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Analytical Method Development and Validation of Brivaracetam In Bulk And Pharmaceutical Dosage Form By RP-HPLC Method

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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-HPLC 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 HPLC. The chromatographic conditions were successfully developed for the separation of Brivaracetam by using Agilent column (4.6×150mm) 5µ, flow rate was 1.0 ml/min, mobile phase ratio was Phosphate buffer: methanol (25:75% v/v), detection wavelength was 270 nm. The instrument used was HPLC with an Auto Sampler, Separation module 2695, photo diode array detector 996, Empower-software version-2. The retention times were found to be 2.182 min. The % purity of Brivaracetam was found to be 98.56%.The system suitability parameters for Brivaracetam such as theoretical plates and tailing factor were found to be 4343 and 1.6 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.
KEY WORDS: Agilent column, Brivaracetam, RP-HPLC

I. INTRODUCTION

Brivaracetam, a third-generation antiepileptic racetam derivative sold under the brand name Briviact, a chemical analog of levetiracetam, is a racetam derivative with anticonvulsant (antiepileptic) properties. 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 $C_{32}H_{45}N_3O_4S$.

Brivaracetam is used to treat partial-onset seizures with or without secondary genera-lisation, in combination with other antiepileptic drugs [1]. Brivaracetam is believed to act by binding to the ubiquitous synaptic vesicle glycoprotein 2A (SV2A) . 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 analog. 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 [2]. 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.

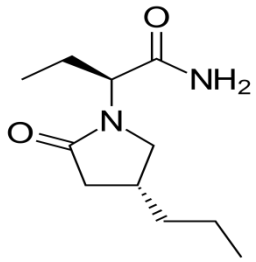


Fig.1. Structure of Brivaracetam

Literature survey revealed that very few methods are available for the analysis of Brivaracetam by HPLC methods which paved the way for us to carry out the analysis in pure as well as marketed dosage form.

COMPARISON OF THE REPORTED METHODS WITH THE PRESENT METHOD:

S.no	Title	Mobile phase	Stationary phase	Flow rate	Range	R_t	r^2
1	HPLC Studies on Degradation Behavior 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
2	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
3.	**Analytical Method Development and Validation Of Brivaracetam In Bulk And Pharmaceutical Dosage Form By RP-HPLC Method**	**Phosphate buffer: MeOH (25:75% v/v)**	**Agilent (4.6×150mm) 5µ**	**1.0 ml/min**	**20-100 µg/ml**	**2.18**	**0.992**

II. MATERIALS AND METHODS:

Instruments Used:

System Alliance Waters 2690 separation module, Pump Analytical HPLC isocratic pump, Detector Photo diode array detector, Software Empower 2 software, Column Agilent (250×4.6mm, 5µ) C- 18 RP-column, Sonicator Analytical Technologies Limited- Ultrasonic cleaner. U.V double beam spectrophotometer LABINDIA, UV 3000^{+}pH meter, Weighing machine

Chemicals Used:

Brivaracetam sample, KH_2PO_4, Water and Methanol for HPLC, Acetonitrile for HPLC, Ortho phosphoric Acid.

CHROMATOGRAPHIC CONDITIONS (OPTIMIZED METHOD):

Column : Agilent (4.6×150mm) 5µ
Mobile phase ratio : Phosphate buffer: MeOH (25:75% v/v)
Detection wavelength : 270 nm
Flow rate : 1.0 ml/min
Injection volume : 10µl
Column temperature : Ambient
Auto sampler temperature : Ambient
Run time : 5 min
Retention time : 2.182 min

