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*Original article*

# *In vitro* Dissolution, *in vivo* Bioavailability, and in *vitro*–in *vivo* Correlation Assessment of Chloroquine Phosphate Tablets Marketed in Afghanistan

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**Abstract**
Malaria remains a major public health challenge in Afghanistan, where *Plasmodium vivax* is the dominant species. Chloroquine (CQ) phosphate is the first-line treatment. Concerns over substandard and falsified anti-malarial medications necessitate rigorous dissolution and bioavailability monitoring. This study evaluated the in vitro quality and in vivo bioavailability of five CQ phosphate tablet brands in Afghanistan and established a Level C in vitro–in vivo correlation (IVIVC). The study was conducted from June, 2025 to August, 2025 on five brands of CQ phosphate (250 mg) tablets. In vitro quality was assessed for weight variation and dissolution using a verified High-Performance Liquid Chromatography with Ultraviolet detection (HPLC-UV) (n=6 tablets/brand), following United States Pharmacopeia (USP) guidelines. The HPLC method was verified for repeatability, linearity, and accuracy as per ICH Q2(R2) guidelines. In vivo bioavailability was assessed by measuring whole blood CQ concentrations at 24 hours post dose in 20 *P. vivax* patients using HPLC-UV. A Level C IVIVC was established by correlating mean dissolution percentage with mean blood concentration. All brands met USP dissolution criteria (≥75% release in 45 min), but inter-brand variability was significant. CQ-004 showed the highest and most consistent dissolution (97.62% ± 1.32%) and bioavailability (362 ± 57 ng/mL), followed by CQ-003 (96.41% ± 2.74%; 359 ± 56 ng/mL) and CQ-002 (96.17% ± 2.55%; 318 ± 53 ng/mL). CQ-005 had the lowest dissolution (89.2% ± 2.99%) and moderate bioavailability (336 ± 58 ng/mL), while CQ-001 showed the lowest bioavailability (291 ± 66 ng/mL) despite acceptable dissolution (94.26% ± 2.83%). A positive Level C IVIVC was observed, confirming that higher in vitro dissolution predicts greater systemic exposure.
All tested brands met minimum standards, but CQ-004 and CQ-003 demonstrated superior dissolution and bioavailability. The established Level C IVIVC confirms that in vitro dissolution can predict in vivo exposure, reinforcing the value of routine dissolution testing in post-market surveillance.
**Keywords.** Chloroquine Phosphate, Dissolution Testing, Bioavailability, IVIVC, Antimalarial Quality.

## Introduction

Malaria is a serious global health challenge, particularly in endemic regions such as Afghanistan [1]. According to the World Malaria Report 2024 by the World Health Organization (WHO), in 2023, globally, there were 263 million estimated malaria cases, 597000 deaths in 83 countries, which shows an increase of 11 million cases compared to 2022 [2]. Afghanistan ranks as the third highest in malaria burden globally, and 5% of infections in Afghanistan are caused by *P.falciparum*, while 95% by *P. vivax* is responsible for the majority of infections in Afghanistan [1,3]. The standard treatment for uncomplicated malaria caused by *P. vivax is* Chloroquine (CQ) (or ACTs in CQ-resistant areas) combined with primaquine, which is the treatment of choice [3–5]. The National Institute of Malarial Control Program of Afghanistan follows WHO guidelines for treatment and recommends CQ or ACTs depending on local resistance [6]. CQ chemical formula $C_{18}H_{26}ClN_3$, molecular weight 319.88 g/mol, and it belongs to the 4-aminoquinoline class of antimalarial drugs. It's a BCS Class I drug with high permeability and high solubility [7,8]. Due to CQ's effectiveness, wide availability, and broad use, it's essential to consistently assess its effectiveness through in vitro and in vivo assessment. [9–11].

Since 2013, the World Health Organization (WHO) has reported around 1,500 cases of substandard and falsified products, most of which involved antimalarial; this highlights the urgent need for regulatory bodies to address the presence of substandard or poor quality anti-malarial medications [12,13]. In Afghanistan, a study analyzed 7,740 various anti-malarial products. A subset of CQ and other anti-malarial tablets was tested for in vitro dissolution. Results showed that 32% of these samples did not meet the USP dissolution tolerance limits, indicating the presence of substandard antimalarials in the country [14]. Dissolution testing is a widely used in vitro method for evaluating the drug release profile of solid dosage forms such as tablets and capsules [15]. A comprehensive review systematically analyzed 97 experimental studies and confirmed that HPLC-UV is the gold standard for CQ quantification in both pharmaceutical products and biological samples, due to its superior sensitivity, selectivity, and ability to separate the drug from excipients, degradation products, and metabolites [16].

