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HPLC Studies on Degradation Behavior of Brivaracetam and Development of Validated Stability – Indicating HPLC Assay Method

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ABSTRACT

This research work paper describes the degradation of brivaracetam under different ICH prescribed conditions (Hydrolysis, oxidation, photolysis and sunlight) and development of a stability-indicating reversed-phase HPLC assay for separation of the standard drug from its degradation products. The drug was found to be degraded in acidic medium, basic conditions, under oxidative stress and in presence of sunlight. Separation of the drug and the degradation products was carried out successfully on a C_{18} column using methanol: water: acetonitrile in the ratio of (30:10:60 *v/v*). The wavelength used for detection was 242 nm. The method was validated with respect to linearity, precision, accuracy, selectivity, specificity and ruggedness. The linear regression analysis data plot showed good linearity with r^2 = 0.999 in the concentration range of 1-6 µg/ml. The limit of detection (LOD) and limit of quantitation (LOQ) were found to be 0.1 and 0.4 µg/ ml respectively.

INTRODUCTION

The present study was aimed to establish the stability of brivaracetam (BVR) by using ICH prescribed conditions and develop a validated stability-indicating HPLC assay method. Brivaracetam (Fig. 1) chemically is a 4-n-propyl analog of levetiracetam and a racetam derivative with anticonvulsant properties [1-2]. Brivaracetam is believed to act by binding to the ubiquitous synaptic vesicle glycoprotein 2A (SV2A) [3]. Phase II clinical trials in adult patients with refractory partial seizures were promising. Positive preliminary results from stage III trials have been recorded, [4-5] along with evidence that it is around 10 times more potent for the prevention of certain types of seizure in mouse models than levetiracetam, of which it is an analog. It acts as a novel high-affinity synaptic vesicle protein 2A (SV2A) ligand, displays inhibitory activity at neuronal voltage-dependent sodium channels, data from animal models suggested potent and broad-spectrum antiepileptic activities.

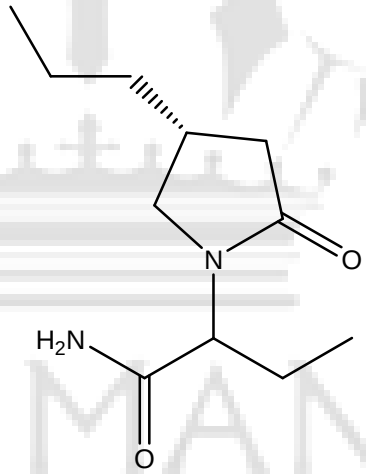

Figure 1: Structure of Brivaracetam

There is no known information in the literature about the degradation of brivaracetam. Literature survey 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 are reported for the drug [6-10].

The primary aim was to develop and validate a stability indicating HPLC method for the rapid quantitation of the drug. The present study illustrates development and validation of simple,

accurate, economical and reproducible SIM method for determination of brivaracetam by HPLC as a bulk drug. The proposed method was validated as per ICH guidelines [11-12].

MATERIALS AND METHODS

Materials:

Pharmaceutical grade brivaracetam was kindly procured from Manus Akttevva Biopharma LLP, Ahmedabad, India. It was certified to contain 99.85% (w/w) on a dry basis and was used further without purification. All chemicals and reagents of analytical grade were purchased from Merck Chemicals, Mumbai, India.

Instrumentation:

The HPLC system (Jasco Corporation, Tokyo, Japan) consisted of a Pump (model Jasco PU-2080 Plus) along with manual injector sampler programmed at 20 µl capacity per injection was used. The detector consisted of UV/ VIS (model Jasco UV 2075). LC separations were performed on a Hypersil Gold C_{18} analytical column Dim. (mm) 250 × 4.6, Particle Sz. (µ) 5 (Thermo Scientific, Waltham, USA). Data was integrated using Jasco-Borwin version 1.5, LC-Net II/ADC system. The mobile phase consisted of a mixture of methanol: water: acetonitrile in the ratio of (30:10:60 *v/v*). The mobile phase was degassed and filtered by passing through a 0.45 mm pore size membrane filter (Millipore, Milford, MA, USA) prior to use. The flow rate was 1 ml/min. All determinations were performed at ambient temperature with a detection wavelength of 242 nm.

