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DEVELOPMENT OF A NEW, RAPID AND SENSITIVE HPTLC-DERIVATIZATION METHOD FOR ESTIMATION OF BRIVARACETAM IN BULK AND PHARMACEUTICAL DOSAGE FORM

Pankaj Bhamare, Rupal Dubey*, Neeraj Upmanyu, Bhaskar Kumar Gupta, Umadoss Pothuvan

Faculty of Pharmacy School of Pharmacy and Research, People's University, Bhopal, Madhya Pradesh, India 462037.

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***Corresponding Author**
Dr. Rupal Dubey
Faculty of Pharmacy School of Pharmacy and Research, People's University, Bhopal, Madhya Pradesh, India 462037.

ABSTRACT

Brivaracetam is a racetam derivative of levetiracetam. The official High performance thin layer chromatography (HPTLC) method for Brivaracetam has not been published yet. A new, simple, accurate, and precise high-performance thin-layer chromatographic method has been established for analysis of Brivaracetam in tablet formulations. Standard and sample solutions of Brivaracetam were applied to precoated silica gel G 60 F_{254} HPTLC plates and the plates were developed with Toluene: methanol: triethylamine in the ratio (4:1:0.5 *v/v/v*), as mobile phase. Ninhydrin solution was used for derivatization purpose. Ultra-Violet detection was performed densitometrically at 278 nm. The percentage relative standard deviation for precision and accuracy of the method was found to be less than 2%. The retention factor of Brivaracetam was 0.45. The linear range was 1000–6000 ng/spot for Brivaracetam; the correlation coefficient, r^2, was 0.9988. The method was validated in accordance with the requirements of International Conference on Harmonization guidelines and was shown to be suitable for quantitative estimation of Brivaracetam. The method was successfully used for determination of the drug in tablets. Tablet excipients did not interfere with the high-performance thin-layer chromatographic analysis.

KEYWORDS: Epilepsy, Brivaracetam, Chromatographic separation, Validation.

INTRODUCTION

The chemical name of brivaracetam is (S)-2-((R)-2-Oxo-4-propylpyrrolidin-1-yl) butanamide. Brivaracetam is a racetam derivative of levetiracetam used in the treatment of partial-onset seizures. Synaptic vesicle glycoprotein 2A is a specific binding site for Brivaracetam, which is having 20 times higher affinity than levetiracetam.[1-2] It is available under the brand name Briviact made by Union Chimique Belge (UCB). Briviact received FDA approval in February 2016. Very few analytical methods are available in literature regarding brivaracetam, few research reveals pharmacokinetics and metabolism of ^{14}C-brivaracetam, metabolism studies of brivaracetam and gemfibrozil, clinical trials of adjunctive brivaracetam for refractory partial onset seizures, identification of drug metabolites in human plasma or serum integrating metabolite prediction, by LC–HRMS methods which are reported for the brivaracetam.[3-9] HPTLC method using derivatization has not been published yet for quantitative estimation of Brivaracetam.

We have developed a rapid, selective and sensitive HPTLC method of analysis. Regulatory requirements for the identification, quantification, and control of impurities in drug substances and their formulated products are now being explicitly defined, particularly by the International Conference on Harmonization (ICH).[10-11] From the preceding details it is apparent that a validated method is required to be developed which would provide determination of brivaracetam and also provide an indication of brivaracetam stability.

HPTLC is a sophisticated instrumental technique based on the full capabilities of thin layer chromatography, the advantages of automation, scanning, full optimization, selective detection principle, minimum sample preparation, hyphenation, etc. enable it to be a powerful analytical tool for chromatographic information of complex mixtures of inorganic, organic, and biomolecules.[12] HPTLC is a powerful analytical technique having wide range of applications such as phytochemical and biomedical analysis, herbal drug quantification, active ingredient quantification, fingerprinting of formulations, and check for adulterants in formulations.[13,14]

HPTLC is a concept that includes a widely standardized methodology based on scientific facts as well as the use of validated methods for qualitative and quantitative analysis.[15] HPTLC meets all quality requirements for today's analytical labs, to increase the resolution and to allow more accurate quantitative measurements.[16] HPTLC is also an ideal screening

tool for adulterations and is highly suitable for evaluation and monitoring of cultivation, harvesting, and extraction processes and testing of stability.[17]

