IJBPAS, June, 2024, 13(6): 2872-2881

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

BIOANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR DETERMINATION OF BRIVARACETAM IN HUMAN PLASMA

DAMLE MC*, MADGULKAR AR AND KAITKAR PY

All India Shri Shivaji Memorial Society's College of Pharmacy, Kennedy Road, Near R.T.O Office, Pune-411001

***Corresponding Author: Prof. Dr. Mrinalini C. Damle; E Mail: damle_mc@aissmscop.com**

Received 15th April 2023; Revised 8th June 2023; Accepted 18th Sept. 2023; Available online 1st June 2024

https://doi.org/10.31032/IJBPAS/2024/13.6.8096

ABSTRACT

A simple and rapid high-performance liquid chromatographic method for the determination of an anti-epileptic drug Brivaracetam in human plasma has been developed, optimized, and validated, with an azole anti-fungal Fluconazole as the internal standard (I.S). A protein precipitation technique was used for plasma sample pre-treatment before injection into the HPLC system with a UV detector. The mobile phase consisted of Phosphate Buffer (0.05M, pH=5) and Acetonitrile in the ratio of 70:30 *v/v*. The detection wavelength chosen was 220 nm. The method was found to be linear over the concentration range of 1-8 µg/mL. It was validated as per ICH M10 guidelines for the parameter's selectivity, specificity, linearity, accuracy, precision, stability, carry-over, recovery, and matrix effect. The stability of Brivaracetam in plasma was studied in terms of bench-top stability, freeze-thaw stability, and long-term stability. The results were found to be within the acceptable limits stated by ICH. The developed method can be applied during clinical trials, therapeutic drug monitoring in human plasma and bio-equivalence studies.

Keywords: Brivaracetam, Fluconazole, HPLC, human plasma, bioanalytical, validation

INTRODUCTION:

Brivaracetam is an anti-epileptic drug (AED) that has efficacy for focal and secondary seizures. The chemical name of Brivaracetam is (2S)-2-[(4R)-2-oxo-4-propyltetrahydro-

1H-pyrrol-1-yl] butanamide **[1, 2]**. Its molecular formula is $C_{11}H_{20}N_2O_2$ and its molecular weight is 212.29 g/mol. The exact mechanism of action of Brivaracetam is unclear, but it is recognized as an anti-convulsant too. It has a selective affinity for synaptic vesicle protein SV2A **[3-5]**. Epilepsy is the syndrome consisting of two or more unprovoked seizures that occur more than 24 hours apart. Seizures affect people in many different ways. Seizures are disruptive and may cause injuries to patients. Epilepsy patients have higher rates of psychiatrically co-existing conditions **[6]**. There are certain clinically relevant interactions of AEDs, which involve other classes of drugs and their effect on plasma concentration. The drug Brivaracetam is a white amorphous powder that is freely soluble in water. Cmax of the drug is 3.5 µg/ml **[7]**. According to the literature survey, a few bioanalytical methods are reported **[8]**, UPLC coupled with MS/MS **[9]**, LC-MS/MS using Liquid-Liquid Extraction **[10]** and to the best of our knowledge, there was scope for a simple HPLC bioanalytical method to be developed on Brivaracetam in human plasma. The present study aims to develop a simple and rapid high-performance liquid chromatography HPLC – UV method of bioanalysis to estimate Brivaracetam. To confirm the reliability of the extraction and quantitation, validation was performed at concentration ranges LLOQ, LQC, MQC, and HQC.

MATERIALS AND METHODS:

Reagents and Chemicals: Brivaracetam used as a working standard, was received as a gift sample from ZYDUS Pharmaceuticals. Fluconazole used as an internal standard was received as a gift sample from CIPLA Ltd. Water (HPLC grade) generated through the ELGA system, chemicals and reagents used, Acetonitrile (HPLC grade), Potassium dihydrogen orthophosphate, Trichloroacetic acid were purchased from Loba Chemie.

