ABSTRACT

A simple, precise and accurate difference spectroscopic method has been developed for the estimation of lincomycin hydrochloride (LMH) in bulk and pharmaceutical dosage form. The proposed method is based on the measurement of absorbance of Lincomycin hydrochloride at maxima 209 nm and minima 220 nm. The measurement value is the amplitude of maxima and minima between two equimolar solutions of the analyte in different chemical forms, which exhibit different spectral characteristics. Since the drug was freely soluble in distilled water, a stock solution (1mg/ml) was prepared with distilled water. Further dilution was made by using 0.1N sodium hydroxide and phosphate buffer of pH6.8 separately. The maxima and minima in the difference spectra of lincomycin hydrochloride were at 209 nm and 220 nm, respectively. Difference in absorbance between these maxima and minima was calculated to find out the amplitude. This amplitude was plotted against concentrations. The drug obeyed the beer’s law in the concentration range 5-25μg/ml and showed good correlation. The result of analysis was validated by recovery studies.

Keywords: Lincomycin hydrochloride; difference spectroscopy; pharmaceutical dosage forms.

INTRODUCTION

Lincomycin hydrochloride is systemic antibiotic, belongs to the group of lincosamides, which is active against most common gram positive bacteria. It inhibits cell growth and microbial protein synthesis, by interacting strongly and specifically with the 50 S ribosomal subunit, at mutually related sites. Lincomycin hydrochloride mainly consists of methyl 6-amino-6,8-dideoxy-N-[(2S,4R)-1-methyl-4-propylprolyl]-1-thio-D-erythro-α-galacto-octopyranoside hydrochloride monohydrate, an antimicrobial substance produced by Streptomyces lincolnensis var. lincolnensis or by any other means. It has approved to be an excellent antibiotic for the treatment of infectious disease like acne, anthrax, pneumonia and also for the treatment of furunculosis, carbuncles, impetigo, burns and wounds.

It is official in Indian Pharmacopeia, British Pharmacopoeia, United States Pharmacopeia and The National Formulary. A few analytical method have been reported for its quantitative estimation in pharmaceutical formulations like liquid chromatography with pulsed electrochemical detection, UV spectrophotometry, Colorimetry, and atomic absorption spectroscopy. In view of the above fact, some rapid and sensitive analytical methods are in need for its quantitative estimation.

METHODS

Shimadzu UV-1700UV/VIS Spectrophotometer with 1 cm matched quartz cells was used for spectral and absorbance measurements. As this drug has no marketed tablet formulations yet, we have formulated the tablets by varying the ratio of polymers using most commonly used excipients by keeping the strength as constant (750mg of LMH) and analysed the drug. Freshly prepared 0.1 N NaOH and phosphate buffer of pH6.8 and distilled water were used in the present study.

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PREPARATION OF LINCOMYCIN HYDROCHLORIDE

Preparation of standard stock solution

100mg of lincomycin hydrochloride was weighed accurately and dissolved in distilled water made up the volume to 100ml in a volumetric flask. Further the solution was diluted with 0.1 N NaOH separately and phosphate buffer of pH6.8 separately to get the concentration of 100 µg/ml (working standards). Different aliquots were taken from working standards and diluted with 0.1N NaOH and phosphate buffer pH6.8 respectively to prepare a series of concentrations from 5-25 µg/ml as reference and test solutions, respectively. Difference spectrum was recorded by placing lincomycin hydrochloride in 0.1 N NaOH in reference cell and phosphate buffer pH6.8 in a sample cell.

The difference in the absorbance between 209 nm and 220 nm was calculated to find out the amplitude. The calibration curve was prepared by plotting amplitude versus concentrations.

Preparation of standard stock solution of tablet formulation

Twenty tablets of lincomycin hydrochloride containing 750 mg were weighed and their average net weight was calculated. The tablets were triturated to get a fine powder equivalent to 100mg of lincomycin was weighed and transferred into 50 ml volumetric flask. Dissolved in distilled water and made up to volume with the same. The solution was filtered through Whatman filter paper No.41 from the stock solution, 10µg/ml solution was prepared separately by using 0.1 N NaOH and phosphate buffer pH6.8. The amplitude was calculated by measuring the absorbance of the equimolar concentrations at maxima and minima in the difference spectrum. The amount of lincomycin was calculated. The procedure was repeated for six times to perform precision.

