Original Article

SPECTROPHOTOMETRIC DETERMINATION OF AN ANTIMALARIAL DRUG CHLOROQUINE IN BULK AND PHARMACEUTICAL FORMULATIONS

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ABSTRACT

Objective: The aim of this paper was to develop the spectrophotometric method of an antimalarial drug chloroquine in bulk and pharmaceutical formulations.

Methods: A simple, sensitive and accurate spectrophotometric method have been developed for the determination of chloroquine in pure and dosage forms which is based on oxidation of the drug with Fe(III)-1, 10 phenanthroline complex producing orange colored chromogen which is measured at 510 nm. Different experimental parameters affecting the color development and stability of the colored product are carefully studied and optimized.

Results: Beer's law is obeyed in the concentration range of 20-320 µg/ml for the developed method. The molar absorptivity and sandell sensitivity are found to be 666.66 L mol^{-1} cm^{-1} and 0.77 µg/cm^2 respectively. The regression equation for chloroquine was found to be y = 0.0013X+0.005 and the correlation coefficient for the regression line were 0.999.

Conclusion: The developed method could be successfully applied for determination of chloroquine in pharmaceutical formulations. The results obtained are in good agreement with those obtained by using the standard method.

Keywords: Chloroquine, Spectrophotometry, Dosage forms, 1, 10-phenanthroline (O-PHEN), Fe (III).

INTRODUCTION

Chloroquine (CQ)

Chloroquine, chemically,7-chloro-4-(4-diethylamino-1-methyl butyl amino) quinoline, was originally synthesised in 1934 in Germany as Resochin. As the diphosphate and renamed chloroquine. It was extensively studied in America. It is soluble in water; but very slightly soluble in ethanol. It is practically insoluble in chloroform. The structural formula of chloroquine phosphate is as given in fig. I.
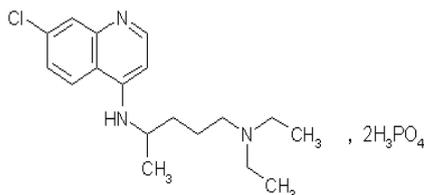
Uses

1. Chloroquine is the drug of choice for clinical cure and suppressive prophylaxis of all types of malaria, except that caused by resistant p. falciparum. It completely cures sensitive falciparum disease, but relapses in vivax and ovale malaria are not prevented, though interval between relapses may be increased.

2. Extra intestinal amoebiasis.

3. Rheumatoid arthritis.

4. Dicoid lupus erythematosus very effective; less valuable in systemic LE.

5. Lepra reactions.

6. Photogenic reactions.

7. Inectious mononucleosis; affords symptomatic relief. Doses In the suppression of malaria, 500 mg once weekly; in the treatment of malaria, 0.5 to 1.5 g daily. In the treatment of malaria, by intravenous or intra muscular injection; for an adult, the equivalent of 200 to 300 mg of chloroquine base. In the treatment of hypatic amoebiasis, 0.5 to 1.0 g daily in divided doses. Chloroquine phosphate tablet should not be chewed. Chloroquine base 150 mg 2 tablets BD. RESOCHIN, CLOQUIN, LARIAGO, NIVAQUINP 150 mg and 300 mg, 100 mg per 10 ml in oral susp., 40 mg/ml injections are available in pharmaceutical formulations.

Side effects

Toxicity of chloroquine is low, but side effects [1] are frequent and unpleasant: nausea, vomiting, anorexia, uncontrollable itching, epigastria pain, uneasiness difficulty in accommodation and headache. Suppressive doses have been safely given for 3 years. Parenteral administration can cause hypotension, cardiac depression, arrhythmias and CNS toxicity including convulsions (more likely in children).

Prolonged use of high doses may cause loss of vision due to retinal damage. Corneal deposits may also occur and graying of hair cans reversible on discontinuation. Only a few methods viz, HPLC, Spectro fluirimetry, electrophoresis, UV-visible spectrophotometry appeared in the literature for the determination of CQ in bulk and pharmaceutical formulations. There is a need for simple spectrophotometric method for the analysis of Chloroquine in pharmaceutical formulations.

The present method is simple, sensitive, accurate and economic. This method can be successfully applied for analysis of pharmaceutical formulations in any laboratory. No interference was observed in the analysis of Chloroquine from common excipients found in pharmaceutical formulation. The proposed method is economic when compared with HPLC methods

Experimental

Instrumentation

An ELICO SL-159 model, 2 nm high resolution, double beam, 1 cm length quartz coated optics; wavelength range 190-1100 nm; High stability, linearity, precision instrument is used for all the spectral measurements. All chemicals and reagents used in the analysis are of analytical grade and doubly distilled water is used for the preparation of all the solutions.

MATERIALS AND METHODS

Preparation of standard solution of drug

An accurately weighed 250 mg of chloroquine (CQ) is dissolved in 125 ml of ethanol. The final volume is adjusted to 250 ml with distilled water in the standard flask. One ml of this solution contains 1 mg/ml.

