PULSATILE DELIVERY OF METHYLPHENIDATE HYDROCHLORIDE PULSINCAP BY BOX-BEHNKEN DESIGN

ABSTRACT

The objective of this study was to prepare and evaluate Methylphenidate hydrochloride pulsatile drug delivery system using pulsincap technique by applying Box-Behnken design. The drug is a central nervous system (CNS) stimulant used in the treatment of attention deficit hyperactivity disorder (ADHD). Pulsincap system was prepared by using formaldehyde cross linked capsules. Capsules were filled with methylphenidate hydrochloride granules and hydrogel plug made of HPMC K100M is placed over granules to achieve desired drug release after lag time. The untreated cap was then fitted and sealed using 5% ethyl cellulose ethanolic solution to the formaldehyde treated capsule body. Granules were prepared by wet granulation technique using two polymers Ethyl cellulose and Eudragit RS100. Box-Behnken design was applied for optimization in which three independent variables, X1 = Drug: polymer ratio, X2 = Polymer: polymer ratio (Ethyl cellulose: Eudragit RS 100) and X3 = Plug weight were selected. Two dependent variables Y1 = lag time and Y2 = percent release were selected. The empty formaldehyde treated capsules were evaluated for physical appearance, solubility, capsule dimensions and formaldehyde content. Hydrogel plugs were evaluated for hardness & thickness of the plug, lag time and swelling index. Granules were evaluated for percentage yield, assay and flow properties. The prepared pulsincap formulations were evaluated for weight variation, content uniformity, capsule lock length, in-vitro dissolutions studies, drug kinetics and stability studies. Contour plots and Response surface plots indicated that with the increase in X1 and X3 there is increase in Lag time and decrease in % drug release and whereas with the increase in X2 the lag time was at moderate level and % drug release was increased. From this observation, formulation F11 was optimized as it provided desired lag time of 4.2±0.03 hours and least drug release of 72±0.14% for 8 hrs. The formulations were found to be physically compatible with excipients and stable.

Keywords: Box-Behnken design, pulsatile drug delivery, pulsincap, methylphenidate.

INTRODUCTION

Pulsatile drug delivery systems (PDDS) are gaining popularity as they ensure spatial, temporal and, smart delivery by delivering drugs at the right place in right amount at right time thereby improving patient compliance.[1] These devices are meant to provide drugs in accordance with disease's circadian behaviour. This means that these systems will deliver drug at a point in the circadian cycle when disease is at its most severe and fatal (24 hrs.). The pulsatile drug delivery technique is most useful for drugs administered to treat disorders with a chronopharmacological pattern. [2]

Attention Deficit Hyperactivity Disorder (ADHD) is a disorder that is characterised by a pattern of inattention (inability to concentrate) sometimes combined with hyperactivity-impulsivity that is persistent, developmentally inappropriate, and occurs in two different settings before seven years age. Neurological imbalances symptoms exhibited by ADHD patients are because of dopamine imbalances, wherein higher levels of dopamine in brain are seen in the afternoon time. Certain neurotransmitters are low in quantity or lacking in patients with ADHD is shown in scientific studies. Methylphenidate hydrochloride is a central nervous system (CNS) stimulant used in the treatment of Attention Deficit Hyperactivity Disorder (ADHD). The drug blocks dopamine transporters and increases the neurotransmitters (dopamine and noradrenalin) at synapse. Marketed formulations of Methylphenidate hydrochloride like RITALIN and CONCERTA provides both immediate release followed by a delayed release of the drug. Ritalin LA® is a methylphenidate hydrochloride extended-release capsule showing bi-modal release profile. CONCERTA® is an extended-release tablet of methylphenidate HCl USP and delivers drug at a controlled rate using osmotic pressure. Ritalin LA® uses the SODAS® (Spheroidal Oral Drug Absorption System) technology. From research envisaged we found that the pulsincap technology was not used. So in the present study Methylphenidate HCl capsules using “pulsincap technique”

was formulated to obtain delayed release of the drug (after lag time). In this ADHD condition, there is a necessity for the drug to release in afternoon time during which the symptoms are highly observed. When administered to a child in the morning around 8 to 9 A.M, prepared pulsincap releases in the afternoon around 12 to 1 P.M, reducing the symptoms of ADHD in children. This avoids dosing during day time which is highly beneficial for school children. Generally for children drug dose cannot be given accurately at afternoon time. So, the drug can be given in the morning hours which tend to release in the afternoon time. Pulsatile drug delivery of Methylphenidate hydrochloride is required as the symptoms of ADHD are highly observed in the afternoon time. "Pulsincap" technique is used to prepare Methylphenidate hydrochloride pulsatile- release capsules by applying Box-Behnken Design. Formaldehyde treated capsule bodies are used for preparing pulsincap system. Highly retarding polymers like Eudragit RS 100 and Ethyl cellulose N22 are chosen to prepare the methylphenidate HCL granules. HPMC K100M and HPMC K4M are chosen to prepare hydrogel plugs. The pulsincap is formulated by filling the methylphenidate HCL granules in to formaldehyde cross-linked capsule bodies and by placing a hydrogel plug upon it. Lag time is influenced by the polymers and hydrogel plug used.

MATERIALS AND METHODS

Chemicals and Reagents:

Methylphenidate Hydrochloride was obtained from Dr. Reddy's laboratories, Hyderabad, India. Size "0" capsules were obtained from S.D. Fine Chemicals Ltd, Hyderabad, India. Eudragit RS 100 was obtained from Mumbai, Maharashtra, India. Ethyl cellulose N22 was obtained from Balaji Drugs, Hyderabad, India. HPMC K100M and HPMC K4M were obtained from Yarrow Chemical Products Mumbai, Maharashtra, India. All the chemicals utilised were of analytical standards.

Methods:

Formulation of Pulsincap drug delivery system: Includes various steps

Cross linking of empty capsules: In order to prepare a pulsincap system size '0' capsules were chosen and the solubility of these gelatin capsules is modified by cross linking them with formaldehyde. Hard gelatin capsules of size '0' were taken and their bodies were separated from caps. 25 ml of 15% (v/v) formaldehyde was prepared, taken into petri plate and placed at the bottom of the desiccator. Capsules bodies were evenly spread on the mesh and the mesh is placed above the petri plate containing formaldehyde. The desiccators were tightly closed and empty bodies of capsules were exposed to formaldehyde vapours. The reaction time is then optimised by removing capsule bodies at different time intervals from desiccator i.e., capsule bodies were collected at every 1 hour until 6 hours. They are dried at 50°C for 30 minutes after being removed from the desiccator to ensure that the reaction between gelatin and formaldehyde vapours is completed. Residual formaldehyde was removed by drying capsules bodies at room temperature. These capsule bodies were sealed with untreated caps and stored in self-sealing covers.[3,4]

Preparation of hydrogel plug: Two different swellable hydrophilic polymers HPMC K100M and HPMC K4M were initially selected as they can control the lag time. Required amounts of HPMC K100M and HPMC K4M were weighed and compressed using '6mm punch' in tablet compression machine.[3]

Preparation of granules: Wet granulation method was used for preparation of Methylphenidate HCl granules using PVPK30 as granulating agent. The wet mass was passed through a 20#mesh to obtain granules and dried at room temperature.

Filling of granules in capsule: Granules equivalent to 40mg of the drug dose were weighed and filled into formaldehyde treated capsule body and locked by hydrogel plug and fixed with untreated cap.[3]

Sealing of capsules: The cap was sealed with the body using 5 % ethyl cellulose ethanolic solution.[3]

Drug-excipient compatibility study

FTIR studies: The spectrum analysis of pure drug and physical mixture of drug with different excipients were studied by FTIR. Potassium bromide disk was mounted in a holder and IR spectrum was recorded from 4000cm to 500 cm in a scan time of 12 minutes using a shimadzu (Koyto, Japan) IR spectrophotometer (model-8400S). The spectra were observed for the presence of characteristic peaks.

