AMERICAN JOURNAL OF PHARMTECH RESEARCH

Formulation and In Vivo Studies of Solid Lipid Nanoparticles of Bortezomib

K. Taraka Sunil Kumar*[1, 2], M. Mohan Varma[2], P. Ravi Prakash[3]

1.Research scholar, Jawaharlal Nehru Technological University, Kukatpally, Hyderabad-500072, Telangana, India.

2.Department of Pharmaceutics, Shri Vishnu College of Pharmacy, Bhimavaram-534202, Andhra Pradesh, India.

3.Department of Pharmaceutics, Creative Educational Society College of Pharmacy, Kurnool-518218, Andhra Pradesh, India.

ABSTRACT

Bortezomib is formulated as the solid lipid nanoparticle (SLN) system with the use of a 3-factor, 3-level Box–Behnken design, by hot homogenization followed by an ultra sonication method. Trimyristin (Dynasan-114), tripalmitin (Dynasan116) and tristearin (Dynasan-118) were used as lipids and based on the results from the initial studies tripalmitin (Dynasan116) was selected as the lipid for the further studies along with phosphate dylcholine as surfactant and Poloxamer 188 as stabilizer. The optimized formulation (F1) was obtained with minimum particle size (204 nm), maximum entrapment efficiency (70.24) and drug loading (21.24). *In vitro* release studies showed that maximum cumulative drug release was obtained for F1 (99.74%). The optimized formulation Bortezomib followed zero-order release kinetics with a strong correlation coefficient (R^2= 0.9994). The pharmacokinetic studies in rabbits demonstrated that SLN formulation could be used for increasing the oral bioavailability of the drug for more than 2 fold when compared with pure drug. SLNs of Bortezomib were successfully developed to yield an optimized formulation with lowest particle size and highest entrapment efficiency that could sustain the release of drug.

Key words: Bortezomib, SLN, Cancer, Tripalmitin, Box-Behnken design, Pharmacokinetic studies.

*Corresponding Author Email: sunil123pharma@gmail.com

Received 01 January 2019, Accepted 07 January 2019

Please cite this article as: Taraka S *et al.*, Formulation and in Vivo Studies of Solid Lipid Nanoparticles of Bortezomib . American Journal of PharmTech Research 2019.

INTRODUCTION

Solid lipid nanoparticles (SLNs) are the first generation colloidal drug carrier systems developed at the beginning of the 1990s [1]. SLN can be defined as colloidal drug delivery system consisting of solid lipid and stabilized with surfactant and particle size ranging from 10 nm to 1000 nm [2].During the preparation of SLN, several factors like effect of lipids and surfactants [3], processing parameters such as homogenization and sonication, formulation parameters such as drug to lipid ratio, lipid type and concentration and surfactant type and concentration [4] are to be considered. Bortezomib, a proteasome inhibitor, is clinically used for the treatment of multiple myeloma and mantle cell lymphoma via intravenous or subcutaneous administration [5].In the present study, Bortezomib loaded SLNs were prepared by hot homogenization followed by the ultra sonication, optimization of Bortezomib loaded SLN was done by Box-Behnken design.

MATERIALS AND METHOD

Materials

Trimyristin (Dynasan-114), tripalmitin (Dynasan116) and tristearin (Dynasan-118) were purchased from Sigma-Aldrich Chemicals, Hyderabad, India. Bortezomib was gifted by Dr. Reddy's labs, India. Egg Lecithin and Poloxamer-188 were gift samples from Aurobindo Labs, India. All other chemicals and solvents were of analytical grade and were used without further purification.

Methods

Design of experiments

According to Box-Behnken design, a total number of 17 experiments were conducted (Table 1) and the obtained responses for the dependent variables recorded On the basis of preliminary studies, the factors like drug to lipid ratio (1:10 - 1:20), concentration of phosphatidylcholine (50-100 mg) and concentration of poloxamer 188 (100-200 mg) were identified as the formulation variables(Table 2). Based on particle size, PDI and ZP results (Table 3) tripalmitin (Dynasan116) was selected as the lipid of choice for further investigations along with phosphatidylcholine as surfactant and Poloxamer 188 as stabilizer. The optimized formulation was obtained which have the minimum particle size with maximum entrapment efficiency and drug loading**.** The optimized formulation was prepared and evaluated for particle size, entrapment efficiency and percentage drug loading. Observed response value of the optimized formulation was compared with predicted value.

The effect of the independent variables on each response parameters was visualized from the perturbation plots [6-8].

