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VALIDATED SPECTROPHOMETRIC METHOD FOR THE ESTIMATION OF CLINDAMYCIN IN PHARMACEUTICAL FORMULATION VIA ION-PAIR REACTION USING METHYL ORANGE DYE

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ABSTRACT

In present work a simple, accurate, precise, reproducible, method was developed for the estimation of Clindamycin in bulk drug and pharmaceutical formulation by spectrophotometer. Spectrophotometric methods are described for determination of Clindamycin in bulk and pharmaceutical dosage forms using methyl orange as chromogenic agents. Calibration curves have correlation coefficients (r) 0.999 indicating good linearity over a concentration range of 10-50 µg/mL. The %RSD was less than 2%, showing high degree of precision of the proposed method. The methods were satisfactory applied for the determination of drugs in both bulk and pharmaceutical dosage forms.

Key words: Clindamycin, Methyl orange, Spectrophotometric method.

INTRODUCTION:

Lincosamide antibiotics are one of the classes of antibiotics most associated with pseudomembranous colitis caused by Clastridium difficile. Lincosamides prevent bacteria replicating by interfering with the synthesis of proteins. They bind to the 23s portion of the 50S subunit of bacterial ribosomes and cause premature dissociation of the peptidyl-tRNA from the ribosome (Tanel, T et al..2003).

Clindamycin chemically as (2S,4R)-N-{2-chloro-1-[(2R,3R,4S,5R,6R)-3,4,5 trihydroxy-6-(methylsulfanyl)oxan-2-yl]propyl}-1- ethyl-4-propylpyrrolidine-2-carboxamide (Fig.1) is a semi-synthetic derivative of lincomycin. It is synthesized from microbially fermented lincomycin by replacing a hydroxyl group at the 7-position of lincomycin by a chlorine group, that significantly increases its activity. The effect of clindamycin, which is primarily bacteriostatic, is exerted by its binding to the 50S ribosomal subunit and the consequent inhibition of bacterial protein synthesis (D.W. Boothe et al. 2001). It is active against aerobic Gram-positive and anaerobic bacteria, mycoplasmas and some protozoa. In companion animal medicine, clindamycin is

mainly used in the treatment of diseases like staphylococcal skin infections and osteomyelitis, periodontal disease, bacterial prostatitis, *toxoplasmosis*, and *neosporosis* (J.F. Prescott et al..2000) Several methods have been published for the determination of clindamycin in bulk drugs and formulations. Microbiological (L.W. Brown et al..1981) and Spectrophotometric (F.A. El-Yazbi et al..1993) assays both suffer from lack of specificity and accuracy. Gas–liquid chromatography (GLC) (Brown,1974and Oesterling,1970) compensates for the previous, but requires relatively complicated sample manipulation. HPLC methods (Brown, 1978, Landis et al..,1980, A. Hornedo-Nuñes et al..;1990, J.A. Orwa et al. 1999) are much more accurate and precise. Refractive index (L.W. Brown, 1978 and J.B. Landis et al..; 1980) electrochemical (A. Hornedo-Nuñes, 1990) and UV (J.B. Landis et al..;1980 and A. Hornedo-Nuñes et al..;1990, J.A. Orwa et al..1999), detection have been applied. UV detection seems to offer more, in terms of sensitivity and stability. Determination of clindamycin in biological samples has been performed by microbiological (Metzler et al..;1973.) RIA (Duckworth et al..;1993,), GLC (Gatti et al..;1993) HPLC/UV (La Follette et al..1988, C.-M. Liu et al.,1997; Fieger-Büschges et al., (1999) and HPLC/MS (L.-L. Yu et al..,1999; Lobenhoffer et al.,2001; Cherlet et al.,2002; G.N. Rechberger et al.,2003) methods. The aim of this study was to develop a sensitive and accurate spectrophotometric method for determination of clindamycin in raw material and pharmaceutical dosage forms through ion-pair complex formation between the drugs and methyl orange. The reaction conditions and the application of the method are presented. The constructed calibration curves were utilized in determining the concentration of these drugs in different pharmaceutical preparations available.

