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Spectrophotometric determination of amodiaquine in amodiaquine-containing tablets

Authors: S. T. Abdullahi[1*,A,C&D], O. M. Ogunniyi[1,B&F], O. I. Eniayewu[1,E&F], O. D. Bamidele[1,E&F], A. Sanusi[1,E&F], F. M. Madaki[2,E&F], N. S. Njinga[1,E&F], M. T. Bakare-Odunola[1,E&F]

[1]*Department of Pharmaceutical & Medicinal Chemistry, University of Ilorin, Ilorin, Nigeria*

[2]*Department of Biochemistry, Federal University of Technology, Minna, Nigeria*

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

Abstract

Background: Amodiaquine (AQ) is a medication on the World Health Organisation's List of Essential Medicines and is recommended for the treatment of malaria in combination with artesunate (ASAQ) or sulphadoxine-pyrimethamine (SPAQ) to reduce the risk of resistance. This study evaluated the amodiaquine potency of amodiaquine-containing formulations.

Methods: Identity and potency were established using amodiaquine dihydrochloride dihydrate analytical reference standard. The amodiaquine content of each sample was measured by assessing its absorbance after dissolution and proper dilution in 0.1N HCl using ultraviolet (UV) spectrophotometry (USP Monograph).

Results: A total of ten co-blistered and fixed-dose combination amodiaquine-containing tablets were randomly collected and tested. The mean percentage amodiaquine content of the tablets was determined from the absorbance values at 223, 237 and 342 nm. All the samples were found to contain amodiaquine as one of the active pharmaceutical ingredients of the tablets. Validation tests indicate excellent linearity (correlation coefficient, $r > 0.999$), good precision (relative standard deviation (%), < 2), and accuracy [recovery (%) for AQ (100.16, 99.89 and 100.06); ASAQ (100.14, 100.12 and 100.14) and SPAQ (99.16, 100.11 and 99.21) at 223, 237 and 342 nm,

respectively], of the method. The active amodiaquine % contents of the samples were evaluated and found to be in the range of 101.58 – 106.95%.

Conclusion: All the samples passed the identification and potency tests based on the USP acceptance criteria of 93.0 – 107.0% and are of good quality with respect to the active amodiaquine content of the formulations.

Keywords: amodiaquine, ultraviolet spectrophotometry, potency, validation

INTRODUCTION

Substandard and falsified medicinal products pose a significant global health issue, affecting millions of people and undermining health systems worldwide (WHO, 2024). These products are present in all countries and include various types of pharmaceuticals, such as life-saving medicines like vaccines, antibiotics, and cancer therapies. The World Health Organisation (WHO) estimated that about 10% of medicines in low- and middle-income countries failed quality control tests, indicating they are substandard or falsified (WHO, 2024). Sub-Saharan Africa has one of the highest prevalence rates (Webb, 2014; Petersen et al., 2017; Ozawa et al., 2018). Generally, antimalarials and antibiotics attract more attention (Zabala et al. 2022). This situation can lead to serious health risks, treatment failures, and even death (WHO, 2024).

Amodiaquine is an orally active 4-aminoquinoline derivative with antimalarial and anti-inflammatory properties (Parhizgar and Tahghighi, 2017). It is chemically a quinoline (Figure 1) having a chloro group at the 7-position and an aryl amino group at the 4-position (PubChem, 2025a; Hawley et al., 1996). It is a yellow, odourless (or almost odourless), crystalline powder, practically insoluble in water, with a molecular formula of $C_{20}H_{22}ClN_3O$ and a relative molecular mass of 355.861. Its International Union of Pure and Applied Chemistry (IUPAC) name is 4-[(7-chloroquinolin-4-yl)amino]-2-(diethylaminomethyl)phenol (PubChem, 2025a). It is a diprotic weak base with a pKa of 7.08 and 8.14 at 25°C (Parhizgar and Tahghighi, 2017).

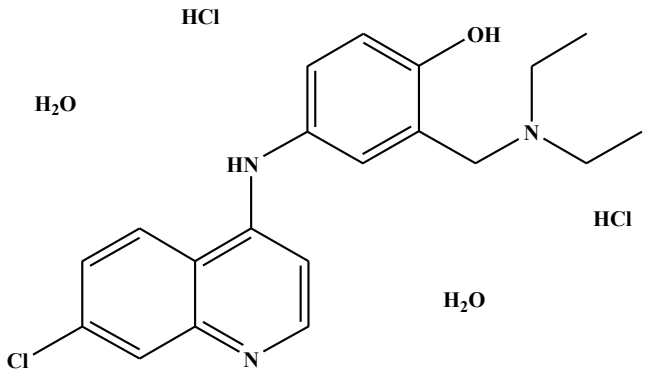


Figure 1: Amodiaquine dihydrochloride dihydrate ($C_{20}H_{22}ClN_3O.2HCl.2H_2O$) chemical structure

The WHO recommends the use of ADQ together with artesunate for the treatment of uncomplicated falciparum malaria, to reduce the risk of drug resistance compared to monotherapy (WHO, 2023; WHO, 2022). Thus, Amodiaquine is widely used as an antimalarial drug, particularly in combination therapies, such as artesunate-amodiaquine (ASAQ), as a first-line treatment for uncomplicated Plasmodium falciparum

malaria in many endemic regions. Amodiaquine is also used in seasonal malaria chemoprevention (SMC) for children aged 3 to 59 months in areas where malaria transmission is seasonal, notably in Africa's Sahel region (WHO, 2023). This intervention consists of full antimalarial treatment courses of sulfadoxine-pyrimethamine and amodiaquine (SPAQ), administered monthly (28 days) during the high-transmission period in the rainy season, generally, for up to five months per year (MMV, 2024).

