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DEVELOPMENT AND VALIDATION OF SPECTROSCOPIC METHOD FOR ESTIMATION OF LEVETIRACETAM IN TABLET DOSAGE FORM

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ABSTRACT

The present work aims at developing newer analytical methods that are simple, rapid, sensitive, precise, reliable and accurate for analytical method development and validation of Levetiracetam in tablet dosage form. The Levetiracetam is a nootropic agents, anticonvulsants, the drug binds to a synaptic vesicle glycoprotein and inhibits pre synaptic calcium channels and reducing neurotransmitter release and acting as a neuromodulator and is safely used in the treatment of epilepsy.From the solubility profile glacial acetic acid was chosen as common solvent for the estimation Levetiracetamat 221 nm. The optimum conc. of the Levetiracetam was found to be 65µg/ml and it was shown good absorbance valve which was found to be 0.4738. Results of the analysis were validated statistically as per the ICH guidelines.Linearity studies were carried out and the range was found to be 30 – 90 □g/ml. The regression coefficient value of Levetiracetam in glacial acetic acid was found to be 0.99978.The accuracy of the method was performed by recovery studies.The percentage recovery was found to be in the range of 99.73–100.08%.The precision was performed by analyzing standard and sample solutions of Levetiracetam (65□g/ml)at working concentration level for 6 times.Further the precision of the method was confirmed by intra-day and inter-day analysis.The low RSD values indicate that the method is precise. The Robustness was performed at different wavelength by using working standard solutions of Levetiracetam. The % RSD values for wavelength variation were found to be 0.7403 (standard), 0.7357 (sample) in glacial acetic acid.

Keywords: ICH Guidelines, Levetiracetam, epilepsy, anticonvulsants, neurotransmitter.

INTRODUCTION:

Levetiracetam, chemically (2R)-2-(2-oxopyrrolidin-1-yl) butanamide(Fig. No.1), is anootropicagents, anti-convulsants, the drug binds to a synaptic vesicle glycoprotein, SV2A, and inhibits pre synaptic calcium channels and reducing neurotransmitter release and acting as a neuromodulator.Levetiracetam may selectively prevent hyper synchronization of epileptiform burst firing and propagation of seizure activity. Levetiracetam binds to the synaptic vesicle protein SV2A, which is thought to be involved in the regulation of vesicle exocytosis. Literature survey revealed that there are few analytical methods have been reported for the determination of Levetiracetam in pure drug, pharmaceutical dosage forms and biological samples using Visible Spectrophotometry, High Performance Liquid Chromatography and Mass Spectroscopy. But UV Visible spectroscopic methods are not available for the determination of Levetiracetam and in bulk as well as in their formulations. Hence an attempt was made to develop and validate simple, rapid and reliable analytical method for estimation of Levetiracetam. The present work aims to develop and validate a simple, reliable,

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workable and economical method for the estimation of Levetiracetamin table dosage form.

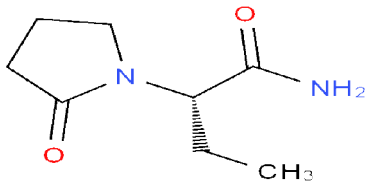

Figure 1. Structure of Levitracetam

MATERIALS AND METHODS

Materials:

Drug Samples

Levetiracetam was obtained as a gift sample from RA ChemPharma Pvt. Ltd. Hyderabad.

Reference standards

Levetiracetam -RA ChemPharma Pvt. Ltd.

Percentage purity - 99.86 %

Instruments used:

Different instruments used to carry out the present work, Electronic Weighing balance – single pan balance, Model Axis LC/GC. Digital pH meter - Model-Systronics. Sonicator- Ultra Sonicator – Model-Bandelinsonorex. Double Beam UV-Visible spectrophotometer A Schimadzu version 1.12-Double Beam UV Visible spectrophotometer. UV spectra of standard and sample solutions were recorded in 1cm quartz cells at the wavelength ranges of 200-400 nm.

