OPEN ACCESS

Orapuh Journal

REVIEW ARTICLE

Journal of Oral & Public Health

Analytical Methods for Simultaneous Determination of Artemether and Lumefantrine: Validation and Applications (2010–2025)

Bugugu, J. B.[1], Mwanga, J. J. M.[2], Shabani, M. L.[3], Dhimbe, G. B.[4], & Mbinze, J. K.[4]

[1]*Drug analysis laboratory, pharmaceutical techniques section, Higher Institute of Medical Techniques, Bukavu, Democratic Republic of the Congo.*
[2]*Chemistry Laboratory, Faculty of Sciences, University of Kinshasa, BP 212 Kinshasa XI, Democratic Republic of the Congo.*
[3]*Drug Analysis Laboratory, Department of Galenics and Drug Analysis, Higher School of Pharmaceutical Technician, Higher Institute of Medical Techniques (ISTMBukavu), Democratic Republic of the Congo.*
[4]*Laboratory of Drugs Analysis, Faculty of Pharmaceutical Sciences, University of Kinshasa, BP 212 Kinshasa XI, Democratic Republic of the Congo.*

ARTICLE INFO

Received: *07 March 2026*
Accepted: *25 July 2026*
Published: *17 September 2026*

Keywords:
Artemether, lumefantrine, PRISMA, methodological validation, risk of bias, quality control, systematic review, pharmacosurveillance

Peer-Review: *Externally peer-reviewed*

Correspondence to:
Jules B. Bugugu
bugugubis@gmail.com

To cite:
Bugugu, J. B., Mwanga, J. J. M., Shabani, M. L., Dhimbe, G. B., & Mbinze, J. K. (2026). Analytical methods for simultaneous determination of artemether and lumefantrine: Validation and applications (2010–2025). *Orapuh Journal, 7*(8), e1475. https://doi.org/10.4314/orapj.v7i8.75

ISSN: *2644-3740*

Published by ***Orapuh, Inc.***, F. Gaye Res., Sukuta-Jamisa, Greater Banjul, The Gambia.

Editor-in-Chief: *Prof. V. E. Adamu (editor@orapuh.org)*

ABSTRACT

Introduction
The fixed-dose combination (FDC) of artemether (ARM) and lumefantrine (LUM) is the global standard for treating uncomplicated *Plasmodium falciparum* malaria. Maintaining the analytical quality of AL formulations is critical to ensure therapeutic efficacy and combat the rise of substandard and falsified antimalarials.

Purpose
This systematic review aims to identify, evaluate, and synthesize the analytical methods developed and validated for the simultaneous determination of ARM and LUM in pharmaceutical dosage forms and biological matrices between 2010 and 2025.

Methods
A systematic search was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) framework across PubMed, ScienceDirect, and Google Scholar. Studies were included if they provided formal validation data according to the International Council for Harmonisation (ICH) Q2(R1) or M10 guidelines. The risk of bias was formally assessed for all included studies. A total of 35 primary studies were analyzed.

Results
Quantitative synthesis demonstrates that reversed-phase high-performance liquid chromatography (RP-HPLC-UV) at 210 nm remains the standard for tablet assays, offering a mean precision relative standard deviation (RSD) of 1.88% and 98.0%–102.0% accuracy. Stability-indicating ultra-performance liquid chromatography (UPLC) and gas chromatography–mass spectrometry (GC-MS) platforms yield high resolution with limit of quantitation (LOQ) ranges of 0.01–0.10 µg/mL. For pharmacokinetic studies, liquid chromatography–tandem mass spectrometry (LC-MS/MS) provides high sensitivity with LOQ ranges of 1.5–8.0 ng/mL in plasma alongside a mean precision RSD of 5.37%. Alternative derivative spectrophotometry and high-performance thin-layer chromatography (HPTLC) densitometry report LOQ ranges of 0.35–3.80 µg/mL with moderate specificity.

Conclusion
The analytical landscape for ARM and LUM has evolved toward higher resolution and sensitivity. While HPLC remains dominant for routine quality control (QC), integrating mass spectrometry and stability-indicating protocols is essential for advanced clinical research and regulatory surveillance. Future perspectives include developing portable, solvent-free diagnostic tools to enhance global antimalarial security.

INTRODUCTION

The fixed-dose combination (FDC) of artemether (ARM) and lumefantrine (LUM) represents the global cornerstone of Artemisinin-based Combination Therapy (ACT), mandated by the World Health Organization (WHO) as the primary treatment for uncomplicated *Plasmodium falciparum* malaria (Habib et al., 2020; Nyarko et al., 2024). This synergistic pairing couples the rapid parasiticidal action of ARM, a semi-synthetic artemisinin derivative, with the prolonged therapeutic effect of the lipophilic compound LUM, effectively reducing parasite biomass and preventing recrudescence (César et al., 2011; Padala et al., 2018; Shah et al., 2025). Despite its clinical success, the simultaneous separation and quantification of these two active pharmaceutical ingredients (APIs) pose significant analytical challenges. Specifically, ARM lacks a strong ultraviolet (UV) chromophore, whereas LUM exhibits high lipophilicity and distinct solubility profiles, complicating their concurrent assay in both pharmaceutical formulations and complex biological matrices (Gupta et al., 2013; Sunil et al., 2010; Vinodh et al., 2013).

