Int. J. Pharm. Investigation, 2019;9(3):135-140 Original Article

Quality by Design based Developed and Validation of RP-HPLC Method for Simultaneous Estimation of Pazopanib in Bulk and Pharmaceutical Dosage Forms

Kiran Kumar Buralla[1], Varadarajan Parthasarathy[2,*]
[1]Department of Pharmacy, Annamalai University, Annamalainagar, Chidambaram, Tamil Nadu, INDIA.
[2]Department of Pharmacy, Centre for Cell Biology and Drug Discovery, Annamalai University, Annamalainagar, Chidambaram, Tamil Nadu, INDIA.

ABSTRACT

Objectives: This paper describes the development of an accurate, precise, robust, sensitive, economical and rapid isocratic RP-HPLC technique complying quality by design and validate according to ICH guidelines. The simultaneous estimation of the Pazopanib with Dasatinib as internal standard in bulk and pharmaceutical dosage forms with the help of chemo metrics, multicriteria decision-making approach. The main objective was to identify the robust chromatographic conditions where an adequate separation of the components with quality peaks, within acceptable run time can be achieved. Target Analytical Profile (TAP) was defined and systematic risk analysis was carried out to identify Critical Method Attributes (CMA) having an impact on Critical Quality Attributes (CQA). % Acetonitrile (ACN), KH_2PO_4, pH and Flow rate was identified as CMA. **Methods:** The effective chromatographic separation was achieved by utilizing Phenomenex Enable C_{18} column and mobile phase consisted of ACN and Phosphate buffer (60:40 %v/v, pH 5) adjusted with orthophosphoric acid and the flow rate of 1.2 ml/min. the elution was monitored at 290nm using a PDA-UV detector. **Results:** The developed method resulted in eluting the drug at 2.190 min, respectively. The regression coefficients were observed to be 0.998 for all models. The LOD was about 10.74 nano-gram/ml and LOQ were about 31.74 nano-gram/ml. The relative standard deviation was observed to be 0.5079%. **Conclusion:** The results of the study demonstrated that the suggested RP-HPLC method is rapid, simple, accurate and precise. The method was validated by determining its accuracy, precision and system stability.

Key words: RP-HPLC, Tyrosine kinase inhibitors, Pazopanib, Dasatinib, Quality by Design.

Correspondence

Dr. V. Parthasarathy, Ph.D., Post. Doc. Res.,
Professor, Department of Pharmacy, Director, Centre for Cell Biology and Drug Discovery Annamalai University, Annamalainagar-608002, Chidambaram, Tamil Nadu, INDIA.

Phone no: +919443512724

Email: vapartha@yahoo.com

DOI: 10.5530/ijpi.2019.3.25

INTRODUCTION

Pazopanib (PAZ) is a second-generation tyrosine kinase inhibitor (TKI).[1] It is generally presented as white to yellow solid form. The molecular formula is $C_{21}H_{23}N_7O_2S$ and the chemical name is 5[{4(2,3-dimethyl-2H-indazol-6-yl)methylamino}2-pyrimidinyl]2-methylbenzenesulfonamide (Figure 1) and the molecular weight of 437.52 gm/mol.

Figure 1: Response Surface Methodology (RSM) is a statistically designed experimental tool where large numbers of factors are simultaneously studied.[2,3] The multivariate method has advantages, including a reduction in the number of trial runs, improves statistical explanation possibilities and indicates whether parameters interact or not. CCD is known as a multivariate experimental design which is used to optimize the chromatographic parameters and their interaction effects and quadratic effects of the mobile phase composition, pH and flow rate on the peak area.[4] The validation of the proposed method was carried out according to ICH guideline ICH Q2 (R1).[5] AQbD plays a vital role in developing a robust method as an early risk assessment and helps to identify the critical analytical parameters and to focus on these factors in method development.[6,7] Hence, the present study aimed at the development of a new, rapid, sensitive and validated RP-HPLC method for the analysis of PAZ in bulk and pharmaceutical dosage forms.

