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FORMULATION AND OPTIMIZATION OF SUSTAINED RELEASE TABLETS OF DALFAMPRIDINE BY FACTORIAL DESIGN MODEL

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ABSTARCT

Objective To investigate and optimize the sustained release tablets of Dalfampridine by using various hydrophilic and hydrophobic polymers as dissolution controlled release agents by using software design 3 level factorial models. **Method** Initially, preliminary experiments were performed to determine the main factors and the appropriate ranges in which the optima lie. The effects of polymer concentrations as independent variables (Eudragit RSPO, Eudragit RLPO, HPMC for Dalfampridine on the various times of invitro drug release were tested. Through preliminary screening the retardant polymers concentrations were identified as the most significant variables. On the basis of the preliminary trials a 3-factor, 3-level Box-Behnken design [Table 18 & 20] was employed to study the effect of each independent variable on dependent variables (various drug release times like D1, D6, D12, and T50). This design is suitable for exploring quadratic response surfaces and constructing second-order polynomial models. **Results:** As per the Box behnken 3 level factorial designs, the optimization of Dalfampridine sustained release tablets were done. The software provided each drug 17 formulations by varying concentration of retardating polymers. The granules were prepared as per the designed formulas and all the Precompression parameters were compliance the sustained release tablets criteria as per IP specifications. The granules were compressed as sustained release tablets and subjected to post compression parameters to assess the quality of tablets. The post compression parameters were successfully compliance the sustained release tablets specifications. The formulations were controlled the drug release over the period of 12 hours. The best formulations were optimized by using statistical tools analysing various dissolution parameters like D1, D6, D12 and T50. From the above design, we found the optimized formulations from each category of drugs. The selected formulations were stable for short time stability studies. By using the pharmacokinetics and bioavailability parameters, the relative bioavailability and AUC, AUMC, Cmax, Tmax, t1/2, MRT & clearances was found. **Conclusion** The results of our study reveal that statistically design optimized formulations of Dalfampridine sustained release formulations provide more bioavailable than normal conventional tablets.

Keywords: Dalfampridine, Sustained release tablets, Box behnken factorial designs

INTRODUCTION

Oral drug delivery has been known for decades as the most widely utilized route of administration among all the routes that have been explored for the systemic delivery of drugs via various pharmaceutical products of different dosage forms. The oral route is the most preferred method of administration the reasons that the oral route achieved such popularity may be in part due to its ease of administration as well as the traditional belief that by oral administration the drug is well absorbed along with the gastrointestinal tract along with food stuff [1]. Oral drug delivery systems can range from relatively simple immediate release (IR) formulation to complex extended or modified release dosage forms. The rate limiting step in most of the solid dosage forms is bioavailability, the rate and extent of drug absorption through biological membrane to the systemic circulation in unchanged form. For a drug to elicit its desired pharmacological activity the drug concentration in the plasma should reach minimum effect concentration. Ideal drug delivery system is one which delivers the drug at the site of action in sufficient amount and at the appropriate rate. Sustained releases denote the system which is able to provide some therapeutic control either it is of temporal or spatial nature or both. In other words, the system attempts to provide a constant drug concentration in the target tissue [2]. It is this nature of this system that makes it different from sustained release systems. Sustained release products are formulations that release active drug compounds into the body gradually and predictably over a 6-12 h period and that can be taken once or twice a day. Typically these products provide numerous benefits compared with immediate release drugs, greater effectiveness in

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the treatment of chronic conditions, reduced side effects, greater convenience and higher levels of patient compliance due to a simplified dosing schedule. The aim of the study was to formulate and optimize the Dalfampridine matrix tablets by using Eudragit RSPO, Eudragit RLPO and HPMC K110M.

METHODOLOGY

Factorial design of optimization of Dalfampridine SR Tablets:

Initially, preliminary experiments (one factor at a time approach) were performed to determine the main factors and the appropriate ranges in which the optima lie. The effects of polymer concentrations as independent variables (Eudragit RSPO, Eudragit RLPO, HPMC for Dalfampridine) on the various times of invitro drug release were tested. Through preliminary screening the retardant polymers concentrations were identified as the most significant variables. On the basis of the preliminary trials a 3-factor, 3-level Box-Behnken [3] design [Table 2] was employed to study the effect of each independent variable on dependent variables (various drug release times like D1, D6, D12, and T50). This design is suitable for exploring quadratic response surfaces and constructing second-order polynomial models. The design consists of replicated centre points and the set of points lying at the midpoint of each edge of the multidimensional cube that defines the region of interest. The independent factors and the dependent variables used in the design are listed in Table1**.**

Three independent variables are Eudragit RSPO, Eudragit RLPO, HPMC for Dalfampridine. The percentage levels of each variable were determined to develop sustained release tablets. The levels were set as low, and high. Then a 3^2 factorial design was constructed to study the effect of concentration of retardant polymers. Various invitro drug release times (D1, D6, D12, T50) were selected as dependent variables. A statistical model incorporating interactive and polynomial terms was developed to evaluate the responses.

Y = b0 + b1 X1 + b2 X2 + b1.2 X1 X2 + b1.1 X1 2 + b2.2 X2 2

Where: y is dependent variable b0 is the arithmetic mean response of the 17 runs, b1 is the estimated coefficient for the factor X1 X2 X3 is the main effects represent the average result of changing one factor at a time from its low to high value. The interaction terms X1 X2 X3 demonstrate how the response changes when 3 factors are changed simultaneously. X1 2 X2 X3 is used to investigate nonlinearity.

Table 2: Design Summary for Dalfampridine sustained release tablets

	Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3	R. 4
Run	A:Eudragit RSPO	B:Eudragit RLPO	C:HPMC	D1	D6	D12	T50
	mg	mg	mg	%	%	%	hours
1	50	50	44.5	31	74	87	2.53
2	62.5	50	29	30	70	81	3.07
3	50	50	44.5	32	75	86	2.49
4	50	37.5	29	40	76	93	1.909
5	37.5	50	29	35	69	85	2.75
6	50	50	44.5	33	75	86	2.47
7	62.5	62.5	44.5	31	71	82	2.93
8	50	50	44.5	32	74	84	2.315
9	37.5	62.5	44.5	34	70	87	2.71
10	50	50	44.5	33	74	89	2.41
11	50	62.5	29	35	74	90	2.85
12	62.5	37.5	44.5	31	70	84	3.1
13	50	37.5	60	32	78	91	2.95
14	37.5	37.5	44.5	36	69	89	2.8
15	37.5	50	60	34	70	85	2.74
16	50	62.5	60	33	78	90	2.54
17	62.5	50	60	32	72	81	2.98

Table 2: List of dependent and independent variables in Box-Behnken design for Dalfampridine sustained release tablet

Factor	Independent variables	Units	Minimum	Maximum
A	Eudragit RSPO	mg	37.5	62.5
B	Eudragit RLPO	mg	37.5	62.5
C	HPMC	mg	29	60

Response	dependent variables	Units
R1	D1	%
R2	D6	%
R3	D12	%
R4	T50	hours

FORMULATION

Dalfampridine matrix tablets were prepared employing Eudragit RSPO, Eudragit RLPO, HPMCK100M as matrix former polymers by direct compression technique using microcrystalline cellulose (avicel pH 102) as diluents. All the ingredients were passed through the sieve no 40 separately and shifted in plastic bag to attain uniformity.The powder blend was lubricated with 1% w/w magnesium Stearate which was previously passed through the sieve no 60. Lubricated blend was then compressed into tablets of weigh 250 mg using B tooling in a rotary tablet press [4].