Preparation of reagents

0.241%(w/v) Fe (III) solution is prepared by dissolving 241 mg of anhydrous ferric ammonium sulphate in 100 ml of double distilled water, 0.991% (w/v) o-phenanthroline is prepared by dissolving 991 mg of the reagent in 100 ml of alcohol and 0.15% (v/v) O-phosphoric acid solution is prepared by diluting 0.15 mL of laboratory reagent (AR Grade) of o-phosphoric acid to 100 ml with distilled water.

Experimental procedure

Different portions (1.0-8.0 ml, 1000μg/ml) of standard CQ solution is delivered into a series of 25 ml calibrated standard flask and then 1.0 mL of $5.0x10^{-3}$M of Fe (III) solution, 1.0 ml of $5.0x10^{-2}$M o-phenanthroline are added successively. The total volume in each flask is brought to 16 ml with distilled water. The flasks are kept on a boiling water bath for 30 minutes. The flasks are removed and cooled to room temperature.2.0 ml of $2.0x10^{-2}$M of o-phosphoric acid is added and volume in each flask is made up to the mark with distilled water. The absorbance of the colored complex solution is measured after 5 minutes against a reagent blank prepared at 510 nm (fig. 1). The amount of the CQ is computed from the appropriate calibration graph (fig. 2).
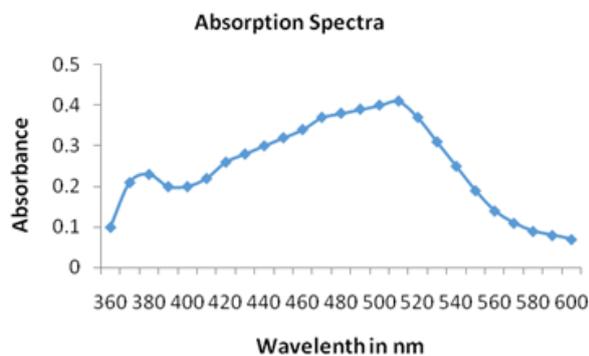

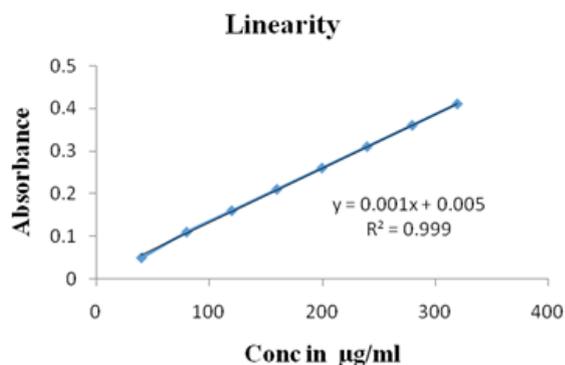Fig. 1: Absorption spectra of chloroquine with Fe (III)/O-PHEN

Fig. 2: Linear plot of chloroquine with Fe (III)/O-PHEN

Analysis of pharmaceutical sample

Tablets powdered equivalent to250 mg of the drug is weighed accurately and transferred into 250 ml beakers and shaken with 125 ml ethanol by following standard method. The standard solution is filtered into 250 ml standard flasks and volume is adjusted with 50%

ethanol. Suitable aliquots of this solution used for the determination of chloroquine contents by procedure describe earlier.

Optimization of experimental conditions

The optimization of experimental conditions is accomplished by sequentially optimizing one variable at a time while keeping all other variables constant. In this work, the influence of concentration of reagents (H_3PO_4 and 1, 10 Phenanthroline) and heating time required for maximum and stable color development was studied to obtain the optimum conditions. (fig. 3-fig. 5).
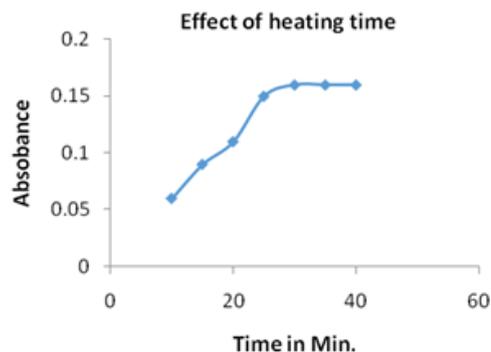

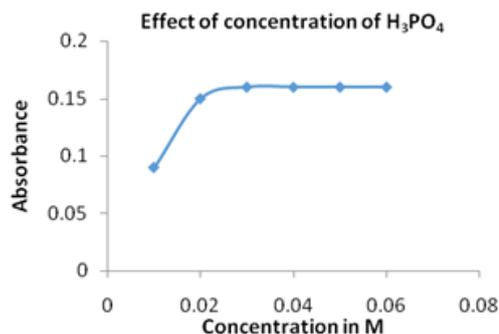Fig. 3: Effect of heating time on absorbance of developed system

