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DEVELOPMENT OF THE UV SPECTROPHOTOMETRIC METHOD OF LEVETIRACETAM IN BULK DRUG DOSAGE FORM AND STRESS DEGRADATION STUDY

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ABSTRACT

In the present study simple, precise, accurate, economical and reliable UV spectrophotometric method has been developed for the estimation of Levetiracetam in tablet dosage form. The drug shows maximum absorption (maximum λ_{max}) at 265.0 nm in distill water and the method exhibited high sensitivity, with linearity which obeys Beer's law in the concentration in the range of 2-12 µg /mL with good correlation coefficient (r^2=0.9982). The limit of detection and limit of quantitation were found to be 4.875µg/ml and 14.77µg/ml respectively for Levetiracetam. The results of analysis were validated by recovery studies. The recovery was found to be 82.46-104.85%. The relative standard deviation was found to be < 2.0 % in all cases. The proposed spectrophotometric method was validated statically as per the ICH Q_2 (R_1) guidelines with respect to linearity, specificity, accuracy, precision and robustness. The proposed method validation were found to be accurate, highly specific and can be used for the reliable quantification of Levetiracetam in bulk form and routine analysis of pharmaceutical formulations

KEY WORDS

Levetiracetam, Spectrophotometry, Validation, Stress Degradation Study,

1. INTRODUCTION

Levetiracetam [1,2] is a new antiepileptic drug [3] recently approved by the U.S. Food and Drug Administration as or as an adjunct in partial on set seizures, myoclonic seizures and primary generalized tonic-clonic seizures and mono therapy for partial seizures with or without secondary generalization. Levetiracetam has possible benefits for other psychiatric and neurologic conditions [4] such as Tourette syndrome, autism, and anxiety disorders. Levetiracetam seems to be a safe and sound and effective treatment for migraine [5] with aura. Chemically it is (α_S)-α-ethyl-2oxo-1-pyrrolidineacetamide with a molecular formula of $C_8H_{14}N_2O_2$ and a molecular weight of 170.20 g/mol. This is a structural analog of piracetam, which binds to a synaptic vesicle protein SV2A and is believed to impede nerve conduction across synapses. The exact mechanism by which Levetiracetam shows its antiepileptic effect is still unknown. However, it is believed that it binds to a synaptic vesicle protein, thus slowing down nerve conduction across synapses. Stability studies suggest proper formulations, design manufacturing processes, and selecting proper storage condition and packaging for the drug product.

Furthermore, it aids in establishing shelf life of product [6-8]. Literature review reveals that various analytical methods in pure and pharmaceutical dosage forms like Visible spectrophotometric method [9] Liquid Chromatographic and megabore Gas-Liquid Chromatographic determination [10] HPLC-UV [11-18] Capillary electrophoresis [19] and LC-MS [20,21,22] and other analytical methods have been developed individually. However, no UV spectrophotometric method is available for the quantitative determination of

Levetiracetam. The objective of the current study was to develop an accurate, simple, and economical analytical UV- spectrophotometric method for the estimation of Levetiracetam in pharmaceutical dosage forms. The proposed spectrophotometric method was validated statically as per the ICH Q2 (R1) guidelines. However, the requirement of very simple, fast, efficient, precise, time saving and highly reliable analytical UV spectrophotometric method for routine quality control purpose always necessities to see a new and better method. Hence, it was proposed to develop a simple, trouble-free, fast, perfect, and sensitive UV method for the concurrent estimation of Levetiracetam in pure form and pharmaceutical formulations. The structural formula of Levetiracetam is shown in Figure 1.

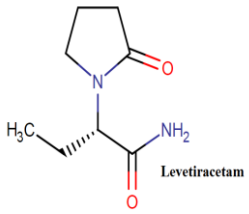

Fig.1. Chemical structure of Levetiracetam

2. EXPERIMENTAL

2.1. Selection of solvent: A number of trails were made to find out the ideal solvent system for dissolving the drug. The solvents such as water, methanol and acetonitrile were tried based on the solubility of the drug. Better absorption maximum was found to be 265 nm with distill water, so distill water was selected as optimized solvent in this spectrophotometric method.

