International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 12, Issue 5, 2020

Original Article

PART II: OPTIMIZATION OF ISOSORBIDE DINITRATE SUSTAINED RELEASE LAYER IN ANTIHYPERTENSIVE BILAYER TABLET

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Received: 30 Apr 2020, Revised and Accepted: 06 Jul 2020

ABSTRACT

Objective: Aim of the present study was the optimization of the sustained release (SR) layer of isosorbide dinitrate (ISDN) 40 mg and compressed with the immediate-release (IR) layer of hydralazine hydrochloride (HHC) 25 mg to decrease the dosing frequency and development of a novel b. i. d dosage form.

Methods: Drug excipients compatibility study was carried out by FT-IR and a preliminary study was conducted for screening of polymer. The amount of HPMC K100M (X_1) and the amount of Polyox^{tm} WSR303 (X_2) were chosen as independent variables in 3^2full factorial designs. While % cumulative drug releases at 1 h (Q_1) (Y_1), % cumulative drug release at 2 h (Q_2) (Y_2), % cumulative drug release at 4 h (Q_4) (Y_3) and % cumulative drug release at 6 h (Q_6) (Y_4), were taken as dependent variables and statistically evaluation by using sigma plot 13.0. In the present study, according to the U. S. P. 2007 the following constraints were used for the selection of an optimized batch: Q_1=15% to 30%, Q_2=50% to 70%, Q_4=65% to 85% and Q_6>75%. To validate the evolved mathematical models, a checkpoint batch was selected from its desirability value.

Results: FT-IR spectra show that the drug and excipients were compatible with each other. The calculated F values found for Q_1, Q_2, Q_4, and Q_6 were 084.583, 038.188, 057.719, and 118.396, respectively. All Calculated F values are greater than the tabulated value for all dependent variables. Prepared checkpoint batch selected from its desirability value 1 and it gives a 93.40±1.29 % drug release within 6 h.

Conclusion: This bilayer formulation of anti-hypertensive drugs decreases the dosing frequency of HHC and ISDN.

Keywords: Bilayer tablet, 3^2 Full factorial design, Hydralazine hydrochloride, Isosorbide dinitrate, Sustained release layer

DOI: http://dx.doi.org/10.22159/ijap.2020v12i5.38098. Journal homepage: https://innovareacademics.in/journals/index.php/ijap

INTRODUCTION

Hypertension affects around half of the adult population worldwide, being one of the most common cardiovascular disorders (CVD). It occurs when the high cardiac output exerts pressure on the arterial wall as the blood flow increases. The conventional dosage form used in the treatment of hypertension cannot produce the desired therapeutic effect for a prolonged period and thus dose fluctuation and missing dose chances are more. The rationale for using fixed-dose combination therapy is to obtain increased blood pressure control by employing two antihypertensive drugs with different modes of action and enhance compliance by using a single tablet. Bilayer tablet is suitable for the sequential release of two drugs in combination, separate, and for sustained release [1, 2].

HHC, directly acting as potent peripheral vasodilator, is widely prescribed in the treatment of hypertension and congestive heart failure by direct relaxation of arteriolar smooth muscle. ISDN is relaxation of vascular smooth muscle and consequent dilatation of peripheral arteries and veins, especially the latter. Dilatation of the veins promotes peripheral pooling of blood and decreases venous return to the heart, thereby reducing left ventricular end-diastolic pressure and pulmonary capillary wedge pressure. The combination consists of an immediate-release layer of hydralazine hydrochloride 25 mg and SR layer of ISDN 40 mg fixed-dose that functions as a nitric oxide enhancer and an antioxidant that helps to prevent tolerance to the prolonged use of nitrate. This combination also balanced after-load and pre-load reduction with a lowering of ventricular filling pressure and systemic and pulmonic vascular resistance. The hemodynamic effects of the combination drug in heart failure include increased cardiac output [3, 4]. In the present study, according to the U. S. P. 2007 the following constraints were used for the selection of an optimized batch: Q_1=15% to 30%, Q_2= 50% to 70%, Q_4= 65% to 85% and Q_6>75%.

Aim of the present study was the optimization of the SR layer of ISDN 40 mg and compressed with a pre-optimized IR layer of HHC 25 mg to decrease the dosing frequency and develops twice a day formulation.

MATERIALS AND METHODS

Materials

HHC was kindly supplied as gift samples by Torrent Pharmaceuticals, Ahmedabad, Gujarat India. ISDN was supplied as gift samples from Cadila Pharmaceuticals Ltd, Ahmadabad, India. Microcrystalline cellulose (MCC), Polyox^{tm} WSR 303, Ethylcellulose (EC) were procured from Colorcon Asia Pvt. Ltd., Ahmedabad, Gujarat, India. Magnesium stearates, talc, sodium starch glycolate (SSG), croscarmellose sodium (CCS) were purchased from SD Fine Chemicals, Mumbai, India. All other materials and chemicals used were of either pharmaceutical or analytical grade.

