

Quality by Design (QbD) Assisted Development and Validation of a Robust RP-HPLC Method for the Estimation of Brivaracetam in Bulk and Dosage Forms

S. Fulmali¹, A. Jain¹ and S. Kadam^{1*}

^{1*}Department of Quality Assurance, Shri. D. D. Vispute college of pharmacy and Research center, Panvel.

Correspondence: Sakshi Kadam/ sakshinitinkadam8828@gmail.com

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ABSTRACT

Brivaracetam is a new antiepileptic agent used to treat seizures that happen partially from multiple different sites in the brain. One of the objectives of this research was to develop accurate, reliable, precise methods using UV spectrophotometry (ultraviolet-visible spectrophotometry) and high-performance liquid chromatography (HPLC) to quantify brivaracetam in drug product and pharmaceutical formulations. To accomplish this goal, preliminary studies were performed to measure solubility, identify melting point, and perform Fourier transforms infrared (FTIR) spectroscopic analysis. The maximum absorbance observed at UV spectroscopy with brivaracetam was 210 nm, and the linearity of the method was established within a mass concentration range of 10 to 60 µg/mL. An HPLC method was developed using a BDS Hypersil column (250 mm x 4.6 mm 5 µm) with a mobile phase of water and acetonitrile (65:35 v/v) at a flowrate of 1.0 mL/min with a detection wavelength of 210 nm. To optimize the chromatographic conditions used to quantify brivaracetam, Analytical Quality by Design (AQbD), and Central Composite Design were used. In addition, both methods were validated according to ICH Q2 (R1) guidelines to verify their specificity, linearity, accuracy, precision, robustness, limit of detection (LOD), and limit of quantification (LOQ). Validation results confirmed that UV spectrophotometric and HPLC methods were reliable, reproducible, and appropriate for use as routine methods for quality control testing of brivaracetam in unprocessed and tablet pharmaceutical formulations. Our developed methods can be used successfully in pharmaceutical companies for quality assurance and regulatory compliance.

Keywords: Antiepileptic agent, seizures, Fourier transforms infrared (FTIR), Brivaracetam, Limit of qualification (LOQ), Quality By Design (AQbD).

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1. INTRODUCTION

A seizure is a sudden change in brain function that happens due to exaggerated bursts of activity from groups of neurons within your brain. To differentiate between a seizure caused by abnormal activity of your brain cells versus an event such as a psychogenic seizure (non-seizure), the term "epileptic seizure" will be used. With recurrent spontaneous seizures, you have epilepsy¹. "Epilepsy" encompasses many different types of conditions, all of which indicate there is an underlying problem with how the brain functions. An individual can have a seizure as a result of a reversible issue (ex., fever or low blood sugar); the seizure will not meet the definition of epilepsy because it would only be considered to be a single or short-term condition rather than the chronic condition of epilepsy². When there is a cluster of clinical indicators that occur together in relation to epileptic seizure types, age of onset, EEG data, trivial factors, genetics, natural history, prognosis, and AED response it is

termed an "epilepsy syndrome." Avoid using ambiguous phrases such as "seizure disorder"³.

1.1 Types of Seizures:

There are three types of seizures as follows:

- **Partial seizures (focal)**⁴: Begin in one area of one hemisphere of the brain. Consciousness may be preserved or impaired, with symptoms such as localized muscle movements, sensory changes, or altered behavior.
- **Generalized Seizures**⁵: Involve both hemispheres of the brain from onset and usually cause loss of consciousness. They include tonic-clonic, absence, myoclonic, tonic, clonic, and atonic seizures.
- **Unclassified Seizures**⁶: Cannot be classified as focal or generalized due to insufficient clinical information or an unknown seizure onset.

*Author for Correspondence: sakshinitinkadam8828@gmail.com

1.2 Analytical Methods for Brivaracetam:

Analytical methods are critical for establishing the quality, safety, and efficacy of pharmaceuticals across their lifecycle. They are important to drug development, drug formulation, quality control, stability testing and regulatory compliance. Proper analytical methods have the characteristics of simplicity, accuracy, precision, robustness, sensitivity, and cost-effectiveness, allowing for their use in pharmaceutical and quality control laboratories⁷.

Various analytical methods have been applied to Brivaracetam determination (bulk drug, formulation or biological specimen) such as UV Spectrophotometry, HPLC, LC-MS/MS and HPTLC, yet these methods typically involve complex sample preparation, expensive instrumentation (capital and operational), long analysis time, or high solvent consumption, limiting their use as routine techniques. In addition, only a limited number of studies have implemented an Analytical Quality by Design (AQbD) approach to systematically develop the RP-HPLC conditions^{8,9}.

The aim of this study is to develop and validate methods for determining Brivaracetam in both bulk powders and

dosage forms with high levels of simplicity, rapidness, accuracy, precision, and economy. Both the UV spectrophotometric and AQbD-based RP-HPLC methods will be specifically validated to be following the International Conference on Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines¹⁰. The RP-HPLC method was created using a central composite design under an AQbD framework and validated according to ICH Q2(R1) requirements. The resulting methods will be useful in supporting the baseline quality assurance of pharmaceutical products through the development and validation of appropriate analytical testing methods¹¹.

Brivaracetam (also known as n-Propyl-Levetiracetam) is a new anti-epileptic drug (AED) and is the first selective ligands for the synaptic vesicular protein 2A (SV2A) which are part of a larger family of drugs referred to as "racetams"¹². After the secondary structural-based molecular modelling of the SV2A protein, [using 12,000 structurally diverse, small molecule compounds] as part of an innovative, targeted approach to finding new ligands to SV2A, brivaracetam was found to be the only compound from the series of tested ligands to be (a) a clinically active AED and (b) a selective SV2A ligands¹³.

2. MATERIALS AND METHODS

2.1 Instruments and Materials

Sr. No.	Item	Category	Grade/Model/Specification	Supplier/Manufacturer
1	Brivaracetam	API	API	Glenmark Pharmaceuticals, Mumbai
2	Methanol	Chemical	HPLC Grade	Merck Life Science Pvt. Ltd.
3	Acetonitrile	Chemical	HPLC Grade	Merck Life Science Pvt. Ltd.
4	Water	Chemical	HPLC Grade	Lab Link Filtration Unit
5	Ultra Sonicator	Instrument	Life Care Equipment	Life Care Equipment
6	Weighing Balance	Instrument	Shimadzu (Sensitivity: 0.001 g)	Shimadzu
7	HPLC System	Instrument	JASCO Extrema LC 4000	JASCO
8	HPLC Column	Instrument	Hypersil C18 (250 × 4.6 mm, 5 µm)	Hypersil
9	UV-Visible Spectrophotometer	Instrument	Shimadzu 1800	Shimadzu

2.2 Methods and Preliminary studies:

2.2.1 Solubility Measurements: Abiraterone acetate and Prednisolone were solubilized in water, methanol and acetonitrile for solubility testing.

2.2.2 Measuring melting point: A small quantity of Brivaracetam was filled into a capillary tube and heated gradually in a melting point apparatus. The temperature at which the drug melted was recorded and compared with the reported melting point range to confirm its identity and purity.

2.2.3. FTIR Analysis of Brivaracetam: FTIR spectroscopy was performed to characterize

Brivaracetam by recording its infrared spectrum over the range of **4000–400 cm⁻¹**. The obtained spectrum was used to identify the characteristic functional groups and confirm the identity of the drug.

2.3 Methodology For Analytical Method By Uv Spectrophotometry

2.3.1. UV Spectrophotometric Method Development Solvent Selection: Methanol was selected as the solvent for Brivaracetam based on its excellent solubility and suitability for UV spectrophotometric analysis.

2.3.2. Preparation of Standard Solution: A stock solution of Brivaracetam (1000 µg/mL) was prepared by dissolving 100 mg of the drug in methanol and making the volume up to 100 mL. A working standard solution (100 µg/mL) was prepared by diluting 10 mL of the stock solution to 100 mL with methanol.

2.3.3. Preparation of Sample Solution: 20 tablets were weighed and powdered. An amount equivalent to 100 mg of Brivaracetam was transferred to a 100 mL volumetric flask, dissolved in methanol, sonicated for 15 min, and diluted to volume. A working sample solution (100 µg/mL) was prepared by further dilution.

2.3.4. Selection of Detection Wavelength: A 10 µg/mL Brivaracetam solution was scanned over 200–400 nm using methanol as blank. The maximum absorbance (λ_{max}) was observed at 210 nm, which was selected for analysis.

2.4 UV Method Validation

2.4.1. Linearity: Linearity was evaluated over the concentration range of 10–60 µg/mL by plotting absorbance against concentration and calculating the regression equation and correlation coefficient.

2.4.2. Limit of Detection (LOD) and Limit of Quantification (LOQ): LOD and LOQ were calculated using the standard deviation of the response and the slope of the calibration curve according to ICH guidelines.

2.4.3. Precision: Intraday and interday precision were assessed by analysing Brivaracetam solutions at different time intervals. The results were expressed as %RSD, which was found to be less than 2%.

2.4.4. Accuracy: Accuracy was determined by the standard addition method at 80%, 100%, and 120% concentration levels. Recovery studies were performed in triplicate, and the percentage recovery was calculated.

2.4.5. Robustness: Robustness was evaluated by introducing small deliberate changes in the detection wavelength (210 ± 2 nm). The method showed consistent performance, indicating its reliability under minor variations in analytical conditions.

2.4.6. Assay: Equal volumes of the standard and sample solutions were diluted with methanol to the required concentration and analysed using a UV spectrophotometer at 210 nm, with methanol as the blank. The assay of Brivaracetam was calculated by comparing the absorbance of the sample with that of the standard.

The formula used to calculate the Assay (%) of Brivaracetam is as follows:

$$\% \text{ Assay} = (\text{AT} \times \text{WS} \times \text{DT} \times \text{P} \times \text{AW} \times 100) / (\text{AS} \times \text{WT} \times \text{DS} \times 100 \times \text{LC})$$

Where: AT = Average absorbance of assay preparation

AS = Average absorbance of standard preparation

WS = Weight of standard sample

WT = Weight of sample in assay preparation

DS = Dilution factor of standard preparation

DT = Dilution factor of assay preparation

P = Potency

LC = Label claim

AW = Average weight

2.5 HPLC METHOD DEVELOPMENT AND OPTIMIZATION:

2.5.1. Preliminary Solubility Studies: The solubility profile of Brivaracetam was evaluated in different solvents: Water, methanol, and acetonitrile by making distinct solutions and carrying out analysis by UV spectroscopy. The solvent with the best solubility was selected for further analytical work.

2.5.2. Selection of a Suitable Solvent: Based on the solubility study results, it appears that methanol has good solubility with Brivaracetam, thus being the solvent used for all additional analyses in the current methodology development process.

2.5.3. Preparation of Standard Stock Solution: To prepare a standard stock solution of Brivaracetam 2000 µg/mL, an accurate amount (200 mg) of the drug was weighed into a 100 mL volumetric flask. The flask was filled with approximately 70 mL of methanol and was sonicated until the drug completely dissolved in the methanol. The volume in the flask was then made up to the mark with methanol.

2.5.4. Preparation of Sample Solution: 20 Brivaracetam tablets were weighed, powdered, and an amount equivalent to 200 mg of the drug was transferred to a 100 mL volumetric flask. The powder was dissolved in methanol with sonication, and the volume was made up to the mark to obtain a 2000 µg/mL sample stock solution.

2.5.5. Selection of Detection Wavelength: A standard Brivaracetam solution was scanned in the range of 200–400 nm using a UV spectrophotometer. The drug exhibited maximum absorbance (λ_{max}) at 210 nm, which was selected as the detection wavelength for analysis.

2.5.6. Selection of Mobile Phase: Various mobile phase compositions were evaluated to achieve optimum peak resolution, symmetry, and minimum tailing. The mobile phase providing the best chromatographic performance was selected for the RP-HPLC method.

Table 2. Mobile phase trial data

Trial No.	Mobile Phase	Composition (%v/v)
1	Acetonitrile: Methanol	50:50
2	Water: Acetonitrile	10:90
3	Water: Methanol	10:90
4	Acetonitrile: Methanol	40:60
5	Acetonitrile: Methanol	10:90

2.6 Optimized Chromatographic Conditions for HPLC by QbD Approach:

Table 3. Optimized Chromatographic Conditions

Sr.No.	Specifications	Description
1	Equipment	JASCO Extrema LC 4000 system
2	Software	CHROMNAV
3	Column	BDS Hypersil C18 column (250 mm × 4.6mm) having 5.0µm particle size
4	Mobile phase	Water: Acetonitrile (65:35)
5	Run time	10 min
6	Flow rate	1 mL/min
7	Column temperature	25°C
8	Injection volume	10 µL
9	Detection wavelength	210 nm
10	Diluent	Methanol
11	Elution mode	Isocratic

2.7 Assay

A suitable volume of the standard stock solution and sample stock solution was pipetted out and diluted to obtain the desired concentration. The prepared solutions were injected into the HPLC system six times. The peak areas were recorded, and the % purity (assay) of each drug in the sample was calculated using the following formula:

$$\% \text{Assay} = \frac{AT \times WS \times DT \times 100}{AS \times DS \times WT \times LC}$$

Where,

AT = Average absorbance of assay preparation

AS = Average absorbance of assay preparation

WS = Weight of standard sample

WS = Weight of standard sample

DS = Dilution factor of standard preparation

DT = Dilution factor of assay preparation

P=Potency

LC=Label Claim

AW=Average Weight

3. RESULTS AND DISCUSSION

3.1 Solubility Study

Pre-formulation studies indicated that Brivaracetam was sparingly soluble in water. Several solvents were screened during preliminary solubility testing, and methanol exhibited the highest solubility for the drug. Hence, methanol was selected as the solvent for subsequent analytical investigations.

Table 4. Solubility Study for Brivaracetam

Sr.No.	Solvent	Solubility Status
1.	Water	Sparingly soluble
2.	Acetonitrile	Freely soluble
3.	Methanol	Freely soluble

3.2 Melting Point:

The melting point Brivaracetam was determined using the open capillary method, and it was found to be 75 °C.

