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Research Article

RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF BRIVARACETAM IN BULK AND FORMULATION

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ABSTRACT
The analytical method was developed and validated for estimation of Brivaracetam in bulk and formulation by Reverse Phase High performance liquid chromatography. The separation was carried out on Luna C8 (150 × 4.6mm, 5µm) column. The mobile phase consists of ACN: Water at flow rate 1 ml/min at 208nm. The column temperature was adjusted at 30°C with injection volume 20µl. The retention time of Brivaracetam was 3.1 min. The linearity of the calibration curve was linear over the concentration range 10-50 µg/ml. The developed method was validated according to the International Council for Harmonization (ICH) Guidelines. The developed method was easy, rapid, linear, precise, accurate and consistent. So, the method can be successfully applied for the routine analysis of Brivaracetam in pharmaceutical formulation.

Keywords: Brivaracetam, RP-HPLC, Method Validation, Estimation, Chromatogram.

1. INTRODUCTION
Brivaracetam-(2S)-2-[(4R)-2-oxo-4-propyltetrahydro-1H -pyrrol-1-yl] butanamide is an antiepileptic drug [1]. Brivaracetam is used along with other medications to control partial seizures in adults and children. Brivaracetam operates by decreasing abnormal electrical activity in the brain [2].

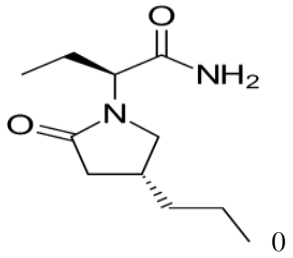

Fig. 1: Structure of Brivaracetam [3]

Brivaracetam chemically is a 4-n-propyl analog of levetiracetam and a racetam derivative with anticonvulsant properties [4]. Brivaracetam is believed to act by binding to the ubiquitous synaptic vesicles glycoprotein to 2A (SV2A). Phase II clinical trials in adult patients with refractory limited seizures were promising. It acts as a new high-affinity synaptic vesicle protein 2A (SV2A) ligand, displays inhibitory activity at neuronal voltage-dependent sodium channels, data from animal models suggested potent and broad-spectrum antiepileptic activities [5]. It is believed to play a role in the regulation of neurotransmission by stimulating vesicle fusion and maintaining a reverse of secretoryvesicles [6].
The pharmacokinetics of Brivaracetam exhibits linear pharmacokinetics over a wide dose range and is rapidly and completely absorbed after oral administration. Brivaracetam has an elimination half-life of 7-8 hours and has plasma protein binding of less than 20% [7]. Literature survey revealed that few methods are available for the analysis of Brivaracetam by RP-HPLC method. A few methods have been reported for the determination of the particular drug. The reported methods for the estimation of Brivaracetam were UV-Spectrophotometric methods [8], HPLC [9-11], UPLC [12], TLC [13]. This paved the way for us to carry out the analysis in pure as well as formulation.

2. MATERIAL AND METHODS
2.1. Chemicals and Reagents
The drug Brivaracetam was obtained as gift sample from Micro labs Ltd. Brevipil ™ (25mg) manufactured in India

by Sun Pharma Laboratories Ltd. East-Sikkim. Water (HPLC grade), Acetonitrile (HPLC grade) and Methano l (HPLC grade) were obtained from Merck Pharmaceutical Pvt. Ltd., Mumbai, India. 0.45 µm Millipore syringe filters (Ultipor® N_{66}®Nylon Membrane) were from PALL Life sciences.

2.2. Instruments

Analytical balance (Shimadzu AY220), UV-Spectrophotometer (Systronic-2201), Sonicator (Oscar Microclean 103). High Performance Liquid Chromatography (Younglin Acme 9000) were used for varied purposes.

2.3. Chromatographic equipment and conditions

Chromatographic separation was achieved on a Luna C8 (150 × 4.6mm, 5µm). The mobile phase used for the separation of Brivaracetam was Acetonitrile: Water in the ratio of 40:60. Flow rate was set at a 1ml/min at ambient temperature 30°C, an injection volume of 20 µL using UV-visible detector (UV730D) at wavelength of 208nm. The mobile phase was ultrasonicated for degassing and filtered through 0.45µm membrane nylon filter using vacuum pump before pumping into HPLC system.