Chemicals used:

Water - Milli Q water in house, Glacial acetic acid - Finar, Sodium hydroxide - GR/Merck, Potassium dibasic anhydrous - Molychem

Method Development

Standard preparation:

Weigh accurately about 100.0 mg of standard Levetiracetam, dissolve in glacial acetic acidand make up the volume to 100ml with the same. Pipette out 6.5 ml andmake up to 100 ml with glacial acetic acid. The final conc. of Levetiracetam standard was 65□g/ml.The solutions were scanned in UV region in the wavelength range from 200 to 400 nm.(Fig. No. 2)

Sample preparation:

Weigh equivalent weight of 131.6 mg of Levetiracetamtablet contents, dissolve in glacial acetic acidand make up the volume to 100ml with the same. Pipette out 6.5 ml andmake up to 100 ml with glacial acetic acid. The final conc. of Levetiracetam sample was 65□g/ml.The solutions were scanned in UV region in the wavelength range from 200 to 400 nm.(Fig. No. 3)

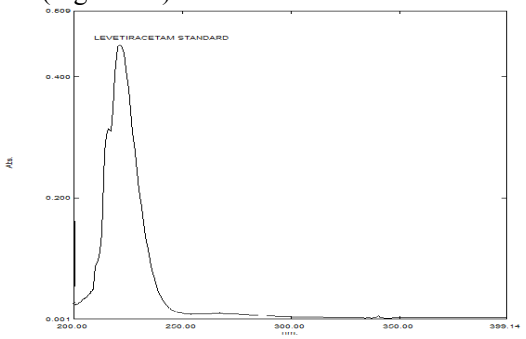

Figure 2. Standard Spectra

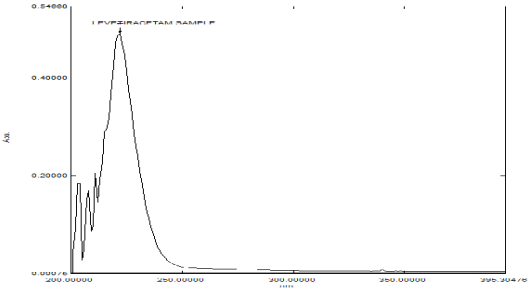

Figure 3. Standard Spectra

RESULTS AND DISCUSSION

Development of the spectrophotometric method

Proper wave length selection of the methods depends upon the nature of the sample and its solubility. To develop a rugged and suitable spectrophotometric method for the quantitative determination of Levetiracetam, the analytical condition were selected after testing the different parameters such as diluents, diluents concentration, diluents pH and other conditions.From the solubility profile glacial acetic acid was chosen as common solvent for the estimation Levetiracetam.

Table 1. Calibration Data for Levetiracetam

Concentration (µg/ml)	Absorbance
30	0.222
40	0.293
50	0.364
60	0.440
70	0.504
80	0.576
90	0.646

Selection of wavelength

By scanning the standard solution of Levetiracetam in UV spectrophotometer between 200 nm to 400 nm on spectrum mode, using glacial acetic acid as a blank, thewavelength of analysis (λmax), 221 nm was selected. Sample and standard solution absorbance was measured at 221 nm.

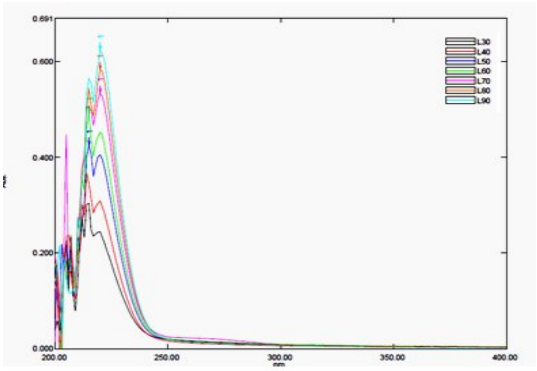

Figure 4. Overlay Levetiracetam spectrum

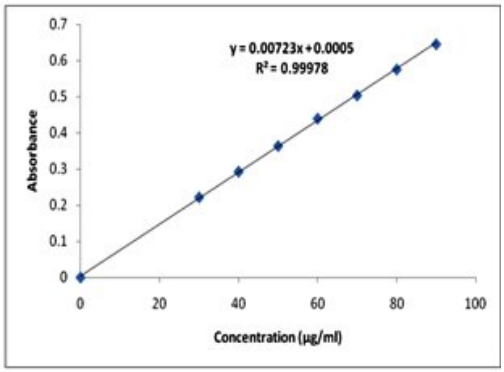

Figure 5. Linearity Curve

Validation of developed method

Linearity and range:

The linearity of an analytical method is its ability (within a given range) to obtain the test results which are directly proportional to the concentration (amount) of analyte in the samples within a given range.The calibration curve constructed was evaluated by using correlation coefficient. The absorbances of Levetiracetam were linear over the range of 30-90□g/ml(Fig No. 4& 5). The average absorbance of each concentration obtained was plotted against the concentration of the analyte. The correlation coefficient for the data was calculated as 0.99978. The regression line were observed to be in the form of y = 0.00723 x + 0.0001. The results are summarized in Table No 1.The experiments indicated that there was a linear relationship between the amount of analyte and the absorbances within the range studied.