Drug-excipients compatibility study

Drug excipient interaction plays a vital role in achieving the stability of the drug in the dosage form. Fourier transform infrared spectroscopy (FT-IR) was used to study the physical and chemical interactions between drugs and excipients. FT-IR spectra of ISDN and mixtures of drugs with other excipients were obtained by using the FT-IR instrument. (FT-IR-1700, Shimadzu, Kyoto, Japan) [5].

Preliminary screening of polymer for SR layer

The development of a SR layer containing ISDN 40 mg by selecting ingredients in the appropriate amount and polymer was optimized thereafter. The SR layer of ISDN was prepared by the direct compression method. HPMC K4M, HPMC K100M, polyox^{tm}WSR 301 and Polyox^{tm} WSR 303 were used in various amounts as shown in table 1. Batch T1 was prepared with HPMC K4M and polyox^{tm}WSR 301. Batch T2 prepared to check the effect of HPMC K4M with polyox^{tm}WSR 301. Batch T3 and T4 were prepared with HPMC K15M with different grades of polyox^{tm}WSR. Batch T5 and T6 were developed to check the effect of HPMC K100M on different grades of polyox^{tm}WSR. Prepared layer was evaluated for weight variation, thickness, hardness, friability, disintegration time, drug content, and % cumulative drug release [6, 7].

Table 1: Preliminary screening of polymer for SR layer

Ingredients (mg)	T1	T2	T3	T4	T5	T6
ISDN	40	40	40	40	40	40
MCCPH102	88.8	88.8	88.8	88.8	88.8	88.8
HPMC K4M	60	60	-	-	-	-
HPMC K15M	-	-	60	60	-	-
HPMC K100M	-	-	-	-	60	60
Polyox^{tm}WSR301	60	-	60	-	60	-
Polyox^{tm}WSR303	-	60	-	60	-	60
Quinoline yellow	0.2	0.2	0.2	0.2	0.2	0.2
Magnesium stearate	1	1	1	1	1	1
Total (mg/tablet)	250					

Optimization of polymer by using 3^2full factorial designs

A 3^2full factorial design was used for the optimization of the SR layer. The formulation of factorial batches was shown in table 2. Based on preliminary results, the amount of HPMC K100M (X_1) and the amount of polyox^{tm}WSR 303 (X_2) were chosen as independent variables in 3^2 full factorial designs. In this study, Q_1 (Y_1), Q_2 (Y_2), Q_4 (Y_3) and Q_6 (Y_4) were taken as dependent variables. Multiple linear regression analysis, ANOVA and graphical representation of the influence of factor by contour plots were performed using sigma plot 13.0 [8, 9]. The experimental runs and measured responses of 3^2 full factorial design batches of SR layer of ISDN 40 mg were depleted in table 4.

Table 2: Formulation of factorial design batches for the immediate release layer

Ingredients (mg)	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8	F_9
ISDN	40	40	40	40	40	40	40	40	40
MCCPH102	88.8	78.8	68.8	78.8	68.8	58.8	68.8	58.8	48.8
HPMC K100M	60	70	80	60	70	80	60	70	80
polyox^{tm} wsr303	60	60	60	70	70	70	80	80	80
Quinoline yellow	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Magnesium stearate	1	1	1	1	1	1	1	1	1
Total	250	250	250	250	250	250	250	250	250

Development of bilayer tablet

The bilayer tablet was prepared using by direct compression method. In this method IR layer HHN 25 mg and SR layer ISDN 40 mg was were prepared separately and the two layers were compressed together. IR layer of HHN was prepared using a direct compression method. IR layer prepared by using a rotary tablet punching machine by using 8 mm flat punch on a 12-station rotary tablet machine [10, 11]. SR layer of ISDN was optimized by using 3^2full factorial designs and it was prepared using a direct compression method. In this method ISDN, HPMC K100M and polyox WSR 303 were passed through 40# mesh and mixed well. Other excipients like MCC PH102 and quinoline yellow were added to the above mixture. Finally magnesium stearate was added to the above blend and mixed homogenously. The whole powder blend was collected for compression and 250 mg tablets were compressed using a rotary tablet punching machine by using 8 mm flat punch on a 12-station rotary tablet machine [12, 13].

Evaluation of bilayer tablet

The prepared tablets were evaluated for thickness, hardness, friability, and disintegration time of the SR layer were measured as described by Nivedithaa VR *et al.* and Fridrun P *et al.* [14, 15].

HPLC was used for estimation of HHN and ISDN: The drug concentration was measured according to the reverse-phase high-performance liquid chromatography. Analysis of sample was performed using a cyber lab HPLC system equipped with an LCP-100 pump, cyber lab LC-UV100 UV detector, and RP C18 column (250 × 4.6 mm ID, particle size 5µ) at ambient temperature. The mobile phase used was a mixture of methanol and distilled water 1000 ml containing 0.1 ml TEA each (60: 40). The pH was adjusted to 6.5. The flow rate was 1.0 ml per min. The detection was carried out at 215 nm. A calibration curve was plotted for HHN and ISDN. A good linear relationship was observed between the concentration of the drug and the peak area of the drug with a correlation coefficient [16].

