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Application of Derivative Spectrophotometer for Analysis of Chloroquine Phosphate Dosage Forms

Siham Abdallah[1*], E. E. Kamal[2], M. E. M. Hagga[3] and Ishraqa Mohamed[4]

[1]Qassim University, KSA.
[2]University of Khartoum, Sudan.
[3]Khartoum Technology College, Sudan.
[4]National Drug Quality Control Laboratory, Sudan.

Authors' contributions

This work was carried out in collaboration between all authors. Author SA designed the study, wrote the protocol, performed the spectroscopy analysis and wrote the first draft of the manuscript. Authors EEK and MEMH managed the literature searches and analyses the results. Author IM managed the experimental process. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Afirst derivative spectrophotometric method was developed for the determination of chloroquine phosphate in formulations.
The aim of the present work is to develop and optimize a derivative spectrophotometric method for the analysis of chloroquine phosphate substance and to study of the expected interference of pharmaceutical excipent used in chloroquine phosphate formulations on the developed method, to employ the developed method for analysis of chloroquine phosphate in pharmaceutical dosage forms, and to compare between the developed method and the official methods for analysis of chloroquine phosphate formulations.
The zero order absorption and first derivative spectra were measured for chloroquine phosphate working standard and formulation using UV spectrophotometer.
The derivative procedure was based on the linear relationship between the concentration of

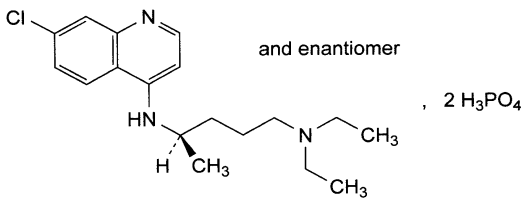
**Corresponding author: E-mail: siham99100@gmail.com;*

chloroquine phosphate and first order derivative amplitude at 225 nm, 239 nm, 260 nm, and 349 nm. The first derivative spectra were developed between 200 nm- 400 nm. The developed method was compared with high performance liquid chromatography (HPLC) and the official non-aqueous titration methods (BP and USP).
The results obtained showed good linearity and selectivity in the concentration range 2.5 -50 µg/ml. The developed method was successfully applied in the quantitative assay of commercial tablets, injections and syrup formulations.
The method was simple, rapid and suitable for quality control application.

Keywords: Chloroquine phosphate; derivative; spectrophotometer; absorbance; spectral selectivity; pharmaceutical dosage forms; interference; excipent.

1. INTRODUCTION

Chloroquine phosphate; is 4- aminoquinolone-derivative, has the following chemical structure [1].



7-chloro-4-(4-diethyl amino-1-methyl butylamino) quinolone; $C_{18}H_{26}ClN_3$, $2H_3PO_4$.

It also exists as chloroquine hydrochloride; $C_{18}H_{26}ClN_3$, 2HCl.

And chloroquine sulphate; $C_{18}H_{26}ClN_3$, H_2SO_4 H_2O.

It is a antimalarial drug and found effective against erythrocytic forms of *Plasmodium vivax, P. ovale* and *P. malariae*; it also used in the treatment of amebiasis, rheumatoid arthritis, discoid lupus erythematosus and photosensitive diseases [2].

Choroquine phosphate tablets and injections are official drugs available in both the British and the United State Pharmacopoeia. While chloroquine phosphate syrup is not official drugs [1,3].

The method of analysis of tablets and injections in both pharmacopoeias is non-aqueous titration. A high performance liquid chromatography (HPLC) method, which is not pharmacopeial, but a validated one is also available [4].

The technique of ultra violet-visible spectrophotometry is one of the most commonly used technique in pharmaceutical and biomedical analysis. It is employed in quantitative purposes and with certain limitations for characterization of drugs, impurities, metabolites and related substances.

The technique basically involves measurement the amount of a substance in solution absorbed by ultraviolet (190-380 nm) or visible (380-800 nm) radiation.

