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Method Development and Validation of Levetiracetam by UHPLC in Bulk and Tablet Dosage Form

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ABSTRACT:
The new, rapid, sensitive, simple, precise and accurate Ultra High Performance Liquid Chromatography (UHPLC) method was development and validation of levetiracetam by UHPLC in bulk and tablet dosage form. The column used was Agilent C_{18} column (4.6mm x 100mm; 2.5µm) with mobile phase containing the MEOH: Water (0.1% TEA with PH 5.5 with OPA) in the ration of 20:80% v/v. The detection wavelength was 207nm and flow rate were 0.7ml/min. The retention time of levetiracetam were found to be 4.424min According to the ICH guidelines, linearity, accuracy, range, and robustness were all within acceptable limits.

Keywords: Column, Levetiracetam, Simple, Tablet, Validation,,UHPLC.

INTRODUCTION

Levetiracetam is an antiepileptic drug used in treatment of epilepsy, partial onset, myoclonic or tonic-clonic seizures.[1] They are distinguished by their capacity to prevent partial seizures. Each drug has a different mode of action.[2] LEV has the empirical formula C8 H14N2O2 and is chemically known as (-)-(s)-a-ethyl-2-oxo-1-pyrrolidine acetamide.[3] It was marketed as Keppra and used to treat epilepsy. Both monotherapy and adjunctive treatment are used with levetiracetam [4] Other mental and neurological problems such Tourette syndrome, autism, and anxiety disorders may be helped by levetiracetam.[5]

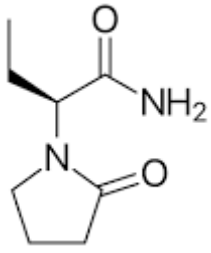

Fig.1.structure of levetiracetam

which binds to the synaptic vesicle protein SV2A and is thought to slow nerve conduction across synapses The exact mechanism by which Levetiracetam exerts its antiepileptic effect is unknown, but it is thought to bind to a synaptic vesicle protein, thus slowing nerve conduction across synapses.[6]

METHOD

Chemical and reagents-

HPLC grade tea, Orthophosphoric acid (OPA) were purchased from Avantor Performance material India Ltd. Thane, Maharashtra and methanol,water from Merck Specialties Pvt. Ltd. Shiv Sager Estate 'A' Worli, Mumbai.the Dosage forms of the studied drugs were purchased from local pharmacies,including Levepsy (labelled to contain 500mg LEV)

Instrumentation-The analysis of the drug was carried out on Agilent (S.K.) Gradient System UV Detector. Prepared with reverse stage (Agilent) C_{18} column (4.6mm x 100mm; 2.5µm), a SP930Dpump, a 20µl injection loop and UV730D (DAD) absorbance detector and running Chemstation software.

- **Preparation of std. Levetiracetam solution:**
  An accurately weighed quantity, 10 mg of Levetiracetam was dissolved in methanol in a 10 ml volumetric flask and volume made up to 10.0 ml to produce a solution of 1000ug/ml
- **Preparation of Stock Standard Solution :** Weight and transfer precisely An aliquot of the Levetiracetam stock solution was mixed in a volumetric flask in 10 ml, and the volume was adjusted up to mark with mobile phase after adding 10 mg of Levetiracetam working standard into a 10 ml flask as about diluent methanol completely and making volume up to the mark with the same solvent to get 1000 g/ml standard (stock solution). Next, 15 minutes were spent sonicating the volumetric to dissolve the volume was built up to the required level with MEOH:Water (0.05% OPA), produced in (20 ml MEOH: 80 ml Water (0.1% TEA) pH 5.5 with OPA, and the resultant solution, which was 0.1 ml, was transferred to a 10 ml volumetric flask.
- **Selection of mobile phase:** Mobile phase was prepared by mixing the MEOH: Water (0.1% TEA with PH 5.5 with OPA) in the ration of 20:80% v/v and the mixture degasified by vacuum filtration using 0.45µ filter and sonication.
- **6.6.1. Optimization of Chromatographic condition:**
- Column : C18 (100 mm× 4.6mm)
- Particle size packing : 2.5µm
- Detection wavelength : 207 nm
- Flow rate : 0.7 ml/min
- Temperature : Ambient
- Sample size : 20 µl

METHOD VALIDATION

The method was validated in accordance with ICH Q2 requirements for linearity, precision, accuracy, system adaptability, robustness, ruggedness, LOD, and LOQ.(7)

Linearity:

The capacity of an analytical method to produce test findings that are proportionate to the analyte concentration in samples falling within a specified range, either directly or through a well stated mathematical transformation, is known as linearity.