Bioavailability, the fraction of the administered drug that reaches systemic circulation in its active form, is crucial for therapeutic efficacy. In case of CQ phosphate tablets, optimal bioavailability ensures effective


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blood concentrations for parasiticidal effects while avoiding subtherapeutic levels that contribute to resistance's development [17]. Drug release from its formulation is a key determinant of its bioavailability 18. In formulation techniques, the choice of excipients such as binders, solvents, fillers, preservatives, and stabilizers can significantly alter a drug's absorption rate or extent of drug release profile and bioavailability [19–21]. In vitro-in vivo correlation (IVIVC) refers to a predictive mathematical relationship between a drug's in vitro dissolution profile and in vivo bioavailability. Regulatory guidance from the USP chapter defines three correlation levels: A, B, and C, with Level C representing a single-point relationship suitable for quality control in resource-limited settings [22].

## Materials and Methods

All chemicals and reagents utilized in this study were analytical and HPLC grade. Reference Standard (RS) from Sigma Aldrich. Acetonitrile HPLC grade, Ortho-phosphoric acid 85%, Perchloric acid 60%, tert-Butyl methyl ether (TBME) are purchased from Merck, Germany; Triethylamine (TEA) 99.5% from Sigma Aldrich, Germany; Hydrochloric acid 37% from Avantor Performance, Poland; and Hexane from Thermo-Fisher, Germany.

### *In vitro drug release assessment*

This method involved the inclusion of five brands of CQ phosphate tablets (CQ-001, CQ-002, CQ-003, CQ-004, and CQ-005) sourced from Kabul pharmacies; for ethical reasons, the commercial brand names were replaced with coded identifiers (CQ-001 – CQ-005) throughout this study. Twenty tablets per brand were weighed individually. Weight variation limits followed guidelines 5% for ≥250 mg for uncoated and film-coated tablets [23,24].

### *Dissolution test*

The dissolution of 250 mg CQ phosphate tablets of five selected brands and USP RS was conducted using a dissolution apparatus ERWEKA DT 820. Each tablet was injected into a 900 mL vessel of deionized water maintained at 37°C ± 0.5°C, with paddles rotating at 100 rpm for 45 minutes [25,26].

### *Liquid chromatography-UV analysis*

A ten µL injection volume was used after baseline stabilization. The Agilent 1260 Infinity II HPLC system was used; chromatographic separation was performed on a $C_{18}$ column 4.6 × 100 mm, 5 µm, L1 packing with an isocratic mobile phase of methanol: phosphate buffer pH 2.5, 22:78 v/v pumped at a flow rate of 1.2 mL/min, UV detection at 224 nm, in accordance with [26].

### *Mobile Phase Preparation*

The isocratic mobile phase consisted of methanol and phosphate buffer (22:78 v/v). The phosphate buffer was prepared by adding 0.05Mol in DI water, and 1.0 mL of perchloric acid 60%, then the pH was adjusted to 2.5 using orthophosphoric acid. The buffer was then filtered through a 0.45-µm nylon membrane filter and degassed by sonication for 10 minutes before use. A stock solution of CQ phosphate USP RS with a concentration of 0.27 mg/mL was prepared by dissolving 250 mg of the reference standard in 900 mL of dissolution medium. From this stock solution, working standards with concentrations of 0.06, 0.09, 0.12, 0.15, and 0.18 mg/mL were prepared. These solutions were degassed by sonication for 10 minutes and filtered through 0.2 µm nylon syringe filters into HPLC vials.

For each analysis, a 10 µL aliquot was injected into the HPLC system using an autosampler [26,27]. One 250 mg CQ phosphate tablet was added to a dissolution test (DT) vessel. After exactly 45 minutes, an aliquot was withdrawn and diluted with dissolution medium to achieve a target concentration of 0.15 mg/mL of CQ phosphate, as specified in the USP monograph. The sample was mixed for 15 minutes, degassed by ultra-sonication for 10 minutes, filtered through 0.2µm nylon syringe filters into HPLC vials, and 10 µL was injected into the HPLC system [26,27]. Dissolution with the HPLC method, focusing on three critical parameters: repeatability, linearity, and accuracy verification. Sample repeatability was assessed by analyzing six homogeneous samples from the same dissolution vessel; the linearity was assessed by five concentration levels: 0.06, 0.09, 0.12, 0.15, and 0.18 mg/mL, using CQ Phosphate RS; each concentration was analyzed in triplicate over three days (n=9 per level); and accuracy was determined through recovery studies at each linearity level 26,28,29.