Preparation of Standard Solution

A standard stock solution containing 1.00 mg/ml of brivaracetam was prepared in acetonitrile. The working standard was prepared by diluting the above stock solution in the mobile phase to reach a concentration range of 1-10 µg/ml. The stock solution was stored at 2–8^{o}C protected from light.

Degradation Studies

Acid and Base Induced Degradation:

Acid decomposition studies were performed by exposing the solution of drug to 0.01N hydrochloric acid refluxed at 50°C for 15 mins. The studies in alkaline conditions were carried out in 0.01N sodium hydroxide and the solution was kept at a room temperature. The resultant solutions were diluted in the mobile phase and injected into the Hypersil Gold C_{18} analytical column and the chromatogram was observed.

Hydrogen Peroxide-Induced Degradation:

To study hydrogen peroxide-induced degradation, the sample was exposed to 3% hydrogen peroxide at room temperature for a period of 2 hours. The resultant solution was diluted in the mobile phase and injected into the Hypersil Gold C_{18} analytical column and the chromatogram was observed.

Photochemical Degradation:

The photochemical stability of the drug was studied by exposing the stock solution (1000 µg/ml) to direct sunlight for 48 hrs. The resultant solution was diluted in the mobile phase and injected into the Hypersil Gold C_{18} analytical column.
The drug solution was also kept in the Photostability chamber for 48 hours. The diluted solution was injected in the column to observe the degradation pattern.

Separation Studies:

The mixed standard stock solution was diluted in the mobile phase to a concentration containing 10 µg/ml of brivaracetam. Then, the stock solution is injected into the Hypersil Gold C_{18} analytical column. Different ratios of methanol: water and acetonitrile were tried. Methanol-water resulted in wider peaks. Thus methanol: water: acetonitrile was selected as the mobile phase. Different ratios of mobile phases were tried to get a retention time which allowed sufficient separation of the standard from its degradant peaks. The optimum mobile phase was thus, found to consist of methanol: water: acetonitrile in the ratio of (30:10:60 *v/v*). The separation was carried out at ambient temperature with a flow rate of 1.0 ml/min. The drug in

presence of their degradation products was satisfactorily resolved with retention times for 7.9+0.2. Acceptable retention time (R_T), plates, asymmetry and good resolution for brivaracetam were obtained.

Validation of Method

Limit of Detection and Limit of Quantification:

Limit of detection (LOD) and limit of quantitation (LOQ) were separately determined at a signal to noise ratio of 0.1 and 0.4. LOD and LOQ were determined by using the visual method of detection. A known concentration of the sample was injected into the column and the resulting peak was observed for the lowest amount of detection and quantitation.

Linearity and Range:

The linearity of the method was studied by injecting the standard solution diluted in the mobile phase to get the concentration range of 1-6 µg/ml six times into the LC system keeping the injection volume constant. The peak areas were plotted against the corresponding concentrations to obtain the calibration graphs.

Precision:

The precision of the proposed method was evaluated by carrying out six independent assays of the test sample. RSD (%) of six assay values obtained was calculated. Repeatability studies were performed by analysis of three different concentrations by HPLC (2, 4 and 6 µg/ml for brivaracetam) six times on the same day. Intermediate precision was carried out by analyzing the samples on three different days.

Accuracy:

Accuracy of the method was carried out by applying the method to drug sample to which known amounts of standard powder corresponding to 80, 100 and 120% had been added (standard addition method), mixed and the powder was extracted and analyzed by running chromatograms in optimized mobile phase. The mixture was analyzed by the proposed method. The experiment was performed in triplicate and recovery (%), RSD (%) was calculated.

Robustness:

The robustness was studied by evaluating the effect of small but deliberate variations in the chromatographic conditions. The conditions studied were flow rate (altered by ±0.1 ml/min); mobile phase composition (Acetonitrile ±1 ml). Robustness of the method was done at three different concentration levels for HPLC (2, 4, 6 µg/ml).

RESULTS AND DISCUSSION

Selection of Scanning Wavelength:

UV spectrum of brivaracetam (Fig. 2) showed maximum absorbance at 242 nm and the same was selected as the scanning wavelength.