Preparation of Bortezomib loaded SLN

Bortezomib loaded SLNs were prepared by hot homogenization followed by the ultrasonication. Bortezomib (dose 3.5 mg), lipid and phosphatidylcholine were dissolved in 5ml of 1:1 mixture of chloroform and methanol. Organic solvents were completely removed using a rota evaporator (Heidolph, Schwabach, Germany). The drug embedded lipid layer was melted by heating to 5°C above melting point of the lipid. Aqueous phase was prepared by dissolving Poloxamer 188 in double distilled water and heated to same temperature (based on lipid melting point) of oil phase. Hot aqueous phase was added to the oil phase, homogenization was carried out (at 12000 rpm) using homogenizer (Diax900, Heidolph, Germany) for 4 min. The coarse hot oil in water emulsion so obtained was ultrasonicated using a 12 T probe Sonicator (Vibracell, Sonics, CT, USA) for 20 min. Bortezomib loaded solid lipid nanoparticles were obtained by allowing hot nanoemulsion to cool to room temperature.

Characterization of solid lipid nanoparticles

The size, polydispersity index (PDI) and zeta potential (ZP) of the SLNs were measured by using Zetasizer (Nano ZS90, Malvern, Worcestershire, UK). Characterization of crystallinity carried out by powder X-ray diffractometry (Multiflex, M/s. Rigaku, Tokyo, Japan).The morphology of nanoparticles were studied by Scanning Electron Microscope (SEM, Hitachi, Tokyo, Japan).

***In vitro* drug release studies**

In vitro release studies were performed using dialysis bag method. Dialysis membrane (molecular weight cut-off between 12,000 -14,000) was soaked overnight in double distilled water prior to the release studies. Hydrochloric acid (0.1N) and phosphate buffer pH 6.8 was used as release media. The experimental unit consists of a donor and receptor compartment. Donor compartment consists of a boiling tube which was cut open at one end and tied with dialysis membrane at the other end into which SLN dispersion of 3 ml was taken for release study. Receptor compartment consists of a 250 ml beaker which was filled with 100 ml release medium and the temperature of it was maintained at 37±0.5°C. At 0.5, 1, 2, 3, 4, 6, 8, 10, 12 and 24 h time points, 3 ml sample each were withdrawn from receiver compartment and replenished with the same volume of release medium. The collected samples were suitably diluted and analyzed by UV-Visible Spectrophotometer at 270 nm.

Drug release kinetics

To elucidate the mode and mechanism of drug release, the data from the *in vitro* release study were fitted into various kinetic models like zero order, first order, Higuchi's and Korsemeyer Peppa's model.

Stability studies

Stability of Bortezomib nanoparticles suspension in screw-capped glass vials was evaluated over a time period of 60 days. Six samples were divided into two groups and stored at 25°C and 4°C. Drug leakage from nanoparticles and mean particle size of the samples were determined at the end of 1, 7, 15, 30, 45, and 60 days.

Pharmacokinetic studies

Animal Preparation

Male Rabbits were (weighing 2-3 kg) selected for this study, all the animals were healthy during the period of the experiment. Animals were maintained at room temperature 25^0C, Relative Humidity 45% and 12 h alternate light and dark cycle with 100 % fresh air exchange in animal rooms, uninterrupted power and water supply and rabbits were fed with standard diet and water ad libitum. The protocol of animal study was approved by the institutional animal ethics committee with IAEC NO: 439/PO/01/a/CPCSEA.

***In vivo* study design**

The Rabbits were randomly divided into two groups each group contains six animals[9]. The group A was received prepared optimized solid lipid nanoparticles equivalent to 0.4375 mg Bortezomib. Pure drug suspension of Bortezomib was administered group B with equivalent dose of animal body weight (0.4375 mg). Blood samples (approximately 0.5 ml) were obtained with syringes by marginal ear vein at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 20 and 24 h post dose. During collection, blood sample has been mixed thoroughly with heparin in order to prevent blood clotting. Plasma was separated by centrifugation of the blood at 5000 rpm in cooling centrifuge for 5 min to 10 min and stored frozen at −20°C until analysis.

HPLC study

Bortezomib was measured in plasma using a validated a HPLC method with UV detector at 270 nm chromatographic peaks were separated on 5 µm intensil, C18 column (4.6x250 mmx5 µm) using a mixture of water-Acetonitrile-formic acid (71: 28: 1, v/v/v) as mobile phase at a flow rate of 1 ml/min, pH 6 was adjusted with triethyllamime. The chromatograms showed good resolution and no interference from plasma. The retention time of Bortezomib and internal standard (Atazanavir) were approximately 5.9±0.05 min and 10.19± 0.03 min respectively [10].

