Development & Validation of Stability Indicating RP-HPLC Method for Linezolid Immediate Release Tablet Dosage Form

Vasant D. Khasia*, Hetal V. Khasia, Dhara Desai, Dharmishtha N. Bhakhar, Ashok R. Parmar

ABSTRACT
Stability indicating RP-HPLC method has been developed and subsequently validated for the determination of Linezolid. The proposed RP-HPLC method utilizes Phenomenex Luna C18 (250×4.6×5 µm) at 300C and mobile phase consisting of buffer: methanol (65: 35 v/v) at a flow rate 1.5 ml/min. Linezolid was separated in less than 60 minutes with good resolution and minimal tailing, without interference of excipients. The method was validated according to ICH guidelines. All validation parameters were within the acceptable range. The linear regression analysis data for calibration plots shows good linear relationship with R=0.999 in the concentration range of 1-6 µg/ml for a Linezolid. The limit of detection and limit for quantitation for Linezolid were found to be of 0.15 µg/ml and 0.4 µg/ml. Intraday and Interday precision (% relative standard deviation) is less than 2%. The method shows the % recovery of Linezolid 96.5 – 115.1%. The drug is subjected to different stress conditions and the resulting degradation products obtained did not interfere with the detection of Linezolid and the assay is thus stability indicating.

Key words: RH-HPLC, Linezolid, ICH Guideline, Validation, Stability-indicating.

INTRODUCTION
Linezolid is N-[(5S)-3-[3-fluoro-4-(4-morpholinyl) phenyl]-2-oxo-5 oxazolidinyl] methyl] acetamide a potent Antibacterial-Anti-infective. It inhibits bacterial protein synthesis by binding to the P site of the 50S ribosomal subunit and preventing formation of the larger ribosomal-ternary N-formylmethionine-t-RNA (t-RNA^fMet) - 70S complex that initiates protein synthesis [1-2].

Literature survey revealed several methods based on different techniques, viz, RP-HPLC [3-4], LC-MS-MS [5], RP-LC [6], HPLC [7,11], UV [8], HPTLC [9] and Chiral HPLC [10] methods are reported for the estimation of Linezolid alone or in combination with other agents. No method related to the stability-indicating RP-HPLC determination of Linezolid Immediate Release Tablet dosage form was found. The aim of the present work was to develop an accurate, selective, precise, robust, and stability-indicating RP-HPLC method for estimation of Linezolid Immediate Release Tablet dosage form in the presence of its degradation product.

MATERIALS AND METHODS

Instruments
The Waters (Alliance-2695, HPLC) instrument was equipped with PDA Detector, Empower software with gradient pump, injected with Rheodyne valve with 20 µ fixed loop and Phenomenex Luna C18 (250 x 4.6 mm i.d., 5.0 µm particle size) , Mettle Toledo (AX-205 analytical balance), Compact ultrasonic (Oscar Ultra Sonics, OU-35, sonicator) and Indian Lab. (pH meter) were used during the study.

Reagents and materials
The reagents, materials, gift sample of analytically pure Linezolid was provided by Cadila Healthcare Ltd, Ahmedabad , HPLC grade Methanol, Acetonitrile, Water and AR Grade Triethanolamine, Ammonium dihydrogen phosphate acid, Ortho-Phosphoric acid, H O , Sodium hydroxide from Merck, Mumbai, India and all other AR grade réagents from Merck chemicals, India. Marketed preparation Zyvox Tablet IR (Cadila Healthcare Ltd, India) 100mg.

Chromatographic conditions
The mobile phase consisted of Buffer:Methanol (65:35 v/v) and filtered through 0.22 µm filter and degassed before use. The flow rate was 1.5 ml/min and Phenomenex Luna, C18 (250 x 4.6 mm i.d., 5.0µm particle size) column at 30°C was used. The runtime was 55 min. The elution was monitored with a PDA detector in the UV range and the injection volume was 20 µl.

Preparation of buffer
Dissolve 1.36gm Potassium dihydrogen phosphate in 1000 ml Milli-Q water. Adjust PH 4.6 with Orthophosphoric acid. Filter through 0.22 µm membrane filter paper.

Preparation of mobile phase
Buffer
Methanol (65:35 v/v) were used and filtered through 0.22 µm filter, sonicated for 10min and used as mobile phase. Mobile phase only is used as diluents.

Preparation of Linezolid Standard stock solution
100mg of standard Linezolid was weighed and transferred to 100ml volumetric flask containing a few ml of diluent. It was sonicated for few minutes, and volume was made up to the mark to give a stock solution having strength 1 mg/ml (1000 µg/ml). From this an aliquote of 1ml was taken and diluted to 10ml to obtain the working standard solution of concentration 100µg/ml.

