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Asian Journal of Pharmaceutical Research and Development

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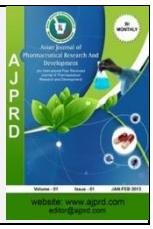

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Research Article

A Combine Spectrophotometric and Chromatographic Method Development and Validation of Levetiracetam Bulk And Tablet Dosage Form

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A B S T R A C T

Objective: Objective of the present analytical research work was to develop and validate Spectrophotometric method and High Performance Liquid Chromatographic method (HPLC Method) for the Levetiracetam bulk and tablet dosage form

Methods: A spectrophotometric method and a HPLC method have been developed and validated for estimation of Levetiracetam in bulk.

Method A (UV SPECTROMETRY Method): Methanol was used for the preparation of stock and working standard solutions of the drugs. 400-200nm UV range was used to scanned standard solutions of drugs using UV spectrophotometer. The λ_{max} of Levetiracetam was found to be 220 nm.

Method B (HPLC Method): The HPLC method for Levetiracetam was developed using cosmosil C18 (4.6 mm x 250 mm, particle size: 5 µm), as stationary particle, isocratic mode. Methanol: ACN: Water (60:20:20) pH3 as a mobile phase. The mobile phase was maintained at a flow rate of 1 ml / min and the detection was carried out at 220 nm. Both the methods were validated according to the ICH guidelines.

Results: Levetiracetam was found to be linear in the concentration range of 10-50 µg/ml for spectrophotometric and HPLC method. Retention time was found to be 4.5 min for Levetiracetam.

Interpretation and Conclusion: Results of validation study were found to be satisfactory. So, the methods can be successfully applied for the routine analysis of Levetiracetam.

Keywords: UV Spectrophotometric Method, HPLC Method, Levetiracetam

A R T I C L E I N F O: Received 18 July 2021; Review Complete; 15 August 2021 Accepted; 28 Sept. 2021 Available online 15 Oct. 2021

Cite this article as:

Pimpale A, Pagare R, Aher S, Phadtare D, A Combine Spectrophotometric and Chromatographic Method Development and Validation of Levetiracetam Bulk And Tablet Dosage Form , Asian Journal of Pharmaceutical Research and Development. 2021; 9(5):40-39.

DOI: http://dx.doi.org/10.22270/ajprd.v9i51000

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INTRODUCTION

Levetiracetam is a medication within the pyrrolidine class that is used to treat different types of seizures that originate in epileptic issues. He was supported for the first time for use in the United States in 1999 and is mainly with another enemy of epileptic drugs (AEDs). Levetiracetam has a large restorative file and virtually no possibility of delivering, or relying on pharmacokinetic communications, these

qualities are settled in a positive decision on other AEDs, a class of infamous drugs by having thinner records useful and an inclination to the association in Drug collaborations. [15,16]

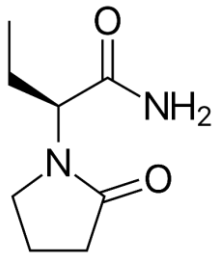

Figure 1: Structure of Levetiracetam

By chemically Levetiracetam is (*S*)-2-(2-Oxopyrrolidin-1-yl)butanamide with molecular formula and weight of $C_8H_{14}N_2O_2$ and 170.212 g/mole respectively. This investigation strategy follows ICH approval rules. This research strategy follows the approval rules of the ICH. This research tries to promote the new fast and convincing technique to guarantee levetiracetam in the massive structure, as indicated by the rules of ICH Q2 R1.[15,16]

MATERIALS AND METHODS

Chemicals and Reagents

Analytically pure samples of Levetiracetam api were kindly provided by Invochem labratories, Water (HPLC Grade), Acetonitrile and MeOH (AR grade) Merck specialities private limited, Mumbai.

Instrument Used

Ultrasonicator (Wenser pvt ltd PGB-100), Electronic Weighing Balance (Shimadzu AY-220), Cellulose Acetate Filter, 0.45 µm (Nylon 66), UV VIS Spectrophotometer (Shimadzu UV-1800), HPLC System (Analytical Technologies).

1. Spectrophotometric Method

1.1 Development of Spectrophotometric Method

Selection of Solvent

Solutions of Levetiracetam (1000 µg/ml) were prepared in various solvents like Acetonitrile, methanol and water. These tests solutions were scanned under UV-Visible Region between (200 nm to 800 nm) and intensity of absorption and wavelength of absorption were calculated.

