RESEARCH PAPER

Analytical Method Development and Validation of Brivaracetam using RP-HPLC

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ABSTRACT

The present study aimed to develop and validate a simple, sensitive, accurate, and cost-effective Reversed-Phase High-Performance Liquid Chromatographic (RP-HPLC) method for the quantitative estimation of Brivaracetam in bulk drug and pharmaceutical Tablet dosage form. Chromatographic separation was achieved on a Grace C18 column (150 × 4.6 mm, 3.5 µm) using an isocratic mobile phase of Methanol:0.1% Orthophosphoric Acid in water (70:30 v/v) at a flow rate of 1.0 mL/min, with PDA detection at 216 nm and an injection volume of 20 µL. The drug was characterized by physical appearance, solubility, DSC, FTIR, and UV spectroscopy prior to method development. The method was validated as per ICH Q2 (R1) guidelines for system suitability, specificity, linearity, accuracy, precision (intraday and interday), LOD, LOQ, and robustness. Brivaracetam eluted at a retention time of 4.848 ± 0.243 min with an asymmetry factor of 1.13 and theoretical plate count of 3246. The method showed excellent linearity over 2–12 µg/mL (R^2 = 0.9994, y = 50043x − 1847.5). Mean percentage recoveries at 50%, 100%, and 150% spiking levels were 100.987 ± 0.920%, 99.802 ± 0.202%, and 100.180 ± 0.350%, respectively and all are within 98.0–102.0%. Intraday and interday precision studies yielded % RSD values below 0.714% and 0.620%, respectively. LOD and LOQ were calculated to be 0.331 µg/mL and 1.002 µg/mL. The assay of commercial Brevipil 50 tablets gave a mean recovery of 100.248 ± 0.567% (%RSD 0.566%). The developed RP-HPLC method is simple, rapid, accurate, precise, and fully validated as per ICH Q2 (R1) guidelines. It is suitable for routine quality control of Brivaracetam in both bulk API and tablet dosage form.

Keywords: Brivaracetam, RP-HPLC, Method Development, Method Validation, ICH Q2 (R1), Antiepileptic, SV2A, Quality Control, Pharmaceutical Analysis.

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Conflict of interest: None

1. INTRODUCTION:

Epilepsy, a chronic disease of the neurological system, can be defined as an unexpected onset of two or more seizures, more than 24h apart. The estimated number of people who are currently affected by epilepsy worldwide is 50 million, thus defining it as a prevalent disease of the neurological system throughout the globe [1]. Usually, a seizure causes harm and may even lead to psychoemotional distress to a patient and it is well established that patients with epilepsy also show higher rate of related psychiatric disorders [2]. Despite discovery of different types of anticonvulsants acting through different mechanisms, drug resistance is the major difficulty which leads to one of the diagnostic and treatment issues in epilepsy, affecting 30% of patients and the unmet need of designing drugs that target to defeat this problem led to the discovery of a new group of antiepileptic agents with high specificity, potency and better tolerability profiles.

Development of brivaracetam (BRV) was started to overcome these limitations. BRV was approved by the United States Food and Drug Administration (US FDA) in February 2016 for the adjunctive treatment of partial onset seizures in patients 16 years of age and older with drug resistant epilepsy [3]. High levels of tolerability and efficacy was observed in adults with refractory focal seizures, confirmed by positive phase II study [4, 5].

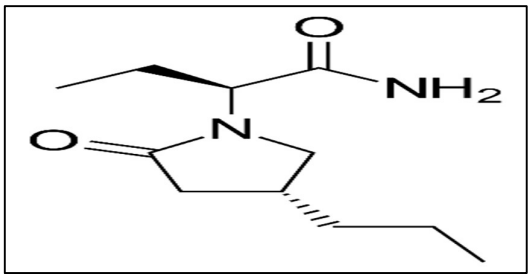

Figure 1: Chemical Structure of Brivaracetam

Brivaracetam is a third-generation antiepileptic drug and the 4-n-propyl analogue of the drug levetiracetam; the drug is a racetam and is named (2S)-2-[(4R)-2-oxo-4-propylpyrrolidin-1-yl] butanamide in IUPAC. From analysis it is clear there are two sections of the molecule that have chiral centers and these are the Pyrrolidinone ring and the butanamide chain giving 3 other stereoisomers than the required molecule; these three are analytically important as impurity molecules in drug development [6,7]. Brivaracetam is sold by the name Briviact and is produced by UCB [3].