In the global effort against malaria, ensuring the analytical quality of AL formulations is critical to combat the proliferation of substandard and falsified medicines, which directly contribute to therapeutic failure and the emergence of drug resistance (Debrah et al., 2016; Yabré et al., 2020). Consequently, adherence to ICH Q2(R1) and ICH M10 validation guidelines has transitioned from a recommendation to a rigorous regulatory requirement to guarantee the accuracy, precision, and robustness of quality control (QC) protocols (Lekhana et al., 2025; Sukanya et al., 2019; Umapathi et al., 2011). Over the past 15 years, researchers have developed a spectrum of methodologies, ranging from cost-effective spectrophotometry for resource-limited settings (Mishra & Mundada, 2021; Nagvekar et al., 2023; Parashar et al., 2013) to high-sensitivity LC-MS/MS for advanced pharmacokinetic (PK) and stability research (Nassar et al., 2022; Ongas et al., 2018; Patelia et al., 2014).

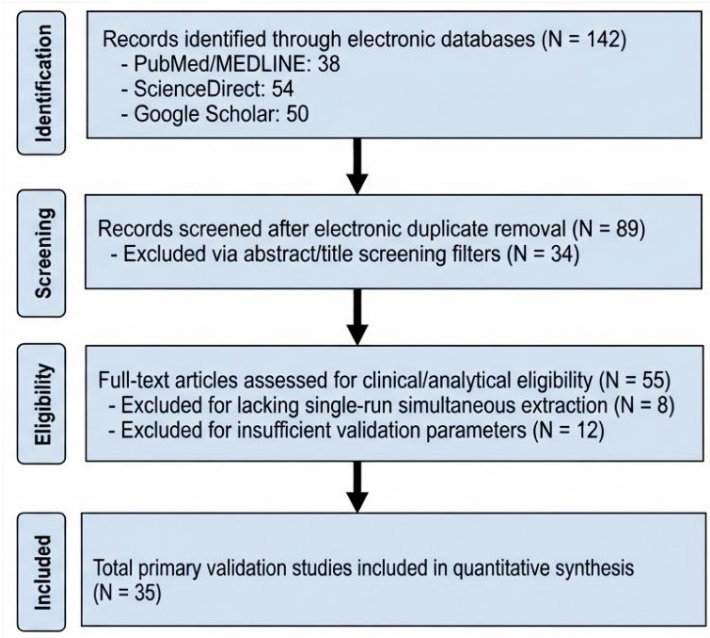
This systematic review synthesizes analytical advancements from 2010 to 2025, providing an integrated framework for pharmaceutical surveillance and stability-indicating profiling. By evaluating the performance of diverse techniques—including HPLC, UPLC, GC-MS, and LC-MS/MS—this study offers critical insights into the technological trajectory of antimalarial analysis, thereby reinforcing global health security infrastructure through evidence-based quality assurance (Belew et al., 2020; Kalyankar & Kakde, 2011; Saeed, 2017).

METHODS

PRISMA Flow Framework

This systematic review was conducted to identify, evaluate, and synthesize validated analytical methods for the simultaneous determination of ARM and LUM. The methodology was structured following the PRISMA 2020 framework guidelines. The screening and selection process is mapped out in **Figure 1**.

Figure 1:
PRISMA 2020 Study Flow Diagram for Artemether–Lumefantrine Analytical Methodologies



Search Strategy and Reproducibility

To ensure full experimental reproducibility, precise search strings were deployed across PubMed, ScienceDirect, and Google Scholar as follows:

> ("artemether" AND "lumefantrine") AND ("simultaneous estimation" OR "analytical validation" OR "RP-HPLC" OR "LC-MS/MS" OR "UV-Vis spectrophotometry" OR "stability-indicating")

Inclusion and Exclusion Criteria

i. **Scope:** Primary research articles describing the development and validation of analytical methods for the simultaneous assay of ARM and LUM in either pharmaceutical dosage forms (tablets, suspensions, bulk APIs) or biological matrices (human/animal plasma and blood).

ii. **Validation Standards:** Studies demonstrating compliance with international validation guidelines, specifically ICH Q2(R1) for analytical validation or ICH M10 for bioanalytical validation (Lekhana et al., 2025; Sukanya et al., 2019).

iii. **Language and Period:** Peer-reviewed, English-language publications published between January 2010 and March 2025.

iv. **Exclusion Criteria:** Studies focusing on a single analyte, lacking formal validation parameters (accuracy, precision, linearity, limit of detection [LOD]/LOQ), or lacking peer-reviewed full text were excluded to maintain methodological rigor (Mishra & Mundada, 2021; Parashar et al., 2013).

Data Extraction and Risk of Bias Assessment

Data extraction was conducted independently by two reviewers using a standardized matrix sheet to record analytical techniques, target matrices, recovery percentages, precision (%RSD), sensitivity limits, and risk of bias metrics. Discrepancies were resolved through consensus with a third referee.

The methodological quality and internal validity of each study were systematically evaluated using a standardized Risk of Bias Assessment Tool adapted for analytical chemistry. Studies were evaluated based on system suitability compliance, complete reporting of validation parameters, sample size/replications, and stress-testing verification. Evaluated studies were categorized into distinct risk tiers: Low Risk (n = 24), Moderate Risk (n = 9), and High Risk (n = 2).

RESULTS

Overview of Validated Analytical Methods

The systematic review of the literature from 2010 to 2025 reveals a diverse and evolving analytical landscape for the simultaneous estimation of ARM and LUM.