Spectral selectivity can be induced and/or enhanced by a number of chemicals or by instrumental technique such as difference, higher-derivative and dual wavelength spectrophotometry [5].

In derivative spectrophotometry the absorbance (A) of a sample is differentiated with respect to wavelength (λ) to generate the first, second, or higher order derivatives. In the context of derivative spectrophotometry, the normal absorption spectrum is referred to as the fundamental, zero order or ^0D spectrum [6].

The aim of the present work is to develop and optimize a derivative spectrophotometric method for the analysis of chloroquine phosphate substance and to employ the developed method for analysis of chloroquine phosphate in pharmaceutical dosage forms, to assess the selectivity of the developed method in presence of pharmaceutical excipent and to compare between the developed and the official methods for analysis of chloroquine phosphate formulations.

2. MATERIALS AND METHODS

2.1 Materials and Equipments

2.1.1 Materials

2.1.1.1 Authentic standard

Working standard of chloroquine phosphate was provided by Amipharma Laboratories, Sudan.

2.1.1.2 Pharmaceuticals preparation

The pharmaceutical formulations used included both branded and generic products, these include four tablet dosage forms (Amiquine tablets, Efroquine tablets, Lariago tablets and Chloroquine Phosphate tablets), two syrup dosage forms (Lariago syrup and Chioroquine Phosphate syrup) and two injectable (Balsaquine injections and Chloroquine Phosphate injections).

2.1.1.3 Chemical

Acetonitrile HPLC grade, Phosphoric acid and Sodium hydroxide.

2.1.2 Equipment

- **A double-beam UV-VIS. Spectrophotometer, Unicam Heyios,** model UVA 082017 with software and epson printer/Germany, was used in the quantitative analysis of different samples of chloroquine phosphate.
- **Syknm, High Performance Liquid Chromatograph** (HPLC) connected to a UV/Visible dector S3200, Laboratory Computing Integrator and S1121 pump / Japan was used for HPLC analysis.
- **Memotitrator** was used for non-aqueous titrations.

2.2 Methods

2.2.1 Calibration curve

- Standard stock solutions of chloroquine phosphate (1.0 mg ml^{-1}) were prepared by dissolving the chloroquine phosphate powder in: (1) Water and (2) 0.01 M HCI
- A calibration curve was constructed by assaying standard solutions of Chloroquine Phosphate in the range 2.5-50 µg/ ml in water and 0.01 M HCI.
- The zero order absorption, first derivative spectra and high performance liquid chromatography were measured for these concentrations.

2.2.2 Analysis of chloroquine phosphate in pharmaceutical dosage form

Samples of different dosage forms of chloroquine phosphate, that covered the 23 excipents used in chloroquine phosphate formulation were analyzed by dissolving a quantity equivalent to 100 mg of chloroquine phosphate of each formulation in 100 ml of water, 5 ml of this solution was diluted to 100 ml with water, the solution was analyzed using zero order absorption, first derivative spectra and high performance liquid chromatography.

The first derivative spectra were recorded over the range 200-400 nm with a speed of 1200 nm/min, band width 2 nm and medium smoothing to the curve.

The chromatographic procedure was carried out using a mobile phase consisting of a mixture of phosphate buffer (pH 3.0) and acetonitrile in the ratio (3:2). The mobile phase was filtered and degassed before use [4].

The phosphate buffer pH 3 was prepared by adding 7 ml of phosphoric acid (85%w/w) to 100 ml of water; 700 ml of water were then added, the pH adjusted to 3.0 using 10 M NaOH and the volume completed to 1000 ml with water.

The separation was carried out using a C18 column with a flow rate of 1.5 ml min^{-1}, at wavelength 349 nm.

2.2.3 Analysis of chloroquine phosphate in presence of excipent

The possibility of interference of excipent used on the analysis of chloroquine phosphate was investigated. This was carried out by accurately weighing aquantity of each excipent equivalent to the concentration in 100 mg chloroquine phosphate in the formulation in 100 ml volumetric flask and diluting to volume with water. Five ml of this solution were accurately transefered to 100 ml volumetric flask containing 5 ml of the stock standard chloroquine phosphate solution and the volume was completed with water. The resultant mixture was analyzed by the three methods.

