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Development and Validation of UV Spectroscopic and RP-HPLC Method for Determination of Levetiracetam in Bulk and Combined Dosage Form

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Abstract: The objective of present research was to develop and validate suitable UV Spectroscopic and RP-HPLC method for analyzing Levetiracetam in single and combined dosage form. The UV method was performed at the wavelength of 206 nm using water as solvent and various validation parameters such as, Linearity, Range, Accuracy, Precision, Limit of detection, Limit of Quantification, Ruggedness and Robustness were studied, similarly the RP-HPLC method was performed using Potassium dihydrogen phosphate: Acetonitrile in the ratio of 80:20 and various validation parameters as evaluated for UV spectroscopic method above were also evaluated for Levetiracetam by RP-HPLC method. Both the developed methods were found to be accurate and precise with all the validation parameters within the range. The regression coefficient values were found to be 0.9999 and 0.9999 for UV and RP-HPLC method respectively. Both these method can be effectively used in determination of Levetiracetam in single or combine form.

Keywords: Levetiracetam, UV Spectroscopy, RP-HPLC, Validation

1 Introduction

Levetiracetam is an antiepileptic drug used in treatment of epilepsy, partial onset, myoclonic or tonic-clonic seizures. It is S-enantiomer of Etiracetam. Its chemical name is (S)-2-(2-oxopyrrolidin-1-yl) butanamide. It acts by binding to SV2A (synaptic vesicle glycoprotein 2A) and inhibits presynaptic calcium channels reducing neurotransmitter release and acting as a neuromodulator. Fig.1 [1-3].

The UV spectroscopic method is simple and rapid method for the detection of various dosage forms. UV-Visible spectroscopy is the measurement of the wavelength and intensity of absorption of near ultraviolet and visible light by a sample. Ultraviolet and a visible light are energetic enough to promote outer electrons to higher energy levels. By using this UV spectroscopic method Levetiracetam determination is carried out and all validation parameters are performed [4-6].

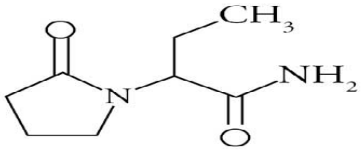

Fig. 1: Chemical Structure of Levetiracetam.

High performance liquid chromatography (HPLC) is the major and integral analytical tool applied in all stages of drug discovery, development and production. HPLC is the method of choice for checking peak purity of new chemical entities, monitoring reaction changes is in synthetic procedures or scale up, evaluating new formulations and carrying out quality control / assurance of final drug product. The goal of HPLC method is to separate and quantify the main drug, any reaction impurities, all available synthetic intermediates and any degrades [7-12].

2 Experimental Sections

2.1 UV Spectroscopic Method

2.1.1 Materials

UV-Visible Double Beam Spectrophotometer Shimadzu, Model UV-1800-240 V, Wavelength Range 190-1100 nm, Band Width 2 nm, 1 cm Matched Quartz Cells, UV Probe software. Levetiracetam was received as gift sample from Lupin ltd., Aurangabad. Tablet formulation manufactured by ucb pharma was purchased from local market Keppra 250 mg per tablets.

2.1.2 Preparation of Standard Drug Solution

10 mg of Levetiracetam was weighed and is dissolved in water and the final volume was adjusted to 100 mL to obtain stock solution (100 µg/mL) 100 mL.

2.1.3 Determination of Analytical Wavelength

1mL is taken from working stock solutions and volume adjusted to 10 mL using water and the samples were scanned to get good results. The wavelength selected should be such that at wavelength the absorbance of component should be as large as possible. So the wavelength chosen was 206 nm for Levetiracetam. (λmax)

2.1.4. Preparation of Calibration Curve

Appropriate aliquots were pipette out from the standard stock solution into a series of 10 mL volumetric flasks. The volume was made up to the mark with water to get a set of solutions for each drug having the concentration 2, 4, 6, 8, 10 µg/mL. The absorbance of each of these solutions were measured at the selected wavelength 206 nm and plotted against concentration.

2.1.5. UV Method Validation

2.1.5.1 Linearity and Range

For quantitative analysis of Levetiracetam, the calibration curves were plotted for each concentration ranges. The linearity ranges for Levetiracetam was found to be 2-10 µg/mL.

2.1.5.2. Precision

The reproducibility of proposed method was determined by performing tablet assay at different time intervals (3 hour interval) on same day (Intra-day precision) and on three consecutive days (Inter-day precision) .

