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Stability-indicating UV-spectrophotometric assay of chloroquine phosphate in pharmaceuticals

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ABSTRACT

Chloroquine phosphate (CQP) is a 4-aminoquinolein antimalarial drug. One spectrophotometric method which is cost-effective and rapid was described for the determination of CQP in bulk drug and dosage forms. This method is based on the measurement of the absorbance of CQP solution in 0.1 M HCl at 342 nm. Beer's law was obeyed over the concentration range, 2.5-25 µg mL^{-1} (r=0.9998) with limits of detection (LOD) and quantification (LOQ) values of 0.39 and 1.18 µg mL^{-1}, respectively. The apparent molar absorptivity was calculated to be 8.88×10^3 L mol^{-1} cm^{-1} and the Sandell sensitivity was 0.0401 µg cm^{-2}. Intraday and inter-day accuracy expressed as percent relative error was better than 1.5% and the corresponding precision reported as percent relative standard deviation (%RSD) was < 2%. Method, when applied to commercial tablets, yielded % found values in the range, 98.24 – 100.6% with an RSD of < 1.5%. Accuracy of the method was confirmed by recovery study *via* standard-addition procedure. The method was validated for selectivity, robustness and ruggedness also. The method was evaluated for its stability-indicating ability by forced degradation study. As a part of this, the drug was subjected to acid-, base-, peroxide-, heat- and light-induced stress conditions before recording the spectrum; and the drug was found undergo significant degradation under base hydrolysis and insignificant degradation in oxidative stress conditions, and remained stable to other conditions.

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INTRODUCTION

Chloroquine phosphate (CQP), chemically known as 7-chloro-4[{4-(diethylamino)-1-methylbutyl} amino] quinoline, is a 4-aminoquinolein antimalarial drug. It is the prototype synthetic antimalarial drug most widely used to treat all types of malarial infections. The drug is also prescribed to decrease the symptoms of rheumatoid arthritis and to treat systemic and discod lupus erythematosus in adults [1-3]. Determination of chloroquine concentrations in biological samples is important for several reasons, such as assessment of patient compliance, evaluation of pharmacokinetic data and prevention of toxic blood concentrations after prolonged use, especially in the case of treatment of rheumatoid arthritis. Reliable analysis methods are also required for quality control of chloroquine preparations.

A variety of analytical approaches have been employed to quantify chloroquine in bulk durg and formulations. Chloroquine (CLQ) is officially listed in the United states pharmacopoeia [4] which has adopted high performance liquid chromatographic method, where as British pharmacopoeia [5] has adopted a titrimetric procedure for the assay of drug in formulations. Other analytical methods reported for the assay of CLQ in pharmaceuticals include visible spectrophotometry [6-21], spectrofluorimetry [22], high performance liquid chromatography [23-29], titrimetry [30,31], gravimetry [32], membrane electrode-based potentiometry [33-36], flowinjection chemiluminescence spectrometry [37,38], and bio assay [39-41].

Despite its simplicity and cost-effectiveness, uv-spectrophotometry has been sparsely applied to determine CQP in pharmaceuticals. Two reported methods, which are based on difference absorptivity [42] and derivative [43] modes, are indirect and cumbersome besides being less sensitive with narrow linear dynamic ranges. In addition, none of the uv-spectrophotometric methods [42,43] is stability-indicating, which is mandatory according the current ICH guidelines.

Stress testing of a drug substance can help to identify the potential degradation products, which can inturn help to establish degradation path ways, and the intrinsic stability of the molecule, and to validate the stability-indicating ability of the analytical methods used. The current drug stability test guideline Q1A(R2) and photostability testing Q1B is issued by the international conference on harmonization (ICH) [44,45] suggest that stress studies should be carriedout on a drug to establish its inherent stability, leading to identification of degradation products and help in validation of analytical methods (ICH Q2 R1) to used in stability studies [46].

