DEVELOPMENT AND VALIDATION OF SPECTROSCOPIC METHOD FOR ESTIMATION OF LEVETIRACETAM IN TABLET DOSAGE FORM

ABSTRACT

The present work aims at developing newer analytical methods that are simple, rapid, sensitive, precise, reliable and accurate for analytical method development and validation of Levetiracetam in tablet dosage form. The Levetiracetam is a nootropic agents, anticonvulsants, the drug binds to a synaptic vesicle glycoprotein and inhibits pre synaptic calcium channels and reducing neurotransmitter release and acting as a neuromodulator and is safely used in the treatment of epilepsy. From the solubility profile glacial acetic acid was chosen as common solvent for the estimation Levetiracetamat 221 nm. The optimum conc. of the Levetiracetam was found to be 65µg/ml and it was shown good absorbance valve which was found to be 0.4738. Results of the analysis were validated statistically as per the ICH guidelines. Linearity studies were carried out and the range was found to be 30–90 µg/ml. The regression coefficient value of Levetiracetam in glacial acetic acid was found to be 0.99978. The accuracy of the method was performed by recovery studies. The percentage recovery was found to be in the range of 99.73–100.08%. The precision was performed by analyzing standard and sample solutions of Levetiracetam (65µg/ml) at working concentration level for 6 times. Further the precision of the method was confirmed by intra-day and inter-day analysis. The low RSD values indicate that the method is precise. The Robustness was performed at different wavelength by using working standard solutions of Levetiracetam. The % RSD values for wavelength variation were found to be 0.7403 (standard), 0.7357 (sample) in glacial acetic acid.

Keywords: ICH Guidelines, Levetiracetam, epilepsy, anticonvulsants, neurotransmitter.

INTRODUCTION:

Levetiracetam, chemically (2R)-2-(2-oxopyrrolidin-1-yl) butanamide(Fig. No.1), is anotropics agents, anticonvulsants, the drug binds to a synaptic vesicle glycoprotein, SV2A, and inhibits pre synaptic calcium channels and reducing neurotransmitter release and acting as a neuromodulator. Levetiracetam may selectively prevent hyper synchronization of epileptiform burst firing and propagation of seizure activity. Levetiracetam binds to the synaptic vesicle protein SV2A, which is thought to be involved in the regulation of vesicle exocytosis. Literature survey revealed that there are few analytical methods have been reported for the determination of Levetiracetam in pure drug, pharmaceutical dosage forms and biological samples using Visible Spectrophotometry, High Performance Liquid Chromatography and Mass Spectroscopy. But UV Visible spectroscopic methods are not available for the determination of Levetiracetam and in bulk as well as in their formulations. Hence an attempt was made to develop and validate simple, rapid and reliable analytical method for estimation of Levetiracetam. The present work aims to develop and validate a simple, reliable,
workable and economical method for the estimation of Levetiracetamin table dosage form.

![Figure 1. Structure of Levitracetam](image)

**MATERIALS AND METHODS**

**Materials:**

*Drug Samples*

Levetiracetam was obtained as a gift sample from RA ChemPharma Pvt. Ltd. Hyderabad.

*Reference standards*

Levetiracetam -RA ChemPharma Pvt. Ltd.

Percentage purity - 99.86 %

*Instruments used:*

Different instruments used to carry out the present work, Electronic Weighing balance – single pan balance, Model Axis LC/GC. Digital pH meter - Model-Systronics. Sonicator- Ultra Sonicator – Model-Bandelinsonorex. Double Beam UV-Visible spectrophotometer A Schimadzu version 1.12-Double Beam UV Visible spectrophotometer. UV spectra of standard and sample solutions were recorded in 1cm quartz cells at the wavelength ranges of 200-400 nm.

