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# Role of UV-Visible Spectrophotometry in the Determination of Lincomycin Hydrochloride in Bulk and Tablet Dosage Forms

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**Abstract: Objective:** A simple and sensitive visible spectrophotometric method has been developed for the quantitative estimation of Lincomycin hydrochloride in bulk and pharmaceutical dosage forms. **Method:**This method is based on the reaction of Lincomycin hydrochloride with 1 ml of 1% w/v 3-methyl 1, 2 benzthiazolinehydrazide hydrochloride reagent (MBTH reagent) in presence 1% w/v ferric chloride solution, to yield a green colour. This colour has a characteristic light absorption in the visible region, with absorption maximum 463nm. This method is validated for their linearity, accuracy and precision, recovery and ruggedness according to the ICH guidelines.
**Results:**The methods obey beers law in the concentration range of 1 to 5 µg/ml, respectively. The limit of detection (LOD) and the limit of quantitation (LOQ) of the methods varied from0.301 to 0.293.The intra and inter batch accuracy (%recovery) and precision (%RSD) ranged from 0.811 to 0.705 and 0.258 to 0.854 respectively.
**Conclusion:**The proposed method is applied to pharmaceutical formulation with acceptable accuracy and precision without any interference from commonly used excipients and additives. The results obtained were statistically validated and found to be reproducible.
**Key words:** Lincomycin hydrochloride, 3-methyl 1, 2 benzthiazolinehydrazide hydrochloride reagent, Ferric chloride, Spectrophotometric method.

## Introduction

Methyl 6,8 – dideoxy -[(2S,4R)-1-methyl -4-propyl pyrrolidine-2-carboxamido]-1-thio-D-erythro-α-D-galacto-octopyranoside monohydrochloride, monohydrate, an antimicrobial substances produced by other means[1]. LMH is a systemic antibiotic, belongs to the group of lincosamide, which is active against most common gram positive bacteria. LMH inhibit cell growth and microbial protein synthesis, by interacting subunit, at mutually related sites. It has approved to be excellent for infectious disease like acne, anthrax, pneumonia and also for the treatment of furunculosis, carbuncles, impetigo, burns and wounds[2-3]. It is an orally effective antibiotic[4].

It is official India and British pharmacopoeia. A few analytical method have been reported for its quantitative estimation in pharmaceutical formulations which includes biological fluids using HPLC , UV spectrophotometry, liquid chromatography and electrophoresis methods[5-10] .In view of the above fact, some rapid and sensitive analytical methods are in need its quantitative estimation.

The present work aims to develop a simple, precise, accurate and validated visible spectroscopic method for the estimation of LMH in bulk and pharmaceutical dosage form.

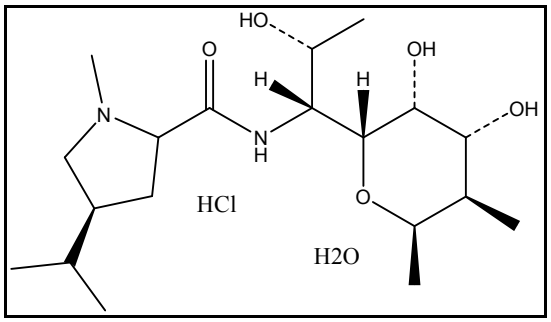



**Fig.1.Structure of lincomycin hydrochloride**

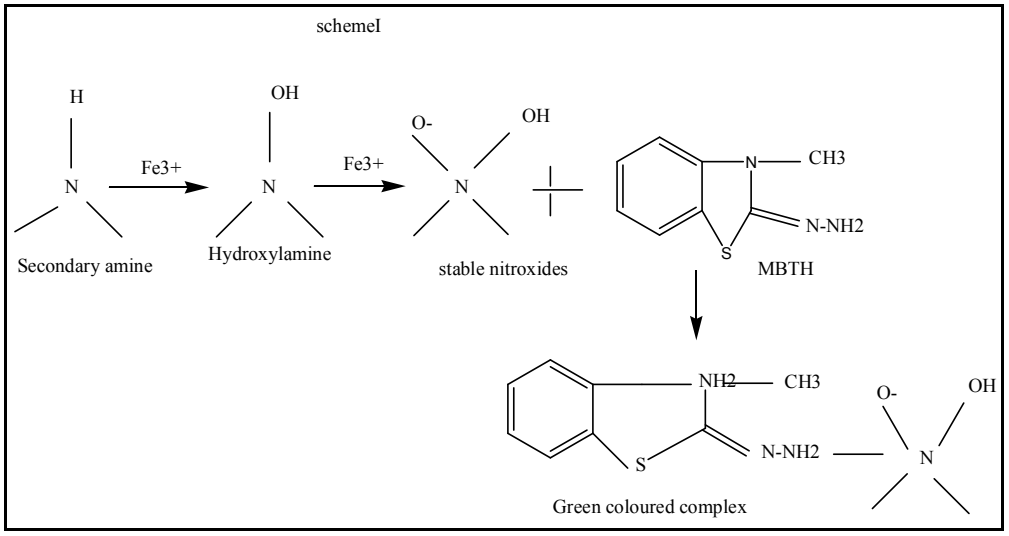
## Experimental

Shimadzu UV-1700UV/VIS Spectrophotometer with 1 cm matched quartz cells was used for spectral measurements. Whatmann filter paper No: 41 was used to filter the solution. Ultrasonicator was used in the initial steps of extraction. All the chemicals used were of analytical grade procured from Lobo chemicals, Mumbai. Aqueous solution of MBTH (1%w/v) and ferric chloride (1%w/v) was prepared freshly. The pharmaceutical grade of LMH was supplied as a gift sample by Wallace pharmaceutical Pvt. Ltd. As this drug has no marketed formulation yet, we have formulated tablets by varying the ratio of polymers with most commonly used excipients of label claim as 750 mg.