DSC Studies: The instrument was calibrated with indium standard 3-5mg samples were weighed and placed in a closed, hermetic sample pans with pin hole. Thermograms were produced by heating the sample at a constant rate 10°C/min from 0°C to 210.0°C. A dry purge of nitrogen gas of 50 ml/min was used for all runs. Samples were heated. The heat of fusion, melting point, appearance of any new peak, disappearance of the crystalline sharp peak and peak shape were recorded. The thermogram of the optimized Methylphenidate HCl formulation was superimposed with that of pure drug.

Evaluation of empty formaldehyde treated capsules

Physical Examination: Capsules were visually examined for any defects after 6 hrs. of formaldehyde cross linking.

Solubility studies: The solubility of capsule bodies which were cross linked using 15% v/v formaldehyde solution was checked in orbital shaker bath. This was performed in order to optimize the crosslinking time. Capsules were collected at the end of every 1 hour of cross linking and checked for their solubility in 0.1N HCL. Deformation of capsule body shape is considered as end point.[3,5,6]

Measurement of dimensions of capsule bodies: Capsules are subjected to measurement of dimensions in order to compare the differences between plain capsules and formaldehyde

cross-linked capsules. Dimensions like total capsule length, capsule body diameter and capsule body length of both plain capsules and formaldehyde cross-linked capsules were measured by using screw gauge and compared (4, 6).[3,5]

Quantitative test for free formaldehyde content: To prove that the formaldehyde content in the cross-linked capsules is within the limits a quantitative test was employed. The sample was accurately weighed (about 3 g of formaldehyde treated capsules) and added to a mixture of H_2O_2 (25ml) and 1M sodium hydroxide (50ml) in a conical flask. Heated on the water bath until effervescence ceases (usually about 30 mins), cooled and excess of alkali was titrated with 1M hydrochloric acid, using phenolphthalein as indicator.[7]

Equivalent factor: 0.03003g of HCHO ≡ 1ml of M NaOH

Evaluation of Hydrogel Plugs

Hardness and thickness: The hardness and thickness of 50mg, 75mg and 100mg HPMC K 100M hydrogel plug were measured by using Monsanto hardness tester and screw gauge respectively.[3]

Lag time: Lag time was determined during in vitro dissolution studies of formulations. The prepared hydrogel plugs were plugged to capsule bodies containing formulated granules and the caps were closed. The lag time test was conducted using USP I dissolution testing apparatus using 0.1N HCl for 2 hours, followed by 4.5 pH phosphate buffer for 3 hours and then followed by 6.8 pH phosphate buffer for 3 hrs. The drug release was observed. The graphs were plotted by taking time on x-axis vs. % drug release on y-axis and by the extrapolation of drug release line on to the x-axis, lag time was obtained.[5]

Swelling Index: Individually weighed plugs (W1) were placed in glass beakers with 200 ml of 0.1 N HCl and incubated at 37°C1°C. At regular 1 hour time intervals until 4 hrs. the plugs were removed from the beakers, and the excess surface liquid was removed carefully using

the filter paper. The swollen plugs we reweighed again (W2) and swelling index (SI) was calculated using the formula.[8]

$$\text{Swelling Index} = \frac{W2-W1}{W1}$$

Evaluation of granules

Percentage yield: The prepared granules were collected and weighed (practical yield).The percentage yield of granules was calculated using formula.[9]

$$\boldsymbol{Percentage\ yield = \frac{Practical\ yield}{Theoretical\ yield} x100}$$

Assay: Granules were accurately weighed equivalent to the drug dose and dissolved in 10ml of acetone. After required dilutions the drug was analysed by UV-Visible spectrophotometric method at 208nm.[10]

Flow properties: Angle of repose, Bulk density, Tapped density, Compressibility index, and Hausner's ratio were determined.

Angle of repose: Funnel method was used to determine angle of repose. Granules were accurately weighed and placed in a funnel, with the funnel's height adjusted so that its tip touched the apex of the granules inside. The granules were allowed to pass through the funnel and drop on the surface. The diameter of the pile of the granules was measured and the angle of repose was calculated using the equation:

$$\tan \theta = h/r$$

Where, θ = angle of repose, h = height of the heap (in cm) and r = radius of the base (in cm).[11]

Bulk density (ρ_b): It is the mass of the powder divided by the bulk volume.[11]

Tapped density (ρt): It is the mass of the powder divided by the tapped volume.[11]

Compressibility index: Carr's index was calculated from the following equation using the values of bulk density (ρ_b) and tapped density (ρ_t).[11]

$C = (\rho_t - \rho_b / \rho_t) \times 100$

Hausner's ratio: It is an indirect index of ease of powder flow. It is calculated using formula.[10,11,12]

Hausner's ratio = ρ_t / ρ_b

ρt is tapped density and ρb is bulk density.

Optimization using Box-Behnken design

A three-factor, three-level Box-Behnken design (BBD) was selected for the optimization procedure to explore quadratic response surfaces and construct second order polynomial models using Design Expert 11 (Version 11; Stat-Ease Inc, Minneapolis, MN). The Box-Behnken design is an independent quadratic design used to optimize the formulation where, the treatment combinations are taken at midpoints of edges and at the center of the process space. Initial preliminary trials were carried out to evaluate the formulations and for the processing of pulsatile capsules. It was observed that variations in the quantities of polymers affect the lag time and percent release. When the polymers ethyl cellulose and eudragit RS 100 were used in ratio the release of methylphenidate was delayed and the lag time was found to be increased and also when the 100mg of hydrogel plug (HPMCK100M) was used it was found that the lag time increased. Based on this, three independent variables, X1 = Drug: polymer ratio, X2 = Polymer: polymer ratio (Ethyl cellulose: Eudragit RS 100) and X3 = Plug weight were selected at three levels (low, medium, and high). The levels for these three parameters were determined from the preliminary trials.Y1 = lag time in hrs and Y2 = percent release after 8 hrs were selected as dependent factors.[13] The independent and dependent factors, are given in table no.1. A three level three factor Box-Behnken design (BBD) was generated using Design Expert 11 software. The design gives us the coded values which are converted in to the actual values for all the factor levels to obtain the formulations according to Box- Behnken design, this is given in table no. 2.

Table 1: Factor and Factor levels of Box-Behnken experimental design

Independent factors	Levels		
	Low	Medium	High
	-1	0	1
X1 = Drug: polymer ratio	1:1	1:2	1:3
X2 = Polymer: polymer ratio (Ethyl cellulose: Eudragit RS 100)	1:1	1:2	1:3
X3 = Plug weight (mg)	50	75	100
Responses (Dependent factors)			
Y1 = lag time in (hrs)			
Y2 = percent release of methylphenidate after 8hours (%)			

Table 2: Formulations table according to Box Behnken experimental design

Formulation Code	Drug (mg)	Polymer (mg)	Ethyl cellulose (mg)	Eudragit RS 100 (mg)	HPMC K 100M Plug (mg)
F1	40	80	40	40	100
F2	40	120	40	80	100
F3	40	120	30	90	75
F4	40	80	26.66	53.33	75
F5	40	80	40	40	50
F6	40	40	13.33	26.66	100
F7	40	40	20	20	75
F8	40	40	13.33	26.66	50
F9	40	80	26.66	53.33	75
F10	40	80	26.66	53.33	75
F11	40	80	20	60	100
F12	40	40	10	30	75
F13	40	120	40	80	50
F14	40	80	20	60	50
F15	40	120	60	60	75

Evaluation of pulsincap

Weight variation: Twenty capsules were selected randomly and weighed collectively and individually. Average weight was calculated. The % weight variation was calculated.[3]

Content Uniformity: Twenty capsules were randomly selected from each batch and their contents were removed and powdered. From this sample, 40mg of powder (equivalent to drug dose) was accurately transferred to 10ml volumetric flask. The volume was made up with acetone and sonicated for 30 mins. Then, 1ml of the above solution was transferred to 10ml volumetric flask and the volume was made up to the mark with 0.1N HCL. The resulted solution was filtered through Whatman filter paper, suitably diluted and the drug content was estimated spectrophotometrically by measuring absorbance at 208nm.[3]

Measurement of capsule lock length: Lock length of capsules was measured using screw gauge and the values were noted.