### *In vivo bioavailability*

The bioavailability of CQ phosphate tablets for therapeutic efficacy was evaluated through HPLC-UV analysis of whole blood samples collected at 24 hours on the second day of treatment. Samples were obtained from 20 consenting *P. vivax* patients who received CQ according to National Institute of Malaria and Leishmaniasis Control Program (NILMCP) guidelines at a dose of 25 mg base/kg BW 30Four patients per brand.


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***Mobile phase***
DI water adjusted to pH 2.8 with orthophosphoric acid, and acetonitrile in a ratio of 85:15 (v/v) with 1% triethylamine; Column: $C_{18}$ 150 × 4.6 mm, 5 µm; Flow rate 3 mL/min; Detection: 340 nm Injection volume 50 µL [31].

***Standard preparation for calibration***
Stock solutions of CQ were prepared in DI water, while the internal standard (IS), quinine sulfate (QS), was dissolved in methanol. Calibration standards were prepared by Drug-free whole blood spiked with known concentrations of CQ 150, 300, 750, 1000, and 1500 ng/mL, along with a fixed amount of QS as the internal standard [31]. A blood sample of 150 µL volume was subjected to an extraction process that included acid-base treatment, liquid–liquid extraction, evaporation, and reconstitution using the mobile phase. After reconstitution, a 50 µL aliquot of the final solution was injected into the HPLC system for analysis [31].

***Extraction procedure***
A 150 µL sample of whole blood was transferred into borosilicate glass tubes and spiked with 750 ng of QS as the internal standard. Then 500 µL of 0.2 M hydrochloric acid was added, and the mixture was incubated for 2 minutes, followed by the addition of 1 mL of 20% sodium hydroxide. CQ and QS were extracted using 5 mL of hexane:tert-butyl methyl ether mixture (1:1, v/v) with gentle mixing for 30 minutes. After centrifugation at 3000 g for 10 minutes, the organic layer was collected, evaporated to dryness under a nitrogen stream at 37°C, and the residue was reconstituted in 100 µL of the mobile phase. Finally, 50 µL of the reconstituted solution was injected into the HPLC system [31].

## Results
The quality and performance of five CQ phosphate 250 mg tablet brands (CQ-001 to CQ-005) were evaluated through weight variation, dissolution, and bioavailability testing (LC-UV). The ±5% acceptance limit for tablets weighing ≥250 mg, CQ-001 exhibited the greatest variability, ranging from 4.77% to 5.90%, whereas CQ-003 showed the most uniform tablet weights between 1.44% and 1.73%.

***Table 1. weight variation for five brands of CQ phosphate (250 mg) tablet (n = 20) from each brand***

| Weight | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Brands** | **N** | **Mean** | **Std. Deviation** | **95% Confidence Interval for Mean** | | **Minimum** | **Maximum** |
| | | | | **Lower Bound** | **Upper Bound** | | |
| Tab CQ-001 | 20 | 543.9000 | 14.58875 | 537.0723 | 550.7277 | 518.00 | 576.00 |
| Tab CQ-002 | 20 | 517.6500 | 6.89222 | 514.4243 | 520.8757 | 507.00 | 533.00 |
| Tab CQ-003 | 20 | 345.4000 | 3.34664 | 343.8337 | 346.9663 | 339.00 | 350.00 |
| Tab CQ-004 | 20 | 409.0000 | 5.01576 | 406.6525 | 411.3475 | 401.00 | 420.00 |
| Tab CQ-005 | 20 | 464.2500 | 16.81752 | 456.3792 | 472.1208 | 435.00 | 485.00 |

***HPLC-UV Dissolution Method Verification***
The HPLC-UV method employed for in vitro dissolution analysis was verified in accordance with ICH Q2 (R2) guidelines and showed satisfactory performance.