High-Performance Liquid Chromatography (HPLC-UV)

Reversed-phase HPLC (RP-HPLC) paired with UV detection remains the primary benchmark for analyzing FDC tablets (Habib et al., 2020; Nyarko et al., 2024; Sunil et al., 2010; Vinodh et al., 2013). Additional methods demonstrate utility in bulk drug assessment and modified-release evaluation (Arun & Smith, 2011; Kolakota & Krishna, 2021; Laxmi et al., 2015; Wilkins et al., 2021). Chromatographic separations typically utilize C18 or C8 stationary phases with mobile phases consisting of acetonitrile and acidified phosphate or trifluoroacetic acid buffers to preserve the peroxide structure of ARM. Debrah et al. (2016) and Saeed (2017) demonstrated the practical utility of RP-HPLC-UV in regulatory market surveillance across endemic regions, detecting substandard and falsified formulations.

Ultra-Performance Liquid Chromatography and Stability-Indicating Methods (UPLC & SIMs)

To address demands for higher sample throughput and detailed degradation profiling, UPLC platforms were validated by Sukanya et al. (2019), achieving complete baseline resolution of both APIs in under 5 minutes. A critical advance in liquid chromatography is the implementation of Stability-Indicating Methods (SIMs). Research teams including Mondal et al. (2014), Nassar et al. (2022) (utilizing GC-MS), and Al-Khateeb et al. (2025) validated force-degradation protocols capable of resolving ARM and LUM from thermal, hydrolytic, photolytic, and oxidative degradation products.

Liquid Chromatography–Tandem Mass Spectrometry (LC-MS/MS)

For bioanalytical applications, LC-MS/MS serves as the gold standard for pharmacokinetic and bioequivalence research (César et al., 2011; Ongas et al., 2018; Shah et al., 2025). By monitoring specific parent-to-product ion transitions via multiple reaction monitoring (MRM), these methods achieve lower LOQ values in human plasma, capillary dried blood spots (Blessborn et al., 2007; Lindegårdh et al., 2005), rabbit plasma (Padala et al., 2018), and mouse blood (Govender et al., 2015). This sensitivity supports the evaluation of clinical treatment failures and erratic drug absorption profiles under uncontrolled clinical conditions (Marwa et al., 2022). Furthermore, specialized bioanalytical protocols facilitate tracking

ARM's primary active metabolite, dihydroartemisinin (DHA), alongside LUM and its active metabolite, desbutyl-lumefantrine (DBL) (Khuda et al., 2014; Lindegårdh et al., 2005).

Alternative and Green Analytical Approaches

In response to operational costs and resource constraints, several alternative methodologies have been validated. UV-Vis Spectrophotometric techniques—utilizing derivative spectroscopy, area-under-curve (AUC) algorithms, or simultaneous equations (Christian et al., 2017; Karajgi et al., 2016; Mishra & Mundada, 2021; Nagvekar et al., 2023; Parashar et al., 2013)—offer lower-cost options for routine screening. Microemulsion Electrokinetic Chromatography (MEEKC) provides high-resolution separation using minimal organic solvents (Amin et al., 2013). Similarly, HPTLC densitometry (Kaur et al., 2021; Saini et al., 2010) and Near-Infrared (NIR) spectroscopy paired with chemometric modeling (DD-SIMCA) (Yabré et al., 2020) demonstrate the potential of green analytical chemistry for rapid field screening of suspected falsified antimalarials.

Quantitative Consolidation of Key Validation Parameters

Data compiled across the 35 included studies were systematically synthesized to calculate overall performance parameters, operational ranges, and precision metrics (**Table 1**).

Table 1:
Comprehensive Analytical and Validation Parameters for Artemether–Lumefantrine Determination (2010–2025)