MATERIALS

Chemicals and reagents

Pazopanib references sample was a gift from MSN Labs Ltd, Hyderabad, India. The chemicals and reagents include Acetonitrile, Pottasium dihydrogen orthophosphate Buffer and Orthophosphoric acid AR grade was obtained from Sd Fine-Chem Ltd and Milli Q water (Merk) were of HPLC grade. It is commercially available as Votrient® marketed by GSK Rx India with a labeled claim of 200mg per tablet.

Instrumentation

The HPLC analysis was carried out with a Shimadzu HPLC system, with two LC-20AD separation modules and SPD-m20A PDA detector, a Rheodyne injector. The chromatographic and integrated data were recorded using LC solution data acquisition software. Absorbance spectra were recorded using a UV-VIS spectrophotometer.

Statistical software

Experimental design, data analysis and desirability function estimate were performed by utilizing version 11 of Design-Expert® programming.

Preparation of Buffer

Weighed 0.680gm of KH_2PO_4 transferred into a 500ml volumetric flask and added 400ml of Milli Q water and mix well and make up the fi-

nal volume by using solvent. Adjusted the pH of the buffer solution to 5 (±0.5) with orthophosphoric acid.

Preparation of Standard Solution

Stock standard solution of PAZ was prepared in the mobile phase. The stock solution was stored at 4°C ± 0.05 and protected from light. Working standard solution was PAZ freshly prepared by diluting the stock solution with a mobile phase before the analysis. Calibration curves revealing peak area ratios of PAZ were prepared at the range of 2, 4, 6, 8 and 10µg/ml.

Preparation of Sample

Ten tablets (Votrient) were weighed and then powdered, which is equivalent to 100mg of PAZ into a 10ml of volumetric flask and added 8ml of mobile phase and sonicated for 30 min with occasional shaking. The volume made up to 10mL with mobile phase and mixed. Filtered the solution through the 0.45µm membrane filter. Transferred 1ml of above solution into a 10ml volumetric flask, diluted to the final volume of 10ml with mobile phase.

Preparation of Internal Standard Solution

The internal standard "Dasatinib" (DTN) was taken for achieving chromatographic differentiation for separation at particular retention time for working standard PAZ. For this, 10mg of DTN dissolved in 2ml of mobile phase and volume was adjusted to 10ml with mobile phase to form the internal standard solution. Further, the solution was suitably diluted to 5mg/ml concentration and the precisely 1 ml solution was added to each dilution of the standard solution of PAZ.[8]

Selection of detection wavelength

For RP-HPLC method analytical wavelength was determined from UV-spectra of PAZ and DTN recorded by using UV-VIS spectrophotometer. Solutions of both the drugs were scanned in the UV range between 200 to 400nm against blank. PAZ and DTN showed significant absorbance at 290nm using PDA detector.

Method development by using the QbD concept

Step 1: Analytical target profile (ATP)

The first step was to clearly define the objectives of the method development, called the ATP. The HPLC method should allow the simultaneous separation of the two drugs, in reasonable analysis time. The main objectives were, firstly, to optimize the chromatographic conditions to improve the quality of chromatogram in terms of resolution, capacity and separation factor. Secondly, to successfully apply the developed method for estimation of PAZ and DTN.[9,10]

Step 2: Risk assessment

In HPLC, numerous factors can influence the quality of separation, mainly the column configuration and the mobile phase. Other factors, such as column temperature, detection parameters, sample composition and injection volume, can also affect method performances. Each analytical step was examined and risk assessment was performed based on the risk severity, occurrence and detect ability. Although risk assessment appears to be straightforward based on mathematical calculation, correct assessment of each factor was ensured by referring chromatographic science and practical experience in the field.[11]

Step 3: Performing experimental design

Following risk analysis, preliminary method development trials were conducted to select appropriate ranges of method variables. DOE methodology was used and for this purpose, an efficient and comprehensive CCD was selected. This design is based on three factors at three levels requiring 20 experimental runs plus 6 central point replications. Central replicates help to establish reproducibility and improve the validity of experimental design.[12] The key independent variables of the HPLC method were selected as % ACN, pH and flow rate variable as retention time, capacity factor, resolution factor, separation factor.