EVALUATION

Precompression parameters:

Angle of Repose, Bulk Density and Tapped Density, Carr's Compressibility Index (%) & Hausner's Ratio (USP) was calculated by official standard procedures.

POST COMPRESSIONAL PARAMETERS

Hardness test:

Hardness (diametric crushing strength) is a force required to break a tablet across the diameter. The hardness of a tablet is an indication of its strength. The tablet should be stable to mechanical stress during coating, packaging transportation and also during patient handling. The degree of hardness varies with the different manufactures and with the different types of tablets. The permissible limit for sustained release tablets is 4-12 kg/cm^2. The hardness was tested using Pfizer hardness tester.

Weight variation test:

This is an important in-process quality control test to be checked frequently (every half an hour). Corrections were made during the compression of tablets. Any variation in the weight of tablet (for any reason) leads to either under medication or overdose. So, every tablet in each batch should have a uniform weight. 20 tablets were weighed individually. Average weight was calculated from the total weight of all tablets. The individual weights were compared with the average weight. The percentage difference in the weight variation should be within the permissible limits (7.5%).

Friability test:

Friability is the loss of weight of tablet in the container/package, due to removal of fine particles from the surface. This in-process quality control test is performed to ensure the ability of tablets to withstand the shocks during processing, handling, transportation, and shipment. Roche friabilator was used to measure the friability of the tablets. It was rotated at a rate of 25 rpm. Five tablets were weighed collectively and placed in the chamber of the friabilator. In the friabilator, the tablets were exposed to rolling, resulting from free fall of tablets within the chamber of the friabilator. After 100 rotations (i.e. in 4 minutes), the tablets were taken out from the friabilator and intact tablets were again weighed collectively. Permitted friability limit is 1.0%.

Dissolution Studies:
Dissolution was performed using United States Pharmacopoeia (USP) type II (paddle) apparatus, 900 ml of phosphate buffer, pH 6.8 at 37 ± 0.5°C and 50 rpm. Aliquots sample (5 ml) of the solution was withdrawn from the dissolution apparatus at the appropriate time intervals, and the samples were replaced with fresh dissolution medium. Absorbance of the samples was measured at 262 nm for Dalfampridine and 270nm for tramadol tabltes using UV-Visible double-beam spectrophotometer. The drug content was calculated using the equation generated from standard calibration curve. The cumulative % drug release was calculated [5].

IN-VIVO STUDIES

Pharmacokinetics analysis:
Adult SD rats (180 g ± 10 g) of either gender will be obtained from Central animal house. The animals will be housed in large, spacious polyacrylic cages at an ambient room temperature with 12-h light/12-h dark cycle with free access of food and water ad libitum. All the animals experimental procedure were carried out as per Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guideline.

Pharmacokinetic analysis of SR tablet:
The rats will divided into 2 groups each of 6 animals.

Group I: Pure drug
Group III : Optimized formulation

The rats will be fasted for 12 h prior to the experiment and 30 min before drug administration water also removed from the animal cage. The pure drug (Group I and II) and drug matrix formulation (Group III and IV) will be suspended in 0.5% carboxymethyl cellulose. The drug will be administered orally and monitored for any abnormal signs in 10 min. After 2 h of drug administration animals will allow to take food and water ad libitum. The sample for pharmacokinetic analysis will be collected at 0 h, 0.5 h before to drug administration and 1, 2, 4, 8 and 12 h after drug administration.

Collection of blood:
To avoid fluctuations in hormone levels due to circadian rhythms, Rats were made to bleed at 09:00 a.m. and 10:00 a.m. The sample (700 µl) will be collected through retro orbital sinus under mild anaesthesia. The samples will be collected in a sample collection tube coated with sodium EDTA. Samples will be centrifuged at 3000 rpm at 4°C. The plasma will be separated and stored at <-20°C until analysis.

Sample preparation:
During the analysis, the 2 ml of blood samples were centrifuged at 6000rpm at 15 minute to separate the plasma. 0.5ml of plasma was pipetted into 2.0 ml centrifuge tube with this 0.5 ml of precipitating agent (10% Perchloric acid) was added. The superannuated solution was vortexes for 5 minutes and centrifuged at 5000 rpm for 7mins and used for the analysis [6].

Analysis of drug concentration in plasma:
Instrumentation:
Isocratic chromatographic separation was performed on a Shimadzu liquid chromatographic system equipped with a LC-20AD solvent delivery system (pump), SPD-20A photo diode array detector, and SIL-20ACHT injector. All chromatographic experiments were carried out in the isocratic mode. The Thermo C_{18} (250 x 4.6 mm i.d. 5µ) column was used for the analysis. The version was used 1.25 with applied for data collecting and processing (Shimadzu, Japan). Methanol and Buffer (30:70). Buffer:About 0.92 g/L of octane sulfonic acid sodium salt and 0.77 g/L of ammonium acetate in water. Add 1 mL of trimethylamine per L of mixture, and adjust with phosphoric acid to a pH of 4.0. Pass through a suitable filter in Diluent of 0.45-µm pore size.Detection wavelength: 240 nm.Flow rate: 1.0 ml/min.

Pharmacokinetics parameters:
Based on the drug concentration in the plasma, the pharmacokinetic parameters like AUC, AUMC, C_{max}, T_{max}, $t_{1/2}$, MRT & clearances were also evaluated by using PK Solver Version 4.0 software.

RESULT AND DISCUSSION

Design of experiments (DOE) has been used as a powerful approach to reduce the variation in a process and, ultimately, to produce high product yield with good sustained release of drug from the formulation. Among various design approaches, the Box-Behnken design was used to optimize and evaluate main effects, interaction effects and quadratic effects of the process variables on the various dissolution time parameters like D1, D6, D12, T50. This design is suitable for exploring quadratic response surfaces and constructing second order polynomial models. The design consists of replicated centre points and the set of points lying at the midpoint of each edge of the multidimensional cube. These designs are rotatable (or near rotatable) and require 3 levels of each factor. Seventeen experiments were required for the response surface methodology based on the

Box-Behnken design. Based on the experimental design, the factor combinations yielded different responses as presented in Table 2. These results clearly indicate that all the dependent variables are strongly dependent on the selected independent variables as they show a wide variation among the 17 batches.

