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ANALYTICAL METHODE DEVELOPMENT AND VALIDATION OF "LEVETIRACETAM" IN BULK AND DOSAGE FORM

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ABSTRACT

A simple, sensitive, specific, accurate, and precise reverse phase high performance liquid chromatographic method was developed and validated for the estimation of Levetiracetam in tablet dosage forms. A C18column having 250 × 4.6 mm and mobile phase containing phosphate buffer: acetonitrile (75:25v/v) was used. The flow rate was 0.90 ml/min and effluents are monitored at 206 nm. The retention time of Levetiracetam is 3.63 min. The method was validated for specificity, linearity, accuracy, precision, limit of quantification, limit of detection, robustness in accordance with ICH guidelines. Limit of detection and limit of quantification for estimation of Levetiracetam found to be 0.59 µg/ml and 1.79 µg/ml. Recovery of Levetiracetam in tablet formulation was found to be 101.4%. Proposed method was successfully applied for the quantitative determination of Levetiracetam in commercially available tablet dosage forms.

Keywords: - Levetiracetam, RP-HPLC Method development, Validation.

1. INTRODUCTION

Analytical chemistry is defined as "The science and the art of determining the composition of materials, which deals with both theoretical, practical science. In analytical chemistry it is of prime importance to gain information about the qualitative and quantitative composition of substances and chemical species. Pharmaceutical analysis deals medicaments and their precursors. Quality is important in every product. Quality control is a concept, which strives to produce a perfect product. Physico-chemical methods are used to study the physical phenomenon that occurs as a result of chemical reactions. Physico-chemical methods are optical, photometry (photocolorimetry and spectrophotometry covering UV-Visible, IR Spectroscopy and nepheloturbidimetry) and chromatographic (column, paper, thin layer, gas liquid and high performance liquid chromatography) methods. Modern pharmaceutical analysis must need the following requirements. 1. The analysis should take a minimal time. 2. The accuracy of the analysis should meet the demands of Pharmacopoeia. 3. The analysis should be economical. 4. The selected method should be precise and selective. Chromatography: The term

chromatography was first used by the Russian chemist and botanist Michael Tswett in 1906. The term chromatography is derived from the Greek words: Chroma for color and Graphein to write. "Chromatography is a physical method of separation in which the components to be separated are distributed between two phases, one of which is stationary while the other moves in a definite direction.

1.1 Drug profile

Name: Levetiracetam

Structure:

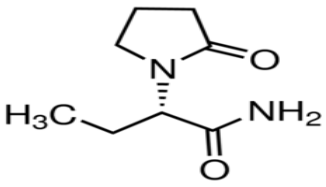

Fig 1- Structure of Levetiracetam

Description: Levetiracetam, sold under the brand name Keppra among others, is a medication used to treat epilepsy. It is used for partial-onset, myoclonic, or tonic-clonic seizures and is taken either by mouth as an immediate or extended release formulation or by injection into a vein. Common side effects of levetiracetam include sleepiness, dizziness, feeling tired, and aggression. It is unclear if levetiracetam is safe for use during pregnancy, but it appears safe for use while breastfeeding.

IUPAC name: (2S)-1-{2-[(3-hydroxyadamantan-1-yl) amino] acetyl1}] pyrrolidone-2-carboniytile
Chemical formula: $C_8H_{14}N_2O_2$
Molecular mass: 170.209 g/mol
Physical state: Solid
Melting point: 115-118℃
Solubility: Soluble in water, methanol, ethanol, acetonitrile and chloroform
pka: The pka of levetiracetam is <-2

Mechanism of action: the drug binds to SV2A, a synaptic vesicle glycoprotein, and inhibits presynaptic calcium channels, reducing neurotransmitter release and acting as a neuromodulator.

2. MATERIAL AND METHOD

2.1 Materials

Levetiracetam obtained from swapnroop drug agency, Aurangabad.

2.2 Instrument

The analysis of the drug was carried out on thermofisher gradient system UV detector. Equipedv with C18 column (250 × 4.6 mm) and running chromoquest 4.1 software.

