FORMULATION AND EVALUATION OF MOUTH DISSOLVING TABLET OF AMBRISENTAN BY INCLUSION COMPLEX USING B-CYCLODEXTRIN

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Article History: **Received:** 18.04.2023 **Revised:** 07.05.2023 **Accepted:** 16.06.2023

Abstract: *Ambrisentan is an US FDA approved (2007) drug, belongs to BCS Class II, second oral endothelin A - receptor antagonist known for the treatment of pulmonary arterial hypertension, but its oral administration is limited as it is insoluble in water. The present investigation was aimed towards developing a β-cyclodextrin (β-CD) inclusion complex (IC) based mouth dissolving tablet (MDT) of Ambrisentan (AMB), for improving the solubility and dissolution characteristics, and also providing fast onset of antihypertensive action. Inclusion complex were prepared by kneading method and evaluated for percentage yield, saturation solubility, drug content, in-vitro dissolution studies, X-ray diffraction (XRD) and FTIR. IC (1:1), which has shown more significant results, was compressed into MDT by direct compression method using superdisintegrants and various tablet excipients. The powder blends evaluated for pre compression parameters has shown excellent and good flow properties. The observations and results obtained from post compression parameters for MDTs are within the acceptable limits.*
MDTs formulated with sodium starch glycolate (8%) and crosspovidone (5%) as a superdisintegrants has shown fastest disintegration within 44.71 ± 1.58 seconds and improved drug dissolution i.e., 98.90% drug release in 30 minutes. The short-term stability studies of optimized formulation (F3) were carried out at two different temperature and humidity conditions viz. RT and 40 ± 20C / 75 ± 5% RH for 3 months. During stability studies, samples were tested initially and thereafter for hardness, disintegration time, drug contents and in-vitro drug release at different time intervals. No profound changes were observed in the tablet integrity throughout the stability studies. Thus, it was concluded that, IC based MDT provides faster onset of action with good therapeutic response. MDT of Ambrisentan can be helpful for the patient in managing onset of symptoms of PAH.

Keywords: *Ambrisentan, β-cyclodextrin, Inclusion Complex, Solubility, Dissolution, Mouth Dissolving Tablet.*

INTRODUCTION

In order to design new dosage formulations, a variety of pharmaceutical research has been done recently. When considering quality of life, the majority of these efforts have been focused on medication convenience(1). Mouth dissolving tablet system is one which refers to a tablet that dissolves quickly in saliva within a few seconds without the need of water or chewing(2).Although drug delivery technology has advanced greatly, the oral route is still the best way to administer therapeutic agents since it is inexpensive, simple to administer, accurate, allows patients to self-medicate, helps them avoid pain, and has a high rate of patient compliance. The most common dosage forms are tablets and capsules; however, the main disadvantage of these dosage forms is dysphasia, or trouble swallowing. This issue prompted the creation of novel solid dose forms, such as mouth-dissolving tablets that quickly dissolve in saliva without the need for water(3). Ambrisentan is a selective propanoic acid endothelin receptor antagonist that has received US and EU approval for the treatment of pulmonary arterial hypertension (PAH), a

disease characterized by a progressive rise in pulmonary vascular resistance that results in right ventricular heart failure and premature death(4)(5). Ambrisentan is BCS Class II drug of the Biopharmaceutics Classification System (BSC), and consequently, it has low solubility and high permeability. Ambrisentan is practically insoluble in water due to its highly lipophilic structure. One of the most effective methods to enhance its solubility is to be included in the cavities of the cyclodextrins.
Cyclodextrins (CDs) are a class of cyclic oligosaccharides that are produced by the enzymatic action of starch molecules and are composed of glucopyranose units joined by a (α-1, 4) linkage. The molecular structure of cyclodextrins is distinctive because it has glucose-containing ethereal oxygens on the inside and skeletal carbons on the outside (6)(7)(8).
Drugs physicochemical characteristics, such as stability, solubility, and rate of dissolution, as well as their pharmacologic characteristics, such as drug bioavailability, are changed as a result of complex formation between cyclodextrin and guests or drug molecules(9)(10)(11)**.**
Cyclodextrins can form host guest inclusion complexes by weak intermolecular interactions with a wide variety of guests including organic molecules, inorganic ions, and coordination compounds. This phenomenon was shown by researchers as one of the techniques of alleviating the issue of solubility of water insoluble drugs. (12) (13) (14).
There are several water-insoluble drugs products that have received US FDA (U.S. Food and drugs Administration) approval and make use of the cyclodextrin inclusion complex technology. Additionally enhancing the solubility and taste-masking of drugs(15). CDs also improve their physical and chemical stability(16), absorption and bioavailability(17).Thus, development of an MDT might be beneficial for an Antihypertensive drug like Ambrisentan to provide fast onset of action. Hence, an attempt was made in the present investigation to improve the solubility of Ambrisentan through the formulation of Inclusion complex using β-cyclodextrin and to convert the optimized IC into mouth dissolving tablet formulations.
To the best of our knowledge, no report mentioning the evaluation of the solubility of Ambrisentan tablets containing AMB–β-CD inclusion complex in the form of an MDT has been published.

Materials

Ambrisentan was obtained as a gift sample from Lupin Limited (Research Park), Pune. β-Cyclodextrin (βCD) was obtained as a gift sample from R. P. Chemicals, Mumbai. All other chemicals and materials used were of pharmaceutical grade.

Method

Analysis of API by UV visible spectrophotometer

Preparation of buffer solution-

Dissolve 28.80 gm of Disodium Hydrogen Phosphate (Na_2HPO_4) and 11.45 gm of Potassium Dihydrogen Phosphate (KH_2PO_4) in sufficient amount of distilled water to produce 1000 ml. phosphate buffer pH 6.8.

Determination of λ max and Plot of calibration Curve of Ambrisentan in Phosphate Buffer pH 6.8

Preparation of Stock Solution

Stock I

Ambrisentan (100mg) was accurately weighed and transferred into the 100 ml volumetric flask. It was dissolved in phosphate buffer pH 6.8 and volume was made up to the mark (To get a 1000 µg/ml solution).

Stock II

From stock solution I, 10 ml solution was withdrawn and diluted upto 100 ml using phosphate buffer pH 6.8 (100 µg/ml).

Stock III

From stock solution II, 2, 4, 6, 8 and 10 ml were withdrawn and diluted to 10 ml using phosphate buffer pH 6.8 to obtain dilutions as 2, 4, 6, 8 and 10 µg/ml respectively.

Determination of λ max
The dilution from Stock III solution with highest concentration i.e., 10 µg/ml concentration was scanned over UV-visible range of 200-400 nm in UV Visible spectrophotometer (V-530, Jasco) for λmax determination.