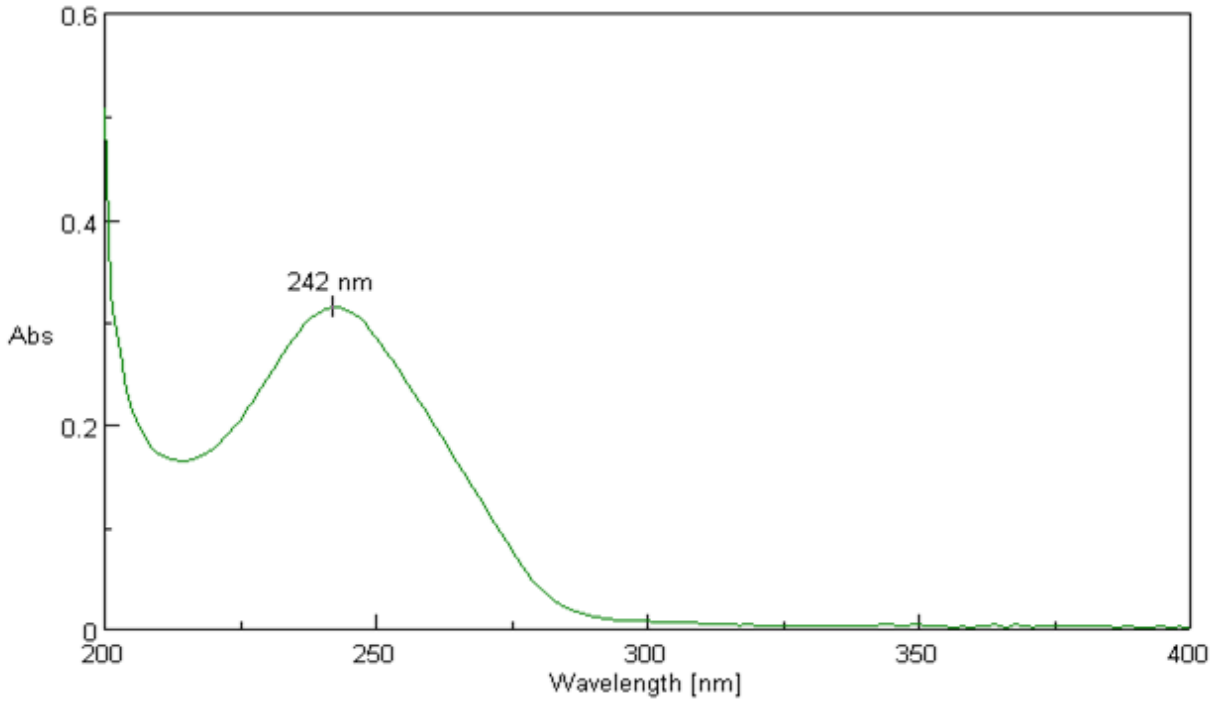

Figure 2: UV spectra for Brivaracetam

Optimization of HPLC Method:

The mixed standard stock solution was diluted in the mobile phase to a concentration containing 10 µg/ml of brivaracetam. Then, the stock solution was injected into the Hypersil Gold C_{18} analytical column. Different ratios of methanol: water and acetonitrile: water was tried. Methanol: water resulted in wider peaks. Thus methanol: water: acetonitrile was selected as the

mobile phase. Different ratios of mobile phases were tried to get a retention time which allowed sufficient separation of the standard from its degradant peaks. The optimum mobile phase was thus found to consist of methanol: water: acetonitrile in the ratio of (30:10:60 *v/v*). The separation was carried out at ambient temperature with a flow rate of 1.0 ml/min. The drug in presence of their degradation products was satisfactorily resolved with retention times for 7.9+0.2 (Fig 3). Acceptable retention time (R_T), plates, asymmetry and good resolution for brivaracetam were obtained.