The aim of this study was, therefore, to develop a simple, rapid and direct uv-spectrophotometric method, which is stability-indicating, for the quantification of CQP in pharmaceuticals; and to study forced degradation of the drug under various stress conditions like acid- and base-hydrolysis, oxidation, photolysis and thermolysis, performed according to ICH guidelines.

EXPERIMENTAL

Apparatus

Shimadzu Pharmaspec 1700 uv/visible spectrophotometer provided with 1-cm quartz cells was used for absorbance measurement**.**

Reagents

All chemicals used were of analytical reagent grade. Doubly-distilled water was used to prepare solutions wherever required. Pharmaceutical grade CQP (certified to be 99.95% pure) was procured from Cipla India Ltd., Mumbai, India, and used as received. Cadiquin 200 mg (Zydus Cadila Healthcare Ltd., Bangalore), Maliago 500 mg (Cipla ltd, Bangalore) tablets, Cloquin 40 mg/mL injection (Indoco Remedies Ltd, Baddi, India) and Emquin 100 mg/10 mL suspension (Merck Ltd, Mumbai, India) were purchased from local market and chloroform (spectroscopic grade) was purchased from Merck, Mumbai, India.

Hydrochloric acid (5 M): Prepared by diluting concentrated acid (Merck, Mumbai, India, Sp. gr. 1.18) with water.

Hydrogen peroxide (H_2O_2, 5% v/v): Prepared by diluting 9 ml of the commercially available 30% reagent (Merck, Mumbai, India,) to 50 ml with water in a volumetric flask.

Sodium hydroxide solution (NaOH, 5 M): Prepared by dissolving required amount of pellets (Merck, Mumbai, India,) in water.

Standard drug solution

A stock standard solution of 100 µg ml^{-1} CQP was prepared by dissolving 10 mg of pure CQP in 0.1 M HCl and diluted to 100 ml with the same solvent in a calibrated flask.

General Procedures

Preparation of calibration curve

Into a series of 10 ml calibration flasks, aliquots of standard drug solution (0.25 to 2.5 ml of 100 µg ml^{-1}) equivalent to 2.5-25.0 µg ml^{-1} CQP were accurately transferred and the volume was made up to the mark with 0.1 M HCl. The absorbance of each solution was then measured at 342 nm against 0.1 M HCl as the blank.

A calibration curve was prepared by plotting the absorbance *versus* concentration of drug. The concentration of the unknown was computed from the regression equation derived using the Beer's law data.

Procedure for dosage forms

Tablets: Twenty tablets were weighed and pulverized. An amount of tablet powder containing 10 mg CQP was transferred into a 100 ml volumetric flask. The content was shaken well with about 60 ml of 0.1 M HCl for 20 min and the extract was diluted to the mark with the same solvent. It was filtered using Whatman No 42 filter paper. First 10 ml portion of the filtrate was discarded and a subsequent portion (1-5 mL) was subjected to analysis following the general procedure described earlier.

Injection: Chloroquine injection solution equivalent to 10 mg CQP was diluted to 100 mL with 0.1M HCl in a calibrated flask. A known aliquot (1.5 mL) was used for assay.

Suspension: A quantity of suspension containing 10 mg of CQP was quantitatively transferred into a 100 mL calibrated flask, 50 mL 0.1M HCl was added and the contents were shaken for 5 min, and diluted to the mark with 0.1M HCl and mixed. The content was filtered using a whatman No 42 filter paper and 1.5 mL of filtrate was taken for assay in 5 replicates.

Placebo blank and synthetic mixture analyses

A placebo blank containing lactose (20mg), starch (40 mg), acacia (35 mg), sodium citrate (35 mg), hydroxyl cellulose (35 mg), magnesium stearate (35 mg), talc (40 mg) and sodium alginate (35 mg) was prepared by mixing all the components into a homogeneous mixture. Thirty mg of the placebo blank was taken and its solution prepared as described under '*Procedure for tablets*' and then analyzed using the procedures described above.

To 20 mg of the placebo blank, 10 mg of CQP was added and homogenized, transferred to 50 ml volumetric flask and the solution was prepared as described under "*Procedure for tablets*". A convenient aliquot was diluted and then subjected to analysis by the procedures described above.

