RESEARCH PAPER

Development and Validation of a Stability-Indicating Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) Method for the Determination of Brivaracetam

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ABSTRACT
The present study describes development of a stability indicating RP-HPLC method for quantitative estimation of Brivaracetam in bulk drug and tablet form medications. Conventional solvent systems produced peak behavior in unsymmetrical form and inconsistent retention characteristics at the time of preliminary optimization. In my experimental generation of symmetrical and stable peak, incorporation of dilute formic acid resulted in better chromatographic performance. I have used Kromacil C18 column with Methanol and 0.1% Formic acid in water (50:50 v/v) at 1.0 ml/min flow rate for 210 nm UV detection for chromatographic separation. Optimized Brivaracetam was eluted at ~5.49 min into the good chromatographic response.
I had conducted my experimental forced degradation studies under the acidic (pH 5.0-8.5), alkaline (pH 10.0-13.0), oxidative (H_2O_2), thermal (70^oC), and photolytic (UV, sunlight) conditions. In contrast, significant peak shifts and degradation were seen under hydrolytic conditions while similar degradation but lower were seen under thermal, oxidative and photolytic conditions. Degradation peaks remained well resolved from the principal analyte peak giving the method a good stability indicating capacity. Validation studies demonstrated proportional detector response over the concentration range of 80-120 µg/ml with correlation coefficient of 0.99941. Analytical performance was shown to be consistent in repeated analysis in recovery and precision experimental studies. The developed method was successfully applied to assay determination of marketed Brivaracetam tablet formulation and may additionally be useful for future LC-MS/MS-based degradation studies.

Keywords: Brivaracetam, RP-HPLC, Antiepileptic, Stability-indicating method, Method validation, Forced degradation,

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1. INTRODUCTION

Epilepsy affecting nearly 50 million individuals globally, including around 12 million individuals in India, which is associated with recurrent seizure episodes resulting from excessive and abnormal neuronal electrical discharges within the brain [1,2]. Such seizure activity mainly develops due to an imbalance between excitatory glutamatergic pathways and inhibitory GABAergic neurotransmission. The symptoms of seizures can vary and may include temporary loss of consciousness, muscle spasms, confusion, or problems with memory [1]. Based on the affected brain region, researchers commonly categorized seizures as focal seizures involving a localized brain region and generalized seizures affecting both cerebral hemispheres [1,2]. Although considerable advancement has been achieved in antiepileptic therapy, nearly 30% of patients still show inadequate response to currently available medications, indicating the need for the development of more effective therapies [3].

Brivaracetam is a third-generation antiepileptic medication which is identified chemically as (2S)-2[(4R)-2-oxo-propylpyrrolidin-yl] butanamide. It was

approved in February 2016 by USFDA for management of focal seizures in both adults and pediatric patients [2,20]. It was developed as an improved analogue of Levetiracetam (LEV), with modifications aimed at enhancing its therapeutic activity. When we comparing Levetiracetam and Brivaracetam drug, Studies have reported that Brivaracetam possesses 15-30 fold greater binding affinity toward Synaptic Vesicle Protein 2A (SV2A) than LEV [9].

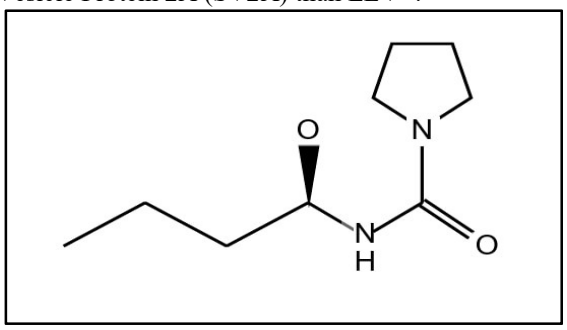


Figure 1 Chemical Structure of Brivaracetam

SV2A is a glycoprotein present in the presynaptic membrane involved in neurotransmitter release through synaptic vesicle exocytosis. By selectively binding to SV2A, BRV reduces neuronal hyperexcitability and controls the abnormal electrical activity responsible for seizures [1,9]. BRV is commercially available in multiple dosage forms including tablets containing 10-100 mg strength, oral solution preparations of 10 mg/ml, and intravenous injections with a concentration equivalent to 50 mg/ml. This range formulations allows flexible dosing and is suitable for different patient groups, including pediatric and critically ill patients.

From a pharmacokinetic point of view, BRV shows rapid and almost complete oral absorption, with bioavailability greater than 95%. It exhibits low plasma protein binding of less than 20% and possesses plasma half -life of approximately 9 hours. BRV primarily undergoes hepatic metabolism through CYP2C19 mediated pathways, producing inactive metabolites that are eliminated mainly through urine [2,20]. It has moderate lipophilicity (log P ~0.86) and 212.29 g/mol molecular weight with molecular formula $C_{11}H_{20}N_2O_2$. The drug is categorized under BCS Class I, which indicates high permeability and high solubility, which supports its formulation into different dosage forms.

From a structural perspective, BRV contains a chiral center and an amide functional group [2]. The presence of a chiral center requires careful control over enantiomeric purity, pronounced degradation observed during hydrolysis studies may be associated with susceptibility of the amide linkage toward acidic and alkaline conditions, leading to the formation of carboxylic acid and amide degradation products [25]. Due to these characteristics, stability-indicating analytical methods capable of separating degradation products from the intact drug become essential during pharmaceutical analysis under stress conditions [25]. A review of the reported methods for the estimation of BRV shows that a range of analytical techniques have been used, including spectrophotometry [23], RP-HPLC [7,26], UPLC [28,31], chiral HPLC [2], LC-MS/MS [29], and LC-QTOF-MS [32]. However, several of the available HPLC methods have certain limitations. For example, some methods use non-volatile inorganic buffers such as phosphate or acetate, which are not suitable for LC-MS analysis and may lead to instrument contamination due to salt deposition [8,18].

In addition, acetonitrile is commonly used as the organic solvent in many methods, which increases both the cost of analysis and environmental impact [8,18]. Furthermore, several reported analytical approaches lack the extensive forced degradation studies necessary to meet all ICH recommended stress conditions, and do not provide evidence of the method being a clear stability indicating method [24]. The present investigation was therefore undertaken to develop a comparatively economical and stability-indicating RP-HPLC method for Brivaracetam using Methanol and dilute Formic acid, thereby reducing dependence on non-volatile buffer systems during routine chromatographic analysis. As a result, formic acid was chosen as a volatile acidic modifier for two main reasons. It helps maintain the acidic conditions required for proper performance of the C18 column and good peak shape, and at the same time, it is compatible with LC-MS/MS system, which is useful for future identification of degradation products [8,27]. Validation experiments confirmed consistent analytical performance of the developed chromatographic method, which was further employed for assay determination of marketed Brivaracetam tablets [11].

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Brivaracetam working standard obtained from Vidisha Analytical. Merck (Mumbai, India) provided HPLC-grade methanol and acetonitrile. Siddhi Lab provided HPLC-grade water. Formic acid, orthophosphoric acid, hydrogen peroxide (H_2O_2), sodium hydroxide (NaOH), and hydrochloric acid (HCl), were among the analytical quality reagents that were purchased from Thermo Fisher Scientific, Mumbai, India. Excipients used for Placebo preparation included lactose, starch, magnesium stearate, talc, and crospovidone. The highest quality of commercially available chemicals and reagents were utilized in the investigation without any additional purification.

2.2 Instrumentation

By using an Agilent 1260 Infinity II HPLC system, chromatographic analysis was carried out which was fitted with autosampler, quaternary gradient pump, column oven compartment, and UV detector controlled by Openlab EZ Chrome software. UV-Visible spectrophotometry for wavelength selection was performed in a Jasco V-550 double-beam spectrophotometer with Spectra Manager Software. Weighing operations used an Azcet CY 223C high-precision analytical balance. A LabMan digital pH meter was used for pH measurement. Ultrasonication during sample preparation was performed using a Biotechnic sonicator, while filtration was carried out using 0.45 µm nylon or PVDF membrane syringe filters.

3. CHROMATOGRAPHIC CONDITIONS

Optimized chromatographic conditions were established after systematic screening of five mobile phase systems, are summarized in Table 1. Optimized chromatographic separation of Brivaracetam was achieved on a Kromacil C18 column using Methanol and 0.1% Formic acid in water (50:50, v/v) under isocratic mode. Before chromatographic use, prior to analysis, the prepared mobile phase was filtered through a 0.45 µm nylon membrane filter and degassed by sonication for 15 minutes. Fresh mobile phase was prepared daily to maintain chromatographic consistency and minimize possible degradation or contamination. Isocratic elution was delivered at a flow rate of 1.0 ml/min. Chromatographic detection was performed at 210 nm with the column oven maintained at 40ºC. A sample injection volume of 20 µl was employed throughout the study, chromatographic analysis was completed within a total run time of 10 min.

