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Studies in Stability Indicating Chromatographic Method Development and Validation

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KEYWORDS

HPLC, Brivaracetam,

method development, validation, forced degradation

ABSTRACT:

Rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validation of Brivaracetam in its pure form as well as in tablet dosage form. Chromatography was carried out on Inertsil ODS-3, 250 mm x 4.6 mm, 5µm column using a mixture of phosphate buffer: acetonitrile (60:40%v/v) as the mobile phase at a flow rate of 1.0 ml/min, the detection was carried out at 205 nm. The retention time of the brivaracetam was 3.267 min. The method produces linear responses in the concentration range of 100-300 µg/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.

Introduction: Analysis is necessary for all goods and services, but it is especially important for medications because they deal with human life. Analytical chemistry is the study of chemical additives' separation, measurement, and identification. Analytical knowledge is divided into two kinds.

Objectives: To develop UV and HPLC method for determination of Brivaracetam in the Pharmaceutical Dosage form ,To study stability indicating method of drug

Methods: Understanding the Physicochemical properties of drug molecule. ➢ Selection of chromatographic conditions.➢ Developing the approach of analysis. ➢ Sample preparation ➢ Method optimization➢ Method validation.

Results: HPLC method development and validation involves a systematic approach to designing and verifying an analytical method for separation, detection, and quantification of analytes. The process typically includes understanding the sample's characteristics, selecting appropriate chromatographic conditions, optimizing the method, and then validating the method's performance.

Conclusions: A simple, rapid, selective, precise and accurate HPLC method has been developed for the estimation of Brivaracetam in bulk drugs and its Tablet dosage forms.The test method is validated for Specificity (Selectivity), Linearity (Range), Precision (System, Method), Accuracy (Recovery), Stability of analytical solution and Filter suitability found to be within the specified limit.

1. Introduction

Worldwide, around 50 million people suffer with epilepsy. In addition to being approved for the treatment of multiple seizure or syndrome types, more than 20 antiepileptic medications (AEDs) are available for the treatment of epilepsy. A third or more of patients, nevertheless, do not improve with AED therapy. Epileptic seizures, which are caused by an aberrant

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synchronized firing of excitatory neurons in the brain, are a defining feature of this illness. [1]

A novel antiepileptic medication (AED) called brivaracetam [(2S)-2-[(4R)-2-oxo-4-propylpyrrolidin-1-yl] butanamide] (figure 1) has been authorized for the adjunctive treatment of focal (partial onset) seizures in adults. Within minutes of treatment, the target molecule, SV2A, is engaged, demonstrating its high lipid solubility and quick brain penetration.[2] Brivaracetam is a 4-n-propyl analogue of levetiracetam and a third-generation antiepileptic racetam derivative.[3,4] Brivaracetam's precise mode of action is unknown, although it is thought to have an anticonvulsant effect because of its strong affinity for the brain's synaptic vesicle protein 2A (SV2A). A protein-coding gene involved in synaptic signal transmission is the SV2A glycoprotein. [5]

In the United States (UCB Inc., 2018), brivaracetam is prescribed as monotherapy or adjunctive therapy for focal seizures in patients aged ≥4 years, and in the European Union (EU), it is prescribed as adjunctive therapy for focal seizures with or without secondary generalization in patients aged ≥4 years (UCB Pharma SA, 2018). For the best seizure management, many epileptic patients will need multiple AEDs, and many more have comorbidities that necessitate medication. Understanding the possible drug-drug interactions (DDIs) that may arise when using brivaracetam is crucial since they might impact therapeutic drug levels and, consequently, a medicine's efficacy or safety. Hepatic and extra-hepatic cytochrome P450 (CYP450) enzymes metabolize a wide range of medications; medications that inhibit or induce these enzymes may result in toxicity, adverse events, or increased or decreased efficacy; in the end, dose adjustments may be necessary to maintain appropriate therapeutic drug levels. Drugs that impact other metabolizing enzymes (such epoxide hydrolase) or drug transporters (like P-glycoprotein [P-gp]) may have comparable effects.[6]

The pharmacokinetic properties of brivaracetam are considered while determining the probability of DDIs. Brivaracetam has almost full oral bioavailability and is quickly absorbed. Brivaracetam has a distribution volume of 0.49 ± 0.05 L/kg, which is comparable to the total volume of bodily water, and minimal plasma protein binding (<20%).[7]