Plot of calibration curve
The absorbance of blank and all the dilutions was measured at λmax 262 nm in UV-visible spectrophotometer (V-530, Jasco). The calibration curve between concentration (X- axis) and absorbance (Y-axis) was plotted.

Solubility Study of an API
Solubility of an API was determined in different solvents like Water, phosphate buffer pH 6.8, phosphate buffer pH 7.4. Solubility was determined by sonication method. 100 mg of an API was dissolved in 100 ml of solvent and sonicated for 30 min. Then, solution was filtered through Whatman Filter Paper. The filtrate was analyzed by UV-visible spectrophotometer at λmax 262 nm (V-530, Jasco).

Drug-Excipient Compatibility Study
The compatibility studies of the drug with carrier and excipients were studied using FTIR spectroscopy. FTIR spectroscopy was carried out to check the compatibility between drug and excipients. FTIR spectra of drug samples were recorded using potassium bromide (KBr) pellets at resolution of 4000 - 400 cm^{-1} for its authentication and to study principal peaks using FTIR spectrophotometer (Tensor-37 Bruker). Dry sample of drug and potassium bromide was mixed uniformly and filled into the die cavity of sample holder in order to record an IR spectrum. The observed peaks were compared with the standard peaks.

DSC Analysis
Differential Scanning Calorimetric (DSC) thermograms of Ambrisentan, were recorded (DSC-60, Shimadzu, Japan). Nitrogen gas flowed at 20 psi to create an inert atmosphere to prevent any oxidation reaction with the sample holder (made of aluminium). The equipment was calibrated for baseline and temperature with indium metal. The sample was hermetically sealed in an aluminium pan and scanned from 10 to 200 °C at a rate of 10°C/min.

Preparation of Amb: β-CD Inclusion Complex
Ambrisentan-β-CD inclusion complex was prepared by Kneading method in ratio of 1:1, 1:2, 1:3, 1:4 using distilled water and ethanol (Ratio 1:1) as wetting agent, as shown in Table 1. Accurately weighed quantities of Ambrisentan and β-CD, in different ratios, were transferred to a glass mortar. Then wetting agent was added gradually and the mixture was kneaded thoroughly for 30 min in a glass mortar until the thick slurry was formed. Then slurry was collected and dried in hot air oven at 45°C for 24 hours. The dried mass was scrapped and passed through sieve no #60 to obtain uniform dispersion. The prepared dispersion was stored in desiccators for further studies.

Table 1: Formulation code for Inclusion Complex

Sr. No	Method	Drug: Carrier Complex	Drug: Carrier Ratio	Code for Inclusion Complex
1	Kneading Method	Ambrisentan: β-CD	1:1	IC1
2			1:2	IC2
3			1:3	IC3
4			1:4	IC4

Evaluation of Inclusion Complex

Determination of Percentage Yield

Percentage yield evaluation helps to understand the efficiency of any formulation method and also the formulation losses. Percentage yield was calculated using following formula,

$$\text{Percentage Yield}(\%) = \frac{\text{Weight of Inclusion Complex}}{\text{Weight of Drug} + \text{weight of Carrier}} \times 100$$

Saturation Solubility Studies

To evaluate the rise in solubility of Ambrisentan from Inclusion Complex, saturation solubility measurements were conducted and compared with that of pure drug. The known quantity of Inclusion Complex was added to the volumetric flask containing 10ml solvent of water, phosphate buffer pH 6.8 and phosphate buffer pH 7.4 separately to obtain a saturated solution. After 24 hours, this saturated solution was filtered, suitably diluted, and analyzed by UV-visible spectrophotometer (V-530, Jasco) at 262nm (19).

Determination of Drug Content

10 mg equivalent API- Inclusion Complex was accurately weighed and dissolved in 10 ml phosphate buffer pH 6.8. The solution was sonicated for 30 mins. The resulting solution was filtered through Whatman Filter paper and further diluted. Then absorbance was measured by using UV-visible spectrophotometer (V-530, Jasco) at λmax 262 nm, using phosphate buffer pH 6.8 as a blank. Then % drug content was calculated.(20).

$$\text{Drug content}(\%) = \frac{\text{Absorbance of test}}{\text{Absorbance of standard at the same dilution}} \times 100$$

In-vitro Dissolution Test

In-vitro dissolution test of a Plain drug (API) and IC1, IC2, IC3, IC4 were carried out by using USP dissolution test apparatus II (Paddle type, DS 8000 Labindia). The dissolution medium used was 900 ml of pH 6.8 Phosphate buffer, equilibrated at 37±0.5 °C and paddle rotation speed maintained at 50 RPM. Aliquots of 5 ml were withdrawn at specified time and replaced with equal volume of fresh dissolution medium at 37±0.5 °C. Aliquots withdrawn were filtered and analyzed at λmax 262 nm using UV-visible spectrophotometer (V-530, Jasco).

X-ray diffraction (XRD)

X-ray diffraction pattern of API and its β-cyclodextrin Inclusion Complex were recorded individually using X-ray diffractometer (Ultima IV Rigaku), using Cu ka2 rays with a voltage of 40 kV and a current of 40 mA, scan axis 2θ/θ, scan ranges between 5 to 80° and signals were collected for 20 min. For XRD analysis, 100 mg of an API and API-inclusion complex equivalent to 100 mg were placed in the sample holder groove and tightly packed.

XRD studies are used to determine degree of crystallinity and/or any changes in the degree of crystallinity of an API. It is one of the factors influencing the solubility and dissolution rate of an API. Thus, the crystalline nature of an API and extent of its conversion to amorphous form was studied by using XRD studies.

Fourier transform infrared spectroscopy

FTIR spectrum of Inclusion complex (IC1) recorded using Fourier transform infrared spectrophotometer (Tensor-37 Bruker).

Factorial Design

Central composite design (3^2) was used to design Ambrisentan-β-CD Inclusion complex containing Mouth dissolving tablets (MDT) as per shown in Table 2. In this design, 2 factors were evaluated at 3 levels and experimental trials were conducted at 9 possible combinations. The concentration of superdisintegrants like

Sodium Starch Glycolate (X1) and Crospovidone (X2), were selected as independent variables at three (low, medium and high) levels of each variable (-1, 0 and +1) as shown in Table 2 and 3. The in-vitro drug release (Y1) and disintegration time(Y2) were selected as dependent variables. The Design Expert 13 software was used for generating the experimental design.