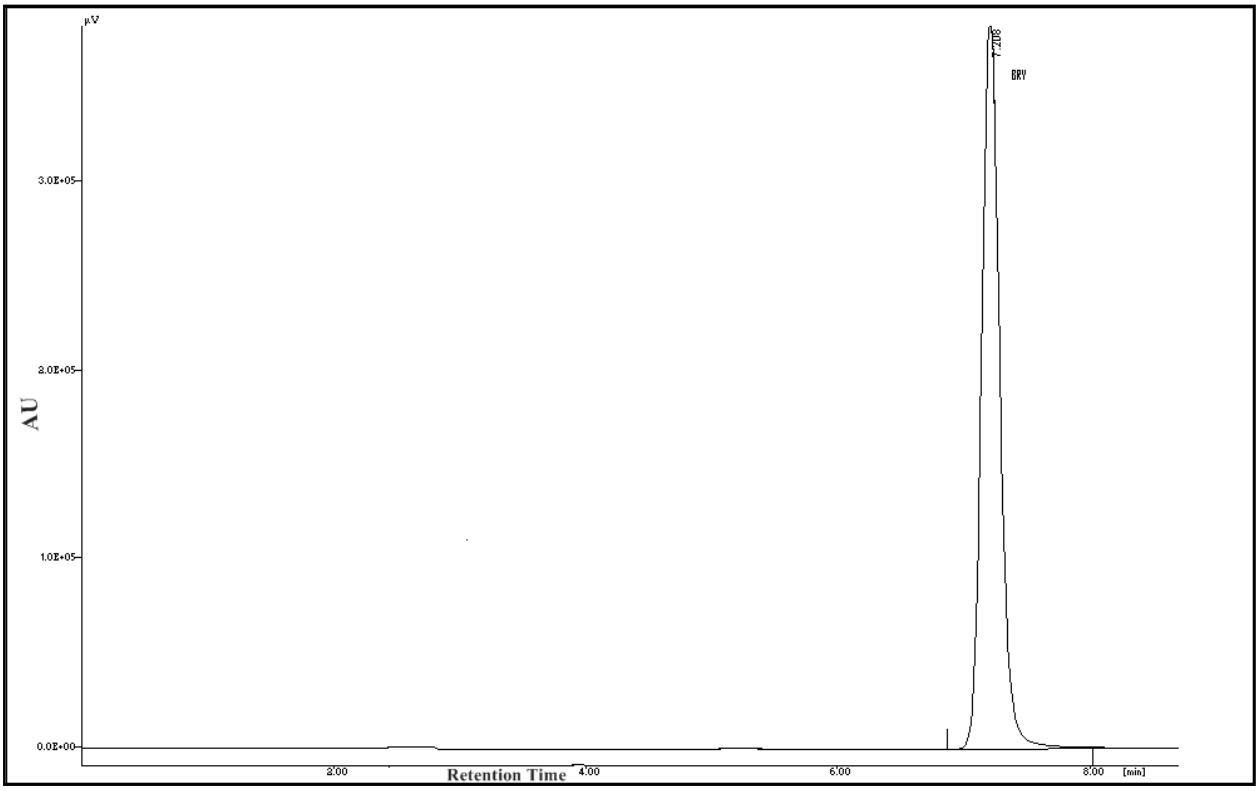

Figure 3: Chromatogram for standard drug

Forced Degradation Studies

Acid and Base Induced Degradation:

The optimized condition for acid degradation was achieved by exposing the solution to 0.01N hydrochloric acid refluxed at 50^{o}C for 15 mins. The solution was then diluted with mobile phase

and injected into the column. The following chromatogram was obtained for degradation pattern (Fig. 4). The degradation peaks were observed at 2.092 mins and 3.808 mins.

The drug was found to undergo rapid degradation in alkaline conditions. Initially, when refluxed with 0.01N sodium hydroxide, it resulted in 90% degradation of the drug. Upon reducing the degradation conditions, it gave 50% degradation with 0.01N sodium hydroxide at room temperature in 15 mins. Thus, it can be concluded that brivaracetam is unstable in alkaline conditions.