Table 3: Pre Compression parameters of formulation F1-F8

Code	Bulk Density (gm/mL) ± SD*	Tapped Density (gm/mL) ± SD*	Compressibility Index (%) ± SD*	Hausner' s Ratio ± SD*	Angle of Repose (θ) ± SD*
F1	0.82±1.1	0.941±0.2	13.05±0.2	1.13±0.9	25.51±0.35
F2	0.80±1.4	0.936±0.8	14.525±0.4	1.16±0.4	25.83±0.78
F3	0.78±1	0.935±0.3	15.936±0.8	1.14±0.3	26.12±0.91
F4	0.81±0.7	0.923±0.1	12.24±0.3	1.13±1.9	26.96±0.78
F5	0.80±0.2	0.925±0.5	13.40±0.7	1.15±0.7	25.25±0.23
F6	0.81±0.7	0.928±0.9	12.60±0.4	1.14±0.5	26.22±0.59
F7	0.81±0.9	0.932±0.1	13.19±0.6	1.15±0.3	26.55±0.99
F8	0.81±0.3	0.929±0.2	13.24±0.8	1.13±0.9	25.23±0.43

***represents mean ± standard deviation (n = 3)**

Table 4: Pre Compression parameters of formulation F9-F17

Code	Bulk Density (gm/mL) ± SD*	Tapped Density (gm/mL) ± SD*	Compressibility Index (%) ± SD*	Hausner' s Ratio ± SD*	Angle of Repose (θ) ± SD*
F9	0.82±1.2	0.921±0.2	14.10±0.2	1.15±0.4	25.30±0.15
F10	0.80±0.8	0.926±0.2	14.142±0.4	1.14±0.2	26.13±0.48
F11	0.82±1.2	0.950±0.1	14.906±0.8	1.14±0.4	26.44±0.40
F12	0.81±0.2	0.920±0.1	12.04±0.3	1.13±1.2	26.04±0.70
F13	0.81±0.8	0.925±0.2	12.10±0.4	1.10±0.5	25.45±0.43
F14	0.81±0.2	0.948±0.5	12.82±0.2	1.10±0.5	26.10±0.41
F15	0.81±0.5	0.920±0.1	14.24±0.2	1.18±0.3	25.51±0.79
F16	0.82±0.4	0.934±0.2	12.14±0.4	1.12±0.5	25.40±0.13
F17	0.81±0.7	0.955±0.3	13.40±0.7	1.13±1.9	26.96±0.78

Table 5: Post Compression parameters of formulation F1-F8

Formulation Code	Parameters			
	Average Weight of Tablet in (mg) ± SD*	Hardness in (Kg/cm^2)± SD**	Thickness (in mm) ± SD**	Friability (%)± SD***
F1	250.30±0.0033	7.94±0.219	4.232±0.0130	0.406±0.406
F2	250.19±0.0032	7.84±0.114	4.244±0.0167	0.56±0.421
F3	250.26±0.0028	8.08±0.130	4.234±0.0114	0.06±0.007
F4	250.01±0.0027	8.08±0.164	4.248±0.013	0.108±0.013
F5	250.24±0.0025	7.98±0.148	4.234±0.011	0.108±0.013
F6	250.17±0.0027	8.02±0.836	4.256±0.013	0.06±0.007
F7	250.22±0.0025	8.04±0.1	4.244±0.013	0.56±0.421
F8	250.34±0.0025	8.14±0.054	4.246±0.011	0.048±0.008

Table 6: Post Compression parameters of formulation F9-F17

Formulation Code	Parameters			
	Average Weight of Tablet in (mg) ± SD*	Hardness in (Kg/cm^2)± SD**	Thickness (in mm) ± SD**	Friability (%)± SD***
F9	250.12±0.0030	7.50±0.210	4.242±0.0142	0.410±0.441
F10	250.20±0.0031	7.42±0.124	4.242±0.0110	0.50±0.420
F11	250.14±0.0024	8.10±0.114	4.210±0.0142	0.12±0.014
F12	250.24±0.0021	8.54±0.152	4.234±0.024	0.110±0.023
F13	250.12±0.0020	7.84±0.124	4.220±0.0124	0.124±0.021
F14	250.22±0.0025	8.14±0.875	4.214±0.0131	0.142±0.024
F15	250.14±0.0022	8.10±0.102	4.213±0.0120	0.451±0.424
F16	250.24±0.0022	8.21±0.015	4.214±0.0142	0.102±0.020
F17	250.10±0.0022	8.25±0.174	4.242±0.0142	0.201±0.024

The bulk density was in range of 0.786-0.82 g/ml.The tapped density was found to be in range of 0.920-0.955 g/ml.The compressibility index and hausner's ratio were found to be between 12.10%-15.93% & 1.10-1.18 respectively.The angle of repose was in range of 22 ^{O}.5' - 26 ^{O}.9' (Table 3&4).From the above results it was found to be the powder blend has good-excellent flow properties.

Post compression results were shown in table 36&37.Weight variation results were found to be within specifications ± 7.5 % as per I.P.Hardness of all the formulations lies between 7.42 – 8.54 kg/ cm^2 and all the 17 formulation hardness were compliance the IP limit.Thicknesses of the entire tablets were found to be 4.210 mm - 4.456 mm.Friability of all the formulations were found to be < 0.10 % and were within specifications and for optimized formulation the friability was found to be 0.56% (Table 5&6).

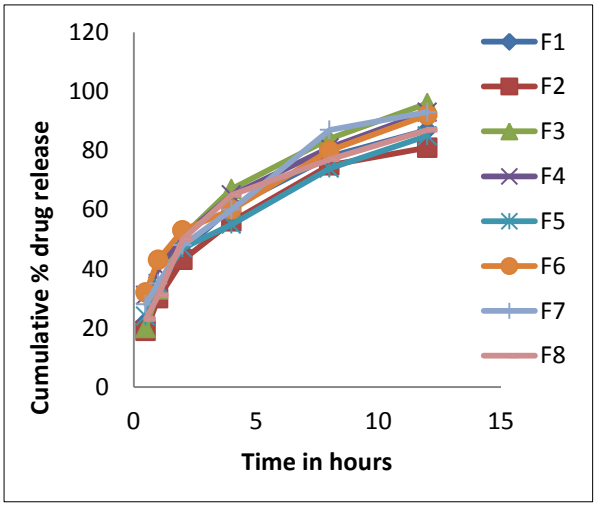


Figure 1: *In vitro* dissolution profiles of the formulations F1-F8

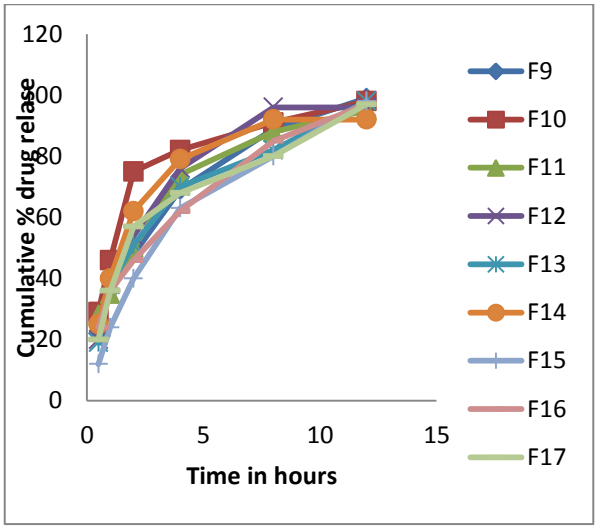


Figure 2: *In vitro* dissolution profiles of the formulations F9-F17

In vitro drug release data Dalfampridine sustained release tablets:

From the in vitro data it was concluded that all the formulations were able to extend the release for duration of 12 hours.But the formulations with high concentration of HPMC were not able to control the burst release (> 30% release in 1hour), whereas the formulations with high concentration of eudragit RLPO were also not able to met the sustained release criteria in 1st hour & 6th hour (F3, F4).Formulations with high concentration of eudragit RSPO, low concentration of RLPO & HPMC met the extended release criteria i.e., NMT 30% release in 1st hour, 30-70% release within 6 hours & NLT 80% release in 12 hours.The regression coefficient values obtained from the various release kinetic models reveled that all the formulations follows the first order release with Higuchi diffusion (r^2 near to 0.99)& follows Fickian diffusion (k value ≤ 0.5) and the plots were shown in Fig. 1&2.

