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Analytical Method Development and Validation for the Estimation of Brivaracetam in Bulk and Its Dosage Form by RP-HPLC

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ABSTRACT

A simple reverse phase liquid Chromatographic method has been developed and subsequently validated for determination of Brivaracetam. The mobile composition of Potassium Dihydrogen orthophosphate (0.02M): Methanol 40:60v/v Buffer pH 6.0 adjusted with ortho phosphoric acid, waters column of C18 (250X4.6 ID) 5µm and flow rate 1.2 ml/min, using UV detection at 290 nm. run time 4.0 min efficient and reproducible method was developed for determination of Brivaracetam in tablet dosage form. The retention time of Brivaracetam were found to be 2.35 min and Results of analysis were validated statistically and by recovery studies. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise and accurate, which is useful for the routine determination of Brivaracetam bulk drug and in its pharmaceutical dosage form.

Keywords: Brivaracetam, Development, Forced degradation, HPLC, Validation

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1. Introduction

High performance liquid chromatography is a very sensitive analytical technique most widely used for quantitative and qualitative analysis of pharmaceuticals. The principle advantage of HPLC compared to classical column chromatography is improved resolution of the separated substance, faster separation times and the increased accuracy, precision and sensitivity.

In the modern pharmaceutical industry, high-performance liquid chromatography (HPLC) is the major and integral analytical tool applied in all stages of drug discovery, development, and production. The development of new chemical entities (NCEs) is comprised of two major activities: drug discovery and drug development. The goal of the drug discovery program is to investigate a plethora of

compounds employing fast screening approaches, leading to generation of lead compounds and then narrowing the selection through targeted synthesis and selective screening (lead optimization).

Types of HPLC Techniques:

Based on modes of chromatography:

- Normal phase chromatography
- Reverse phase chromatography

Based on principle of separation:

- Adsorption chromatography
- Ion exchange chromatography
- Size exclusion chromatography
- Affinity chromatography
- Chiral phase chromatography

Base on elution technique:

- Isocratic separation
- Gradient separation

Based on the scale of operation:

- Analytical HPLC
- Preparative HPLC

HPLC Instrumentation:

The following parts are present in high performance liquid chromatography [9]

- Mobile Phase reservoirs and solvent treatment systems
- Pumping System
- Sample injection systems
- Liquid chromatographic columns
- Types of column packing
- Detectors

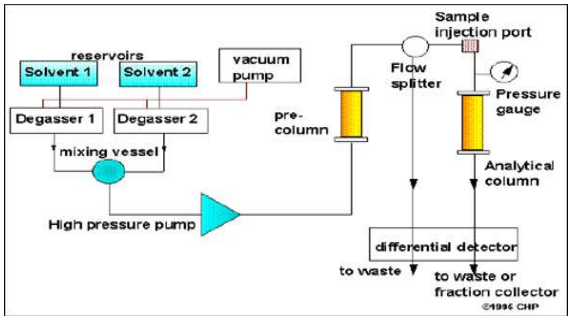

Fig 1: Instrumentation of HPLC

2. Materials and Methods

Table 1: List of Instruments

S.No	Equipment's	Model	Company
1	Electronic Balance	ER200A	ASCOSET
2	Ultra-Sonicator	SE60US	ENERTECH
3	Heating Mantle	BTI	BIO TECHNICS INDIA
4	Thermal oven	---------	NARANG
5	pH Meter	AD102U	ADWA
6	Filter Paper 0.45 microns	---------	MILLI PORE

Table 2: List of Chemicals and Reagents

S. No.	Chemicals/standards and reagents	Grade	Make
1	Ortho-Phosphoric Acid	AR	Finar
2	Methanol	HPLC	Merck
3	Acetonitrile	HPLC	Merck
4	Water	HPLC	LobaChemi
5	Brivaracetam	API	Torrent Pharma, Ahmadabad, India.

Method Procedure:

Method development for estimation of Brivaracetam in Pharmaceutical dosage forms includes the following steps:

1. Detection wavelength (λmax)
2. Column
3. Selection of mobile phase
5. Preparations and procedures

Analytical Method Development and Optimization:

The present study was carried out to develop and validate RP-HPLC method for the estimation of Brivaracetam in bulk and application of the developed methods for the determination of assay (% purity) of the marketed formulation.

