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A novel RP-HPLC method for the analysis of levetiracetam in formulations

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ABSTRACT

A simple, rapid RP-HPLC method was developed and validated for assay of Levetiracetam in formulations. Isocratic elution at a flow rate of 1.0ml/min was employed on a Chromosil C18 (250x4.6mm, 5µm in particle size) at room temperature. The mobile phase consisted of Methanol: Water:TEA 75:25:05 (V/V). The UV detection wavelength was 214 nm and 20µl sample was injected. The retention time for Levetiracetam was 2.59 min. The percentage RSD for precision and accuracy of the method was found to be less than 2%. The method was validated as per the ICH guidelines. The method was successfully applied for routine analysis of Levetiracetam in tablet dosage form and bulk drug.

Key Words: Levetiracetam, RP-HPLC, UV detection, recovery, precise, 214 nm.

INTRODUCTION

Levetiracetam is an anticonvulsant medication used to treat epilepsy.[1] It is the S-enantiomer of etiracetam, structurally similar to the prototypical nootropic drug piracetam. Along with other anticonvulsants like gabapentin, it is also sometimes used to treat neuropathic pain.Levetiracetam has been approved in the European Union as a monotherapy treatment for epilepsy in the case of partial seizures, or as an adjunctive therapy for partial, myoclonic and tonic-clonic seizures.[2] It is also used in veterinary medicine for similar purposes.

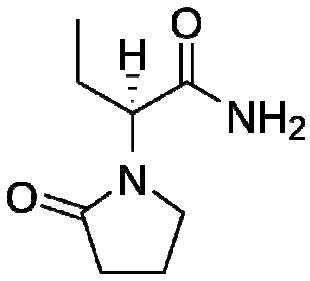


FIGURE.1

Levetiracetam has potential benefits for other psychiatric and neurologic conditions such as Tourette syndrome, autism, bipolar disorder and anxiety disorder, but its most serious adverse

effects are behavioral and its benefit-risk ratio in these conditions is not well understood.[3] The exact mechanism by which levetiracetam acts to treat epilepsy is unknown. However, the drug binds to a synaptic vesicle protein, SV2A,[4] which is believed to impede nerve conduction across synapses.[5] Levetiracetam is, in general, well tolerated[6] but may cause drowsiness, weakness, unsteady walking, coordination problems, headache, pain, forgetfulness, anxiety, irritability or hostility, dizziness, moodiness, nervousness, loss of appetite, vomiting, diarrhea, constipation, and changes in skin color.Some serious side effects can be depression, hallucinating (hearing voices or seeing visions that do not exist), suicidal thoughts, seizures that are worse or different, fever, sore throat, and other signs of infection, double vision, itching, rash, swelling of the face. A study published in 2005 suggests that the addition of pyridoxine (vitamin B6) may curtail some of the psychiatric symptoms.[7]

MATERIALS AND METHODS

Materials

Working standard of Levetiracetam was obtained from well reputed research laboratories. HPLC grade water, methanol, Tri ethyl amine (TEA)was purchased from E. Merck

Apparatus

A Series HPLC system PEAK LC7000 isocratic HPLC with PEAK 7000 delivery system. Rheodyne manual sample injector with switch (77251), Analytical column Chromosil C18. 250×4.6mm, Electronic balance-DENVER (SI234), A manual Rheodyne injector with a 20 µl loop was used for the injection of sample., PEAK LC software were used. UV 2301 SPECOPHOTOMETER was used to determine the wavelength of maximum absorbance

Determination of wavelength of maximum absorbance

The standard solutions of Levetiracetam were scanned in the range of 200 -400 nm against mobile phase as a blank. Levetiracetam showed maximum absorbance at 260 nm. So the wavelength selected for the determination of Levetiracetam was 214 nm.

Chromatographic equipment and conditions

The development and validation of the assay was performed on A Series 200 HPLC system PEAK LC7000 isocratic HPLC with PEAK 7000 delivery system. Rheodyne manual sample injector with switch (77251),Analytical column Chromosil 100-5 C18. 250×4.6mm, , manual injector rheodyne valve) with 20µL fixed loop, PEAK LC software were used.

The mobile phase consisted of a Methanol , water,TEA 75:25;5 (v/v). Injections were carried out using a 20 µl loop at room temperature (20 + 2 °C) and the flow rate was 1.0 ml/min. Detection was performed at 214 nm with 5.0 min runtime.

Standard and sample solutions

A 10 mg amount of Levetiracetam reference substance was accurately weighed, dissolved in mobile phase and diluted to volume in a 100 ml volumetric flask to obtain 100 ppm concentrated solition. From standard solution by the serial dilution we prepared required concentrations. A composite of 20 tablets was prepared by grinding them to a fine, uniform size powder. 10 mg of, Levetiracetam was accurately weighted and quantitatively transferred into a 100 ml volumetric flask. Approximately 30 ml mobile phase were added and the solution was sonicated for 15 min. The flask was filled to volume with mobile phase, and mixed. After filtration, an amount of the solution was diluted with mobile phase to a concentration of 10 µg/ml.

