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DEVELOPMENT OF DIFFERENCE SPECTROSCOPIC METHOD FOR THE ESTIMATION OF LINCOMYCIN HYDROCHLORIDE IN BULK AND PHARMACEUTICAL SOLID DOSAGE FORMS

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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 exhibits 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 5-25ì g/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[1]. Lincomycin hydrochloride mainly consists of methyl 6-amino-6,8-dideoxy-N-[(2S,4R)-1-methyl-4-propylprolyl]-1-thio-D-erythro-á-galacto-octopyranoside hydrochloride monohydrate,[2] 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[3].

It is official in Indian Pharmacopeia[2], British Pharmacopoeia[4], Unites States Pharmacopeia and The National Formulary[5]. A few analytical method have been reported for its quantitative estimation in pharmaceutical formulations like liquid chromatography with pulsed electrochemical detection[6], UV spectrophotometry[7], Colorimetry[8], and atomic absorption spectroscopy[9] .In view of the above fact, some rapid and sensitive analytical methods are in need for its quantitative estimation.

In view of the above, some simple analytical methods are in need for its quantitative estimation. Hence the present work aims to develop a simple, precise, accurate and validated difference spectroscopic method for the estimation of lincomycin hydrochloride in bulk and tablet dosage form.

MATERIALS AND METHODS

Materials

Lincomycin hydrochloride was a gift sample from Wallace Pharmaceutical Pvt. Ltd, Goa. Sodium hydroxide, Potassium-di-hydrogen phosphate used was of AR grade procured from Hi media chemicals, Mumbai.

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 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 (ÄA) between two equimolar solutions of the analyte in different chemical forms which exhibit different spectral characteristics[10]. 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).

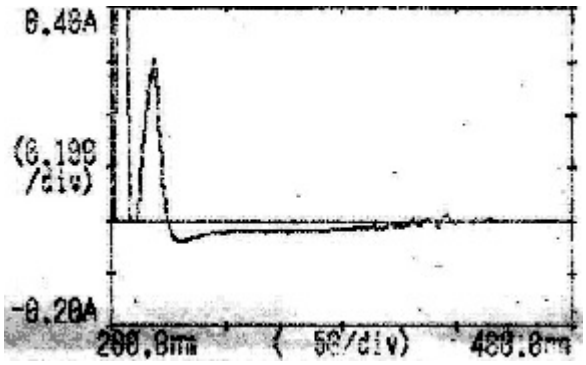

Fig.1: The difference spectrum showed that the maxima at 209 and minima at 220 nm.

In alkaline solution, drug shows more intense peak than acidic peak. Therefore ÄA 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 (r^2) exceeded 0.9998.

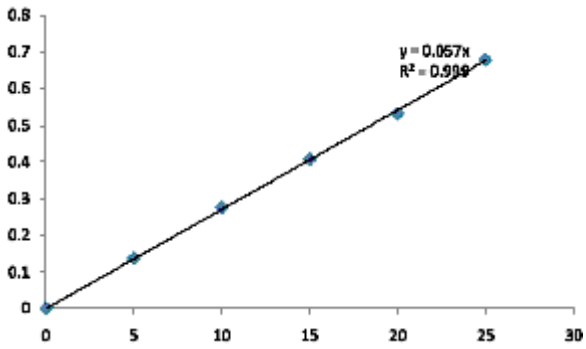

Fig.2:*Calibration plot for Lincomycin Hydrochloride*

The regression equation for the method was found to be y= 0.05768x +0.0092 and found to be linear over beer's range 5-25µg/ml respectively. The molar absorptivity (lit/mol.cm) was found to be $1.0034x10^4$ (Table 1).

Table 1: Optical characteristics of difference spectrophotometric method

Parameters	Difference spectroscopic method
λmax (nm)	209[a], 220[b]
Beer's law limit (µg/ml)	5 – 25
Molar absorptivity (L mol^{-1} cm^{-1})	1.0034×10^{4}
Correlation coefficient(r)	0.99986
% Relative standard deviation, n=6	0.2651
Regression equation (y = mx+c)	Y= 0.05768X +0.0092
Slope (m)	0.05768
Intercept (c)	0.0092
LOD (µg/ml)	0.1325
LOQ (µg/ml)	0.4014

a and b are the maxima and minima ,respectively.

