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ORIGINAL ARTICLE

QbD-Driven Development and Validation of a U-HPLC Method for Estimation of Brivaracetam Bulk and its Formulation

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ABSTRACT

The current studies entail Quality by Design (QbD)enabled development of a simple, rapid, sensitive and cost-effective ultra high-performance liquid chromatographic method for estimation of Brivaracetam (BR).Quality by Design approach to method development uses statistical design of experiments to develop a robust method 'design space'. The design space defines the experimental region in which changes to method parameters will not significantly affect the results. The present study describes the development of a comprehensive science and risk based HPLC method and subsequent validation for the analysis of BR drug substances and drug products using a quality by design approach. The optimal chromatographic separation was achieved using Methanol and 0.1% OPA in ratio of 60:40v/v (pH 2.8) as the mobile phase with a flow rate of 1 mL/min by using a DAD detector at 224 nm. The developed method was validated as per international conference on Harmonization guidelines with respect to specificity, limit of detection, limit of quantification, precision, linearity, accuracy, robustness and ruggedness.

Keywords: *Brivaracetam, Quality By Design approach,U-HPLC,DAD.*

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INTRODUCTION

Brivaracetam [(2S)-2-[(4R)-2-oxo-4-propyl-tetrahydro-1H-pyrrol-1-yl]butanamide] used in the treatment of the partial-onset seizures[3]. Brivaracetam is believed to act by binding to the ubiquitous synaptic vesicle glycoprotein [4]. Brivaracetam (Briviact), a chemical analog of levetiracetam [5]. Chemical Structure of Brivaracetam was shown in the Figure 1:

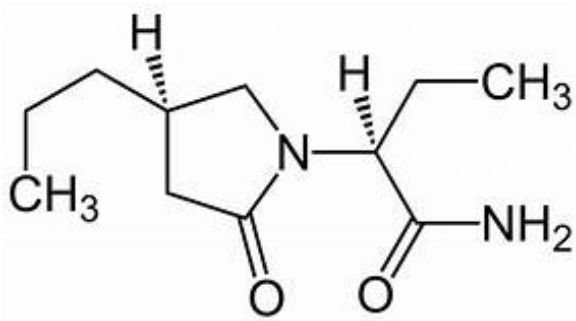

Fig.-1: Chemical structure of Brivaracetam

Method validation as per the recommended ICH guidelines does not provide much reliability with respect to reduction in method variability beyond the conventional robust testing. Thus,
Implementation of Quality by Design (QbD) principles for analytical method development has now been practiced quite popularly for attaining high robustness and enhanced method performance (11). The AQbD approach facilitates science and risk-based understanding of the major sources of variability,

followed by identification of CMPs using risk assessment and factor screening studies to find out the highrisk variables with critical impact on the analytical performance followed by optimization of them using suitable experimental designs for augmenting method performance (12). In the past few decades, literature reports on diverse drugs have vouched the phenomenal benefits of the AQbD approach for developing the analytical methods for the drug substances (13), impurities and degradation products in an effective and cost-effective manner. Thus, attempts, therefore, were made for the development of a simple, rapid, sensitive, robust, effective and economical U-HPLC method by employing analytical QbD principles for estimation of BR in bulk drug and pharmaceutical formulations. The rational use of experimental design has been explored for comprehensive understanding of the factor–response relationship followed by method validation Studies for ensuring the robust performance.

MATERIAL AND METHODS

Reagents and Chemicals:

Water, Methanol,Ortho-phosphoric acid,was used in the study.

Instrumentation:

Agilent (1100series) with Auto sampler and DAD detector with Chemstation software were used.

Chromatographic condition:

A High performance liquid chromatogram equipped with DAD detector, the purity determination performed on a Agilent C18 (100mm x 4.6 ID, Particle size: 2.5 micron) at ambient temperature using mobile phase consisting of Methanol: 0.1% ortho phosphoric acid (pH2.8) in the ratio of 60:40v/v respectively. The UHPLC system operated with an isocratic elution mode at flow rate of 1.0 mL/min. The injection volume was 20 µl. UV detection was carried out at 224 nm. Diluent was prepared by mixing in the ration of Methanol: 0.1% OPA in Water (60:40v/v).

