A validated HPLC Method for Quantitative Analysis of Lincomycin Hydrochloride

Mohamed Shamakh\(^1\), Taha Attia\(^2\) and Hanan Azoz\(^1\)

(1) Department of Biochemistry and Feed Deficiency, Pharmacology Unit, Animal Health Research Institute, Dokki, Giza, Egypt.

(2) Department of Pharmacology, Faculty of Veterinary Medicine. University of Sadat City, Egypt.

*Corresponding author: pharm.shamakh90@gmail.com  Received: 17/12/2021  Accepted: 1/1/2022

ABSTRACT

A precise, simple and sensitive high-performance liquid chromatographic (HPLC) method has been developed and validated for qualitative and quantitative estimation of lincomycin hydrochloride in combined drug substance and in sterile powder for oral solution. Chromatographic separation was performed by Shimadzu Prominence-i LC 2030 C with ZORBAX SB-C18 column (3.5µm, 4.6 mm×75 mm) at ambient temperature with isocratic mobile phase consisting of 67:33 (v/v) mixture of 0.023 M orthophosphoric acid (pH adjusted to 2.3) and acetonitril as mobile phase, at a flow rate of 1.0 ml/min with UV detection at 210 nm. The method was validated for linearity and range, accuracy, specificity, robustness, precision (intra and inter-day) and limit of quantification and limit of detection. The calibration plot was linear from 5.0 -100 µg/ml with regression equations \( y = 33.605x + 5.6529 \) and correlation coefficient (\( R^2 \)) of 0.9999. In conclusion, the developed method was precise, simple and rapid for estimation of lincomycin hydrochloride.

Keywords: Lincomycin, Validated, HPLC, Isocratic

INTRODUCTION

Lincomycin is a lincosamide antibiotic closely related to macrolide. The chemical name for lincomycin hydrochloride is Methyl 6,8-dideoxy -6- (1-methyl- trans -4- propyl -L2-pyrolidinecarboxamido)-1 -thio- D- erythro- α - D - galacto - octopyranoside - mono hydrochloride monohydrate (Fig.1) (Budavari S., 2001; Sweetman S.C., 2002). The molecular formula of lincomycin hcl is C18H34N2O6S.HCl.H2O. lincomycin was produced by Streptomyces lincolnensis (Family Streptomycetaceae) (Kumar P.R. et al., 2017). It inhibits protein synthesis through binding to the 50S bacterial ribosome subunit (Goodman and Gilman’s 2001). It is mainly active against Gram-positive bacteria, obligate anaerobes and against mycoplasmas (Giguère S., et al., 2006). A few analytical hplc method have been previously described for lincomycin quantitative analysis (Kumar P.R. et al., 2017; Sharma M. et al., 2019; Gouri S.S. et al., 2014; Sharma M. et al., 2019; Nielsen and Gyrd-Hansen, 1998). Lincomycin distributes well into tissues and is known to produce high intracellular concentrations. It was widely metabolized by hepatic microsomal enzymes to inactive metabolites which was eliminated through bile and urine (Brown R.B., et al. 1975; Hornish R.E., et al., 1987). It is used alone or in combination with other drugs like spectinomycin in poultry for oral treatment of bacterial enteric infections, control of respiratory infections and growth promoters. Using lincomycin in veterinary treatment has been shown to be effective in cattle, sheep and horses at various doses (Plenderleith R.W., 1988).
MATERIAL AND METHODS

1. Materials

Reagent and chemicals
Acetonitrile was of HPLC grade, while orthophosphoric acid (85%) was analytical grade which were purchased from Merck, Germany.

De-ionized water was from a Milli-Q- System (Millipore, Bedford, MA, USA).

Lincomycin hydrochloride reference substance (purity ≥ 95.0%) was purchased from Sigma Aldrich (3050 Spruce Street, Saint Louis, MO 63103, USA).

Pharmaceutical product containing lincomycin Hcl was obtained commercially (Lincoprima®, primavet, Egypt).

Preparation of standard solutions
Stock standard solution of lincomycin was prepared by dissolving an equivalent amount to 10 mg lincomycin in 10 mL deionized water (1000 µg/mL). Intermediate solution of lincomycin was prepared by transfer 1 mL of stock solution to 10 mL volumetric flask and diluted with deionized water to give a final concentration (100 µg/mL) and from which working solutions were prepared (5, 10, 15, 25, 50, 100 µg/mL).

Preparation of sample solutions
25 mg of drug powder was accurately weighed and dissolved in to 10 mL volumetric flask with deionized water to give a final concentration (1000 µg/mL) which considered stock solution. Intermediate solution was prepared by transfer 1 mL of stock solution to 10 mL volumetric flask and diluted with deionized water to give a final concentration (100 µg/mL).

Instrumentation and chromatographic parameters
Shimadzu Prominence-i LC 2030 C apparatus equipped with degassing unit, quaternary pump, Shimadzu UV- Vis (Diode array) detector, auto sampler and agilent column ZORBAX SB-C18 (3.5µm, 4.6 mmx75 mm).

Cooling centrifuge, ultrasonic bath, vortex mixer.

Chromatographic parameters:
Mobile phase consisting of 0.023 M orthophosphoric acid (pH adjusted to 2.3) and acetonitrile 67:33 (v/v). Flow rate of 1.0 ml/min with UV detection at 210 nm at room temperature according to (Nielsen and Gyrd-Hansen, 1998).

Method Validation

The method was validated by evaluation of precision (inter and intra day), linearity and range, accuracy, robustness, specificity, limit of detection and quantification to verify that these method performance characters fullfil the requirements described by International Conference on Harmonization of technical requirements for registration of pharmaceuticals for human use (ICH, 2005).