Step 4: Analysis of experimental results and optimization of the method

Systematic statistical analysis of experimental results was carried out using Design expert Software. After specifying variable constraints, numerical and graphical optimization was carried out. Statistical tools like predicted vs. actual plot, ANOVA, lack of fit, prediction equations and contour plots were used to analyze each individual response parameter and design space was generated.

Step 5: Defining the analytical method performance strategy

Overall understanding of method performance under various experimental conditions enabled to set the control strategy to manage risk and ensure that the method delivers desirable quality attributes.[13]

Chromatographic Conditions

The composition of mobile phase was ACN: KH_2PO_4 (pH 5) at the ratio of 60:40 v/v was used in isocratic mode at a flow rate of 1.2 ml/min. The mobile phase was filtered through a 0.45 µm membrane filter and sonicated for 20 min before use. Injection volume was 20µl and detection was performed at 290nm at ambient temperature.

METHODS

Linearity

Linearity, a concentration ranges from 2 to 10 µg/ml of PAZ was prepared. The calibrated graph was plotted by taking the peak area versus concentration. The correlation coefficient, intercept, slope and linear regression analysis were done.

Accuracy

Standard mixtures of PAZ and DTN were analyzed by HPLC using optimized chromatographic conditions. Accuracy was tested for three different concentrations within the calibration range in triplicate (n = 3X3). Percentage recoveries and RSD were calculated.

Precision

The precision of the developed method was assessed by determining intra-day variation and reported as percentage RSD. For evaluation of intra-day precision of the optimized method, three different concentrations of drug were analyzed in triplicate.

Specificity

The specificity of the developed analytical method was assessed for PAZ and DTN in combination and was further established by carrying out forced degradation analysis.

Quantification Limits

Limit of Detection (DL) was established with the help of the following equation: 3.3 σ/S and limit of Quantitation (QL) was determined using the equation: 10 σ/S, where, σ is the standard deviation of the responses and S is the slope of the calibration curve.

RESULTS

Method development and optimization

In this experimental work, firstly, the ultraviolet absorption spectrum was obtained and the maximum absorption peak was found at 290 nm as (Figure 2A). Therefore, the detection wavelength of the detector was set at this wavelength for further analysis. Various solvent systems have been reported in the literature for the chromatographic analysis of PAZ and DTN individually. By taking into account the nature of drugs under study, the method development trials were initiated by using C_{18} column and ACN: KH_2PO_4 as mobile phase. Figure 3B shows representative chromatogram generated over 6 min showing peaks of both the drugs. These initial trials were used as a basis to decide experimental ranges of three CMAs, pH, flow rate and %ACN in the mobile phase. Therefore, these factors were chosen as independent variables for experimental design. Central Composite Design values of the 20 experimental runs and their results are shown in Table 1 and 2. The excellent resolution was obtained between two major peaks. The collected data were subjected to statistical analyses using Design-Expert software.

A steepest slope or curvature indicates the sensitiveness of the response to a particular factor (Figure 3A). (a) The flow rate (factor C) had the most important effect on a retention time tR_2, followed by factor A and B. (b) The factors %ACN and pH (A and B) had a significant effect on K_1, followed by factor C. (c) The pH (factor B) had the most important effect on an $Rs_{(1,2)}$ followed by factor A and C. (d) The factors pH and flow rate (B and C) had a significant effect on $S_{(1,2)}$ followed by factor A.

Response surfaces plots for K_1, $Rs_{(1,2)}$ and $S_{(1,2)}$ and tR_2 are illustrated in Figure 3B (% ACN concentration was plotted against the pH. Flow rate held at constant at the center value).

Design space constitutes the ranges of variables as 30-70 % of ACN content in the mobile phase, 0.6-1.2 ml/min of flow rate and 3-5 pH of aqueous phase to get desired retention time 1.736 min. of DTN peak. Indicated design space was explored and the working point was selected on the basis of highest desirability and with sufficient surrounding design space. The predicted experimental conditions were verified experimentally and the resulting chromatogram was identical to Figure 2B. Peaks at retention time 1.736 and 2.190 min. correspond to DTN and PAZ, respectively.