Forced degradation studies

Ten µg ml^{-1} CQP (2.5 ml of 100 µg ml^{-1} CQP) was taken in three 25 mL volumetric flasks and mixed with 5 ml of 5 M HCl (acid hydrolysis) or 5 M NaOH (alkaline hydrolysis) or 5% H_2O_2 (oxidative degradation) and the flasks were kept in a hot water bath maintained at 80°C, for 2h. The solution was cooled to room temperature and diluted to the mark with 0.1 M HCl after neutralization with 5.0 ml of 5 M NaOH (for acid hydrolysis) and 5 ml of 5 M HCl (for alkaline hydrolysis). In thermal degradation, solid drug was kept in Petri

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dish in oven at 100 °C for 24 h. After cooling to room temperature, 10 µg ml^{-1} CQP solution in 0.1 M HCl was prepared and spectrum recorded. For uv degradation study, the solid drug in a petridish was exposed to 200 watt hr. m^{-2}, UV-radiation and 1.2 million lux hr. of visible radiation in a photostability chamber. Post-irradiation, a 10 µg mL^{-1} CQP solution in 0.1 M HCl was prepared and its absorption spectrum recorded.

RESULTS AND DISCUSSION

Spectral characteristics

The absorption spectrum of 10 µg ml^{-1} CQP solution in 0.1 M HCl recorded between 200 and 400 nm showed an absorption maximum at 342 nm, and at this wavelength 0.1 M HCl had insignificant absorbance. Therefore, 342 nm was used as analytical wavelength (λ_{max}). Figure 1 represents the absorption spectrum of CQP in 0.1 M HCl along with blank.

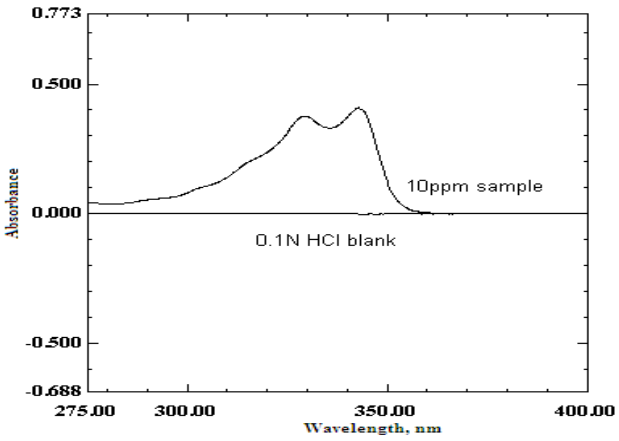

Figure 1: Absorption spectrum of CQP (10 µg ml^{-1}) in 0.1 M HCl

Method validation

Linearity and sensitivity

A linear correlation was found between absorbance at λ_{max} and concentration of CQP (Figure 3). The slope (b), intercept (a) and correlation coefficient (r) were evaluated by using the method of least squares. Optical characteristics such as Beer's law limits, molar absorptivity and Sandell sensitivity values are compiled in Table 2. The limits of detection (LOD) and quantitation (LOQ) values calculated according to ICH guidelines, are also presented in Table 2.

Table 2 Sensitivity and regression parameters

Parameter
λ_{max}, nm
Linear range, µg ml^{-1}
Molar absorptivity(ε), l mol^{-1} cm^{-1}
Sandell sensitivity*, µg/cm^2
Limit of detection (LOD), µg ml^{-1}
Limit of quantification (LOQ), µg ml^{-1}
Regression equation**
Intercept (a)
Slope (b)
Regression coefficient (r)

