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DEVELOPMENT AND VALIDATION OF LC METHOD FOR THE ESTIMATION OF LINCOMYCIN IN PHARMACEUTICAL DOSAGEFORMS

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Abstract: A simple, specific, accurate and precise reverse phase high performance liquid chromatographic method was developed and validated for the estimation of Lincomycin in tablet dosage form. An Inertsil ODS C-18, 5µm column having 250 x 4.6mm internal diameter in isocratic mode with mobile phase containing methanol: Acetonitrile: 1%OPA (10:65:25v/v/v) was employed. The flow rate was 1.0ml/min and effluents were monitored at 258nm. Ornidazole was used as an internal standard. The retention time for Lincomycin and Ornidazole were 8.8min and 2.6 min. The method was validated for linearity, accuracy, precision, specificity, limit of detection, limit of quantification and robustness. Limit of detection and limit of quantification were found to be 0.05ppm and 0.15ppm respectively and recovery of Lincomycin from tablet formulation was found to be 101.307%. The proposed method was successfully applied for the quantitative determination of Lincomycin in tablet formulation.

Keywords: Lincomycin, HPLC, Internal standard, Linearity, Validation, 258nm.

INTRODUCTION

Lincomycin is a lincosamide antibiotic that comes from the actinomyces *Streptomyces lincolnensis*. It has been structurally modified by thionyl chloride to its more commonly known 7-chloro-7-deoxy derivative, clindamycin. Although similar in structure, antibacterial spectrum, and in mechanism of action to macrolides, they are also effective against other species as well, i.e., actinomycetes, mycoplasma, and some species of *Plasmodium*. However, because of its adverse effects and toxicity, it is rarely used today.

The I.U.P.A.C name of Licomycin is (2*S*,4*R*)-*N*-[(1*R*,2*R*)-2-hydroxy-1-[(2*R*,3*R*,4*S*,5*R*,6*R*)-3,4,5-trihydroxy-6-(methylsulfanyl)oxan-2-yl]propyl]-1-methyl-4-propylpyrrolidine-2-carboxamide

Molecular formula: $C_{18}H_{34}N_2O_6S$
Molecular weight: 406.538 g/mol

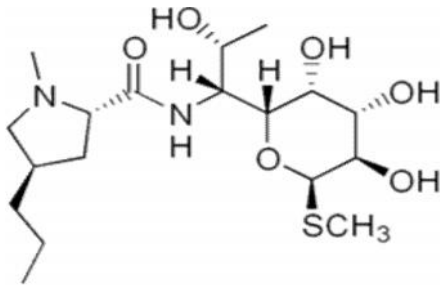


Figure.1: Molecular Structure of Lincomycin

Literature survey[1-18] revealed that numerous methods have been reported for estimation of Lincomycin in pharmaceutical formulations has been reported.

Present study involves development of LC method using simple mobile phase which is sensitive and rapid for quantification of Lincomycin in tablet dosage forms as well as subsequent validation of developed method according to ICH guide lines. The present sample was received from M/sWallace Pharmaceuticals Ltd., GOA as gesture.

EXPERIMENTAL

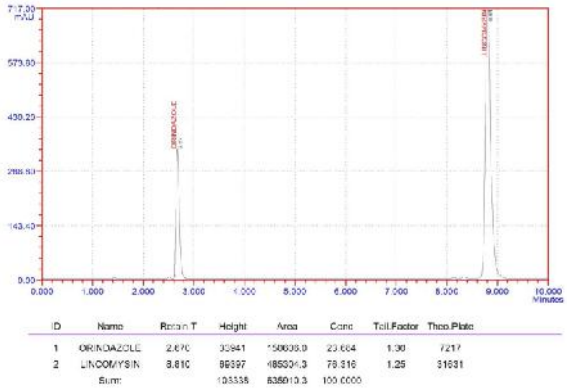
Instrument: The liquid chromatographic system consisted of Shimadzu HPLC model (VP series) containing LC-10AT (VP series) pump, variable wave length programmable UV/visible detector SPD-10AVP and rheodyne injector (7725i) with 20µl fixed loop. Chromatographic analysis was performed using Intersil ODS C-18 column with 250 x 4.6mm internal diameter and 5µm particle size. Shimadzu electronic balance (AX-200) was used for weighing purpose.