Drug content for ISDN: The drug content was carried out by weighing 10 tablets from each batch and calculated the average weight. Then the tablets were triturated and weighed accurately, which was equivalent to 100 mg ISDN. It was dissolved in a 100 ml volumetric flask containing 100 ml of 0.1N HCl and volume was made to 100 ml with solvent. The volumetric flask was shaken using a sonicator for 1 h and after suitable dilution, with 0.1N HCl the drug content was determined using HPLC at 215 nm.

In vitro drug release study: *In vitro* release of bilayer tablets was determined using a USP type-II dissolution test apparatus at 100 rpm. The dissolution was studied using 900 ml of simulated gastric fluid 0.1N HCl (without enzyme, pH 1.2) for the first 2 h and followed by a simulated intestinal fluid (without enzyme pH 6.8) for the remaining 10 h. The temperature was maintained at 37±0.5 °C. 5 ml sample was taken at different time intervals up to 12 h. filter through Whatman filter paper and replaced by an equal volume of dissolution medium sample were suitably diluted and analyzed by HPLC at 215 nm.

Stability study: Optimized batch was packed in aluminum foil and was placed for stability study at 40°C/75% RH for 6 mo. The sample was evaluated after 6 mo for physical parameters and *In vitro* dissolution. The dissolution profile of the product compared using the similarity factor, f_2, which was calculated by the following formula.

$$f_2 = 50 \log \left[\{1+\frac{1}{n}\sum_{t=1}^{n}(R_t - T_t)^2\}^{-0.5} x100\right]$$

Where a log is a logarithm to the base 10, n is the number of time points, $\sum$ is the summation over all time points, R_t is the mean dissolution value of the reference profile at time t and T_t is the mean dissolution value of the test profile at the same time point. The USFDA draft guidance document contains more information on the similarity factor (f_2). The value of similarity factor (f_2) between 50 and 100 suggests that the two dissolution profiles are similar [17, 18].

RESULTS AND DISCUSSION

Drug-excipients compatibility study by FT-IR

Fourier transform infrared spectroscopy (FT-IR) study was used to the physical and chemical interactions between drugs and

excipients. FT-IR spectra of ISDN with excipients were recorded using KBr mixing method on the FT-IR instrument. The FT-IR spectra of ISDN and the mixture of drugs with excipient are shown in fig. 1(A) and fig. 1(B). ISDN exhibited peaks due to C-O, O=NO_2, C-H_2–CH_2, and N=O stretching as shown in fig. 1. It was observed that there were no or very minor changes in main drug peaks in the IR spectra of the mixture and pure drug. The FT-IR study revealed no physical or chemical interaction of drugs with excipient [19].

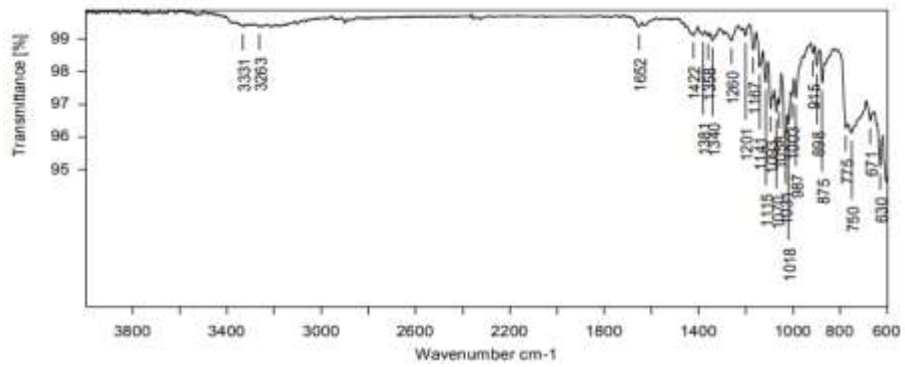


Fig. 1(A): FT-IRof ISDN (Indian pharmacopeia)

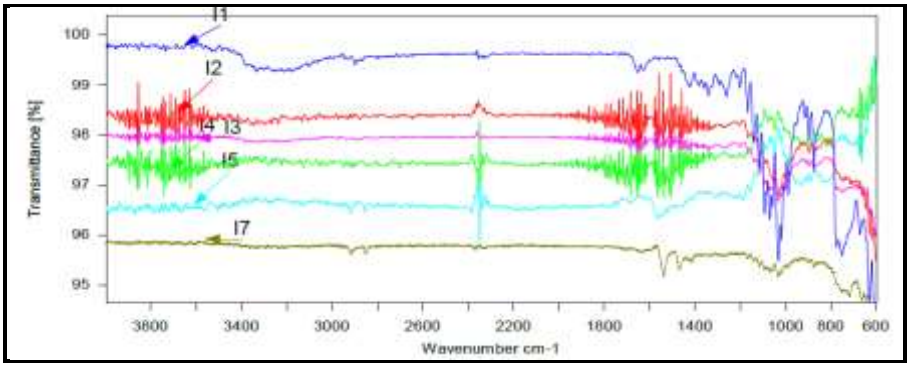


Fig. 1(B): Overlay spectra of ISDNwith all excipients, (I1 = ISDN, I2 = ISDN+MCCPH102, I3 = ISDN+HPMC K100M, I4 = ISDN+polyox^{tm} WSR303, I4 = ISDN+HPMC K15M, I7 = ISDN+Magnesium Stearate)