Preparation of standard solution BR:

Weighed accurately about 10 mg of BR standard and transffered into 20mL of volumetric flask, added about 20 mL of diluent, shaked to dissolved and volume was made up to the mark with diluent. (concentration of BR is 1000 µg/ml) A-grade bulb pipette into 10 ml volumetric flasks and the solutions were made up to volume with mobile phase to give final concentrations of 10,20,30,40 and 50 µg/ml for BR.

Preparation of Sample solution BR:

Twenty tablets were weighed and finely powdered. An accurately weighed amount of powder equivalent to 10 mg of BR was transferred into a 20.0 ml volumetric flask. Then 20 ml of diluent was added in it. The flask contents were sonicated for 10 min to make the contents homogeneous. This solution was then diluted up to the mark with diluents. The resultant solution was filtered through Whatman Grade I filter paper. One milliliter of the filtrate was transferred to a 10 ml volumetric flask and then the volume was made up to the mark with diluent to furnish a sample solution containing 40 µg/ml of BR.

Experimental Design

The experimental design (regular Two level full factorial), desirability function and statistical data analysis calculations were performed by using Design-Expert® version 9.0.6 (Stat Ease Stat-Ease, Inc., Minneapolis, MN, USA). Several types of experimental designs (e.g. two levels full factorial, three level fractional factorial, Placket- Burman, mixed level designs) are available and these designs allow the simultaneous examination of qualitative, quantitative and mixture related factors.

RESULTS AND DISCUSSION

Initial method development

The main objective of the chromatographic method was to separate of BR, from each other and from the placebo peaks. A blend solution prepared from the tablets containing 1000 µg/mL of BR. A placebo solution was prepared as per test preparation and used to identify the placebo peaks. Before starting the development impurity mix, placebo and degradation samples analyzed with different HPLC method, it was observed that base line was not good. To achieve shorter run time and good baseline different organic solvents along with different compositions in different columns were tried for the separation. The chromatographic separation was achieved on Agilent C18, 100 x 4.6mm, 2.5 µm column with mobile phase containing a mixture of 0.1% OPA in water (pH 2.8): Methanol, in the ratio of 40:60 v/v respectively. Flow rate was 1.0 mL/min and the column oven temperature was maintained at Ambient. The injection volume was 20 µl and UV detection was carried out at 224 nm. After this initial optimization, method was subjected to factorial design to study the variables which can influence the resolution between BR.

Method Optimization by Design of Experiments

A two level full factorial was selected for the present study to determine the main effects and all interactions between the factors, leading 2f experiments, where f is factors. During the preliminary study, factors (f) which could have significant affects were extracted for further analysis. Based on the initial separation flow rate and Methanol ratio,were selected as critical parameters (Table-1) to evaluate the quality target method profiles (resolution and RRT) and critical quality attributes. Evaluating all of these parameters with A two level full factorial would involve 9 = 9 trials. Total 9 runs were performed. In all the experiments RS 1, were monitored. These experiments were performed and the results are summarized in the (Table-2)

Table-1: Factors and Critical Quality Attributes

CMPs	Range of Each Parameter Used for DOE			QTMP	CQA
	Original condition	Low Level	High Level		
%Methanol	60%	55 %	65%	Resolution NLT 2	RS 3:BR
Flow Rate	1 ml/min	0.9 ml/min	1.1 ml/min	RRT of BR	

RRT: Relative retention time; Critical Method Parameters (CMPs); Quality Target Method Profile (QTMP); Critical Quality Attributes (CQA)

Table-2: Matrix of Experiments for 2 Full Factorial Designs

		Factor 1	Factor 2
Std	Run	A:Flow rate	B:Methanol
		ml/min	%
8	1	1	65
1	2	0.9	55
3	3	1.1	55
5	4	1	60
6	5	1.1	60
4	6	0.9	60
7	7	0.9	65
2	8	1	55
9	9	1.1	65

The results (Table-3) after completion of the 9 experiments were analyzed through Design Expert ®software. The effect on the two dependent variables with the independent variables was explained by using Cubical graphs (Figures- 2 and 4).