Preparation of Plasma Samples for HPLC Analysis

Rabbit plasma (0.5 ml) samples were prepared for chromatography by precipitating proteins with 2.5 ml of ice-cold absolute ethanol for each 0.5 ml of plasma. After centrifugation the ethanol was transferred into a clean tube. The precipitate was re suspended with 1 ml of Acetonitrile by vortexing for 1 min. After centrifugation (5000 – 6000 rpm for 10 min), the Acetonitrile was added to the

ethanol and the organic mixture was taken to near dryness by a steam of nitrogen at room temperature**.**

Pharmacokinetic analysis

The pharmacokinetic parameters, peak plasma concentrations (C_{max}) and time to reach peak concentration (t_{max}) were directly obtained from concentration time data. In the present study, AUC_{0-t} refers to the AUC from 0 to 24 h, which was determined by linear trapezoidal rule and $AUC_{0-\alpha}$ refers to the AUC from time at zero hours to infinity.

The pharmacokinetic parameters were performed by a non compartmental analysis using Win Nonlin 3.3® pharmacokinetic software (Pharsight Mountain View, CA USA). All values are expressed as the mean ± standard deviation (SD). Statistical analysis was performed with Graph Pad In Stat software (version 3.00, Graph Pad Software, San Diego, CA, USA) using one-way analysis of variance (ANOVA) followed by Tukey–Kramer multiple comparison test. Difference with $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Preliminary experiments

Preliminary experiments conducted using three different lipids Trimyristin (Dynasan-114), tripalmitin (Dynasan116) and tristearin (Dynasan-118). Based on the results from the initial studies tripalmitin (Dynasan116) was selected as the lipid for the further studies.

The possible intermolecular interaction between Bortezomib and the selected lipid tripalmitin (Dynasan116) was checked by FTIR. Some additional peaks were observed with physical mixture, which could be due to the presence of functional groups of lipid. The characteristic DSC peak of drug is slightly becomes asymmetric and the almost vertical line after peak maximum could be due to two possible degradation processes of Bortezomib.

Seventeen experiments were required for the response surface methodology based on the Box–Behnken design (Table 1).

Table 1: Box–Behnken experimental design and observed responses

Run	Factor A Drug to lipid ratio	Factor B Conc. of phosphatidylcholine	Factor C Conc. of poloxamer188	Response Y1 Particle size	Response Y2 Entrapment efficiency	Response Y3 Drug loading
1	1:15	75	150	191.82	71.23	19.26
2	1:20	50	150	413.56	73.82	23.42
3	1:15	100	200	363.72	47.46	16.72
4	1:10	100	150	212.62	68.93	20.26
5	1:20	75	100	432.42	70.76	21.36
6	1: 15	50	200	243.62	58.12	18.32

7	1: 15	75	150	192.24	70.86	19.48
8	1:10	75	100	204.32	54.32	14.12
9	1: 15	75	150	191.32	71.36	19.12
10	1:20	100	150	442.56	69.82	18.38
11	1:10	75	200	206.72	59.62	17.98
12	1:20	75	200	424.24	70.36	17.24
13	1: 15	100	100	218.32	69.13	21.26
14	1:10	50	150	190.12	49.36	12.28
15	1: 15	75	150	192.06	69.76	20.12
16	1: 15	75	150	192.96	69.32	19.82
17	1: 15	50	100	283.46	51.72	14.13

Data were analyzed using Stat-Ease Design Expert ® software V8.0.1 to obtain analysis of variance (ANOVA), regression coefficients and regression equation. Mathematical relationships were generated using multiple linear regression analysis for the mentioned. Constraints like minimizing the particle size in addition to maximizing the entrapment efficiency and drug loading were set as goals to locate the optimum settings of independent variables. The optimized levels and predicted values of Y1, Y2 and Y3 are shown in Table 2.