3.3 FTIR:

FTIR spectroscopy was used to identify and confirm Brivaracetam, which is shown in Table 5 and in Figure 2

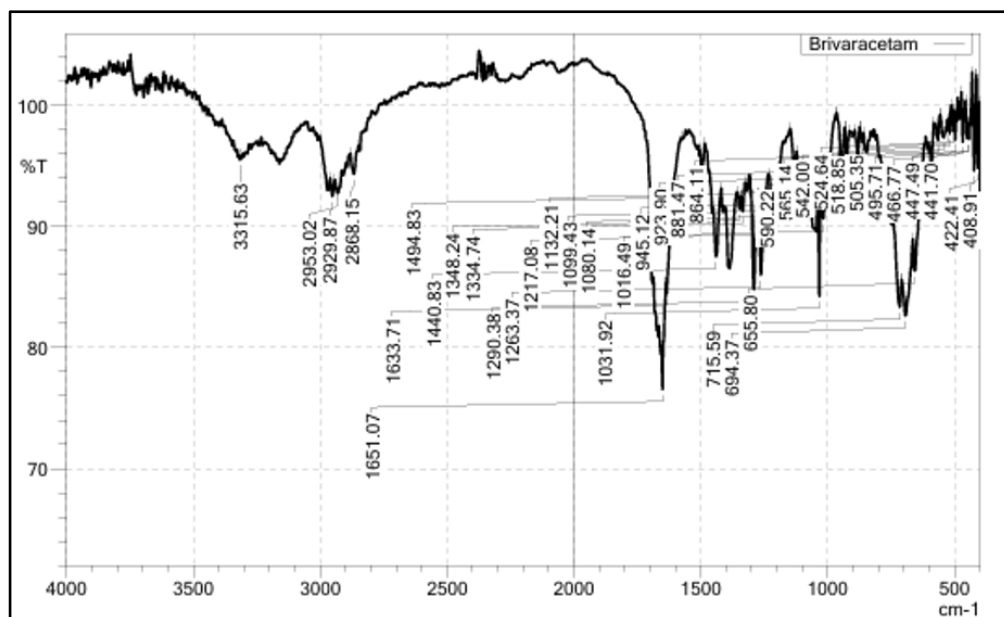


Figure 2. FTIR spectrum of Brivaracetam

Table 5. FT-IR Interpretation of Brivaracetam

Observed Peak (cm ⁻¹)	Standard Range (cm ⁻¹)	Assignment / Functional Group	Structural Inference
3315.63	3300–3500	N–H stretching	Presence of amide group
2953.02, 2875.30	2850–2960	C–H stretching (alkane)	Aliphatic hydrocarbon chains
1683.74	1650–1700	C=O stretching (amide)	Confirms amide carbonyl group
1651.07	1600–1680	C=C / amide I band	Conjugation or amide linkage
1494.83	1450–1500	C–C aromatic stretching	Aromatic ring presence
1448.31	1400–1470	CH ₂ bending	Alkyl group confirmation
1384.24, 1343.34	1300–1380	C–N stretching	Amine/amide linkage
1290.83, 1263.37	1250–1310	C–N / C–O stretching	Secondary amide or ether linkage
1171.21, 1099.42	1050–1200	C–O stretching	Ether or alcohol functionality
1031.92, 1016.49	1000–1100	C–O / C–N stretching	Supporting heteroatom bonds
881.47	850–900	=C–H bending	Alkene/aromatic substitution
741.65, 693.47	690–900	Aromatic C–H bending	Substituted aromatic ring
658.8	600–700	C–Cl / ring deformation	Possible halogen or ring vibration
509.84, 456.20	400–600	Skeletal vibrations	Fingerprint region confirmation

3.4 Selection of Detection Wavelength: Brivaracetam showed maximum absorption at 210nm, as shown in Figures 3.

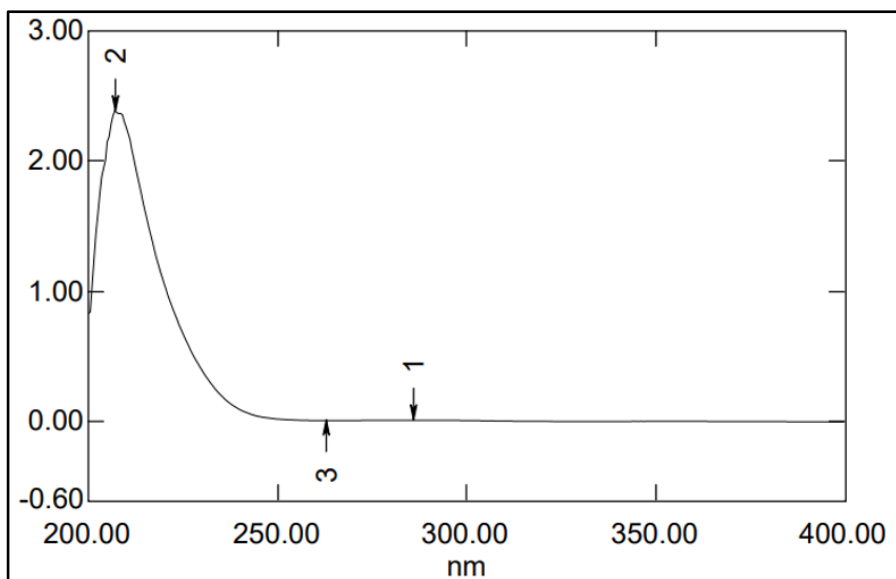


Figure 3 : UV absorption spectra of Brivaracetam

3.5 Validation Parameters Evaluated Using the UV Method:

3.5.1 Linearity

For linearity, 10 - 60 µg/mL solutions of Brivaracetam were prepared from 100 µg/mL stock solutions of

Brivaracetam. Absorbance of the solution was recorded at 210nm as shown in Table 6. Calibration curves are shown in Figure 4

Table 6. Linearity study of Brivaracetam

Sr. No.	Concentration (µg/mL)	Absorbance
1	0	0
2	10	0.134
3	20	0.307
4	30	0.492
5	40	0.658
6	50	0.826
7	60	0.993

The linearity curve plotted between concentration and absorbance for Brivaracetam showed a correlation coefficient (R^2) of 0.999, which was within the acceptable

limit, indicating excellent linearity of the developed method over the studied concentration range.

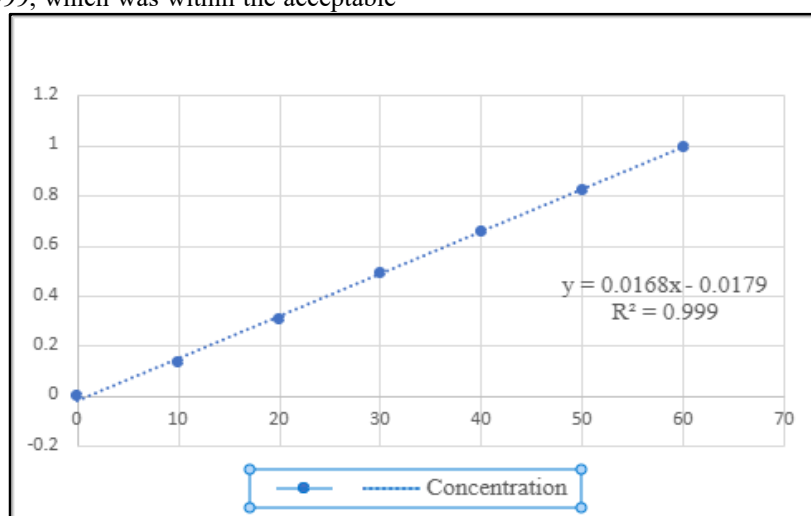


Figure 4. Calibration curve of Brivaracetam by UV

3.5.2 LOD & LOQ:

For Brivaracetam, LOD and LOQ were found to be 0.896242 µg/mL and 1.895955 µg/mL

Intraday and interday analyses were carried out for the brivaracetam to evaluate the precision of the method. The drug was measured six times at concentrations of 20 µg/mL for Brivaracetam, with the results presented in Tables 7 and 8. The % RSD should not exceed 2.

3.5.3 Precision:

Table 7. Intraday Precision of Brivaracetam by UV

Sr.No.	Concentration (µg/mL)	Absorbance	Calculated Conc (x)	(x- $\bar{x}$) ²	Standard Deviation (SD)	%RSD
1	20	0.324	20.35	0.0484	0.192	0.95%
2	20	0.316	19.87	0.0676		
3	20	0.32	20.11	0.0004		
4	20	0.318	19.99	0.0196		
5	20	0.32	20.11	0.0004		
6	20	0.324	20.35	0.0484		

Table 8. Interday Precision of Brivaracetam by UV

Sr. No.	Concentration (µg/mL)	Absorbance	Calculated Conc (x)	(x- $\bar{x}$) ²	Standard Deviation(SD)	%RSD
1	20	0.311	19.57	0.019	0.275	1.39%
2	20	0.307	19.33	0.144		
3	20	0.316	19.87	0.025		
4	20	0.311	19.57	0.019		
5	20	0.315	19.81	0.01		
6	20	0.32	20.11	0.16		

3.5.4 Accuracy:

The accuracy of the proposed method was evaluated through recovery studies. Standard drug solutions were

added to the sample at three different concentration levels (80%, 100%, and 120%) within the linearity range for the drug, and the percentage recovery was calculated. The values for % recovery and %RSD are shown in Tables 9.

Table 9. Accuracy data of Brivaracetam by UV

Level	Standard added (µg/mL)	Sample added (µg/mL)	Total concentration (µg/mL)	Absorbance	Calculated concentration (µg/mL)	Mean concentration (µg/mL)	% Recovery	SD	%RSD
80%	20	16	36	0.594	36.42	35.96	1.17%	0.42	99.89%
	20	16	36	0.581	35.64				
	20	16	36	0.584	35.82				
100%	20	20	40	0.652	39.87	40.22	0.87%	0.35	100.55%
	20	20	40	0.664	40.58				
	20	20	40	0.658	40.23				
120%	20	24	42	0.718	43.80	44.06	0.52%	0.23	100.1%
	20	24	42	0.724	44.16				
	20	24	42	0.725	44.22				

The percentage recovery of Brivaracetam was found to be within the acceptable limits, demonstrating the accuracy of the developed analytical method.

minor variations in wavelength did not produce any significant change in absorbance. The %RSD values remained below 2%, indicating that the method was robust and the results were within acceptable limits as given in Table 10.

3.5.5 Robustness:

The sample solutions of Brivaracetam were prepared and analyzed at different wavelengths. It was observed that

Table 10. Robustness data of Brivaracetam by UV

Sr. No.	Concentration (µg/mL)	Wavelength (nm)	Absorbance	SD	%RSD
1	20	206	0.299	0.00587	1.91%
2	20	208	0.303		
3	20	210	0.307		
4	20	212	0.310		
5	20	214	0.314		

3.5.6 Assay

The assay was carried out using a UV spectrophotometric method to accurately determine the content of

Brivaracetam in the dosage form. Six replicates of standard and sample solutions were prepared, and the % Assay was calculated for the drug as given in Table 11.

Table 11. Assay of Brivaracetam by UV

Sr. No.	Concentration (µg/mL)	Absorbance of Standard Solution	Absorbance of Sample Solution
1.	20	0.311	0.309
2.	20	0.307	0.312
3.	20	0.316	0.314
4.	20	0.311	0.318
5.	20	0.315	0.312
6.	20	0.32	0.319
	Average	0.313	0.314
		Tablet Avg Weight	199.44 mg
		Std. Weight	199.44mg
		Sample Weight	100 mg
		Label Amount	100 mg
		Std. Purity (%)	99%
		Assay (%)	99.29%

3.6 HPLC METHOD DEVELOPMENT AND OPTIMIZATION

Selection of quality target product profile

The QbD approach was applied for the method development of Brivaracetam using central composite design. The QTPPs chosen for optimization of HPLC

chromatographic conditions were retention time, theoretical plates, and tailing factor.

Selection of Critical Quality Attributes

Mobile phase, flow rate, and column temperature were three critical quality attributes that required control. QTPPs of both drugs were monitored using Design-Expert software® (Version 8.0.7.1) as given in Table 12.

Table 12. Selection of CQA and Response

CQA	QTPPs
Mobile phase	Retention time
Flow rate	Theoretical plates
Column temperature	Symmetry Factor
	Area

Experimental Design Plan

By applying CCD, 20 trials, 3 factors, and 4 responses method had been selected for methodDevelopment for Brivaracetam as per Table 13.

Table 13. Design Plan and Observed Data

Sr.No.	Factor-1 Mobile phase	Factor-2 Flow rate	Factor-3 Column temperature	Response-1 Retention of Brivaracetam	Response-2 NTP of Brivaracetam	Response-3 The Symmetry factor of Brivaracetam	Response-4 Peak Area of Brivaracetam
1	30	1	20	6.147	7889	1.431	161021
2.	32.5	1.25	16.59	4.187	5633	1.476	137226
3.	32.5	1.25	25	4.267	6256	1.607	140552
4.	32.5	1.25	25	4.267	6256	1.607	140552
5.	32.5	1.25	25	4.267	6256	1.607	140552
6.	32.5	1.67	25	3.197	5462	1.36	99745
7.	32.5	1.25	25	4.267	6256	1.607	140552
8.	35	1	20	4.73	6129	1.461	161657
9.	36.7	1.25	25	3.5	4218	1.314	135272
10.	32.5	1.25	25	4.267	6256	1.607	140552
11.	35	1	30	4.63	5159	1.261	170184
12.	28.3	1.25	25	5.627	7164	1.329	127430
13.	32.5	0.83	25	6.24	6445	1.337	195551

14.	30	1.5	30	4.137	6425	1.586	112755
15.	35	1.5	30	3.133	4722	1.239	106552
16.	32.5	1.25	33.41	4.263	5631	1.476	134460
17.	30	1	30	6.143	7681	1.414	166281
18.	30	1.5	20	4.16	6247	1.347	104050
19.	32.5	1.25	25	4.267	6256	1.607	140552
20.	35	1.5	20	3.14	4694	1.332	117343

Design Space:

After defining the QTPP and CQAs, a Central Composite Design (CCD) was employed using Design-Expert® software (Version 8.0.7.1) to optimize the RP-HPLC method. The effects of mobile phase (A), flow rate (B), and column temperature (C) on retention time, theoretical plates, tailing factor, and peak area were evaluated. The experimental data were fitted to a second-order quadratic model for method optimization.

$$Y = \beta_0 + \beta_1A + \beta_2B + \beta_3C + \beta_{12}AB + \beta_{13}AC + \beta_{23}BC + \beta_{11}A^2 + \beta_{22}B^2 + \beta_{33}C^2$$

Where:

- Y = Response variable (e.g., retention time, theoretical plates, peak symmetry and area)
- A, B, C = Coded independent variables (Mobile phase, flow rate, and column temperature)
- β_0 = Intercept (average response across the design space)
- $\beta_1, \beta_2, \beta_3$ = Linear coefficients for A, B, and C, respectively

- $\beta_{12}, \beta_{13}, \beta_{23}$ = Interaction coefficients between the factors A and B, A and C, and B and C, respectively

respectively

- $\beta_{11}, \beta_{22}, \beta_{33}$ = Quadratic coefficients for A, B, and C, respectively

Response 1: Retention Time

The **Central Composite Design (CCD)** generated response surface and contour plots to evaluate the effects of **mobile phase (A), flow rate (B), and column temperature (C)** on the retention time of Brivaracetam. The quadratic model indicated that **flow rate (B)** had the greatest influence by significantly reducing retention time, followed by the **mobile phase (A)**, while **column temperature (C)** showed a minimal effect. The interaction terms demonstrated minor combined effects between the variables, confirming the suitability of the model for optimizing chromatographic conditions as shown in Figure 5.

$$RT \text{ OF BRV} = +4.27 - 0.62 * A - 0.89 * B - 4.528E-004 * C + 0.11 * A * B - 1.000E-002 * A * C + 9.250E-003 * B * C + 0.11 * A^2 + 0.16 * B^2 - 0.013 * C^2$$

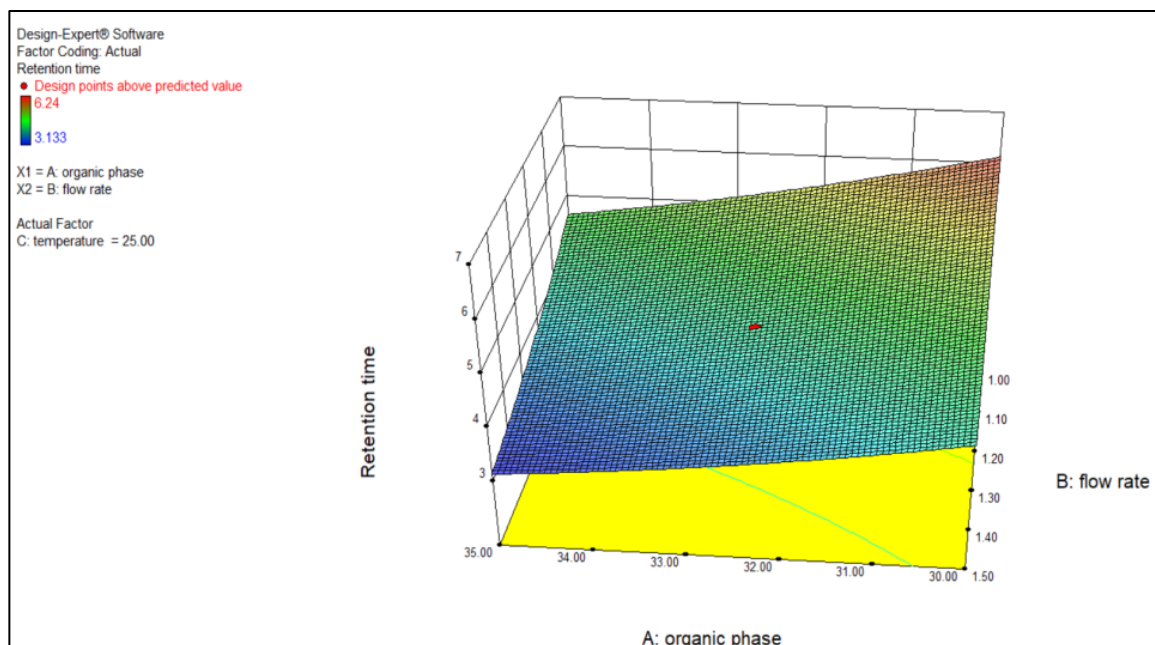


Figure 5. plot for Retention time for Brivaracetam

The QbD quadratic model shows that the retention time (RT) of Brivaracetam is governed by factors A, B, and C with both linear and non-linear effects.

