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## Validated UV-Visible spectrometry using water as a solvent for determination of chloroquine in tablet samples

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Reported methods including the official methods for determination of chloroquine in tablet samples use carcinogenic organic solvents. In this study, UV-Vis spectrometry using water as a solvent was developed for determination of chloroquine phosphate in pharmaceutical tablet dosage forms. The method was validated according to the International Conference on Harmonization (ICH) and USP guidelines. The absorbance of chloroquine phosphate in distilled water at  $\lambda_{\max}$  of 343 nm showed linear dependence on concentration in the range 10.88-30.56  $\mu\text{g mL}^{-1}$  with determination coefficient of 0.99972. Recovery results in the range 98.79–101.20% and low coefficient of variation values for intra-day and inter-day precisions (0.37% and 0.76%, respectively) showed the accuracy and reproducibility of the method. The method was used for determination of chloroquine phosphate in tablet formulations of different brands. Results in the range 100.63–103.52% of the labeled chloroquine phosphate in tablet formulations confirmed the applicability of the developed method for real sample analysis. Hence, the developed UV-Vis method using environmentally friendly water as a solvent, with an excellent accuracy and precision showed that the developed method can be a potential substituent for the official reference methods.

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**Capsule Summary:** UV-Vis spectrophotometry using water as a solvent showed an excellent precision and accuracy for determination of chloroquine in pharmaceutical formulations.

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### INTRODUCTION

Chloroquine phosphate (CQ) is an anti-malarial drug, which is absorbed rapidly and almost completely through gastrointestinal tract (Patel et al., 2005). It is the proto-type synthetic anti-malaria drug most widely used to treat all

types of malaria infections, and when administrated orally; it is usually well tolerated and effective (Ali et al, 2009).

The problem of malaria as a public health concern has continued to be highlighted by the high morbidity and mortality, and negative impact on socio-economic development in Africa (Chaulet et al., 1994; Trape, 2001; Celestino et al., 2003). It is a serious disease that can cause

death if not treated right away. It is caused by a parasite that can infect a certain type of mosquito which feeds on humans (Taneja et al., 2013). The outcome of treatment in malaria depends on the right diagnosis, selection of the right drug and its efficacy, correctness of the advice given to the patient and compliance of the patient (Bajpai and Jyoti, 2006; Anbarasan et al., 2013).

Chloroquine phosphate has marked and rapid schizontocidal activity against all infections of Plasmodium malariae and Plasmodium ovale and against Chloroquine-sensitive infections of Plasmodium falciparum and Plasmodium vivax. It is also gametocytocidal against Plasmodium vivax, Plasmodium malariae, and Plasmodium ovale as well as immature gametocytes of Plasmodium falciparum (Lawal et al., 2012). The drug is also indicated for the treatment of extra intestinal amoebiasis (Patel et al., 2005).

While the official British Pharmacopeia (BP) method for determination of chloroquine phosphate from pharmaceutical tablet formulation is potentiometric titration (Nelson et al., 2010; BP, 2013), the official United State Pharmacopeia (USP) method is UV-Vis spectrophotometric method (USP, 2007). Both official methods require extraction of chloroquine base from tablet samples using chloroform which is expensive and carcinogenic chemical besides lengthy procedure for sample treatment prior to analysis.

On top of the reference official methods, many analytical methods; Spectrofluorimetry (Vogel and Konigk, 1975; Adelusi and Salako, 1980; Anbarasan et al., 2013), Spectrophotometry (Patel et al., 2005; Ofokansi et al., 2009; Aitha, 2013), HPLC (Vogel and Konigk, 1975; Chaulet et al., 1993; Abdelrahman et al., 1994; Walker and Ademowo, 1996; Paci et al., 2002; Patel et al., 2005; Samanidou et al., 2005), Gas chromatography (Bergqvist and Eckerbom, 1984), and Calorimetry (Kaseje and Brandling-Bennett, 1988) have been reported for determination of chloroquine phosphate from different dosage forms.

Both the official methods and the other reported methods are all time consuming, environmental unfriendly, and cost-ineffective. Moreover, many of the reported methods involve several steps making them tedious and time consuming for routine quality control analysis of the drug from its pharmaceutical formulations in manufacturing and regulatory laboratories. The solvents and reagents these methods use are expensive and/or environmentally hazardous. Thus, development of simple, environmentally friendly, and cost-effective method that could be used for the determination of chloroquine in complex matrix is vital.

