Available online at www.ijpsdronline.com
International Journal of Pharmaceutical Sciences and Drug Research 2011; 3(1): 13-18

Research Article ISSN 0975-248X

Optimization and Validation of Modulated Release Formulation of Ranitidine HCl by Response Surface Methodology

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ABSTRACT
The objective of the present study was, 1) to systematically device a model of factors that would yield an optimized sustained release dosage form of model drug (Ranitidine HCl), 2) to validate the models using R^2 values, 3) to optimize the formulation by response surface methodology (RSM). A three - factor, three - level Box-Behnken design was used for the optimization procedure, with the amounts of HPMC K100M (X_1), MCC (X_2) and Compression Force (X_3) as independent variables. Three dependent variables were considered: percentage of drug release at 1 h, 12 h and $T_{50\%}$. The regression equation obtained from experiment i. e $Y_2 = 92.41 + 3.18X_1 + 2.05\ X_2 + 2.14X_3 + 2.41X_1X_2 + 0.24\ X_1X_3 + 0.11\ X_2X_3 - 3.82X_1^2 - 2.59X_2^2 - 0.46X_3^2$, explained the main and interaction effects of factors that influenced the drug release. Optimization was performed by maximizing the drug release in 12 hrs and placing constraints on Y_1, Y_2 and Y_3. Validation of optimization by carrying out by performing 8 experimental runs showed high degree of prognostic ability of response surface methodology. The results showed that the optimized formulation provided a dissolution pattern similar to the predicted curve, which indicated that the optimal formulation could be obtained using RSM. A simple high performance liquid chromatography method was developed and the dissolution samples were analysed by this procedure.

Keywords: Optimization, sustained release, Ranitidine HCl, Response surface methodology (RSM), Validation.

INTRODUCTION

In the past few years, modulated release systems have become increasingly important, because these systems can maintain the pharmacologic effect for an appropriate extended time. Hydrophilic gel forming matrix tablets are extensively used for an oral extended release dosage forms due to their simplicity, cost effectiveness and reduction of risk of toxicity due to dose dumping. [1-4] In the development of an extended release dosage form an important issue was to design an optimized formulation with minimum number of trials in short time. For this a computer optimization technique, based on response surface methodology (RSM) utilizing a polynomial equation has been widely used. Many statistical experimental designs have been recognized as useful techniques to optimize process variables. RSM is widely used when only a few significant factors are involved in optimization. Various types of RSM designs include 3^2 full factorial designs, central composite design [5-6] and Box-Behnken design. [7] Box - Behnken design is an independent, rotatable or nearly rotatable quadratic design (contains no embedded factorial or fractional factorial design in which the treatment combinations are at the midpoints of the edges of the process space and at the center. A three factor, three level designs would require a total of 27 runs without any repetitions and 30 runs with 3 repetitions. Box-Behnken design requires fewer runs (15 runs) in a three factor experimental design. Hence this design was used to optimize Ranitidine hydrochloride extended release tablets.

Ranitidine hydrochloride (RHCl) is a hydrophilic H_2-receptor antagonist. It is widely prescribed in active duodenal ulcers, gastric ulcers, Zollinger-Ellison syndrome, gastro-esophageal reflux disease, and erosive- esophagitis. The maintenance of uniform plasma levels of a cardiovascular drug is important in ensuring the desired therapeutic response. The half life of R HCl is 2.5 - 3 hours and multiple doses are required to maintain uniform plasma levels to elicit a good therapeutic response. [8]

The current study aimed at developing and optimizing an oral sustained release dosage form of RHCl using computer aided optimization technique i.e. Box-Behnken statistical design with constraints on cumulative percentage of drug release after 1 h (Y_1, NMT 30%) and 12 h (Y_2, NLT 85%). The independent variables chosen for the present study were: amount of release retardant polymers – HPMC K100M (X_1), MCC (X_2), compression force (X_3). The dependent variables

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studied were cumulative percentage of drug release after 1 h (Y_1) and 12 h (Y_2); time required for 50% dissolution –$T_{50\%}$ (Y_3).

