



Simultaneous determination of Brivaracetam and its isomers in the Brivaracetam drug by RP-HPLC

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ABSTRACT

It is clear from the literature that there are only a few methods reported with limitations of sophisticated instruments and poor sensitivity for the assay of Brivaracetam, and, particularly, no report on the simultaneous determination of the assay of Brivaracetam and its enantiomers (En) and diastereomers (DS) in a single method. Hence, it was our goal to build on a robust high-performance liquid chromatography (HPLC) process to the simultaneous assessment of Brivaracetam, and its En and DS, from the Brivaracetam drug. Accordingly, the reverse-phase chiral HPLC method development was established in a constructive way, such as buffer selection, column selection, mobile phase composition, organic modifier compositions, and column temperature variation. As a consequence, the simultaneous assessments were achieved in Daicel CHIRALPAK IG-3 (250 mm x 4.6 mm x 3 μm) with reverse-phase setup conditions consisting of 10 mM (NH₄)HCO₃ buffer: CH₃CN (1:1) %v/v in an isocratic elution. High sensitivity was obtained for Brivaracetam and its stereoisomers at Limit of quantitation (LOQ) 0.25 μg/ml and Limit of detection (LOD) 0.08 μg/ml. This method is systematically validated by the International Council for Harmonization procedures. Thus, the method is applicable in quality control, in drug analysis, and in the investigation of counterfeit drugs as well.

INTRODUCTION

High-performance liquid chromatography (HPLC) is an influential technique for separating and comparing components to a greater extent than other techniques, which is uniquely held as an inevitable technique in the pharmaceutical domain. Reverse-phase chromatography (RP-HPLC) is a sub-technique that consists of both stationary and liquid phases where the components interact between phases, thus separating into possible individual components based on their interaction differences (Ahuja, 2007; Bailey, 1976).

Brivaracetam is an additional drug for the treatment of epilepsy (Khaleghi, 2017). Epilepsy is defined as an intellectual disarray that causes an individual to have persistent seizures

at 16 years of age and to grow up with epilepsy. This is an unending medicinal disarray (typically consequential in erratic, malicious, repeated seizures which affect a range of physical and psychological functions), affecting more than 50 million people across the globe annually. BRIVIACT is used in combination with other antiepileptic drugs to treat the same type of seizures (Matagne *et al.*, 2008; Rogawski, 2016).

To the best of our knowledge, there are no effective HPLC methods for the simultaneous quantification of assays and isomers of the Brivaracetam drug substance. Such a capable method to determine the assay free from isomers (Liao *et al.*, 2020; Qiu *et al.*, 2016) is more authenticated to estimate the content of active ingredients (Abram *et al.*, 2019; Blackstone *et al.*, 2014). Even the few reported methods are also limited to either lesser sensitivity (Baksam *et al.*, 2020; Bhamare *et al.*, 2021; Jessica *et al.*, 2021; Mali and Mhaske, 2016; Mansour *et al.*, 2021; Pankaj *et al.*, 2020; Vasanth and Rajkamal, 2018; Zhu *et al.*, 2017) or the need of sophisticated sensitive instruments (i.e., ultra-performance liquid chromatography) than HPLC (Iqbal *et al.*, 2017; Mohamed *et al.*, 2020; Vishweshwar *et al.*, 2018). It is essential to develop an

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economic, simple, and single method to address all the issues with easy accessibility. Accordingly, the present research focused on developing/validating a highly sensitive/robust RP chiral HPLC approach to determine the Brivaracetam isomers (Fig. 1) from the Brivaracetam drug in the presence of related substances and its degradants.

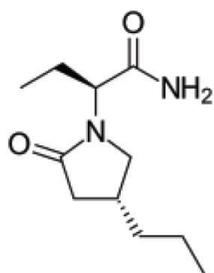
MATERIALS AND METHODS

Chemicals

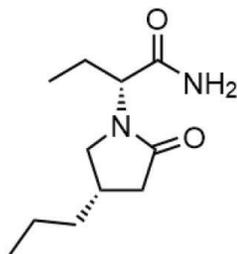
Analytical reagent grade ammonium bicarbonate, ammonium formate, ammonium acetate, and chromatography solvents were procured from Honeywell (Hyderabad). Milli-Q water equipment was used as the water source to prepare all solutions. The active ingredients and related components were procured from authenticated sources.

Instrumentation

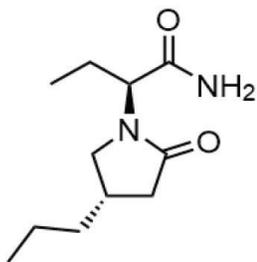
The system used for the method development and validation in this study was Agilent 1260 Infinity II HPLC, with a low pressure pump, a degasser, an ultraviolet detector, autosampler, column thermostat, and a controller with Empower3 operating system from Waters. The same conditions were used for the MS study in the Liquid chromatography and mass spectrometry (LCMS) system using Agilent 6470B triple quadrupole LCMS, controlled by MassHunter workstation software.



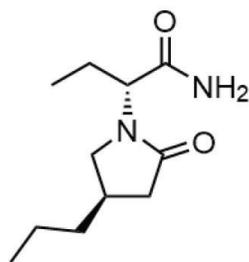
Brivaracetam (α S,4R)
(Drug substance)



Brivaracetam (α R,4S)
(Enantiomer)



Brivaracetam (α S,4S)
(Diastereomer-1)



Brivaracetam(α R,4R)
(Diastereomer-2)

Figure 1. Brivaracetam and its isomers (Note: Related compounds 1–3 are process related impurities of Brivaracetam, which are not provided here).

Chromatographic conditions

The required resolution was successfully attained using CHIRALPAK IG-3 (250 mm \times 4.6 mm \times 3 μ m) with a 10 mM (NH₄)HCO₃ buffer: CH₃CN (1:1) %v/v with a constant separation mode. The eluent was driven as 1 ml/minute in a constant flow mode with column thermostat at 25°C and detection wavelength at 210 nm. The analyte solution was introduced using the 100 μ l loop with the accurate volume of 10 μ l. The DS 1–2, enantiomer, and drug were identified on Agilent 6470B triple quadrupole LCMS. The MS parameters (ESI +ve) were capillary voltage (2.0 kV), cone voltage (15.0 V), extractor voltage (2.0 V), source and desolvation temperatures (150°C and 700°C, respectively), and gas flow (600 l/hour). The developed method was validated by the International Council for Harmonization (ICH, 2005) guidelines.