*Chemicals used:*

Water - Milli Q water in house,

Glacial acetic acid - Finar, Sodium hydroxide - GR/Merck, Potassium dibasic anhydrous - Molychem

**Method Development**

*Standard preparation:*

Weigh accurately about 100.0 mg of standard Levetiracetam, dissolve in glacial acetic acidand make up the volume to 100ml with the same. Pipette out 6.5 ml and make up to 100 ml with glacial acetic acid. The final conc. of Levetiracetam sample was 65.6 g/ml. The solutions were scanned in UV region in the wavelength range from 200 to 400 nm.(Fig. No. 3)

**Sample preparation:*

Weigh equivalent weight of 131.6 mg of Leviteracetam tablet contents, dissolve in glacial acetic acidand make up the volume to 100ml with the same. Pipette out 6.5 ml and make up to 100 ml with glacial acetic acid. The final conc. of Levetiracetam sample was 65.6 g/ml. The solutions were scanned in UV region in the wavelength range from 200 to 400 nm.(Fig. No. 3)

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>0.222</td>
</tr>
<tr>
<td>40</td>
<td>0.293</td>
</tr>
<tr>
<td>50</td>
<td>0.364</td>
</tr>
<tr>
<td>60</td>
<td>0.440</td>
</tr>
<tr>
<td>70</td>
<td>0.504</td>
</tr>
<tr>
<td>80</td>
<td>0.576</td>
</tr>
<tr>
<td>90</td>
<td>0.646</td>
</tr>
</tbody>
</table>

**RESULTS AND DISCUSSION**

*Development of the spectrophotometric method*

Proper wave length selection of the methods depends upon the nature of the sample and its solubility. To develop a rugged and suitable spectrophotometric method for the quantitative determination of Levetiracetam, the analytical condition were selected after testing the different parameters such as diluents, diluents concentration, diluents pH and other conditions. From the solubility profile glacial acetic acid was chosen as common solvent for the estimation Leviteracetam.
Selection of wavelength

By scanning the standard solution of Levetiracetam in UV spectrophotometer between 200 nm to 400 nm on spectrum mode, using glacial acetic acid as a blank, the wavelength of analysis (λmax), 221 nm was selected. Sample and standard solution absorbance was measured at 221 nm.

Validation of developed method

Linearity and range:

The linearity of an analytical method is its ability (within a given range) to obtain the test results which are directly proportional to the concentration (amount) of analyte in the samples within a given range. The calibration curve constructed was evaluated by using correlation coefficient. The absorbances of Levetiracetam were linear over the range of 30-90 μg/ml (Fig. No. 4 & 5). The average absorbance of each concentration obtained was plotted against the concentration of the analyte. The correlation coefficient for the data was calculated as 0.99978. The regression line was observed to be in the form of \( y = 0.00723 \times + 0.0001 \). The results are summarized in Table No 1. The experiments indicated that there was a linear relationship between the amount of analyte and the absorbances within the range studied.

Precision

The precision of the method was calculated from the reproducibility of percentage assay of six Levetiracetam samples. The results are summarized in Table No 2. The results showed that the precision of the method is good.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Levetiracetam Standard Absorbance values at 221 nm in glacial acetic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Standard Sample</td>
</tr>
<tr>
<td>1</td>
<td>0.476 0.485</td>
</tr>
<tr>
<td>2</td>
<td>0.473 0.481</td>
</tr>
<tr>
<td>3</td>
<td>0.474 0.478</td>
</tr>
<tr>
<td>4</td>
<td>0.475 0.477</td>
</tr>
<tr>
<td>5</td>
<td>0.471 0.477</td>
</tr>
<tr>
<td>6</td>
<td>0.474 0.478</td>
</tr>
<tr>
<td>Mean</td>
<td>0.4738 0.4793</td>
</tr>
<tr>
<td>SD</td>
<td>0.0017 0.0031</td>
</tr>
<tr>
<td>% RSD</td>
<td>0.3635 0.6553</td>
</tr>
</tbody>
</table>