In HPTC, Derivatization method can be defined as a procedural technique that primarily modifies an analyte's functionality in order to enable chromatographic separations. Derivatization can be performed either by immersing the plates or by spraying the plates with a suitable reagent.[18-21] Optimization of Mass spectrometry parameters for a particular molecule can be optimized using TLC. LC–MS and TLC–MS are the complimentary analytical techniques.[22] With the advancements in the stationary phases and the introduction of densitometers as detection equipment, the technique achieves for given applications a precision and trueness comparable to high performance liquid chromatography.[23] In quantitative evaluation during HPTLC analysis, Scanning has been done by two methods i.e. Slit Scanning and Video Scanning.[24]

The main objective of this work is to establish and validate a simple accurate and reproducible method for quantitative TLC analysis of brivaracetam in bulk and tablet dosage form as per ICH guidelines in continuation with the present research work (25). Novelty of proposed method is in application of derivatization methodology for the rapid estimation of Brivaracetam in finished pharmaceutical product using TLC-Densitometry. For chemical structure of Brivaracetam refer **Figure 1**.

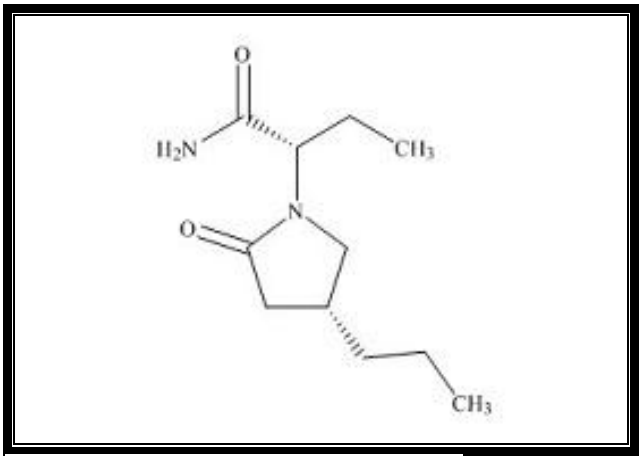

Figure 1: Chemical structure of brivaracetam.

METHODOLOGY

Selection of solvent

Methanol was selected as the common solvent for dissolving brivaracetam.

Selection of stationary phase

Identification and determination of drug was performed on (10 cm x 10 cm, layer thickness of 0.2mm, E-Merck, Darmstadt, Germany) TLC aluminium plates precoated with silica gel 60 F_{254}.

Selection and Optimization of the mobile phase

During mobile phase optimization, initially, toluene was used as a single component mobile phase (on the basis of their polarity) for resolution of the spot. Later, the mixtures of solvents were used for spot separation purpose. In case of toluene, peak was not resolved. Using toluene and methanol (in the ratio of 4.5:0.5 *v/v*) tailing was observed with R_f of 0.32 for Brivaracetam. Later mobile phase ratio for toluene and methanol, has been changed to 3.5:1.5 *v/v*, result shows slight tailing with high R_f of 0.61. The band was developed in mixture of toluene and methanol in the ratio of 4:1 *v/v*. In this mobile phase the drug was separated with good resolution but slight tailing was observed. Hence, to reduce the tailing, triethylamine was added in the solvent system. Thus, the final mobile phase consisted of Toluene: Methanol: Triethylamine in the ratio of 4:1:0.5 *v/v/v*, spot has been resolved with R_f of 0.45 for Brivaracetam.

Preparation of ninhydrin solution (For derivatization)

0.2% w/v ninhydrin in methanol was used for derivatization purpose.

Preparation of standard solution

An accurately weighed quantity of 10mg brivaracetam was transferred to 10ml volumetric flask, dissolved in methanol and volume was made up to mark with the same solvent to obtain concentration 1000ng/µL.

Derivatization methodology

Derivatization of brivaracetam involved 10 mL of standard stock solution of brivaracetam by dissolving 10 mg of drug in 10 mL methanol and mixed with 10 mL of 0.2% ninhydrin solution. Heat the resultant mixture for 20 minutes at temperature 60°C on water bath and further use this mixture for method development and validation.

