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Design of Experiment - Pulsatile Drug Delivery of Anti-Hypertensive Agent Enalapril Maleate

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Abstract **Original Research Article**

The aim of the present investigation was to design and optimize pulsatile drug delivery of model antihypertensive drug enalapril. The developed formulations were optimized using 4-factor 3- level Box-Behnken statistical design. The rationale behind this Box Behnken design based on the salient principles of design of experiments and quality by design approach. Three independent variables viz., ethyl cellulose (X1), sodium alginate (X2), HPMC (X3) and three dependent variables viz., percentage drug release at 4 hrs (Y1), percentage drug release at 8 hrs (Y2) and percentage drug release at12 hrs (Y3) were selected for the study. Contour plots were constructed to further elucidate the relationship between the independent variables and dependent variables. The polynominal equations and contour plots developed using Box Behnken design allowed us to prepare Pulsatile tablets with optimum responses.

Keywords: Enalapril, Box Behnken, Contour plot, optimization, Pulsatile, DoE- Design of experiment.

INTRODUCTION

Cardiovascular disease (CVD) is the most leading cause for the death and disability and associated with high cost of health care [1]. In cardiovascular diseases hypertension is the most dangerous cause for the mortality rate which increases during early morning hours of the day or just before waking up is majorly responsible for heart attacks, or if it is severe which causes death. In order to satisfy this need novel technology is developed that is called pulsatile release. Drug delivery based on the circadian rhythm i.e., Chronopharmaceutics, which is presently gaining lot of attention throughout the world. Many diseases show circadian variation e.g. arthritis, hypertension and bronchial asthma, these demands time scheduled drug delivery for the required pharmacological action. Many epidemiological studies revealed that there is high risk of pathological conditions during 24 hr cycle. Now a days many patients suffering from CVD like sudden cardiac arrest, stroke and myocardial infarction are very high in the early morning hours because blood pressure rises just before waking period. Catecholamines secretion increases whenever the blood pressure rises therefore plasma rennin activity also increases. So it can be well treated by chronotherapeutic approach or by preparing pulsatile core in cup tablets [2]. Pulsatile tablet release the drug after a predetermined lag time means during certain period of time there is no release of drug after that rapid and complete of drug that ideally match the circadian pathophysiology of particular disease.

Thus present study attempts to design and optimize a chronomodulated drug delivery system of enalapril maleate which is used for the treatment of hypertension. It was aimed to have a lag time of 6 hrs means if the tablet is taken at the bed time around 10 pm then it is expected to release the drug after a period of 6 hrs, at the 4 am when the blood pressure is more at that time. Such time controlled pulsatile tablet can be formulated mainly with drug containing core tablet coated with rupturable and swellable polymers. Because coating of polymer to the core, it protect core from the environment e.g. water, acidic pH enzymes until the drug is released after a predetermined lag phase. The coatings can rupture/swell or alter their permeability at the required time [3].

Enalapril maleate is an anti hypertensive agent which is used to treat hypertension and it's an ACE (angiotensin converting enzyme) inhibitor. It lowers the hypertension/blood pressure by reducing peripheral vascular resistance without increasing heart rate, contractility and cardiac output. Many types of hypertension in patients suffering from diabetes and severe kidney failures can be easily treated with enalapril. It is best suitable drug for the treatment of heart failure. The bioavailability is 55% and its half life

is 11 hrs; the absorption of enalapril maleate will not be affected by taking food [4].

In the present investigation enalapril maleate core in cup tablets were prepared by direct compression method which delivers the drug by chronotherapeutic approach and optimized. The core in cup tablets are thus needed to be optimized for the desired response, many statistical experimental designs have been recognized as useful techniques to optimize the formulation and process variables [5].

Response surface methodology is a collection of mathematical and statistical techniques useful for designing and optimizing of pharmaceutical formulation, by minimum experimentation. Based on the principle of design of experiments, the methodology objective is to optimize a response which is influenced by several independent variables. The response surface methodology encompasses the use of various types of experimental designs, generation of polynominal equations and mapping of the response over the experimental domain to determine the optimum formulation [6].