2.4. Preparation of Mobile Phase

HPLC grade Acetonitrile and Water were used for the preparation of mobile phase in the ratio 40:60. Each component of mobile phase was filtered twice using 0.45µm membrane filter and degassed for 15min by sonicating each components of mobile phase.

2.5. Preparation of Solution

2.5.1. Preparation of Standard stock solution- I

Brivaracetam API (10 mg) was transferred into 10 ml of volumetric flask containing 5ml of diluent (Methanol). The volume was made up to the mark with diluent and sonicated for 5min. (Conc=1000µg/ml). From the stock solution, 3ml was pipetted out and transferred in to 10 ml volumetric flask and further made up the volume with water as a diluent and added further 20 ml with same (water) diluent. The final concentration of sample was 100µg/ml.

2.5.2. Preparation of sample solution

Twenty tablets of film coated Brevipil (25mg) tablets were weighed, crushed, finely powdered and mixed well. A portion of tablet powder equivalent to weight of 10 mg of Brivaracetam was transferred to a 10 ml of volumetric flask and volume was made upto the mark with same diluent (Water) and sonicated for 15 minutes to complete dissolution of drug. The solution was filtered through whatman filter paper no.1 to remove insoluble residue.

The above prepared solution was further diluted to get required concentrations and then analyzed. The content of the tablet was calculated from plotted calibration graph by using regression equation.

2.6. Validation of Analytical Method

The proposed method of analysis was validated by following ICH Q2 (R1) guidelines of studying the parameters like specificity, linearity & Range, Accuracy, Assay, Precision, LOD, LOQ and Robustness.

3. RESULTS AND DISCUSSION

3.1. Method Development

The proposed RP-HPLC method was developed and optimized for a series of trials in terms of mobile phase selection, composition, wavelength, choice of stationary phase of column, flow rate and column temperature. Brivaracetam showed the absorbance maxima at 208nm. Hence this wavelength was selected as working wavelength for the proposed RP-HPLC method.

3.2. Specificity

Blank, Standard and tablet solution were injected and chromatograms were examined. Specificity was determined by comparing test result obtained from analysis of sample solution containing excipient with that of test results those obtained from standard drug. There was no interference detected at retention time of Brivaracetam dummy solution. The retention time obtained from standard and sample solutions were presented in Fig. 2.

