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A RAPID LIQUID CHROMATOGRAPHIC ESTIMATION OF BRIVARACETAM AND ITS RELATED IMPURITIES

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ABSTRACT

A rapid High Performance Liquid Chromatography (HPLC) method for assay of Brivaracetam in Brivaracetam Tablets (10mg and 100mg) has been developed and validated by using In-house methodology. Inertsil ODS 3V, 150mm x 4.6mm, 5µ HPLC column was used with variable wavelength detector (VWD) and photo diode array detector (PDA). Chromatographic elution was carried out using a mixture of 0.1%v/v Trifluoroacetic acid solution and acetonitrile (60:40 v/v). The wavelength used for detection was 210nm. The method was found to be precise, linear (with r^2 = 0.99981), accurate, specific, robust, rugged, stability indicating and suitable for its intended use. Brivaracetam was found to be degraded in greater extent under basic condition. This method was found to be suitable for identification purpose.

Key words: HPLC, Brivaracetam, Degradation, Validation.

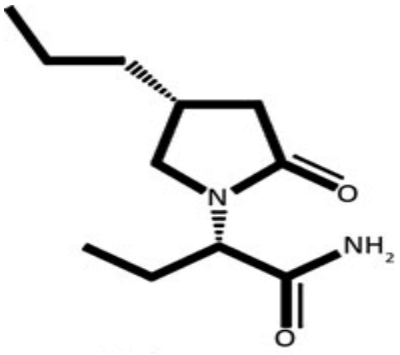
INTRODUCTION

The present study was aimed to develop and validate rapid HPLC method of analysis for Brivaracetam and it's related impurities by using ICH guidelines. Brivaracetam is a racetam derivative of levetiracetam used in the treatment of partial-onset seizures. Brivaracetam binds SV2A (Synaptic vesicle glycoprotein 2A modulator, Epoxide Hydrolase Inhibitor) with 20 times higher affinity than levetiracetam [1-2]. It is available under the brand name Briviact made by UCB. Briviact received FDA approval in Feb 2016. Literature survey reveals very few analytical methods are available for Brivaracetam, it also 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 [3-7]. As compared to existing analytical methods, we have developed a rapid, selective and sensitive HPLC method of analysis (which can be used for determination of assay as well as it's related substances).

The objective of this study is to demonstrate that the proposed method is suitable for its intended use. Along with the method development, this validation study covers following parameters,

a) Precision (System precision, Method precision, Intermediate precision i.e. Ruggedness)
b) Specificity
c) Forced degradation
d) Stability in analytical solution
e) Linearity
f) Accuracy
g) Range
h) Filter paper selection study
i) Robustness

Fig 1. Structure of Brivaracetam



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IUPAC Nomenclature: (2S)-2-[(4R)-2-oxo-4-propylpyrrolidin-1-yl] butanamide)

The proposed method of analysis was validated as per ICH guidelines. In analytical method validation study following parameters were covered [8-10].

a) Precision (System precision, Method precision, Intermediate precision i.e. Ruggedness),
b) Linearity and range, Accuracy
c) Filter paper selection study, Solution stability
d) Specificity, Forced degradation
e) Robustness

Materials

Sample for Brivaracetam (Brivaracetam Tablets) was procured from UCB Pharma SA, Brussels, Belgium. All the chemicals and analytical grade reagents were purchased from Merck Chemicals, Mumbai, India.

Instrumentation

Agilent 1200 Series HPLC was equipped with pump, injector and PDA Detector. Liquid chromatographic seperations were performed on Inertsil ODS 3V, 150mm x 4.6mm, 5µ HPLC column. All the determinations were performed at ambient temperature with run time of 5 minutes, 10µl injection volume, 1ml/minute flow rate and column oven temperature of 30°C. The wavelength used for detection was 210nm. Mobile phase consisted of a mixture of 0.1%v/v Trifluoroacetic acid solution and acetonitrile (60:40 v/v). Mobile phase was degassed and filtered by passing through a 0.45µ Ultipor Nylon 6, 6 membrane filter (Pall Lifesciences). Mettler Toledo Analytical balance, hot air oven of Thermolab Scientific Equipments and Newtronics Photostability chamber were used.

Preparation of Brivaracetam Standard Solution

Weighed accurately about 50mg of Brivaracetam working standard and transferred into a 250ml volumetric flask, added 200ml of mobile phase, sonicated to dissolve and make up the volume with mobile phase. (Note: The standard preparation is stable upto 24 hours at 25°C)

Preparation of Sample Solution

Weighed and powdered 20 tablets. Weighed accurately a quantity of the sample equivalent to about 50 mg of Brivaracetam into a 250 ml volumetric flask. Added 200ml of mobile phase sonicated for 15minutes with intermittent shaking and make up the volume with mobile phase. Filtered through 0.45µ PVDF (Millipore) filter or 0.45µ Nylon filter. (Note: The sample solution is stable upto 24 hours at 25°C)

Procedure

Washed the column and equilibrated with mobile phase. Separately injected equal volumes (10µl) of blank, standard preparation (five replicates injections) and sample solution (duplicate injections) into the chromatograph. Recorded the chromatograms and measured the peak responses for Brivaracetam. The System suitability parameters was found to be satisfactory. From the peak responses, calculated the content of Brivaracetam in the sample.