2.2. Instruments used: Shimadzu, Double beam Model: UV-1800 spectrophotometer was used to record absorption spectra with the UV-VIS spectrophotometer with 1 cm matched quartz cells were used for estimation. The experiment was performed at room temperature.

2.3. Reagents and Materials: Levetiracetam (API) standards gift sample was obtained from Amoli organics, vadodara, India. Keppra tablets containing 250 mg of Levetiracetam tablets are obtained from local pharmacy. All the chemicals and reagents used were analytical grade. distill water was used throughout the experiment which was provided by Channabasweshwar Pharmacy College, Department of Quality Assurance, Latur (MS), India

2.4. Selection of detection wavelength: Appropriate dilutions of Levetiracetam were prepared from the standard stock solution. The standard solution of Levetiracetam in distill water was scanned over wavelength range 200 to 400 nm by using UV-Visible spectrophotometer. Wavelength 265nm was selected for analysis where Levetiracetam showed higher absorbance. The absorption spectrum of Levetiracetam is shown in Figure 2.

2.5. Preparation of standard drug solutions: 10 mg of Levetiracetam pure drug was accurately weighed and transferred into a 100 mL volumetric flask containing distilled water. The volume was made up to the mark with distilled water to get the stock solution (100 µg/mL). This solution was further diluted with the same to get the working standard solutions.

2.6. Preparation of Calibration curve: Aliquots of standard drug (0.2 mL to 1.2 mL, 100 µg/mL) solution in triple distilled water were transferred into a series of 10 mL volumetric flasks and the solution was made up to 10 mL with water. The absorbance of solutions was measured at 265.0 nm against reagent blank and the calibration curve of Levetiracetam was constructed. Calibration data is presented in Table 1. Calibration curve was prepared by plotting concentration of Levetiracetam on x-axis and their respective absorbance's on y-axis. The calibration curve is shown in Figure 3.

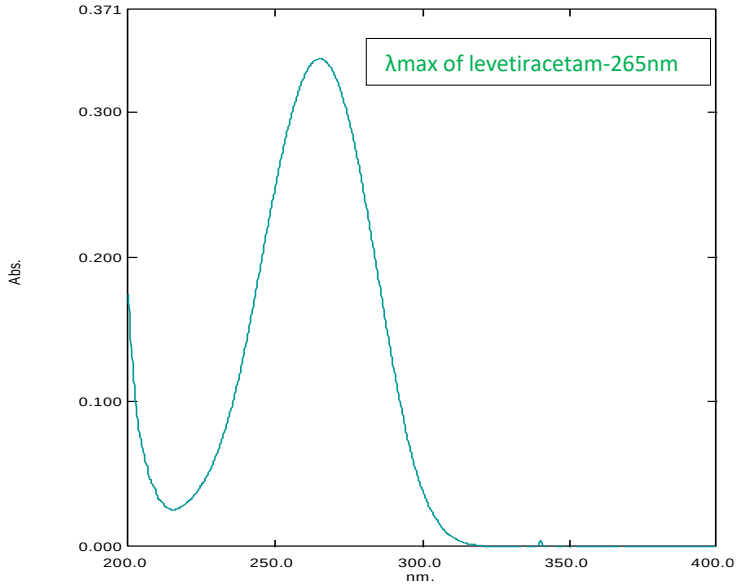

Figure 2. UV Spectrum of levetiracetam.