Amodiaquine is formulated as a phosphate, sulphate, or hydrochloride salt and is dosed by base content (Garner et al., 2009). Amodiaquine Hydrochloride, which is the hydrochloride salt of amodiaquine with the IUPAC name 4-[(7-chloroquinolin-4-yl)amino]-2-(diethylaminomethyl)phenol dihydrochloride dihydrate, structural formula: $C_{20}H_{22}ClN_3O.2HCl.2H_2O$ (Figure 1) and a relative molecular mass of 464.812 (PubChem, 2025b). Amodiaquine Hydrochloride is soluble in water (1 in 22 parts) and ethanol (96%) (1 in 70 parts) but practically insoluble in benzene, chloroform, and ether (TMI, 1983). Because ADQ is generally administered in combination with artesunate, it is available in co-blistered packs as kits and also as fixed-dose combinations (FDCs). The FDCs are formulated as bilayered tablets with ADQ in one layer and artesunate in the other layer (Nair et al., 2012).

Assays of pharmaceutical products are a critical part of the Quality Assurance process and play a crucial role in ensuring the quality, safety, and efficacy of drugs (Kretchy et al., 2025). The definition of drug quality, according to the US Food and Drug Administration International Conference on Harmonisation (FDA ICH) document, is the suitability of either a drug substance or drug product for its intended use. (FDA, 2019; ICH, 1999). Drug quality includes the three main attributes: identity, strength, and purity. Of the three attributes, identification and strength tests are of great importance for drug formulations, while purity is of prominent importance in the case of bulk (Parhizgar and Tahghighi, 2017) drug materials (Görög, 2018).

Tests that verify the identity of a drug product and confirm the presence of the correct active pharmaceutical ingredient (API) are referred to as identification tests. In contrast, tests that determine whether the drug product contains the appropriate quantity of the active ingredient, ensuring patients are not underdosed or overdosed, are known as quantitative tests (Görög, 2008; Görög, 2015). These assays are vital for demonstrating

compliance with pharmacopeial standards (such as BP, USP, EP, IP) and regulatory agencies (National Agency for Food and Drug Administration and Control [NAFDAC], FDA, European Medicines Agency [EMA], WHO). This study was conducted to determine whether the amodiaquine-containing formulations currently marketed and used in Ilorin, North Central Nigeria, comply with pharmacopeial requirements with respect to the identity and amodiaquine potency of the formulations as part of the WHO's strategies to detect any substandard and falsified pharmaceuticals already in the supply chain.

METHODOLOGY

Instrumentation

The active pharmaceutical ingredient was analysed using a UV-vis double beam spectrophotometer [model: GS-UV61(PC); serial No: UQC1212006 by General Scientific Hong Kong Limited], which was equipped with 1 cm matched quartz cells for sample and reference solutions. The spectrophotometer had a spectral bandwidth of 0.1 nm and a wavelength accuracy of ±0.5 nm. Scanning of the samples was performed at a speed of 50 nm/min by adjusting the slit width to 1 nm. A calibrated analytical balance (PA214) by Ohaus, USA, was used for weighing. Precision measurements were facilitated by micro pipettes (P20: 2–20 µL) from Switzerland.

Chemicals and Reagents

The amodiaquine dihydrochloride dihydrate analytical reference standard was procured from Toronto Research Chemicals Inc, Toronto, Canada. The concentrated hydrochloric acid (HCl) (37%) used in the study was of analytical reagent grade without further purification. The 0.1N HCl assay solvent was prepared by dissolving the appropriate volume of HCl in distilled water. Double-distilled water, prepared in the laboratory using the Water Pure-Hit Still (BASIC/PH4 model), was used throughout the experiments.

Sample Collections

Amodiaquine-containing tablet (fixed-dose combination and co-blistered) samples were procured from various community pharmacy outlets using a simple random sampling technique, and coded to conceal the brand names. The samples were subjected to visual inspection, and descriptive features of each were documented. All the samples were visually examined for the descriptive characteristics of the tablets, packaging, and labelling information.

Preparation of Assay Solvent

Hydrochloric Acid Solvent: About 8.4 mL of concentrated HCl (purity of 37%$^{w}/_{w}$ and specific gravity of 1.18 at 25 °C) was transferred into a 1L volumetric flask containing some distilled water and made up to the mark with distilled water to produce approximately 0.1N HCl solution.

Preparation of Standard Solutions

Calibrated volumetric flasks (50 and 100 mL) and pipettes (1, 5, and 10 mL) were used to prepare standard solutions. Standard or sample stock solution (C1: 1000 µg/mL) of amodiaquine hydrochloride in 0.1N HCl was prepared in triplicate by accurately weighing and transferring 100 mg of the standard powder into a 100 mL volumetric flask. The powder was dissolved and brought to volume with the solvent, and the flask was allowed to stand for at least 15 minutes. Working standard solution (C2: 100 µg/mL) was prepared by pipetting 10 mL of the stock solution (C1), transferring it into a 100 mL volumetric flask, and diluting to volume with the solvent. A standard solution (C3: 20 µg/mL) was prepared by pipetting 10 mL of the working standard solution and diluting to the 50 mL mark in a volumetric flask with the solvent, as shown in Figure 2.