In-vitro dissolution studies: *In-vitro* drug dissolution studies were carried out using USP type-I (basket type) apparatus. The release of methylphenidate HCl from the pulsincap system was studied using three different dissolution media (900ml) in order to simulate pH changes across the GI tract. 0.1 N HCl (1.2 pH Buffer) for 2hrs, 4.5 pH phosphate buffer for 3hrs and 6.8 pH phosphate buffer. The rotating basket stirrer is set at a stirring speed of 100 rpm and a temperature is adjusted to 37 ± 0.5°C.Samples were withdrawn at predetermined time intervals of 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7 and 8hrs and replaced with 5ml of fresh dissolution medium. The withdrawn samples were assayed at 208 nm for drug content using a UV visible spectrophotometer.[3,14]

Stability studies: Pulsincap formulations were tested for their stability in amber colored bottle containers. Optimized formulation was stored at accelerated stability conditions (40°C ±2°C /75%± 5%RH) as per ICH guidelines over a period of 1 month in a humidity chamber and the capsules were evaluated for physical appearance, drug content, lag time and in-vitro drug release every week.[15]

RESULTS AND DISCUSSION

Drug-excipient compatibility study

FTIR study was done to verify if there was any interaction between the pure drug and the various excipients employed in the study. From FTIR spectra of the pure drug and optimised formulation figure no. 1, the peaks identified in the pure drug (as shown in table no. 3) were relatively same when compared with the blend, indicating no drug polymer interaction. The pure drug was found to be compatible with polymers and not altered functionally.

DSC thermograms of pure drug showed a sharp peak at 225°C and in optimized formulation peak was observed at 211.2°C (figure no2). A small peak observed in optimized formulation

at 268°C is due to the polymer ethyl cellulose used in the formulation. Indicating no interaction between drug and excipients.

Evaluation of empty formaldehyde cross linked capsules

Physical appearance of formaldehyde treated capsules showed no significant changes after exposing to formaldehyde vapours for 6 hours.[3]

The solubility of capsule bodies was checked in orbital shaker bath and it was found that 15% v/v formaldehyde solution and 6 hours exposure time was optimum as the capsule bodies remained intact up to 8 hours in 0.1 N HCL. Capsules bodies collected from the desiccator at the end of each hour were checked for their solubility and the observation is given in table no. 4nand figure no. 3.[5]

The dimensions of the capsules (n=6) were measured and it was observed that there was a slight decrease in diameter and length of capsules after formaldehyde cross linking (4, 6). The measured dimensions of capsule bodies are given in table no. 5.

Titration was carried out according to the procedure and it was observed that 1ml of HCL was consumed during the titration. It was calculated according to the equivalent factor and obtained as:

1ml HCl ≡ 1ml NaoH ≡ 0.03003g of HCHO ≡ 30.03 mg of HCHO

Thus, the amount of formaldehyde present in sample (10 formaldehyde treated capsules) was found to be 30.03mg. So, in one capsule it was found to be 3.003mgwhich is within the limits (50mg/day) according to ICH guidelines for impurities. (ICH Harmonised tripartite guideline impurities: guideline for residual solvents -Q3CR5, 2011).

Evaluation of the hydrogel plug

Hardness and thickness of hydrogel plugs of three different weights were measured and the plugs were evaluated for their lag time and swelling index. The results are given in table no. 6 and 7. Results showed that at all polymer levels, drug release from the higher viscosity grade,

K100M was slower as compared to the lower viscosity grade, K4M.The low viscosity grade HPMC was not found to be so effective to sustain the release of drugs from their matrices since medium can penetrate easily. Upon increasing the viscosity grade of the polymer, i.e., by using HPMC K100M plug the release rate has been found to be sustained. This is because when the viscosity of the polymer increases, the penetration of medium decreases due to the increased viscosity, resulting in delay in drug release. Hence, HPMC K100M plug was optimized. It is considered as the ideal hydrogel plug and was used in the final formulation.[16]

Evaluation of granules

The Percentage yield of all the formulations was calculated and it was observed that formulation F2 showed the highest percentage yield of 85.91%, formulation F6 showed the lowest percentage yield of 52.92% while rest of the formulations showed percentage yield in the range of 60 – 80% **(**10). Assay was performed for the granules of all the formulations (11) and the results are shown in the table no. 8.

Flow properties were determined and the results are given in table no.9.The results indicate that all the formulations show excellent flow properties.[10,11,12]

Evaluation of pulsincap system

Weight variation, Content Uniformity, Measurement of capsule lock length was done and results are shown in table no. 10. The results were within pharmacoepeial limits.

In-vitro drug release studies were conducted for all the 15 formulations obtained by applying experimental design. The % drug release of all the formulations is shown in figure no.4, 5 and 6.[3,14,17] It is observed that 100% of drug release was obtained for pure drug within 1 hour whereas for all the formulations lag time ranging from 2.4 hours to 4.2 hours was observed after which drug release was seen. Formulations F1, F2 and F11 prepared using higher drug: polymer ratio of 1:2 & 1:3 and higher plug weight of 100mg of HPMC K100M,

showed higher lag time of 4 to 4.2 hours and lower drug release at end of 8 hours whereas for other formulations F3, F4, F5, F6, F7, F8, F9, F10, F12, F13, F14 and F15 which were prepared using lower drug: polymer ratios of 1:1 and lower plug weights of 50 mg and 75 mg of HPMC K100M, it was found that at end of 8 hours there is higher amount of drug release and even the lag time was found to be less (2.4 to 3.5 hrs).

Optimization by Box-Behnken design

Three independent variables (factors) X1 = Drug: polymer ratio, X2 = Polymer: polymer ratio (Ethyl cellulose: Eudragit RS 100) and X3 = Plug weight were selected at three levels (low, medium, and high). Two dependent factorsY1 = lag time in hrs and Y2 = percent release after 8 hrs were selected.

It is observed that the Y1 response, i.e., lag time followed three models: linear model, quadratic model, 2FI model whereas, Y2 response for all formulations followed a linear model. On overall linear model is selected for both the responses as the suitable statistical model for formulation optimization since it is the suggested model by the design expert software. This is shown in table no. 11.

