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Development and Validation of Area under the Curve and first derivative methods for the Estimation of Levetiracetam in Bulk and Tablet Dosage Form by using UV Spectrophotometry

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Abstract

A simple, selective, precise and accurate "AUC" and "FIRST DERIVATIVE" methods for estimation of Levetiracetam drug in bulk and formulation by using UV spectrophotometric method was developed and validated. The solvent used was 0.1N sodium hydroxide and water. Levetiracetam was detected at 282 nm at room temperature. The linear regression analysis data for the linearity plot showed good linear relationship with correlation coefficient value, R2 = 0.998 in the concentration range 10 – 60 µg/ml with slope 0.008, intercept -0.021. The method was validated according to the International Conference on Harmonization (ICH) guidelines for linearity, range, accuracy, precision and specificity and applied on bulk powder and pharmaceutical formulations. Levetiracetam was determined in sterile dosage form in range of 99.42% with 0.131 standard deviation. The accuracy of the method was validated by recovery studies and was found to be significant and under specification limits, with % Recovery 85 – 103.8 (within acceptable range (85 -103%).

Keywords: UV spectrophotometer, Levetiracetam, sodium hydroxide, validation

Contents

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1. Introduction

Levetiracetam is an anti-epileptic drug which exerts its antiepileptic effect is unknown. The antiepileptic activity of Levetiracetam was assessed in a number of animal models of epileptic seizures. Levetiracetam did not inhibit single seizures induced by maximal stimulation with electrical current or different chemo convulsants and showed only minimal activity in sub maximal stimulation and in threshold tests. Protection was observed, however, against secondarily generalized activity from focal seizures induced by pilocarpine and kainic acid. It is white, off white

colour crystalline powder with faint odour. Chemically it is, (2R)-2-(2-Oxopyrrolidin-1-Yl) Butanamide with molecular formula $C_8H_{14}N_2O_2$.

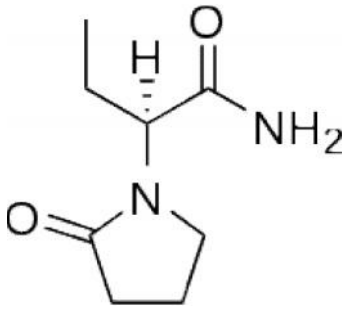

Figure 1. Structure of Levetiracetam

Literature survey reveals that various analytical methods have been reported for the estimation of Levetiracetam based on different technique, such as; HPLC with UV detection, UV detection with RP-HPLC used for drug monitoring and toxicology of levetiracetam. The aim of present work is to develop a simple, specific, sensitive and accurate "AUC" and "FIRST DERIVATIVE" methods for estimation of Levetiracetam drug in bulk and formulation by using UV spectrophotometric method and and validate as per ICH guidelines [3].

2. Experimental

2.1 Reagents and materials

Levetiracetam working standard was supplied by Reddy's laboratory and formulation (Label claim: 150mg, and manufacturer Cipla) were procured from the local market. Sodium hydroxide was obtained from laboratory chemicals.

2.2 Apparatus

The UV -Vis spectrophotometer (Shimadzu model) was employed in the method development and assay method validation.

2.3 Methods

2.3.1. Selection of solvent

Solubility of the drug in different solvents was performed. Levetiracetam was easily soluble in sodium hydroxide. The drug showed good spectrum and was stable in sodium hydroxide, so it was selected as a solvent of choice. The absorption maxima of levetiracetam were found to be 282nm.

2.3.2. Preparation of standard stock solution

Accurately weighed quantity of 100 mg Levetiracetam reference standard was transferred into 100 ml volumetric flask and dissolved and diluted up to the mark with sodium hydroxide to give a stock solution having strength 1mg/ml. From this prepare working standards.

2.4 Method Validation

2.4.1. Linearity

Working standard solutions for the drug having concentration 10, 20, 30, 40, 50, and up to 60µg/ml were prepared from the standard stock solution. The absorbance of resulting solutions were measured at wavelength of 282nm against solvent blank and a calibration curve was plotted to get the linearity and regression equation.