Preparation of 0.02 M Potassium Dihydrogen orthophosphate Buffer:

2.72 g of Potassium Dihydrogen orthophosphate was weighed into a 1000 ml beaker, dissolved and diluted to 1000 ml with HPLC water. The pH was adjusted to 6.0 with OPA.

Preparation of mobile phase:

400 mL (40%) of Methanol, 100 mL (10%) of HPLC grade water and 500 mL (50%) Potassium Dihydrogen orthophosphate buffer pH 6.0 were mixed in a 1000 ml volumetric flask and kept for sonication in an ultrasonic water bath for 5 minutes. The solution was filter through 0.45 µ filter under vacuum filtration. Mobile phase was used as diluent.

Standard preparation:

100 mg of Brivaracetam was accurately weighed, transferred to a 100 ml volumetric flask, dissolved in the diluent and final volume was made upto the mark with the same to get a standard stock solution of1 mg/ml.

Preparation of working standard solution:

2 ml of the standard stock solution was diluted to, in a 25 ml volumetric flask to get 80 µg/ml working standard solution.

Preparation of solutions for linearity study: 50-150 %

Linearity Level 50%:

1 ml of stock solution was pipetted out and transferred into 25 ml volumetric flask. The solution was diluted upto the mark with diluent. The concentration obtained is about 40 µg/ml of Brivaracetam.

Linearity Level 75%:

1.5 ml of stock solution was pipetted out and transferred into 25 ml volumetric flask. The solution was diluted upto the mark with diluent. The concentration obtained is about 60 µg/ml of Brivaracetam.

Linearity Level 100%:
2 ml of stock solution was pipetted out and transferred into 25 ml volumetric flask. The solution was diluted upto the mark with diluent. The concentration obtained is about 80 µg/ml of LAF.

Linearity Level 125%:
2.5 ml of stock solution was pipetted out and transferred into 25 ml volumetric flask. The solution was diluted upto the mark with diluent. The concentration obtained is about 100 µg/ml of Brivaracetam.

Linearity Level 150%:
3.0 ml of stock solution was pipetted out and transferred into 25 ml volumetric flask. The solution was diluted upto the mark with diluent. The concentration obtained is about 120 µg/ml of Brivaracetam.

Determination of wave length
The working standard solution of Brivaracetam (80 µg/ml) was scanned in the range of 200-400 nm using mobile phase as blank. The drug showed maximum absorbance at 290 nm, which was selected for the determination.

Assay of Tablet dosage form
Twenty tablets were weighed and average weight was calculated. The tablets were ground to fine powder and a quantity of powder equivalent of 25 mg was accurately weighed and transferred to a volumetric flask of 25 ml capacity. 15 ml of mobile phase was transferred to volumetric flask and sonicated for 10 mins. The final volume was made upto the mark with the same to get the sample stock solution of 1000 µg/ml. The solution was filtered through 0.45 µ membrane filter. An aliquot of 2 ml was transferred to a 25 ml volumetric flask and diluted upto the mark with mobile phase to get a final concentration of 80 µg/ml. 10 µl of this solution was injected into the chromatograph and the chromatogram was recorded. The peak area was determined and the amount of Brivaracetam was calculated.

Method Validation
The developed method was validated as per ICH guidelines Q2 (R_1). The validation parameters studied were specificity, linearity, accuracy, precision, limit of detection, limit of quantitation and robustness.

System suitability:
Prior to the validation study, system suitability tests were performed by measurement of general characteristics such as peak symmetry, number of theoretical plates, retention time, tailing factor etc. The results obtained were satisfactory and in accordance with guidelines.