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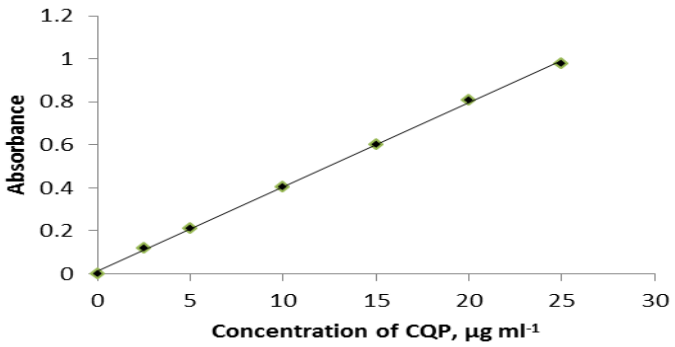

Figure 3 Calibration curve

Accuracy and precision

Accuracy was evaluated as relative error (%) between the measured concentrations and the concentrations taken (Bias %). The results obtained are compiled in Table 3. Precision of the method was calculated in terms of intermediate precision (intra-day and inter-day). Three different concentrations of CQP were analysed in seven replicates during the same day (intra-day precision) and for five consecutive days (inter-day precision). The results of this study are contained in Table 3.

Table 3 Results of intra-day and inter-day accuracy and precision study

CQP taken, µg ml^{-1}	Intra-day accuracy and precision (n=7)			Inter-day accuracy and precision (n=5)		
	CQP found, µg ml^{-1}	%RE	%RSD	CQP found, µg ml^{-1}	%RE	%RSD
10.0	09.95	0.46	1.93	9.82	0.72	2.48
15.0	15.08	0.58	1.54	15.13	0.89	1.76
20.0	19.73	1.34	1.37	19.70	1.47	1.58

RE: Relative error and RSD: Relative standard deviation.

Robustness and ruggedness

Method robustness was tested by measuring the absorbance at 341, 342 and 343 nm whereas the method ruggedness was tested by comparing the RSD values of the results obtained by three different analysts, and also with three different cuvettes by a single analyst. The intermediate precision, expressed as percent RSD, which is a measure of robustness and ruggedness was within the acceptable limits as shown in the Table 4.

Table 4 Results of robustness and ruggedness expressed as intermediate precision(%RSD)

CQP taken, µg ml^{-1}	Method robustness	Method ruggedness	
	Parameter altered		
	Wavelength*, nm, RSD % (n = 3)	Inter-analysts' RSD, % (n = 3)	Inter-cuvettes' RSD, % (n = 3)
10.0	1.78	0.97	1.18
15.0	2.16	1.12	1.39
20.0	2.63	1.03	1.07

*Wavelengths used were 341, 342 and 343 nm.

Selectivity

The proposed method was tested for selectivity by placebo blank and synthetic mixture analyses. The placebo blank solution was subjected to analysis according to the recommended procedure and found that

there was no interference from the inactive ingredients, indicating a high selectivity for determining CQP in its tablets. When the synthetic mixture solution was subjected to analyses at 10, 15 and 20 µg ml^{-1} levels, the percent recoveries were 98.48, 97.36 and 102.7 respectively, with % RSD being less than 2.5 implying that the assay procedure is free from matrix interference.

Application to dosage forms

In order to evaluate the analytical applicability of the proposed method CQP in commercial tablets, injection and suspension was determined and the results obtained by the proposed method were compared with those of the reference method [5] by applying Student's t-test for accuracy and F-test for precision. The results (Table 5) showed that the Student's t- and F-values at 95 % confidence level did not exceed the tabulated values, which confirmed that there is a good agreement between the results obtained by the proposed method and the reference method with respect to accuracy and precision.

Table 5 Results of analysis of tablets by the proposed methods and statistical comparison of the results with the reference method

Tablet brand name[b]	Label claim	Found[a] (Percent of label claim ±SD)	
		Reference method	Proposed method
Cadiquin (Tablet)	200 mg/tablet	98.11±1.21	98.62±1.81 **t**=0.52 **F**=2.24
Maliago (Tablet)	500 mg/tablet	98.11±1.21	97.51±1.16 **t**=0.80 **F**=1.09
Cloquin (Injection)	40 mg/mL	98.11±1.21	96.91±1.56 **t**=1.36 **F**=1.66
Emquin (suspension)	100 mg/10 mL	98.11±1.21	97.11±1.28 **t**=1.26 **F**=1.12

Mean value of five determinations,

The value of t and F (tabulated) at 95 % confidence level and for four degrees of freedom are 2.77 and 6.39, respectively.