Brivaracetam works as an anticonvulsant by binding with a selective, high affinity, to Synaptic Vesicle Protein 2A (SV2A). Widely distributed across the synaptic membrane, this protein plays a crucial role in regulating neurotransmitter release following nerve activation. By modulating the release of GABA, it may also play a key role in the onset and development of epilepsy [8, 9]. The binding affinity to SV2A is nearly 20 times that for brivaracetam compared to levetiracetam which therefore explains the need for a lower therapeutic dose range [8]. Brivaracetam binds to the intraluminal side of synaptic vesicle recycling endocytic receptor, blocking the synaptic exocytosis and excitatory neurotransmission following the high frequency firing of neurons, therefore abolishing the conduction of the impulses, controlling neuronal hyperexcitability and regulating epileptogenesis [9]. Besides that, Brivaracetam can block the voltage-gated Na+ channel which could also contribute to its antiepileptic activity, however, to which degree is still not determined [8].

Pharmacokinetically Brivaracetam shows a dose independent, linear kinetics, it is readily and rapidly absorbed orally with bioavailability approaching 100% [10]. Volume of distribution is about 0.5 L/kg with <20% plasma protein binding of Brivaracetam, suggesting broad tissue distribution [10, 11]. Elimination half-life is around 7-8 hours indicating the need for 2X daily dosing. Major metabolic route is the cleavage of acetamide moiety by hydrolysis and some hydroxylation occurs at the second level by CYP2C19. Mainly via the kidneys, the substance is eliminated [10].

Since closely related stereo isomeric impurities can compromise drug safety, we need precise testing methods to quantify Brivaracetam as both a bulk substance and a finished product. HPLC is utilized throughout drug discovery, development and manufacture for reasons such as studies of peak purity, reaction progress monitoring, formulation analysis and quality control of the final product [12]. RP-HPLC is the most frequent type of HPLC. It uses a non-polar stationary phase (C18-bonded) and a polar aqueous organic mobile phase. It covers 65-80% of pharmaceutical HPLC separation and this is mainly due to its versatility, simplicity of development and its compatibility with most drugs substances [12, 13]. Brivaracetam is a water-soluble, somewhat polar molecule so it can be tested by RP-HPLC during normal quality control analysis.

2. MATERIALS AND METHOD:

2.1. Chemicals:

Brivaracetam was procured from Moleculochem pvt. Ltd. HPLC grade methanol was purchased from S D fine-chem limited, Mumbai. HPLC grade water generated using Lab Link system. Orthophosphoric acid was bought from merck laboratories pvt. Ltd., Mumbai. Brevipil 50 Tablets (Sun Pharmaceutical Industries Ltd., label claim 50 mg) were purchased from a local pharmacy.

2.2. Instrumentation:

All instruments were calibrated and performance-qualified prior to use. The instruments and equipment used in this study are listed in Table 1.

Table 1: Instruments and Equipment Used in the Study

Instrument / Equipment	Make and Model
HPLC Pump	JASCO PU-2080 Plus
PDA Detector	JASCO MD-2010 Plus
Analytical Column	Grace C18 150 × 4.6 mm, 3.5 µm particle size
Sample Injector	Rheodyne injector
HPLC Software	Borwin-PDA Version 1.50
UV-Visible Spectrophotometer	Shimadzu Double-beam UV-1900
Analytical Balance	Shimadzu AY-120
Sonicator / Ultrasonic Bath	Prama Solutions
Water Purification System	Lab Link XTRA PURE
DSC Instrument	Mettler Toledo STAR SW 19.00
FTIR Spectrometer	JASCO FTIR-4600 A

3. EXPERIMENTAL WORK:

3.1. Preformulation Studies:

3.1.1. Physical Appearance:

The bulk drug sample of Brivaracetam was examined for its colour, odour, and physical state. Its melting point was determined using melting point apparatus. A small amount of material was placed into a capillary that was connected to a calibrated thermometer in order to determine the melting point. The assembly was then continuously heated while it was suspended in the paraffin bath.