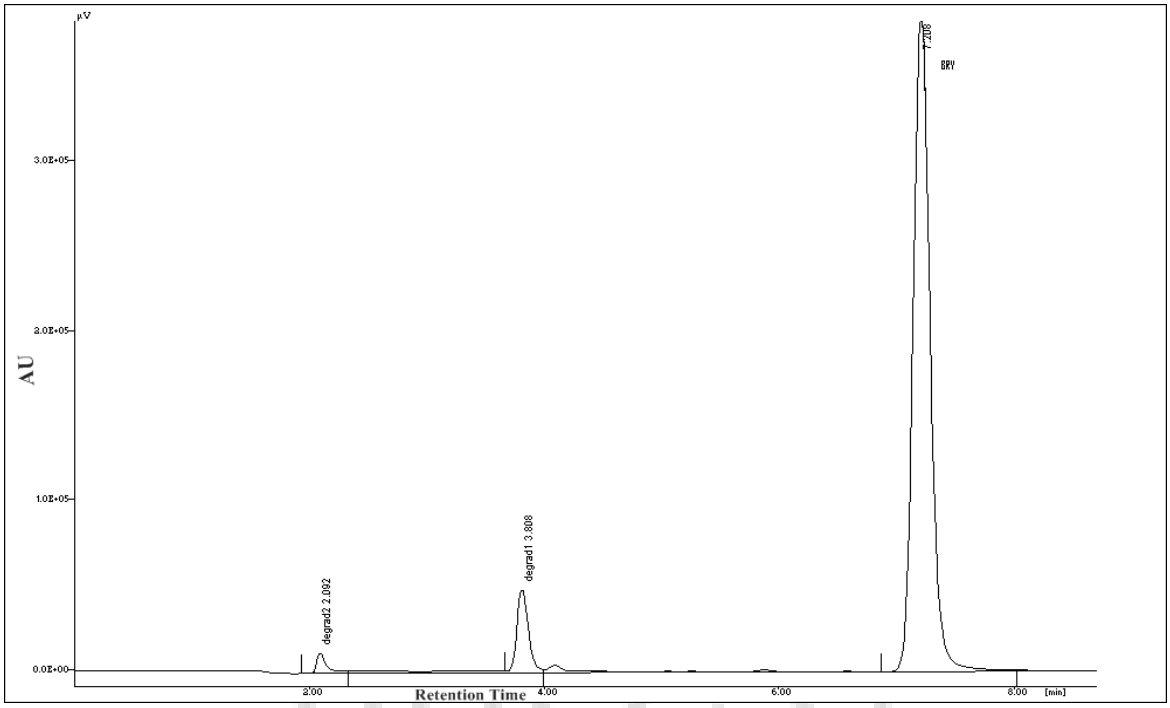

Figure 4: Chromatogram for Acid Induced Degradation

Hydrogen Peroxide-Induced Degradation:

The drug was found to be highly labile in oxidative conditions. When exposed to 3% H_2O_2 at room temperature for 2 hrs it showed a degradation of 10% with two degradation products at 3.7 mins and 4 mins (Fig. 5).