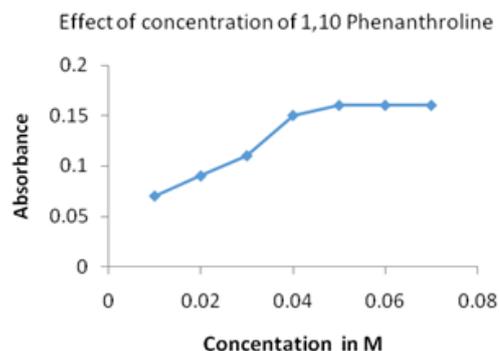Fig. 4: Effect of concentration of H_3PO_4 on colour development

Fig. 5: Effect of concentration of 1, 10 phenanthroline on absorbance of developed system

RESULTS AND DISCUSSION

In order to test whether the colored product formed in this method adhere to Beer's law, the absorbance at maximum wavelength of a series of eight concentrations are plotted against concentration of the drug in μg/mL (Fig2). Beer's law is obeyed within the limits 20-320 μg/mL of chloroquine, molar absorptivity and sandell sensitivity

is found to be 666.66L mol-1 cm -1 and 0.77 μg /cm2.Regression analysis of the Beer's law plots at λ max reveals a good correlation. The graphs show negligible intercept and are described by the regression equation y = 0.0013X + 0.005 (where Y is the absorbance of 1 cm layer, b is the slope, a is intercept and C is the concentration of the measured solution in μg/mL). The high molar absorptivity of the resulting colored complex indicates the high sensitivity of the method.

Precision of the developed method is ascertained from the absorbance values obtained by actual determination of ten replicates of a fixed amount of the test in total solution. The percent of relative standard deviation and Variation from mean at 95% level confidence limit percentage calculated for the developed method. To determine the accuracy of the method, three different amounts of drug sample within the linearity limits are prepared and analyzed by the developed method. The percent recoveries of the drug by this method is found to be within the range which indicates that the developed method is accurate. Optical characteristics, linear regression parameters, precision and accuracy of the proposed method is shown in Table-1. The method has been successfully applied for the determination of chloroquine in pharmaceutical preparations.

Table 1: Optical characteristics, Regression parameters, Precision and accuracy of the proposed method

Parameters [3-5]	Method
Maximum Wavelength λ_{max}	510 nm
Beer's Law Limits μg/ml	20-320
Sandell's Sensitivity (μg/cm^2/0.0001 Absorbance)	0.77
Molar Absorptivity Lt/mole/cm	666.66
Slope(b)^a	0.0013
Intercept(a)^a	0.005
Standard Deviation on intercept(S_a)	.0029
Standard Deviation on slope (S_b)	.0008
Correlation Coefficient (r)	0.999
Standard Deviation (S)	1.1067
%Relative Standard Deviation	1.1050
Variation from mean at 95% level confidence limit	±0.791
Limit of Detection (LOD)μg/ml	.1915
Limit of Quantification (LOQ)μg/ml	.5801

^{a}Regression equation Y=a+bC, Where Y stands for absorbance and C is concentration in μg/ml ^{b}% Relative standard deviation is calculated for ten determination

The proposed method has been used for the analysis of chloroquine. The result obtained is comparable with standard method [2] (table 2).

Table 2: Analysis of pharmaceutical formulations of chloroquine

Drug	Manufacturing company	Labelled amount(mg)	*Amount found by Proposed Method	*Amount found by Referrence method
Chloroquine phosphate tablet	IPCA Laboraories Ltd.	250	249.85	249.92

* Average of three determination
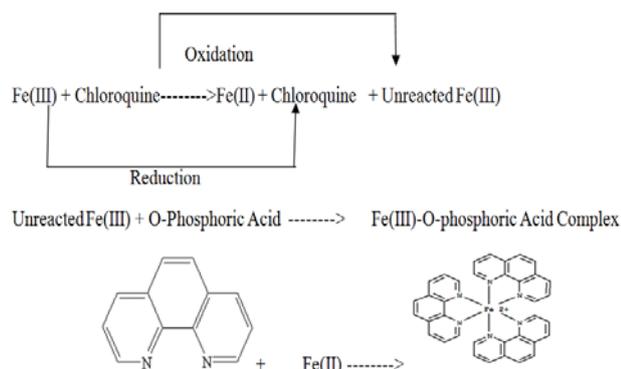
Scheme of coloured product

Ferric salt converts into a ferrous salt upon oxidation and can be easily detected by the usual reagent ophenanthroline. The reduction product is tris complex of Fe (II), well known as ferroin. The colored product of the reaction is given below [6].

CONCLUSION

The developed method is simple, sensitive, accurate and reproducible. This method can be successfully applied for the analysis of pharmaceutical formulations in any laboratory.

CONFLICT OF INTERESTS

Declared None

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