Validation

Linear regression analysis obtained the r2 values as 0.998 for PAZ (Table 3A), confirming the linear relationship between the peak area and the concentration of the drug. In accuracy and precision study percent recoveries were found to be in the range of 99.65 to 100.65 for PAZ (Table 3C). Both intraday precisions measured in terms of %RSD was less than 2.5% over the chosen range of both the drugs (Table 3B).

Application of the developed method

The developed RP-HPLC method is sensitive and specific for the quantitative assurance of PAZ. The technique was approved for various parameters and, consequently, has been applied for the estimation of the drug in pharmaceutical dosage forms, such as tablets. Each tablet was analyzed in triplicate. The recovered amount of PAZ was 99.78% (Table 3D). None of the ingredients of tablet interfered with the analyte peak.

The measured signal was shown to be precise, accurate and linear over the concentration range tested with a retention time of 2.190 min and made the method economical due to lower solvent consumption. The % RSD for all parameters was observed under 2, which shows the validity

Table 1: Central Composite Design with measured response.

Run	Factor A (ACN %v/v)	Factor B (pH)	Factor C (Flow rate)	Response 1 (tR)	Response 2 (K_1)	Response 3 (Rs)	Response 4 (S)
1	50	4	0.9	2.996	0.178	1.183	1.414
2	50	2.3	0.9	2.382	0.504	2.538	1.263
3	50	4	0.39	6.763	0.141	1.326	0.973
4	16.3	4	0.9	6.541	5.38	1.596	1.235
5	70	5	0.6	4.361	0.316	4.506	2.251
6	30	5	1.2	1.573	0.046	1.362	1.863
7	83.6	4	0.9	2.421	7.242	1.64	1.619
8	70	3	0.6	2.825	1.121	0.506	1.117
9	70	3	1.2	1.422	0.138	0.468	1.558
10	50	5.6	0.9	5.577	0.48	5.381	1.623
11	50	4	0.9	3.004	0.181	1.141	1.416
12	30	3	1.2	3.095	0.677	0.305	1.561
13	30	3	0.6	5.081	0.062	2.572	1.247
14	50	4	0.9	2.988	0.171	1.191	1.524
15	50	4	0.9	3.017	0.189	1.121	1.496
16	50	4	0.9	2.996	0.193	1.145	1.419
17	50	4	1.4	1.92	0.874	1.132	1.721
18	70	5	1.2	2.117	0.253	3.859	2.535
19	50	4	0.9	2.992	0.175	1.129	1.422
20	30	5	0.6	3.259	0.037	0.707	2.631

tR= Retention Time, K1=Capacity, Rs= Resolution and S= Separation

Table 2: ANOVA for response surface reduced quadratic model.

Source	Sum of Squares				d_f				Mean Square				F-Value				*p*-Value				
	tR	K_1	Rs	S	tR	K_1	Rs	S	tR	K_1	Rs	S	tR	K_1	Rs	S	tR	K_1	Rs	S	Sig
Model	25.05	50.63	32.13	1.64	3	9	9	3	8.35	5.63	3.57	0.54	7.0	3.26	9.7	4.4	0.0032	0.040	0.0007	0.0185	Sig
%ACN	6.21	1.26	1.46	0.04	1	1	1	1	6.21	1.26	1.46	0.04	5.2	0.72	3.9	0.3	0.0365	0.414	0.0736	0.5423	
pH	1.33	0.141	9.46	1.42	1	1	1	1	1.33	0.141	9.46	1.42	1.1	0.08	25	11	0.3068	0.780	0.0005	0.0036	
Flow rate	17.51	0.047	0.503	0.17	1	1	1	1	17.51	0.047	0.503	0.17	14	0.02	1.3	1.4	0.0015	0.871	0.2679	0.2542	
Residual	19.08	17.28	3.66	1.96	16	10	10	16	1.19	1.73	0.366	0.122									
Lack of Fit	19.08	17.28	3.66	1.95	11	5	5	11	1.73	3.46	0.731	0.176					0.0001	0.0001	0.0001	0.0001	Sig
Pure Error	0.0005	0.0004	0.004	0.01	5	5	5	5	0.0001	0.0001	0.0008	0.002									
Cor Total	44.13	67.91	35.79	3.60	19	19	19	19													