Related components and isomers' preparation

5 mg each of the related compounds 1–3, DS 1–2, and enantiomer was added to separate 5 ml standard flasks with the diluents to prepare stock-1 solutions. 1 ml of each stock-1 solutions was diluted to 50 ml to obtain stock-2 solutions. Then, 5 ml of each stock-2 solutions was diluted to 100 ml (the resulting solutions contained 0.1% each of related compounds 1–3, DS 1–2, and En). 5 mg of the Brivaracetam racemate was diluted to 5 ml for the purpose of method development.

Standard solution preparation

10 mg of the Brivaracetam standard was diluted to 10 ml, which is known as the assay standard solution (standard stock-1). 1 ml of standard stock-1 was diluted to 50 ml (standard stock-2); furthermore, 5 ml of this was diluted to 100 ml (the resulting solution contained 0.1% Brivaracetam, with a sample strength of 1.0 mg/ml).

Test solution preparation for assay and isomer contents

About 10 mg of the test sample was dissolved and diluted to 10 ml with diluents.

RESULTS AND DISCUSSION

Method development and optimization

Selection of the phases

Choice of the separation method is vital as all components are isomers; therefore, RP was chosen for simple operation. For the separation of chiral and isomeric compounds, chiral phase will be the best choice and RP separation will provide a range of selectivity and easy handling. Columns tested in the study include CHIRALPAK IC, CHIRALPAK IG-3, and CHIRALCEL OJ-3R, with various organic modifiers. The initial experimental data are shown in Table 1, which indicate the importance of stationary phase selection because the selectivity of each analyte really depends on the stationary phase.

The method development was initiated with the volatile 10 mM ammonium bicarbonate, ammonium acetate, and ammonium formate as buffers, and acetonitrile and methanol as organic modifiers. Based on the preliminary trials, it was concluded that CHIRALPAK IG-3 with 10 mM ammonium bicarbonate as the buffer and acetonitrile as the organic modifier

in 50% v/v provided favorable separation for all the isomers, as shown in Figure 2.

Method optimization

The method was optimized with the selective separation of all the isomers and Brivaracetam from other related compounds and unknown impurities using 10 mM ammonium bicarbonate buffer with acetonitrile as the organic modifier in the isocratic condition on CHIRALPAK IG-3. The optimization comprised buffer strength, thermostat, and mobile phase flow. The stationary

phase was recognized based on the trials and column compatibility according to the separation requirements and required system suitability criteria. The method was further optimized with three individual factors at two stages (*viz.* buffer at 10 and 30 mM; thermostat at 20°C and 30°C; and flow at 0.8 and 1.2 ml/minute).

Finally, the modest buffer concentration of 10 mM with a 1.0 ml/minute flow rate was opted as the best condition to obtain the finest resolution with quality peaks. Column thermostat is another important parameter that did not show any notable effect on the selectivity, and hence kept at 25°C. The final chromatograms

Table 1. Stationary phase and mobile phase screening

Column	Buffer (10 mM solution)	Organic modifier	Observation
CHIRALPAK IC	Ammonium bicarbonate	CH ₃ CN	
		CH ₃ OH	
	Ammonium acetate	CH ₃ CN	
		CH ₃ OH	
		CH ₃ CN	
	Ammonium formate	CH ₃ OH	
CH ₃ OH			
CHIRALPAK IG-3	Ammonium bicarbonate	CH ₃ CN	
		CH ₃ OH	
	Ammonium acetate	CH ₃ CN	
		CH ₃ OH	
		CH ₃ CN	
	Ammonium formate	CH ₃ OH	
CH ₃ OH			
CHIRALCEL OI-3R	Ammonium bicarbonate	CH ₃ CN	
		CH ₃ OH	
	Ammonium acetate	CH ₃ CN	
		CH ₃ OH	
		CH ₃ CN	
	Ammonium formate	CH ₃ OH	
CH ₃ OH			

Note: Since the screening was involved with gradient elution baseline deflection observed in the above chromatograms. CH₃CN: Acetonitrile, CH₃OH: Methanol.

are shown in Figures 3–6 and the data of the spiked sample chromatogram are provided in Table 2.

Forced degradation and specificity

The sample was exposed to solid state stress, such as thermal degradation (7 days at 70°C), humidity (7 days at 98% RH/25°C), aerial oxidation exposure (7 days open), and photolytic degradation (UV 200 Watt-hour/m², 1.2 million lux hours), and it was observed that there was no interference at the desired peak. The exposed conditions did not show any major degradation. Peak purity (purity angle < purity threshold) of the exposed samples was verified in the Photo diode array detector (PDA) detector, whereas the peak homogeneity was verified in the mass spectrometer. In MS, three Brivaracetam (BRVC) samples and one batch sample (spiked with all the isomers and related compounds) were analyzed under the

same HPLC conditions with the PDA detector to check the quality of the isomers. All the isomers were confirmed and demonstrated that there are no other impurities co-eluting on the isomer peaks. Because the desired peaks are pure and homogeneous, the method is specific. The absence of interference at the retention time of any isomers from its related compounds and degradation products is witnessed by the data. The peak purity was examined by PDA (purity angle is lesser than the threshold), and the chromatogram and data are shown in Figure 7 and Table 3. The mass spectra with *m/z* values of sodium adduct are shown in Figures 8–12.