Table 2: Optimized values obtained by the constraints applies on Y1, Y2 and Y3

Independent variable	**Nominal values**	**Predicted values**			**Observed values**			
		Particle size (Y1)	**Entrapment efficiency (Y2)**	**Drug loading (Y3)**	**Batch**	**Particle size (Y1)**	**Entrapment efficiency (Y2)**	**Drug loading (Y3)**
Drug lipid ratio (A)	1:11.35	190.11	69.41	21.17	F1	204	70.24	21.24
					F2	192.8	68.46	20.62
Conc. of phosphatidylcholine (B)	100				F3	173.3	69.72	20.84
Conc. of poloxomer 188 (C)	123.5							

Optimization and confirmation experiments

All the prepared formulations were analyzed in order to determine their particle size distribution and zeta potential. The mean size of all the formulations was ranging from 173.3 ± 9.8 nm to 204 ±12.9 nm (Table 3). The PDI was ranging from 0.210 to 0.284, indicating the narrow size distribution. The SLN formulations exhibited negative surface charge with the inclusion of Bortezomib which clearly suggested the orientation of Bortezomib in the lipid matrix. The surface charge is a key factor for the stability of colloidal dispersion. In our case, the zeta potential values of SLN formulations were found to be in between -27.7 ± 5.48 mV to -29.3 ± 4.89 mV. ZP is an important factor that affects the stability of colloidal systems. Total entrapment efficiency of the nanoparticles formulations was

determined and found to be ranging from 68.46 ± 0.37 % to 70.24 ± 0.18 %. The precent drug loading of the formulations was found to be in the range from 20.62 ± 2.12 % to 21.24 ± 1.72 %.

Table 3:**The mean particle size, PDI, zeta potential, entrapment efficiency and % drug loading of optimized formulations**

Batch	MPS ± SD (nm)	PDI	ZP ± SD (mV)	% EE ± SD	% DR ± SD
1	204 ±12.9	0.284	-27.7 ± 5.48	70.24 ± 0.18	21.24 ± 1.72
2	192.8 ±11.32	0.210	-28.9 ± 7.79	68.46 ± 0.37	20.62 ± 2.12
3	173.3 ± 9.8	0.260	-29.3 ± 4.89	69.72 ± 0.82	20.84 ± 0.94

n = 3 ($p < 0.05$)

***In vitro* drug release studies**

Cumulative release (%) of pure drug Bortezomib (Control) within 24 h was obtained to be 21.08 which indicate a slow release pattern and very less drug release for the control. The slow release of Bortezomib can be attributed to the limited solubility. The complete and controlled release of the drug from the optimized SLN formulations can be due to the reduced particle size and enhanced effective surface area (Figure 1 and Table 4).

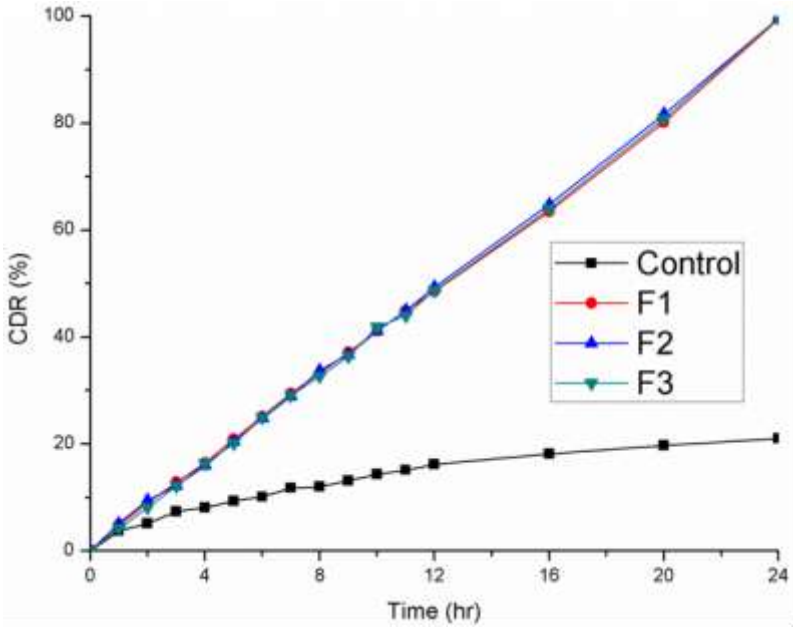

Figure 1: *In-vitro* release of Bortezomib from nanoformulation

Table 4: Dissolution profile of Bortezomib solid lipid nanoparticles (Optimized batches)

Time (h)	% CDR Control (pure drug)	F1	F2	F3
0	0	0	0	0
1	5.72	4.88	5.10	4.23
2	6.16	8.92	9.46	8.16
3	7.36	12.88	12.17	12.13
4	8.12	16.45	15.94	16.36
5	9.34	20.96	20.46	20.12
6	10.12	25.12	24.78	24.98
7	11.78	29.46	28.94	29.12
8	12.04	33.23	33.76	32.65
9	13.13	37.14	36.73	36.36
10	14.34	41.16	41.17	41.86
11	15.12	44.92	44.93	44.07
12	16.23	48.56	49.32	48.76
16	18.13	63.47	64.76	63.96
20	19.72	80.84	81.65	80.12
24	21.08	99.74	99.65	99.49

Drug release kinetics

Release data for optimized formulation (F1) was fitted into various kinetic equations to find out the order and mechanism of drug release .Kinetic analysis of drug release profiles showed that the systems predominantly released Bortezomib in a zero-order manner with a strong correlation coefficient (R^2= 0.9994).