Data Analysis Dalfampridine sustained release tablets:

These equations represent the quantitative effect of polymer concentrations as independent variables like Eudragit RSPO, Eudragit RLPO, HPMC one Dalfampridine sustained release tablets dissolution time parameters like D1, D6, D12 and T50. The values of the coefficient A, B, C and D are related to the effect of these variables on the responses R1,R2, R3 and R4. Coefficients with more than one factor term and those with higher order terms represent interaction terms and quadratic relationship respectively. A positive sign represents a synergistic effect, while a negative sign indicates an antagonistic effect. A backward elimination procedure was adopted to fit the data to the quadratic model. All the polynomial equations were found to be statistically significant (P <0.05), as determined using ANOVA (Table 2), as per the provision of Design Expert software.

Effect of sustained release polymers concentration on dissolution rat at 1st hour:

The mathematical model generated for D1 (R1) was found to be significant with F-value of 7.45 ($p < 0.0013$) and R^2 value of 0.9448. The independent variables A, B, C and the residual values have significant effects on the D1, since the P-values less than 0.05 represent the significant model terms as shown in Table 7.

Table 42 shows the results of ANOVA, which was carried out to identify insignificant factors. From the results of ANOVA for the measured responses, it was found that the amount of Eudragit RSPO, Eudragit RLPO and HPMC had a significant effect on dissolution rates of sustained release tablets ($p < 0.05$). The calculated value of F are less than their critical value, it may be concluded that those interaction term do not contribute significantly can be omitted from the full model.

The polynomial equations can be used to draw conclusions after considering the magnitude of coefficient and the mathematical sign it carries, i.e. positive or negative. The coefficient obtained shows that coefficient A, B & C were positive (Table 7). As the concentration of polymers increased, dissolution rate time decreased. The magnitude of coefficient showed that D1 has more effect than D2 on dissolution rate. This is due to water swelling effect of Eudragit polymers. The response surface plot and contour plot of effect of Eudragit RSPO and Eudragit RLPO on dissolution rate are shown in Figure 3 respectively.

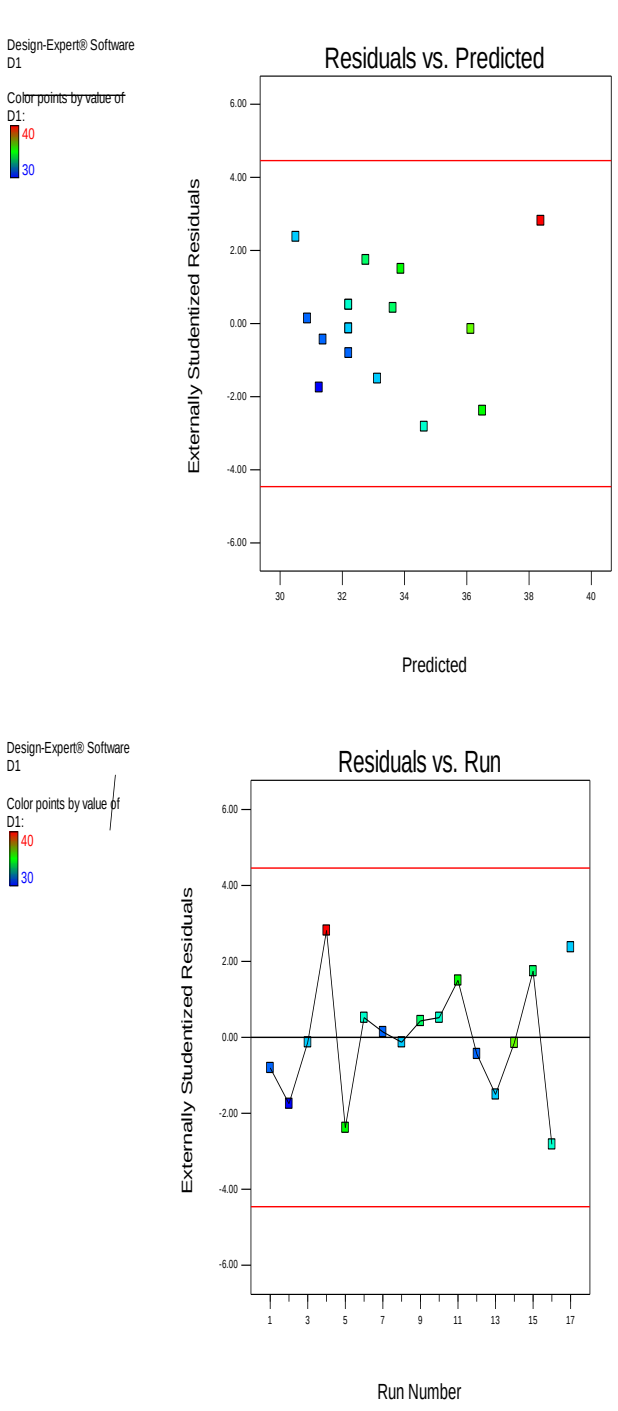
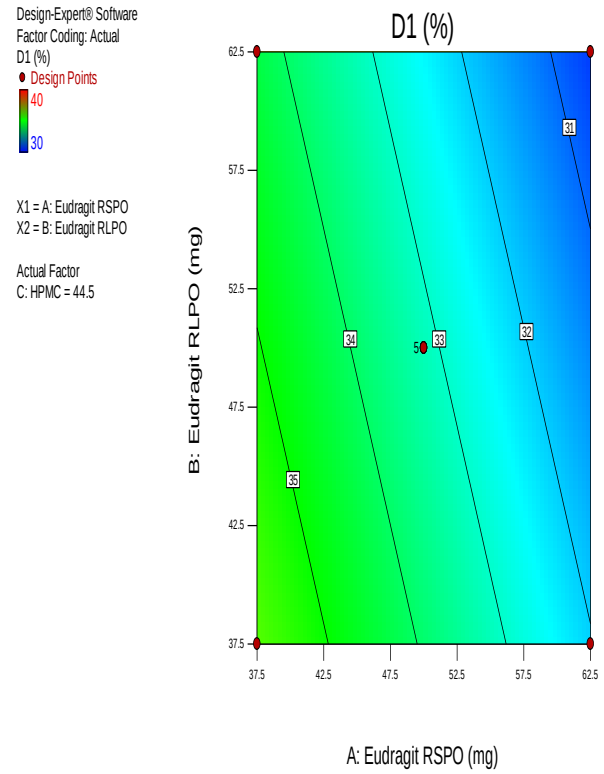


Figure 3a: Residuals vs. Run plot for dissolution rate at 1st hour. b) Predicted vs. actual plot for dissolution rate at 1st hour

This is a plot of the residuals versus the experimental run order. A random scatter data shows no lurking variability during experiment (Fig 4).