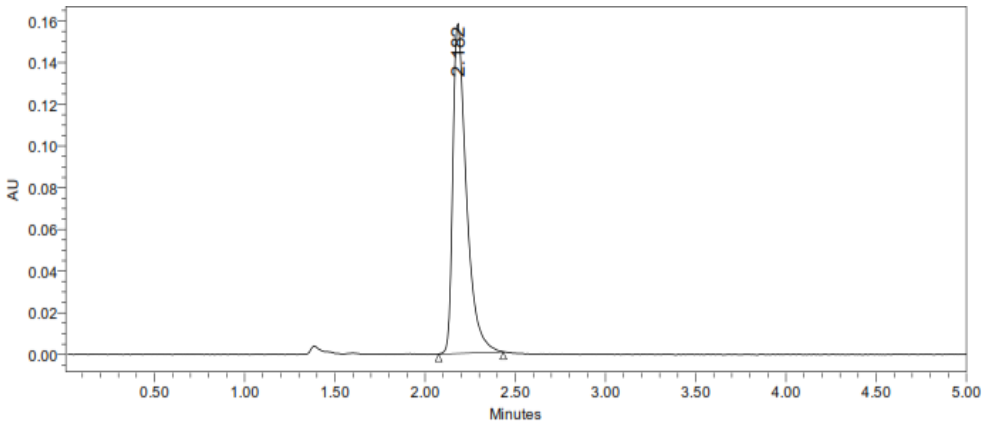


Fig.2. Chromatogram showing optimized method

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:
25 volumes of phosphate buffer is mixed with 75 volumes of methanol, sonicated and filtered through a membrane filter.

Preparation of the Brivaracetam standard and sample solution:

Sample solution preparation:
10 mg of Brivaracetam tablet powder was accurately weighed and transferred into a 10 ml clean dry volumetric flask, add about 2ml of diluent and sonicate to dissolve it completely and making volume up to the mark with the same solvent(Stock solution). Further pipette 10ml of the above stock solution into a 100ml volumetric flask and was diluted up to the mark with diluent.

Standard solution preparation
10 mg Brivaracetam working standard was accurately weighed and transferred into a 10ml 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).Further pipette out 1ml of the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent.

METHOD DEVELOPMENT [4, 5]:

- 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 isobestic 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 [6, 7]:
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.

III. RESULTS AND DISCUSSION

Linearity:

The method was found to be linear in the range of 20-100 ppm. The results are tabulated in the following table:

Table 1: Linearity data of Brivaracetam

S.no	Concentration	Area
1	20 ppm	905957
2	40 ppm	1033632
3	60 ppm	1200130
4	80 ppm	1403642
5	100 ppm	1608820
	Correlation coefficient: 0.992	

