International e-Conference on New Horizons And Multidisciplinary Applications In Science And Technology
In Association withInternational Journal of Scientific Research in Science and Technology
Volume 9 | Issue 6 | Print ISSN: 2395-6011 | Online ISSN: 2395-602X (www.ijsrst.com)

Development and Validation of Stability Indicating RP-HPLC Method for the Estimation of Levetiracetam by Forced Degradation Studies

Akshay R. Kale[1], Dr. Shirish P. Jain[2], Dr. Vijay S. Borkar[3], Madhav Chakolkar[4], Dr. Swati Tathod*[5]

[1]Principal, Department of Quality Assurance Techniques, RajarshiShahu College of Pharmacy, Buldhana – 44 , Maharashtra, India

[2]Associate Professor, Department of Pharmaeceutical Chemistry, RajarshiShahu College of Pharmacy, Buldhana – 44 , Maharashtra, India

[3]Assistant Professor, Department of Pharmacognosy & Phytochemistry,RajarshiShahu College of Pharmacy, Buldhana – 44 , Maharashtra, India

[4]Associate Professor, Head Department of Quality Assurance Techniques, RajarshiShahu College of Pharmacy, Buldhana – 44 , Maharashtra, India

*[5]Assistant Professor & Head Department of Botany, Shri.VitthalRukhaminiArts,Commerce& Science College, Sawana, Maharashtra, India

ABSTRACT

Literature survey reveals the availability of various analytical methods for the analysis of Levetiracetam in biological samples by RP-HPLC. And there is no spectrophotometric method is available for estimation of Levetiracetam in bulk and pharmaceutical dosage form. There are few RP-HPLC methods are in simultaneous estimation available for this Levetiracetam. Hence there is a need to develop spectrophotmetric and RP-HPLC method for the estimation of Levetiracetam in bulk and pharmaceutical dosage form.

The present a novel developed analytical method for utilizing the Methanol: OPA: Distilled Water (80:10:10) on a Hi Q Sil C-18 (250×4.6mm, 5.0µm)column by using a flow rate 0.7 mL/min where an excellent resolution with sharp peaks of Levetiracetam was obtained.

The UV method employed was absorption maxima method having absorbance measurement at 221 nm.Retention time was found to be 2.7 min for Levetiracetam. Linearity was observed in the concentration range of 10-90µg/ml (r^2 = 0.9999) for UV spectrophotometry and RP-HPLC. Levetiracetam solutions were exposed to acid and alkali hydrolysis, oxidation by hydrogen peroxide, neutral hydrolysis and photo degradation. Statistical analysis proves that the method is repeatable, selective and accurate for the estimation of Levetiracetam.The methods were successfully validated as per ICH guidelines in terms of precision, robustness and recovery.The methodcan be used in pharmaceutical industries for routine analysis of drugs in pharmaceutical dosage forms.

Keywords: Levetiracetam, Method development, Validation, UV-spectrophotometry, RP-HPLC, force degradation, Levetiracetam and ICH guidelines.

I. INTRODUCTION

Levetiracetam is for the treatment of treat epilepsy. Chemically it is (2R)-2-(2-oxopyrrolidin-1-yl) butanamide. The drug binds to synaptic vesicle glycoprotein, SV2A, and inhibits pre synaptic calcium channels and reducing neurotransmitter release and acting as a neuromodulator.

Levetiracetam is a drug within t pyrrolidine class that is used to treat various types of seizures stemming from epileptic disorders. It was first approved for use in the United States in 1999 and is structurally and mechanistically unrelated to other anti-epileptic drugs (AEDs). Levetiracetam possesses a wide therapeutic index and little-to-no potential to produce, or be subject to, pharmacokinetic interactions these characteristics make it a desirable choice over other AEDs, a class of drugs notorious for having generally narrow therapeutic indexes and a propensity for involvement in drug interactions.

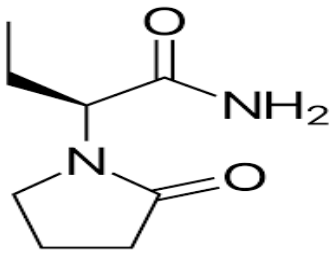

Fig. 1: Structure of Levetiracetam

II. MATERIALS AND METHODS

Levetiracetam was obtained as a gift sample from Aurobindo Pharma.and as such without further purification. All chemicals and reagents used were spectrophotometric and HPLC grade. The solvent used methanol was of AR grade.

Reagents: Methanol, Water, 1N HCl, 1N NaOH, 6% H_2O_2, OPA.

