ORIGINAL ARTICLE

Design and Optimization of Rivaroxaban Lipid Solid Dispersion for Dissolution Enhancement using Statistical Experimental Design

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Abstract

Aim: The purpose of the present study was to understand the effect of formulation variables of lipid solid dispersion on the dissolution of a model drug, rivaroxaban. **Method:** A three-factor, three-level Box–Behnken design was used to explore the main and interaction effect of several independent formulation variables including the amount of Gelucire 48/16 (X_1), Compitrol HD5 ATO (X_2), and Labrasol (X_3). Particle size (Y_1) and dissolution percentage of rivaroxaban (Y_2) were the dependent variables. **Statistical Analysis:** A mathematical relationship was obtained to explain the effect of all factors and their colinearities on the dissolution of rivaroxaban. **Results:** A formulation optimization was then performed to maximize dissolution percentage of rivaroxaban (Y_2). The optimized formulation was predicted to dissolution 62.4% of rivaroxaban at 5 min, when X_1, X_2, and X_3 values were 20.0, 30.0, and 2.0 mg, respectively. **Conclusion:** In conclusion, the Box–Behnken experimental design allowed us to understand the effect of formulation variables on the dissolution of rivaroxaban from lipid solid dispersion, and optimize the formulation to obtain drug dissolution.

Key words: Box–Behnken design, compitrol HD5 ATO Labrasol, dissolution, Gelucire 48/16, Rivaroxaban, lipid solid dispersion

INTRODUCTION

Rivaroxaban drug used in acute coronary syndrome, prevention of cardiovascular death, myocardial infarction and stent thrombosis.[1] Rivaroxaban is a potent selective oral direct factor Xa inhibitor, it belongs Biopharmaceutics Classification System Class II drug, having low solubility and high permeability. Therefore, the limited aqueous solubility (20.0 µg/mL) of rivaroxaban is the main hurdle of its *in vitro* dissolution profile, and thus the oral bioavailability. Depending on the choice of excipient(s) and formulation techniques, it is possible to obtain a variety of systems including physical mixtures, liquid/solid solutions, solid dispersions, and self-micro or self-nano emulsifying drug delivery systems.[2] Lipid-based formulations including lipid solid dispersion formulations offer the potential for enhancing the dissolution and absorption of poorly soluble and/or poorly permeable compounds. Lipid solid dispersions are one of the most successful formulations to improve the *in-vitro* dissolution and *in-vivo* absorption of poorly soluble drugs and their bioavailability.[3]

The dissolution of drug from lipid solid dispersion is controlled by the relative amount of combinations of hydrophilic lipids in the formulation. However, it is time-consuming to obtain an optimized formulation with rapid and complete dissolution by traditional formulation screening and optimization process. The application of statistical experimental design to pharmaceutical formulation development has been demonstrated to be efficient and satisfactory to acquire the necessary information to understand the relationship between independent and dependent variables in a formulation.

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Response surface methodology (RSM) is often used when only a few significant factors are involved in optimization. Box–Behnken design, one of RSM design, was applied herein because it requires fewer runs (17 runs) in a three-factor experimental design among all the RSM designs and is particularly useful when extreme treatment combinations should be avoided.[4] The independent variables for the present study were the following: The amount of Gelucire 48/16 (X_1), Compitrol HD5 (X_2) and Labrasol (X_3). The dependent variables included drug dissolution profile and particle size. As part of the optimization process, the main effects, interaction effects and quadratic effects of the formulation ingredients were investigated to understand the factors influencing the dissolution of rivaroxaban from the lipid solid dispersion. Spray drying method was chosen for the preparation of solid dispersions.[5] The spray drying method was selected for the preparation of rivaroxaban lipid solid dispersion, which were formulated by dissolving accurately weighed amounts of rivaroxaban, Gelucire 48/16, Compitrol HD5 and Labrasol in dichloromethane (DCM) processed for spray drying and collects the dried sample from the drying chamber, cyclone 1 and cyclone 2 then dried material was packed in an airtight container.[6]

MATERIALS AND METHODS

Materials

Rivaroxaban drug was obtained as gift sample from MSN Laboratories, Hyderabad, India. Macrogol stearate polyoxyl stearate (Type I) NF (Gelucire 48/16, HLB = 12), Behenoyl polyoxyl-8 glycerides NF (Compitrol HD5, HLB = 5) and Caprylocaproyl polyoxyl-8 glycerides NF (Labrasol, HLB = 12) provided by Gattefosse (Saint-Priest Cedex, France) as a gift samples. High-performance liquid chromatography (HPLC) grade acetonitrile and phosphoric acid were purchased from Tedia. Co., Ltd. (Fairfield, OH, USA). Gelatin capsules (size 1) were obtained from ACG Capsules. (Mumbai, Maharashtra, India). Reagents were of analytical grade, and preparation of HPLC mobile phase was done with Millie-Q demineralized double-distilled water.