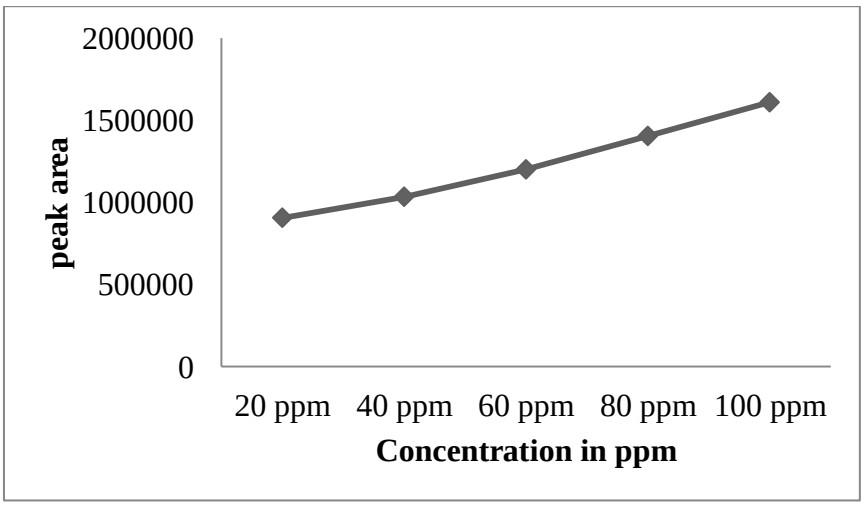
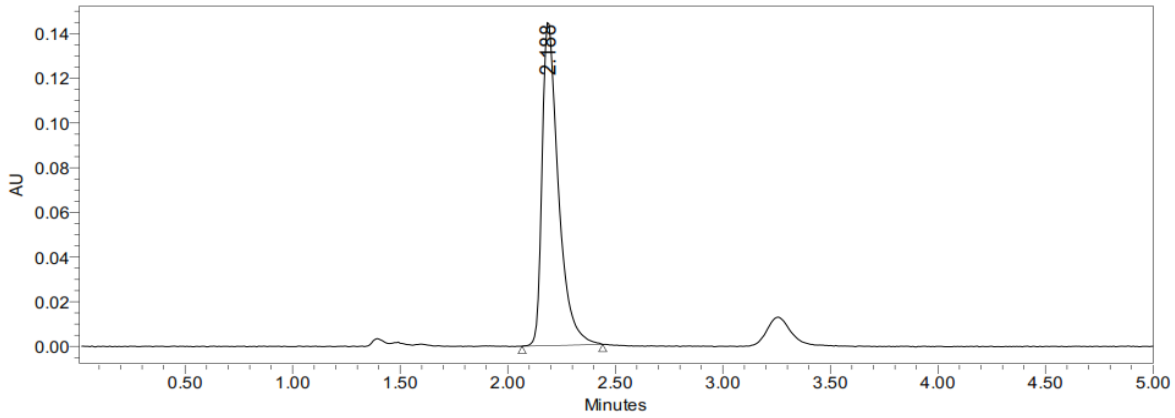
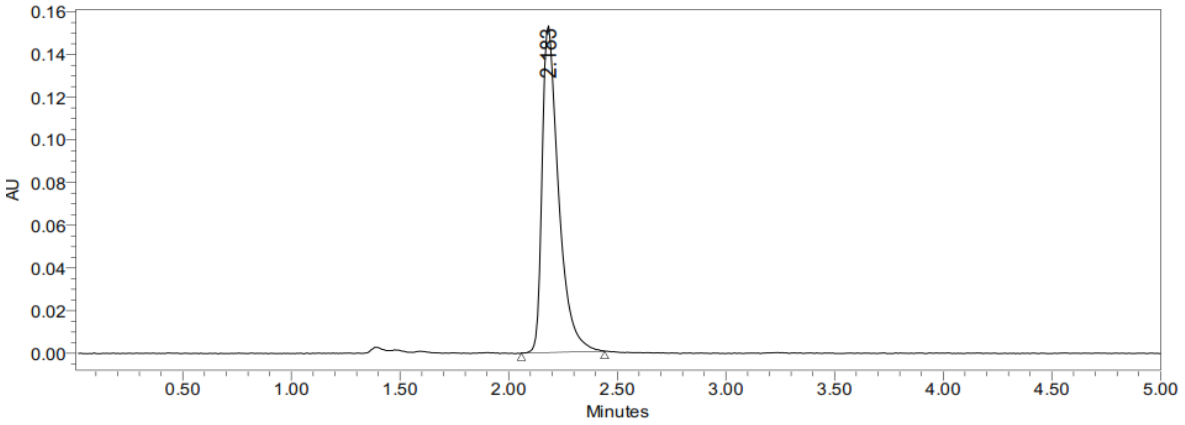