Detector and Detection wavelength selection

Stock solution of the drug was prepared in methanol and spectral scanning was performed using TLC Scanner 3. The UV spectrum of the drug showed wavelength at 278nm, and the drug showed better sensitivity; so, it was used for detection of the drug.

Instrumentation and Chromatographic conditions

Densitometry scanning was performed on Camag TLC Scanner equipped with winCATS software version 1.3.0 at 278nm. The source of radiation utilized was deuterium lamp. All the that were used are of analytical reagent grade, purchased from Merck Chemicals Limited, Worli, Mumbai, India. Water used for analysis purposes was of HPLC grade (filtered through an ELGA PURELAB classic water purification system). Water bath used for analysis was of Thermo Fisher Scientific (Precision water bath). All weighing was done on Shimadzu balance (Model: AY-120), while Ultrasonicator used during analysis was of Trans-O-Sonic make.

The samples were spotted in the form of bands of width 6mm with 100 µl sample syringe on pre-coated silica gel aluminium plates 60 F_{254} as a stationary phase (having dimensions of 10cm x 10cm with 250mm thickness, E-Merck, Darmstadt, Germany) using Camag Linomat 5 (Switzerland) sample applicator. The plates were pre-washed with methanol and activated at 110˚C for 15 min, prior to the chromatography. The space between two bands was maintained at 14mm. The slit dimension was kept at 6mm×0.45mm. The mobile phase consisted of Toluene: Methanol: Triethylamine (in the ratio of 4:1:0.5 *v/v/v*). Linear ascending development was carried out in 20cm×10cm twin trough glass chamber. The optimized chamber saturation time for mobile phase was 25 minutes. Evaluation was performed using peak area with linear regression. A typical chromatogram of brivaracetam was shown in **Figure 2.**

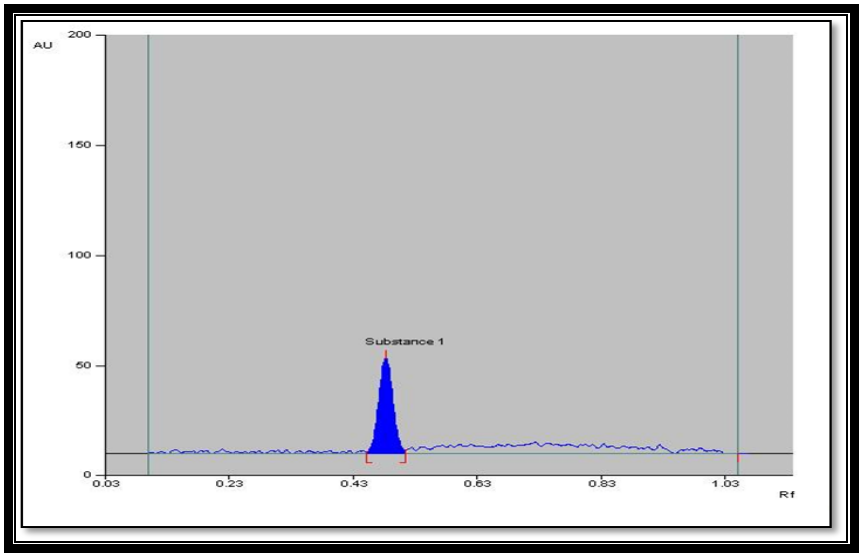

Figure 2: Typical HPTLC densitogram for brivaracetam.

Linearity study

Aliquots of derivatized solutions 2, 4, 6, 8, 10 and 12µL of brivaracetam was applied on TLC plate with the help of microlitre syringe, using Linomat 5 sample applicator to obtained the concentration of 1000, 2000, 3000, 4000, 5000 and 6000ng per band. The standard curves were evaluated for within a day and day-to-day reproducibility. Each experiment was repeated five times. For evaluation of linearity, peak area and concentration were subjected to least square regression analysis to calculate calibration equation and correlation coefficient (r^2). The results are shown in **Table 1 and Figure 3, 4.**

Table 1: Linearity studies (Linearity and Range).