Intermediate Precision

Further the precision of the method was confirmed by intra-day and inter-day analysis. The analysis of formulation was carried out for three times in the same day and one time in the three consecutive days. The % RSD value of intraday analysis was shown in Table No 3, 4.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Intraday Precision</th>
<th>Inter day Precision</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Standard</td>
<td>Sample</td>
</tr>
<tr>
<td></td>
<td>Day-1</td>
<td>Day-2</td>
</tr>
<tr>
<td>Absorbance at λ max</td>
<td>0.472 0.474 0.475</td>
<td>0.470 0.473 0.471</td>
</tr>
<tr>
<td>Mean</td>
<td>0.4736 0.47415</td>
<td>0.4713 0.4715</td>
</tr>
<tr>
<td>SD</td>
<td>0.0015 0.0015</td>
<td>0.0015 0.0020</td>
</tr>
<tr>
<td>%RSD</td>
<td>0.3225 0.3177</td>
<td>0.3240 0.4397</td>
</tr>
</tbody>
</table>

Madhu et al, JGTPS, 2015, Vol. 6(4): 2956 - 2962
The results were well within acceptable limits of % RSD less than 2.0% for all parameters viz., intraday, inter day and analyst to analyst variation. These results indicated that the developed method is rugged.

**Accuracy**

Accuracy of the method was expressed in terms of recovery of added compound at 80%, 100% and 120% level of sample. Mean % recovery and % RSD were calculated and were summarized in Table No 5. The result shown that best recoveries (99.6 – 101.4%) of the drug were obtained at each added concentration, indicating that the method was accurate.Evaluation data of accuracy study of Levetiracetam was shown in (Fig. No. 6)

**Robustness**

The evaluation of robustness should show the reliability of an analysis with respect to deliberate variations in method parameters. If measurements are susceptible to variation in analytical conditions, the analytical condition should be suitably controlled or a precautionary statement should be included in the procedure. The Robustness was performed at different wave length by using working standard solutions of Levetiracetam. The result of robustness study of the developed assay method was established in Table No 6. The result shown that during all variance conditions, assay value of the test preparation solution was not affected and it was in accordance with that of actual. System suitability parameters were also found satisfactory; hence the analytical method would be concluded as robust.

**System suitability**

A system suitability test of the spectrophotometric system was performed before each validation run. Six replicate reading of standard preparation were taken and % RSD of standard reading were taken for same. Acceptance criteria for system suitability, % RSD of standard reading not more than 2.0%, were full fill during all validation parameter.

Table 4. Ruggedness Data for Analyst to Analyst

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Levetiracetam Standard</th>
<th>Levetiracetam Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Analyst 1</td>
<td>Analyst 2</td>
</tr>
<tr>
<td>Analyst to Analyst</td>
<td>0.472</td>
<td>0.471</td>
</tr>
<tr>
<td></td>
<td>0.474</td>
<td>0.474</td>
</tr>
<tr>
<td></td>
<td>0.475</td>
<td>0.475</td>
</tr>
<tr>
<td>Mean</td>
<td>0.4736</td>
<td>0.4733</td>
</tr>
<tr>
<td>SD</td>
<td>0.0015</td>
<td>0.0015</td>
</tr>
<tr>
<td>%RSD</td>
<td>0.3225</td>
<td>0.4397</td>
</tr>
</tbody>
</table>

Table 5. Evaluation Data of Accuracy Study

<table>
<thead>
<tr>
<th>% Recovery Level</th>
<th>% Recovery</th>
<th>Mean % Recovery</th>
<th>SD</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>80%</td>
<td>0.376</td>
<td>99.73</td>
<td>0.2700</td>
<td>0.2707</td>
</tr>
<tr>
<td></td>
<td>0.375</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.374</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100%</td>
<td>0.474</td>
<td>100.08</td>
<td>0.4200</td>
<td>0.4196</td>
</tr>
<tr>
<td></td>
<td>0.472</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.476</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>120%</td>
<td>0.543</td>
<td>99.88</td>
<td>0.3843</td>
<td>0.3847</td>
</tr>
<tr>
<td></td>
<td>0.546</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.542</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 6. Robustness Data for Wavelength Variation