Evaluation of System Suitability

1. The % RSD (relative standard deviation) for five replicate injections of standard preparation should be not more than 2.
2. Tailing factor for Brivaracetam peak should be not more than 2.
3. The column efficiency for Brivaracetam peak should be not less than 2000 theoretical plates.

Formula used for assay calculation

$$\%\ \text{Content of Brivaracetam (As \% LA)} = \frac{AT}{AS} \times \frac{Wstd}{250} \times \frac{250}{W_{test}} \times \frac{\text{Avg. wt. (mg)}}{L.C} \times \frac{P}{100}$$

Where,

AT = Average of the area counts of the Brivaracetam peak obtained from the chromatograms of the assay Preparation.

AS = Average of the area counts of the Brivaracetam peak obtained from the chromatograms of the standard Preparation.

Wstd = Weight of Brivaracetam working standard in mg.

Wtest = Weight of sample in mg.

L.C. = Label claim of Brivaracetam in mg/tablet.

P = Potency of Brivaracetam working standard (% on as is basis).

Analytical method validation (Stability indicating method)

Precision

System precision

Prepared standard preparation as per test method and injected for 6 times into HPLC system. The mean, SD and % RSD for peak areas of Brivaracetam were calculated. The results are tabulated in table – 1. Acceptance criteria is the % relative standard deviation (% RSD) for peak area of Brivaracetam should not be more than 2.0

Method precision

Six sample solutions were analysed as per test method. The % assay for six assay preparations was calculated and the results are tabulated in table-1. Acceptance criteria is the % relative standard deviation (%RSD) for % assay of Brivaracetam for the six assay preparations should not be more than 2.0.

Intermediate precision (Ruggedness)

Ruggedness of the method was verified by analysing the six assay preparations of 100mg strength of same batch which was used for method precision as per

test method by different analyst using different instrument and different column on different day. The %assay of Brivaracetam was determined. Calculated % RSD for % assay of Brivaracetam in six assay preparations and overall %RSD for ruggedness results with the method precision results. The results are tabulated in table-2. Acceptance criteria is the % relative standard deviation (%RSD) for % assay of the six assay preparations should not be more than 2 and the overall % RSD should not be more than 2.

Specificity

Specificity of the method was evaluated by injecting the blank, Placebo, standard preparation and the assay preparation (control sample) prepared as per the proposed method into HPLC system to check for the interference if any at the retention time of Brivaracetam peak. There was no interference from the blank and Placebo at the retention time of Brivaracetam peak. Acceptance criteria is no peaks shall be eluted at the retention time of Brivaracetam peak in blank and placebo; Peak purity of Brivaracetam shall be passed for assay preparation (control sample). Retention time of standard preparation and assay preparation were found to be 3.126 minute for both. Purity match for Brivaracetam peak in assay preparation was found to be 999.492. (Peak found to be pure as purity match factor was more than 980). Thus proposed analytical method was found to be specific. Refer Figure 2a, 2b and 2c.

Forced degradation

Forced degradation study was carried out by treating the sample under the following conditions.

a) Degradation by hydrochloric acid (Acid treated sample)

Assay preparation and placebo were treated with 5 ml of 0.1N hydrochloric acid solution for 24 hours. Treated assay preparation was neutralized with 5ml of 0.1N sodium hydroxide solution. Treated samples solutions were analyzed as per the test method.

b) Degradation by sodium hydroxide (Base treated sample)

Assay preparation and placebo were treated with 5 ml of 0.1N sodium hydroxide solution for 24 hours. Treated assay preparation was neutralized with 5ml of 0.1N hydrochloric acid solution. Treated assay preparations were analyzed as per the test method.

c) Degradation by hydrogen peroxide (Peroxide treated sample)

Assay preparation and placebo were treated with 5ml of 3% hydrogen peroxide solution for 24 hours. Treated assay preparation was neutralized with 5ml of 3% Sodium bisulfite solution. Treated assay preparation were analyzed as per the test method.

d) Degradation by thermal (Heat treated sample)

Assay preparation and placebo were kept in oven at 60°C for about 24 hours. Treated assay preparation were analysed as per the test method.

e) Degradation by UV –Visible light (UV-visible treated sample)

Assay preparation and placebo were exposed to UV light of about 200 watt hours/square meter and to visible light for about 1.2 million lux hours in Photostability chamber. Treated assay preparation were analysed as per the test method.

f) Degradation by water hydrolysis

Assay preparation and placebo were treated with 5ml of water for 24 hours. Treated assay preparation was analyzed as per the test method.

g) Degradation by humidity

Assay preparation and placebo were exposed at 25ºC temperature and 90% relative humidity prepared with supersaturated solution of potassium nitrate for 24 hours. Treated assay preparations were analyzed as per the test method.

The results of forced degradation studies are summarized in table 3. Acceptance criteria is the peak purity for Brivaracetam peaks shall be passed.