The temperature was recorded as 77.46^0C. The observations were recorded and compared with the pharmacopoeial description.

3.1.2. Solubility Study:

The solubility of Brivaracetam was assessed qualitatively in a series of solvents at room temperature (25 ± 2°C). Approximately 10mg of Brivaracetam was added to 10 mL of each solvent in a stoppered glass tube and agitated by gentle shaking for 5 minutes, followed by visual observation. The solvents evaluated were water, methanol, ethanol, acetonitrile, and chloroform.

3.1.3. Differential Scanning Calorimeter (DSC):

Thermal determination of Brivaracetam was performed using (Mettler Toledo STAR DSC). An accurately weighed amount of about 5.3 mg of drug was sealed hermetically in an aluminum DSC pan. The sample was heated from 25°C to 300°C at a heating rate of 10.00 °C/min under nitrogen purge atmosphere. The resulting thermogram was analyzed to determine the onset temperature and the peak melting temperature. The characteristic endothermic peak temperature was observed at 77.46^0C and compared with the reported melting point of Brivaracetam (76-78.7°C) to confirm the identity and purity of drug substance [14].

3.1.4. FTIR Spectroscopy:

Infrared spectroscopic determination of Brivaracetam was carried out using a (JASCO FTIR-4600 A) spectrometer. A drug sample was directly applied on the diamond ATR crystal and recorded between 650- 4000cm-1 with 4cm-1 resolution. The obtained IR spectrum was interpreted for characteristic functional group absorption bands and the observed peaks were compared with the reported spectral data of Brivaracetam to confirm the structural identity.

3.1.5. UV Spectroscopy Selection of Analytical Wavelength (λmax):

A standard solution of Brivaracetam (10 µg/mL) was prepared by diluting the standard stock solution (1000 µg/mL in methanol) with methanol to the appropriate concentration. The solution was scanned in the wavelength range of 200–400 nm using a (Shimadzu UV-1900 double-beam UV-visible spectrophotometer) and methanol was used as blank reference. The wavelength of maximum absorbance (λmax) was shown at 216 nm.

3.2. Preparation of Standard Solutions:

3.2.1. Mobile Phase Preparation:

The mobile phase was prepared by mixing Methanol and 0.1% v/v Orthophosphoric Acid in HPLC grade water in the ratio of 70:30 v/v. The aqueous component (0.1% OPA solution) was made by adding 1.0 mL of orthophosphoric acid to 1000 mL of HPLC grade water and mixing thoroughly. Both components were individually vacuum filtered using a 0.45 µm PVDF membrane filter and degassed by sonication for 15 minutes.

3.2.2. Preparation of Standard Stock Solution:

An accurately weighed 10 mg of Brivaracetam was transferred to 10 ml volumetric flask, and the volume was made up to 10 ml with methanol, to get standard stock solution of Brivaracetam (1000 µg/mL). From the standard stock solution, working standard solution was prepared using mobile phase as final diluent.

3.2.3. Working Standard Solution (4µg/mL):

A suitable aliquot was transferred from the stock standard solution (1000 µg/mL) into a 10 mL volumetric flask and diluted to volume with the mobile phase (Methanol: 0.1% OPA, 70:30 v/v) to prepare a working standard solution at a final concentration of 4 µg/mL. This concentration was used for system suitability evaluation, specificity testing, and assay and precision studies.

3.2.4. Sample Solution (Tablet Assay):

Twenty tablets of Brevipil 50 were weighed and the average weight was calculated. Tablet powder equivalent to 10 mg of Brivaracetam was transferred to a 10 mL volumetric flask, dissolved in methanol with sonication, filtered, and diluted to yield a working concentration of 4µg/mL with mobile phase.