Instrumentation

Absorbance measurements were made on UV-visible spectrophotometer Jasco (Japan) V-730 with band width of 1.5 and 1mm quartz cell was used as sample holder. In addition electronic balance, micropipette and sonicator were used in this study. For weighing electronic balance belonging to Metller Toledo model ME204 was used. It had a working range of 0.1 miligrams to 100 grams of substance.

The RP-HPLC system (Jasco HPLC) consisted of quaternary gradient pump with manual injection facility. The capacity of loop was 20 µl. The detector consisted of a PDA operated at whole wavelength range. The software used was ChromNAV. The column used was Hi Q sil C-18 (250mm×4.6mm, 5µm). Absorbance measurements were made on PDA detector. The pH meter used was of electronic. For an IR spectrum of drug sample was recorded in KBr pellets on Shimadzu IR Affinity-1.

Preparation of mobile phase

90 ml Methanol mixed with 10 ml of Water. The solution was degrassed in an ultrasonic sonicator for 15 minutes and filtered through 0.45 µm whatmann's filter paper under vaccum.

Preparation of Standard stock and Working solutions

Standard stock solutions of Levetiracetam was prepared by dissolving 100 mg of drug in 100 ml of methanol to get standard stock solution of 1000 µg/ml by sonication for 15 min. From the standard stock solution, 1 ml was

further diluted to 10 ml with mobile phase to get 100 µg/ml solution of Levetiracetam. Further dilutions were made from that to reach a concentration range of 10-90 µg/ml for Levetiracetam.

Selection of wavelength detection

Levetiracetamstandard solution of 10 ppm was scanned at 200-400 nm and UV spectrum was recorded. The observation of spectrum of standard solution, λmax of 221 nm was taken to develop the proposed method. UV-spectrum of Levetiracetam is shown in figure 2.

Chromatographic conditions

Parameter	Condition
Mobile phase	Methanol:OPA: Distilled Water (80:10:10 v/v)
Diluent	Methanol
Column	Hi Q sil C_{18} Column (250mm×4.6mm,5µm)
Column temperature	24°C
Wavelength	220 nm
Injection volume	20 µl
Flow rate	0.7 ml/min
Run time	10 min

Validation of the Proposed Method

The proposed analytical method was comprehensively validated by the guidelines put forward by the ICH. The validation was in accordance with the ICH guidelines Q2A and Q2B, by following the FDA guidance and also as per USP.

System suitability

System suitability tests are a fundamental part of liquid chromatographic method. It ensures that the system is working properly. Parameters such as number of theoretical plates, retention time, and tailing factor were evaluated by system suitability.

Linearity and Range

The standard solutions were prepared by dilution of the stock solution with methanol to reach a concentration range 10-90 µg/ml for Levetiracetam. Nine different concentrations were prepared and linearity graph was plotted for absorbance vs. concentration. The linearity data was analyzed statistically for regression coefficient and statistical significance. The linear correlation coefficient of concentration was found to be 0.9999. The result for linearity of drug is shown in table no.

Accuracy

The accuracy of the method was established by spiking pre-analyzed sample with known amounts of the corresponding drug at three different levels i.e, 80%, 100% and 120% of the drug in tablet. At each level, three determinations were performed. The in-house formulation having 1000 µg/ml concentration of Levetiracetam was diluted with methanol. From 1000 µg/ml stock solution (20µg/ml) was spiked with 16, 20 and 24µg/ml of standard drug solution (1000 µg/ml), separately to get three levels viz. 80%, 100% and 120%respectively to estimate % recovery.

Precision

Intraday precision was determined by analyzing 20, 60, 90 µg/ml concentration of drug three times on the same day with specific time interval. Similarly inter-day precision was determined by analyzing 20, 60, 90 µg/ml concentration of drug three times on three consecutive days. Precision was expressed as % relative standard deviation values.

Robustness

Each factor selected to examine were changed at three levels (-1, 0, 1). One factor at the time was changed to estimate the effect of solution. Robustness of the method was done at concentration level of 20µg/ml for Levetiracetam. One factor was changed at one time to estimate the effect on chromatogram.

Limit of detection (LOD) and limit of quantification (LOQ)

Known concentration (10-90 µg/ml) was prepared. Calibration curves were plotted for each set. LOD and LOQ were calculated using the regression equation and following formula as:

LOD = 3.3* SD/S

LOQ = 10* SD/S

Where,

SD is standard deviation of y- intercept of the calibration curves

S is slope of calibration curves.