Stability in analytical solution

Stability of Brivaracetam in analytical solution was verified by analysing the standard preparation and assay preparation initially and also at different time intervals. Calculated the assay for both the standard preparation and assay preparation. Acceptance criteria is the solution should be considered as stable if the differences in the assay are not more than 2 at each time interval. The difference of assay in the standard and sample solutions of Brivaracetam within the acceptance criteria, hence it was concluded that standard preparation and assay preparation are stable upto 24 hours at 25°C. The results are tabulated in table – 4.

Linearity

The linearity of Brivaracetam was performed using standard preparation in the range of 140.972 mcg/ml to 261.804 mcg/ml of Brivaracetam (about 70% - 130% of test concentration). A graph was plotted with concentration (in mcg/ml) on x-axis and peak areas of Brivaracetam y-axis. Slope, y-intercept, correlation coefficient (r-value) and residual sum of squares (RSS) were determined. The results are tabulated in table – 5 and graphically represented in fig. 4a and 4b. Acceptance criteria is the correlation coefficient (r) value should not be less than 0.999. The detector response of Brivaracetam is directly proportional to concentration ranging from 70% to 130%

of test concentration i.e. 140.972 mcg/ml to 261.804 mcg/ml of Brivaracetam.

Accuracy (Recovery by drug addition method)

Placebo was spiked with the known amount of Brivaracetam in triplicate at 70%, 100% and 130% of test concentration. The amount of Brivaracetam was quantified as per the test method. The % recovery was calculated from the amount found and actual amount added. The results are tabulated in table – 6. Acceptance criteria is the % recovery of Brivaracetam at each spiking level should be in between 98% and 102% and % RSD should not be more than 2. The overall average % Recovery should be between 98% to 102% with %RSD not more than 2.0, The analytical method meets the pre-established acceptance criteria for accuracy study as per ICH guidelines. Hence the method was found to be accurate for the assay of Brivaracetam in Brivaracetam Tablets (10mg and 100mg). For comparative screenshot of recovery study of Brivaracetam refere Figure 5.

Range

Range inferred from the data of linearity, accuracy and precision experiments. The method was found to be linear in the range of 70% to 130% test concentration i.e. 140.972 mcg/ml to 261.804 mcg/ml of Brivaracetam. The method was found to be accurate in the range of 70% to 130% of the test concentration.

Filter paper selection study

Selection of filter paper was evaluated by preparing the assay preparations in triplicate as per test method. Filtered the assay preparations through 0.45µ PVDF filters and 0.45µ Nylon filter and centrifuged a portion of a sample. Calculated % assay difference between centrifuged and filtered assay preparations. The results are tabulated in table – 7. Acceptance criteria is the difference in the results shall not be more than 2.0, from the results obtained, it was concluded that, 0.45µ PVDF filter and 0.45µ Nylon filters are suitable for filtering the sample solution of Brivaracetam Tablets.

Robustness

Robustness of the method was verified by deliberately varying the following instrumental conditions.

a. By changing the flow rate by ± 10%.
b. By changing the temperature by ± 5 °C.
c. By changing the organic content by ± 2 % absolute.

System suitability was evaluated in each condition and assay preparation was analysed in triplicate. The results were compared with the method precision data and tabulated in table-8a and 8b. The system suitability parameters are tabulated in table-9. Acceptance criteria is the overall %RSD for %assay of above results with method precision results should not be more than 2.0, The method was found to be robust for change in flow rate, change in organic content in mobile phase and change in column oven temperature.

Summary of system suitability

System suitability was evaluated by injecting standard preparation during various days of validation experiments. Tailing factor and theoretical plates for Brivaracetam peak from standard preparation was determined. The % relative standard deviation for the peak areas of Brivaracetam peak from five replicate injections of standard preparation were verified at every stage. The results are tabulated in table 9.

Acceptance Criteria

From the standard preparation:
1. Relative standard deviation of five replicate injections of standard preparation for Brivaracetam peak should not be more than 2.0%
2. The tailing factor for Brivaracetam peak should not be more than 2.
3. The theoretical plates for Brivaracetam peak should not be less than 2000.

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.

Table 1. System Precision and Method Precision

System Precision		Method Precision	
Sr. No.	**Peak area of Brivaracetam**	**10mg strength**	**100mg strength**
1	1727620	100.77	102.56
2	1738912	100.87	102.80
3	1708100	101.11	102.48
4	1704439	100.42	102.11
5	1709553	101.15	103.79
6	1733163	101.14	104.06
Mean	1720298	100.91	102.97

SD	14706.2	0.287	0.779
% RSD	0.85	0.28	0.76

Table 2. Intermediate Precision

Sample No.	% Assay (100mg strength)	
	Analyst-I	Analyst-II
1	102.56	102.40
2	102.80	102.44
3	102.48	101.92
4	102.11	102.52
5	103.79	103.17
6	104.06	101.84
Mean	102.97	102.38
SD	0.779	0.480
%RSD	0.76	0.47
Over all Mean	102.67	
Over all SD	0.689	
Over all %RSD	0.67	

