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Simple and selective spectrophotometric assay of chloroquine in pharmaceuticals using two nitrophenols as chromogenic agents.

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Abstract: *Two simple, sensitive and selective spectrophotometric methods are presented for the determination of chloroquine phosphate (CQP) in bulk drug and dosage forms. The methods are based on the interaction of chloroquine (CLQ) with either 2,4-dinitrophenol (DNP) or picric acid (PA) in chloroform resulting in the formation of intensely yellow coloured radical anion which was measured at 430 nm (DNP method) or 420 nm (PA method). The experimental conditions for better performance characteristics of the methods were carefully studied and optimised. Beer's law is obeyed over the concentration ranges of 3 – 70 and 1.2 – 30 µg mL^{-1} for DNP method and PA method, respectively, with corresponding molar absorptivity values of 4.7 × 10^3 and 1.1 × 10^4 l mol^{-1} cm^{-1}. The limits of detection (LOD) and quantification (LOQ) were 1.1 and 3.0 µg mL^{-1} (DNP method), and 0.3 and 1.2 µg mL^{-1} (PA method). Intra-day and inter day precisions expressed as relative standard deviation (RSD) were less than 2 and 2.3 %, respectively, whereas, the corresponding accuracies expressed as relative error (%RE) were under 2.5% and 2.3%. The real samples were successfully analysed by using the proposed methods with the results agreeing with the label claim and those of the reference method.*

I. Introduction:

Malaria is still one of the most severe infectious diseases globally, which is widespread mainly in the tropical and subtropical regions. It kills more people each year than any other infectious diseases except AIDS and tuberculosis [1]. Although it is difficult to obtain an exact figure of the malaria cases, the World Health Organization (WHO) estimates that malaria is responsible for over 300 million clinical cases and over one million deaths annually. About 40% of the global population is estimated to be at risk. Malaria is not just a disease commonly associated with poverty, but is also a cause of poverty and a major hindrance to economic development [1,2]. Chloroquine phosphate is (RS) – 4 – (7 – chloro – 4 – quinolylamino) pentyldiethylamine diorthophosphate. It contains not less than 98.5% and not more than 101.0% of $C_{18}H_{26}ClN_3$, $2H_3PO_4$, calculated with reference to the anhydrous substances [3]. Chloroquine is a 4 – aminoquinoline which has marked and rapid schizontocidal activity against all infections of *P. malariae* and *P. ovale* and against chloroquine-sensitive infections of *P. falciparum* and *P. vivax* [4]. It is also gametocytocidal against *P. Vivax, P. malariae,* and *P. ovale* as well as immature gametocytes of *P. falciparum* and it is not active against intrahepatic form [5].

Reliable analytical methods are required for quality control of CQP preparations. In tune with this requirement, a variety of analytical approaches have been published to quantify CQP in bulk drug and dosage forms. The drug is officially listed in the US pharmacopoeia, which contains a potentiometric titration procedure for the determination of the drug in bulk and dosage forms [6]. In the open literature, methods based on several techniques have been reported for the determination of CQP in pharmaceutical and include: titrimetry [7,8], uv-spectrophotometry [9-11], spectrofluorimetry [12], membrane electrode-based potentiometry [13-16], flow injection chemiluminescence spectrometry [17,18], high performance liquid chromatography [19-26], thin layer chromatography [27], high performance thin layer chromatography [28], gravimetry [29] and bioassay [30-32].

Visible spectrophotometry can be used in pharmaceutical quality control laboratories, because many substances can be selectively converted to coloured products. In addition, the instrument is readily available and fairly easy to operate. Considering these advantages, many visible spectrophotometric methods based on several reaction chemistries are found in the literature for the determination of CQP in pharmaceuticals. CQP has been determined based on its reaction with bromothymol blue [19,33], bromocresol purple [34], rose bengal [35], cobalt thiocyanate [36,37], molybdenum thiocyanate [38,39] or tetrbromophenolphthalein ethylester [40] as ion-pair agent; chloranalic acid [41,42], and dichloro-dicyano-p-benzoquinone or iodine [43] as charge-transfer complexing agent; bromate-bromide [22], and N-bromosuccinimide [44] as brominating agents and ammonium molybdate as an oxidant [45]. Two kinetic spectrophotometric methods have also been described to quantify CQP using $KBrO_3$ or KIO_3 [46] as an oxidant.

Many visible spectrophotometric methods cited above suffer from disadvantages such as critical pH adjustment, tedious and time-consuming liquid-liquid extraction step, longer contact time, poor sensitivity and

selectivity, and/or longer analysis time. Therefore, the development of rapid, simple, sensitive and selective spectrophotometric methods for quantifying CQP in pharmaceuticals was found desirable.