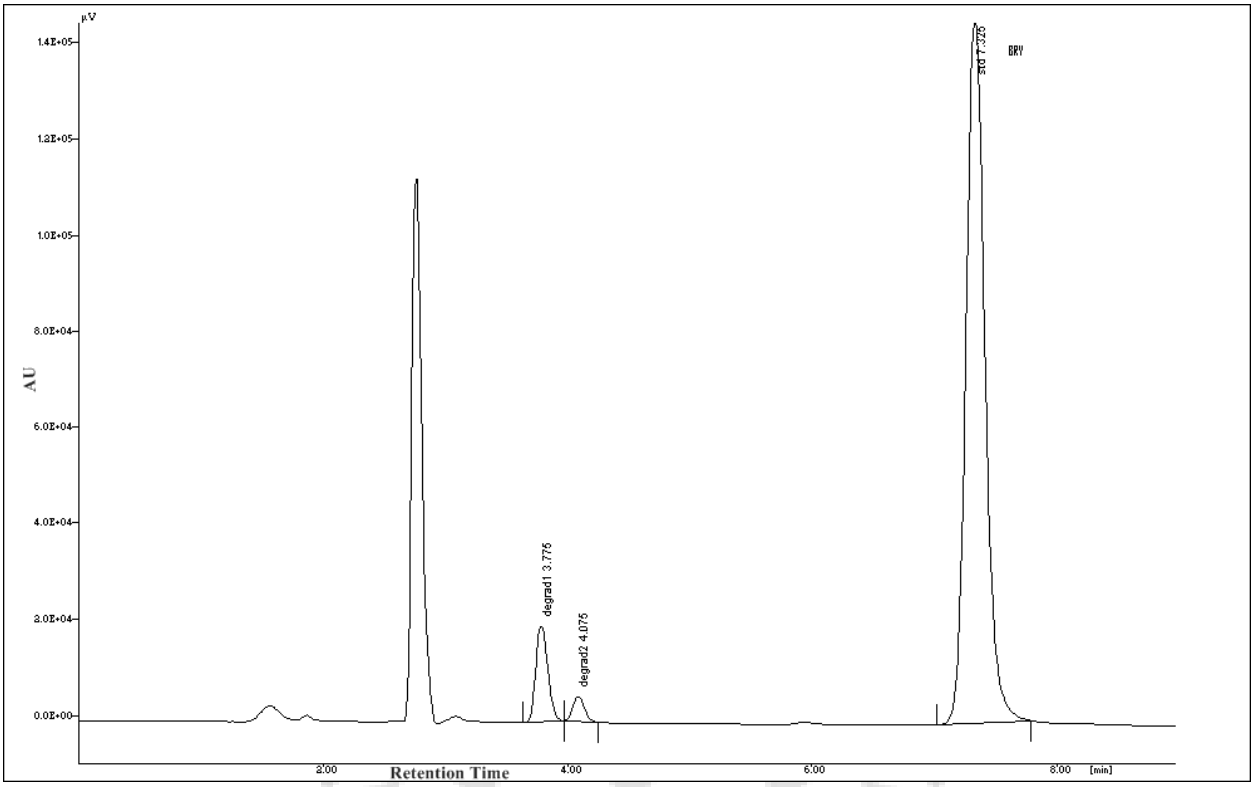

Figure 5: Chromatogram for Hydrogen Peroxide-Induced Degradation

Photolytic Induced Degradation:

The photochemical stability of the drug was studied by exposing the stock solution (1000 µg/ml) to direct sunlight for 48 hrs and the samples were collected over a time period of 1 hour. The resultant solution was diluted in the mobile phase and injected into the Hypersil Gold C_{18} analytical column. The degradation was observed after 2 hrs (Fig. 6). On exposing the drug to sunlight it showed a degradation of 22% within 48 hrs (Fig. 7). Summary of degradation is depicted in (Table 1).