Table-3: Response factors Values in 3 Factorial Design

		Factor 1	Factor 2	Response 1	Response 2
Std	Run	A:Flow rate	B:Methanol	RT	Peak Area
		ml/min	%	Min	
8	1	1	65	6.1	3758.04
1	2	0.9	55	11.12	4272.7
3	3	1.1	55	9.2	3229.32
5	4	1	60	7.8	3742.55
6	5	1.1	60	7.1	3341.53
4	6	0.9	60	8.7	4278.26
7	7	0.9	65	6.9	4285.84
2	8	1	55	10.2	3742.55
9	9	1.1	65	5.6	3337.77

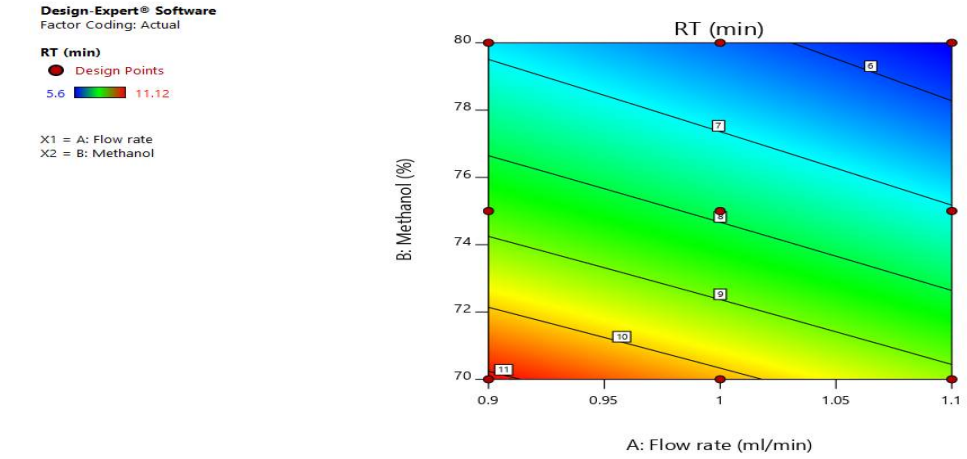

Fig.2- Graph for Response 3 (RT)

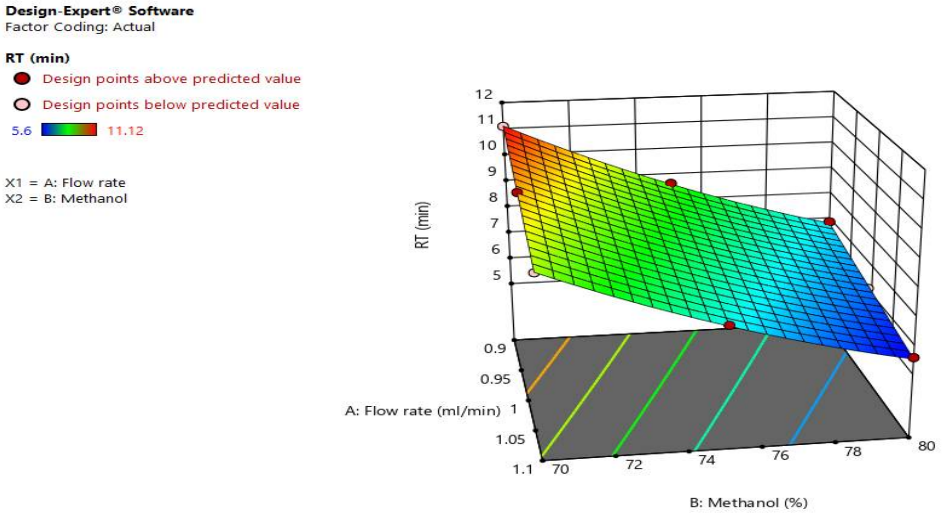

Fig.-3: 3D Surface Model Graph for Response 3 (Resolution 1)