Table 1. Optimized Chromatographic Conditions

Parameter	Specification
Column	Kromacil C18, 250 mm × 4.6 mm i.d., 5 µm
Mobile Phase	Methanol: 0.1% Formic Acid in Water (50:50, v/v)
Elution Mode	Isocratic
Rate of flow	1.0 ml/min
Wavelength of detection	210 nm (UV)
Temperature of column	40°C
Volume of injection	20 µl
Time of run	10 minutes
HPLC System	Agilent 1260 Infinity II

4. PREPARATION OF ANALYTICAL SOLUTIONS

A. Standard Stock Solution

Perfectly weighed Brivaracetam standard equivalent to 20 mg was placed into a 20 ml volumetric flask. The solution was diluted with approximately 15 ml HPLC-grade water and sonicated prior to analysis. The final volume was made up with water to achieve a stock solution containing 1000 µg/ml of Brivaracetam.

B. Working Standard Solution

One milliliter of the standard stock solution was diluted with mobile phase to a final volume of 10 ml, yielding a Brivaracetam concentration of 100 µg/ml. For all chromatographic trials, system suitability testing, and linearity studies, this solution was used.

C. Tablet Sample Solution

Finely powdered Brivamax tablets equivalent to 50 mg of Brivaracetam were introduced into a 50 ml volumetric flask containing approximately 35 ml water. Sonication was continued for 15 min to ensure efficient drug extraction from the tablet matrix. After volume adjustment with water, the solution was filtered through a 0.45 µm membrane filter. Approximate dilution was performed using mobile phase, yielding a final concentration of 100 µg/ml.

5. METHOD DEVELOPMENT AND OPTIMIZATION

Different solvent combinations and chromatographic conditions were investigated to obtain symmetrical peak profile, consistent retention characteristics and

system suitability characteristics [5,24]. Initial trials employing methanol: water and acetonitrile: water systems without pH modifier produced unsatisfactory peak shape and fronting behavior. To minimize silanol interactions and improve chromatographic performance, 0.1% formic acid was incorporated into aqueous phase [15].

Among various combinations evaluated, methanol and 0.1% formic acid in water (50:50, v/v) provided optimum chromatographic behavior with acceptable retention time, asymmetry factor, and theoretical plate count [8]. The developed method generated a sharp and symmetrical peak for Brivaracetam at approximately 5.49 min. Therefore, these chromatographic conditions were selected for further validation studies.

Table 2 List of Trials Taken for Method Development

Trial	Mobile Phase (v/v)	Retention Time (min)	Asymmetry	Plates (N)	Outcome
1	Methanol: Water (70:30)	2.40	-	-	Rejected-fronting hump
2	Methanol: Water (40:60)	2.83	2.29	3,148	Rejected-poor symmetry
3	Acetonitrile: Water (70:30)	2.31	0.61	951	Rejected-fronting low N
4	Methanol: 0.1% FA/ Water (70:30)	1.91	0.92	1,825	Rejected-hump, low N
5	Methanol: 0.1% FA/ Water (50:50)	5.49	1.15	16,155	Accepted

6. FORCED DEGRADATION STUDIES

Stress degradation experiments were conducted to examine the ability of the proposed RP-HPLC method to separate Brivaracetam from its degradation products [10,11]. Brivaracetam samples were subjected to thermal, photolytic, acidic, alkaline, and oxidative stress conditions [16,24].

Thermal degradation was performed by subjecting the drug solution at 80°C for 48 h. Photolytic degradation studies involved exposure of samples to direct sunlight for 72 h. Acidic hydrolysis was performed using 5N hydrochloric acid, whereas alkaline degradation was induced using 5N sodium hydroxide. Oxidative degradation studies were conducted using 30% hydrogen peroxide solution [18,31].

Hydrolytic stress studies revealed comparatively higher degradation in the presence of acidic and alkaline conditions. Acidic stress produced degradation ranging from 17.64% to 33.85%, while alkaline hydrolysis resulted in degradation between 14.82% and 38.14%. In contrast, minimal degradation was observed under thermal, oxidative, and photolytic conditions. Additional degradation peaks generated under hydrolytic stress conditions were adequately resolved from principal drug peak, thereby confirming stability-indicating capability of the method [4,25].

7. ANALYTICAL METHOD VALIDATION

Validation experiments were carried out to assess specificity, linearity, precision, accuracy, robustness, and sensitivity of the designed chromatographic method:

1) **Linearity:**
   A linear detector response was observed across the concentration range of 80-120 µg/ml with correlation coefficient (R^2) of 0.99941 [5,25].
   Regression equation obtained was:
   $Y = 888209.72X – 2599087.80$
   Where:
   Y represents peak area response and
   X represents concentration of Brivaracetam in µg/ml
2) **Accuracy:** Recovery experiments carried out at 80%, 100%, and 120% concentration levels demonstrated reliable analyte quantification, with an average recovery of 99.84%, %RSD below 2%, indicating satisfactory accuracy [19].
3) **Precision:** There are six independent sample prepared solutions were analyzed during repeatability and intermediate precision studies. %RSD values obtained for both studies were below 2%, confirming acceptable precision and reproducibility [26].
4) **Specificity:** Blank and placebo solutions were injected to evaluate possible chromatographic interference at the retention time to evaluate potential interference at retention time of Brivaracetam. No

interfering peaks were observed, confirming specificity of the analytical method [15].

5) **LOD and LOQ:** Calculated from calibration curve using IUCH Q2(R1) equations: LOD = 3.3 σ/S and LOQ = 10 σ/S, where σ = residual standard deviation and S = slope of the regression line [11].
6) **Robustness:** Evaluated by deliberately varying detection wavelength (±2 nm), flow rate (±10%), and column temperature (±2°C) and monitoring system suitability characteristics [25].
7) **System suitability:** working standard five identical injections were employed; acceptability criteria: %RSD of peak area ≤ 2.0%, theoretical plate count ≥ 2000, tailing factor ≤ 2.0 [11,31].

8. RESULTS

A. Preliminary Characterization of Brivaracetam

Brivaracetam drug was observed as a white, odorless, slightly amorphous powder. The drug's identification and purity were confirmed by the experimentally observed melting point of 79°C, which stayed within standard pharmacopeial range of 72-77°C. The drug showed freely solubility in water, methanol, and ethanol, with water selected as the diluent for stock solution preparation due to its compatibility with the mobile system's aqueous phase. Using a 20 µg/ml aqueous BRV solution, UV spectrophotometric scanning (200-400 nm) revealed maximum absorbance at 210 nm; hence, this wavelength optimized for further HPLC examinations.

B. Method Optimization

The optimized conditions produced a sharp peak with consistent symmetry and resolution for BRV with a retention time of 5.49 minutes was produced by the adjusted chromatographic conditions (Trial 5). The asymmetry factor was found to be 1.15, and the theoretical plate count was 16,155; which is significantly more than the minimum acceptability criterion of N ≥ 2000. The optimization of 0.1% formic acid to the aqueous phase helped to reduce silanol interactions on the C18 column, leading to a clear improvement in a peak symmetry compared to earlier trials (Trials 1-4). An isocratic mode of elution was selected instead of gradient elution, as it offers better simplicity and reproducibility for r quality control analysis.

Trial 1:

Chromatogram:

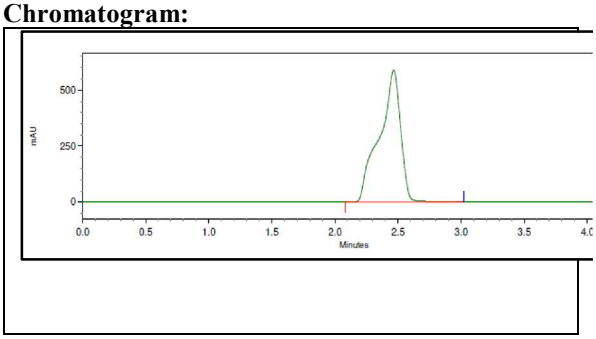


Figure 2 Representative chromatogram of Trial 1

Observation: Brivaracetam eluted at 2.4 minutes with unacceptable chromatography Hump observed at fronting side.
Conclusion: The trial condition was not selected.
Trial 2:
Chromatogram:

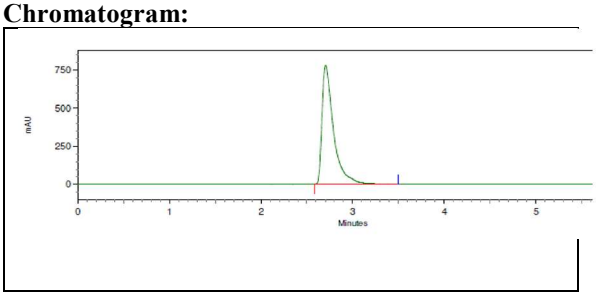


Figure 3 Representative chromatogram of Trial 2
Observation: Brivaracetam eluted at 2.83 minutes with unacceptable chromatography. (Asymmetry: 2.29 and Theoretical plates 3148)
Conclusion: The evaluated solvent system was excluded from further studies.
Trial 3:
Chromatogram:

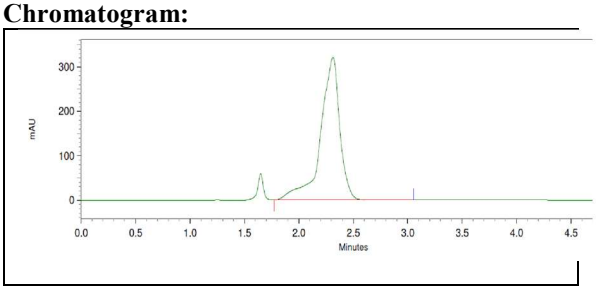


Figure 4 Representative chromatogram of Trial 3

Observation: Brivaracetam eluted at 2.31 minutes with unacceptable chromatography (Asymmetry: 0.61 and Theoretical plates 951) Hump observed prior to peak.
Conclusion: The chromatographic condition was not considered suitable for subsequent analysis.
Trial 4:
Chromatogram:

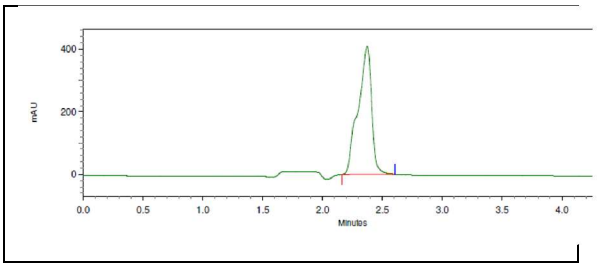


Figure 5 Representative chromatogram of Trial 4

Observation: Brivaracetam eluted at 1.91 Minutes but chromatography not Acceptable. (Asymmetry: 0.92 and Theoretical plates 1825) Hump observed

Conclusion: Method Rejected.

Trial 5: Chromatogram:

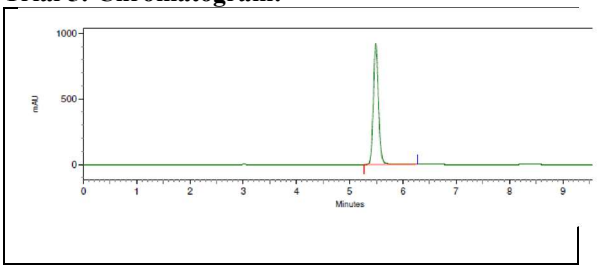


Figure 6 Representative chromatogram of Trial 5

Observation: The optimized system produced a well-defined Brivaracetam peak at 5.49 min with acceptable peak symmetry and theoretical plate count of 16155.

Conclusion: based on peak symmetry, retention behavior, and system suitability performance, the method was accepted for further analytical evaluation.

Conclusion: It was found from the results of Trials 1 to 5, that the chromatographic conditions of Trial 5 yielded the best peak symmetry, adequate retention time and satisfactory tailing factor. The results obtained in Trial 5 thus allowed for the optimization of the chromatographic system that were chosen for further method validation studies.

Table 3. Optimized Chromatographic Conditions

Parameter	**Description**
Elution Mode	Isocratic
Name of column	Kromasil C18**,** 250 mm X 4.6mm ID, 5 µm
Name of Detector	UV Detector
Volume of injection	20 µl
Wavelength detection	210 nm
Temperature of Column Oven	40ºC
Mobile Phase	Methanol: 0.1 % Formic acid in Water (50:50%V/V)
Rate of flow	1.0 ml/min
Time of run	10 minutes

C. Forced Degradation of Brivaracetam API

The forced degradation results are given in Table 4 BRV demonstrated stability under thermal (80ºC/48 hours; 1.71% degradation) and photolytic (direct sunlight/72 hours; 0.84% degradation) conditions. Similarly, oxidative stress with 30% H_2O_2 produced negligible degradation (< 2%) at both 24-hour and 48-hour exposure times. In contrast, significant hydrolytic degradation was observed under acidic conditions: 17.64% after 12 hours and 33.85% after 24 hours of exposure to 5N HCl. Alkaline conditions produced comparably substantial degradation: 14.82% after 10 hours and 38.14% after 24 hours with 5N NaOH. New chromatographic peaks appeared in the acid and base stressed chromatograms, all resolved from the BRV peak, confirming the stability-indicating capability. A peak at approximately 2.4 min observed exclusively in peroxide-treated samples was identified as arising from H_2O_2 itself (confirmed by blank peroxide injection), not from BRV degradation.

Table 4 Forced Degradation Results for Brivaracetam APIStress condition	Exposure	% Assay	% Degradation	Remarks
Thermal (80ºC)	48 h	98.29	1.71%	Stable, no new peaks
Photolytic (sunlight)	72 h	99.16	0.84%	Stable, no new peaks
Acid - 5N HCl (Trial 2)	12 h, RT	82.36	17.64%	Degradation peaks observed
Acid - 5N HCl (Trial 1)	24 h, RT	66.15	33.85%	Significant degradation
Base - 5N NaOH (Trial 2)	10 h, RT	85.18	14.82%	Degradation peaks observed
Base - 5N NaOH (Trial 1)	24 h, RT	61.86	38.14%	Significant degradation
Oxidative - 30% H_2O_2 (Trial 1)	24 h, RT	98.75	1.25%	Stable; H_2O_2 peak at 2.4 min
Oxidative - 30% H_2O_2 (Trial 2)	48 h, RT	98.46	1.54%	Stable; H_2O_2 peak at 2.4 min

1) Physical degradation:

a. Thermal degradation:

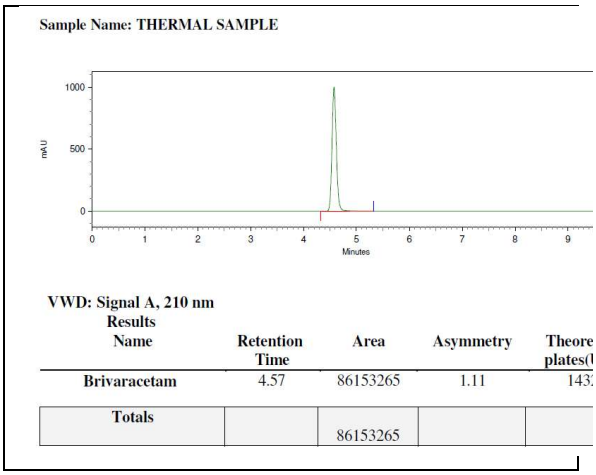


Figure 7 Representative chromatogram of Thermal sample

Result: No appreciable change in chromatographic response was observed after maintaining the sample at 80°C for 48 hours.

b. Photolytic degradation:

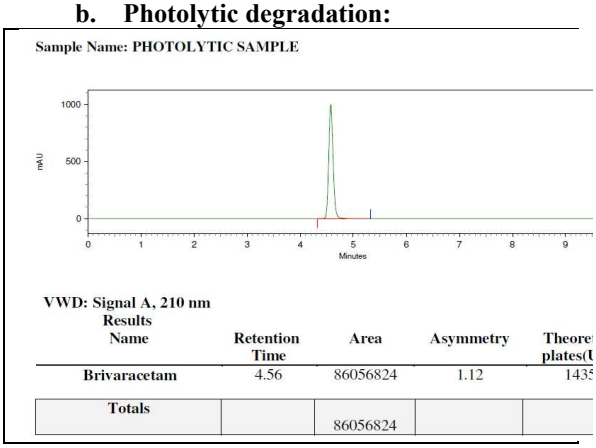


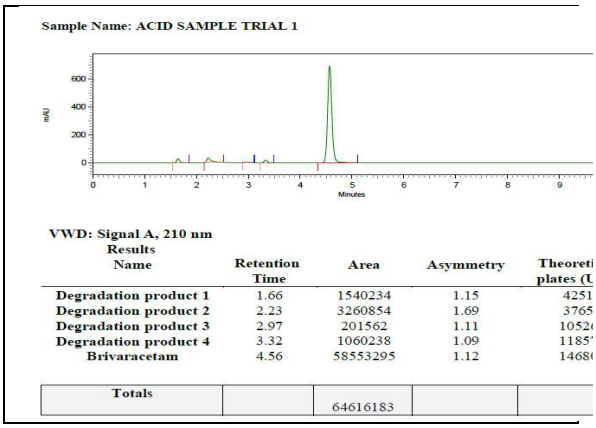
Figure 8 Representative chromatogram of Photolytic sample

Result: After exposing the sample at direct sun light for 72 hours, No degradation found.