Fig.3. Calibration curve of Brivaracetam

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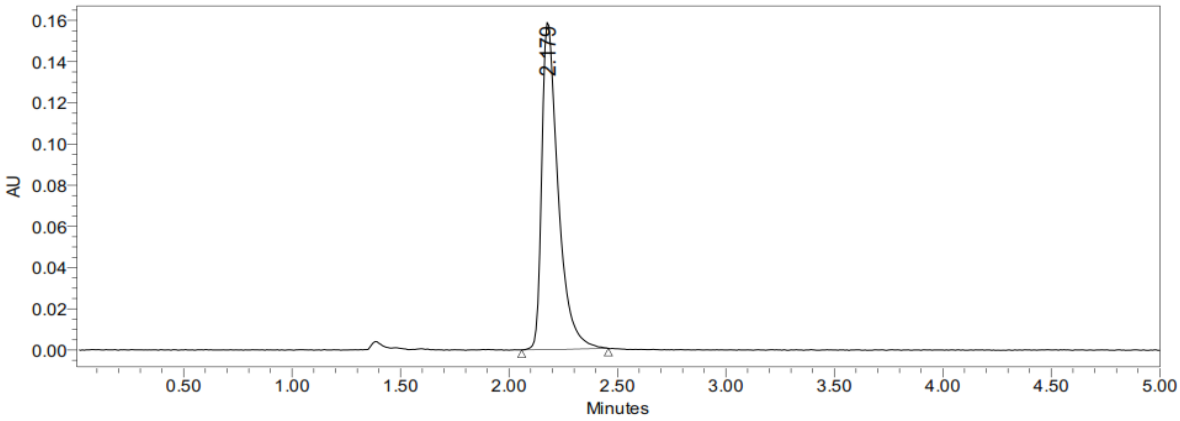
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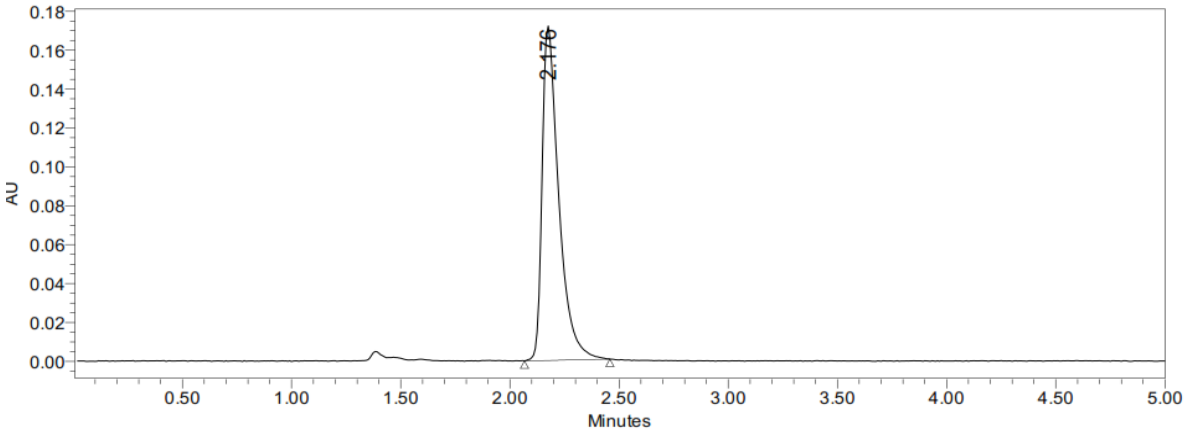
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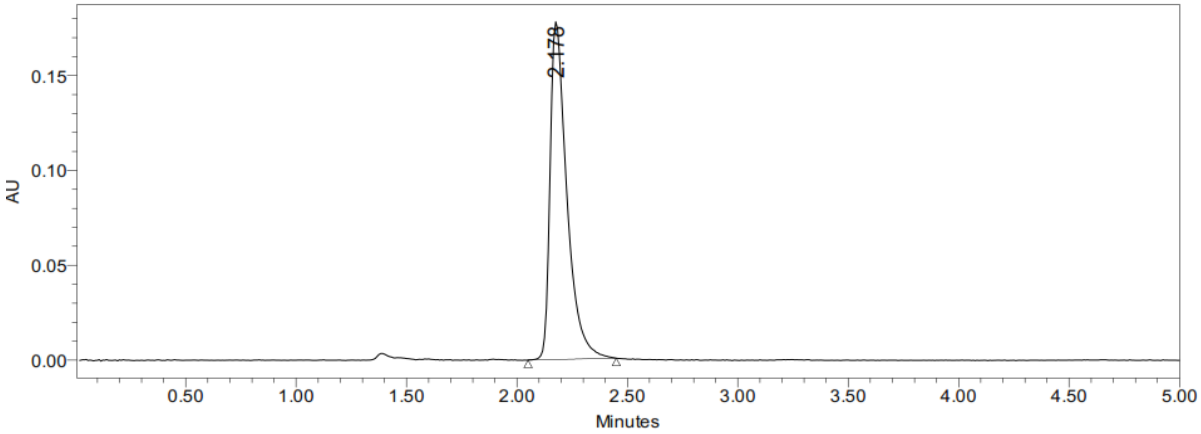