3.3. Chromatographic Method Development and Optimization:

The chromatographic conditions for Brivaracetam determination were optimized using a trial-based systematic approach. A Grace C18 column (150 × 4.6 mm, 3.5 µm) was used during the development phase. Different mobile phase compositions were tried to obtain well resolved symmetrical peak having acceptable retention time, satisfactory peak shape and adequate column efficiency. Three chromatographic trials were conducted as summarised in Table 2.

Table 2: Chromatographic Trials Conducted during Mobile Phase Optimization

Trial No.	Mobile Phase Composition	Flow Rate	Observation
1	Methanol : 0.05M Phosphate Buffer (50:50 v/v)	1.0 mL/min	Retention time and peak shape was unsatisfactory, the theoretical plate count was insufficient
2	Methanol : 0.05M Phosphate Buffer (80:20 v/v)	1.0 mL/min	Retention time was improved however, significant peak tailing was observed
3	Methanol : 0.1% OPA in Water (70:30 v/v)	1.0 mL/min	Well-resolved symmetrical peak observed having appropriate RT and satisfactory system suitability parameters

- **Trial 3** - The optimized mobile phase composition was selected as using Methanol:0.1% OPA in Water (70:30 v/v), which produced a symmetric and well-resolved Brivaracetam peak, with a retention time of 4.848 ± 0.243 min and system suitability parameters within acceptance limits.

3.4. Analytical Method Validation [16, 17]:

The developed RP-HPLC method was validated as per ICH Harmonized Guideline Q2 (R1) Validation of Analytical Procedures. The validation parameters were examined for the following:

- System suitability
- Specificity
- Linearity and range
- Accuracy
- Precision (intraday and interday)
- Limit of detection (LOD)
- Limit of quantification (LOQ)
- Robustness.

4. RESULTS AND DISCUSSION:

4.1. Physical Appearance:

The brivaracetam bulk drug was visually inspected and was found to be white to off-white, odorless, crystalline powder. This observation matched with the description given in Indian Pharmacopoeia 2022 and literature confirming the identity of the procured drug substance [15].

4.2. Solubility Profile:

The solubility of Brivaracetam was qualitatively evaluated by visual observation in five solvents at room temperature (25 ± 2°C). The results are summarised in Table 3.

Table 3: Solubility Profile of Brivaracetam in Various Solvents

Solvent	Solubility Observed
Methanol	Freely soluble
Ethanol	Freely soluble
Acetonitrile	Soluble
Water	Slightly soluble
Chloroform	Slightly soluble

4.3. Differential Scanning Calorimetry (DSC):

The DSC thermogram of Brivaracetam exhibited sharp single endothermic peak with onset temperature of 75.45°C and melting point (extrapolated peak) of 77.46°C with enthalpy of fusion of −89.27 J/g. The observed melting point is in agreement with the reported literature melting point of 76.0–78.7°C, which confirms the identity of the drug substance [14]. The thermogram of Brivaracetam is shown in Figure 2.

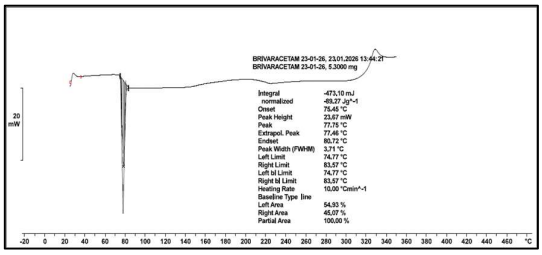

Figure 2: DSC Thermogram of Brivaracetam

4.4. FTIR Spectroscopy:

The FTIR spectrum of Brivaracetam was recorded using ATR mode (JASCO FTIR-4600, 4000–650 cm^{-1}).The obtained spectrum was compared with the standard reference spectrum of Brivaracetam and all principal peaks were found to be in agreement, confirming the identity of the drug substance. The characteristic peaks are summarized in Table 4 and the spectrum is shown in Figure 3.

