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Research Article

Analytical Method Development and Validation of Brivaracetam in API and Marketed Formulation by RP-HPLC

Banothu Bhadru*[1], Peddinti Naveen Kumar[2], Damera Sujatha[3], Pranay Renukuntla[4], Narender Boggula[1]

[1]CMR College of Pharmacy, Kandlakoya, Medchal, Hyderabad, Telangana, India.
[2]Research Scholar, JSS College of Pharmacy, Mysuru, Karnataka, India.
[3]University College of Pharmaceutical Sciences, Kakatiya University, Hanamakonda, Warangal, Telangana, India.
[4]School of Pharmacy, Anurag University, Venkatapur, Ghatkesar, Medchal-Malkajgiri, Hyderabad, Telangana, India.

A B S T R A C T

A rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validated of brivaracetam in its pure form as well as in tablet dosage form. Chromatography was carried out on a Symmetry ODS RP C_{18},5µm, 15mm x 4.6mm column using a mixture of phosphate buffer: methanol: acetonitrile (30:35:35% v/v) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 285 nm. The retention time of the brivaracetam was 2.183. The method produces linear responses in the concentration range of 60-140µg/ml of brivaracetam. The method precision for the determination of assay was below 2.0%RSD. The method was found to be sensitive, accurate and precise useful in the quality control of bulk and pharmaceutical formulations.

Keywords: Brivaracetam, RP-HPLC, validation, linearity, anti-epileptic.

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*Address for Correspondence:
Dr .Banothu Bhadru, Associate Professor Department Of Pharmaceutical Analysis, Cmr College Of Pharmacy, Kandlakoya, Medchal, Hyderabad, Telangana, India-50401

INTRODUCTION

The HPLC is the most important and essential analytical instrument used in all phases of drug discovery, development, and production. HPLC is the method of choice for determining the peak purity of new chemical entities, monitoring reaction changes during synthetic procedures or scale, assessing new formulations, and performing quality control/assurance of the finished therapeutic product. The major objective of the HPLC approach is to extract and measure the active substance as well as any contaminants from the process, accessible synthetic intermediates, and degradation[1,2].

Brivaracetam is a racetam derivative of levetiracetam having prominent activity against epilepsy. Brivaracetam is a third-generation anti-epileptic racetam derivative of a 4-n-propyl analogue of levetiracetam.It is soluble in water, buffer(pH-1.2,4.5 and 7.4), ethanol, methanol, glacial acetic acid, freely soluble in acetonitrile and acetone. The chemical formula is $C_{11}H_{20}N_2O_2$. IUPAC name is (2S)-2-[(4R)-2-oxo-4-propylpyrrolidin-1-yl] butanamide. Brivaracetam is a white to off-white amorphous powder with a melting point of 72-78 ^0C[3,4].

Brivaracetam is a racetam derivative of levetiracetam which is used in the treatment of epileptic seizures. It binds with Synaptic vesicle glycoprotein 2A modulator with 20 times higher affinity than levetiracetam. Briviact received FDA approval in February 2016. It is available under the brand name Briviact made by Union Chimique Belge (UCB), a multinational biopharmaceutical company headquartered in Brussels, Belgium. Although the exact mechanism through which Brivaracetam exerts its effects is not fully known, this agent targets and binds to synaptic vesicle protein 2A (SV2A) in the brain. This prevents

synaptic vesicle exocytosis and the synaptic release of certain, as of yet not fully known, excitatory neurotransmitters[1,5-7].

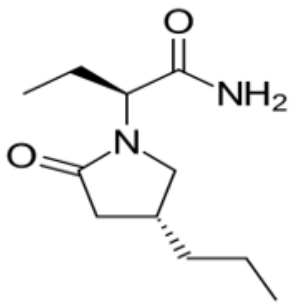

Figure 1: Chemical structure of brivaracetam

The aim of this study is to develop a simple, accurate and precise HPLC method for the analysis of brivaracetam in bulk and tablet dosage form.

MATERIALS AND METHODS

Instruments

HPLC, UV-Visible spectrometer, electronic balance, ultra sonicator.