Table 2: Independent variables

Level of Variable	Low (-1)	Medium (0)	High (+1)
Sodium starch glycolate (%)	2	5	8
Crospovidone (%)	1	3	5

Table 3: Central composite design layout for MDT

Formulation Batch	Factors (Superdisintegrants)			
	Factor 1		Factor 2	
	Sodium starch glycolate (X1)		Crospovidone (X2)	
	Coded level of variable	Actual level of variable (mg)	Coded level of variable	Actual level of variable (mg)
F1	0	10	0	7
F2	+1	16	-1	4
F3	+1	16	+1	10
F4	-1	4	+1	10
F5	0	10	+1	10
F6	-1	4	-1	4
F7	+1	16	0	7
F8	-1	4	0	7
F9	0	10	-1	4

Pre-Compression Evaluation of Powder Blend

Bulk density

The bulk density indicates the pore volume within the powder blend. Accurately weighed quantity of powder (M in gm) was transferred to measuring cylinder, and bulk volumes (Vb in ml) were measured. Bulk density was calculated by using formula,(21),

$$\text{Bulk density} = \frac{\text{Mass of powder(M)}}{\text{Bulk volume of the powder (Vb)}}$$

Tapped density

The tapped density of a powder blend is calculated to exclude the volume occupied by the voids and pores within the powder blend. Accurately weighted quantity of powder (M in gm) was transferred to measuring cylinder. The measuring cylinder was mounted on bulk density apparatus, and run for 100 tapings. The tapped volume of powders was measured (Vt in ml), and tapped density was calculated using formula(22),

$$\text{Tapped density} = \frac{\text{Mass of powder(M)}}{\text{Tapped volume of the powder (Vt)}}$$

Carr's index

It is a quick and straightforward indirect method to measure the relative strength of interparticle and frictional forces of bulk powders. It is expressed in % and given by

$$\text{Carr's index (\%)} = \frac{\text{Tapped Density(TD)} - \text{Bulk Density(BD)}}{\text{Tapped Density(TD)}} \times 100$$

Hausner's ratio

Hausner's ratio is an indirect index of ease of powder flow(23),

$$\text{Hausner's ratio} = \frac{\text{Tapped density(TD)}}{\text{Bulk density(BD)}}$$

Angle of repose

The angle of repose is defined as, maximum angle that can be formed between a pile of powder's surface and a horizontal plane. The angle of repose is a useful tool for calculating the frictional force in loose powder or granules. To calculate the powder's angle of repose funnel method was used and angle of repose was calculated using the following equation.

$$\tan\theta = \frac{h}{r}$$

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

Where,θ is the angle of repose, h is height of pile and r is radius of the base of pile.

Formulation of Inclusion Complex into Mouth Dissolving Tablet

Optimized batch of Ambrisentan-β-CD Inclusion Complex was selected for the formulation of mouth dissolving tablets. All materials were accurately weighed and passed through sieve #60 to get uniform particle size distribution of powder blend. The powder blend was evaluated for pre-compression parameters followed by addition of lubricant and glidant. Then powder blend was mixed and compressed into tablets by using RIMEK MINI PRESS-II MT single station tablet punching machine, with punch size of 8 mm, as shown in Table 4. The compressed tablets were evaluated for post compression parameters.

Table 4: Formulation table for Ambrisentan: β-CD inclusion complex based mouth dissolving tablet

Ingredients (mg)	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**
Ambrisentan + β-CD IC1	20	20	20	20	20	20	20	20	20
Sodium Starch Glycolate	10	16	16	4	10	4	16	4	10
Crospovidone	7	4	10	10	10	4	7	7	4
MCC (Avicel pH 101)	30	30	30	30	30	30	30	30	30
Sodium saccharin	8	8	8	8	8	8	8	8	8
Vanillin	5	5	5	5	5	5	5	5	5
Magnesium stearate	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Talc	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Mannitol	115	112	106	118	112	124	109	121	118
Total	200	200	200	200	200	200	200	200	200

***All ingredients are taken in mg**

***IC equivalent to 10 mg was taken**

Post-compression evaluation of MDT

Tablet Appearance

The general appearance of tablets includes shape, colour, surface texture.

Weight variation
The average weight of 20 tablets was calculated after a random selection. The weight of each tablet was then measured, and the results were compared to an average weight.

Thickness
For uniformity in tablet size, the thickness of the tablet is important. Thickness was measured using Vernier Calliper's. It was determined by checking the thickness of tablets of each formulation.

Hardness
The force needed to break a tablet across its diameter is referred to as the tablet's hardness. Under the conditions of handling, transformation and storage prior to usage the hardness of the tablet determines how it resists to abrasion, chipping or breaking. The tablet hardness of all formulation was studied using the Monsanto hardness tester.

Friability
Friability test is performed to determine the weight loss from the tablet and comparing the final weight with the original tablet. This test is important to obtain the surface resistance during the packaging and transport. The tablet friability was checked by using Friability tester (FT 1020 Labindia). The Friability was calculated using formula,

$$\text{Friability (\%)} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

Drug content
Ten tablets were randomly selected, crushed and powder blend equivalent to 10 mg of drug was dissolved in pH 6.8 phosphate buffer, and volume was made up upto 100 ml with pH 6.8 phosphate buffer. The solution was sonicated for 1 hour. From this solution, 1ml aliquot was withdrawn and diluted to 10 ml with pH 6.8 phosphate buffer. Solution was filtered and absorbance was measured spectrophotometrically (V-530, Jasco) at λmax 262 nm against by using pH 6.8 phosphate buffer as a blank. Total amount of drug present in each tablet was calculated using formula,

$$\text{Drug content(\%)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard at the same dilution}} \times 100$$

Wetting time
A piece of tissue paper was folded twice in a 6.5 cm glass petri dish containing 5 ml distilled water. The tablet was placed on the paper and the time for complete wetting of the tablet was measured in seconds (24).

Water absorption ratio
The wetted tablets were reweighed and the water absorption ratio R, was determined according to the following formula,

$$R = \frac{Wa - Wb}{Wb} \times 100$$

Where, Wa and Wb are weights of tablets after and before test.

In-vitro disintegration time
The disintegration time of the tablet was measured in six-glass tube USP disintegration test apparatus (DT 1000 Labindia) containing 900 ml phosphate buffer pH 6.8 (37 ± 0.5°C). Each glass tube containing 1 tablet was placed in 900 ml beaker of phosphate buffer pH 6.8, such that the tablets remain below the surface of liquid on their upward movement and descend not closer than 2.5 cm from the bottom of the beaker. The time in seconds taken for the complete disintegration of the tablet with no palpable mass in the apparatus was measured.

In-vitro dissolution time

In vitro dissolution studies will carry out by USP II (paddle type) dissolution test apparatus (DS 8000 Labindia) at 50 rpm. The dissolution medium consists of 900ml of 6.8 pH Phosphate buffer. This is maintained at 37±0.5 °C. Aliquots of 5 ml were withdrawn at specified time and equivalent amount of fresh dissolution medium is added. Aliquots withdrawn were filtered and analyzed at 262 nm using UV visible spectrophotometer (V-530, Jasco).