Table 4: FTIR Spectral Interpretation of Brivaracetam

Sr. No.	Functional Group	Standard IR Range (cm^{-1})	Observed IR Range (cm^{-1})
1	N–H stretching (Primary amide, –$CONH_2$)	3500–3100	3316.96, 3158.83
2	C–H stretching (Alkyl chain, –CH_2 / –CH_3)	3000–2850	2966.93, 2844.49
3	C=O stretching (Lactam + Amide, most diagnostic)	1700–1630	1652.7
4	C–N stretching (Pyrrolidinone ring)	1400–1200	1347.03, 1217.83
5	C–O / C–C stretching (Fingerprint region)	1300–1000	1054.87, 1014.87

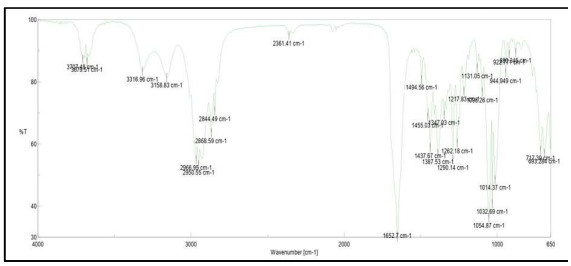

Figure 3: FTIR of Brivaracetam

4.5. UV Spectroscopic Analysis:

The solution of Brivaracetam (10 µg/mL in methanol) was scanned in the wavelength range of 200-400 nm using (Shimadzu UV-1900 double beam spectrophotometer). The UV absorption spectrum showed a characteristic maximum absorbance (λmax) at 216 nm and showed in Figure 4.

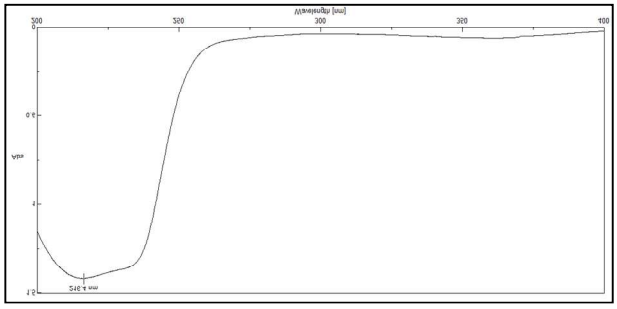

Figure 4: UV Spectrum of Brivaracetam in Methanol

4.6. Optimized Chromatographic Conditions for Brivaracetam:

The final optimized chromatographic conditions are presented in Table 5.

Table 5: Optimized Chromatographic Conditions for Brivaracetam

Chromatographic Parameter	Optimized Condition
Column	Grace C18 (150 × 4.6 mm, 3.5 µm particle size)
Mobile Phase	Methanol : 0.1% OPA in Water (70:30 v/v); isocratic
Flow Rate	1.0 mL/min
Detection Wavelength	216 nm (PDA detector)
Injection Volume	20 µL
Column Temperature	Ambient
Diluent	Methanol
Retention Time (BRV)	4.848 ± 0.243 min

4.7. System Suitability:

System suitability tests are method specific tests to confirm that the analytical system is suitable to use just before committing the samples for analysis.

System suitability was checked by injecting five replicates of standard working solution of Brivaracetam (4µg/mL) under optimized chromatographic conditions. The results are shown in Table 6.

Table 6: System Suitability Parameters of the Developed RP-HPLC Method

Parameter	Obtained values
RT (min)	4.848 ± 0.243
Area	199663.939
Asymmetry	1.13
Plates (N)	3246

4.8. Specificity:

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically, these might include impurities, degradants, matrix, etc. For specificity, blank solution interference at the retention time of Brivaracetam peak was checked. For that chromatograms of Blank and spiked drug are shown in Figure 5 and Figure 6.