Instrumentation and chromatographic conditions: Bioanalytical method of Brivaracetam was performed on HPLC system equipped with HPLC Pump (Make/Model- JASCO/PU2080 plus) Hypersil Thermo C8 Column of dimension 250*4.6mm (Make- Thermo Fisher), Rheodyne Injector and UV detector (Make/Model- JASCO/UV2075 plus). The data acquisition was done using Borwin Chromatography Software (version 1.5). Digital balance (Make/Model- Shimadzu/ ATX 224R) was used for weighing. The mobile phase was filtered through membrane filter of 0.45µm pore size, using vaccum pump (Make/Model- BIOMEDIA/JETVAC-JI), an

Ultra-sonic bath sonicator was used for degassing of the mobile phase. The centrifuge used was from REMI and vortexing was done on a cyclo mixer (Make/Model-REMI/CM101).

Selection of Wavelength: From the standard stock solution, further dilutions were done using acetonitrile and it was scanned over the range of 200-400 nm.

Selection of Internal Standard (I.S.): Internal standard (I.S.) is a compound added to samples to monitor the accuracy and precision of sample pre-treatment as well as the robustness of the bioanalytical method. Atenolol, Bedaquiline, Teriflunomide, Metformin., Nebivolol HCl, and Fluconazole were analyzed for internal standard trials. Amongst all these, Fluconazole was selected as the internal standard as peaks of the drug and I.S. were well resolved.

Mobile Phase Preparation: HPLC grade ACN was added to Phosphate Buffer (0.05 M, pH=5) made in HPLC grade water (30:70 *v/v*). The solution was further filtered through a 0.45 µm membrane filter and sonicated for 10 minutes.

Preparation of stock solution: The stock solution of Brivaracetam and Fluconazole (I.S) was prepared separately by transferring accurately weighed 10 mg of the drug into a 10ml volumetric flask and making up the volume with acetonitrile to obtain a concentration of 1000 µg/ml. Working solution for Brivaracetam was prepared by diluting appropriate stock solution to get a final concentration of 1-8 µg/ml and for Fluconazole to get a final concentration of 50 µg/ml.

Preparation of Spiked plasma sample: The reported clinical Cmax of Brivaracetam is 3.5µg/ml [(7)]. On this basis, the linearity range was chosen as 1-8 µg/ml. Spiked plasma was prepared by spiking 4.8 ml plasma with 0.1 ml from each stock solution (50, 100, 150, 200, 250, 300, 350, 400 µg/ml) and 0.1 ml of Fluconazole (250 µg/ml) as an internal standard separately. The content was vortexed for 2 minutes each. The vortexed solution and precipitating agent were taken in a ratio of 1:1. Acetonitrile along with Trichloroacetic acid in the ratio of 9:1 was added as the precipitating agent. These solutions were vortexed again and then centrifuged for 3 minutes. The similar procedure was followed for blank plasma (only plasma), zero plasma (plasma and internal standard), and spiked plasma.

Bio-analytical Method Validation: [11]

1) **Selectivity:** Selectivity of the bio-analytical method is the ability of the proposed method to quantify and differentiate the working standard

drug in the presence of an interfering substance.

2) **Calibration Curve:** The correlation between the known concentration and response was evaluated through a regression analysis of the calibration curve constructed using an eight-point (1, 2, 3, 4, 5, 6, 7, and 8 µg/ml) standard calibration curve. The calibration curve was constructed with ratio of area drug/I.S. called as response factor on Y-axis and concentration on X-axis.
3) **Accuracy:** The percent mean accuracy was determined for all QC samples. The response factors were substituted in the regression equation to get the concentration of the given sample. The deviation of the average from the theoretical value served as the estimation of accuracy.
4) **Precision:** The closeness of the individual measured value of the drug analyte among all aliquots of the same volume of the plasma was assessed by injecting six replicates at LLOQ, LQC, MQC, and HQC levels. The precision of the method performed on the HPLC system was evaluated by determining the % CV of the repeated injections.
5) **Recovery:** Recovery studies were performed by comparing the chromatographic response for samples after extraction at LLOQ, LQC, MQC, and HQC with the standard in three replicates.
6) **Carry Over:** Carryover is the impact of the previous injection on the next injection of the analyte. It was determined by injecting blank samples after HQC injection of 6 µg/ml.
7) **Matrix Effect**: The method was performed using a pooled plasma sample. This indeed marks that the study results were carried out in a matrix condition.
8) **Stability Studies:** The stability procedure should evaluate the stability of the analyte during sample collection and handling after long-term (frozen at intended storage temperature) and short-term (room temperature) storage conditions.