Preparation of Standard Stock Solution

Standard stock solution was prepared in methanol by dissolving 10 mg drug into 10 ml methanol to obtain 1000 µg/ml strength.

Selection of Wavelength Range

From the stock solutions, 0.1 ml of Levetiracetam standard stock solution was transferred to 10 ml volumetric flask and the volume was adjusted to the mark with MeOH to obtain Strength 10µg/ml. The solution was scanned under the UV range 200-400 nm.

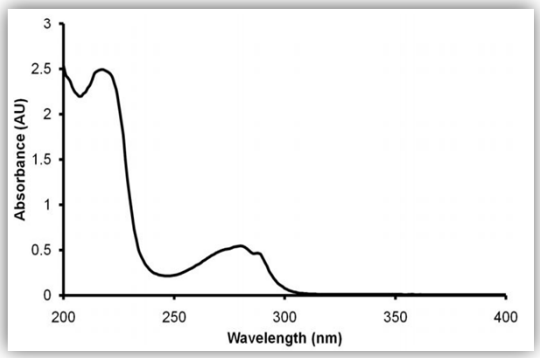

Figure 2: UV spectra of Levetiracetam

Preparation for Calibration Curve

Calibration curve were prepared and graph was plotted by absorbance vs Wavelength.

Analysis of Tablets

For the analysis of the commercial formulation, twenty tablets were weighed, the average weight determined and crushed in fine powder. A heavy amount with powder precision equivalent to 10 mg of levetiracetam was transferred into a 10 ml volumetric flask containing 5 ml of methanol, stirred manually for 10 min, the volume was adjusted to mark with the same solvent and filtered to Through Whatman Filter Paper. The absorbance of the sample solution was recorded to the registered at 220 nm.[18]

1.2. Validation of Spectrophotometric Method[1,3]

Linearity and Range

The linearity of the analytical method for the levetiracetam was evaluating by studying standard calibration curves. The analytical method range was decided from the interval between the upper and lower level of the calibration curves when tracing the registration curve.[11,13,15]

Accuracy

Accuracy of the method was determined by standard addition method at three different concentration levels i.e. 50%, 100%, 150%. Standard concentration of 10,20 and 30 µg/ml was added into 10 µg/ml of tablet concentration. The % recovery was then calculated by using formula

% Recovery = A – B/ C,

Where,

A = Total amount of drug estimated

B = Amount of drug found on pre analysed basis

C = Amount of Pure drug added

Precision

The precision of an analytical method was studied by performing intraday and interday precision.

Intra-day Precision

Intra-day precision was carried out by analyzing the 10, 20, 30 µg/ml of Levetiracetam solution for three times in the same day.

Inter-day Precision

Inter-day precision was carried out by measuring the the 10, 20, 30 µg/ml of Levetiracetam solution for three consecutive days.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Detection limit and Quantitation limit were determined depend on the standard deviation of y-intercepts of calibration curves and average slope of calibration curves.

$$LOD = 3.3 \times \frac{\text{Standard deviation of intercept}}{\text{Slope}}$$

$$LOQ = 10 \times \frac{\text{Standard deviation of intercept}}{\text{Slope}}$$

Ruggedness

Ruggedness of the method was determined by two different analysts keeping same experimental and environmental conditions. An appropriate concentration 30 and 50 µg/ml of Levetiracetam solution was subjected to analysis and % RSD was determined. This procedure was repeated three times for each analyst.[4,5]

2. Chromatographic Method

2.1. Development of Chromatographic method

Description

The sample of Levetiracetam pure drug was observed for its color, texture and nature.

Solubility

The small amount sample of Levetiracetam was taken in test tubes and observed its solubility in various solvents like Acetonitrile, Methanol and water.

Selection of Mobile Phase

The selection was carried out on the basis of literature survey. After measuring the solubility of drug in different solvents as well on the basis of literature review; Acetonitrile, Methanol and water were selected as a first choice.