Precision

The precision of the method was calculated from the reproducibility of percentage assay of six Levetiracetamsamples. The results are summarized in Table No 2. The results showed that the precision of the method is good.

Table 2. Evaluation data of Precision Study

S. No	LevetiracetamStandard Absorbance values at 221 nm in glacial acetic acid	
	Standard	Sample
1	0.476	0.485
2	0.473	0.481
3	0.474	0.478
4	0.475	0.477
5	0.471	0.477
6	0.474	0.478
Mean	0.4738	0.4793
SD	0.0017	0.0031
% RSD	0.3635	0.6553

Intermediate Precision

Further the precision of the method was confirmed by intra-day and inter-day analysis. The analysis of formulation was carried out for three times in the same day and one time in the three consecutive days. The % RSD value of intraday analysis was shown in Table No 3, 4.

Table 3. Intraday & Interday Precision Data

Parameter	Intraday Precision		Inter day Precision			
			Standard		Sample	
	Standard	Sample	Day-1	Day-2	Day-1	Day-2
Absorbance at λ max	0.472	0.482	0.470	0.471	0.481	0.482
	0.474	0.481	0.473	0.474	0.479	0.481
	0.475	0.479	0.471	0.475	0.478	0.478
Mean	0.4736	0.4806	0.4713	0.4733	0.4793	0.4803
SD	0.0015	0.0015	0.0015	0.0020	0.0015	0.0020
%RSD	0.3225	0.3177	0.3240	0.4397	0.3186	0.4333

The results were well within acceptable limits of % RSD less than 2.0% for all parameters viz., intraday, inter day and analyst to analyst variation. These results indicated that the developed method is rugged.

Accuracy

Accuracy of the method was expressed in terms of recovery of added compound at 80%, 100% and 120% level of sample. Mean % recovery and % RSD were calculated and were summarized in Table No 5. The result shown that best recoveries (99.6 – 101.4%) of the drug were obtained at each added concentration, indicating that the method was accurate.Evaluation data of accuracy study of Levetiracetam was shown in (Fig. No. 6)

Robustness

The evaluation of robustness should show the reliability of an analysis with respect to deliberate variations in method parameters.If measurements are susceptible to variation in analytical conditions, the analytical condition should be suitably controlled or a precautionary statement should be included in the procedure. The Robustness was performed at different wave length by using working standard solutions of Levetiracetam. The result of robustness study of the developed assay method was established in Table No 6.The result shown that during all variance conditions, assay value of the test preparation solution was not affected and it was in accordance with that of actual. System suitability parameters were also found satisfactory; hence the analytical method would be concluded as robust.

System suitability

A system suitability test of the spectrophotometric system was performed before each validation run. Six replicate reading of standard preparation were taken and % RSD of standard reading were taken for same. Acceptance criteria for system suitability, % RSD of standard reading not more than 2.0%, were full fill during all validation parameter.

Table 4. Ruggedness Data for Analyst to Analyst

Parameter	Levetiracetam Standard			Levetiracetam Sample		
	Analyst 1	Analyst 2	Analyst 3	Analyst 1	Analyst 2	Analyst 3
Analyst to Analyst	0.472	0.471	0.470	0.482	0.481	0.482
	0.474	0.474	0.473	0.481	0.479	0.481
	0.475	0.475	0.471	0.479	0.478	0.478
Mean	0.4736	0.4733	0.4713	0.4806	0.4793	0.4803
SD	0.0015	0.0020	0.0015	0.0015	0.0015	0.0020
%RSD	0.3225	0.4397	0.3240	0.3177	0.3186	0.4333