2.1.5.3. Detection Limit and Quantification Limit (LOD and LOQ)

The limit of detection (LOD) and limit of quantification (LOQ) of Levetiracetam by the proposed methods were determined using calibration standards. LOD and LOQ values were calculated as 3.3 SD/D and 10 SD/D respectively, where D is the slope of the calibration curve and SD is the standard deviation.

2.1.5.4 Accuracy

The accuracy was determined by standard addition method. Three different levels (80%, 100% and 120%) of standards were spiked to commercial tablet in triplicate. The mean of percentage recoveries and the % relative standard deviation (%RSD) was calculated.

2.1.5.5 Robustness and Ruggedness

Table 01: Instrumentation of HPLC.

Instrument	**Manufacturing company**
HPLC	Shimadzu
Detector & pump no.	UV 730 D & SP 930 D
Software	Class VP 6.13 SP2
Column	4.6 x 250 mm
Particle size packing	5 µ
Stationary phase	C18 (Luna)
Mobile phase	0.136% w/v KH_2PO_4: Acetonitrile (80:20)
Injection volume	20 µl

The robustness study was carried out by determining effect of small variation in wavelength and in ruggedness; sample was analyzed by two different analysts.

2.2 HPLC Method

The instrumentation of HPLC method was provided in Table 01 with specifications.

2.2.1 Reagents and Chemicals

HPLC Grade Acetonitrile and Potassium dihydrogen phosphate were purchased from Merk Mumbai, India.

2.2.2 Selection of Stationary Phase

Luna 5µ 4.6 x 250 mm C18 column was selected for analysis.

2.2.3 Selection of Mobile Phase

Mobile phase consisting 0.136% w/v KH_2PO_4: acetonitrile in a ratio (80: 20) was selected with a flow rate 1mL/min.

2.2.4 Selection of Detection Wavelength

The selection of wavelength was carried out by obtaining the UV-Visible Spectra of 60 µg/mL solution of Levetiracetam in mobile phase, and the wavelength of maximum absorption was found to be 206 nm.

2.2.5 Preparation of Standard Solution

10 mg of Levetiracetam active pharmaceutical ingredient (API) were weighed and transferred into 10 mL volumetric flask. Then drug is dissolved in 5 mL of mobile phase by vigorous shaking and then volume was made up to the mark with mobile phase to obtain final concentration of 1000

µg/mL. 10, 20, 30, 40, 50, 60 µg/mL standard working solutions were prepared by serially diluting the stock solution.

2.2.6. HPLC Method Validation

2.2.6.1 Linearity

For quantitative analysis of Levetiracetam, the calibration curves were plotted for each concentration ranges. The linearity ranges for Levetiracetam found to be 10-60 µg/mL respectively.

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

The LOD and LOQ of Levetiracetam by the proposed methods were determined using calibration standards. LOD and LOQ values were calculated as 3.3 SD/D and 10 SD/D respectively, where D is the slope of the calibration curve and SD is the standard deviation.

2.2.6.3 Accuracy

The accuracy was determined by standard addition method. Three different levels (80%, 100% and 120%) of standards were spiked to commercial tablet in triplicate. The mean of percentage recoveries and the %RSD was calculated.

2.2.6.4 Precision

The reproducibility of proposed method was determined by performing tablet assay at different time intervals (3 hour interval) on same day (Intra-day precision) and on three consecutive days (Inter-day precision) .

2.2.6.5 System Suitability

System suitability was done to verify the repeatability of HPLC method. Theoretical plate, repeatability of retention time and peak area were determined and compared.

2.2.6.6 Robustness and Ruggedness

The robustness study was carried out by determining effect of small variation in wavelength and in ruggedness; sample was analyzed by two different analysts.