The aim of this work, therefore, is to develop a validated simple, time saving, environmentally friendly, and cost-effective UV-Vis spectrophotometric analytical method for the quantitative determination of chloroquine phosphate in its pharmaceutical tablet formulations using water as a solvent.

## Material and Methods

### Chemicals

All reagents and chemicals used were of analytical grade which were used without further purification. Hydrochloric acid (36.5% w/v, Sigma-Aldrich, Germany) and Sulphuric acid (95-98%, Himedia laboratories Pvt. Ltd, India) were used. The active pharmaceutical ingredient in the tablet formulation, standard chloroquine phosphate (99.21%, B# 3100C3RJ; IPCA laboratories Ltd, India), the inactive pharmaceutical ingredients in the tablet formulation; Maize Starch (B# STEX1408373; Devats, India ), Magnesium stearate (B# 20131109; Huzhou Zhammong pharmaceutical Co. Ltd, China) and purified Talc (B# 177; Neelkanth Mine Chem., India) were used.

### Instruments

A double-beam UV-Vis spectrophotometer (UV-1700 Shimadzu, Japan) was used for absorbance measurement.

### Solution preparation

**Standard solution:** A 3.40 mg mL<sup>-1</sup> stock solution of chloroquine phosphate in water was prepared by taking 0.34 g of standard chloroquine phosphate in 100 mL volumetric flask. About 60 mL of distilled water was first added and swirled for 5 minutes until the chloroquine phosphate is totally dissolved and finally diluted with the same solvent up to the mark. A clear solution of chloroquine phosphate in distilled water was obtained after filtration. 100 mL of 340 µg mL<sup>-1</sup> intermediate standard solution of chloroquine phosphate in distilled water was prepared from the filtered stock solution.

Working standard solutions of different concentrations (10.88, 12.24, 13.60, 14.96, 16.32, 21.24, and 30.56 µg mL<sup>-1</sup>) were prepared from the intermediate standard solution by serial dilution with distilled water.

**Tablet sample solution:** Chloroquine phosphate tablet samples of different brands (B#16968; APF, and B#3080723; EPHARM, both Ethiopian brands) labeled as 250 mg/tablet were purchased from local pharmacies. Twenty tablets of each brand were accurately weighed and powdered using a clean mortar and pestle. For each brand, a quantity of the powder containing 0.34 g of chloroquine phosphate according to the label was transferred to 100 mL volumetric flask, dissolved in about 60 mL of distilled water and then diluted up to the mark with distilled water. A clear solution of tablet chloroquine phosphate in distilled water was obtained by filtration. A working tablet solution of 13.60 µg mL<sup>-1</sup> was prepared by dissolving 10 mL of the filtrate in 100 mL of distilled water.

A synthetic mixture was prepared by mixing appropriate amounts of the active pharmaceutical substance (chloroquine phosphate) and the In-active pharmaceutical substances (excipients). The sample solution of the synthetic mixture was prepared in the same way as tablet sample.

### Analytical method

In order to determine the wave length of maximum absorbance ( $\lambda_{max}$ ) of chloroquine phosphate, 13.60 µg mL<sup>-1</sup> of the standard solution was scanned in the wave length range

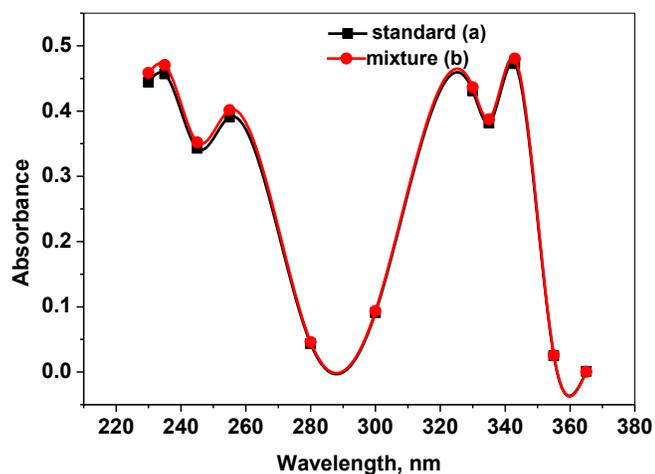


Fig. 1: UV-Vis spectrum

400 to 230 nm against distilled water as a blank. The wavelength corresponding to maximum absorbance ( $\lambda_{\max}$ ) was found to be 343 nm which was used as the working wavelength ( $\lambda_{\max}$ ) for determination of chloroquine phosphate in pharmaceutical formulations.