It was concluded that Factor A decreases RT ($\beta_1 = -0.62$), while its quadratic term ($A^2 = +0.11$) indicates curvature at extreme levels. Factor B has the strongest effect, significantly decreasing RT ($\beta_2 = -0.89$) with noticeable curvature ($B^2 = +0.16$). Factor C has a very small effect,

slightly decreasing RT ($\beta_3 = -4.528 \times 10^{-4}$) with a mild quadratic influence ($C^2 = -0.013$).

Interaction terms show combined effects: AB (+0.11) slightly increases RT when A and B increase together; AC

(-0.01) slightly decreases RT, and BC (+0.00925) shows a minor positive influence. Overall, the model confirms a non-linear response surface where B is the most influential factor affecting RT as shown in Figure 6.

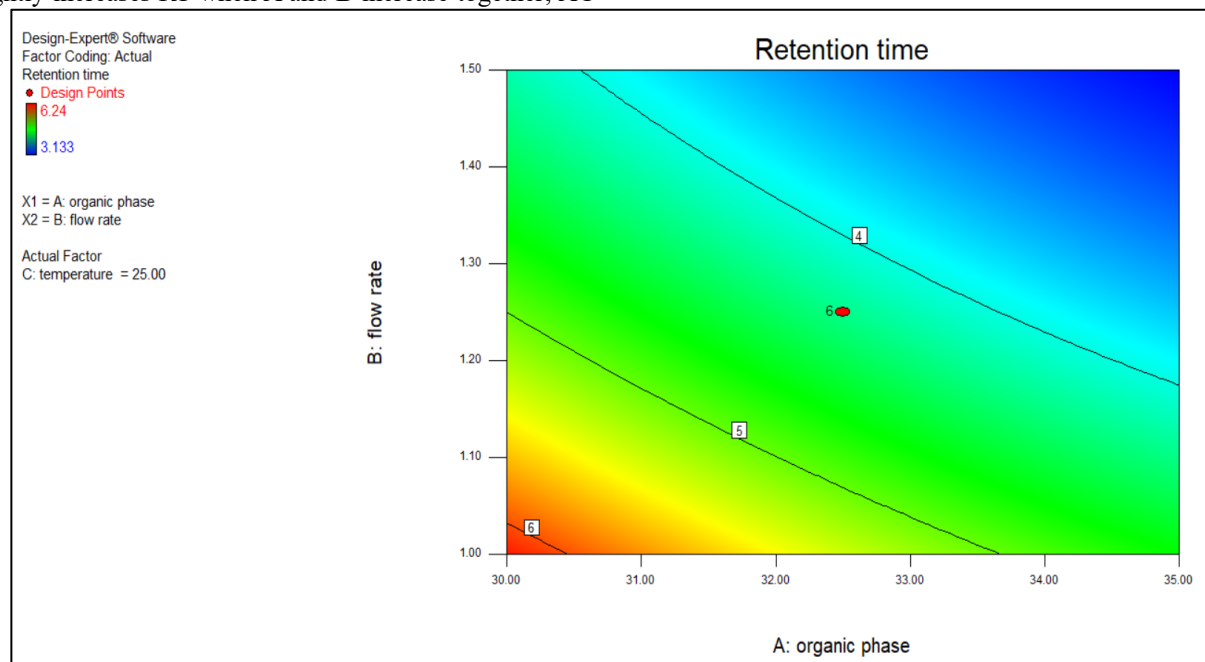


Figure 6. Contour plot for Retention time for Brivaracetam

Response 2: NTP for Brivaracetam

The quadratic Polynomial equation for the measured response for the NTP of Brivaracetam is given below:

$$\text{NTP OF BRV} = +6051.75 - 914.75 * A - 470.33 * B - 71.42 * C$$

It was concluded that NTP decreases with an increase in factors A, B, and C. Among these factors, A has the

greatest negative effect (-914.75) on NTP, followed by B (-470.33), while C (-71.42) has the least effect. Therefore, factor A is the most critical factor influencing column efficiency, and increasing its level significantly reduces the number of theoretical plates as shown in Figure 7 and Figure 8.

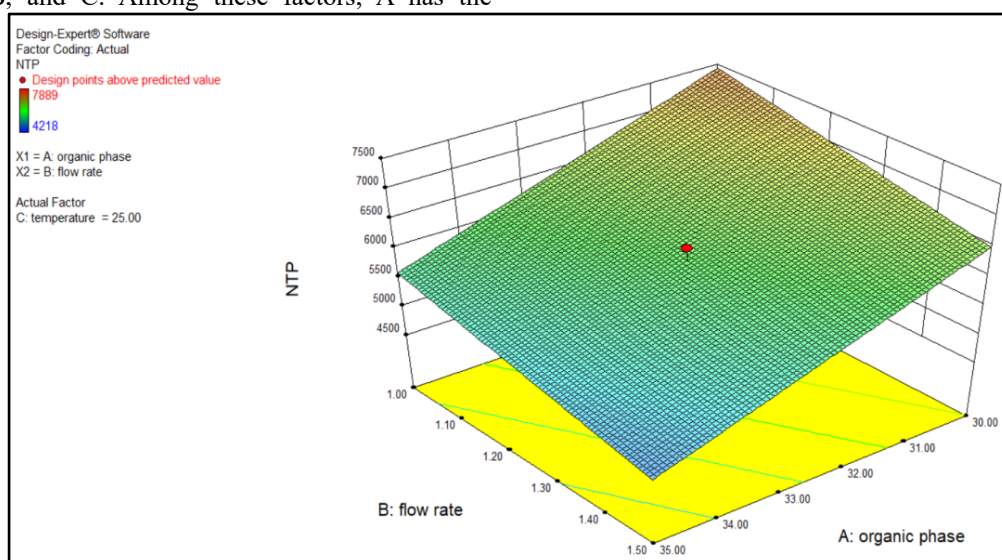


Figure 7.3D plot for NTP for Brivaracetam

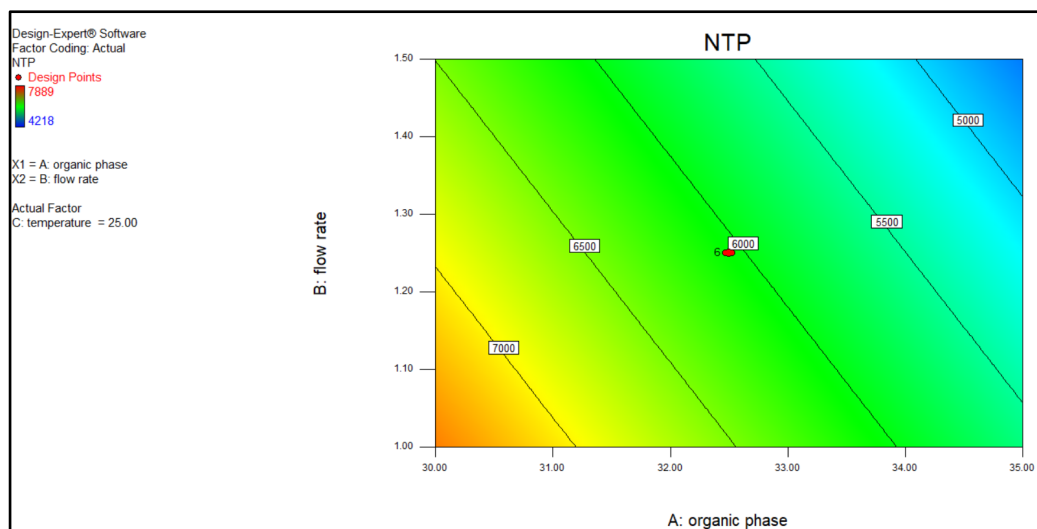


Figure 8. Contour plot for NTP for Brivaracetam

Response 3: Symmetry Factor for Brivaracetam

The quadratic Polynomial equation for the measured response for symmetry factor of Brivaracetam is given below:

$$\text{Symmetry Factor for BRV} = +1.61 - 0.037 * A - 1.781E-003 * B - 5.199E-003 * C - 0.030 * A * B - 0.064 * A * C + 0.045 * B * C - 0.098 * A^2 - 0.088 * B^2 - 0.043 * C^2$$

It was concluded that the symmetry factor is slightly reduced by increasing A, B, and C, with A having the greatest influence. The interaction term BC (+0.045) slightly improves symmetry, while the negative quadratic terms ($A^2 = -0.098$, $B^2 = -0.088$, $C^2 = -0.043$) confirm a non-linear response. Overall, factor A is the most critical variable affecting peak symmetry as shown in Figure 9 and Figure 10.

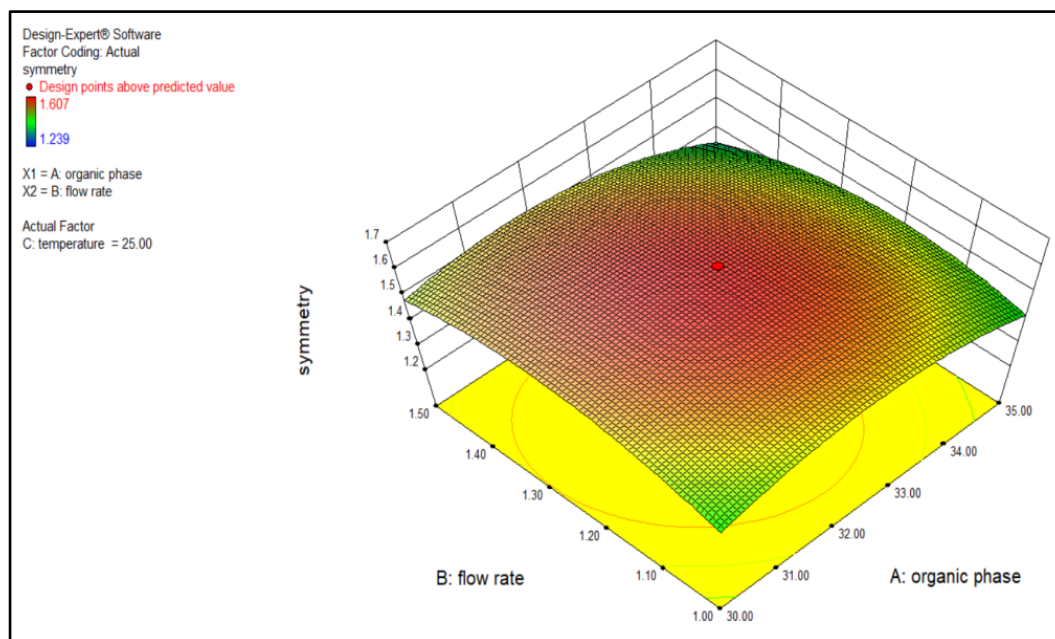


Figure 9. 3D plot for Symmetry Factor for Brivaracetam

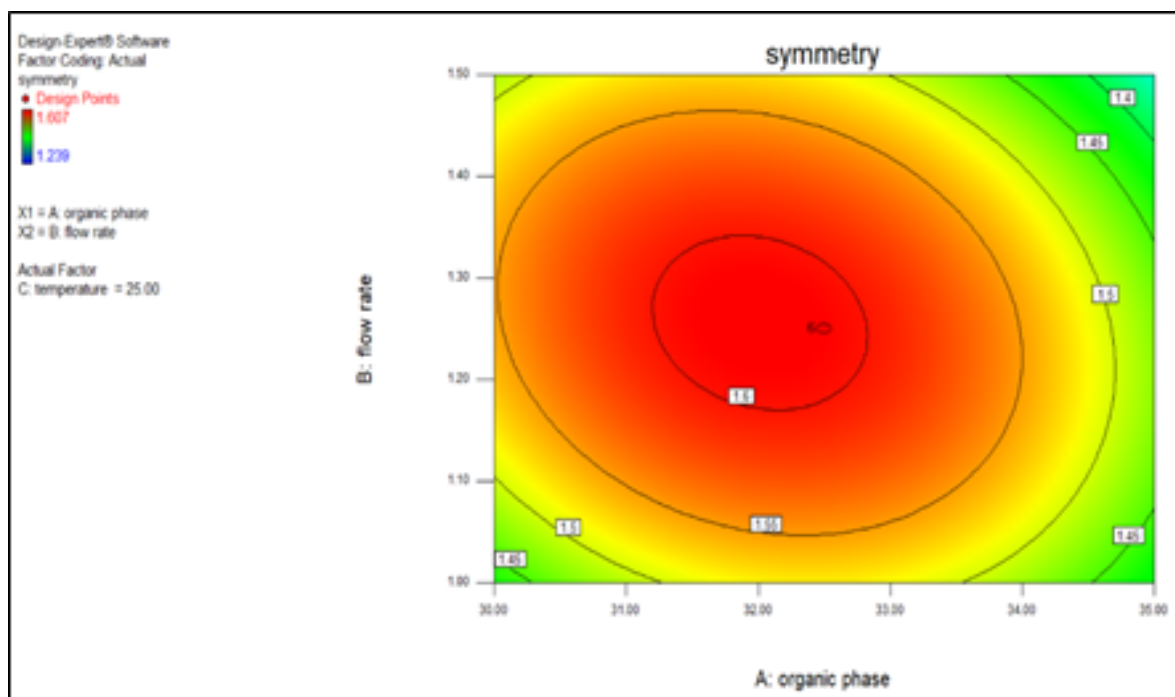


Figure 10. Contour plot for Symmetry factor for Brivaracetam

Response 4: Peak Area for Brivaracetam

The quadratic Polynomial equation for the measured response for peak area of Brivaracetam is given below:

$$\text{Area for BRV} = +1.406\text{E}+005 + 1817.23 * A - 27793.29 * B + 516.16 * C + 318.88 * A * B - 2028.62 * A * C - 1984.12 * B * C - 3392.85 * A^2 + 2369.01 * B^2 - 1804.69 * C^2$$

It was concluded that peak area is mainly affected by factor B (-27793.29), which significantly reduces the response when increased. Factors A ($+1817.23$) and C ($+516.16$) contribute positively to peak area. The interaction and quadratic terms confirm a non-linear relationship, indicating that proper optimization of A, B, and C is necessary to achieve maximum peak area and better chromatographic performance as shown in Figure 11 and Figure 12.