Stability testing

Stability studies were carried out on optimized formulation. Randomly selected tablets were stored in amber colored rubber stoppered vials, at room temperature and elevated temperature i.e., 40 ± 2^0C / 75 ± 5% RH, in a Stability chamber (Bio-Technics India) for a period of 3 months. At intervals of 0 month and 1 month thereafter, the tablets were examined for Hardness, Disintegration time, Drug content and In-vitro drug release.

RESULTS AND DISCUSSION

Determination of λ max

The λ max of the Ambrisentan was found to be 262 nm in Phosphate Buffer pH 6.8. As shown in Figure 1.

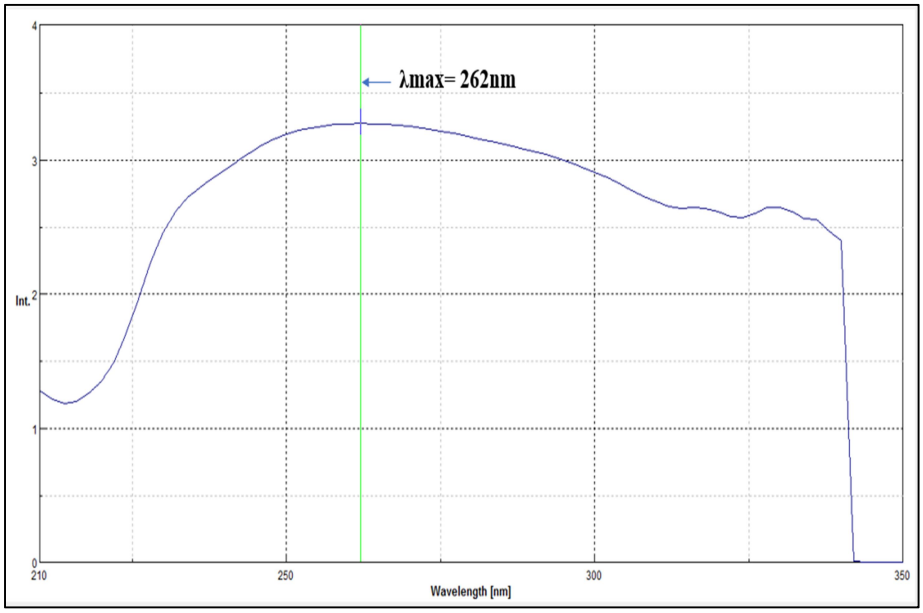

Figure 1: UV spectrum of Ambrisentan

Plot of calibration curve

The absorbance of Ambrisentan was measured by UV spectrophotometer at 262 nm against phosphate buffer pH 6.8 as blank. As shown in Table 5 and Figure 2.

Table 5: Absorbance of Ambrisentan in Phosphate Buffer pH 6.8

Concentration	Absorbance
0	0
2	0.0594

4	0.1195
6	0.1788
8	0.2374
10	0.2931

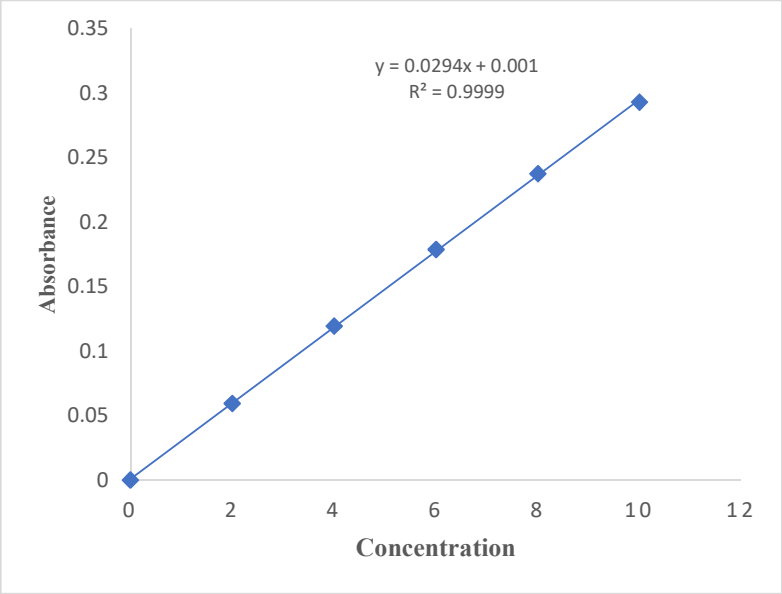

Figure 2: Calibration curve of Ambrisentan in Phosphate Buffer pH 6.8

Solubility Study

Solubility profile of Ambrisentan in different solvents is shown in table 6.

Table 6: Solubility profile of Ambrisentan

Sr. No	Solvent	Solubility (mg/ml)
1	Water	0.0185 ± 0.0019
2	Phosphate Buffer pH 6.8	0.0198 ± 0.0038
3	Phosphate Buffer pH 7.4	0.0210 ± 0.0049

*** All values are ± SD (n=3)**

Drug Excipient Compatibility Study

FTIR Spectroscopic analysis

The FTIR of Ambrisentan shows intense band at 3477.41 cm^{-1}, 2964.31 cm^{-1}, 1376.50 cm^{-1} and 1302.11 cm^{-1} and 1112.93 cm^{-1} corresponding to the functional groups COOH, C-H (Aromatic), C-H (Aliphatic), C-N (Aromatic) and C-O-C bending (Figure 3) The FTIR of drug with excipients and IC1 shown intense band similar to the FTIR of Ambrisentan indicates no change in the functional groups. From the FTIR spectra it is observed that no major change in the frequencies of functional groups, hence it is concluded that there is no interaction between drug and excipients.