Table 3: Preliminary screening of polymer

Batch	Hardness (Kg/cm^2)	Friability (%)	Q_1	Q_2	Q_4	Q_6
T1	3.30±0.08	0.40±0.02	51.09±2.35	90.80±1.57	98.80±.57	99.90±1.89
T2	3.22±0.06	0.42±0.05	46.03±2.85	85.60±2.47	97.20±1.51	98.60±2.15
T3	3.18±0.03	0.48±0.03	43.09±2.35	80.80±1.57	96.08±1.57	98.09±1.89
T4	3.26±0.02	0.46±0.05	38.30±2.85	75.60±2.47	96.07±2.32	98.18±1.65
T5	3.35±0.04	0.42±0.04	35.20±1.38	74.05±2.48	93.01±1.79	98.58±2.38
T6	3.28±0.01	0.44±0.07	32.70±2.67	70.08±1.41	90.07±1.81	99.08±1.34

*All values are mean±SD, (n=6)

Table 4: Runs and measured responses of 3^{2}factorial design batches

Batch code	HPMC K100M (X_1)	POLYOX™ WSR303 ® (X_2)	Q_1 (Y_1)	Q_2 (Y_2)	$4Q_4$ (Y_3)	Q_6 (Y_4)
F_1	-1	-1	32.70±2.67	70.08±1.41	90.07±1.81	99.83±1.124
F_2	0	-1	31.33±0.57	69.20±0.29	87.9±2.33	98.11±1.32
F_3	1	-1	28.62±0.87	67.23±014	85.3±1.55	97.12±1.544
F_4	-1	0	24.66±0.87	62.34±0.27	82.5±1.89	95.18±1.42
F_5	0	0	21.34±0.59	57.54±0.24	79.3±1.67	94.68±1.21
F_6	1	0	18.66±0.32	53.34±0.17	75.3±2.57	93.43±1.04
F_7	-1	1	15.33±0.25	50.54±0.14	70.4±2.89	92.78±1.67
F_8	0	1	14.63±0.78	48.43±0.27	69.3±2.40	91.38±1.2334
F_9	1	1	13.16±0.85	46.46±0.89	68.9±1.97	90.58±1.31
Factors and the levels in the design						
Independent variables			**Low (-1)**	**Medium (0)**	**High (1)**	
HPMC K100M (X_1)			60	70	80	
POLYOX™ WSR303 (X_2)			60	70	80	

*All values are mean±SD, (n=6)

Table 5: Evaluation of 3^2factorial design batches

Batch	Hausner's ratio	Angle of repose(Θ)	Weight variation (mg)	Thickness (mm)	Friability %	Drug content %
F_1	1.15±0.05	23.54±0.02	249.40±0.18	4.22±0.03	0.73±0.03	99.40±0.36
F_2	1.14±0.01	22.36±0.09	250.60±0.07	4.57±0.04	0.68±0.15	99.45±1.18
F_3	1.14±0.05	22.38±0.09	251.83±1.07	4.36±0.04	0.73±0.04	99.86±1.52
F_4	1.16±0.05	23.82±0.07	250.50±0.56	4.30±0.05	0.53±0.05	98.13±2.23
F_5	1.18±0.07	25.38±0.06	250.70±0.57	4.28±0.07	0.53±0.03	99.33±1.52
F_6	1.17±0.01	24.80±0.05	250.90±0.78	4.41±0.09	0.44±0.017	99.26±1.32
F_7	1.16±0.02	23.81±0.03	251.43±1.55	4.48±0.02	0.62±0.02	98.60±1.87
F_8	1.16±0.02	23.86±0.31	250.45±0.67	4.42±0.01	0.81±0.45	99.70±0.71
F_9	1.17±0.01	24.85±0.07	250.70±0.56	4.25±0.32	0.59±0.45	99.57±1.47

*All values are mean±SD, (n=6)

Preliminary screening of polymers for SR layer

The batches T1 to T6 were prepared to achieve an optimized concentration of polymer and the most efficacious one among incorporated to prepare SR layer of ISDN 40 mg, as shown by table 1. Preliminary trial batches results were shown in table 3. Batch T1 containing HPMC K4M and Polyox^{tm} WSR 301 showed 51.09±2.35 % drug release at 1 h. Batch T2 and batch T3 were shown 46.03±2.85 % and 43.09±2.35 % drug release in 1 h, respectively. While batch T4 and T5 give, 75.60±2.48% and 74.05±2.48% release in 2 h. Batch T6 contains HPMC K100M and Polyox^{tm} WSR 303 that shows 32.70±2.67 % and 70.08±1.41% drug release at 1 h and 2 h, respectively. Hence, further trials were carried out by using a combination of HPMC K100M and polyox^{tm}WSR 303 to understand their effect and optimize the concentration of both for the desired release profile [20].