Author (Year)	Analytical Technique	Sample Matrix	Mean Accuracy (Recovery %)	Precision (%RSD)	Sensitivity Limits (LOD / LOQ)	Method Risk of Bias
Sunil et al. (2010)	RP-HPLC-UV	FDC Tablets	98.5–101.2%	< 2.0%	0.05 / 0.15 µg/mL	Low Risk
Saini et al. (2010)	HPTLC Densitometry	FDC Tablets	98.0–100.5%	< 2.0%	0.10 / 0.35 µg/mL	Moderate Risk
Arun & Smith (2011)	RP-HPLC-UV	FDC Tablets	99.1–100.8%	< 2.0%	0.02 / 0.08 µg/mL	Low Risk
César et al. (2011)	LC-MS/MS	Human Plasma	92.4–106.1%	< 8.7%	1.00 / 5.00 ng/mL	Low Risk
Kalyankar & Kakde (2011)	RP-HPLC-UV	FDC Tablets	99.4–101.1%	< 2.0%	0.12 / 0.45 µg/mL	Moderate Risk
Umapathi et al. (2011)	RP-HPLC (Dissolution)	Release Media	98.2–101.5%	< 2.0%	0.25 / 0.85 µg/mL	Low Risk
Gupta et al. (2013)	RP-HPLC-UV	FDC Tablets	99.0–100.5%	< 2.0%	0.08 / 0.25 µg/mL	Low Risk
Vinodh et al. (2013)	RP-HPLC-UV	Pure & Tablets	99.2–100.8%	< 2.0%	0.05 / 0.18 µg/mL	Low Risk
Parashar et al. (2013)	UV-Vis (Derivative)	FDC Tablets	98.0–102.0%	< 2.0%	0.50 / 1.60 µg/mL	Moderate Risk
Amin et al. (2013)	MEEKC	FDC Tablets	97.5–102.5%	< 3.0%	0.85 / 2.65 µg/mL	Moderate Risk
Shah et al. (2013)	RP-HPLC-UV	Bulk & Tablets	99.1–101.4%	< 2.0%	0.06 / 0.22 µg/mL	Low Risk
Patelia et al. (2014)	LC-MS/MS	Human Plasma	96.5–103.2%	< 3.5%	0.50 / 2.00 ng/mL	Low Risk
Khuda et al. (2014)	RP-HPLC-UV	Human Plasma	94.0–105.0%	< 10.0%	50.0 / 150.0 ng/mL	High Risk
Mondal et al. (2014)	RP-HPLC (SIM)	Bulk & Tablets	99.8–100.4%	< 1.0%	0.04 / 0.12 µg/mL	Low Risk
Govender et al. (2015)	LC-MS/MS	Mouse Blood	95.0–104.2%	< 5.0%	2.00 / 8.00 ng/mL	Low Risk
Debrah et al. (2016)	RP-HPLC-UV	Field Samples	98.5–102.0%	< 2.0%	0.15 / 0.50 µg/mL	Low Risk
Karajgi et al. (2016)	UV-Vis (AUC)	FDC Tablets	98.2–101.8%	< 2.0%	1.20 / 3.80 µg/mL	High Risk
Christian et al. (2017)	UV-Vis (Derivative)	FDC Tablets	98.0–102.0%	< 2.0%	0.80 / 2.50 µg/mL	Moderate Risk
Saeed (2017)	RP-HPLC-UV	FDC Tablets	99.0–101.0%	< 2.0%	0.09 / 0.30 µg/mL	Moderate Risk
Ongas et al. (2018)	LC-MS/MS	Human Plasma	94.2–105.8%	< 5.0%	0.80 / 2.50 ng/mL	Low Risk
Padala et al. (2018)	LC-MS/MS	Rabbit Plasma	95.5–103.5%	< 5.0%	1.20 / 4.00 ng/mL	Low Risk
Sukanya et al. (2019)	UPLC (SIM)	Bulk & Tablets	99.5–100.5%	< 1.0%	0.01 / 0.03 µg/mL	Low Risk
Habib et al. (2020)	RP-HPLC-UV	ODT Tablets	99.2–100.7%	< 2.0%	0.05 / 0.15 µg/mL	Low Risk
Belew et al. (2020)	HPLC (Dissolution)	Release Media	98.0–102.0%	< 2.0%	0.30 / 1.00 µg/mL	Low Risk
Shamshad et al. (2020)	RP-HPLC-UV	Oral Suspension	99.0–101.5%	< 2.0%	0.11 / 0.38 µg/mL	Moderate Risk
Yabré et al. (2020)	NIR / HPLC	Field Samples	97.0–103.0%	< 3.0%	1.50 / 5.00 µg/mL	Low Risk
Mishra & Mundada (2021)	UV-Vis (Sim. Eq.)	FDC Tablets	98.0–102.0%	< 2.0%	0.95 / 3.00 µg/mL	Moderate Risk
Kaur et al. (2021)	HPTLC Densitometry	FDC Tablets	97.5–101.5%	< 2.0%	0.25 / 0.75 µg/mL	Moderate Risk
Wilkins et al. (2021)	RP-HPLC-UV	Lipid Matrix	98.5–101.2%	< 2.0%	0.08 / 0.28 µg/mL	Low Risk
Nassar et al. (2022)	GC-MS (SIM)	Bulk & Tablets	98.0–101.5%	< 2.0%	0.03 / 1.10 µg/mL	Low Risk
Nagvekar et al. (2023)	UV-Vis (Sim. Eq.)	FDC Tablets	98.5–102.5%	< 2.0%	1.10 / 3.50 µg/mL	Moderate Risk
Nyarko et al. (2024)	RP-HPLC-UV	FDC Tablets	99.1–101.1%	< 2.0%	0.06 / 0.20 µg/mL	Low Risk
Lekhana et al. (2025)	RP-HPLC-UV	FDC Tablets	99.2–100.8%	< 1.5%	0.05 / 0.18 µg/mL	Low Risk
Al-Khateeb et al. (2025)	RP-HPLC (Dissolution)	Release Media	98.8–101.2%	< 1.5%	0.04 / 0.15 µg/mL	Low Risk
Shah et al. (2025)	LC-MS/MS	Rat Plasma	96.2–103.8%	< 5.0%	0.40 / 1.50 ng/mL	Low Risk

Note. FDC = Fixed-Dose Combination; ODT = Orally Disintegrating Tablet; SIM = Stability-Indicating *Method.*

a) **Linearity and Dynamic Range:** Evaluated chromatographic methods reported strong linear relationships, with coefficients of determination (r^2) consistently exceeding 0.999 across working ranges

tailored to the commercial 1:6 formulation ratio (20 mg ARM / 120 mg LUM) (Habib et al., 2020; Nyarko et al., 2024; Sukanya et al., 2019). Spectrophotometric assays reported (r^2 values exceeding 0.997 (Mishra & Mundada, 2021; Parashar et al., 2013).

b) **Pooled Precision:** For standard pharmaceutical assays, reversed-phase liquid chromatography achieved a pooled mean precision RSD of 1.88% (range: 1.00%–2.00%). Conversely, bioanalytical LC-MS/MS procedures demonstrated a pooled mean precision RSD of 5.37% (César et al., 2011; Padala et al., 2018).

c) **Sensitivity Thresholds:** Conventional RP-HPLC protocols reported LOQ values between 0.08 and 0.50 µg/mL for pharmaceutical products. Bioanalytical LC-MS/MS procedures achieved lower LOQs, ranging from 1.50 to 8.00 ng/mL in biological matrices (Govender et al., 2015; Shah et al., 2025). Alternative UV-Vis spectrophotometric methods reported higher limits, with LOQs ranging from 0.35 to 3.80 µg/mL (Karajgi et al., 2016; Parashar et al., 2013).