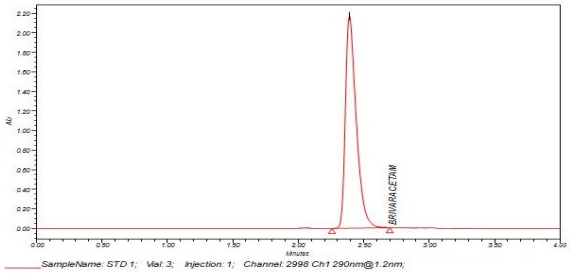

Fig 2: Standard Chromatogram of Brivaracetam

Specificity:
Specificity of an analytical method is its capability to measure the analyte precisely and particularly in presence of parts that may be likely to be present in the sample matrix. Chromatograms of standard and sample prove that the method was specific.

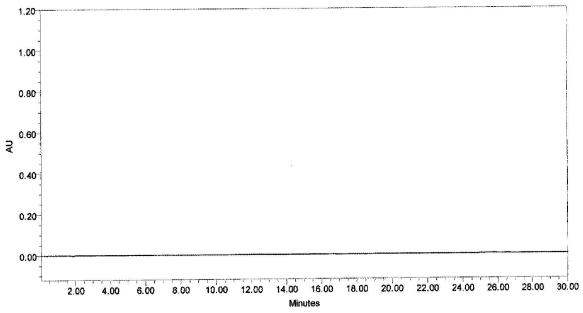

Fig 3: Blank Chromatogram of Brivaracetam

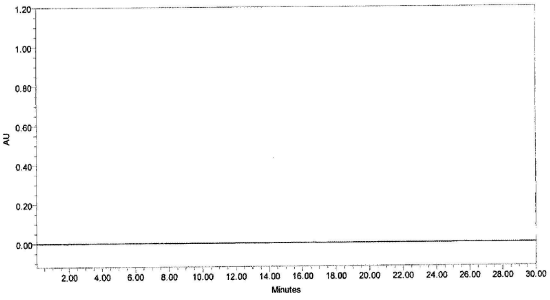

Fig 4: Placebo Chromatogram of Brivaracetam

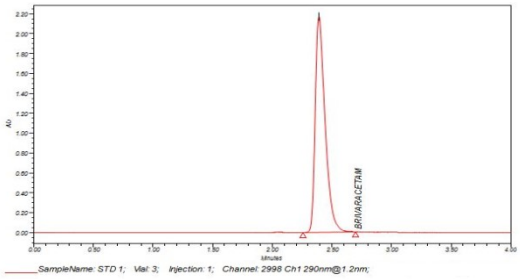

Fig 5: Standard Chromatogram of Brivaracetam

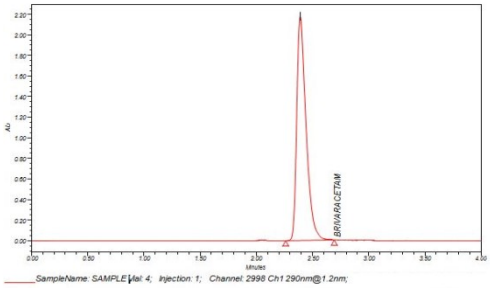

Fig 6: Sample Chromatogram of Brivaracetam

Linearity:
The linearity plot was constructed with five concentration at the level of 50-150% (40, 60, 80, 100, 120 µg/ml of Brivaracetam). The response of the drug was found to be linear in the studied concentration range and the linear regression equation was y = 95602x - 9465.4. The correlation coefficient was found to be 0.9997.