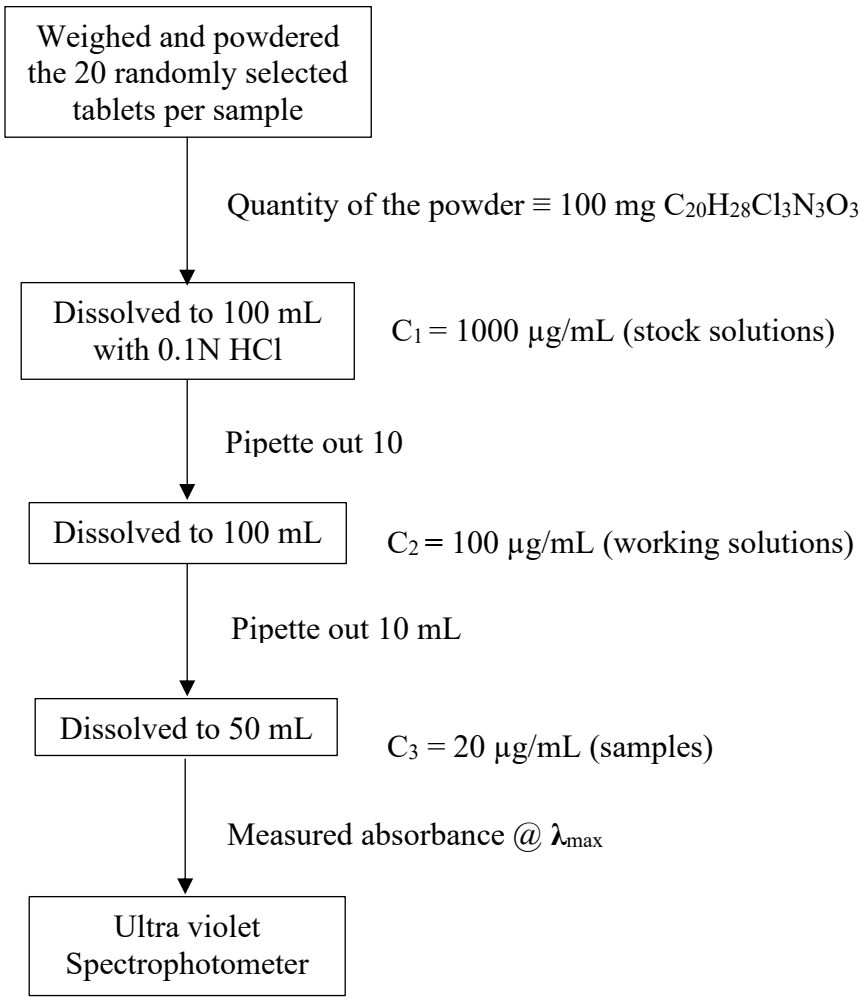


Figure 2: Summary of the study design for preparation of the amodiaquine dihydrochloride dihydrate test solutions

Qualitative Analysis

For the spectrophotometric identity test, the reference standard and the test samples were prepared using identical procedures in a 0.1N HCl solution and scanned from 400 nm to 200 nm. The spectrum of amodiaquine hydrochloride in solutions of the test samples was compared to confirm that they exhibit absorption maxima only at the same detection wavelengths as those shown by the reference standard solutions. Additionally, the molar absorptivity values of the analyte at the detection wavelengths of absorption maxima (λmax) of the spectrum in 0.1N HCl were calculated and compared between standard and test samples to ensure they do not differ above ± 2% (USP, 2000).

Quantitative Analysis

Generation of a standard calibration curve

From the 100 µg/mL working standard solutions, suitable aliquots (2.5, 5, 7.5, 10, 12.5, and 15 mL) were taken in 50 mL volumetric flasks and made up to volume with the solvent to prepare a series of standard solutions of the strengths (5, 10, 15, 20, 25, and 30 µg/mL) respectively. Each concentration level was prepared in triplicate, and their absorbances in the UV region were measured immediately against the blank (0.1N HCl). The concentration of each test solution was determined from the corresponding calibration curve.

Validation

Method validation parameters, including linearity, limit of detection (LOD), limit of quantification (LOQ), repeatability, intermediate precision, and accuracy, were performed according to the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines (ICH, 2023).

Linearity: Calibration curves were obtained by plotting absorbance or amplitude against the corresponding concentration using linear regression analysis.

Limit of Detection (LOD): is the lowest possible concentration at which the method can detect but not quantify the analyte within the matrix with a certain level of confidence. This was calculated based on the standard deviation of the y-intercept and the slope of the calibration curve using the equation:

$$\mathrm{LOD} = \frac{3.3\sigma}{S}$$

Where σ is the standard deviation of the y intercept, and S is the slope of the calibration curve (ICH, 2023).

Limit of Quantification (LOQ): represents the lowest concentration of the analyte that can be reliably quantified by the method. Similarly, this was calculated based on the standard deviation of the y-intercept and the slope of the calibration curve using the equation (ICH, 2023).

$$\mathrm{LOQ} = \frac{10\sigma}{S}$$

Precision: The repeatability and intermediate (interday and ruggedness) precision studies were carried out by estimating the working concentration of amodiaquine HCl standard (i.e., 20 μg/mL) at three different intervals (i.e., 0, 6, and 12 hours) on the same day (intraday) to demonstrate repeatability. The intermediate precision on two different days (after 24 and 48 hours) and analyses of the test solutions by two different analysts (I and II) to demonstrate intermediate precision of the method. A robustness test was also carried out to assess the effect of deliberate, slight changes in spectrophotometric conditions on the determination of amodiaquine hydrochloride

in the tablets. Absorbance values were recorded for the variations of ± 1 nm in the detection wavelength of maxima. The absorbance, observed concentrations, and % RSDs (i.e., the standard deviation expressed as a percentage of the mean) were calculated.

Accuracy (% Recovery): This measures the accuracy of the analytical method and is expressed as the percentage of the true value (termed as % recovery). It was evaluated using the standard addition method at three different concentration levels of 80%, 100%, and 120% of the 20 µg/mL test solution (i.e., 16, 20, and 24 µg/mL) in triplicate (n = 3).[21] Each of the pre-quantified concentration levels of the tablet samples was spiked with approximately 20 µL of the stock standard solutions, thoroughly mixed by vigorous shaking, and the absorbance re-measured at the detection wavelengths of maximum absorption. The amount of added analyte concentrations was calculated, and the total amount of amodiaquine hydrochloride was determined using the corresponding regression equations. The percentage recoveries at each level were calculated using the formula:

$$\text{Recovery} = \frac{Observed\ concentration}{Calculated\ concentration} x\ 100$$