Selection of Analytical Wavelength

To find out the appropriate wavelength for the determination of Levetiracetam, the solution of the same in the MeOH were scanned separately by UV–Visible spectrophotometer in the range of 190-400 nm and the spectrum were recorded.[9,10]

Preparation of Mobile Phase

***Mobile Phase A*:** AR grade Acetonitrile (60%)

***Mobile Phase B*:** AR grade MeOH (20%)

***Mobile Phase C*:** AR grade water (20%)

All the solvents were degassed in ultra-sonicator for 15 min

Preparation of Standard Stock Solution

Standard stock solution was prepared by dissolving 10 mg of Levetiracetam in 10 ml methanol that gives concentration of 1000 g/ml of Levetiracetam and labeled as Standard stock Levetiracetam.

Preparation of Calibration Curve

Analysis of tablets

To determine the content of levetiracetam in conventional tablets; Twenty tablets were weighed, their medium weight determined and finely fed and 10.0 mg of levetiracetam were transferred to a 10 ml volumetric flask containing 5 ml of methanol, sonicated for 30 minutes and diluted at 1000 ml with Methanol. The resulting solution was filtered, using a filter of 0.22 µm and 15 µg / ml was injected into the system. The amount of levetiracetam was determined. The test procedure was repeated for six times and calculated using the following equation.

$$Ct = \frac{Rt \times Cs}{Rs}$$

Where, Ct and Cs = Concentration of Sample and Standard Solution, respectively.

Rt and Rs = Peak Area for Sample and Standard Solution, respectively.

2.2 Validation of HPLC Method[1,2]

Linearity

The linearity of analytical method for Levetiracetam was evaluated by studying standard calibration curves. The range of analytical method was find out from the interval between upper and lower level of concentrations of calibration curves by plotting the Area obtained vs Concentration.

Accuracy

Accuracy of the method was determined by standard addition method at three different concentration levels i.e. 50%, 100, 150%. Standard concentration of 10,20 and

30 µg/ml was added into 10 µg/ml of tablet concentration. The % Recoveries was determined by applying regression equation on it.

Precision

The precision of an analytical method was evaluated by performing intraday and interday precision.

Intra-day Precision

Intra-day precision was calculated by analyzing the standard solutions of Levetiracetam (10, 30, 50 µg/ml) and at three different time intervals on same day.

Inter-day Precision

Inter-day precision was calculated by analyzing the combined standard solution of Levetiracetam (10, 30, 50 µg/ml) on three consecutive days. The results were reported in form of % RSD.

Limit of Detection and Limit of Quantitation

Detection limit and Quantitation limit were calculated based on the standard deviation of y-intercepts of calibration curves and average slope of calibration curves.

$$LOD = 3.3 \times \frac{\text{Standard deviation of intercept}}{\text{Slope}}$$

$$LOQ = 10 \times \frac{\text{Standard deviation of intercept}}{\text{Slope}}$$

Robustness

Standard sample solution of Levetiracetam (20 µg/ml)) were used and analysis carried out at different flow rate (0.7,0.8,0.9 ml/min) and wavelength (218,220,222 nm).

Ruggedness

Ruggedness of the method was evaluated by two different analysts keeping same experimental and environmental conditions. An appropriate concentration 30 µg/ml of Levetiracetam sample solution was subjected to analysis and % RSD was determined. This procedure was repeated three times.

System Suitability

Standard solution of Levetiracetam (30 µg/ml) was prepared and analyzed. Chromatograms were studied for different parameters such as retention time , Asymmetry factor and No. of theoretical plates to determined that whether they are complies with the recommended limit by guidelines or not.[11,13]

RESULT AND DISCUSSION

1. UV-Visible Spectrophotometric Methods

Linearity study

Standard solution having concentration range of 10, 20,30,40,50 µg/ml of Levetiracetam solution was prepared from standard stock solution. Absorbances of these solutions were recorded at 220 nm wavelength. Calibration curve was plotted by absorbance *vs* concentration.

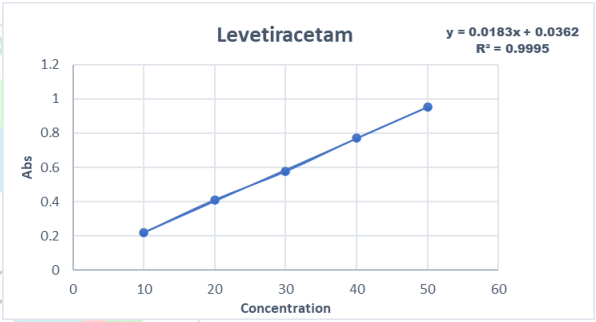

Figure 3: Calibration curve by UV

Table 1: Data of calibration curve by UV

Sr. No.	Conc. (µg/mL)	Absorbance
1	10	0.2179
2	20	0.4108
3	30	0.5764
4	40	0.7710
5	50	0.9542