RRESULT:

Detection Wavelength: It was observed that Brivaracetam showed considerable absorbance at 220 nm. Hence, this wavelength was chosen for detection **(Figure 1)**.

Selectivity: The evaluation was done using blank plasma samples. The absence of

interference at Brivaracetam and Fluconazole retention time was confirmed **(Figure 2-4)**.

Linearity: The result is mentioned in **Table 2, Figure 5**. The R^2 value was found to be 0.9832.

Accuracy: : Accuracy was estimated by using a minimum of 5 replicates of 4 concentrations i.e. at LLOQ , LQC, MQC, HQC. The result is mentioned in **Table 3**.

Precision: The precision was assessed by injecting six replicates at LLOQ, LQC, MQC, and HQC levels. Intraday precision was evaluated by determining the % CV of the response of the repeated injections injected on the same day. On the contrary, inter-day precision was calculated after a comparison of the measured values of the samples injected on three different days. The result is mentioned in **Table 4 and 5**.

Recovery:

Recovery from human plasma samples was evaluated in triplicate for 4 concentrations of Brivaracetam (1, 2, 4, and 6 µg/ml). The overall mean recovery of Brivaracetam was found to be 65.56%. The results are mentioned in **Table 6**.

Carry Over: Response of blank samples was below the LLOQ, hence there was no carry over.

Stability Studies: Brivaracetam stability was evaluated using two concentration levels i.e. at LQC and HQC. For each sample to be tested mean of 3 samples was taken. These were processed, stored, and analyzed. The results are summarized in **Table 7**.

1. **Freeze and thaw stability:** The freeze and thaw stability of spiked quality control samples was determined after 3 freeze and thaw cycles stored at -20 ºC, and compared to the freshly spiked quality control sample to assess stability. The mean percent stability for HQC (6 µg/ml) and LQC (2 µg/ml) was found to be 95.48% and 94.43% respectively.
2. **Long-Term Stability:** The long-term stability of the LQC and HQC was determined after 7 days stored at -20 ºC, comparing them against the freshly prepared stock solution assessed for stability. The percent mean stability for HQC (6 µg/ml) and LQC (2 µg/ml) are found to be 98.42% and 97.45% respectively.
3. **Short-Term Stability:** The short-term temperature stability of spiked quality control samples was determined for a period of 4 hrs. stored at room temperature. Comparing them against the freshly spiked quality control samples assessed stability. The percent mean stability for HQC (6 µg/ml) and LQC (2 µg/ml) are found to be 96.66% and 95.07% respectively.

4. **Stock solution stability:** The stock solution stability of the drug and I.S. was determined for 2 hrs at room temperature. Comparing them against the freshly weighed stock solution assessed for stability. The percent mean stability for Brivaracetam at HQC (6 µg/ml) and LQC (2 µg/ml) levels are found to be 94.15% and 95.20% respectively.

