Pharmaceutica Analytica Acta

Nagib Qarah et al., Pharm Anal Acta 2017, 8:3
DOI: 10.4172/2153-2435.1000539

Research Article Open Access

Sensitive and Selective Extraction-Free Spectrophotometric Assay of Chloroquine Phosphate in Pharmaceuticals Based on Ion-Pair Reaction with Bromocresol Green and Bromocresol Purple

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Received date: February 22, 2017; **Accepted date:** March 20, 2017; **Published date:** March 22, 2017

Abstract

Chloroquine Phosphate (CQP) is an antimalarial agent extensively used in the treatment of malaria. Two spectrophotometric methods, which are rapid, simple, selective and sensitive, are presented for the determination of CQP in bulk and dosage forms using two sulphonphthalein dyes: Bromocresol Green (BCG method) and Bromocresol Purple (BCP method). The methods are based on the formation of chloroform-soluble ion-pairs, when CQP is reacted with either dye, suitable for measurement at 420 nm in both the methods. The effects of reaction time, dye concentration and reaction medium were carefully studied and optimized. Under the optimum reaction conditions, Beer's law is obeyed overconcentration ranges 1-20 and 0.5-12 μg mL^{-1} CQP (base) for BCG method and BCP method respectively, with corresponding molar absorptivity values of 1.79 × 10^4 and 3.09 × 10^4 L mol^{-1} cm^{-1}. The calculated limits of detection (LOD) and quantification (LOQ) are 0.27 and 0.82 μg mL^{-1} (BCG method); 0.15 and 0.46 μg mL^{-1} (BCP method). Intra-day and inter-day %RSD values were ≤1.56% and ≤1.83% whereas the respective %RE values were better than 2%. Robustness of the methods was determined by performing analysis with slightly altered optimum conditions while ruggedness was tested by inter-personnel as well as inter-equipment variations; the %RSD values were within the accepted limits in both instances. Method selectivity was as ascertained by placebo blank and synthetic mixture analysis with no detectable interference from co-formulated substances in the assays. The methods were applied to the determination of CQP in tablets, suspension and injections with satisfactory results. Accuracy was also confirmed by recovery test via standard-addition procedure.

Keywords: Chloroquine phosphate; Assay; Spectrophotometry; Dyes; Ion-pair; Pharmaceuticals

Introduction

Chloroquine Phosphate (CQP) is chemically known as 7-chloro-4[[4-(diethylamino)-1-methylbutyl]amino]quinoline phosphate, 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 discoid lupus erythematosus in adults [1-3]. bCPQ is official in the US pharmacopeia, which contains a uv-spectrophotometric method [2]. In the open literature, few methods are found for the determination of CQP in pharmaceuticals and include titrimetry [4-6], gravimetry [7], potentiometry [8-11], spectrofluorimetry [12-17], uv-spectrophotometry [18-21], HPLC [22-32], HPTLC [26] and bioassay [33].

As visible spectrophotometric methods offer significant advantages over the reported methods with respect to selectivity and sensitivity [4-7], simplicity and ease of performance [8-33] and cost-effectiveness [11-32], several such methods have been developed for the determination of CQP in pharmaceuticals. The drug has been determined as chloroform/methylene chloride-soluble ion-pair with and dyes such as bromocresol purple [34], Rose Bengal [35], wool fast blue and bromocresol green [36], complexes formed at acidic pH buffers were extracted into suitable organic solvents and measured at wavelengths of maximum absorption. Ion-pairs formed with complex ions such as, $[Co(SCN)_4]2-$ [37], $[Mo(SCN)_6]-$ [38] were extracted into organic solvent and measured, serving as basis of assay of CQP. Methods based on measurement of chare-transfer complexes formed by the drug with chloranilic acid [39], iodine and 2,3-dichloro-5,6-dicyano-p-benzoquinone (DDQ) [40] in polar organic solvents have been published by several researchers. The yellow coloured product resulting from bromination of CQP with bromate-bromide mixture [41] in acid medium was also used for the determination of CQP. Measurement of orange-red coloured ferroin, formed as a result of redox-complexation reactions involving CQP, iron (III) and 1,10-phenanthroline, has also been employed for the quantification of CQP by visible spectrophotometry [42]. It was also determined by kinetic spectrophotometry [43] using bromate and iodate as oxidimetric agents.