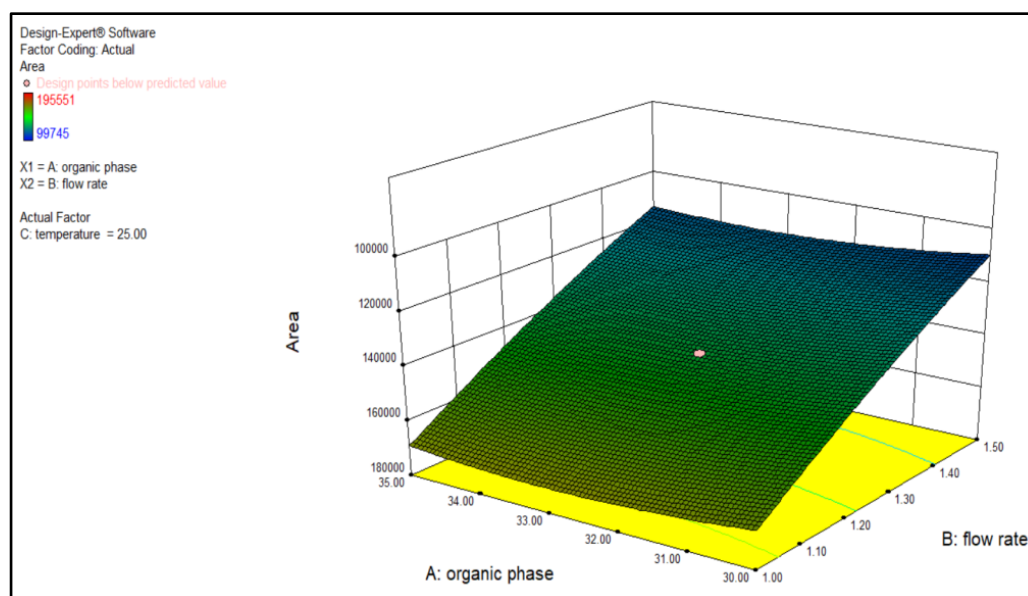


Figure 11. 3D plot for Peak Area for Brivaracetam

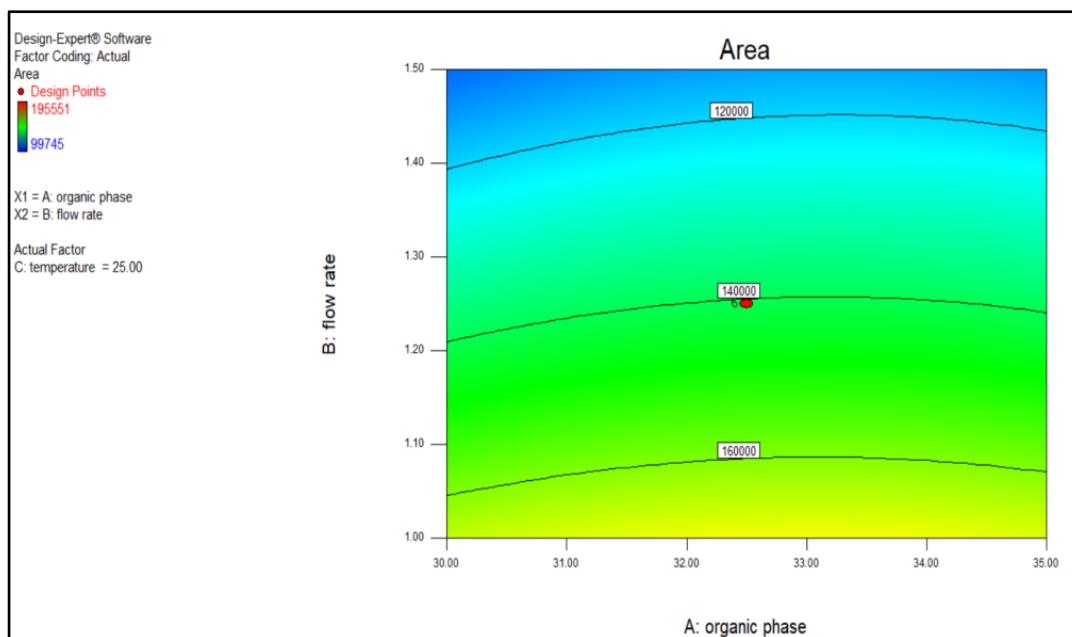


Figure 12. Contour plot for Peak area for Brivaracetam

Optimized Solution for Method Development

(Version 8.0.7.1) as given in Table 14,15 and 16 and in figure 13 and Figure 14.

It was obtained by studying all responses in different experimental conditions using Design-Expert software®

Table 14. Solutions Given by Software

Number	Mobile phase	Flow Rate(mL/min)	Temperature(°C)	Desirability	
1	35	1.00	25.00	1.000	Selected
2	35	1.00	25.00	0.998	

Table 15. Confirmation Report Analysis

Factor	Name	Level	Low Level	High Level	Std. Dev.	Coding
A	Mobile Phase	35	30	35	0.000	Actual
B	Flow Rate	1.00	1.00	1.50	0.000	Actual
C	Temperature	25.00	25.00	30.00	0.000	Actual

Table 16. Optimized Solution for Method Development

Mobile Phase	Flow rate (mL/min)	Temp. (°C)	Retention time of Brivaracetam (min)	NTP of Brivaracetam	Symmetry factor of Brivaracetam	Peak area of Brivaracetam
35	1.00	25°C	4.69	5607	1.41	168841

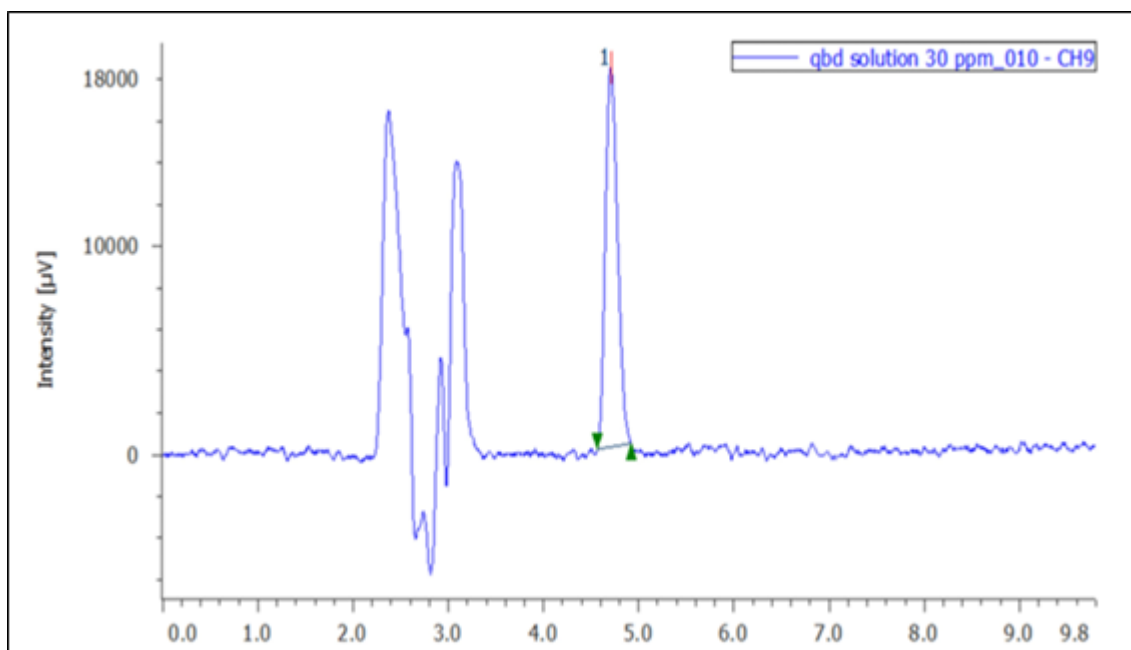


Figure 13. Chromatogram of Optimized Method

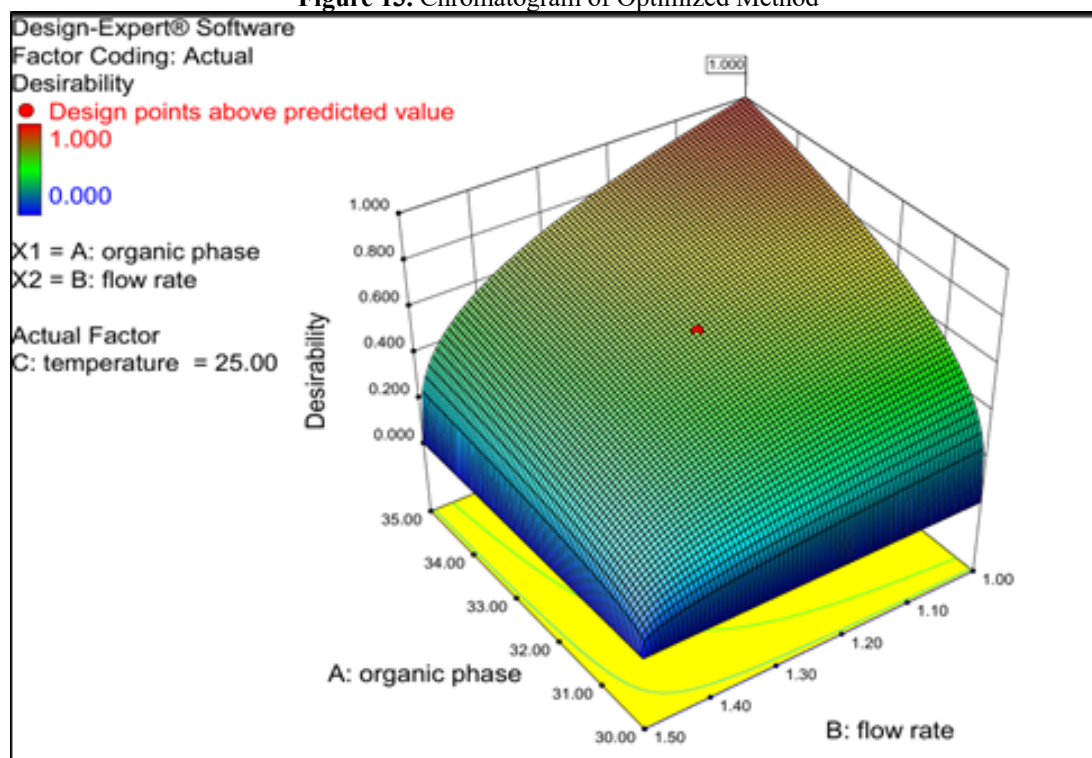


Figure 14. 3D Surface Plot of Desirability for the Optimized Method

Validation of RP-HPLC Method

Specificity

Specificity is the ability of the method to unequivocally assess the analyte in the presence of impurities,

degradation of products, matrix components, or other potential interferences and shown in Figure 15.

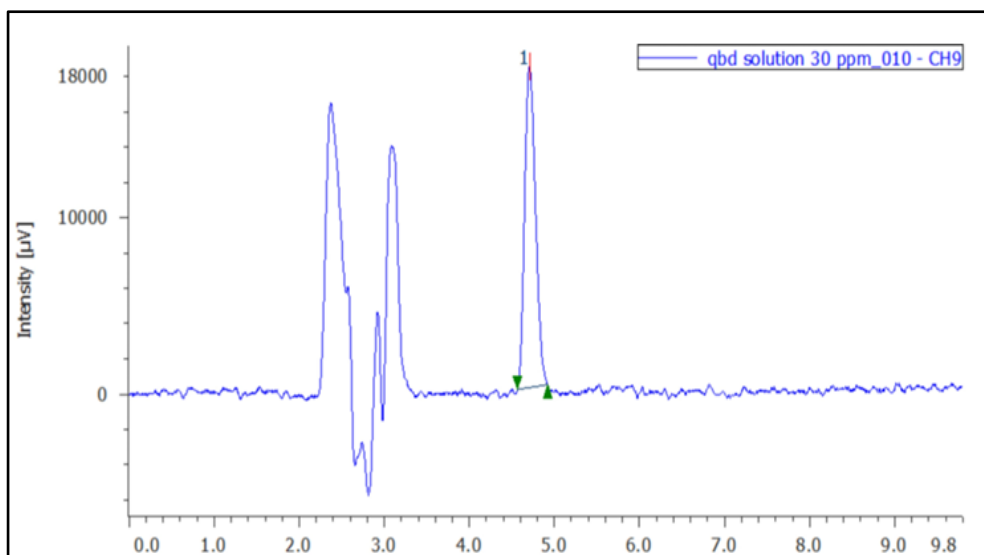


Figure 15. Chromatogram for Specificity of Brivaracetam

Observation:

A sharp and well-resolved peak for Brivaracetam was observed at approximately 4.8 min retention time with no interfering 16 peaks at the analyte retention time, confirming the specificity of the developed RP-HPLC method.

Linearity

A linear regression curve of area response versus concentration was constructed for Brivaracetam in the range of 10-60 μg/mL as given in Table 17.

Table 17. Linearity of Brivaracetam

Sr no.	Brivaracetam	
	Concentration (μg/mL)	Peak area
1	0	0
2	10	47598
3	20	108317
4	30	161911
5	40	226148
6	50	264637
7	60	336618

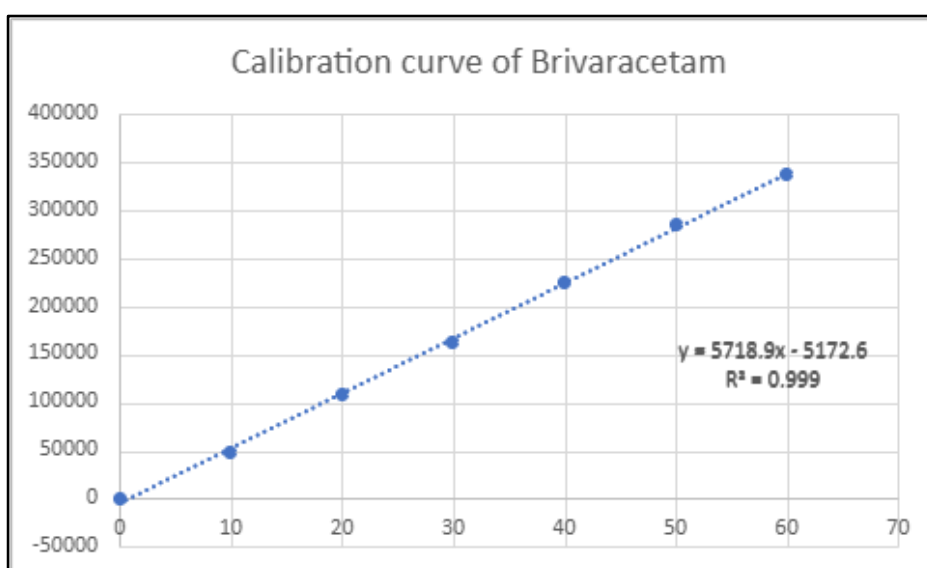


Figure 16. Calibration Curve of Brivaracetam at 210nm

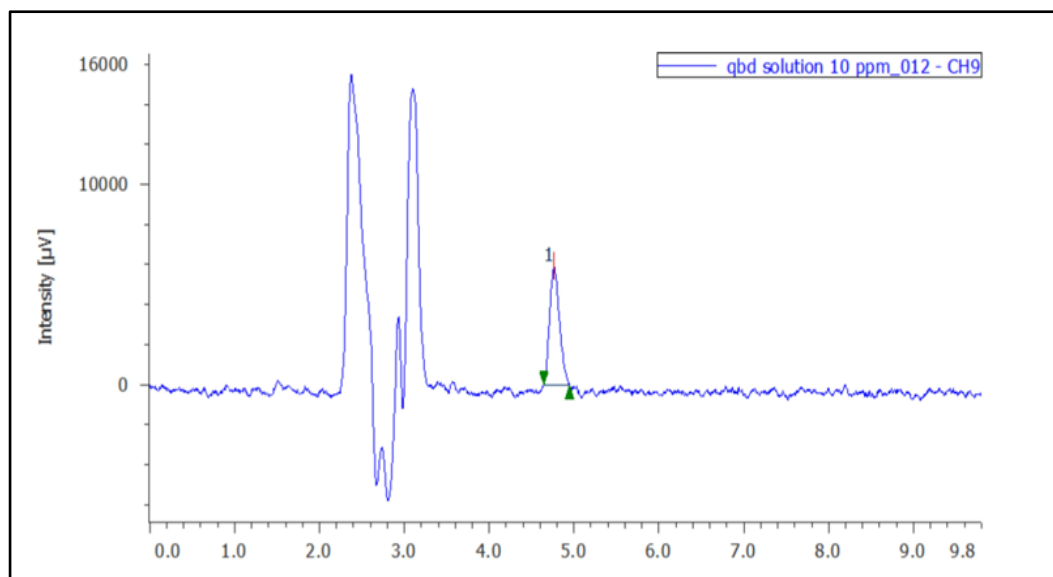


Figure 17. Chromatogram for Specificity of Brivaracetam

Observation:

A sharp and well-resolved peak for Brivaracetam was observed at approximately 4.8 min retention time with no interfering peaks at the analyte retention time, confirming the specificity of the developed RP-HPLC method as shown in Figure 16 and Figure 17.

