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ORIGINAL ARTICLE **OPEN ACCESS**

Development and Validation RP-UFLC Method for the Estimation of Brivaracetam in Bulk and Pharmaceutical Dosage Form

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ABSTRACT

The aim of the present work is to develop a simple and cost-effective new method for the estimation of Brivaracetam by RP-UFLC method. After literature survey we found that very few methods are reported for the estimation of the drug by HPLC, which aimed us to develop a method by UFLC. The chromatographic conditions were successfully developed for the separation of Brivaracetam by using Shim-pack GIST C-18 column(4.6×250mm) 5µ, flow rate was 1.0 ml/min, mobile phase ratio was water: acetonitrile (50:50% v/v), detection wavelength was 242nm. The instrument used was SHIMADZU UFLC with an Auto Sampler, UV/VIS detector, Lab Solutions software. The retention time were found to be 3.98min. The % purity of Brivaracetam was found to be 98.24%.The system suitability parameters for Brivaracetam such as theoretical plates and tailing factor were found to be 10718 and 1.08 respectively. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)) with respect to all the validation parameters and found to be within the acceptable limits.

Keywords: *Brivaracetam, RP-UFLC, Validation, ICH guideline*

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INTRODUCTION

Brivaracetam, a third-generation antiepileptic racetam derivative sold under the brand name Briviact, a chemical analogue of levetiracetam, is a racetam derivative with anticonvulsant (antiepileptic) properties.[2-3]Chemically it is (2*S*)-2-[(4*R*)-2-oxo-4-propylpyrrolidin-1-yl] butanamide, with a molecular weight of 212.15 g/mol and chemical formula C32H45N3O4S.

Brivaracetam is used to treat partial-onset seizures with or without secondary generalization, in combination with other antiepileptic drugs. Brivaracetam is believed to act by binding to the ubiquitous synaptic vesicle glycoprotein 2A (SV2A).[1]Phase II clinical trials in adult patients with refractory partial seizures were promising. Positive preliminary results from stage III trials have been recorded, 4-5 along with evidence that it is around 10 times more potent for the prevention of certain types of seizure in mouse models than levetiracetam, of which it is an analogue. It acts as a novel 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. The marketed formulation is available in various dosage forms such as tablet, oral solution and injection for single use. It is available in dosage of 10, 25, 50, 75 and 100 mg tablet.

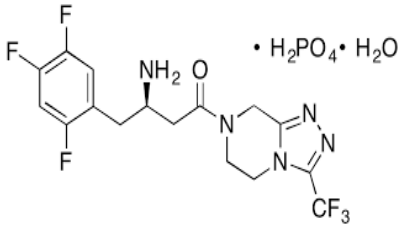

Fig.1. Structure of Brivaracetam

Literature survey revealed that very few methods are available for the analysis of Brivaracetam by HPLC methods. Hence present work targeted to developed a new precise, accurate and sensitive RP-UFLC method for the determination of Brivaracetam in API and formulation.

Table 1: COMPARISON OF THE REPORTED METHODS WITH THE PRESENT METHOD:

Title	Mobile phase	Stationary phase	Flow rate	Range	Rt	r^2
HPLC Studies on Degradation Behaviour of Brivaracetam and Development of Validated Stability – Indicating HPLC Assay Method	Methanol: water: acetonitrile (30:10:60 v/v)	Hypersil Gold C18 analytical column n Dim. (mm) 250 × 4.6, Particle Size (µ) 5	1.0 ml/min	1-6 µg/ml	7.19	0.999
Development and validation of stability-indicating UPLC method for the determination of Brivaracetam, its related impurities and degradation products	Solvent-A: Degassed buffer Solvent-B : Water : Acetonitrile: 20: 80 (v/v)	UPLC BEH SHIELD RP18 (100 mm x 2.1 mm, 1.7 µm) column	0.3 ml/min	0.06-0.4 µg/ml	1.00	0.993
Analytical Method Development and Validation of Brivaracetam in Bulk and Pharmaceutical Dosage Form By RP-UFLC Method	Water: acetonitrile (50:50% v/v)	Shim-pack GIST C-18 column (4.6×250mm) 5µ	1.0 ml/min	1-6 µg/ml	3.98	0.996

MATERIAL AND METHODS

Instruments Used:

SHIMADZU UFLC, Pump Analytical UFLC isocratic pump, UV/VIS Detector, Lab Solutions software, Shim-pack GIST C-18 column (4.6×250mm) 5µ, Solicitor Analytical Technologies Limited- Ultrasonic cleaner. U.V double beam spectrophotometer SHIMADZU, UV1800+pH meter, Weighing machine

Materials:

Pharmaceutical grade Brivaracetam was kindly procured from Glenmark Pharma.