This method is based on the reaction of LMH with 3-methyl-2-benzothiazolinone hydrazone hydrochloride(MBTH) reagent and ferric chloride to form green colored chromogen (scheme1) with aborption maximum at 423nm and Beer's law is obeyed in the concentration range of 1 to 5 µg/ml.

It known that secondary amine undergoes oxidation to give hydroxylamine which converts into stable nitroxides to give a green colour complex with MBTH reagent[6-9].The probable reactions involved in this method have been shown in scheme I.

**Reaction:**




**Preparation of standard and sample solutions:**

About 100 mg of LMH pure drug was accurately weighed and dissolved in water and volume made up to 100mL with water (1 mg/ml).The final concentration of LMH was made to 100mcg/ml with distilled water. For sample solutions, the tablets of LMH were accurately weighed and average weight per tablet was

determined. The tablets were powdered and powder equivalent to 100mg of drug was taken and treated in a similar manner that of standard.

**Preparation of calibration curve:**

Different aliquots of drug standard solution ranging from 1 to 5 ml (1ml=100µg/ml) were transferred separately into a series of 10ml volumetric flasks. To each, 1ml of 1%w/v MBTH and 1 ml 1%w/v ferric chloride solution was added and kept aside for 5min for the completion of reaction at the room temperature. The volumes were made up to the mark with distilled water. The absorbance of the resulting green chromogen was measured at λmax 423nm against the reagent blank. Calibration curve was prepared by plotting concentration versus absorbance and found to be linear in the concentration range of 1 to 5µg/ml. Similarly absorbance of sample solution was measured and amount of LMH was determined from standard calibration curve.

To test the accuracy and reproducibility of proposed methods recovery experiments were performed by adding known amount of drug to the preanalysed formulation and reanalyzing the mixture by proposed method[10,11].

**Assay method**

For formulation analysis, twenty tablets of LMH each containing 750mg were accurately weighed and powdered. The powder equivalent to 100mg LMH was weighed and dissolved in distilled water, sonicated and the filterate were taken further diluted with the water to get the concentration with in the linearity range of standard curve. To each, 1ml of 1%w/v MBTH and 1ml 1%w/v ferric chloride solution was added and kept aside for 5min for the completion of reaction at the room temperature. The volumes were made up to the mark with distilled water. The absorbance of the resulting green chromogen was measured at λmax 423nm against the reagent blank.

## Results and Discussion

The method approached gives novelty to the development procedure. Presence of nitro group in sample structure enables for reduction in the primary stage of reaction and then further reaction proceeds with MBTH[12-14]. Optical characteristics show results which obeys beers lamberts law limit and is summarized in Table 1. The regression analysis using method of least squares was made for the slope (b), intercept (a) and correlation (r) obtained from different concentrations and the results are shown in Table 1. The percent relative standard deviation and percent range of error (0.05 and 0.01 level of confidence limits) of LMH are presented in Table 1. The results showed that this method has reasonable precision. The results of analysis of tablet formulations are done at three levels recorded in Table 2. To study accuracy, reproducibility and precision of the method, recovery studies were carried out by adding known amount of pure drugs to the analyzed sample of tablet powder and mixture was reanalyzed for the drug content using the proposed method. The result of recovery of pure drug was found to be with in the limit and standard deviation is 0.694 calculated from statistical analysis which has been summarized in Table 2. Interference studies revealed that the common excipients and other additives are usually present in the tablet dosage forms and they did not interfere at their regularly added levels.

**Table-1 Optical Characteristics and Precision**

| Parameters | Method |
|---|---|
| λmax (nm) | 463 |
| Beer's law limits (mcg/mL) | 1-5 |
| Molar absorptivity ($L/mol^{-1}.cm^{-1}$) | $2.6836 \times 10^4$ |
| Sandell's sensitivity ($mcg/mL/cm^2$/0.001 absorbance unit) | 0.0137 |
| Limit of detection | 0.293 |
| Limit of quantitation | 0.301 |
| Accuracy | 99.6±0.81 |
| | |
| Regression equation (Y*) | |
| Slope (b) | 0.0725 |

| | |
|---|---|
| Intercept (a) | 0.00329 |
| Correlation corfficient (r) | 0.99984 |
| Precsion (% RSD)**<br>Interday<br>Intraday | <br>0.258<br>0.854 |
| Range of errors** | |
| Confidence limits with 0.05 level | 0.00771 |
| Confidence limits with 0.01 level | 0.00632 |

Y=bC + a

*Where C is the concentration of Lincomycin hydrochloride in mcg/mL and Y is the absorbance at the respective* $\lambda_{max}$, ***for six measurements*

**Table-2 Evaluation of Lincomycin Hydrochloride in Pharmaceutical Dosage Forms**

| Formulation | Labelled amount(mg/tab) | Amount obtained by proposed method | Percentage recovery** ±S.D |
|---|---|---|---|
| Tablet I | 750 | 749.98 | 99.99±0.103 |
| Tablet II | 750 | 748.79 | 99.84±0.329 |

***Average ±S.D. of six determinations*

## Conclusion

A new colorimetric method has been developed for the analysis of LMH and its tablet formulation. It has been shown above that the method is cost effective and less time consuming, accurate, reproducible, repeatable, linear, precise and selective, providing the reliability of the method. The scan results were very clear and obeys beers law to a certain extend, which enables rapid quantities of many samples in routine quality control. A minimal interference was observed from excipient. These results show the method could find practical application as a quality control tool for analysis of LMH from their different pharmaceutical dosage forms in quality control laboratory.

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