MATERIALS AND METHODS

Ranitidine HCl was obtained as a gift sample from Albert-David Limited (Kolkata, India). Other materials used were Potassium dihydrogen phosphate, Acetonitrile (Merck Ltd, Mumbai), HPMC K100M, MCC (Stadmed private limited, Kolkata, India). Talc, magnesium stearate and dicalcium phosphate, (Loba chemicals, Mumbai). All other chemicals used were of analytical grade throughout the analysis.

Preparation of matrix tablets

The drug and polymer were sifted through #22 meshes and mixed well to ensure the uniformity of the premix blend. The premix blends was then mixed with MCC and were lubricated with talc and magnesium stearate. The tablets were prepared by directly compressing the mass at an average weight of 600 mg on a 10 station Lab Press compression machine (Cip machineries Pvt. Ltd., Ahmedabad) using 11.9 mm circular, concave punches. Various formulations of Ranitidine HCl sustained release matrix tablets were prepared using the following excipients. HPMCK-100 M, MCC, dicalcium phosphate, talc, and magnesium stearate.

Experimental design

A three factor, three levels Box-Behnken design was used for the optimization procedure. The design consists of a replicated center points and a set of points lying at the midpoint of each edge of the multidimensional cube that defines the region of interest. The non linear computer generated quadratic model is given as:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + E \quad (1)$$

Where y is the measured response associated with each factor level combination; b_0 is an intercept; b_1 to b_{33} are regression coefficients computed from the observed experimental values of Y; and X_1, X_2 and X_3 are the coded levels of independent variables and E is the error term. The independent and dependent variables used in the design are listed in Table 1. A total of 15 runs with triplicate center points are given in Table 2 along with the observed responses and other release parameters.

Table 1: Variables in Box-Behnken design

Factor	Levels used (coded)		
	Low	medium	High
X_1 = HPMC K4M (%)	20	30	40
X_2 = PVPK-90 (%)	5	10	15
X_3 = Compression (Tons)	1	3	5
Response			Constraints
Y_1 = Cumulative % drug released in 1 h			20 – 30%
Y_2 = Cumulative % drug released in 12 h			> 85%
Y_3 = Time for 50 % dissolution ($T_{50\%}$)			> 4 hrs

Tablet physical evaluation

Tablets were also evaluated for their hardness (n=6) (Monsanto hardness tester), friability (n=6) (Roche friabilator, 100 rpm), weight variation (n = 20) and thickness (n = 10) (Mitutoyo digital vernier caliper).

Determination of release profiles

An automated tablet dissolution tester (USP XXIII), with a basket speed of 100 rpm and 900 ml of simulated gastric fluid without enzymes as the dissolution medium at 37ºC was employed. Samples were withdrawn at different time points (1, 2, 4, 8, and 12 h), suitably diluted and assayed by High performance liquid chromatography (HPLC) using UV detector at 320 nm. Samples were filtered using 0.45 µm Millipore filter. The dissolution experiments were carried out in triplicate. The cumulative percent drug release was calculated for the formulations and the drug release data was curve fitted to various kinetic models to study the mechanism of drug release from the matrices.

HPLC analysis

The HPLC apparatus (Knauer, Germany) adjusted with HPLC pump (Knauer 1000), Rheodyne injector (D-14163 Berlin), UV detector (Knauer 2500) and EZChrom (version 3.1.6) software. Reverse phase-HPLC analysis was performed isocratically at room temperature using a cyano, 250 × 4.6mm, 5µ particle size stainless steel column. A mixture of dihydrogen phosphate buffer and acetonitrile in the ratio of 50:50 (v/v) was used as mobile phase. The mobile phase was filtered through 0.45 µm membrane filter. The eluent was monitored with a UV detector set at 320 nm at a flow rate of 1.0 ml min^{-1} and a sample size of 50 µl was injected through the Rheodyne injector.

Statistical analysis and Optimization

The application of mathematical optimization in the pharmaceutical field was first reported by Fonner *et al*. Later developments in the computer science have enabled the incorporation of the optimization algorithm into the experimental design software. For this research article, Design- Expert Trial version 7.1.1 software (Stat - Ease Inc. Minneapolis) was used for optimization.