Table 3. Forced Degradation

S. No.	Condition	% Assay	% Degradation with respect to Untreated sample	Purity Match
1	Untreated Sample	102.11	---	999.463
2	Acid Treated sample	99.10	2.95	999.504
3	Base Treated sample	90.23	11.63	999.526
4	Peroxide Treated sample	100.68	1.40	999.337
5	Heat Treated sample	100.56	1.52	999.671
6	Hydrolysis Treated sample	99.46	2.60	999.465
7	UV-Visible Treated sample	102.86	$	999.502
8	Humidity Treated Sample	103.59	$	999.450

Note: Chromeleon software: The Purity match should be more than 980; $ Difference less than or equal to zero

Table 4. Solution stability of standard and assay preparation

Time (hours)	Standard Preparation		Assay Preparation	
	% Assay	Difference	% Assay	Difference
Initial	101.36	-	102.31	-
2	100.27	1.09	102.34	0.03
4	101.08	0.28	102.71	0.40
8	100.65	0.71	102.84	0.53
12	100.69	0.67	103.00	0.69
16	101.04	0.32	103.18	0.87
20	101.22	0.14	103.32	1.01
24	101.65	0.29	103.70	1.39

Table 5. Linearity of Brivaracetam

Linearity level (%)	Concentration of Brivaracetam (mcg/ml)	Peak areas
70	140.972	1212890
80	161.110	1376299
90	181.249	1555747
100	201.388	1711538
110	221.527	1878529
120	241.666	2031704
130	261.804	2215860
Slope	8233	

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y-intercept	53758
r-value	0.99981
Residual sum of squares	294721470

Table 6. Accuracy (Recovery by drug addition method)

Recovery level (%)	Actual Amount of Brivaracetam added in mg	Amount of Brivaracetam found in mg	% Recovery	Mean	SD	% RSD
Level – 1 (70%)	36.02	36.08	100.17	100.22	0.821	0.82
	35.83	36.21	101.06			
	36.19	35.98	99.42			
Level – 2 (100%)	50.00	49.63	99.26	100.02	0.775	0.77
	49.54	49.94	100.81			
	50.02	50.02	100.00			
Level – 3 (130%)	65.28	64.24	98.41	99.14	1.453	1.47
	64.54	65.06	100.81			
	65.28	64.10	98.19			
Over all mean			99.79			
Over all SD			1.047			
Over all % RSD			1.05			

Table 7. Filter Paper Selection Study

Preparation details	Sample No.	% Assay	Difference
Centrifuged sample	1	103.57	-
	2	103.99	-
	3	102.98	-
0.45µ PVDF filter	1	102.56	1.01
	2	102.80	1.19
	3	102.48	0.50
0.45µ Nylon filter	1	103.15	0.42
	2	103.82	0.17
	3	103.40	0.42

Table 8a. Results of Robustness study

Sr. No.	(I)	(II)	(III)	(IV)	(V)	(VI)	(VII)
1	102.56	102.30	102.18	101.06	100.32	101.00	100.94
2	102.80	101.30	101.20	100.90	100.66	101.10	100.93
3	102.48	100.79	100.75	100.58	100.67	100.66	100.52
4	102.11	-	-	-	-	-	-
5	103.79	-	-	-	-	-	-
6	104.06	-	-	-	-	-	-
Over all Mean		102.47	102.44	102.26	102.16	102.28	102.24
Over all SD		1.045	1.070	1.232	1.360	1.200	1.253
Overall % RSD		1.02	1.04	1.20	1.33	1.17	1.23

Table 8b. Experimental parameter details (Robustness study)

Sr. No	Experiment (Actual Values related to parameter)
(I)	Method Precision
(II)	Minus flow (0.9ml)
(III)	Plus flow (1.1ml)
(IV)	Minus temperature (25°C)
(V)	Plus Temperature (35°C)

(VI)	Minus organic content (– 2 % absolute)
(VII)	Plus organic content (+ 2% absolute)

Table 9. Summary of system suitability

Sr. No.	Name of Experiment	Tailing factor	Theoretical plates	% RSD
1	System precision, Method precision, Solution stability, Filter selection study	1.4	5152	0.85
2	Linearity and Range	1.3	5600	0.53
3	Recovery	1.3	5591	0.75
4	Robustness (Plus Temperature)	1.4	4912	0.73
5	Robustness (Minus Temperature)	1.3	5593	0.27
6	Robustness (Plus Flow rate)	1.3	5301	0.24
7	Robustness (Minus Flow rate)	1.3	6069	0.14
8	Robustness (Plus Organic)	1.3	5498	0.10
9	Robustness (Minus Organic)	1.2	5730	0.13
10	Ruggedness, Specificity	1.1	7737	0.42
11	Forced degradation	1.1	7589	0.11