It was certified to contain 100.5(w/w) on a dry basis and was used further without purification. All chemicals and reagents of analytical grade were purchased from Vishal Institute of Pharmaceutical Education and Research, Ale.

Chemicals Used:

Brivaracetam sample, Water and Methanol for HPLC, Acetonitrile for HPLC

CHROMATOGRAPHIC CONDITIONS (OPTIMIZED METHOD):

Column:	Shim-pack GIST C-18 column (4.6×250mm) 5µ
Mobile phase ratio:	Water: Acetonitrile (50:50% v/v)
Detection wavelength:	242 nm
Flow rate:	1.0 ml/min
Injection volume:	20µl
Column temperature:	40
Auto sampler temperature:	Ambient
Run time:	7 min
Retention time:	3.98 min

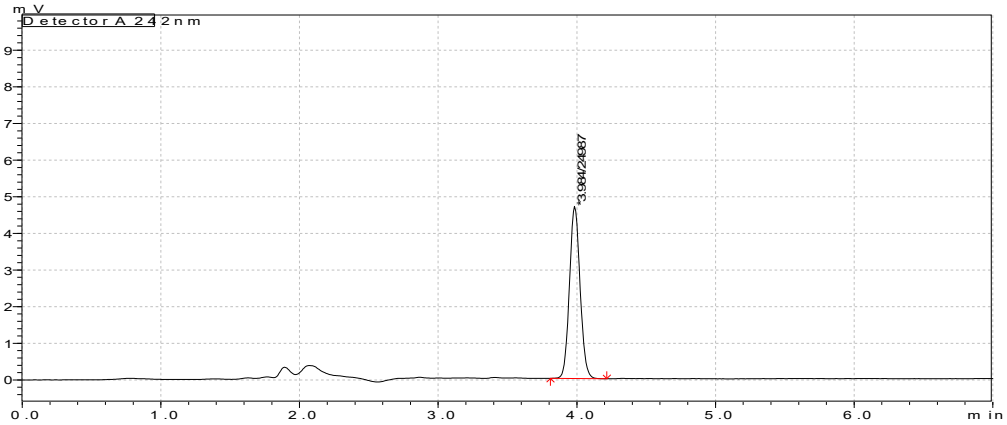

Fig.2. Chromatogram showing optimized method for standard solution.

The above chromatogram shows good peak shape with a minimum retention time when compared to that of other trials; hence we conclude that this is the optimized method for carrying out further analysis

Mobile phase preparation:

500 ml volumes of water is mixed with 500 ml volumes of Acetonitrile, sonicated and filtered through a membrane filter.

Preparation of the Brivaracetam standard and sample solution:

Sample solution preparation:

20 tablets of Brivaracetam (Briviact) were weighed and average weight of tablet was calculated. The tablet was crushed into a fine powder using mortar and pestle.25 mg Brivaracetam equivalent tablet powder was accurately weighed and transferred into a 25 ml clean dry volumetric flask, add about 10ml of diluent and sonicate to dissolve it completely and making volume up to the mark with the same solvent (Stock solution). Filter the solution through 0.45µ Whatmann filter paper. Further pipette out 10ml of the above stock solution into a 100ml volumetric flask and was diluted up to the mark with diluent(100µg/ml) then7.5 ml of this solution is transferred into 25 ml volumetric flask and made up to volume to obtained 30µg/ml.

Standard stock solution preparation

25 mg Brivaracetam working standard was accurately weighed and transferred into a 25 ml clean dry volumetric flask and about 2ml of diluent is added and sonicated to dissolve it completely and make volume up to the mark with the same solvent (Stock solution) (1000ppm). From above stock solution 30 ppm of final standard solution was prepared by serial dilution.