1. Linearity and Range

Linearity was established by preparing six concentrations of standard lincomycin hydrochloride in the range 5.0 - 100.0 µg/mL. Peak area of lincomycin hydrochloride was plotted versus it’s concentration to obtain the calibration curve and calculate correlation coefficient, y-intercept and regression line equation.

2. Precision

2.1. Repeatability

It is also called intra day precision and assessed by preparing aminumin of six determinants (10 µg/ml) on the same day. % relative standard deviations (% RSD) of peak areas for each concentration was calculated.

2.2. Intermediate precision

It is also called inter day precision (within laboratory variations) and assessed by preparing aminumin of six determinants (10 µg/ml) on six different days. % relative standard deviations (% RSD) of peak areas for each concentration was calculated.

3. Specificity

It is the ability to determine unequivocally the analyte in the presence of impurities and/or excipients.

4. Accuracy and Recovery

Accuracy of the method was assessed by mean recoveries % calculation. It was determined by standard addition at three concentrations levels (50%, 100%, 120%). It was prepared by adding known amounts of the reference standard to fixed amount of drug sample, then these samples (in triplicate) was analyzed against the same concentration of standard reference.

5. Robustness

It means the degree to which the method remains unaffected by minor but deliberate changes in the method parameter. Changing in mobile phase pH, column temperature (°C) and wavelength (nm) were studied. Relative standard deviation was calculated and should
not more 2% for every change parameter.

6. Limit of detection and quantification

They were calculated from standard deviation of the intercept (σ) and the slope (S).

LOD = 3.3 × (σ/S), LOQ = 10 × (σ/S)

RESULTS AND DISCUSSION

1. Linearity and Range

Calibration curve was obtained between peak area responses versus drug concentrations in the range of 5.0 - 100.0 μg/mL. The linearity of calibration curve was demonstrated by linear regression equations \( y = 33.605x + 5.6529 \) with correlation coefficient \( (R^2) \) of 0.9999.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correlation coefficient ( (r^2) )</td>
<td>0.9999</td>
</tr>
<tr>
<td>Slope</td>
<td>33.605</td>
</tr>
<tr>
<td>y-intercept</td>
<td>+5.6529</td>
</tr>
<tr>
<td>Regression equation</td>
<td>( y = 33.605x + 5.6529 )</td>
</tr>
</tbody>
</table>

![Fig. (2). Calibration curve of lincomycin hydrochloride.](image)

2. Precision

Intra-day and inter-day precision were assessed by preparing a minimum of six determinants on the same day (repeatability) and on six different days (intermediate precision) respectively. Relative standard deviation % was calculated as following \( \text{RSD} = \frac{\text{SD} \times 100}{x} \) and should not >2% as described in (Table 2) which confirm that the method was precise.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avg. areas</td>
<td>335.98</td>
</tr>
<tr>
<td>Inter-day precision</td>
<td>335.73</td>
</tr>
</tbody>
</table>

3. Specificity

In the presence of impurities and/or excipients, the chromatogram of lincomycin hydrochloride showed no interference with other peaks at the same retention time of lincomycin hydrochloride as showed in (Figure 3).

![Fig. (3). Chromatogram of lincomycin hydrochloride (Retention Time = 3.875 min) at concentration (20 µg/ml).](image)

4. Accuracy and Recovery

At three concentrations levels (50%, 100%, 120%), samples were prepared by standard addition (5µg/ml, 10µg/ml, 12µg/ml). The samples (in triplicate) was analyzed against the same concentration of standard reference. Mean recoveries for lincomycin were ranged from 98.33% to 99.83% (Table 3) and these results indicate that the method was accurate.

<table>
<thead>
<tr>
<th>Level conc.</th>
<th>5 µg/ml</th>
<th>10 µg/ml</th>
<th>12 µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (n=3)</td>
<td>4.92</td>
<td>9.90</td>
<td>11.98</td>
</tr>
<tr>
<td>SD</td>
<td>0.031</td>
<td>0.05</td>
<td>0.027</td>
</tr>
</tbody>
</table>
Robustness
Deliberate changes in mobile phase pH (2.1 - 2.5), column temperature (23ºC - 27ºC) and wavelength (208 nm - 212 nm) were studied. It was observed that there were no changes in chromographic behavior of lincomycin so, the method is robust.

Table (4). Robustness results of lincomycin hydrochloride.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Variation</th>
<th>Mean area</th>
<th>RSD%</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH of mobile phase</td>
<td>+0.2</td>
<td>335.90</td>
<td>0.125</td>
</tr>
<tr>
<td></td>
<td>-0.2</td>
<td>335.86</td>
<td>0.093</td>
</tr>
<tr>
<td>Column temperature</td>
<td>+2</td>
<td>336.08</td>
<td>0.393</td>
</tr>
<tr>
<td></td>
<td>-2</td>
<td>335.85</td>
<td>0.219</td>
</tr>
<tr>
<td>Wavelength</td>
<td>+2</td>
<td>336.18</td>
<td>0.143</td>
</tr>
<tr>
<td></td>
<td>-2</td>
<td>336.37</td>
<td>0.176</td>
</tr>
</tbody>
</table>

6. Limit of detection and quantification
They were calculated from standard deviation of the intercept (σ) and the slope (S). LOD of lincomycin was 1.41 μg/ ml and LOQ was 4.29 μg/ ml.

CONCLUSION
In the current study, a new validated HPLC method was simple, accurate, robust, precise and applicable for quantitative analysis of lincomycin hydrochloride without any interference. Therefore, this method can be used for quantitative analysis of lincomycin hydrochloride.

REFERENCES
ICH (2005): International conference on harmonization of technical requirements for registration of pharmaceuticals, validation of analytical procedures; methodology, harmonized tripartite guideline.ICH, Geneva.