ANOVA indicates analysis of variance; df-degrees of freedom, F-Fischers ratio and Sig- Significant

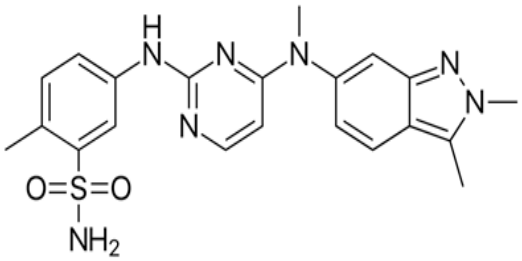


Figure 1: Chemical structure of Pazopanib.

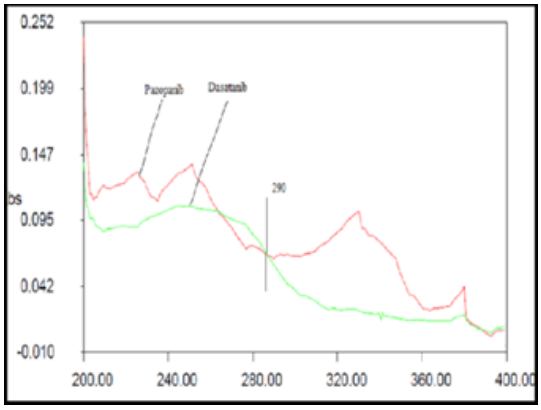


Figure 2A: Result of UV overlay spectra of (a) Pazopanib, (b) Dasatinib.

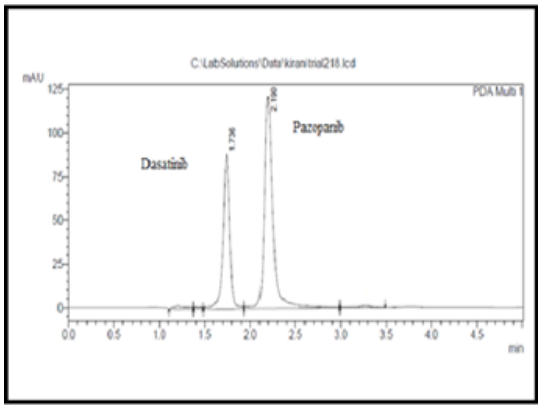


Figure 2B: Optimized chromatogram of Pazopanib.

of technique and assay results obtained by this method are in reasonable agreement. Chromatogram of PAZ is given in Figure 2B.

DISCUSSION

In this study, AQbD concept was used in the development of HPLC method for the simultaneous analysis of PAZ and DTN in the presence of samples. CQA was identified as capacity, resolution, separation factor and retention time. On the basis of risk priority number, mobile phase parameters were found to be most critical for the given analysis. Therefore, three parameters, pH, flow rate and % ACN in the mobile phase were selected as CMA.

A Central Composite experimental design with three independent variables at four levels was employed to optimize critical method parameters. All of the 20 experiments were performed randomly and chromatograms

Table 3: (A, B, C, and D): Results of Linearity, Precision, Accuracy, and Assay for Pazopanib

Table 3A: Linearity values for PAZ.

Conc. (µg/ml)	Avg Area
2	0.322196
4	0.635566
6	0.964382
8	1.244366
10	1.622547

Table 3B: Precision values for PAZ.