Concentration of brivaracetam (ng/band)	Peak area (Mean ± SD)	% RSD (n = 6)
1000	784.2 ± 9.07	1.24
2000	884.12 ± 13.66	1.54
3000	976.48 ± 7.93	0.81
4000	1083.12 ±14.28	1.33
5000	1161.92 ± 11.66	0.95
6000	1255.86 ± 10.29	0.80

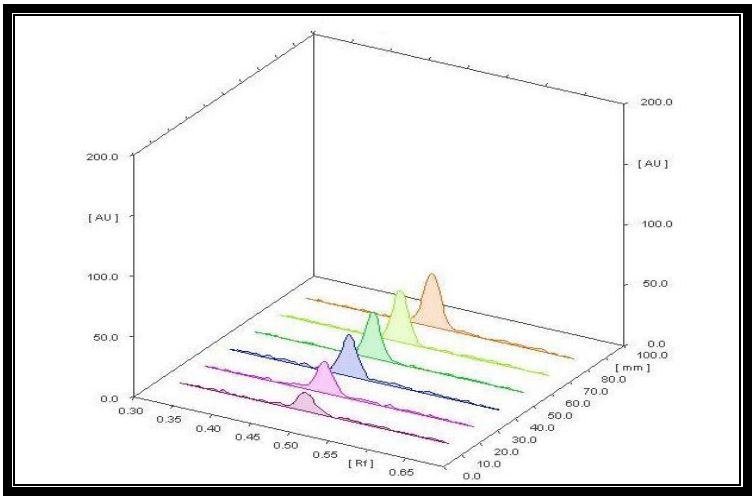

Figure 3: 3-Dimensional overlay plot for brivaracetam.

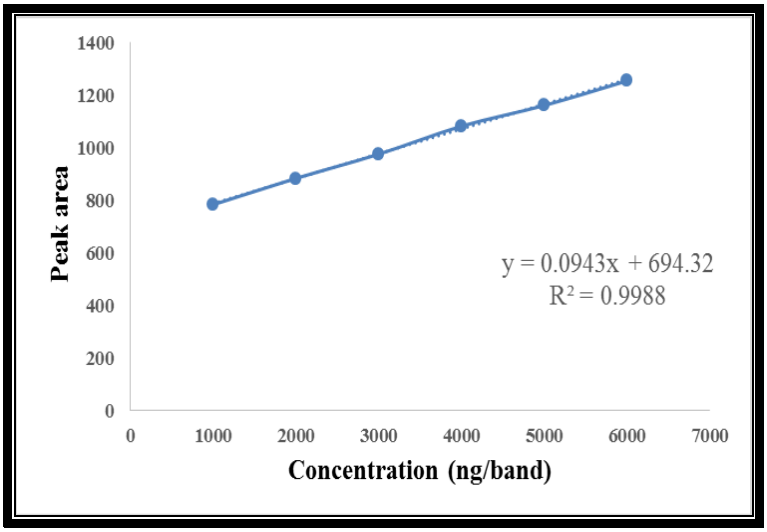

Figure 4: Calibration curve for brivaracetam (Linearity and Range).

Analysis of bulk material

Accurately weighed 10mg of brivaracetam was transferred into 10mL volumetric flask containing 5mL of methanol, shaken manually and volume was adjusted to mark using same solvent. Aliquots, 10mL and add 10 ml ninhydrin solution, an appropriate volume 8µL was applied on TLC plate to obtain concentration 4000ng/band. The drug concentration was determined using regression equation and the results are shown in **Table 2.**

Table 2: Analysis of brivaracetam in bulk material.

Component	Amount taken (ng/band)	Amount found ± SD	Amount found (%)	%RSD (n = 6)
Brivaracetam	4000	4018.699 ± 61.52	100.46	0.86

Analysis of marketed tablet formulation

The proposed validated HPTLC method was applied for the determination of brivaracetam in Briviact tablets (marketed sample). Satisfactory results were obtained with respect to the label claim. Ten Brivaracetam tablets were accurately weighed and ground into fine power. An accurately weighed quantity of tablet powder equivalent to 10mg was transferred into10mL of volumetric flask containing 5ml of methanol, shaken manually for 20 minutes and volume was made up to the mark using methanol. It was then filtered using Whatmann filter paper no. 41, add 10mL ninhydrin solution heat for 20min. An appropriate volume 8µL was applied on TLC plate, developed and scanned. The concentration was determined using linear regression equation. The results are shown in **Table 3.**

Table 3: Analysis of Brivaracetam in marketed tablet formulation.