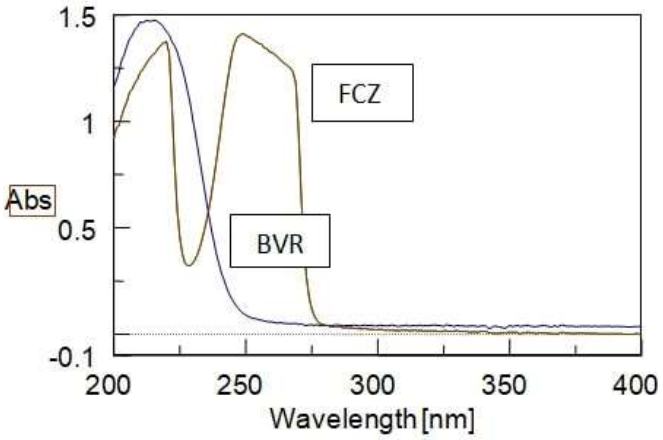

Figure 1: UV-Spectral Overlay of Brivaracetam and Fluconazole

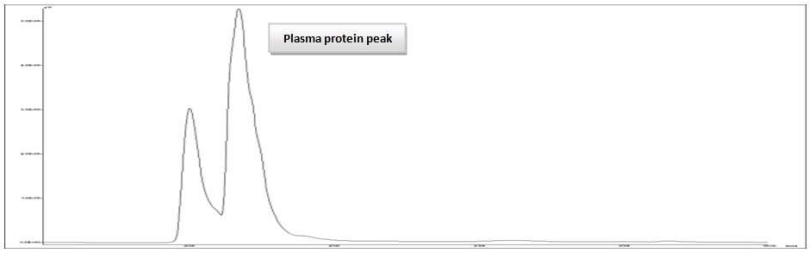

Figure 2: Chromatogram of blank human plasma

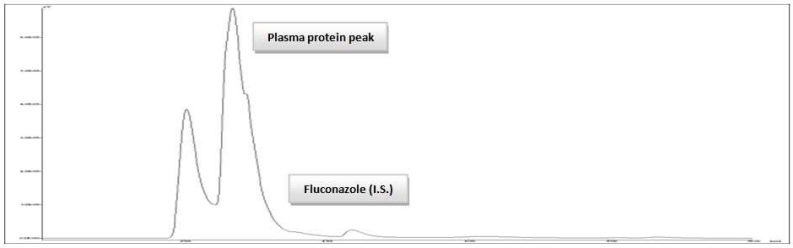

Figure 3: Chromatogram of zero plasma, Fluconazole (I.S.) peak at retention time (RT) = 4.35 min

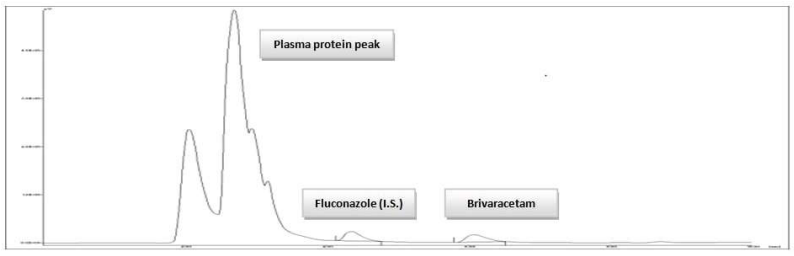

Figure 4: Chromatogram of HQC (6 µg/ml), Fluconazole (I.S.) peak at RT = 4.35, Brivaracetam peak at RT = 6.258 min

Table 2: Linearity Studies

Replicate No.	Concentration (µg/ml)							
	1	2	3	4	5	6	7	8
	Response Factor							
1	0.0417	0.1136	0.1332	0.2093	0.2872	0.3827	0.4420	0.5144
2	0.0431	0.1166	0.1320	0.2042	0.2558	0.3794	0.4545	0.5332
3	0.0403	0.1081	0.1310	0.2114	0.2764	0.3860	0.4403	0.5711
4	0.0398	0.1125	0.1517	0.2218	0.2772	0.3815	0.4528	0.5364
5	0.0424	0.1117	0.1298	0.2218	0.2557	0.3841	0.4341	0.5304
Mean	0.0415	0.1125	0.1355	0.2137	0.2705	0.3827	0.4447	0.5371
SD	0.0013	0.0030	0.0091	0.0078	0.0125	0.0022	0.0077	0.0186
%CV	3.284	2.740	6.7231	3.6632	4.6573	0.5862	1.7430	3.4661