The chloroquine phosphate content in two brands of tablet samples and synthetic tablet sample was determined spectrophotometrically using water as a solvent. For comparison purpose, the marketed tablet samples and laboratory prepared sample were analyzed following the standard reference procedure (USP, 2007). Briefly: twenty 250 mg labeled tablets of each brand were weighed and finely powdered. An accurately weighed portion of the powder, equivalent to about 800 mg of chloroquine phosphate, was transferred to a 200 mL volumetric flask to which about 100 mL of distilled water was added and swirled mechanically for about 20 minutes. Distilled water was then added up to the mark, mixed, and then filtered using Whatman filter paper. 50 mL of the clear filtrate was pipette out into 250 mL separatory funnel to which 5 mL of 6 N ammonium hydroxide was added. After agitation, chloroquine phosphate was extracted using five successive 25 mL portions of chloroform. The combined chloroform extract was washed with 10 mL of water, and the washing water was further extracted with 10 mL of chloroform. The combined chloroform extract was evaporated on a steam bath to about 10 mL, then added 50 mL of dilute hydrochloric acid (1 in 250), and continued heating on the steam bath until the odor of chloroform was no longer perceptible. The solution was transferred to a 200 mL volumetric flask, the evaporating vessel was washed with portions of dilute hydrochloric acid (1 in 1000), the washings was added to the volumetric flask and diluted stepwise up to the mark with dilute hydrochloric acid (1 in 1000) to obtain an estimated concentration of  $10 \mu\text{g mL}^{-1}$ . In the same manner, a standard sample solution was prepared by dissolving an accurately weighed, 400 mg, of USP chloroquine phosphate reference substance (purity = 99.21%) in dilute hydrochloric acid (1 in

1000), and diluted quantitatively and stepwise with the same dilute hydrochloric acid to obtain a standard solution of  $10 \mu\text{g mL}^{-1}$  concentration. Concomitantly, the absorbance of both solutions was determined in 1cm cell at the maximum absorbance wavelength (343 nm) using dilute hydrochloric acid (1 in 1000) as the blank. The amount of chloroquine phosphate in the dosage formulations was calculated and recorded.

### Validation of the proposed method

After selection of the suitable solvent and the wave length of maximum absorbance, the developed method was validated for the parameters; specificity, linearity, precision, accuracy, limit of detection and quantification, robustness / ruggedness and stability of analyte solution as per the International Conference on Harmonization (ICH)<sup>24</sup> and USP<sup>12</sup> guidelines.

## RESULTS AND DISCUSSION

### Selection of suitable solvent

Solubility of a drug is one of its important physico-chemical properties (Abolghasem and Mohammad, 2016). The solvent system should provide homogeneous solution where by all the solute substance under investigation is completely dissolved. The same concentrations of chloroquine phosphate were prepared using three different solvents (distilled water, 0.1 M HCl and 0.05 M  $\text{H}_2\text{SO}_4$ ) and measured spectrophotometric absorbances are summarized in Table 1.

As can be seen from the table, the mean absorbance of the analyte in distilled water is reasonable large with an improved precision than in the other solvents making distilled water as a best candidate solvent for the determination of chloroquine phosphate using UV-Vis spectrophotometer.

### Method validation

**Interference study:** In this study, the effect of excipients such as talc, starch, and magnesium stearate in the determination of chloroquine phosphate was investigated. Two solutions; one containing only chloroquine phosphate and the other containing a mixture of chloroquine phosphate and the excipients (placebo) both with similar chloroquine phosphate concentration were prepared. The UV-Vis absorbance for the two samples was recorded by scanning between 230 and 365 nm. The spectra were compared in terms of both the  $\lambda_{\max}$  and absorbance intensity (Fig. 1). As can be seen from the Figure, presence of the excipients (curve b of figure 1) did not show any additional  $\lambda_{\max}$  nor significantly affect the absorbance intensity of the analyte at its characteristic  $\lambda_{\max}$  (343 nm). The presence of the potential interferents (excipients) caused an error of less than 2% which still is tolerable (Tulasamma and Venkateswarlu, 2012). Therefore, the proposed UV-Vis

**Table 1:** Selection of suitable solvent for UV-Vis spectrophotometric determination of chloroquine phosphate

Solvent	Absorbance			Mean	RSD (%)
	Trial-1*	Trial-2*	Trial-3*		
Distilled water	0.4827	0.4814	0.4829	0.4823	0.166
0.1 M HCl	0.4778	0.4778	0.4799	0.4785	0.251
0.05 M H <sub>2</sub> SO <sub>4</sub>	0.4941	0.4956	0.4974	0.4957	0.343