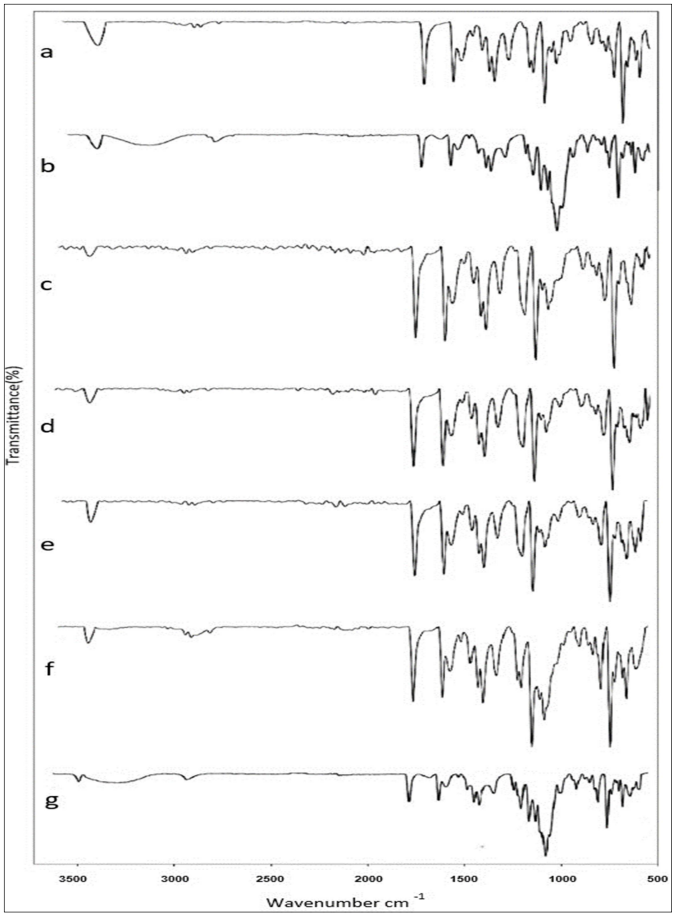

Figure 3: FTIR spectra of a) Ambrisentan b) Ambrisentan-βCD c) Ambrisentan-Sodium starch glycolate d) Ambrisentan-Crospovidone e) Ambrisentan-MCC (Avicel PH 101) f) Physical Mixture g) IC1

Differential Scanning Calorimetry Analysis

The DSC thermogram of Ambrisentan is as shown in Figure 4. It showed a sharp endothermic peak at 170.87°C, Onset at 163.40 °C and Endset at 173.86 °C indicating purity of drug.

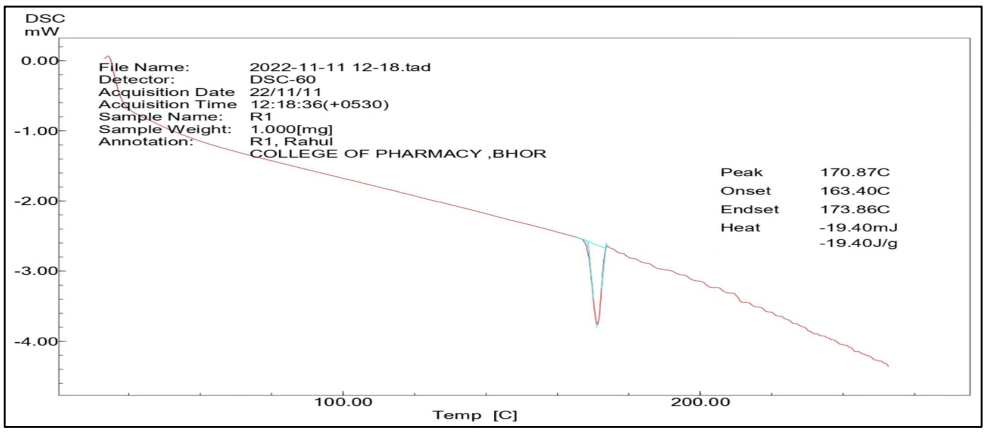

Figure 4: DSC of Ambrisentan

Preparation and evaluation of Inclusion Complex

Inclusion complex of Ambrisentan with β-CD prepared by kneading method in 1:1, 1:2 and 1:3 and 1:4 molars ratios were evaluated by Percentage yield, saturation solubility studies, Percentage drug content, FTIR studies and XRD studies.

Determination of Percentage Yield

The percentage yield of all Ambrisentan: β-CD IC ratios by kneading method was found to be in the range of 97.00 ± 0.12 to 99.52 ± 0.12%. The results are shown in Table 7.

Table 7: Percentage yield of IC

Sr. No.	Inclusion complex ratio	Percentage yield (%)
1.	IC1	99.52 ± 0.12
2.	IC2	98.16 ± 0.76
3.	IC3	97.43 ± 0.08
4.	IC4	97.00 ± 0.12

*** All values are in % ± SD**

Saturation Solubility studies

The effect on solubility of Ambrisentan after preparation of their inclusion complex using kneading method, saturation solubility studies were performed in water, phosphate buffer pH 6.8 and phosphate buffer pH 7.4. A 13.56-fold in Water, 16.97-fold in phosphate buffer pH 6.8 and 19.82-fold in phosphate buffer pH 7.4 increase was evident. It was studied that an increase in solubility with β-CD in ratio of Ambrisentan: β-CD (1:1) was achieved when compared to the solubility of pure drug and observed that the solubility decreased with increasing concentration of the carrier (Table 8). Hence, saturation solubility of different ratios was calculated, that shows 1:1 complex is highest. This might be an indicative reason for an improvement of wetting of drug particles and localized solubilization by the water-soluble carrier.

Table 8: Saturation Solubility

Inclusion Complex Code	Solubility in Different Solvent (mg/ml)		
	Water	Phosphate buffer pH 6.8	Phosphate buffer pH 7.4
Pure Drug	0.0185 ± 0.0019	0.0198 ± 0.0038	0.0210 ± 0.0049
IC1	0.2510 ± 0.0055	0.3362 ± 0.0042	0.4164 ± 0.0021
IC2	0.2057 ± 0.0044	0.2318 ± 0.0039	0.3624 ± 0.0034
IC3	0.1689 ± 0.0034	0.2158 ± 0.0024	0.2682 ± 0.0015
IC4	0.1006 ± 0.0021	0.1427 ± 0.0043	0.2243 ± 0.0057

*** All values are ± SD (n=3)**

Determination of Drug content

The drug content study was carried out to determine the percent amount of drug incorporated in the Inclusion Complex. The drug content for all Inclusion Complex batches was found to be in the range of 95.85 ± 0.84 - 98.79 ± 0.25. The results are shown in Table 9.

Table 9: Drug content of Inclusion Complex

Sr. No.	Inclusion complex ratio	Drug content (%)
1.	IC1	98.79 ± 0.25
2.	IC2	97.93 ± 0.74
3.	IC3	96.70 ± 1.12
4.	IC4	95.85 ± 0.84

*** All values are ± SD (n=3)**

In vitro Dissolution of Inclusion complex

The In vitro Dissolution profile of plain drug (Ambrisentan) and Inclusion complex (IC1 - IC4) are shown in Table 10 and Figure 5. It was found that IC1 has shown highest cumulative % drug release as compared to plain drug (Ambrisentan). The cumulative % drug release of plain drug was found to be 23.29 % in 30 minutes. While cumulative % drug release of IC1 - IC4 were found to be 81.67 - 50.67 % respectively in 30 minutes.

The improvement in the dissolution rate of Ambrisentan in its Inclusion complex can be attributed to its enhanced solubility due to reduction in particle size in kneading process and improvement in hydrophilicity &/or wettability in presence of β-CD.