Lack of fit is an undesirable characteristic for a model. If the model does not fit the data well, the test will show a significant lack of fit. For a well-fitted model, lack of fit will be insignificant ($P > 0.10$).In this case, lack of fit was insignificant, so the model fits the data generated. Fit summary given in figure no. 7 suggests linear model for both the responses by the design expert software.[13]

A mathematical relationship between factors and responses were generated using multiple linear regression analysis in the form of equations. These equations represent the quantitative effect of variables (X1, X2, and X3) and their interactions on the response Y. Coefficients with more than one factor term represent interaction terms whereas, those with higher order

terms represent quadratic relationships. A positive sign represents a synergistic effect and a negative sign indicates an antagonistic effect.[13]

Linear model equation for Y_1:

$Y_1 = 3.1933 + 0.55X_1 + 0.15X_2 + 0.42X_3$

Linear model equation for Y2:

$Y2 = 86.89 - 9.47X_1 + 2.40\ X_2 - 0.73X_3$

To study the effect of independent variables on dependent variables 2-D contour plot and 3-D response surface analysis was done using software Design expert 11. These plots provide information about effect of two independent variables on one dependent variable at a time by keeping third independent variable at middle level.[18]

Effect on Lag time (R1)

2-D contour plot (figure no.8**)** shows that, as the level ofX1 (Drug: Polymer ratio) was increased from -1 to 1 at centre level of X3(Plug weight), the lag time increased from 2.6 to 3.8 hours and as X2(Polymer: Polymer ratio) was increased from -1 to 1 at centre level of X3(Plug weight), the lag time was found be moderately affected. Response surface plot as shown in figure no.9 depicts similar agonistic effect of X1 and X3on lag time. Thus, it was observed that with the increase in Drug: Polymer ratio (X1) there was increase in Lag time and with the increase in Polymer: Polymer ratio (X2) the lag time was at moderate level. With further increase in X3 (Plug weight) i.e., at higher level of X3, the Lag time was observed to be increased up to 4.1 hours.[18]

Effect on % drug release (R2)

Figure no.8 depicts that as the level ofX1 (Drug: Polymer ratio) was increased from -1 to 1 at centre level of X3(Plug weight) the % drug release was found to be decreased from 95% to 80%. And with the increase in X2 (Polymer: Polymer ratio), the % drug release was found to be increased as seen through the contour plot. Thus, it was observed that with the increase in

Drug: Polymer ratio (X1) there was decrease in % drug release whereas, with the increase in Polymer: Polymer ratio (X2) also the % drug release was increased. With further increase in X3 (Plug weight) i.e., at higher level of X3, the % drug release was observed to be decreased up to75%. 3-D response surface plot as shown in figure no.9also shows similar declining trend of % drug release with increase in X1 (Drug: Polymer ratio) and X3 (Plug weight) (19).

One-way ANOVA was performed in order to determine the effect of factor on the responses. The results of the ANOVA were applied to identify insignificant factors. Values of Probability less than 0.0500 indicates factors are significant and have a significant effect on the responses (14, 19).One-way ANOVA for the factors A, B, C versus Lag time is given in table no 12. From this table it is observed that, for factor A Drug: Polymer ratio P value was found to be (P=0.017) which indicates that Drug: Polymer ratio is a significant factor and it has significant effect on lag time whereas Factor B Polymer: Polymer ratio with P value (P=0.588) and Factor C Plug weight with P value (P=0.089) are found to be insignificant factors on the response, lag time.

In the similar way, One-way ANOVA was performed for the factors A, B, C versus % Drug release in order to determine the effect of the factors on % Drug release. The data is given in table no 13. From this table it is observed that, for factor A Drug: Polymer ratio P value was found to be (P=0.042) which indicates that Drug: Polymer ratio is a significant factor and it has significant effect on % Drug release whereas Factor B Polymer: Polymer ratio with P value (P=0.857) and Factor C Plug weight with P value (P=0.746) are found to be insignificant factors on the response% drug release. From this generated data, we can hypothesize that as the Drug: Polymer ratio increases lag time increases and % drug release decreases.

The polynomial equation for dependent and independent variables was generated, the formulation was optimized for the responses. The optimum variables were obtained by the numerical analysis based on

the criterion of desirability. The graphs obtained for Predicted vs. Actual responses are shown in figure no 10. (20). Based on the predicted values obtained for responses by considering highest lag time and least % drug release at the end of 8 hours, three formulations F1, F2 and F11 were selected and the in vitro dissolution studies were carried out again to confirm the validity of the optimization procedure and the results were given in the following table no.14. As the results obtained for invitro dissolution studies were close to the predicted responses, it was proved that the design applied is significantly fitting the data and thus the design is validated.

The lag time of4 to 5 hours and minimum % drug release at the end of 8 hrs from the pulsatile capsules was selected as target response for the optimization. Based on this, F11 was selected as optimized formulation as it showed the highest lag time of 4.2 hours and least % drug release of 72% at the end of 8 hours.

Optimized Formulation F11 followed zero order drug release kinetics and it was found to follow korsemeyer-peppas drug release mechanism. Model dependent kinetics of F11 formulation is shown in Figure no.11.

The Stability study results are given in table no.15. The formulation was found to be stable for one month, with no significant change in physical appearance, drug content, lag time and in-vitro drug release, we conclude that the pulsincaps are stable.[15]

Table 3: FTIR Interpretationof Methylphenidate hydrochloride

Sl.no	Region in (cm^{-1})	Bond	Functional group
1	1820-1660	C=O stretching	Carbonyl group
2	3400-2400	O-H	Acid
3	1600 and1475	C=C stretching	Aromatic ring
4	3400	N-H stretching	Amide
5	1375	CH_3	Methyl group

Table 4: Optimization of formaldehyde concentration and formaldehyde exposure time

Formaldehyde vapor exposure time (Cross linking time)	Observation (in 0.1N HCL)
1 hr	Formed a palpable mass in 1hr 10mins.
2 hr	Formed a palpable mass in 3hrs
3 hr	Formed a palpable mass in 4hr 30mins
4 hr	Deformation of capsule body shape in 5 hours
5 hr	Deformation of capsule body shape in 5hr 30mins
6 hr	Intact up to 8 hours.

Table 5: Comparison of dimensions of capsule bodies

Parameter(mm)	Before treatment	After treatment
Avg. Capsule length	20.73±0.14	19.84±0.15
Avg. Capsule body diameter	7.56±0.22	7.21±0.17
Avg. Capsule body length	17.41±0.26	16.96±0.18

Table 6: Evaluation of the hydrogel plug

Hydrogel plug (mg)	HPMC K100M			HPMC K4M		
	Hardness (kg/cm^2)	Thickness (mm)	Lag time (hrs)	Hardness (kg/cm^2)	Thickness (mm)	Lag time (hrs)
50	1.5±0.05	1±0.13	2.4±0.01	1.5±0.04	1±0.21	1.5±0.09
75	5.8±0.06	1.5±0.01	3±0.20	5.5±0.05	1.5±0.06	2±0.12
100	6.1±0.11	2±0.07	4.1±0.12	6±0.15	2±0.14	2.8±0.05

Table 7: Swelling Index of the hydrogel plugs (at the end of 4 hours) in three different media

Media	HPMC K100M Swelling Index (%)			HPMC K4M Swelling Index (%)		
	50 mg	75mg	100mg	50 mg	75mg	100mg
0.1 N HCL	135.4±0.18	101±0.24	125±0.13	57.4±0.15	98±0.11	120±0.14
4.5 pH phosphate buffer	97.8±0.17	109±0.13	170±0.15	79.8±0.21	90±0.19	114±0.11
6.8 pH phosphate buffer	100.6±0.19	117±0.23	190±0.16	85.45±0.19	92±0.24	120±0.17

Table 8: Percentage yield and assay of granules

Formulation Code	Percentage yield (%)	Assay
F1	72.20±0.89	96.80 ±0.44
F2	85.91±0.88	95.00±0.31
F3	76.38±0.71	98.90±0.35
F4	77.54±0.57	94.70±0.15
F5	71.70±0.43	100.0±0.11
F6	52.92±0.71	96.30±0.25
F7	60.64±0.81	99.00±0.31
F8	67.10±0.82	99.80±0.36
F9	77.54±0.57	94.70±0.15
F10	77.54±0.57	94.70±0.15
F11	73.64±0.44	100.1±0.32
F12	60.78±0.91	100.0±0.47
F13	65.70±0.21	99.30±0.23
F14	70.30±0.63	91.70±0.20
F15	77.22±0.52	97.90±0.31

