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Research Article

ESTIMATION OF CLOFAZIMINE IN CAPSULE DOSAGE FORM BY USING UV-VIS SPECTROSCOPY

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ABSTRACT

Objective: The present research work discusses the estimation of Clofazimine in capsule dosage form by using UV-VIS Spectroscopy.

Method: A simple, accurate, sensitive and precise Ultraviolet specrophotometric method has been developed for the determination of clofazimine in capsule dosage form. The solutions of standard and sample were prepared in benzene.

Results: In the UV specrophotometric method, the quantitative determination of the drug was carried at 452 nm and the linearity range was found to be 1-6 µg/ml. The calibration graphs constructed at their wavelength of determination were found to be linear for UV specrophotometric methods.

Conclusion: The proposed methods have been extensively validated statistically that included parameters such as linearity, accuracy, precision, LOD, LOQ, recovery and robustness. There was no significant difference between the performance of the proposed method regarding the mean values and standard deviations. The described methods can be readily utilized for analysis of pharmaceutical formulation.

Keywords: Method development; Validation; Clofazimine.

INTRODUCTION

Clofazimine is chemically 3-(4-chloroanilino)-10-(4-chlorophenyl)-2,10- dihydro-2 (isopropylimino) phenazine. Its molecular formula is $C_{27}H_{22}Cl_2N_4$ with molecular weight of 473.4. The tissue half-life after a single dose has been reported to be about 10 days; that after multiple oral doses has been variously estimated to be between 25 and 90 days [1]. Clofazimine is a reddish-brown powder. It is readily soluble in benzene; soluble in chloroform; poorly soluble in acetone and in ethyl acetate; sparingly soluble in methanol and in ethanol; and virtually insoluble in water [2, 3, 4, 6]. Clofazimine is absorbed from the gastrointestinal tract in amounts varying from 45 to 62%. Absorption is greatest when clofazimine is given in microcrystalline formulations and when it is taken immediately after food [4]. Clofazimine, a lipophilic riminophenazine antibiotic, possesses both antimycobacterial and anti-inflammatory activities. Clofazimine is mainly indicated in the treatment of lepromatous leprosy, including dapsone-resistant lepromatous leprosy and lepromatous leprosy complicated by erythema nodosum leprosum, and has been included as an anti-leprosy medicine in the current WHO Model Lists of Essential Medicines for adults and children [5]. The drug has been shown to bind to cytosine - guanine DNA base pairs in vitro. The binding is specific for guanine residues only. The DNA of *Mycobacterium Zeprue* has a high guanine - cytosine content, consequently this binding may disrupt the template function of the DNA, causing inhibition of protein synthesis. [6]. It is official in Indian Pharmacopoeia [7].

Review of literature suggest that very few [6,8] UV-Vis spectrophotometric and high performance liquid chromatographic method have been developed for the analysis of Clofazimine. Hence present research work has been done to develop a simple and robust UV-VIS spectrophotometric method and validation for the quantification of Clofazimine in bulk fluids and capsules.

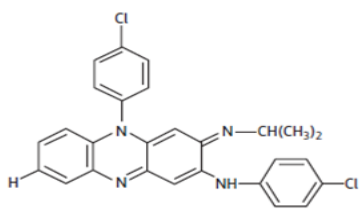

Fig. 1: Chemical structure of clofazimine

MATERIALS AND METHODS

Instrumentation

Analysis carried out on Lab India UV-Vis spectrophotometer (200-800 nm), a double beam high speed scanning spectrophotometer (200-800) with a photomultiplier tube detector and having variable spectral bandwidth (0.5-5.0 nm).

Chemical and reagents

All chemical used were of A.R grade. clofazimine and market formulation CLOFROS 100 mg was procured from Sangrose Labratories Pvt Ltd. Kerela as a gift sample. Benzene was procured from Himedia, Labratories Pvt. Ltd, Mumbai

Method (Development of simple spectroscopic method)

Standard stock solution

10 mg of clofazimine was accurately weighed and transferred to 10 ml volumetric flask. To this benzene was added to dissolve the drug and the volume was made up to 10 ml with the benzene. The concentration of this resulting solution was 1000 µg/ml. 5 ml was taken from this solution and transferred to 50 ml volumetric flask and volume was made upto the mark with benzene (100 µg/ml) to produce final standard stock solution.