3. RESULTS AND DISCUSSION

3.1 Spectrophotometric Method

Derivative spectroscopy has been widely applied in the analysis of different pharmaceutical dosage forms. It solves the problem of analysis associated with drug combinations, stability studies of drugs, degradation products, drug impurities, interference of excipent in drugs, topical preparations analysis like creams and ointments and it also employed for analysis of drugs in biological fluids.

Derivative spectroscopy has also been used for the analysis of binary mixtures as study done by Nevin Erk for analysis of lostran potassium and hydrochlorothiazide and the assay of ephedrine hydrochloride and theophylline in pharmaceutical formulations, [7,8].

3.1.1 Derivative spectrophotometry calibration curves

The calibration curves, obtained by plotting the value of first derivative versus concentration at wavelength 225 nm, 239 nm, 260 nm, 333 nm and 349 nm were measured (amplitude) for each concentration. The regression, intercept and slope were calculated for each wavelength. The results are shown in Table 1.

From the results obtained it is evident that there is no significant difference in linearity of chloroquine phosphate calibration curve in water and 0.01 M HCl. It was therefore decided to use water as the solvent in the analysis carried out in this work.

The chloroquine calibration curves were linear in all selected wavelengths. However, the best linearity was observed at wavelength 349 nm.

3.1.2 Absorbance spectrophotometry calibration curves

The calibration curves, obtained by plotting absorbencies values versus concentration at the wavelengths 221 nm, 236 nm, 256 nm, 331 nm and 343 nm, showed linear relationships in the concentration range 2.5-50 µg ml^{-1}.

The calibration curves were in agreement with the Beer Lamber's law.

The results obtained using water and 0.01 M HCl was shown in Table 2.

It seen from the results that there is no significant difference when water or 0.01 M HCl were used. So water was used as the solvent.

3.2 High Performance Liquid Chromatography Method

3.2.1 Calibration curve

A calibration curve was constructed by assaying standard solutions of chloroquine phosphate in the range 2.5-50 µg ml^{-1} in water. The peak height was plotted against concentration. The calibration graphs of the high performance liquid chromatography for chloroquine phosphate standard solutions gave an intercept of 1.75 X 10^{-2}, a slope of 2.59 X 10^{-1} and a regression of 0.999.

It was seen from Table 3 that; the linear relationship was obtained in the concentration range of 2.5-50 µg ml^{-1}.

The linearity of calibration curve was validated by the values of correlation coefficient of the regression equation which were 0.99 to 1.0 for all methods which signify the adherent of the systems to Beer Lamber's law.

The values of intercept were close to zero in all methods.

3.3 Analysis of Chloroquine Phosphate in Pharmaceutical Dosage Forms

The results of analysis of chloroquine phosphate in the different dosage forms by first derivative spectrophotometer were compared with results obtained by the other three methods (Table 4). F and t-test were used to compare precision and accuracy of the first derivative spectrophotometry method to the other three methods at 349 nm (Table 5).

Table 1. Statistical analysis of the calibration curve of chloroquine phosphate using first-derivative spectrophotometry

Wavelength	Solvent	Intereept	Slope	Regression (r)
225 nm	Water	1.5X10^{-2}	1.5 X 10^{-2}	0.988
225 nm	0.01M HCl	-4.5 X 10^{-2}	1.6 X 10^{-2}	0.987
239 nm	Water	5 X 10^{-4}	-6.8 X 10^{-3}	0.999
239 nm	0.01M HCl	1.2 X 10^{-3}	-6.9 X 10^{-3}	0.999
260 nm	Water	-2 X 10^{-3}	-6.8 X 10^{-3}	0.999
260 nm	0.01M HCl	1.2 X 10^{-3}	-6.9 X 10^{-3}	0.999
333 nm	Water	-7 X 10^{-4}	-3.1 X 10^{-3}	0.997
333 nm	0.01M HCl	-7 X 10^{-4}	-3.1 X 10^{-3}	0.995
349 nm	Water	-4.1 X 10^{-3}	-1.71 X 10^{-2}	1.000
349 nm	0.01M HCl	-7.3 X 10^{-3}	-1.73 X 10^{-2}	0.999