2.4.2. Accuracy

Accuracy of the method was determined by recovery experiments. To the formulation, the reference standards of the drug were added at the level of 50%, 100%, 150%. The recovery studies were carried out three times and the percentage recovery and %RSD of the recovery were calculated.

2.4.3. Precision

The precision of the method was demonstrated by method precision, system precision, inter-day and intra-day variation studies. The precision studies were performed for the standard concentration.

2.4.4. LOD

Limit of detection is the lowest concentration of analyte in a sample that can be detected, but not necessarily quantified. LOD was performed at a wavelength of 282 nm for 30µg/ml and the value is 1.33.

2.4.5 LOQ

Limit of quantitation is the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions. LOQ was performed at a wavelength of 282 nm for 10µg/ml and the value is 0.239.

2.5 Assay (method-A)

Weigh accurately about 10 tablets and take 100mg equivalent quantity of Levetiracetam and transfer into a 100ml standard flask. And dissolve the formulation in sodium hydroxides by using ultrasonication. Then pipette out 10ml of

solution and make up to 100ml leads to 10µg/ml concentration solution. This solution can be estimated in UV spectrophotometer by using Water as blank at 282nm.

$$P \quad p = \frac{t \quad al}{s} \quad X\,d \quad f \quad X\,1$$

2.6. Area under Curve Method (method-B)

Area under the curve method is applicable when there is no sharp peak and when broad spectra are obtained. Area calculation processing item calculation the area bound by the curve and horizontal axis. For the selection of analytical wavelength, 10 µg/ml solution of Levetiracetam was prepared by appropriate dilution of standard stock solution and scanned in the range of 200-400 nm. From the spectra of drug, area under curve in the range of 277-287 nm was selected for the analysis. The calibration curve was prepared in the concentration range of 10-60 µg/ml.By using the calibration curve, the concentration of the sample solution can be determined.

2.7. First Derivative Spectroscopy (method-C)

In this method, 10 µg/ml solution of Levetiracetam was prepared by appropriate dilution of standard stock solution and scanned in the spectrum mode from 200-400 nm. The absorption spectra thus obtained were derivatized for first order. First order derivative spectra of drug showed a sharp peak at 214 nm, which was selected for its quantification. The calibration curve for Levetiracetam was plotted in the concentration range of 10-60 µg/ml at wavelength 214 nm. The concentration of drug in test solution was determined against the calibration curve in quantitation mode.

3. Results and Discussion

The present work was aimed comparatively to the earlier literature report in connection to the priority of developing ‘‘AUC’’ and ”FIRST DERIVATIVE” methods for estimation of Levetiracetam drug in bulk and formulation by using UV spectrophotometric validated method as per ICH guidelines.In this current study, the API named levetiracetam (anti -epileptic) which is the most essential therapeutic agent in treatment of seizures. Among the analytical techniques available for quantification, the “AUC” and ”FIRST DERIVATIVE” methods is an emerging technique reliable in vast areas of research that incited the author to undertake method development and validation as per ICH guidelines for the API. The method followed here was external standard method in which sodium hydroxide was used as a solvent, and absorbance wavelength was 282nm.

The method was validated for all validation parameters as per ICH guidelines. The linearity range for levetiracetam was 10 – 60 µg/ml. with R^2 value of 0.998. The % RSD for method and system precision was < 2%. The method has been validated for assay of sterile dosage forms with 97%. The accuracy of the method was validated by recovery studies and was found to significant and under specification limits, with % Recovery 95.5 – 103.8 (within acceptable range (95 -103%). The assay results were found to be 97% (i.e with in 95.5-103.8%).