3^2 full factorial design model evaluation

A statistical model incorporating interactive and polynomial terms used to evaluate the responses:

$$Y=b_0+b_1X_1+b_2X_2+b_{11}X_1^2+b_{22}X_2^2+b_{12}X_1X_2$$

Where Y is the dependent variable, b_o is the arithmetic mean response of the 9 runs and any bi is the estimated coefficients for the related factor X_i. The main effects (X_1 and X_2) represent the average result of changing one factor at a time from its low to high value. The polynomial terms (X_1^2 and X_2^2) are included to investigate nonlinearity. The interaction term "X_1X_2" shows how the response changes when the two factors change simultaneously. Evaluation data for SR layer of ISDN 40 mg were presented in table 4 and table 5. The fitted equations relating the responses that are, Q_1 (Y_1), % Q_2 (Y_2), Q_4 (Y_3) and % Q_6 (Y_4) were to the transformed factor are shown in table 7. The polynomial equation used to conclude after considering the magnitude of coefficient and mathematical sign it carries (i.e. positive or negative). The results of ANOVA suggested that calculated F values for Q_1, Q_2, Q_4, and Q_6 were 084.583, 038.188, 057.719, and 118.396, respectively, shown in table 6. Tabulated F value was found to be 9.013 at α = 0.05. Calculated F values are greater than tabulated for all dependent variables. Therefore, the factors selected have shown significant effects. From the results of multiple regression analysis, it was found that all factors had a statistically significant influence on all dependent variables as $p<0.05$ [21, 22].

Table 6: Results of the ANOVA for dependent variables

Source of variation	DF	SS	MS	F value	P value
Q_1*					
Regression	5	437.224	087.445	084.583	0.002
Residual	3	003.102	001.034		
Total	8	440.325	055.041		
Q_2#					
Regression	5	666.154	133.231	038.188	0.006
Residual	3	010.467	003.489		
Total	8	676.621	084.578		
Q_4@					
Regression	5	528.985	105.797	057.719	0.004
Residual	3	005.499	001.833		
Total	8	534.484	066.810		
$Q_6$$					
Regression	5	76.869	15.374	118.396	0.001
Residual	3	0.390	0.130		
Total	8	77.259	9.657		

*Q_1= % cumulative drug releases at 1 h, #Q_2= % cumulative drug releases at 2 h, @Q_4= % cumulative drug releases at 4 h, $$Q_6$= % cumulative drug releases at 6 h

Table 7: Summary of regression output of factors for measured responses

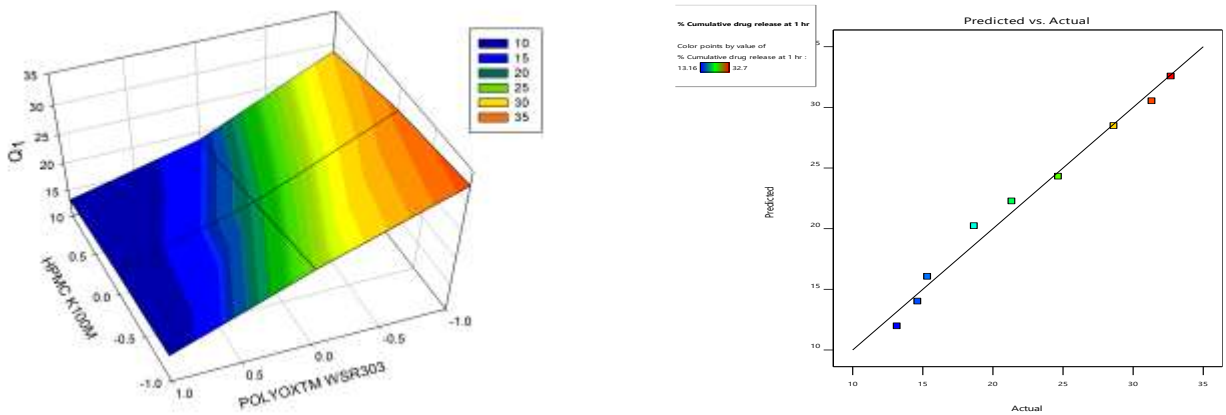
Responses	Model	Coefficient of regression parameters						
		b_0	b_1	b_2	b_{11}	b_{22}	b_{12}	R^2
Q_1	Full	21.717	2.042	8.255	0.245	1.075	0.478	0.993
	Reduced	21.717	2.042	8.255	-	-	-	
Q_2	Full	57.779	2.655	10.180	0.0583	0.917	0.308	0.959
	Reduced	57.779	2.655	10.180	-	-	-	
Q_4	Full	79.143	2.248	9.090	0.105	0.400	0.797	0.990
	Reduced	79.143	2.248	9.090	-	-	-	
Q_6	Full	94.366	1.110	3.387	0.0967	0.537	0.127	0.995
	Reduced	94.366	1.110	3.387	-	-	-	

*Q_1= % cumulative drug releases at 1 h, #Q_2= % cumulative drug releases at 2 h, @Q_4= % cumulative drug releases at 4 h, $$Q_6$= % cumulative drug releases at 6 h

