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SENSTIVITY OF LEVETIRACETAM IN DIFFERENT SOLVENTS BY UV METHOD

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Abstract : Levetiracetam is a medicament used to treat epilepsy. Two simple UV spectrophotometric methods have been developed for the estimation of Levetiracetam in bulk and pharmaceutical dosage forms. The absorption maxima (λmax) of Levetiracetam was found to be 209 nm in Distilled water and in 0.01N Sodium hydroxide and Beer-Lambert's law was obeyed over the concentration range 2-10µg/ml. LOD and LOQ values of Levetriacetam was found to be 0.0479 µg/ml, 0.0594 µg/ml and 0.1595 µg/ml, 0.1981µg/ml in Distilled water and 0.01N Sodium hydroxide respectively. The validation parameters were treated statistically with 't' test and significant differences were observed. The study clearly revealed that the solvents influence the determination of Levetiracetam. The methods developed were rapid and easy can be applied for the estimation of Levetiracetam in bulk and pharmaceutical formulations.

Keywords: Levetiracetam, Distilled water, Sodium hydroxide, UV Spectrophotometry.

1. Introduction

Levetiracetam, sold under the brand name Keppra among others, is a medication used to treat epilepsy. It is used for partial-onset, myoclonic, or tonic–clonic seizures and is taken either by mouth as an immediate or extended release formulation or by injection into a vein. Levetiracetam is about as effective as phenytoin for prevention of early seizures after traumatic brain injury. It may be effective for prevention of seizures associated with subarachnoid hemorrhages. The exact mechanism by which levetiracetam acts to treat epilepsy is unknown. Levetiracetam does not exhibit pharmacologic actions similar to that of classical anticonvulsants. It does not inhibit voltage-dependent Na+ channels, does not affect GABAergic transmission, and does not bind to GABAergic or glutamatergic receptors. However, the drug binds to SV2A**,** a synaptic vsicle glycoprotein, and inhibits presynaptic calcium channels**,** reducing neurotransmitter release and acting as a neuromodulator. This is believed to impede impulse conduction across synapses. The absorption of levetiracetam tablets and oral solution is rapid and essentially complete. The bioavailability of levetiracetam is close to 100 percent, and the effect of food on absorption is minor. Levetiracetam chemically, (S)-2-(2-oxopyrrolidin-1-yl)butanamide The molecular formula of Levetiracetam is $C_8H_{14}N_2O_2$ and its molecular weight is 170.209 g/mol . Choice of solvent can shift peaks to shorter or longer λ depends on their nature of the interaction of the particular solvent with the environment of the chromophore in the excited state of the molecule. Solvents can affect the fine structure of absorption curves as well as the intensities and wavelengths of maxima. There are various methods are available for the estimation of Levetiracetam. Among these methods High performance liquid chromatography (HPLC), spectrophotometric methods and LC/MS reported for the quantification of Levetiracetam. The effect of solvents for the determination of Levetiracetam has not been reported. In the present study two UV spectrophotometric methods were developed for estimation of Levetiracetam in bulk and pharmaceutical formulations among these the sensitive one can be confirmed by calculating limit of detection (LOD) and limit of quantification (LOQ) as per ICH guidelines.

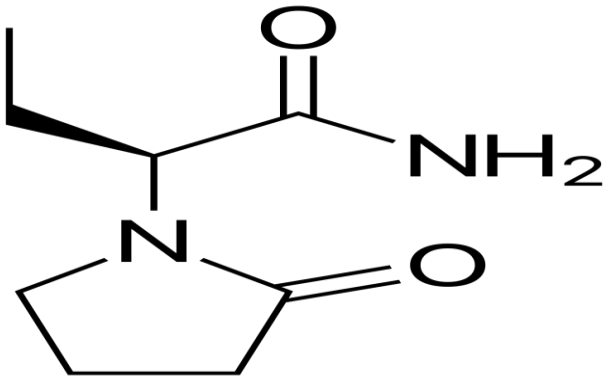

Fig. 1: Structure of Levetiracetam

2. Materials and methods

Absorbance measurements were made on double beam UV-Visible spectrophotometer with spectral band width of 0.5nm and wavelength accuracy of ± 0.3nm with 10 mm matched quartz cuvettes (ELICO SL 244) were employed. Levetriacetam reference standard was gifted by Dr.Reddy's Laboratories, Hyderabad. Sodium hydroxide used was of analytical grade purchased from National scientific Laboratories Vijayawada and Millipore Q distilled water.

3.1.Preparation of standard stock solutions of Levetriacetam

50 mg of Levetriacetam pharmaceutical grade was accurately weighed two times separately and transferred into two different 50ml volumetric flasks and dissolved in Distilled water, Sodium hydroxide volume was made up to the mark with the same solvents the stock solution obtained was 1000ppm(1mg/ml).