Stability study

The stability study indicates that no significant difference ($p < 0.05$) was found in entrapment efficiency and particle size of optimized formulation stored at refrigerated conditions and at room temperature.

Pharmacokinetics studies

Mean plasma concentration profiles of prepared Bortezomib optimized formulation and Bortezomib Pure drug are presented in Figure 2.Bortezomib optimized formulation exhibited higher oral bioavailability in controlled manner when compared with pure drug. All the pharmacokinetics parameters displayed in Table 5.

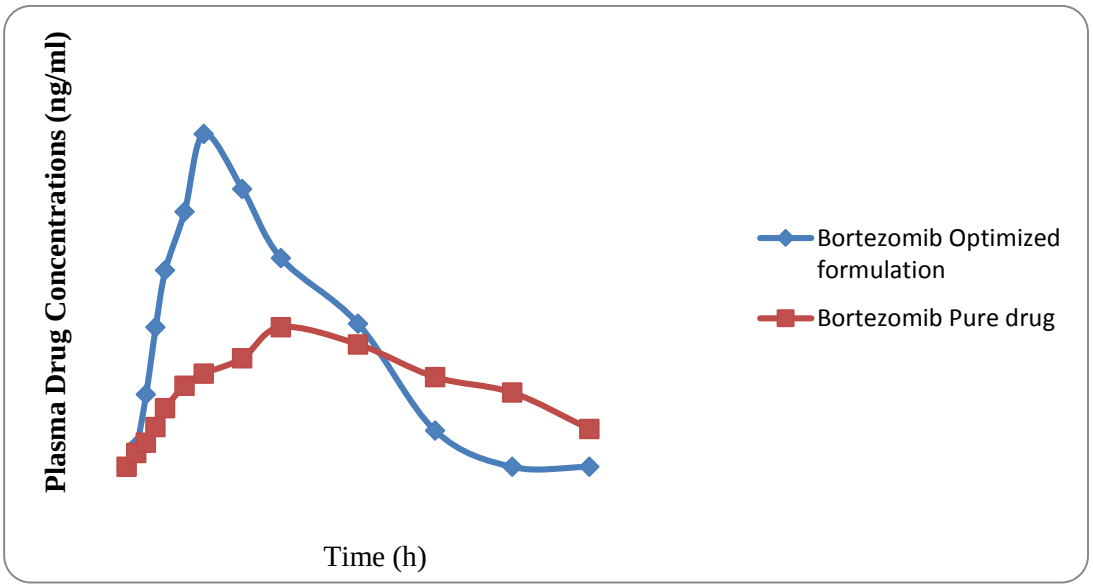

Figure 2: Plasma Concentrations of Bortezomib optimized formulation (F1) and Bortezomib Pure drug at different time intervals

The T_{max} of the Bortezomib optimized formulation (F1) and pure drug was 4.00±0.01 h and 8.00±0.01h respectively. The C_{max} of Test formulation (1.93±0.31ng/ml) was significantly higher compared with pure drug (0.81±0.05ng/ml). However, the $AUC_{0-\infty}$ values for the optimized (10.12±1.32ng h/ml) was higher when compared with pure drug (5.18±1.42ng h/ml), this suggests that the Enzalutamide contained in the form of SLN was completely absorbed showing more than 2 fold bioavailability when compared with pure drug.