Estimation of the In-house tablet formulation:

The tablets of Levetiracetam are not available in Indian market; hence in-house tablet was prepared. The tablet was prepared by using 5 mg of Levetiracetam and 10 mg of lactose. The quantity of each ingredient was calculated & weighed for 20 tablets. Twenty tablets of Levetiracetam were weighed and the average weight was calculated. Quantity equivalent to 10 mg and volume make up to 100 ml. The resulting solution was then filtered using 0.45µ Whatmann filter paper. The original stock solution was further diluted to get sample solution of drug concentration of 20 µg/ml Levetiracetam. A 20 µl volume of sample solution was injected into UV-visible spectroscopy, six times, under the conditions described above. The absorbance for the drug was measured at 220 nm and concentrations in the samples were determined using UV-visible spectroscopy system under the same conditions using linear regression equation.

Degradation study

Stress degradation studies were carried under conditions of acid, base, neutral hydrolysis, oxidation and photolysis. For each study, two samples were prepared (Blank and of Levetiracetam). The blank solution subjected to stress in the same manner as the drug solution. Dry heat and photolytic degradation was carried out in solid state.

Alkaline hydrolysis

5 ml working standard solution of Levetiracetam (2000 µg/ml) was mixed with 5 ml of 1 N methanolicNaOH. The solution was kept for 24 hr in dark place. The resulting solution was neutralized and 2ml was diluted with mobile phase to 10 ml and was injected (20 µg/ml).

Acidic hydrolysis

5 ml working standard solution of Levetiracetam (2000 µg/ml) was mixed with 5 ml of 1 N HCl. The solution was kept for 24 hr in dark place. The resulting solution was neutralized and 2 ml was diluted with mobile phase to 10 ml and was injected (20 µg/ml).

Oxidation

5 ml working standard solution of Levetiracetam (2000 µg/ml) was mixed with 5 ml of 6% H_2O_2 solution. The solution was kept for 24 hr in dark place. The resulting solution was neutralized and 2 ml was diluted with mobile phase to 10 ml and was injected (20 µg/ml).

Neutral hydrolysis

5 ml working standard solution of Levetiracetam (2000 µg/ml) was mixed with 5 ml of distilled water. The solution was kept for 24 hr in dark place. The resulting solution was neutralized and 2 ml was diluted with mobile phase to 10 ml and was injected (20 µg/ml).

Photo-degradation studies:

Photolytic studies were carried out by exposure of drug to sunlight for 24 hr at room temperature. Sample was withdrawn after exposure and processed as per standard solution preparation procedure to get 20 µg/ml as final concentration and was injected.

Degradation under dry heat

Dry heat studies were performed by keeping drug sample in oven (60°C) for a period of 24 hr. Sample was withdrawn after 24 hour and processed as per standard solution preparation procedure mentioned under 1.5 to get 20 µg/ml as final concentration and were injected.

III. RESULTS AND DISCUSSION

HPLC method development

As per the ICH guidelines the method was developed and validated properly. Linearity was observed over a concentration range of 10 to 90 µg/ml. The mobile phase consisting of Methanol:OPA: Distilled Water (80:10:10 v/v) with 0.7 ml/min in as flow rate was optimized as it was found to give best system suitability parameters.

System suitability

The system suitability was performed by injecting standard solution of Levetiracetam. The system suitability parameters were satisfactory and the theoretical plates were obtained above 2000. Tailing factor was found below 2. % RSD also found below 2%. Shown in figure 3, and table no. 1.

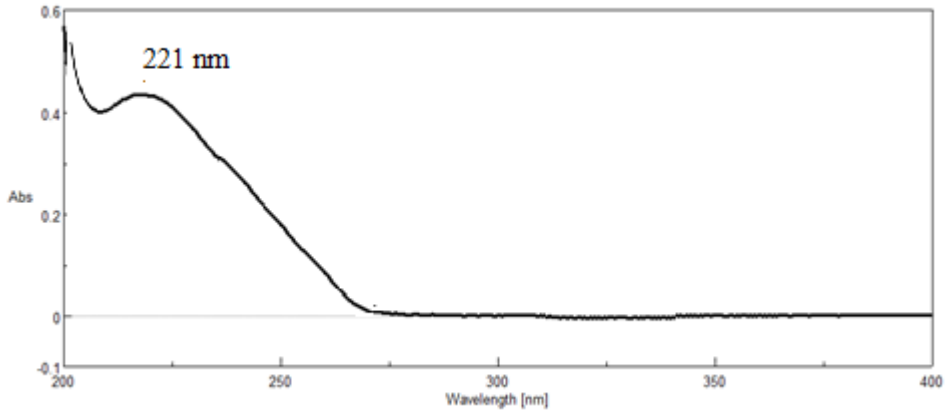

Fig. 2: UV spectrum of Levetiracetam in methanol (10 µg/ml)