Charge-transfer(C-T) complexes, also called electron-donor-acceptor (EDA) complexes, may be formed when one interactant can perform as the electron donor and the other as the electron acceptor. The appearance of a new electronic absorption band, not attributable to either the donor or the acceptor, often, is taken as evidence for charge-transfer complexing [47]. Charge transfer phenomenon was introduced first by Mulliken [48, 49] and widely discussed by Foster [50] to define a new type of adduct to explain the behavior of certain classes of molecules which do not conform to classical patterns of ionic, covalent, and coordination of hydrogen bonding components. While such adducts largely retain some of the properties of the components, some changes are apparent, e.g., its solubility, the diamagnetic and paramagnetic susceptibility. The charge-transfer complexation arises from the partial transfer of an electron from a donating molecule having sufficient low ionization potential to an accepting one having sufficient high electron affinity and as a result, formation of intensely colored charge-transfer complexes which absorb radiation in the visible region [51] occurs. The source molecule from which the charge is transferred is called the electron donor (D) and the receiving molecule is called the electron acceptor (A).

$$\mathbf{D + A \rightarrow DA}$$

Compounds with unshared pairs of electrons may interact with other compounds through the donation of such electrons in a manner different from the traditional dative bond formation. Those interactions giving rise to intermolecular forces may be sufficiently strong to show features that do not exactly fit the definition of the classical dipole–dipole, dipole-induced dipole and/or van der Waals interactions. Depending upon the orbital that accepts these electrons, these acceptors may be described as δ - or π -acceptors [52].

Amines are excellent electron donors because of their low ionization potentials and can strongly interact with electron acceptors [53, 54]. Charge transfer complexation reactions have been extensively utilized for the determination of many pharmaceutical compounds containing amino group [55-68].

From the literature survey presented previously, it is clear that there is no report dealing with the determination of CQP in pharmaceutical formulations, based on its reaction with nitrophenols: 2,4-dinitrophenol (DNP) or 2,4,6-trinitrophenol (picric acid; PA) The reagents under study have numerous applications as analytical reagents and they have been used for the spectrophotometric determination of many drugs in pharmaceutical formulations [69-72]. In this, we describe the use of DNP and PA as chromogenic agents, for spectrophotometric determination of CQP in pure drug and in its formulations. Since CQP is a diphosphate salt, transfer of non-bonding electrons is restricted. Hence, it was found necessary to convert CQP to base form, and the free base form (CRQ) was extracted into chloroform. The methods involved the charge-transfer(C-T) complex formation reaction of the base form of the CQP with PA or DNP in chloroform to form intensely colored radical anion measurable at 420 nm or 430 nm.

II. Experimental

Apparatus

A Systronics, model 106 digital spectrophotometer (Systronica Ltd, Ahmedabad, India) with 1 cm path length quartz cells, was used for absorbance measurement.

Reagents and chemicals.

All chemicals used were of analytical reagent-grade and doubly distilled water was used throughout the study.

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), 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.

Dinitrophenol (0.1%): Prepared by dissolving 0.1 g of dinitrophenol (S.D. Fine Chem Ltd, Mumbai, India) in 100 ml of chloroform and used for the assay in method A.

Picric acid (0.025%): Prepared by dissolving 0.025 g of picric acid (S.D. Fine Chem Ltd, Mumbai, India) in 100 ml of chloroform and used for the assay in method B.

Sodium hydroxide (1.0 M): Accurately weighed 4 g of the pure NaOH (Merck, Mumbai, India) was dissolved in water, the solution was made up to 100 ml with water.

Preparation of CQP base (CRQ) solution

Into a 125 ml separating funnel, an accurately weighed 32.5 mg of pure CQP was transferred and dissolved in about 30 ml of water, and the solution rendered alkaline by adding 5 ml of 1 M NaOH and the

content was shaken for 5 min. The free base (CRQ) formed was extracted with three 20 ml portions of chloroform, the extract was passed over anhydrous sodium sulphate and collected in a 100 ml volumetric flask. The volume was made up to mark with chloroform and the resulting solution (200 µg ml^{-1} CRQ) was further diluted with chloroform to get working concentrations of 100 and 50 µg mL^{-1} CRQ for method A and method B, respectively.

General procedures

Preparation of standard graph.