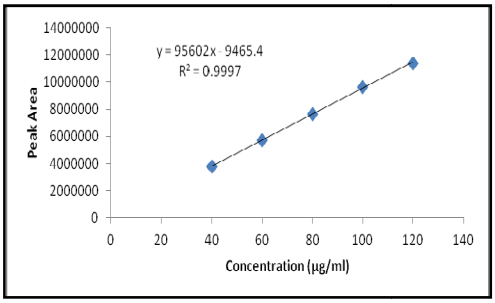

Fig 7: Linearity curve for Brivaracetam

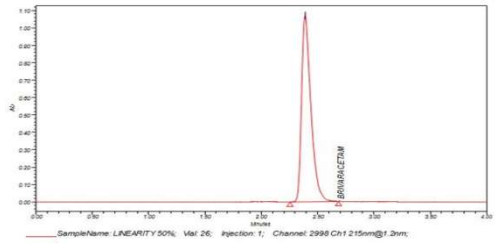

Fig 8: Linearity Chromatogram of 50%

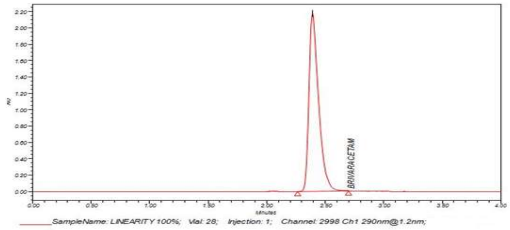

Fig 9: Linearity Chromatogram of 100%

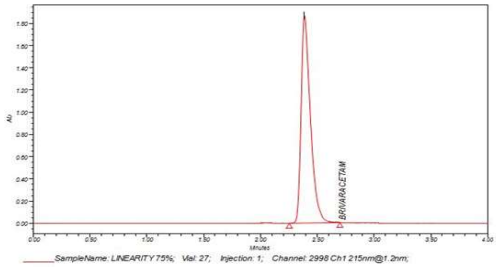

Fig 10: Linearity Chromatogram of 75%

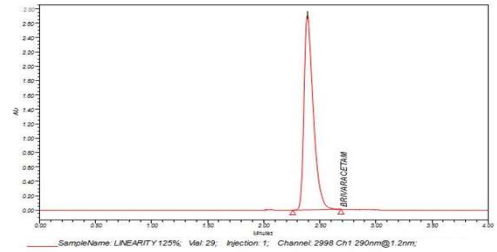

Fig 11: Linearity Chromatogram of 125%

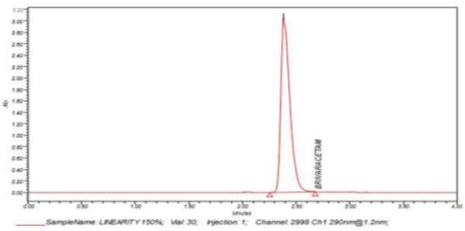

Fig 12: Linearity Chromatogram of 150%

Precision:

Intra and inter-day precision of the analytical method was determined by performing method precision for three times in same day and followed by three consequent days. %RSD was calculated and found to be within the specified limits (<2 %).

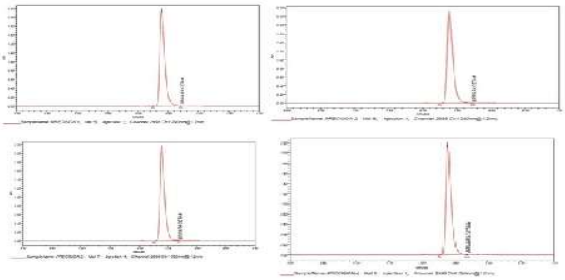

Fig 13: Chromatograms for Precision

Accuracy:

The accuracy of the method was assessed by standard addition method. % Recovery for three concentrations (corresponding to 50, 100 and 150 % of test solution concentration) were determined. For each concentration three replicates were prepared. The mean recovery of Brivaracetam was found to be 100 %.

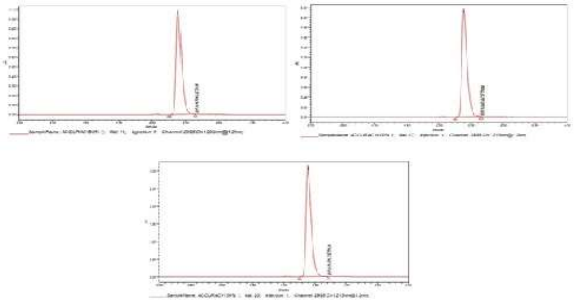

Fig 14: Chromatograms for Accuracy

Limit of detection and Limit of quantitation:

The limit of detection (LOD) and limit of quantitation (LOQ) were determined by using standard deviation of response and slope of the calibration curve. The LOD and LOQ of the proposed method were found to be 0.0006 and 0.002 µg/ml respectively. The representative chromatograms were shown.