Validation of optimization model

Statistical validity of the polynomials was established on the basis of ANOVA provision in the Design Expert Software. Subsequently, the feasibility and grid searches were performed to locate the composition of optimum formulations. The 3-D response surface plots were drawn using this software. Eight optimum check points were selected by intensive grid search performed over the entire experimental domain to validate the chosen experimental design and polynomial equations. The formulations corresponding to these check points were prepared and evaluated for various response properties. The resultant experimental data of response properties were compared with that of the predicted values. Linear regression plots between the observed and predicted values of the response properties were drawn.

RESULTS AND DISCUSSION

Drug content and physical evaluation

Evaluation of the matrix tablets yielded a drug content ranging from 98.22 to104.13% of the desired amount, which justifies an even quantity of drug in all formulations. The homogeneity of the drug in the physical mixtures allows the preparation of tablets with uniform weight. The weight of the tablets ranged between 588.40 mg to 615.80 mg. The hardness of the different formulations studied was in the range of 5 - 7 Kg/cm^2. The thickness of the tablets was found in the range of 4.78 mm to 5.36 mm. The tablets also passed the friability test (F < 1%), showing that all the formulations lie within the limits.

Data fitting to the model and ANOVA

For the response surface methodology based on Box-Behnken design, 15 experiments were required. The experimental runs and the observed responses for the 15 formulations are given in Table 2. Based on the experimental design, the factor combinations resulted in different release

rates. The range of responses Y_1, the cumulative % drug released after 1 h was 45.83% in formulation No. 13 (maximum) and 23.51 % in formulation No 6. Similarly, the response Y_2 was maximum in formulation No. 13 and minimum in formulation No. 15.

Mathematical relationship in the form of polynomial equations for the measured responses obtained with the statistical package Design Expert version 7.1.1 are listed in Table 3. These equations represent the quantitative effect of variables (X_1, X_2, X_3) and their interactions on the response Y_2. Coefficients with more than one factor term and those with higher order terms represent interaction terms and quadratic relationships respectively. A positive sign represents a synergistic effect, while a negative sign indicates an antagonistic effect. The values of $X_1 – X_3$ were substituted in the equation to obtain the theoretical values of Y_2. The predicted and the experimental values were in reasonably good agreement.

ANOVA was performed to estimate the significance of the model. At 5% level of significance, a model is considered significant if the p-value is less than 0.05. The ANOVA analysis for all the three responses is shown in Table 3. ANOVA analysis of Y_1 showed that coefficients b1 and b3 had significant effect with F value of 12.07 (p = 0.0052) and 19.01 (p = 0.0011) respectively. For Y_2 and Y_3, the main coefficients b_1, b_2, b_3 and interaction coefficients b_1^2, b_2^2 had significant effect with p value less than 0.05. It was observed that increase in the polymer concentration of HPMC K100M increased the $T_{50\%}$ due to more retarded release of the drug.

Table 2: Observed responses in Box – Behnken design and release parameters

Run	Independent factors			Response			Release parameters		
	X_1	X_2	X_3	Y_1	Y_2	Y_3	n	K_H	R^2
1	0	-1	1	24.56	88.56	3.9	0.4893	21.34	0.9803
2	1	0	-1	34.61	88.64	3.9	0.4821	22.34	0.9831
3	0	0	0	25.86	92.11	5.1	0.4984	25.92	0.9861
4	-1	0	1	27.44	87.15	3.6	0.4633	23.82	0.9752
5	1	1	0	32.56	93.15	4.8	0.4264	24.38	0.9889
6	1	0	1	23.51	95.61	5.0	0.5341	27.03	0.9658
7	0	1	-1	35.16	89.96	4.1	0.5394	22.63	0.9821
8	0	1	1	28.64	94.95	5.2	0.4916	25.84	0.9873
9	-1	-1	0	39.46	94.57	3.4	0.5333	19.37	0.9511
10	-1	1	0	40.56	100.89	3.0	0.4892	21.11	0.9362
11	0	0	0	29.98	90.57	4.3	0.4834	25.71	0.9889
12	1	-1	0	27.71	86.61	3.5	0.5551	23.29	0.9732
13	-1	0	-1	45.83	101.14	3.2	0.4806	17.41	0.9884
14	0	0	0	29.45	94.56	5.2	0.4865	24.04	0.9888
15	0	-1	-1	33.55	87.89	3.1	0.5041	25.13	0.9638

