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Research Article

Quality evaluation of antimalarial drugs used in Djibouti: A study of Quinine, Primaquine, Artesunate, and Coartem formulations

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Abstract

Malaria remains a significant public health challenge in Djibouti, with cases rising in recent years. The quality of antimalarial drugs, particularly those imported from countries with less stringent regulatory frameworks, is a critical concern. This study aimed to evaluate the quality of commonly used antimalarial drugs in Djibouti, including quinine, primaquine, artesunate, and Coartem. A series of analytical techniques, such as thin-layer chromatography (TLC), spectrophotometry and high-performance liquid chromatography–mass spectrometry (HPLC-MS), were employed to assess the composition, purity, and dosage accuracy of these medications. The findings revealed that some samples contained impurities and the content of active pharmaceutical ingredients often deviated from the labelled amounts. Specifically, quinine tablets were found to be 29% underdosed compared with the labelled 300 mg, with an actual content of 212.69 mg per tablet. In the LC-MS analysis, besides the active ingredients listed on the labels, derivatives such as dihydroquinine and artemisinin were identified. These discrepancies compared with the labels may be attributed to these medicines' production and/or storage conditions.

Keywords: Analysis; Antibiotic; Djibouti; HPLC; spectrophotometer; TLC

1. INTRODUCTION

Malaria is a parasitic disease caused by the genus Plasmodium and transmitted through the bites of infected female Anopheles mosquitoes. Among the over 100 species of Plasmodium, five infect humans: *Plasmodium falciparum* (the most dangerous), *P. vivax, P. ovale, P. malariae*, and *P. knowlesi* [1].

The Plasmodium lifecycle consists of an asexual phase in humans (liver and then red blood cells) and a sexual phase in the mosquito. After several replications, gametocytes are transmitted to the mosquito during a bite, ensuring the continuation of the cycle. Malaria presents with various symptoms, including fever, headache, and vomiting, and may lead to complications such as anemia or cerebral malaria [2]. Treatment has evolved from the use of plants to quinine, and more recently, to modern drugs based on artemisinin [3]. In Djibouti, malaria remains a serious public health issue, with an increase in cases between 2017 and 2018 [4]. Medicinal plant preparations continue to be the first-line

remedy [5–7]. The country relies on imports of conventional medicines from abroad in its fight against malaria.

This study aims to conduct quality control of antimalarial medicines, employing two types of analyses: qualitative analysis to identify the components present in a drug sample, and quantitative analysis to determine the specific amounts or concentrations of those components. Both analyses are crucial for ensuring the safety, efficacy, and quality of pharmaceutical products. These analyses are particularly essential for products from countries without stringent regulatory policies, which are highly competitive in price and, during periods of high inflation, are very popular among Djiboutian patients.

2. MATERIALS AND METHODS

2.1. Materials

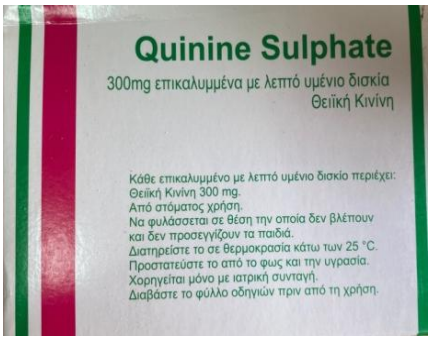
2.1.1. Drugs analyzed

We received antimalarial samples from CAMME (Central Purchasing Agency for Medicines and Essential Materials), a state-run center responsible for storing and distributing medicines to public healthcare facilities. These antimalarials are distributed free of charge and are funded by the government of Djibouti. A visual inspection showed no abnormalities in packaging or labeling. All necessary information was included on the packaging (Schemes 1-4).

A-Quinine

Quinine, an alkaloid derived from the bark of Cinchona trees (native to South America), is widely used for its antimalarial action against *Plasmodium falciparum*, the primary causative agent of malaria in Djibouti. Its molecular formula is $C_{20}H_{24}N_2O_2$, and it is available in tablet form (300 mg) or as an injectable solution (245 mg/mL) (Scheme 1). The tablet form is manufactured by the Cypriot company Remedica, while the injectable form is produced by the French laboratory Renaudin.