Table 2: linear regression analysis by UV

Sr.No	Parameters	Zero Order spectrophotometric method
1	λmax (nm)	220
2	Beer's law limit (µg/mL)	10-50
3	Regression equation[y]	y = 0.0183x + 0.0362
4	Slope[m]	0.0.18
5	Intercept [c]	0.0362
6	Correlation coefficient [r2]	0.9995
7	Limit of detection (LOD) (µg/mL)	0.0719
8	Limit of quantitation (LOQ) (µg/mL)	0.218

Validation Parameters

Validation of the developed method was carried out accordingly to the ICH guidelines. Accuracy of method was performed at 50%, 100% and 150% level by standard addition method

and % recovery method. Levetiracetam were found to be in the range of 99.57 – 100.15 %. Precision of the method was determined by % RSD of intra-day precision, inter-day precision. The LOD and LOQ of Levetiracetam was found to be 0.0719 and 0.218 µg/ml, respectively.[1,4]

Table 4: Result of Accuracy study

Level of addition	% Mean recovery*	SD	% RSD
50%	99.57	0.12	0.18
100%	100.15	0.08	0.12
150%	100.01	0.12	0.27

Table 5A: Result of intraday precision

Sr. No.	Conc. (µg/mL)	Abs	Mean	SD	% RSD
1	10	0.2169	0.2118	0.0013	0.6013
2	10	0.2179			
3	10	0.2195			
4	20	0.4123	0.4111	0.0010	0.2816
5	20	0.4108			
6	20	0.4102			
7	30	0.5758	0.5764	0.0007	0.1218
8	30	0.5764			
9	30	0.5772			

Table 5B: Result of interday precision

S.no.	Conc. (µg/mL)	Abs	Mean	SD	%RSD
1	10	0.2148	0.2154	0.0022	1.0299
2	10	0.2179			
3	10	0.2136			
4	20	0.4136	0.4119	0.0014	0.3537
5	20	0.4108			
6	20	0.4115			
7	30	0.5743	0.5756	0.0011	0.2013
8	30	0.5764			
9	30	0.5762			

Table 6A: Result of robustness study

Parameters	Change In Wavelength(±2 nm)			
	Wavelength (218nm)		Wavelength (222nm)	
	15ppm	25ppm	15ppm	25ppm
Mean(n=3)		0.9538	0.5759	0.9540
SD	0.0007	0.0006	0.0001	0.0016
% RSD	0.1917	0.0988	0.4818	0.2634

Table 6 B: Result of robustness study

Parameters	Change In Solvent			
	Water		0.1N NaOH	
	15ppm	25ppm	15ppm	25ppm
Mean(n=3)	0.5765	0.9552	0.5763	0.9545
SD	0.00079	0.0015	0.0006	0.0008
% RSD	0.250	0.3614	0.2086	0.2091

Table 7: Result of ruggedness study

Parameters	Change In Analyst			
	Analyst I		Analyst II	
	15ppm	25ppm	15ppm	25ppm
Mean(n=3)	0.4112	0.7732	0.4108	0.7710
SD	0.0006	0.0008	0.0011	0.0006
% RSD	0.2807	0.2076	0.5467	0.1460

2. Chromatographic Method

Selection of Analytical Wavelength

The standard sample solutions of Levetiracetam (10 µg/ml) were scanned between the UV region of 190 - 400 nm and the UV spectra were recorded. It was found that Levetiracetam drug showed the maximum absorbance at 220 nm. So, the wavelength of detection used was 220 nm.