Component	Amount taken (ng/band)	Amount found ± SD	Amount found (%)	%RSD (n =6)
Brivaracetam	4000	3967.59 ± 2.88	99.18	1.83

Analytical method validation

The developed method was validated as per the International Conference on Harmonization (ICH) guidelines with respect to linearity and range, precision, accuracy, limit of detection and limit of quantification.

Precision

Precision studies were performed as repeatability, Intra-day and Inter–day precision.

The repeatability of sample application was performed using concentration 4000ng/band. The results are shown in **Table 4a.**

Table 4a: Repeatability studies.

Concentration (ng/band)	Amount Found ± S.D (n = 6)	Amount Found (%)	% RSD (n = 6)
4000	4002.04 ± 2.89	100.01	1.68

The intra-day precision study was performed by analysing three different concentrations 3000, 4000 and 5000ng/band for three times within a day.

For inter-day precision studies same concentration were analysed for three different days over a period of week. The results are shown in **Table 4b.**

Table 4b: Precision studies.

Concentration (ng/band)	Intra-day precision		Inter-day precision	
	Amount found ± SD (n = 3)	% RSD	% Amount found ± SD (n = 3)	% RSD
3000	2982.6 ± 11.28	1.15	2990.7 ± 10.33	1.06
4000	4013.5 ± 19.71	1.83	4003.6 ± 11.10	1.07
5000	4966.1 ± 16.70	1.43	4998.5 ± 03.32	0.26

Accuracy (Recovery)

The accuracy of the method was assessed by recovery experiment. It was performed at three different concentration levels i.e. at 80%, 100% and 120% level. To the pre-analysed sample solution 2000ng/band, a known amount drug standard was over spotted at three different concentration levels and re-analysed using proposed method. Results are shown in **Table 5.**

Table 5: Accuracy studies (Recovery).

Drug	Initial amount (ng/band)	Amount added (ng/band)	Recovery (%)	%RSD (n = 3)
Brivaracetam	2000	80	98.25	0.15
	2000	100	100.02	0.13
	2000	120	99.40	0.23

Specificity

The specificity of the method was ascertained by analysing standard brivaracetam and brivaracetam extracted from tablets. The band for brivaracetam in sample was confirmed by comparing the R_f and spectra of the band with those obtained from standard. The peak purity of brivaracetam was assessed by comparing spectra acquired at three different positions on the band, i.e. peak -start (S), peak- apex (M), and peak- end (E). The mobile phase designed for the method resolved the drug very efficiently. The R_f value of Brivaracetam was found to be 0.45±0.03. The peak purity of brivaracetam was assessed by correlating the spectra of brivaracetam extracted from Tablet and brivaracetam standard at the peak-start (S), peak-apex (M) and at the peak-end (E) positions of the band. Correlation between these spectra indicated purity of brivaracetam peak {correlation *r*(S, M) = 0.996 *r* (M, E) = 0.998{. The peak purity spectra were shown in **Figure 5.**

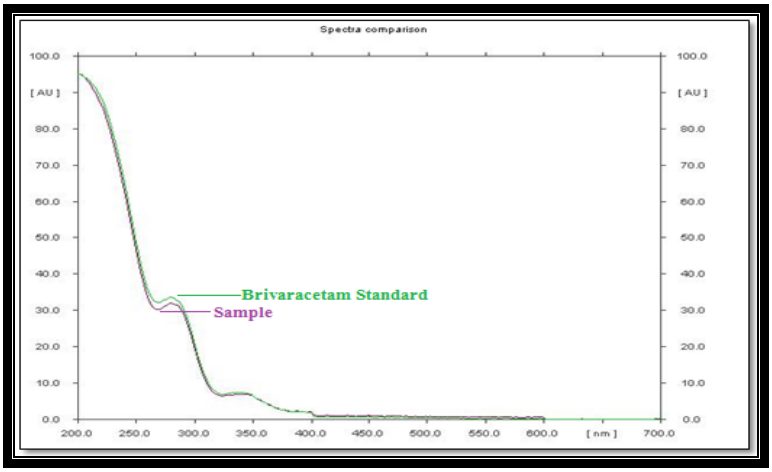

Figure 5: Overlay of peak purity spectra for brivaracetam extracted from Tablet and Brivaracetam standard.