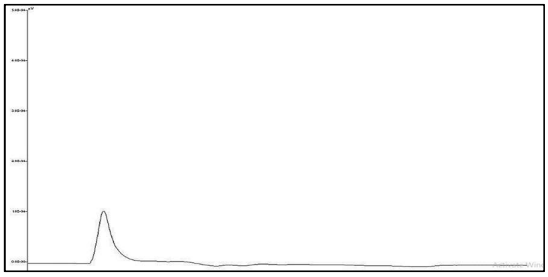

Figure 5: Chromatogram of Blank (MP)

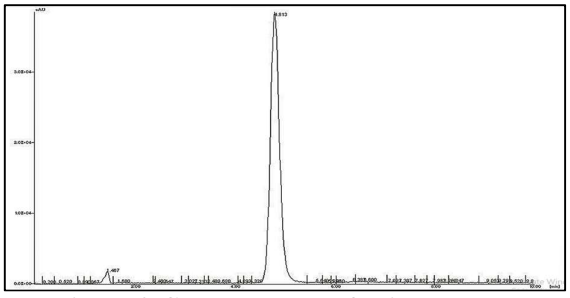

Figure 6: Chromatogram of Brivaracetam

4.9. Linearity and Range:

Linearity is the ability of the analytical procedure to produce a test results that are directly proportional to the conc. of the analyte in sample, within specific range. The acceptance criteria is (R^2) = 0.999 or more. Range is the interval between Upper and Lower Levels that have been demonstrated to be determined with precision, accuracy and linearity.

The linearity of the method was estimated by taking six concentrations of the solutes in the range of 2 – 12µg/mL for Brivaracetam. The solutions were prepared and injected into the HPLC system to determine the peak area. A linearity graph was plotted between the concentration and average area for each solute. The regression coefficient value (R^2) was also determined. The results obtained are summarized in Table 7. Calibration Curve and overlay is shown in Figure 7 and 8 respectively.

Table 7: Linearity of Brivaracetam

Replicates	Concentrations of Brivaracetam (µg/mL)					
	2	4	6	8	10	12
1	102299.244	195532.445	292939.296	381551.982	508842.132	613902.383
2	101959.598	195746.305	287660.138	393168.460	487396.168	600317.666
3	99973.027	199301.539	296716.921	389369.866	495557.607	591743.981
4	102526.071	195604.958	300120.618	388355.526	496454.395	610509.538
5	105256.946	199663.939	295104.279	402594.640	514503.660	604681.505
6	104696.803	204297.138	295229.130	395155.570	501201.426	594402.381
Average	102785.281	198357.721	294628.397	391699.341	500659.231	602592.909
SD	1931.683	3471.399	4159.891	7104.756	9783.027	8773.827
% RSD	1.879	1.750	1.412	1.814	1.954	1.456

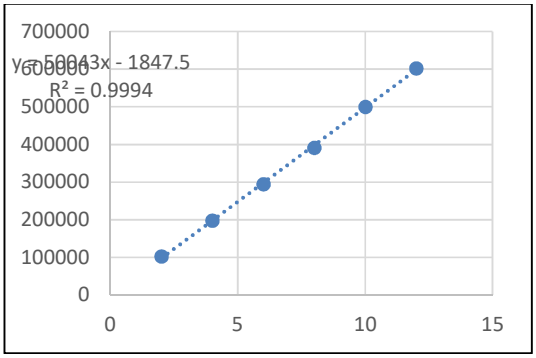

Figure 7: Calibration curve for Brivaracetam

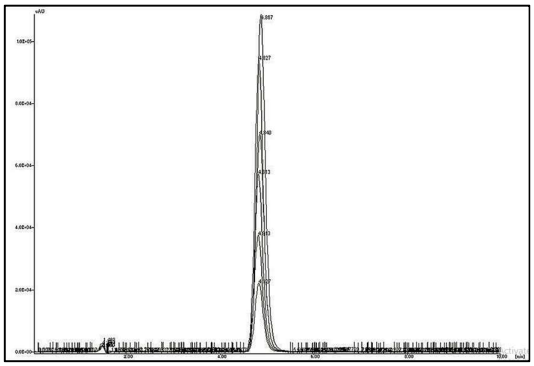

Figure 8: Overlay of Chromatogram of Linearity

4.10. Accuracy:

Accuracy expresses the closeness of the value of analytical method to the true value of analytical method. The acceptance criterion is 98% to 102%.

The accuracy (recovery) was determined by spiking the standard drug solutions at concentrations of 50%, 100%, and 150% in the HPLC system. The study was performed in a triplicate way with data expressed in the form of % recovery ± % relative standard deviation based on definite concentrations. The results obtained are summarized in Table 8.