Table 3: ANOVA summary of all responses (Y_1, Y_2, Y_3)

Source	Y_1 (Linear)		Y_2 (Quadratic)		Y_3 (Quadratic)	
	F value	p -value	F value	p -value	F value	p -value
Model	10.85	0.0013	11.23	0.0080	10.09	0.0102
X_1	12.07	0.0052	32.96	0.0032	23.56	0.0047
X_2	1.48	0.248	13.72	0.0139	9.77	0.0261
X_3	19.01	0.0011	14.97	0.0118	10.52	0.0228
$X_1.X_2$	-	-	9.44	0.0277	6.31	0.0537
$X_1.X_3$	-	-	0.094	0.7717	1.01	0.3612
$X_2.X_3$	-	-	0.019	0.8962	0.32	0.5938
X_1^2	-	-	21.93	0.0054	23.60	0.0046
X_2^2	-	-	10.06	0.0248	15.92	0.0104
X_3^2	-	-	0.32	0.5948	4.93	0.0771

Regression equations of the fitted model

$Y_1 = 32.184 – 4.237.X_1 + 1.322.X_2 – 5.632.X_3$

$Y_2 = 92.41 + 3.18.X_1 + 2.05.X_2 + 2.14.X_3 + 2.41.X_1X_2 + 0.24.X_1X_3 + 0.11.X_2X_3 – 3.82.X_1^2 – 2.59X_2^2 – 0.46X_3^2$

$Y_3 = 5.00 + 0.51.X_1 + 0.33.X_2 + 0.34.X_3 + 0.38.X_1X_2 + 0.15.X_2X_3 + 0.0085.X_1X_3 - 0.75.X_1^2 – 0.62.X_2^2 – 0.34.X_3^2$

Standardized main effects and reliability of the models

Table 4 shows the standardized main effects (SME) obtained by dividing the main effects with the standard error of the main effects. [9-11] Factor X_1 showed a larger SME value of 5.78 indicating the significant effect of HPMC K100M on drug release. Factors X_2 and X_3 showed almost same effect on % release at 12 h and $T_{50\%}$ as observed from their SME values. The reliability of the model was further supported by high R^2 values. Also the p – values of lack of fit (0.3249, 0.8012, 0.9860) above 0.05 also justifies the reliability of the model because for a particular model, p value for lack of fit should be non significant.

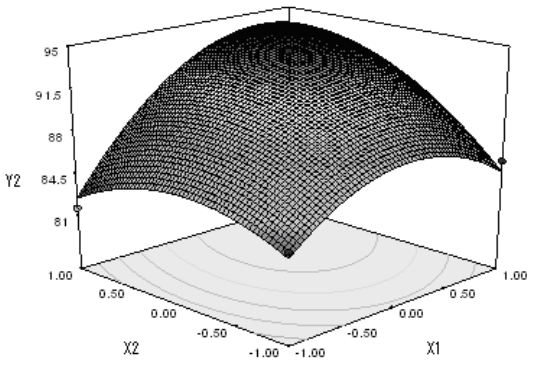
Table 4: Standardized main effects of the factors on the responses

Factor	Standardized main effects (SME)		
	Y_1 (Linear model)	Y_2 (Quadratic model)	Y_3 (Quadratic model)
X_1	- 3.18	5.78	4.63
X_2	0.99	3.72	3.0
X_3	- 4.23	3.89	3.09
$X_1.X_2$	-	3.08	2.53
$X_1.X_3$	-	0.30	1.0
$X_2.X_3$	-	0.14	0.56
X_1^2	-	- 4.65	4.68
X_2^2	-	- 3.15	- 3.87
X_3^2	-	-0.56	- 2.125
R^2	93.14%	95.29%	94.78%
p – Value of lack of fit	0.3249	0.8012	0.9860

Response surface analysis

Contour plots (Fig. 1B, 2B, 3B) are two dimensional representations of the responses for the selected factors. Three dimensional (3-D) surface plots (Fig. 1A, 2A, 3A) for the obtained responses were drawn based on the model polynomial functions to assess the change of the response surface. These plots explain the relationship between the dependent and independent variables. Response surface plots for the responses Y_2 are given in Fig. 1-3 along with their corresponding contour plots (Fig. 1B, 2B, 3B).