Sensitivity

The sensitivity of measurement of brivaracetam by the use of the proposed method was determined in terms of the LOD and LOQ. The LOD and LOQ were calculated using equation LOD = 3.3 X N/B and LOQ = 10 X N/B; Where, 'N' is standard deviation of the peak areas of the drugs (n=3), taken as a measure of noise, and 'B' is the slope of the corresponding calibration curve.

To the study the LOD and LOQ of brivaracetam, lower part of the linearity curve was considered. Different concentrations i.e. 1000, 1200, 1400, 1600, 1800 and 2000ng/band were applied on the TLC plate developed and scanned. The standard deviation of peak areas was determined. For determination of LOD and LOQ slope of corresponding calibration curve was considered. Limit of detection was found to be 182.6 ng/band and Limit of quantification was found to be 552.8 ng/band for Brivaracetam.

Ruggedness (Intermediate precision)

Ruggedness of the proposed method was studied by two different analysts using same experimental and environmental conditions. The 4000ng/band Brivaracetam was applied on TLC plate developed and scanned. Procedure was repeated for 6 times. The results are shown in **Table 6.**

Table 6: Ruggedness studies (Intermediate precision).

Drug	Amount in (ng/band)	% Amount found (n = 6)		% RSD	
		Analyst (I)	Analyst (II)	Analyst (I)	Analyst (II)
Brivaracetam	4000	100.55	99.18	0.63	1.72

Robustness

The robustness of an analytical procedure is defined as a measure of its capacity to remain unaffected by small but deliberate variations in experimental parameters, providing an indication of the analytical method's suitability and reliability during normal use. Some important parameters for testing the robustness of HPTLC methods include small but deliberate variations in mobile phase composition, mobile phase volume, development distance and saturation time. Robustness was studied at the concentration level of 4000 ng/band. In this study, Initially, mobile phase ratio was, Toluene: Methanol: Triethylamine in the ratio of 4:1:0.5 *v/v/v*, few parameters like mobile phase composition (± 0.5mL), mobile phase volume (± 2mL), solvent migration distance in chromatography (± 0.5cm) and duration of saturation time (± 5minutes), were changed deliberately and the effects on analytical results were examined. The method was found to be unaffected by small changes with % RSD for all the parameters less than 2% indicating that proposed analytical method is robust. This robustness study helps to establish system suitability parameters to make sure the validity of the entire system is maintained throughout implementation and use. The SD and % RSD values of the peak areas were calculated. The results of the studies are shown in **Table 7.**

Table 7: Robustness study.

Robustness parameter (Deliberate change)	Standard deviation of peak area	% R.S.D. (n = 3)
Mobile phase composition (± 0.5mL)	41.48	1.02
Mobile phase volume (± 2mL)	50.28	1.23
Solvent migration distance (± 0.5cm)	72.81	1.55
HPTLC Plate saturation time (± 5min)	70.59	1.08

Ethical consideration

Ethical consideration has been completely observed by the authors with respect to the research. The present study not required any investigations/interventions to be conducted on the human subjects/patients; project does not involve any drug trial on animals.

Conflict of interest

There is no conflict of interest among the authors.

CONCLUSION

It is found that the developed HPTLC technique is quite simple, authentic, reproducible, definite sensitive, favourable, economical and specific. It can become efficient analytical tool

for routine quality control of brivaracetam in bulk drug and its pharmaceutical dosage forms. The proposed TLC densitometry method was validated as per ICH guidelines. The standard deviation, % RSD and standard error calculated for the method are low, indicating a high degree of precision of the method. The results of the recovery studies performed show a high degree of accuracy of the proposed method. The method can be used to determine the purity. Statistical tests indicate that the proposed HPTLC method appear to be suitable for routine determination of brivaracetam. Hence, it can be concluded that the developed TLC-densitometric method of analysis is accurate, precise, and selective and can be employed successfully in the estimation of Brivaracetam in bulk and in pharmaceutical formulation.

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