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Table 8: Optimized Parameters

Mobile phase	Methanol : ACN: Water (60:20:20) pH3
Selection of column	Cosmosil C18 (4.6mm x 250mm, Particle size: 5µm)
Injection volume	20 µL
Flow rate	0.8 ml/min
Column temperature	Room Temperature
Detection wavelength	220nm
Retention time	4.5 min

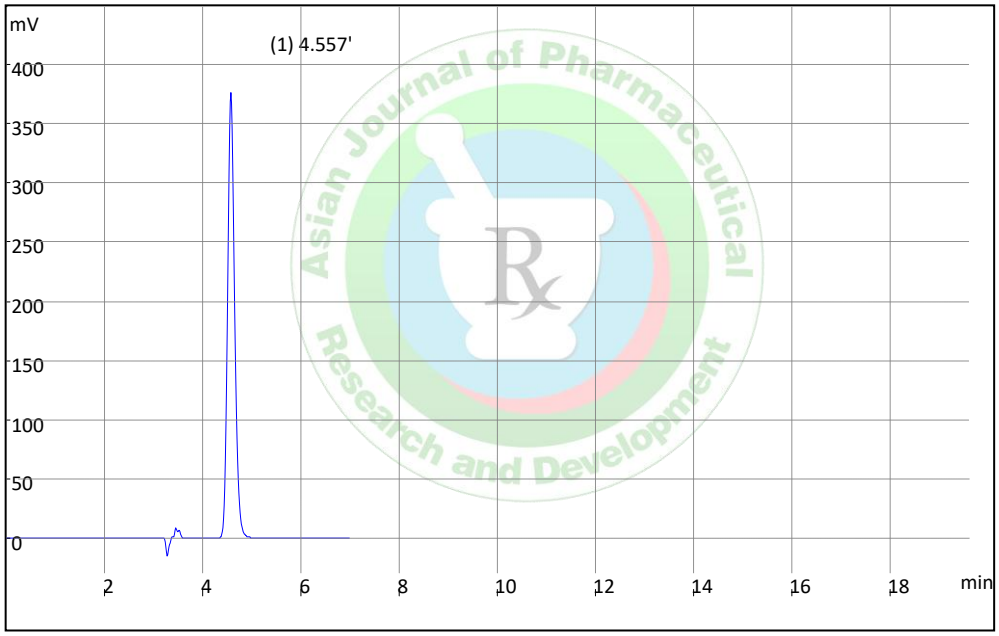

Figure 4: Typical chromatograph of Levetiracetam by HPLC at Optimized condition

Linearity Study

Levetiracetam solution was found to be linear between the concentration range of 10-50 µg/ml.

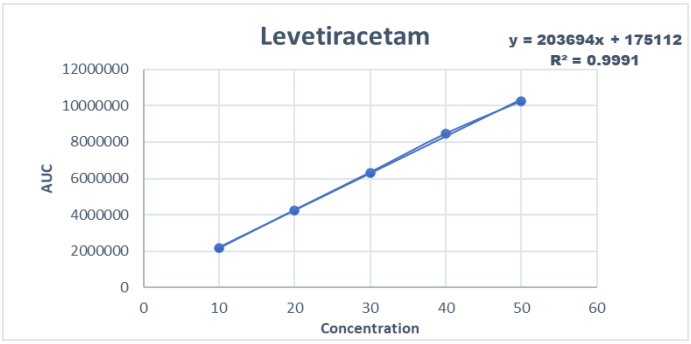

Figure 5: Calibration curve by HPLC

Table 9: Result of calibration curve

Sr. No.	Conc.(µg/ml)	Area
1	10	215263
2	20	425659
3	30	632564
4	40	845826
5	50	102364

Table 10: Linear regression analysis

Sr. No	Parameters	High performance liquid
1	λmax (nm)	220
2	Beer's law limit (µg/mL)	10-50
3	Regression equation[y]	y = 203694x + 175112
4	Slope[m]	203694
5	Intercept [c]	175112
6	Correlation coefficient [r^2]	0.9991
7	Limit of detection (LOD) (µg/mL)	0.1407
8	Limit of quantitation (LOQ) (µg/mL)	0.4265

Validation Parameters

This developed method was validated according to the ICH guidelines. The accuracy by % Recovery method of Levetiracetam was found in the range of 98.96-101.2%. From the precision study it was found that for the both the parameters i.e. intraday and interday are within the limits which is below 2 % of RSD. LOD and LOQ of Levetiracetam were found 0.1407 and 0.4265 µg / ml, respectively. For the study of robustness, the effect of the change in the wavelength (± 2 nm) and the change in flowrate (± 0.1 ml / min) in the middle peak area, were studied % RSD. RSD percentage of each peak in both the variables was found to be less than 2%. [1,5,9]

Accuracy

Accuracy was determined by standard addition method and % recovery found was within acceptable limit by the guidelines.