Recovery study

To a fixed amount of drug in formulation (pre-analysed): pure drug at three different levels was added, and the total was found by the proposed method. Each test was repeated three times. The results compiled in Table 6 show that recoveries were in the range from 98.51 to 102.5% indicating that commonly added excipients to tablets did not interfere in the determination

Table 6 Results of recovery study by standard addition method

Dosage Studied	CQP in dosage fom µg ml^{-1}	Pure CQP added µg ml^{-1}	Total found µg ml^{-1}	CQP recovered Percent ± SD
Cadiquin (Tablet)	9.86	5	14.72	99.06±0.92
	9.86	10	19.51	98.24±1.58
	9.86	15	24.12	97.02±1.38
Maliago (Tablet)	9.75	5	14.41	97.69 ±1.81
	9.75	10	19.14	96.91±1.92
	9.75	15	24.31	98.22±1.56
Cloquin (Injection)	9.69	5	14.35	97.69±1.63
	9.69	10	19.53	99.19±1.48
	9.69	15	24.25	98.22±1.78
Emquin (suspension)	9.71	5	14.62	99.39±1.82
	9.71	10	19.26	97.72±1.72
	9.71	15	24.26	98.18±1.26

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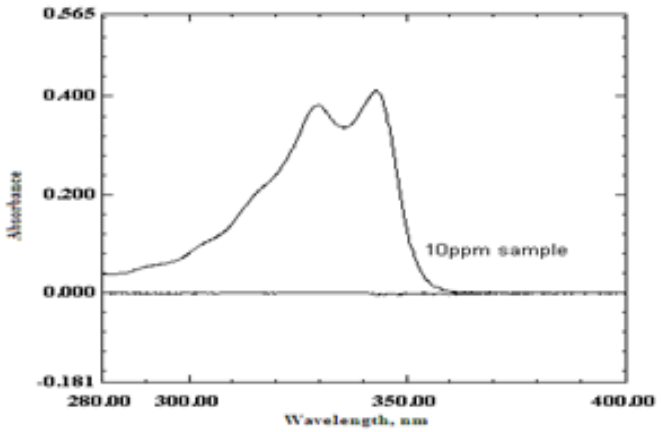
Forced degradation studies:

The CQP was subjected to acid, base and hydrogen peroxide induced degradation in solution state, and photo and thermal degradation in solid state. The study was performed by measuring the absorbance of CQP solution after subjecting to forced degradation. The results of this are presented in Table 1. The results revealed that, the drug was stable under the acid hydrolysis, photo and thermal degradation. There was significant change in the absorbance after base hydrolysis and insignificant degradation under oxidative degradation. The absorption spectra (Figure 2) recorded for this degraded CQP.

Table 1 Results of degradation study

Degradation condition	% Degradation
No degradation (control)	No degradation
Acid hydrolysis (5 M HCl , 80°C, 2 hours)	No degradation
Base hydrolysis (5 M NaOH , 80°C, 2 hours)	51
Oxidation (5% H_2O_2 , 80°C, 2 hours)	5
Thermal (100°C, 24 hours)	No degradation
Photolytic (1.2 K flux, 48 hours)	No degradation



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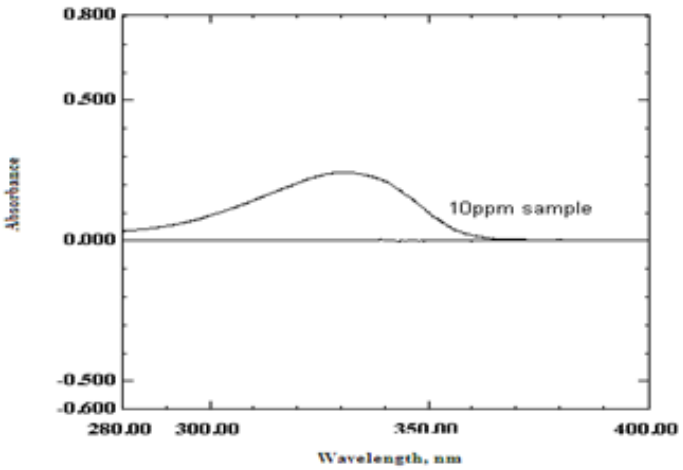