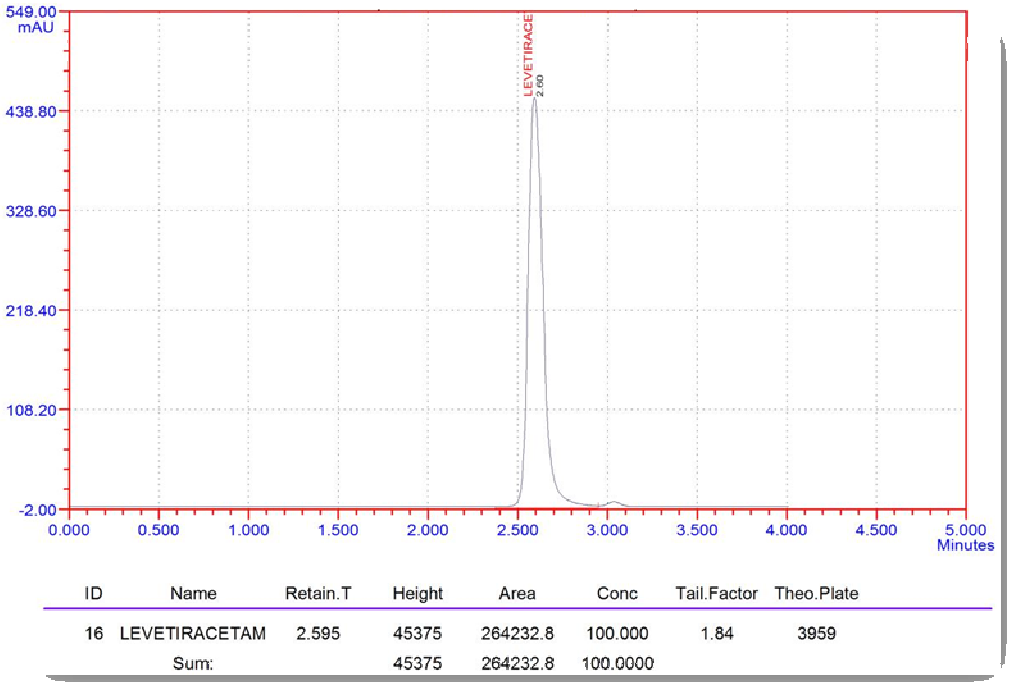
Method validation

Method validation was performed following ICH specifications for specificity, range of linearity, accuracy, precision and robustness

RESULTS AND DISCUSSION

System Suitability

Having optimized the efficiency of a chromatographic separation the quality of the chromatography was monitored by applying the following system suitability tests: capacity factor, tailing factor and theoretical plates. The system suitability method acceptance criteria set in each validation run were: capacity factor >2.0, tailing factor ≤2.0 and theoretical plates >2000 13. In all cases, the relative standard deviation (R.S.D) for the analytic peak area for two consecutive injections was < 2.0%. A chromatogram obtained from reference substance solution is presented. System suitability parameters were shown in Table.1. Standard chromatogram was given in Figure.2



ID	Name	Retain.T	Height	Area	Conc	Tail.Factor	Theo.Plate
16	LEVETIRACETAM	2.595	45375	264232.8	100.000	1.84	3959
	Sum:		45375	264232.8	100.0000		

Figure.2

Range of linearity

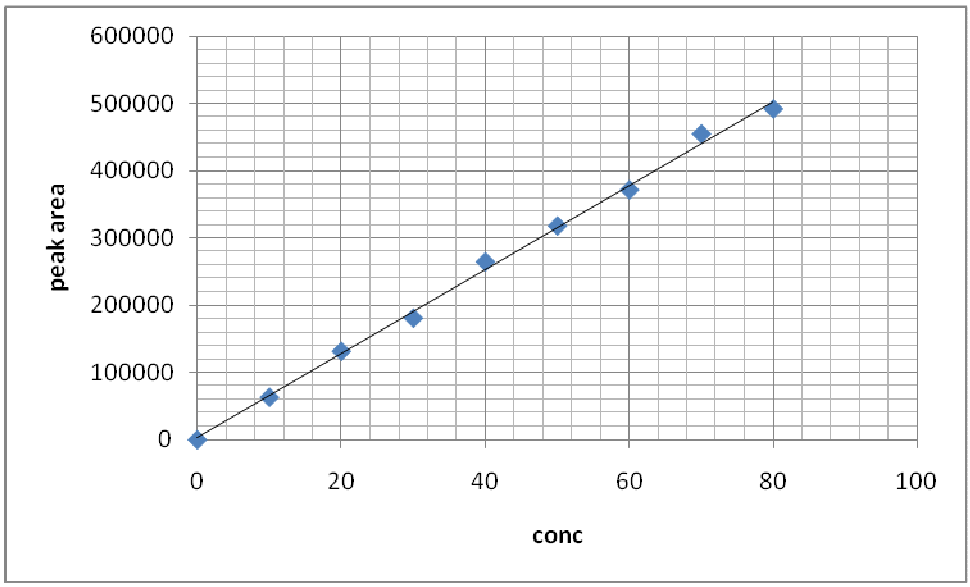
Standard curves were constructed daily, for three consecutive days, using seven standard concentrations in a range of 10,20,30,40,50,60,70,80 µg/ml. for Levetiracetam. The linearity of peak area responses versus concentrations was demonstrated by linear least square regression analysis. The linear regression equation was y = 2294.97 + 6258.86x (r= 0.997). Linearity values can shown in Table: 2