Objectives

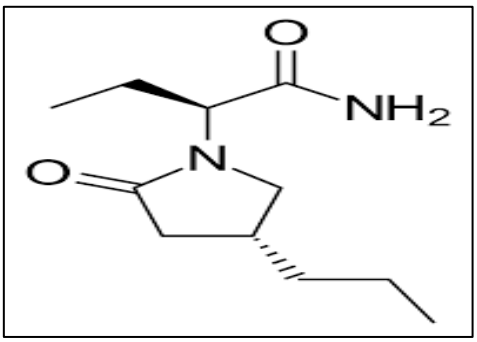

Figure 1: Structure of Brivaracetam

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2. Materials &Methods

Brivaracetam API was procured from Zenvision Pharma LLP. The other chemicals such HPLC grade acetonitrile and OPA was purchased from Merck India Pvt Ltd, Mumbai. AR grade potassium dihydrogen phosphate was

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purchased from Merck India Pvt Ltd, Mumbai. Milli Q water was prepared in-house.

Preparation of Buffer and Mobile phase:

Preparation of Buffer:

Weighed and transferred about 2.72 g of Potassium Di-hydrogen Phosphate into 1000 ml volumetric flask. Added 700 ml of water, sonicated to dissolve and mixed well properly. Diluted up to the mark with water and mixed well. Adjusted pH 3.0 properly with diluted ortho phosphoric acid solution. Filtered through 0.45-micron membrane filter.

Preparation of Mobile phase:

Prepared mixture of buffer pH 3.0 and Acetonitrile in the ratio of 60:40 (v/v) and mixed well. Sonicated to degas.

Preparation of Diluted Ortho Phosphoric acid solution:

Diluted 2.0 ml of ortho phosphoric acid in 100 ml of water and mixed well.

Preparation of Diluent:

Prepared mixture of Buffer pH 3.0 and Acetonitrile in the ratio of 50:50 (v/v) and mixed well. Sonicated to degas.

Blank: Used Diluent as blank solution.

Instrumentation and chromatographic conditions:

Analytical method development and validation was performed on System Alliance Waters 2690 separation module, waters 2996 photo diode array detector (PDA). Empower 3 software was used for data acquisition and data integration. Inertsil ODS-3, 250 mm x 4.6 mm, 5µm column was used for chromatographic separation having column temperature 35°C. Mobile phase used was pH 3.0 buffer solution: acetonitrile in the ratio 60:40 % v/v with flow rate 1.5 ml/min. The detection wavelength used was 205 nm.

Standard stock solution (10 ppm):

Weighed and transferred about 10.0 mg of Brivaracetam working standard into 10 mL of volumetric flask, added 5.0 mL of diluent and sonicated to dissolve. Cooled to room temperature and diluted up to the mark with diluent. Shake well to mix properly.

Preparation of working standard solution:

This solution was prepared by withdrawing 0.1 mL standard stock solution in 10 mL volumetric flask. Made up the volume with diluent. Shake well to mix properly.

Analytical Method Validation

The objective of the validation of an analytical procedure is to demonstrate that the method is suitable for its intended purpose. According to ICH Q2(R1) guidelines, following parameters are performed to validate the method.

1. System suitability
2. Linearity
3. Accuracy
4. Precision
   a) System Precision
   b) Method Precision
5. Robustness

1. System suitability

System suitability tests are method specific test to decide if the analytical system is fit to use immediately before committing the samples for analysis.

Standard solution of Brivaracetam was injected five times into HPLC system as per test procedure. The system suitability parameters were evaluated from standard chromatograms obtained, by calculating the % RSD of retention times, tailing factor, theoretical plates and peak areas from three replicate injections.

Acceptance criteria - % RSD for the retention times of principal peak from 5 replicate injections of each Standard solution should not be more than 2.0 %. The number of theoretical plates (N) for the peaks should be not less than 2000. The Tailing factor (T) for the peaks should not be more than 2.0.

2. Linearity

Performed linearity at five levels over the range of 50% to 150% of the working concentration. Prepared standard stock solution of Brivaracetam standard and diluted suitably to obtain desired concentration at about 50%, 80%, 100%, 120% and 150%. Blank, standard solution and linearity levels solutions were prepared and analysed as per method validation protocol. Correlation coefficient observed must be greater than 0.995.(8)

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3. Accuracy

Accuracy was determined at three levels (50%, 100% and 150%). The accuracy of the method for Brivaracetam should be determined by spiking Brivaracetam API in presence of placebo which is prepared as per test methodology. Each level prepared in triplicate and performed the assay as per test methodology.