METHOD DEVELOPMENT: [6-7]

- Studying the physiochemical properties of the drug.
- Selection of wavelength: Sample solution of Brivaracetam was taken and scanned in the range of 200-400 nm and the spectrum was obtained. The overlay spectrum was used for selection of wavelength for Brivaracetam. The isosbestic point was taken as detection wavelength.
- Selection of chromatographic conditions
    1. Mobile Phase.
    2. Selection of stationary Phase
    3. Selection of flow rate
    4. Selection of column temperature
    5. Selection of elution temperature
    6. Selection of detector
- Sample preparation
- Method optimization

METHOD VALIDATION:[5] [8]

Validation of the method is carried out as per ICH guidelines for the following parameters such as system Suitability, linearity, specificity, precision, accuracy, limit of detection, limit of quantification and robustness.

System suitability

This parameter used to know whether the UFLC system is suitable for actual chromatographic conditions or not. System suitability was estimated by injecting six standard solutions of Brivaracetam and from the chromatograms %RSD, theoretical plates and peak symmetry were calculated.

Specificity This parameter performed by injecting blank, standard and sample solutions and any interference with excipient and mobile phase were observed.

Linearity and range

These parameters were studied by injecting 10,20,30,40 and 50µg/mL solutions (prepared from standard stock solution) into UFLC system and observed the linear relationship between concentration and peak area in the concentration range of 10-50µg/ml.

Accuracy

This parameter studied by preparing 50%, 100% and 150% concentration solutions of Brivaracetamin triplicate by spiking the standard drug to the placebo and calculated the percentage recovery of Brivaracetam.

Precision

System precision:

This parameter carried out to determine whether the HPLC instrument working perfectly or not. System precision studied by injecting standard Brivaracetam 30µg/ml solution six times and %RSD was calculated from peak areas.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ were determined as per ICH guidelines by using the formulas 3.3×SD/S and 10×SD/S respectively. In the formulas SD is the response standard deviation and S is the calibration curve slope. A signal-to noise ratio for LOD is between 3 or 2:1 and LOQ is 10:1.

Robustness

Robustness was done by changing the actual chromatographic conditions like mobile phase ratio and flow rate. Results were determined by calculating the %RSD for six injections peak area values of each change in condition.

III. RESULTS AND DISCUSSION

Selection of Scanning Wavelength:

UV spectrum of Brivaracetam (Fig. 3) showed maximum absorbance at 242 nm and the same was selected as the scanning wavelength.

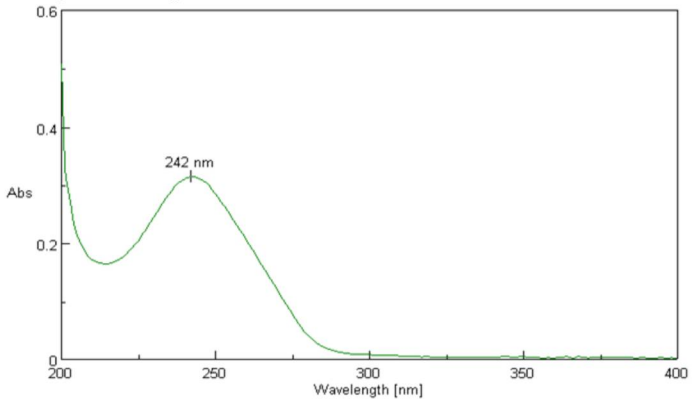

Figure 3: UV spectra for Brivaracetam

Optimization of HPLC Method:

The mixed standard stock solution was diluted in the mobile phase to a concentration containing30 µg/ml of Brivaracetam. Then, the stock solution was injected into the Shim-pack GIST C-18 column (250mm×4.6mm,5µm). Different ratios of methanol: water and acetonitrile: water was tried. Methanol: water resulted in wider peaks. Thus water: acetonitrile was selected as the mobile phase. Different ratios of mobile phases were tried to get a retention time which allowed sufficient separation of the standard from its degradant peaks. The optimum mobile phase was thus found to consist of water: acetonitrile in the ratio of (50:50 *v/v*). This preparation was carried out at ambient temperature with a flow rate of 1.0 ml/min with retention times is 3.9±0. 02. Acceptable retention time (RT), theoretical plates, asymmetry and good resolution for Brivaracetam were obtained. The method was found to be linear in the range of 10-50ppm. The results are tabulated in the following table no:2 and fig 5-9 shown the chromatogram of Brivaracetam linearity.