Sample preparation

The content of 20 capsules CLOFROS 100 mg were mixed and the average weight of one capsule was caliculated. The contents of capsules were taken equivalent to 10 mg of clofazimine in to a 100ml volumetric flask. The formulation was first dissolved in benzene (25 ml) and sonicated for about 10-15 min and finally the volume made up to the mark with benzene. The solution was filtered and final dilution of the sample (6µg/ml) was prepared and measured the absorbance against blank at 452nm. The amount of clofazimine was computed by using the equation referring to the calibration curve. (Table 3)

Recovery study

To check the accuracy of the developed method recovery study was carried out as per ICH norms. Where to a reanalyzed sample solution, standard solutions of all the two drugs were added equivalent to 80, 100 and 120% of its drug content. Recovery study was carried by doing replicate study. (Table 4)

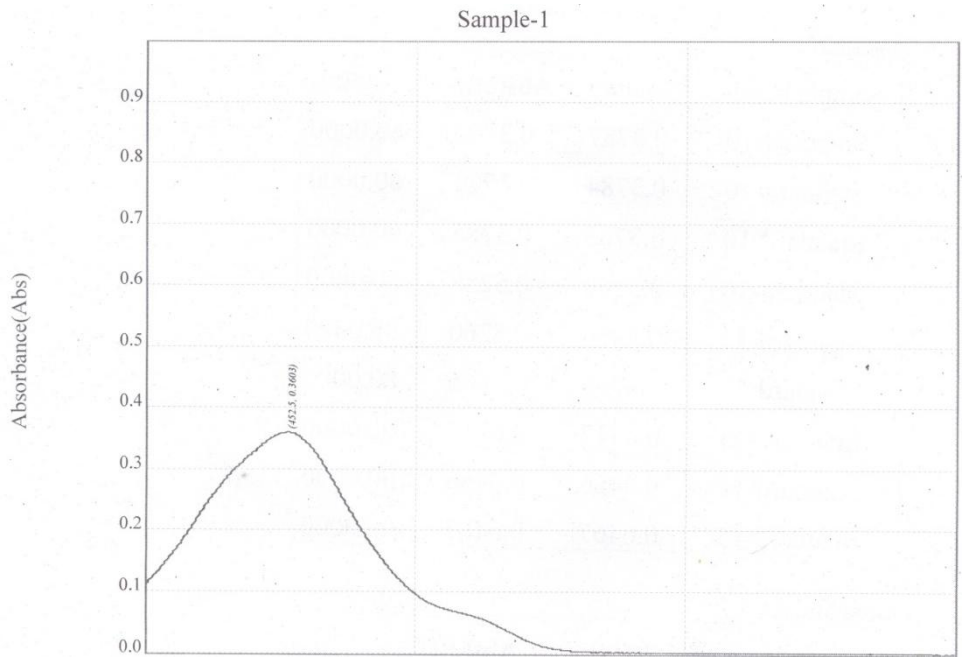

Fig. 2: Overline spectra of clofazimine

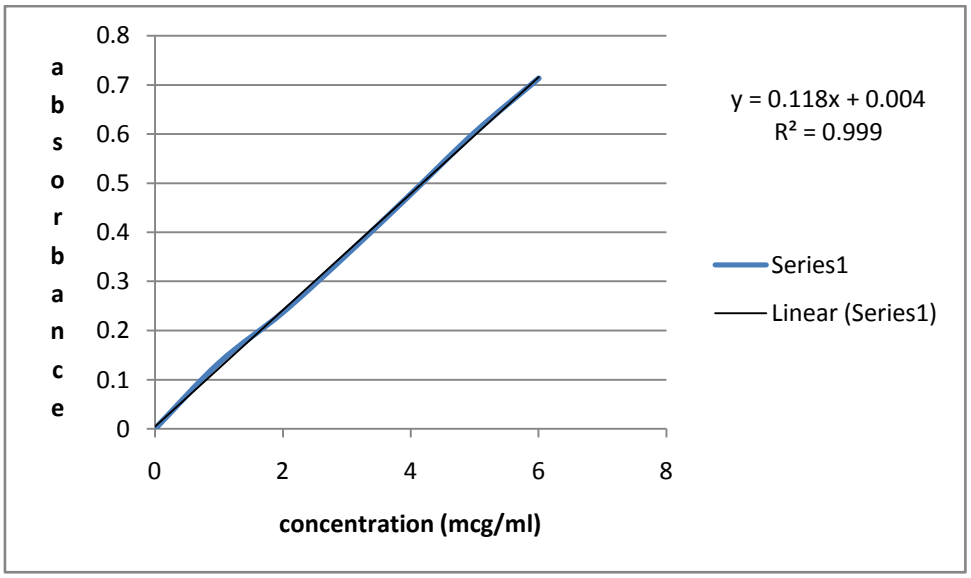

Fig. 3: Calibration curve for clofazimine

Method validation

The analytical method was validated with respect to parameters such as linearity, limit of detection (LOD), limit of quantitation (LOQ), precision, accuracy, robustness, and recovery. (ICH Q2R1 2003)

