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A VALIDATED RP-HPLC METHOD FOR THE ESTIMATION OF LEVETIRACETAM IN BULK AND PHARMACEUTICAL FORMULATION

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ABSTRACT

A new sensitive, specific, linear, precise and accurate RP-HPLC method was developed and validated for estimation of Levetiracetam in Bulk and Pharmaceutical Tablet Formulations. An isocratic, reversed phase HPLC method was developed to separate the drug from the degradation products, Phenomenex Gemini 5µ C18 (2) 100A (250 x 4.60mm, 5 µ) column. Hamilton syringe (705 NR, 50 µL) was used for injecting sample and standard solution. Data was compiled using Spinchrom software. Mobile phase consists of mixture of Methanol:Acetonitrile in the ratio (90:10 v/v) at a flow rate of 1.0 mL /min. UV detection was performed at 210 nm. The Linearity was established for Levetiracetam in the range of 5- 30µg/ml with correlation coefficient of 0.9997. LOD and LOQ were found to be 0.076µg/ml and 0.23µg/ml respectively. Retention time of Levetiracetam were found to be 2.281min and 2.274min. % Recovery was found to be 99.78-100.45 and %RSD was found with in ± 2. The method has been validated according to ICH guidelines for linearity, precision, accuracy, robustness, ruggedness, LOD and LOQ. The developed validated method was successfully applied for reliable quantification of Levetiracetam in bulk and pharmaceutical dosage form.

KEYWORDS: Levetiracetam, RP- HPLC, validation, pharmaceutical formulations.

INTRODUCTION

Levetiracetam is an anticonvulsant drug used treat the epilepsy.[1] Levetiracetam is a drug with in the pyrrolidine class that is used to treat various types of seizures.[2] Chemically it is known as pyrrolidinone and acetamide derivatives. Levetiracetam may selectively prevent synchronization of epileptic form bust firing and propagation of seizure activity. It is also used to treat neuropathic pain.[3] The chemical name of Levetiracetam is (S)-2-(2-oxopyrollidin-1- yl) butanamide with molecular weight of 170.20 g/ml.

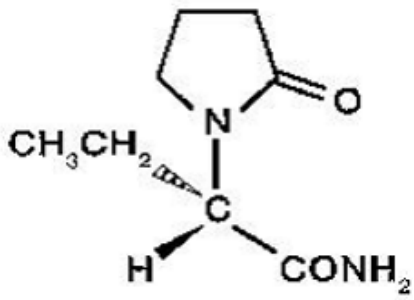

Fig. 1: Chemical structure of levetiracetam.

Literature survey revealed that there were few analytical methods have been reported for the determination of the Levetiracetam in pure drug and pharmaceutical dosage form by using UV spectrophotometric,[4-6] HPLC,[7-12] and HPTLC.[13]

The aim of the present work is to develop and validate a novel, rapid, precise and specific Area under curve UV spectrophotometric method for estimation of Levetiracetam in bulk and tablet dosage form.

MATERIALS AND METHODS

Material and reagents: The Levetiracetam was obtained as a gift sample from the pharmaceutical industry and Levipil tablet obtained from Pharmacy store. Methanol and distilled water were obtained Bharathi College of pharmacy, Bharathinagara, KM Doddi, Maddur Taluk, Mandya District, India. All chemicals used are of HPLC grade. Distilled water was used throughout the experiment.

Instrumentation: Chromatographic separation was performed on a Shimadzu LC-20AT HPLC system comprising a variable wavelength programmable UV/ VIS detector SPD-20A (VP- series), Shimadzu LC-20AT (VP series) pump and Phenomenex Gemini 5µ C18 (2) 100A (250 x 4.60mm, 5 µ) column. Hamilton syringe (705 NR, 50 µL) was used for injecting sample and standard solution. Data was compiled using Spinchrom software.

Chromatographic conditions

Table 1: HPLC method development parameters.

HPLC method development parameters	
Column	C18, 250 X 4.6 mm, 5µ
Flow rate	1.0 mL / min
Wavelength	210 nm
Column temperature	30°C
Injection volume	50 µL
Run time	7 minutes
Diluents	Mobile phase
Elution	Isocratic

Preparation of solutions Mobile phase preparation

The Mobile phase consisted of a mixture of Methanol (90%), Acetonitrile (10%) in the ratio of v/v, which was filtered through a membrane and degassed before use. pH Adjusted to 3.5 with 0.1% Ortho phosphoric acid.

Preparation of sample Standard Solution:

The formulation tablets of Levetiracetam (Levipil-500mg) were crushed to give finely powdered material. From the Powder prepared a 100mg of Levetiracetam was accurately weighed, transferred in a 100 ml volumetric flask, add 30 ml of diluents and sonicate to dissolve and dilute to volume with diluent. Transfer 10 mL of standard stock solution into 100 ml volumetric flask and dilute to volume with diluent. And an appropriate concentration of sample was prepared at the time of analysis. 50µl of these solutions were injected in triplicate into HPLC system and the peak areas were recorded.