Fig. 1A shows the 3-D plot of the effect of factors X_1 and X_2 on the response Y_2. At the lowest level of X_1 and X_2, Y_1 was 34.51 and Y_2 was 82.68. The decrease in % drug release was polymer concentration dependent. The % release at 12 hrs (Y_2) obtained was 93.91 when X_1 was 0.28 and X_2 was 0.48. Fig. 2 explains the effect of factors X_1, X_3 on the response Y_2. At a level of 0.70:-1.00 for X1, X3, the % release at 12 hrs was 90.17. Fig. 3 explains the effect of factors X_2, X_3 on the response Y_2. At the lowest levels of both X_2 and X_3, the % release at 12 hrs was 85.31%. The % release (Y_2) was 90.09% when CF was 0.62 and X_2 was kept minimum.



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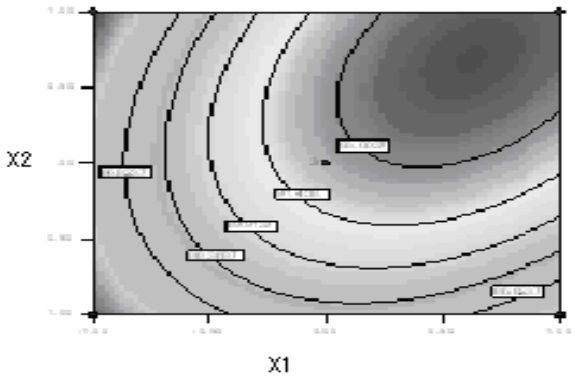
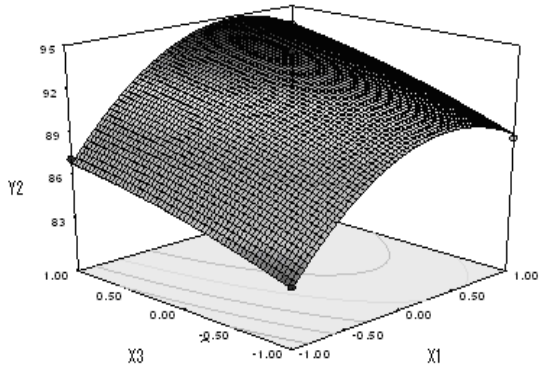


B)

Fig. 1: A) Response surface plot and B) Contour plot showing the effect of HPMC K100M (X_1) and MCC (X_2) on response Y_2



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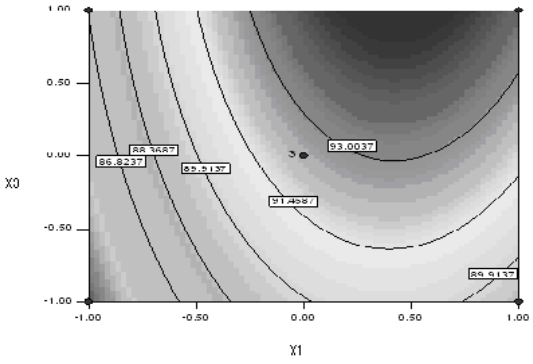

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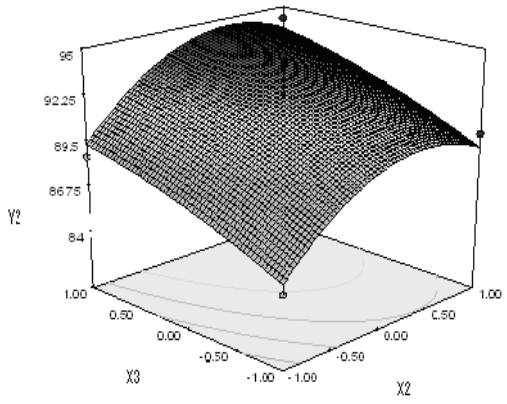
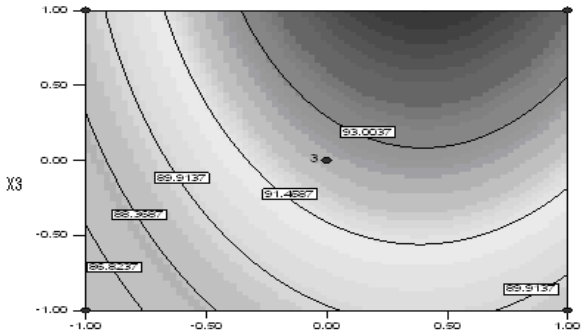
Fig. 2: A) Response surface plot and B) Contour plot showing the effect of HPMC K100M (X_1) and Compression Force (X_3) on response Y_2.