***Repeatability***
was performed using six homogeneous samples collected from the same vessel at the target concentration of 0.15 mg/ml with a standard deviation of 0.983 and relative standard deviation (RSD) of 0.393%, which were within the acceptable limits.

***Linearity***
The calibration curve constructed using five concentration levels (0.06, 0.09, 0.12, 0.15, and 0.18 mg/mL) demonstrated excellent linearity, with a regression equation of $y = 1394.6x - 2.3667$ and a correlation coefficient ($R^2$) of 0.9992 (Fig 1).


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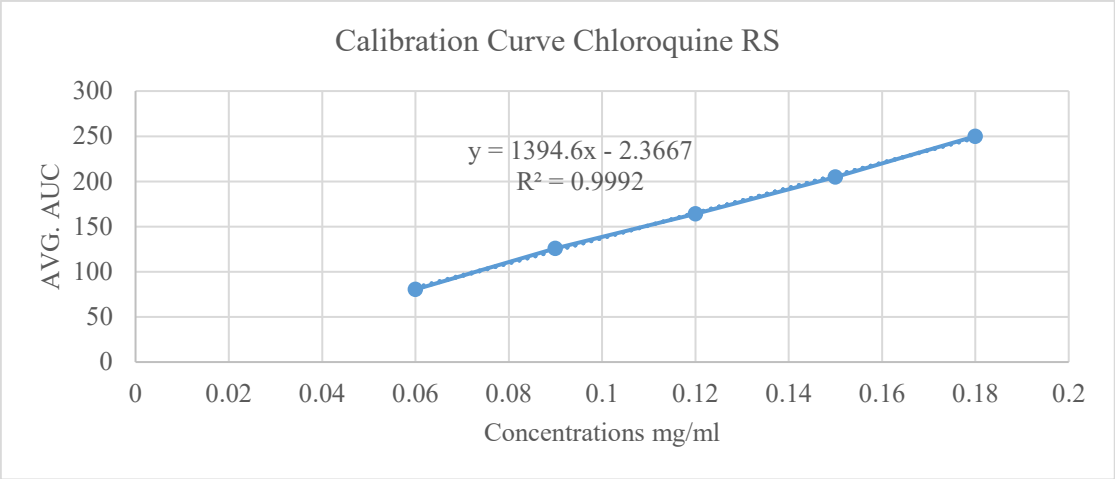


***Fig 1. Calibration curve for CQ 250mg RS demonstrating the relationship between concentration and average AUC***

Accuracy assessment of the HPLC-UV method for CQ phosphate at five concentration levels (n = 9 per level) showed mean recovery values ranging from 99.56% to 100.27%. All individual recovery results were within the acceptance range of 93.0%–107.0%, confirming the accuracy of the method. Retention time of CQ reference standard is shown in (Fig 2).

