Research Article

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF BELINOSTAT IN PHARMACEUTICAL FORMULATION BY RP-HPLC

Arun Kumar Kuna *, S. Ganapathy and G. V. Radha
Gitam Institute of Pharmacy, Gitam University, Gandhi Nagar, Rushikonda, Visakhapatnam, Andhra Pradesh, India
*Corresponding Author Email: kunaarun@yahoo.co.in

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ABSTRACT

A simple, specific and accurate reverse phase high performance liquid chromatographic method was developed for the determination of Belinostat in pharmaceutical dosage form. The column used was ALTIMA C18, 150 x 4.6 mm, 5µ with mobile phase containing Buffer and Acetonitrile were taken in the ratio of 40:60 the flow rate was 1.0 mL/min and eluent was monitored at 266nm. The retention time of Belinostat was 2.320 min. The linearity of the drug was designed at a range for which correlation coefficient was 0.999. The proposed method was validated and successfully applied to the estimation of Belinostat in formulations. Forced degradation studies were conducted to identify reactions which may occur to degrade a processed product.

Keywords: Belinostat, RP-HPLC, Assay Development and Validation, Degradation.

INTRODUCTION

Belinostat (trade name Beleodaq, previously known as PXD101) is a histone deacetylase inhibitor drug developed by TopoTarget for the treatment of hematological malignancies and solid tumors. It was approved in July 2014 by the US FDA to treat peripheral T-cell lymphoma. In 2007 preliminary results were released from the Phase II clinical trial of intravenous belinostat in combination with carboplatin and paclitaxel for relapsed ovarian cancer. Final results in late 2009 of a phase II trial for T-cell lymphoma were encouraging. Belinostat has been granted orphan drug and fast track designation by the FDA, and was approved in the US for the use against peripheral T-cell lymphoma on 3 July 2014. It is not approved in Europe as of August 2014. Structure of Belinostat shown in Figure 1.

![Figure 1: Structure of Belinostat](Image)

MATERIALS AND METHODS

A Waters HPLC system consisting of a quaternary pump, an inbuilt auto sampler, a column oven and Waters PDA detector was employed throughout the analysis. The data was acquired using Empower 2 software and the column used was ALTIMA C18, 150 x 4.6 mm, 5µ. Double beam Uv-Visible spectroscopy (Make: T60). Labmal sonicator was used for enhancing dissolution of the compounds. A Metsar make pH meter was used for pH adjustment. Analytically pure Belinostat was obtained as gift samples from Spectrum Pharma Research Solutions, Hyderabad. Solvents were of Analytical grade used for the processing of experiment and for the preparation of mobile phase.

Preparation of Mobile phase, Standard Preparation & Sample Preparation

Mobile Phase: Buffer (1ML of Ortho phosphoric acid solution in a 1000ml of volumetric flask add about 100ml of milli-Q water and final volume make up to 1000 ml with milli-Q water) and Acetonitrile were taken in the ratio of 40:60.

Standard Preparation: Accurately Weighed and transferred 10mg of Belinostat working Standards into a 10 ml clean dry volumetric flask, add 7ml of diluent, sonicated for 30 minutes and make up to the final volume with diluents (100µg/ml Belinostat). From the above stock solution, 1 ml was pipetted out in to a 10ml Volumetric flask and then make up to the final volume with diluent.

Sample Preparation: 1 vial were in 500 mL volumetric flask, 300mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 1ml was pipetted out into a 10 ml volumetric flask and made upto 10ml with diluent.

Chromatographic conditions

A reverse phase C18 column equilibrated with mobile phase Buffer and Acetonitrile were taken in the ratio of 40:60 was used. Mobile phase flow rate was maintained at 1.0 mL/min and eluents was monitored at 210 nm. The sample was injected using a 10 µL fixed loop, and the total run time was 6.0 min. Appropriate aliquot of Belinostat stock solutions was taken in different volumetric flasks and diluted up to the mark with mobile phase to obtain final concentrations of Belinostat. The solution was injected and chromatograms were recorded.
Calibration curve was constructed by plotting average peak area versus concentrations and regression equation was calculated for Belinostat.