A



B

Scheme 1: (A) Tablets and (B) injectable quinine.

B-Primaquine

Primaquine, with the molecular formula $C_{15}H_{21}N_3O$, is a medication used to treat malaria forms caused by *Plasmodium vivax* and *Plasmodium ovale*. Available in tablet form, it is also used to eliminate the hypnozoite forms of the parasite, which are responsible for malaria relapses. It is also produced by the Cypriot company Remedica.

Scheme 2: Primaquine

C-Artesunate

Artesunate, a derivative of artemisinin, is used to treat severe malaria. Its molecular formula is $C_{19}H_{28}O_8$, and it is administered in injectable form, primarily via intravenous injection, to ensure rapid action in severe cases of the disease. It is produced in China by the pharmaceutical group FOSUN PHARMA.

Scheme 3: Artesun injectable.

D-Artemether/Luméfantrine

Artemether, with the molecular formula $C_{16}H_{26}O_5$, is a derivative of artemisinin, a compound highly effective against malaria, particularly in treating infections caused by *Plasmodium falciparum*. In combination with artemether, lumefantrine—an active ingredient with the molecular formula $C_{30}H_{32}Cl_3NO$—is also administered in tablet form. Lumefantrine complements the action of artemether by effectively targeting *Plasmodium falciparum*, providing a combined therapeutic option for managing severe forms of the disease. The product analyzed in this study is manufactured by the Indian pharmaceutical company IPCA and is formulated in a fixed-dose combination of 20 mg artemether and 120 mg lumefantrine.

Scheme 4: Artemether/Luméfantrine.

2.1.2. Analysis materials

The equipment used in this study was primarily installed in the chemistry laboratory of the Institute of Medicinal Research (IRM) at the Center for Studies and Research of Djibouti (CERD). The equipment includes an Agilent LCMS, a DU® 800 UV–Vis spectrophotometer, a high-precision balance, a MEMMERT oven, a dark room, and thin-layer chromatographic (TLC) plates with their accessories.

2.1.3. Solvents and pharmaceuticals standards

The organic solvents and the USP pharmaceutical references were purchased from Sigma Aldrich, Germany.

2.2. Qualitative analysis

2.2.1. Thin layer chromatography (TLC)

Thin layer chromatography (TLC) is an essential technique in drug analysis, offering critical insights into the composition of pharmaceutical products, identifying potential impurities, and ensuring quality control. Widely employed in the pharmaceutical industry and drug testing laboratories, TLC serves as a key method for assessing the quality of antibiotics. In this study, we used TLC to analyze all samples. The experimental protocols were adapted from existing literature with some adaptations (see Table 1). The retention factors (Rf) are compared with those in the literature and the standard for quinine.

Table 1: Mobile phases used in TLC analysis of different drugs.

Drugs	Mobile phase	$R_{experimetal}/R_{reference}$	Reference
Q. tablet	Diéthylamine-Toluene-Isopropanol		Standard
Q. injectable	1/4/5	0.82/ 0.82	
Coartem (L-A)	Toluene - Ethyl Acetate - Acetic Anhydride 18/4/2	L. 0.24/0.4 A. 0.5/0.78	[8]
Primaquine	Hexane - Diethylether - Méthanol - Diethylamine 37,5/37,5/25/0,5	0.27/0.22	[9]
Artesunate	Méthanol - Toluene - Ethyl Acetate - Acid Acetic glacial 1/4,5/4/0,1	0.42/0.51	[10]

2.2.2. Spectrophotometric profile

The characteristic absorbance curve of a compound at different wavelengths is a unique absorption signature, making it a common identification method. We dissolved the standards and drugs under the same conditions and performed a scan of their UV and visible absorbances using a spectrophotometer. The protocol is based on our previous publication on antibiotics in Djibouti [11].