3 Results and Discussion

3.1 UV Spectroscopic Method:

3.1.1. Analytical Wavelength:

The maximum absorption observed at the wavelength of 206 nm hence the wavelength for Levetiracetam is 206 nm hence the wavelength of Levetiracetam complies with pharmacopoeial standards as per USP-NF (2004). (Figure 2)

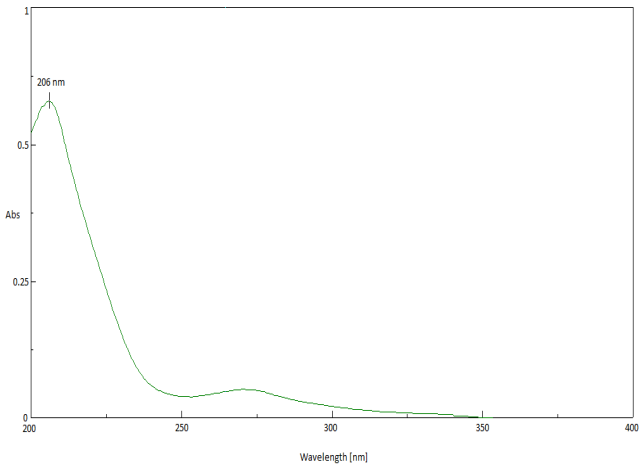

Fig. 2: UV Spectra of Levetiracetam.

3.1.2 Linearity and Range

The linearity was determined by using working standard solutions between 2-10 µg/mL. The absorbances of these solutions were recorded (table 02). Calibration curve of absorbance vs concentration plotted on excel sheet linear regression was performed. The correlation coefficient, regression equation of Levetiracetam was calculated by using list square method and it was found that the method was linear at the desired concentration range (figure 3). Syed et al (2014) performed similar type of study for determination of atenolol.

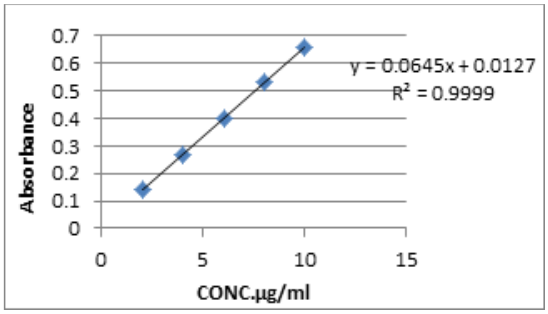

Fig. 3: Calibration Curve for Levetiracetam by UV.

Table 2: Calibration curve data for Levetiracetam by UV.

Conc. (µg/ml)	Abs.
2	0.142
4	*0.268*
6	*0.401*
8	*0.531*
10	0.655

3.1.3 Limit of Detection and Limit of Quantification

Limit of detection is the lowest amount of analyte which can be detected but not necessarily quantified and limit of

quantification is the lowest possible concentration that can be quantified. LOD and LOQ of Levetiracetam was found to be 0.13 µg/mL and 0.41 µg/mL respectively as calculated by Linearity curve. Lower value indicate that 0.13 µg/mL can be detected in bulk or combined form for Levetiracetam and 0.41 µg/mL can be quantified in bulk or combined form for Levetiracetam. Syed et al (2014) performed similar type of study for determination of atenolol.

3.1.4 Accuracy

The accuracy of analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted true value. The accuracy for the analytical method was evaluated at 80%, 100% and 120% levels. Absorbance was measured at 206 nm wavelength and results were obtained in terms of percent recovery. Three determinations at each level were performed and % RSD was calculated for each level and found to be 0.8125(Table 3). Ravishankar et al (2015) performed similar study for levetiracetam but the recovery was low.

Table 3: Recovery Studies.

Statistics	Level of Recovery		
	80%	100%	120%
Amount present (µg/ml)	2	2	2
Amount of standard added (µg/ml)	1.6	2	2.4
Total amount recover	3.52	3.93	4.31
%recovery	95.41	96.94	96.48
Mean	96.28		
SD	0.7823		
%RSD	0.8125		

3.1.5 Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogenous sample under prescribed conditions. Intra-day precision was studied by recording absorbance of standard solution of 6 µg/mL concentration at three independent series in the same day (Table 4). Inter-day precision studies were performed by recording absorbance of standard solution of 6 µg/mL concentration on three consequent days (Table 5). The % RSD was calculated. syed et al(2014) and ravishankar et al (2015) performed similar type of study for different drugs but the results obtained in the current study were within the limit.

Table 4: Intra – day Precision by UV

Analyte	Absorbance		
	0 Hr.	3 Hr.	6 Hr.
Mean	0.4066	0.4005	0.3757
SD	0.0028	0.0012	0.0044
%RSD	0.6913	0.3020	1.1891

Table 5: Inter – day Precision by UV

Analyte	Absorbance		
	0 Hr.	24 Hr.	48 Hr.
Mean	0.4066	0.3832	0.3663
SD	0.0028	0.0041	0.0029
%RSD	0.6913	1.0756	0.7921

3.1.6 Robustness of the Method

The concept of robustness of an analytical procedure has been defined by the ICH as "a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters". Robustness was studied by determining effects of small variation of wavelength (±1) no significant change in SD value and RSD of absorbance indicating the robustness of method. Syed et al performed similar type of study.