The data points should be split evenly by the 45 degree line. So there are no values, which are not easily predicted by the model (Fig 24)



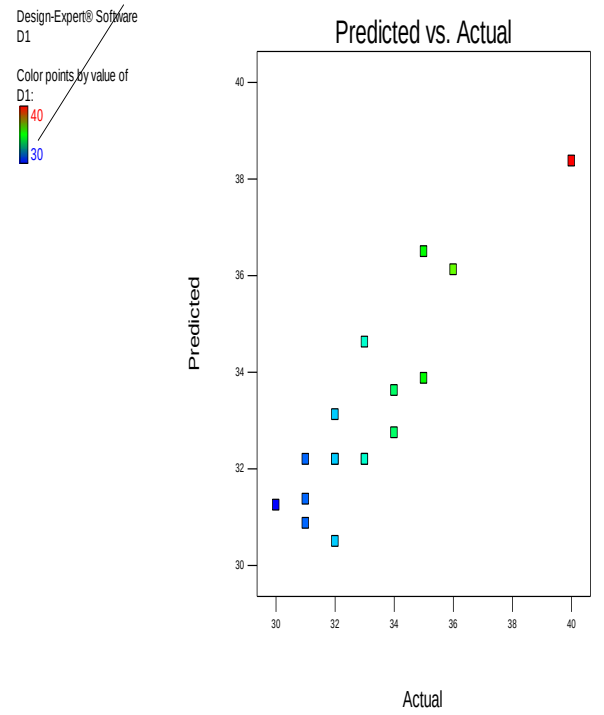


Figure 4a: Externally studentized residuals plot for dissolution rate at 1st hour. b) Response surface plot showing effect of Eudragit RLPO and RSPO on Dissolution rate at 1st hour.

This plot helps the experimenter detect outliers in the data. There are no data points that are outside the red lines. So, there are no data points that are not fit well by the current model (Fig 5).

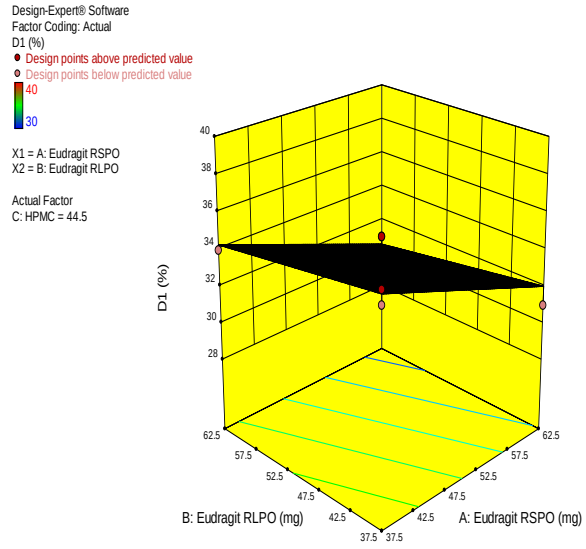


Figure 5: Contour plot showing effect of Eudragit RSPO and RLPO on dissolution rate at 1st hour.

Results of the equation indicate that the effect of A is more significant than B and C. The influence of the main and interactive effects of independent variables on the D1 was further elucidated using the perturbation and 3D response surface plots. The Contour plot (Figure 27) showing the main effects of A, B and C on the D1 (R1) of sustained tablets. This figure clearly shows that A has the main and the major effect on D1 followed by B which has a moderate effect on D1 followed by C which has a little effect on D1. The relationship between the dependent and independent variables was further elucidated using response surface plots. Figure 26 shows the interactive effect of A and B on the D1 (R1) at fixed level of C. At low levels of A (Eudragit RSPO), D1 increases from 34 to 36%. Similarly, at high levels of A, D1 increases from 30 to 32% dissolution of analysis of sustained release tablets was found to be in the range of 30 to 40% as shown in Table 7.

Effect of sustained release polymers concentration on dissolution rat at 6th hour:

In the case of D6, at the low level of A dissolution was increased from 69 to 70% and at high level 70 to 72%. At the low concentration of variable of A, the responses D12 and T50 showed 85 to 89% and 2.71 to 2.8 hours. At the high concentration 81 to 84% and 2.93 to 3.1 hours. The factorial equation for dissolution at 1 hour exhibited a good correlation coefficient (0.9448) and the Model F value of 24.35which implies the model is significant. Values of "Prob> F" less than 0.0500 indicate model terms are significant (Table 8).

D1=33.176470588235-1.875 * A

Table 7 : Results of Regression Analysis D1

Sum of		Mean	F	p-value	
Source	**Squares**	**df**	**Square**	**Value**	**Prob > F**
Model	42.75	3	14.25	3.73	0.0393	significant
A-Eudragit RSPO	28.12	1	28.12	7.35	0.0178	
B-Eudragit RLPO	4.50	1	4.50	1.18	0.0978	
C-HPMC	10.13	1	10.13	2.65	0.0277	
Residual	49.72	13	3.82			
Lack of Fit	46.92	9	5.21	7.45	0.0344	significant
Pure Error	2.80	4	0.70			

Table 8: Results of Regression Analysis of D6

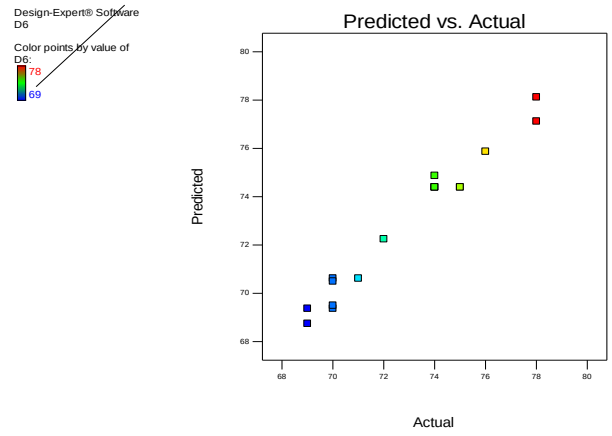
	Sum of		Mean	F	p-value	
Source	Squares	df	Square	Value	Prob > F	
Model	139.31	9	15.48	24.35	0.0002	significant
A-Eudragit RSPO	3.13	1	3.13	4.92	0.0621	
B-Eudragit RLPO	0.000	1	0.000	0.000	1.0000	
C-HPMC	10.12	1	10.12	15.93	0.0053	
AB	0.000	1	0.000	0.000	1.0000	
AC	0.25	1	0.25	0.39	0.5505	
BC	1.00	1	1.00	1.57	0.2500	
A^2	119.39	1	119.39	187.81	< 0.0001	
B^2	3.60	1	3.60	5.67	0.0488	
C^2	5.81	1	5.81	9.14	0.0193	
Residual	4.45	7	0.64			
Lack of Fit	3.25	3	1.08	3.61	0.0034	significant
Pure Error	1.20	4	0.30			