Diluted Standard Preparation
Dilute 2.0 ml of the working standard solution to 50.0 ml with diluents and mix.
Preparation of Sample solution of Linezolid
Accurately 20 tablets were weighed to determine average weight of tablets. Then tablets were finely crushed and tablet powder equivalent to 100 mg of Linezolid was transferred into 100 ml volumetric flask. Then 50 ml diluents was added to flask and sonicated for 40 minute with intermittent shaking. Make up volume upto 100 ml than solution was filtered and the final concentration of test sample solution was made upto 100 µg/ml of Linezolid.

Method Validation
The method was validated for accuracy, precision, specificity, detection limit, quantitation limit and robustness. The specificity of this method was evaluated to ensure there was no interference from placebo components (prepared in solution) or from products resulting from forced degradation[12].

Specificity
A blank preparation, standard preparation and sample preparation were prepared as per method. Peak area for the main peak in standard preparation and sample preparation were determined and recorded.

Linearity and range
Linearity was determined at five levels over the range of 50% to 150% with respect to the test concentration. A standard stock solution was prepared and further diluted to attain concentration of about 50%, 75%, 100%, 125% and 150% of sample concentration.

Accuracy
The accuracy of the method was assessed by determination of the recovery of the method at 3 different concentrations (corresponding to 50%, 100% and 150% of test solution concentration) by addition of known amounts of standard to the placebo. For each concentration three sets were prepared.

Precision
Method precision was established by assaying six sample preparations under same conditions. For intermediate precision the procedure followed for method precision was repeated on a different day, by a different analyst, using a different RP-HPLC system.

Reproducibility
The procedure followed for reproducibility was repeated in different laboratory.

Limit of detection and quantification
LOD and LOQ were calculated using following equation as per ICH guidelines.

\[
LOD = 3.3 \times \frac{\sigma}{S} \quad \text{and} \quad LOQ = 10 \times \frac{\sigma}{S},
\]

where \(\sigma\) is the standard deviation of response and \(S\) is the slope of the calibration curve.

Robustness
The robustness of the method was established by making deliberate minor variations in the experimental conditions like Flow rate (±10.0 %) and Organic phase ratio (±2.0 %).

Solution Stability
Stability of the standard solution was evaluated after storing for 12, 24, 36, 48 hrs. The response of the aged solution was evaluated by comparing with freshly prepared solutions.

System suitability
The suitability of the chromatographic system was tested before each stage of validation. Six replicate injections of standard preparation were injected and asymmetry, number of theoretical plates and RSD of peak area was determined.

Analysis of marketed formulations
The sample solution was injected at above chromatographic conditions and peak areas were measured. The quantification was carried out by keeping these values in the straight line equation of calibration curve.

Forced degradation study
To perform the forced degradation study 100 mg of the standard dissolved in few ml of the diluents was subjected to acidic, alkaline, oxidizing, humidity, thermal and photolysis conditions. For acidic degradation 5 ml 1N HCl was added to the drug, at 70°C for 2hrs and the mixture was neutralized. For alkaline degradation 5ml 0.1N NaOH was added to the drug, kept at 70°C for 20 min. and the mixture was neutralized. For degradation under oxidizing conditions the drug allowed to stand with 5ml (30% v/v) H O at 70°C 2hrs [13]. For thermal degradation the powdered drug was exposed at 100°C for 3 days. Sample solution and placebo was also subjected to the same stress conditions to determine whether any peaks arise from the excipients. After completion of the treatments the solutions was diluted with the diluents to furnish 100µg/ml of Linezolid solutions. The chromatogram for each sample was recorded to detect any type of peak likely to appear the purity of the drug peak.

RESULTS AND DISCUSSION
In this work an analytical RP-HPLC method for assay of Linezolid in Tablet formulation was developed and validated. The basic chromatographic conditions were designed to be simple and easy to use, and were selected after testing the different conditions that affect RP-HPLC analysis, for example column, aqueous and organic components of the mobile phase, proportion of mobile phase components, detection wavelength, diluents and concentration of analyte. The mobile phase consisting of Buffer: Methanol (65:35 v/v) was selected which give a sharp peak of Linezolid (Fig. 1, 2). The flow rate was maintained 1.5 ml/min. The retention time was 60 min and detection wavelength was 251nm.
Validation

The method was specific as there was no interference from any peaks due to excipients. The calibration curve was found to be linear over the range of 1-6 µg/ml Linezolid (Fig. 3). The data of regression analysis of the calibration curves are shown in table I. The accuracy of the method was determined by calculating recoveries of Linezolid by spiking known quantities of standard drug to placebo preparations. The recoveries were found to be 96.5-115.1% for Linezolid. The high values indicate that the method is accurate. The Precision was performed and the RSD value for Linezolid was found to be 0.269-0.74%. Reproducibility was performed and the RSD value was found to be 1.093% for Linezolid. The low RSD values indicate that the method is precise and reproducible. The detection limit for Linezolid was 0.15 µg/ml while quantitation limit for Linezolid was 0.4 µg/ml. The validation parameters are summarized in table II. In all deliberately varied conditions, the percentage RSD for the peak areas of the main analyte peak of Linezolid was found to be well within the acceptance limit of 2% thus indicating the method robustness. The solution stability study revealed that Linezolid solution was stable for 48hrs.