Table 5. Evaluation Data of Accuracy Study

% Recovery Level	% Recovery	Mean % Recovery	SD	% RSD
80%	0.376	99.73	0.2700	0.2707
	0.375			
	0.374			
100%	0.474	100.08	0.4200	0.4196
	0.472			
	0.476			
120%	0.543	99.88	0.3843	0.3847
	0.546			
	0.542			

Table 6. Robustness Data for Wavelength Variation

Wavelength(nm)	Levetiracetam inglacial acetic acid	
	Standard	Sample
220	0.471	0.477
221	0.474	0.481
222	0.478	0.474
Mean	0.4743	0.4773
SD	0.0035	0.0035

The optical parameters like molar absorptivity, correlation coefficient, slope, intercept, LOD, LOQ and standard error were calculated and results were shown in Table No 7.

Table 7. Robustness Data for Wavelength Variation

Wavelength(nm)	Levetiracetam in glacial acetic acid	
	Standard	Sample
220	0.471	0.477
221	0.474	0.481
222	0.478	0.474
Mean	0.4743	0.4773
SD	0.0035	0.0035
%RSD	0.7403	0.7357

Table 8. Validation Dataof Levetiracetam

Parameters	Levetiracetam in glacial acetic acid
Beers law limit (µg/ml)	20-120
Molar absorptivity (L mol^{-1} $cm^{-1)}$	0.00728
Sandell's sensitivity (µg/cm^2/0.001 A.U)	0.00728
Correlation coefficient (r^2)	0.99978
Regression equation (y = mx+c)	y = 0.00723x + 0.0001 R^2 = 0.99978
Slope (m)	0.00723
Intercept (c)	0.0001
LOD (µg/ml)	0.7759
LOQ (µg/ml)	2.3513
Standard Error	0.00069

CONCLUSION

The present analytical method was validated as per ICH Q2 (R1) guideline and it meets to specific acceptance criteria. It is concluded that the analytical method was specific, precise, linear, accurate, economic, and sensitive, and hence the present developed analytical method can be used for its intended purpose.

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REFERENCES:

1. International Conference on Harmonisation Guideline on Validation of Analytical Procedures: Text and Methodology; Q2 (R1), 2005.
2. ICH guidelines Q2B. In; Validation of analytical Procedures; Methodology, International Conference on Harmonization, Geneva 1996.
3. Code Q2A, Text on Validation of Analytical Procedures. ICH Harmonized Tripartite Guidelines Geneva, Switzerland 27th October, 1994, 1 - 5.
4. United States of pharmacopoeia and national formulary. 24th Asian edition. The United States Pharmacopoeia convention Inc; 2001, 2149-52.
5. Code Q2B, Validation of Analytical Procedures; Methodology. ICH Harmonized Tripartite Guidelines Geneva, Switzerland, 6th November, 1996, 1 - 8.
6. Abou-Khalil B, "Levetiracetam in the treatment of epilepsy". Neuropsychiatr Dis Treat Vol. 4 (3), June 2008, 507–23.
7. TR Browne, GK Szabo., IE Leppik. Absence of pharmacokinetic drug interaction of Levetiracetam with Phenytoin in patients with epilepsy determined by new technique. J ClinPharmacol. , Vol. 40, 2000, 590–595.
8. Lynch BA., Lambeng N., Nocka K. "The synaptic vesicle protein SV2A is the binding site for the antiepileptic drug levetiracetam". ProcNatlAcadSci USA. Vol. 101 (26): June 2004, 9861–9866.