2.2.3. LC-MS analysis

We performed the LC-MS analysis of the two most used substances: Quinine and Coartem (Lumefantrine and artemether). Detection and identification of these two molecules were conducted using by high performance liquid chromatography with tandem mass spectrometry (LC-MS/MS) according to [12].

2.3. Quantitaive analysis

2.3.1. Mass uniformity

According to international pharmacopoeia standards, mass uniformity refers to the average weight of 20 tablets. The deviation between the weight of individual tablets and the average weight should not exceed ±5% for at least 18 of the 20 tablets, and must not exceed ±10% for any of them [13].

The test involves weighing 20 tablets and calculating the mean, standard deviation, and coefficient of variation. The latter measures the variability among the samples, even when the means differ. Table 2 provides the acceptable and tolerated deviation limits for two specific tablets.

2.3.2. Assay by spectrophotometry

The assay is based on Quinine and the method employs is spectrophotometry via calibration. Samples are prepared by dissolving 100 mg of standard quinine in 100 ml of a 0.1 N HCl solution (S1). Progressive dilutions are then performed: S2 (0.08 mg/ml), S3 (0.064 mg/ml), S4 (0.05 mg/ml), S5 (0.032 mg/ml), and S6 (0.016 mg/ml). These dilutions provide a range of concentrations suitable for analysis. Absorbance was measured in a 1 cm cuvette using a UV-VIS spectrophotometer at the maximum absorbance wavelength of 348 nm [14].

2.4. Statistical analysis

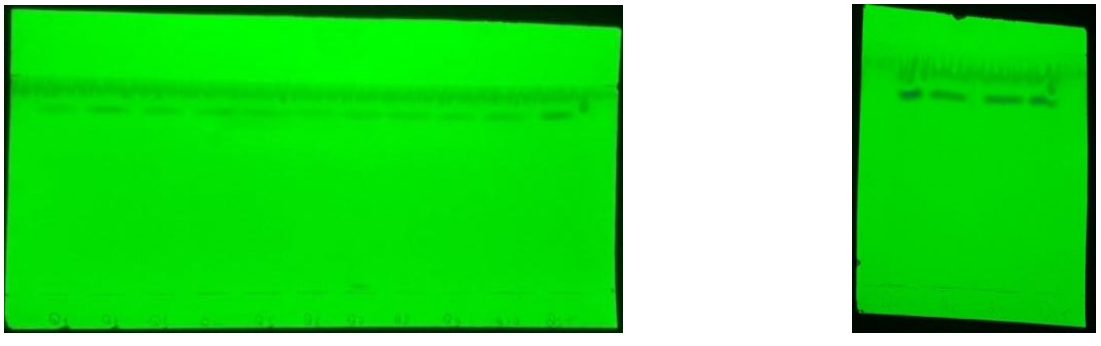
The analyses were carried out in triplicate and the statistical processing (mean, standard deviation) was done with Excell. The linearity of the calibration curves is evaluated with the squared correlation factor (only $R^2 \geq 0.98$ is considered in the calculation of contents). For the tablet dosage form, the results relate to 10 different tablets.

3. RESULTS AND DISCUSSIONS

3.1. Qualitative analysis

3.1.1 TLC analysis

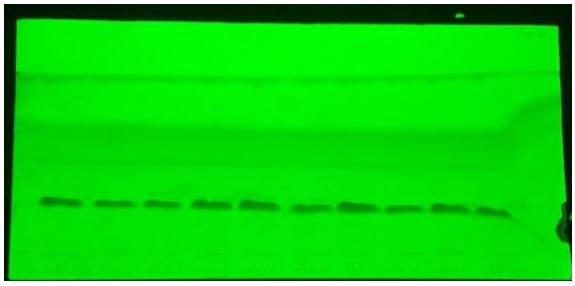
We performed 10 applications corresponding to the 10 quinine tablets from our sample, along with a final spot corresponding to the quinine reference standard (TLC 1-A). The spots migrated to the same height as the standard, with a retention factor (Rf) of 0.82. Similarly, for the injectable quinine (TLC 1-B), we performed 3 applications, with the final one being the reference standard. An Rf value of 0.81 was observed, and the spots from the samples showed similar intensity to that of the reference.