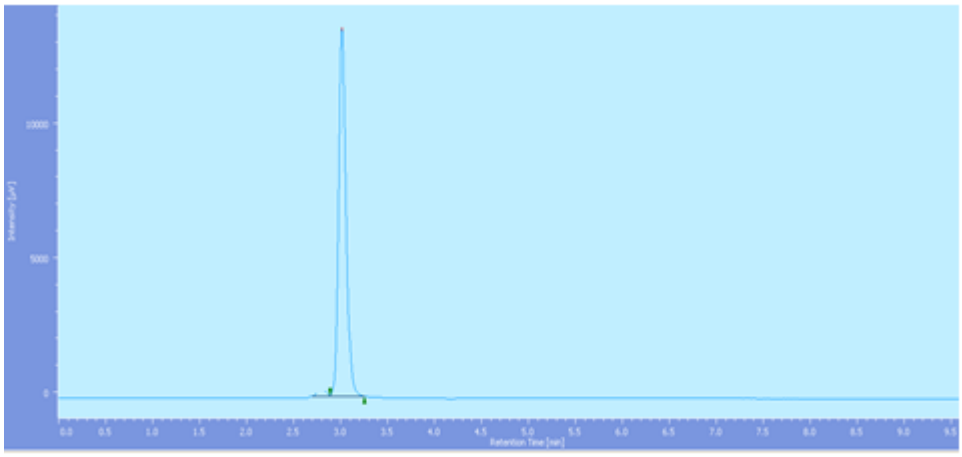

Fig. 3: Optimized chromatogram of Levetiracetam

Table 1: System suitability parameters

Sr. No.	Parameter	Levetiracetam	Formula	Limits
1	Retention time (min)	2.7	-------	1<K<20
2	No. Of theoretical plates (N)	17219	N=16(t/W)	N>2000
3	Tailing factor	1.4	$T_f=W_{0.05\%}/2f$	$T_f<2$

Report: The system suitability parameters were determined for Levetiracetam and were found to be within acceptance criteria.

Linearity and Range

Table 2: Calibration data of Levetiracetam

Sr.no.	Concentration (µg/ml)	Mean peak area
1	10	90074
2	20	180357
3	30	269156
4	40	354128
5	50	445233
6	60	532357
7	70	609468
8	80	702315
9	90	782355

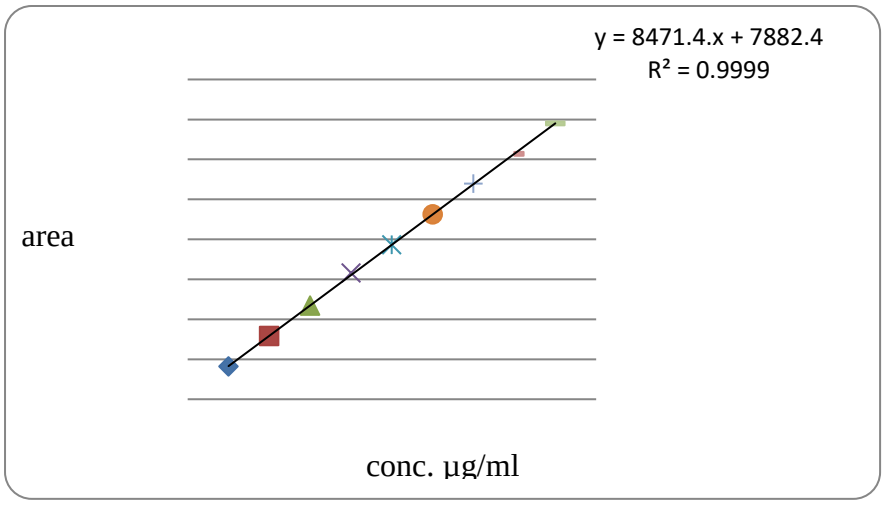

Fig. 4: Standard calibration curve of Levetiracetam for RP-HPLC method (10-90 µg/ml)

Table 3: Linearity parameters for RP-HPLC

Sr. No.	Parameters	Observation
1	Linearity range (µg/ml)	10-90
2	Regression equation	Y = 8471.4x + 7882.4
3	Coefficient (r^2)	0.9999
4	Intercept	8471.4
5	Slope	7882.4

Report: The proposed method was found to be linear over the concentration range of 10-90 µg/ml. The regression coefficient (r^2) for Levetiracetam was found to be 0.9999 which is well within the acceptance limits.

Accuracy

Table 4: Recovery study of Levetiracetam

Level of recovery (%)	Mean	SD	%RSD
80%	99.94	0.4615	0.4688
100%	99.36	0.9136	0.9251
120%	99.70	0.3241	0.3325

Report: The percentage recovery of Levetiracetam was well within the limit. Hence, the method was found to be accurate.

