

Full Length Research Paper

**RAPID SPECTROFLUORIMETRIC DETERMINATION OF CHLOROQUINE
PHOSPHATE TABLETS**

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ABSTRACT

Purpose: To develop a rapid and cost effective spectrofluorimetric method for the assay of chloroquine phosphate tablets.

Methods: Spectrofluorimetric assays of chloroquine phosphate tablets were done in dilute acids and following extraction in chloroform. Results obtained were compared statistically with the official UV/VIS spectrophotometric method. Method A comprises direct spectrofluorimetric assay of chloroquine tablets in 0.05 M H₂SO₄ and method B involves prior extraction of chloroquine base in chloroform.

Results: The accuracy of determination of chloroquine phosphate tablets in 0.05M H₂SO₄ was similar to the official USP XXII spectrophotometric method (P>0.05). Other solvents of comparison were water and 0.1NHCL. There was no obvious advantage in extracting chloroquine into chloroform prior to analysis (P> 0.05) as recommended in the USP. Judging from the stoke's shift, true fluorescence was also found with 0.05M H₂SO₄. The assays were linear over the concentration range of 1-10 µg/ml in 0.05M H₂SO₄ with a limit of detection of 0.77µg/ml. The percent recoveries of 100.76 ± 1.60 and 101.24 ± 0.77% were obtained following assay of chloroquine without extraction and after extraction with chloroform, respectively. Dilute sulphuric acid (0.05M) was found to give the best correlation between fluorescence intensity and concentration of chloroquine phosphate.

Conclusion: the spectrofluorimetric method developed is rapid and simple and could find application as a cost-effective technique for the assay of chloroquine phosphate tablets.

Keywords: spectrofluorimetry, chloroquine phosphate tablets, dilute acids, extraction, UV-VIS spectrophotometry.

INTRODUCTION

Chloroquine [7 - chloro- 4 - (diethylamino - 1 - methyl butylamino) quinoline] is still used for the treatment of malaria, where there is no chloroquine resistance. Falciparum malaria; and its usually given orally and by parenteral routes. Adverse effects include headache and gastrointestinal disturbances, while Ocular and cardiotoxicities are experienced at high doses.^[1] Chloroquine is official in both the BP ^[2] and USP ^[3]. The USP assays chloroquine by non-aqueous titration while the dosage forms are determined by UV spectrophotometric method at maximum absorption wavelength of 343nm. The BP

method is non-aqueous titration after chloroform extraction of chloroquine base.^[4-7] Many analytical procedures have been reported for chloroquine in dosage forms and biological fluids. Majority of the procedures are chromatographic techniques. The few spectrophotometric methods involve back extraction from chloroform into dilute acids before measurements are made.^[8-10] Although, good accuracies have been reported the procedures are often time-consuming and cost-ineffective. Liquid chromatographic techniques with UV detection have been reported, radioimmunoassay ^[8], gas chromatography ^[9-10], and TLC ^[12] have also been used for the assay of chloroquine in dosage forms or biological samples. Chloroquine belongs to the class of inherently fluorescent

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compounds^[13] and this characteristic has been utilized for its assay in biological fluids. Some previously reported fluorimetric methods include those of Chaulet et al^[14], Samanidou et al^[15] and that of Adelusì and Salako^[16]. The possibility for an improved and rapid spectrofluorimetric analysis of chloroquine in tablets was explored in this work. Results obtained were comparable to the official spectrophotometric method. Subsequently, a modification to fluorimetric method has been developed, validated using calibration curve over a range of concentrations and applied to the assay of chloroquine tablets.

MATERIALS AND METHODS

Chemicals and reagents:

Analytical grades of Ammonia, Chloroform, sulphuric acid, Hydrochloric acid and ammonium chloride (all analytical reagent grades from BDH, poole, England,), chloroquine phosphate CRS (Sigma, USA) and chloroquine phosphate tablets.

Equipment:

Shimadzu spectrofluorimeter (V. RF-1501), Shimadzu spectrophotometer, Milli-Q plus ultra pure water system, analytical balance (Mettler H80), etc were among the basic equipment used during the study.

Instrument Set up:

A self-assessment test for baseline correction and instrument suitability were carried out as well as Peak height and signal-to-noise ratio assessment for the spectrofluorimeter using bidistilled water produced with the Milli-Q plus system. A peak height in the range 35±15 and signal-to-noise ratio above 300 were adjudged adequate for successful analysis.

Spectra measurement:

Chloroquine phosphate stock solutions (1 mg/ml) were prepared in water, 0.1M HCL and 0.05M H₂SO₄. The excitation and emission wavelength for the spectrofluorimetric analysis were determined by setting the

scanning range at 220-900nm. The wavelengths were automatically selected by the self-searching synchronous motors of the equipment. The excitation and emission wavelengths obtained with the excitation spectrum type energy were confirmed as representing the true values by selecting the excitation wavelength maximum and then scanning for the two parameters using the emission spectrum type energy. The excitation and emission maxima obtained were used for subsequent preparation of calibration curves and sample analyses.