Table 9: Flow properties of granules

Formulation	Bulk density (gm/ml)	Tapped density (gm/ml)	Carr's index (%)	Hausner ratio	Angle of repose (º)
F1	0.463±0.11	0.530±0.18	12.6±0.21	1.14±0.27	15.12±0.16
F2	0.592±0.12	0.632±0.11	6.32±0.12	1.06±0.22	13.39±0.32
F3	0.617±0.10	0.722±0.16	14.4±0.32	1.17±0.11	13.70±0.12
F4	0.432±0.16	0.480±0.19	11.1±0.11	1.12±0.16	14.80±0.28
F5	0.601±0.14	0.632±0.12	4.90±0.18	1.05±0.30	13.60±0.21
F6	0.484±0.10	0.518±0.20	6.67±0.23	1.07±0.16	11.72±0.14
F7	0.475±0.15	0.554±0.13	14.2±0.11	1.16±0.29	12.26±0.25
F8	0.460±0.20	0.525±0.15	12.3±0.37	1.14±0.23	13.83±0.12
F9	0.432±0.16	0.480±0.19	11.1±0.11	1.12±0.16	14.80±0.28
F10	0.432±0.16	0.480±0.19	11.1±0.11	1.12±0.16	14.80±0.28
F11	0.529±0.11	0.597±0.22	11.4±0.20	1.12±0.19	13.70±0.22
F12	0.417±0.21	0.476±0.12	12.5±0.31	1.14±0.22	15.28±0.16
F13	0.483±0.19	0.544±0.13	11.2±0.10	1.12±0.38	13.3±0.15
F14	0.505±0.14	0.544±0.18	7.14±0.20	1.07±0.14	12.63±0.20
F15	0.568±0.17	0.640±0.13	11.25±0.18	1.12±0.10	14.31±0.32

Table 10: Weight variation, content uniformity and capsule lock length of pulsincap formulation

Formulation	Weight variation (mg)	Content Uniformity (%)	Capsule lock length (mm)
F1	312.00±2.65	92.30±0.47	20±0.01
F2	372.67±3.21	91.00±0.50	19±0.03
F3	333.00±3.61	95.19±0.57	19.5±0.01
F4	302.33±2.08	92.67±0.53	20±0.07
F5	273.67±4.93	98.93±0.49	19±0.02
F6	305.00±3.00	94.59±0.57	19±0.05
F7	288.33±2.08	100.6±0.70	19.2±0.01
F8	275.67±4.16	101.23±0.44	20±0.01
F9	302.33±2.08	92.67±0.53	19.7±0.08
F10	302.33±2.08	92.67±0.53	19±0.06
F11	328.00±2.65	104.3±0.61	20±0.02
F12	293.33±2.52	99.89±0.59	20±0.05
F13	294.00±2.65	97.25±0.71	20±0.01
F14	273.67±2.52	93.84±0.58	19±0.04
F15	336.33±2.52	95.45±0.38	19±0.01

Table11: ANOVA summary for responses Y_1 and Y_2

Response	Model	F- value	P-value	R squared
	Linear	9.36	0.0023	0.7186
Y_1(lag time)	Quadratic	7.07	0.0211	0.9272
	2FI	3.89	0.0404	0.7448
Y_2(%drug release)	Linear	1.48	0.27	0.2874

Table 12: One-way ANOVA of factors A, B, C versus lag time

Factor	Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor A (Drug: Polymer ratio)	A	2	2.772	1.3861	5.82	0.017
	Error	12	2.857	0.2381		
	Total	14	5.629			
Factor B (Polymer: Polymer ratio)	B	2	0.4772	0.2386	0.56	0.588
	Error	12	5.1521	0.4293		
	Total	14	5.6293			
Factor C (Plug weight)	C	2	1.866	0.9329	2.97	0.089
	Error	12	3.764	0.3136		
	Total	14	5.629			

Table 13: One-way ANOVA of factors A, B, C versus % Drug release

Factor	Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor A (Drug: Polymer ratio)	A	2	1096	548.1	4.17	0.042
	Error	12	1578	131.5		
	Total	14	2674			
Factor B (Polymer: Polymer ratio)	B	2	67.82	33.91	0.16	0.857
	Error	12	2606.08	217.17		
	Total	14	2673.90			
Factor C (Plug weight)	C	2	127.4	63.69	0.30	0.746
	Error	12	2546.5	212.21		
	Total	14	2673.9			

Table 14: Validation

Formulation	R_1(Lag time in hrs)		R_2 (% drug release)	
	Predicted	Obtained	Predicted	Obtained
F1	3.468	3.2±0.19	83.76	81.79±0.23
F2	4.16	4.1±0.04	84.615	85.34±0.16
F11	3.768	4.2±0.27	76.04	72±0.09

Table 15 Stability study of optimized formulation (F11)

Test	Initial	1st week	2nd week	1 month
Physical appearance	Capsules are intact	Intact	Intact	Intact
Drug content	100.1±0.32	100.05±0.40	99.96±0.64	99.92±0.44
Lag time	4.2±0.03	4.18±0.01	4.18±0.06	4.16±0.04
% Drug release	72±0.15	71.84±0.52	71.62±0.53	71.2±0.43