Chemicals

Brivaracetam (API & tablets), KH_2PO_4, water and methanol for HPLC, acetonitrile for HPLC, ortho phosphoric acid.

Preparation of standard

Weigh accurately about 100 mg of brivaracetam standard and transfers into a 100ml of volumetric flask to this add few ml of methanol dissolve it and make up the volume. From the above solution pipette out 10ml and transfer into 100ml volumetric flask and make up the volume with methanol.

Preparation of sample

Collect 10 tablets and powder it. Accurately weigh equivalent weight to 100 mg of brivaracetam powder and transfers into a 100ml of volumetric flask to this add few ml of methanol dissolve it and make up the volume, filter it. From the above solution pipette out 10ml and transfer into 100ml volumetric flask and make up the volume with methanol.

Method validation[8-10]

Linearity

The ability of the method to produce results those are directly or indirectly proportional to the concentration of the analyte in samples within a given range.

Precision

The degree of closeness of the agreement among individual test results when the method is applied to multiple samplings of a homogeneous sample. It is a measure of either the degree of reproducibility (agreement under different conditions) or repeatability (agreement under the same conditions) of the method.

Accuracy

The closeness of results was obtained by a method to the true value. It is a measure of the exactness of the method.

Limit of detection (LOD) and limit of quantification (LOQ)

The detection of limit and quantification limit for each analyte were determined based on a signal-to-noise concept, as the lowest concentration.

RESULTS AND DISCUSSION

The aim of this study was to develop a simple, accurate and precise HPLC method for the analysis of brivaracetam in bulk and tablet dosage forms using mobile phase and commonly employed Symmetry C_{18} column with UV detector at 285 nm. The typical chromatogram of brivaracetam was shown in Figure 2.

Chromatographic conditions (optimizedmethod):

Column	: Symmetry C18 (150 x 4.6mm; 5µm)
Mobilephaseratio	: Phosphatebuffer: Methanol: Acetonitrile (30:35:35%v/v)
Detectionwavelength	: 285nm
Flow rate: 1.0ml/min	
Injection volume	: 10µl
Columntemperature	: Ambient
Runtime	: 5min
Retentiontime	: 2.182min

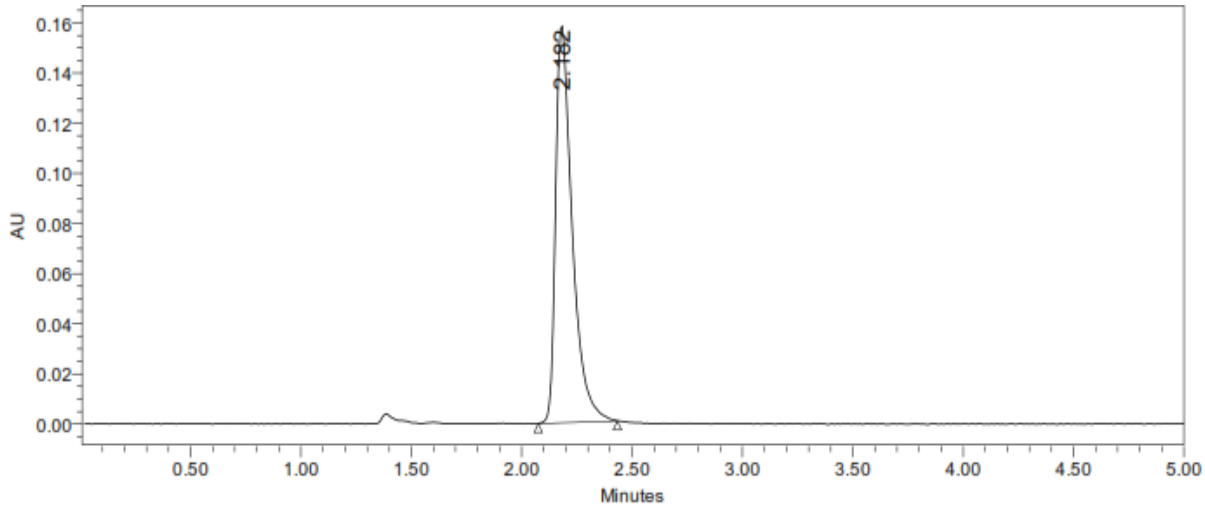

Figure 2: Chromatogram of brivaracetam in optimized condition

Table1: Summary of method optimization

Column used	Mobile phase	Retention time	Peak area	Plate count	Tailing factor	Flow rate
Symmetry ODS (C_{18}) RP Column, 250 mm x 4.6 mm, 5µm	Water: methanol 15: 85	2.182	742946	2896	1.37	1.5ml/min

Method validation

In this method, linearity, precision, accuracy, robustness, LOD and LOQ were validated for the selected brivaracetam drug by RP-HPLC.