Methodology

Formulation of rivaroxaban lipid solid dispersion

The preparation method includes the dispersion of rivaroxaban in the lipid matrix consisting of Gelucire 48/16, Compritol HD5 and Labrasol at different weight ratios. The drug dispersed lipid matrix is dissolved in the DCM to obtain a clear solution. The ratio of lipids in the optimized and finalized composition present in the ratio of 10:15:1 of Gelucire 48/16, Compritol HD5 and Labrasol, respectively. The resulting solution was subjected to spray drying using Labultima spray dryer. The total solid contents concentration was maintained at 5 w/v %. The dried rivaroxaban drug loaded lipid-solid dispersions were collected from the vessel, solid mass was grounded and passed through sieve no. 100 and stored in desiccated environment until further study.

To develop free flowing fine particles by spray drying method, different adsorbents were selected and evaluated.[7,8] The flow propertied of resulting dried powder from the trails Gelucire 48/16 in combination with Compritol HD5 and Labrasol was found to be satisfactory. The optimum drug to excipient(s) ratio was identified on the basis of drug content, drug solubility, and physical form.

Box–Behnken experimental design

Box–Behnken statistical screening design was used to evaluate main effects, interaction effects, and quadratic effects of the formulation ingredients on the *in vitro* performance of rivaroxaban lipid solid dispersion and optimize the formulation. The non-linear quadratic model generated by the design is of the form:

$$Y = A_0 + A_1X_1 + A_2X_2 + A_3X_3 + A_4X_1X_2 + A_5X_2X_3 + A_7X_1^2 + A_8X_2^2 + A_9X_3^2$$

in which Y is the measured response of the dependent variables associated with each factor-level combination; A_0 is the intercept; A_1–A_9 are the regression coefficients; X_1, X_2 and X_3 are the independent variables studied, which were listed in Table 1. A three-factor, three-level Box–Behnken design was generated by an experimental design software Design-Expert version 9.0 (Stasease). The amounts of Gelucire 48/16, Compitrol HD5 and Labrasol in each of the 17 formulations were given in Table 2.

Finally, the 17 formulations with different concentrations of Gelucire 48/16, Compitrol HD5 and Labrasol, each containing rivaroxaban at a final loading of 10 mg, were filled into size 1 gelatine capsules. The final drug concentrations in these formulations varied from 16.20% to 24.00%.

Laser diffraction particle size analysis

The particle size and particle size distribution of the developed formulations are measured using a laser diffraction

Table 1: Variables in the Box–Behnken design

Independent variables	Level		
	Low	Middle	High
X_1: Amount of Gelucire 48/16 added (mg)	15	20	25
X_2: Amount of Compitrol HD5 added (mg)	25	30	35
X_3: Amount of Labrasol added (mg)	1.5	2.0	2.5

Table 2: Box-Behnken design: Independent (X) and dependent variables (Y)

Formulations	Gelucire 48/16 (X_1)	CompitrolHD5 (X_2)	Labrasol (X_3)	PSD (Y_1)	Dissolution at 5 min (Y_2)	Dissolution at 30 min (Y_3)
1	20	35	1.5	36	51.7	94.2
2	15	30	2.5	67	48.9	89.4
3	25	30	1.5	36	50.6	95.3
4	20	35	2.5	72	52.7	94.8
5	20	25	1.5	29	58.9	94.6
6	25	25	2	55	52.9	98.5
7	25	30	2.5	70	47.7	97.8
8	20	25	2.5	67	50.8	98.3
9	20	30	2	49	64.7	95.3
10	20	30	2	52	63.8	95.0
11	25	35	2	55	51.7	96.1
12	20	30	2	51	62.9	96.7
13	15	30	1.5	78	46.8	86.7
14	20	30	2	44	62.4	94.8
15	15	35	2	48	48.9	92.3
16	15	25	2	40	51.6	93.2
17	20	30	2	43	64.3	97.5

PSD: Particle size distribution

size analyzer (HELOS Germany). Samples are suspended in water and two to three drops of isopropyl alcohol is added to disperse the particles and ultrasonicated at 50% amplitude. The particle size and distribution is measured at a measurement range of 10 s in 500 ms time base and at the optimum concentration of 10%.