**UV –Vis Spectroscopy Conditions**

The sample was prepared using mobile phase as a diluent and inserted into UV –Vis spectroscopy. Then record the spectrum it’s monitored at 210 nm. Appropriate aliquot of Belinostat stock solutions was taken in different volumetric flasks and diluted up to the mark with mobile phase to obtain final concentrations of Belinostat.

**Determination of Belinostat in dosage form**

For the estimation of Belinostat from a vial, as the sample preparations mentioned above respective solutions were prepared. The solution was injected at above chromatographic conditions and peak areas were measured. The quantification was carried out by keeping these values to the straight line equation of calibration curve. The method was validated for accuracy, precision, LOD, LOQ, Specificity, Robustness and Ruggedness.

**Accuracy**

The accuracy of the method was determined by calculating recovery of Belinostat by the spiked method. Known amount of Belinostat was added to a pre quantified sample solution, and the amount of Belinostat was estimated by measuring the peak areas and by fitting these values to the straight-line equation of calibration curve.

**Precision**

Precision studies were performed (Method & day to day). The results are reported in terms of relative standard deviation. The Repeatability studies were carried out by estimating response of 6 different concentrations of Belinostat and results are reported in terms of relative standard deviation (%RSD). %RSD was 1.21, for Method precision it was 0.98 and for day to day precision it was 1.10.

**LOD and LOQ Limits**

The level of quantification (LOQ) and detection (LOD) were conducted on the basis of signal to noise ratio method.

**Specificity**

It is the ability to assess unequivocally the analyte in the presence of components that may be expected to be present. Commonly used excipients were spiked into a pre weighed quantity of drugs. The chromatogram was taken by appropriate dilutions and the quantities of drugs were determined.

**Robustness**

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Robustness of the method was studied by changing change in the chromatographic parameters; Effect of Variation in column oven temperature ± % 10 and the flow 0.9 and 1.1 ml/min instead of 1.0 ml/min.

**Ruggedness**

The ruggedness of test method was demonstrated by carrying out precision study in six preparation of sample on a single batch sample by different analyst. The results of the study are tabulated.

**DEGRADATION**

To understand the degradation behavior, degradation studies performed.

**Oxidation**

To 1 ml of stock solution of Belinostat, 1 ml of 20% hydrogen peroxide (H2O2) was added separately. The solutions were kept for 30 min at 600c. For HPLC study, the resultant solution was diluted to obtain 100µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

**Acid Degradation Studies**

To 1 ml of stock solution Belinostat, 1ml of 2N Hydrochloric acid was added and refluxed for 30mins at 600c. The resultant solution was diluted to obtain 100µg/ml l solution and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

**Alkali Degradation Studies**

To 1 ml of stock solution Belinostat, 1 ml of 2 N sodium hydroxide was added and refluxed for 30mins at 600c. The resultant solution was diluted to obtain 100µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

**Dry Heat Degradation Studies**

The standard drug solution was placed in oven at 1050c for 6 h to study dry heat degradation. For HPLC study, the resultant solution was diluted to obtain 100µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

**Photo Stability studies**

The photochemical stability of the drug was also studied by exposing the 120µg/ml solution to UV Light by keeping the beaker in UV Chamber for 7days or 200 Watt hours/m2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 100µg/ml solutions and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

**Neutral Degradation Studies**

Stress testing under neutral conditions was studied by refluxing the drug in water for 6hrs at a temperature of 60ºc. For HPLC study, the resultant solution was diluted to 100µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.
Figure 2: HPLC chromatogram of Belinostat in optimized chromatographic conditions

Figure 3: Calibration curve of Belinostat

Table 1: Validation parameters and data for proposed methods

<table>
<thead>
<tr>
<th>Validation parameter</th>
<th>Results (HPLC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity</td>
<td>25-150 µg/mL</td>
</tr>
<tr>
<td>Regression coefficient ($r^2$)</td>
<td>0.999</td>
</tr>
<tr>
<td>* Accuracy (% recovery)</td>
<td>99.66%</td>
</tr>
<tr>
<td>** Method precision (%RSD)</td>
<td>0.50</td>
</tr>
<tr>
<td>** Ruggedness (%RSD)</td>
<td>99.26%</td>
</tr>
</tbody>
</table>

* Replicates of three concentration levels (in three determinations); ** Six repetitive injections of same homogeneous sample.