DNP method: Different aliquots (0.1, 0.25, 0.5……3.5 ml) of standard CQP solution (100 µg ml^{-1}) were accurately transferred into a series of 5 ml calibration flasks using a micro burette. One ml of 0.1% DNP solution was added to each flask and diluted to volume with chloroform. The content was mixed well and the absorbance was measured at 420 nm against a reagent blank.

PA method: Aliquots (0.1, 0.25, 0.5……..3.0 ml) of a standard CQP (50 µg ml^{-1}) solution were accurately transferred into a series of 5 ml calibration flasks. To each flask, 1 ml of 0.025% PA solution was added and the solution made up to volume with chloroform. The content was mixed well and the absorbance was measured at 430 nm against a reagent blank.

Standard graph was prepared by plotting the absorbance *versus* drug concentration, and the concentration of the unknown was computed from the respective regression equation derived using the Beer's law data.

Procedure for tablets dosage forms

Tablets: Twenty tablets were weighed and pulverized. The amount of tablet powder equivalent to 32.5 mg of CQP was transferred into a 100 ml volumetric flask containing 30 ml of water. The content was shaken well for 20 min. The resulting solution was filtered through Whatman No 42 filter paper and the filtrate was collected in to a 125 ml separating funnel. The salt was converted to free base as described earlier, CRQ solutions of concentrations 100 and 50 µg ml^{-1} for method A and method B, respectively, were prepared as described under the general procedure for pure drug, and a suitable aliquot was used for assay by applying procedures described earlier.

Injections: An aliquot of injection solution equivalent to 10 mg of base was measured accurately and transferred quantitatively to a separating funnel and diluted to 20 mL with water. The salt was converted to base and extracted with 5 × 10 mL portions of chloroform, the separated chloroform extracts were dried over anhydrous Na_2SO_4 and collected in a 50 mL standard flask. A convenient aliquot was then subjected to assay using the recommended procedures.

Suspension: A portion of the syrup containing 10 mg of CRQ was quantitatively transferred into a separating funnel, mixed and shaken with 20 mL of water and steps described under injections were then followed.

Procedure for placebo blank synthetic mixture analyses

A placebo blank containing lactose (20 mg), starch (40 mg), gum 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. A 20 mg of the placebo blank was accurately weighed and its solution prepared as described under procedure for 'tablets', and then subjected to analysis by following the general procedures.

To 30 mg of the placebo blank, 32.5 mg of CQP was added and homogenized, transferred to 100 ml volumetric flask, and the solution was prepared as described under "*Procedure for tablets*". The extract was diluted to 100 and 50 µg mL^{-1} levels then subjected to analysis by the procedures described above.

III. Results and Discussion

Absorption spectra

The reaction of chloroquine base (CRQ) as n-electron donor and the π-acceptors: DNP and PA result in the formation of yellow C-T complexes having absorption maxima at 420 and 430 nm, respectively (Figure 1). The respective blanks had negligible absorbance at this wavelength.

Reaction pathway

Charge-transfer complex is a complex formed between an electron-donor and an electron-acceptor and is characterized by electronic transition(s) to an excited state in which there is a partial transfer of electronic charge from the donor to the acceptor moiety. As a result, the excitation energy of this resonance occurs very frequently in the visible region of the electro-magnetic spectrum [50]. This produces the usually intense colors

characteristic for these complexes. Therefore, CRQ, a nitrogenous base, a n-donor, was made to react with DNP or PA to form a coloured charge transfer complex in chloroform.

DNP and PA were earlier used for the determination of some amine derivatives through formation of intense yellow coloured complexes [68,69,73,74]. When an amine is reacted with a polynitrophenol, one type of force field produces an acid-base interaction, and the other, an electron donor-acceptor interaction. The former interaction leads to the formation of true phenolate by proton-transfer, and the latter, to a true molecular compound by charge-transfer [70]. The explanation for the produced color in both methods lies in the formation of complexes between the pairs of molecules CRQ-DNP and CRQ-PA, and this complex formation leads to the production of two new molecular orbitals and, consequently, to a new electronic transition [75].