Method validation

According to the ICH guidelines, the developed RP chiral method (to estimate the isomers and Brivaracetam in the Brivaracetam drug) was validated. System suitability, specificity,

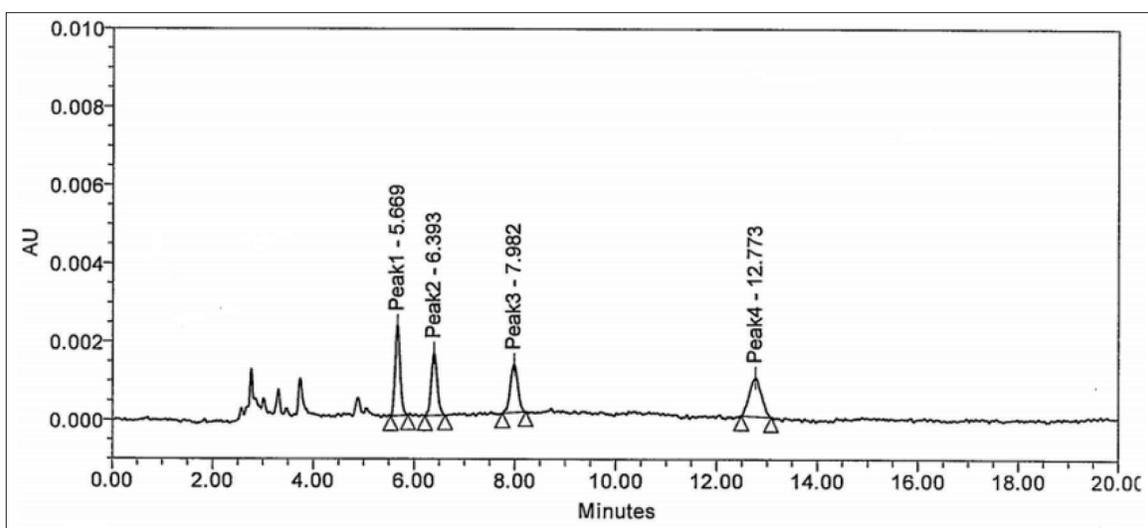


Figure 2. Reverse phase chiral separation of all isomers and Brivaracetam. Elution order is, Peak-1: Enantiomer (En), Peak-2: Diastereomer-2 (DS-2), Peak-3: Diastereomer-1 (DS-1), Peak-4: Drug (Brivaracetam).

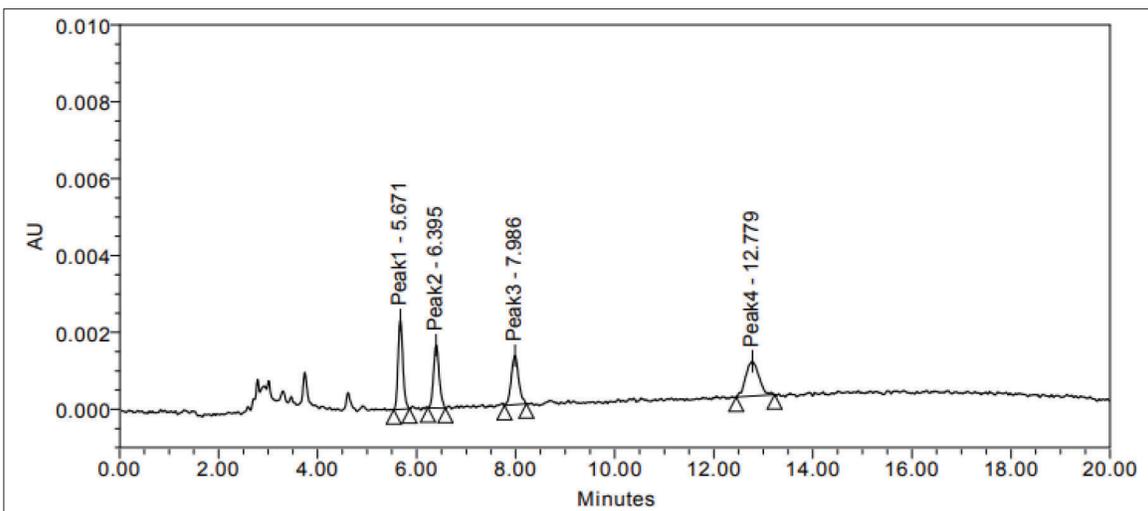


Figure 3. Standard impurity mixture at 1.0 µg/ml level.

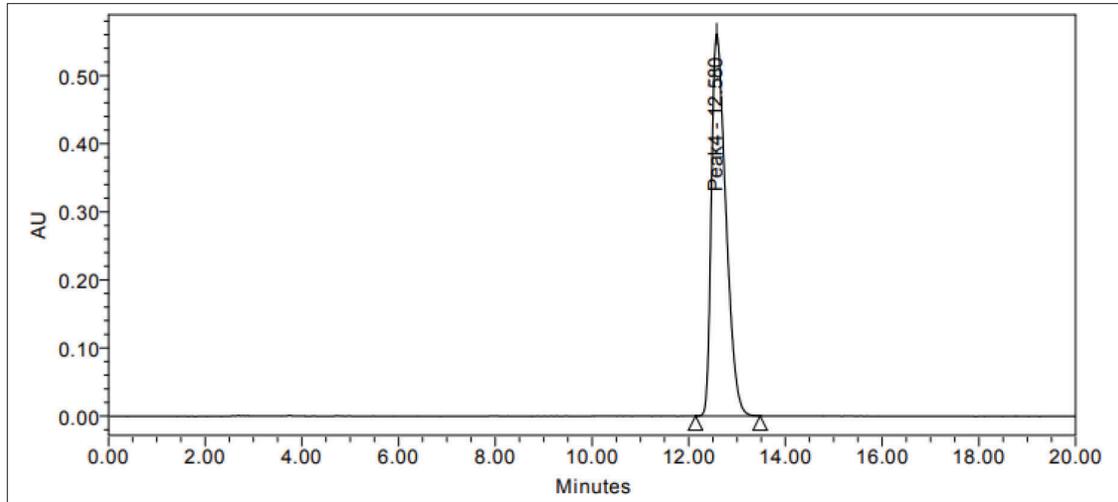


Figure 4. Assay standard chromatogram.