Evaluation of Amodiaquine Hydrochloride Content of Tablets

Twenty tablets from each of the ten samples were weighed together and triturated into a fine powder. The powder equivalent to 100 mg of amodiaquine hydrochloride was calculated, weighed, and transferred to a 100 mL volumetric flask in triplicate using 0.1N HCl solvent to dissolve and fill to the mark. The resulting mixtures were allowed to stand for 15 minutes at room temperature, then thoroughly shaken to ensure complete dissolution of the analyte. The resulting 1000 µg/mL sample stock solutions (C1) were filtered through Whatman filter paper Grade 42 (125 mm). Exactly 10 mL of each of the triplicate solutions (C1) was transferred into another 100 mL flask and diluted to volume with the appropriate solvent to get a 100 µg/mL working solution (C2). Precisely 10 mL of this working solution (C2) was pipetted into a 50 mL volumetric flask, and the final volume was adjusted to mark to produce a 20 µg/mL sample (C3), as shown in Figure 2. The absorption spectra were scanned over the range of 200–400 nm, and absorbance values were recorded. The amodiaquine hydrochloride concentrations were calculated from the regression equations derived from the standard calibration curve.

Statistical Analysis

The recovery results are presented as means (% relative standard deviation of the mean) of

triplicate measurements, and precision as the mean of % relative standard deviation (range). For the potency test of samples, amodiaquine hydrochloride tablets contain not less than 93.0% and not more than 107.0% of the labelled amount of amodiaquine, i.e., within a range (the difference between the highest and lowest values) of 14% (USP, 2000). Three quality categories were defined based on the percentage of labelled amodiaquine content in the samples. A content of 93- 107% of the labelled claim indicates good quality in accordance with USP specifications. Values of 83–93% or 107–117% were classified as low quality, while contents <83% or >117% were considered substandard and/or falsified products (Antignac et al., 2017). All statistical calculations were carried out using Excel 2021 and SPSS version 27.

RESULTS

Descriptive Characteristics of the Analysed Samples

A total of 10 samples were collected in June 2023 from drug retail outlets and hospitals located in Ilorin metropolitan, North Central Nigeria. The descriptive features of all ten samples analysed are shown in Table 1. All the study brands were within the expiry dates and are registered with the NAFDAC, with 70% of them manufactured in China. The first four samples (i.e., 40%) are artesunate + amodiaquine, i.e., ASAQ fixed-dose combination formulation, except the first one, which is co-blistered, and the fourth one, with an amodiaquine strength of 270 mg instead of 300mg. The remaining 60% of the samples are sulphadoxine + pyrimethamine + amodiaquine, i.e. SPAQ, with the amodiaquine base strengths of: 76.5 mg for ADQ-5; 153 mg for ADQ-6 and ADQ-7; 75 mg for ADQ-8 and 150 mg for ADQ-9 and ADQ-10. The shelf life of 60% of the samples is three years, while the remaining 40% (i.e., ADQ-4 and ADQ-8-10) have a shelf life of two years.

Table 1: Descriptive characteristics of sampled brands of amodiaquine dihydrochloride dihydrate containing tablets

Brand samples	Batches	NAFDAC numbers	Manufactured countries	Manufactured dates	Expiry dates
*ADQ-1	221121	04-9254	China	11/2021	11/2024

ADQ-2	230821	04-7848	China	08/2021	08/2024
ADQ-3	150721	04-7849	China	07/2021	07/2024
ADQ-4	SH201003	A4-6252	China	10/2020	10/2022
ADQ-5	LF220416	B4-6481	China	12/03/2022	11/03/2025
ADQ-6	LF220419	B4-6482	China	15/03/2022	14/03/2025
ADQ-7	LF220418	B4-6482	China	14/03/2022	13/03/2025
ADQ-8	B.WSB22011	B4-9356	India	12/2022	11/2024
ADQ-9	B.WSA22056	B4-9357	India	12/2022	11/2024
ADQ-10	B.WSA22055	B4-9357	India	12/2022	11/2024

*Coblistered tablet; Abbreviation: NAFDAC - National Agency for Food and Drug Administration and Control

Identification Tests

This test revealed that all samples contain amodiaquine as the active or one of the active pharmaceutical ingredients per the label. The individual UV spectra of all test samples for the ten brands compared favourably with the spectrum of the amodiaquine hydrochloride analytical reference standard and showed maximum absorption wavelength (λ_{max}) of 223, 237, and 342 nm (Figure 3). Additionally, comparison of the computed molar absorptivity values of the test samples at various detection wavelengths with reference standards indicated that the percentage differences were approximately 0.5% at 223 (0.51 – 0.54) and 342 (0,43 – 0.46) nm and less than 1% at 237 (0.90 – 0,95) nm. This is well within the USP specification of a ± 2% difference limit (Table 2).

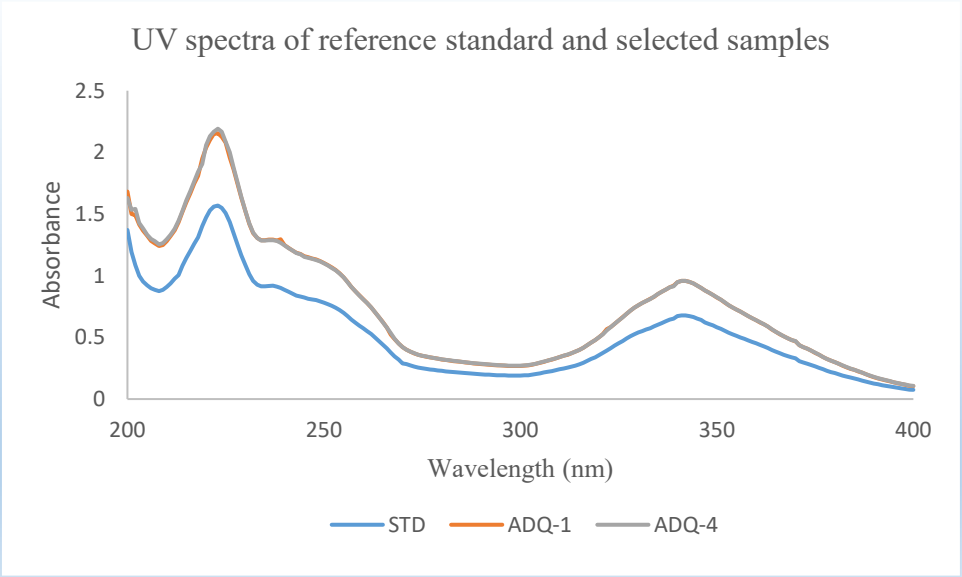


Figure 3: Comparison of the UV absorption spectra of selected samples with the spectrum of the analytical reference standard amodiaquine dihydrochloride dihydrate.