The interaction between CRQ (D), an n-donor, and nitrophenols (A), π-acceptors, is a charge transfer complexation reaction followed by the formation of radical ion [76] according to the Scheme 1.

$$D^{\cdot\cdot} + A \rightarrow [D^{\cdot\cdot} \rightarrow A] \rightarrow D^{\cdot+} + A^{\cdot-}$$

[Donor + Acceptor → Complex → Radical anion, which is coloured]

Optimization of reaction conditions

Choice of solvent

Several organic solvents such as chloroform, dichloromethane, 1,2-dichloroethane were tried for the extraction of base form of CQP. Only chloroform favored the quantitative extraction of the drug in its base form. In order to select a suitable solvent for preparation of the reagent solutions used in the study, the reagents were prepared separately in different solvents such as chloroform, acetonitrile, acetone, 2-propanol and dichloromethane, and the reaction of CRQ with DNP or PA was followed. The chloroform solvent was found to be the ideal solvent for preparation of DNP and PA reagents. Similarly, the effect of the diluting solvent was studied and the results showed that the ideal diluting solvent to achieve maximum sensitivity was chloroform in both methods.

Effect of reagent concentration

The optimum concentration of the reagent required to achieve maximum sensitivity of the developed colored species in each method was ascertained by adding different amounts of the reagent to a fixed concentration of CRQ. The results showed that 1.0 ml of 0.1% DNP or 0.025% PA solution was optimum for the production of maximum and reproducible color intensity (Figure 2).

Effect of reaction time and stability of the C-T complexes

The optimum reaction times were determined by measuring the absorbance of the complex formed upon the addition of reagent solution to CRQ solution at room temperature. The reaction in both methods was instantaneous. The absorbance of the resulting C-T complexes remained stable for at least 45 and 90 min, in DNP method and PA method, respectively.

Composition of the C-T complexes

The composition of the C-T complex was established by Job's method of continuous variations [77] using equimolar concentrations of the drug and reagents (6.25×10^{-4} M in DNP method, 8.99×10^{-4} M in PA method). Five solutions containing CRQ and the reagent (DNP or PA) in various molar ratios with a total volume of 5 ml in both the methods were prepared. The absorbance of solutions was subsequently measured at 420 or 430 nm. The graphs of the results obtained (Figure 3) gave a maximum at a molar ratio of $X_{max} = 0.5$ in both the methods which indicated the formation of a 1:1 C-T complex between CQP and reagent DNP or PA.

Method validation

Linearity and sensitivity

Under the optimized experimental conditions, the standard calibration curves for CQP with DNP and PA were constructed by plotting absorbance *versus* concentration **(**Figure 4**)**. The linear regression equations were obtained by the method of least squares and the Beer's law range, molar absorptivity, Sandell's sensitivity, correlation coefficient, limits of detection and quantification for both methods are calculated according to ICH guidelines [78] and are summarized in Table 1.

Accuracy and precision

In order to determine the accuracy and precision of the proposed methods, pure drug (CRQ) solution at three different concentration levels (within the working range) were prepared and analyzed during the same day (intra-day precision) and on five consecutive days(inter-day precision) and the results are presented in Table 2.

Selectivity

The recommended procedures were applied to the analysis of placebo blank and the resulting absorbance readings in both methods were same as that of the reagent blank, confirming non interference from the placebo. The analysis of synthetic mixture solution prepared as described earlier yielded percent recoveries of 99.8±1.13 and 99.1±1.06 (n=5) for method A, and method B, respectively. The results of this study showed that the inactive ingredients did not interfere in the assay indicating the high selectivity of the proposed methods and its utility for routine determination in pure drug and in tablets form

Robustness and ruggedness

To evaluate the robustness of the methods, an important experimental variable i.e., volume of reagent in both the methods were altered incrementally and the effect of this change on the absorbance of the C-T complexes was studied. The results of this study are presented in Table 3 and indicated that the proposed methods are robust. Method ruggedness was evaluated by performing the analysis following the recommended procedures by three different analysts and on three different cuvettes by the same analyst. From the %RSD values presented in Table 3, one it can be concluded that the proposed methods are rugged.

Application to tablets

The proposed methods were applied to the determination of CQP in dosageforms and the results are compiled in Table 4. The results obtained were statistically compared with those obtained by the reference method [3], by applying the Student's t-test for accuracy and F-test for precision at 95% confidence level. The reference method involved the potentiometric titration of the drug with perchloric acid. As can be seen from the Table 4, the calculated t- and F- values at 95% confidence level did not exceed the tabulated values for four degrees of freedom. This indicates that there are no significant differences between the proposed methods and the reference method with respect to accuracy and precision.

Recovery studies

To further ascertain the accuracy of the proposed methods, a standard addition procedure was followed. To a fixed amount of pre-analyzed tablet powder, syrup or injection solution pure drug at three different levels was added. The total was found by the proposed methods. The determination at each level was repeated three times and the percent recovery of the added standard was calculated. Results of this study presented in Table 5 reveal that the accuracy of methods was unaffected by the various excipients present in the formulations.