A St B St

TLC 1: The profile of quinine tablet (A)/injectable (B) and their standard (St).

However, regarding the chromatogram of Coartem, two distinct spots were observed for each application under UV light (TLC-2). One of the spots, located lower on the plate, exhibited a higher intensity than the other. To reveal the transparent components, the plate was sprayed with a mixture of sulfuric acid R/methanol (10:90) and then heated at 180°C for 10 minutes.



A

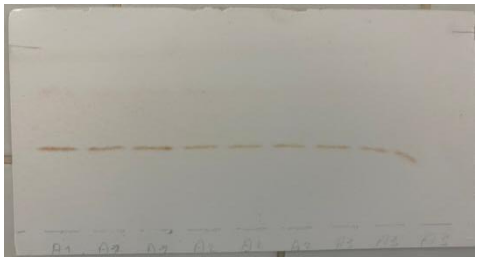
B

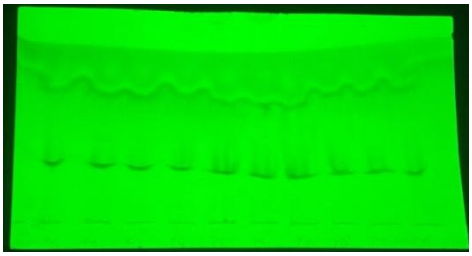
TLC 2: Luméfantrine (A) observed at 254 nm and artemether (B) observed at daylight.

After this treatment, orange-colored spots became visible to the naked eye. In the absence of a direct reference standard in our study, we compared the results with those obtained in a previous study [8]. In that study, the reported Rf values were 0.4 for lumefantrine and 0.78 for artemether. A discrepancy was noted between these reference values and those obtained from our samples. Specifically, the Rf values in our analysis were lower, with lumefantrine at 0.24 and artemether at 0.50 (Table 1).

On the chromatoplate of primaquine, a single spot was observed under UV light (TLC-3A) with an Rf value of 0.27. This was compared with a previously reported study, which noted an Rf value was of 0.22, indicating a slight differences [9].

For artesunate (TLC- 3B), orange-colored spots were observed with an Rf value of 0.42. However, it is important to note that this differs from the reference study, in which the reported Rf value was 0.51 [10]. This discrepancy may suggest variations in the composition or purity of our sample compared to the reference used in the previous study. Additionally, It could be attributed to environmental conditions during the analysis, such as temperature, humidity, or the analytical instrument used. Further investigation may be required to better understand the cause of this variation and to ensure the reliability of our results.





TLC 3: Primaquine observed at 254 nm **(A) and artesunate (B)** observed at daylight.

No absence of active pharmaceutical ingredients was observed. The results indicate the presence of quinine in the analyzed samples, as similarities were noted between the sample spots and the quinine reference standard. However, discrepancies were observed between the Rf values of other antimalarial samples and those reported in reference studies. These variations may be attributed to differences in composition, purity, or experimental conditions. Nonetheless, the consistency of spot heights across each batch confirms the presence of the active ingredient. To enhance the understanding of these discrepancies and ensure the reliability of the results, a more comprehensive analysis is recommended. This should include a thorough review of experimental conditions, an assessment of sample purity, and a more precise comparison with reference standards.

3.1.2 Absorbance profils

In the figures below, we observe the absorbance curves as a function of wavelength for quinine and Coartem (Figure 1). The figure presents three curves corresponding to the absorbance of quinine in tablet form, injectable form, and the reference standard. At 250 nm, the reference quinine and the tablet form exhibit a maximum absorption peak, indicating that this wavelength is particularly suitable for detecting the

presence of quinine in the analyzed samples. However, the maximum absorption peak for the injectable form is slightly shifted to around 240 nm. The maximum absorption peak for Coartem is observed to be shifted toward 260 nm, which is also where the absorbance peaks for artesunate and primaquine occur.