Table 2: Identification test by comparison of the calculated absorptivity of the test sample with the reference standard.

Amodiaquine	ε_{223} (% Diff)	ε_{237} (% Diff)	ε_{342} (% Diff)
Standard	36,591	21,412	15,792
ADQ-1	36,788 (0.54)	21,211 (-0.94)	15,720 (-0.46)
ADQ-2	36,778 (0.51)	21,220 (-0.90)	15,724 (-0.43)
ADQ-3	36,788 (0.54)	21,210 (-0.95)	15720 (-0.45)
ADQ-4	36,788 (0.54)	21,212 (-0.94)	15720 (-0.46)
ADQ-5	36,788 (0.54)	21,211 (-0.94)	15,720 (-0.46)
ADQ-6	36,790 (0.54)	21,211 (-0.94)	15,720 (-0.46)
ADQ-7	36,790 (0.54)	21,210 (-0.95)	15,720 (-0.46)
ADQ-8	36,790 (0.54)	21,211 (-0.94)	15,720 (-0.46)
ADQ-9	36,789 (0.54)	21,211 (-0.94)	15,720 (-0.46)
ADQ-10	36,788 (0.54)	21,210 (-0.95)	15,720 (-0.46)

Abbreviation: A:- Absorbance; ε:-molar absorptivites; Diff:- Difference

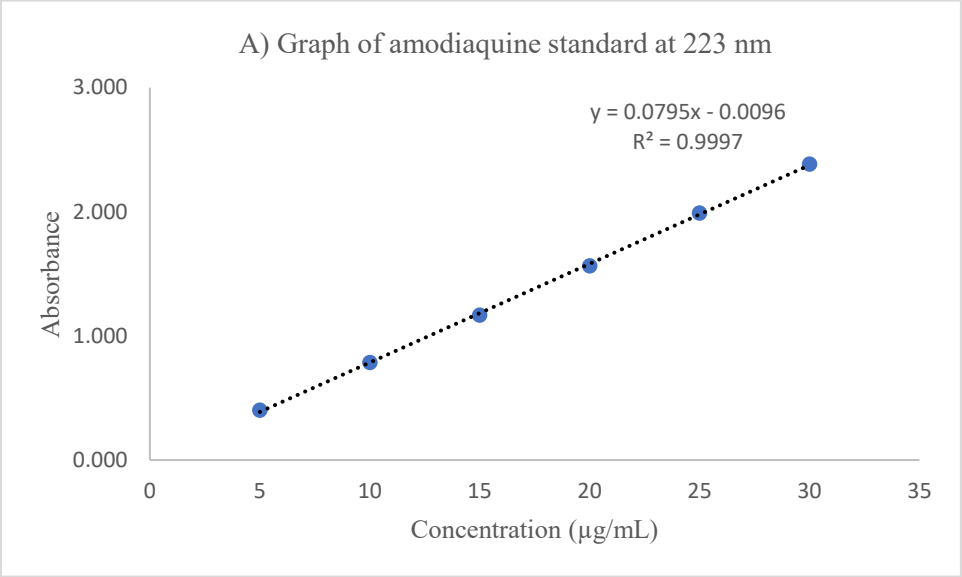
Linearity and Range

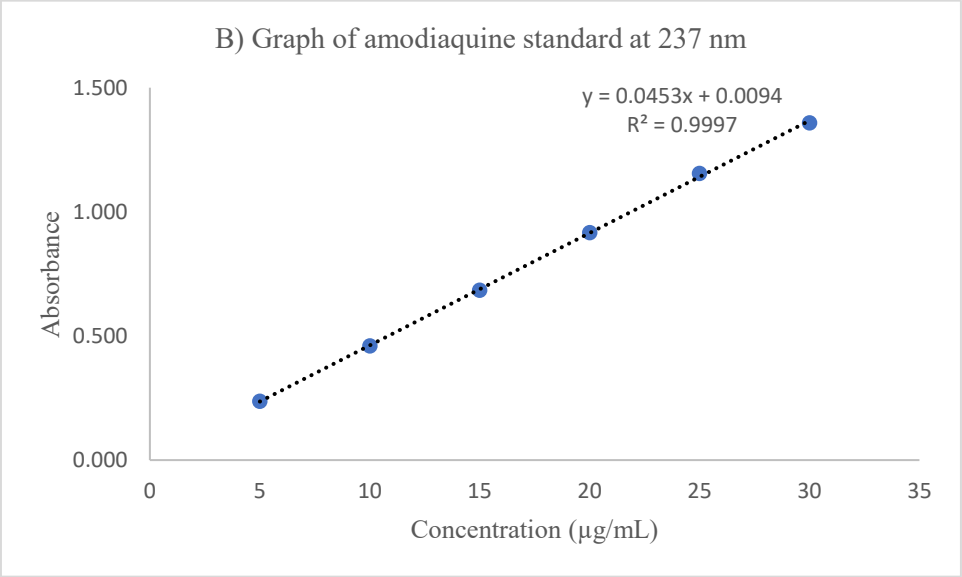
Calibration curves of the amodiaquine hydrochloride analytical reference standard solutions at the three detection wavelengths were constructed by plotting absorbance versus concentration. Linearity was observed within the concentration range of 5 – 30 μg/mL. The regression equations showed the same correlation coefficient (r) of 0.9997 across the three wavelengths, indicating excellent linearity. The % RSD of the absorbance in triplicate analyses was less than 2.0% (range: 0.15 – 1.76% as indicated in Table 3). The concentrations obtained from the calibration plots demonstrated remarkable recovery [range (%) of 4.82 for 223 nm; 1.89 for 223 nm, and 2.80 for 342 nm]. The standard calibration curves of amodiaquine hydrochloride at the three wavelengths are shown in Figure 4.