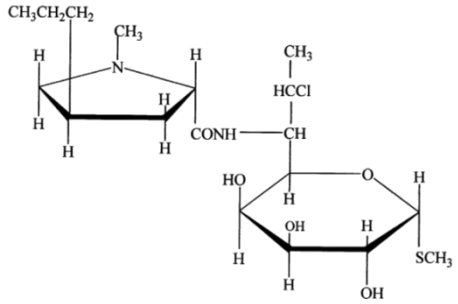

Fig. 1. Chemical structure of clindamycin.

MATERIALS AND METHODS

Chemicals and Reagents

Clindamycin was received as gift sample from IPCA Laboratories, Mumbai India. All solvents and reagents were of analytical grade. All the solutions were protected for light and were analyzed on the day of preparations. Triple distilled water was generated in house. Capsule, Dalacin C 150 mg was purchased from local market.

Methyl orange dye solution: 2% dye solution was prepared by dissolving 2g of Methyl orange in100ml of distilled water.

The present work was carried out on UV visible spectrophotometer. The absorption spectra of reference and test solution were carried out in a 1 cm quartz cell over the range of 200-800nm.

Preparation of calibration curve

The absorption maxima of Clindamycin were determined by running the spectrum of drug solution in double beam ultraviolet spectrophotometer. Accurately weighed 10 mg of clindamycin separately and dissolved in 10 ml of 0.1N HCL in 10 ml of volumetric flask

and prepared suitable dilution to make different concentration of standard with concentration range of 10-50µg/ml. 2 ml of standard drug solution and add 1 ml of methyl orange dye solution and 3 ml of chloroform, pipette out the coloured layer and analyzed for drug content by UV spectrophotometer at a λmax of 666 nm using of 0.1 N HCl as blank.

Assay of tablet formulation

Twenty capsules were taken and average weight of capsules was determined. The capsules were crushed in a mortar and the powder equivalent to 150 mg of drug was transferred to 100ml standard flask. The powder was dissolved in 50 ml of 0.1 N HCl and made up to volume with of 0.1 N HCL. The sample was mixed thoroughly and filtered through a 0.45µ membrane filter. The filtered solution was diluted suitably and add 1 ml of methyl orange dye solution and 3 ml of chloroform pipette out the coloured layer and analyzed for drug content by UV spectrophotometer at a λmax of 666 nm using of 0.1 N HCl as blank.

Method Validation

The developed method was validated as per ICH guideline(ICH,2005) with respect to linearity, precision, selectivity, recovery, accuracy and stability.

Linearity and construction of calibration curve

Solutions containing 10, 20, 30, 40 and 50µg / ml of clindamycin were prepared from standard solution to determine the linearity range. The detection was carried out at 666 nm. Spectrums were recorded and absorbance was recorded for all the concentrations. A calibration plot of concentration over the absorbance was constructed and was shown in Fig 2. The optical characteristics such as Beer's law limits, regression equation and correlation coefficient, mean absorbance value, and statistical data of the calibration curve were calculated and results are presented in Table 1 & 2.

Accuracy

The accuracy of the proposed methods was assessed by recovery studies at three different levels i.e. 80%, 100%, 120%. The recovery studies were carried out by adding known amount of standard solution of clindamycin to pre analysed tablet solutions. The resulting solutions were then reanalyzed by proposed methods. Whole analysis procedure was repeated to find out the recovery of the added drug sample. This recovery analysis was repeated at 3 replicate of 5 concentrations levels.

Precision

Precision of the methods was studied at three level as at repeatability, intermediate precision (Day to Day and analyst to analyst) and reproducibility. Repeatability was performed by analyzing same concentration of drugs for five times. Day to Day was performed by analyzing 5 different concentration of the drug for three days in a week.