Table.1 System suitability parameters

Mobile phase	Methanol : water: TEA 70:25:5 (v/v)
Pump mode	Isocratic
pH	4.6
Diluents	Methanol
Column	Chromosil C18 column (250 X 4.6 mm, 5µ)
Column Temp	Ambient
Wavelength	214nm
Injection Volume	20 µl
Flow rate	1.0 ml/min
Run time	5 minutes
Retention Time	2.59 minutes

Table.2

Level	**Concentration of Levetiracetam In ppm**	**peak area**
Level - 1	10	63199
Level - 2	20	131353
Level - 3	30	180619
Level - 4	40	264232
Level - 5	50	317567
Level - 6	60	371251
Level - 7	70	454326
	80	491298
Range: 6ppm to 12ppm	Slope Intercept Correlation coefficient	6258.86 2294.97 0.9986



Graph.1

Precision

To study precision, six replicate standard solutions of Levetiracetam(40 ppm) were prepared and analyzed using the proposed method. The percent relative standard deviation (% RSD) for peak responses was calculated and it was found to be 0.75which is well within the acceptance criteria of not more than 2.0%. Results of system precision studies are shown in Table.3

Table.3 Precision Results for Levetiracetam

Sample	Conc. (in ppm)	Injection No.	Peak Areas	RSD (Acceptance criteria ≤ 2.0%)
Levetiracetam	9	1	264453	**0.75**
		2	264502	
		3	264767	
		4	265208	
		5	261500	
		6	260413	

Limit of Detection and Limit of Quantification:

To determine the Limit of Detection (LOD) sample was dissolved by using Mobile phase and injected until peak was disappeared. After 0.05 ppm dilution Peak was not clearly observed, based on which 0.06 ppm is considered as Limit of Detection and Limit of Quantification is 0.15 ppm.

Table.4

Parameter	Measured Value
Limit of Quantification	0.15 ppm
Limit of Detection	0.05 ppm

Robustness

Typical variations in liquid chromatography conditions were used to evaluate the robustness of the assay method. In this study, the chromatographic parameters monitored were retention time, area, capacity factor, tailing factor and theoretical plates. The robustness acceptance criteria set in the validation were the same established on system suitability test describe above.

Table.5

S.NO	PARAMETER	CONDITION	AREA
1	Standard	Standard conditions	264509
2	Mobile phase	Methanol 60%,ACN 40%	265907
3	Mobile phase pH	5.2	265037
4	Wavelength	258 nm	264710

Recovery

Recover test was performed at 3 different concentrations i.e. 20ppm,40ppm,60ppm. Results are given in table.6

Table.6

Amount of Levetiracetam (in ppm)	Amount found in sample	% Recovery	% RSD
20	19.92	99.96	**0.83**
20	19.86	99.30	
20	19.91	99.55	
40	40.02	100.05	**0.27**
40	39.85	99.62	
40	39.99	99.90	
60	59.98	99.96	**0.48**
60	59.93	99.88	
60	59.97	99.95	
		Mean Recovery 99.49	**Mean 0.52**

Table.7 FORMULATION ANALYSIS

S.NO	Tablet	Dosage	Sample conc	Saple estimated	% of Drug Estimated in Tablet
1	KEPPRA	500mg	50 ppm	49.925 ppm	99.85

CONCLUSION

The proposed method for the assay of Levetiracetam in formulations is very simple and rapid. It should be emphasized it is isocratic and the mobile phase do not contain any buffer. The method was validated for specificity, linearity, precision, accuracy and robustness. Although the method could effectively separate the drug from its products, further studies should be performed in order to use it to evaluate the stability of pharmaceutical formulations. It Runtime of analysis is very short, so we can complete the analysis very rapidly. Due to absence of salt buffer we will protect column efficiency also.

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