Table 8: Results of Accuracy Studies

Spiked Level (%)	Conc. of Drug added (µg/mL)	Amount of Drug added (µg/mL)	Area	Amount recovered (µg/mL)	% Recovery	Mean ± %RSD
50	4	2	302945.944	6.091	101.511	100.987 ± 0.920
	4	2	298150.870	5.995	99.914	
	4	2	303021.256	6.092	101.536	
100	4	4	396771.636	7.966	99.569	99.802 ± 0.202
	4	4	398155.560	7.993	99.915	
	4	4	398184.927	7.994	99.922	
150	4	6	499557.607	10.019	100.195	100.180 ± 0.350
	4	6	497695.531	9.982	99.823	
	4	6	501201.426	10.052	100.523	

4.11. Precision:

Precision expresses the closeness of agreement between a series of measurements obtained from multiple sampling of same homogeneous sample under prescribed condition. The acceptance criteria is %RSD should be ≤2%.

The precision of the developed method was estimated in terms of inter-day and intra-day variability by spiking the concentrations six times in a single day (intra-day) and on three different days (inter-day). The data were expressed in precision ± % relative standard deviation. The results obtained are summarized in Table 9 and 10 respectively.

4.11.1. Intraday Precision Studies:

Table 9: Intraday Precision Studies

Theoretical Conc(µg/mL)	Peak Area	Amount Recovered (µg/mL)	%Recovery	Mean± SD	SD	%RSD
4	209659.750	3.975	99.365	100.126	0.7143	0.714
4	211386.791	4.009	100.231			
4	212484.077	4.031	100.782			
8	411008.255	8.016	100.198	100.462	0.2426	0.243
8	412264.585	8.041	100.513			
8	412908.882	8.054	100.675			
12	607988.095	11.969	99.745	99.709	0.2822	0.282
12	609341.902	11.997	99.972			
12	605989.045	11.929	99.411			

4.11.2. Interday Precision:

Table 10: Interday Precision Studies

Theoretical Conc(µg/mL)	Peak Area	Amount Recovered (µg/mL)	%Recovery	Mean± SD	SD	%RSD
4	212105.205	4.024	100.592	100.438	0.2187	0.218
4	211301.337	4.008	100.188			
4	211991.995	4.021	100.535			
8	408914.341	7.974	99.673	99.372	0.6209	0.620
8	404870.776	7.893	98.658			
8	409359.326	7.983	99.785			
12	615022	12.111	100.922		0.36	

	.288			100.626	89	0.368
12	613956.825	12.089	100.744			
12	610780.689	12.026	100.213			

4.12. Limit of Detection and Limit of Quantification:

The limit of detection (LOD) may be defined as the lowest detectable concentration by any analytical method, but not necessarily to measure the exact amount.

The LOD was determined by the formula:

$$LOD = 3.3 \times \left(\frac{\sigma}{S}\right)$$

Where σ = standard deviation of response

S = slope of the calibration curve.

The slope S may be estimated from the calibration curve of the analyte.

The limit of quantification (LOQ) may be defined as the lowest detectable concentration by any analytical method with a particular level of accuracy and precision.

The LOQ is determined by the formula:

$$LOQ = 10 \times \left(\frac{\sigma}{S}\right)$$

Where σ = standard deviation of response

S = slope of the calibration curve.

The slope S may be estimated from the calibration curve of the analyte.

The results obtained are summarized in Table 11.

Table 11: LOD & LOQ Studies

Parameter	Formula	S.D of Y-intercept (*σ*)	Slope (m)	Concentration (µg/mL)
LOD	3.3 (*σ*/*S*)	5015.916	5004 3.052	0.331
LOQ	10 (*σ*/*S*)	5015.916	5004 3.052	1.002

4.13. Assay:

Accurately weigh powdered marketed tablets (Brevipil 50 by Sun Pharmaceutical Industries Ltd; Label claim - 50 mg) which are equivalent to 10 mg of drug content and diluted appropriately to 1000 µg/mL with methanol. Then the solution is sonicated and filtered. From that stock solution dilution of 4µg/mL was prepared and volume is made with mobile phase. To get the test results 6 replicates of same concentration are evaluated. The results obtained are summarized in Table 12.