(b)

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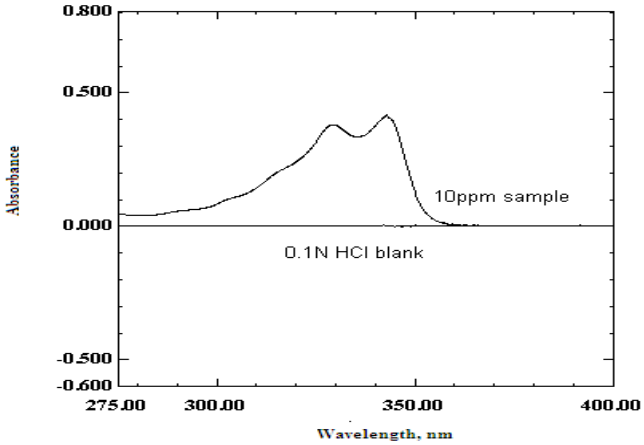

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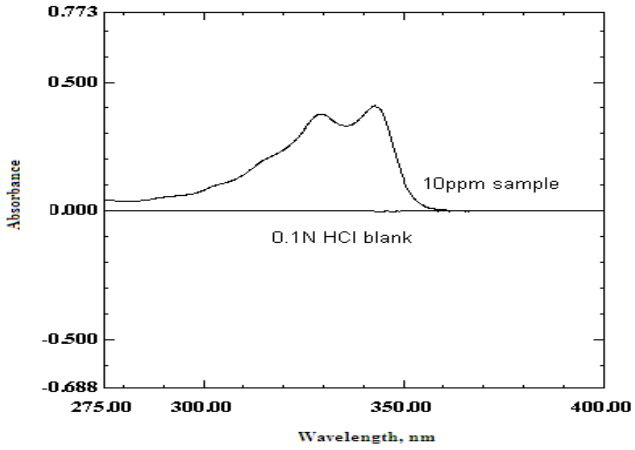

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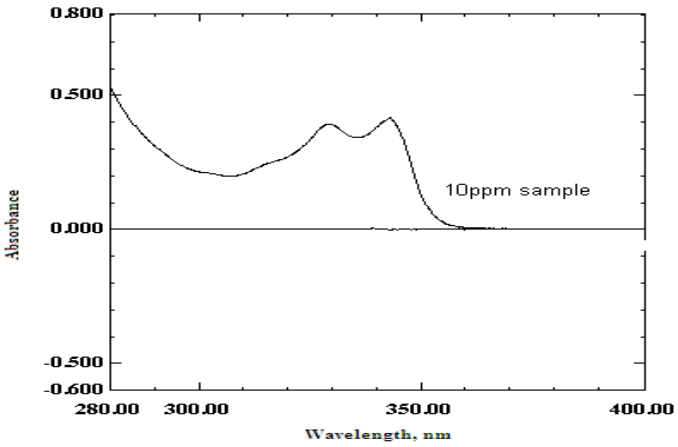

(e)

Figure 2: Absorption spectra of 10 µg ml^{-1} CQP after
(a) acid hydrolysis, (b) base hydrolysis, (c) photo degradation (d) thermal degradation and (e) peroxide degradation.

CONCLUSION

A direct uv-spectrophotometric method was developed and validated for the determination of chloroquine phosphate in pharmaceuticals. The hall mark of the method is the simplicity compared to the

reported uv-spectrophotometric methods, and also several visible spectrophotometric methods. Other advantages are high sensitivity and wide linear dynamic range. The method is stability-indicating and can be used in industrial quality control lab for rapid assay.

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