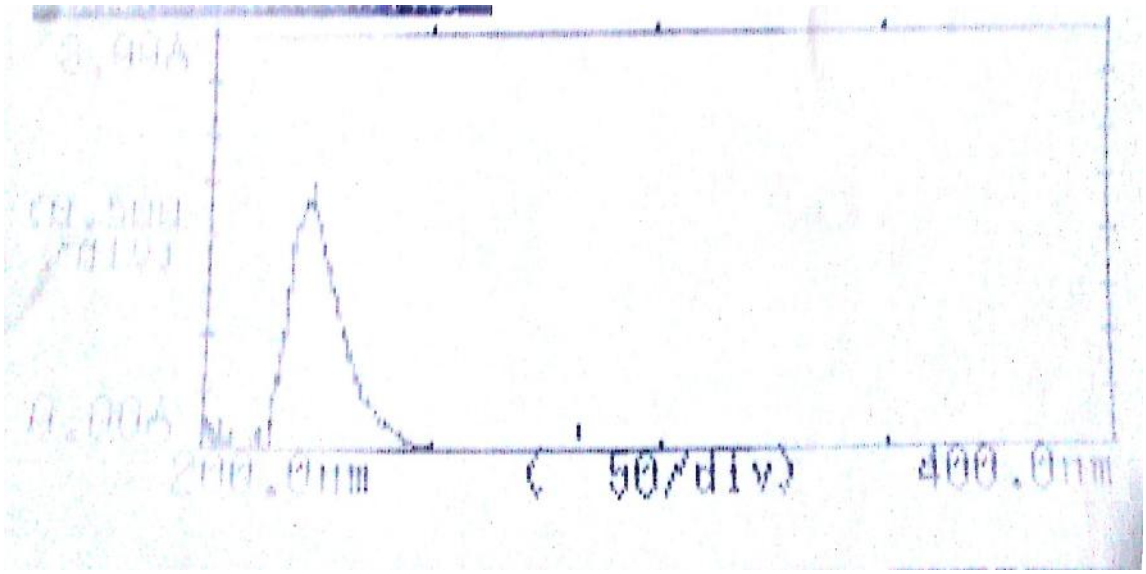

Figure 1. UV Spectrum of Levetiracetam

Table 1. Standard curve values

Concentration(µg/mL)	Absorbance
0	0
10	0.004
20	0.009
30	0.014
40	0.019
50	0.023
60	0.028