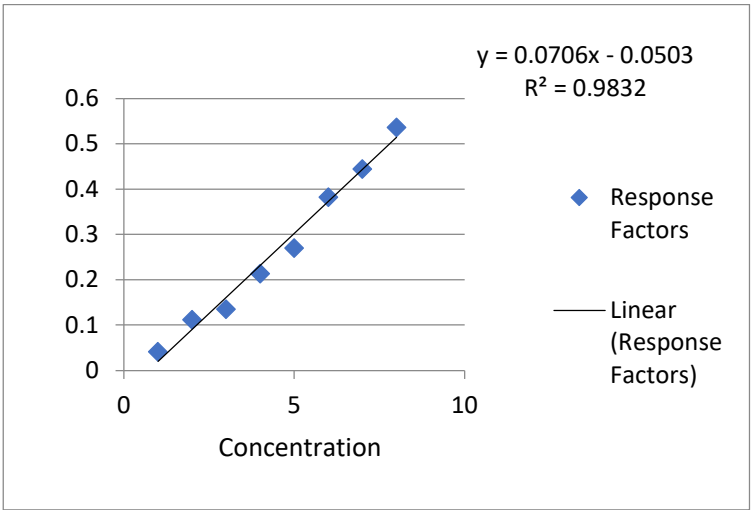

Figure 5: Regression Curve for linearity

Table 3: Accuracy Studies

Replicates	Calculated concentration			
	LLOQ	LQC	MQC	HQC
1	1.1932	1.9786	3.8496	5.7601
2	1.1927	2.0051	3.8282	6.076
3	1.1785	1.9575	3.8940	6.0018
4	1.1902	1.9584	3.9457	5.9886
5	1.1734	1.9954	3.9457	6.004
Mean	1.1856	1.9790	3.8926	5.9661
SD	0.0090	0.0214	0.0539	0.1200
%CV	0.7651	1.0828	1.3852	2.013
%Mean Accuracy	118.56	98.95	97.31	99.43
Acceptance Criteria: The accuracy at each concentration level should be within ±15% of the nominal concentration, except at the LLOQ, where it should be within ±20%.				

Table 4: Results of Intraday Precision

Concentation Level	LLOQ	LQC	MQC	HQC
Mean Conc.	1.1843	1.9876	3.8901	5.9131
Standard Deviation	0.0056	0.0169	0.0256	0.0811
%CV	0.4801	0.8541	0.6597	1.3717
Acceptance Criteria: The precision (%CV) of the concentrations determined at each level should not exceed 15%, except at the LLOQ, where it should not exceed 20%.				

Table 5: Results of Interday Precision

Concentration Level	% CV		
	Day 1	Day 2	Day 3
LLOQ	1.2327	0.2952	0.9337
LQC	0.6801	0.3868	1.0137
MQC	1.3840	0.5641	0.8019
HQC	1.2291	1.3773	1.2962
Acceptance Criteria: The precision (%CV) of the concentrations determined at each level should not exceed 15%, except at the LLOQ, where it should not exceed 20%.			

Table 6: Results of Recovery studies

Conc. level	Area of Brivaracetam		Recovery	Area of Fluconazole (I.S)		Recovery
	Standard	Spiked plasma	%Mean Recovery	Standard	Spiked plasma	%Mean Recovery
LLOQ	38973	24399	62.60	466164.7	330817	70.96
LQC	55134	37561	68.12	567491.2	350587	66.75
MQC	107716	70940	65.85	551324.5	370793	67.25
HQC	184508	121177	65.67	569512.1	378885	66.52
Overall	Overall % Mean Recovery		65.56	Overall% Mean Recovery		67.87

Table 7: Summary of stability studies

Stability	Conc.(µg/ml)	Mean Stability (%)	% CV
Freeze thaw stability(three cycles)	LQC	94.43	0.9614
	HQC	95.48	0.5478
Short term stability(for 4h at R.T.)	LQC	97.45	0.7129
	HQC	98.42	0.2214
Long term stability (for 7 days at 4^0 C)	LQC	95.07	1.0165
	HQC	96.66	0.2214
Stock solution stability(for 2h)	LQC	95.20	1.3253
	HQC	94.15	0.2214
Acceptance Criteria: The % mean stability for drug and I.S. should be within range 85-115%.			≤ 15%

DISCUSSIONS:

The previously reported methods were complex and tedious in nature involving techniques like liquid-liquid extraction and UPLC coupled with MS/MS, **[9, 10]**. Thus, a simple, faster and optimized, bioanalytical method is developed and validated. The extraction is done using protein precipitation in human plasma and later response factor of Brivaracetam was subsequently calculated. The results of validation parameters were found to meet all the acceptance limits of the parameters stated by ICH. The study results here state that Brivaracetam can be effectively extracted from human plasma and for ensuring the extraction efficiency Fluconazole can be used as internal standard. There were no interfering peaks amongst the working standard and internal standard. The method is optimized by achieving the retention time, such that peaks are well resolved. Apart from other reported methods, like analytical method development by RP-HPLC on bulk and formulation **[8]**, UPLC coupled with MS/MS **[9]**, LC-MS/MS using Liquid-Liquid Extraction **[10]** instead, there can be a simpler

HPLC estimation of Brivaracetam from human plasma at a laboratory scale by employing a protein precipitation method. The stability of Brivaracetam in human plasma, is a function of storage conditions, chemical properties of the drug, the matrix, and the storage system. The drug was found to be quite stable in human plasma.