Table 2. Statistical analysis of the calibration curve of chloroquine phosphate using zero-order absorbance spectrophotometry

Wavelength	Solvent	Intercept	Slope	Regression (r)
221 nm	Water	5.33×10^{-2}	4.3×10^{-2}	0.997
221 nm	0.01M HCl	1.1×10^{-2}	5.59×10^{-2}	1.000
236 nm	Water	-2.29×10^{-2}	3.45×10^{-2}	1.000
236 nm	0.01M HCl	-1.9×10^{-2}	3.47×10^{-2}	0.999
256 nm	Water	-1.40×10^{-2}	2.93×10^{-2}	1.000
256 nm	0.01M HCl	-1.22×10^{-2}	2.93×10^{-2}	1.000
231 nm	Water	-5.90×10^{-3}	3.16×10^{-2}	1.000
231 nm	0.01M HCl	-2.50×10^{-3}	3.16×10^{-2}	1.000
246 nm	Water	4.20×10^{-3}	3.31×10^{-2}	1.000
246 nm	0.01M HCl	1.13×10^{-2}	3.35×10^{-2}	0.999

Table 3. Comparison of the calibration graphs of chloroquine phosphate using first-order derivative, zero-order absorbance spectrophotometry and HPLC methods

Method	Wavelength	Intercept	Slope	Regression (r)
Absorbance	221 nm	5.33×10^{-2}	4.3×10^{-2}	0.997
Derivative	225 nm	1.5×10^{-2}	1.5×10^{-2}	0.988
Absorbance	236 nm	-2.29×10^{-2}	3.45×10^{-2}	1.000
Derivative	239 nm	5×10^{-4}	-6.8×10^{-3}	0.999
Absorbance	256 nm	-1.40×10^{-2}	2.93×10^{-2}	1.000
Derivative	260 nm	-2×10^{-3}	-6.8×10^{-3}	0.999
Absorbance	331 nm	-5.90×10^{-3}	3.16×10^{-2}	1.000
Derivative	333 nm	-7×10^{-4}	-3.1×10^{-3}	0.997
Absorbance	346 nm	4.20×10^{-3}	3.31×10^{-2}	1.000
Derivative	349 nm	-4.1×10^{-3}	-1.71×10^{-2}	1.000
HPLC	349 nm	1.76×10^{-2}	2.59×10^{-1}	0.999

The results of analysis of chloroquine phosphate injection and tablet dosage forms showed no significant differences when carried by either of the first order derivative, zero order absorption spectrophotometry, HPLC, and non-aqueous titration methods. The calculated F and t values were smaller than the tabulated ones, It was therefore concluded that first order derivative spectrophotometry was precise and accurate at all wavelengths; result being almost identical (as F–value and t-value were almost zero).

Statistical analysis of the results obtained for chloroquine phosphate in syrup dosage forms showed that there were significant differences between first order derivative spectrophotometry, zero order absorption and non-aqueous titration methods of analysis at all wavelengths except at wavelength 333 nm and 349 nm. However comparison of the results obtained by the first order derivative method and the HPLC method revealed no significant differences when analysis was conducted at wavelength 260, 333, 349 nm.

The results of derivative technique obtained were in agree with results reported by Hassan et al for the determination of cisapride in pharmaceutical preparations [9], Lei and Mandan [10] for determination of simvasatin in tablet dosage form and Ozer and Senel [11], for the determination of lisinopril in pharmaceutical preparations.

Application for derivative technique is extremely wide used, it solve problem of overlap of peaks as study done by Skujins and Varian [12] for determination of the exact number and location of peaks in the UV-Visible spectrum of the uranyl ions.