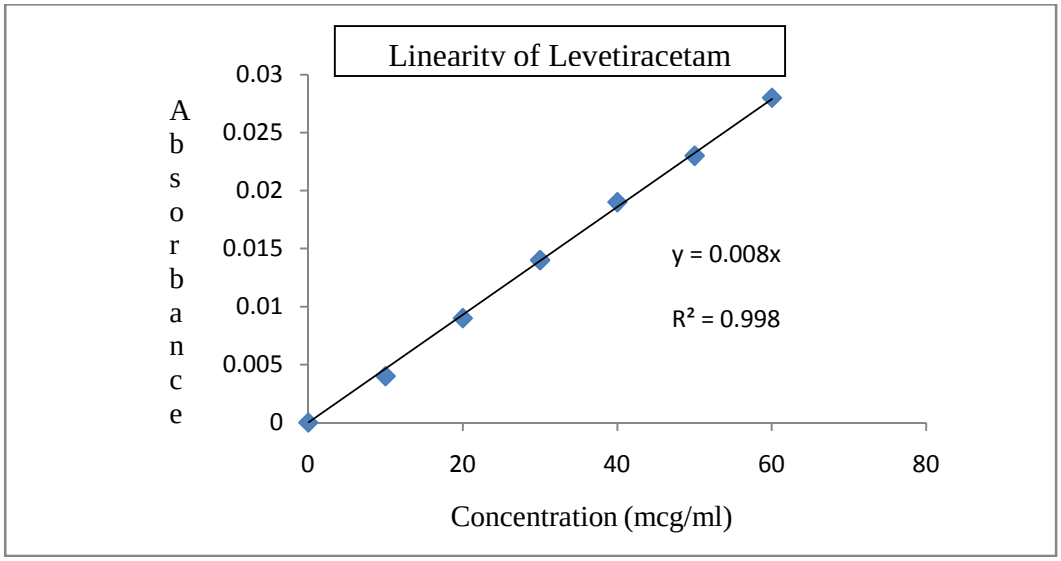

Figure 2. Standard curve of Levetiracetam

Table 2. Characteristics (or) parameters of Levetiracetam

Parameters	Method-A	Method-B	Method-C
λ_{max}	282	287-277	214
Beer's law limit	10-60µg/ml	10-40µg/ml	10-60µg/ml
Molar absorptivity	1.054	1.054	1.054
Regression equation (Y = mx + c)	0.042	0.042	0.025
Slope (m)	0.042	0.042	0.25
Correlation coefficient (r)	0.981	0.981	0.981
Relative standard deviation (%)	0.131	0.923	1.343
LOD Value	1.33	1.33	1.33
LOQ Value	0.239	0.239	0.239

Table 3. Assay of Levetiracetam

Drug	Label claim mg/tab	Amount found mg/tab	%Purity
Levetiracetam	150	140	97

Table 4. Levetiracetam intra-day and inter-day precision

Sample (µg/ml)	Intra-day precision	%RSD	Inter-day Precision	%RSD
10	0.007 0.021 0.011	1.78	0.011 0.019 0.028	0.89
30	0.022 0.015 0.027	1.23	0.015 0.021 0.031	1.57
60	0.039 0.027 0.032	0.982	0.019 0.032 0.034	0.987

Table 5. Recovery studies (accuracy parameter) of Levetiracetam

Test µg/ml	Level %	Amount of standard drug added (µg/ml)	%Recovery	Standard deviation	% RSD
10	50 100 150	0.027 0,011 0.005	95.5% 103% 95%	0.0117	1.8

Table 6. Area under curve Values of Levetiracetam

Concentration(µg/ml)	AUC values
0	0
10	0.4267
20	0.8057
30	1.1865
40	1.8795
Slope	0.043