CONCLUSION:

Brivaracetam could potentially be extracted from human plasma and quantified using a simple HPLC bioanalytical approach. The process of extraction was made easier and took less time overall by the use of protein precipitation. The method's dependability and accuracy were ensured by the validation parameters compliance with the ICH acceptance limits. The specificity and effectiveness of the approach were proven by the absence of interfering peaks and the optimised retention times. The future prospect of this method is that it could be used for routine bioanalysis, clinical trials, therapeutic drug monitoring, as well as bioavailability and bioequivalence investigations of Brivaracetam.

ACKNOWLEDGEMENT:

The authors extend their gratitude to ZYDUS Pharmaceuticals Ltd. and CIPLA Ltd. for providing gift samples of Brivaracetam and Fluconazole respectively. The authors are also thankful to Management, A.I.S.S.M.S College of Pharmacy for providing necessary facilities to carry out the research work.

REFERENCES:

[1] O'Neil, M.J., ed. The Merck Index: An Encyclopedia of Chemicals, Drugs, and Biologicals, 15th ed. New Jersey: Merck, 2013.

[2] Pankaj Bhamare, Rupal Dubey, Neeraj Upmanyu, Pothuvan Umadoss. A simple HPLC Method for In-Vitro Dissolution study of Brivaracetam in Pharmaceutical Dosage Form. Asian Journal of Pharmaceutical Analysis. 2021, 11(1): 1-8.

[3] Gillard M, Fuks B, Leclercq K, Matagne A. Binding characteristics of Brivaracetam, a selective, high affinity SV2A ligand in rat, mouse and human brain: relationship to anti-convulsant properties. European Journal of Pharmacology. 2011 August 16; 664 (1-3): 36-44.

[4] Mhaske D.V, Mali N.V. HPLC Studies on degradation behaviour of Brivaracetam and development of validated stability-indicating HPLC Assay method. International Journal of Scientific Research and Management. 2016 Sept; 4(3): 43-57.

[5] Imam Pasha S, Murali Balaram Varanasi, Ibrahim Mohammed. Chromogenic spectrophotometric estimation of Brivaracetam in bulk drug and it's formulation with FolinCiocalteu reagent. IOSR Journal of Pharmacy. 2017; 7(12): 44-48.

[6] Mula M. Brivaracetam for the treatment of epilepsy in adults. Expert Review Neurotherapeutics. 2014, 14: 361-5.

[7] Assessment report: Briviact, International non-proprietary name: Brivaracetam Procedure No. EMEA/H/C/003898/0000. European Medicines Agency (EMA), 19 November 2015 EMA/CHMP/822086/2015.

[8] K.V.L.D. Spandana, et.al. Analytical Method Development and Validation of Brivaracetam in bulk and pharmaceutical dosage form by RP-HPLC method. IOSR Journal of Pharmacy. 2021, 11(05): 26-33.

[9] Aalapati, Kiran K, Singh, Amit; Patnaik, Ranjana S. Method development and validation of a novel UHPLC coupled with MS/MS system for the estimation of Brivaracetam in human (k2edta) plasma samples and its application to pharmacokinetic study. Current Pharmaceutical Analysis. 2022 June, 5(9): 504-512.

[10] D. Atul Vasanth, B. Rajkamal. A validated LC-MS/MS method for pharmacokinetic study of brivaracetam in healthy rabbits. International Journal of Pharmacy and Pharmaceutical Sciences. 2018, 10(2): 24-29.

[11] International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use, ICH Harmonised Guideline. Bioanalytical Method Validation and Study Sample Analysis M10. Final Version Adopted On 24 May 2022, 1-23.