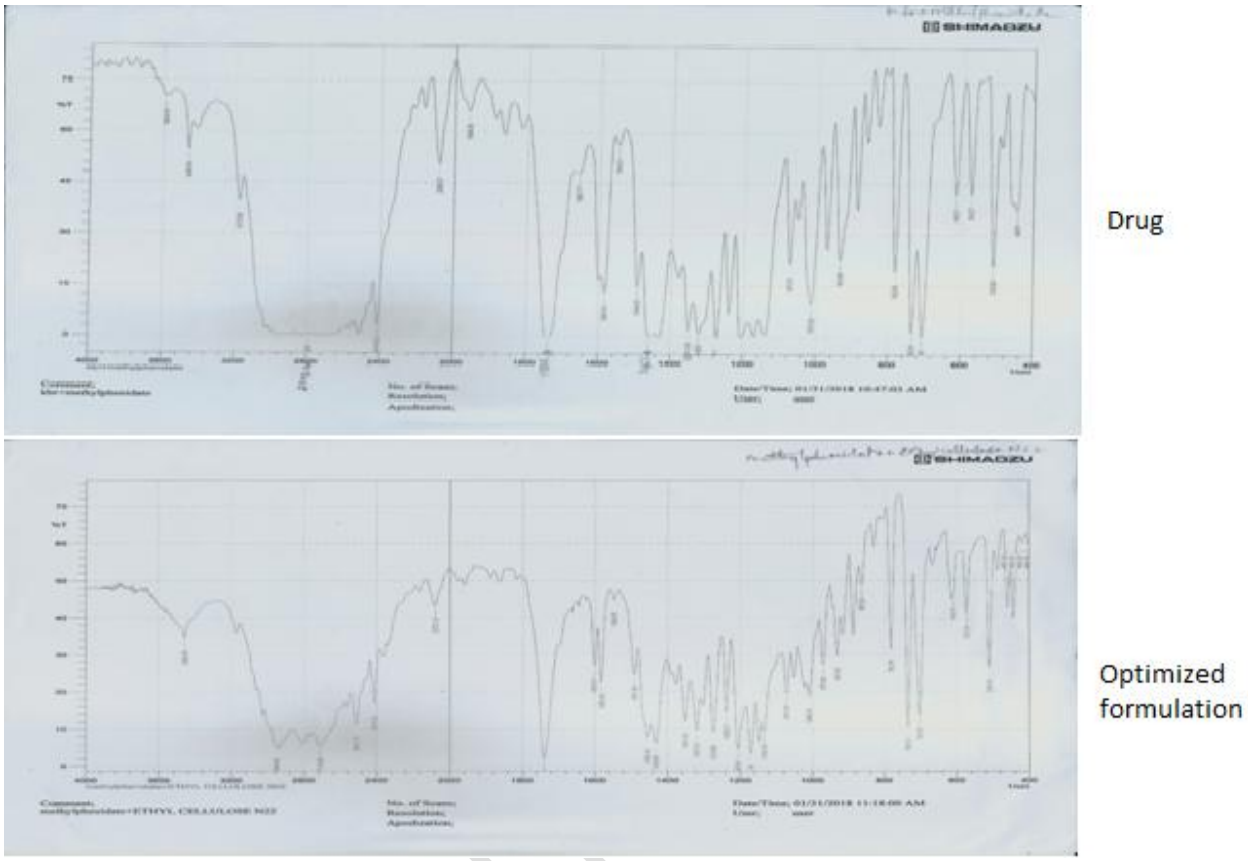

Fig 1: FTIR graph of pure drug and optimized formulation

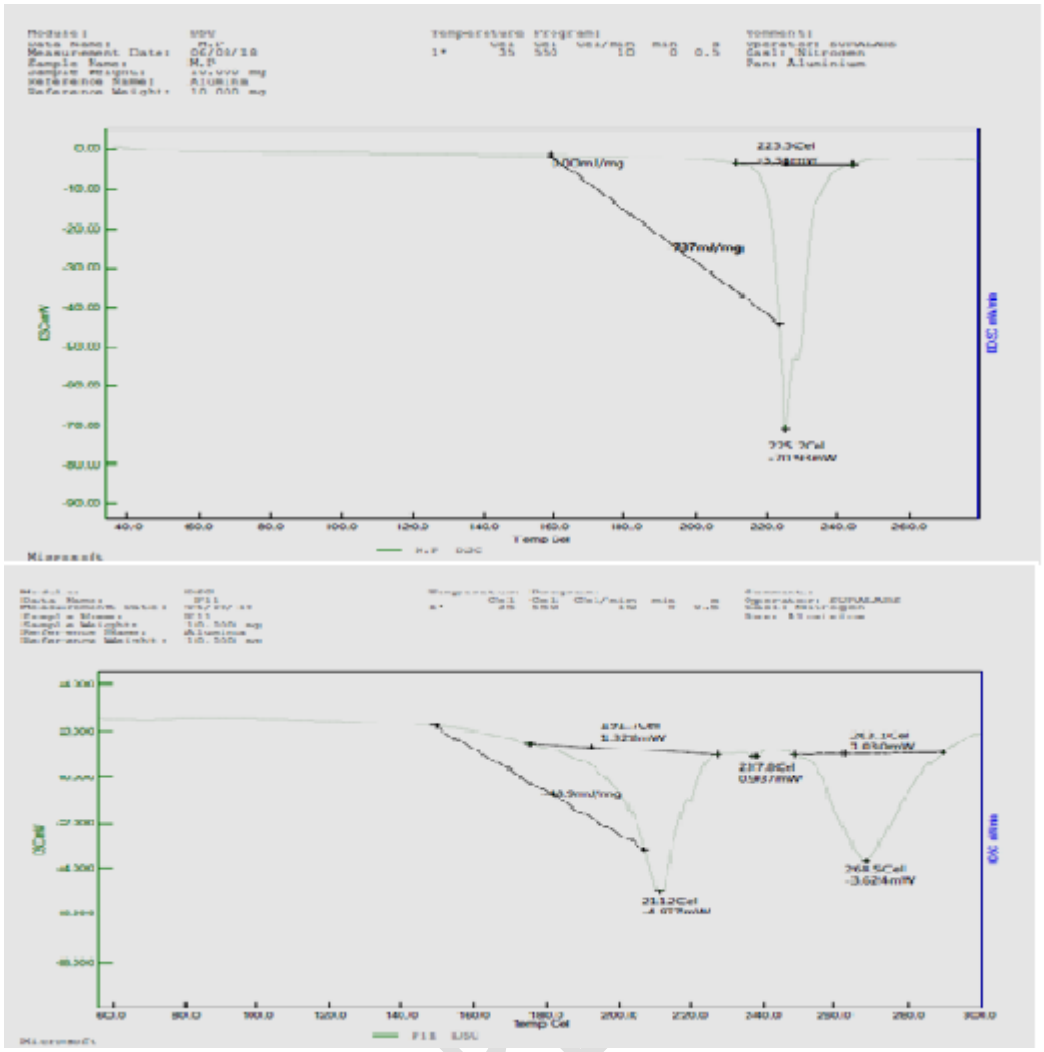

Fig 2: DSC of pure drug and optimized formulation

Capsule body (Initially)

Capsule body turned in to a soft fluffy mass

Fig 3: Solubility of formaldehyde treated capsule body (showing end point)

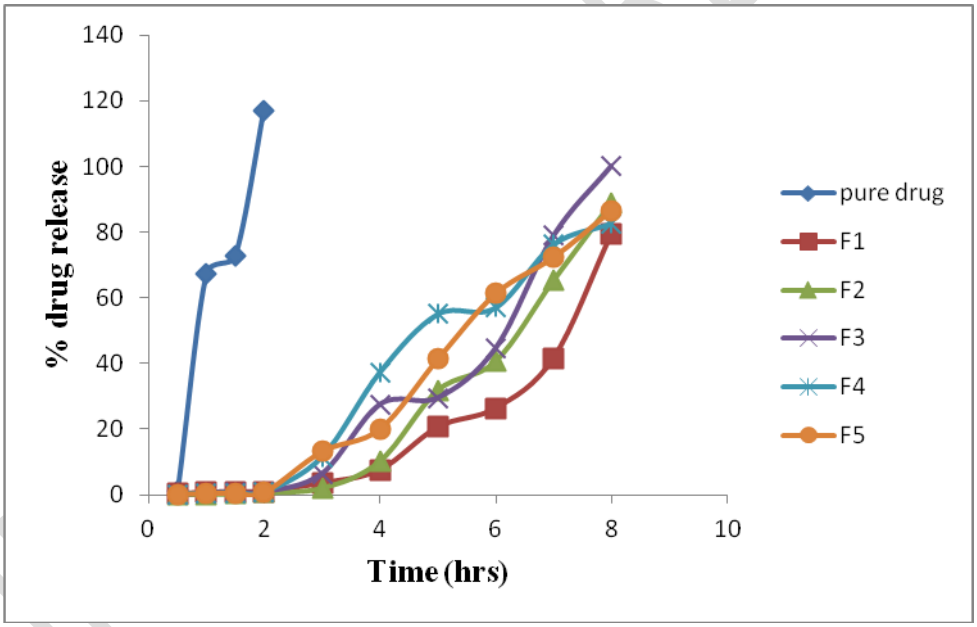

Fig 4: In-vitro drug dissolution of pulsincapin comparison with pure drug (F1 – F5)

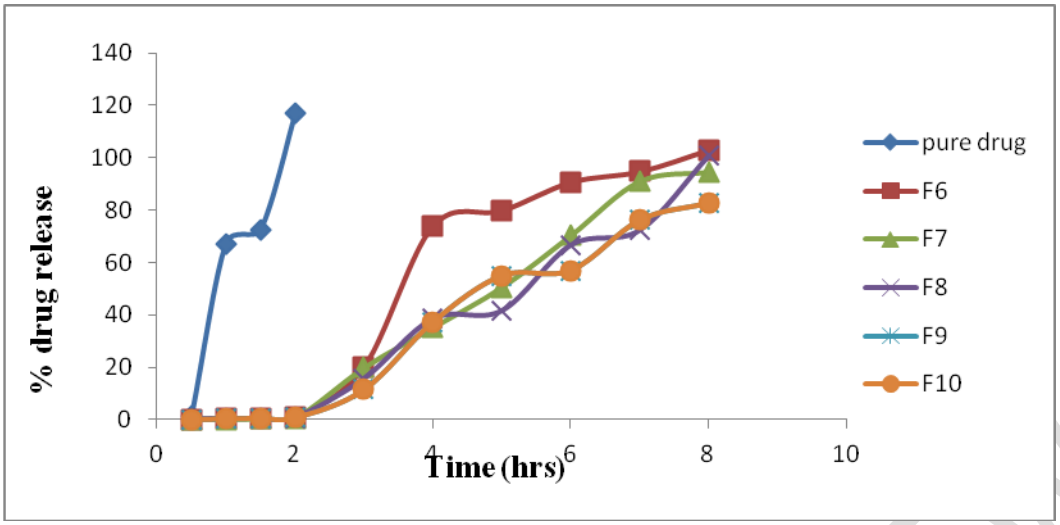

Fig 5: In-vitro drug dissolution of pulsincapin comparison with pure drug (F6 – F10)