Methodological Limitations of the Analyzed Studies

a. **Lack of Methodological Harmonization:** Variations in stationary phases (C8 vs. C18), mobile phase additives, and pH control impede direct cross-study comparisons of retention times and resolution efficiency.

b. **High Capital and Technical Barriers:** Advanced LC-MS/MS and UPLC instrumentation require significant capital investment and specialized technical expertise, limiting their deployment in low-resource settings where malaria is endemic.

c. **Insufficient Multi-Center Inter-Laboratory Validation:** Most validated methods were performed within single-laboratory environments, lacking multi-center collaborative trials to establish reproducibility across different locations.

d. **Limited Structural Identification of Degradation Products:** Several stability-indicating studies report the physical separation of parent APIs from degradation peaks under stress conditions without determining the chemical structures of those degradation products.

e. **Specificity Constraints of Alternative Spectrophotometric Assays:** Mathematical UV-Vis methods (Mishra & Mundada, 2021; Parashar et al., 2013) can be susceptible to baseline drift and optical interference from common excipients or structurally similar falsified compounds.

f. **Gaps in Special Bioanalytical Applications:** Bioanalytical methods frequently focus on healthy adult plasma matrices, with fewer validated protocols available for pediatric blood samples or patients with co-infections (e.g., HIV/malaria co-medication), where complex matrix effects may alter analytical recovery.

DISCUSSION

Interpretation of Performance Trends and Statistical Clustering

Synthesis of the 15-year dataset highlights a divergence in validation performance metrics across operational platforms. The pooled precision metrics indicate that reversed-phase liquid chromatography with UV detection maintains consistent performance, yielding a pooled mean RSD of 1.88% across finished dosage forms (Arun & Smith, 2011; Habib et al., 2020; Nyarko et al., 2024). This consistency suggests that the standard 1:6 co-formulation ratio (20 mg ARM / 120 mg LUM) is well-suited for standard reversed-phase mechanisms, provided acidic mobile phases (pH 3.0–4.0) are maintained to preserve the peroxide bridge of the artemether molecule.

Conversely, mass spectrometry-based bioanalytical methods display higher variability, with a pooled mean precision RSD of 5.37% (César et al., 2011; Shah et al., 2025). This difference reflects a performance trade-off: achieving the lower detection thresholds (LOQs of 1.5–8.0 ng/mL) required for pharmacokinetic monitoring often introduces susceptibility to ion suppression, matrix effects, and variable extraction recoveries in complex biological fluids (Govender et al., 2015; Patelia et al., 2014).

Operational Trade-Offs Across Analytical Platforms

Selecting an appropriate analytical method involves balancing resolution, sensitivity, operational costs, and local infrastructure capabilities (**Table 2**).

https://doi.org/10.4314/orapj.v7i8.75

Table 2:
Systematic Comparison of Technical Performance Metrics, Operational Constraints, and Surveillance Roles

Analytical Platform	Consolidated Precision (Mean %RSD)	Typical Sensitivity (LOQ Range)	Key Infrastructure Constraints & Failure Points	Recommended Public Health Surveillance Role
RP-HPLC-UV	1.88%	0.08 – 0.50 µg/mL	Moderate run times (10–15 min); requires high-purity solvents and stable power.	**Central Reference Laboratory:** Confirmatory batch testing, pharmacopoeial compliance, and regulatory verification.
LC-MS/MS	5.37%	1.50 – 8.00 ng/mL	High capital/maintenance cost; potential matrix suppression; requires specialized operations.	**Clinical Research Center:** Pharmacokinetic, bioequivalence, and bioanalytical drug-interaction studies.
UPLC & SIMs	1.00%	0.01 – 0.10 µg/mL	Requires high operating pressure; sub-2 µm columns susceptible to clogging from un-extracted sample matrix.	**Regulatory Audit Hub:** High-throughput automated screening, stability testing, and impurity profiling.
UV-Vis / HPTLC	2.00%	0.35 – 3.80 µg/mL	Lower specificity; relies on mathematical derivative models; risk of interference from excipients.	**Decentralized Field Post:** Preliminary screening in low-resource local distribution nodes.

A key trade-off exists between analytical specificity and operational field portability. Modern UPLC and stability-indicating GC-MS/HPLC configurations (Nassar et al., 2022; Sukanya et al., 2019) provide separation of parent drugs from degradation products, but they carry higher maintenance requirements and consumable costs. Conversely, derivative and simultaneous-equation UV-Vis spectrophotometry (Mishra & Mundada, 2021; Parashar et al., 2013) reduce solvent consumption and equipment costs, but rely on mathematical resolution, which can limit their capacity to resolve unexpected degradation products or novel falsified adulterants.

Regulatory Impact and Applicability in Endemic Settings

The technical capabilities established across the 2010–2025 literature directly support post-market surveillance networks and compliance with WHO quality specifications. While RP-HPLC-UV remains the standard for regulatory batch verification, regions facing high malaria burdens may experience resource constraints—such as limited continuous power, technical support, or solvent supply—that affect laboratory operations.

To address these operational challenges, the validation literature supports a tiered, multi-level surveillance model. Portable, non-destructive tools—such as handheld NIR spectrometers (Yabré et al., 2020)—can be deployed at border checkpoints and regional distribution points for rapid, low-cost initial screening. Samples flagged for potential dosage discrepancies or unusual spectra can then be referred to centralized reference laboratories equipped with validated RP-HPLC or UPLC platforms for confirmatory testing. Aligning field screening with centralized laboratory infrastructure can help optimize resource allocation while maintaining regulatory oversight across the antimalarial supply chain.