Full and reduced model for Q_1

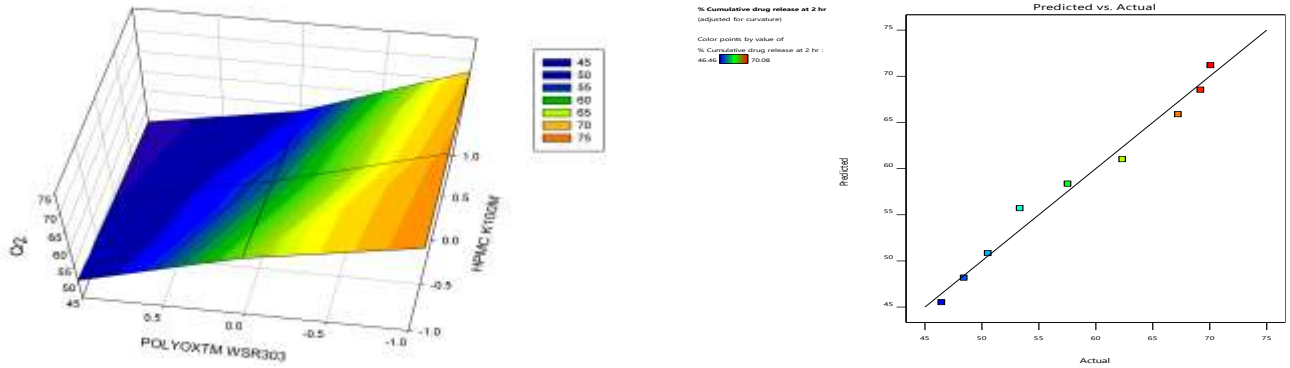
$$Q_1 = 21.717-(2.042 * X_1)-(8.255 * X_2)-(0.245 * X_1^2)+(1.075 * X_2^2)+(0.478 * X_1 X_2)$$

In this analysis, Q_1 ranges were observed between 13.16±0.85% to 32.7±2.67 %. Based on the analysis of variance (ANOVA) the result showed that the developed linear model was highly significant, as was evident from a very low probability value 0.002. The value of R^2 was found to be 0.993. The plot of the observed value of Q_1 versus the predicted value of Q_1 (fig. 3B) shows a straight line. Therefore, it concluded that the equation has good predictive ability. From the 3D plot (fig. 3A) and regression coefficient values of factors, it was concluded that when the amount of polymer was increased, drug releases was decrease. The polyether chains of polyox^{tm}WSR303 can form strong hydrogen bonds with water; polyox^{tm}WSR303 upon exposure to water or gastric juices, they hydrate and swell rapidly to form hydrogels. Therefore, polyox^{tm} WSR 303 was given a more significant effect on drug releases. Interaction and nonlinearity was not observed. For Q_1, the significance levels of the coefficients b_{11}, b_{22}, and b_{12} were found to be P= 0.756, 0.232, and 0.417, respectively, so they were omitted from the full model to generate a reduced model. The coefficients b_1, and b_2 were found to be significant at P<0.05; hence, it was retained in the reduced model. The reduced model for Q_1 $Q_1 = 21.717-(2.042 * X_1)-(8.255 * X_2)$



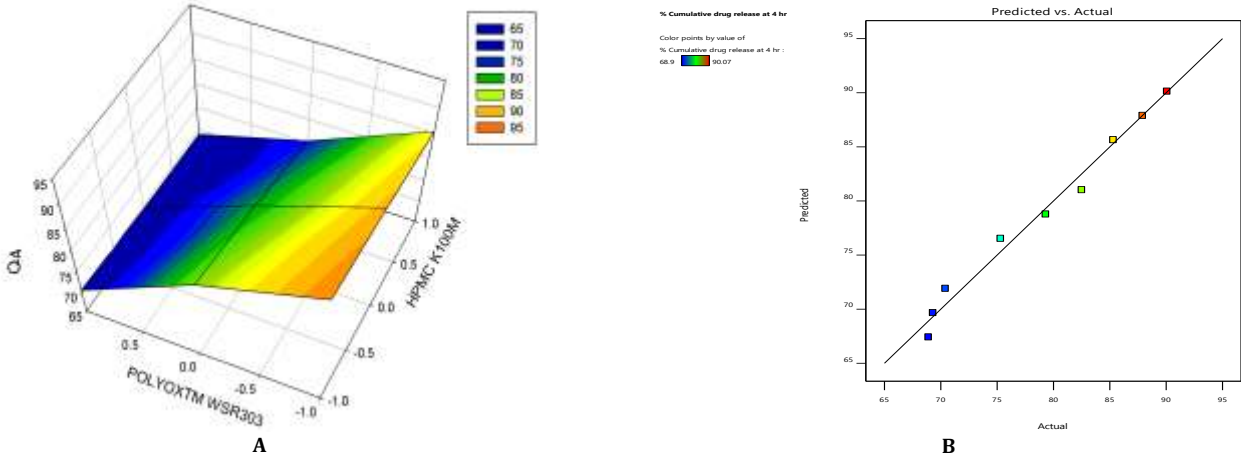
A B

Fig. 2: (A) 3D plot showing the effect of HPMC K100M and polyox^{tm}WSR303 on % Q_1 (B) Predicted Vs actual value of Q_1