2.3 Selection of detection of wavelength

The UV spectrum of diluted solution of various concentration of Levetiracetam in mobile phase was recorded using a UV spectrophotometer. The wavelength of maximum absorbance was observed at 206nm. This wavelength was used for detection of Levetiracetam.

2.4 Preparation of Mobile phase

The aim is to find the correct concentration of the mobile phase. The mobile phase and its strength is a measure ability to pull analytes from the column. In reverse phase HPLC with aqueous mobile phases such as phosphate buffer and acetonitrile (75:25v/v). The retention time is also important criteria for selection of mobile phase.

2.5 Preparation of standard stock solution

An accurately weighed quantity pure powder of Levetiracetam (10 mg) was transferred to 10ml volumetric flask dissolved and diluted to the mark with mixture of phosphate buffer and acetonitrile in the ratio of 75:25v/v. The volume was made up to the mark using same mixture of mobile phase to get final concentration1000µg/ml.

2.6 Preparation of sample solution

10 tablet label claim 500mg Levetiracetam IP (LEVIPIL, Sunpharma laboratories ltd.) were weighed and crushed into fine powder. The amount of powder equivalent to 25mg of Levetiracetam was weighed and transferred into the 10ml of mobile phase. The resulting solution was filtered through 0.45µ membrane filter and sonicated for 20 min in two cycles each of 10 min. from the sample stock solution.

2.7 Optimized method for Levetiracetam

Table 1: Chromatographic condition

Parameter	Optimized Condition
Column	:C18 column(4.6m×250mm)
Mobile phase	:Phosphate buffer: Acetonitrile (75:25 v/v)
Detection wavelength	:206nm
Flow rate	:0.90 ml/min
Column temperature	:Ambient
Sample size	:10µl
Run time	:7.0 min

3. System suitability testing

3.1 Preparation of working solution

From freshly prepared standard stock solution (1000µg/ml), 1.0ml stock solution was pipetted out and diluted upto 10ml to obtain consequential solution of 10µg/ml. The resulting solution was filtered through 0.45µ membrane filter and sonicated for three cycles each of 10min.Three replicates of this solution was injected and result were recorded for RT, area, tailing factor, theoretical plates, SD, %RSD were calculated for the results and other parameters are shown in Table 1.

3.2 Method Validation

3.2.1 Linearity

The linearity of an analytical procedure is its ability to obtain test results, which are directly proportional to the concentration of analyte in the sample. A linear relationship should be evaluated across the range of the analytical procedure. It is demonstrated directly on the drug substance by dilution of a standard stock solution of the drug product components, using the proposed procedure. For the establishment of linearity, minimum of five concentrations is recommended by ICH guideline. The value of correlation co-efficient should fall around 0.99. The regression equation and correlation coefficient was calculated and found to be within the required limits as shown in Tables 2and 3 respectively.

3.2.2 Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample. The precision of an analytical procedure is usually expressed as the variance, standard deviation or

coefficient of variation of a series of measurements. The intra-day and inter-day precision results were shown in Table 4.

3.2.3 Accuracy/Recovery

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. The evaluation of accuracy has got very prime importance as it deliberately forces the method to extract the drug and impurities at higher and lower level. The recovery results for accuracy study of Levetiracetam were represented in Table 5.

3.2.4 Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. The results of robustness study were shown in Tables 6 & 7 respectively.

3.2.5 Limit of detection and limit of quantification

Limit of detection is the lowest concentration in a sample that can be detected, but not necessarily quantified under the stated experimental conditions. The limit of quantification is the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy. Limit of detection and limit of quantification was calculated using following formula LOD= 3.3SD/S and LOQ= 10SD/S, where the SD= standard deviation of response (peak area) and S= slope of the calibration curve.

4. RESULT AND DISCUSSION

4.1 System suitability test

To optimize the chromatographic conditions, the effect of chromatographic variables such as composition of mobile phase, flow rate and the column were studied. The resulting chromatograms were recorded and the chromatographic parameters such as peak area, resolution and theoretical plates were integrated. The conditions obtained most excellent resolution; symmetry factor and theoretical plate were selected for further estimation.