Table 1: Readings for Linearity of clindamycin

Conc. (µg/ml) / Rep.	Absorbance at 666 nm					
	0	**10**	**20**	**30**	**40**	**50**
Replicate-1	0.00	0.108	0.218	0.322	0.424	0.530
Replicate-2	0.00	0.110	0.215	0.310	0.430	0.538
Replicate-3	0.00	0.104	0.210	0.316	0.421	0.532
Replicate-4	0.00	0.107	0.220	0.323	0.428	0.528
Replicate-5	0.00	0.109	0.217	0.318	0.423	0.531
Replicate-6	0.00	0.112	0.216	0.325	0.426	0.534
Mean	0.00	0.108	0.216	0.318	0.425	0.532
S.D.	0.00	0.003	0.003	0.006	0.003	0.004
% R.S.D.	0.00	2.49	1.57	1.72	0.77	0.65

Reading of 5concentrations and 5 replicate

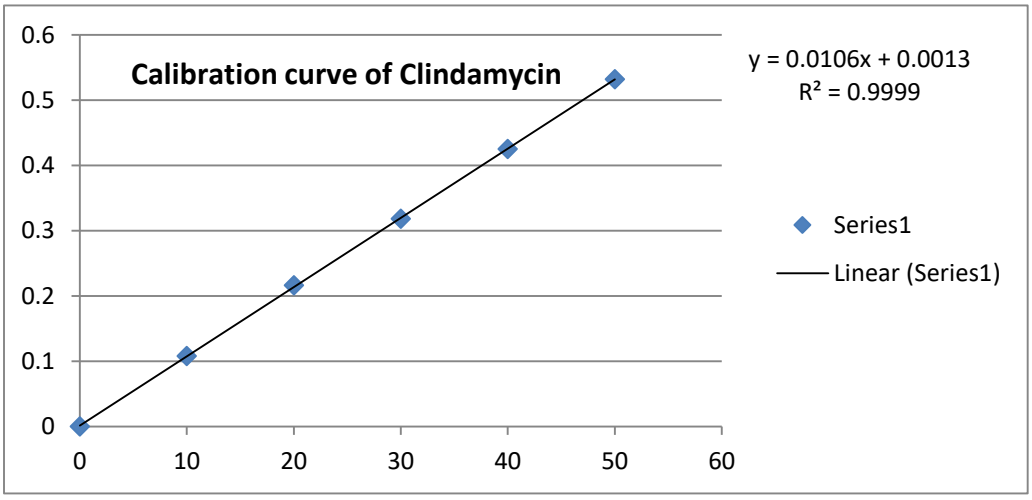

Figure 2: Calibration curve of clindamycin at 666nm

Table 2: Optical characteristics of the proposed method

Parameters	Results
Wavelength	666nm
Beer's law limit (µg/mL)	10-50
Regression equation (Y=mx+c)	y = 0.010x + 0.001
Slope (m)	0.010
Intercept(c)	0.001
Correlation Coeffient (r)	0.999

Table 3: Results of recovery studies on marketed formulations

Recovery level %	% Recovery (Mean±SD)*
80	98.34±0.146
100	99.14±0.134
120	99.21±0.234

*Average of five determination

Table 4: Results of Precision (% R.S.D.)

Parameter	(Mean±SD)*	% RSD
Repeatability	98.30±0.07	0.074
Day to Day	99.38±0.12	0.57
Analyst to Analyst	98.29±0.08	0.083
Reproducibility	99.03±0.21	1.20

*Average of five determination

RESULTS AND DISCUSSION

The proposed spectrophotometric methods are indirect and based on the determination of the clindamycin in marketed capsule formulation using methyl orange as chromogenic agents. Calibration curves have correlation coefficients (r) 0.999 indicating good linearity over a concentration range of 10-50 µg/mL. The regression characteristics were reported in Table 2.The accuracy of the methods was determined by investigating the recovery of drugs at concentration levels covering the specified range (five replicates of each concentration) Table 3. The %RSD was less than 2%, showing high degree of precision of the proposed method Table 4. The results of the method lie within the prescribed limit, showing that method is free from interference from excipients.

CONCLUSION

The proposed method is simple, precise, accurate and convenient. Therefore, it can be useful for routine analyses and quality control assay of the examined drugs in raw material and in capsules without fear of interference caused by the excipients expected to be present in capsules. This is for the first time that visible spectrophotometric method is being reported for the assay of clindamycin. The spectrophotometric method can be applied routinely because it does not require high cost reagents and equipment when it is compared with HPLC analysis.

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DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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