Table.1. Linearity data for levetiracetam

Sr.No.	Concentration (µg/ml)	Absorbance
1	0	0
2	0.2	0.256
3	0.4	0.322
4	0.6	0.378
5	0.8	0.429
6	1.0	0.498
7	1.2	0.552

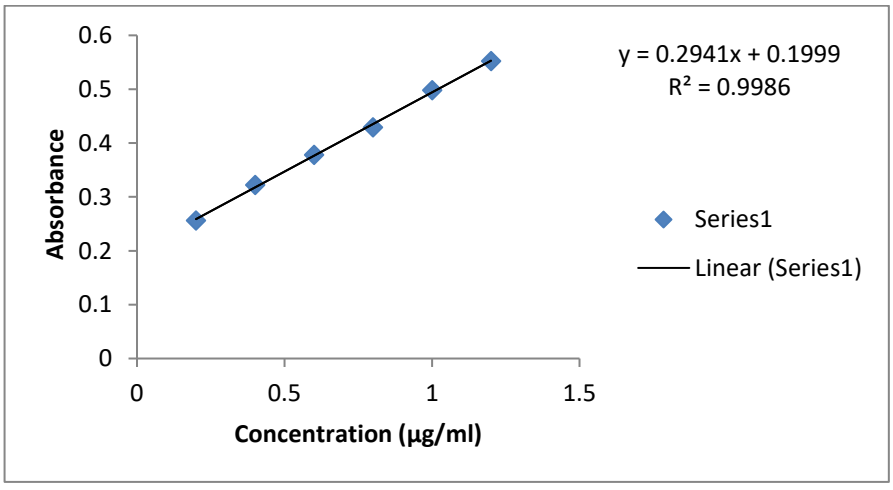

Figure.3 Calibration curve of Levetiracetam UV Spectrum method.

2.7. Procedure for assay of pharmaceutical formulations: 20 tablets of Levetiracetam marketed formulations were weighed and powdered in a glass mortar. A quantity of tablet powder equivalent to 100 mg of Levetiracetam was transferred into a 100 mL volumetric flask and to this 25 ml of distilled water was added and the solution was sonicated for 25 minutes and solution was then filtered through a whatman filter paper and the final volume was made up to 100 ml to obtain concentration of 1 mg/mL Levetiracetam. This solution was further diluted to obtain concentration 10 µg/mL and was used for analysis.

2.8. Validation of the developed method:

2.8.1. Linearity:

The linearity of an analytical procedure is its ability to obtain test results, which are directly proportional to the concentration of analyte in the sample. Linearity can be assessed by performing single measurements at several analyte concentrations. The linearity was established across the range and the absorbance of standard stock solutions in the range of 0.2-1.2 µg/ml was measured at 265.0 nm. The calibration curves was prepared by plotting graph between absorbance and concentration. Linearity was determined by least square regression method.

2.8.2. Precision:

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogenous sample under prescribed conditions. Precision was determined by intra-day and inter-day study. The repeatability of the method was calculated by carrying out the assay 3 times on the same day and intermediate precision was evaluated by carrying out the assay on 3 consecutive days for the sample solution. It is expressed as % RSD. The percent relative standard deviation (% RSD) was calculated.

2.8.3. Specificity:

The specificity of the method was analyzed by UV scan of the standard stock solutions in distilled water to determine the wave length of maximum absorption (λ max). The scanning can be helpful to specificity of the method by evaluating interaction study obtained from scan of individual standard drug solutions with other excipients of different formulations. In the present study no interfering peak was observed from the standard drug with different concentrations. Thus, scanning can be helpful to specificity. The specificity study shown in Figure 4.