Precision

Precision was performed by intraday and interday precision.

Table 5: Intraday Precision studies

Sr. No.	Amount claimed	Area	Amt found	%Amt found	Mean	SD	%RSD
1	20	177275	19.79	98.98	99.45	0.5335	0.5365
2	20	178971	20.01	100.03			
3	20	177832	19.86	99.34			
4	60	531568	59.54	99.23			

5	60	532460	60.21	100.36	99.66	0.6071	0.6091
6	60	531986	59.64	99.41			
7	90	785916	90.83	100.92	100.16	0.6653	0.6642
8	90	781234	89.91	99.90			
9	90	780956	89.70	99.67			

Table 6: Interday Precision studies

Sr. No.	Amount claimed	Area	Amt found	%Amt found	Mean	SD	%RSD
1	20	176975	19.77	98.85	99.64	0.7851	0.7879
2	20	179452	20.08	100.42			
3	20	178956	19.93	99.66			
4	60	532241	60.10	100.17	99.85	0.3883	0.3889
5	60	531415	59.65	99.42			
6	60	531989	59.98	99.97			
7	90	780589	89.67	99.63	99.27	0.6914	0.6964
8	90	779858	88.64	98.48			
9	90	786895	89.75	99.72			

Report: The % RSD values of peak areas for reported injections of Levetiracetam were found to be <2%, which indicates that the proposed method was precise.

Robustness

Table 7: Robustness data of Levetiracetam

Factor	Level	Levetiracetam (Retention Time)
A: Flow rate (ml/min)		
0.6	-0.1	2.65
0.7	0	2.7
0.8	+0.1	2.83
B: Wavelength change		
219	-0.1	2.56
220	0	2.7
221	+0.1	2.83

Report: From the above observation, it can be concluded that, the method is robust with respect to change in flow rate.

LOD and LOQ

Table 8: Summary of the validation parameters

Parameters	Levetiracetam
Regression equation	Y=8471.4x+7882.4
Correlation coefficient	0.9999
Linearity	10-90 µg/ml
Recovery	99.52-99.98%

LOD	0.6134
LOQ	1.8163
Robustness	Robust
% Assay	0.6569

Estimation of the In-house tablet formulation

Assay of In-house tablet formulation is represented in table no 31 and the overlain chromatogram is shown in fig. no.

Table 8: Estimation of the In-house tablet formulation

Sr. No.	Amount taken (µg/ml)	Peak Area	Amount of drug found (µg/ml)	% Amount found
1	20	178695	19.94	99.72
2	20	179245	20.01	100.03
3	20	177985	19.79	98.98
4	20	179312	20.09	100.49
5	20	177991	19.80	99.02
6	20	179346	20.08	100.42
Mean				99.77
Standard Deviation (S.D)				0.6629
%Relative Standard Deviation (%R.S.D)				0.6569

Report: Levetiracetam is not less than 98 % and not more than 102 %

Forced degradation studies

The forced degradation studies of Levetiracetam were carried out under various stress conditions, and the resultant chromatogram of alkaline hydrolysis shown in figures.

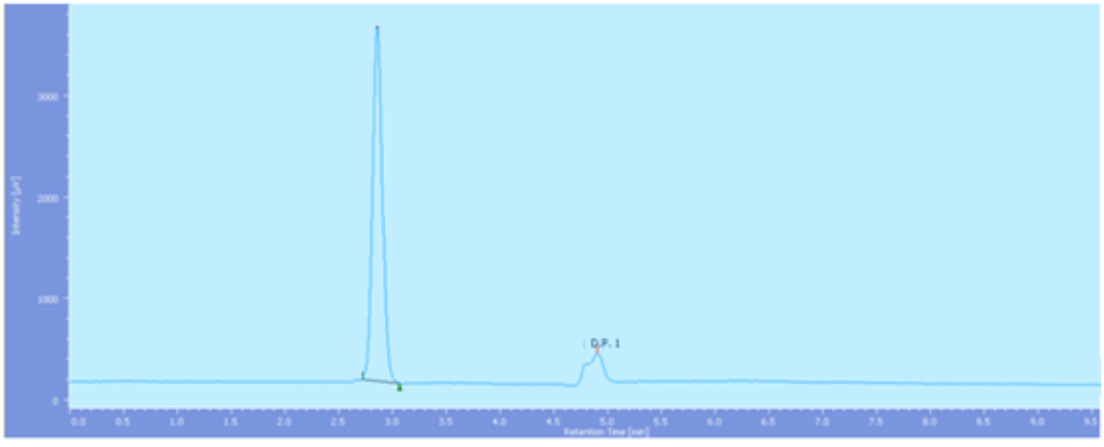

Fig 5. Chromatogram of Levetiracetam after alkaline hydrolysis (20 µg/ml)