Fig 2a. Specimen chromatogram of Blank

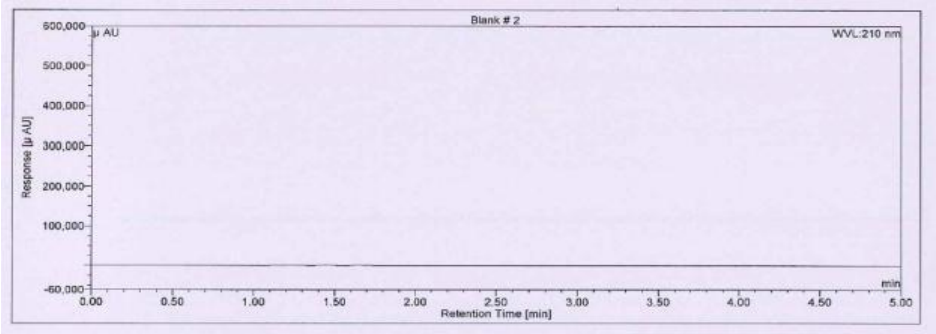
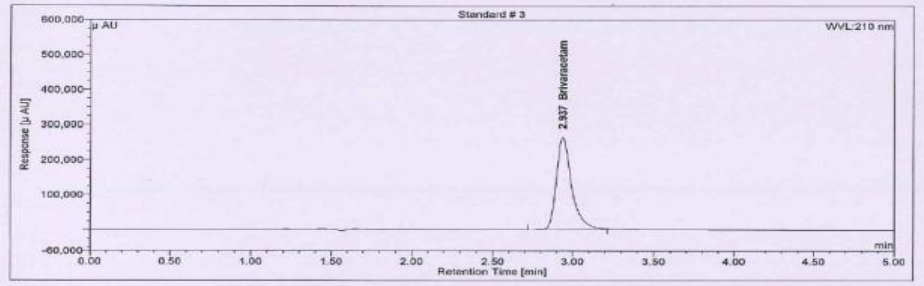
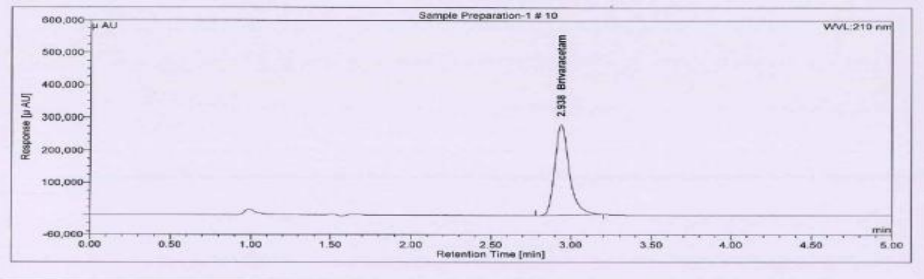


Fig 2b. Specimen chromatogram of Standard



Peak No.	Ret.Time min	Peak Name	Area µ AU*sec	Area %	Theoretical Plates	Tailing	Capacity factor	Resolution
1	2.937	Brivaracetam	1727620	100.000	5152	1.4	n.a.	n.a.

Fig 2c. Specimen chromatogram of Sample



Peak No.	Ret.Time min	Peak Name	Area µ AU*sec	Area %	Theoretical Plates	Tailing	Capacity factor	Resolution
1	2.938	Brivaracetam	1748521	100.000	5508	1.4	n.a.	n.a.

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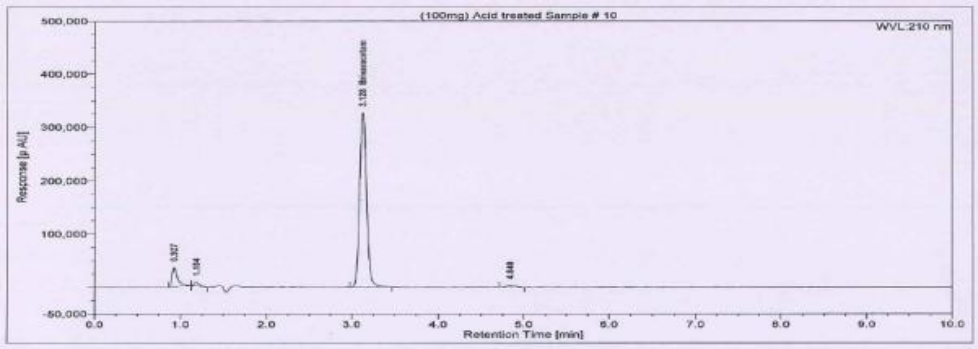
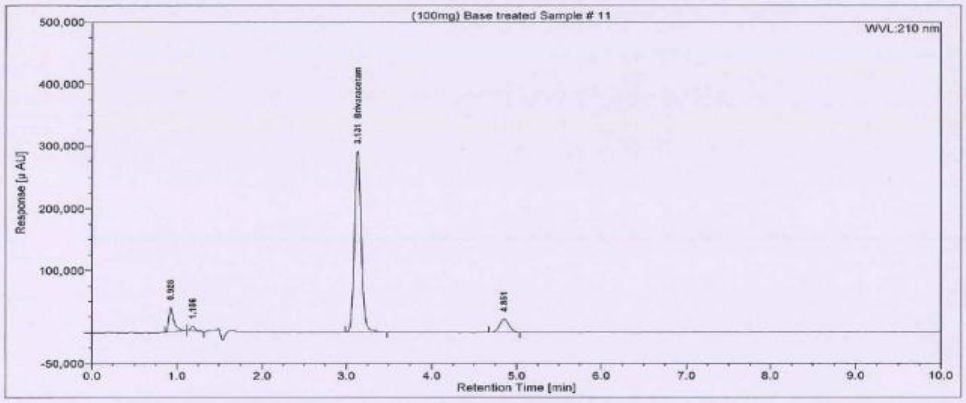
Fig 3a. Specimen chromatogram of 0.1N HCl treated sample