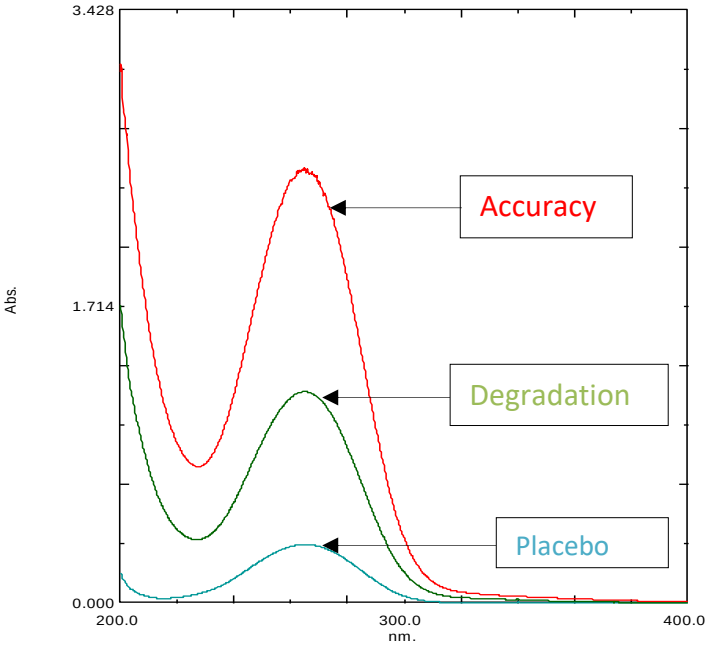

Figure.4 Specificity of the method determined by comparing the spectra of accuracy sample, placebo and degradation products.

2.8.4. Accuracy (Recovery studies)

The accuracy of analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted true value. The accuracy of the method was determined by preparing solutions of different concentrations, that is, 80, 100, and 120%, in which the amount of marketed formulation was kept constant (5 mg) and the amount of pure drug was varied, that is, 4 mg, 5 mg, and 6 mg for 80, 100, and 120%, respectively. The solutions were prepared in triplicate and the accuracy was indicated by % recovery. Recovery study were evaluated on same day at three different times and on three different days for intra-day and inter-day precision.

2.8.5. Ruggedness

Method ruggedness is defined as the reproducibility of results when the method is performed under actual use

conditions. This includes different analysts, laboratories, columns, instruments, sources of reagents, chemicals, solvents and so on. Method ruggedness may not be known when a method is first developed, but insight is obtained during subsequent use of that method.

2.8.6. Robustness

The concept of robustness of an analytical procedure has been defined by the ICH as "a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters". The most important aspect of robustness is to develop methods that allow for expected variations in the separation parameters. For the determination of a method's robustness, parameters such as variation in detector wavelength are varied within a realistic range and the quantitative influence of the variables is determined. If the influence of the parameter is within a previously specified tolerance, the parameter is said to be within the method's robustness range. The absorbance was measured, and assay was calculated for six times.

2.8.7. LOD and LOQ

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantified as an exact value. The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. Limit of Detection and Limit of Quantitation were calculated using the formula

LOD= 3.3(SD) / S and LOQ= 10 (SD) / S

where SD=standard deviation of response (absorbance) and S= slope of the calibration.

3. STRESS DEGRADATION STUDIES

3.1. Photolytic Degradation

Specific amount of drug Levetiracetam was weighed accurately and putted into the daylight for 8 hrs. after 8hrs 10mg drug was weighed and made stock solution (100µg/mL) with diluents. Then an appropriate concentration (10µg/mL) was prepared and absorbance was measured in UV spectrophotometer.

3.2. Thermal Degradation

Drug was taken in a Petri dish which was previously cleaned and dried then was put it into the oven for 48 hrs then it was taken out and weighted 10mg drug was weighed and made stock solution (100µg/mL) with diluents. Then an appropriate concentration (10µg/mL) was prepared and absorbance was measured in UV spectrophotometer.

3.3. Acid Degradation

0.01N HCL was taken in a 10ml volumetric flask then accurately weighed 10mg drug Levetiracetam was dissolved in it. Then the solution was refluxed for 2hrs then from this solution an appropriate concentration (10µg/mL) was prepared using diluents and absorbance was measured in UV spectrophotometer.

3.4. Alkali Degradation

0.01N NAOH was taken in a 10ml volumetric flask then accurately weighed 10mg drug Levetiracetam was dissolved in it. Then the solution was refluxed for 2hrs then from this solution an appropriate concentration (10µg/mL) was prepared using diluents and absorbance was measured in UV spectrophotometer.

3.5. Oxidation with H_2O_2

3% H_2O_2 solution was taken in a 10ml volumetric flask then accurately weighed 10mg drug Levetiracetam was dissolved in it. Then the solution was kept in dark for 2hrs then from this solution an appropriate concentration (10µg/mL) was prepared using diluents and absorbance was measured in UV spectrophotometer.