Linearity:

A linear regression curve of area response versus concentration was constructed for Brivaracetam in the range of 10-60 µg/mL as given in Table 18.

Table 18. Linearity of Brivaracetam

Sr no.	Brivaracetam	
	Concentration (µg/mL)	Peak area
1	0	0
2	10	47598
3	20	108317
4	30	161911
5	40	226148
6	50	264637
7	60	336618

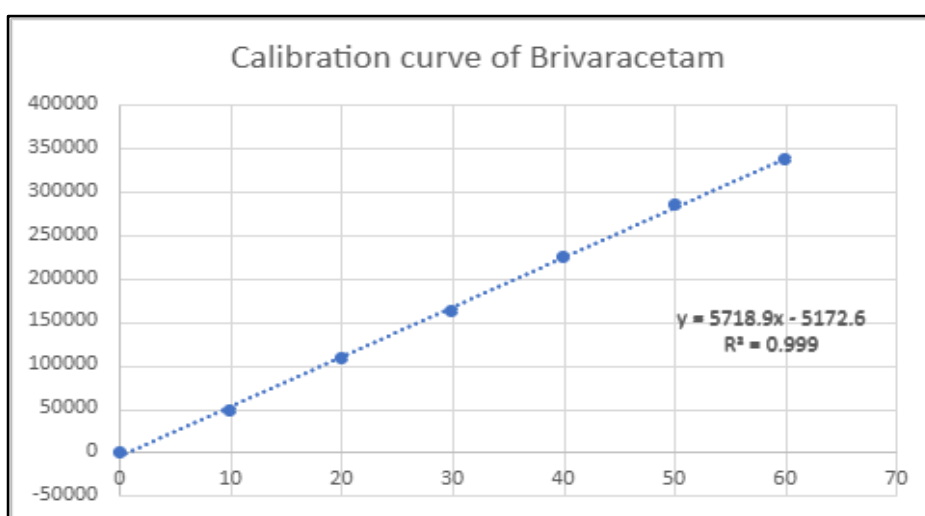


Figure 18. Calibration Curve of Brivaracetam at 210nm

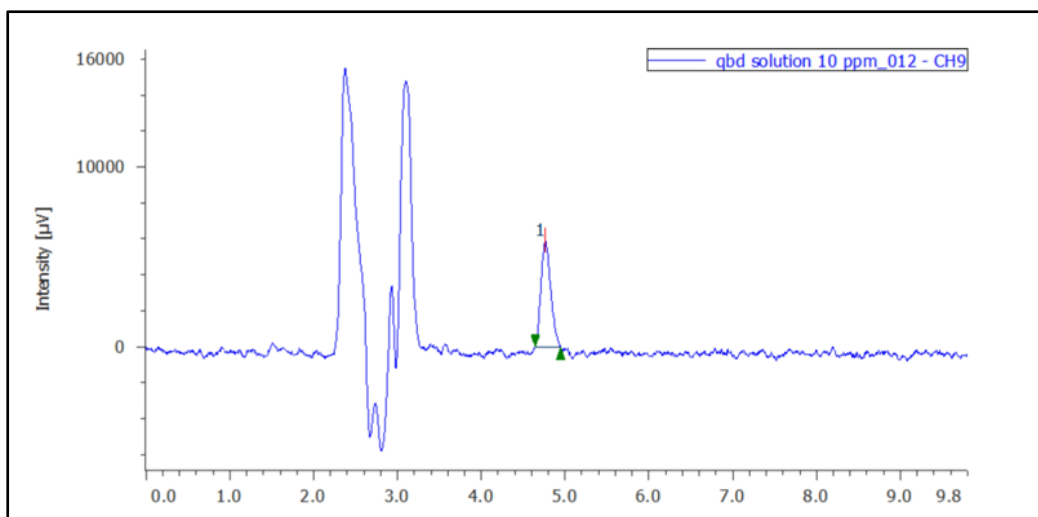


Figure 19. Chromatogram of Brivaracetam (10 µg/mL) for Linearity

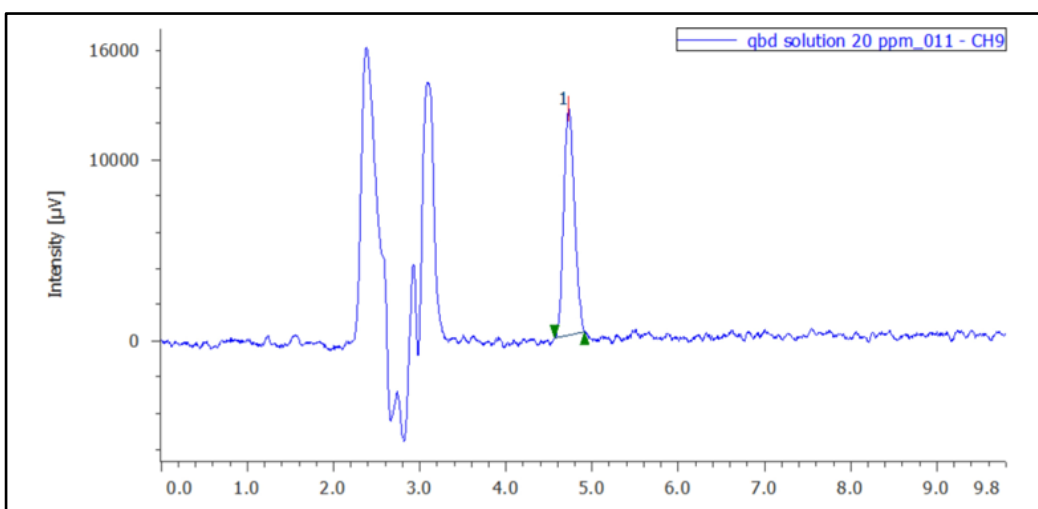


Figure 20. Chromatogram of Brivaracetam (20 µg/mL) for Linearity

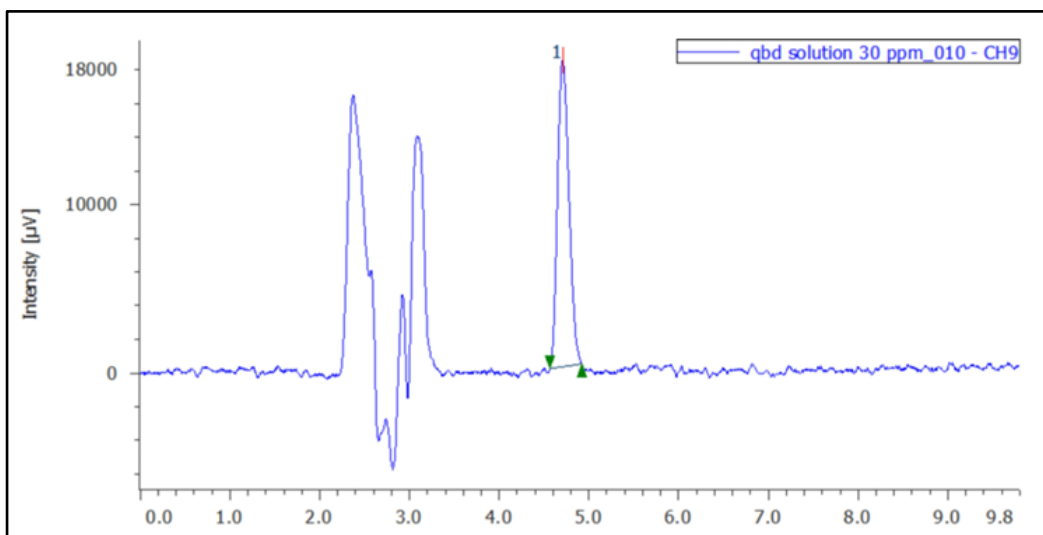


Figure 21. Chromatogram of Brivaracetam (30 µg/mL) for Linearity

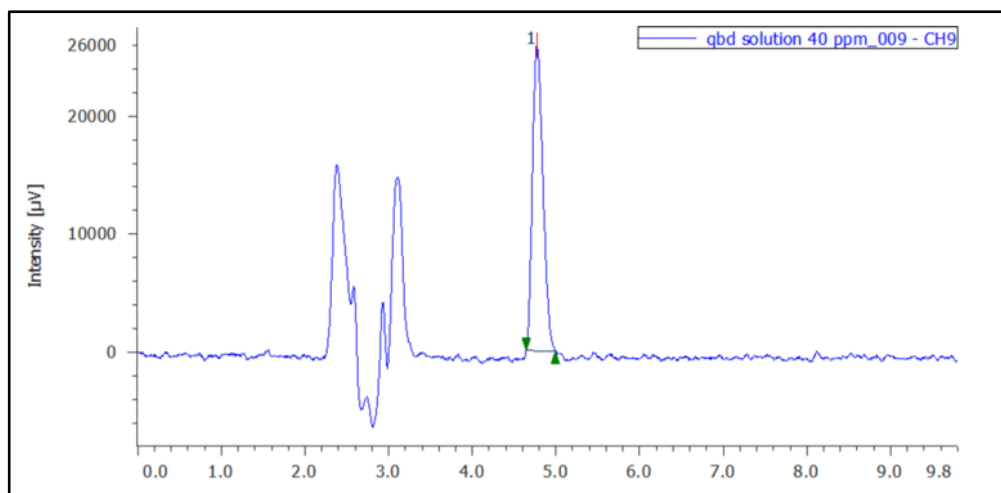


Figure 22. Chromatogram of Brivaracetam (40 µg/mL) for Linearity

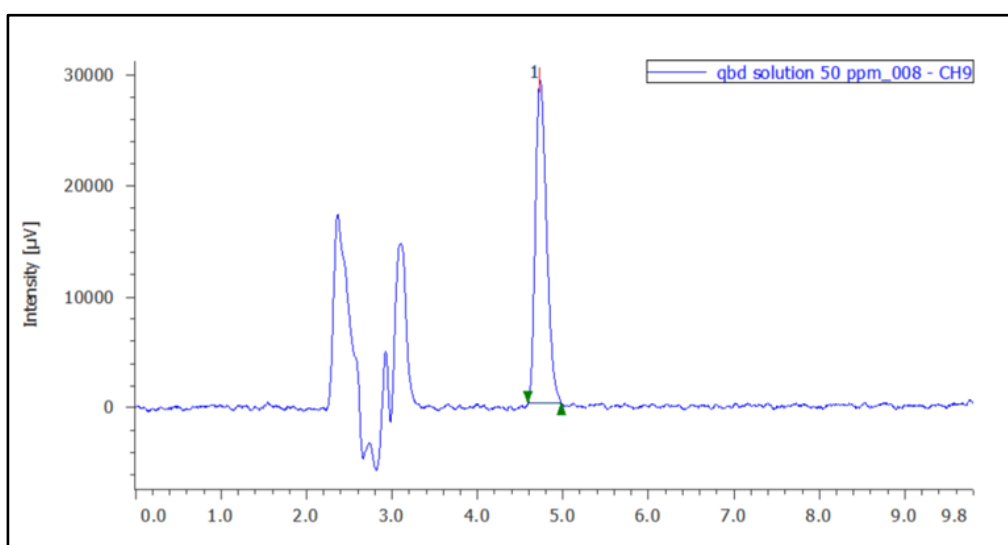


Figure 23. Chromatogram of Brivaracetam (50 µg/mL) for Linearity

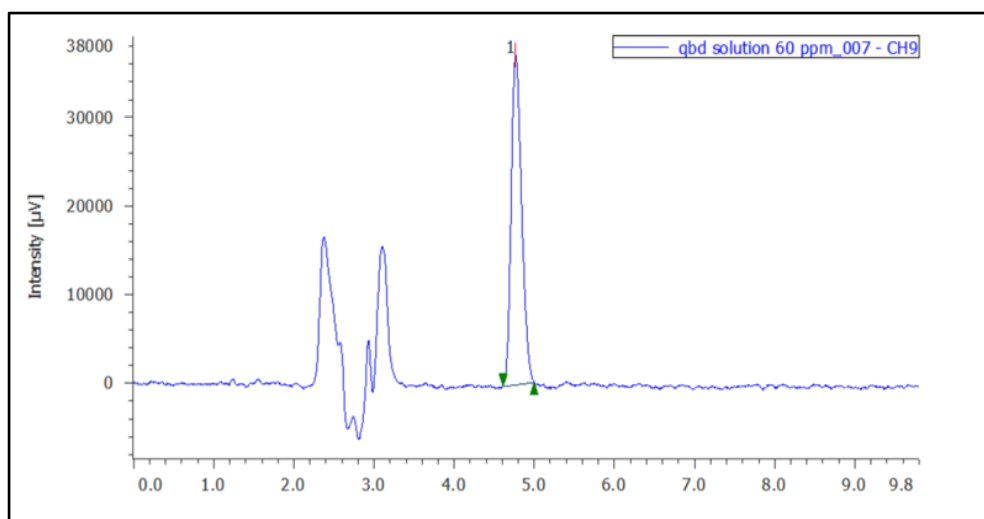


Figure 24. Chromatogram of Brivaracetam (60 µg/mL) for Linearity

Observations: A linear regression analysis was performed, and the calibration curve for the drug showed good correlation between peak area and concentration. The correlation coefficient was found to be 0.999 for

Brivaracetam, indicating excellent linearity within the specified concentration ranges as shown in Figure 18-24.

Precision: Six replicate measurements were conducted for the drug at concentrations of 30 µg/mL for BRV as given

in Table 19 and Table 20.