Dissolution studies

Dissolution profile performed for rivaroxaban lipid solid dispersion formulations derived from Box-Behnken design. The dissolution rate of rivaroxaban from the prepared lipid solid dispersion (F1 to F17) is measured using a Disso-2000 model dissolution test system (Labindia, India) USP apparatus II (paddle) method in pH 4.5 acetate buffer. The resulted solid dispersion was filled into hard gelatin capsule equivalent to 10 mg of rivaroxaban. In each dissolution vessel, drug-filled capsules are added to 900 ml dissolution medium. Bath temperature and paddle rotation speed are maintained at 37°C and stirred at 75 rpm. Samples were collected periodically and replaced with a fresh dissolution medium. After collection of 30 min sample, recovery study is conducted by stirring the paddle at 200 rpm for 5 min and sample is collected. Samples are filtered through filters (10 µm) and analyzed using HPLC.

Optimization of formulation components

After generating the polynomial equations relating the dependent and independent variables, optimization of Y_2 (dissolution percentage of rivaroxaban at 5 min) was performed using a desirability function to obtain the levels of X_1, X_2, and X_3 which maximized Y_2.

Solubility studies

Rivaroxaban solubility in Gelucire 48/16, Compitrol HD5 and Labrasol were measured as follows: Excess amount of Rivaroxan was added into cap tubes each containing 1 g of each component and slightly heated. After sealing, the tubes were shaken with an isothermal shaker (Taicang Laboratorial Equipment Factory, Jiangsu, China) at 37°C for 72 h. After reaching equilibrium, each tube was centrifuged at 10,000 g for 10 min.

An appropriate amount of the samples were taken from the supernatant and then diluted with acetonitrile. The concentrations of rivaroxaban were then determined by the HPLC method as shown in Table 3.

RESULTS AND DISCUSSION

Saturation solubility of drug in lipid component

Saturation solubility performed for drug in lipid component, and saturation solubility is shown in Table 3.

Rivaroxaban shows good solubility in Gelucire 48/16 and Labrasol as compare to the Compitrol HD5. The rivaroxaban

incorporated solid-lipid dispersions are prepared dissolving the drug, Gelucire 48/16, Compitrol HD5 and Labrasol in dichloromethane (DCM) and processed for spray drying with the optimized process parameters and collects the dried sample from the drying chamber, cyclone 1 and cyclone 2. By preparing the rivaroxaban incorporated solid lipid particles, it is assumed to have a higher aqueous dissolution of the drug due to the morphological conversion of the drug, hydrophilic lipids and reduced particle size.

A series of experiments were then conducted based on the generated Box–Behnken design. The observed responses for the 17 formulations are given in Table 2. To avoid the possible undesirable interaction, the dissolution tests were carried out immediately after the loading of contents. The dissolution profiles of the 17 formulations were presented in Table 4a and b and Figure 1a-c.

Although all formulations demonstrated a full dissolution of rivaroxaban at 30 min, the dissolution percentage of rivaroxaban at 5 min varied from 42.70% to 64.70% with no particular pattern being found.

The mathematical relationship in the form of a polynomial equation for the measured response, Y_2, is listed below. The above equation represents the quantitative effect of independent variables (X_1, X_2, and X_3) and their interactions on the response (Y_2). Concerning the P value of the coefficients, X_{12}, X_2, X_{22} and X_1X_3 were found to have a significant effect on Y_2. The theoretical values of Y_2 were obtained by substituting the values of X_1-X_3 into the above equation, which was in reasonably good agreement with the observed values as shown in Table 5.

Table 3: Rivaroxaban solubility in lipid component

Composition	Saturation solubility (µg/ml)
Drug+Gelucire 48/16	169.5±3.1
Drug+Compitrol HD5	85.35±4.7
Drug+Labrasol	155.5±9.5

The model fit summary statistics given in Table 6, and the model suggested the design is quadratic. The analysis of variance (ANOVA) for Y_2 was summarized in Table 7. With all the $P < 0.05$, we further confirmed that the linear, square and interaction effect of the independent variables were significant in predicting the response (Y_2). The relationship between the dependent and independent variables was further elucidated using contour plots.