Reagents and materials: Methanol (HPLC grade) was purchased from E. Merck, Mumbai, India. LC grade water was obtained by double distillation and purification through milli – Q water purification system. Ortho phosphoric acid of analytical grade was procured from qualigens, Mumbai, India.

Preparation of Standard Stock Solution: A stock solution of Lincomycin was prepared by accurately weighing 10mg of drug, transferring to 100ml of volumetric flask, dissolving in 25ml of solvent and diluting up to mark with solvent. Appropriate aliquot of this solution was further diluted with solvent to obtain final standard solution of 2ppm of Lincomycin. In this method development and validation 2ppm of Orindazole also added as internal standard. Resultant solution was filtered through Ultipor N_{66} Nylon 6, 6 membrane sample filter paper.

Preparation of sample Solution: The formulation tablets of Lincomycin were crushed to give finely powdered material. Powder equivalent to 10mg of Lincomycin was taken in 10 ml of volumetric flask containing 5ml of mobile phase and was shaken to dissolve the drug, 2ppm Orindazole solution added as internal standard then filtered through Ultipor N_{66} Nylon 6, 6 membrane sample filter paper. Volume of the filtrate was adjusted to the mark with the same solvent to obtain concentration of 2ppm.

Figure.2: HPLC chromatogram of Lincomycin formulation



Chromatographic conditions: The mobile phase consisting of Methanol: Acetonitrile: OPA were filtered through 0.45µ Ultipor N_{66} Nylon 6, 6 membrane solvent filter, degassed and were pumped from the solvent reservoir in the ratio of 10:65:25, v/v/v and was pumped into the column. The flow rate of mobile phase was maintained at 1.0ml/min and detection wavelength was set at 258nm with a run time of 10min. The volume of injection loop was 20µl prior to injection of the drug solution the column was equilibrated for at least 30min with the mobile phase flowing through the system. The column and the HPLC system were kept in ambient temperature.

Calibration curve: Appropriate aliquots of standard Lincomycin stock solution were taken in different volumetric flasks and resultant solution was diluted up to the mark with mobile phase to obtain final concentration of 0.5, 1.0, 1.5, 2.0 and 2.5ppm of Lincomycin. These solutions were injected into chromatographic system, chromatograms were obtained and peak area ratio was determined for each concentration of drug solution. Calibration curve of Lincomycin was constructed by plotting peak area ratio versus applied concentration of Lincomycin and regression equation was computed. Similarly the sample solution was chromatographed and concentration of Lincomycin in tablet sample was found out using regression equation.

Method validation: The method was validated for accuracy, precision, linearity, specificity, limit of detection, limit of quantification and robustness by following procedures.

Accuracy: The accuracy of the method was determined by calculating recovery of Lincomycin by the method of standard addition. Known amount of Lincomycin (1ppm and 0.5, 1.0, 1.5ppm) was added to a pre quantified sample solution and the amount of Lincomycin was estimated by measuring the peak area ratios and by fitting these values to the straight line equation of calibration curve. The recovery studies were carried out three times over the specified concentration range and amount of Lincomycin was estimated by measuring the peak area ratio. From the above determination, percentage recovery and standard deviation of percentage recovery were calculated.

Precision: The intra-day precision study of Lincomycin was carried out by estimating the correspondence responses six times on the same day with 2ppm concentration and inter-day precision study of Lincomycin was carried out by estimating the correspondence responses six times next day with 2ppm concentration.

Linearity and range: The linearity of the method was determined at six concentration levels ranging from 0.5-2.5ppm for Lincomycin.

Specificity: Commonly used excipients (colloidal silicon dioxide, lactose, magnesium stearate, povidone, starch and talc) were spiked into a pre-weighed quantity of drug. The chromatogram was taken by appropriate dilutions and the quantity of drug was determined.