CONCLUSION

The analytical methodologies for artemether–lumefantrine combinations continue to support both routine quality assurance and advanced research. High-throughput UPLC and sensitive LC-MS/MS platforms enable detailed clinical, bioanalytical, and stability investigations, whereas validated RP-HPLC-UV protocols remain the foundation for regulatory compliance and product quality testing. This systematic review provides a consolidated synthesis of 15 years of validation data to inform method selection across differing laboratory capabilities. To further strengthen antimalarial surveillance, future work should focus on multi-center collaborative validations and the refinement of portable, environmentally sustainable analytical tools suitable for deployment in malaria-endemic regions.

Acknowledgments: The authors express their gratitude to the Faculty of Pharmaceutical Sciences at the University of Kisangani and the Pharmaceutical Techniques Section at the Higher Institute of Medical Techniques of Bukavu for providing database and digital library access. We also thank our academic colleagues and reviewers for their constructive feedback during the preparation of this manuscript.

Use of Artificial Intelligence (AI) Declaration: Google Gemini (1.5 Pro) was utilized for initial language translation assistance (French to English) and text formatting. The authors critically reviewed, revised, and verified all content, and maintain full responsibility for the final manuscript.

Authors' Contributions:

i. **Conceptualization:** J. B. Bugugu, J. J. M. Mwanga, and J. K. Mbinze.
ii. **Methodology:** J. B. Bugugu, J. J. M. Mwanga, M. L. Shabani, and G. B. Dhimbe.
iii. **Formal analysis:** J. B. Bugugu and J. J. M. Mwanga.

iv. **Investigation:** J. B. Bugugu, M. L. Shabani, and G. B. Dhimbe.
v. **Resources:** J. B. Bugugu and J. K. Mbinze.
vi. **Writing – original draft:** J. B. Bugugu.
vii. **Writing – review & editing:** J. B. Bugugu, J. J. M. Mwanga, M. L. Shabani, G. B. Dhimbe, and J. K. Mbinze.
viii. **Supervision:** J. K. Mbinze.

Data Availability Statement: All data analyzed during this systematic review are included within this published article.

Ethical Approval: This study is a systematic review of secondary published literature and did not involve human participants, animal experimentation, or primary biological data collection. Formal ethical review board approval was not required.

Funding: This research received no external grant funding from public, commercial, or non-profit sectors.

Conflicts of Interest: The authors declare that they have no conflicts of interest.

ORCID iDs:

Bugugu, J. B.[1]: Nil identified.
Mwanga, J. J. M.[2]: Nil identified.
Shabani, M. L.[3]: Nil identified.
Dhimbe, G. B.[4]: Nil identified.
Mbinze, J. K.[4]: Nil identified.

REFERENCES

Al-Khateeb, L. A., Alluhayb, A. H., Saleh, S. M., Alsehli, B. R., Binsaleh, A. Y., & Mohamed, M. A. (2025). Integrated RP-HPLC approach for concurrent assay and dissolution analysis of artemether and lumefantrine: Leveraging MoGSA and RAPI. *Acta Chromatographica*, *37*(4), 605–615. https://doi.org/10.1556/1326.2025.01387

Amin, N. C., Fabre, H., Blanchin, M. D., & Montels, J. (2013). Determination of artemether and lumefantrine in anti-malarial fixed-dose combination tablets by microemulsion electrokinetic chromatography with short-end injection procedure. *Malaria Journal*, *12*, Article 202. https://doi.org/10.1186/1475-2875-12-202

Arun, R., & Smith, A. A. (2011). Simultaneous HPLC-UV method for the estimation of artemether and lumefantrine in tablet dosage form. *International Journal of Pharmaceutical and Biomedical Research*, *2*(3), 201–205.

Belew, S., Suleman, S., Duguma, M., Teshome, H., Wynendaele, E., Duchateau, L., & De Spiegeleer, B. (2020). Development of a dissolution method for lumefantrine and artemether in immediate release fixed dose artemether/lumefantrine tablets. *Malaria Journal*, *19*, Article 139. https://doi.org/10.1186/s12936-020-03209-5

Blessborn, D., Römsing, S., Annerberg, A., Sundquist, D., Björkman, A., Lindegardh, N., & Bergqvist, Y. (2007). Development and validation of an automated solid-phase extraction and liquid chromatographic method for determination of lumefantrine in capillary blood on sampling paper. *Journal of Pharmaceutical and Biomedical Analysis*, *45*(2), 282–287. https://doi.org/10.1016/j.jpba.2007.07.015

César, I. C., de Aquino Ribeiro, J. A., de Souza Teixeira, L., Bellorio, K. B., de Abreu, F. C., Moreira, J. M., Chellini, P. R., & Pianetti, G. A. (2011). Liquid chromatography–tandem mass spectrometry for the simultaneous quantitation of artemether and lumefantrine in human plasma: Application for a pharmacokinetic study. *Journal of Pharmaceutical and Biomedical Analysis*, *54*(1), 114–120. https://doi.org/10.1016/j.jpba.2010.07.027

Christian, J., Shah, P., Patel, M., Patel, K., & Gandhi, T. (2017). Optimizing derivatization conditions using an experimental design and simultaneous estimation of artemether and lumefantrine by ratio first order derivative spectrophotometric method. *Journal of Taibah University for Science*, *11*(5), 729–740. https://doi.org/10.1016/j.jtusci.2016.08.003