2) Chemical Degradation:

a) Acid Degradation:

Trial 1:

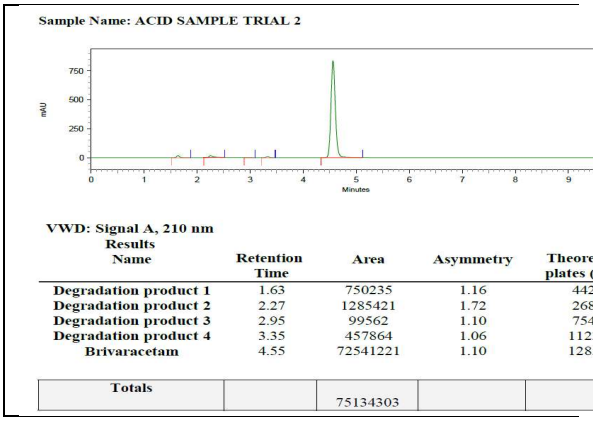


Name	Retention Time	Area	Asymmetry	Theoreti plates (U
Degradation product 1	1.66	1540234	1.15	4251
Degradation product 2	2.23	3260854	1.69	3765
Degradation product 3	2.97	201562	1.11	1052
Degradation product 4	3.32	1060238	1.09	1185
Brivaracetam	4.56	58553295	1.12	1468
Totals		64616183		

Figure 9 Representative chromatogram of sample exposed under Acid trial 1

Result: After exposing the sample under acid trial 1, 33.85% degradation found.

Trial 2:



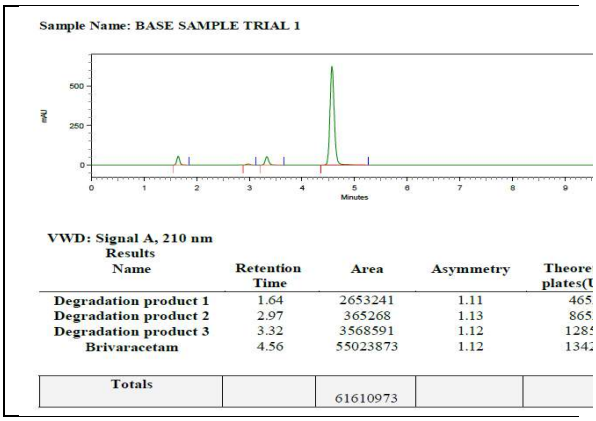
Name	Retention Time	Area	Asymmetry	Theore plates (
Degradation product 1	1.63	750235	1.16	442
Degradation product 2	2.27	1285421	1.72	268
Degradation product 3	2.95	99562	1.10	754
Degradation product 4	3.35	457864	1.06	112
Brivaracetam	4.55	72541221	1.10	128
Totals		75134303		

Figure 10 Chromatographic response of Brivaracetam after exposure to acidic stress conditions (Trial 2)

Result: After exposing the sample under acid trial 2, 17.64% degradation found.

b. Base Degradation:

Trial 1:

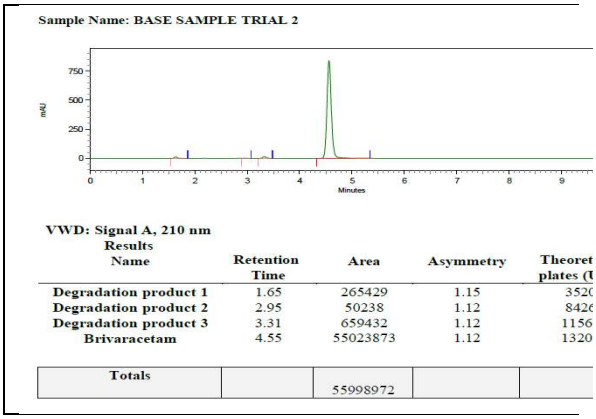


Name	Retention Time	Area	Asymmetry	Theore plates(U
Degradation product 1	1.64	2653241	1.11	465
Degradation product 2	2.97	365268	1.13	865
Degradation product 3	3.32	3568591	1.12	1285
Brivaracetam	4.56	55023873	1.12	1342
Totals		61610973		

Figure 11 Chromatographic profile of Brivaracetam following alkaline degradation study (Trial 1)

Result: After exposing the sample under Base trial 1, 38.14 % degradation found.

Trial 2:



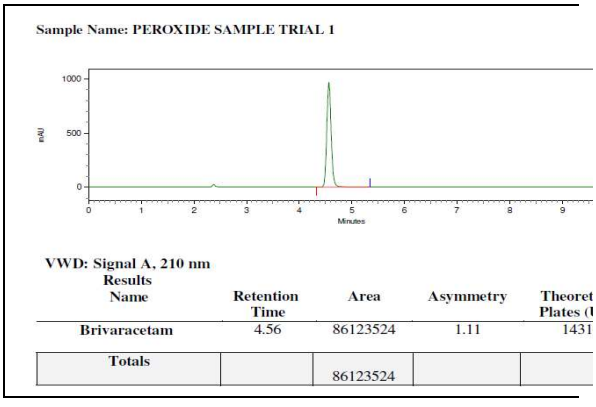
Name	Retention Time	Area	Asymmetry	Theoret plates (U
Degradation product 1	1.65	265429	1.15	3520
Degradation product 2	2.95	50238	1.12	8426
Degradation product 3	3.31	659432	1.12	1156
Brivaracetam	4.55	55023873	1.12	1320
Totals		55998972		

Figure 12 Representative chromatogram obtained after alkaline stress study (Trial 2)

Result: After exposing the sample under Base trial 2, 14.82 % degradation found.

c. Peroxide degradation:

Trial 1:



Name	Retention Time	Area	Asymmetry	Theoret Plates (U
Brivaracetam	4.56	86123524	1.11	1431
Totals		86123524		

Figure 13 Chromatographic profile of the sample after exposure to peroxide-induced oxidative stress (Trial 1)

Result: After exposing the sample under Peroxide trial 1, No degradation found.

Trial 2:

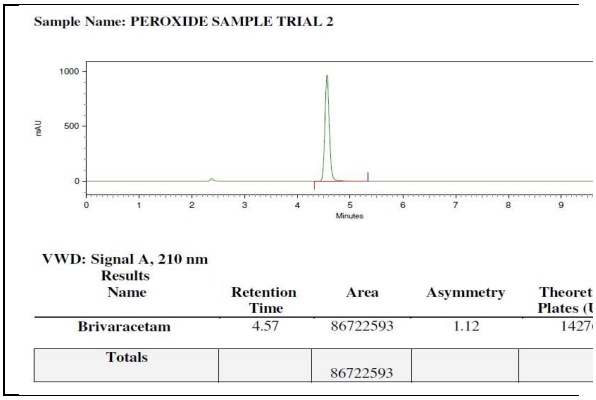


Figure 14 Chromatographic profile of the sample after exposure to peroxide-induced oxidative stress (Trial 1)

Result: After exposing the sample under Peroxide trial 2, No degradation found.

Note: Peak at 2.4 minutes is of Peroxide peak. (Detected in peroxide trials)

Blank Peroxide chromatogram:

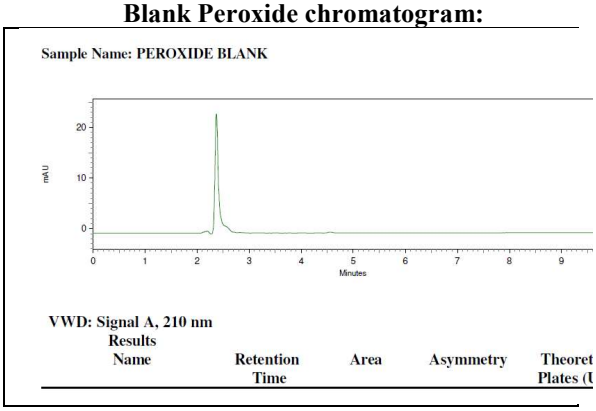


Figure 15 Representative chromatogram of Blank Peroxide

D. Validation of RP-HPLC Method

1) Specificity:

Blank & Placebo solution were prepared and introduced separately to evaluate any possible interference at R.T. of Brivaracetam.

Table 5 Result of Specificity

Description	Observation
Blank	No interference was observed at the R.T. of Brivaracetam from the blank solution.
Placebo	No interference was observed at the R.T. of Brivaracetam due to placebo solution.