Table 9: Results of Regression Analysis of D12

	Sum of		Mean	F	p-value	
Source	Squares	df	Square	Value	Prob > F	
Model	184.04	9	20.45	10.08	0.0030	significant
A-Eudragit RSPO	40.50	1	40.50	19.96	0.0029	
B-Eudragit RLPO	8.00	1	8.00	3.94	0.0874	
C-HPMC	0.50	1	0.50	0.25	0.0348	
AB	0.000	1	0.000	0.000	1.0000	
AC	0.000	1	0.000	0.000	1.0000	
BC	1.00	1	1.00	0.49	0.0053	
A^2	83.38	1	83.38	41.10	0.0004	
B^2	53.06	1	53.06	26.16	0.0014	
C^2	4.64	1	4.64	2.29	0.0241	
Residual	14.20	7	2.03			
Lack of Fit	1.00	3	0.33	0.10	0.0453	significant
Pure Error	13.20	4	3.30			

The polynomial equations can be used to draw conclusions after considering the magnitude of coefficient and the mathematical sign it carries, i.e. positive or negative. The coefficient obtained shows that coefficient A, B,C,AB,AC,BC,A^2,B^2 were positive (Table 8). The positive sign for A^2 indicated that as the concentration of Eudragit RSPO increased, dissolution rate decreased. This is due to water uptake and swelling property of Eudragit RSPO. The positive sign for B^2 indicated that as the Eudragit RLPO concentration increased, dissolution also increased. Eudragit RLPO is the hydrophobic nature, hence reduces surface area and decrease solubilization time. The effect of Eudragit RLPO on dissolution rate at 6th hours depends on its swelling property. The magnitude of coefficient showed that R2 has more effect than R1 on dissolution rate. The response surface plot and contour plot of effect of Eudragit concentration on dissolution rate at 6th hours.

This is a plot of the residuals versus the experimental run order. A random scatter data shows no lurking variability during experiment (Fig 6).



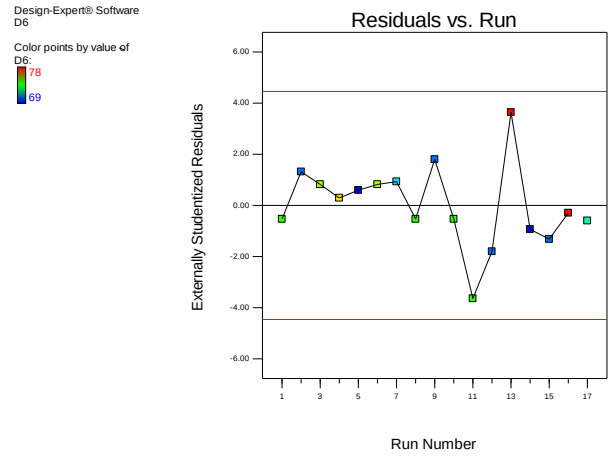
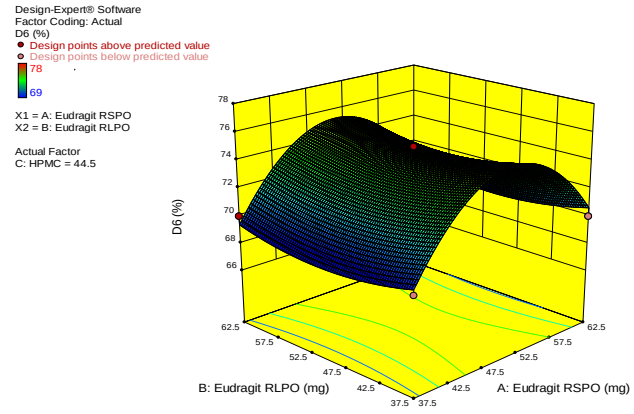


Figure 6a: Residuals vs. Run plot for dissolution rate at 6th hours. b)Predicted vs. actual plot for dissolution rate at 6th hours

The data points should be split evenly by the 45 degree line. So there are no values, which are not easily predicted by the model (Fig 7).

This plot helps the experimenter detect outliers in the data. There are no data points that are outside the red lines. So, there are no data points that are not fit well by the current model (Fig 8).



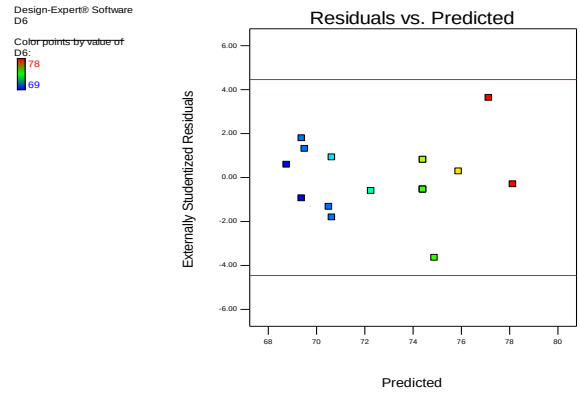


Figure 7a: Externally studentized residuals plot for dissolution rate at 6th hours. b) Response surface plot showing effect of Eudragit RSPO and RLPO on dissolution rate at 6th hour.

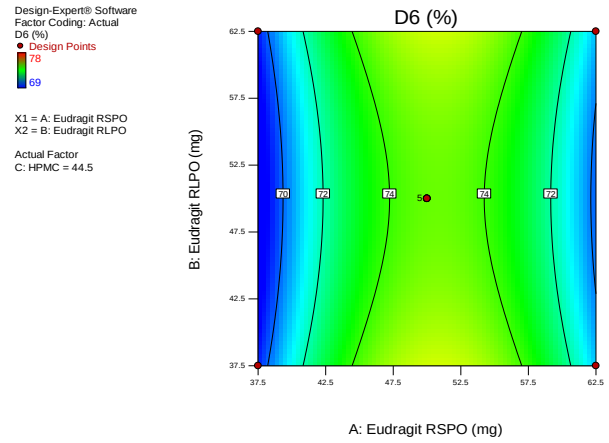


Figure 8: Contour plot showing effect of Eudragit RSPO and RLPO on dissolution rate at 6th hours

Effect of sustained release polymers concentration on dissolution rat at 12th hour:

At the low concentration of variable of A, the responses D12 showed 85 to 89% and at the high concentration it showed 81 to 84%. The factorial equation for dissolution at 12 hour exhibited a good correlation coefficient (0.9448) and the Model F value of 7.45which implies the model is significant. Values of "Prob> F" less than 0.0500 indicate model terms are significant (Table 9).

The polynomial equations can be used to draw conclusions after considering the magnitude of coefficient and the mathematical sign it carries, i.e. positive or negative. The coefficient obtained shows that coefficient A, B,C,AB,AC,BC,A^2,B^2 and C^2 were positive (Table 44). The positive sign for A^2 indicated that as the concentration of Eudragit RSPO increase, retardation of dissolution rate increase. This is due to water uptake and swelling property of Eudragit RSPO. The positive sign for B^2 indicated that as the Eudragit RLPO concentration increased, retardation of dissolution also increased. Eudragit RLPO is the hydrophobic nature, hence reduces surface area and decrease solubilization time. The effect of Eudragit RLPO on dissolution rate at 12th hours depends on its swelling property. The C^2 concentration insignificantly affecting rate of retardation of dissolution rate of sustained release tablets. The magnitude of coefficient showed that R3 has more effect than R1 & R2 on dissolution rate. The response surface plot and contour plot of effect of Eudragit concentration on dissolution rate at 12 th hours.