Preparation of calibration curves:

The relative fluorescent intensities obtained from the spectra measurement provided a guide for the selection of the concentration range within which the sensitivity and response factor of the spectrofluorimeter was to be maintained. Calibration curves were obtained using water, 0.1M HCL and 0.05M H₂SO₄ with their respective excitation and emission wavelengths.

Accuracy and Repeatability Assessment:

A two-day assessment of the accuracy and repeatability of the spectrofluorimetric method was carried out in 0.05M H₂SO₄ using concentrations of 1.4 and 8µg/ml. Percent recoveries were calculated and results expressed as mean recoveries ± S.D. The accuracy and repeatability assessment were carried out for direct analysis in 0.05M H₂SO₄ (Method A) and following back extraction from chloroform (Method B). Sample measurements were done using λ_{max} of 368nm.

Tablet Analysis:

Five batches of chloroquine phosphate tablets produced by a Pharmaceutical Manufacturing Company in Nigeria were analyzed for their content of chloroquine phosphate. An amount equivalent to 0.01g of chloroquine phosphate was weighed and dissolved in 10ml bidistilled water, filtered and then aliquots were withdrawn and diluted in 0.05M H₂SO₄ to give final concentrations of 5µg/ml The procedure was repeated by basifying the solution,

extracting liberated chloroquine into chloroform, concentrated and back extracted into 0.05M H₂SO₄. Absorbance measurements were done at 339nm excitation and 368nm emission maxima.

Spectrophotometric Determination:

Sample determinations were repeated for the five batches of chloroquine phosphate tablets using the USP [3] spectrophotometric method. The absorbance values were recorded at wavelength 343nm.

Statistical Analysis:

Simple linear regression and correlation analysis were used to judge the suitability of the calibration ranges of chloroquine in water, 0.1N HCl and 0.05M H₂SO₄. Comparisons of % recoveries from tablets were done using t-test and ANOVA. P values less than or equal to 0.05 were taken as significant.

RESULTS

Chloroquine phosphate fluoresced significantly in the three solvents adopted. However, of the three solvents; water, 0.1N HCl and 0.05M H₂SO₄, the best Stoke's shift (difference between emission and excitation maxima) was found with 0.05M H₂SO₄. The spectrofluorimetric data is presented in Table 1. The coefficient of determination for the calibration curves was also found to be highest using 0.05M H₂SO₄. The limit of detection was determined to be 0.77µg/ml while 95% confidence intervals for the slope and intercept were respectively 69.20, ±4.8 and 13.03 ±2.7, the overall recovery using Method A (direct analysis in 0.05M H₂SO₄) was 100.80 ±1.06 (RSD 1.05%) while that of method B (prior extraction into chloroform) was 101.24 ±0.77 (RSD 0.76%). There was no obvious advantage in extracting chloroquine into chloroform prior to analysis (p> 0.05). all the three methods (USP, Method A and Method B) were found to give equivalent results (Table 2).

DISCUSSION

The highly soluble nature of chloroquine phosphate in water coupled with its inherent fluorescent property enabled direct analysis in water and diluted acids. However, the correlation of fluorescence intensity with concentration was low when water and 0.1M HCL were adopted as diluting solvents. Thus 0.05M H₂SO₄ was experimented as the diluting solvent. Excellent linear relationship was obtained using this solvent (r=0.9980) and the spectral measurement gave Stoke's shift less than 30nm, which represents true fluorescent behaviour¹⁷. However, all the calibration curves prepared in the three solvents provided correlation coefficients that were significantly different from tabulated $t_{0.001}$ and were adjudged as being different from zero correlation. The test of significant difference between the results of spectrofluorimetric methods A and B showed that at all levels of probability; there was no significant difference between the percent recoveries. Method A had a better advantage in terms of convenience of analysis due to the higher cost-benefit ratio and the speed of analysis. The spectrofluorimetric method A, which was a direct spectrofluorimetric analysis in 0.05M H₂SO₄, provided the same advantage of convenience over the USP spectrophotometric method. Both method B and USP method involved back extractions. Extraction processes are often fraught with inaccuracies due to greater possibility of loss of materials during extraction and concentrating steps which method A had by-passed. Method A also possessed this advantage over most of the previously reported fluorimetric methods [14-16]. High level of fluorescence quenching was observed when water and 0.1N HCl were used as diluting solvents. This may account for the low correlation obtained between fluorescence intensity and concentration. The phosphate moiety may have had a significant fluorescence quenching property due to its large size and thus accounted for the non-suitability of water as a solvent. The markedly displaced emission maximum further corroborated this result. The non-suitability of 0.1N HCL as a diluting solvent could be explained based on the property of certain inorganic anions as noted by Glasstone

[17]. The halogens are among the most powerful fluorescence quenchers known and may have been responsible for the low recoveries of chloroquine following the extraction process. The new spectrofluorimetric methods A and B were successfully applied to the assay of chloroquine phosphate in tablets. Similar accuracies were obtained when the results were compared to the USP spectrophotometric method. This is obvious from the results tabulated in Table 2.