Table 11: Result of Accuracy by HPLC

Level of addition	% Mean recovery*	SD	% RSD
50%	100.2	0.1581	0.157813
100%	101.2	0.7693	0.759967
150%	98.96	1.0415	1.052409

Precision

Intraday and interday precision provides assurity the repeatability of test results. The % RSD found was below 2

Table 12A: Result of intraday precision

Sr. No.	Conc. (µg/mL)	Area	Mean	SD	%RSD
1	10	2163582	2156577	12082.82	0.560278
2	10	2163524			
3	10	2142625			
4	30	6335698	6326949.67	15287.54	0.241626
5	30	6321562			
6	30	6323589			
7	50	10123658	10157598.7	153912.8	1.515248
8	50	10325649			
9	50	10023489			

Table 12B: Result of Interday precision

Sr. No.	Conc. (µg/mL)	Area	Mean	SD	%RSD
1	10	2178552	2173556	8687.98987	0.39971318
2	10	2163524			
3	10	2178592			
4	30	6356215	6336780.67	17706.9615	0.2794315
5	30	6321562			
6	30	6332565			
7	50	10023659	10157514.7	153885.543	1.51499208
8	50	10325649			
9	50	10123236			

Robustness

Robustness was determined by different deliberate variations in the chromatographic conditions i.e. change in flowrate and change in wavelength.

Table 13: Result of robustness study

Sr.No	Parameter	Condition	Area	Mean	SD	%RSD
1	Change in Flow rate (ml/min)	0.7	4236598	4256272	19515.5	0.45851
2		0.8	4256592			
3		0.9	4275625			
1	Change in Wavelength (nm)	218	4286523	4262247	22000.5	0.51617
2		220	4256592			
3		222	4243626			

Ruggedness

Ruggedness was studied by different analyst.

Table 14: Result of Ruggedness

Sr.No	Analyst	Conc. (µg/ml)	Area	Mean area*	SD	% RSD
1	Analyst-I	30	6325252	6331348.33	22463.7341	0.35480174
			6356231			
			6312562			
2	Analyst-II	30	6336258	6332698.33	6448.15108	0.10182312
			6336582			
			6325255			

% Assay of Marketed formulation

rmulation	**ea of Standard**	**ea of degraded Sample**	**Assay**
vera 500	25645	10514	**17**

Specificity

Excipients and impurities were not interacting with the standard drug, hence method is specific.

Table No.23: Data for specificity study

Drug conc. (µg/ml)	Excipients (µg/ml)	Total conc. (µg/ml)	Area	Mean	SD	%RSD
10	20	30	2156325	2166129.33	9664.6669	0.4461722
10	20	30	2166415			
10	20	30	2175648			
20	20	40	4225639	4269212	51253.2918	1.20053283
20	20	40	4256315			
20	20	40	4325682			
30	20	50	6325689	6323468.67	9983.42738	0.15787897
30	20	50	6332155			
30	20	50	6312562			

Table 15: Result of system Suitability

Sr. No.	conc. (µg/ml)	Retention Time (min)	Theoretical plates	Asymmetry Factor
1	30	4.51	8152	1.25
2	30	4.52	8124	1.24
3	30	4.5	8031	1.25
4	30	4.53	8264	1.23
5	30	4.51	8362	1.24
6	30	4.52	8215	1.25
Mean		4.515	8191.33333	1.2433333
SD		0.010488088	115.546816	0.008165
%RSD		0.232294319	1.41059839	0.6566997

CONCLUSION

In the present investigation, it was found that the UV spectrophotometric method is successfully developed and validated, and it was found to be simple, economical and fast. It was found that HPLC was more accurate, precise, robust and robust to the estimation of Levetiracetam in bulk form and tablet dosage forms. The excipients generally present in the pharmaceutical formulation did not interfere with the estimation of levetiracetam. This method is also beneficial for the formulation and drug Development. These methods are always useful for analysis, purity tests and testing of levetiracetam. The consumption of time and chemical products is less compared to another tedious method. This is a new concept for the validation of the development of the method and the transfer of methods in pharmaceutical companies. The results and statistical parameters demonstrate that the proposed UV spectrophotometric method and HPLC is simple, fast, specific, precise and robust for the routine analysis of levetiracetam in bulk form and pharmaceutical dosage forms.

ACKNOWLEDGEMENT

No conflict of interest

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