The Box-Behnken design was particularly selected because it needs less runs than a central composite design in cases of three or four independent variables. Three independent variables viz., ethyl cellulose (X1), sodium alginate (X2), HPMC (X3) and three dependent variables viz., percentage drug release at 4 hrs (Y1), percentage drug release at 8 hrs (Y2) and percentage drug release at12 hrs (Y3) were selected for the study. The optimization is done using Design expert 11 (Trial version 11, Stat-Ease Inc., and Minneapolis, MN) to interpret the results and easy scale up.

MATERIALS AND METHODS

Materials

Enalapril maleate (API) was a gift sample from Cipla pharmaceutical company, Mumbai. Sodium starch glycolate, cross povidone, croscarmellose sodium, talc, PVP K90, magnesium stearate were procured from SD fine chemicals, Mumbai. Galen IQ 72 was used as directly compressible diluent and it was obtained from Beneo palatinit industry, Germany. All other reagents used were of analytical grade throughout the study.

Methods

Box-Behnken Experimental design

The developed formulations were optimized using 4-factor 3- level Box-Behnken statistical design. The rationale behind this Box Behnken design based on the salient principles of Design of experiments and quality by design (QbD) approach. The design was particularly selected because it needs less runs than a central composite design in cases of three or four independent variables. It provides understanding of the plausible interaction(s) among the different levels of variables and helps in selecting "the best" formulation with minimal expenditure of time, effort and developmental cost vis-a'-vis the traditional one factor at a time (OFAT) approach [7]. The QbD methodology involves defining the critical process parameters using screening and risk assessment, optimization data analysis and optimum search through response surface methodology to embark upon the design space and postulation of control strategy for continuous improvement [8, 9]. This property prevents a potential loss of data in those cases. The design matrix generated the nonlinear quadratic equation for the response as shown below;

$$\mathbf{Y = b_0 + b_1A + b_2B + b_3C + b_{12}AB + b_{13}AC + b_{23}BC + b_{11}A^2 + b_{22}B^2 + b_{33}C^2 \ldots\ldots\ldots}$$

Where Y is the response related with each factor level combination; b_0 is constant; b_1, b_2, b_3 are linear coefficients, b_{12}, b_{13}, b_{23} are interaction coefficients while b_{11}, b_{22}, b_{33} are quadratic coefficients generated from the observed experimental values of response from experimental runs, while A, B and C are the coded intensity of independent variables. The terms A^2, B^2 and C^2 (i = 1, 2 or 3) represent the interaction and quadratic terms, respectively [10].

Preparation of enalapril maleate core tablets

Core tablet formulations were prepared using PVP K90, SSG, CCS and crospovidone in different ratios by direct compression method. Calculated quantities of selected drug and polymers and directly compressible vehicle Galen IQ 72 were mixed in a polybag for 10min to achieve uniform mixing. The mixture was subjected for direct compression using 8mm flat punch in Cad mach 10 station rotary punching machine. During compression weight variation, hardness was checked.

Preparation of press coated core in cup tablets

These were prepared by direct compression method. Each core in cup tablets consisting of an impermeable layer ethyl cellulose, swellable and rupturable polymer comprising of ethyl cellulose, sodium alginate and HPMC K15M, HPMC K100M at different ratios along with directly compressible vehicle galen IQ. A mixture of galen IQ and ethyl cellulose was filled in the die cavity of 10mm diameter then gently compact the powder bed with a flat surface spatula. The core tablet was carefully placed in the center of the powder bed then the die wall was filled with the mixture of swellable and rupturable polymers such as ethyl cellulose, sodium alginate, HPMC K15M and HPMC K100M at different ratios, so that the surrounding surfaces of the core tablet were fully covered. The powder bed was compressed directly by using 10 mm flat punch in Cadmach-10 station rotary punching machine.

RESULTS AND DISCUSSION

Table-1: Variables and their levels in Box-Behnken design for Set-I studies

Independent variables	levels		
	Low(-1)	Medium(1)	High(+1)
Ethyl cellulose-X_1	5	10	15
Sodium alginate-X_2	10	15	20
HPMC-X_3	10	15	20
Dependent variables			
Percentage drug release at 4hrs-Y_1			
Percentage drug release at 8hrs-Y_2			
Percentage drug release at 12hrs-Y_3			

Table-2: Variables and their levels in Box-Behnken design for Set-II studies.