Table 1: Regression analysis of Calibration curve.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Linezolid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity range</td>
<td>1-6 µg/ml</td>
</tr>
<tr>
<td>Slope</td>
<td>304.17</td>
</tr>
<tr>
<td>Intercept</td>
<td>4557</td>
</tr>
<tr>
<td>Correlation Coefficient</td>
<td>0.999</td>
</tr>
<tr>
<td>Wavelength</td>
<td>251 nm</td>
</tr>
</tbody>
</table>

Table 2: Summary of validation and system suitability parameters.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Linezolid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retention Time</td>
<td>15.147</td>
</tr>
<tr>
<td>Asymmetry</td>
<td>1.04</td>
</tr>
<tr>
<td>Theoretical Plates</td>
<td>7204</td>
</tr>
<tr>
<td>Detection limit (µg/ml)</td>
<td>0.0495</td>
</tr>
<tr>
<td>Quantitation limit (µg/ml)</td>
<td>0.1499</td>
</tr>
<tr>
<td>Accuracy (% recovery)</td>
<td>99.57-100.12 %</td>
</tr>
<tr>
<td>Method Precision (% RSD)</td>
<td></td>
</tr>
<tr>
<td>Repeatability</td>
<td>0.64-2.99</td>
</tr>
<tr>
<td>Intraday Precision</td>
<td>0.31-0.87</td>
</tr>
<tr>
<td>Interday Precision</td>
<td>0.36-0.77</td>
</tr>
<tr>
<td>Specificity Specific</td>
<td>Specific</td>
</tr>
<tr>
<td>Selectivity Selective</td>
<td>Selective</td>
</tr>
<tr>
<td>Robustness (% RSD)</td>
<td>0.53</td>
</tr>
<tr>
<td>Solution Stability</td>
<td>Stable for 48 hrs</td>
</tr>
</tbody>
</table>

Forced degradation study

The study revealed that there was no interference by the degraded products formed (Fig. 4). There was also no interference from blank and placebo at the retention time of the analyte peak. The specificity of the method was determined by peak purity of the peak obtained by Linezolid in the stressed sample. The peak purity index was found out to be > 0.999. Percentage degradation achieved for Linezolid standard and sample is shown in table-3.
Vasant D. Khasia et al. / Journal of Pharmacy Research 2012,5(8),4115-4118

Fig. 4. Chromatogram of forced degradation study. Chromatographic profiles of Linezolid after subjecting them to (A) acidic, (B) alkaline, (C) oxidative, (D) humidity (E) thermal degradation and (F) photo stability

Table 3: Forced degradation study of for Azelnidipine proposed RP-HPLC method.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Time (hrs)</th>
<th>% Degradation Linezolid</th>
<th>Peak area Sample (µg/ml)</th>
<th>Standard (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1N HCl (70°C)</td>
<td>2hrs</td>
<td>42.32</td>
<td>2636799</td>
<td>3187126.5</td>
</tr>
<tr>
<td>0.5N NaOH (70°C)</td>
<td>20min</td>
<td>7.13</td>
<td>2605101</td>
<td></td>
</tr>
<tr>
<td>30% peroxide(70°C)</td>
<td>3day</td>
<td>11.5</td>
<td>2605701</td>
<td></td>
</tr>
<tr>
<td>Humidity</td>
<td>24</td>
<td>0.061</td>
<td>2824571</td>
<td></td>
</tr>
<tr>
<td>Thermal (100°C)</td>
<td>24</td>
<td>0.064</td>
<td>2790511</td>
<td></td>
</tr>
<tr>
<td>Photolysis</td>
<td>0.063</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Analysis of marketed formulations
The proposed method was applied to the determination of Linezolid in pharmaceutical dosage form (tablet). The result for Linezolid was comparable with the corresponding labeled amount (table 4).

Table 4: Assay Results of Pharmaceutical Dosage Form (tablet).

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Actual amount (µg/ml)</th>
<th>Amount Found (µg/ml)</th>
<th>% of Drug Found</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azelnidipine</td>
<td>100</td>
<td>100.12</td>
<td>101.75</td>
</tr>
</tbody>
</table>

CONCLUSION
This RP-HPLC method for assay of Linezolid in pharmaceutical dosage form (Tablet) was successfully developed and validated for its intended purpose. The method was shown to be specific, linear, precise, accurate, reproducible and robust. Because the method separates linezolid and the degradation products formed under the variety of stress conditions, it can be regarded as stability indicating.

REFERENCES

Source of support: Nil, Conflict of interest: None Declared