Table 10: In vitro dissolution data of Plain drug and its Inclusion complex

Time (min)	Cumulative % Drug Release				
	Plain Drug	IC1	IC2	IC3	IC4
0	0	0	0	0	0
3	2.25	15.14	12.29	10.23	7.87
6	5.69	28.76	23.45	17.47	12.69
9	7.47	39.34	31.34	23.29	19.34
12	10.29	45.87	38.19	31.16	24.28
15	13.17	52.19	44.48	37.27	29.19
18	14.34	58.28	51.34	44.39	33.47
21	17.13	65.27	59.49	49.89	39.84
24	19.26	73.73	65.31	53.26	45.24
27	21.05	78.31	69.76	57.37	48.16
30	23.29	81.67	72.28	59.19	50.67

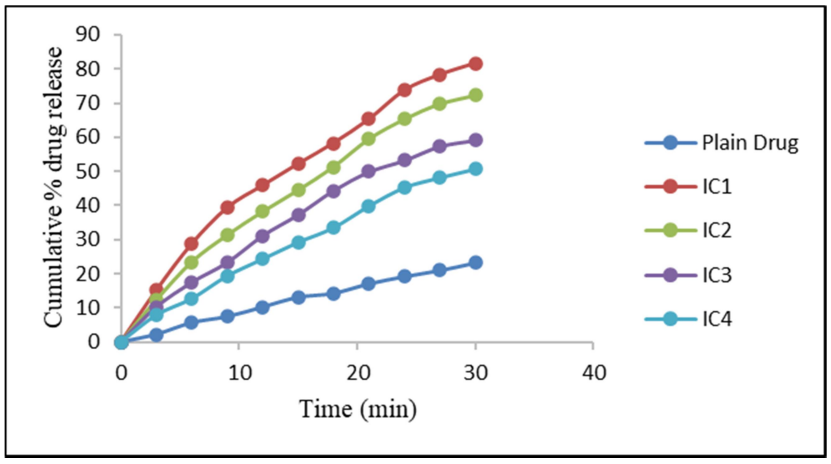

Fig 5: In vitro dissolution Profile of Plain drug and its Inclusion complex

X-ray diffraction (XRD) studies

XRD is a useful method for the detection of efficiency of β-CD encapsulation and to assess the degree of crystallinity of a given sample. The formation of an inclusion complex can be confirmed by comparison of XRD pattern of drug and IC, because the changes in crystallinity of Ambrisentan can be attributed to its partial amorphization or improved wettability in presence of hydrophilic carrier i.e., βCD.

The XRD patterns of the Ambrisentan and IC1 are as shown in Figure 6a and Figure 6b. XRD patterns of the Ambrisentan (Figure 6a) shows characteristic peaks at 8.86°, 12.30°, 14.12°, 18.28°, 20.54°, 24.24°, 26.84° of 2θ. While XRD patterns of IC1 (Figure 6b**)** has shown reduction in intensity of characteristic

peaks of Ambrisentan. This is indicating that the drug was partially amorphized. Also, the distinctive halo pattern confirms the conversion of drug from its crystalline form to amorphous form in its complex with β-CD.

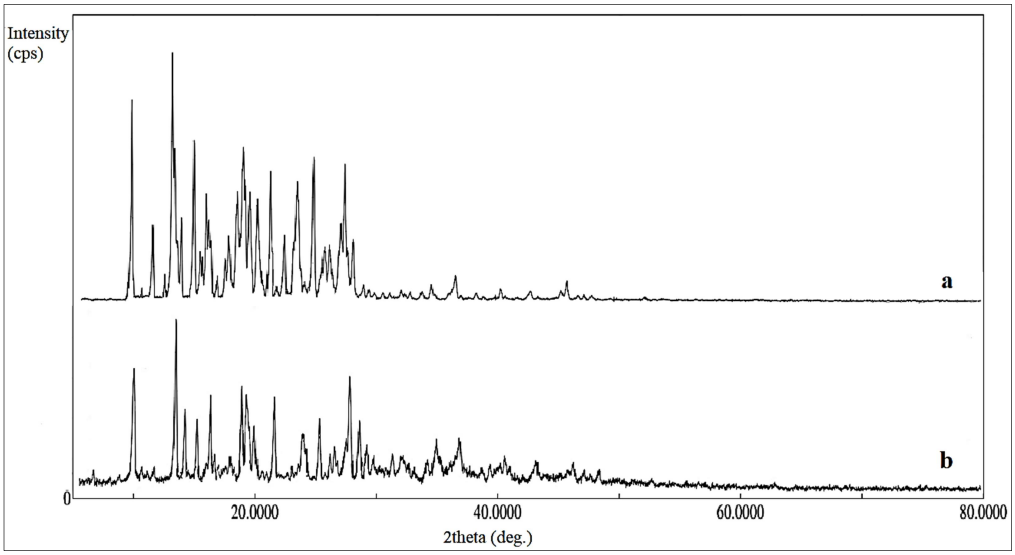

Figure 6: XRD of a) Ambrisentan and b) IC1

FTIR Spectroscopic analysis

The FTIR Spectrum of Ambrisentan and IC1 is as shown in Figure 3. It was found that no profound changes occurred in characteristic peaks of Ambrisentan and all original peaks were retained in inclusion complex with β-CD.

Formulation and evaluation of Mouth Dissolving Tablet by Direct Compression Method

The Inclusion complex-based mouth dissolving tablets were formulated as per central composite design using superdisintegrants viz. sodium starch glycolate (2%, 5% and 8% concentration) crospovidone (1%, 3.5% and 5% concentration), that aid rapid disintegration of tablet when placed in mouth. Also, Mannitol is used as bulking agent, it has cooling effect in the mouth, sweet taste and better water absorption capacity helps in faster disintegration of the tablet. Microcrystalline cellulose was used as disintegrant and to aid direct compression process. Vanillin and sodium saccharin were used as flavouring agent and sweetening agent respectively, to improves tablet's palatability by masking bitter taste of drug.

Pre-compression evaluation of powder blend

The blend of all batches was evaluated for precompression parameters like bulk density and was found to be between 0.4416 ± 0.05 - 0.4984 ± 0.03 and tapped density between 0.5059 ± 0.05 - 0.5711 ± 0.06. Carr's index was found to be in between 10.37±0.09 - 15.00 ± 0.41, Hausner's ratio ranged between 1.11 ± 0.02 to 1.17 ± 0.02. Angle of repose was found to be between 30.25 ± 0.46 - 35.15 ± 0.21. All the formulations showed excellent and good flow properties. The results are shown in Table 11.