Table 19. Intraday Precision of Brivaracetam

Sr.no.	Sample Name	Concentration (ug/mL)	Peak area	Theoretical plates	Symmetry factor	Retention time (min)
1	Test_01	30	160401	5783	1.328	4.770
2	Test_02	30	162175	5860	1.359	4.760
3	Test_03	30	160844	5778	1.329	4.750
4	Test_04	30	166307	5673	1.351	4.710
5	Test_05	30	167370	5860	1.342	4.747
6	Test_06	30	163127	5946	1.391	4.773
Mean6.3			163370	5816	1.350	4.751
Standard deviation			2857.48	93.31	0.024	0.024
% RSD			1.75	1.60	1.77	0.50

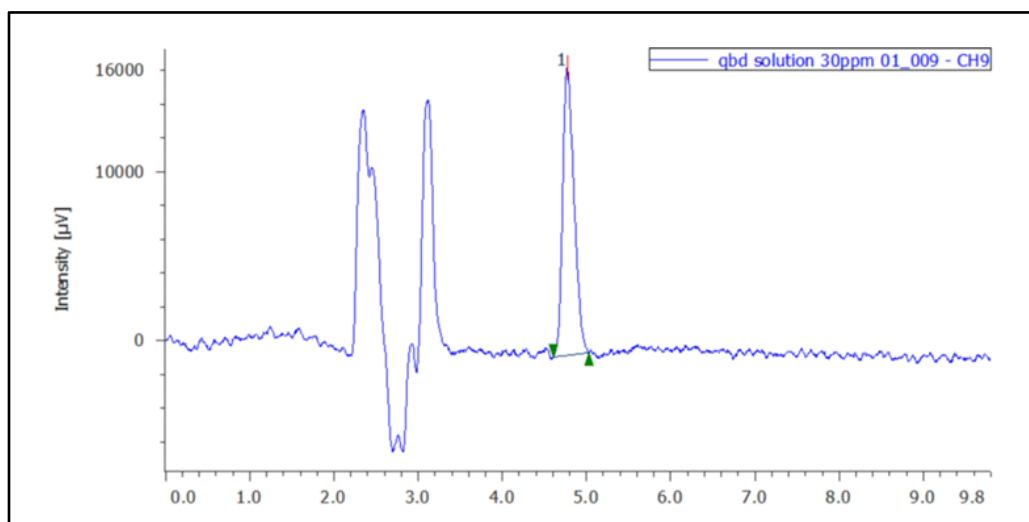


Figure 25. Chromatogram of Brivaracetam for Intraday precision (01)

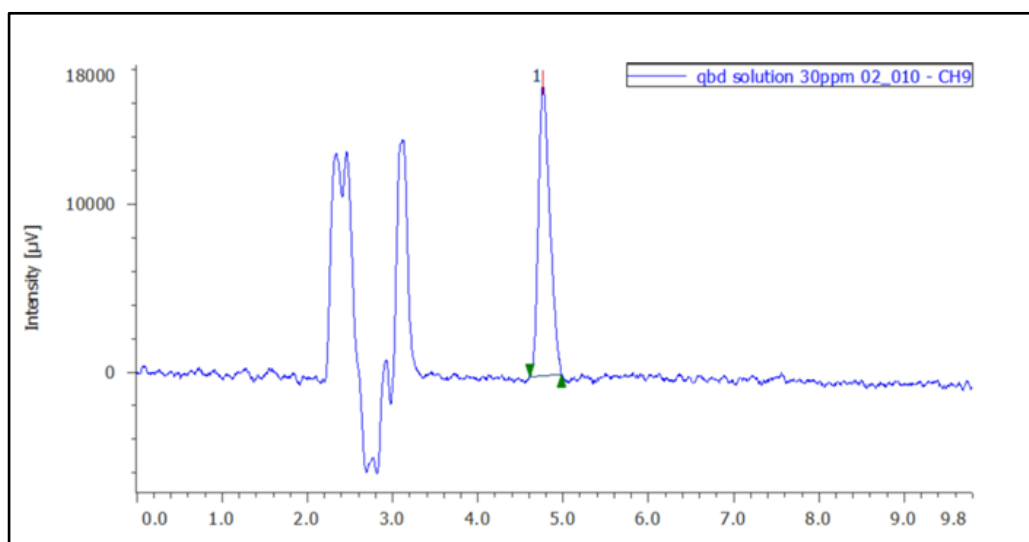


Figure 26. Chromatogram of Brivaracetam for Intraday precision (02)

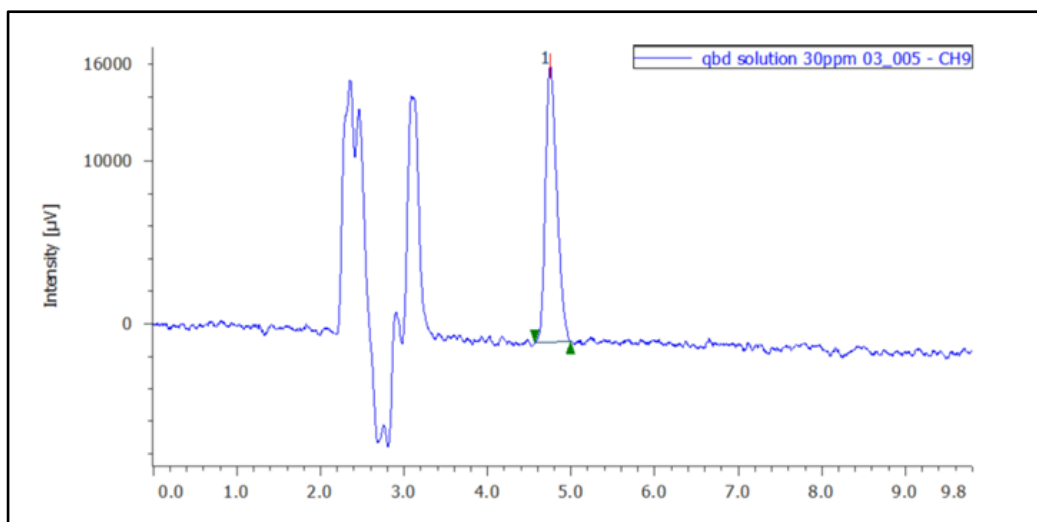


Figure 27. Chromatogram of Brivaracetam for Intraday precision (03)

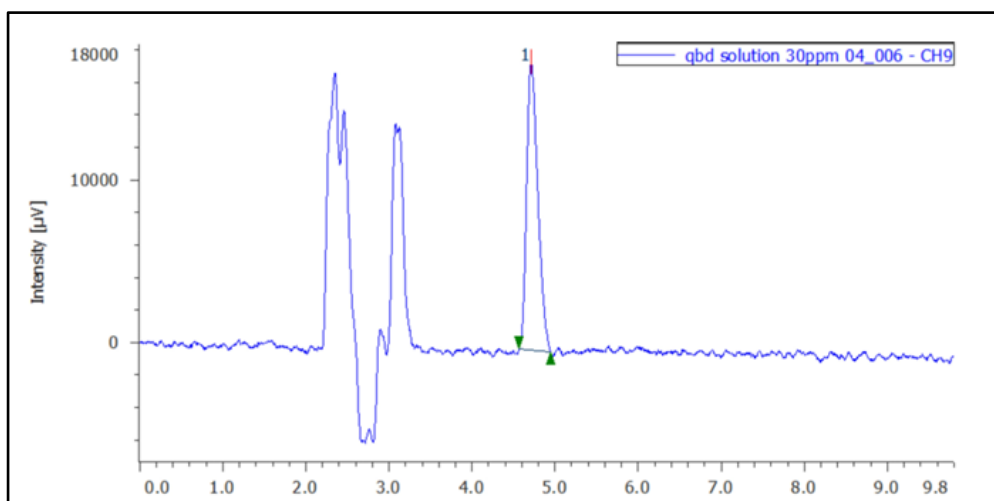


Figure 28. Chromatogram of Brivaracetam for Intraday precision (04)

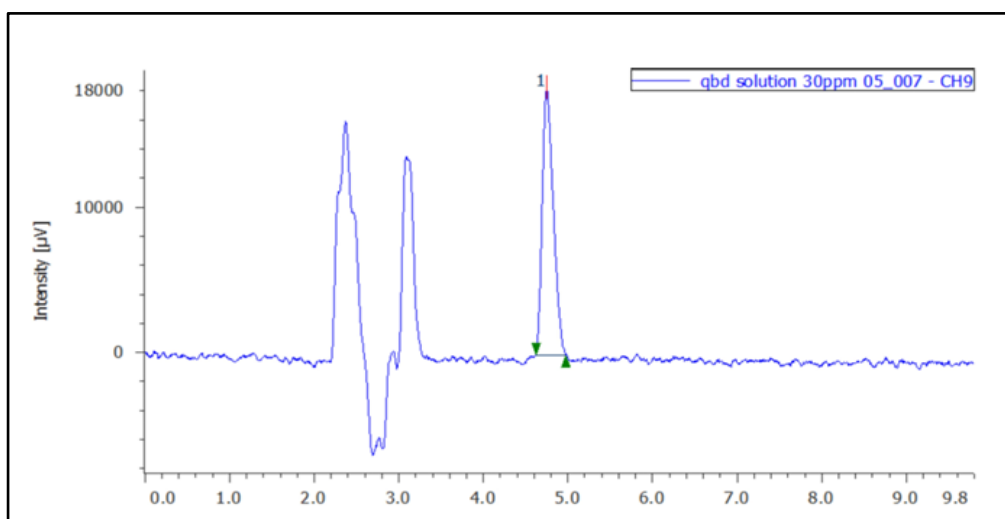


Figure 29. Chromatogram of Brivaracetam for Intraday precision (05)

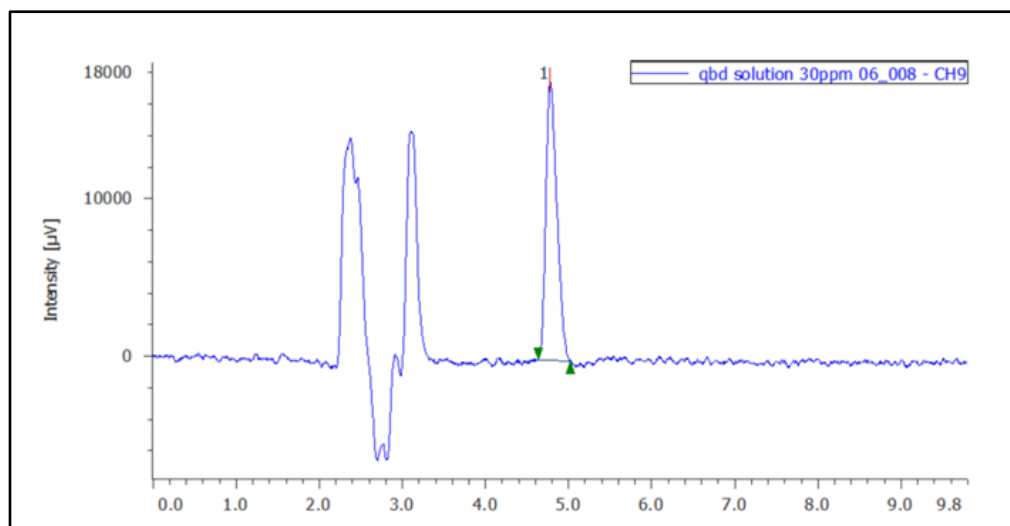


Figure 30. Chromatogram of Brivaracetam for Intraday precision (06)

Table 20. Interday Precision of Brivaracetam

Sr.no.	Sample Name	Concentration (ug/mL)	Peak area	Theoretical plates	Symmetry factor	Retention time (min)
1	Test_01	30	160484	5836	1.362	4.703
2	Test_02	30	165424	5721	1.311	4.707
3	Test_03	30	157711	6248	1.368	4.710
4	Test_04	30	160922	6245	1.253	4.730
5	Test_05	30	164928	5376	1.355	4.650
6	Test_06	30	161046	6091	1.375	4.710
Mean			161752	6206	1.36	4.702
Standard deviation			2930.38	114.22	0.027	0.027
% RSD			1.81	1.84	1.96	0.58

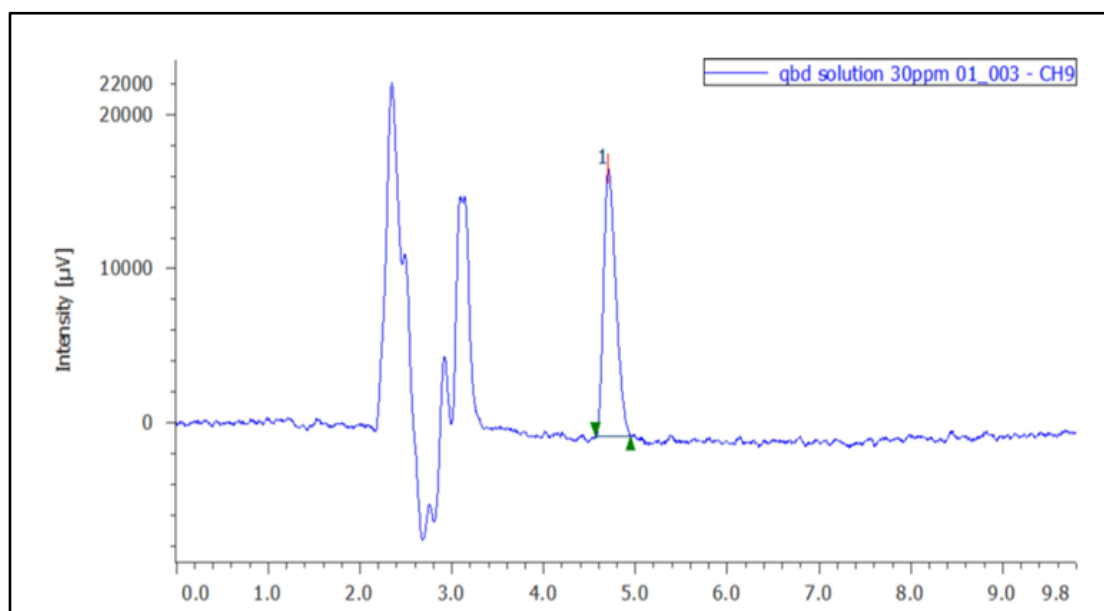


Figure 31. Chromatogram of Brivaracetam for Interday precision (01)

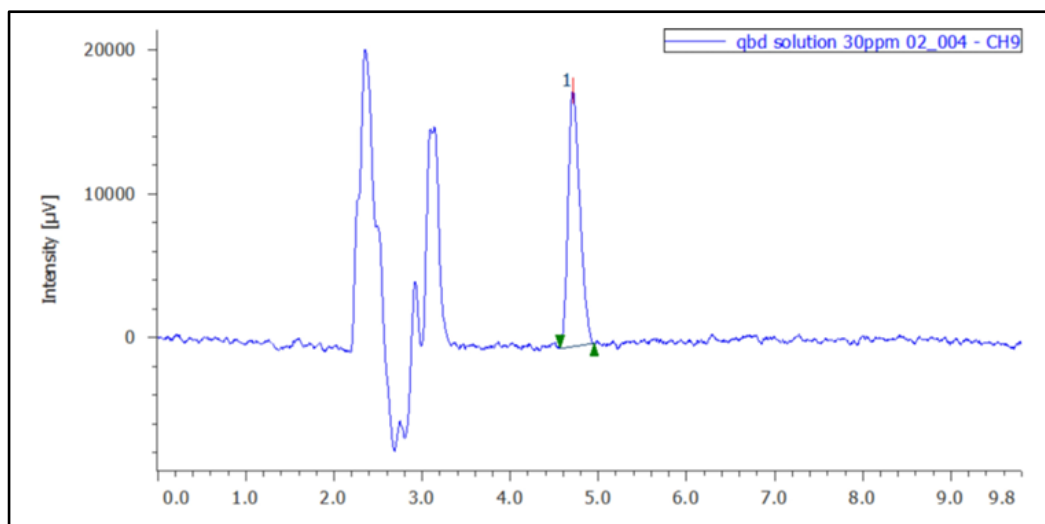


Figure 32. Chromatogram of Brivaracetam for Interday precision (02)