A B

Fig. 3: (A) 3D plot showing the effect of HPMC K100M and polyox^{tm} wsr303 on % Q_2 (B) Predicted Vs actual value of Q_2



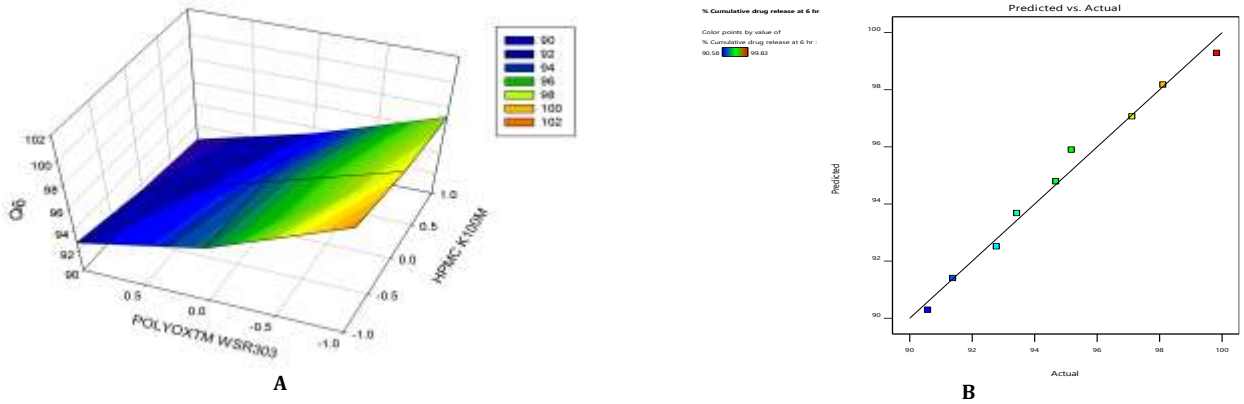
A B

Fig. 4 (A): 3D plot showing the effect of HPMC K100M and POLYOX™ WSR303 on Q_4 (B) Predicted Vs actual value of Q_4

Full and reduced model for Q_4

$$Q_4 = 79.143-(2.248 * X_1)-(9.090 * X_2)-(0.105 * X_1^2)-(0.400 * X_2^2)+(0.797 * X_1 X_2)$$

In this analysis, the Q_4 ranges were observed between 68.90±1.97% to 90.70±1.81%. The value of R^2 was found to be 0.985. ANOVA analysis indicates that the developed linear model was significant, and it shows a very low probability value of 0.004. The plot of the observed value of Q_4 versus the predicted value of Q_4 (fig. 4B) shows a straight line. From the 3D plot (fig. 4A) and the regression coefficient values of factors, it was concluded that when Polyox^{tm} WSR303 amount was increased Q_4 more decrease compared to the HPMC K100M. It was found that higher polymeric content in the matrix decreased the release rate of the drug. Interaction and nonlinearity was not observed. For Q_4, the significance levels of the coefficients b_{11}, b_{22}, and b_{12} were found to be P= 0.920, 0.704, and 0.324, respectively, so they were omitted from the full model to generate a reduced model [22]. The coefficients b_1 ,and b_2 were found to be significant at P<0.05; hence, it was retained in the reduced model. The reduced model for Q_4 $Q_4 = 79.143-(2.248 * X_1)-(9.090 * X_2)$



A B

Fig. 5: (A) 3D plot showing the effect of HPMC K100M and Polyox^{tm} WSR303 on Q_6 (B) Predicted Vs actual value of Q_6

Full and reduced model for Q_6

$$Q_6 = 94.366-(1.110 * X_1)-(3.387 * X_2)+(0.0967 * X_1^2)+(0.537 * X_2^2)+(0.127 * X_1 X_2)$$

In this analysis, the Q_6 ranges were observed between 90.58±1.34% to 99.83±1.12 %. Based on the analysis of variance (ANOVA) the result showed that the developed linear model was highly significant, as was evident from a very low probability value 0.001. The value of R^2 was found to be 0.995. The plot of the observed value of Q_6 versus the predicted value of Q_6 (fig. 5B) shows a straight line. Therefore, it concluded that the equation has good predictive ability. Interaction and nonlinearity was not observed. From the 3D plot (fig. 5A) and regression coefficient values of factors, it was concluded that when the amount of polymer was increased, drug releases was decreased. The results also indicated that Polyox^{tm} WSR 303 was given a more significant effect on drug releases. For Q_6, the significance levels of the coefficients b_{11}, b_{22}, and b_{12} were found to be P= 0.730, 0.126 and 0.530 respectively, so they were omitted from the full model to generate a reduced model. The coefficients b_1, and b_2 were found to be significant at P<0.05; hence, it was retained in the reduced model

$$Q_6 = 94.366-(1.110 * X_1)-(3.387 * X_2)$$

Search for the selection of optimized formulation

The optimization of the SR layer of ISDN 40 mg by numerical optimization. According to the U. S. P. 2007 the following constraints were used for the selection of an optimized batch: Q_1=15% to 30%, Q_2=50% to 70%, Q_4=65% to 85% and Q_6>75%. Further, the optimized SR layer was demarcated in the design space (overlay plot) shown in fig. 6. To validate the evolved mathematical models, a check-point was selected from its desirability value 1. Check-point batch CP1 was prepared and evaluated. The observed and predicted values for batch CP1 as shown in table 8. A good correlation was found between observed and predicted values. Hence, it was concluding that the evolved models might be used for the theoretical prediction of responses within the factor space. Optimized SR layer gives a 93.40±1.29 % drug release within 6 h. It was kept for stability study and *in vitro* release profile at initial and after 6 mo was compared using similarity factor, f_2, value that was found to be 93.078% for SR layer of ISDN. There is no significant difference in the similarity factor.