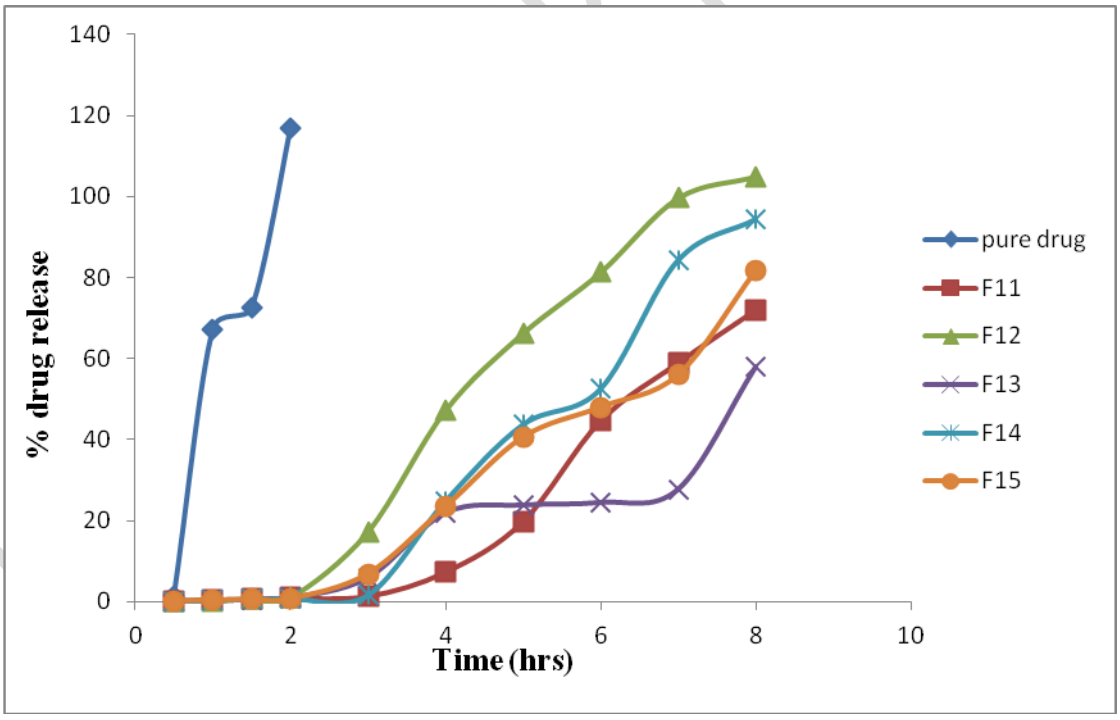

Fig 6: In-vitro drug dissolution of pulsincapin comparison with pure drug. (F11– F15)

Fit Summary

Response 1: R1 (Lag time)

Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	
Linear	**0.0023**		**0.6418**	**0.4171**	**Suggested**
2FI	0.8428		0.5533	-0.2761	
Quadratic	0.0790		0.7961	-0.1653	
Cubic			1.0000		Aliased

Fit Summary

Response 2: R2 (% drug release)

Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	
Linear	**0.2742**		**0.0930**	**-0.5066**	**Suggested**
2FI	0.6069		-0.0039	-1.9726	
Quadratic	0.5407		-0.0811	-5.1779	
Cubic			1.0000		Aliased

Fig 7: Fit summary for responses Y_1 and Y_2.

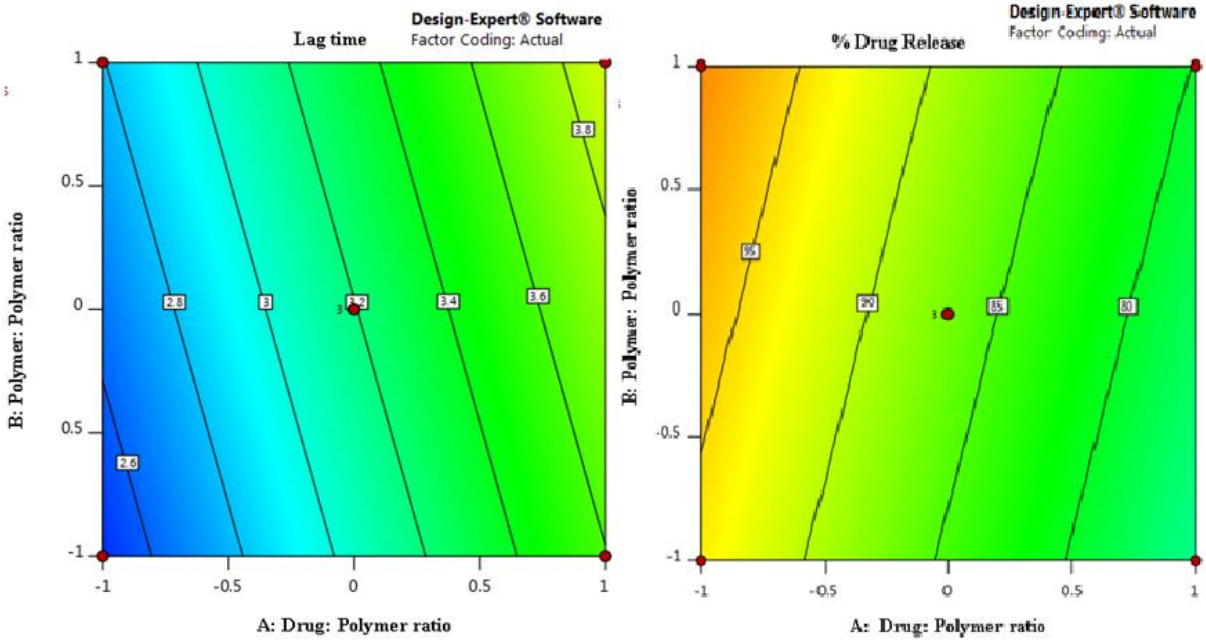

Fig 8: Contour plot for the effect of Drug: polymer ratio and Polymer: polymer ratio on Lag time and % Drug release at centre level of X3 (Plug weight).