Table 2: Linearity data of Brivaracetam

Sr.no	Concentration	Area
1	10 ppm	8566
2	20ppm	17195
3	30 ppm	24987
4	40 ppm	36338
5	50 ppm	44779
	Correlation coefficient: 0.996	

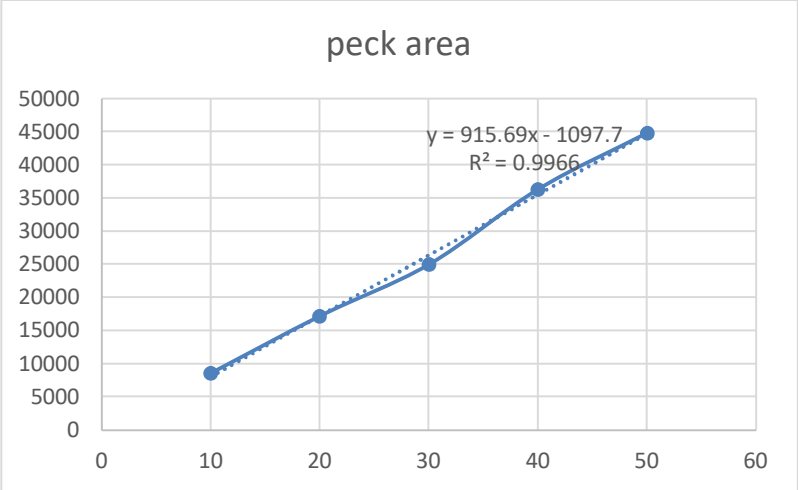

Fig 4: calibration graph of Brivaracetam by RP-UFLC.

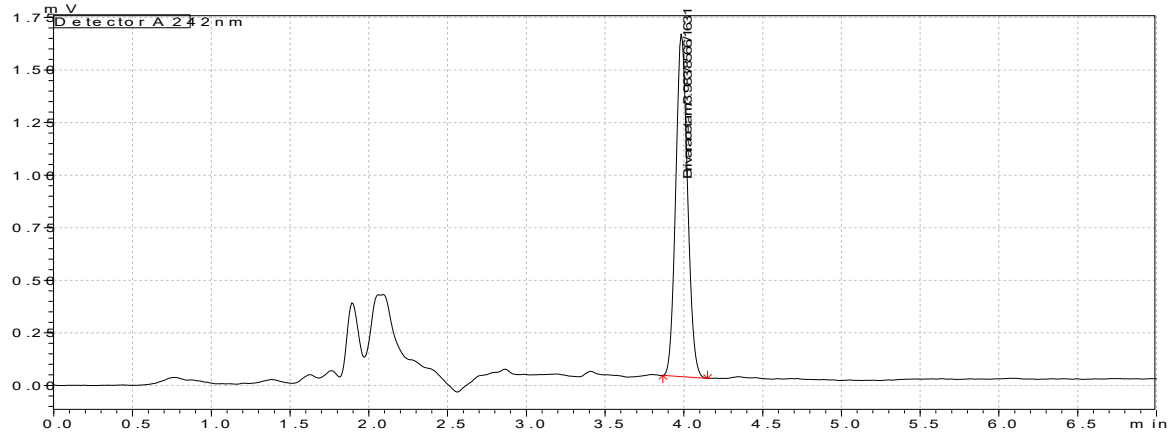

Fig 5: standard chromatogram of Brivaracetam (10µg/ml)

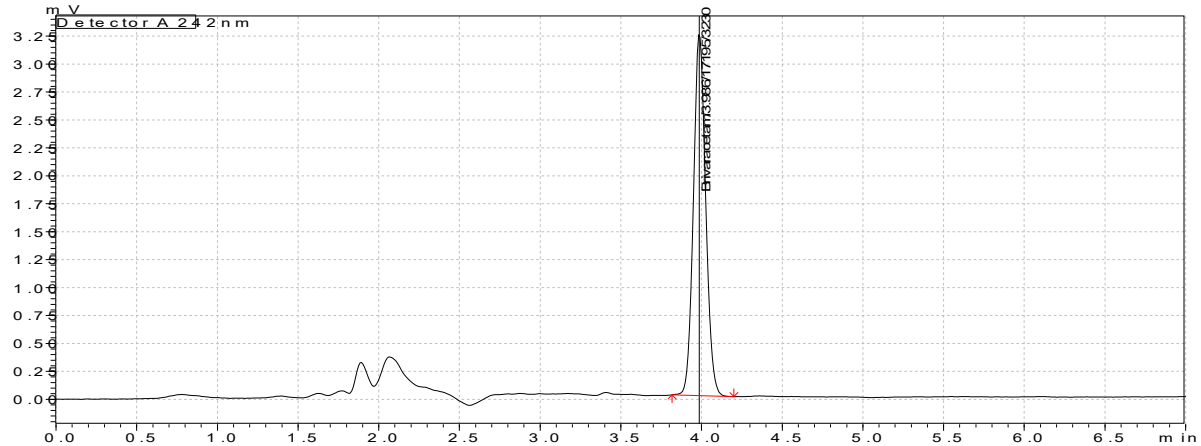

Fig 6: standard chromatogram of Brivaracetam (20µg/ml)