Fig 4-8: chromatograms showing linearity of Brivaracetam(20-100 ppm)

Accuracy:

Accuracy was calculated by taking three concentrations in the range of 5-15 mg/ml and the mean recovery of the sample was calculated and was found to be within the limits (98-102%). The results for accuracy of the method were given below:

Table 2: Results for accuracy and % recovery of Brivaracetam

% concentration	Average area	Amount added	Amount found	% recovery	Mean recovery
50%	1143519	5	4.86	98.81%	98.96%
100%	2938342	10	9.88	99.08%	
150%	4452758	15	15	100.0%	

Precision:

Precision studies were studied by intraday and inter-day analysis of the samples and the % RSD was found to be 0.3 and 0.8 respectively.

Table 3: Intra-day precision results of Brivaracetam

S.no	Concentration	Rt	Peak area	Peak height
1.	20	2.185	824170	158772
2.	40	2.191	826053	157336
3.	60	2.204	823442	156124
4.	80	2.207	818967	155674
5.	100	2.210	823476	156033
Mean		823221.9		
Standard deviation		2604.2		
% RSD		0.3		

Table 4: Inter-day precision results of Brivaracetam

S.no	Concentration	Rt	Peak area	Peak height
1.	20	2.180	830760	155678
2.	40	2.184	832532	157536
3.	60	2.185	823385	155124
4.	80	2.188	840724	155674
5.	100	2.188	829385	153083
Mean		831357.9		
Standard deviation		6263.2		
% RSD		0.8		

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:

$$LOD = 3.3 * \frac{\sigma}{S} \quad \& \quad LOQ = 10 * \frac{\sigma}{S}$$

The LOD and LOQ of the method were found to be 3.67 and 8.87 µg 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 tabulated below:

Table 5: Results for robustness of Brivaracetam

S.no	Parameter	Variation	Results	% RSD
1.	Flow rate	0.8	4517	1.6
		1.0	4343	1.6
		1.2	4209	1.6
2.	Mobile phase composition	73	4623	1.6
		75	4543	1.6
		77	4864	1.6

System suitability:
It is calculated in order to assess the suitability of the chromatographic system with the proposed method. Parameters analyzed include theoretical plates, LOD and LOQ of the method.

Table 6: System suitability results of Brivaracetam

S.no	Parameter	Results
1.	Theoretical plates	4343
2.	LOD (µg/ml)	0.439
3.	LOQ (µg/ml)	1.466

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

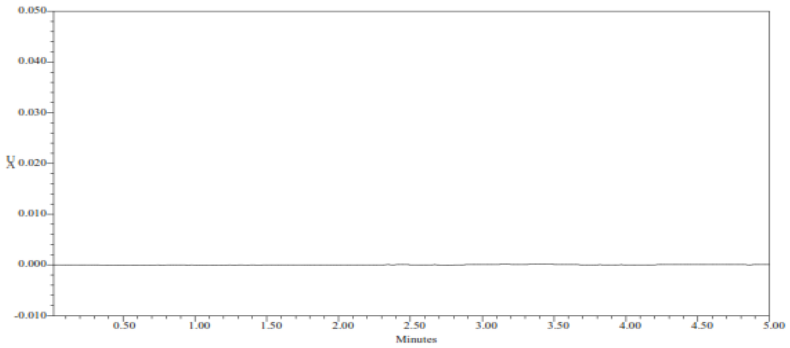

Fig.9. Chromatogram showing blank preparation (Mobile Phase)

Assay:
Assay was carried out with the proposed method by taking the formulation available from the market. The results were found to be within the limits and the % assay of the formulation was found to be 99.94.