However, many of these methods suffer from limitations and include low sensitivity [39,41], narrow linear range [34], pH dependency, use of tedious and time-consuming step, extraction step [34,36] and/or longer contact time [41]. Therefore, need for simple, facile, and easy to perform spectrophotometric methods for the determination of CQP in pharmaceuticals was strongly felt. Previously, BCP [34] and BCG [36] have been used as ion-pair agents for the spectrophotometric determination of CQP. However, these procedures are tedious and time consuming since the ion-pair formed at a specific pH in aqueous medium need to be extracted and less accurate due to possible incomplete recovery of analyte from the aqueous phase methods based on the measurement of ion-pairs bromocresol purple

(BCP method) in chloroform, without involving extract step. The new methods exhibited advantageous features with respect to sensitivity, linear range, speed and ease of performance compared to the existing methods.

Experimental

Apparatus

A Systronics model 166 digital spectrophotometer (Systronics, Ahmedabad, and Gujarat, India) with matched 1 cm quartz cells was used for absorbance measurements.

Reagents and materials

The chemicals used were of analytical-reagent grade, and spectroscopic-grade organic solvents were used in the assay. Chloroformic solutions of bromocresol green (BCG) and Bromocresol Purple (BCP) (both from Loba Chemie Ltd., Mumbai, India; both 0.05%) were prepared.

High purity chloroquine phosphate (99.95%) was procured from Cipla India Ltd., Mumbai, India, and used as received. Cadiquin 200 mg (Zydus Cadila Healthcare Ltd., Bangalore, India), Maliago 500 mg (Cipla Ltd., Bangalore, India) tablets; Cloquin 40 mg/mL injection (Indoco Remedies Ltd., Baddi, India) and Emquin 160 mg/10 mL suspension (Merck Biopharm India) were purchased from local market.

Preparation of chloroquine base (CQB) solution

An amount of chloroquine phosphate CQP equivalent to 10 mg CQB was dissolved in about 20 mL of water and pH of aqueous solution was raised to about 9.5-10 by adding 5 mL of 1 M NaOH solution. CQB formed was extracted by shaking with 4 × 10 mL portions of chloroform; extracts were passed over anhydrous sulphate and collected in a dry 50 mL volumetric flask. The extracts were made up to the mark with chloroform and mixed. The resulting 200 μg mL^{-1} CQB solution was diluted to 40 and 20 μg mL^{-1} levels with chloroform.

Procedures

Procedure for bulk drug

Preparation of calibration graphs: BCG method: Aliquots (0.25-5.0 mL) of 40 μg mL^{-1} CQB solution were transferred accurately into several 10 mL volumetric flasks and added 2 mL of 0.05% BCG solution to each flask. The contents were diluted to the mark with chloroform and absorbance of each solution measured at 420 nm vs the blank, after 5 min.

BCP method: Different volumes, from 0.25 to 6.0 mL, of 20 μg mL^{-1} CQB solution were accurately measured and placed in 10 mL volumetric flasks. Two mL of 0.05% BCP solution were added to each flask and the contents diluted to the mark with chloroform, and absorbance measured after 5 min at 420 nm against the reagent blank.

In each case, calibration curve was prepared by plotting absorbance vs concentration, and concentration of the unknown was deduced from the regression equation derived from Beer's law data.