Table 3: Calibration curve plots for the three wavelengths of maximum absorbances (λ_{max}) at different aliquots of the assay solvent

Conc (µg/mL)	Absorbance [RSD (%)]	Observed conc. (µg/mL)	Recovery (%)	Absorbance [RSD (%)]	Observed conc. (µg/mL)	Recovery (%)	Absorbance [RSD (%)]	Observed conc. (µg/mL)	Recovery (%)
		λ_{max} of 223 nm			λ_{max} of 237 nm			λ_{max} of 342 nm	
5	0.402 (1.04)	5.17	103.46	0.237 (1.76)	5.02	100.34	0.174 (1.75)	5.07	101.44
10	0.786 (1.04)	10.01	100.08	0.460 (1.12)	9.94	99.40	0.339 (0.78)	9.96	99.58
15	1.167 (1.00)	14.80	98.64	0.684 (1.47)	14.90	99.33	0.502 (1.04)	14.80	98.64
20	1.565 (0.52)	19.81	99.05	0.916 (0.48)	20.01	100.07	0.678 (0.15)	20.02	100.09
25	1.989 (1.01)	25.14	100.56	1.154 (1.22)	25.27	101.10	0.853 (1.02)	25.22	100.88
30	2.383 (0.36)	30.10	100.33	1.358 (0.56)	29.76	99.21	1.009 (0.55)	29.84	99.47

Abbreviations: Conc:- concentration; RSD:- relative standard deviation


A) Graph of amodiaquine standard at 223 nm
y = 0.0795x - 0.0096
R² = 0.9997
Absorbance
3.000
2.000
1.000
0.000
0
5
10
15
20
25
30
35
Concentration (µg/mL)


B) Graph of amodiaquine standard at 237 nm
y = 0.0453x + 0.0094
R² = 0.9997
Absorbance
1.500
1.000
0.500
0.000
0
5
10
15
20
25
30
35
Concentration (µg/mL)

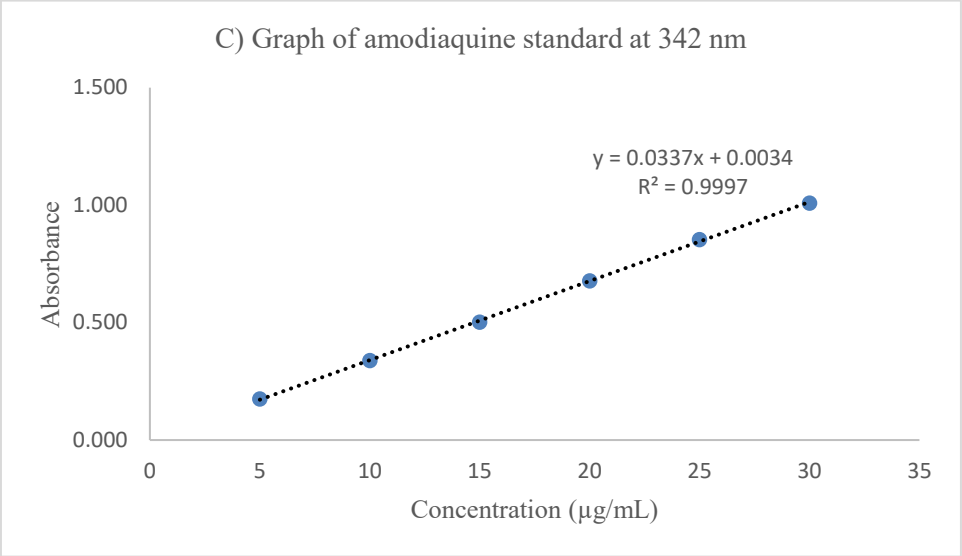


Figure 4: Standard calibration curves showing the linear relationship between concentration and absorbance for amodiaquine dihydrochloride dihydrate measured at A) 223 nm, B) 237 nm, and C) 342 nm.

Precision [RSD (%)]

Precision measures the level of agreement among individual test results when the method is applied repeatedly to triplicate analyses of a homogeneous sample. Repeatability and intermediate precision of the method showed the smallest % RSD ranges at 223 nm (0.86 and 0.90%) compared to the values at 237 nm (2.06 and 2.43%) and 342 nm (1.44 and 2.08%), respectively, as summarised in Table 4. Comparison of the robustness of the method as a result of the slight variations (±1) in the detection wavelengths demonstrated the smallest % RSD range at 237 nm (0.03%) versus 223 nm (0.29% and 342 nm (0.99%), as presented in Table 5.

Table 4: Methods' Precision

Parameters	Detection λmax (nm)	Time (h)	Concentration (µg/mL)	RSD (%)	Recovery (%)
Repeatability	223	0	19.81	0.51	99.05
		6	19.73	0.95	98.65
		12	19.64	1.14	98.19
	237	0	20.01	0.48	100.07
		6	19.60	0.66	98.01
		12	19.73	0.59	98.63
	342	0	20.02	0.15	100.09
		6	19.73	1.42	98.65
		12	19.73	1.65	98.65
Intermediate	Interday				
	223	0	19.73	0.78	98.65
		24	19.86	1.05	99.28
		48	19.68	1.28	98.38
	237	0	20.01	0.48	100.07
		24	19.84	1.17	99.18
		48	19.53	0.49	97.64
	342	0	20.02	0.15	100.09
		24	19.82	1.31	99.10
		48	19.74	1.40	98.70
	Ruggedness	Analysts			
	223	I	19.77	0.87	98.84
		II	19.78	1.56	98.91
	237	I	19.92	1.38	99.62
		II	19.88	1.09	99.40
	342	I	19.62	1.06	98.11
		II	20.04	1.88	100.19