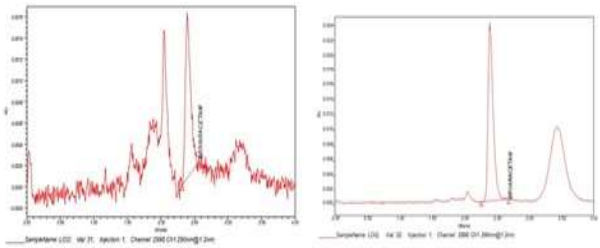

Fig 15: LOD and LOQ Chromatograms of Brivaracetam

Robustness study:

The robustness of the method was evaluated by assaying test solutions after slight but deliberate changes in the analytical conditions like flow rate (± 0.1 ml/min), the

column temperature (± 2°C) and in wave length (± 2nm). System suitability data was found to be satisfactory during variation of the analytical conditions. Results of system suitability show that the analytical method remained unaffected by slight but deliberate changes in the analytical conditions.

Assay:

The proposed method was applied for the tablet of Brivaracetam and the mean % assay was found to be 100 %. The chromatogram shows that no interference from excipients.

Degradation studies (Stress testing):

Forced degradation study was performed to evaluate the stability of the developed method using the stress conditions like exposure of sample solution to acid (0.1 N HCl), base (0.1 N NaOH), peroxide (H_2O_2), UV light, and water. Investigation was done for the degradation products. For acid treatment 5 ml of stock solution of Brivaracetam was taken in a 25 ml volumetric flask; to it 5 ml of 0.1 N HCl was added and kept a side at room temperature for 24 hrs. This solution was neutralized with alkali 0.1 N NaOH and diluted suitably to a final concentration of 400 µg/ml. Same procedure was followed for alkali (0.1 N NaOH) treated sample and neutralized with acid (0.1 N HCl) and sample was treated with 10% solution of H_2O_2 for peroxide treated samples. 20µl all the sample solutions were injected into the chromatographand chromatograms were recorded.

3. Results and Discussion

RP-HPLC method was developed and validated for the determination of Brivaracetam in tablet doses form.In the process of HPLC method development the optimization was done by changing the mobile phase, mobile phase ratio, column and flow rate. Method development focuses on identifying buffer type, strength and pH of organic solvent. Implementing small changes to optimize selectivity enhanced resolution. Different trials were performed and finally the optimized method was found to be suitable. The mobile composition of Potassium Di hydrogen ortho phosphate (0.02M): Methanol 40:60v/v Buffer pH 6.0 adjusted with ortho phosphoric acid, waters column of C18 (250_X4.6 ID) 5μm and flow rate 1.2 ml/min, run time 4.0 min efficient and reproducible method was developed for determination ofBrivaracetam in tablet dosage form and optimized chromatogram is obtained with good resolution.

When a method has been used, it must validation before practical use. By following the ICH guidelines for analytical method validation, the system suitability test was performed and the validation characteristics were addressed.The system suitability test ensures the validity of the analytical procedure as well as confirms the resolution. System suitability parameters like retention time, tailing factor, efficiency, capacity factor and resolution was performed. The results were found to be within the limits.

The specificity study was performed and the acid, base, oxidative, photolytic and thermal degradation studies were done to the mother sample. The precision study for system as well as method was conducted for Brivaracetam and the standard stock solutions. The samples were injected six times in to the HPLC system and the retention time was recorded. It was found to be with the specified limit according to ICH guide lines, it shows that the drugs are having good precision. The accuracy was confirmed by recovery studies by adding known amount of pure drug to the previously analyzed formulation and the mixture was analysed by the proposed method. The percentage recovery ofBrivaracetam was 100.00%. The linearity studies were conducted for Brivaracetam standard stock solutions. For the constructions of calibration curves, five different known concentrations of standard solutions of Brivaracetam were selected. The peak areas of those solutions over the concentration range of 40 to 120 μg/ml were observed and recorded.Therefore the developed method was specific, accurate, precise, linear, robust, simple and rapid. Hence, the RP-HPLC method may be applied forBrivaracetam.