Linearity

Linearity is established by least squares linear regression analysis of the calibration curve. The constructed calibration curve is linear over the concentration range 1-6 µg/ml. (Table 1, 4)

Accuracy

Accuracy was studied by adding two different amounts (corresponding to 80%, 100% and 120% of the test preparation concentrations) of clofazimine to the placebo preparation and comparing the actual and measured concentrations. For each level, three solutions were prepared and each was used in duplicate (Table 4)

Precision

The precision of the method, as intra-day repeatability was evaluated by performing six independent assays of the test sample preparation and calculating the standard deviation. The intermediate (interday) precision of the method was checked by performing same procedure on different days by another person under the same experimental conditions. (Table 4)

LOD and LOQ

The LOD and LOQ of clofazimine were calculated by Mathematical equation.

$$LOD = \frac{3.3 \times \text{standard deviation } (\sigma)}{\text{slope}}$$

$$LOQ = \frac{10 \times \text{standard deviation } (\sigma)}{\text{slope}}$$

Robustness

Robustness of proposed method was performed by changing UV analyst and the remaining conditions (solvent, dilution, UV Spectrophotometer) were same. (Table 4)

RESULTS AND DISCUSSION

In UV spectroscopic method, the spectra were utilized for developing the equations for analysis. and linearity data (Table 1) it was found to that clofazimine obeys beer's law in the range of 1-6 µg / ml. Clofazimine showed maximum absorbance at 452 nm in benzene (Figure 1) with a good correlation coefficient 0.999 (Table 2)and calibration curve was shown in Figure 3. The percentage purity and relative standard deviation from the assay of the capsule dosage forms (Table 3) were found to be within the limits. The accuracy data of the drug (Table 4) was shown good percentage recovery and Standard deviation within the range of 98.36 to 99.45 respectively. The Inter-day and Intra-day(Table 4) precision values were found to be 100.26±0.0004, 99.09±0.0005 respectively, which indicates that the proposed method is accurate and also reveals that there is no interference of the commonly used excipients and additives in the formulation.

CONCLUSION

The proposed method for the estimation of clofazimine was found to be simple, sensitive and reliable with good precision and accuracy. The method is specific while estimating the commercial formulations without interference of excipients and other additives. Hence this method can be used for the routine analysis of clofazimine in pure and pharmaceutical formulations.

Table 1: Linearity study of Clofazimine

Conc. µg/ml	Dilution1	Dilution 2	Dilution3	Dilution4	Dilution5	Dilution6	Mean±SD
1	0.1365	0.1329	0.1378	0.1346	0.1310	0.1309	0.1339±0.0033
2	0.2346	0.2389	0.2308	0.2401	0.2406	0.2378	0.2371±0.0037
3	0.3578	0.3576	0.3521	0.3576	0.3532	0.3501	0.3547±0.0033
4	0.4780	0.4711	0.4781	0.4781	0.4776	0.4739	0.4776±0.0006
5	0.6029	0.5999	0.6021	0.6021	0.6078	0.6033	0.6030±0.0026
6	0.7144	0.7142	0.7111	0.7139	0.7126	0.7126	0.7131±0.0012

Table 2: Calibration curve for Clofazimine

S. No.	Parameter (Units)	Clofazimine
1	Linearity range (µg/ml)	1-6
2	Correlation coefficient (r^2)	0.999
3	Slope	0.118
4	Intercept	0.004

Table 3: Market formulation analysis

Formulation	Drug	Label claim (mg)	%Amount found±S.D
Capsule	Clofazimine	100mg	101.27±0.0019

Table 4: Validation parameters

S. No.	Validation parameter	Mean±S.D
1	Linearity	1-6µg/ml
2	Correlation coefficient r^2	0.999
3	Slope	0.118
4	Intercept	0.004
5	Precision	
	Interday 1st day	99.09±0.0005
	2nd day	100.7±0.0004
	3rd day	101.0±0.0003
	Intraday 1st hrs	98.65±0.0001
	2nd hrs	99.05±0.0006
	3rd hrs	99.57±0.0009
6	Recovery 80%	99.45±0.0004
	100%	98.92±0.0014
	120%	98.36±0.0004
7	LOD (µg/ml)	0.1016
8	LOQ (µg/ml)	0.3389
9	Robustness	99.49±0.0004

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