG 6000	20	20	20	20	20	20
3	MCC	142.7	140.8	138.9	-	-	-
4	DCP	-			142.7	140.8	138.9
5	CP	3.8	5.7	7.6	3.8	5.7	7.6
6	MS	1.75	1.75	1.75	1.75	1.75	1.75
7	Talc	1.75	1.75	1.75	1.75	1.75	1.75
	Total weight	175	175	175	175	175	175

MCC: Microcrystalline cellulose, DCP: Dibasic calcium phosphate, CP: Cross povidone, MS: Magnesium stearate.

4.6. Analysis of flowing characteristics of powder blends

The powder blends obtained after weighing all the ingredients were firstly subjected to evaluation of flow characteristics before compression into the tablets. The powders were estimated for bulk density, tapped density (Bulk density apparatus), consolidation index, angle of repose (fixed funnel method) and Hausner's ratio [23] [24].

4.7. Evaluation of core tablets

4.7.1. Weight variation test

The prepared tablets around 20 were randomly picked and accurately weighed. The mean weight of an individual tablet was recorded with standard derivations. The weight variation test passes within 2.5 % variations from the average tablets [25].

4.7.2. Crushing strength

The crushing strength of tablets from each batch was tested by a Monsanto hardness tester and values were reported by an average of three [26].

4.7.3. Friability

The tablets were subjected to friability using a Roche friabilator. The tablets from each batch were weighed accurately equivalent to 6.5 g and further kept in the friabilator which was rotated at a speed of 25 rpm for 100 rotations. After rotation, tablets were collected and reweighed. The percentage of the friabilator was calculated by subtracting the weight of the initial from the final weight [27].

4.7.4. Disintegration

The randomly selected 6 tablets were kept in the disintegration test apparatus and the time required to pass all the particles from the sieves was noted. This test was carried out at 37±0.5° C using 900 ml of simulated gastric fluid [28].

4.7.5. In-vitro dissolution

The dissolution study of Ambrisentan tablets was performed with USP Dissolution apparatus II (Paddle) using pH 7.4 phosphate buffer. The paddle was allowed to rotate at a speed of 50 rpm, at 37±0.5° C. The samples were withdrawn at an interval of 5 minutes for core tablets and 1 h for pulsatile coated tablets

respectively, diluted, was filtered through a 0.45 µm membrane filter and analyzed spectrophotometrically at 264 nm [29].

4.7.6. Content uniformity

The prepared tablets were randomly selected and converted into powder after crushing. The average weight of the tablet containing a powder was taken and dissolved with pH 7.4 phosphate buffer. The solution was further diluted and filter through a 0.45 µ membrane filter and analyzed spectrophotometrically at 264 nm [30].

4.8. Optimization of core tablets

The core tablets were evaluated for post-compression analysis and an optimized batch was selected based on the least disintegration time and greater dissolution release [31].

4.9. Development of pulsatile tablets of Ambrisentan

The pulsatile tablets of Ambrisentan were formulated using core tablets by compression coating method. In this method, various proportions of coating agents such as Eudragit L 100 and ethyl cellulose grades (EC 7, and 10 cps) in different proportions were utilized. As depicted in Table 6 and 7, various proportions of coating agents were subjected to blending in a double cone blender and this mixture was utilized for compression after the addition of lubricant. The optimized tablet was placed on the half-portion of the coating agent and remaining half quantity was added further to compress it as pulse tablets [32] [33].

Table 6: Composition of a coating material containing Eudragit L100+ EC 7

Sr. No	Ingredients	PC1	PC2	PC3	PC4	PC5
1	Core tablet	175	175	175	175	175
2	Eudragit L100	250	62.5	125	187.5	-
3	EC 7	-	187.5	125	62.5	250
4	MS	2.5	2.5	2.5	2.5	2.5
5	Talc	2.5	2.5	2.5	2.5	2.5
	Total	430	430	430	430	430

EC: Ethyl cellulose, MS: Magnesium stearate

Table 7: Composition of coating material containing Eudragit L100+ EC 10

Sr. No	Ingredients	PC6	PC7	PC8	PC9
1	Core tablet	175	175	175	175
2	Eudragit L100	62.5	125	187.5	
3	EC 10	187.5	125	62.5	250
4	MS	2.5	2.5	2.5	2.5
5	Talc	2.5	2.5	2.5	2.5
	Total	430	430	430	430

EC: Ethyl cellulose, MS: Magnesium stearate

4.10. Evaluation of pulsatile tablets of Ambrisentan

The post-compression evaluation was performed for compression coated tablets in exactly a similar fashion to that of core tablets which includes weight variation, crushing strength, friability, in-vitro dissolution and content uniformity. Being coated tablets, the disintegration test was not applicable for pulsatile tablets [34].

4.11. Preliminary study

The stability study of an optimized formulation was carried out according to the ICH guidelines. The optimized batch was kept at 40 ^{0}C and 75 % RH for about 3 months. The samples were withdrawn at an interval of one month and estimated for their physical appearances like any changes in size, color and shape of the tablets. Moreover, friability, drug content, and dissolution time were also assessed [35].

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