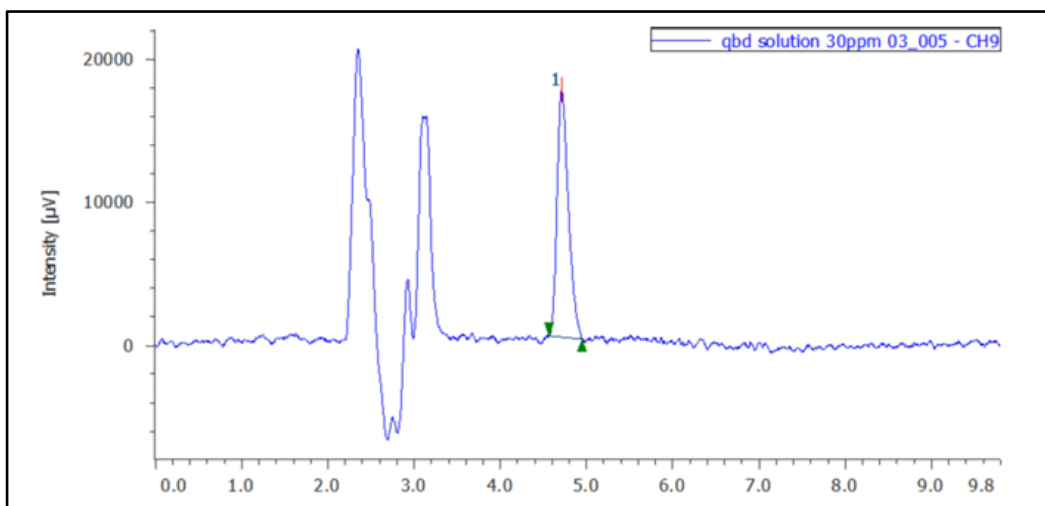


Figure 33. Chromatogram of Brivaracetam for Interday precision (03)

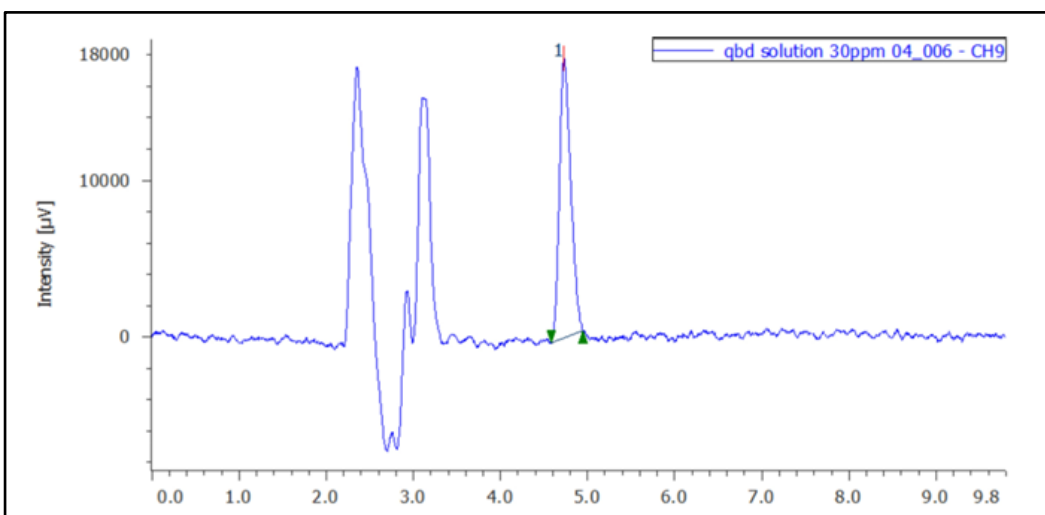


Figure 34. Chromatogram of Brivaracetam for Interday precision (04)

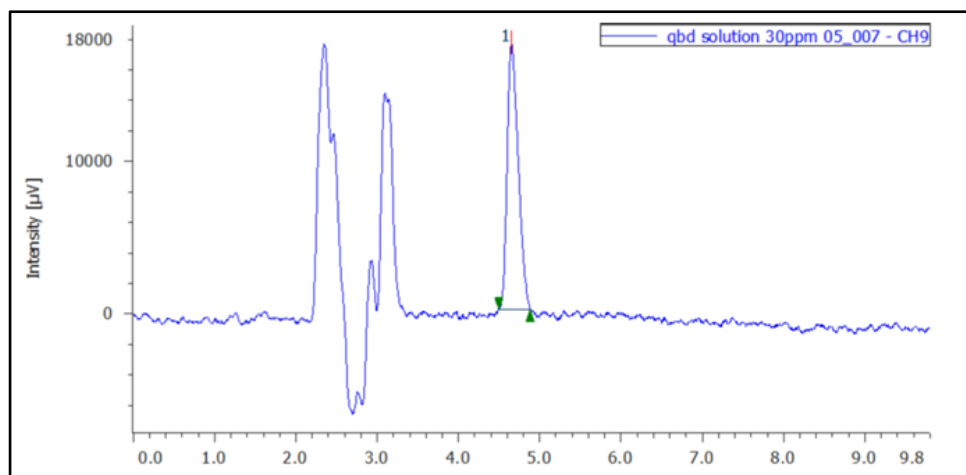


Figure 35. Chromatogram of Brivaracetam for Interday precision (05)

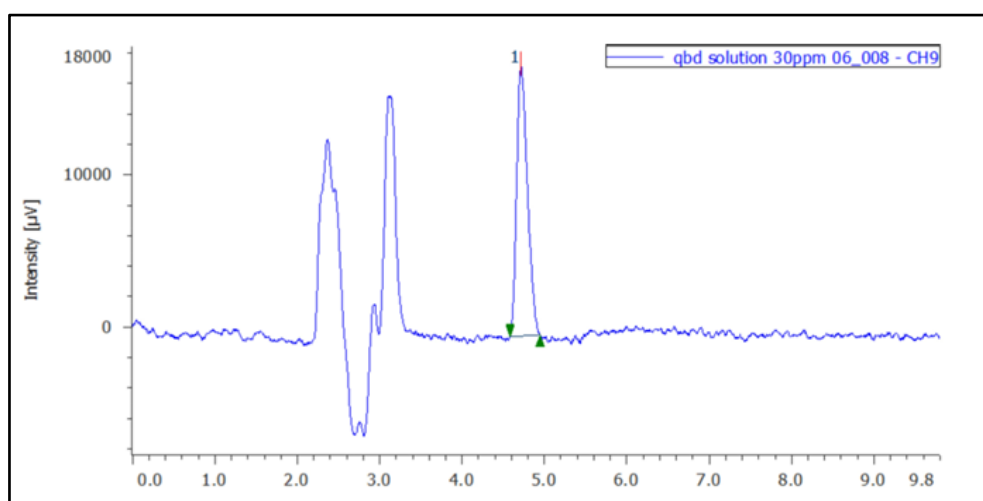


Figure 36. Chromatogram of Brivaracetam for Interday precision (06)

Observations: The %RSD for intraday precision was 1.75 for Brivaracetam, while the %RSD for interday precision was 1.81 for Brivaracetam as shown in figure 25-36.

Acceptance Criteria: The %RSD should not be more than 2%.

Accuracy: The method's accuracy was assessed by measuring the % Recovery of both Abiraterone acetate and Prednisolone at three levels: 80%, 100%, and 120% as given in Table 21.

Table 21. Accuracy Data of Brivaracetam

Level	Standard Solution	Sample Solution	Total Conc (ug/mL)	Calculated conc.	%Recovery	Standard deviation	%RSD
80%	30	24	54	53.8253	99.01617638	654.9628488	0.21704928 2
	30	24	54	53.8345			
	30	24	54	53.6317			
100%	30	30	60	59.7374	98.54047301	1534.350134	0.45812801 6
	30	30	60	59.2532			
	30	30	60	59.6956			
120%	30	36	66	65.2107	98.22835653	751.1060733	0.20405648 5
	30	36	66	65.4317			
	30	36	66	65.4441			

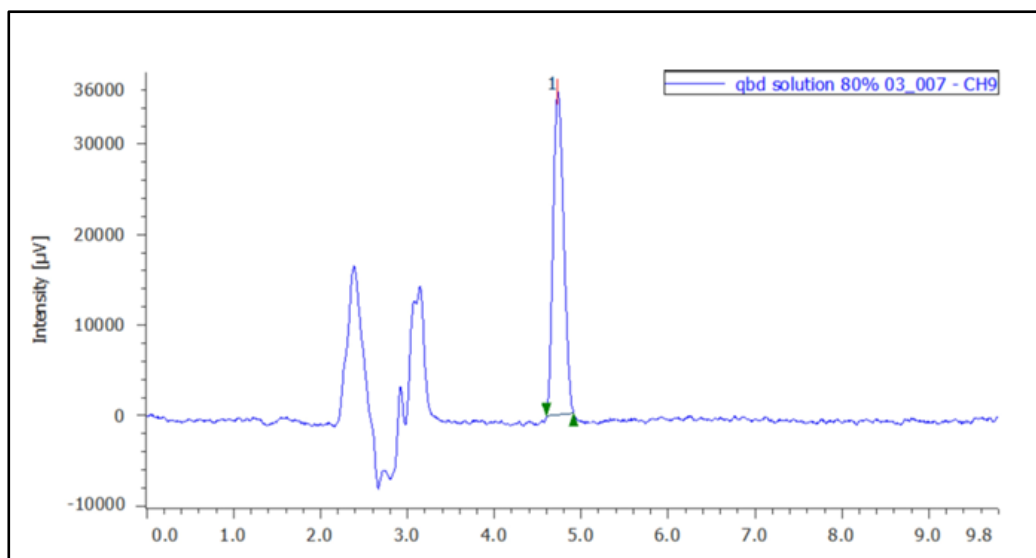


Figure 37. Chromatogram of Brivaracetam for Accuracy (80%)

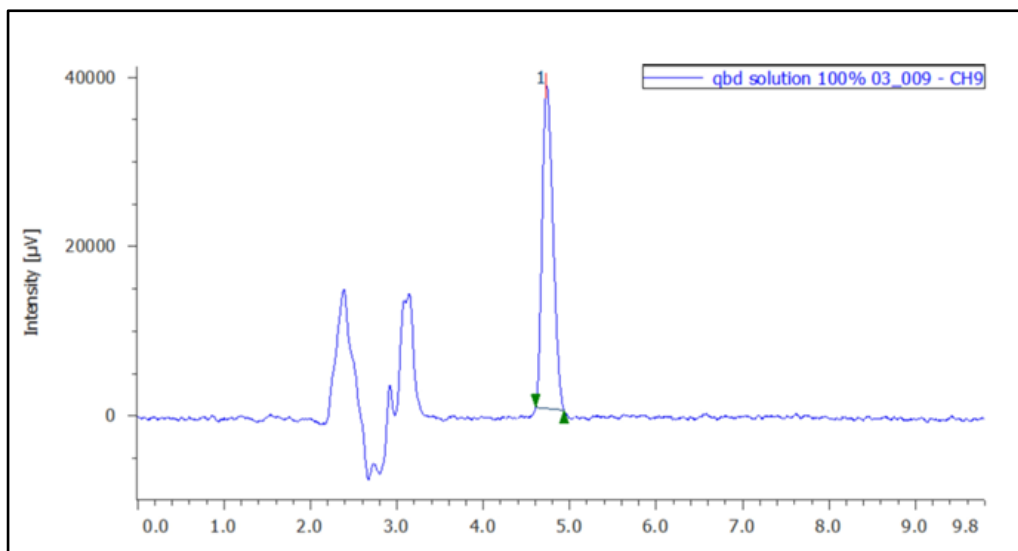


Figure 38. Chromatogram of Brivaracetam for Accuracy (100%)

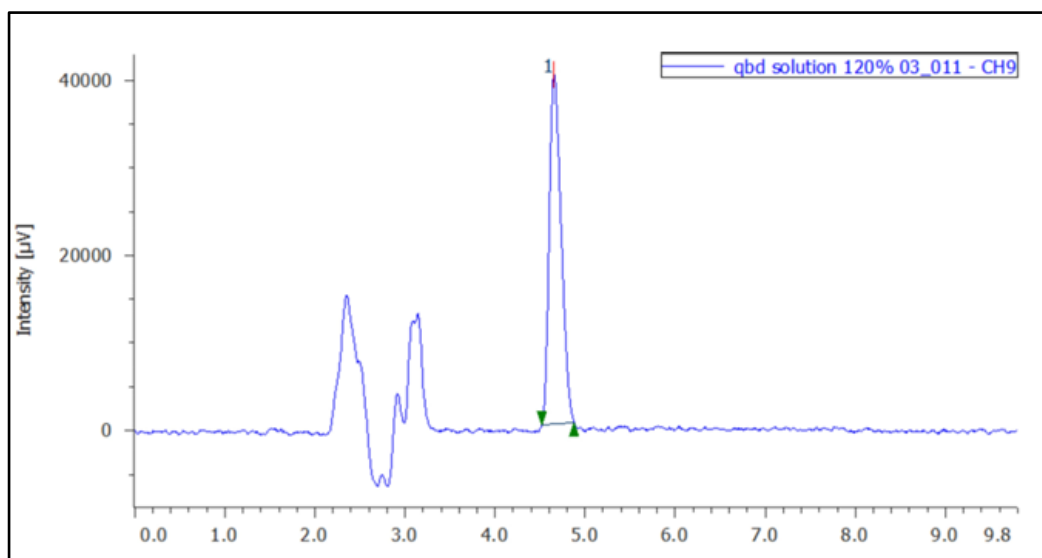


Figure 39. Chromatogram of Brivaracetam for Accuracy (120%)

Observations: The results obtained indicate that %Recovery ranged from 98.0 to 102.0%, and the %RSD was less than 2% for the drug as shown in Figure 37-39.

Acceptance Criteria: % Recovery should be in the range of 98-102%.

3.6.1 LOD & LOQ

The method's limit of detection and quantification was

evaluated through the analysis of six duplicates of standard solutions of Brivaracetam.

3.6.2 Robustness

The experimental conditions (flow rate, Temperature, and Wavelength) were deliberately altered at three different levels, and retention time and other chromatographic responses were evaluated as given in Table 22.