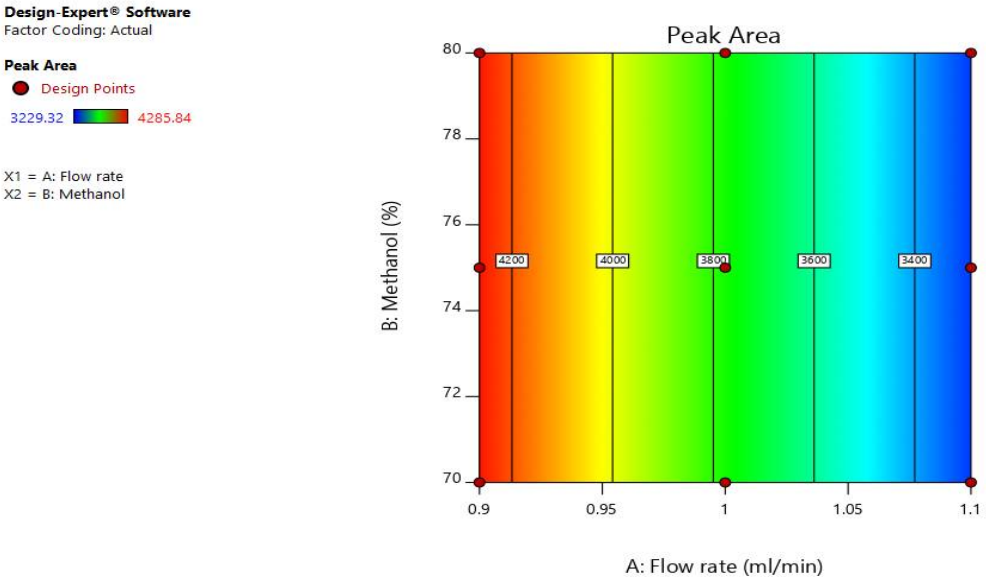

Fig.4- Graph for Response 3 (Area)

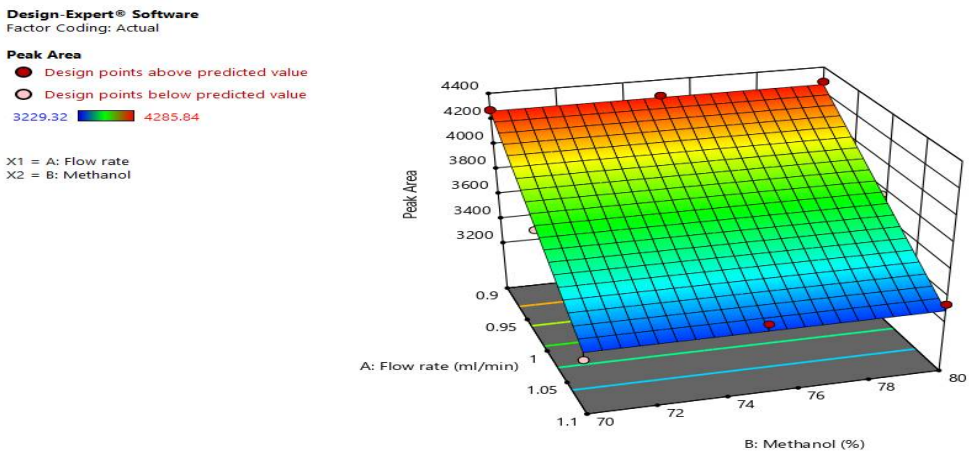

Fig.-5: 3D Surface Model Graph for Response 4 (Resolution 2)