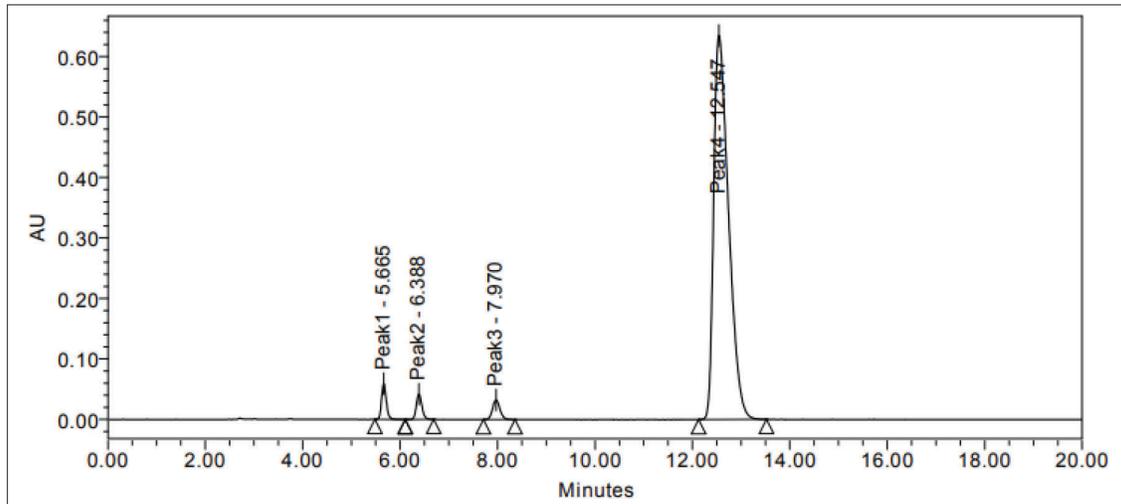


Figure 5. Spiked sample chromatogram.

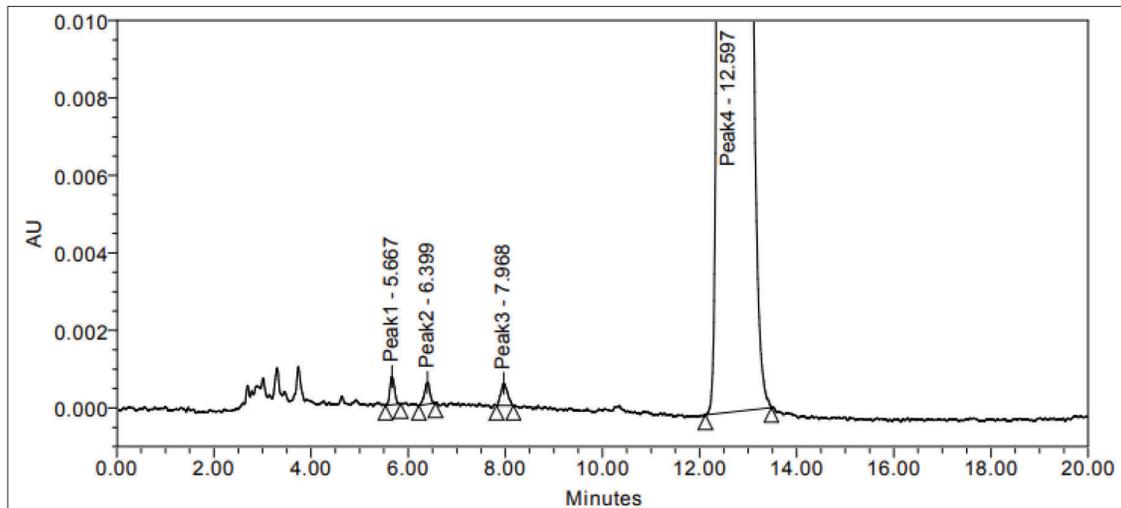


Figure 6. LOQ level isomers spiked sample chromatogram.

system precision, method precision, intermediate precision, recovery, robustness, linearity, LOQ, LOQ precision, LOD, and range were determined.

Specificity

Method specificity was determined by spiking all the related substances in the sample by forced degradation study. In addition, peak homogeneity was confirmed using the LCMS study of the same spiked and unspiked samples by confirming *m/z* pureness of all those peaks.

Linearity and range

The linearity was measured by six levels, i.e., LOQ to 150% level of limit concentration (1 µg/ml) for DS-1, DS-2, En, and Brivaracetam. The linearity study solutions were prepared from LOQ to 150% of specification level for isomers and drug in the isomer quantification, whereas for the assay quantification, 80–120% of the test concentration solutions were injected to

demonstrate the method linearity and range. The correlation was drawn for each analyte concentration against its response.

Precision

Precision was ascertained by determining the repeatability and ruggedness of recoveries (DS-1, DS-2, and En). Repeatability of the method was carried out by the spiked test sample preparations six times with all the components of the drug substance. For the assay, six replicate preparations were made and the % Related standard deviation (RSD) as an evaluation parameter was checked. The ruggedness was also estimated by performing intermediate precision with similar spiked sample preparations on different days under similar conditions and atmosphere.

Accuracy

Accuracy was verified at four concentrations (viz., LOQ, 50%, 100%, and 150%) by spiking all the components (DS-1, DS-2, and En) of Brivaracetam at each level, and calculating the amount recovered and standard deviation at each level in

Table 2. Data of the spiked sample chromatogram.

Name	Retention Time	USP Resolution	RT Ratio
Peak1	5.665	-	-
Peak2	6.388	3.66	0.451
Peak3	7.970	6.38	0.509
Peak4	12.547	10.61	0.635