A)



B)

Fig. 3: A) Response surface plot and B) Contour plot showing the effect of MCC (X_2) and Compression Force (X_3) on response Y_2.

Optimization

After generating the model polynomial equations to relate the dependent and independent variables, the process was optimized for all three responses. Optimum formulation was selected based on the constraints set on independent variables: Y_1 (20 – 30%), Y_2 (85 – 100%), Y_3 (> 4hrs). The final optimal experimental parameters were calculated using the extensive grid search and feasibility search provided in the Design Expert software. From the various solutions provided by the software, the formulation containing 189 mg of HPMC K100M, 50.4 mg of MCC and 4.06 tons of Compression force was found to fulfill the maximum requisite of an optimum formulation because of the better regulation between the initial release after 1 h and release at the end of 12 h. The release profile of the optimized formulation is shown in Fig. 4.

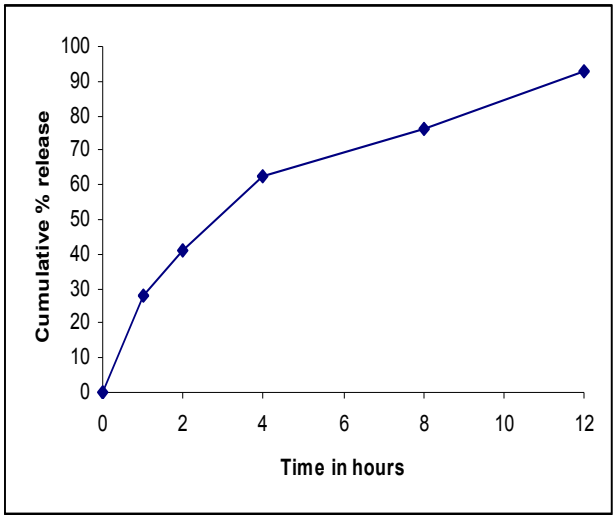


Fig. 4: Dissolution profile of the optimized formulation

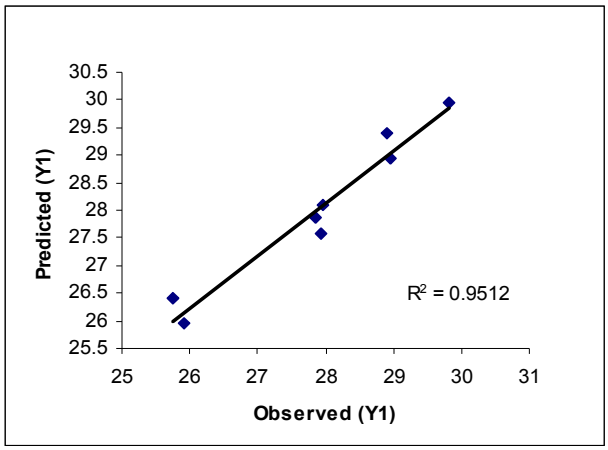
Validation of the RSM results

Eight check point formulations were selected, for which the results of all the dependent variables were found to be within the limits. Table 5 lists the obtained and predicted values of the check point formulations along with the % prediction error. Linearity correlation plots between the observed experimental values and the predicted values are shown in Fig. 5 (A, C, E). The residual plots showing the scatter of the residual values versus the actual values are shown in Fig. 5

(B, D, F). High R^2 values of 0.9171 to 0.9613 explain the linearity between the observed and the predicted values. The low % prediction error of -0.042 to 2.34 indicate the high prognostic ability of RSM.