Recovery studies

The accuracy of the proposed method was examined by determining the recovery of the drug by standard addition technique. To the preanalysed formulation, a known amount of lincomycin hydrochloride raw material was added in different concentration viz., 25%, 50%, 75%, 100% and 125% in both the reference and sample solutions. The procedure was repeated as per the analysis of formulation. The amplitude was calculated and the amount of lincomycin hydrochloride recovered was determined. This was repeated for six times.

RESULTS AND DISCUSSION

A simple, precise, accurate difference spectrophotometric method has been developed for the estimation of lincomycin hydrochloride in pure form and in tablet formulation. In this study, the measured value is the difference in absorbance (ΔA) between two equimolar solutions of the analyte in different chemical forms which exhibit different spectral characteristics. The different spectrum of LMH in 0.1N NaOH was recorded by taking Lincomycin hydrochloride in phosphate buffer pH 6.8 solutions as blank. The difference spectrum showed that the maxima at 209 nm and minima at 220 nm (Fig.1).

In alkaline solution, drug shows more intense peak than acidic peak. Therefore ΔA is positive. Six point calibration graphs were constructed covering a concentration range 5-25 µg/ml. Six independent determinations were performed at each concentration. Linear relationship between amplitude of maxima and minima of difference spectra versus the corresponding drug concentrations were observed (Fig.2). The standard deviation of the slope and intercept were low. The correlation co-efficient (r²) exceeded 0.9998.

The regression equation for the method was found to be \( y = 0.05768x + 0.0092 \) and found to be linear over beer’s range 5-25 µg/ml respectively. The molar absorptivity (lit/mole.cm) was found to be 1.0034x10⁴ (Table 1).
LOD and LOQ were calculated for the sensitivity of the method. They were quantified based on the signal to noise ratio. The LOD is lowest detectable concentration of the analyte by the method and while LOQ is the minimum quantifiable concentration. LOD and LOQ were calculated according to ICH guidelines and shown in (Table 1).

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\text{LOD} = 3 \times \frac{\text{SD}}{\text{slope}}
\]

\[
\text{LOQ} = 10 \times \frac{\text{SD}}{\text{slope}}
\]

The repeatability study (n=6) was carried out and the amount of Lincomycin hydrochloride was found to be 99.23±0.239 with %RSD value of 0.242. It showed that the method was precise and equipment used for the study worked correctly for the developed analytical method and being highly repetitive (Table 2). The intra-day and inter-day studies precision were expressed as % relative standard deviation and found to be 0.375 and 0.398 respectively (Table 3). All the formulations contained excipients, lubricating agents and binders which were added along with the active drug constituents was determined by accuracy. The data for accuracy expressed in terms of percentage recovery of Lincomycin hydrochloride in the real samples were within the range of 99.39% and 99.82% mean %RSD was 0.3292, satisfying the acceptance criteria for the study (Table 4). All the above validation parameters were performed as per ICH guidelines.

**CONCLUSION**

The proposed method is simple, accurate, precise and selective for the estimation of Lincomycin hydrochloride in bulk and tablet dosage forms. The method is economical, rapid and do not require any sophisticated instrument. Hence it can be effectively applied for the routine analysis of Lincomycin hydrochloride in bulk and tablet dosage forms.

**ACKNOWLEDGEMENT**

The authors wish to thank Department of Pharmaceutical Technology, Anna University Tiruchirappalli and Karuna College of Pharmacy, Thirumittacode, Palakkad (Kerala), India for providing necessary facilities and to carry out the work. The authors wish to express their gratitude to Wallace Pharmaceutical Pvt. Ltd, Goa for providing the authentic sample (Lincomycin Hydrochloride).

**REFERENCES**