3.2.Determination of λmax

The two different stock solutions were suitably diluted with Distilled water and Sodium hydroxide to get a concentration of 10 ppm (10µg/ml) and scanned in the UV region ranges from 200nm-400nm. The wave length at which maximum absorbance observed was noted. The absorbance of the standard solutions containing the Distilled water and Sodium hydroxide was observed at 209 nm .

3.3.Beer's Lambert law

The two different stock solutions were suitably diluted to get concentration range from 2-10µg/ml and their absorbance were measured at λmax 209 nm against distilled water as blank and λmax 209 nm against sodium hydroxide as blank.. Calibration curve constructed for Levetriacetam in different solvents by taking concentration (µg/ml) on x-axis and their absorbance on y-axis.

The proposed methods are validated for the following parameters

4.1 Linearity

Linearity ranges of the proposed UV methods were found out. In order to find out the linearity range of proposed UV methods a curve was constructed by plotting absorbance obtained for the analyte against its concentrations in distilled water and sodium hydroxide. A series of 2(µg/ml), 4(µg/ml), 6(µg/ml), 8(µg/ml), and 10(µg/ml) were prepared for standard calibration curve and absorbance were observed. The results were subjected to regression analysis by the least squares method to calculate slope (m), intercept(c) and

regression coefficient (R^2).

4.2.Precision

Precision of method was determined in terms of repeatability (with in run precision), intermediate precision and reproducibility (between run precision).

4.2.1.Repeatability

Repeatability of the method was determined by analyzing three samples of 4(µg/ml), 6(µg/ml), 8(µg/ml) concentration in two different solvents and the %RSD and SE were calculated.

4.2.2.Intermediate precision

4.2.2.1 Intraday precision

It was calculated by analyzing six test samples of Levetriacetam on the same day, and the intraday precision of the method was determined by evaluating the samples of Levetriacetam on different days or and on two different spectrophotometers in the same laboratory.

4.2.2.2Reproducibility

The sample solutions were prepared and analyzed in different labs.

4.3.Sensitivity

The sensitivity of the proposed UV method was measured in terms of limit of detection (LOD) and limit of quantification (LOQ).The LOD and LOQ were calculated using formula:

$$LOD = 3.3\ \sigma/s \ \&\ LOQ = 10\ \sigma/s$$

Where

σ= standard deviation of Y-intercepts of regression lines.

S= slope of the calibration curve.

Sandell's sensitivity and Molar absorption coefficient

It is calculated by using the following formula

$$S = \epsilon . p$$

Where

S = sandell's sensitivity

ϵ = specific extinction coefficient

P = concentration of substance in mg/liter.

4.4.Robustness

To determine the robustness of the method, the experimental conditions were altered and assay was evaluated. Sample solutions were prepared and absorbance were observed at ± 5nm from absorption maxima.

4.5. Accuracy

Accuracy of the methods were confirmed by studying recovery at three different concentrations for 80, 100, 120% of these expected, in accordance with ICH guidelines by replicate analysis. Standard drug solutions were added to a pre analyzed sample solution and %drug content was measured.

4.6.Analysis of tablet formulations

Twenty tablets from each brand were weighed and grained into a fine powder using a Pestle and Mortar. An amount of tablet powder equivalent to 50 mg of Levetriacetam and dilutions were made with respective solvents, filtered using Whatmann No.42 filter. Analyze these solutions by using proposed UV methods.

4.7.Recovery study

To further ascertain the accuracy and reliability of the proposed methods, recovery experiments were performed via standard-addition procedure. Pre-analyzed tablet powder was spiked with pure Levetriacetam at three different levels and the total was found by the proposed methods. Each determination was repeated three times. The percent recovery of pure Levetriacetam added was within the permissible limits indicating the absence of inactive ingredients in the assay. These results are depicted in Table.No.6

5. Results and discussion

The wavelength maxima obtained for Levetriacetam in two different solvents were 209 nm in Distilled water and in sodium hydroxide . The developed UV spectrophotometric methods followed beer's law in the range of 2-10µg/ml.The relative standard deviation values were observed less than '1' indicates precision of the method, the lower standard error value indicates the accuracy of the method. The molar extinction coefficient, sandell's sensitivity, and LOD & LOQ values were calculated as per ICH guide lines, the values are depicted in table No.5. Based on LOD and LOQ values, the solvents were ranked Distilled water > Sodium hydroxide. The study clearly revealed the sensitivity of the method improved in the presence of Distilled water than in Sodium hydroxide. Distilled water was found to be more sensitive as it offered lowest LOD and LOQ values. LOD and LOQ values are subjected to statistically and data shown in Table.No:3&4. Significant differences were observed between the solvents used for the determination of drug. Lowest LOD and LOQ values were observed with the solvent i.e. Distilled water. The LOD and LOQ values of Levetriacetam in Distilled water and Sodium hydroxide were subjected to **t**-test. The calculated 't value compared with t- table value, the observed value was greater than the table value indicated that the significant differences between the LOD and LOQ values of Levetriacetam in two different solvents.