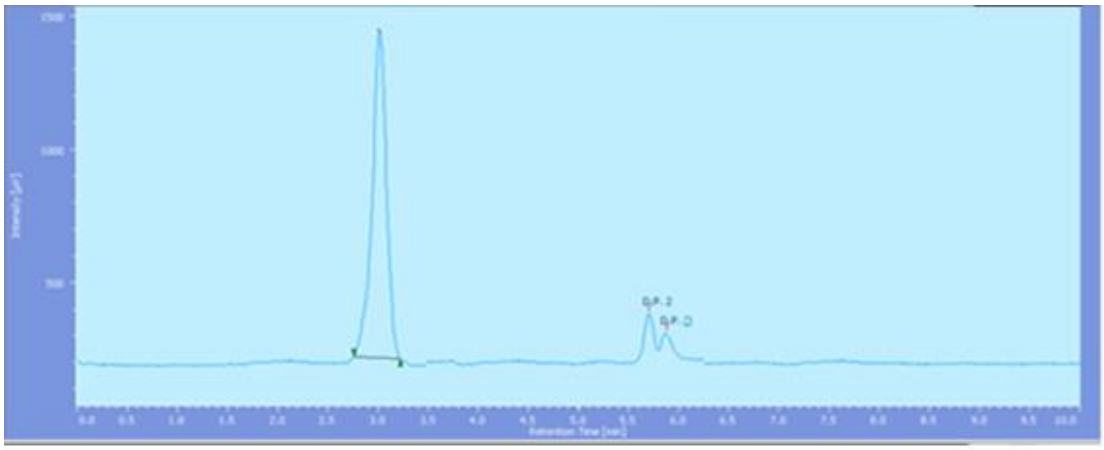

Fig 6. Chromatogram of Levetiracetam after acid hydrolysis (20 µg/ml)

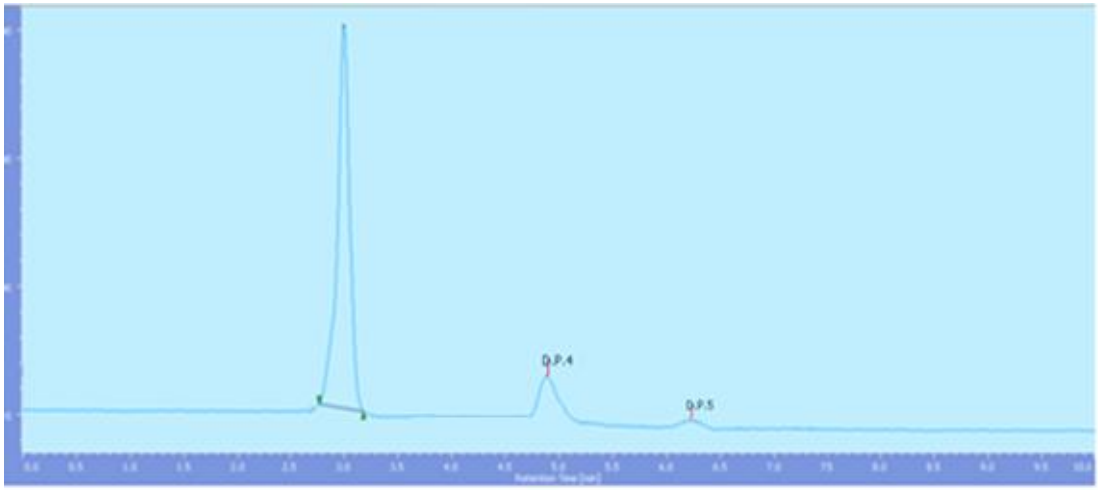

Fig 7. Chromatogram of Levetiracetam after oxidation (6% H_2O_2) (20 µg/ml)