The effect of X_1 and X_2 and their interaction on Y_2 at a middle level of X_3 is given in Figure 2. As illustrated in Figure 2, when the amount of Gelucire 48/16 (X_1) increased from 15 to 25 mg and Compitrol HD 5 (X_2) increased from 25 to 35 mg, the dissolution at 5 min (Y_2) increased to a certain level and then decreased to original value. This phenomenon might be explained by the action mechanism of Gelucire 48/16 and Compitrol HD5 in the lipid solid dispersion. Compitrol HD5 having a low HLB value, drug release from the formulation retard as the concentration of Compitrol HD5 increased after a particular concentration, result in decrease the dissolution.

Figure 3 response surface plots (three-dimensional) showing the effect of the amounts of Gelucire (X_1) and Labrasol (X_3) on the response dissolution at 5 min (Y_2). Thus, at low Gelucire 48/16 concentration, the increased amount of Gelucire 48/16 favored the drug dissolution by emulsifying action of Gelucire 48/16 might be

Table 4a: Dissolution profiles of rivaroxaban lipid solid dispersion formulation 1=8

Time (min)	Dissolution profile of rivaroxaban lipid solid dispersion formulation F1-F8							
	F1	F2	F3	F4	F5	F6	F7	F8
5	51.7±9.8	48.9±8.6	50.6±7.9	52.7±8.1	58.9±7.8	52.9±6.1	47.7±8.3	50.8±9.8
10	62.4±8.4	57.2±6.8	63.3±6.8	66.8±7.2	67.9±6.3	73.9±5.6	67.4±8.2	69.2±8.4
15	74.5±6.9	66.2±5.6	73.5±5.2	76.2±6.6	78.4±5.2	86.4±4.6	78.5±5.9	80.2±6.3
20	86.9±4.7	79.5±3.5	85.4±3.1	89.4±3.8	88.2±3.8	91.8±3.2	86.8±2.9	84.5±4.1
30	94.2±2.8	89.4±2.1	95.3±0.8	94.8±1.2	94.6±0.9	98.5±0.5	97.8±1.1	98.3±2.9

Mean±SD, *n*=6. SD: Standard deviation

Table 4b: Dissolution profiles of rivaroxaban lipid solid dispersion formulation 9-17

Time (min)	Dissolution profile of rivaroxaban lipid solid dispersion formulation F9-F17								
	F9	F10	F11	F12	F13	F14	F15	F16	F17
5	64.7±6.8	63.8±8.5	51.7±6.6	62.9±8.1	46.8±8.9	62.4±5.8	48.9±6.8	51.6±7.8	64.3±8.1
10	77.6±5.4	72.2±7.2	65.9±4.8	76.8±6.8	54.3±7.2	71.8±4.4	58.7±4.6	62.7±6.5	72.7±7.3
15	84.3±3.9	81.9±5.3	77.9±3.3	83.2±5.5	65.2±5.8	81.7±3.2	68.2±3.7	72.6±5.8	83.2±5.4
20	89.4±2.8	85.5±3.2	87.5±1.8	88.5±4.2	77.4±4.1	88.7±2.7	80.1±1.9	81.5±4.4	88.9±3.8
30	95.3±1.6	95.0±2.2	96.1±0.6	96.7±2.8	86.7±1.9	94.8±0.9	92.3±0.7	93.2±2.2	97.5±1.8

Mean±SD, *n*=6, SD: Standard deviation

responsible for the improvement in drug release properties from the prepared solid dispersions. At higher level of Gelucire 48/16, the formation of liquid crystal was observed. The role of added Gelucire 48/16 (X_1) and its interaction with Labrasol (X_3) on rivaroxaban dissolution at 5 min (Y_2) can be discussed with the help of Figure 3. As shown in this Figure 3, with a low level of the Gelucire 48/16 added, Y_2 levels increased from 46.80 to 64.70% when X_3 increased from 1.5 to 2.5 mg, while Y_2 decreased from 58.90% to 47.70% at high X_1 with the same increase in X_3.

Hence, it is clear that the ratio of Gelucire 48/16 and Labrasol has a major effect on the dissolution percentage of rivaroxaban at 5 min, which is in agreement with the obtained P value of the coefficient of X_1X_3.