Independent variables	levels		
	Low(-1)	Medium(1)	High(+1)
Ethyl cellulose-X_1	5	10	15
Sodium alginate-X_1	5	10	15
Ethyl cellulose:HPMCK4M-X_3	10	15	20
Dependent variables			
Percentage drug release at 4hrs-Y_1			
Percentage drug release at 8hrs-Y_2			
Percentage drug release at 12hrs-Y_3			

Table-3: Observed Box-Behnken Design Enalapril maleate core-in-cup tablet runs with their actual and predicted experimental value

Run Order	Dependent variables					
	Y_1- After 4hrs		Y_2- After 8hrs		Y_3- After 12hrs	
	Actual Value	Predicted Value	Actual Value	Predicted Value	Actual Value	Predicted Value
1	10.00	9.40	66.00	65.80	91.00	91.18
2	11.00	10.25	70.00	69.88	84.00	85.68
3	15.00	15.13	71.00	71.38	89.00	88.43
4	10.00	9.40	66.00	65.80	92.00	91.18
5	8.00	8.75	62.00	62.13	98.00	96.68
6	14.00	13.88	68.00	67.63	94.00	93.93
7	10.00	10.00	68.00	68.38	86.00	87.18
8	10.00	10.00	65.00	64.62	95.00	95.18
9	9.00	8.38	60.00	59.88	89.00	89.93
10	10.00	10.13	65.00	65.00	89.00	88.43
11	10.00	9.40	66.00	65.80	91.00	91.18
12	7.00	9.40	66.00	65.80	92.00	91.18
13	5.00	5.63	70.00	69.75	86.00	84.43
14	10.00	9.40	65.00	65.80	91.00	91.18
15	5.00	4.38	62.00	62.25	97.00	97.93
16	8.00	8.63	60.00	60.13	93.00	92.43
17	10.00	9.88	61.00	61.00	93.00	93.93

Table-4: ANOVA for response surface quadratic model for drug release at 4hrs

Parameters	Sum of Squares	df	Mean Square	F-value	p-value	Remark
Drug release at 4 hrs (Quadratic model)	96.29	9	10.70	7.53	0.0072	significant
X1	1.13	1	1.13	0.7915	0.4032	
X2	1.13	1	1.13	0.7915	0.4032	
X3	0.5000	1	0.5000	0.3518	0.5718	
Residual	9.95	7	1.42			
Lack of fit	2.75	3	0.9167	0.5093	0.6970	
Pure error	7.20	4	1.80			
Cor total	106.24	16				

Table-5: ANOVA for response surface quadratic model for drug release at 8hrs

Parameters	Sum of Squares	df	Mean Square	F-value	p-value	Remark
Drug release at 8 hrs (Quadratic model)	188.33	9	20.93	94.50	< 0.0001	Significant
X1	66.13	1	66.13	298.63	< 0.0001	
X2	8.00	1	8.00	36.13	0.0005	
X3	6.13	1	6.13	27.66	0.0012	
Residual	1.55	7	0.2214			
Lack of fit	0.7500	3	0.2500	1.25	0.4028	
Pure error	0.8000	4	0.2000			
Cor total	189.88	16				

Table-6: ANOVA for response surface quadratic model for drug release at 12hrs

Parameters	Sum of Squares	df	Mean Square	F-value	p-value	Remark
Drug release at 12 hrs (Quadratic model)	217.00	3	72.33	69.81	<0.0001	significant
X1	180.50	1	180.50	174.19	< 0.0001	
X2	4.50	1	4.50	4.34	0.0575	
X3	32.00	1	32.00	30.88	< 0.0001	
Residual	13.47	13	1.04			
Lack of fit	12.27	9	1.36	4.54	0.0794	
Pure error	1.20	4	0.3000			
Cor total	230.47	16				