Table 11: Pre-compression evaluation of powder blend

Batch	Bulk Density (gm/ml)	Tapped Density (gm/ml)	Carr's Index (%)	Hausner's Ratio	Angle of Repose(^{0})
F1	0.4954 ± 0.05	0.5711 ± 0.06	13.25 ± 0.23	1.15 ± 0.02	32.48 ± 0.22
F2	0.4810 ± 0.02	0.5492 ± 0.03	12.41 ± 0.87	1.14 ± 0.01	31.98 ± 0.75
F3	0.4984 ± 0.03	0.5561 ± 0.04	10.37 ± 0.09	1.11 ± 0.02	30.25 ± 0.46
F4	0.4810 ± 0.06	0.5659 ± 0.06	15.00 ± 0.41	1.17 ± 0.02	34.25 ± 0.46

F5	0.4831 ± 0.03	0.5493 ± 0.03	12.05 ± 0.94	1.13 ± 0.01	31.87 ± 0.8
F6	0.4752 ± 0.04	0.5565 ± 0.05	14.57 ± 0.36	1.17 ± 0.01	35.15 ± 0.21
F7	0.4675 ± 0.03	0.5241 ± 0.03	10.79 ± 0.20	1.12 ± 0.01	30.76 ± 0.96
F8	0.4416 ± 0.05	0.5059 ± 0.05	14.54 ± 0.75	1.14 ± 0.02	32.86 ± 0.93
F9	0.4572 ± 0.02	0.5343 ± 0.02	14.43 ± 0.60	1.16 ± 0.01	32.51 ± 0.14

*** All values are ± SD (n=3)**

Post-compression evaluation of MDT

The results obtained from post compression tests such as Thickness, Weight variation test, Hardness and Friability are shown in Table 12. Tablets of all formulation appears as white to off white colour, round shape with upper and lower surface smooth and circular biconvex without any deformities. The hardness of the tablet indicated good mechanical strength. Less than 1% friability indicated that the tablet had good resistance to external forces like shock and abrasion.

Table 12: Post-compression evaluation of MDT

Batch	Thickness(mm)	Weight variation test(mg)	Hardness(kg/cm$^{2)}$	Friability (%)
F1	3.56 ± 0.13	195.72 ± 0.55	3.78 ± 0.20	0.72 ± 0.06
F2	3.45 ± 0.17	200.41 ± 0.85	3.76 ± 0.04	0.70 ± 0.15
F3	3.37 ± 0.08	202.89 ± 1.87	3.69 ± 0.03	0.57 ± 0.06
F4	3.78 ± 0.10	198.53 ± 0.88	3.86 ± 0.11	0.77 ± 0.15
F5	3.44 ± 0.10	197.69 ± 1.03	3.74 ± 0.13	0.62 ± 0.12
F6	3.79 ± 0.02	200.67 ± 1.65	3.81 ± 0.26	0.77 ± 0.19
F7	3.43 ± 0.08	205.86 ± 0.91	3.73 ± 0.07	0.58 ± 0.09
F8	3.77 ± 0.11	200.06 ± 1.91	3.87 ± 0.02	0.76 ± 0.19
F9	3.64 ± 0.24	199.69 ± 1.97	3.84 ± 0.10	0.74 ± 0.11

*** All values are ± SD (n=3)**

Drug content

The drug content of all formulations was found to be 96.93±0.68 - 99.31±0.34% respectively. The results are shown in Table 13.

Wetting time

All the formulations showed quick wetting between the range of 34.06 ± 1.00 - 59.42 ± 2.15 seconds respectively. This result was due to the capacity of absorption of water and swelling. The results are shown in Table 13.

Water absorption ratio

Water absorptions ratio of formulation F1-F9 was obtained in the ranges of 40.81 ± 0.13 % to 61.29 ± 0.62 % respectively. The results are shown in Table 13. Water absorption ratio of tablets increases with increase in the concentration of superdisintegrants. More the super disintegrant concentration greater was the water uptake and therefore increases in water absorptions ratio.

In-vitro disintegration test

The most important parameter that needs to be optimized in the development of mouth dissolving tablets is the disintergration time of tablets. In present study all tablets disintegrated within the range of 44.71 ±

1.58 - 79.81 ± 1.76 seconds fulfilling the official requirement (less than 3 min) for mouth dissolving tablets. The results are shown in Table 13.

Table 13: Post-compression evaluation of MDT

Batch	Drug Content (%)	Wetting Time (sec)	Water Absorption Ratio (%)	In-vitro disintegration Time(sec)
F1	98.40 ± 1.28	45.21 ± 2.08	51.24 ± 0.43	60.82 ± 1.78
F2	98.63 ± 0.34	42.14 ± 1.52	56.82 ± 0.15	56.62 ± 2.30
F3	99.31 ± 0.34	34.06 ± 1.00	61.29 ± 0.62	44.71 ± 1.58
F4	96.93 ± 0.68	56.35 ± 2.64	42.30 ± 0.48	74.50 ± 2.64
F5	98.85 ± 0.51	41.08 ± 2.64	57.46 ± 0.51	51.15 ± 2.37
F6	95.45 ± 0.85	59.42 ± 2.15	40.81 ± 0.13	79.81 ± 1.76
F7	99.08 ± 0.70	38.01 ± 2.00	59.63 ± 0.34	48.94 ± 2.32
F8	97.15 ± 1.03	53.47 ± 2.04	44.21 ± 0.10	68.20 ± 2.73
F9	97.72 ± 0.70	49.10 ± 1.52	48.34 ± 0.58	65.94 ± 2.05

*** All values are ± SD (n=3)**

In-vitro dissolution test

In-vitro dissolution profiles of all F1 to F9 tablet batches is as shown in Table 14 and Figure 7. The percentage drug release was found to be in the range of 85.16 to 98.90 (unit %). The dissolution rate was found to be improved with increasing concentrations of superdisintegrants. Amongst all batches the optimized F3 batch has shown highest cumulative percentage drug release of about 98.90% in 30 minutes. This may be attributed to presence of Sodium starch glycolate (8%) and Crospovidone (5%) as superdisintegrants.