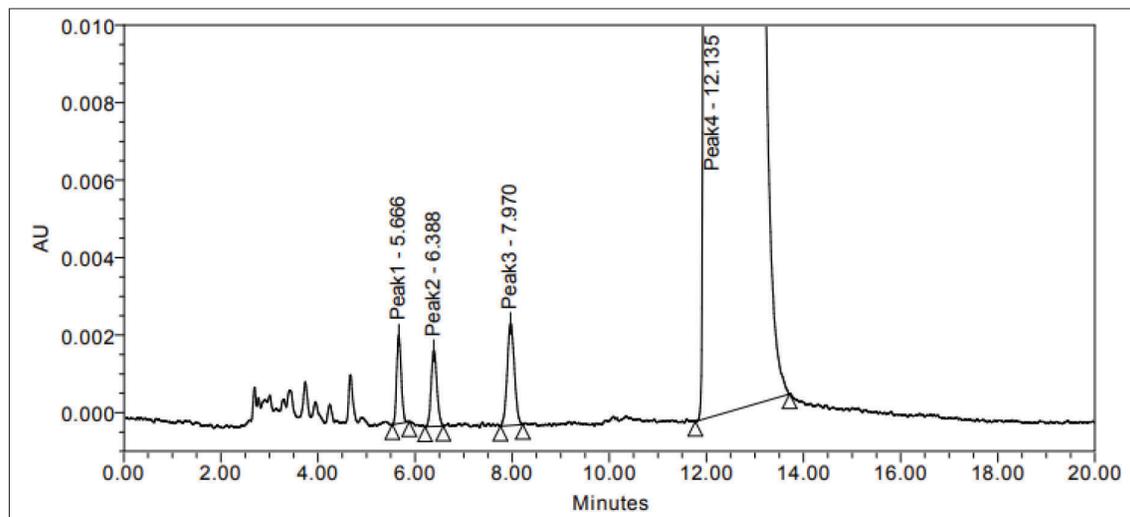


Figure 7. Spiked sample LC chromatogram data for peak purity.

Table 3. Data of the spiked sample LC chromatogram.

Name	Retention time	Purity angle	Purity threshold
Peak1	5.666	0.331	1.340
Peak2	6.388	0.392	1.427
Peak3	7.970	0.451	1.504
Peak4	12.135	0.822	1.026

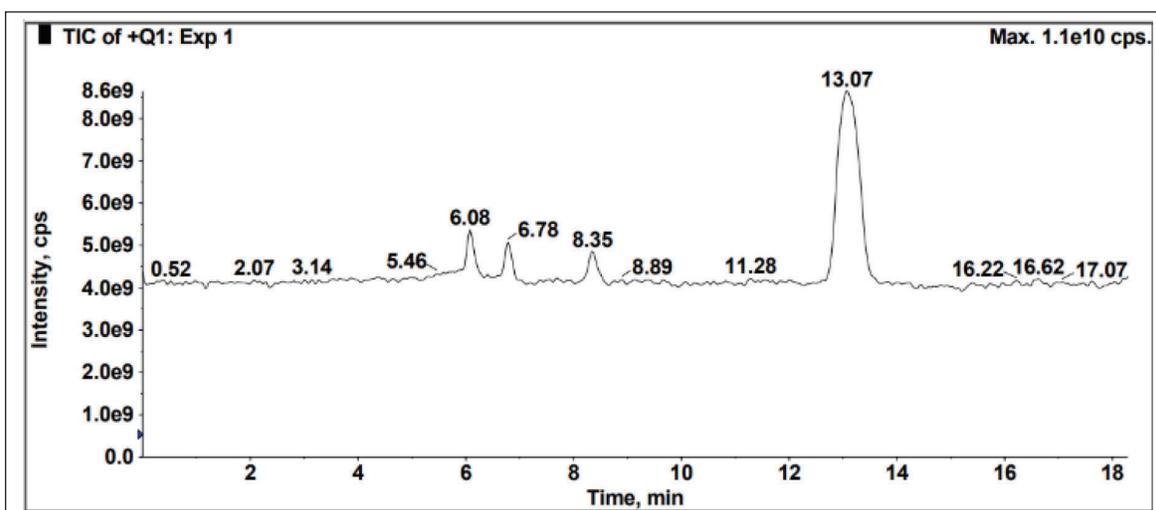


Figure 8. Spiked sample LCMS spectral data Total ion current (TIC) for peak homogeneity.

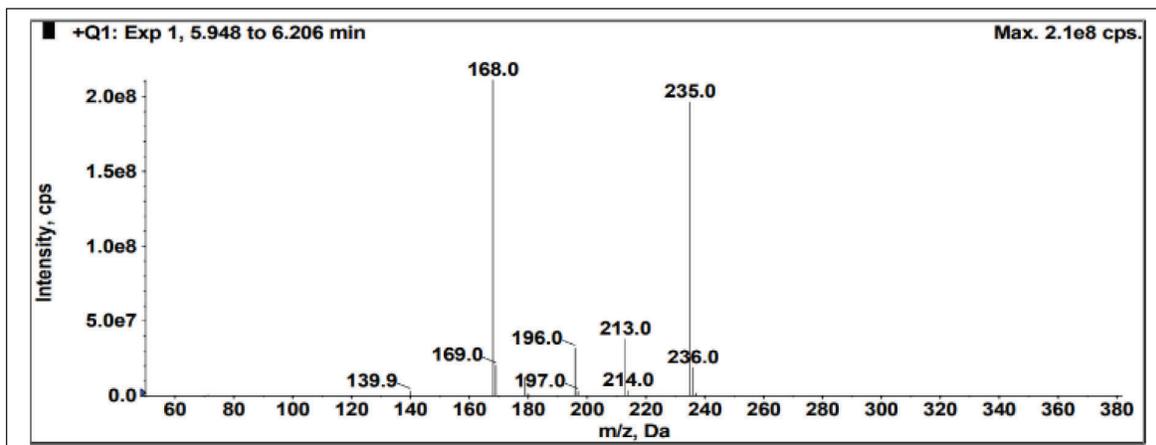


Figure 9. Mass spectrum of Enantiomer (En) from spiked sample.