Procedure for dosage forms

Tablets: An amount of pulverized chloroquine phosphate tablets equivalent to 10 mg of chloroquine base was accurately weighed. Into a 50 mL stoppered flask and shaken with 20 mL water for 20 min. to extract the active content to the aqueous phase, the contents were filtered using a quantitative filter paper. The resulting filtrate and washings were quantitatively transferred into a 125 mL separating funnel and then treated exactly as described under preparation of standard base solution. Tablet extract containing 200 μg mL^{-1}CQB was diluted to 40 and 20 μg mL^{-1} levels and assayed in five replicates by 2 mL and 3 mL aliquots, respectively, by BCG method and BCP method.

Injection: Aliquot of injectable product equivalent to 20 mg Chloroquine base was transferred accurately to a 125 mL separating funnel containing 10 mL of water and then steps described under preparation of standard solution were followed. After diluting this solution (200 μg mL^{-1}) to 40 and 20 μg mL^{-1} levels, general procedures were applied.

Suspension: Five mL of suspension containing 80 mg CQP was diluted accurately with water to 50 mL in a calibration flask. An aliquot of diluted suspension (containing 10 mg CQB) was transferred into a 125 mL separating funnel containing 10 mL water, the salt was converted to base and finally extracted as chloroformic solution as described earlier. The extract was diluted with chloroform and subjected to analysis following the general procedure.

Procedure for placebo and synthetic mixture

A placebo blank of the composition: 25 mg sucrose, 10 mg talc, 15 mg lactose, 20 mg starch, 10 mg gelatin, 20 mg magnesium stearate, 25 mg sodium alginate and 25 mg methyl cellulose was prepared by homogeneous mixing in a mortar. 10 mg of placebo was placed in a 50 mL calibration flask and its extract prepared as described under "procedure for tablets". Two mL of the extract was subject to analysis following the general procedures. To 10 mg of the placebo blank prepared above, CQP equivalent to 10 mg CQB was added, mixed thoroughly and the mixture was quantitatively transferred into a 50 mL stoppered bottle; and then steps described under "procedure for tablets" were followed.

Procedure for stoichiometric relationship

Job's method of continuous variations of equimolar solutions was employed. CQB and dye solutions (BCG or BCP) equivalent to 3.75 × 10^{-5} M each were prepared in chloroform. CQB and BCG or BCP solutions mixed in complementary ratios keeping the total volume at 10 mL, and the absorbance was measured at 420 nm. A plot of absorbance versus mole fraction of the drug was prepared in each case.

Procedures for method validation

The assay validation procedures were carried out according to current ICH guidelines [44], and the validation parameters included linearity, the Limits of Detection (LOD), Limits of Quantification (LOQ), precision, accuracy, robustness, ruggedness, selectivity and accuracy by recovery test.

Linearity

The linearity was assessed by the calibration graph, which was constructed by plotting the absorbance (Y) versus concentration of

CQB (X) and the regression equation was calculated. The LOD and LOQ were calculated using the formulae:

$$LOD = \frac{3S}{k} and\ LOD = \frac{10S}{k}$$

Where S is the standard deviation of the replicate blank absorbance values and k is the slope of the calibration curve. The Beer's law range, the regression equation, LOD, LOQ, molar absorptivity and Sandell sensitivity values are computed in Table 1.

Accuracy and precision

The accuracy of the methods was determined on the basis of the difference in mean calculated and amount/concentration taken (% deviation from the actual concentration, DFA); and the precision was determined by calculating the intra-day and inter-day relative standard deviation. These were computed by analyzing standard solution of CQB at three levels seven times on the same day (intra-day) and on five consecutive days (inter-day). The results of this study expressed as relative error (%RE) and relative standard deviation (%RSD) are presented in Table 2.

Robustness and ruggedness

Robustness was evaluated by assaying the standard solutions after slight but deliberate variations in the analytical conditions like contact time and volume of dyes. Ruggedness, on the other hand, was assessed by a study in which the determination was performed by three analysts and also by a single analyst using three different instruments in the same laboratory and also by three analysts using a single instrument. The results of this study reported as intermediate precision and expressed as %RSD are computed in Table 3.

Selectivity

The placebo blank and synthetic mixtures were analyzed by the developed methods and the results compared with those obtained on standard drug solution.