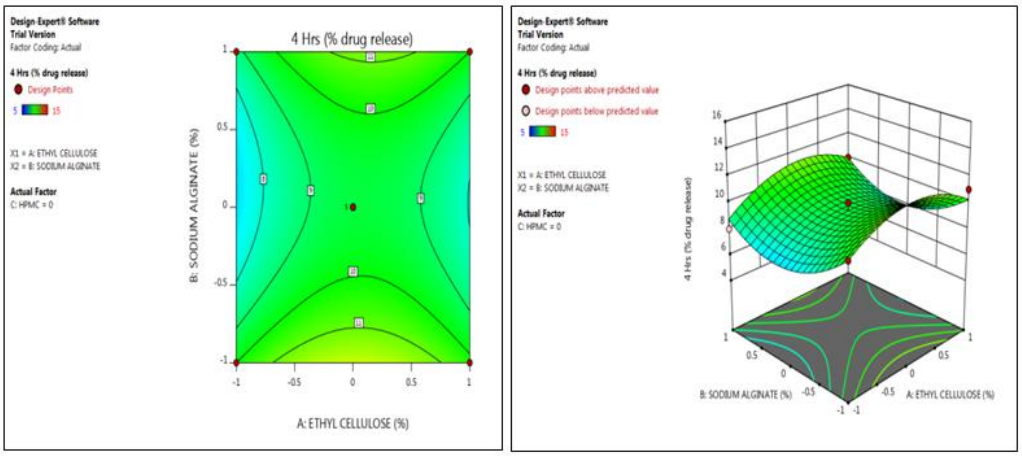

Fig-1: Contour plots and 3D response plot for variables studied at 4hrs

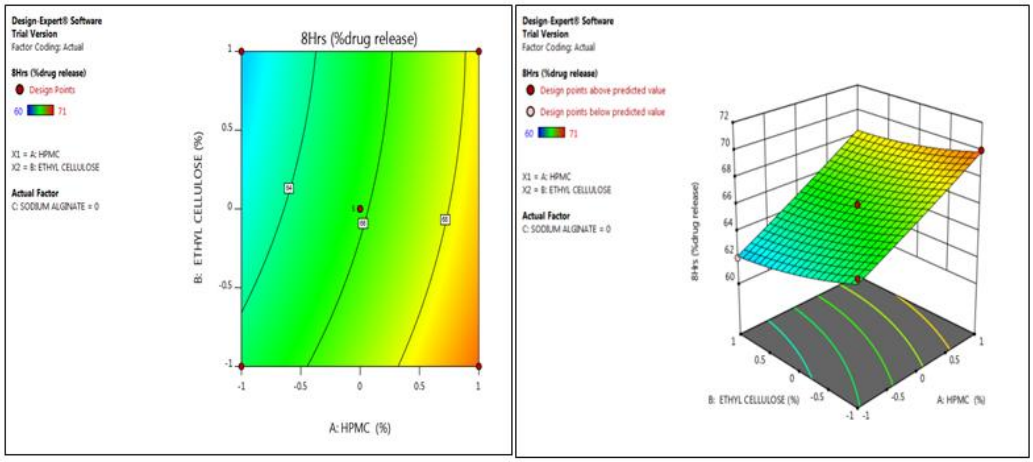

Fig-2: Contour plots and 3D response plot for variables studied at 8hrs

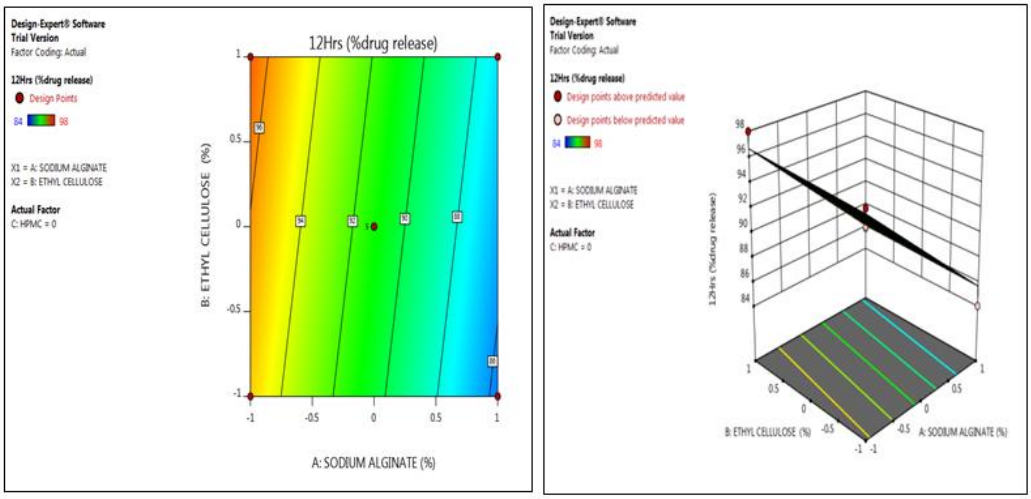

Fig-3: Contour plots and 3D response plot for variables studied at 12hrs

DISCUSSION

Effect of formulation variables on drug release at 4hrs: In trial runs F1–F17 drug release was in the range of 5 to15% after 4hrs, maximum drug release 15% was shown by formulation F3, where as minimum drug release was shown by 5%, was shown by formulation F13 and F15. The effect of the variables on the drug release in formulations F1–F17 are shown in figures respectively

$$Y1 = 9.4 + 0.375X_1 - 0.365X_2$$

All the polynomial equations were found to be statistically significant (P<0.0010), as determined using ANOVA, as per the provision of Design Expert software. The combined effect of concentration of HPMC, sodium alginate and Ethyl cellulose on drug release at 4hs was shown in counter plot and in 3D response surface plot. In above equation X_1 Bears / having positive sign and X_2 also Bears / having negative sign which shows that increased/decreased / at lower concentration of HPMC and Ethyl cellulose is required for get optimum drug release at 4hs. From the plots and polynomial equation it can concluded that both factor HPMC (X_1), Ethyl cellulose(X_2) and Sodium alginate (X_3) having significant effect on drug release at 4hrs.

Effect of formulation variables on drug release at 8hrs:

Amount of polymer used in formulation having significant effect on drug release. In formulations, F1–F17 drug release was in the range of 60 to 71 at 8 h, maximum drug release 71 was shown by formulation F3, where as minimum drug release was shown by 60, was shown by formulation F9 and F16. The effect of the variables on the drug release in formulations F1–F17 are shown figures

$$Y_2 = 65.80+2.88X_1 - 1.00X_2+0.875X_1X_2+0.25X_1+1.50X_2$$

All the polynomial equations were found to be statistically significant (P<0.0010), as determined using ANOVA, as per the provision of Design Expert software. The combined effect of concentration of HPMC, sodium alginate and Ethyl cellulose on drug release at 8hs was shown in counter plot and in 3D response surface plot. In above equation X_1 Bears / having positive sign and X_2 also Bears / having negative sign which shows that increased/decreased at lower concentration of HPMC, sodium alginate and Ethyl cellulose is required for get optimum drug release at 8hrs. From the plots and polynomial equation it can concluded that both factor HPMC (X_1) and Ethyl cellulose(X_2) having significant effect on drug release at 8hrs.