IV. Conclusions:

The two visible spectrophotometric methods developed were validated for quantitative determination of CQP. Both methods are simple, rapid, selective and sensitive and show good linearity, precision, accuracy and robustness. The methods were completely validated showing satisfactory data for all the validation parameters tested as per the ICH guidelines . Unlike many reported spectrophotometric methods, the present methods are free from such limitations as pH control extraction step, and longer analysis time. The methods are featured by wide linear dynamic ranges and high sensitivity compared to several published methods as indicated in Table-6. Thus, the methods can be used for the assay of CQP in bulk and dosage forms.

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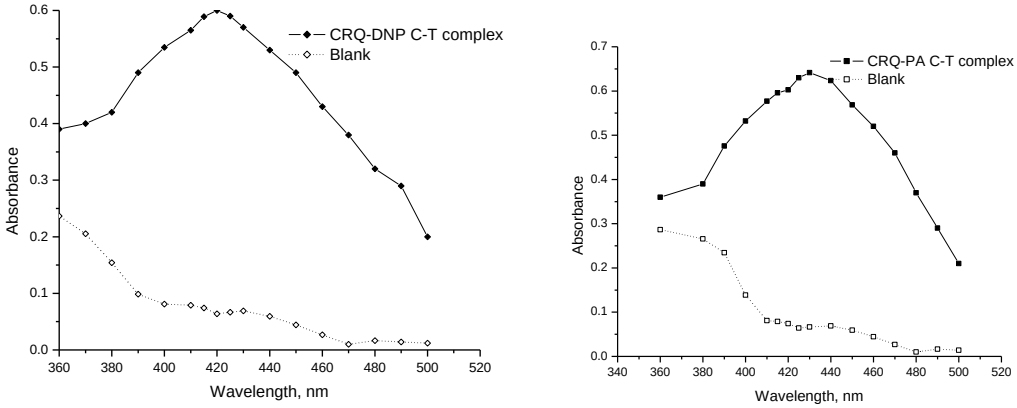


(a) (b)

Figure 1: Absorption spectra of: (a) CRQ(40 µg ml^{-1})-DNP; (b) CRQ(20 µg ml^{-1})-PA CT complexes.

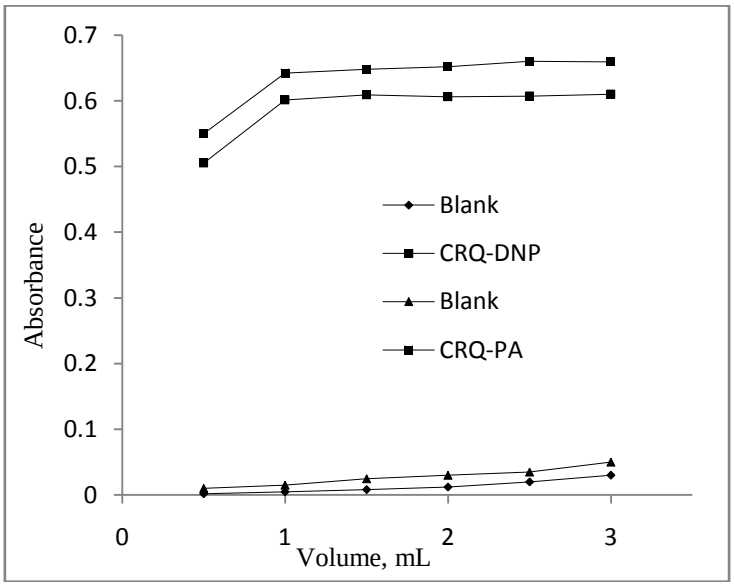
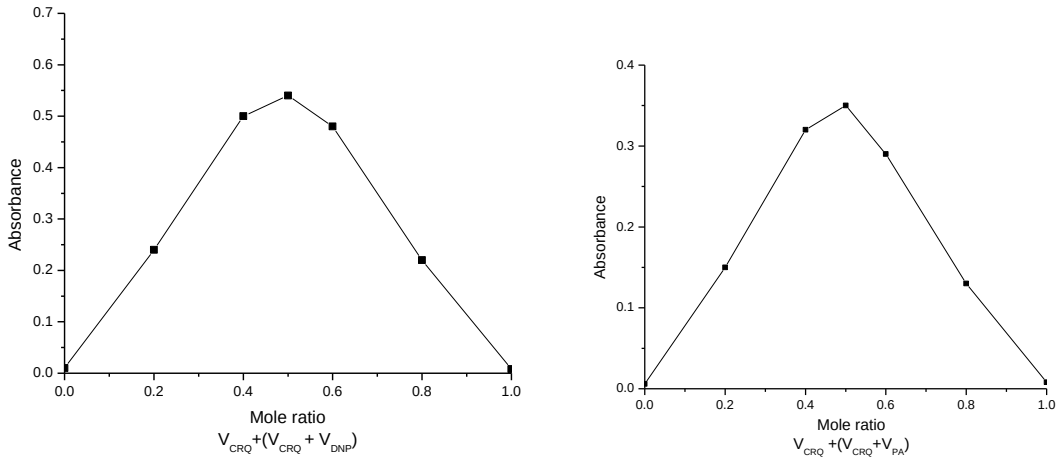
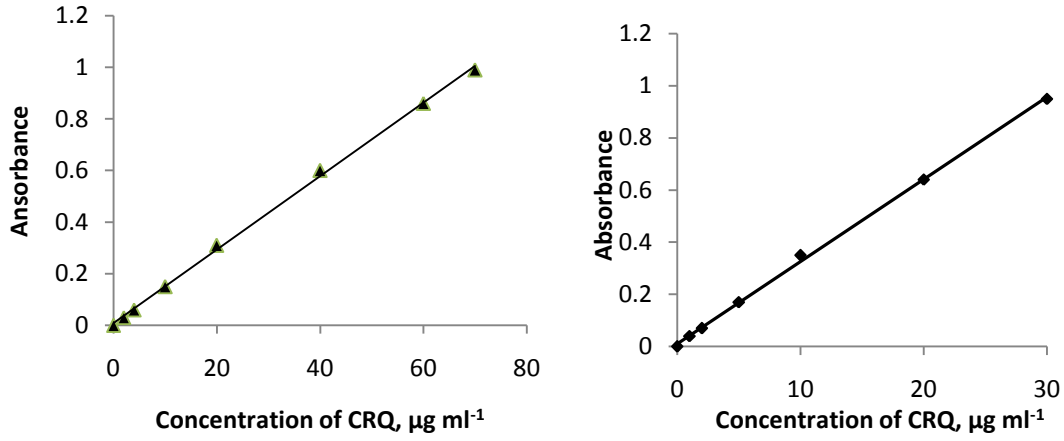