This is a plot of the residuals versus the experimental run order. A random scatter data shows no lurking variability during experiment (fig 9).

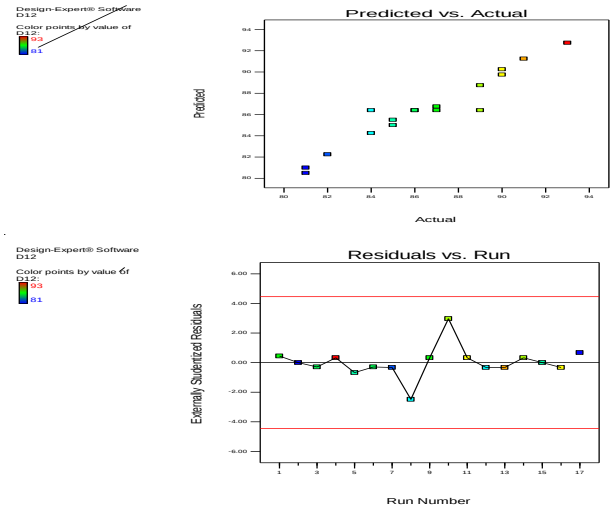


Figure 9a: Residuals vs. Run plot for dissolution rate at 12th hours. b)Predicted vs. actual plot for dissolution rate at 12th hours

The data points should be split evenly by the 45 degree line. So there are no values, which are not easily predicted by the model (Fig 10).

This plot helps the experimenter detect outliers in the data. There are no data points that are outside the red lines. So, there are no data points that are not fit well by the current model (Fig 11).

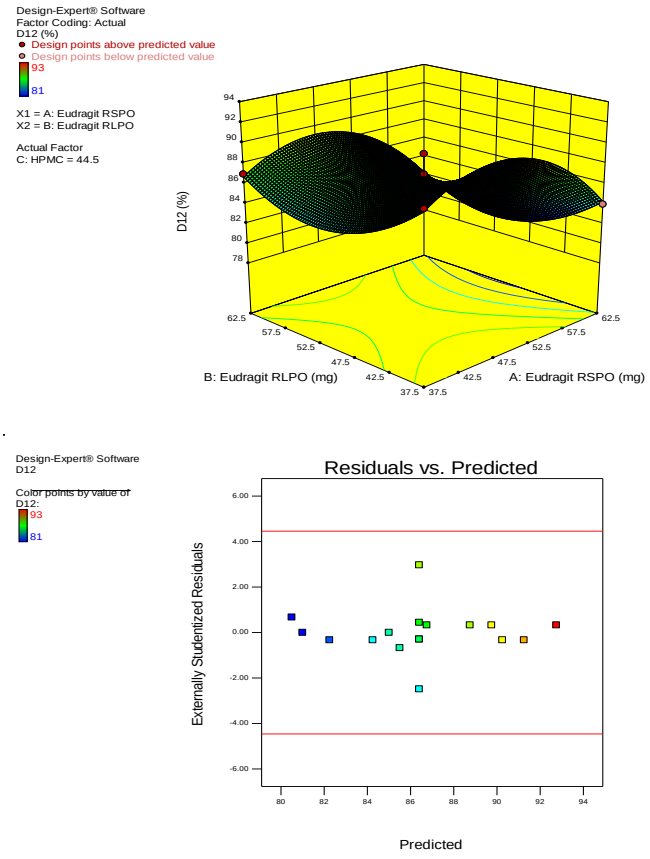


Figure 10a: Externally studentized residuals plot for dissolution rate at 12th hours. b)Response surface plot showing effect of Eudragit RSPO and RLPO on dissolution rate at 12th hours

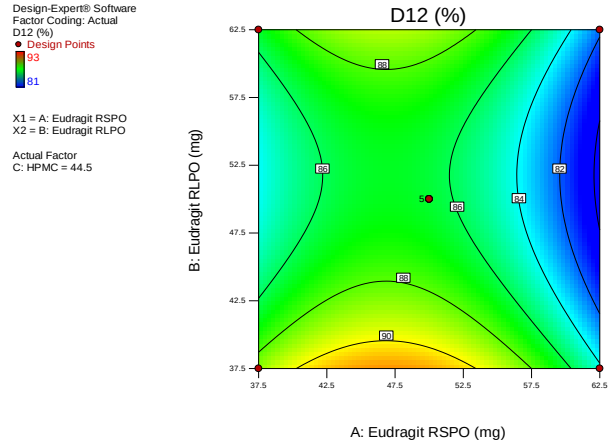


Figure 11. Contour plot showing effect of Eudragit RSPO and RLPO on dissolution rate at 12 th hours

Effect of sustained release polymers concentration on time of 50% drug release:

At the low concentration of variable of A, the responses T50 showed 2.71 to 2.8 hours. At the high concentration showed 2.93 to 3.1 hours. The factorial equation for dissolution of 50% of drug release exhibited a good correlation coefficient (0.9448) and the Model F value of 7.45which implies the model is significant. Values of "Prob> F" less than 0.0500 indicate model terms are significant (Table 10).

Table 10: Results of Regression Analysis of T50

	Sum of		Mean	F	p-value	
Source	Squares	df	Square	Value	Prob > F	
Model	1.34	9	0.15	5.40	0.0185	significant
A-Eudragit RSPO	0.15	1	0.15	5.30	0.0549	
B-Eudragit RLPO	9.180E-003	1	9.180E-003	0.33	0.0517	
C-HPMC	0.050	1	0.050	1.81	0.2207	
AB	1.600E-003	1	1.600E-003	0.058	0.0164	
AC	1.600E-003	1	1.600E-003	0.058	0.0164	
BC	0.46	1	0.46	16.57	0.0047	
A^2	0.62	1	0.62	22.36	0.0021	
B^2	0.015	1	0.015	0.54	0.0449	
C^2	0.015	1	0.015	0.54	0.0449	
Residual	0.19	7	0.028			
Lack of Fit	0.16	3	0.055	7.85	0.0376	significant
Pure Error	0.028	4	6.995E-003			

Table 11: Optimized formula and observed values

Confirmation Report

Factor	Name	Level	Low Level
A	Eudragit RSPO	50.00	37.50
B	Eudragit RLPO	37.50	37.50
C	HPMC	60.00	29.00

Response	Predicted	Observed
D1	32.8015	32.21
D6	77.125	78.43
D12	91.25	90.64
T50	2.945	2.884

The polynomial equations can be used to draw conclusions after considering the magnitude of coefficient and the mathematical sign it carries, i.e. positive or negative. The coefficient obtained shows that coefficient A, C, BC,A^2,B^2 and C^2 were positive (Table 45). B, AB and AC showed negative response. The positive sign for A^2 indicated that as the concentration of Eudragit RSPO increase, retardation of dissolution rate increase and reduce the drug leach from the matrix of tablets. This is due to water uptake and swelling property of Eudragit RSPO. The positive sign for B indicated that as the Eudragit RLPO concentration increased, retardation of dissolution also decreased and increase the T50. Eudragit RLPO is the hydrophobic nature, hence reduces surface area and decrease solubilization time. The effect of Eudragit RLPO on 50% drug leached depends on its swelling property. The C^2 concentration insignificantly affecting rate of retardation of dissolution rate of sustained release tablets. The magnitude of coefficient showed that R2 has more effect than R1 & R3 on dissolution rate. The response surface plot and contour plot of effect of Eudragit concentration on T50.