Linearity

In order to check the linearity for the developed method, solutions of five different concentrations ranging from 60µg/mL - 140µg/mL were prepared. The chromatograms were recorded, and the peak areas were given in Table 1, and linearity graph was shown in Figure 3.

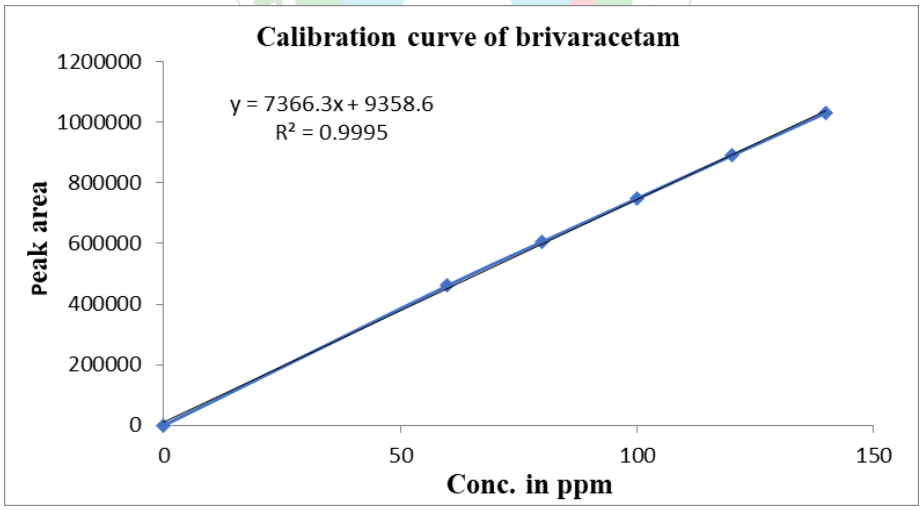

Figure 3: Calibration curve of brivaracetam

Table2: Linearity data for brivaracetam

Conc. (µg/ml)	Area
60	461404
80	606157
100	748506
120	891041
140	1032196

Accuracy

Recovery studies were used to determine the method's accuracy, and the % recovery was calculated. Brivaracetam recovery rates were reported to be in the range of 100.34%.

Table 3: Accuracy of brivaracetam

Accuracy	Amount taken(mg)	Amount added(mg)	Amount recovered	Peak area	% Recovery	Mean recovery
80%	100	80	80.698	603517	100.997	**100.57**
	100	80	80.773	604598	100.841	
	100	80	80.656	605213	100.945	
100%	100	100	99.833	746471	99.933	**100.22**
	100	100	100.083	745574	100.083	
	100	100	100.565	747652	100.365	
120%	100	120	120.390	894415	100.241	**100.25**
	100	120	120.301	896762	100.167	
	100	120	120.242	895541	100.368	

Precision

Repeatability

The peak areas and retention periods acquired by real determination of six replicates of a given quantity of medication were used to determine the precision of each approach individually. Brivaracetam is a type of brivaracetam that (API). The % relative standard deviation for brivaracetam was calculated and is shown in Table4.

Table 4: Repeatability data for brivaracetam

S. No.	Injection	Peak Area
1	Injection 1	743826
2	Injection 2	745277
3	Injection 3	742506
4	Injection 4	747576
5	Injection 5	746715
6	Injection 6	741278
7	**Average**	**744529.6667**
8	**SD**	**2440.4116**
9	**% RSD**	**0.32777**

Intermediate precision:

The Intermediate precision consists of two methods. They are

Intra and interday:

Inintra and interday process, the 80%, 100% and 120% concentration are injected at different intervals of time in same and different days.