Preparation of Standard solution

Levetiracetam weigh and transfer the tablet powder equal to 100 mg of Levetiracetam into 100 ml volumetric flask add 30 ml of diluent, sonicate to dissolve for 10 minutes and dilute to volume with diluent. Further filter the solution through 0.45µ filter. And an appropriate concentration of sample (was prepared at the time of analysis. 50µl of these solutions were injected in triplicate into HPLC system and preceded as said for the standard respectively.

System suitability requirements from stock and standard solutions

a) Tailing factor: NMT 2.0
b) Theoretical Plates: NLT 2000

RESULTS AND DISCUSSION

Validation of the proposed method

The proposed method was validated as per ICH guidelines.[14] The parameters studied for validation were specificity, linearity, precision, accuracy, robustness, system suitability, limit of detection, limit of quantification, and solution stability.

Specificity: By comparing the chromatograms of blank, standard and sample (Prepared from Formulation). It was found that there is no interference due to excipients in the tablet formulation and also found good correlation between the retention times of standard and sample. The specificity results are shown in Table 2.

Table 2: Specificity of Levetiracetam.

Name of the solution	Retention time in min
Blank	0
Levetiracetam(Standard)	2.274
Levetiracetam(Sample)	2.281

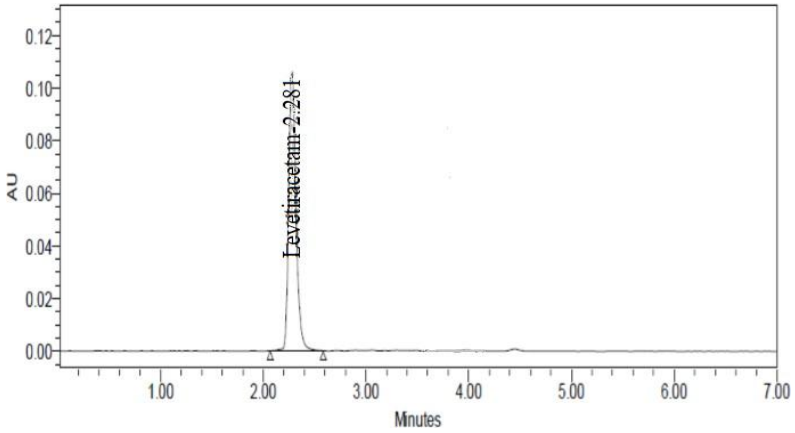

Figure 2: Chromatogram of Sample solution of Levetiracetam.

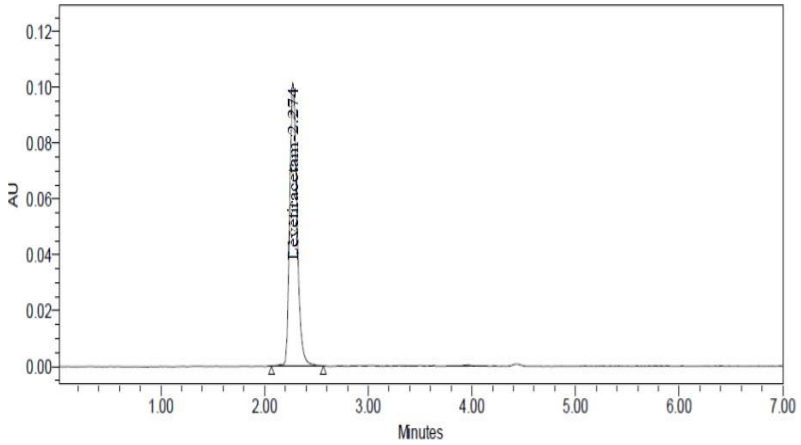

Figure 3: Chromatogram of standard solution of Levetiracetam.

Linearity: The linearity of the response of the drug was verified at six concentration levels, ranging from 5-30µg/ml of Levetiracetam in each linearity level were prepared. 50µl of each concentration was injected in duplicate into the HPLC system. The response was read at 210 nm and the corresponding chromatograms were recorded. From these chromatograms, the mean peak areas were presented in Table-3.

Table 3: Linearity of Levetiracetam.