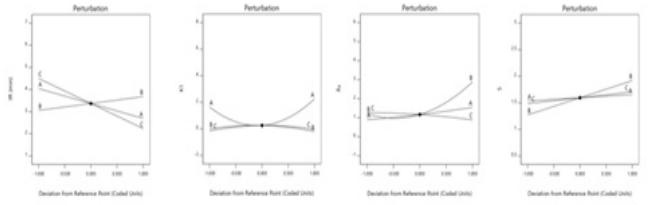
Conc	Drug Area	Internal Std	Drug/IS	Avg	Sdv	%RSD
	26685	81111	0.328994			
2	26268	81528	0.322196	0.322219	0.006763	2.098877
	25851	81945	0.315468			
	51329	79688	0.644125			
4	50912	80105	0.635566	0.635604	0.008502	1.33764
	50497	80522	0.627121			
6	79181	81259	0.974427	0.964405	0.010012	1.038142

Table 3C: Accuracy study for PAZ.

Percentage	PAZ	IS	Pazo/IS	AVG	Sdv	%RSD	%Recovery
	231527	81847	2.828778				
80%	233286	81049	2.878333	2.865816	0.032632	1.138665	99.65
	235045	81321	2.890336				
	261165	82592	3.16211				
100%	262924	81877	3.211207	3.18823	0.024699	0.774692	99.78
	264683	82937	3.191374				
	287433	81721	3.517248				
120%	289192	81639	3.542327	3.538202	0.019227	0.543418	100.65
	290951	81842	3.555033				

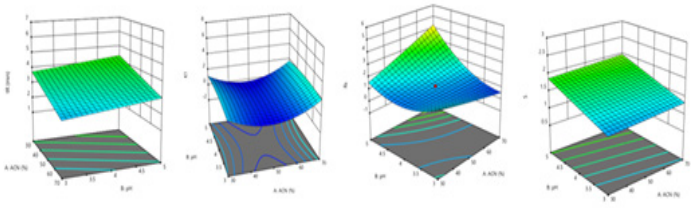
Table 3D: Assay for PAZ in tablet formulation.

Conc	PAZ	IS	PAZ/IS	Obt. Amt	Mean	SD	%RSD	Lab.Amt	%Recovery
	131622	82368	1.597975	10.018					
10	131215	82775	1.585201	9.938	9.978	0.04	0.40088	200 mg	99.78
	130808	82182	1.591687	9.978					



(a) (b) (c) (d)

Figure 3A: Perturbation plots showing the effect of each independent variables on (a) tR (b) K_1 (c) Rs, (d) S, where A is acetonitrile concentration, B is the pH of buffer, C is the flow rate.



(i) (ii) (iii) (iv)

Figure 3B: Response surfaces related to acetonitrile (A), pH (B) and flow rate (C): (a) retention time of the last peak (tR2); (b) capacity factor first peak (K_1); (c) resolution factor (Rs); and (d) separation factor (S).

Figure 3: (A&B): Results of Perturbation and Response surface Method.

obtained were evaluated for capacity, resolution, separation and retention time for peaks of both the drugs.

Analysis of perturbation plots and response plots of optimization models revealed that the factor A and B had the significant effect on separation of the analytes, whereas the factor C, i.e., the Flow rate, is of less significance.

The identified point is characterized by the specific CMA combination as mobile phase consisting of Acetonitrile: KH_2PO_4 to pH 5 using orthophosphoric acid (60:40, %v/v) at ambient temperature. The design space presents the operable method region where the changes will not affect the quality of analysis.

Specificity was assessed by percent recovery of both the drugs when analyzed in combination. Percent recoveries of PAZ were within statistical limits. It was observed that the peaks of each of the drugs were well separated and not interfering. Thus, it can be said that the method is specific to each of the two drugs in combination. The estimated LOD and LOQ values confirmed that the methods are sufficiently sensitive. Moreover, percent recoveries of the drugs were found to be acceptable. Hence, the developed method can be suitable, utilized for concurrent, quantitative analysis of PAZ and DTN.

The method was validated for linearity, precision, accuracy, sensitivity, system suitability, as well as robustness. The developed method is convenient and effective for quality control as well as simultaneous routine analysis of PAZ in pharmaceutical dosage forms.