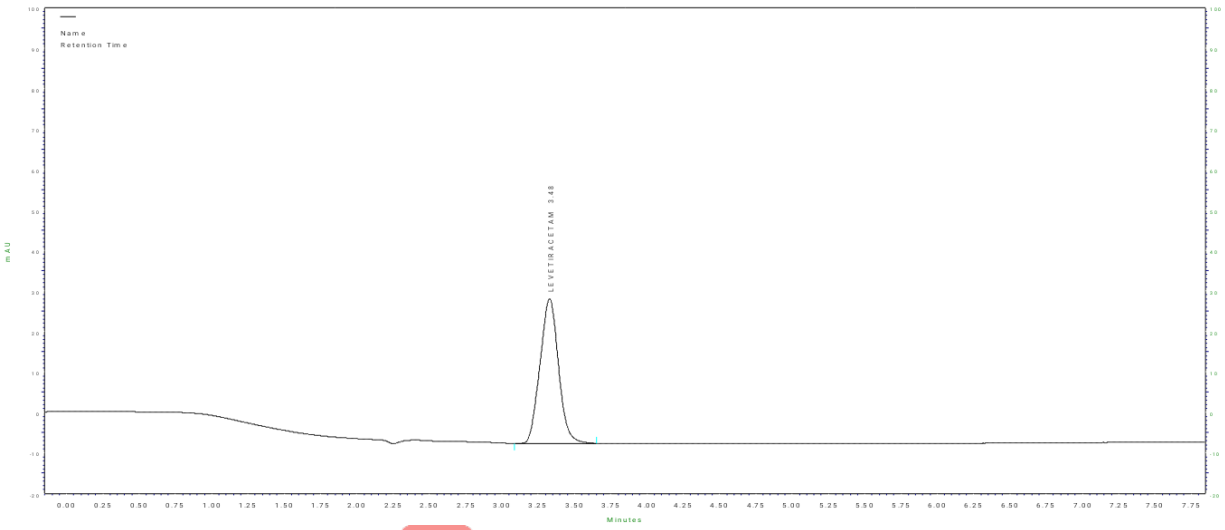

Fig1: Chromatogram of system suitability

Table 2: System suitability parameter

Sr.no	System suitability parameter	Mean observation	Standard limits	Inference
1	Retention time	3.63	NLT 2.0min	Passed
2	Area	312686	NLT 2000	Passed
3	Theoretical plate	33625	NLT 2000	Passed
4	Tailing factor	0.88	NMT 2.0	Passed
5	% RSD	0.57	NMT2.0%	Passed

4.2 Linearity

The standard calibration curve was constructed between concentration Vs peak area and linearity was found in the range from 10µg/ml to 60µg/ml. The regression equation and correlation coefficient was calculated and found to be within the required limit.

Table 3: Linearity data of Levetiracetam

Sr. no.	Conc. µg/ml	Mean Area*
1	10	314711
2	20	615815
3	30	929321
4	40	1245798
5	50	1545873
6	60	1849155

Table 4: Results for Linearity

Parameters	Values
Concentration range	10µg/ml to 60µg/ml
Regression equation (Y)	y = 30797x +5558.4.
Slope (m)	30797
Intercept (c)	5558.4
Correlation coefficient	0.9999