Table 5: Results of intra and inter day assay

Conc. of brivaracetam (API in µg/ml)	Observed conc. of brivaracetam (µg/ml) by the proposed method			
	Intraday		Interday	
	Mean(n=6)	%RSD	Mean(n=6)	%RSD
80	80.38	0.56	80.45	0.56
100	100.17	0.71	100.50	0.77
120	120.89	0.89	120.91	0.85

LOD and LOQ

The slope of line and variance acquired from accuracy studies were used to evaluate the limit of detection(LOD) and the limit of quantization (LOQ) parameters.

LOD = 3.3(SD/S)

LOQ= 10(SD/S)

Where;

SD =Standard deviation,

S =Slope.

The lowest concentration level at which the analyte can be reliably detected (LOD) and quantified (LOQ) was found to be 0.07 g/ml and 0.21 g/ml, respectively.

System suitability

Many analytical processes include system suitability testing as part of the process. The tests are founded on the idea that the equipment, electronics, analytical activities, and samples to be studied are all part of a larger system that may be evaluated. The parameters for the system suitability test were established as follows. Table 6 displays the information.

Table6: System suitability parameter

S.No.	Parameter	Limit	Result
1	Retention time	Rt > 2	4.783
2	Asymmetry	T ≤ 2	1.35
3	Theoretical plates	N > 2000	2865
4	Tailing factor	T < 2	1.37

Robustness

Minute changes in chromatographic conditions such as flow rate 1.0 ml (0.1 ml/min), Wavelength of detection 284 (2nm), and organic phase content in mobile phase (5%) were studied to determine the method's robustness, and the results of (percent RSD 2%) were in shown in Table 7.

Table7: Robustness

Changes in parameter	%RSD
Flow(1.1ml/min)	0.45
Flow(0.9ml/min)	0.38
More organic	0.76
Less organic	0.65
Wavelength of detection(286nm)	0.98
More organic	0.93

Estimation of brivaracetam in pharmaceutical dosage form

To determine the average weight of20tablets,crush with mortar and pestle. A quantity of powder equivalent to 25 mg of powder was transfer into 25 ml volumetric flask and sonicated for 15 min, and the volume was made up to 25 ml with the mobile phase. Then, 10 mL of the aforementioned solution was diluted to 100 mL. The results were recorded. The data are shown in Table 8.

Table 8: Recovery data for estimation of brivaracetam

Brand name	Labelled amount(mg)	Mean(±SD)	Assay%
Briviact	50mg	50.10 (±0.468)	100.34

The amount of drug in Briviact Tablet was found to be 49.867 (± 0.468) mg/tab for brivaracetam & % purity was found to be 99.825%.

Different chromatographic settings were used to establish a precise, linear, specific, and acceptable stability indicating RP-HPLC method for analysis of brivaracetam, and the results observed are described in preceding chapters.Isocratic elution is straightforward, requiring only one pump and a flat baseline separation for consistent results. As a result, it was chosen over gradient elution for the current investigation.

CONCLUSION

For the analysis of brivaracetam API, a sensitive and selective RP-HPLC technique has been designed and validated.Various columns are available for RP-HPLC, but the Symmetry ODS (C_{18}) RP Column, 250 mm x 4.6 mm, 5m column was chosen since the peak shape, resolution, and absorbance were good while using this column. After scanning the standard drug solution over 200 to 400nm, the detection wavelength was chosen. The UV spectrum of brivaracetam shows that the majority of HPLC work may be done conveniently in the 284 nm wavelength region. Furthermore, the optimal analysis was discovered to be a flow rate of 1 ml/min and an injection volume of 10µl.The developed RP-HPLC method has high sensitivity, precision, and repeatability.The results suggest that the developed method is yet another suitable assay, purity, and analysis method for brivaracetam in various formulations.

Author contributions

All authors contributed toexperimental work, data collection, drafting or revising the article, gave final approval of the version to be published, and agreed to be accountable for all aspects of the work.

Competing interest statement

All authors declare that there is no conflict of interests regarding publication of this paper.

Ethical approval

Not required.

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