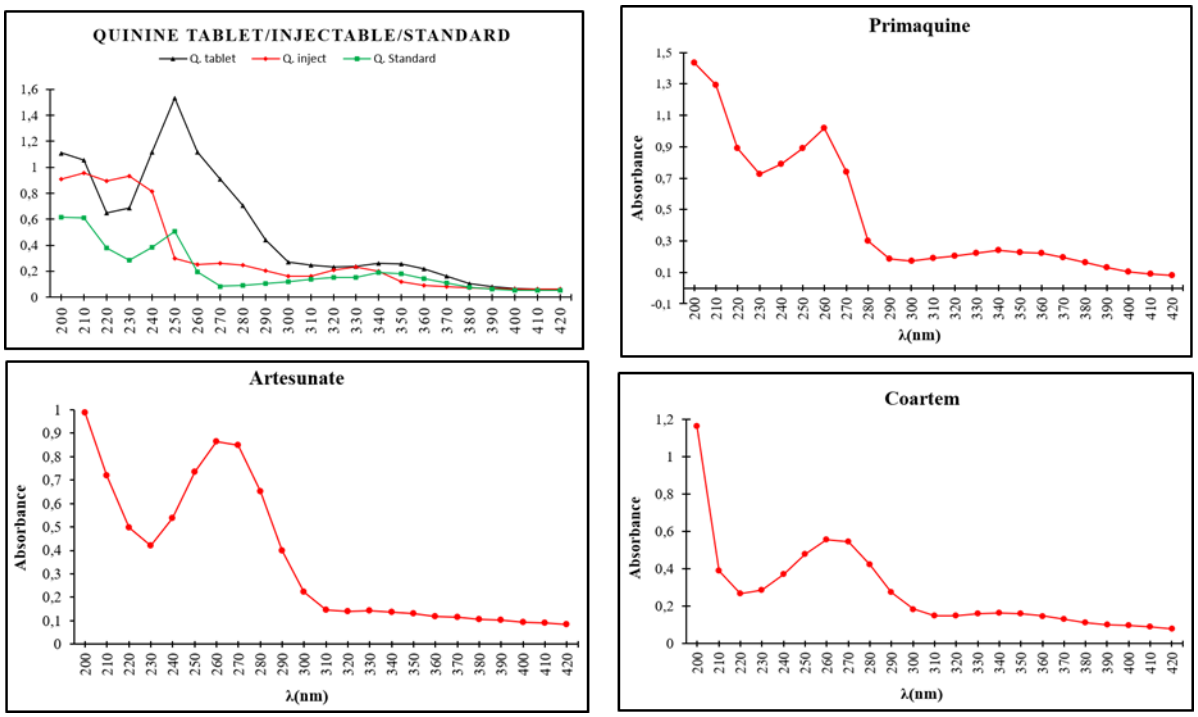


Figure 1: Spectrophotometric profile of quinine (with standard), Coartem, Artesunate and primaquine.

3.1.3 MS identification: The case of Coartem and quinine.

The LC-MS analysis (liquid chromatography coupled with mass spectrometry) of the Coartem drug, which contains artemether and lumefantrine, as well as quinine, facilitated the identification of the main active compounds and certain impurities.

The chromatogram in Figure 2 highlights three distinct peaks (A, B, and C) corresponding to substances present in the formulation. Each peak was analyzed in detail based on its retention time and mass spectrum, enabling reliable identification of the molecules through comparison with known fragmentation data and bibliographic references.

Spectrum A shows a molecular ion peak with an exact m/z value of 528.938, corresponding to the intact lumefantrine compound. This peak was observed at a retention time of 22.8 minutes in the chromatogram.

The LC-MS analysis (liquid chromatography coupled with mass spectrometry) of the Coartem drug, containing artemether and lumefantrine, as well as quinine, enabled the identification of the main active compounds along with certain impurities.

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Spectrum A shows a molecular ion peak with an exact m/z value of 528.938, which corresponds to the intact lumefantrine compound. This peak was observed at a retention time of 22.8 minutes in the chromatogram.