Chromatograms:

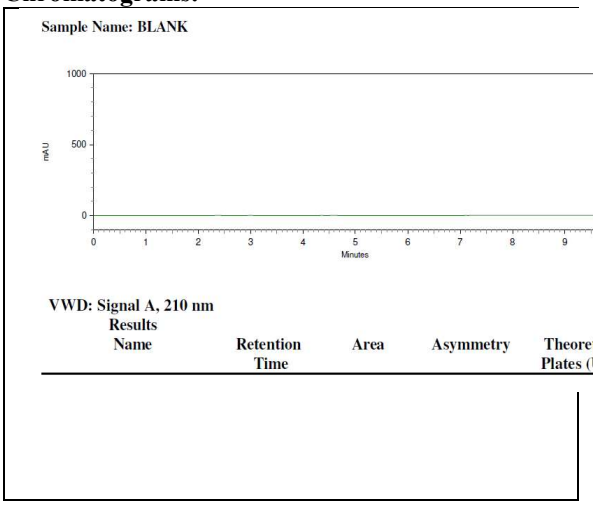


Figure 16 Chromatogram of Blank solution

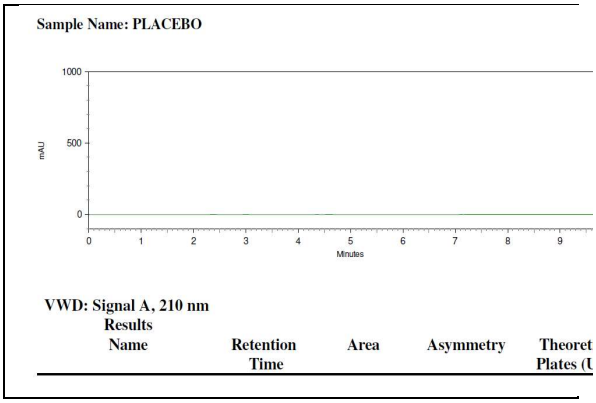


Figure 17 Chromatogram of Placebo solution

Acceptance criteria:

No peak attributable to Brivaracetam should be observed in blank and placebo chromatograms at the retention time of BRV .

Data interpretation: No interfering peaks were observed at the retention time of BRV in either blank or placebo chromatograms. Hence, the developed chromatographic method satisfied the specificity acceptability criterion.

2) Linearity and Range

By examining the correlation between analyte concentration and matching chromatographic response within the chosen concentration range,

the linearity of the devised analytical technique was assessed.

Table 6 Brivaracetam Linearity Data

Level	Conc (µg/mL)	Area	Mean	% RSD
80%	80.00	68625361	68612443	0.264
		68425429		
		68786539		
90%	90.00	76812416	76840072	0.169
		76726538		
		76981263		
100 %	100.00	86350184	86321019	0.065
		86256425		
		86356449		
110 %	110.00	95821662	95785844	0.152
		95625631		
		95910238		
120 %	120.00	103540653	103550043	0.095
		103456251		
		103653224		

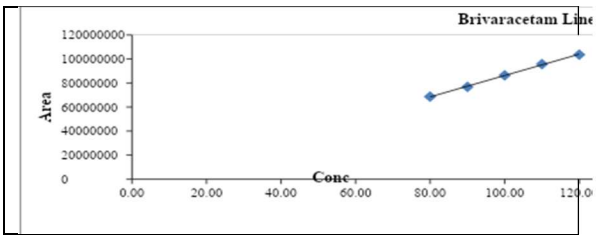


Figure 18 Calibration curve of Brivaracetam

Sr. no.	Parameter	Result value	Acceptance criteria
1	Beer's linearity range	80.0 - 120.0 µg/mL	NA
2	Correlation coefficient (R^2)	0.99941	NLT 0.98
3	Intercept	-2599087.80	To be report
4	Slope	888209.72	To be report
5	% RSD for area at each level	NA	NMT 2.0

Table 7 Results of linearity of drug

The respective linear equation for Brivaracetam was:

Y = M X + C

Y = 888209.72 X + -2599087.80

Where, X represents concentration of analyte in µg/mL

Y represents is area of peak.

M represents the slope

C represents an intercept

Chromatograms:

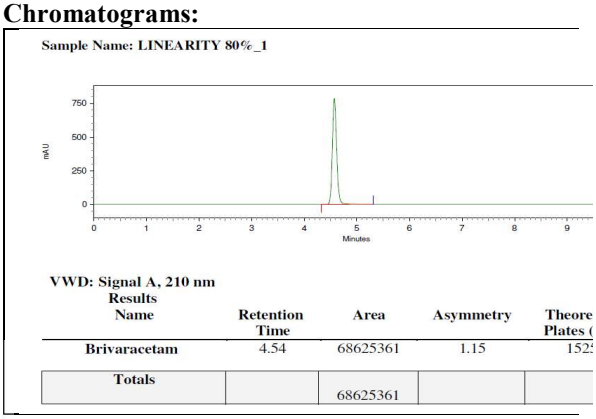


Figure 19 Representative chromatogram of Linearity 80%

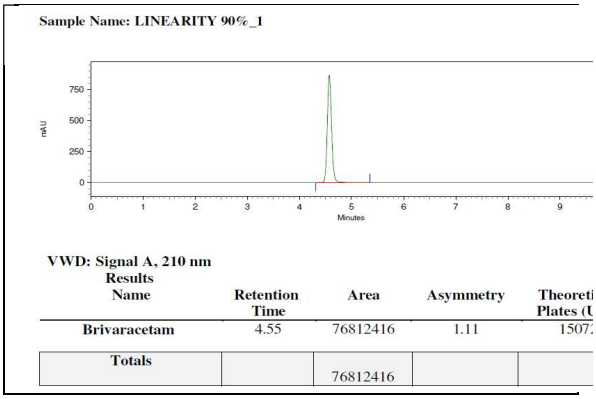


Figure 20 Representative chromatogram of Linearity 90%

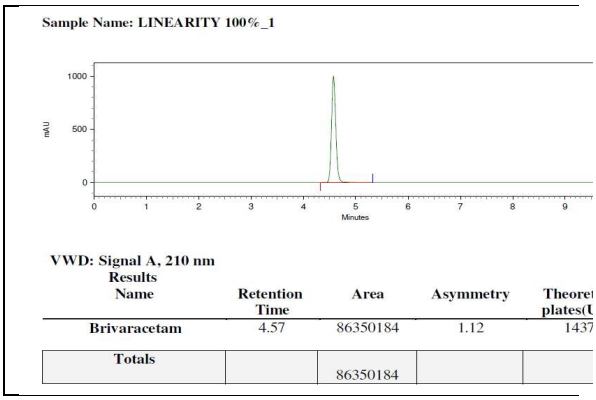


Figure 21 Representative chromatogram of Linearity 100%

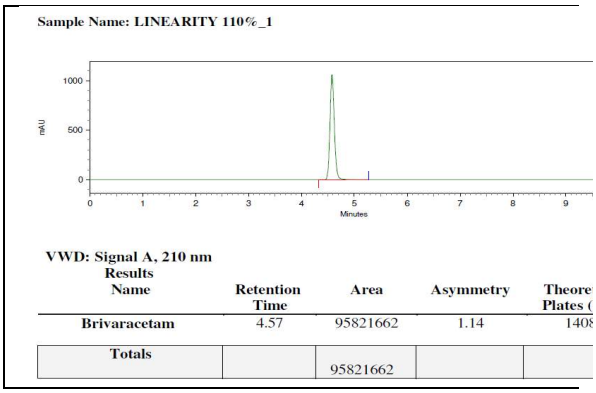


Figure 22 Representative chromatogram of Linearity 110%

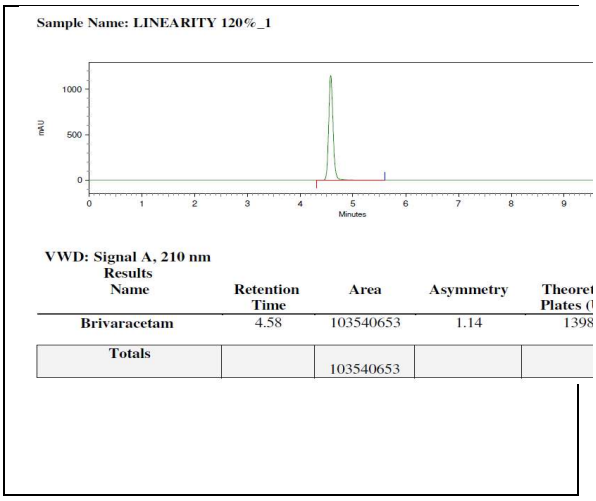


Figure 23 Representative chromatogram of Linearity 120%

Conclusion:

Brivaracetam showed a linear chromatographic response over the concentration range of 80.0 - 120.0 µg /ml, according to the calibration curve. The regression result that was obtained fell within acceptable analytical bounds.

3) Limit of Detection (LOD) and Limit of Quantitation (LOQ):

σ = 484395.24 (Residual standard deviation of a regression line)

s = 888209.72 (Slope)

Detection limit (LOD):

LOD = 3.3 σ / S

LOD = 3.3 x 484395.24 / 888209.72

LOD = 1.80 µg/mL

Quantitation limit (LOQ):

LOQ = 10 σ / S

LOQ = 10 x 484395.24 / 888209.72

LOQ = 5.45 µg/mL

4) Accuracy (Recovery):

An analytical method's accuracy is determined by how closely its test findings match the true value. An analytical method's accuracy is assessed by using it to examine samples that have known concentrations of analyte added.