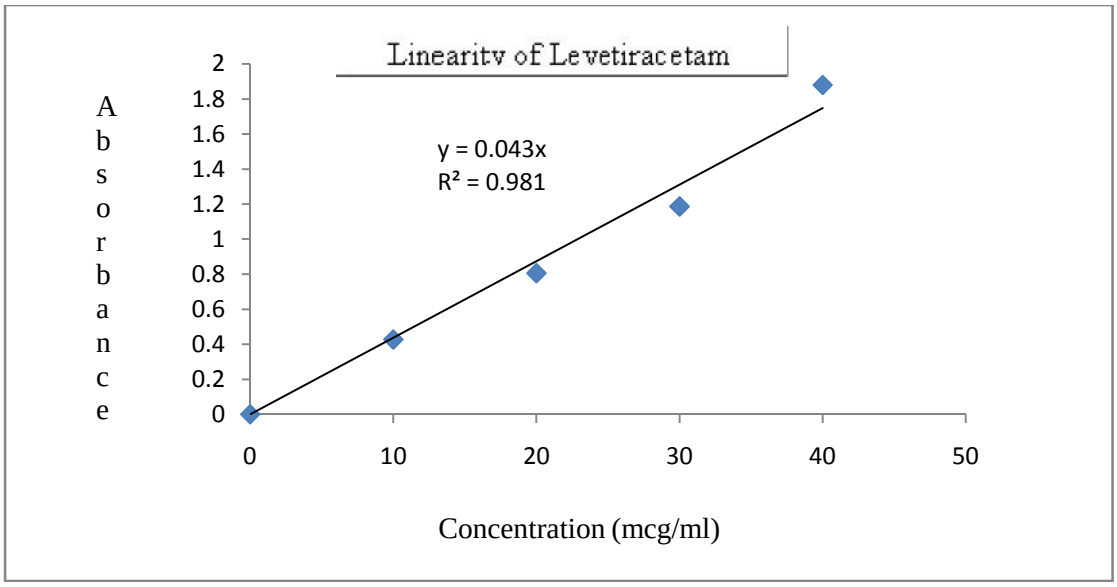

Figure 3. AUC Calibration Graph of Levetiracetam

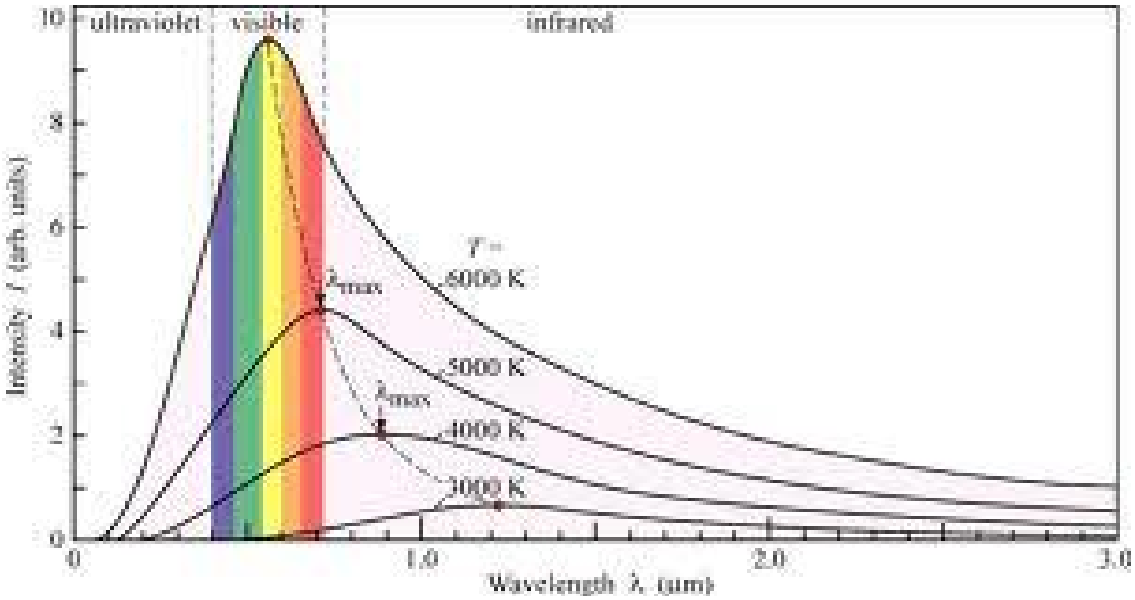

Figure 4. Area under Curve spectrum of Levetiracetam

Table 7. First Derivative Values of Levetiracetam

Concentration (µg/ml)	Absorbance
0	0
10	0.386
20	0.588
30	0.828
40	1.026
50	1.306
60	1.471
Slope	0.0258