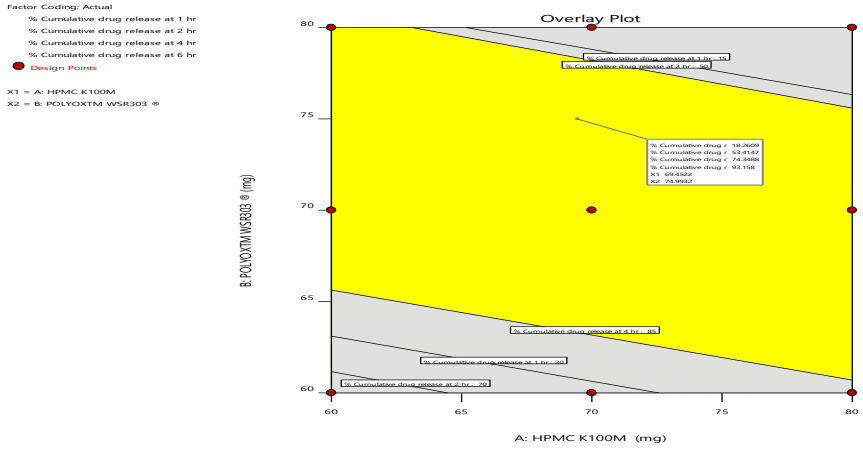


Fig. 6: Overlay plot depicting yellow color region design space and flagged point as the optimized SR layer of ISDN 40 mg

Table 8: Formulation and evaluation of check point batches

Formulation of check point batches				
Batch Code	Variable Level			
	Coded Value		Actual Value	
	X_1	X_2	X_1(mg)	X_2(mg)
CP1	0	1.11	70.00	77.94
Evaluation of check point batches and comparison with predicted value				
Parameter		Actual value	Predicted value	
% Cumulative drug releases at 1 h (Q_1)		14.87±0.36	15.708	
% Cumulative drug release at 2 h (Q_2)		52.40±1.24	50.259	
% Cumulative drug release at 4 h (Q_4		79.40±0.68	71.531	
% Cumulative drug release at 6 h (Q_6)		93.40±1.29	92.096	

(n=6)

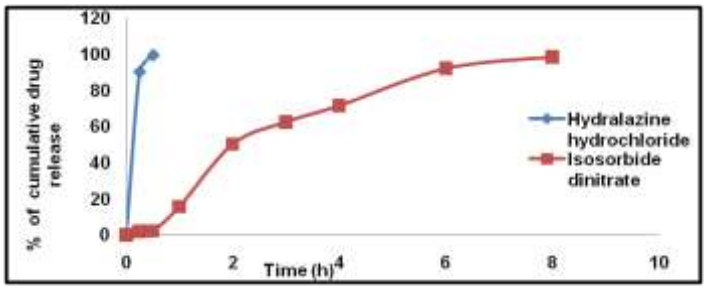


Fig. 7: *In vitro* drug release study of bilayer antihypertensive tablet

Evaluation of bilayer tablets

Finally, the optimized IR layer HHC 25 mg and SR layer ISDN 40 mg were prepared separately and two layers compressed together. *In vitro* Release of the bilayer tablet was shown in fig. 7. HHC gives 100% drug release in 30 min. While ISDN gives 91.67±0.68% in a simulated intestinal fluid in 6 h.

CONCLUSION

The present investigation was to formulate, evaluate, and optimize SR layer containing ISDN 40 mg in a bilayer tablet. There was no Drug-excipient interaction in the FT-IR study. From the results of preliminary studies, HPMC K100M (X_1) and the amount of Polyox^{tm} WSR303 (X_2) was chosen as independent variables in 3^2 full factorial design. While Q_1 (Y_1), Q_2 (Y_2), Q_4 (Y_3) and Q_6 (Y_4), were taken as dependent variables. The effect of independent variables on dependent variables was studied by analyzing the response surface plot and polynomial equation. Optimization of the SR layer of ISDN was performed by the overlay plot. A checkpoint batch was designed according to the results of the desirability value and evaluated for all the parameters. The results of the comparison of predicted response and obtained responses were found in good agreement. The formulation was found to be stable during accelerated stability study. SR of ISDN was helped to prevent tolerance to the prolonged use of nitrate. Finally, optimize layers of HHC and optimize layer of ISDN were prepared separately, and the two layers compressed together. This fixed-dose two times a day therapy has functioned as a nitric oxide enhancer and an antioxidant that helps to prevent tolerance to the prolonged use of nitrate.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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