Table 3: System suitability data for Brivaracetam

S. No	Parameter	Result	Acceptance Limit
1.	Retention time (Rt)*	2.35 min	--
2.	Resolution factor*	--	--
3.	Number of theoretical plates (N)*	6755	More than 4000
4.	Tailing factor (T)*	1.00	Less than 2
5.	Capacity factor (K)*	-	0.5<K<20
* Number of injections: 6 replicates			

Table 4: Linearity Data of Brivaracetam

Linearity Level	Concentration (µg/ml)	Peak Area
50%	40	3793458
75%	60	5716681
100%	80	7661150
125%	100	9620341
150%	120	11401823
Slope		95602
Intercept		9465.4
Regression coefficient		0.9997

Table 5: Summary of precision study for Brivaracetam

S. No	Intraday precision% Assay		Inter day precision	
	Peak Area	% Assay	Peak Area	% Assay
1.	7522699	99.05	7622699	100.37
2.	7642606	100.63	7642606	100.63
3.	7618974	100.32	7618974	100.32
4.	7676367	101.07	7676367	101.07
5.	7765881	102.25	7665881	100.94
6.	7532006	99.17	7632006	100.49
Average	7626422	100.42	7643089	100.63
STDEV	91578.98	1.21	23440.36	0.31
% RSD	1.20	1.20	0.31	0.31

Table 6: Evaluation data of accuracy study for Brivaracetam

Sample No.	Spiked Level	Sample Weight (mg)	Sample Area	µg/ml added	µg/ml found	% Recovery	% Mean Recovery
1	**50%**	197.50	3831749	40.00	40.36	100.90	100
2	**50%**	197.50	3811606	40.0000	40.1493	100.37	
3	**50%**	197.50	3773683	40.0000	39.7499	99.37	
4	**50%**	197.50	3730428	40.0000	39.2942	98.24	
5	**50%**	197.50	3813458	40.0000	40.1688	100.42	
6	**50%**	197.50	3752716	40.0000	39.5290	98.82	
1	**100%**	395.00	7594479	80.0000	79.9960	100	100
2	**100%**	395.00	7499061	80.0000	78.9909	99	
3	**100%**	395.00	7761150	80.0000	81.7516	102	
1	**150%**	592.50	11362905	120.0000	119.6904	99.74	100
2	**150%**	592.50	11401823	120.0000	120.1004	100.08	
3	**150%**	592.50	11382080	120.0000	119.8924	99.91	
4	**150%**	592.50	11392046	120.0000	119.9974	100.00	
5	**150%**	592.50	11400193	120.0000	120.0832	100.07	
6	**150%**	592.50	11390759	120.0000	119.9838	99.99	

Table 7: Evaluation data of robustness study for Brivaracetam

Robust conditions	Rt (min)	Peak area	% Assay
1.3 ml/min flow rate	2.383	7592252	99.97
Column temp at 28°C	2.378	7642606	100.63
Column temp at 32°C	2.375	7418974	97.68
Wave length 268 nm	2.378	7676367	101.07
Wave length 292 nm	2.377	7665881	100.94
Average	2.38	7591680.33	99.96
SD	0.001	96455.64	1.27
%RSD	0.09	1.27	1.27

Table 8: Assay data of Brivaracetam Tablets

Sample No.	Sample Weight	Sample Area	% Assay
1	395.0	7522699	99.05
2	395.0	7642606	100.63
3	395.0	7618974	100.32
4	395.0	7676367	101.07
5	395.0	7765881	102.25
6	395.0	7532006	99.17
Average			100.41
STD			1.21
% RSD			1.20

Table 9: Data of degradation studies of Brivaracetam

Nature of the Sample	Sample Weight	Sample Area	% Assay	Difference of Assay
Acid	395.0	7295881	96.06	3.94
Base	395.0	7312006	96.28	3.72
Peroxide	395.0	7592006	99.96	0.04
Water	395.0	7532006	99.17	0.83
Light	395.0	7496367	98.70	1.3

4. Conclusion

The study was focused to develop and validate HPLC method for estimation of Brivaracetam in tablet dosage form.For routine analytical purpose it is desirable to establish methods capable of analyzing huge number of samples in a short time period with good robustness, Accuracy and precision without any prior separation steps.HPLC method generates large amount of quality data, which serve as highly powerful and convenient analytical tool.The method shows good reproducibility and good recovery. From the specificity studies,it was found that the developed methods were specific for Brivaracetam.

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