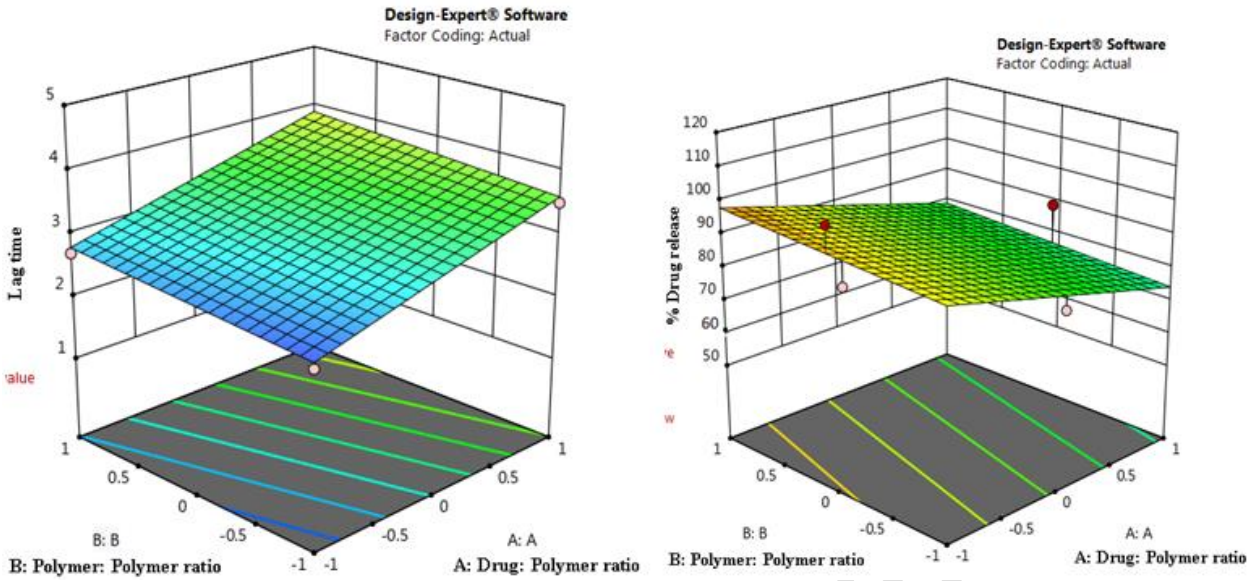

Figure 9: Response surface plot showing influence on Lag time and % Drug release

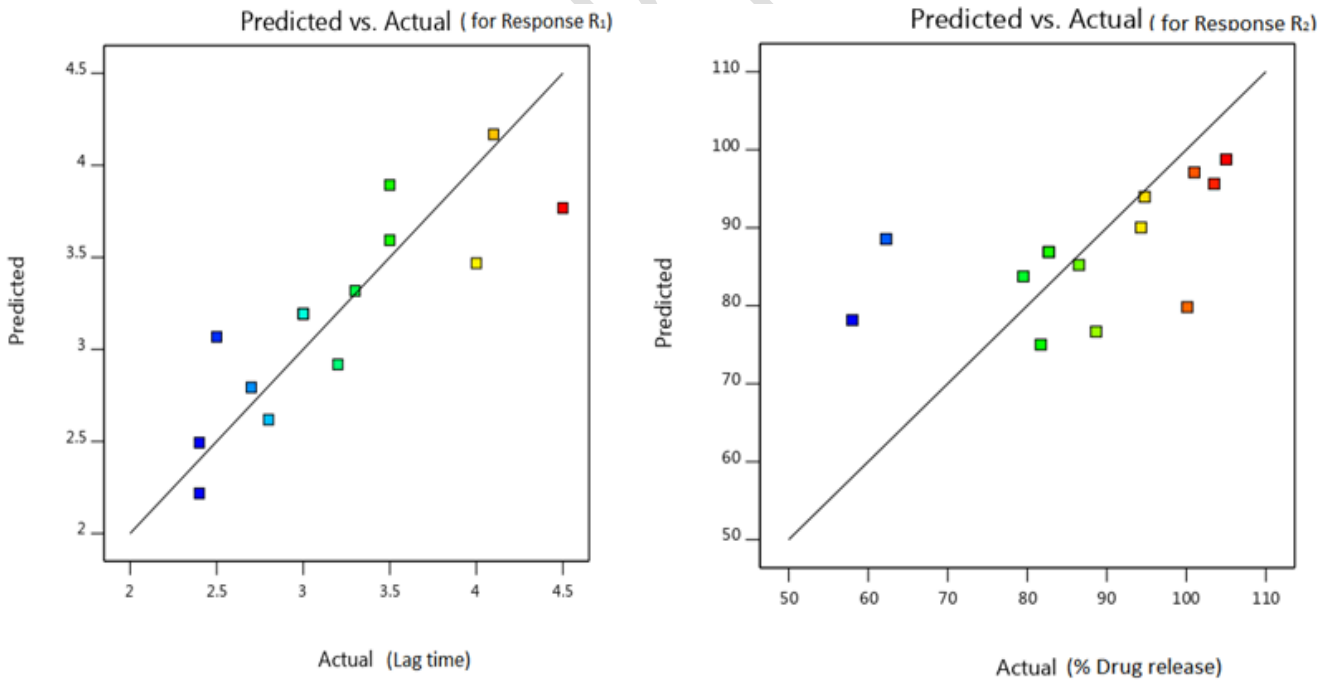

Fig 10: Predicted vs. Actual response for R_1 (Lag time) and R_2 (% Drug release)

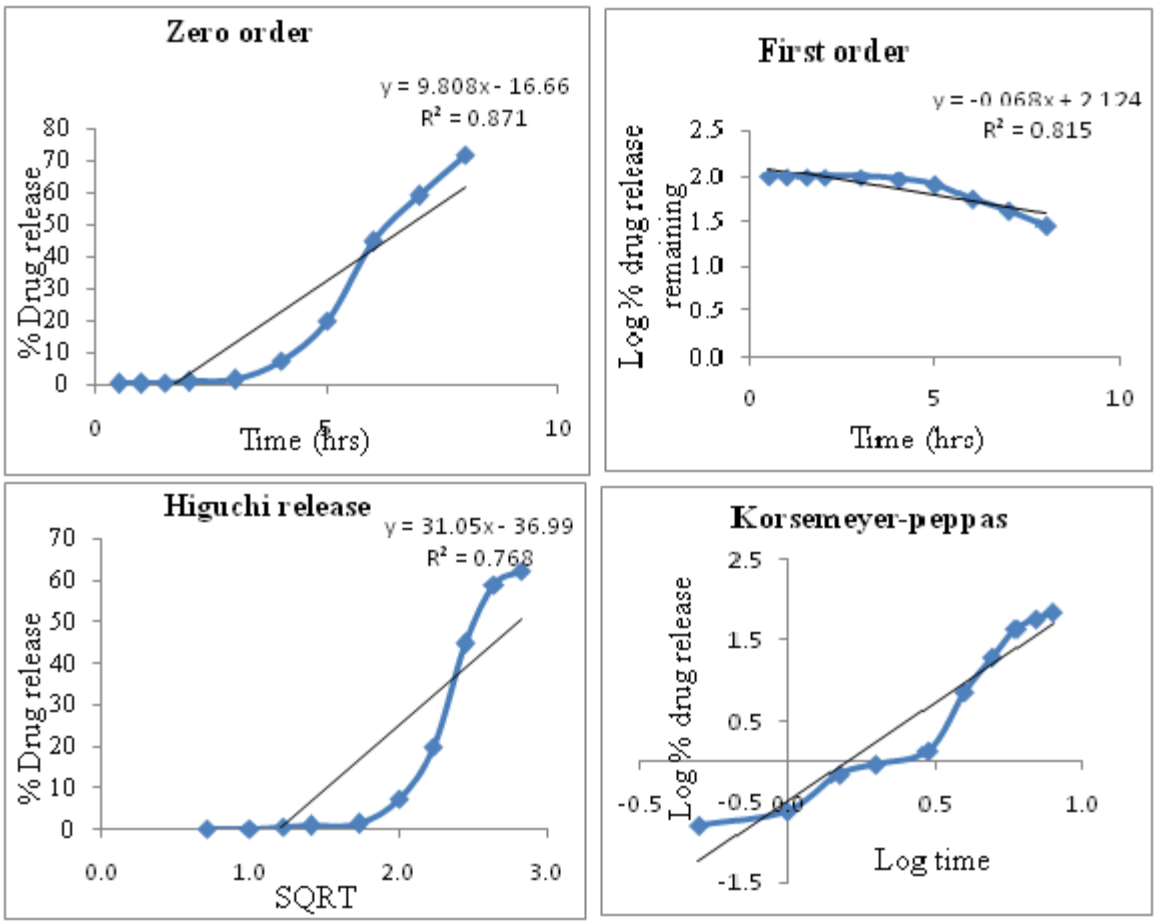

Fig 11: Model dependent kinetics for the formulation F11

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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