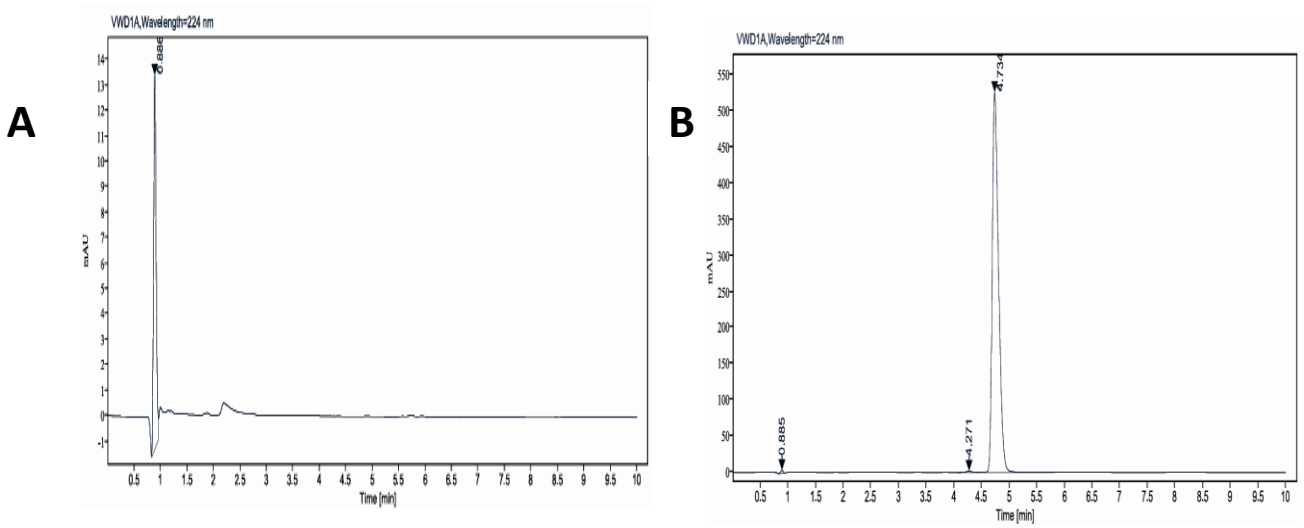


***Fig 2. HPLC chromatograms of (A) blank dissolution medium, deionized water, and (B) CQ phosphate reference standard (0.15 mg/mL) with retention time 4.7 min. Chromatographic conditions: $C_{18}$ column, methanol: phosphate buffer pH 2.5 (22:78 v/v), 1.2 mL/min, λ=224 nm, injection volume 10 µL***

***In Vivo Bioavailability HPLC-UV Method Verification***

***Linearity***

The HPLC-UV method for whole blood analysis demonstrated acceptable linearity across the calibration range of 150–1500 ng/mL, with a correlation coefficient ($R^2$) of 0.98.

***Recovery***

Extraction efficiency was evaluated using drug-free whole blood samples spiked with CQ at five concentration levels. Mean recovery values ranged from 96.1% to 109.4%, indicating satisfactory accuracy for biological sample analysis.

***In Vitro Dissolution Analysis (HPLC-UV)***

All five evaluated brands met the pharmacopeial requirement of ≥75% drug release at 45 minutes. The mean dissolution percentages were 94.26% ± 2.83% for CQ-001, 96.17% ± 2.56% for CQ-002, 96.41% ± 2.74% for CQ-003, 97.63% ± 1.33% for CQ-004, and 89.20% ± 2.99% for CQ-005. Among the tested brands, CQ-004 showed the highest mean dissolution with the lowest variability, whereas CQ-005 exhibited the lowest mean dissolution value.


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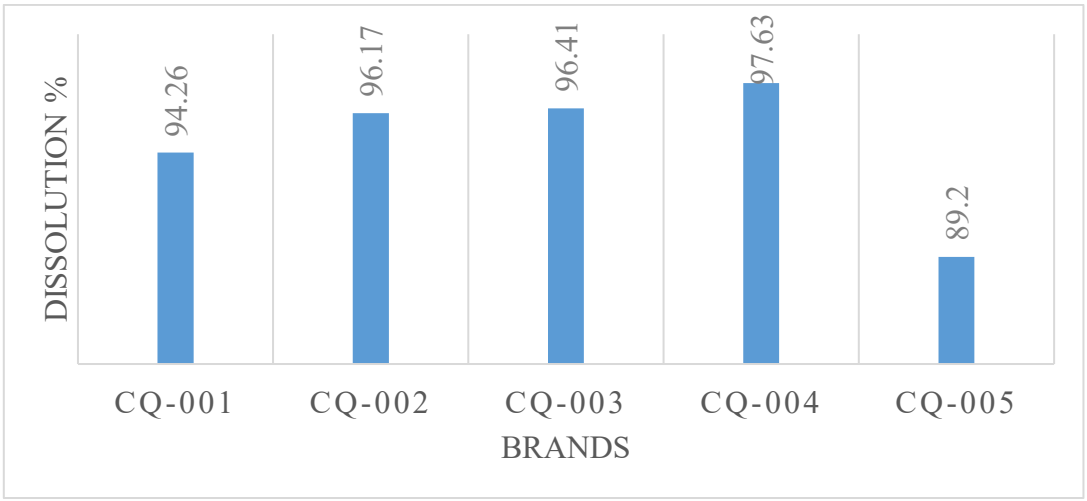