This is a plot of the residuals versus the experimental run order. A random scatter data shows no lurking variability during experiment (Fig 12).

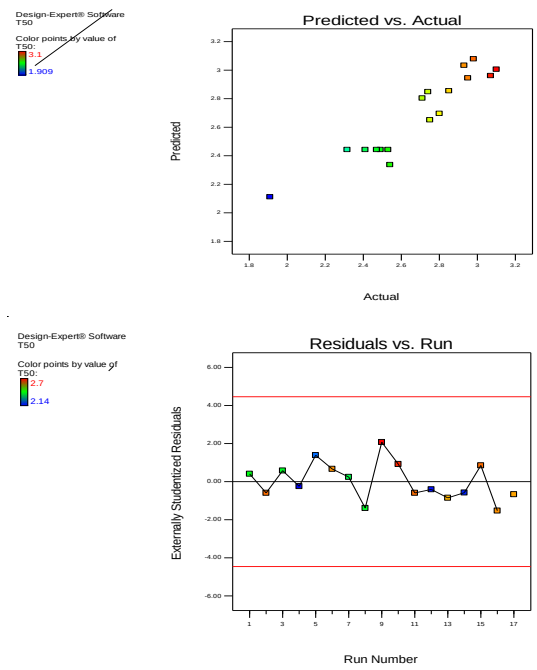


Figure 12a: Residuals vs. Run plot for T50. b)Predicted vs. actual plot for T50

The data points should be split evenly by the 45 degree line. So there are no values, which are not easily predicted by the model (Fig 13).

This plot helps the experimenter detect outliers in the data. There are no data points that are outside the red lines. So, there are no data points that are not fit well by the current model (Fig 14).

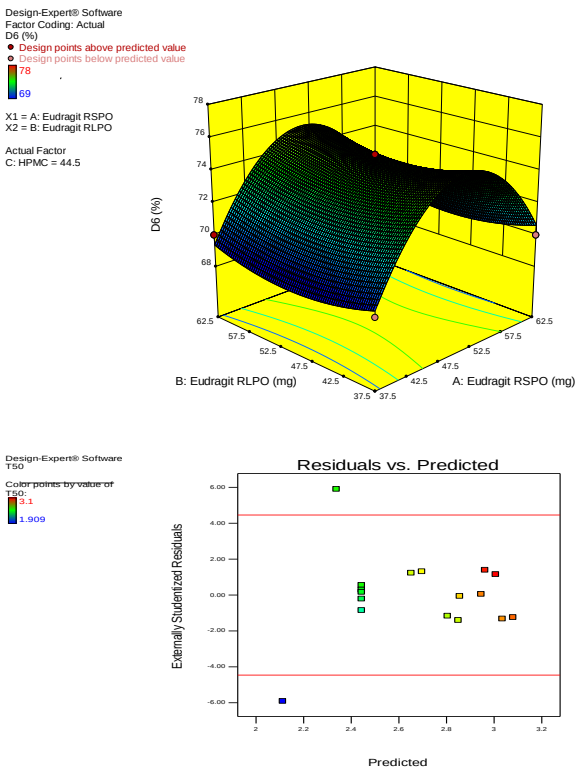


Figure 13a: Externally studentized residuals plot for T50. b)Response surface plot showing effect of Eudragit RSPO and RLPO on T50.

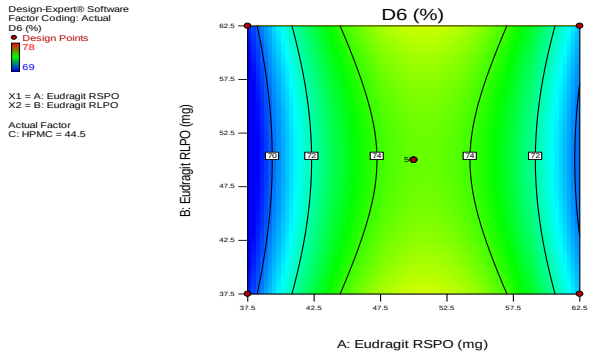


Figure 14: Contour plot showing effect of Eudragit RSPO and RLPO on T50

After generating the polynomial equations relating the dependent and independent variables, the process was optimized for the responses. Numerical optimization using the desirability approach was employed to locate the optimal settings of the process variables to obtain the desired responses. Optimized conditions were obtained by setting constraints on the dependent and independent variables. Optimization was performed to obtain the levels of A-C which maximize or minimize the results. The optimized levels and predicted values of R1, R2, R3 and R4 are shown in Table 10.

Based on the factorial design studies, the optimized formulation of Dalfampridine sustained release tablets was founded. The optimized Dalfampridine sustained release tablets formulation shows in table 46. The optimized formula showed better D1, D6, D12 and T50 i.e.32.8% and 77.12%, 91.25% & 2.94 hours respectively. To verify these values, three batches of Dalfampridine sustained release tablets were prepared according to the predicted level of A, B and C. obtained R1, R2, R3 & R4 values were in a closed agreement with the predicted values as shown in table 11. This demonstrated the reliability of optimized procedure in predicting the operating parameters for the preparation of Dalfampridine sustained release tablets. The optimized formulation was observed dissolution parameters D1, D6, D12 and T50 i.e.32.21% and 78.43%, 90.64% & 2.884 hours respectively. From the observed results, the values were subjected to factorial design of 17 formulations. The observed values are very closely matches with formulation 13.

Pharmacokinetics and Bioavailability parameters:

The plasma concentration of Dalfampridine and time was plotted in trapezoidal method and pharmacokinetic parameters were calculated [7]. The AUG,AUMC Cmax, Tmax, MRT, T1/2 and clearance values of oral administered Dalfampridine were 17.2 µIU/mL/h, 655.0

μIU/mL/h, 12.22 μIU/Ml, 2 h, 8.7 h, 0.1328 μg/ml/h respectively. In the case of Dalfampridine AUG, AUMC, Cmax, Tmax, MRT, T1/2 and clearance were 532 μIU/mL/h,17116 μIU/mL/h, 16.14 μIU/mL, 4 h, 32.1 h, 0.0187 μg/ml/h respectively**.** The values were significantly compared with oral Dalfampridine administration. Measurable concentration of Dalfampridine was observed immediately after administration and relatively steady plasma Dalfampridine concentration over 12 h [8]. The relative bioavailability of Dalfampridine SR tablets was 30.9, when compared with oral administration. Therefore Dalfampridine SR tablets maintained 31 fold more bioavailability, compared with conventional tablets.

CONCLUSION

The present study was focused on the formulation and optimization of the Dalfampridine sustained release tablets to improve the versatility & patient compliance. The sustained release tablets were formulated by direct compression and the formulations optimized using the Design Expert Software. Factorial design was the best tool to optimize the formulations.

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