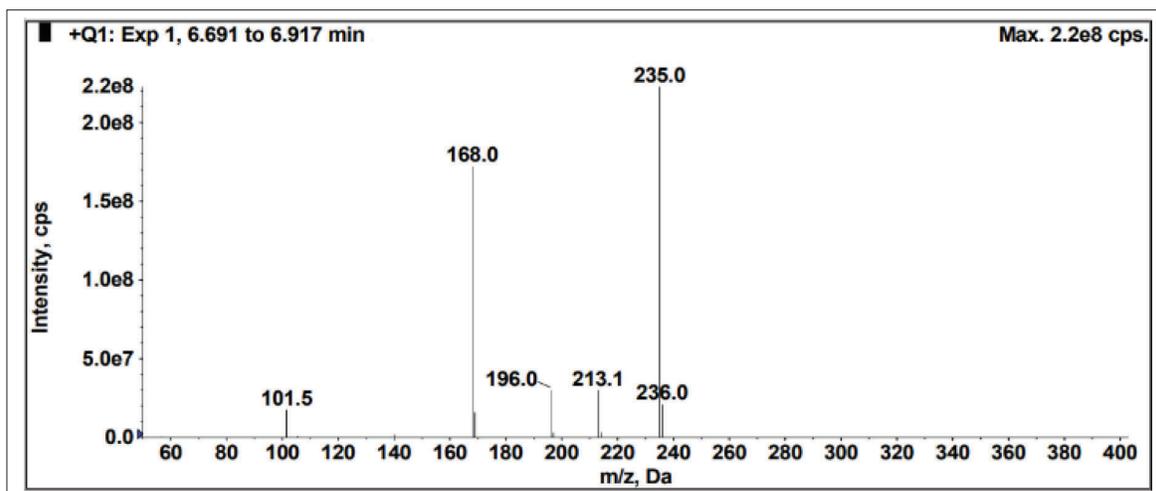


Figure 10. Mass spectrum of diastereomer-2 (DS-2) from spiked sample.

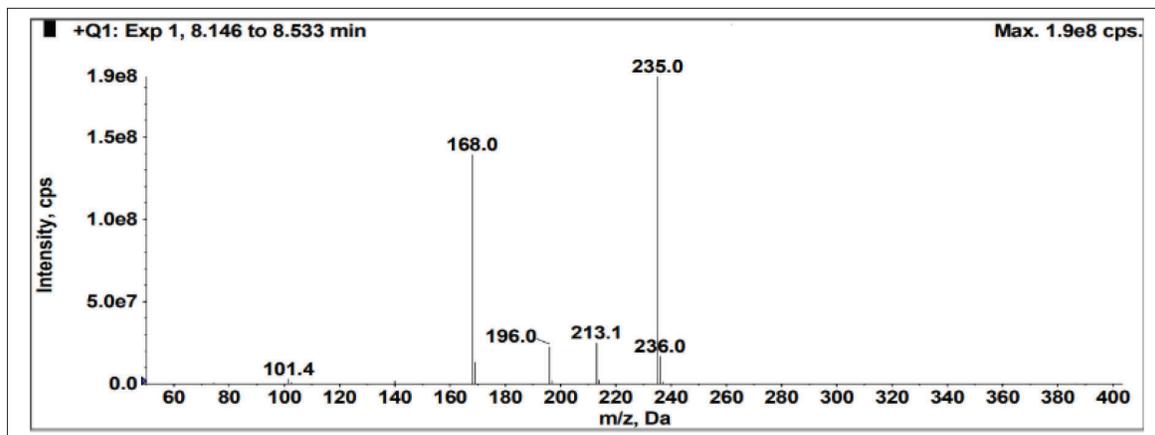


Figure 11. Mass spectrum of diastereomer-1 (DS-1) from spiked sample.

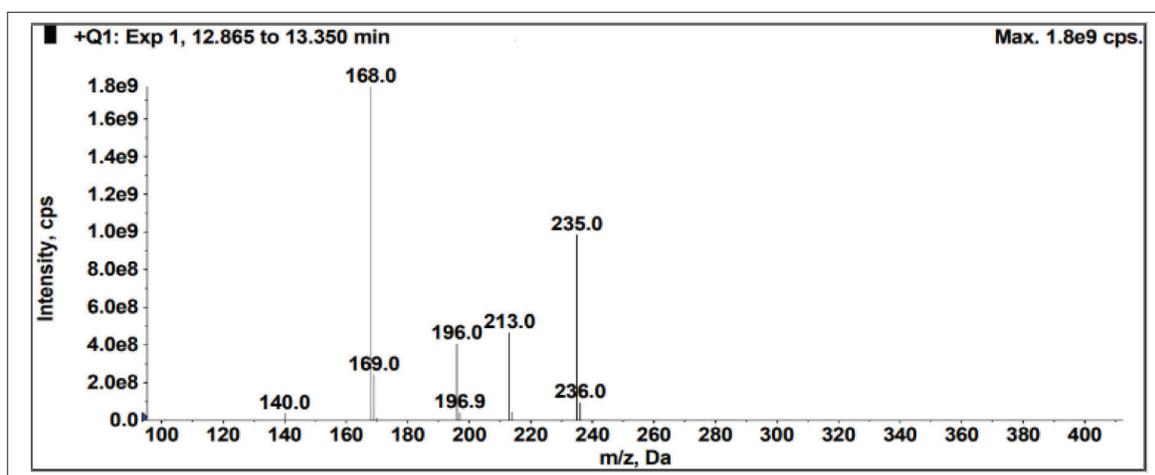


Figure 12. Mass spectrum of drug (Brivaracetam) from spiked sample.

Table 4. Validation data of the proposed method for stereoisomers determination.

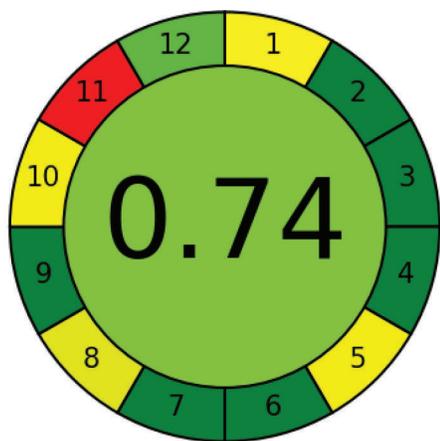
Parameters	DS-1	DS-2	En	Brivaracetam
System precision 1.0 (µg/ml) level standard solutions %RSD	2.5	1.8	1.1	1.3
Linearity and Range (µg/ml)	0.25–150	0.25–1.5	0.25–1.5	0.25–1.5
Slope	174,632	157,500	166,094	154,879
Intercept	0.0059	0.0038	0.0048	0.0074
Correlation-coefficient	0.9999	0.9998	0.9997	0.9997
LOQ (µg/ml)	0.249	0.251	0.248	0.255
LOD (µg/ml)	0.082	0.083	0.082	0.084
Repeatability				
%Content (Spiked sample)	0.11	0.10	0.10	Nil
%RSD	3.3	4.4	4.0	Nil
Intermediate Precision	0.10	0.10	0.10	Nil
%Content (Spiked sample)	2.9	3.5	4.7	Nil

Table 5. Validation of the proposed method for Brivaracetam determination.