3.3.1 Analysis of chloroquine phosphate standard in presence of excipents

Analysis of chloroquine phosphate standard solutions containing methyl paraben, propyl paraben and saccharin sodium showed significant differences at all wave lengths except 333 nm and 349 nm between first derivative and HPLC method and between absorbance spectroscopy and HPLC. It can be concluded that first derivative and absorption spectroscopy methods at these wave length are not suitable for analysis of chloroquine phosphate formulation containing these excipents. This is understandable since one expects high selectivity at high wave length.

The results of analysis of chloroquine phosphate standard with all other excipents showed no significant difference at all wave lengths compared to the HPLC method (Table 6).

The application of derivative spectroscopy in stability studies has been reported by Castro D. Moreo et al. [13] for the determination of omeprazole tablet in aqueous solution.

Analysis of Acebutolol HCl in presence of it is acid –induced degradation products, has been reported by derivative spectroscopy method. Similar work has been reported for the determination of benoxinate hydrochloride and its degradation product by first derivative spectrophotometry [14,15].

Derivative spectroscopy has been applied in the determination of drugs in pharmaceutical dosage form such as creams and ointments, as study done by Nilgun Gundan Goger and Lerzan Gokcen [16] for determination of micanazole in pharmaceutical creams by derivative spectroscopy without prior extraction of active ingredient.

Derivative spectroscopy has reported for the analysis of the drugs in biological fluids.

The determination of cefuroxime and cefadroxil in urine is an example of application of derivative spectroscopic analysis of drug in biological fluids [17].

In the present work, a derivative spectroscopy method has been found to be linear and selective. It was employed for analysis of chloroquine phosphate in different pharmaceutical dosage forms. The results obtained showed that the developed method is similar in selectivity to HPLC method and superior to the zero order and the official non-aqueous titration methods. While it has, advantage over the HPLC method being less time consuming and less expensive.

Table 4. Content (%w/w) of chloroquine phosphate in pharmaceutical dosage forms analyzed by the three methods

Dosage form	Derivative	HPLC	Non-aqueous titration	Absorbance
	Average (%w/w) ± sd	Average (%w/w) ± sd	Average (%w/) ± sd	Average (%w/w) ± sd
Lariago syrup	103.93±1.07	103.7±0.91	103.9±1.208	98.86±0.97
Lariago tablet	104.36±0.46	104.6±0.57	102.3±0.13	99.73±0.12
Amiquine tablet	100.96±1.99	100.56±0.99	100.53±1.14	101.06±1.02
Chloroquine tablet	105.5±1.02	105.3±0.78	105.4±0.86	105.16±0.90
Efroquine tablet	98.4±0.29	98.1±0.43	98.16±0.69	98.93±0.17
Chloroquine injection	101.8±0.24	101.9±0.71	101.83±0.33	101.33±0.54
Balsaquine injection	102.1±0.16	101.8±0.29	101.43±0.36	102.13±0.41

Table 5. Calculated t-test and F– test for comparison of the results of analysis of chloroquine phosphate in pharmaceutical dosage form by 1st derivative at 349 nm to the other three methods

Dosage form	Absorbance		HPLC		Non-aqueous titration	
	*t-test	**F-test	*t-test	**F-test	*t-test	**F-test
Chloroquine syrup	0.007	0.579	0.317	0.886	0.170	0.573
Lariago syrup	0.371	0.842	0.385	0.723	0.031	0.931
Lariago tablet	0.340	0.795	0.038	0.970	0.002	0.090
Amiquine tablet	0.475	0.579	0.373	0.744	0.439	0.810
Chloroquine tablet	0.432	0.736	0.467	0.857	0.467	0.857
Efroquine tablet	0.047	0.634	0.376	0.558	0.066	0.268
Chloroquineinjection	0.445	0.211	0.458	0.353	0.285	0.736
Balsaquine injection	0.211	0.211	0.262	0.735	0.262	0.211

**The tabulated T at the 95% confidence level and degree of freedom 2 = 4.303*

***The tabulated F at the 95% confidence level and degree of freedom 2 = 19.0*

Table 6. Results of analysis of chloroquine phosphate standard (%w/w) in presence of exiepents