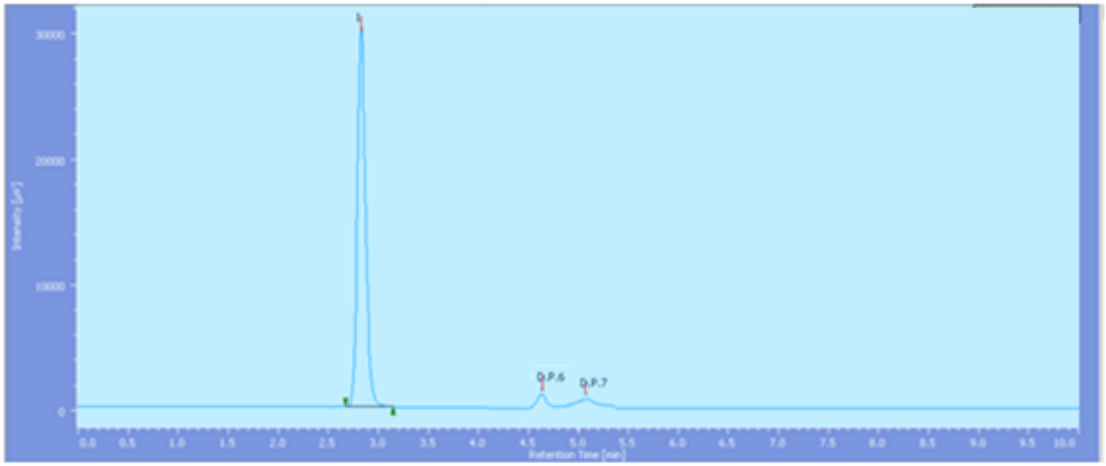

Fig 8. Chromatogram of Levetiracetam after neutral hydrolysis (20µg/ml)

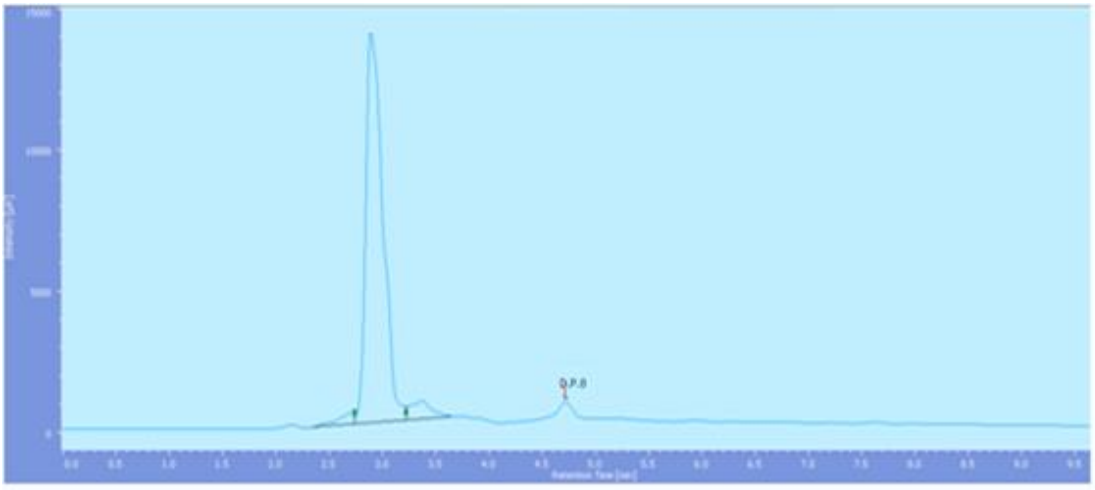

Fig. 9. Chromatogram of Levetiracetam after photo-degradation (20 µg/ml)

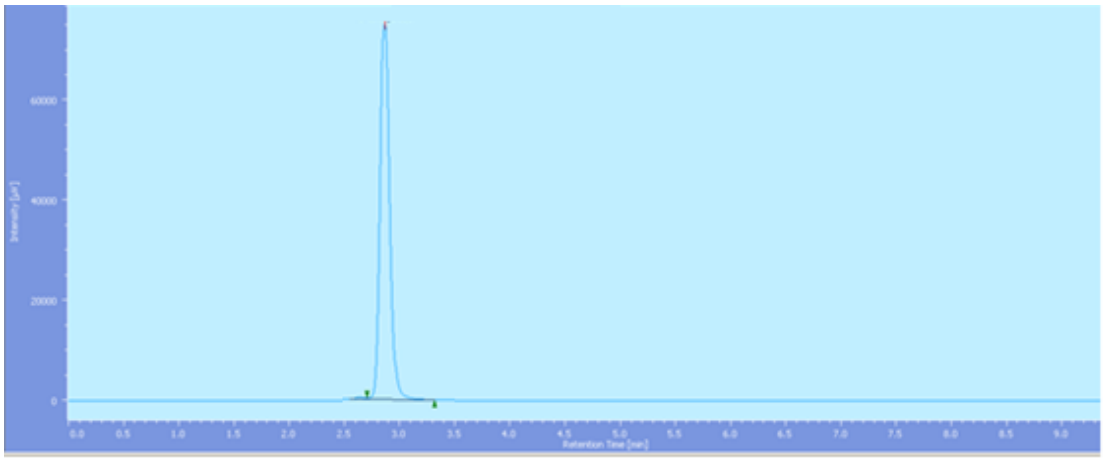

Fig 10. Chromatogram of Levetiracetam after dry heat (20 µg/ml)