Abbreviations: SD:- standard deviation; RSD:- relative standard deviation

Table 5: Methods' Robustness

Parameters	Detection λmax (nm)	Settings	Concentration (µg/mL)	RSD (%)	Recovery (%)
		Robustness			
λ_{max} (± 1)	223	222	19.74	0.27	99
		223	19.81	0.50	99
		224	19.59	0.21	98
	237	236	19.97	0.48	100
		237	20.01	0.45	100
		238	19.87	0.45	99
	342	341	19.94	0.70	100
		342	20.02	0.15	100
		343	19.82	1.14	99

Abbreviations: RSD:- relative standard deviation; λ_{max}: wavelength of maximum absorbance

Accuracy [Recovery (%)]

Accuracy evaluates the ability of the method to correctly identify the API in the presence of excipients under the spectrophotometric conditions used for analysing amodiaquine formulations. It reflects how closely the test results match the true value. Accuracy was assessed for three formulation types: amodiaquine alone (co-blistered), amodiaquine plus artesunate (ASAQ), and amodiaquine plus sulphadoxine plus pyrimethamine (SPAQ) in fixed-dose combinations. The mean recovery values obtained at the detection wavelengths were all below ±2%, confirming good accuracy of the method. Specifically, recovery values were AQ (100.2%, 99.9%, 100.1%), ASAQ (100.1%, 100.1%, 100.1%), and SPAQ (99.2%, 100.1%, 99.2%) at 223, 237, and 342 nm, respectively, as presented in Table 6.

Table 6: % Recovery of added amount to samples

Detection λmax (nm)	Before addition Concentration (µg/mL) Predicted	Observed	RSD (%)	After addition Concentration (µg/mL) Calculated	Observed	RSD (%)	Recovery (%)
			Amodiaquine tablet				
223	16	17.19	0.68	17.50	17.55	0.48	100.31
	20	21.10	0.67	21.40	21.39	0.32	99.93
	24	24.76	1.21	25.07	25.13	0.41	100.25
237	16	17.62	0.72	17.93	17.90	0.47	99.83
	20	21.90	0.60	22.21	22.20	0.43	99.94
	24	26.35	0.69	26.66	26.63	0.53	99.89
342	16	17.61	0.56	17.92	17.91	0.44	99.94
	20	21.85	0.49	22.16	22.19	0.39	100.16
	24	26.31	0.69	26.62	26.64	0.64	100.07
			Artesunate + Amodiaquine (ASAQ) tablet				
223	16	17.11	0.71	17.41	17.50	0.83	100.51
	20	21.30	1.00	21.61	21.72	0.52	100.55
	24	25.51	1.64	25.82	25.66	0.40	99.37
237	16	17.47	0.79	17.78	17.83	0.52	100.28
	20	21.82	1.04	22.13	22.17	1.01	100.17
	24	26.10	1.03	26.41	26.39	0.69	99.93
342	16	17.49	0.66	17.80	17.86	0.68	100.37
	20	21.87	1.13	22.18	22.20	0.98	100.09
	24	26.13	1.01	26.44	26.43	0.77	99.96
			Sulfadoxine + Pyrimethamine + Amodiaquine (SPAQ) tablet				
223	16	15.64	0.38	16.04	16.03	0.43	99.94
	20	19.81	0.51	20.21	20.10	0.47	99.47
	24	23.63	0.35	24.03	23.57	0.64	98.06
237	16	15.80	0.58	16.20	16.19	0.49	99.94
	20	20.01	0.48	20.41	20.25	0.38	99.19
	24	23.58	0.38	23.98	24.27	1.24	101.19
342	16	15.99	0.50	16.39	16.25	0.37	99.14
	20	20.22	0.15	20.62	20.29	0.30	98.44
	24	23.84	0.33	24.24	24.25	0.86	100.04

Abbreviations: RSD:- relative standard deviation

Other Validation and Optical Parameters

A summary of the optical characteristics of the amodiaquine hydrochloride analytical reference standard and method at three detection wavelengths—specific absorbance, molar absorptivity, and Sandell's sensitivities—is presented in Table 7. Although the linearity range and correlation coefficient (r) were identical, the assay method was more sensitive at 223 nm compared to 237 nm and 342 nm for the limit of detection (µg/mL: 0.2409 versus 0.2910 and 0.2610); molar absorptivity ($L.mol^{-1}.cm^{-1}$: 36,591 versus 21,412 and 15,792); and Sandell's sensitivity ($\mu g.cm^{-2}$: 0.0127 versus 0.0217 and 0.0294). However, the method validation was more accurate [Recovery (%): 100.00 versus 100.15 and 100.10] and precise [RSD (%): 0.65 versus 0.77 and 0.97] at 237 nm compared to 223 nm and 342 nm, respectively, as summarised in Table 7.

Table 7: Comparison of the optical characteristics of $C_{20}H_{28}Cl_3N_3O_3$ and method validation parameters

Parameter (unit)	Value (RSD)/range/equation		
Detection wavelength [(λ_{max}) nm]	223	237	342
Linearity range (µg/mL)		5 - 30	
Regression equation	*y = 0.0795x - 0.0096*	*y = 0.0453x + 0.0094*	*y = 0.0337x + 0.0034*
Correlation coefficient (r)	0.9998	0.9998	0.9998
Limit of detection (µg/mL)	0.2409	0.2910	0.2610
Limit of quantification (µg/mL)	0.7299	0.8818	0.7909
Specific absorbance [RSD (%)]	787 (6)	461 (6)	340 (2)
Molar absorptivity ($L.mol^{-1}.cm^{-1}$)	36,591 (268)	21,412 (273)	15,792 (97)
Sandell's sensitivity ($\mu g.cm^{-2}$)	0.0127	0.0217	0.0294
Accuracy [Recovery (RSD) %]	100.15 (0.49)	100.00 (0.61)	100.10 (0.65)
Precision [RSD (range) %]			
Repeatability	0.87 (0.63)	0.58 (0.18)	1.07 (1.50)
Robustness	0.32 (0.29)	0.46 (0.03)	0.67 (0.99)
Intermediate precision	1.11 (0.78)	0.92 (0.90)	1.16 (1.73)