In summary, this work has shown that chloroquine phosphate tablets and/or capsules can be assayed

quantitatively using the formulation without extraction, but with dilute sulphuric acid solution as the solvent in a spectrofluorimetric determination. This is a much easier method than that of the USP.

CONCLUSION

The spectrofluorimetric method for the assay of chloroquine phosphate described in this work is simple, rapid and convenient and could be used as a cost-effective analytical method for in-process quality control method for chloroquine tablets.

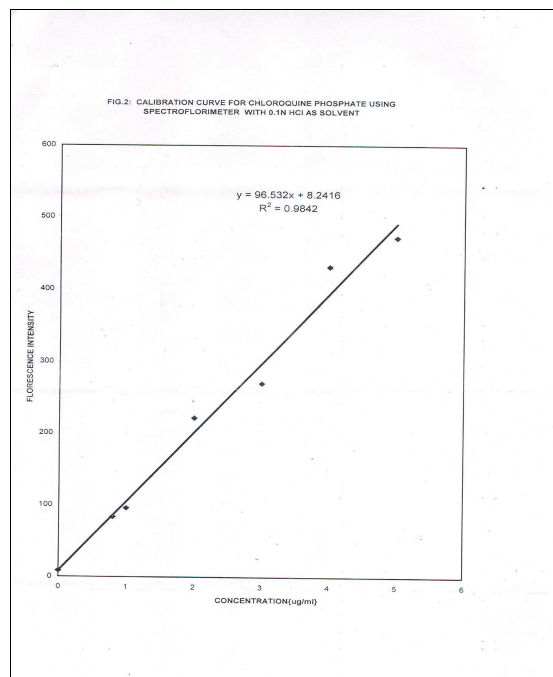
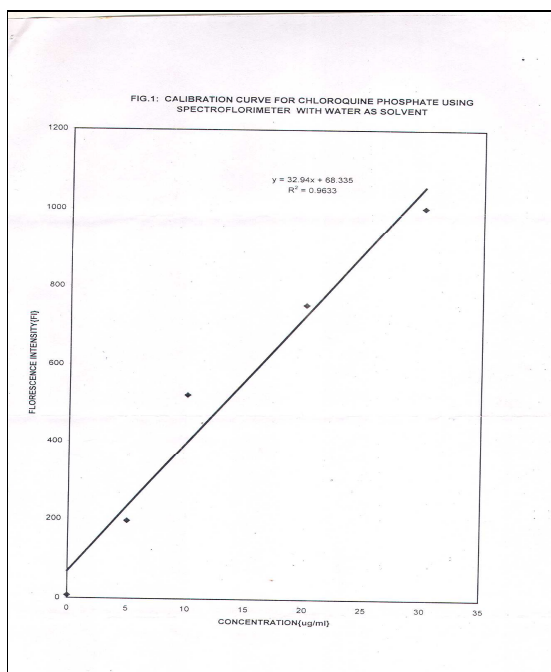


Table I: SPECTROFLUORIMETRIC DATA USING VARIOUS SOLVENTS

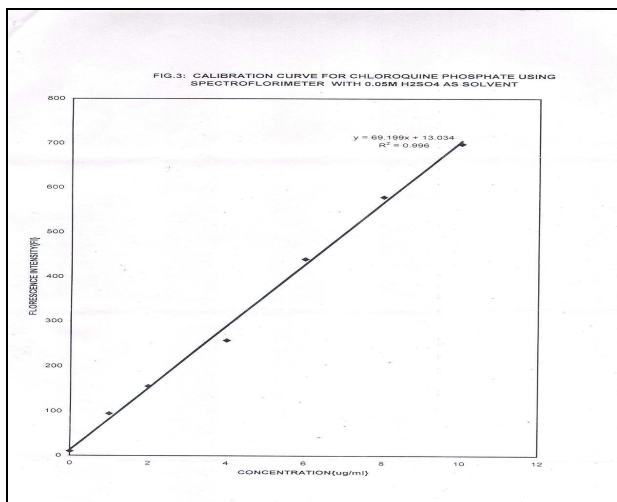
Solvent	Excitation Maximum λ_{ex} (nm)	Emission Maximum λ_{em} (nm)	Stoke's Shift ^a (nm)	Excitation Scanning range (nm)	Emission Scanning range (nm)
Water	254	738	484	220-738	254-770
0.1 M HCL	253	369	116	230-269	253-419
0.05M H ₂ SO ₄	339	368	29	220-368	339-418

^aStoke's shift= Emission maximum – Excitation maximum

Table II: ASSAY OF CHLOROQUINE PHOSPHATE IN TABLETS

Tablet batch	USP method	Spectrofluorimetric Method A	Spectrofluorimetric Method	p-value (ANOVA)
1	102.77±0.08	103.94±0.88	102.17±1.16	0.17
2	104.99±0.08	103.54±0.90	105.8±0.14	0.18
3	106.92±0.19	103.56±0.91	102.97±0.30	0.08
4	106.72±0.35	105.91±1.60	103.33±0.37	0.09
5	106.54±0.41	104.46±0.43	104.82±0.38	0.16

^aUSP limits: 93 – 107% of declared of labelled amount



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