Debrah, P., Nettey, H., Miltersen, K. K., Ayeh-Kumi, P., Brock, B., Sarkodie, J. A., & Hardlei, T. F. (2016). Artemether–lumefantrine concentrations in tablets and powders from Ghana measured by a new high-performance liquid chromatography method. *American Journal of Tropical Medicine and Hygiene*, *95*(1), 158–163. https://doi.org/10.4269/ajtmh.15-0868

Govender, K., Gibhard, L., Du Plessis, L., & Wiesner, L. (2015). Development and validation of a LC–MS/MS method for the quantitation of lumefantrine in mouse whole blood and plasma. *Journal of Chromatography B*, *985*, 6–13. https://doi.org/10.1016/j.jchromb.2015.01.015

Gupta, N. K., Babu, A. M., & Gupta, P. (2013). Simultaneous estimation of artemether and lumefantrine by RP-HPLC method development in pharmaceutical tablet dosage form. *International Journal of Pharmaceutical Erudition*, *3*(1), 10–17.

Habib, R., Shoaib, M. H., Ahmed, F. R., Siddiqui, F., Yousuf, R. I., Saleem, T., & Khan, M. Z. (2020). HPLC-UV method for simultaneous quantitation of artemether and lumefantrine in fixed dose combination orodispersible tablet formulation.

Pakistan Journal of Pharmaceutical Sciences, *33*(4), 1561–1567.

Kalyankar, T. M., & Kakde, R. B. (2011). Reversed-phase liquid chromatographic method for simultaneous determination of artemether and lumefantrine in pharmaceutical preparation. *International Journal of ChemTech Research*, *3*(3), 1722–1727.

Karajgi, S. R., Tanveer, A. R., & Kalyane, N. V. (2016). Simultaneous determination of artemether and lumefantrine by area under curve UV spectrophotometric method. *Journal of Pharmaceutical Sciences and Research*, *8*(6), 506–511.

Kaur, R., Katare, O. P., Singh, G., Mohan, V., Saini, S., & Singh, B. (2021). A derivatization-based densitometric method for simultaneous estimation of artemether and lumefantrine: Method development, validation and applications. *Microchemical Journal*, *171*, Article 106820. https://doi.org/10.1016/j.microc.2021.106820

Khuda, F., Iqbal, Z., Shah, Y., Ahmad, L., Nasir, F., Khan, A. Z., & Shahbaz, N. (2014). Method development and validation for simultaneous determination of lumefantrine and its major metabolite, desbutyl lumefantrine in human plasma using RP-HPLC/UV detection. *Journal of Chromatography B*, *944*, 114–122. https://doi.org/10.1016/j.jchromb.2013.10.037

Kolakota, R., & Krishna, G. S. (2021). A new RP-HPLC method development and validation for simultaneous estimation of lumefantrine & artemether using bulk and pharmaceutical dosage forms. *World Journal of Pharmaceutical and Biotechnology Research*, *8*(2), 45–52.

Laxmi, M., Ghosh, S., & Ravikumar, B. V. V. (2015). Analytical method development & validation of artemether in bulk drug by RP-HPLC method as per ICH guidelines. *International Journal of Pharmaceutical Sciences and Research*, *7*(2), 112–118.

Lekhana, A. M., Richards, M. P., & Vishwanath, B. A. (2025). Simultaneous estimation of artemether and lumefantrine by RP-HPLC method development in pharmaceutical tablet dosage form. *Rajiv Gandhi University of Health Sciences Journal of Pharmaceutical Sciences*, *15*(2), Article 6. https://doi.org/10.26463/rjps.15_2_6

Lindegårdh, N., Annerberg, A., Blessborn, D., Bergqvist, Y., Day, N., & White, N. J. (2005). Development and validation of a bioanalytical method using automated solid-phase extraction and LC-UV for the simultaneous determination of lumefantrine and its desbutyl metabolite in plasma. *Journal of Pharmaceutical and Biomedical Analysis*, *37*(5), 1081–1088. https://doi.org/10.1016/j.jpba.2004.07.041

Marwa, K. J., Liwa, A. C., Konje, E. T., Mwita, S., Kamugisha, E., & Swedberg, G. (2022). Lumefantrine plasma concentrations in uncontrolled conditions among patients treated with artemether-lumefantrine for uncomplicated plasmodium falciparum malaria in Mwanza, Tanzania. *International Journal of Infectious Diseases*, *123*, 192–199. https://doi.org/10.1016/j.ijid.2022.08.020

Mishra, M., & Mundada, A. (2021). Simultaneous estimation and validation of artemether and lumefantrine by UV spectrophotometry in tablet. *Journal of Drug Delivery and Therapeutics*, *11*(2), 16–22. https://doi.org/10.22270/jddt.v11i2.4576

Mondal, P., Rani, S. S., & Ramakrishna, R. (2014). Novel stability indicating validated RP-HPLC method for simultaneous quantification of artemether and lumefantrine in bulk and tablet. *Current Pharmaceutical Analysis*, *10*(4), 271–278. https://doi.org/10.2174/1573412910666140701183927

Nagvekar, U. V., Rapatwar, S. K., Jadhavar, S. M., Bhurewad, K. S., Garad, A. K., Naikwadi, A. Z., & Sanjapure, S. M. (2023). Simultaneous estimation of artemether and lumefantrine in tablet formulation by UV-visible spectroscopy using simultaneous equation method. *International Journal of Advanced Research in Science, Communication and Technology*, *8*(1), 105–112.