3.1.7 Ruggedness of the Method

Method ruggedness is defined as the reproducibility of results when the method is performed under actual use conditions. This includes different analysts, laboratories, columns, instruments, sources of reagents, chemicals, solvents and so on. Tablet sample was analysed by two different analyst at the same concentration no significant change found in absorbance and % RSD of absorbance found less than 2% which represent that method was rugged. Syed et al(2014) performed similar type of study.
All the validation parameters was reported in Table 6

Table 6: Summary of Validation Parameters by UV.

Parameters	Data for Levetiracetam
Linearity range	2-10 µg/ml
Line of regression	y = 0.0645x + 0.0127
Correlations coefficient	R^2 = 0.9999
Limit of detection (µg/ml)	0.13
Limit of Quantification (µg/ml)	0.41
Accuracy (%mean recovery)	96.28%
Intra-day precision	0.7274
Inter-day precision	0.8530
Robustness	Robust
Ruggedness	% RSD is less than 2

3.2 HPLC Method

3.2.1 Linearity

A 80: 20 v/v mixture of 0.136% w/v KH_2PO_4 and acetonitrile was used and dilution was made in the range of 10-60 µg/mL for Levetiracetam (table 07). The calibration graph constructed by plotting concentration of the drug against the peak area was found to be linear in the concentration range of 10-60 µg/mL for Levetiracetam. Beer's law was found to be obeyed over this concentration range. The regression equation was found to be Y = 307.98x + 51.026 and the correlation coefficient (r) of the standard curve was found to be 0.9999(fig 04 and 05). Bhatnagar et al performed similar study for levodopa and carbidopa in combination.

Table 7: Linearity of Levetiracetam by HPLC.

Sr.no.	Conc. (µg/ml)	Mean peak area
1	10	3152.11
2	20	6149.81
3	30	9284.21
4	40	12446.31
5	50	15452.39
6	60	18496.55

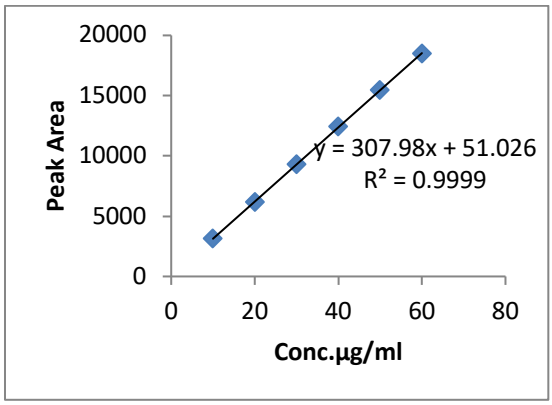

Fig. 4: Calibration Curve of Levetiracetam by HPLC.

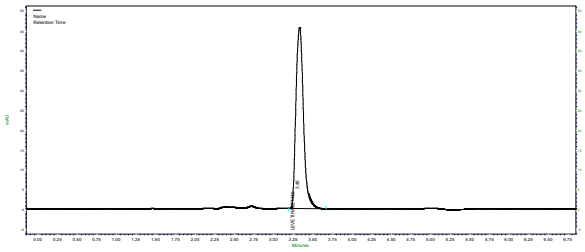

Fig. 5: Typical Chromatogram of Levetiracetam.

3.2.2 LOD and LOQ

The limit of detection and limit of quantification for Levetiracetam was found to be 0.5643 µg/mL and 1.7102 µg/mL respectively, which indicate the sensitivity of the method. Nagaraju et al performed similar study for levetiracetam but the values were deviating from the standard and also the combination of mobile phase was very complex.

3.2.3 Specificity

The specificity of the method was ascertained by analysing standard drug and sample. The spot for Levetiracetam in sample was confirmed by comparing the R*f* and spectra of the spot with that of standards indicating no interference of any another peak of mobile phase, impurity. Rao et al performed the similar work but the results obtained were not specific as per the data.

3.2.4 Precision

The precision of the assay was determined by repeatability (intraday) (table 08) and intermediate precision (inter-day) (table 09) and reported as %RSD. The %RSD values obtained from peak area for Levetiracetam were observed. Rao et al (2010) performed precision study but it was found to be more than 100% and also has not reported SD and %RSD.