Table 22. Robustness Data of Brivaracetam

Sr.No.			1	2	3	Mean	SD	%RSD
Flow Rate	0.8 mL/min	Area	270835	273641	272537	272337	1412.61	0.52
		Rt	5.963	5.96	5.93	5.95	0.018	0.31
		NTP	7102	7065	7012	7059.67	45.12	0.64
	1.2 mL/min	Area	150055	146533	151421	149336	2530.84	1.69
		Rt	3.94	3.893	3.94	3.924	0.027	0.69
		NTP	6719	6710	6752	6727	22.56	0.34
Temperature	20°C	Area	233220	233460	234159	233613	486.18	0.21
		Rt	4.707	4.763	4.737	4.736	0.028	0.6
		NTP	6511	6421	6519	6483	54.79	0.84
	30°C	Area	221844	214819	222327	219663.33	4257.95	1.94
		Rt	4.763	4.763	4.767	4.764	0.002	0.05
		NTP	7374	7592	7371	7445.67	127.02	1.71
Wavelength	208nm	Area	213983	208692	216081	212918.67	3736.9	1.76
		Rt	4.727	4.71	4.723	4.72	0.009	0.19
		NTP	7523	7507	7757	7595.67	141.92	1.87
	212nm	Area	165344	163524	162708	163858.67	1324.79	0.81
		Rt	4.66	4.657	4.733	4.683	0.043	0.92
		NTP	7263	7347	7205	7271	72.76	1.00

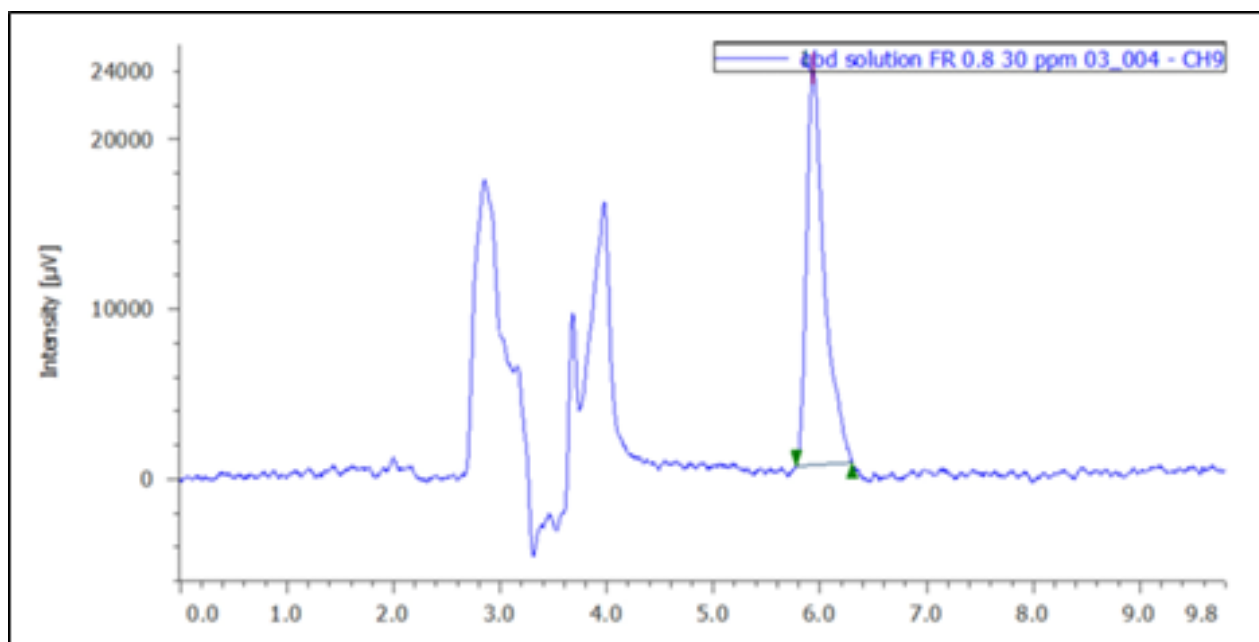


Figure 40. Chromatogram of Brivaracetam for Robustness (0.8 min/mL)

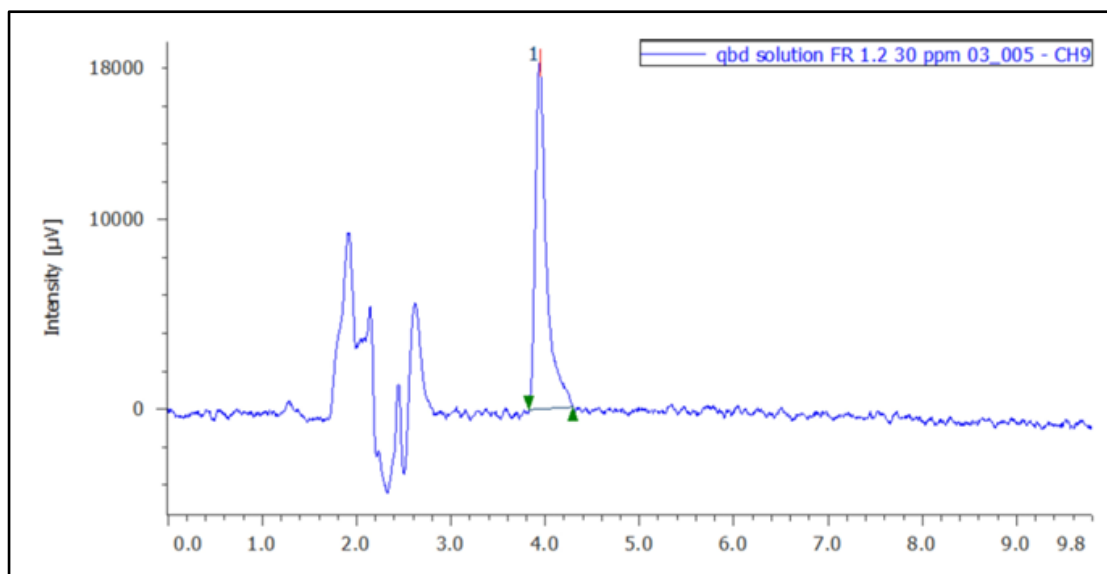


Figure 41. Chromatogram of Brivaracetam for Robustness (1.2 min/mL)

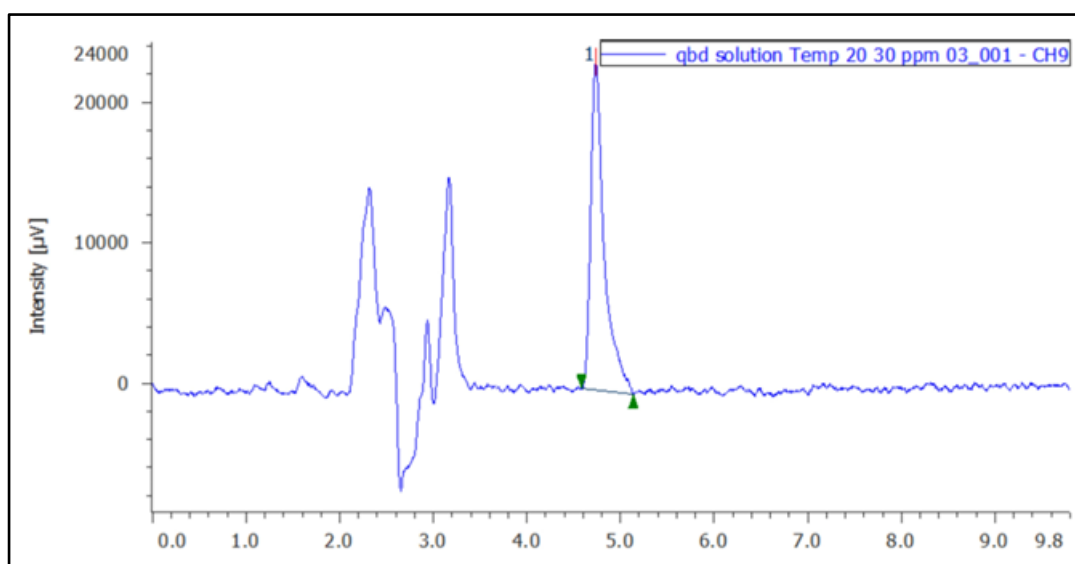


Figure 42. Chromatogram of Brivaracetam for Robustness (20°C)

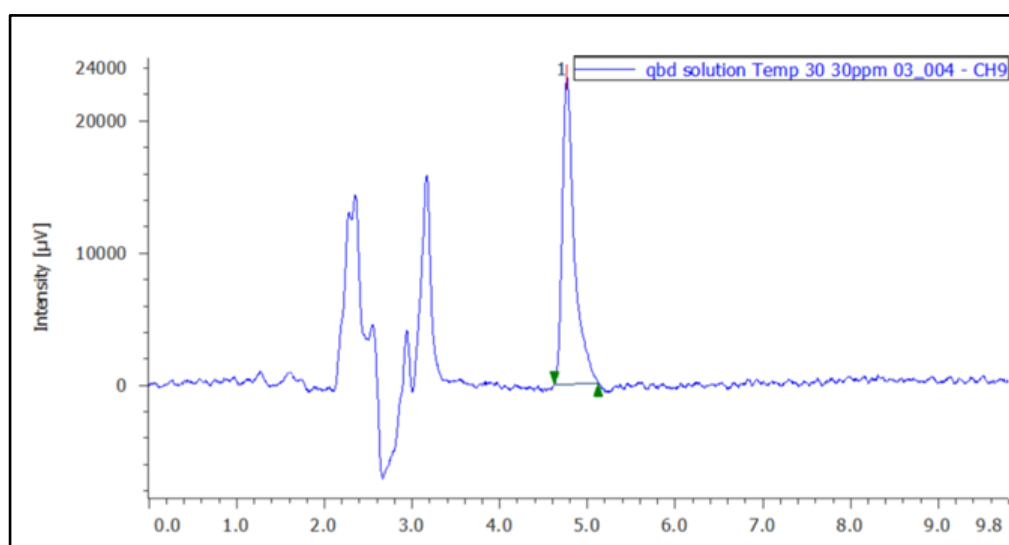


Figure 43. Chromatogram of Brivaracetam for Robustness (30°C)

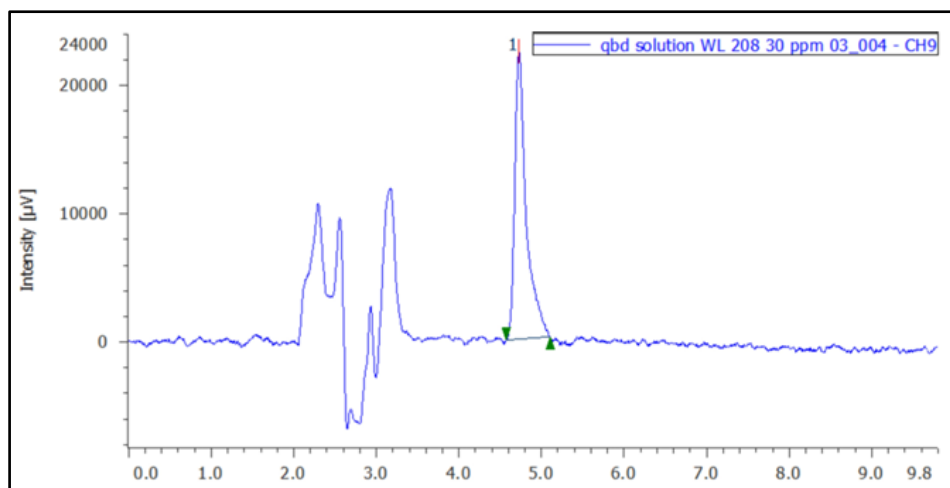


Figure 44. Chromatogram of Brivaracetam for Robustness (208nm)

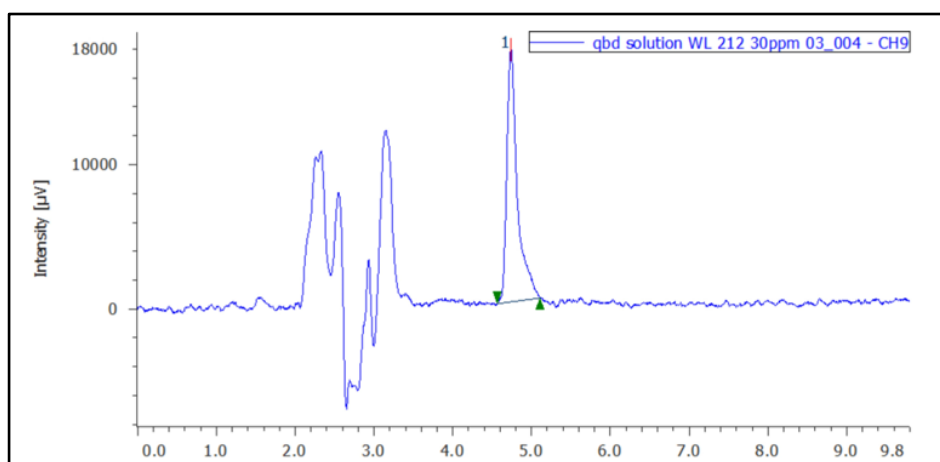


Figure 45. Chromatogram of Brivaracetam for Robustness (212nm)

3.6.3 Observation: The results indicate that the %RSD values were less than 2, which is within the acceptable limits as shown in Figure 40-45.

3.6.4 Acceptance Criteria: % RSD should not be more than 2%.

3.7 Assay

Six replicates of standard and sample solutions were prepared, and the % Assay was calculated for both drugs as given in Table 23.

Table 23. Assay of Abiraterone acetate by RP-HPLC

Sr. No.	Concentration (μg/mL)	Peak Area of Standard Solution
1	30	160401
2	30	162175
3	30	160844
4	30	166307
5	30	167370
6	30	163127
Average		
Tablet Avg Weight		
Std. Weight		
Sample Weight		
Label Amount		
Std. Purity (%)		
Assay (%)		

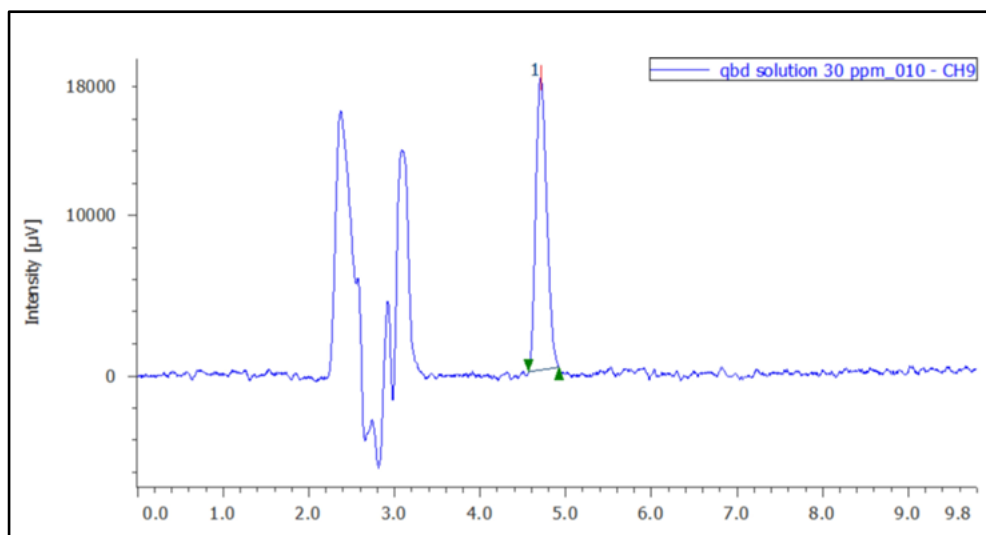


Figure 46. Chromatogram of Brivaracetam for Assay

3.7.1 Acceptance Criteria: % Assay should be in the range of 98-102% as shown in figure 46.

4. CONCLUSION

This research succeeded in establishing accurate and reliable alternative methods for the analysis of Brivaracetam in different pharmaceutical formulations, both tablets and bulk powder. Characterization of Brivaracetam by solubility, melting point, and FTIR analysis substantiated its identity and suitability as a candidate for validation. The choice of formulation method greatly influenced the performance of each analytical method. Also, methanol was chosen to be the best solvent for both methods and 210nm to be the best detection wavelength. The RP-HPLC was aided by employing AQbD principles through Central Composite Design in order to explore the impact that critical method parameters have on chromatographic performance through evaluation of systematic function of the method's parameters using AQbD methodology. Validation of the developed methods was conducted in accordance with Q2(R1) guidelines as well as evaluation of the method's validation characteristics (specificity, linearity, accuracy, precision, robustness, LOQ, and assay) demonstrated good compliance with all criteria. Additionally, the results showed that both validated methods provided accurate, consistent, low-cost analyses with acceptable system suitability and excellent chromatographic performance. Therefore, the validated methods are reliable for routine quality control, assay determination, and regulatory purposes related to Brivaracetam in Pharmaceutical Products.

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