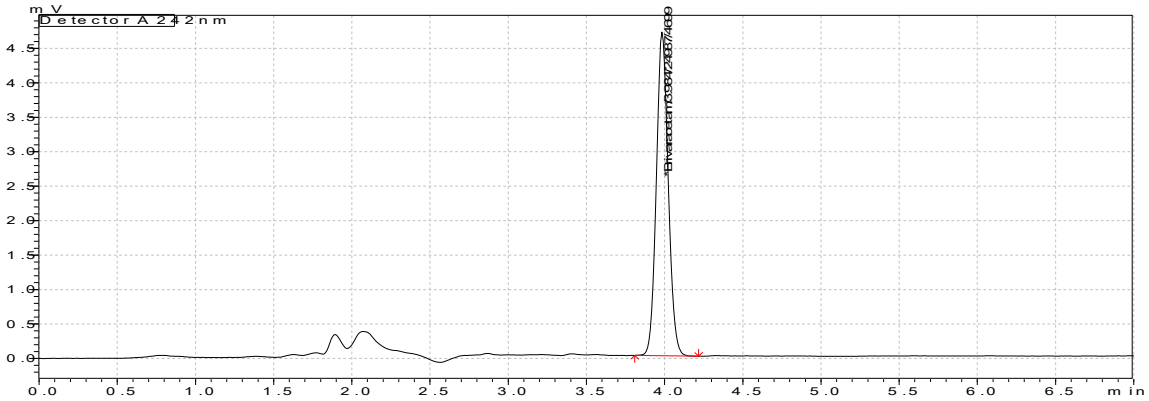

Fig 7: standard chromatogram of Brivaracetam (30µg/ml)

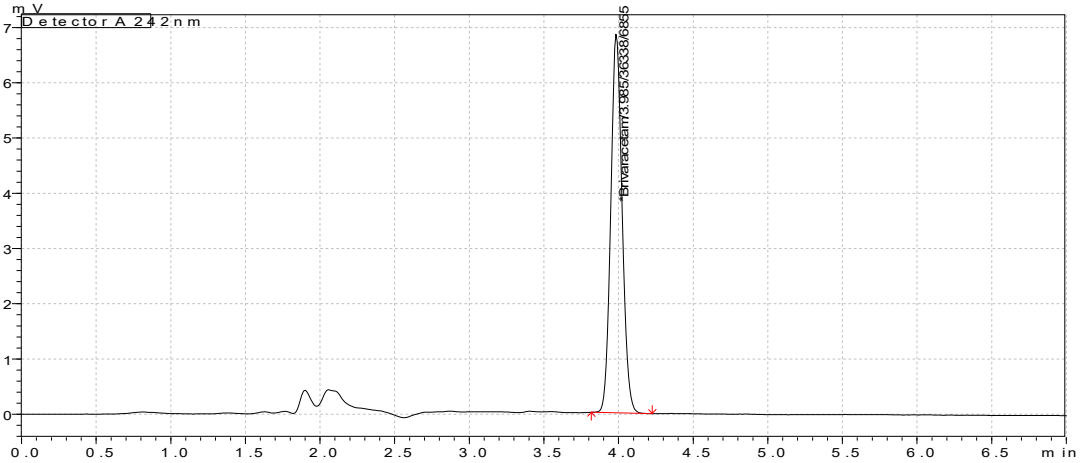

Fig 8: standard chromatogram of Brivaracetam (40µg/ml)