Accuracy

The accuracy of an analytical method is the nearness of test results obtained by that method to the true value. Accuracy may often the expressed as percent recovery by the assay of known added amounts of analyte. The accuracy of an analytical method is determined by applying the method to analysed samples,

to which known amounts of analyte have been added. The accuracy is calculated from the test results as the percentage of analyte recovered by the assay.

Precision:

The degree of consistency between individual test results when a technique is done repeatedly to numerous samplings of a homogenous sample is known as the precision of an analytical method. Standard deviation or relative standard deviation are commonly used to express the precision of an analytical process. Additionally, one-way ANOVA was used to compare the data, and the within-day and between-day mean squares were calculated.

Robustness

Robustness is the evaluation of an analytical method wherein the results obtained are found to be reliable even when performed in a slightly varied condition The mobile phase composition was changed in (±1 ml/ min^{-1}**)** proportion in the composition and the flow rate was (±1ml/ mi^{1}**)**

Limit of Detection and Limit of Quantification-

The Limit of detection of an analytical procedure can be described as the lowest concentration of the analyte in a sample that can be detected by it, but not necessarily quantified as an exact value. The Limit of quantification of an analytical procedure can be described as the lowermost concentration of the analyte in a sample that can be quantified with proper accuracy and precision as an exact value.

Limit of detection LOD = 3.3σ/S

Limit of Quantification LOQ = 10σ/S

Where,

σ = the S.D. of the y-intercepts of regression lines.

S = the slope of the calibration curve

The repeatability of the system was assessed using a sample solution containing 30 g/ml of **Repeatability** levetiracetam. A sample was injected, peak regions were measured, and %RSD was computed.

Result and Discussion-

UV Spectroscopy

UV absorption of 20 mcg solution of Levetiracetam in MEOH was generated and absorbance was taken in the range of 200-400 nm. λ max of Levetiracetam was found to be 207 nm respectively.

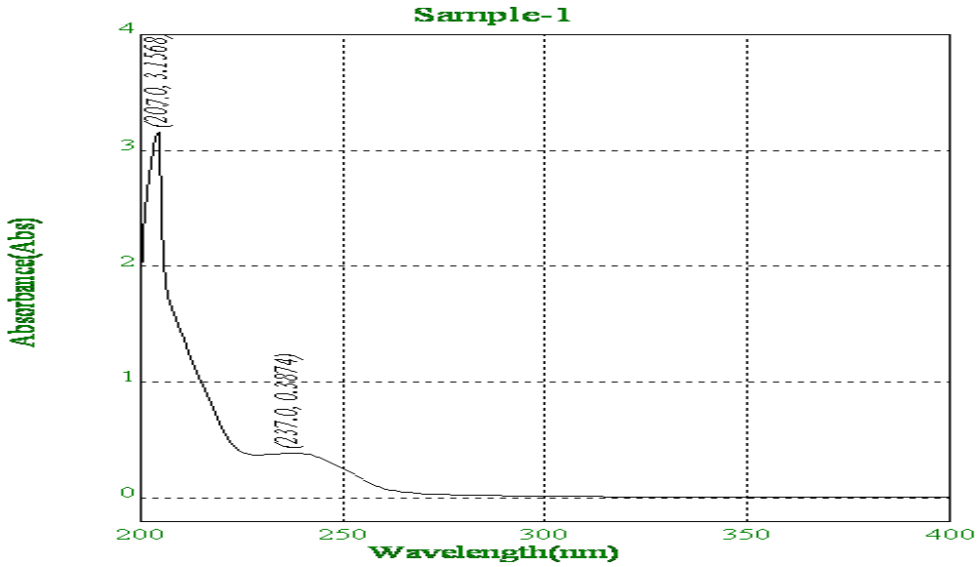

Fig no-2 Uv spectrum of levetiracetam

TABLE NO-1: Studies on the chromatographic behavior of Levetiracetam

Sr No.	Mobile Phase	Retention time	Remark
1.	80 % ACN+20 (0.1%opa) 0.8 ml/min	2.968/4.001	No sharp peak so rejected (Splitting)
2	70% ACN+30(0.1%OPA)0.7 ml/min	2.903/3.528	No sharp peak so rejected (Splitting)
3	50ACN +50(0.1% OPA)MEOH 1.0 ml/min	2.488	No sharp peak so rejected
4	80 % MEOH+20(0.1%OPA) 0.7 ml/min		No sharp peak so rejected
5	75 % MEOH(0.1%OPA) 0.7 ml/min	3.48	No sharp peak so rejected
6	30 % MEOH +70(0.1%TEAPH5.5) 0.7 ml/min	6.7	No sharp peak so rejected (High RT)
7	20 methanol+80 (0.1%TEA PH 5.5 with OPA)	4.424	sharp peak were obtained so selected