<table>
<thead>
<tr>
<th>Wavelength(nm)</th>
<th>Levetiracetam inglacial acetic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Standard Sample</td>
</tr>
<tr>
<td>220</td>
<td>0.471</td>
</tr>
<tr>
<td>221</td>
<td>0.474</td>
</tr>
<tr>
<td>222</td>
<td>0.478</td>
</tr>
<tr>
<td>Mean</td>
<td>0.4743</td>
</tr>
<tr>
<td>SD</td>
<td>0.0035</td>
</tr>
<tr>
<td>220</td>
<td>0.477</td>
</tr>
<tr>
<td>221</td>
<td>0.481</td>
</tr>
<tr>
<td>222</td>
<td>0.474</td>
</tr>
<tr>
<td>Mean</td>
<td>0.4773</td>
</tr>
<tr>
<td>SD</td>
<td>0.0035</td>
</tr>
</tbody>
</table>
The optical parameters like molar absorptivity, correlation coefficient, slope, intercept, LOD, LOQ and standard error were calculated and results were shown in Table No 7.

Table 7. Robustness Data for Wavelength Variation

<table>
<thead>
<tr>
<th>Wavelength(nm)</th>
<th>Levetiracetam in glacial acetic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Standard</td>
</tr>
<tr>
<td>220</td>
<td>0.471</td>
</tr>
<tr>
<td>221</td>
<td>0.474</td>
</tr>
<tr>
<td>222</td>
<td>0.478</td>
</tr>
<tr>
<td>Mean</td>
<td>0.4743</td>
</tr>
<tr>
<td>SD</td>
<td>0.0035</td>
</tr>
<tr>
<td>%RSD</td>
<td>0.7403</td>
</tr>
</tbody>
</table>

Table 8. Validation Data of Levetiracetam

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Levetiracetam in glacial acetic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beers law limit (µg/ml)</td>
<td>20-120</td>
</tr>
<tr>
<td>Molar absorptivity (L mol⁻¹ cm⁻¹)</td>
<td>0.00728</td>
</tr>
<tr>
<td>Sandell’s sensitivity (µg/cm²/0.001 A.U)</td>
<td>0.00728</td>
</tr>
<tr>
<td>Correlation coefficient (r²)</td>
<td>0.99978</td>
</tr>
<tr>
<td>Regression equation (y = mx+c)</td>
<td>y = 0.00723x + 0.0001</td>
</tr>
<tr>
<td>R² = 0.99978</td>
<td></td>
</tr>
<tr>
<td>Slope (m)</td>
<td>0.00723</td>
</tr>
<tr>
<td>Intercept (c)</td>
<td>0.0001</td>
</tr>
<tr>
<td>LOD (µg/ml)</td>
<td>0.7759</td>
</tr>
<tr>
<td>LOQ (µg/ml)</td>
<td>2.3513</td>
</tr>
<tr>
<td>Standard Error</td>
<td>0.00069</td>
</tr>
</tbody>
</table>

CONCLUSION

The present analytical method was validated as per ICH Q2 (R1) guideline and it meets to specific acceptance criteria. It is concluded that the analytical method was specific, precise, linear, accurate, economic, and sensitive, and hence the present developed analytical method can be used for its intended purpose.

ACKNOWLEDGEMENT

The authors thank Sri. C. Gangi Reddy, Founder, Annamacharya Educational Trust for providing all the facilities for graduate studies and dissertation work. They also thank to RA ChemPharma, Pvt. Ltd, Hyderabad, India for providing standard Cyproheptadine HCl. They also thank the faculty of Annamacharya College of Pharmacy for their valuable suggestions and constant encouragement during research work.

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How to cite this article:

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