Table.2 The optical characteristics and the data concerning to the proposed method

Sr. No	Parameters	Data
1.	λ_{max}	265nm
2.	Linearity	2-12µg/ml
3.	Regression equation	Y=0.294 X + 0.199
4.	Correlation coefficient	0.9986
5.	Slop	0.294
6.	Intercept	0.199
7.	LOD	4.875
8.	LOQ	14.77

Table.3 Results of Precision Study

Concentration (µg/ml)	Intra-day precision			Inter-day precision		
	Absorbance mean	SD	%RSD	Absorbance mean	SD	%RSD
4	1.622	0.2232	1.431	1.549	0.0142	0.9205
8	1.183	0.00896	0.7579	1.157	0.00915	0.7909
12	0.867	0.00746	0.8611	0.8545	0.00497	0.5816

Table.4 Results of Accuracy Study

Concentration (µg/ml)	Absorbance mean	%RSD	% Recovery
80%	0.221	0.6635	82.46%
100%	0.270	0.3020	100.74%
120%	0.281	0.3725	104.85%

Table.5 Results for Robustness Study

Sr.No.	λ_{max} 263	λ_{max} 267
Mean	0.3648	0.4888
SD	0.00306	0.00444
% RSD	0.8388	0.9095

Table 6: Content of Levetiracetam in marketed products

Brand	Label claim (mg)	Amount found	Potency
Keppra	250 mg	28.90 mg	99.39%

Table. 7 Stress Degradation Study Results

Stress condition	Degradation %	Remark
Photolytic	0.9924	Stable
Thermal	0.9423	Stable
0.01N HCL	0.9926	Stable
0.01N NAOH	0.9920	Stable
H2O2	0.4839	Stable
Neutral Condition	0.9216	Stable

3. RESULTS AND DISCUSSION

The absorption spectra were recorded in the wavelength region of 200-400 nm in UV method, the absorption maxima curve (265.0nm) was shown in Figure 2. The proposed method obeyed Beer's law in the concentration range of 2-12 µg/mL with good correlation coefficient of $r^2 = 0.9982$. Calibration data is presented in Table 1. Beer's law range was confirmed by the linearity of the calibration curve of Levetiracetam was shown in Figure 3. The optical characteristics and the data concerning to the proposed method is represented in Table 2. Precision of the method was reported in terms of relative standard deviation and it should be evaluated by using a minimum of 3 determinations over which shows % RSD less than 2 shows that the method was precise, and the results are presented in Table 3. Recovery studies were carried out for the developed method by addition of known amount of standard drug solution of Levetiracetam to pre-analyzed tablet sample solution at three different concentration levels. The resulting solutions were

analyzed by the proposed methods. The recovery Table.4 was in the range of 82.46 to 104.85%. The limit of detection and limit of quantitation Table.2 for estimation of Levetiracetam were 4.8753µg/mL, & 14.77 µg/mL respectively. For the determination of a method's robustness, parameters such as variation in detector wavelength are varied within a realistic range and the quantitative influences of the variables were determined. The absorbance was measured, and assay was calculated. The results of robustness are presented in Table 5. The results are within the specified limits which states that this method is robust. The developed method was applied to the analysis of tablet formulations found to be within the proposed limits and the mean % assay value was found to be 98.39 %. The assay results are given in Table 6. The stress degradation studies showed that Levetiracetam stable when exposed to acidic alkaline, dry heat, oxidation, and in photolytic conditions. A summary of the results of the stress degradation studies of levetiracetam are shown in the Table 7. The developed method has good linearity, accuracy and precision results indicates that the high quality of the method.

4. CONCLUSION

The proposed UV spectrophotometric methods of estimation of levetiracetam were found to be accurate, precise and easy. The method can be successfully used for routine analysis of levetiracetam in bulk drug and in pharmaceutical dosage forms without interference.

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