Abbreviations: RSD:- relative standard deviation; λ_{max}:- wavelength of maximum absorption

Potency Evaluation of the Amodiaquine-containing Tablet Samples

The obtained amounts of the amodiaquine dihydrochloride dihydrate salt of the formulations were converted to the base using the factor of 0.7656 (i.e., ratio of molecular mass of amodiaquine base to that of the amodiaquine dihydrochloride dihydrate). The percent contents of the samples were calculated and found to be in the range of 101.6 – 107.0%, which implies that all the samples meet the USP acceptance criteria of 93.0 – 107.0% and are of good quality concerning identity and active amodiaquine content of the formulations (Table 8).

Table 8: Assay of the sampled brands of Amodiaquine-containing tablets from average values of the three λmaxes

Brand	Amount found - salt (mg)	¶ Equivalent amount - base (mg)	RSD (%)	Content (%)	Remarks*
ADQ-1	417.9	319.9	1.3	106.6	passed
ADQ-2	398.0	304.7	1.7	101.6	passed
ADQ-3	417.2	319.4	0.4	106.5	passed
ADQ-4	374.9	287.0	1.6	106.3	passed
ADQ-5	106.8	81.8	0.7	106.9	passed
ADQ-6	213.6	163.6	0.8	106.9	passed
ADQ-7	213.7	163.6	1.4	106.9	passed
ADQ-8	104.2	79.7	0.4	106.3	passed
ADQ-9	209.6	160.4	0.8	107.0	passed
ADQ-10	209.5	160.4	0.7	106.9	passed

Abbreviations: ¶ using the conversion factor of 0.7656; * USP Acceptance Criteria: 93.0 - 107.0%; RSD:- relative standard deviation;

DISCUSSION

The quality of medicines is a topic of global concern. Recent reports indicate that the availability of substandard and falsified drugs has reached disturbing proportions in many resource-limited countries. The present study evaluated the quality of available brands of amodiaquine-containing tablets with respect to the identity and potency of the active amodiaquine ingredient in the samples. The determined potencies of the samples were then compared to the labelled claims to ensure they comply with the pharmacopeial specifications for safety, efficacy, and consistency.

The visual inspection of the samples revealed no indications of substandard and falsified products, as they are all registered and comply with labelling specifications. Identification is the first step in the complex procedure of drug quality control. Suppose a drug fails the identity test to verify the identity of the active pharmaceutical ingredient. In that case, there is no point in proceeding to test for other quality attributes, such as disintegration and dissolution. The results of the present study showed that all the samples passed the identity test for the amodiaquine content of their formulations. The % difference of the comparison of the calculated absorptivity of each of the test samples with the reference standard was lowest at 342 nm (≈0.46%) compared to 223 nm (≈0.54%) and 237 nm (≈0.94%), which probably informed the pharmacopeial choice of 342 nm as the detection λmax. The present study shows that absorbance measurement at any of the three detection wavelengths can be utilised to compute and evaluate the concentration of the active amodiaquine content of the formulations.

The method validation parameters (accuracy and precision) indicated that the results at all three detection wavelengths compared favourably to each other. Assay results were therefore computed as the average of the determinations of the wavelengths. The obtained active amodiaquine contents (101.58 – 106.95%) of the samples are well within the USP specification of acceptance criteria and can be regarded as being of good quality with respect to the amodiaquine potency or strength of the tablets, which was in agreement with reports of previous studies (Adepoju-Bello and Ayim, 1997; Minzi et al., 2003; Amin et al., 2012; Affum et al., 2013; Uzondu and Okafo, 2016). However, this study's results contradict those of previous studies conducted in Nigeria using HPLC and Ghana using UV spectrophotometry, which reported only 53.8 and 25% of the samples failed the assay of amodiaquine content of the tablets, respectively (Owusu-Ansah et al., 2010; Ehianeta et al., 2012). The observed 100% pass of the active amodiaquine content of the samples from the present study may be attributed to improvement in the monitoring activities of the regulatory body (NAFDAC) compared to 2012. The recent NAFDAC's strict enforcement of registration guidelines, implementation of new guidelines to ensure that imported medicines are genuine, as well as raising public awareness and the use of mobile authentication services (e.g. Sproxil®) may be yielding results in curbing the incidence and prevalence of substandard and falsified products in the country.

Although none of the samples failed the assay test, the present study revealed that more of the samples had higher amodiaquine % content than indicated on their labels. This finding aligns with a recent evaluation of 67 antimalarial samples, in which 9 (13.4%) failed due to potency issues, out of which specifically, 3 (33.3%) were found to have excessive amounts of active pharmaceutical ingredients (Maffioli et al., 2024). A major constraint of this study is the inability to assay the potency of the partner drug (artesunate for ASAQ) and drugs (sulphadoxine and pyrimethamine for SPAQ) because the reference standards for these molecules were unavailable. To provide a more comprehensive evaluation of product quality, future studies should include potency testing of all active ingredients in the tablets. While this study offers only a partial view of the quality of amodiaquine-containing tablets available in Nigeria, it demonstrates that the assay method can reliably measure amodiaquine concentrations at 223 and 237 nm, with precision and accuracy comparable to the pharmacopeial standard at 342 nm.

CONCLUSION

The amodiaquine-containing tablets tested met pharmacopeial specifications for identity and potency, showing no evidence of being substandard or falsified. Nonetheless, both the regulatory authority and manufacturers must continue to strengthen regulatory oversight and quality control measures to minimize the circulation of substandard and falsified products in the country.

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*Corresponding author
abdullahi.st@unilorin.edu.ng / +2348035883569

Orcid: 0000-0002-6868-8687

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