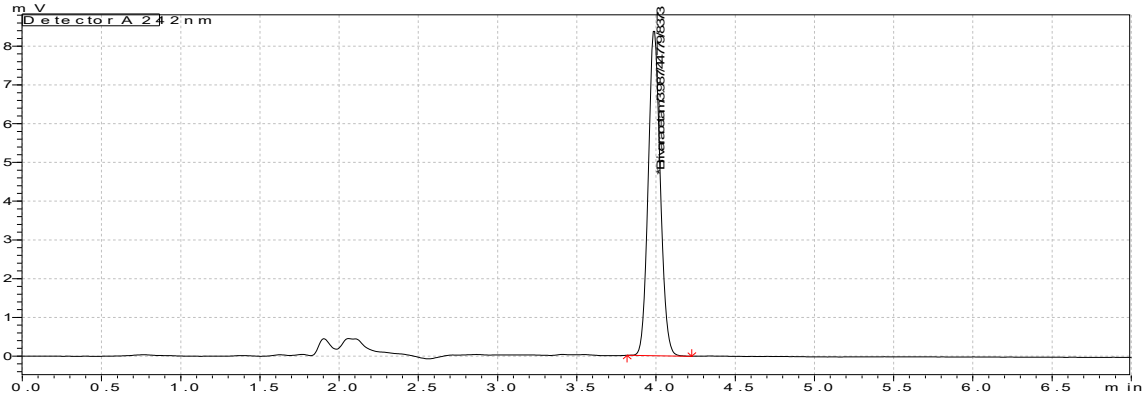

Fig 9: Standard chromatogram of Brivaracetam (50µg/ml)

Precision:

Precision studies were studied and %RSD found to be 0.029

Table 3: System precision results of Brivaracetam

Sr. No.	Concentration	Rt	Peak area	Peak height
1	30	3.98	24987	4699
2	30	3.97	24980	4698
3	30	3.99	24988	4699
4	30	3.98	24987	4699
5	30	3.96	24970	4695
6	30	3.97	24975	4696
Mean	24981.17			
Standard deviation	7.4677			
% RSD	0.029893			

Accuracy:
Accuracy was calculated by taking three concentrations in the range of mg/ml and the mean recovery of the sample was calculated and was found to be within the limits (97.3-100.3%). The results for accuracy of the method were shownin table no:4

Table 4: Results for accuracy and % recovery of Brivaracetam

% Concentration	Amount added	Amount found	% Recovery	%RSD
50%	15	14.7	98	0.678601
50%	15	14.6	97.33	
50%	15	14.8	98.66	
100%	30	29.96	99.86	0.554838
100%	30	29.76	99.2	
100%	30	30.09	100.3	
150%	45	44.8	99.55	0.363463
150%	45	44.7	99.33	
150%	45	44.5	98.88	

Limit of detection and limit of quantification (LOD & LOQ):
The smallest amount of analyte that can be detected by the proposed method is called as limit of detection, while the smallest amount of analyte that can be quantified by the method is called limit of quantification. They can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) according to the formula: The LOD and LOQ of the method were found to be 0.026and0.081µg/ml respectively.

Robustness:
Robustness of the method is analyzed by change in the flow rate or change in the mobile phase composition. The results for robustness of the method are shown in table no:5

Table 5: Results for robustness of Brivaracetam

S. No	Parameter	Variation	Results	% RSD
1.	Flow rate	0.8	24978	0.0189
		1.0	24987	
		1.2	24980	
2.	Mobilephase composition	48:52	24980	0.0144
		50:50	24987	
		52:48	24985	

System suitability:
It is calculated in order to assess the suitability of the chromatographic system with the proposed method. Six standard solutions of Brivaracetam were injected into chromatographic system and from the chromatogram %RSD, theoreticalplate, Retentiontime, tailing factor studied and found to be satisfactory. Result shown in table no.6

Table 6: System suitability results of Brivaracetam

S. No	Parameter	limits	Results
1.	Theoretical plates	NLT 2000	10718
2.	% RSD	NMT 2.0	0.09
3.	Retention time	-	3.98
4.	Tailing factor	≤ 2.0	1.08

Specificity:
The ability of the method to separate the analyte from a given sample in the presence of other components