Table 5: Comparison of pharmacokinetic parameters of Bortezomib Optimized formulation and Bortezomib Pure drug

Parameters	Bortezomib optimized formulation	Bortezomib Pure drug
C_{max} (ng/ml)	1.93±0.81	0.81±0.5
AUC_{0-t} (ng h/ml)	7.12±1.11	3.69±1.21
$AUC_{0-\infty}$ (ng h/ml)	10.12±2.32	5.18±1.42
T_{max} (h)	4.00±1.0	8.00±1.8
$t_{1/2}$ (h)	8.05±1.4	12.12±2.5
K_{el} (h^{-1})	0.086±0.4	0.0571±0.3

CONCLUSION

Bortezomib was chosen to formulate as the solid lipid nanoparticle (SLN) system with the use of a 3-factor, 3-level Box–Behnken design, by hot homogenization followed by an ultrasonication method. Trimyristin (Dynasan-114), tripalmitin (Dynasan-116) and tristearin (Dynasan-118) were used as lipids, based on the results of the initial studies tripalmitin (Dynasan116) was selected as the lipid for the further studies along with phosphatidylcholine and Poloxamer 188. The optimized

formulation (F1) was obtained which have the minimum particle size (204 nm) with maximum entrapment efficiency (70.24) and drug loading (21.24). The optimised batch(s) were further investigated by FTIR, DSC, XRD, SEM and stability. The prepared SLNs were found to have an imperfect crystalline lattice and a spherical morphology. *In vitro* release studies showed maximum cumulative drug release was obtained for F1 (99.74%). The optimized formulation Bortezomib followed zero-order release kinetics with a strong correlation coefficient (R^2= 0.9994). Among all, tripalmitin (Dynasan116) was chosen as the best lipid for formulating SLN because it had high EE and sustained the drug release. The nano formulation prepared under optimized conditions is in concurrence with the expected results. The SLN formulation can be used as a potential carrier for the effective delivery of Bortezomib. The pharmacokinetic results were statistically significant ($p<0.0001$) and indicated that the oral bioavailability of Bortezomib could be improved after incorporation into SLNs when compared to a pure drug suspension. Results of *in vivo* studies in rabbits demonstrated that SLN formulation could be used for increasing the oral bioavailability of this drug. The SLN formulation can be used as a potential carrier for the effective delivery of Bortezomib. SLNs of Bortezomib were successfully developed to yield an optimized formulation with lowest particle size and highest entrapment efficiency that could sustain the release of drug. The pharmacokinetic results were statistically significant, indicated that the oral bioavailability of Bortezomib could be improved after incorporation into SLNs when compared to a pure drug suspension. Results of *in vivo* studies in rabbits demonstrated that SLN formulation could be used for increasing the oral bioavailability of this drug.

ACKNOWLEDGEMENTS

The author Mr. K. T. Sunil Kumar gratefully acknowledge the support rendered by Shri K. V. Vishnu Raju, Chairman, Shri Vishnu Educational Society and Dr D. B. Raju, Director, Shri Vishnu College of Pharmacy for providing facilities to carry out the research work.

REFERENCES

1. Pardeike J, Hommoss A, Muller RH. Lipid nanoparticles (SLN, NLC) in cosmetic and pharmaceutical dermal products. Int J Pharm 2009;366: 170–84.
2. Mehnert W, Mader K. Solid lipid nanoparticles: production, characterization and applications. Adv Drug Deliv Rev 2001; 47: 165e196
3. Soukharev RA. Stability of lipid excipients in solid lipid nanoparticles. Adv Drug Deliv Rev 2007;59: 411e418.

4. Harde H, Das M,Jain S. Solid lipid nanoparticles: an oral bioavailability enhancer vehicle. Expert Opin Drug Deliv 2011; 8: 1407-1424.
5. Mohammad M, Hadi V, Parvin Z. Bortezomib-loaded solid lipid nanoparticles: preparation, characterization and intestinal permeability investigation. Drug Dev Ind Pharm 2018; 44(10): 1598-1605.
6. Montgomery DC. Design and Analysis of Experiments. 8th ed., New York: John Wiley & Sons Inc.; 2007.
7. Myers RH, Montgomery DC, Anderson-Cook CM Response Surface Methodology: Process and Product Optimization Using Designed Experiment. 3rd ed., US: Wiley; 2009.
8. Piepel GF. Programs for generating extreme vertices and centroids of linearly constrained experimental regions. J Qual Technol 1988; 20: 125-139.
9. Muhammad S, Mir Azam, Waheed S, Maqsood-ur-Rehman, Waqar A,Shahzeb K. Fabrication, Characterization, and In Vivo Evaluation of Famotidine Loaded Solid Lipid Nanoparticles for Boosting Oral Bioavailability. Journal of Nanomaterials 2017;1: 1-10.
10. Bhetanabotla C, Rajkamal B. Development and validation of HPLC-UV method for the estimation of Bortezomib in human plasma. Int J of Pharmacy and Analytical Research 2017; 6(3):501-506.