Table 14: In-vitro dissolution data of Ambrisentan: βCD Inclusion complex-based MDT

	Batches (% Cumulative drug release)								
Time(min)	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
3	40.12	42.34	47.21	34.94	45.17	31.35	45.17	28.26	38.06
6	46.56	50.91	56.16	48.16	52.36	51.49	54.26	42.62	48.24
9	51.47	55.16	63.46	56.36	59.71	59.27	61.67	48.74	53.42
12	59.19	62.34	70.61	63.74	67.86	65.16	69.16	52.52	64.74
15	64.24	71.78	80.57	71.34	77.47	67.57	78.41	60.19	71.16
18	72.68	79.38	89.24	75.29	84.68	71.29	87.36	69.56	76.38
21	79.23	88.29	93.63	79.16	87.17	74.74	91.24	74.34	80.61
24	84.15	91.17	96.51	82.27	91.26	80.35	93.16	84.29	83.82
27	89.27	92.19	98.67	85.36	93.13	83.64	95.74	87.17	88.34
30	92.19	93.28	98.90	86.14	95.24	85.16	96.24	88.23	90.23

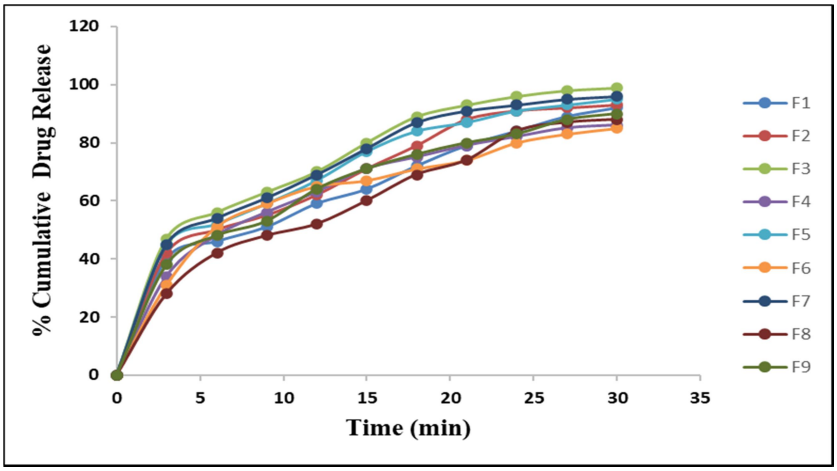

Figure 7: In-vitro dissolution graph of Amb: βCD Inclusion complex-based MDT

The in-vitro dissolution profiles of Plain drug, IC1 and an optimized batch (F3) were compared and the results are as shown in Table 15 and Figure 8.

Table 15: Comparison of in-vitro dissolution data of Plain drug, IC1 and F3 Batch

Time (min)	% Cumulative Drug Release		
	Plain Drug	IC1	F3
0	0	0	0
3	2.25	15.14	47.21
6	5.69	28.76	56.16
9	7.47	39.34	63.46
12	10.29	45.87	70.61
15	13.17	52.19	80.57
18	14.34	58.28	89.24
21	17.13	65.27	93.63
24	19.26	73.73	96.51
27	21.05	78.31	98.67
30	23.29	81.67	98.90

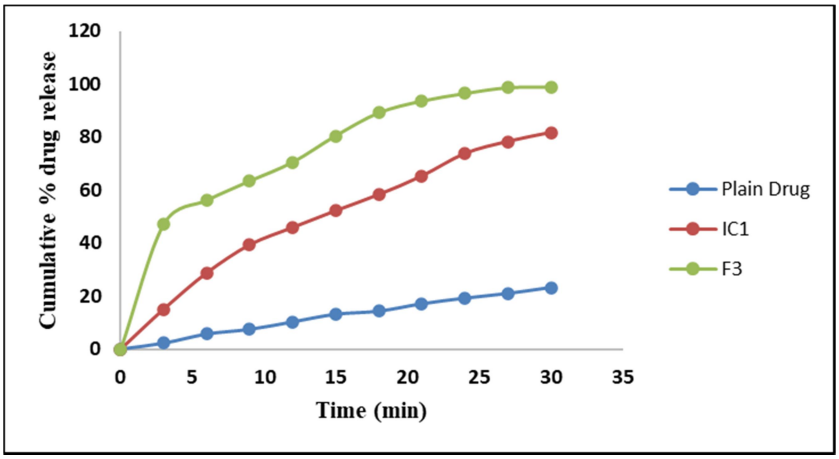

Fig 8: Comparison of in-vitro dissolution profile of Plain drug, IC1 and F3 Batch

Thus, from the results of Post-compression Evaluation of MDTs, it was concluded that, Batch F3 is selected as an Optimized Batch and for further investigation.

Stability studies

The short-term stability study was carried out for the optimized formulation (F3) stored at the temperature and humidity conditions such as, RT and 40 ± 2^0C / 75 ± 5% RH for 3 months. The various parameters such as hardness, disintegration time, drug contents and In-vitro drug release were analyzed at a different time of interval such as 1, 2 and 3 months. There was not much variations observed in any parameters throughout study period of time. The results are shown in Table 16.

Table 16: The stability studies of optimized formulation (F3) at RT and 40 ± 2^0C / 75 ± 5% RH

Stability (Duration)	Hardness (kg/cm^2)		Disintegration time (seconds)		Drug content (%)		In-vitro drug release (%)	
	Storage Condition							
	RT	40 ± 2^0C / 75 ± 5 % RH	RT	40 ± 2^0C / 75 ± 5 % RH	RT	40 ± 2^0C / 75 ± 5 % RH	RT	40 ± 2^0C / 75 ± 5 % RH
Initial	3.69 ± 0.03	3.69 ± 0.03	44.71 ± 1.58	44.71 ± 1.58	99.31 ± 0.34	99.31 ± 0.34	98.90	98.90
1 months	3.69 ± 0.02	3.69 ± 0.01	44.71 ± 1.65	44.71 ± 1.71	99.18 ± 0.23	99.18 ± 0.45	98.48	98.54
2 months	3.69 ± 0.01	3.69 ± 0.00	44.71 ± 1.77	44.71 ± 1.84	99.04 ± 0.28	99.04 ± 0.57	98.32	98.38
3 months	3.69 ± 0.00	3.68 ± 0.01	44.71 ± 1.88	44.71 ± 1.90	98.91 ± 0.37	98.91 ± 0.68	98.18	98.22

*** All values are ± SD**

CONCLUSION

Inclusion complex of AMB was prepared by complexation with β-CD by kneading method. The formation of inclusion complex was confirmed by XRD. The IC was then formulated into an MDT. Sodium starch glycolate, crosspovidone and microcrystalline cellulose reduced the disintegration time of AMB-β-CD IC based MDTs. The tablet parameters complied within the acceptable limit. Amongst all formulation (F1-F9) studied, Formulation F3 which contains sodium starch glycolate (8%) and crospovidone (5%) as a super disintegrant shows minimum disintegration time and percentage cumulative drug release (98.90%) was best and hence F3 formulation considered as the optimized batch as MDT which may provide fast onset of action and better patient compliance for pulmonary arterial hypertension.

ACKNOWLEDGMENTS

The author is grateful to the Founder and President of Rajgad Dnyanpeeth's College of Pharmacy Bhor Pune Shri. Anantraoji Thopte Sir and Shri. Sangram Thopte Sir and Principal of Rajgad Dnyanpeeth's College of Pharmacy Bhor Pune Dr. R. V. Shete Sir for providing the resources needed to complete the research work.

CONFLICT OF INTEREST

The author declares that he does not have any conflict of interest.

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