The spectrum corresponding to peak B reveals an interesting fragmentation pattern, characterized by the absence of the main molecular ion peak (exact m/z), yet it conains several characteristic fragments that facilitate the identification of the compound's structure. The peak at m/z 321 corresponds to a sodium adduct ion [M +

Na]$^+$, which is common in positive-mode mass spectrometry. Thus, the molecular ion of the compound itself has an m/z of 298. The spectrum also displays a series of characteristic fragment ions resulting from successive losses of functional groups. After the loss of the CH_2OH group (hydroxymethyl) at m/z 267, there is a loss of water (H_2O) at m/z 249, followed by a loss of carbon monoxide (CO) at m/z 221.1. Finally, the loss of C_3H_6O (a fragment containing methylated and oxygenated groups) produces a peak at m/z 163. These successive fragmentations enable the identification of the structure of artemether and its fragmented derivatives [15].

The last peak, C, is likely an impurity formed during either manufacturing or storage, and does not correspond to the two active ingredients of Coartem. It represents the mass of artemisinin, a compound derived from artemether. Several characteristic fragments are observed in the spectrum. The first, at m/z 304, results from the sodium adduct [M + Na]$^+$ of artemisinin. Another significant fragment at m/z 265 corresponds to the loss of a water molecule (H_2O) from artemisinin, which is a common feature in the fragmentation spectra of organic compounds [15].