***Fig 3. In-vitro Dissolution Performance of Different Chloroquine Phosphate Brands***

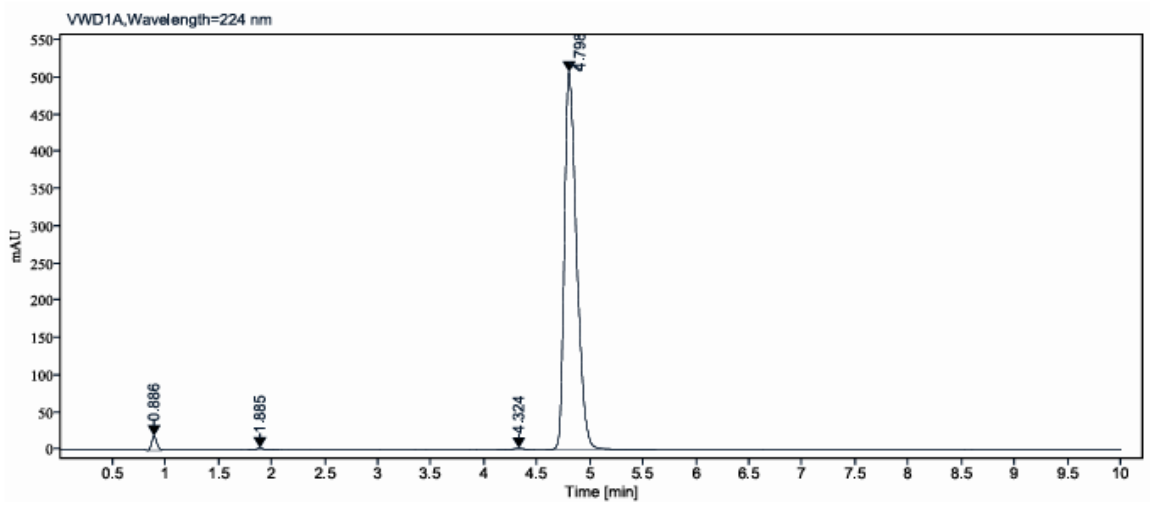


***Fig 4. HPLC chromatograms of e.g., CQ phosphate sample (0.15 mg/mL) with retention time 4.7 min. Chromatographic conditions: $C_{18}$ column, methanol: phosphate buffer pH 2.5 (22:78 v/v), 1.2 mL/min, λ=224 nm, injection volume 10 µL***

***In vivo bioavailability***

The in vivo bioavailability showed that CQ-004 and CQ-003 achieved the highest mean concentrations of 362 ± 57 ng/mL and 359 ± 56 ng/mL, respectively. Intermediate concentrations were observed for CQ-005 (336 ± 58 ng/mL) and CQ-002 (318 ± 53 ng/mL), while CQ-001 showed the lowest mean concentration (291 ± 66 ng/mL).

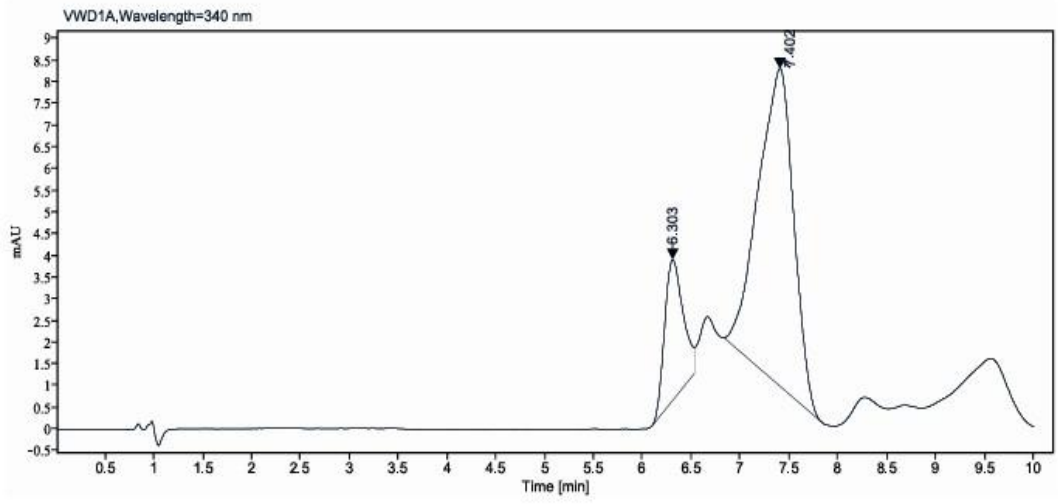


***Fig 5. Chromatograms recorded at 340 nm: CQ peak detected at RT 6.3 min in P. vivax–positive patient whole blood, with IS QS 750ng/ml at RT 7.4***


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***In Vitro–In Vivo Correlation (IVIVC)***