Figure 4 showed the effect of X_2 and X_3 and their interaction on Y_2 at a middle level of X_1. As shown in Figure 4, at all levels of Labrasol, Y_2 levels underwent an increase and then decrease when X_2 increased from 25 to 35 mg, which can be explained by the formation of lipid matrix at higher concentrations decrease the dissolution. On the other hand, at a lower level, Y_2 decreased slightly when X_3 increased from

Table 5: Observed and predicted values and analysis of variance parameters for the response Y_2

Formulation No.	Observed Y_2	Predicted Y_2	Residuals
1	51.7	51.08	0.61
2	48.9	47.92	0.97
3	50.6	51.57	−0.97
4	52.7	53.66	−0.96
5	58.9	57.93	0.96
6	52.9	52.88	0.01
7	47.7	47.10	0.60
8	50.8	51.41	−0.61
9	64.7	63.62	1.08
10	63.8	63.62	0.18
11	51.7	51.33	0.36
12	62.9	63.62	−0.72
13	46.8	47.40	−0.60
14	62.4	63.62	−1.22
15	48.9	48.91	−0.01
16	51.6	51.96	−0.36
17	64.3	63.62	0.68

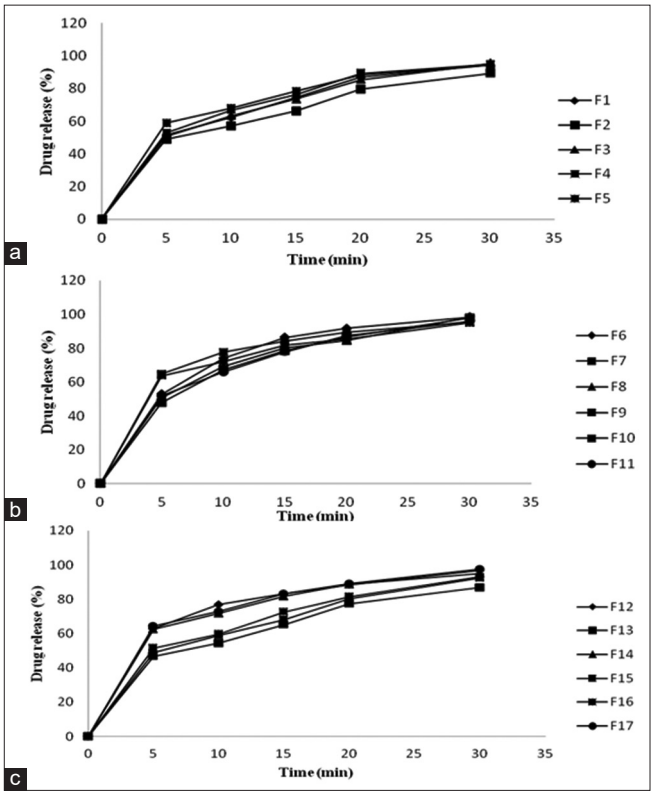

Figure 1: (a) Dissolution profiles of rivaroxaban lipid solid dispersion formulation 1-5 (b) Dissolution profiles of rivaroxaban lipid solid dispersion formulation 6-11 (c) Dissolution profiles of rivaroxaban lipid solid dispersion formulation 12-17.

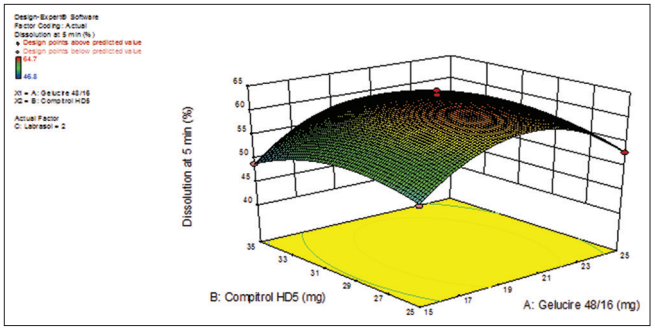

Figure 2: Response surface plots (three-dimensional) showing the effect of the amounts of Gelucire 48/16 (X_1) and Compitrol HD5 (X_2) added on the response Y_2 (dissolution after 5 min)

Table 6: Model summary statistics

Source	Standard deviation	R^2	Adjusted R^2	Predicted R^2	PRESS	
Linear	3.942	0.7326	0.670	0.585	313.484	
2FI	4.277	0.7578	0.612	0.357	485.187	
Quadratic	2.978	0.9178	0.812	0.763	178.8975	Suggested
Cubic	3.755	0.9253	0.701			

Table 7: ANOVA for Y^2

Source	d.f.	Sum of squares	Mean square	*F* ratio	*P* value
Model	6	655.42	72.825	55.68	0.011
A-Gelucire 48/16	1	5.61	5.61	4.290	0.0005
B-Compitrol HD5	1	10.58	10.58	8.089	0.031
C-Labrasol	1	7.80	7.80	5.964	0.047