Figure 2: Effect of reagent concentration on the formation of: CRQ-DNP complex, 40 µg ml^{-1} CRQ) and CRQ-PA complex, 20 µg ml^{-1} CRQ



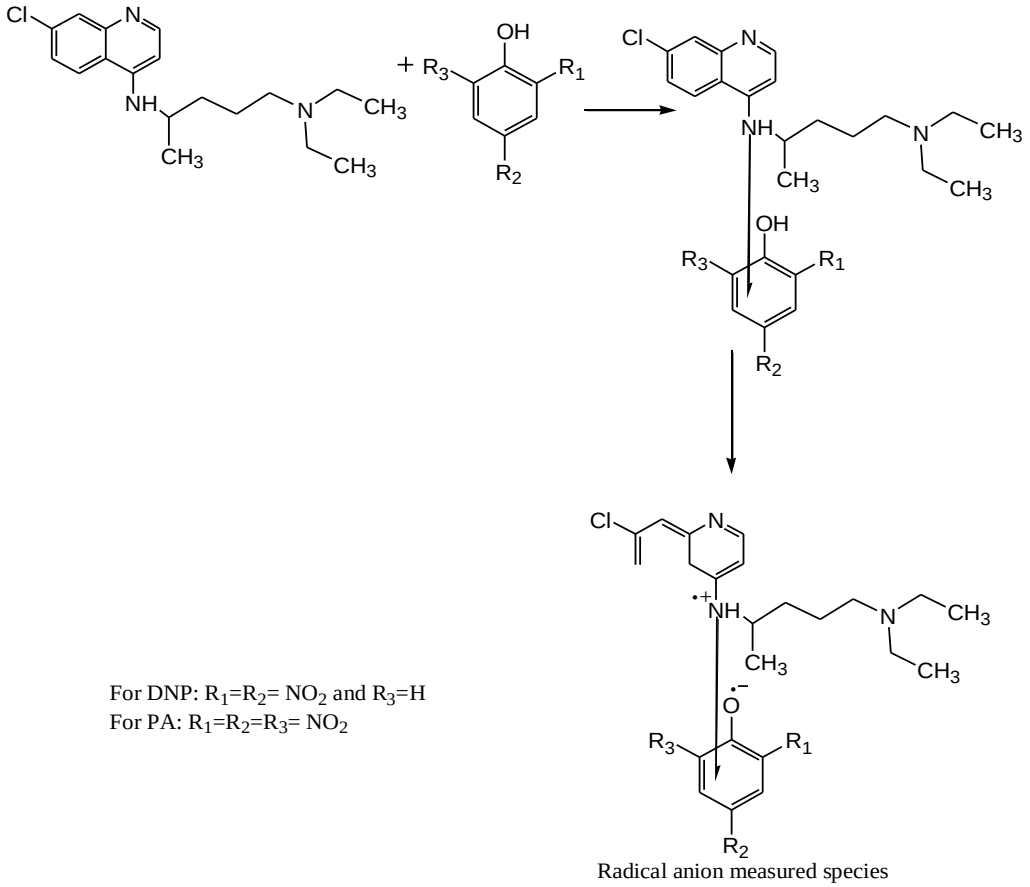
(a) (b)