As shown in (Table 2), a positive association was observed between in vitro dissolution and in vivo exposure. CQ-004 showed the highest dissolution (97.63% ± 1.33%) and bioavailability (362 ± 57 ng/mL), followed by CQ-003 and CQ-002, which exhibited dissolution above 96% and corresponding bioavailability of 359 ± 56 ng/mL and 318 ± 53 ng/mL, respectively. In contrast, CQ-005 demonstrated the lowest dissolution (89.2% ± 2.99%) but moderate bioavailability (336 ± 58 ng/mL), whereas CQ-001 (94.26% dissolution) showed the lowest bioavailability (291 ± 66 ng/mL). The inverse trend observed for CQ-005 warrants further investigation. Overall, the Level C IVIVC supports the use of dissolution testing as a surrogate indicator of in vivo performance.

***Table 2. Summarizes the IVIVC by comparing mean dissolution and bioavailability across brands***

| Brand | Mean In Vitro Dissolution (%) | Mean In Vivo Bioavailability (ng/mL) | Correlation |
|---|---|---|---|
| CQ-001 | 94.26 | 291 | Weak positive |
| CQ-002 | 96.17 | 318 | Moderate positive |
| CQ-003 | 96.41 | 359 | Strong positive |
| CQ-004 | 97.63 | 362 | Strong positive |
| CQ-005 | 89.2 | 336 | Inverse trend |

***Fig 6. Visualizes correlation of CQ mean dissolution percentage and mean 24h blood concentration***

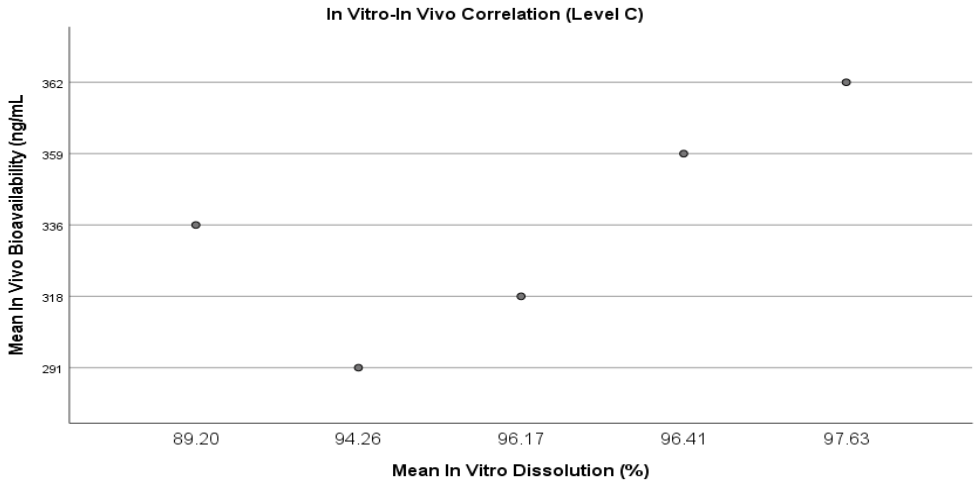


***Fig 7. Visualizes the correlation of CQ mean dissolution percentage and mean 24h blood concentration***

***Table 3. Comparative in vitro and in vivo evaluation of chloroquine phosphate***

| In Vitro Analysis | | Weight Variation | | HPLC Dissolution % | | | In Vivo Bioavailability | HPLC Bioavailability ng/ml | |
|---|---|---|---|---|---|---|---|---|---|
| | Brand | Mean Weight (mg) ± SD | N | Mean Dissolution (%) ± SD | %RSD | N | | Mean Bioavailability ± SD | N |
| | CQ-001 | 543.90 ± 14.59 | 20 | 94.26 ± 2.83 | 3.00% | 6 | | 291 ± 66 | 4 |
| | CQ-002 | 517.65 ± 6.89 | 20 | 96.17 ± 2.55 | 2.63% | 6 | | 318 ± 53 | 4 |
| | CQ-003 | 345.40 ± 3.35 | 20 | 96.41 ± 2.74 | 2.84% | 6 | | 359 ± 56 | 4 |
| | CQ-004 | 409.00 ± 5.02 | 20 | 97.63 ± 1.33 | 1.36% | 6 | | 362 ± 57 | 4 |
| | CQ-005 | 464.25 ± 16.82 | 20 | 89.20 ± 2.99 | 3.35% | 6 | | 336 ± 58 | 4 |