Concentration (µg/ml)	Retention time (min)	Peak area* (mv)
5	2.281	25912
10	2.283	52996
15	2.279	78486
20	2.281	103841
25	2.280	128401
30	2.279	157356

*Average of six determinations

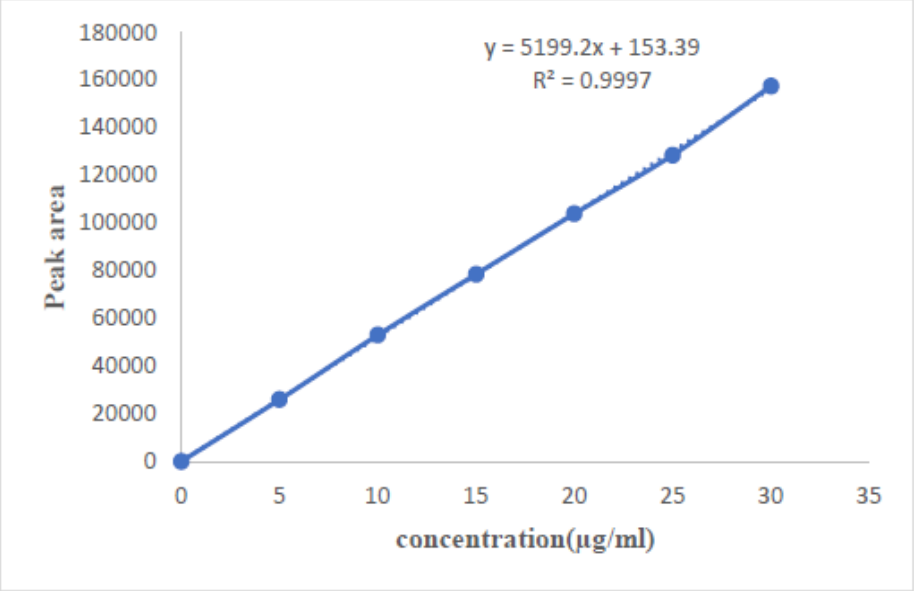

Figure 4: Linearity of Levetiracetam.

Precision: Precision of the method was performed as intraday precision, Inter day precision. To study the intraday precision, six replicate standard solutions (20µg/ml) of Levetiracetam were injected. % RSD was calculated and it was found to be 1.029 and interday precision done same as intraday, six replicate standard solutions (20µg/ml) of Levetiracetam were injected. % RSD was calculated and it was found to be 0.969 which are well within the acceptable criteria of not more than 2.0. Results of system precision studies are shown in Table-4.

Table 4: Results of Precision of Levetiracetam.

SI.NO	Intraday Names	Studies Peak area	Interday Names	Studies Peak area
1	Injection-1	103857	Injection-1	105923
2	Injection-2	103045	Injection-2	104211
3	Injection-3	104756	Injection-3	103782
4	Injection-4	105256	Injection-4	104675
5	Injection-5	106087	Injection-5	103058
6	Injection-6	104168	Injection-6	105136
	AVG	104528.2	**AVG**	104464.2
	STDEV	1076.25	**STDEV**	1012.99
	%RSD	1.029	**%RSD**	0.969

Accuracy: Accuracy of the method was studied by recovery experiments. The recovery experiments were performed by adding known amounts of the drugs in the placebo. The recovery was performed at three levels, 50, 100 and 150% of the label claim of the tablet (500 mg of Levetiracetam). The recovery values for Levetiracetam ranged from 98.0 to 102.0%. The average recoveries of three levels of Levetiracetam were found to be 99.7-100.45%. the results are shown in the Table 5.

Table 5: Results of recovery of Levetiracetam.

Level of addition/ %	Amount added (µg/ml)	Amount found	%Recovery ±Standard deviation*	%RSD
50	10	30.10	99.78±0.704	0.705
		29.91		
		29.70		
100	20	39.89	100.45±0.676	0.666
		40.42		
		40.24		
		49.97		
150	30	50.05	99.65±0.567	0.568
		49.5		

*Average of three determinations

Limit of detection and Limit of quantification: The limit of detection is an analytical method is the smallest amount of analyte in a sample which can be reliable detected by the analytical method. The limit of quantitation is an individual analytical procedure is the smallest amount of the analyte in sample which can be quantitatively determined. LOD and LOQ were calculated using formula LOD = 3.3(SD)/S and LOQ = 10(SD)/S. Results were shown in Table 6.

Table 6: System suitability parameters.

Parameters	Levetiracetam
Linearity	5-30 µg/ml
Regression equation	y = 5199.2x + 153.39
Correlation coefficient	R^2 = 0.9997
Retention time	2.281min
Run time	7 min
Limit of detection(LOD)	0.076 µg/ml
Limit of quantification(LOQ)	0.23 µg/ml
Tailing factor	1.28
Theoretical Plate	5349

Ruggedness

The ruggedness of test method was demonstrated by carrying out precision study in six preparations of sample on a single batch sample by different analysts, the results of the precision study are tabulated as below table-7. The % RSD values are less than 2.

Table 7: Results of Ruggedness of Levetiracetam.

Analysts	Mean area ± Standard deviation*	%RSD
Analyst 1	103932±1001.01	0.96
Analyst 2	103895.3±987.67	0.9

* average of three determinations.

CONCLUSION

The present analytical method was validated as per ICH guidelines and met the acceptance criteria. It was concluded that the developed analytical method was simple, accurate, economical and sensitive, and can be used for routine analysis of Levetiracetam in bulk drug and pharmaceutical dosage forms.

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