Validation parameters	Brivaracetam
Linearity and Range ($\mu\text{g/ml}$)	800–1,200 $\mu\text{g/ml}$
Slope	114,308.7
Intercept	-1.9652
Correlation-coefficient	0.9995
Repeatability	
% Assay	99.64
% RSD	0.44
Intermediate precision	
% Assay	99.32
%RSD	0.33
Accuracy at 80% level	
% Recovery	98.97
% RSD	0.35
Accuracy at 90% level	
% Recovery	99.12
% RSD	0.22
Accuracy at 120% level	
% Recovery	98.78
% RSD	0.47

Table 6. Detailed comparison between the current and literature methods.

S.No	Parameters	Literature report	Current report	Merits/Demerits of current work
1	System	UFLC	HPLC	HPLC instruments are more economical compare to UFLC. 3 Micron column is cheaper than sub 2 micron particle size column.
2	Stationary phase	CHIRALPAK IG-U (100 \times 3.0 mm, 1.6 μm)	CHIRALPAK IG-3 (250 \times 4.6 mm, 3 μm)	Higher ID of the column and particle size requires nominal back pressure; hence, cost effective conventional HPLC is enough.
3	Mobile phase	10 mM ammonium bicarbonate with acetonitrile	10 mM ammonium bicarbonate with acetonitrile	Same mobile phase in both methods. Generally it is volatile, and hence, it can be directly used for orthogonal method verification with LCMS.
4	Total run time	15 minutes	18 minutes	Despite the excess 3 minutes run time, we could achieve interference free En and DS peaks.
5	Detection wavelength	215 nm	210 nm	More sensitive at 210 nm.
6	Linear range	En and DS: 0.8 to 5.6 $\mu\text{g/ml}$	En and DS: 0.25 to 1.5 $\mu\text{g/ml}$	Limit covered up to 150% of specification at 0.10%.
		-	Drug [(S,R)-Brivaracetam]: 0.25 to 150 $\mu\text{g/ml}$	Indicates the method is linear up to 10 fold of limit concentration with respect to drug.
		-	Drug [(S,R)-Brivaracetam]: 800 to 1200 $\mu\text{g/ml}$	Method can be used to report the En and DS results by % area since the linearity was performed for the drug at limit concentration. Assay method linearity proved for 80 to 120% of test concentration.
7	LOQ	0.8 $\mu\text{g/ml}$	0.25 $\mu\text{g/ml}$	Method is highly sensitive to quantify even at very low level.
8	LOD	0.3 $\mu\text{g/ml}$	0.08 $\mu\text{g/ml}$	Method is highly sensitive to detect even at very low level.
9	Resolution	2.0, 3.3 and 4.7	3.7, 6.4 and 10.6	Since the resolutions between the isomers are high it can be employed in any industrial/analytical labs without sample matrix interferences.



1. Sample treatment
2. Sample amount
3. Device positioning
4. Sample prep. stages
5. Automation, miniaturization
6. Derivatization
7. Waste
8. Analysis throughput
9. Energy consumption
10. Source of reagents
11. Toxicity
12. Operator's safety

Criteria	Score
1. Direct analytical techniques should be applied to avoid sample treatment.	0.48
2. Minimal sample size and minimal number of samples are goals.	1.0
3. If possible, measurements should be performed in situ.	1.0
4. Integration of analytical processes and operations saves energy and reduces the use of reagents.	1.0
5. Automated and miniaturized methods should be selected.	0.5
6. Derivatization should be avoided.	1.0
7. Generation of a large volume of analytical waste should be avoided, and proper management of analytical waste should be provided.	1.0
8. Multi-analyte or multi-parameter methods are preferred versus methods using one analyte at a time.	0.55
9. The use of energy should be minimized.	1.0
10. Reagents obtained from renewable sources should be preferred.	0.5
11. Toxic reagents should be eliminated or replaced.	0.0
12. Operator's safety should be increased.	0.8

Figure 13. Greenness chart for the developed HPLC method using AGREE tool. The overall value of 0.74 in the 0 to 1 scale indicates the green analytical procedure of this method. The 12 analytical principles (criteria) and their scores (out of 1) are reproduced in the second chart.

%recovery. The accuracy results were found to be 98–102%, which lies within the acceptable criterion of $\pm 2\%$.

Robustness

Robustness was executed by deliberate changes in the strength of the buffer (10–20 mM), flow (0.8–1.2 ml/minute), and column thermostat (20–30°C) on the optimized method condition. Resolution, tailing of main peak and isomers, and %assay variation between control and modified conditions were used as tools to verify the robustness parameters. The observed results are considered to be acceptable since they are within the allowed limits.

Overall, the adopted approach of method development is based on the consideration of all possible variables (buffer, column chemistry, operating temperature, and organic modifiers and additives selection) to separate the components (DS-1, DS-2, En, and Brivaracetam) effectively from the degradants, related compounds, and unknown impurities using a PDA detector, thus achieving the desired resolution and sensitivity by optimizing all variables of the method.

Based on the validation data (highest sensitivity of 0.25 $\mu\text{g/ml}$ quantification level and 0.08 $\mu\text{g/ml}$ detection level with highest resolutions of 3.7, 6.4, and 10.6), it is clear that this method has higher sensitivity and better resolution than the other reported methods; in addition, the existing reports do not deal with the quantification of isomers and drug substance in single chromatographic conditions as projected by this study. The validation data are presented in Tables 4 and 5, whereas the advantages of this method are compared with a recent analogous method (Baksam *et al.*, 2020) in Table 6 for better comprehension.

Thus, the present study offers a robust and simultaneous RP chiral chromatographic method for the separation of all the components (DS-1, DS-2, En, and Brivaracetam) and assay of Brivaracetam from the degradants, related compounds, and unknown impurities.