\*average of three determinations

**Table 2:** Precision studies of the developed method for determination of chloroquine phosphate

Sample No.	Repeatability	Intra-day Precision	Inter-day precision		
			Day-1	Day-2	Day-3
1	0.4742	0.4807	0.4852	0.4858	0.4916
2	0.4734	0.4855	0.4856	0.4880	0.4888
3	0.4745	0.4823	0.4877	0.4855	0.4907
4	0.4742	0.4806	0.4836	0.4889	0.4946
5	0.4740	0.4816	0.4836	0.4860	0.4943
6	0.4734	0.4819	0.4851	0.4863	0.4950
Mean	0.4740	0.4821	0.4851	0.4868	0.4925
SD*	0.0005	0.0018	0.0015	0.0014	0.0025
RSD** (%)	0.1056	0.3733	0.3092	0.2876	0.5076

\* standard deviation; \*\* relative standard deviation within a day

method for determination of chloroquine phosphate using water as a solvent showed its potential applicability for determining chloroquine phosphate in tablet formulations without significant interference from the drug matrix.

**Linearity:** Five standard concentrations of chloroquine phosphate in distilled water (10.88, 12.24, 13.60, 14.96, 16.32, 21.24, and 30.56  $\mu\text{g mL}^{-1}$ ) in the range 80-120% of the analyte in the tablet (Gandhimathi et al., 2012) were

prepared. For each standard solution, triplicate absorbances at 343 nm against distilled water as a blank were recorded. Mean absorbance showed linear dependence on the concentration of chloroquine phosphate in the range 10.88–30.56  $\mu\text{g mL}^{-1}$  with a linear regression equation and determination coefficient ( $R^2$ ) of  $A = 0.3828C$  ( $\mu\text{g mL}^{-1}$ ) - 0.0391 and 0.99972, respectively.

**Table 3:** Recovery results of spiked chloroquine phosphate from pharmaceutical formulations

Sample No.	added ( $\mu\text{g mL}^{-1}$ )	Measured Absorbance	Found ( $\mu\text{g mL}^{-1}$ )	Mean*( $\mu\text{g mL}^{-1}$ ) ± SD	%Recovery ± RSD
1	6.8	0.2336	6.90	6.87± 0.05	100.98 ± 0.72
		0.2330	6.89		
		0.2302	6.81		
2	13.6	0.4891	13.76	13.76±0.03	101.20 ± 0.19
		0.4882	13.74		
		0.4902	13.79		
3	20.4	0.7284	20.18	20.15± 0.02	98.79 ± 0.11
		0.7269	20.14		
		0.7267	20.14		

\* average of three determinations; SD standard deviation; RSD % relative standard deviation

**Table 4:** Chloroquine phosphate content in APF (tablet-1), EPHARM (tablet-2) brands of tablet formulations and synthetic tablet samples using the proposed and USP official methods

Sample	Proposed method*	Official (USP) method*	**	**
	Assay (%) ± SD	Assay (%) ± SD	t-test	F-test
Tablet-1	101.27 ± 0.22	101.40 ± 0.24	0.694	1.16
Tablet-2	103.52 ± 0.33	101.78 ± 0.12	1.74	8.26
Synthetic mixture	100.63 ± 0.24	101.18 ± 0.17	0.17	1.95

\*mean of three determinations; \*\*tabulated values of t and F at 95% are 4.30 and 19.0, respectively

**Table 5:** Comparison between the proposed and the reference spectrophotometric methods

Parameters	Proposed method	Reference method
Chemicals (solvent) used	Distilled water	$\text{CHCl}_3$ and dil. HCl
Total time of analysis	Max. 30 minutes	Min. 5 hours
Safety of the chemicals used	Environmentally friendly	Environmentally unfriendly
Cost	Cost-effective	Cost-ineffective

**Precision:** Precision of the method was evaluated as discussed under the experimental part. In all cases, six replicate measurements were made for a  $13.6 \mu\text{g mL}^{-1}$  standard chloroquine phosphate solution and the standard and percentage relative standard deviations (%RSD) for each were calculated. As can be seen from Table 2, the calculated coefficient of variation in the three cases (repeatability, intra-day, and inter-day results) was found to be within the acceptable limit range Satisfactorily low

coefficients of variation suggested high level of precision and hence suitability of the developed method for determination of chloroquine phosphate in pharmaceutical formulations using water as a solvent.