4. Precision

a) System precision

It gives degree of reproducibility of analytical procedure for the given method parameters. Blank, standard solution was prepared and analysed as per method validation protocol. Injected blank, standard solution (six replicates) as per methodology and analysed as per method. Calculated % RSD of six replicate standards.(9)

b) Method precision

Prepared the six Assay sample solutions as per test methodology for strength i.e. 100 mg and analyse as per method validation protocol.

5. Robustness

Robustness was done by deliberate varying method parameters (e.g. Column oven Temperature, Mobile phase flow) and measured the effect on the method by monitoring system suitability. All system suitability parameters should be within limit as per test methodology system suitability criteria. The absolute difference % assay value from method precision and robustness should be within ± 2.0 %.(10)

Forced Degradation Studies

A. Acid Degradation
B. Basic Degradation
C. Oxidative Degradation
D. Thermal Degradation

A. Acid degradation:

Weigh 100 mg of Brivaracetam into 50 ml volumetric flask, add 40 ml of diluent, sonicate for 15 min. Add 2 ml of 0.1 N HCl, keep on water bath for 4 hours at 80°C, neutralise and cool the solution. Dilute to volume with diluent and mix well. Filter the solution through 0.45 µm PVDF syringe filter. Keep the solution in dark for 6 hours. Withdraw the sample and analyse using HPLC.

B. Basic Degradation:

Weigh and transfer Brivaracetam API about 100 mg into 50 mL of volumetric flask, add 40 mL of diluent, sonicate for 15 minutes with intermittent shaking and maintain the sonicator temperature at below room temperature throughout the sonication procedure. Add 2 mL of 2N NaOH, keep on water bath for 4 hrs at 80°C, neutralize and cool the solution. Dilute to volume with diluent and mix well. Filter this solution through 0.45µm PVDF syringe filter. Keep the solution in dark for 6 hours. Withdraw the sample and analyse using HPLC.

C. Oxidative Degradation:

Weigh and transfer Brivaracetam API about 100 mg into 50 mL of volumetric flask, add 40 mL of diluent, sonicate for 15 minutes with intermittent shaking and maintain the sonicator temperature at below room temperature throughout the sonication procedure. Add 5 mL of 20% peroxide, keep on water bath for 4 hrs at 80°C. Cool and dilute to volume with diluent and mix well. Filter this solution through 0.45µm PVDF syringe filter. Keep the solution in dark for 6 hours. Withdraw the sample and analyse using HPLC.

D. Thermal Degradation:

Keep sufficient quantity of Brivaracetam API into suitable container at 60°C in oven for 48 hours. Remove container from oven and cool at room temperature. Weigh and transfer Brivaracetam API about 100 mg into 50 mL of volumetric flask, add 40 mL of diluent, sonicate for 15 minutes with intermittent shaking and maintain the sonicator temperature at below room temperature throughout the sonication procedure. Dilute to volume with diluent and mix well. Filter this solution through 0.45µm PVDF syringe filter. Keep the solution in dark for 48 hours. Withdraw the sample and analyse using HPLC.(11)

Acceptance criteria for forced degradation studies: The % degradation should not be more than 30%.

3. Results

Analytical Method Validation

1. System Suitability

No. of theoretical plates were found to be as mean of five replicated as 11715 which is greater than 2000. The mean

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%RSD was found to be 1.002 and mean tailing factor was found to be 1.28 which satisfied the acceptance criteria as shown in table 1 and figure 2.

Injection	Retention time	Peak area	Plate count	Tailing factor
1	3.268	2005499	11517	1.09
2	3.266	2008047	11829	1.06
3	3.270	2011629	11829	1.1
4	3.267	2013172	11821	1.05
5	3.267	2012186	11637	1.09
MEAN	3.267	2010106.6	11715	1.078
SD	0.0015	2881.32	11703.8	0.0139
%RSD	0.046	0.144	1.002	1.28

Table 1. Results for system suitability test

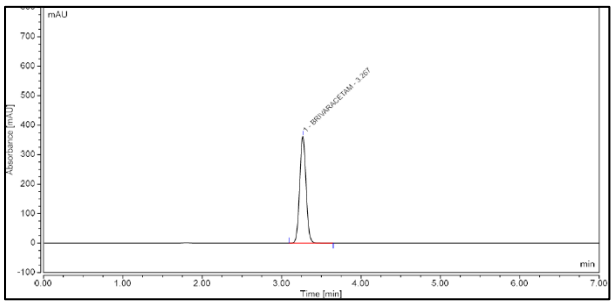

Figure 2: System suitability chromatogram for Brivaracetam

2. Linearity

Linearity was performed at different levels such as 50%, 80%, 100% 120% and 150%. The correlation coefficient was found to be 1.00 which satisfied the criteria as shown in table 2.