Figure 3 Job's continuous variations plots: a) CRQ-DNP and b) CRQ-PA CT complexes



(a) (b)

Figure 4 Calibration curves for the spectrophotometric determination of CQP using DNP (a) and PA (b) as chromogenic agents.



Scheme 1 Possible reaction pathway for the formation of C-T complex between drug (CRQ) and DNP or PA.

Table 1 Sensitivity and regression parameters

Parameter	DNP method	PA method
$\lambda_{max,}$ nm	420	430
Color stability, min	45	90
Linear range, µg ml^{-1}	3 - 70	1.2 - 30
Molar absorptivity(ε), l $mol^{-1}cm^{-1}$	4.7×10^{3}	1.1×10^{4}
Sandell sensitivity*, µg cm^{-2}	0.0673	0.0313
Limit of detection (LOQ), µg ml^{-1}	3.0	1.2
Limit of quantification (LOD), µg ml^{-1}	1.1	0.3
Regression equation, Y**		
Intercept (a)	0.0085	0.0147
Slope (b)	0.0142	0.0313
Regression coefficient (r)	0.9994	0.9995

*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 1.0 cm^2 and l = 1.0 cm.

Y^{**}=a+bX, where Y is the absorbance and X concentration in µg ml^{-1}

Table 2 Results of intra-day and inter-day accuracy and precision study

Method	CRQ taken µg ml^{-1}	Intra-day accuracy and precision (n=5)			Inter-day accuracy and precision(n=5)		
		CRQ found µg ml^{-1}	%RE	%RSD	CRQ found µg ml^{-1}	%RE	%RSD
DNP	20	19.5	2.45	1.92	20.45	2.28	2.26
	40	40.75	1.89	1.5	40.5	1.26	1.64
	60	59.44	0.91	1.37	60.92	1.54	1.34
PA	10	9.88	1.18	1.25	9.83	1.69	1.73
	20	20.17	0.86	0.96	20.21	1.08	1.21
	30	29.6	1.31	1.14	29.52	1.57	1.25

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

Method	CRQ taken µg ml^{-1}	Robustness[a] (%RSD)	Ruggedness	
			Inter-analysts (%RSD), (n=4)	Inter-cuvettes (%RSD), (n=4)
DNP	20	1.26	1.56	2.53
	40	1.21	0.84	3.08
	60	1.17	1.72	2.68
PA	10	1.34	0.76	2.98
	20	1.28	1.26	2.62
	30	1.23	1.01	3.12

DNP,PA volumes used were 0.8, 1.0 and 1.2 ml

Table 4 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 methods	
			DNP method	PA method
Cadiquin (Tablet)	200 mg/tablet	98.56±1.36	98.04±1.86 **t**=0.50 **F**=1.87	99.38±2.51 **t**= 0.64 **F**= 3.41
Maliago (Tablet)	500 mg/tablet	98.56±1.36	100.3±1.04 **t**=2.27 **F**=1.71	99.48±0.95 **t**=1.24 **F**=2.05
Cloquin (Injection)	40 mg/mL	98.56±1.36	99.48±0.98 **t**=1.22 **F**=1.93	97.87±1.64 **t**=0.72 **F**=1.45
Emquin (suspension)	100 mg/10 mL	98.56±1.36	96.37±1.28 **t**=2.62 **F**=1.13	97.66±1.72 **t**=0.92 **F**=1.60

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.