Table 5: Composition of optimum checkpoint formulations, the predicted and experimental values of response variables and percentage prediction error

Formulation composition (X_1 : X_2: X_3)	Response variable	Experimental value	Predicted value	% Error
	Y_1	28.96	28.93	0.103
196.2:51.9:3.54	Y_2	92.29	92.63	-0.752
	Y_3	4.92	4.99	-1.402
	Y_1	27.95	27.99	-0.533
180 : 90 : 5	Y_2	93.71	93.67	0.042
	Y_3	4.81	4.79	2.340
	Y_1	28.9	28.95	-1.734
217.8:44.4:4.8	Y_2	90.14	90.13	0.011
	Y_3	4.59	4.52	1.548
	Y_1	27.93	27.90	1.269
206.4:79.8:4.12	Y_2	95.12	95.15	-0.042
	Y_3	5.21	5.29	-1.512
	Y_1	25.91	25.94	-0.115
206.4: 40.2:4.12	Y_2	91.15	91.19	-0.902
	Y_3	4.56	4.62	-1.298
	Y_1	27.84	27.87	-0.107
189: 50.4:4.06	Y_2	92.83	92.78	0.053
	Y_3	4.99	4.95	-2.156
	Y_1	29.81	29.85	-0.467
182.04:65.1:3.34	Y_2	93.86	93.82	0.042
	Y_3	5.27	5.20	1.151
	Y_1	25.75	25.77	-2.499
193.8:66.9:5	Y_2	95.1	95.16	-0.073
	Y_3	5.09	5.19	-2.115



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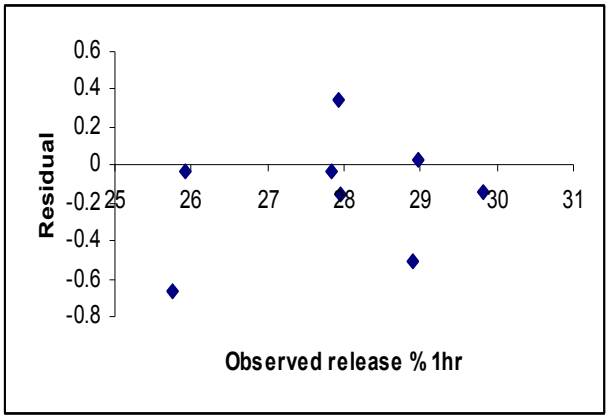

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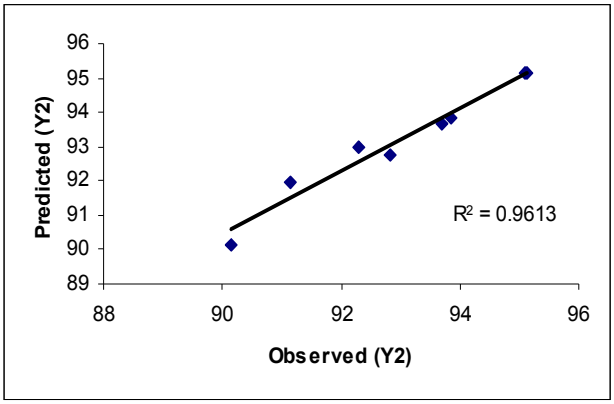

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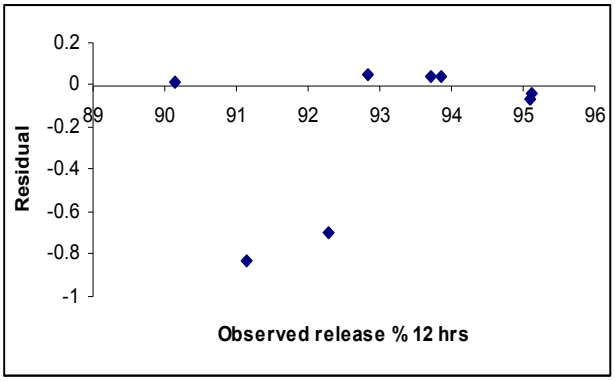