9. Vogl C., Mochida S., Wolff C., "The Synaptic Vesicle Glycoprotein 2A Ligand Levetiracetam Inhibits Presynaptic Ca2+ Channels through an Intracellular Pathway". MolPharmacol. Vol.82 (2), August 2012, 199- 208.
10. Valarmathy J., Samueljoshua L., Rathinavel G., SelvinThanuja C. and Sivakumar T., RP-HPLC Method Development and Validation for Assay of Levetiracetam in Tablet Dosage Form , Research J. Pharm. and Tech. Vol. 1(3): July-Sept. 2008; 395-397.
11. Srinivasu K.,VenkateswaraRao J., Appalaraju N., VanithaPrakash K. and Mukkanti K., Extractive spectrophotometric determination of Levetiracetam in pharmaceutical formulations, Oriental Journal of Chemistry ,Vol. 24(3), 2008, 1013-1016.
12. Tripathi P., Ganesan M., Rauthan SK., Yadav S., Pandey Y., Validated liquid chromatographic ultra violet method for the quantization of Levetiracetam in human plasma using liquid–liquid extraction, International Journal of Pharmaceutical Sciences and Research Vol. 1 (1), 2010, 1-9.
13. Nafiz O Can., GokselArli.,Reversed-Phase HPLC Analysis of Levetiracetam in tablets using Monolithic and conventional C18 silica columns, Journal of AOAC International, Vol. 93(4), Jul-Aug 2010,1077-1085(9).
14. Raju GVH., Ganapathy S., Sankar DG., Naidu PY., Development and Validation of an HPLC Method for Analysis of Levetiracetam in Human Plasma,Asian Journal of Research in Chemistry, Vol. 3 (3) 2010, 1 &776-780.
15. Johnson-Davis K., Juenke JM., Brown P I, McMillin GA.,Simultaneous UPLC-TMS assay for the detection of Levetiracetam and Gabapentin in serum and plasma, Therapeutic Drug Monitoring, Vol. 33 (2), April 2011,209-213.
16. Madhu M, RamyaSmruthi Raj N, Swathi V, Yasmeen R, Mohammed Ishaq B., "Development and validation of UV spectroscopic method for the estimation of Temozolomide in capsule dosage form" International Journal of Biological & Pharmaceutical Research. 2014; 5(8): 701-705.
17. Madhu M, Swathi V, RamyaSmruthi Raj N, Yasmeen R, Pradeep Kumar Y., "Analytical method development and validation of Temozolomide in phosphate buffer using UV spectrophotometer" International Journal of Biological & Pharmaceutical Research. 2014; 5(7): 598-602.
18. Madhu M, Latha S, MadhusudhanaChetty C, Pradeepkumar Y,Hrushikeshreddy Y, Jaya sankar Reddy V., "analytical method development and validation of simultaneousdetermination of Atorvastatin calcium and Amlodipine besilatin tablet dosage form by RP-HPLC" .Journal of Global Trends in Pharmaceutical Sciences, April-June 2011; 2 (2): 149-160.
19. Sravani S, MadhuM ,Vineetkumar, Dipankarkaunakar, Achhrishgoel., "analytical method development & validation for simultaneous estimation of ambroxol hcl and Montelukast sodium in oral syrup by RP-HPLC method"International Journal Of Pharma Professional'sResearch, Oct-2014; 5 (4): 1125-1132.
20. Gireesh Kumar Eri, ChantiNaik M, Padma Y, VenkataRamana M, Madhu M, Gopinath C., "Novel spectroscopic method for the quantitation of Atenolol in bulk and tablet formulations" Journal of Global Trends in Pharmaceutical Sciences, 2014; 5(3):1750–1755.

21. Mohammed Ishaq, ThiruvengadaRajan, AngalaParameswari S, Amruth N, Madhu M and MadhusudanaChetty C., "Analytical Method Development and Validation of Erlotinib by High Performance Liquid Chromatography". Research J. Pharmacy. and Technology, Nov. 2011; 4(11):1787-1790.
22. MadhavaRao M, Gireesh Kumar E, Madhu M, Sushmitha A, Tiruvengadarajan V. S, Gopinath C., " Development and validation of RP-HPLC method for the assay of Tramadol HCl in its capsule formulation" International Journal of Advances in Pharmaceutical Research. July 2014; 5(7): 326– 331.
23. MadhavaRao M, Gireesh Kumar E, Madhu M, Sushmitha A, Tiruvengadarajan V.S, Gopinath C., "development and validation of UV spectrophotometric method for the assay of Tramadol HCl in its capsule formulation". International Journal of Advances in Pharmaceutical Research. Oct.2014; 5(10): 400 – 405.
24. Venkata Prasad G, Sravani S, Mohammed Ishaq B, Madhu M, SreenivasuluMunna,Gopinath C., "Development and Validation of UV-Spectrophotometric Method for Determination of Cephalexin". Asian J. Research Chem., May 2013; 6(5): 490-494.
25. Swetha A. V, Gireesh Kumar Eri, Madhu M, Susmitha A, Gopinath C "Development and validation of new analytical methods for the assay of Mebeverine hydrochloride in tablet dosage form". International Journal of Advances in Pharmaceutical Research Oct. 2015; 6(10): 320 – 331.

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