Table 2: LOD and LOQ data for proposed methods

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOD</td>
<td>LOQ</td>
</tr>
<tr>
<td>RT</td>
<td>2.323</td>
</tr>
<tr>
<td>Area</td>
<td>10054</td>
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<tr>
<td>s/n value</td>
<td>21.7</td>
</tr>
<tr>
<td>Area</td>
<td>6583</td>
</tr>
<tr>
<td>s/n value</td>
<td>16.3</td>
</tr>
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Table 3: Ruggedness data

<table>
<thead>
<tr>
<th>S. No</th>
<th>RT</th>
<th>Area</th>
<th>USP Plate Count</th>
<th>USP Tailing</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.328</td>
<td>788382</td>
<td>3695</td>
<td>1.60</td>
</tr>
<tr>
<td>2</td>
<td>2.331</td>
<td>781653</td>
<td>3763</td>
<td>1.56</td>
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<tr>
<td>3</td>
<td>2.338</td>
<td>792729</td>
<td>3878</td>
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<tr>
<td>4</td>
<td>2.339</td>
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<td>5</td>
<td>2.341</td>
<td>782742</td>
<td>4028</td>
<td>1.57</td>
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<tr>
<td>6</td>
<td>2.343</td>
<td>790084</td>
<td>3852</td>
<td>1.58</td>
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</table>

Table 4: Degradation study data

<table>
<thead>
<tr>
<th>S. No</th>
<th>Study</th>
<th>RT</th>
<th>Area</th>
<th>USP Plate Count</th>
<th>USP Tailing</th>
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<tbody>
<tr>
<td>1</td>
<td>Acid Degradation</td>
<td>2.340</td>
<td>776351</td>
<td>4047</td>
<td>1.6</td>
</tr>
<tr>
<td>2</td>
<td>Base Degradation</td>
<td>2.332</td>
<td>766853</td>
<td>3702</td>
<td>1.5</td>
</tr>
<tr>
<td>3</td>
<td>Peroxide Degradation</td>
<td>2.342</td>
<td>760494</td>
<td>3896</td>
<td>1.6</td>
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<tr>
<td>4</td>
<td>Thermal Degradation</td>
<td>2.335</td>
<td>797578</td>
<td>3827</td>
<td>1.5</td>
</tr>
<tr>
<td>5</td>
<td>UV Degradation</td>
<td>2.332</td>
<td>798161</td>
<td>3800</td>
<td>1.6</td>
</tr>
<tr>
<td>6</td>
<td>Water Degradation</td>
<td>2.330</td>
<td>809089</td>
<td>3692</td>
<td>1.5</td>
</tr>
</tbody>
</table>

RESULTS AND DISCUSSION

The mobile phase Buffer and Acetonitrile were taken in the ratio of 40:60 was found to be satisfactory and gave symmetric peak for Belinostat. The retention time for Belinostat was 2.320 min, shown in Figure 2.

The calibration curve for Belinostat was obtained by plotting the peak area of Belinostat versus the concentration of Belinostat over the range of 25-150 µg/mL, and it was found to be linear with $r^2 = 0.999$, shown in Figure 3. The validation parameters are summarized in Table 1. The recovery Belinostat was found to be 99.66%. The system suitability test parameters are shown in Table 1. The liquid chromatographic method was applied to the determination of Belinostat in dosage form. The results for Belinostat were comparable with the corresponding labeled amount.

System Suitability parameters are detailed in Table 1. The results of both LOD & LOQ values were tabulated in Table 2. Ruggedness details generated after experiment are detailed in Table 3. Degradation details with respect to study are detailed in Table 4.

CONCLUSION

Proposed study describes a RP-HPLC method for the estimation of Belinostat using simple mobile phase when compared to the reported method. The method gives short analysis time (<5 min). The method was validated and found to be simple,
sensitive, accurate and precise by HPLC analytical methods. Percentage of recovery shows that the method is free from interference of the excipients used in the formulation. Therefore, the proposed method can be used for routine analysis of Belinostat in dosage form.

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REFERENCES


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