Table.1: Linearity of Levetriacetam in Siodium hydroxide

Sr. No.	Concentration(ppm)	Absorbance
1.	2	0.2845
2.	4	0.4765
3.	6	0.7014
4.	8	0.9876
5.	10	1.1456

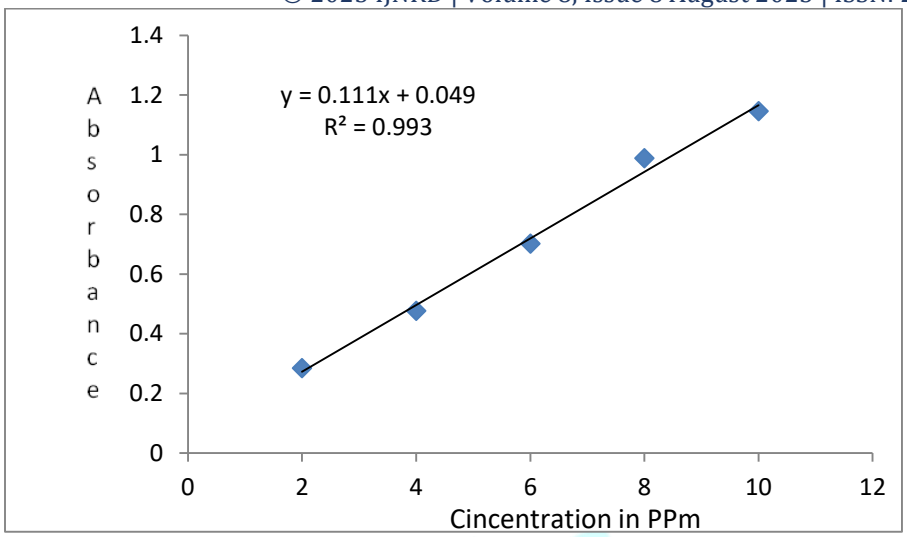

Fig.2: Calibration Curve of Levetriacetam in Sodium hydroxide

Table. 2: Linearity of Levetriacetam in Distilled water

Sr. No.	Concentration(ppm)	Absorbance
1.	2	0.2736
2.	4	0.5498
3.	6	0.8132
4.	8	1.0123
5.	10	1.2836

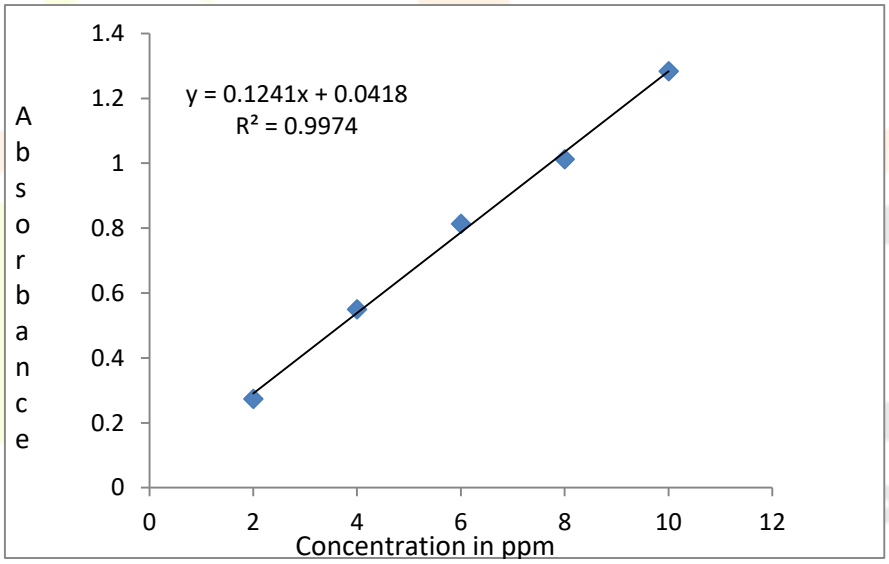

Fig. 3. Calibration Curve of Levetriacetam in Distilled water

Table .3: Limit of Detection (LOD)