Excipent	Absorbance average (%w/w) ±sd	Derivative average (%w/w) ±sd	HPLC average (%w/w) ±sd
Aerosil	101.06±1.01	100.33±1.46	99.86±0.35
Starch	102.13±1.06	102.83±1.72	101.86±1.14
Mg sterate	101.86±0.21	101.06±0.74	100.66±0.56
Avicel	102.76±0.21	103.10±0.49	103.10±0.22
Talc	102.33±0.82	102.53±0.53	102.36±0.54
Lactose	101.76±0.25	101.76±1.18	101.06±0.42
Methyl paraben	100.93±1.11	99.70 ±0.29	99.56±0.57
Acacia	100.70±0.49	101.00±0.91	100.00±0.83
Dicalcium phosphate	100.26±0.34	101.36±0.81	100.26±1.28
Propyl Paraben	98.30±0. 70	97.70±0.91	98.36±0.74
Opadry	100.66±0.98	100.23±0.48	100.93±0.76
Titnium dioxide	104.96±1.59	104.53±1.21	102.9±1.18
Glycol	100.56±0.82	101.03±0.34	100.30±0.65
Glycerin	100.93±1.23	100.30± 1.07	100.30±0.98
Saccharin Na	102.60±0.86	102.50±0.49	102.36±0.69
Sucrose	100.8±0.99	101.46±0.95	100.26±0.87
Citric acid	100.10±0.64	99.93±0.31	100.56±0.53
Na Citrate	100.70±1.30	101.60±0.71	101.40±0.87
Xanthan gum	101.16±2.44	101.26±1.07	101.33±1.16
Sorbitol	101.56±0.86	100.50±0.85	100.76±0.98
Gelatin	100.93±0.86	100.50±0.85	100.76±0.98
Tween 80	101.70±2.29	101.90±1.59	101.26±1.14
Phosphoric A	101.46±2.13	100.20±0.67	100.83±0.77

Table 7. Calculated t-test and F– test for comparison of the results of analysis of chloroquine phosphate standard in presence of excipents by 1st derivative at 349 nm to the absorbance and HPLC Methods

Exipent	Absorbance		HPLC	
	*t-test	**F – test	*t-test	**F – test
Aerosil	0.053	0.646	0.308	0.109
Starch	0.134	0.554	0.071	0.615
Mg sterate	0.134	0.142	0.339	0.720
Avicel	0.257	0.325	0.257	0.325
Talc	0.417	0.589	0.064	0.976
Lactose	0.417	0.084	0.222	0.222
Methyl Paraben	0.411	0.222	0.423	0.417
Acacia	0.336	0.45	0.020	0.907
Dicalcium Phosphate	0.070	0.302	0.106	0.566
Propyl Paraben	0.029	0.741	0.040	0.798
Opadry	0.377	0.445	0.488	0.445
Titnium Dioxide	0.161	0.733	0.191	0.971
Glycerin	0.340	0.860	0.467	0.918
Glycol	0.360	0.390	0.440	0.265
Saccharin Na	0.429	0.489	0.436	0.223
Sucrose	0.372	0.406	0.476	0.331
Citric acid	0.315	0.877	0.347	0.121
Sodium Citrate	0.483	0.757	0.161	0.642
Xanthan Gum	0.484	0.319	0.475	0.916
Sorbitol	0.398	0.372	0.340	0.463
Gelatin	0.481	0.410	0.435	0.373
Tween 80	0.433	0.876	0.335	0.950
Phosphoric Acid	0.308	0.717	0.492	0.380

4. CONCLUSION

The derivative spectroscopic method should been applied in the assay of different pharmaceutical preparations, as it is simple, rapid, precise and accurate, less coasty and less time consuming than either the official non-aqueous titremetric method or the HPLC method.

The present method is suitable as a routine analytical procedure for analysis of chloroquine phosphate in different pharmaceutical dosage forms.

The derivative method can further employed for analysis of chloroquine phosphate in presence of its metabolite i.e. in biological fluids.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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