Nassar, M. W. I., El-Olemy, A., Emara, M. S., & Ahmed, A. M. (2022). Development and validation of stability-indicating GC-MS method for simultaneous determination of lumefantrine and artemether. *Egyptian Journal of Chemistry*, *65*(10), 101–106. https://doi.org/10.21608/ejchem.2022.105371.4856

Nyarko, S., Ofori-Kwakye, K., Johnson, R., Kuntworbe, N., Otu, D. A. B., Yar, D. D., & Osei, Y. A. (2024). Development of the RP-HPLC method for simultaneous determination and quantification of artemether and lumefantrine in fixed-dose combination pharmaceutical dosage forms. *Advances in Pharmacological and Pharmaceutical Sciences*, *2024*, Article 3212298. https://doi.org/10.1155/2024/3212298

Ongas, M. O., Juma, E., Kirimi, C. G., Oloo, F., Kokwaro, G., Aman, R., & Ogutu, B. R. (2018). A selective LC-MS/MS method for simultaneous quantification of artemether, lumefantrine and their principle metabolites in human plasma. *ABC Research Alert*, *6*(3), 12–21. https://doi.org/10.18034/ra.v6i3.335

Padala, N. P., Boggavarapu, R. K., Kunduru, P., Palacharla, R. C., Ajjala, D. R., & Nirogi, R. (2018). LC–MS/MS method for the simultaneous quantification of

artemether, dihydroartemisinin and lumefantrine in rabbit plasma and its application to a PK study. *Drug Metabolism and Pharmacokinetics*, *33*(1), S63. https://doi.org/10.1016/j.dmpk.2017.11.212

Parashar, D., Kumar, A., Aditya, N. P., & Rayasa, R. M. (2013). Simultaneous estimation of artemether and lumefantrine in pharmaceutical dosage forms using derivative spectrophotometry. *Asian Journal of Research in Chemistry*, *6*(3), 232–236.

Patelia, E. M., Thakur, R., & Patel, J. (2014). Bio-analytical method development and validation for estimation of lumefantrine in human plasma by using LC-MS/MS. *International Journal of Biomedical Data Mining*, *3*(1), Article 1000111. https://doi.org/10.4172/2090-4924.1000111

Saeed, N. K. (2017). *Development and validation of HPLC method for determination of artemether and lumefantrine tablets* [Master's thesis, Al-Neelain University]. Al-Neelain University Institutional Repository.

Saini, P. K., Singh, R. M., Mathur, S. C., Singh, G. N., & Lal, B. (2010). A simple and sensitive HPTLC method for quantitative analysis of artemether and lumefantrine in tablets. *Journal of Planar Chromatography – Modern TLC*, *23*(2), 119–122. https://doi.org/10.1556/jpc.23.2010.2.6

Shah, R., Soni, T., Patel, D., & Patel, M. (2025). Simultaneous quantification of artemether and lumefantrine in rat plasma using a validated LC-MS/MS method. *Accreditation and Quality Assurance*, *30*, 429–443. https://doi.org/10.1007/s00769-025-01647-9

Shah, S. R., Bapna, M., Brahmbhatt, K. D., Patel, R. A., & Patel, C. M. (2013). Development and validation of analytical method for simultaneous estimation of artemether and lumefantrine in bulk and marketed fixed dose combination. *Pharma Science Monitor*, *3*(3), 257–266.

Shamshad, K., Gohar, U. F., Mukhtar, H., Zafar, A., & Mubarik, S. (2020). High performance liquid chromatography method development and an innovative technique for validation of artemether and lumefantrine oral dry suspension. *Biomedical Journal of Scientific & Technical Research*, *32*(2), 24860–24867. https://doi.org/10.26717/BJSTR.2020.32.005234

Sukanya, B., Goud, V. M., & Anitha, P. (2019). Stability indicating Ultra Performance Liquid Chromatography method development and validation for simultaneous estimation of artemether and lumefantrine in bulk and pharmaceutical dosage form. *Journal of Drug Delivery and Therapeutics*, *9*(2), 161–168. https://doi.org/10.22270/jddt.v9i2.2408

Sunil, J., Nath, M. S., & Moorthy, U. S. (2010). HPLC method development and validation for simultaneous estimation of artemether and lumefantrine in pharmaceutical dosage forms. *International Journal of Pharmacy and Pharmaceutical Sciences*, *2*(4), 100–104.

Umapathi, P., Ayyappan, J., & Quine, S. D. (2011). Development and validation of a dissolution test method for artemether and lumefantrine in tablets. *Tropical Journal of Pharmaceutical Research*, *10*(5), 643–653. https://doi.org/10.4314/tjpr.v10i5.14

Vinodh, M., Vinayak, M., Pankaj, P., Rahul, K., & Renita, M. (2013). Analytical method development and validation for simultaneous estimation of artemether and lumefantrine in pure and pharmaceutical dosage form using RP-HPLC method. *Malaysian Journal of Analytical Sciences*, *17*(3), 348–358.

Wilkins, C. A., du Plessis, L. H., & Viljoen, J. M. (2021). Investigating in vitro and ex vivo properties of artemether/lumefantrine double-fixed dose combination lipid matrix tablets prepared by hot fusion. *Pharmaceutics*, *13*(7), Article 922. https://doi.org/10.3390/pharmaceutics13070922

Yabré, M., Ferey, L., Sakira, A. K., Bonmatin, C., Fauré, C., Somé, T. I., & Gaudin, K. (2020). Green analytical methods of antimalarial artemether-lumefantrine analysis for falsification detection using a low-cost handheld NIR spectrometer with DD-SIMCA and drug quantification by HPLC. *Molecules*, *25*(15), Article 3397. https://doi.org/10.3390/molecules25153397