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).

LOD = 3 X SD/slope
LOQ = 10 X SD/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[11,12].

Table 2: *Analysis of tablet formulation*

S. No	Labeled amount mg/tablet	Amount found, mg	% Label claim	Average	S.D	%RSD	S. E
1	750	744.95	99.33				
2	750	745.12	99.35				
3	750	742.34	98.98	99.23%	0.239	0.2425	0.211
4	750	742.32	98.98				
5	750	743.55	99.14				
6	750	746.92	99.59				

SD=Standard deviation, RSD =Relative standard deviation, S.E=Standard error

Table 3: *Intraday and Interday analysis of formulation**

S.No	% Label claim		%RSD	
	Intra day	Interday	Intraday	Interday
1	99.53	98.23		
2	99.92	98.14	0.375	0.398
3	99.17	98.87		

**n=3, RSD-Relative standard deviation*

Table 4: *Recovery Studies**

S.No	Amount present mcg/ml	Amount added mcg/ml	Amount found mcg/ml	Amount recovered mcg/ml	Percentage recovery	S.D	RSD	S.E
1	5.1475	2.5845	7.608	2.561	99.39			
2	5.1475	5.1692	10.503	5.150	99.63			
3	5.1475	7.7538	12.929	7.74	99.82	0.329	0.3292	0.169
4	5.1475	10.3384	15.236	10.29	99.54			
5	5.1475	12.923	18.044	12.89	99.77			

**n = 6, S.D =Standard deviation, S.E=Standard error*

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.

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REFERENCES

1. Goodman and Gilman's. The pharmacological basis of therapeutics, 10th edition, Mc-Graw Hill medical publish division, 2001, p.1256.
2. Indian Pharmacopoeia, Ghaziabad; The Indian Pharmacopoeia Commission, Delhi. Manager of Publication, 2010, Vol.2, p.1588.
3. Sweetman S.C.,editor. Martindale: The Complete Drug Reference, 33rd edition, London, Royal Pharmaceutical Society of Great Britain, The Pharmaceutical Press, 2002, p.219.2.
4. British Pharmacopoeia, Ministry of Health and Social Services for Northern Ireland, United Kingdom: The Stationary Office, 1988, Vol.1, p.334.
5. United States Pharmacopoeia: The National Formulary, Rockville, MD: United States Pharmacopoeia Convention, Inc.:1990, XXII, XVII, p.771.
6. Jszunyog. Analysis of a formulation containing Lincomycin and Spectinomycin by liquid chromatography with pulsed electrochemical detection.2002,29(1,2),p.213-220 Doi:10.1016/so731-7085(02)00015-8
7. Feng xue-zhong.,Wu Guang-huil.,Fang bing-huz,Li hua-pengi., Pan yuan-zhil and Wen zi-ming I.: Determination of Lincomycin hydrochloride Injection by UV-Spectrophotometry. DOI:CNKI: SUN:DYJZ:0-2009-12-017.

8. Egutkin N.L., Maidano V.V and Nikitin Yu.E.: Colorimetric determination of lincomycin in the form of a Pd complex. Pharmaceutical Chemistry Journal, 1984, 18(2), p.128-130, DOI: 10.1007/BF00758844.

9. EL Ries M.A. Spectrophotometric and indirect determination of lincomycin by atomic absorption spectroscopy.1994, 27(8), p. 1517-1531, DOI: 10.1080/00032719408006386.

10. Beckett A.H and Stenlake J.B. Practical Pharmaceutical Chemistry, 4th edition, part II, CBS Publishers: 2002, p. 293-296.

11. ICH, Q2 A, Validation of Analytical Procedure: Methodology International Conference on Harmonization, Geneva, October 1994.

12. ICH, Q2 B, Validation of analytical procedure: Methodology International Conference on Harmonization, Geneva, March 1996.