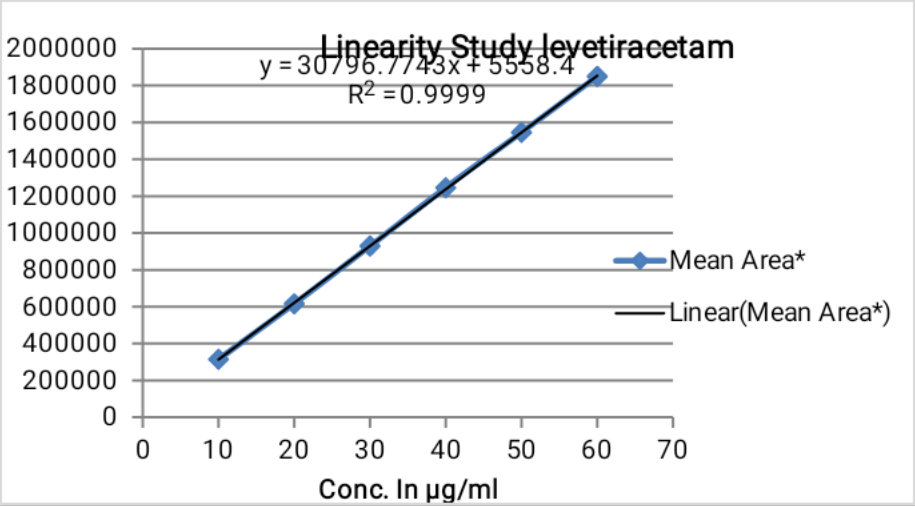

Fig.2: Calibration curve of Levetiracetam

4.3 Precision

Precision Intra-day precision was investigated by replicate applications and measurements of peak area for Levetiracetam for three times on the same day under similar conditions. Inter-day precision was obtained from %RSD values obtained by repeating three times on two different days. The %RSD was calculated which was within the acceptable limits of not more than 2.0.

Table 5: Results of Precision

Conc.(µg/ml)	Intra-day precision		Inter-day Precision	
	Mean± SD	% RSD	Mean± SD	% RSD
15	477447.3± 1584.44	0.33	477263.78±936.59	0.20
35	1079079±8900.57	0.82	1082361.1±11547	1.07
55	1693804.6±6249.0	0.37	1683346±21743	1.29

4.4 Accuracy/ Recovery

The accuracy of the method was tested by triplicate sample at 3 different concentrations equivalent to 80%, 100% and 120% of the active ingredient, by adding a known amount of Levetiracetam standard to a sample with predetermined amount of Levetiracetam. The recovered amount of Levetiracetam, % recovery of each concentration was calculated to determine accuracy.

Table 6: Results of Accuracy/ Recovery

	% Recovery Level	Amount of Standard Taken (µg/ml)	Amount of Sample Spiked (µg/ml)	Mean Area*	Amount Recovered (µg/ml)	% recovery
1	80	10	8	566564	18.31	101.73
2	100	10	10	625069	20.25	101.25
3	120	10	12	689038	22.26	101.22

4.5 Robustness

Robustness is the ability to provide accurate and precise results under a variety of conditions. In order to measure the extent of method robustness, the most critical parameters were interchanged while keeping the other parameters unchanged and in parallel, the chromatographic profile was observed and recorded. The studied parameters were the composition of flow rate, and mobile phase composition. The results of robustness study indicated that the small change in the conditions did not significantly affect the determination of Levetiracetam.

Table 7: Results for Robustness variation wavelength by ±1nm

Parameter	Conc. µg/ml	Mean area	Rt (min)	% Assay Limit (98-102)
Wavelength 205nm	10	312267	3.49	99.60
Wavelength 207nm	10	312452	3.49	99.70

Table 8: Results for Robustness flow rate variation ±0.05ml

Parameter	Conc. µg/ml	Mean area	Rt (min)	% Assay Limit (98-102)
Flow rate 0.85ml	10	309984	3.56	98.80
Flow rate 0.95ml	10	307675	3.49	98.10

4.6 Limit of detection and Limit of quantification

LOD & LOQ: LOD & LOQ Were determined by using following formulas

LOD=3.3xSTEYX/SLOPE
Where,
Steyx= 5514
Slope=30797
LOD=0.59

LOQ=10xSTEYX/SLOPE
Where,
Steyx=5514.91
Slope=30797
LOQ=1.79

Table 9: Result of LOD & LOQ

STEYX	Slope	LOD (µg/ml)	LOQ (µg/ml)
5514.91	30797	0.59	1.79

5. CONCLUSION

The proposed method for the assay of Levetiracetam was simple, rapid, accurate, precise, sensitive and economic for the quantification of Levetiracetam from its pharmaceutical dosage forms. The method was validated for linearity, accuracy, precision, LOD, LOQ, robustness and system suitability. The method was free from interference of other active ingredient and excipients. Hence it can be concluded that this method may be employed for routine quality control analysis of Levetiracetam in Active pharmaceutical ingredient and Formulation product.