Application to formulations

Pharmaceutical formulations solution prepared as described earlier was objected to analysis by applying the developed procedures by taking 3 mL in five replicates, and the measured analytical signal was used to calculate the percent of the label claim. For comparison, the pharmaceutical formulations extract in chloroform were applied the assay by reference method [2]. These finding along with the results of statistical tests are given in Table 4.

Recovery test

Pre-analyzed tablet powder was spiked with pure drug at three levels and the total quantity of the drug was calculated, and finally the percent recovery of the pure drug added was arrived at. The results obtained by this test are found in Table 5.

Results and Discussion

CQB, being a basic nitrogen-containing compound, reacts instantly with BCG and BCP in chloroform, giving characteristic yellow coloured products which exhibit an absorption maximum at 420 nm (Figure 1). The coloured species can be attributed the formation of ion-pair complex between the drug and the dye. The yellow colour is thought to be the result of the proton transfer from the acidic dye to the basic centre of the drug. Subsequently the dye is converted to an open quinoidal yellow-coloured anion [45-51]. The latter forms an ion-pair with the drug cation. The possible reaction pathway is shown in (Figure 2a and 2b).

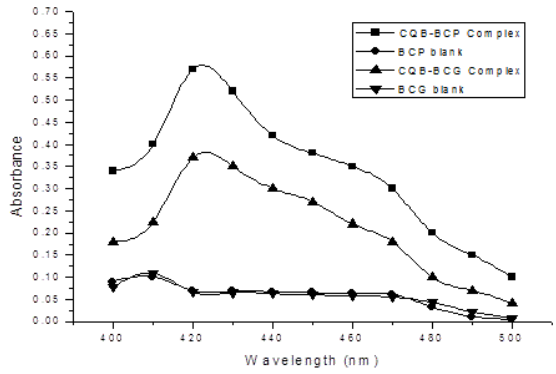

Figure 1: Absorption spectra of CQB-BCG and CQB-BCP complexes (8 µg mL^{-1}) and their blank.

Method development

Optimization of experimental variables: The spectrophotometric properties of colored ion-pair complexes as well as various experimental variables affecting complex formation were carefully studied and optimized to determine the most favorable conditions of assay. All the reactions were studied as a function of reaction medium, reagent volume and reaction time.

Reaction medium: A number of organic solvents such as chloroform, methylene chloride, dichloroethane, carbon tetrachloride, xylene, hexane, etc. were tried as the reaction medium. Chloroform was selected as the ideal medium since it yielded maximum sample absorbance and minimum blank absorbance.

Standing time and stability: The optimum reaction time was investigated from 0.5 to 5 min after mixing the reactants at room temperature (29 ± 2°C). Full colour development was realized almost instantaneously. However, measurement in each instance was made after 5 min to ensure quantitative complexation. The complexes were stable for 1 and 4 h in BCG method and BCP method, respectively.

Effect of dye concentration: The effect of dye concentration on the intensity of the formed yellow coloured ion-pair complexes at the selected wavelengths was tested by measuring the absorbance of solutions containing a constant amount of CQB (8 µg mL^{-1} in both method) and different volumes (0.5-3.0 mL) of dyes. Maximum color intensity of ion-pair was achieved at 2.0 mL in each method (Figure 2). A slight increase in blank absorbance was observed in both methods at larger dye volumes.

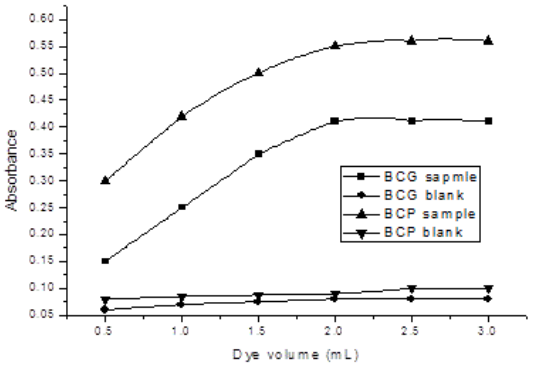

Figure 2: Effect of dye concentration (8 µg mL^{-1} CQB in both methods).