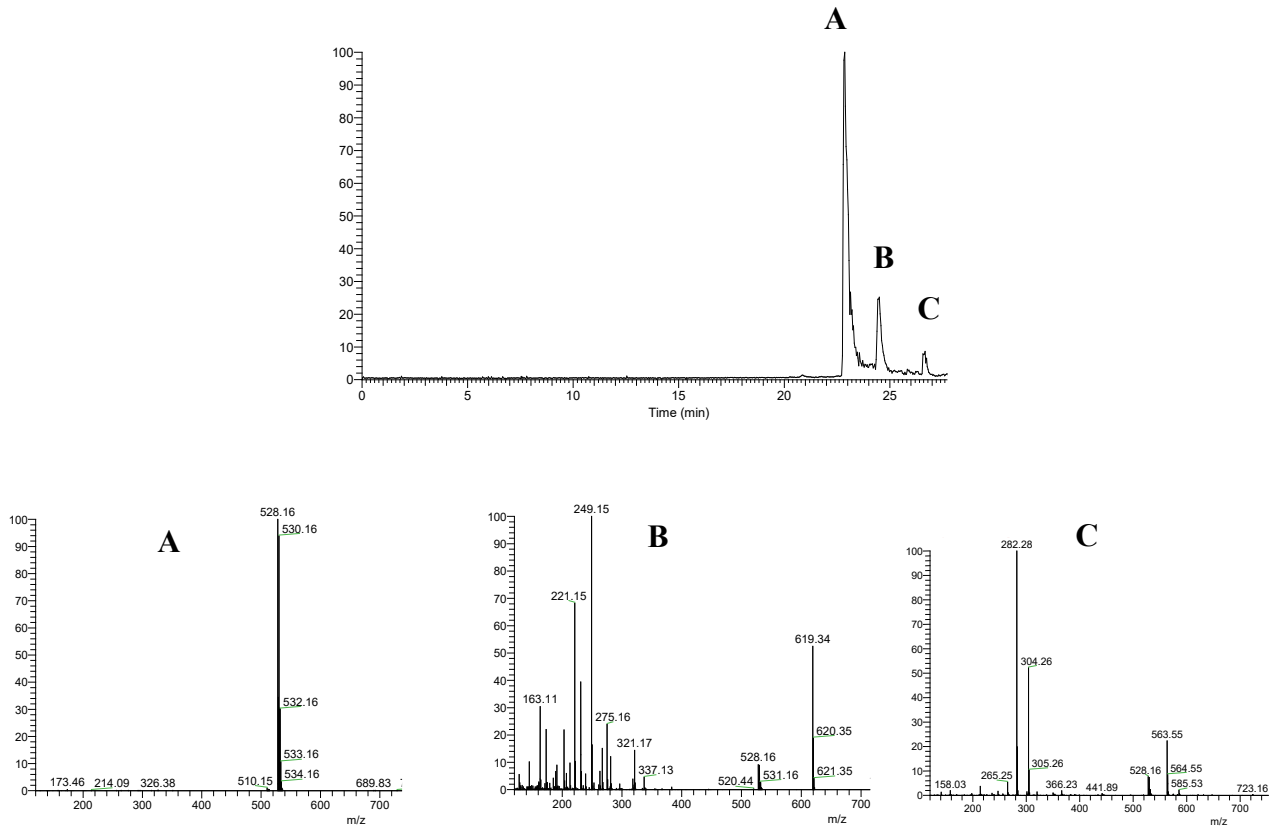

Figure 2: HPLC chromatogram and MS spectra of CoArtem. (A) Mass spectrum of the peak at 22.5 min, identified as lumefantrine ([M+H]$^+$ = 528.16 m/z). (B) Mass spectrum of the peak at 24.6 min, identified as artemether. (D) Mass spectrum of the peak at 26.5 min, corresponding to an impurity or degradation product.

Although the chromatogram of the quinine tablet exhibits significant background noise, the two major peaks, D and E, are clearly identifiable through mass spectrometry. Specifically, the mass of 325.19 Da corresponds to a molecule identified as quinine according to bibliographic references, corresponding to peak D [16]. Similarly, peak E, featuring an [M+H]$^+$ ion at m/z 327, is associated with dihydroquinine, as described in the literature [17].

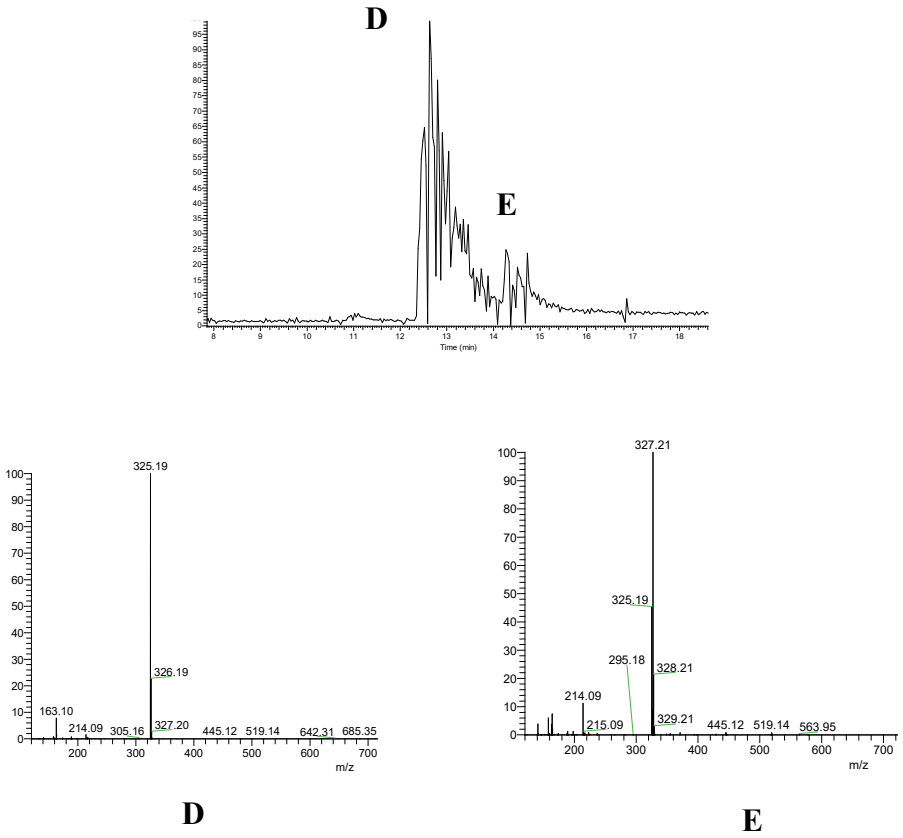

Figure 3: HPLC chromatogram and MS spectra of quinine. (D) Mass spectrum of the peak at 12.8 min, identified as quinine ($[M+H]^+$ = 325.19 m/z). (E) Mass spectrum of the peak at 14.2 min, identified as dihydroquinine.