Table 8 Result and statistical data of Accuracy of Brivaracetam

Level (%)	Area	Recovered conc (µg/mL)	Added conc (µg/mL)	% Recovery	Mean Recovery	% RSD
80	68541352	79.31	80.40	98.64	99.29	0.775
	69023416	79.87	80.60	99.09		
	69235120	80.11	80.00	100.14		
100	86542184	100.14	100.40	99.74	99.74	0.882
	86956835	100.62	100.00	100.62		
	85612568	99.06	100.20	98.86		
120	105542593	122.12	120.80	101.09	100.50	0.946
	105263128	121.80	120.60	101.00		
	103256237	119.48	120.20	99.40		

Total Recovery: 99.84 %

% RSD for Total Recovery: 0.922

Chromatograms:

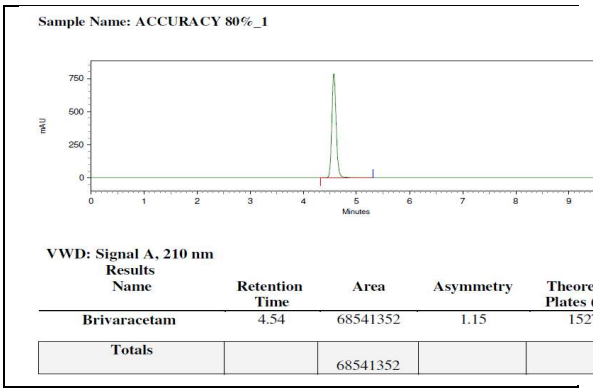


Figure 24 Representative chromatogram of Accuracy 80%

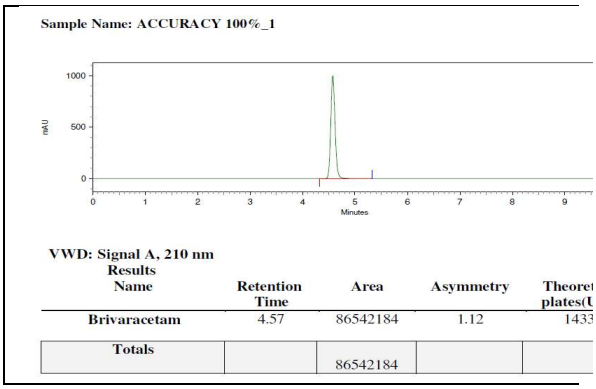


Figure 25 Representative chromatogram of Accuracy 100%

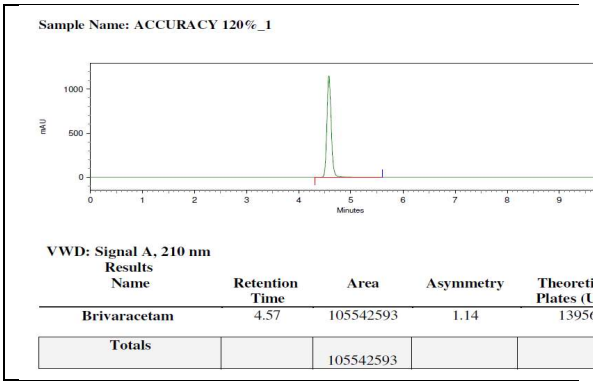


Figure 26 Representative chromatogram of Accuracy 120%

Acceptibility criteria:
% Recovery for each level and total recovery: 98.0 to 102.0%
% RSD for each level and total recovery: NMT 2.0
Data interpretation: At all three levels, the analytical procedure's recovery was found to be well within acceptable requirements. Analyte concentration changes do not hinder recovery.

5) Precision
It refers to "The closeness of agreement between separate test results when the method is applied repeatedly to multiple aliquots of a homogeneous sample."

Table 9 Result of Intra-day and Inter-day Precision for Brivaracetam test sample assay

	Sample	**Test Sample (mg)**	**Area**	**% Assay**
Repeatability	Sample 1	151.5	84923681	98.33
	Sample 2	151.2	85023465	98.64
	Sample 3	151.4	85256731	98.78
	Sample 4	150.6	85062510	99.08
	Sample 5	152.1	85321524	98.40
	Sample 6	151.4	85412456	98.96
	Mean			**98.70**
	STD DEV			**0.2996**
	% RSD			**0.304**
Intermediate precision (Inter-Day)	Sample 1	151.3	85320162	98.92
	Sample 2	151.7	85425686	98.78
	Sample 3	151.6	84215798	97.44
	Sample 4	151.1	84412592	98.00
	Sample 5	152.1	85012831	98.04
	Sample 6	150.9	84945836	98.75
	Mean			**98.32**
	STD DEV			**0.5851**
	% RSD			**0.595**
Repeatability Plus Inter-day	**Mean**			**98.510**
	STD DEV			**0.4849**
	% RSD			**0.492**

Chromatograms:

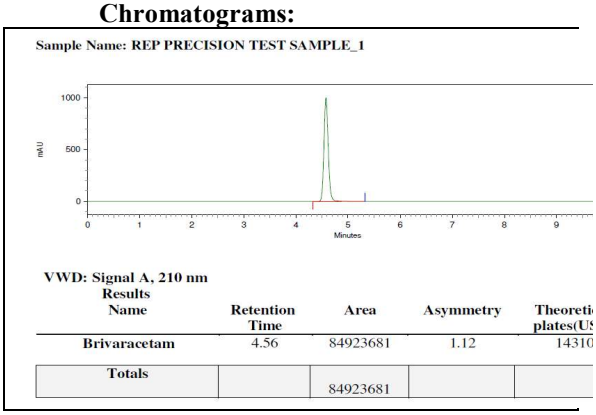


Figure 27 Representative chromatogram of Repeatability precision (Sample 1)

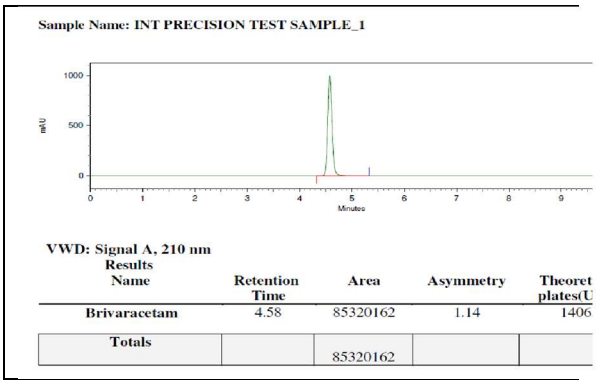


Figure 28 Representative chromatogram of Inter-day precision (Sample 1)

Acceptance criteria:

The assay value for individual samples as well as mean assay value from precision studies (n=6), combine precision (n=6), and combined precision-intermediate precision (n=12) were to be within 90-110%. The %RSD for precision (n=6), intermediate precision (n=6), and combined precision-intermediate precision (n=12) should be no more than 2.0%.

Data interpretation:

The % Assay and % RSD values obtained during the precision and intermediate precision evaluation were found to be within the given limits and thus the analytical method is found to be satisfactory for both precision and intermediate precision.

6) Robustness:

An analytical technique's "Robustness is a measure of its ability to withstand slight but intentional changes in method parameters and gives an indicator of its dependability under typical use." The modifications made under robustness are as follows:

- Wavelength shift
- Variation in flow rate
- Temperature shift in column oven

Table 10 Result of Robustness study

Change in Parameter	R. T.	Standard area	Asymmetry	Theoretical plates
Wavelength by +2 NM (212 NM)	4.57	80352416	1.12	14855
Wavelength by -2 NM (208 NM)	4.57	79653261	1.12	14673
Flow rate by +10% (1.1mL/min)	4.16	74256201	1.10	14230
Flow rate by -10% (0.9mL/min)	5.09	91526351	1.14	11685
Column oven temp by +2ºC (37 ºC)	4.55	95850764	1.12	14560
Column oven temp by -2ºC (33 ºC)	4.56	97023564	1.15	14038

Chromatograms:

A. Change in Wavelength by +2 NM:

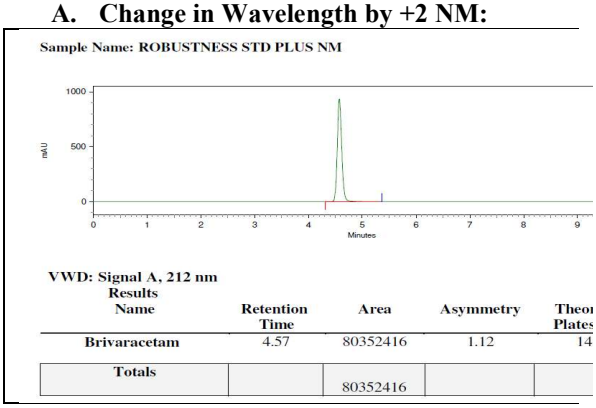


Figure 29 Representative chromatogram of Standard +2 NM

B. Change in Wavelength by -2 NM:

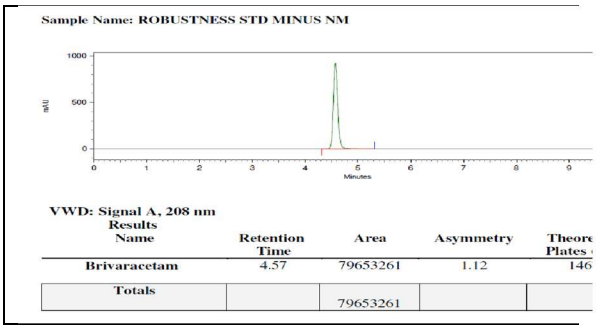


Figure 30 Representative chromatogram of Standard -2 NM

C. Change in Flow rate by + 10% (1.1 mL/min)

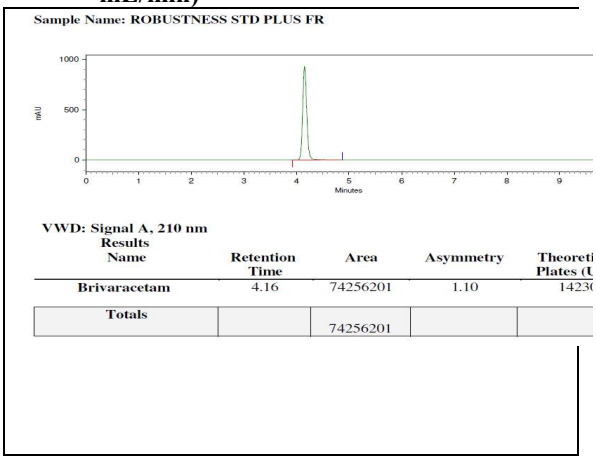


Figure 31 Representative chromatogram of Standard +10% F.R.

D. Change in Flow rate by - 10% (0.9 mL/min)

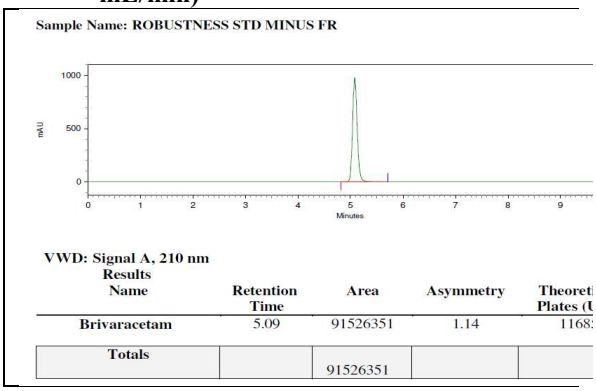


Figure 32 Representative chromatogram of Standard -10% F.R.

E. Change in Column Oven temperature by +2°C:

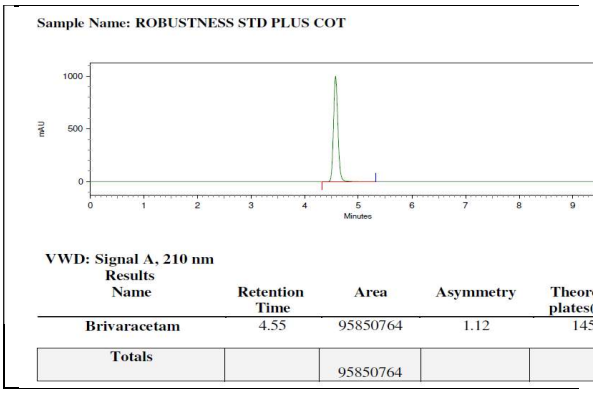


Figure 33 Representative chromatogram of Standard +2°C C.O.T.

F. Change in Column Oven temperature by -2°C:

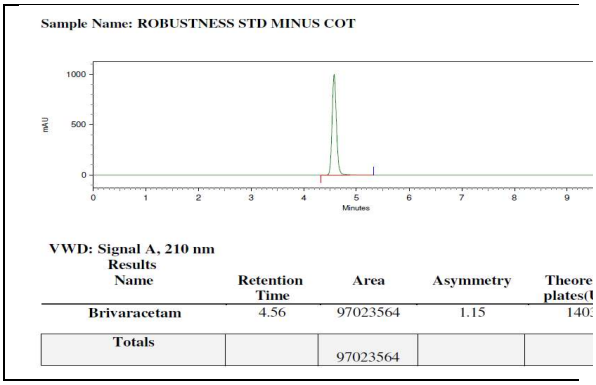


Figure 34 Representative chromatogram of Standard -2°C C.O.T.

Acceptance criteria:

The system suitability requirements for chromatographic analysis should meet accepted system suitability parameters.

Data interpretation: The obtained results demonstrated that all system suitability parameters complied with the predefined acceptance criteria, confirming the robustness and reliability of the developed analytical method.

E) System suitability test

Table 11 System Suitability Results of Brivaracetam

Sr. No.	Standard solution	Area	Asymmetry	Theoretical plates
1	Standard_I	86440265	1.11	14368
2	Standard_II	86485201	1.12	14352
3	Standard_III	86425361	1.11	14348
4	Standard_IV	86420561	1.12	14371
5	Standard_V	86349531	1.11	14346
Mean		**86424184**	**1.11**	**14357**
STD Dev		**48911.284**		
% RSD		**0.06**		

System Suitability Acceptance Criteria:
1. Relative standard deviation of analyte peak areas in standard chromatograms should not exceed 2.0 %.
2. Theoretical plate count for analyte peak in standard chromatograms should not be less than 2000.
3. Tailing Factor (Asymmetry) of analyte peak in Standard Chromatograms should remain below 2.0
Data interpretation: From the results presented above, all system suitability parameters were found within the specified acceptance criteria. Therefore, the developed chromatographic method was considered suitable for the intended analytical application.

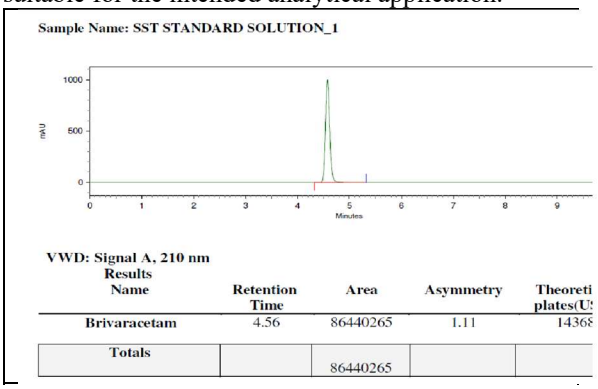


Figure 35 Representative chromatogram Standard solution I of system suitability solution

F) Analysis of Marketed Test Samples (Assay)

Brivamax 50 mg Tablet:
Weight of 20 tablets = 3032.0 gm
Average weight of tablet = 3032.0 / 20 = 151.6 mg
Table 12 Assay results of Brivamax 50 mg Tablet

Sample	Area	% Assay	Mean Assay
Sample 1	85225421	98.81	98.62
Sample 2	85126539	98.43	

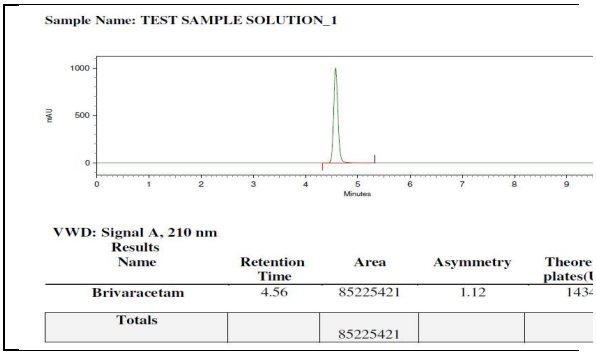


Figure 36 Representative chromatogram Tablet sample I

Acceptance criteria:
1) % Assay found should be in the range of 90-110%.
Data interpretation:
From the obtained results, it was concluded that the assay values of the selected marketed test samples were within acceptable limits, indicating suitability of the samples for further validation studies.

10. DISCUSSION

A straightforward, quick, sensitive, precise, and reproducible stability-indicating RP-HPLC technique has been successfully designed and validated for purpose of quantitative estimation of Brivaracetam in bulk drug and tablet dosage form [11]. In order to avoid the use of non-volatile inorganic buffer systems, lower analytical costs, and maintain compatibility with LC-MS/MS applications for future degradation product characterization, the developed chromatographic method made use of an environmentally friendly mobile phase consisting of Methanol and 0.1% Formic acid in water (50:50, v/v) [8,27].
The method demonstrated robust stability-indicating capability by effectively separating Brivaracetam from its acid- and base-induced degradation products. All ICH Q2(R1) recommendations were determined to be within acceptance limits, confirming that the technique is fit for its intended purpose [11]. The successful assay of the marketed formulation further validates the practical applicability of method [25]. This work contributes a cost-effective, green, and analytically sound platform that meets current regulatory requirements and lays the groundwork for advanced pharmaceutical analysis of Brivaracetam.