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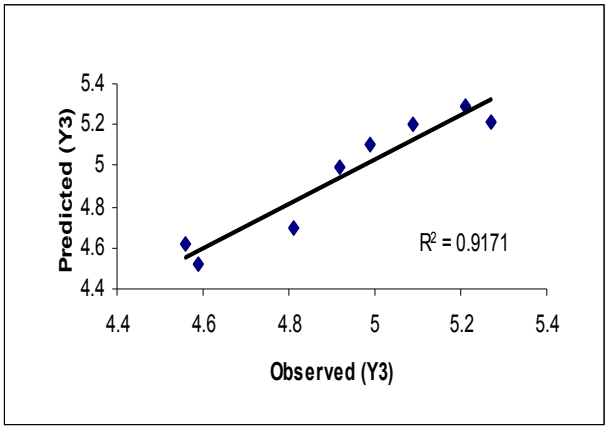

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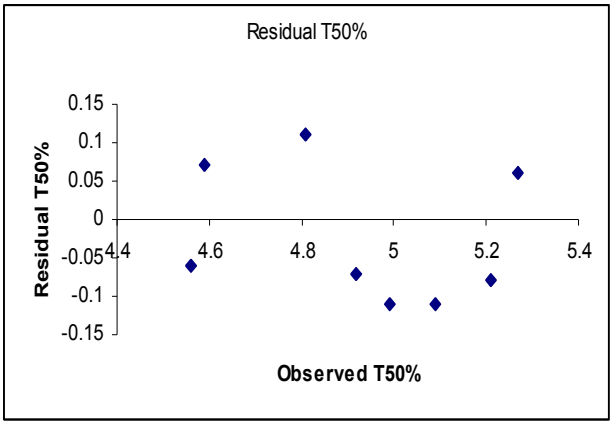

F)

Fig. 5: Linear correlation plots (A, C, E) between observed and the predicted values and corresponding residual plots (B, D, F)

HPLC analysis

The representative HPLC chromatogram obtained after the analysis of the dissolution sample is shown in Figure 6. Under the described chromatographic conditions, RHCl was eluted at a run time of 6.25 minutes. The response obtained in the HPLC system was good and was possible to analyze all the dissolution samples collected at various time points. The HPLC method described is very simple, sensitive and reproducible.

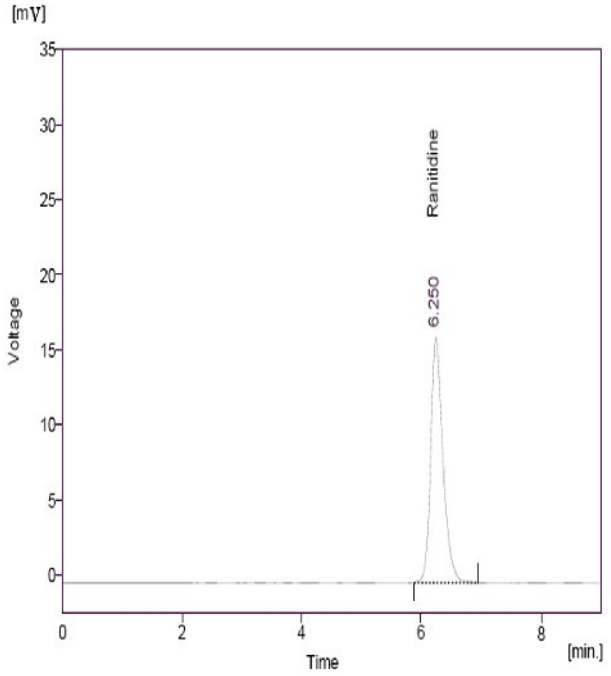

Fig. 6: Representative HPLC chromatogram showing Ranitidine hydrochloride obtained after analysis of dissolution samples

It was concluded that an appropriate statistical design and optimization technique can be successfully used in the development of sustained release tablets of RHCl with predictable drug release properties. Response surface methodology optimization enabled formulation of HPMC matrix tablets with desired RHCl release rate. Validation of the optimization technique demonstrated the reliability of the model. The experimental values of the response variables obtained from the optimized formulations were close and in linear with the predicted values.

ACKNOWLEDGEMENTS

The first author is thankful to Prof (Dr.) T. K Pal of Department of Pharma Technology, Jadavpur University, Kolkata, India for providing a proper platform for carrying out this work.

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