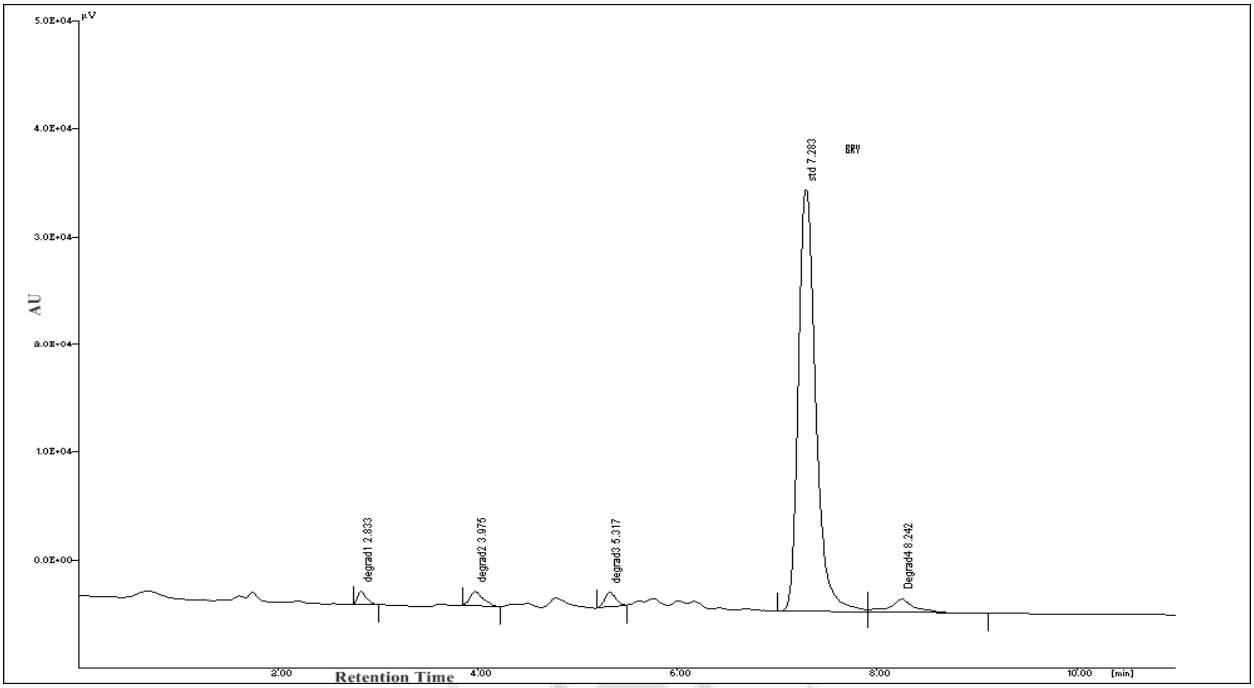

Figure 6: Chromatogram for Photolytic Degradation

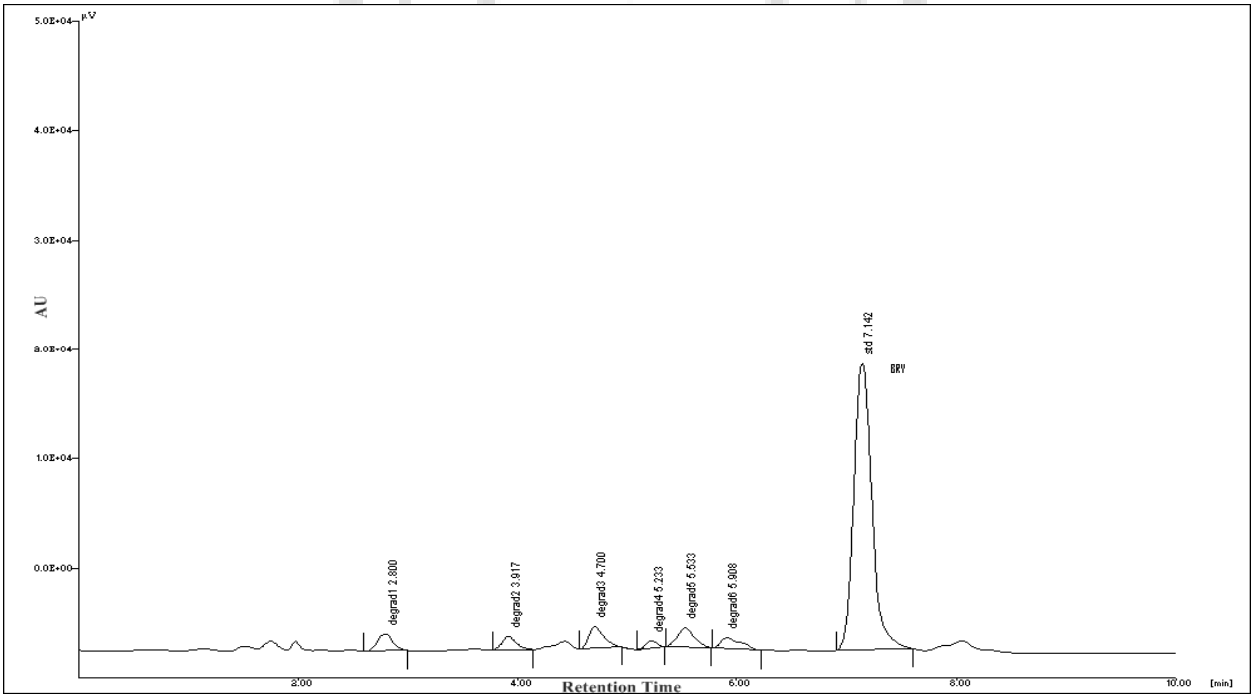

Figure 7: Chromatogram for Photolytic Degradation (Sunlight 48hrs)

Validation of the Developed Method:

Linearity and Range

Brivaracetam showed a linear response in the range of 1-6 µg/ml. The corresponding linear regression equation was y = 35879 x - 7924 with square of correlation coefficient (r^2) of 0.999. An excellent correlation existed between the peak areas and concentration of brivaracetam (Table 2). No significant difference was observed in slopes of Standard curve. Residual analysis was done to ascertain linearity.