## Discussion

Dissolution test is crucial for quality evaluation of in vitro release performance of solid oral tablet forms, and also critical for evaluating the in vivo drug absorption. In this study, the dissolution behavior of the brands tested varied due to differences in formulation and manufacturing processes. These differences can affect drug release kinetics and ultimately impact bioavailability, highlighting the importance of dissolution profiling for the evaluation of comparative products. Weight variation is a fundamental indicator of


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manufacturing precision and dosage unit uniformity. According to the uniformity of mass requirements for single-dose preparations, tablets in the ≥250 mg category must comply with a ±5% deviation from the mean weight. The highest degree of weight uniformity was shown with CQ-003, with minimal deviation from the mean, whereas CQ-001 and CQ-005 demonstrated comparatively higher variability. Although weight variation alone does not confirm content uniformity, particularly for high-dose formulations such as CQ phosphate, it remains an important surrogate marker of batch consistency.

Although all products were labeled 250 mg CQ phosphate, the observed variation in tablet weight e.g., CQ-003: 345.4 mg vs. CQ-001: 543.9 mg is attributable to different formulation strategies employed by manufacturers. CQ-003 appears to contain fewer excipients or different filler types compared to CQ-001. Higher variability may lead to differences in tablet porosity, hardness, and disintegration time, which can subsequently influence dissolution behavior and ultimately bioavailability. The trends observed in this study support this relationship, as brands with better weight uniformity generally exhibited more consistent dissolution and release profiles. Initial dissolution analysis was performed by HPLC using six tablets per brand to assess in vitro drug release. LC-UV results showed mean releases for CQ-001 94.3%, 96.2% for CQ-002, 96.4% for CQ-003, 97.6% for CQ-004, and 89.2% for CQ-005. The relative standard deviation ranged from 1.3% for CQ-004 to 3.0% for CQ-001 and 3.3% for CQ-005 (Table 3).

All brands met the pharmacopeial requirement of not less than 75% drug release within 45 minutes, confirming compliance with minimum quality standards. While HPLC-UV analysis provides accurate quantification of drug release at the batch level, it does not fully capture inter-tablet variability. These findings highlight the importance of stringent quality control measures in pharmaceutical production to ensure consistent therapeutic efficacy and treatment outcomes. The in vivo bioavailability of CQ phosphate tablets was evaluated in patients diagnosed with *Plasmodium vivax* malaria. Blood samples were collected 24 hours post-administration to measure whole-blood CQ concentrations. The mean bioavailability values were 291 ± 66 ng/mL for CQ-001, 318 ± 53 ng/mL for CQ-002, 359 ± 56 ng/mL for CQ-003, 362 ± 57 ng/mL for CQ-004, and 336 ± 58 ng/mL for CQ-005. The highest bioavailability was observed for CQ-004, followed closely by CQ-003. All mean concentrations were within the therapeutic range, indicating acceptable safety and efficacy [31,32]. A Level C IVIVC was established by correlating mean dissolution percentages with mean whole-blood CQ concentrations, in accordance with USP [22].

This single-point correlation demonstrated a positive relationship between in vitro drug release and in vivo systemic exposure, particularly for CQ-003 and CQ-004, which showed both high dissolution and high bioavailability (Fig 6). Notably, CQ-005 exhibited an inverse trend, characterized by lower in vitro dissolution (89.2%) but relatively higher in vivo concentration (336 ng/mL). This finding may be attributed to formulation-related or patient-specific factors, including food intake [33,34], and warrants further investigation. The use of a single 24-hour post-dose sampling point represents a limitation, future studies should include multiple sampling points for more robust pharmacokinetic evaluation.

## Conclusion

The combined in vitro and in vivo findings indicate that although all tested brands met pharmacopeial requirements, inter-brand variability was observed, which may influence patient outcomes if substandard formulations are present in the market. The bioavailability results confirmed therapeutic adequacy and support its continued use in Afghanistan's malaria control program. However, Level C IVIVC supports the use of in vitro dissolution testing as a surrogate marker for in vivo performance in quality control and post-market surveillance of CQ phosphate tablets in Afghanistan. This study has several limitations, including the use of a single post-dose bioavailability sampling time and a limited sample size per brand. Future studies should focus on establishing Level A IVIVC using multiple dissolution time points and full pharmacokinetic profiling to enhance predictive capability and support regulatory decision-making.

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