Table 12: Assay Results of Commercial Tablet

Theoretical Conc(µg/mL)	Peak Area	Amount Recovered (µg/mL)	%Recovery	Mean ± SD	%RSD
4	198741.346	4.008	100.208	100.248 ± 0.567	0.566
4	198258.951	3.999	99.967		
4	199717.444	4.028	100.696		
4	200101.972	4.036	100.888		
4	199174.876	4.017	100.425		
4	196936.002	3.972	99.306		

4.14. Robustness:

Robustness is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters. The acceptance criteria are no change in results.

The robustness of the method was judged by deliberately altering the mobile phase composition by ± 2% v/v (i.e., 68:32% v/v and 72:28% v/v), flow rate by ± 0.05 ml/minute (i.e., 0.95 and 1.05 ml/minute), and wavelength by ± 1 nm (i.e., 215 and 217nm), keeping the other chromatographic parameters constant. The results obtained are summarized in Table 13.

Table 13: Results of Robustness Studies

	Flow Rate (ml/min)		
	0.95	1	1.05
	199678.786	200833.869	196568.275
	202901.519	195780.859	194263.954
	199570.977	197949.388	192510.237
AVG	200717.094	198188.039	194447.489
STD DEV	1892.535	2534.944	2035.235
% RSD	0.943	1.279	1.047
% RSD AVG	**1.090**		
	Mobile Phase (v/v)		
	72:28	70:30	68: 32
	196531.507	197374.066	193584.450
	201199.035	195780.859	202773.766
	200864.072	196593.570	201598.675
AVG	199531.538	196582.832	199318.964
STD DEV	2603.496	796.658	5000.870

% RSD	1.305	0.405	2.509
% RSD AVG	**1.406**		
	Wavelength (nm)		
	215	216	217
	198263.136	197371.216	201563.778
	202947.177	195780.859	199564.853
	198379.355	196593.570	196804.270
AVG	199863.222	196581.882	199310.967
STD DEV	2671.415	795.243	2389.890
% RSD	1.337	0.405	1.199
% RSD AVG	**0.980**		

5. CONCLUSION:

A simple, rapid, accurate, and cost-effective RP-HPLC method was successfully developed and validated for the quantitative estimation of Brivaracetam in bulk drug and Tablet dosage form using a Grace C18 column with an isocratic mobile phase of Methanol: 0.1% OPA in water (70:30 v/v) at a flow rate of 1.0 mL/min with detection at 216 nm. Preformulation studies including DSC and FTIR confirmed the identity and purity of the drug substance, with a melting point of 77.46°C and spectral concordance with the IP 2022 reference. The optimized method produced a clean, symmetrical Brivaracetam peak at a retention time of 4.848 ± 0.243 min with an asymmetry factor of 1.13 and theoretical plate count of 3246.

Validation as per ICH Q2 (R1) guidelines demonstrated excellent linearity over 2–12 µg/mL (R^2 = 0.9994), with mean recoveries of 99.802–100.987% across all accuracy levels. Precision studies yielded % RSD values well below 2.0% for both intraday and interday analyses, confirming outstanding repeatability and reproducibility. LOD and LOQ were established at 0.331µg/mL and 1.002µg/mL, respectively, confirming adequate sensitivity for quality control purposes.

The method was found to be robust under deliberate variations in flow rate, mobile phase composition, and detection wavelength. Assay of commercial Brevipil 50 Tablets gave a mean result of 100.248 ± 0.567%, confirming that formulation excipients do not interfere with drug quantification.

To the best of our knowledge, this represents one of the few fully validated, simple, isocratic RP-HPLC methods for Brivaracetam using conventional laboratory instrumentation. The developed method is therefore suitable and recommended for routine pharmaceutical quality control of Brivaracetam in both bulk API and finished dosage forms.

6. CONFLICT OF INTEREST:

All authors declare that there is no conflict of interests regarding publication of this paper.

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