11. FUTURE SCOPE

The present investigation established a reliable analytical platform for estimation and stability

evaluation of Brivaracetam. Based on the outcomes of the current study, the following areas may be explored in future research:

1) LC-MS/MS for degradation product identification [27,32]
2) Simultaneous estimation with co-administered antiepileptics [8].
3) Bioanalytical method in plasma/urine/CSF [6,30].
4) Chiral HPLC for enantiomeric purity [2,14].
5) Extension to oral solution and IV injection dosage forms [1].
6) Long-term ICH Q1A(R2) formal stability studies [10.]
7) UPLC transfer for high-throughput analysis [28,31].

12. REFERENCES

1. App, D. et al. (2024). A Brief Review of Analytical Methods for the Estimation of Brivaracetam in Pharmaceutical Formulation and Biological Matrices. Asian Journal of Pharmaceutical Research and Development, Vol. 12(2), pp. 154–159.
2. Baksam, V. N., Pocha, V. R., Chakka, V. B., Ummadi, R. R., & Kumar, P. (2020). Development of an effective novel validated stability-indicating HPLC method for the resolution of Brivaracetam stereoisomeric impurities. Chirality, Vol. 32(9), pp. 1208-1219.
3. Bhamare, P., Dubey, R., Upmanyu, N., & Umadoss, P. (2021). A simple HPLC Method for In-Vitro Dissolution study of Brivaracetam in Pharmaceutical Dosage Form. The Asian Journal of Pharmacy and Pharmacology, Vol. 11(1), pp. 1–8.
4. Bhoir, G. C., et al. (2022). Forced degradation study of brivaracetam and development of a validated stability-indicating ultra-performance liquid chromatography method. Journal of Liquid Chromatography & Related Technologies, Vol. 45(15-16), pp. 783-792.
5. Dalal, A., Tegeli, V., & Waghmode, R. (2022). RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF BRIVARACETAM IN BULK AND FORMULATION. International Journal of Pharmaceutical Sciences and Research (IJPSR), Vol. 13(7), pp. 79–83.
6. Damle, M. C. et al. (2024). BIOANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR DETERMINATION OF BRIVARACETAM IN HUMAN PLASMA. International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS), Vol. 13(6), pp. 2872–2881.
7. Devarajan, V., & Ganesan, V. (2019). A validated stability indicating RP-HPLC method for the determination of Brivaracetam in bulk drug and pharmaceutical dosage form. Journal of Research in Pharmacy, Vol. 23(6), pp. 1098-1107.
8. El-hanboushy, S., Marzouk, H. M., & Ayish, N. S. (2025). A sustainable multi-task HPLC – UV method for simultaneous analysis of top neuromodulating agents in diverse pharmaceutical formulations. OPEN Chemistry, Vol. 23, pp. 1–12.
9. Gillard, M., Fuks, B., Leclercq, K., & Matagne, A. (2011). Binding characteristics of brivaracetam, a selective, high affinity SV2A ligand in rat, mouse and human brain: relationship to anti-convulsant properties. European Journal of Pharmacology, Vol. 664(1-3), pp. 36-44.
10. ICH Q1A(R2) Expert Working Group. (2003). Stability Testing of New Drug Substances and Products (R2). ICH Harmonised Tripartite Guideline, N/A.
11. ICH Q2(R1) Expert Working Group. (2005). Validation of Analytical Procedures: Text and Methodology. ICH Harmonised Tripartite Guideline, N/A.
12. ICH Q3B(R2) Expert Working Group. (2006). Impurities in New Drug Products (R2). ICH Harmonised Tripartite Guideline, N/A.
13. ICH Q3C(R8) Expert Working Group. (2021). Impurities: Guideline for Residual Solvents (R8). ICH Harmonised Tripartite Guideline, N/A.
14. Ilayaraja, P., Maniavannan, M., & Parthiban, P. (2022). Simultaneous determination of Brivaracetam and its isomers in the Brivaracetam drug by RP-HPLC. Journal of Applied Pharmaceutical Science (JAPS), Vol. 12(09), pp. 127–138.
15. Jain, P. S., & Jain, N. K. (2019). Development and validation of stability-indicating RP-HPLC method for estimation of Brivaracetam. Journal of Pharmaceutical Analysis, Vol. 9(5), pp. 352-358.
16. Karri, H., Gutta, M., & Thummala, S. (2022). Forced Degradation Study of Brivaracetam and Development of a Novel Validated Stability-Indicating UPLC Method. International Journal of Pharmaceutical

Sciences and Research (IJPSR), Vol. 13(8), pp. 3173-3180.
17. Mestareehi, A. H. (2025). Optimized and Validated Stability-Indicating RP-HPLC Method for Comprehensive Profiling of Process-Related Impurities and Stress-Induced Degradation Products in Rivaroxaban (XARELTO)®. Journal of Advanced Scientific Research, Vol. 18, N/A.
18. Mhaske, D.V., & Mali, N.V. (2016). HPLC Studies on degradation behaviour of Brivaracetam and development of validated stability-indicating HPLC Assay method. International Journal of Science and Research Methodology (IJSRM), Vol. 4(3), pp. 43-57.
19. Mounika, K., Kuchana, V., & Sunitha, P. (2023). Method development and validation for the estimation of Brivaracetam in bulk and marketed tablet formulation by using RP-HPLC method. International Journal of Advanced Research in Medical and Pharmaceutical Sciences, Vol. 8(1), pp. 1-12.
20. Nicolas G.D., Kenda B.M., Matagne A.C., & Michel P. (2016). Brivaracetam: A Selective High-Affinity Ligand for Synaptic Vesicle Protein 2A. CNS Drugs, Vol. 30, pp. 583-594.
21. Padmavathi, Y., Anjali, A., Babu, N. R., & Kumar, P. R. (2017). Development and validation of new FTIR method for quantitative analysis of gliclazide in bulk and pharmaceutical dosage forms. Indian Journal of Pharmaceutical Sciences, Vol. 10(June), pp. 377–382.
22. Padulkar, S., Pingale, R., & Khandave, P. (2025). Studies in Stability Indicating Chromatographic Method Development and Validation. Journal of Chemical Health Risks, Vol. 15, pp. 1538–1545.
23. Pasha, S. I., Varanasi, M. B., & Mohammed, I. (2017). Chromogenic spectrophotometric estimation of Brivaracetam in bulk drug and it's formulation with Folin-Ciocalteu reagent. IOSR Journal of Pharmacy, Vol. 7(12), pp. 44-48.
24. R, N. S., & A, B. D. (2018). Stability Indicating Thin-Layer Chromatographic Determination of Brivarecetam as Bulk Drug: Application to Forced Degradation Study. International Journal of Pharmaceutical Sciences and Research, Vol. 11(04), pp. 351–360.
25. Rathore M., Khanam N., Upadhyay K., & Upadhyay A. (2021). Development and Validation of Stability-Indicating RP-HPLC Method for Estimation of Brivaracetam in Bulk and Tablet Dosage Form. International Journal of Pharmaceutical Sciences Review and Research, Vol. 70(2), pp. 104-110.
26. Saini N., Sharma M., Singh K., Sharma N., & Sharma P.K. (2023). Development and Validation of a Novel Stability Indicating RP-HPLC Method for Determination of Brivaracetam in Bulk and Pharmaceutical Formulation. Journal of Analytical Chemistry, Vol. 78(8), pp. 1083-1090.
27. Sawant, S. D., & Godse, S. D. (2025). LC–ESI-MS Characterization of Degradents of Brivaracetam; Development and Validation of a Stability-Indicating RP-HPLC Method. Research Journal of Pharmacy and Technology, Vol. 18(3), pp. 1218-1223.
28. Shanbhag, V. A., et al. (2020). Development and Validation of UPLC Method for Determination of Brivaracetam in Pharmaceutical Dosage Form. Journal of Drug Delivery and Therapeutics, Vol. 10(4-S), pp. 13-18.
29. Spandana K.V., & Subhashini N.J. (2021). Analytical Method Development and Validation of Brivaracetam in Bulk and Pharmaceutical Dosage Form by RP-HPLC Method. IOSR Journal of Pharmacy, Vol. 11(5), pp. 26-33.
30. Vasanth, D. A., & Rajkamal, B. A. (2017). A validated LC-MS/MS method for pharmacokinetic study of Brivaracetam in healthy rabbits. International Journal of Pharmaceutical Sciences and Research, Vol. 10(2), pp. 24-29.
31. Vishweshwar, V., Babu, J. M., & Muralikrishna, R. (2018). 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 (IJPSR), Vol. 9(6), pp. 2315–2327.
32. Xu, K. et al. (2024). Identification of degradation products of brivaracetam using liquid chromatography quadrupole time-of-flight tandem mass spectrometry: Degradation pathway elucidation. Journal of Pharmaceutical and Biomedical Analysis, Vol. 240, N/A