Linearity levels	Final conc in ppm	Mean area
50%	100	1002750
80%	160	1604399
100%	200	2005499
120%	240	2406599
150%	300	3008249
Correlation coefficient (r)		1.00
Slope		10027
Y-intercept		0.0621
%Y-intercept		0.48

Table 2. Result of linearity

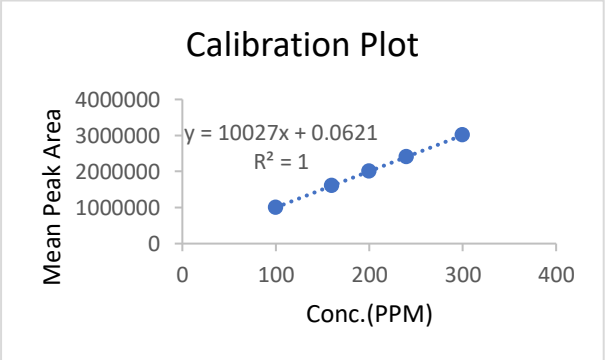

Figure 3: Linearity curve

3. Accuracy

Accuracy was performed by spiking the placebo with standard API at three levels i.e. 50%, 100% and 150% in triplicates. The %RSD for recovery at all three levels was found to be less than 2% as summarised in table 3.

Accuracy levels	Mean % recovery	SD	%RSD
Level-1 (50%)	101.0	0.41	0.40
Level-2 (100%)	100.4	0.14	0.13
Level-3 (150%)	99.5	0.30	0.30
Overall mean		100.3	
Overall SD of recovery for all levels		0.28	
%RSD of recovery for all level		0.27	

Table 3. Result for Accuracy

4. Precision

a) System precision

It was performed to determine the suitability of the used system to produce the required data. The results were determined by injecting six replicates of standard

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solution into the system. The %RSD for retention time and peak area were found to be less than 2%. As in figure 3 and table 4.

Sr. no.	Name of Injection	RT (min)	Area
1.	STD replicate 1	3.268	2005499
2.	STD replicate 2	3.266	2008047
3.	STD replicate 3	3.270	2011629
4.	STD replicate 4	3.267	2013172
5.	STD replicate 5	3.268	2012186
6.	STD replicate 6	3.267	2014638
Mean		**3.267**	0.0015
SD		**0.0015**	0.046
%RSD		**0.046**	3.268

Table 4. Result for system Precision

b) Method precision

Method precision was done to determine whether the proposed method is precise or suitable for analysing the specific drug. For this, %assay was performed. The strength used for the purpose was 100 mg. The %RSD was found to be less than 2% as shown in table 5.

Sample no.	% assay
1	100.2
2	100.1
3	101.6
4	100.5
5	99.2
6	99.5
Mean	**100.2**
SD	**0.84**
%RSD	**0.01**

5. Robustness

Robustness is small but deliberate changes such as change in column oven temp (°C) and flow rate (ml/min) which are made in the set method to determine whether the set method is produces results having %RSD less than 2% and all other parameters such as tailing factor, theoretical plates are as per acceptance criteria as summarized in table 6.

Table 5. Result for Robustness

Parameters	Altered conditions	%RSD	Tailing factor	Theoretical plates	% assay
SST condition	35°C	0.17	1.1	6713	100.2
Column oven temp (°C)	33	0.06	1.02	6481	99.0
	37	0.06	0.97	6658	98.9
SST condition	1.5 ml/min	0.17	1.1	6713	100.2
Flow rate (ml/min)	1.3	0.08	0.98	7599	98.8
	1.7	0.07	1.01	6592	98.7

Forced Degradation Studies:

A. Acid Degradation:

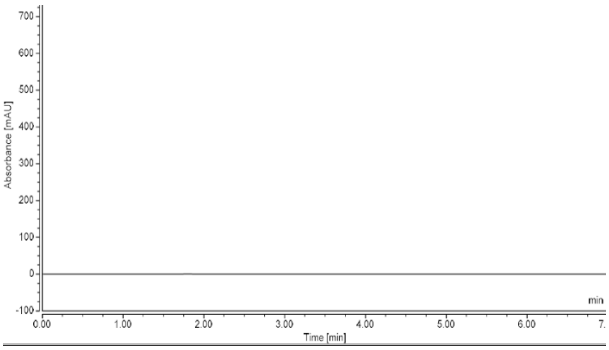

Figure 4: Acid Control

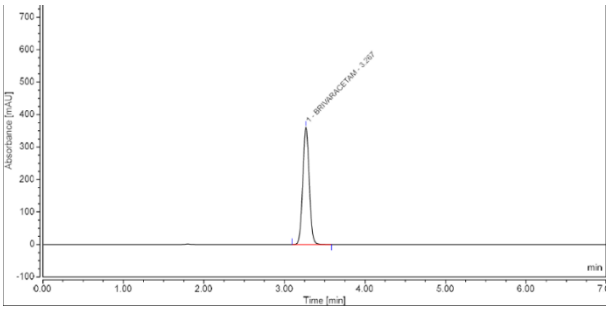

Figure 5: Acid Degradation

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B. Basic Degradation:

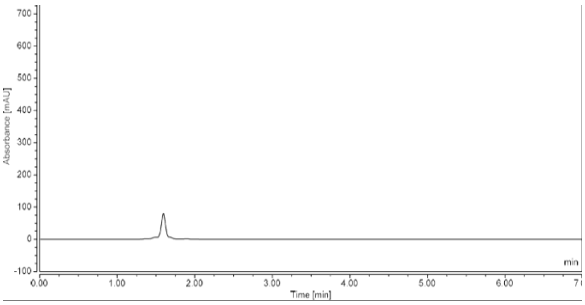

Figure 6: Basic Control

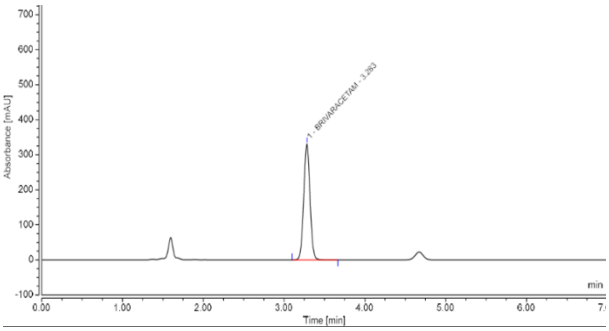

Figure 7: Basic Degradation

C. Oxidative Degradation:

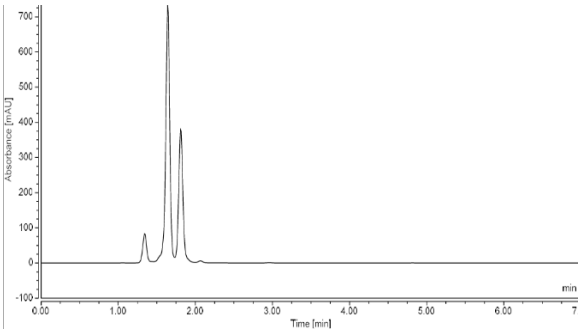

Figure 8: Oxidative Control

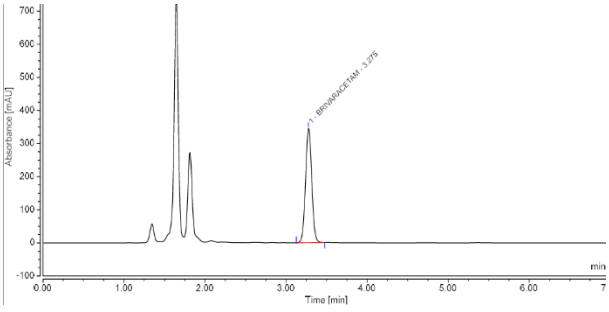

Figure 9: Oxidative Degradation

D. Thermal Degradation

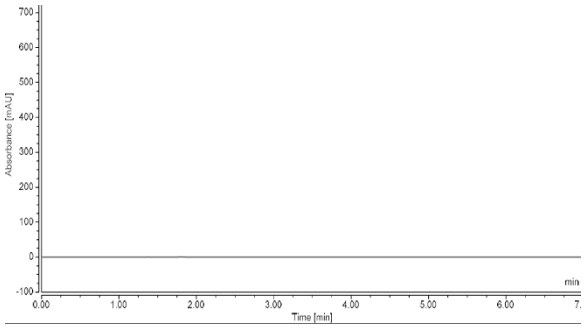

Figure 10: Thermal Control

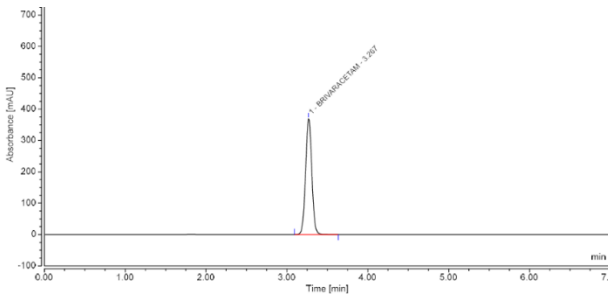

Figure 11: Thermal Degradation

Summary of Forced degradation studies:

Degradation type	Experimental conditions	Sampling time	% degradation	% recovery
Acidic	0.1N HCl	6 hrs	2.81	97.19
Basic	2N NaOH	6 hrs	2.68	97.32
Oxidative	20 %	6 hrs	8.7	91.30
Thermal	60°C	48 hrs	1.46	98.54

4. Conclusion:

Brivaracetam API analysis has been accomplished by the development and validation of a sensitive and selective HPLC technique. Facilitate the suggested HPLC approach, which has excellent repeatability, accuracy, and affectability. The outcome demonstrates that the new approach is yet another appropriate technique for assay, purity and stability that can support the examination of Brivaracetam in tablet formulations. The validation experiments proved the methods to be linear, precise, accurate, specific and selective to the drug in presence of

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degradation products. The drug showed highest degradation in oxidative degradation which was found to be 8.7%, the highest of all. The method has been applied in testing of the commercially available tablets. The suggested method can be used for routine analysis of brivaracetam in quality control laboratories without harming the environment.

5. Ethical consideration

The present study not required any investigations/interventions to be conducted on the human subjects/patients; project does not involve any drug trial on animals.

6. Acknowledgement

The authors are thankful to Zenvision Pharma LLP for providing the sample for brivaracetam.

7. Conflict of interest

The authors declared no conflict of interest.

Refrences

1. Khaleghi F, Nemec EC. Brivaracetam (briviact): a novel adjunctive therapy for partial-onset seizures. Pharm Ther. 2017;42(2):92.

2. Klein P, Diaz A, Gasalla T, Whitesides J. A review of the pharmacology and clinical efficacy of brivaracetam. Clin Pharmacol Adv Appl. 2018 Jan;Volume 10:1–22.

3. Lattanzi S, Cagnetti C, Foschi N, Provinciali L, Silvestrini M. Brivaracetam add-on for refractory focal epilepsy: A systematic review and meta-analysis. Neurology. 2016 Apr 5;86(14):1344–52.

4. Fisher RS, Acevedo C, Arzimanoglou A, Bogacz A, Cross JH, Elger CE, et al. ILAE Official Report: A practical clinical definition of epilepsy. Epilepsia. 2014 Apr;55(4):475–82.

5. Tiwari MN. Brivaracetam: A 3rd Generation Anti-Epileptic With Higher Efficacy And Better Safety Profile. [cited 2025 Feb 20]; Available from: https://www.drsrce.com/article-details-brivaracetama3rdgenerationantiepilepticwithhigherefficacyandbettersafetyprofile

6. Moseley BD, Chanteux H, Nicolas JM, Laloyaux C, Gidal B, Stockis A. A review of the drug−drug interactions of the antiepileptic drug brivaracetam. Epilepsy Res. 2020 Jul 1;163:106327.

7. Sargentini-Maier ML, Espié P, Coquette A, Stockis A. Pharmacokinetics and Metabolism of 14C-Brivaracetam, a Novel SV2A Ligand, in Healthy Subjects. Drug Metab Dispos. 2008 Jan 1;36(1):36–45.

8. Swartz ME, Krull IS, editors. Analytical Method Development and Validation. Boca Raton: CRC Press; 2018. 96 p.

9. Ngwa G. Forced degradation as an integral part of HPLC stability-indicating method development. Drug Deliv Technol. 2010;10(5):56–9. 10. ICH I. Q2 (R1): Validation of analytical procedures: text and methodology. In: International conference on harmonization, Geneva. 2005.

11. Kats M. Forced degradation studies: regulatory considerations and implementation. 2005;