CONCLUSION

This study presents a systematic HPLC method development for the simultaneous analysis of PAZ and DTN, by application of AQbD. The developed method is rapid and reliable for analysis over the method operable design space. Chromatographic peaks of both the drugs were well separated, without any interference from samples. Validation report confirms that the method has good linearity, accuracy, precision, specificity and purity.

ACKNOWLEDGEMENT

Authors extend thanks to UGC for the financial support through UGC BSR Fellowship. I am thankful to Annamalai University, Mr. A. Arenganathan, Asst. Technical Officer, Department of Pharmacy, Annamalai University, Annamalainagar, Chidambaram, Tamilnadu-608002 for providing the necessary laboratory facilities and technical support to carry out this research study.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest. The article does not contain any studies with animals or human participants performed by any of the authors.

ABBREVIATIONS

RP-HPLC: Reverse Phase High Performance Liquid Chromatography; **TAP:** Target Analytical Profile; **CMA:** Critical Method Attributes; **CQA:** Critical Quality Attributes; **ACN:** Acetonitrile; **TKI:** Tyrosine Kinase Inhibitor; **RSM:** Response Surface Methodology; **CCD:** Central Composite Design; **QbD:** Quality by Design; **ATP:** Analytical Target Profile; **DOE:** Design of Expert; **LOD/DL:** Limit of Detection; **LOQ/QL:** Limit of Quantification; **PAZ:** Pazopanib; **DST:** Dasatini.

REFERENCES

1. Escudero-Ortiz V, Perez-Ruixo JJ. Development and validation of an HPLC-UV method for Pazopanib quantification in human plasma and application to patients with cancer in routine clinical practice. The Drug Monit. 2015;37(2):172-9.
2. Montgomery D. Design and analysis of experiment New York. John Wiley and Sons. 1991.
3. Shiow-Ling L, Wen-Chang C. Optimization of medium composition for the production of glycosyltransferase by Aspergillus niger with response surface methodology. Enz Microb Technol. 1997;21(6):436-40.
4. Ferreira SL, Bruns RE, Ferreira HS, Matos GD, David JM, Brandao GC, *et al.* Box-Behnken design: an alternative for the optimization of analytical methods. Anal Chim Acta. 2007;597(2):179-86.
5. ICH Q2 (R1). Validation of analytical procedures: Text and methodology. International Conference on Harmonization Secretariat, Geneva. 2005.
6. Bhutani H, Kurmi M, Singh S, Beg S, Singh B. Quality by Design (QbD) in analytical sciences: An overview. Pharm Times. 2014;46:71-5.
7. Schmidt AH, Stanic M, Molnar I. *In silico* robustness testing of a compendial HPLC purity method by using a multidimensional design space build by chromatography modeling-case study pramipexole. J Pharm Biomed Anal. 2014;91:97-107.
8. Sarwar B, Kanchan K, Suryakanta S, Saquib HM. Development and validation of RP-HPLC method for quantification of Amoxicillin tryhydrate in bulk and pharmaceutical formulations using Box-Behnken experimental design. Journal of Liquid Chromatography and Related Technologies. 2012;35(3):393-406.
9. Guideline IHT. Pharmaceutical development. Q8 (2R) As Revised in August. 2009.
10. Guideline IHT. Quality risk management. Q9, Current Step. 2005;4:408.
11. ICH Q14: Analytical Procedure Development and Revision of Q2(R1) Analytical Validation November. Final Concept Paper. 2018.
12. Kochling J, Wu W, Hua Y, Guan Q, Castaneda-Merced J. A platform Analytical Quality by Design (AQbD) approach for multiple UHPLC-UV and UHPLC-MS methods development for protein analysis. J Pharm Biomed Ana. 2016;125:130-9.
13. Bhatt D, Rane S. QbD approach to analytical method development and its validation. Int J Pharmacy Pharm Sci. 2011;3(1):179-87.

Cite this article: Buralla KK , Parthasarathy V. Quality by Design based Developed and Validation of RP-HPLC Method for Simultaneous Estimation of Pazopanib in Bulk and Pharmaceutical Dosage Forms. Int. J. Pharm. Investigation. 2019;9(3):135-40.