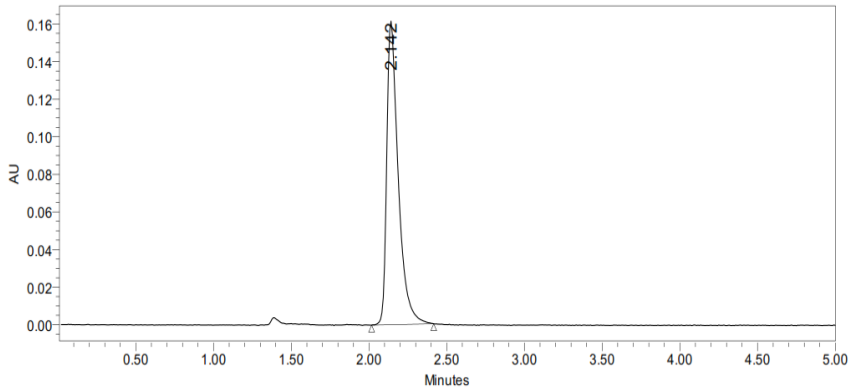

Fig.10. Chromatogram showing assay of Brivaracetam marketed formulation.

IV. SUMMARY AND CONCLUSION

The proposed RP-HPLC method was found to be simple, specific, accurate, precise, robust, rapid and economical. This method gives good resolution of the compound with a short analysis time. Thus the proposed method can be useful for routine analysis of Brivaracetam in API and Pharmaceutical dosage form.

BIBLIOGRAPHY

[1]. Vavilala Vishweshwar, J. Moses Babu and R. Muralikrishna, Development and Validation of Stability-Indicating UPLC Method for the Determination of Brivaracetam, its Related Impurities and Degradation Products, International Journal of Pharmaceutical Sciences and Research, 2018; Vol. 9(6): 2315-2327.

[2]. Pankaj Bhamare, Rupal Dubey, Neeraj Upmanyu, Pothuvan Umadoss, A simple HPLC Method for In-Vitro Dissolution study of Brivaracetam in Pharmaceutical Dosage Form, Asian Journal of Pharmaceutical Analysis, 2021, 11(1).

[3]. N.V Mali, D V Mhaske, HPLC Studies on Degradation Behavior of Brivaracetam and Development of Validated Stability - Indicating HPLC Assay Method, International Journal of Science and Research methodology, September 2016, (4),3: 43-57.

[4]. Practical HPLC Method Development Lloyd R.Snyder, Joseph J. Kirkland, Joseph L. Glajch, Second Edition, 1, 420-430,686-704.

[5]. D. Atul Vasanth*, B. Rajkamal, A validated LC-MS/MS method for pharmacokinetic study of brivaracetam in healthy rabbits, International Journal of Pharmacy and Pharmaceutical Sciences, 2018, 10(2): 24-29.

[6]. Manoj S. Charde, A. S. Welankiwar, Jitendra Kumar Method development by liquid chromatography with validation Int J Pharma Chemistry,2014,4(2), 57-61.

[7]. ICH, Validation of analytical procedures: text and methodology Q2 (R1), in: proceedings of the International Conference on Harmonization, IFPMA, Geneva, 2005.

[8]. Basant Lal, Devesh Kapoor, Manish Jaimini, A review on analytical method validation and its regulatory perspectives, Journal of Drug delivery and therapeutics, 2019; 9(2):501-506.

K.V.L.D.Spandana, et. al. "Analytical Method Development and Validation Of Brivaracetam In Bulk And Pharmaceutical Dosage Form By Rp-Hplc Method." *IOSR Journal of Pharmacy (IOSRPHR)*, 11(05), 2021, pp. 26-33.