Significant affects were observed due to the Flow Rate and Methanol ratio in the mobile phase. No significant affect was observed due to the flow rate of the mobile phase. The desirability zones for Resolution 3 (BR) and RRT of BR, Based on Design Expert analysis, the desirability 3D graphs (Figures-3,5) indicated that the maximum desirability was achieved for (a) amount of Methanol in mobile phase is about 60%, and (b) flow rate is about 1.0 mL/min. The **Predicted R^2** of **0.9945** is in reasonable agreement with the **Adjusted R^2** of **0.9988** for Response 3; RRT is 4.840 for BR was obtained from numerical optimization and point prediction calculations of post analysis (Figure- 6, 7). To confirm the point prediction values, experiments (n = 2) were conducted to determine the mean responses of Resolution 3, Resolution 4 found to be similar as predicted values.

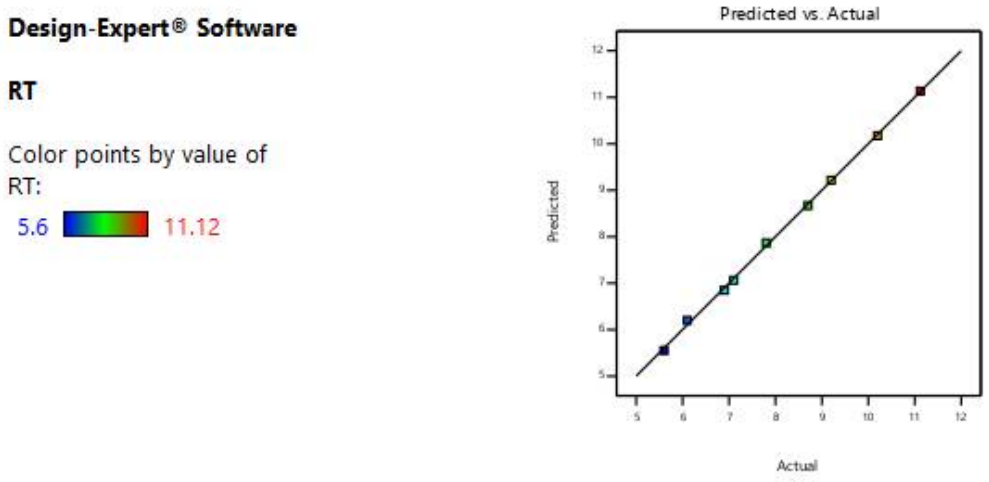

Fig.-6: Box-Cox Plot for Power Graph for Desirability of Response 3

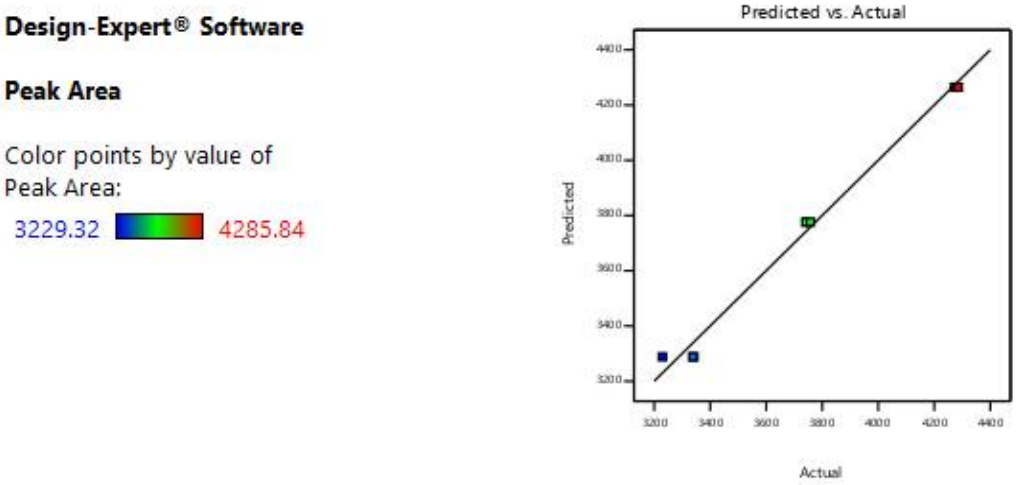

Fig.-7: Box-Cox Plot for Power Graph for Desirability of Response 4

From the pareto chart the resolution between BR was majorly affected by organic phase Methanol ratio, and followed by mixed interaction of flow rate. The definition for design space of a LC method can be "multidimensional combination and interaction of mobile phase (Methanol) and chromatographic parameters (flow rate) that have been demonstrated to provide assurance of result obtained with the method". The initial method development parameters were lying in middle of the design space; hence the initial developed method was finalized and performed method validation. Chromatogram of BR was shown in Figure-8 the overlay chromatogram of BR sample was shown in Figure-9 representing no interference of BR. Also it clearly shows excellent peak is observed.