Limit of detection and limit of quantification:

Limit of detection = 0.05ppm

Limit of quantification = 0.15ppm

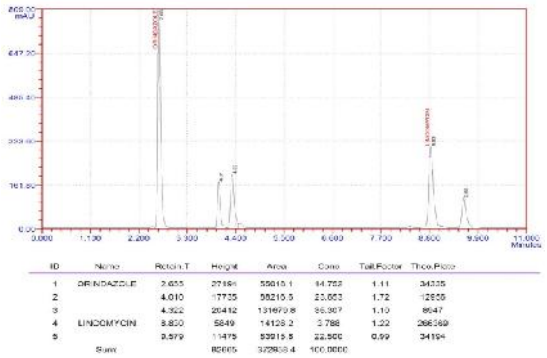
Stability: In order to demonstrate the stability of both standard and sample solutions during analysis, both the solutions were analyzed over a period of 8 hours at room temperature.

Robustness: Robustness of the method was studied by changing the composition of organic phase by ±5% and the pH by ± 0.2, and also by observing the stability of the drugs for 24 hours at ambient temperature in the mobile phase.

RESULTS AND DISCUSSION

The UV spectra of Lincomycin showed that the drug absorbs appreciably at 258nm which was selected as the detection wave length in liquid chromatography. Optimization of mobile phase was performed based on asymmetric factor and peak area obtained. Different mobile phases were tried but satisfactory separation, well resolved and good symmetrical peaks were obtained with the mobile phase methanol: acetonitrile: OPA (10:65:25, v/v/v). The retention time of Lincomycin was found to be 8.8 min, which indicates a good base line

Figure 3: HPLC chromatogram of Lincomycin



The number of theoretical plates was found to be 31631.3, which indicates efficient performance of the column. The asymmetric factor was found to be 1.25, which indicates asymmetric nature of the peak. The calibration curve for Lincomycin was obtained by plotting the peak area ratio versus the concentration of Lincomycin and internal standard area over the range of 0.5-2.5ppm, and it was found to be linear with r^2=0.998. The regression equation of Lincomycin concentration over its peak area ratio was found to be y = 1.556 + 0.0476 x, where x is the concentration of Lincomycin (ppm) and Y is the respective peak area. The data of regression analysis of the calibration curve was shown in table.1. The RSD values for accuracy and precision studies obtained were less than 2% which revealed that developed method was accurate and precise. The limit of detection and limit of quantitation for Lincomycin was found to be 0.05ppm and 0.15ppm, indicates the sensitivity of the method. The system suitability and validation parameters were given in table.2. The high percentage of recovery of Lincomycin was found to be 101.307% indicates that the proposed method is highly accurate. Proposed liquid chromatographic method was applied for the determination of Lincomycin in tablet formulation. The result for Lincomycin was comparable with a corresponding labeled amount (Table.3). The absence of additional peaks indicates no interference of the excipients used in the tablets.

Table 1: Regression analysis of the calibration curve

Parameters	Values
Calibration range (ppm)	0.5-2.5
Slope	1.556
Intercept	0.0476
Correlation coefficient (r^2)	0.998

Table 2: System suitability and validation parameters

Parameters	Results
Theoretical plates (N)	31631.3
Retention time (min)	8.8
Asymmetric factor	1.25
LOD (ppm)	0.05
LOQ (ppm)	0.15
Accuracy (%)	101.3
R.S.D. (%)	0.886

Table 3: Assay results of tablet formulation

Formulation	Labelled claim (mg)	% of Lincomycin in Tablet
Lincocin 500mg	500	55.38%

CONCLUSION

Proposed study describes new LC method for the estimation of Lincomycin in tablet formulation and serum. The method was validated and found to be simple, sensitive, accurate and precise. Percentage of recovery shows that the method is free from interference of the excipients used in the formulation. The proposed method can be used for routine analysis of estimation of Lincomycin in its tablet formulation and serum. We acknowledge with profound thanks, the gift sample received from M/s Wallace pharmaceuticals Ltd., Goa.

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