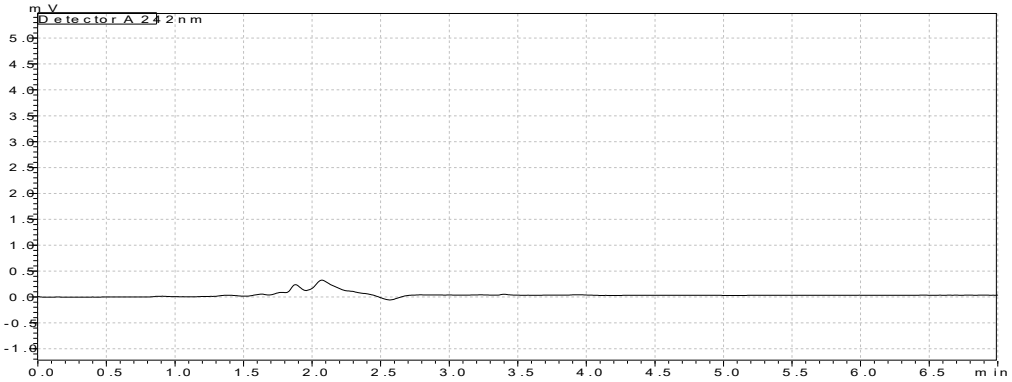

Fig. 10 Chromatogram showing blank preparation (Mobile Phase)

Assay:

Assay was carried out with the proposed method by taking the formulation available from the market, were30 ppm of standard solution and 30 ppm marketed formulation used. The results were found to be within the limits and the % assay of the formulation was found to be 98.24%.

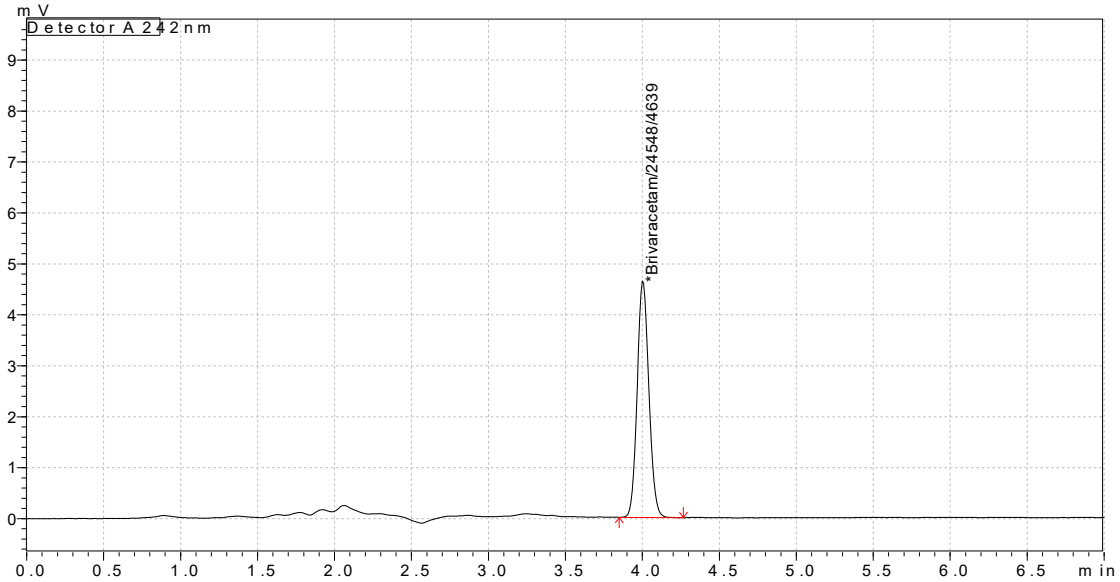

Fig. 11 Chromatogram showing assay of Brivaracetam marketed formulation.

SUMMARY:

Sr.no	Parameter	Result
1.	Linearity range	10-50µg/ml
2	Regression coefficient	Y=915.69x-1097.7
3.	Slope	915.69
4.	Intercept	1097.7
5	Correlation coefficient ($R^{2)}$	0.996
6.	Assay	98.24%
7.	Accuracy (%Recovery)	97.3-100.3%
8.	Precision (%RSD)	System precision-0.029
9.	LOD (µg/ml)	0.026
10.	LOQ (µg/ml)	0.081
11.	Retention time (min)	3.98

CONCLUSION

The current study demonstrated a validated RP-UFLC method for the estimation of Brivaracetam available as the tablet dosage form. The method was completely validated and showed satisfactory results. The method was free from the interference of the other active ingredients and additives used in the formulation. [6]The RP-UFLC method for the determination of Brivaracetam has various advantages like less solvent consumption, low retention time, good peak symmetry accurate, precise and robust. [7] The results of the study indicate that the developed method was found to be accurate, precise, linear, sensitive, simple, economical, and reproducible, which has a short run time, which makes the method

rapid. Hence it can be concluded that this method may be employed for the routine quality control analysis of Brivaracetamin active pharmaceutical preparations.

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