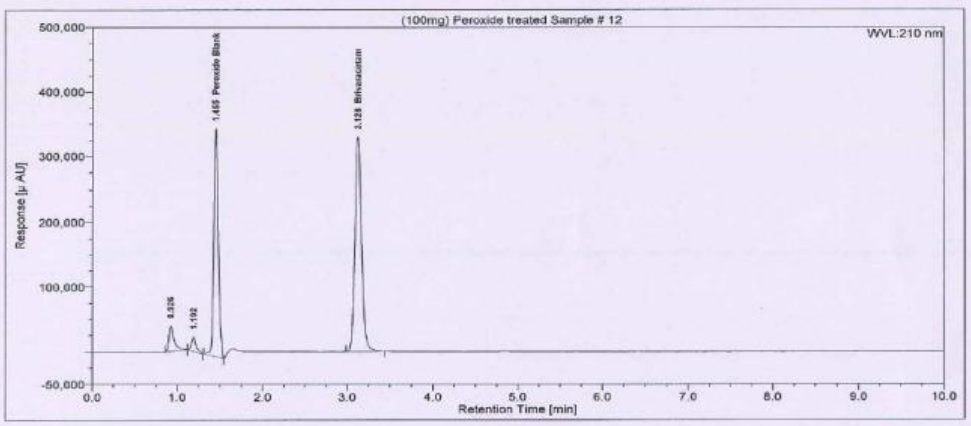
Peak No.	Ret.Time min	Peak Name	Area µ AU*sec	Area %
1	0.927	n.a.	153814	7.70
2	1.184	n.a.	28345	1.42
3	3.128	Brivaracetam	1793015	89.80
4	4.840	n.a.	21504	1.08
Total			1996677	100.00

Fig 3b. Specimen chromatogram of 0.1N NaoH treated sample



Peak No.	Ret.Time min	Peak Name	Area µ AU*sec	Area %
1	0.928	n.a.	166261	8.39
2	1.186	n.a.	27944	1.41
3	3.131	Brivaracetam	1624061	81.93
4	4.851	n.a.	163982	8.27
Total			1982248	100.00

Fig 3c. Specimen chromatogram of 3% Hydrogen peroxide treated sample



Peak No.	Ret.Time min	Peak Name	Area µ AU*sec	Area %
1	0.926	n.a.	167586	5.02
2	1.192	n.a.	88897	2.66
3	1.455	Peroxide Blank	1271377	38.05
4	3.128	Brivaracetam	1813711	54.28
Total			3341571	100.00

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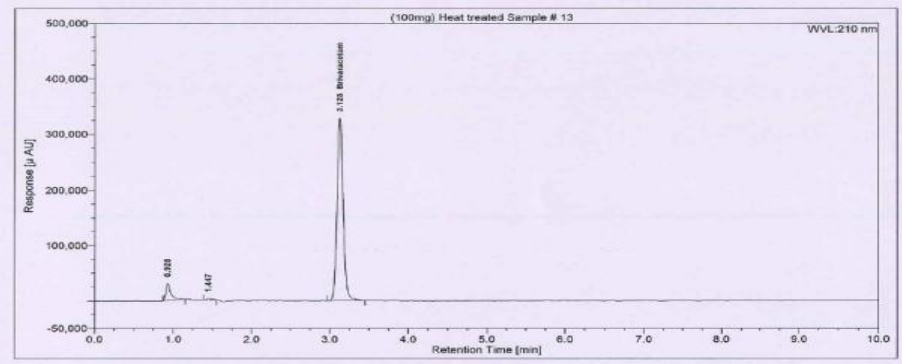
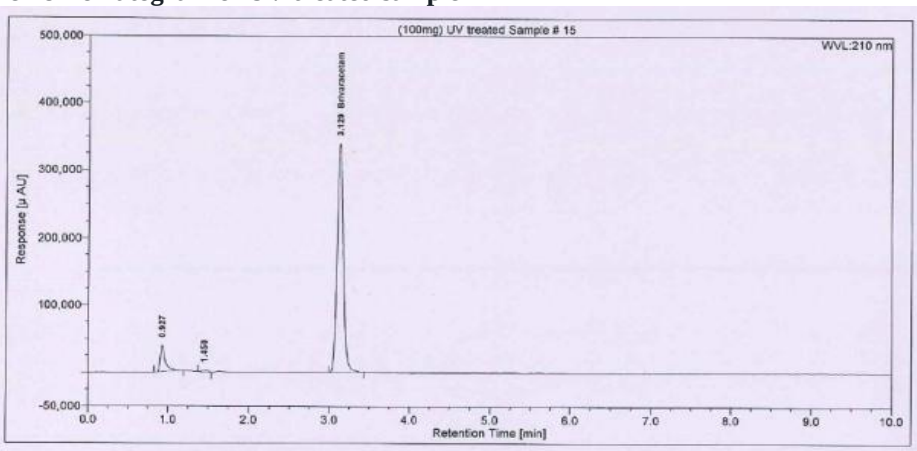
Fig 3d. Specimen chromatogram of Heat (60°C) treated sample