3.2. Quantitaive analysis

3.2.1 Mass uniformity

Table 2 presents the results of the mass uniformity analysis for the tested samples. According to the European Pharmacopoeia, the acceptable variation limits depend on the average tablet weight.

For quinine, with an average weight greater than 250 mg, the coefficient of variation (CV) must not exceed 5%. In our analysis, we obtained a CV of 1.16%, well within the acceptable range. For primaquine, with an average tablet weight between 80 and 250 mg, the allowable variation is up to 7.5%. The observed CV was 0.7%, indicating full compliance with the standard. Regarding Coartem (artemether/lumefantrine), which also has an average weight above 250 mg, the maximum allowable CV is 5%. Our results show a CV of 1.34%, again within acceptable limits.

These findings confirm that the analyzed samples exhibit good mass uniformity, which is essential for ensuring the quality, safety, and therapeutic efficacy of the medications.

Table 2: Mass uniformity (tablets).

Drug	Measured Weight (mg)	CV
Quinine	574.85±6.70	1.16%
Primaquine	83.41±0.58	0.7%
Artemether / Luméfantrine	251.31±3.38	1.34%

3.2.2 Spectrophotometric assay: Case of quinine tablets

Figure 4 shows the calibration curve of quinine. The correlation coefficient (R^2) is close to 1, indicating an excellent linear relationship between absorbance and concentration.

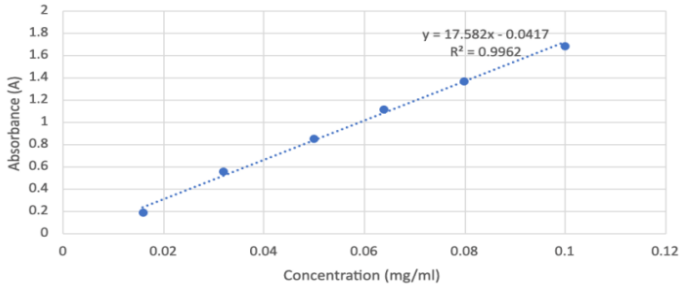

Figure 4: Calibration Curve of Quinine

In this study, the average absorbance of three diluted quinine sample solutions was measured at 0.610. Using the calibration curve equation, the concentration of quinine in the unknown solution was estimated to be 0.037 mg/mL. After applying a dilution factor of 10, the concentration in the original (stock) solution was calculated to be 0.37 mg/mL. By taking a 10 mL aliquot of this solution, the total amount of quinine was found to be 3.7 mg. Assuming an average tablet weight of 574.85 mg, a proportionate calculation indicated that each tablet contains approximately 212.69 mg of quinine—significantly below the 300 mg stated on the label. This represents a deviation of 29%, nearly double the acceptable margin of ±15%.

4. CONCLUSION

The results revealed several concerns, including the presence of impurities and discrepancies between the actual content of active ingredients and the labelled claims. Notably, the quinine tablets were significant underdosed, being 29% below the labelled content. Additionally, variations in the retention factor (Rf) values compared with reference standards suggested differences in the composition or purity of some drugs, including Coartem and artesunate.

This study calls for a more rigorous, systematic approach to drug quality control to mitigate the potential health risks posed by substandard medications.

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Author's contributions: AE conceived the study, collected the samples, performed the analyses, processed the data, and drafted the manuscript. FM contributed to data processing and manuscript writing. DA performed the analyses. AS contributed to data processing and manuscript writing. RR contributed to manuscript writing. IM contributed to manuscript writing. All authors read and approved the final manuscript.

Competing interests: The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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