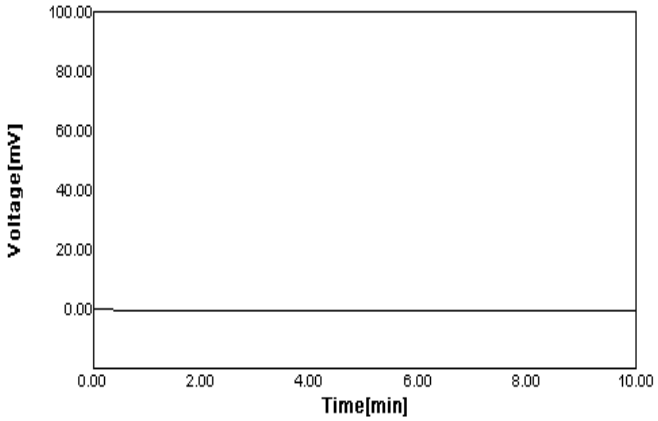

Fig. 2: Chromatogram of Blank in optimized chromatographic condition

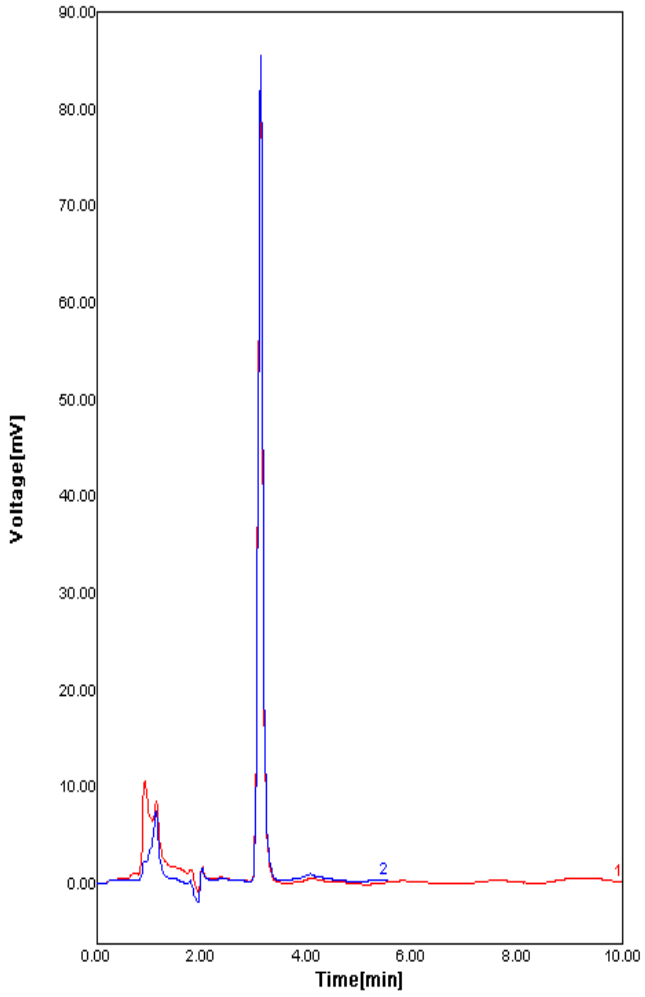

Fig. 3: Overlay of 20µg/ml solution chromatogram (Blue) and Brevipil 25mg tablet chromatogram (Red)

3.3. Linearity and Range

The calibration curve was constructed between concentrations verses peak area by preparing the concentration range of 10-50µg/ml (Table 1). The regression equation was found to be Y = 29.08x-0.4 and correlation coefficient of Brivaracetam was noted at 0.998 (Fig. 4). The overlay of chromatogram of concentration 10-50µg/ml Brivaracetam is given in Fig. 5.