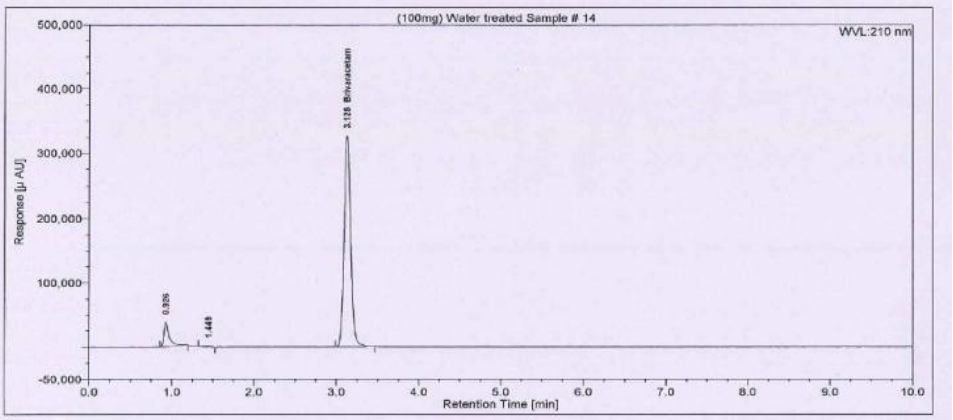
Peak No.	Ret.Time min	Peak Name	Area µ AU*sec	Area %
1	0.928	n.a.	135143	6.88
2	1.447	n.a.	11502	0.59
3	3.128	Brivaracetam	1816911	92.53
Total			1963557	100.00

Figure 3e: Specimen chromatogram of UV treated sample



Peak No.	Ret.Time min	Peak Name	Area µ AU*sec	Area %
1	0.927	n.a.	170388	8.29
2	1.450	n.a.	16956	0.82
3	3.129	Brivaracetam	1869033	90.89
Total			2056376	100.00

Fig 3f. Specimen chromatogram of Water hydrolysis treated sample



Peak No.	Ret.Time min	Peak Name	Area µ AU*sec	Area %
1	0.926	n.a.	169974	8.59
2	1.449	n.a.	10630	0.54
3	3.128	Brivaracetam	1799182	90.88
Total			1979787	100.00

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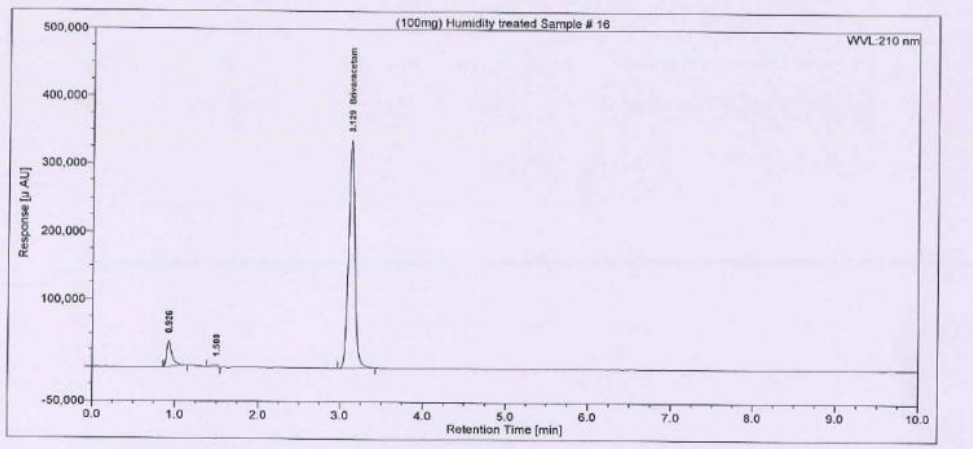
Fig 3g. Specimen chromatogram of Humidity treated sample



Peak No.	Ret.Time min	Peak Name	Area µ AU*sec	Area %
1	0.926	n.a.	162356	7.98
2	1.500	n.a.	17463	0.86
3	3.129	Brivaracetam	1855945	91.17
Total			2035784	100.00