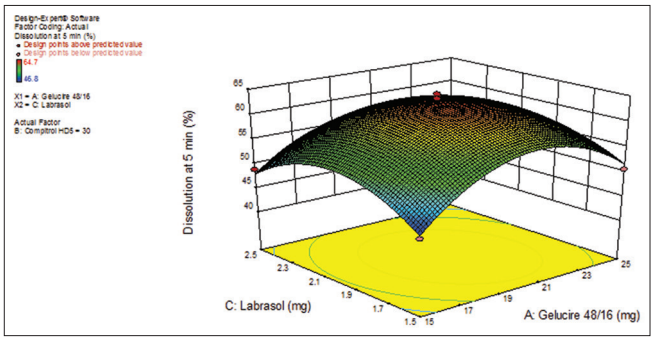

Figure 3: Response surface plots (three-dimensional) showing the effect of the amounts of Gelucire 48/16 (X_1) and Labrasol (X_3) on the response Y_2 (Dissolution at 5 min)

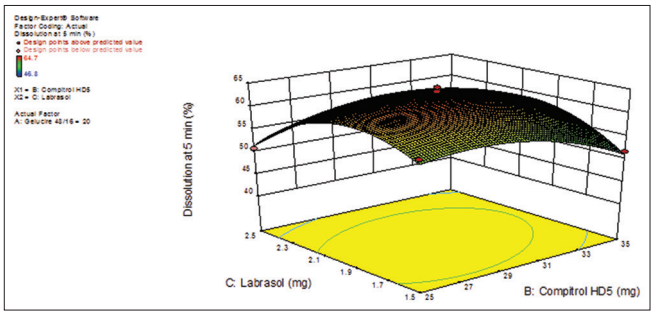

Figure 4: Response surface plots (three-dimensional) showing the effect of the amounts of Compitrol HD5 (X_2) and Labrasol (X_3) added on the response Y_2 (dissolution after 5 min)

1.5 to 2.5 mg, the reason may be Labrasol is a liquid in nature and the concentration at higher level the power obtained not free flowing and the particles obtained in a coarser.

The predicted value of Y_2 was 64.70% at X_1, X_2, and X_3 levels of 20.0, 30.0 and 2.0 mg, respectively.

CONCLUSIONS

Box–Behnken design was successfully utilized to optimize the dissolution of rivaroxaban, a water-insoluble drug, from lipid solid dispersion containing Gelucire 48/16 (X_1), Compitrol HD5 (X_2) and Labrasol (X_3). All the independent variables were found to affect the dissolution of rivaroxaban from the resultant lipid solid dispersion either through linear, quadratic or interaction effects. The optimum formulation prepared provided rivaroxaban dissolution of 64.7% at 5 min. Consequently, through the rigorous analysis of the three independent variables and its effects on the investigated response, this study demonstrated the potential of Box–Behnken design in developing lipid solid dispersion.

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REFERENCES

1. Xarelto Public assessment report,www.ema.europa.eu/EPAR/Publicassessmentreport/EMEA/543519/2008.
2. Palin KJ. Lipids and oral drug delivery. Pharm Int 1985;272-5.
3. Amidon GL, Lennernas H, Shah VP, Crison JR. A theoretical basis for a biopharmaceutic drug classification: The correlation of *in vitro* drug product dissolution and *in vivo* bioavailability. Pharm Res 1995;12:413-20.
4. Box GE, Behnken DW. Some new three level designs for the study of quantitative variables. Technimetrics 1960;2:455-75.
5. Tachibana T, Nakamura NA. A method for preparing an aqueous colloidal dispersion of beta-carotene by polyvinylpyrolidone. Colloid Polym Sci 1965;203:130-3.
6. Xie Y, Li G, Yuan X, Cai Z, Rong R. Preparation and *in vitro* evaluation of solid dispersions of total flavones of *Hippophae rhamnoides* L. AAPS Pharm Sci Tech 2009;10:631-40.
7. Perssutti B, Rubessa F, Princivalle F. Solid dispersions of carbamazepine with gelucire 44/14 and 50/13. STP Pharm Sci 2000;10:479-84.
8. Humberstone AJ, Charman WN. Lipid-based vehicles for the oral delivery of poorly water soluble drugs. Adv Drug Deliv Rev 1997;25:103-28.

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