Table 1: Linearity of Brivaracetam

Sr. no.	Conc.(µg/ml)	Area (mAU)
1	10	309
2	20	556
3	30	882
4	40	1290
5	50	1469

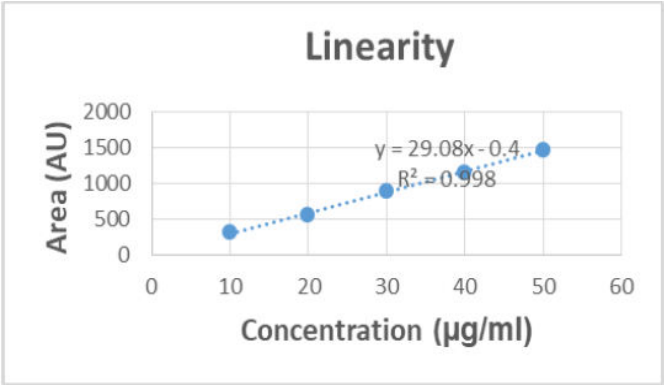

Fig. 4: Calibration curve of Brivaracetam

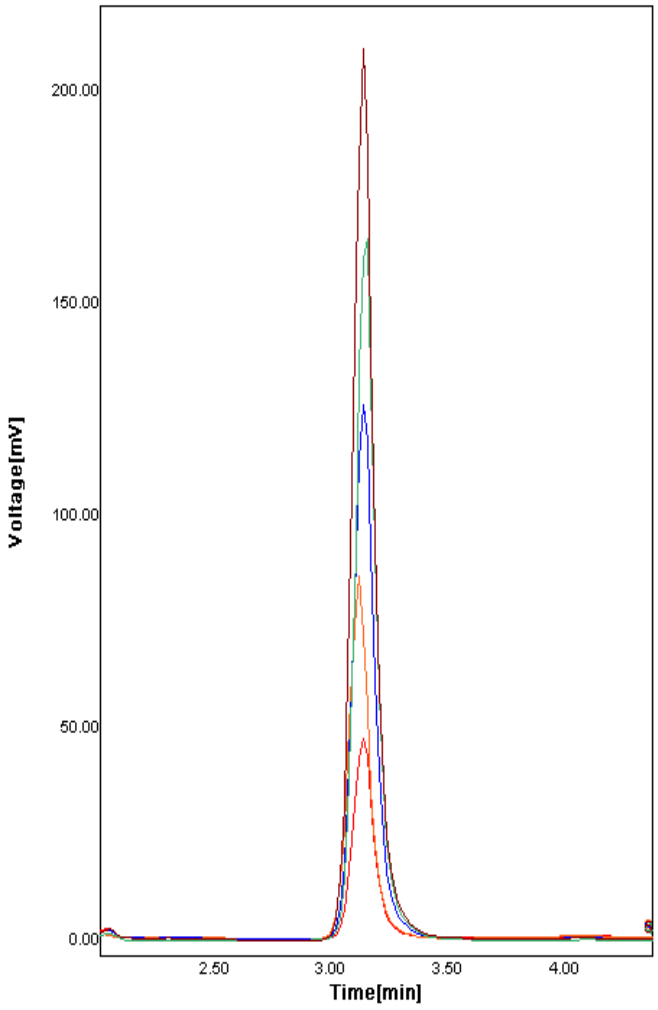

Fig. 5: Overlay of Chromatogram of 10-50µg/ml Brivaracetam

3.4. Precision

The % RSD values for intra-day and inter-day precisions were found to be < 2% for the proposed method which confirms the good precision of the method (Table 2).

3.5. Accuracy

The accuracy of the proposed method was found by standard addition method. A known amount of standard drug was added at 80%, 100% and 120% level. All the solutions were filtered through Millipore syringe and injected into the HPLC system and chromatograms were recorded under the same chromatographic conditions. The concentrations were analyzed with the above described procedure and also the percentage recovery was calculated (Table 3).

3.6. Assay

The method was successfully adopted for the determination of Brivaracetam 25mg film coated tablet. The amount of Brivaracetam was found to be 98.6% of label claim. The method can be employed for routine analysis of estimation of Brivaracetam in the formulation (Table 4).

3.7. Limit of Detection

The developed method was highly sensitivity with LOD of 2.67µg/ml.

3.8. Limit of Quantification

The developed method was highly sensitivity with LOQ of 8.11µg/ml.

3.9. Robustness

The robustness of the developed method was accessed by small changes in method parameter detection wavelength (±1nm). The %RSD of robustness was found to be less than 2% of the purposed method (Table 5).

Table 2: Precision result of Brivaracetam

Sr. No.	Interday Precision		Sr. No.	Intraday Precision	
Sample	Conc. ((µg/ml)	Area	Sample	Conc (µg/ml)	Area
1	20	594	1	20	608
2		605	2		598
3		608	3		588
4		591	4		605
5		580	5		606
6		598	6		595
	Average	596		Average	600
	S.D.	10.13		S.D.	7.72
	%RSD	1.70		%RSD	1.28

Table 3: Accuracy of Brivaracetam

Conc. of spiked level	Amount spiked (µg/ml)	Amount recovery (µg/ml)	% Recovery
80%	8	8.11	101.4
100%	10	10.17	101.7
120%	12	12.27	102.3

Table 4: Assay of Brivaracetam

Brand name	Amount spiked (µg/ml)	Amount recovery (µg/ml)	% Recovery
Brevipil 25mg	20	19.71	98.6

Table 5: Robustness of Brivaracetam

Parameter	Optimized	Used	Area	Average	STDEV	%RSD
Detection wavelength (±1nm)	208	207	525	515.3	9.07	1.76
		208	514			
		209	507			
Mobile phase composition (ACN: Water) v/v	40:60	39:61	535	545.7	10.50	1.92
		40:60	556			
		41:59	546			

Table 6: Summary of Validation Parameter

Parameter	Brivaracetam
Retention time (min)	3.1
Theoretical plate count	10379
Linearity range (µg/ml)	10-50
Regression equation	Y=29.08x-0.4
Slope	29.08
Intercept	0.4
Regression coefficient	0.998
Repeatability(n=6) %RSD	1.7
LOD(µg/ml)	2.67
LOQ(µg/ml)	8.11

4. CONCLUSION

An analytical RP-HPLC method was developed and validated thoroughly for quantitative determination of Brivaracetam in bulk and formulation. The present method was found to be simple, precise, accurate, reproducibility and it gives an acceptable recovery of the analyte, which can be easily applied to the analysis of pharmaceutical formulation of Brivaracetam.

5. ACKNOWLEDGEMENTS

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Conflict of interest

None declatred

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