S.No	Distilled water LOD± S.D(n=6)	Sodium hydroxide LOD ± S.D(n=6)	tCal	tTab	D.F
1.	$0.0479 \pm 3.03X10^{-4}$	$0.0594 \pm 2.56 X10^{-4}$	4.86	2.228	10

D.F = Degrees of freedom, tCal – Calculated t value, tTab – t table value

Table.4: Limit of Quantification (LOQ)

S. No	Distilled water, LOQ±S.D(n=6)	Sodium hydroxide LOQ±S.D(n=6)	tCal	tTab	D.F
1.	$0.1595\pm2.42X10^{-4}$	$0.1981\pm1.45X10^{-4}$	10.48	2.228	10

D.F = Degrees of freedom, tCal - Calculated t value, tTab – t table value

Table. 5: Validation Parameters of Proposed UV methods

S.No	Parameters	Sodium hydroxide	Distilled water
1.	λmax	209 nm	209 nm
2.	Range	2-10µg/ml	2-10 µg/ml
3.	Regression equation	Y=0.111X+0.049	Y=0.124X+0.041
4.	Slope	0.0111	0.0124
5.	Intercept	0.049	0.041
6.	$A^{1\%}$ 1cm	1232	1329
7.	R^2	0.993	0.997
8.	Molar absorption coefficient (litre/mole.cm-1)	2.097×10^4	2.262×10^4
9.	LOD	0.0594 µg/ml	0.0479 µg/ml
10.	LOQ	0.1981µg/ml	0.1595µg/ml
11.	Sandell's sensitivity (µg/cm2/0.001absorbance unit)	0.0090	0.0081

Table .6: Results of recovery study by standard addition method

Brand Name ofTablets	Distilled water				Sodium hydroxide			
	LTC in tablets (µg/ml)	Pure LTC added (µg/ml)	Total found (µg/ml)	Pure LTC recovered percent ±S.D(n=3)	LTC in tablets (µg/ml)	Pure LTC added (µg/ml)	Total found (µg/ml)	Pure LTC recovered percent ± S.D(n=3)
Levera-500	20	6	25.86	99.46 ± 1.34	20	6	25.87	99.5±0.83
	20	9	29.03	100.1±1.32	20	9	28.93	99.75±0.88
	20	12	31.92	99.275± 1.51	20	12	32.04	100.1±0.92
Levimax-500	30	6	35.89	99.69 ±0.79	30	6	35.02	100.0±1.02
	30	9	38.92	99.79± 0.67	30	9	39.05	100.1 ± 1.15
	30	12	42.01	100.0±0.94	30	12	41.91	99.79±0.84

Mean value of three determinations, LTC: Levetriactam, S.D: Standard deviation

6. Conclusion

The proposed methods were easy to perform and applicable for estimation of Levetriacetam in bulk and pharmaceutical formulations in Quality control laboratories.

7.Compliance with ethical standards

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Disclosure of Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

References

[1].Graeme J, Sillsa, John Paul Leacha, Caroline M, Frasera, Gerard Forresta, Neuro chemical studies with the novel anticonvulsant Levetiracetam in mouse brain, European journal of pharmacology, 325, 1997, 35-40.

[2].Carstensen JT, Rhodes CT,A rational approach to stability testing and analytical development for NCE, drug products marketed product stability testing, in Drug Stability: Principles and Practices, G. Wolfgang, Ed., Marcel Dekker, New York, NY, USA, 2000, 415-81.

[3].Contin M, Mohamed S, Albani F, Riva R, Baruzzi A, Simple and validated HPLC-UV analysis of Levetiracetam in deproteinized plasma of patients with epilepsy, Journal of Chromatography B, Analytical Technologies in the Biomedical and Life Sciences, 873, 2008, 129-132.

[4].Jens Martens-Lobenhoffer, Stefanie M, Bode-Boger, Determination of levetiracetam in human plasma with minimal sample pretreatment, jounal of chromatography B, 819, 2005, 197-200.

[5].Mattar KM, Quantification of Levetiracetam in human plasma by liquid chromatography-tandem mass spectrometry: application to therapeutic drug monitoring, Journal of Pharmaceutical and Biomedical Analysis, 48, 2008, 822-828.

[6].ICH Q2 (R1) Validation of analytical procedures, Text and methodology International Conference on Harmonization, Geneva, 2005, 1-17.

[7].VamseekrishnaG, Y.A.Chowdary, S.N.S.Sriram, B.DurgaBhavani, S.Likhiteswari, M.Harshini,UV Spectrophotometric estimation of Sumatriptan: Statstical treatment,Indo American Journal of Pharmaceuticalsciences,2019,06(03):5270-5277.