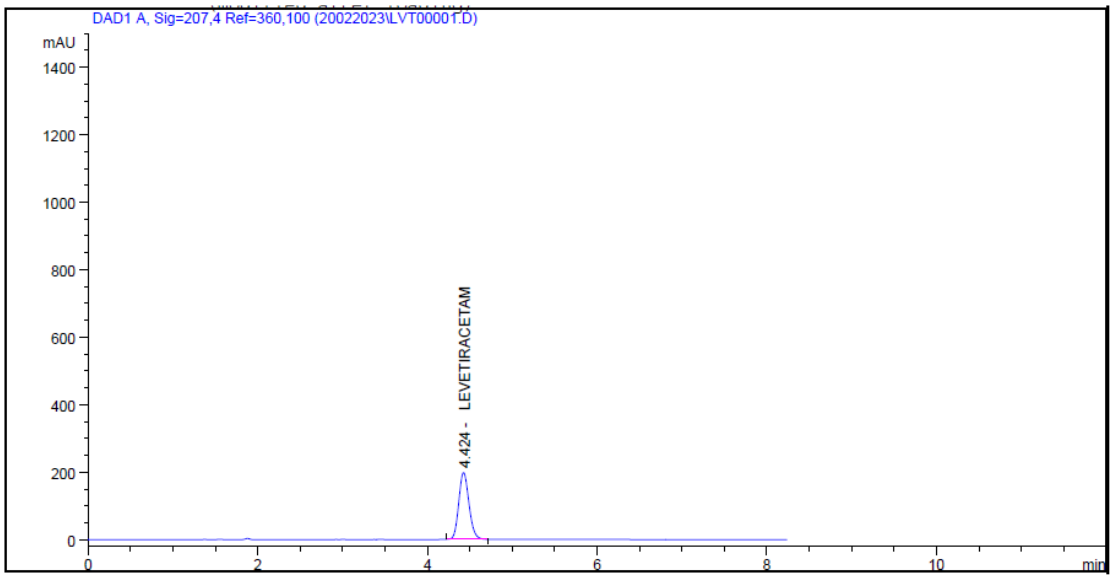

Fig.no.3: Representative Chromatogram of Levetiracetam

METHOD VALIDATION

Linearity:

A fixed volume loop injector was used to inject 20 l of the sample solution into the chromatographic system from different working standard solutions (5-25 g/ml) made from the Levetiracetam standard stock solution. There were chromatograms made. The plot calibration curves are presented in (Fig. 4) and the area of each concentration was recorded and displayed in (Table No. 2)

TABLE NO-2 : Result of linearity studies

Regression Equation Data Y=mx+c	For UHPLC
Slope(m)	152.7
Intercept(c)	114.9
Correlation Coefficient	0.999

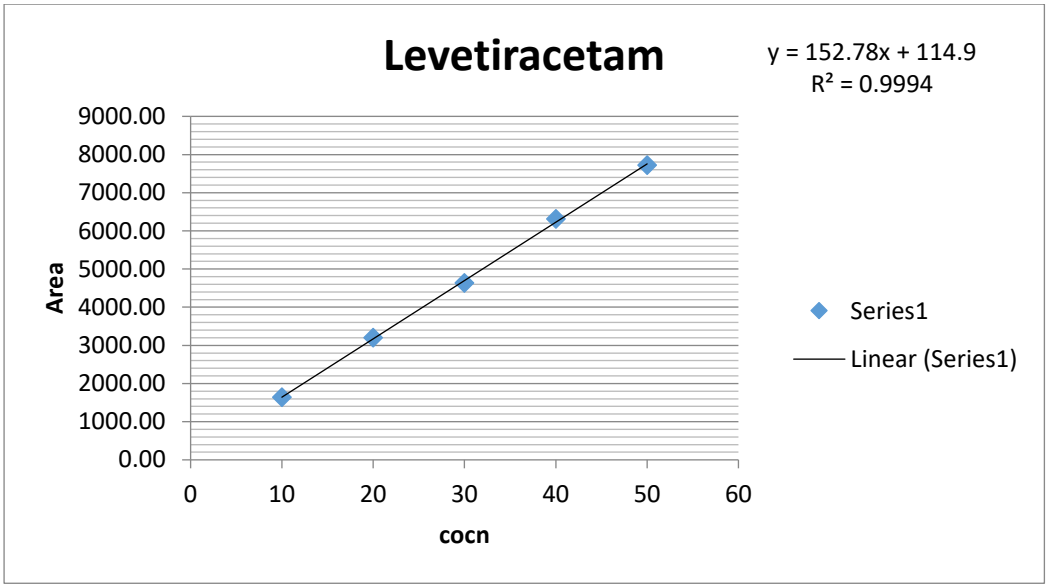

Fig no.4: Linearity graph of levetiracetam

Accuracy:

Recovery studies were carried out to verify the developed method's accuracy. A specific concentration of the standard drug (80%, 100%, and 120%) was added to the before examined tablet solution, and its recovery was then examined. Validation of recovery studies by statistics, as indicated in **(Table No. 3)**

Table.no. 3 Result of aacurancy

Method	**Level of Recovery (%)**	**% RSD**	**Standard Deviation***	**Mean % Recovery**
UHPLC	80 %	0.26	0.26	99.60
	100 %	0.53	0.54	100.95
	120 %	0.15	0.15	99.87

Repeatability)

Levetiracetam system suitability characteristics were estimated using the suggested chromatographic system, and its resolution and repeatability were determined. The outcome is displayed in **(Table No. 4)** below.

TABLE NO:4 Repeatability studies on RP-UHPLC for Levetiracetam

METHOD	**Concentration of Levetiracetam(mg/ml)**	**Peak area**	**Amount found (mg)**	**% Amount found**
UHPLC METHOD	30	4637.98		
	30	4677.33		
	Mean	4657.66	29.75	99.17
	SD	27.82		
	%RSD	0.60		

Precision:

The method was established by analyzing various replicates standards of Levetiracetam. All the solution was analyzed thrice in order to record any intra-day & inter-day variation in the result that concluded. The result obtained for intraday is shown in **(Table No. 5)** respectively.

TABLE NO.5 :Result of precision

Drug	Conc (µg/ml)	Interday Precision		Intraday Precision	
		Mean± SD	%Amt Found	Mean± SD	%Amt Found
Levetiracetam	10	1629.2 ± 9.92	99.17	1648.95 ± 15.1	100.46
	30	4677.4 ± 29.8	99.60	4653.27 ± 2.89	99.07
	50	7736.52 ± 49.9	99.82	7717.74 ± 7.61	99.58

Robustness:

To estimate the robustness of the proposed method, small but deliberate variations in the optimized process parameters were done. The effect of changes in mobile phase composition and flow rate, wavelength on retention period and tailing factor of drug peak was studied.

The mobile phase composition was changed in (±1 ml/min^{-1}**)** proportion and the flow rate was varied by (±1ml/min^{-1}**)**, and wavelength change (±1 ml/min^{-1}**)** of optimized chromatographic condition. The results of robustness studies are shown in **(TableNo.6**).Robustness parameters were also found satisfactory; hence the analytical method would be concluded.

TABLE NO.6: Result of Robustness

Parameters	Conc. (µg/ml)	Amount of detected(mean ±SD)	%RSD
		For Levetiracetam	
Chromatogram of flow change 0.6 ml	30	5447.07	0.82
Chromatogram of flow change 0.8 ml	30	4174.83	1.43
Chromatogram of comp change wavelength change 206 nm	30	5341.0	1.29
Chromatogram of comp change wavelength change 208 nm	30	4215.34	1.49
Chromatogram of mobile phase change79 +21 ml	30	4753.4	0.60
Chromatogram of mobile phase change 81+19 ml	30	4877.78	0.41

LOD and LOQ

Limit of detection = 3.3X28.88/152.7 = 0.6241 (ug/mL)

Limit of Quantitation = 10 X28.88/152.7 = 1.8914 (µg/mL)
The LOD and LOQ of Levetiracetam was found to be 0.6241 (ug/mL) and 1.8914 (ug/mL), analytical method that concluded.

CONCLUSION

Simple, rapid, accurate and precise UHPLC have been developed and validated for the routine analysis of Levetiracetam in API and tablet dosage form .The methods are suitable for the determination of Levetiracetam in Single-component formulations without interference of each other. The developed method is recommended for routine and quality control analysis of the investigated drugs in single component pharmaceutical preparations. The quantity found from the proposed methods was in good agreement with the label claim of the formulation. Also the value of standard deviation and coefficient of variation calculated were satisfactorily low indicating the suitability of the proposed procedures for the routine estimation of tablet dosage forms.

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