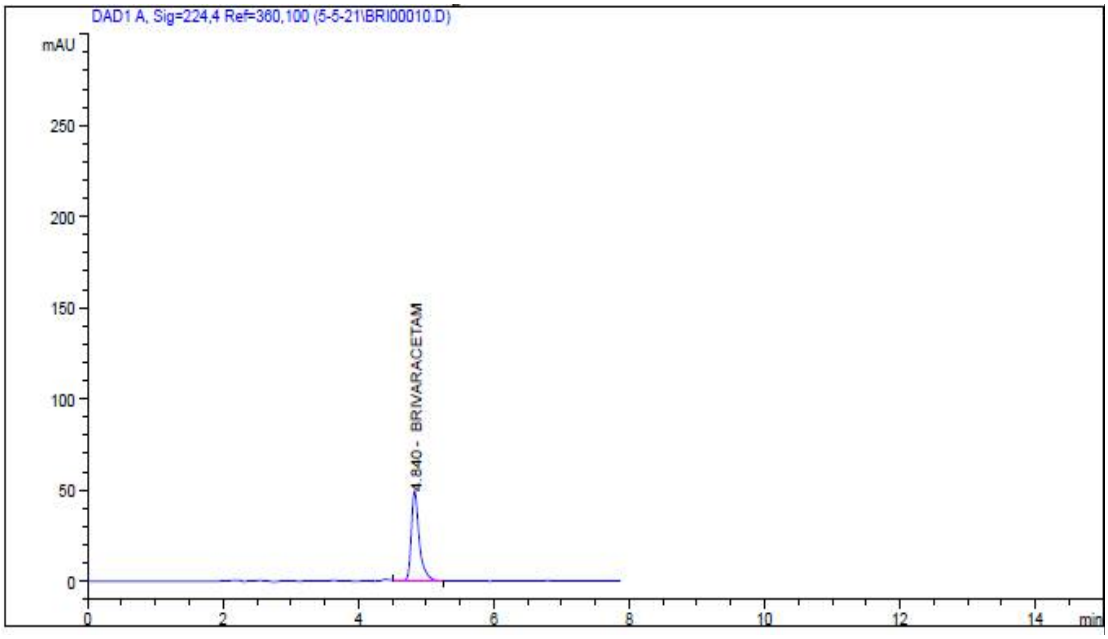

Fig.-8: Chromatograph of mixture of standard BR

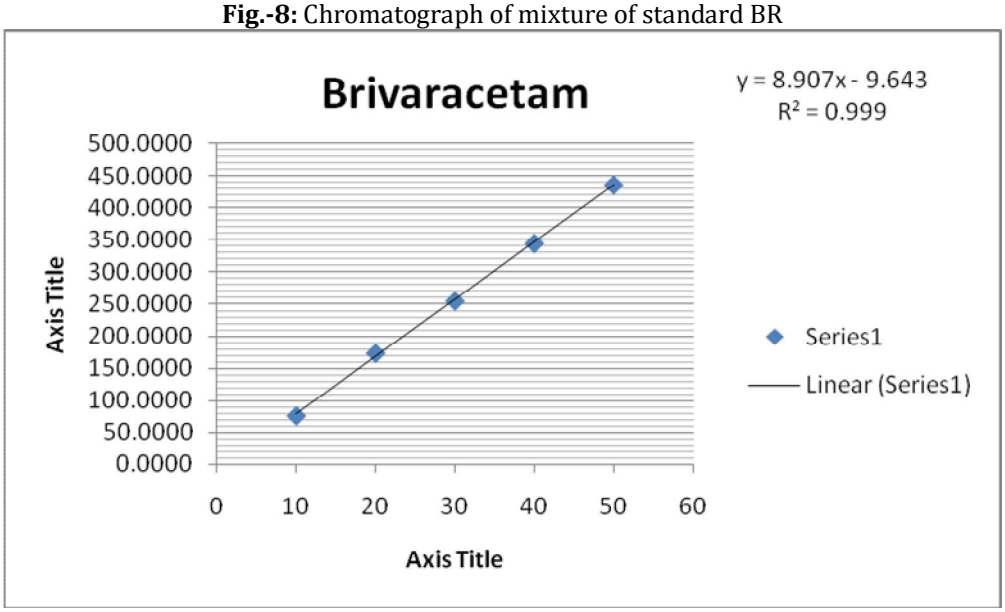

Fig.-9: linear regression for BR

Method Validation

As part of method validation Specificity, Precision, Linearity, LOD-LOQ, Accuracy, Robustness and Solution stability, parameters are verified.

System Suitability:

The suitability of the system was demonstrated by assessing various parameters. It was established by injecting two replicate injections of the standard solution. Theoretical plates were found to be 10048 and 10072, tailing factor of 0.66 and 0.67, and % RSD of peak area was 0.29 for BR. All the system

suitability parameters were well within the limits, indicating that the system was well suitable for performing the analysis.

Linearity

Linearity was established by the least-squares linear regression analysis of the calibration data. Calibration plots were linear over the concentration range of 10-50 µg/ml for BR. Peak areas were plotted against the respective concentrations and linear regression analysis performed on the resulting curves. The linear curve of BR was shown in Fig. 9 and Fig.10. The linear regression equation obtained was Y=8.907-9.643 for BR with correlation coefficient 0.999.

Accuracy:

Accuracy was computed by recoveries studies. The mean percentage recoveries values for three levels were found to be between 99.59-101.45% for BR respectively. The percentage of recoveries values within the limits, indicating the method developed was accurate.

Precision

The %RSD of intraday precision and interday precision were 0.88 and 0.61 for BR. The percentage RSD of system, method, and intermediate precision study was well within the limits (<2%), indicate that the method was precise.

LOD and LOQ:

The LOD was found to be 2.112 µg/ml For BR. The LOQ was found to be 0.7185 µg/ml for BR. The values of LOD and LOQ indicate that the method was greatly sensitive.

Robustness

The robustness of the method was designed by changing the optimized condition adequately. To evaluate the robustness of the developed method, the chromatographic conditions were deliberately altered and the resolution between BR was evaluated. On the assessment of the result it can be deduced that the variation in the changing wavelength, the flow rate does not affect the method significantly. %RSD <2% specifies that the developed method was robust.

Analysis of BR from marketed tablets

The percentage assay of tablet formulation was found to be 100.08% for BR respectively.

CONCLUSIONS

The simple isocratic reverse phase UHPLC method was developed by QBD approach for quantitative analysis of Brivaracetam (BR) in drug products. The method is validated as per ICH guidelines and found to be specific, precise, linear, accurate, rugged, and robust. Satisfactory results were obtained from validation of the method. The method can be used for routine analysis of production samples and to check the stability of samples of BR formulations.

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