Assessment of greenness character of the current method using AGREE tool

AGREE is an analytical tool (Gamal *et al.*, 2021; Pena-Pereira *et al.*, 2020) that can be applied on any analytical procedure to evaluate the environmental compatibility of the developed analytical methods. This tool was constructed based on 12 (SIGNIFICANCE) factors of green analytical chemistry that are related to sample preparation, derivatization, simplicity, automation, solvents toxicity, quantity of samples used for testing, number of components determined in single analysis, consumption of energy per analysis, and analysis time, and the outcome is transformed into a 0–1 scale. The current HPLC method was executed for greenness character using this tool and was found to have a 0.74 score (Fig. 13), which indicates that the developed method is environmentally benign.

CONCLUSION

The proposed and validated RP chiral HPLC method for the simultaneous estimation of assay, isomers, and related impurities has high sensitivity, good resolution, and reliance on simpler HPLC equipment. This method has some other potential advantages, such as shorter run time (18 minutes), with the usage of volatile buffer, good sensitivity, and greener approach. This may

be the first HPLC method with an appreciable chromatographic resolution of all the four components from the degradants, related compounds, and other impurities; and hence, may be considered for the direct application in quality control analysis and counterfeit pharmaceutical drug investigations.

AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

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CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

DATA AVAILABILITY

All data generated and analyzed are included within this research article.

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REFERENCES

- Abram M, Jakubiec M, Kamiński K. Chirality as an important factor for the development of new antiepileptic drugs. *Chem Med Chem*, 2019; 14(20):1744–61.
- Ahuja S. Overview of HPLC method development for pharmaceuticals. *Sep Sci Technol*, 2007; 8:1–11.
- Bailey F. Applications of high-performance liquid chromatography in the pharmaceutical industry. *J Chromatogr A*, 1976; 122:73–84.
- Baksam V, Saritha N, Pocha VR, Chakka VB, Ummadi RR, Kumar P. Development of an effective novel validated stability indicating HPLC method for the resolution of brivaracetam stereoisomeric impurities. *Chirality*, 2020; 32(9):1208–19.
- Bhamare P, Dubey R, Upmanyu N, Umadoss Pothuvan. A simple HPLC method for in-vitro dissolution study of brivaracetam in pharmaceutical dosage form. *Asian J Pharm Anal*, 2021; 11(1):1–8.

Blackstone EA, Fuhr JP Jr, Pociask S. The health and economic effects of counterfeit drugs. *Am Health Drug Benefits*, 2014; 7(4):216–24.

Gamal M, Naguib IA, Panda DS, Abdallah FF. Comparative study of four greenness assessment tools for selection of greenest analytical method for assay of hyoscyne N-butyl bromide. *Anal Methods*, 2021; 13(3):369–80.

ICH Q2 (R1): Validation of analytical procedures: text and methodology. International Council for Harmonisation, Geneva, Switzerland, 2005. [Online]. Available via <https://www.ich.org/page/quality-guidelines> (Accessed 21 January 2021).

Iqbal M, Ezzeldin E, Al-Rashood KA. UPLC–MS/MS assay for identification and quantification of brivaracetam in plasma sample application to pharmacokinetic study in rats. *J Chromatogr B*, 2017; 1060:63–70.

Jessica DA, Cabral LM, Sosua VP. Stability indicating methods for determination of third generation antiepileptic drugs and their related substances. *Crit Rev Anal Chem*, 2021; 1–13; DOI: 10.1080/10408347.2021.1890544.

Khaleghi F. A novel adjunctive therapy for partial-onset seizures. *P T*, 2017; 42(2):92–6.

Liao S, Chen H, Wang G, Wu S, Yang Z, Luo W, Gao X, Qin J, Li HC, Wang ZG. Identification, characterization, synthesis and strategy for minimization of potential impurities observed in the synthesis of brivaracetam. *Tetrahedron*, 2020; 76(26):131273.

Mali NV, Mhaske DV. HPLC studies on degradation behaviour of brivaracetam and development of validated stability indicating HPLC assay method. *IJSRM Human*, 2016; 4(3):43–57.

Mansour NM, Sherbiny DTE, Fawzia A. Ibrahim, Subbagh HIE. Development of an inexpensive sensitive and green HPLC method for the simultaneous determination of brivaracetam, piracetam and carbamazepine application to pharmaceuticals and human plasma. *Microchem J*, 2021; 163:105863. Matagne A, Margineanu DG, Kenda B, Michel P, Klitgaard H. Anticonvulsive and antiepileptic properties of brivaracetam (ucb 34714) a high-affinity ligand for the synaptic vesicle protein SV2A. *Br J Pharmacol*, 2008; 154(8):1662–71.

Mohamed S, Riva R, Contin M. Development and validation of an UHPLC-MS/MS assay for the therapeutic monitoring of brivaracetam plasma concentrations in patients with epilepsy. *Ther Drug Monit*, 2020; 42(3):445–51.

Pankaj B, Umadoss P, Upmanyua N, Dubey R. Identification, isolation, structural characterisation synthesis and in silico toxicity prediction of the alkaline hydrolytic degradation product of Brivaracetam by using LC-PDA preparative HPLC LC/HENI/LTQ FTIR and ¹H NMR. *Anal Methods*, 2020; 12:1868–81.

Pena-Pereira F, Wojnowski W, Tobiszewski M. AGREE-Analytical GREENness metric approach and software. *Anal Chem*, 2020; 92:10076–82.

Qiu S, Tehrani KA, Sergeev S, Bultinck P, Herrebout W, Mathieu B. Stereochemistry of the brivaracetam diastereoisomers. *Chirality*, 2016; 28(3):215–25.

Rogawski MA. A new SV2A ligand for epilepsy. *Cell*, 2016; 167(3):587.

Vasanth DA, Rajkamal B. A validated LC-MS/MS method for pharmacokinetic study of brivaracetam in healthy rabbits. *Int J Pharm Pharm Sci*, 2018; 10(2):24–9.

Vishweshwar V, Babu JM, Muralikrishna R. Development and validation of stability-indicating UPLC method for the determination of brivaracetam its related impurities and degradation products. *Int J Pharm Sci Res*, 2018; 9(6):2315–27.

Zhu LN, Chen D, Chen T, Xu D, Chen SH, Liu L. The adverse event profile of brivaracetam A meta-analysis of randomized controlled trials. *J Seizure*, 2017; 45:7–16.

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