Table 5 Results of recovery study by standard addition method

	DNP method				PA 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**	**CQP in dosage fom µg ml^{-1}**	**Pure CQP added µg ml^{-1}**	**Total found µg ml^{-1}**	**CQP recovered Percent ± SD**
Cadiquin (Tablet)	19.87	10	30.11	100.8±0.84	9.8	5	14.67	99.16 ± 1.78
	19.87	20	39.23	98.40±2.58	9.8	10	20.09	101.5 ± 1.31
	19.87	30	49.52	99.31±1.03	9.8	15	24.46	98.69± 1.24
Maliago (Tablet)	20.06	10	29.81	99.17 ± 1.25	9.9	5	14.81	99.40±1.25
	20.06	20	39.51	98.63 ± 1.56	9.9	10	20.16	101.3±1.58
	20.06	30	49.22	98.32± 1.72	9.9	15	24.66	99.04±1.63
Cloquin (Injection)	19.90	10	30.05	100.50 ± 1.46	9.8	5	14.62	98.78±1.55
	19.90	20	39.81	99.77 ± 1.65	9.8	10	20.11	101.6±1.85
	19.90	30	50.25	100.70± 1.85	9.8	15	25.21	101.7±1.35
Emquin (suspension)	19.27	10	29.63	101.23 ± 1.36	9.8	5	14.71	99.39±1.26
	19.27	20	39.15	99.69 ± 1.90	9.8	10	20.15	101.8±0.91
	19.27	30	49.44	100.35± 1.45	9.8	15	25.16	101.45±1.11

Table 6: Comparison of performance characterics of the proposed methods with the existig methods.

S.No.	Reagent	Methodology	Linear range (µg mL^{-1}) (Є = 1 mol^{-1} cm^{-1})	Remarks	Reference
1	BTB	Drug-dye ion-pair was extracted into $CHCl_3$ and measured at 410 nm.	30 - 150	Strict pH control, tedious and time-consuming extraction step.	19
2	BTB	Drug-dye ion-pair was extracted into $CHCl_3$ and measured at 410 nm.	--	-do-	33
3	BCP	Drug-dye ion-pair was extracted into $CHCl_3$ and measured at 420.	1.25 - 8.75	-do-	34
4	RB	Drug-dye ion-pair was extracted into $CHCl_3$ and measured.	--	-do-	35
5	Co-SCN	Drug-reagent ion pair extracted into nitrobenzene and measured at 625 nm.	2 - 60	-do-	36
6	Co-SCN	Drug-reagent ion pair extracted into isobutyl methyl ketone and measured at 625 nm.	--	-do-	37
7	Mo-SCN	Drug-reagent ion-pair extracted into CH_2Cl_2 and measured	2 - 42	-do-	38
8	Mo-SCN	Drug-reagent ion-pair was extracted into benzene and measured at 465 nm	2 - 20	-do-	39
9	TBPE	Drug-dye ion-pair complex extracted into dichloromethane and measured at 530 nm.	1.6×10^{-6} - 8×10^{-6} M	-do-	40
10	Chloranilic acid	CT complex measured at 520 nm	0.8 - 8	Strict pH control	41
11	Chloranil	CT complex was measured at 450 nm			42
12	DDQ	CT complex measured at 462 nm.	5 - 53 (6.1×10^3)	Moderately sensitive	43
	I_2	CT complex measured at 387 nm	1 - 15 (9.92×10^3)	Measurement at lower analytial wavelength	
13	$KBrO_3$-KBr	Brominated product in H_2SO_4 medium measured at 350 nm.	40 - 200	Longer reaction time, less sensitive	22
14	NBS	Brominated product in acetic acid medium measured at 410nm	10 - 80	Narrow linear range, less sensitive	44

15	Ammonium molybdate-$SnCl_2$	Molybdinum blue formed extracted into isobutanol and measured at 520 nm.	--	--	45
16	$KIO_3/KBrO_3$	Absorbance as a function of time measured at 342/343 nm.	0.2 - 2.0 0.5 - 5.0	Critically dependent on temperature ionic strength and reactants concentration	
17	**DNP**	**Drug-dye ion pair in CHCl3 measured at 420 nm.**	**3 – 70 (CRQ) 4.7×10^3**	**No pH adjustment, no extraction, instantaneous reaction, wide linear dynamic range and fairly sensitive**	**This work**
	PA	**Drug-dye ion pair in CHCl3 measured at 430 nm.**	**1.2– 30 (CRQ) 1.1×10^4**		

Note: BTB-Bromothymol blue; BCP-Bromocresol purple; RB- Rose bengal; TBPE- tetrbromophenolphthalein ethylester; DDQ- dichloro-dicyano-p-benzoquinone; NBS- N-bromosuccinimide; DNP-2,4-dinitrophenol; PA- picric acid.