**Accuracy:** The accuracy of the proposed method for determination of chloroquine in pharmaceutical tablet samples was evaluated by performing recovery studies of standard chloroquine from three synthetic chloroquine

phosphate tablet formulation. 6.8, 13.6, and 20.4  $\mu\text{g mL}^{-1}$  concentration of standard chloroquine (50, 100 and 150% of the chloroquine phosphate tablet label) were prepared and mixed with the excipients. After recording triplicate absorbance measurements for each sample, the corresponding concentration was calculated using the formulated regression equation. As shown in Table 3, excellent recoveries in the range  $98.79 \pm 0.11 - 101.20 \pm 0.19\%$  indicated the accuracy of the developed method and hence its applicability for the determination of chloroquine phosphate in tablet formulations.

**Limit of detection (LOD) and Limit of quantification (LOQ):** Method LOD and LOQ were calculated using the standard deviation of replicate absorbance measurement ( $n = 7$ ) of a blank sample at wave length of 343 nm and the slope of the calibration curve for seven data points as 0.073, and  $0.220 \mu\text{g mL}^{-1}$ , respectively.

**Robustness and ruggedness;** The Robustness/Ruggedness of the developed method was evaluated by comparing the results for the same solution by two analysts. An extremely small variation (0.0012) between the mean results generated by the two analysts each with low %RSD (0.31 and 0.28%) (data not shown) indicated the Robustness / Ruggedness of the method.

**Stability of analyte solution:** The effect of analysis time on both the physical appearance and absorbance of the chloroquine phosphate tablet sample in water was evaluated. Analyses were conducted every 4 hours interval and absorbance results (data not shown) showed no time dependency indicating the stability of chloroquine phosphate in water.

Therefore, absorbance measurements of chloroquine phosphate solution using water as a solvent showed no significant dependence on the analysis time. Hence, the analyte solution was found to be stable up to 24 hours at normal laboratory working conditions with acceptable coefficient of variations (0.7044% and 0.7256% for standard and sample solutions, respectively).

#### ***Determination of chloroquine phosphate in pharmaceutical preparations***

The applicability of the proposed method for the determination of chloroquine phosphate in commercial tablet dosage forms was examined by analyzing marketed products and laboratory prepared sample (synthetic mixtures). The marketed Ethiopian tablet brands were chloroquine-250 mg tablet of Addis pharmaceutical factory (APF) and Ethiopian pharmaceutical manufacturing (EPHARM).

The assay analysis of the marketed tablet and the laboratory prepared synthetic mixture samples were quantitatively analyzed using the new proposed method as per the procedure described under the experimental section of this manuscript. Assay chloroquine phosphate

values for each sample were calculated taking the respective mean absorbance measured using the developed method and the USP official method (USP, 2007).

The results obtained for this analysis (Table 4) were statistically compared with the result obtained using the USP official method by calculating the statistical  $t$  &  $F$  values. As can be seen from the table, the experimental statistical values were less than the respective tabulated theoretical values confirming that the results obtained using the developed method are similar with the result obtained using the official method. Thus, the proposed method which is environmental friendly can be an excellent substituent method for the official method which uses organic solvent for the determination of chloroquine phosphate in pharmaceutical tablet dosage forms.

#### ***Comparison between the proposed and reference spectrophotometric methods***

Comparison between the developed method and the reference method was made in terms of the time needed for analysis and cost of analysis. The comparison showed that the proposed spectrophotometric method was both time and cost effective over the conventional extraction method. The total time required for the analysis of a sample following the proposed method was found to be much shorter and cheaper than the reference (extraction) method as shown in Table 5.

The developed UV-Vis spectrophotometric method uses 572 mL of distilled water for both sample and standard preparations as a solvent whereas the extraction method uses 125 mL of chloroform, 100 mL of distilled water and 720 mL of dilute HCl which makes them to differ greatly in their cost of analysis.

#### **CONCLUSIONS**

A simple UV-Vis spectrophotometric method using cheap and environmental friendly water as a solvent was developed for the determination of chloroquine phosphate in its pharmaceutical tablet dosage form. Its absorbance was measured at maximum absorbance of 343 nm against the corresponding reagent blank. The developed method is simple, precise, reproducible, accurate, specific, economic and less time consuming. The method has also been statistically evaluated and the results obtained are accurate, precise and free from the interferences of other additives or excipients present in the formulation. There was no interference from the excipients used in the tablet formulations and hence the method is suitable for analysis of formulated tablets.

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