Stoichiometry: Job's method continuous variations for establishing the reaction stoichiometry revealed that the reaction between chloroquine (CQB) and the two dyes BCG and BCP, was found to proceed with a ratio of 1:2 (drug: dye), which it is in agreement with the reaction scheme suggested in Figures 3a and 3b.

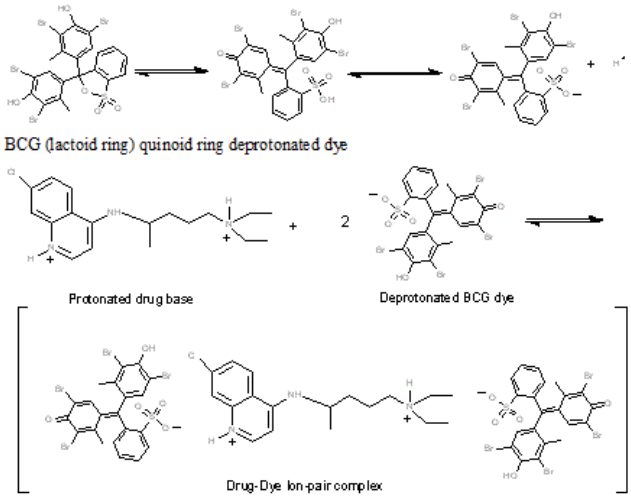

Figure 3a: The possible reaction pathway for the formation of CQB-BCG ion-pair complex.

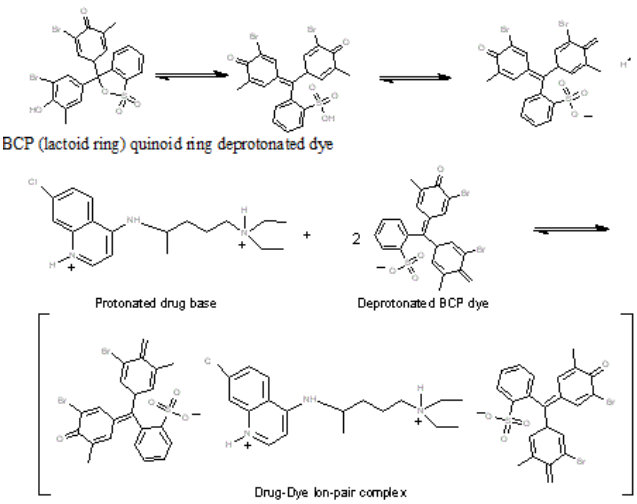

Figure 3b: The possible reaction pathway for the formation of CQB-BCP ion-pair complex.

Method validation: The proposed methods were validated for linearity, sensitivity, accuracy, precision, and selectivity (Figures 4a and 4b).

Linearity and sensitivity: Typical calibration data for the methods were obtained from linear regression analysis of absorbance-concentration data. Beer's law plots were linear over the ranges 1-20 and 0.5-12 µg mL^{-1} for BCG method and BCP method, respectively. The regression parameters and sensitivity indices are computed in Table 1.

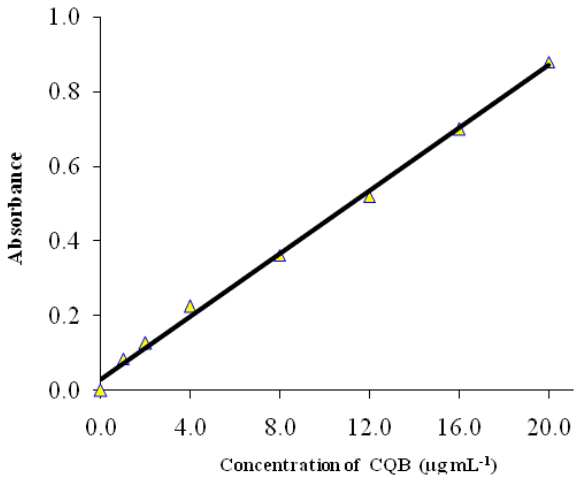

Figure 4a: Calibration curve for BCG method.