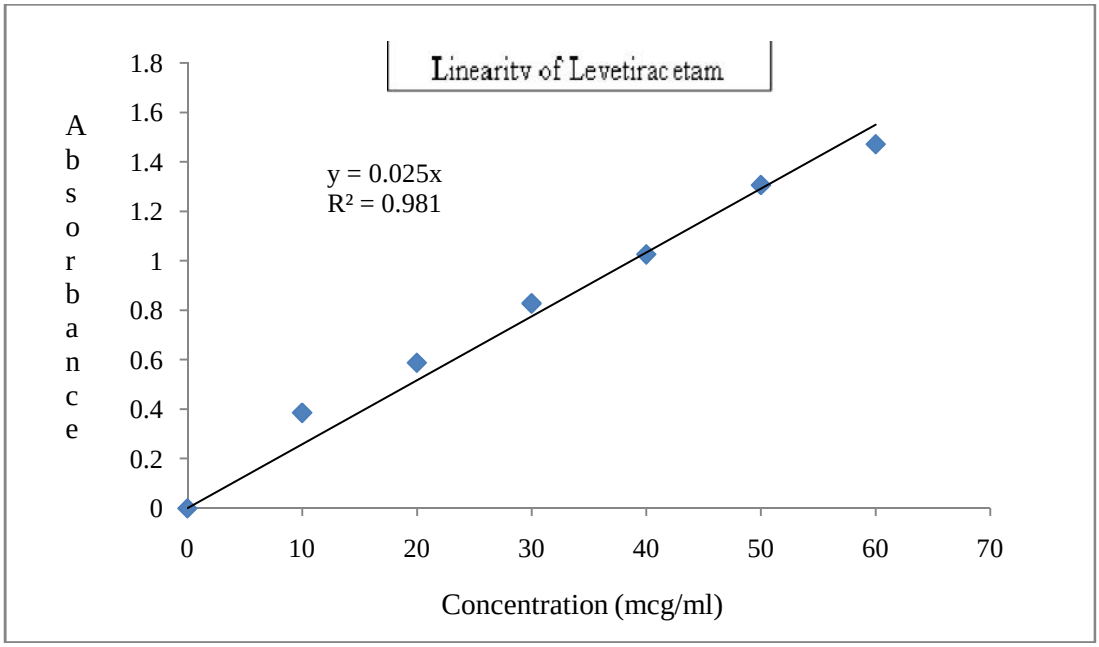

Figure 5: First Derivative calibration graph of Levetiracetam

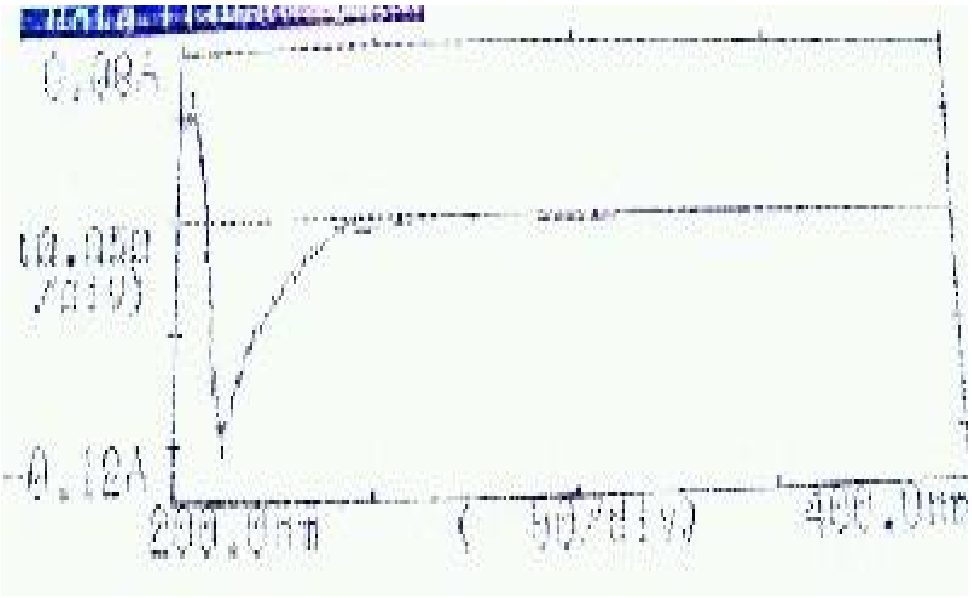

Figure 6. First Derivative curve of Levetiracetam

4. Conclusion

Three methods for the determination of Levetiracetam in the bulk drug and formulation have been developed. In Method-A (Assay) from the spectrum of Levetiracetam, it was found that the maximum absorbance is at 282 nm in Sodium hydroxide. A good linear relationship (0.998) was observed between the concentration ranges of 10-60 µg/ml. The assay of tablet was found to be 97%. The high percentage recovery indicates the high accuracy of the method. Method-B (Area under Curve method) obeyed Beer-Lamberts law in the concentration range 10-60 µg/ml. Method-C (First order derivative spectra) the range is 10-60 µg/ml. The above mentioned methods involves direct analysis without any extraction steps, thus it is performed faster, simple and easier. And this methods showed accurate and précised results. By these results these methods were found to be rapid, simple, accurate, and economic for analysis and quality determination. Thus the three developed methods can be easily applied for the routine quality control of Levetiracetam in bulk and tablet dosage form.

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