Table 9: Result of forced degradation studies of Levetiracetam

Sr. No.	Stress Degradation Condition	Percent recovered (%)	R_t of Levetiracetam	% Degradation	R_t of Degradants
1	Base (1 N NaOH, kept for 24 hr.)	91.41	2.7	8.59	5.88
2	Acid (1 N HCl, kept for 24hr)	90.25	2.7	9.75	5.62, 5.75
3	H_2O_2, 6% (kept for 24hr)	89.61	2.7	10.39	4.83, 6.29
4	Neutral (kept for 24hr.)	96.27	2.7	3.73	4.62, 5.12
5	Photo stability [Sun light for 24hr]	93.28	2.7	6.72	4.79
6	Dry heat (60°C for 4hr)	98.26	2.7	1.74	--

IV. CONCLUSION

The proposed Spectrophotometric and RP-HPLC method was found to be simple, accurate, precise, linear, robust and specific for quantitative estimation of Levetiracetam in bulk and pharmaceutical dosage form. The proposed RP-HPLC method was less cost effective and less time consuming. The values foe system suitability

parameters showed feasibility of this method for routine pharmaceutical application. Hence, the present RP-HPLC method is suitable for routine assay of Levetiracetam in bulk and pharmaceutical dosage form.

Acknowledgments

The author grateful to Aurobindo Pharma for providing the gift sample.

V. REFERENCES

[1] . Jeffery, G.H, J. Bassett, J. Mendham and R. C. Denny, Vogel's "Textbook of Quantitative Chemical Analysis",ELBS; 2005, 5th (ed.) 3-4.

[2] . A.H. Beckett, J.B. Stenlake, Practical Pharmaceutical Chemistry (Part 2), 4th ed., CBS Publishers and Distributors, New Delhi, 1997, 275-295.

[3] . A.V. Kasture, K.R. Mahadik , S.G. Wadodkar, H.N. More, Pharmaceutical Analysis, Vol. 2, NiraliPrakashan, 48-57, 156-168.

[4] . R.J. Hamilton, P.A. Sewell, Introduction to HPLC, 2nd ed., Chapman and Hallondon, 1982, 189

[5] . B.K. Sharma, Instrumental Methods of Chemical Analysis, 21st ed., Goel Publishing House, Meerut, 2002,3-5,10.

[6] . M. Thomas, Analytical Chemistry by Open Learning, Ultraviolet and Visible Spectroscopy, 2nd ed., Wiley-Interscience, New York, 1996, 131-132.

[7] . R.Y. Bauman, Absorption Spectroscopy, Wiley-Interscience, New York, 1975, 405, 569

[8] . D.C. Harris, Quantitative Chemical Analysis, 6th ed., Michelle RusselJulet Publication, 2002, 610-617. 15. Scott P, Principles and Practice of Chromatography, Chrome-ED Book Series, 2003, 1- 2 & 12-14

[9] . P.D. Sethi, HPLC „High Performance Liquid Chromatography", Quantitative Analysis of Pharmaceutical Formulations, 1st ed., CBS Publishers and Distributors, New Delhi, 2001, 3-11, 18-20, 24, 27-28, 116-120.

[10] . R.J. Hamilton, P.A. Sewell, Introduction to HPLC, 2nd ed., Chapman and Hallondon, 1982, 189.

[11] . R. P.W. Scott, Technique and Practice of chromatography, Marcel Dekker, New York, Vol. 70, 1-12.

[12] . P.R. Brown. Advances in Chromatography, Vol. 41, Marcel Dekker, New York, 2001, 1-3.

[13] . Weston A, Brown PR. HPLC and CE – Principles and practice. USA: Academic Press, 1997,1-4, 8-11, 24-26, 29-32, 71.

[14] . Saranjit Singh, Monika Bakshi, Guidance on conduct of: Stress Tests to Determine Inherent Stability of Drugs, April 2000, 1 – 14.

[15] . ICH, Q1A Stability testing of New Drug Substances and Products, In: Proceedings of the International Conference on Harmonization, Geneva, October, 1993.

[16] . ICH, Q1A (R2), Stability testing of new drug substances and products, International Conference on Harmonization, 1996.

[17] . ICH Harmonized Tripartite Guideline Validation of Analytical Procedures: Text and Methodology, Q2 (R1) November 2005, 1-28.

[18] . Gupta S.P, Statistical methods Sultan Chand and Sons Educational Publishers New Delhi, Revised Ed, 2001, 435-453 & 1006-1010.

[19] . ICH Guidance on Analytical Method Validation, in: Proceeding of the International Convention on Quality for the Pharmaceutical Industry, Toronto, Canada, 2002.