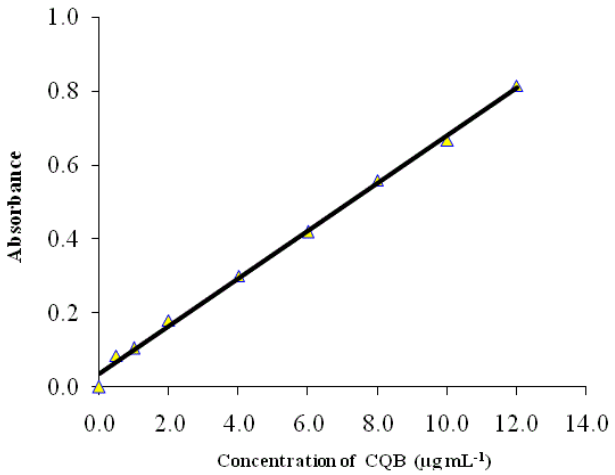

Figure 4b: Calibration curve for BCP method.

Parameter	BCG Method	BCP Method
λ_{max}, nm	420	420
Colour stability	60 min	4 h
Linear range, µg mL^{-1}	1-20	0.5-12
Molar absorptivity (ε), L mol^{-1} cm^{-1}	1.79 × 10^4	3.09 × 10^4
Sandell sensitivity*, µg cm^{-2}	0.0179	0.0104
Limit of Detection (LOD), µg mL^{-1}	0.27	0.15
Limit of Quantification (LOQ), µg mL^{-1}	0.82	0.46
Regression equation, Y**		
Intercept (a)	0.0433	0.048
Slope (b)	0.0412	0.0632
Standard deviation of a (Sa)	0.0998	0.0988
Standard deviation of b (Sb)	0.0051	0.0080
Correlation coefficient (r)	0.9992	0.9996

*Limit of determination as the weight in µg mL^{-1} of solution, which corresponds to an absorbance of A=0.001 measured in a cuvette of cross-sectional area 1 cm^2 and l=1 cm. **Y=a+bX, where Y is the absorbance, X concentration in µg mL^{-1}, a intercept and b slope.

Table 1: Sensitivity and regression parameters.

Precision and accuracy

To determine the precision and accuracy of the methods, standard CQB solutions at three concentration levels, were assayed seven times on the same day and five times each on five successive days. Table 2 show the %Relative Error (RE) and% Relative Standard Deviation (RSD) values, which are the indicators of intra-day as well as inter-day accuracy and precision of the results produced by the methods. Low values (>2%) of both %RE and %RSD reflect the adequate accuracy and precision of the proposed methods.

Method	CQB (µg mL^{-1})	Intra-day accuracy and precision (n=7)			Inter-day accuracy and precision (n=7)		
		CQB found a (µg mL^{-1})	RSDb%	Rec%	CQB found (µg mL^{-1})	RSDb%	Rec%
BCG	6.0	5.89	0.87	1.83	6.08	0.74	1.33
	12.0	12.19	1.26	1.58	12.13	1.33	1.08
	18.0	18.02	0.52	0.12	18.07	1.05	0.39
BCP	3.0	2.96	0.89	1.33	2.95	1.83	1.67
	6.0	5.96	1.56	0.67	6.02	1.36	0.34
	9.0	9.14	0.67	1.56	8.94	0.67	0.33

Table 2: Evaluation of intra-day and inter-day accuracy and precision. (a) Mean value of seven determination; (b) Relative standard deviation (%); (c) Relative error (%).