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7. REFERENCES

1. 2014. The Indian Pharmacopoeia, Vol. IV, Controller of Publications, Government of India, Delhi, pp. 2838-2840.
2. Rockville, MD, 2013. The United States Pharmacopoeia, USP 36, NF 31, Vol. 2, The United States Pharmacopoeia Convention, Inc, pp 5307-5308.
3. Skoog, D., Holler 2007. F. Principle of Instrumental Analysis, ed.1st Thomson Publications, India, pp.1-3, 145-147, 180.
4. Beckett A. H. and Stenlake J. B 2002. In Practical Pharmaceutical Chemistry, 4(2), CBS Publisher and Distributers, New Delhi, 2002, 275-278, 281-300.
5. Validation of Analytical Procedure: Methodology, ICH Harmonized Tripartie Guidelines, November 1996, 1-8

6. ICH, Q2A, Tex on Validation of Analytical Procedures, International Conference on Harmonization, Geneva, October 1994, pp.1-5.
7. P D Sethi, 2001, HPLC Quantitative analysis of pharmaceutical formulation, CBS publishers and distributors, New Delhi, 2nd edition, Pg. No.1-20.
8. Samudrala V., Boovizhikannan T., Ramalingam K.., Gummadi G., Kannan V., AnkireddyV., 2012,5(5), Journal of Pharmacy Research 2834-2836.
9. G. Chaitanya, G. Venkata Ramana, A.K. M. Pawar, 2016, International Journal of Pharmacy and Analytical Research, Vol.5, Issue 1, 190-198.
10. S Gopalkrishnan, B. Jeyashree, A Ramalakshmi, and A Chenthilnathn, 2011, Analytical method development and validation for the determination of Levetiracetam in pharmaceutical formulations by using RP-HPLCRJRS, Vol.3, Issue3, Page No. 194-201.
11. B Rao, AVD Nagedrakumar, BV Raman, JPR 2012, Validated RP-HPLC method for the estimation of Levetiracetam in tablet formulations, Vol.5, Issue1, Page No. 75-78.
12. A L Rao, V N jahanvi, 2010, A Validated RP-HPLC method for the estimation of Levetiracetam in bulk and pharmaceutical formulations, E-J Chem, vol.7, Issue2, Page No. 601-604.
13. K Saikrishna, DVRN Bikshapathi, U Ashok, S Gade & B saikumar, 2011, New RP-HPLC method for determination of Levetiracetam in bulk and prepared tablets, AJBPR, vol.1, Issue3, Page No.294-301.
14. P K Sahu, M Mathrusri, B Rao Aug 2009, Development and validation of an RP-HPLC method for determination of Levetiracetam in bulk and pharmaceutical dosage form, ACAIJ, Vol.8, Issue1, Pg. No. 35-38.
15. Fathima A., Tangadpally R., Kondepudi R., Makula A.,2012, International Research Journal of Pharmacy, ,3(3),177-180.
16. M. Prasad Rao, K. Leelavathi, N. Chandreshta, V. Sowjanya, I Sireesha, I.Rajani, V.V. Naga Premi, 2016, INDO American Journal of Pharmaceutical Sciences, ,3 (5), 492-507.
17. Pani Kumar AD, VenkataRaju Y., Sunith G., Rama Krishna K, Ceema M, Venkteshara Rao 2011, A., International Journal of Research in Pharmaceutical and Biomedical science, Vol.2(1), ,175-181.
18.SreenivasaRaoT, Sukanya K, Chandanam S, Akkamma HG, Sai Kumar S, Manogna 2012, Research Journal of Pharmaceutical and Biological and Chemical science, Vol 3, Issue3, 713-721.
19.K. Ravi Kumar, CH.M.M. PrasadaRao, CH. BabuRao, K.B. ChandraSekhar, P. Gangi Reddy 2010, International Journal of ChemTech Research Vol.2, 307-311.
20.P. Ravisankar, G.DevalaRao, M. Nveen Kumar, M. Krishna Chaitanya, 2013, Scholars Research Library Vol,5(3), 249-260.
21.P D Sethi 2001, HPLC Quantitative analysis of pharmaceutical formulation, CBS publishers and distributors, New Delhi, 2nd edition, Pg. No.1-20.