Fig 4a. Linearity plot for Brivaracetam

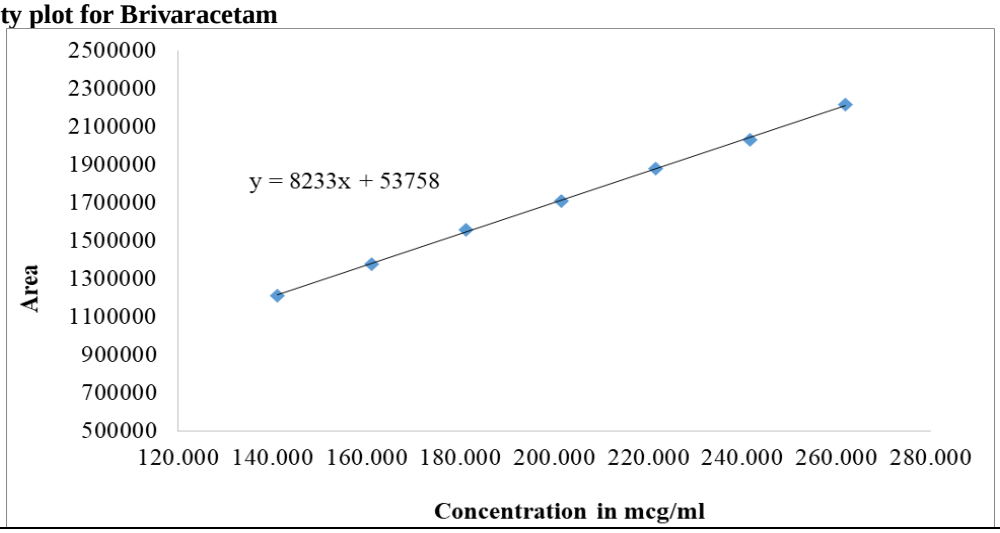


Fig 4b. Comparative screenshot for linearity study of Brivaracetam

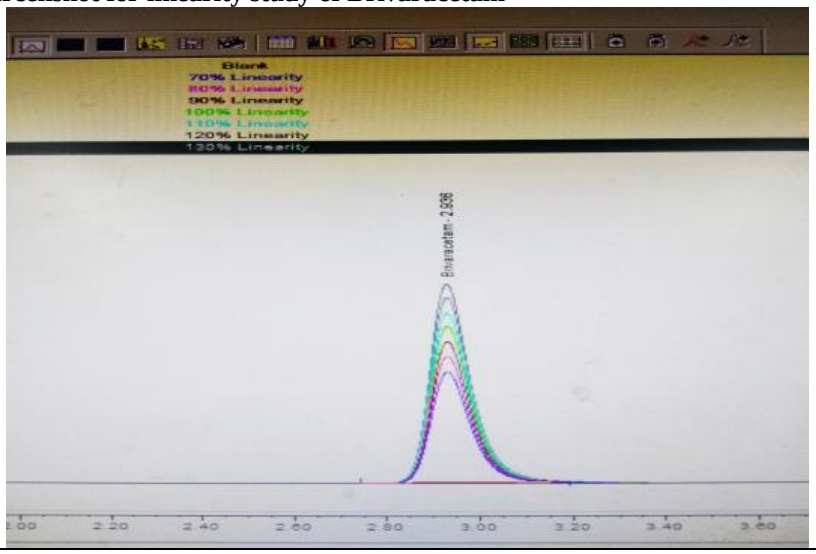


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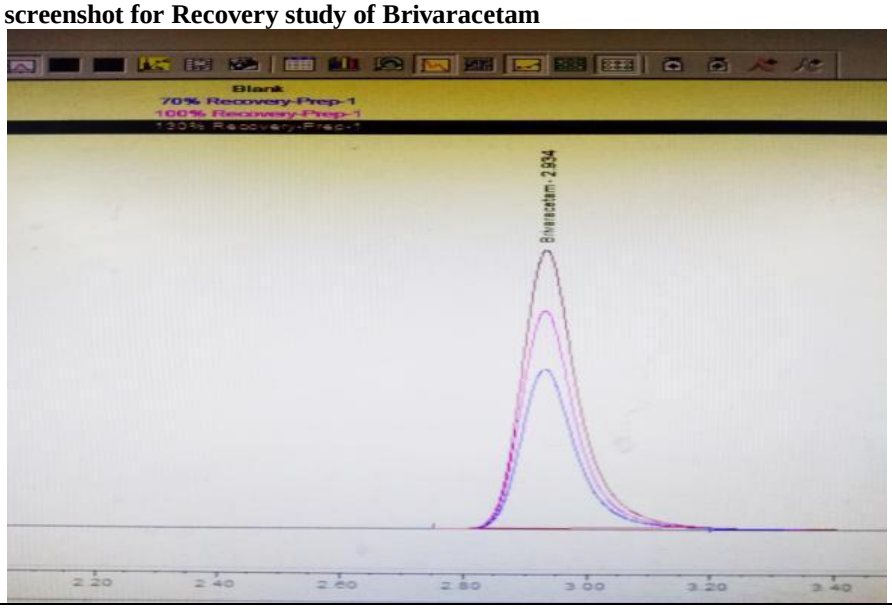
Fig 5. Comparative screenshot for Recovery study of Brivaracetam



CONCLUSION

HPLC is at present one of the most sophisticated tools of analysis. All method validation parameters were performed as per ICH Q2 (R1) guidelines. Therefore method can be employed for routine quality control analysis as per International Conference on Harmonization guidelines.

Expected outcome of the work

The proposed method of analysis is entirely new, simple, fast, sensitive and economical as compared to some reported methods in the literature. The method will be having a suitable application in routine laboratory analysis i.e. in quality control laboratories, with a high degree of accuracy and precision.

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