Robustness and ruggedness

Assay procedure was repeated after making small incremental variations in the optimized conditions such as, reagent volume, contact time, and wavelength, and the effect of these variations on assay results was investigated to assess the robustness of the methods. To evaluate ruggedness, determination was performed by a single analyst using three instruments in the same laboratory and also by three analysts using a single instrument. Each study was performed on three levels of analyte. The results of this study expressed as intermediate precision (%RSD), which are ≤2.23% are compiled in Table 3, reveal that the performance of the method was unaffected by small variations in the optimized experimental variables, and instrument-to-instrument as well as analyst-to-analyst variations.

Method	CQB taken (µg mL-1)	Robustness			Ruggedness	
		Parameters altered*			Inter-analysts (n=3)	Inter-instruments (n=3)
		Volume of dye*	Contact time**	λ_{max}, nmψ		
BCG	6.0	1.05	1.42	1.43	1.35	1.44
	12.0	0.87	1.05	2.01	0.95	1.88
	18.0	1.33	1.28	1.65	1.42	2.21
BCP	3.0	1.53	1.51	1.61	1.24	2.23
	6.0	1.12	1.35	1.55	0.66	1.68
	9.0	1.29	0.86	0.96	1.26	2.04

*Volume of dye was 1.8, 2.0 and 2.2 mL, in both methods.

**Contact time used were 4, 5 and 6 min. ψ Wavelengths used were 418, 420, 422 nm.

Table 3: Results of method robustness and ruggedness expressed as intermediate precision (%RSD).

Selectivity

A systematic study was performed to determine the effect of additives by analyzing the placebo blank and synthetic mixture containing CQP. The absorbance of the placebo solution in each case was almost equal to the absorbance of the reagent blank which revealed no interference. When the methods were applied to determine CQP in the synthetic mixture, at 12 and 8 µg mL^{-1} CQB levels by BCG method and BCP method, respectively, percent recoveries of 97.58 ± 1.65 and 96.46 ± 1.56 were realized, suggesting the selectivity of the methods proposed.

Application to formulations

The proposed methods were applied for the determination of CQB in tablet, injection and suspension and the results are presented in Table 4. The same batch formulations were subjected to the assay by the reference method. For parallel assay by the reference method [2] aqueous tablet extract or diluted injection solution was measured at 343 nm. In the case of suspension, diluted suspension was filtered using a quantitative filter paper before recovering the absorbance and results were statistically evaluated by applying Student's t- and variance ratio F-test. The calculated t- and F-values did not exceed the tabulated values at the 95% confidence level for four degrees of freedom, indicating agreeing accuracy and precision between the proposed methods and the reference method.

Formulation name	Nominal amount	Found* (% of nominal amount ± SD)		
		Official method	Proposed methods	
			BCG Method BCP Method	
Cadiquin tablet	200 mg/tablet	97.46 ± 1.09	98.89 ± 1.35	96.85 ± 1.24
			t =2.24	t=0.82
			F=1.37	F=1.29
Maliago tablet	500 mg/tablet	96.67 ± 1.23	95.96 ± 0.88	94.74 ± 1.18
			t=1.05	t=2.52
			F= 1.95	F= 1.09
Cloquin injection	40 mg per mL	101.4 ± 1.22	102.6 ± 0.73	100.8 ± 1.33
			t= 1.88	t=0.74
			F= 2.79	F=1.19
Emquin suspension	100 mg per mL	97.36 ± 1.44	98.91 ± 0.93	99.28 ± 1.02
			t= 2.03	t=2.43

			F= 2.40	F=1.99
*Mean value of five determinations. (Tabulated t-value at the 95% confidence level and for four degrees of freedom is 2.77). (Tabulated F-value at the 95% confidence level and for four degrees of freedom is 6.39).				

Table 4: Results of analysis of formulations by the proposed methods and statistical comparison of the results with the official method

Accuracy by recovery test

Pre-analyzed tablet powder was spiked with pure CQP at three levels and the total was determined by the proposed methods. The determination each level was replicated thrice. The results of percent recovery of drug which reflect the accuracy are summarized in Table 5, and demonstrate the methods' freedom from interference by the co-formulated substances in the tablets.