Effect of formulation variables on drug release at 12hrs: Amount of polymer used in formulation having significant effect on drug release. In formulations, F1–F17 drug release was in the range of 84 to 98 at 12hrs, maximum drug release 98 was shown by formulation F5, where as minimum drug release was shown by 84, was shown by formulation F3. The effect of the variables on the drug release in formulations F1–F17 are shown in figures.

$$Y_3 = 91.18 - 4.75X_1+0.75X_2$$

All the polynomial equations were found to be statistically significant (P<0.0010), as determined using ANOVA, as per the provision of Design Expert software. The combined effect of concentration of HPMC, sodium alginate and Ethyl cellulose on drug release at 8hrs was shown in counter plot and in 3D response surface plot. In above equation X_1 Bears / having negative sign and X2 Bears / having positive sign which shows that decreased /increased at lower concentration of HPMC, sodium alginate and Ethyl cellulose is required for get optimum drug release at 12hrs.

CONCLUSION

A press coated core in cup pulsatile drug delivery system for Enalapril to mimic the circadian rhythm of the disease by releasing the drug at appropriate time (at the time of symptoms exacerbates). The formulation consisted of a core tablet containing a drug enalapril and outer layer of combination of swellable and rupturable polymers of sodium alginate, ethyl cellulose and HPMC. Direct compression coated tablets proved to be successful to provide the desired pulsed release profile after a programmed lag time. The statistical approach for optimization of formulation is a useful tool, when several variables are to be studied simultaneously. The contour plots and polynomial equations developed by using Box Behnken design allowed us to prepare pulsatile core in cup tablets with optimum characteristics.

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Conflict of interest

The authors declare that there is no conflict of interest

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