Table 8: Intra-day precision by HPLC

Sr no.	Conc.(µg/ml)	SD	% RSD
1	15	0.47	0.46
2	35	0.67	0.68
3	55	0.96	0.99

Table 9: Inter-day precision by HPLC.

Sr no.	Conc.(µg/ml)	SD	% RSD
1	15	1.06	1.03
2	35	1.70	1.70
3	55	0.91	0.92

3.2.2 Accuracy

Recovery studies were performed to judge the accuracy of the method. The studies were carried out by adding a known quantity of pure drug to the pre-analyzed formulation and the proposed method was followed. From the amount of drug found, the percent recovery was calculated. The accuracy of the method was determined by recovery studies and the percentage recovery was calculated. The proposed liquid chromatographic method was applied to the determination of Levetiracetam in their dosage forms (keppra 250 mg tab) (table 10). Masud et al

(2011) performed the accuracy study but the values were found to be not correlating with the results obtained.

Table 10: Accuracy by the recovery study of Levetiracetam.

Stastistics	Level of recovery		
	80%	100%	120%
Amount present(µg/ml)	10	10	10
Amount of standard added (µg/ml)	8	10	12
Mean peak area	5691.65	6286.5	6900.29
Total amount recover(µg/ml)	18.15	20.05	22.00
%recovery	101.91	100.50	100.06
SD	0.91	1.37	1.20
%RSD	0.89	1.36	1.20
Mean % recovery	100.82		
Mean SD	1.16		
Mean % RSD	1.15		

3.2.3 Robustness of the Method

Robustness was studied by determining effects of small variation wavelength (± 1) (Table 11 a) and flow rate (±0.1) (Table 11b). There was no significant change in R*f* value and relative standard deviation of peak area indicating the robustness of method. It was observed that there were no marked changes in the chromatograms, which demonstrated that the proposed method was robust.

3.2.4 Ruggedness of the Method

Table 11a: Robustness Study for Levetiracetam.

Flow rate	Flow- 0.90 ml/min		Flow rate	Flow- 1.1 ml	
Sr no.	Conc. (µg/ml)	Area	Sr no.	Conc.(µg/ml)	Area
1	10	3059.42	1	10	3110.19
2	10	3064.37	2	10	3109.12
3	10	3109.62	3	10	3086.32
Mean		3077.80	Mean		3101.87
SD		27.66	SD		13.48
%RSD		0.89	%RSD		0.43

Tablet sample was analysed by two different analyst at the same concentration the peak area of the sample were not very significant and % RSD of peak area found less than 2% which represent that method was rugged.

Summary of validation parameters by HPLC was as per Table 12.

Table 11b: Robustness Study for Levetiracetam.

Wavelength	205 nm		Wavelength	207 nm	
Sr no.	Conc.(µg/ml)	Area	Sr no.	Conc.(µg/ml)	Area
1	10	3112.48	1	10	3119.73
2	10	3128.17	2	10	3149.28
3	10	3130.26	3	10	3155.26
Mean		3123.63	Mean		3141.42333
SD		9.71	SD		19.02342 33
%RSD		0.31	%RSD		0.60

Table 12: summary of validation parameters by HPLC.

Parameters	**Data for Levetiracetam**
Linearity range	10-60 µg/ml
Line of regression	y = 307.98x + 51.026
Correlations coefficient	R^2 = 0.9999
Retention time (Rf value)	3.48 min
Tailing factor (Tf)	1.24
Limit of detection (µg/ml)	0.56
Limit of Quantification (µg/ml)	1.71
Accuracy (%mean recovery)	100.82
Intra-day precision	0.71
Inter-day precision	1.22
Robustness	Robust
Ruggedness	% RSD is less than 2

4 Conclusions

In the present study suitable UV spectroscopic and RP-HPLC method were developed and validated as per ICH guidelines for determination of Levetiracetam in bulk and combined dosage formulation. It was found that the proposed methods were linear, accurate, reproducible, repeatable, precise, selective, cost effective and specific providing the reliability of the methods.

The developed methods are recommended for routine and quality control analysis of Levetiracetam in bulk or combination. The amounts found from the proposed methods were in good agreement with the label claim of the formulation. The developed method can also be conveniently adopted for dissolution testing and in vivo drug release studies in various pharmaceutical formulations for levetiracetam.

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