Method	Tablet studied	CQB in tablet, μg mL^{-1}	Pure CQB added, μg mL^{-1}	Total found, μg mL^{-1}	Pure CQB recovered Percent ± SD*
BCG	Cadiquin	5.93 5.93 5.93	3.0 6.0 9.0	8.65 11.25 14.28	96.87 ± 1.13 94.32 ± 0.65 95.64 ± 1.03
BCP	Cadiquin	2.91 2.91 2.91	1.5 3.0 4.5	4.55 5.76 7.67	101.41 ± 1.33 97.36 ± 0.85 103.5 ± 1.16
*Mean value of three determinations.					

Table 5: Results of recovery experiment through standard-addition method.

Conclusion

The proposed methods have the advantages of ease of performance, sensitivity as indicated by low LOD values and selectivity as shown by almost 100% recovery values. The methods are free from drastic experimental conditions unlike most of the available methods and simply involve mixing of the reactants followed by absorbance measurement. In contract to the current methods, the proposed methods are characterized by wide linear dynamic ranges enabling their application to samples of wide concentration ranges. The methods have been demonstrated to be both accurate and precise besides being robust and rugged as indicated by low values of relative error and relative standard deviation. Some of the advantageous features of the proposed methods in relation with the existing methods are summed up in Table 6. The main drawback of the methods is their inability to be applied to chloroquine phosphate directly and the need to convert the salt to base form, which of course, is one-step process. However, this limitation is outsmarted by multi-dimensional advantages of the recommended methods, and substantiates their usefulness in quality control laboratories.

S. No	Reagent/s	Methodology	Linear range (μg mL^{-1})	LOD/LOQ (μg mL^{-1})	Remark	Ref. No.
1	BCP	Ion-pair complex formed was extracted in chloroform and measured at 420 nm	1.25-8.75	0.128/0.428	Extraction step, strict pH control	34
2	RB	Ion-pair complex formed was extracted in chloroform and measured at 420 nm	_	_	_	35
3	BCG WTB	Ion-pair complex formed was extracted in chloroform and measured at 420 nm	50-250	_	Extraction step, strict pH control	36
4	$[Co(SCN)_4]2-$	Ion-pair complex formed extracted in nitrobenzene and measured at 625 nm	Feb-60	_	Extraction step, strict pH control	37

5	$[Mo(SCN)_6]$-	Ion-pair complex formed extracted into methylene chloride and measured at 467 nm	Feb-42	_	Extraction step	38
6	Chloranilic acid	CT complex measured at 520 nm	Aug-80	_	Less sensitive	39
7	DDQ	CT complex measured at 462 nm	May-53	_	Moderated sensitive	40
	I2	CT complex measured at 293 nm	15-Jan		Measurement at lower analytical wavelength	
8	$KBrO_3$-KBr	Brominated product in acidic medium measured at 350 nm	40-200	_	Longer reaction time, less sensitive	41
9	Fe(III)-1,10 phenanthroline	Complex product colored measured at 510 nm	20-320	_	Less sensitive	42
10	KBrO3	Recorded the absorbance change with time at 343 nm	0.5-5	0.06	Critical dependent on experimental variables	43
	Kinetic					
11	BCG/BCP	Yellow ion-pair complex formed in chloroform measured at 420 nm	20-Jan	0.27/0.82	No drastic experimental conditions, no extraction step, instantaneous reaction, more sensitive.	Present methods
			0.5-12	0.15/0.46		

Table 6: Comparison of the proposed and the existing methods. BCP: Bromocresol Purple; BCG: Bromocresol Green; RP: Rose Bengal; DDQ: Dichloro-Dicyano-p-Benzoquinone.

Acknowledgements

The authors thank Cipla India Ltd., Mumbai, India for gifting pure chloroquine phosphate sample. Prof. K. Basavaiah thanks the University Grants Commission, New Delhi, India, for financial assistance in the form BSR faculty fellowship.

Declaration of Interest

The authors report no declarations of interest.

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