Precision

The results of the repeatability and intermediate precision experiments are shown in (Table 3). The developed methods were found to be precise as the RSD values for repeatability and intermediate precision studies were < 2%, respectively as recommended by ICH guidelines. From the P value, it can be stated that there is no significant difference between the precision study results thus proving that the method developed is repeatable and reproducible.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

LOD and LOQ were determined by the visual detection method. LOD and LOQ of the drug were found to be 0.1 and 0.4 µg/ml respectively after studying a range of concentrations made from the stock solution.

Robustness of the method

Robustness of the method was studied by deliberately varying parameters like flow rate (±0.1 ml/min) and mobile phase composition (±1 ml).The low values of the RSD %, as shown in (Table 4) indicated the robustness of the proposed method.

Accuracy

Accuracy of the method was carried out by applying the method to drug sample to which known amounts standard powder corresponding to 80, 100 and 120% had been added (standard addition method), mixed and the powder was extracted and analyzed by running chromatograms

in optimized mobile phase. The mixture was analyzed by the proposed method. The experiment was performed in triplicate and recovery (%); RSD (%) was calculated.

CONCLUSION

The validated stability-indicating HPLC method was developed as per the recommended ICH guidelines. In this study, all different degradation conditions were carried out and the products were resolved in a single isocratic run using a simple, validated method using methanol: water: acetonitrile in the ratio of (30:10:60 *v/v*). The drug was found to degrade under acidic conditions, photochemical conditions and is highly unstable in alkaline conditions. The drug was analyzed using Hypersil Gold C_{18} analytical column Dim. (mm) 250 × 4.6, Particle Sz. (µ) 5 (Thermo Scientific, Waltham, USA).

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Table 1: Linearity Regression Data for calibration curve

Parameters	Drug
Linearity range	1-6 µg/ml
r^2	0.999
Slope	35880 ± 530.1
Intercept	-7925 + 064
Confidence limit of slope [a]	34410 to 37350
Confidence limit of intercept [a]	13660 to 2194
Sy.x [b]	2218
P value [c]	< 0.0001

[a] 95% Confidence intervals

[b] Standard deviation of residuals from the line

[c] P value is < 0.0001, considered extremely significant

Table 2: Inter-Day and Intra-Day precision of brivaracetam

Conc. µg/ml	Repeatability (n=6)			Intermediate Precision (n=6)		
	Found conc. ± SD	RSD (%)	S.E.	Found conc. ± SD	RSD (%)	S.E.
2	1.92±0.005	0.26	0.002	2.008±0.003	1.49	0.0012
4	4.05±0.012	0.29	0.004	4.105±0.015	0.36	0.006
6	5.85±0.09	1.53	0.036	5.95±0.003	0.5	0.0012

Table 3: Robustness of the method

Level	Factor	Retention Time	SD of Peak Area	% RSD
Change in Conc. of Acetonitrile in Mobile Phase (n=3)				
-1	44	7.93	0.12	1.51
0	45	7.90	0.09	1.13
1	46	7.88	0.1	1.26
Change in Flow Rate (n=3)				
-1	0.9	7.86	0.05	0.63
0	1	7.90	0.08	1.01
1	1.1	7.92	0.07	0.88

Table 4: Accuracy study of the method

Drug	Amount added in mg (%)	Total amount (mg)	For HPLC (n= 6)		
			Amount Recovered (mg) ± SD	RSD (%)	Recovery (%)
Brivaracetam	8 (80)	18	18.03±0.2	1.82	101.66
	10 (100)	20	19.63±0.09	0.76	98.16
	12 (120)	26.4	26.05±0.22	1.66	100.37

Table 5: Summary of Degradation Studies:

Conditions	Number of Peaks	Retention Time
Acidic (0.01N HCl)	Peak 1 Peak 2	2.092 3.808
Oxidation (3% H_2O_2)	Peak 1 Peak 2	3.792 4.092
Photolytic	Peak 1 Peak 2 Peak 3 Peak 4	2.833 3.975 5.317 8.242
Photolytic (Sunlight)	Peak 1 Peak 2 Peak 3 Peak 4 Peak 5 Peak 6	2.800 3.917 4.700 5.233 5.533 5.908