Evaluation of stability and TLC Fingerprinting of the Artemether Component in Artemether-Lumefantrine Combination Suspension Formulations available in market

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Abstract

Artemether is the main component of the artemisinin-based combination therapy (ACT), used in the management of malaria infection caused by *Plasmodium falciparum*. Hence, its stability and conformation to pharmacopeia standards are significant to the safety of the end users. The study aimed to review the first-order derivative spectrophotometric method for simultaneous estimation of artemether and related substances in pure and combined formulations, and their stability profiles, then develop a simple, precise, and fast technique for their rapid identification. Thin-layer chromatographic (TLC) Fingerprinting principles were used in the analysis. Artemether and its derivatives were spotted on the TLC chromatograms after adding 25 mL of the suspension to a mixture of 100 mL of distilled water and 4 mL of NaOH, extracting the mixture with 60 mL of DCM, drying, and sonicating the residue with 20 mL of the solvent. It was centrifuged, and the clear supernatant was spotted on the TLC plates. The color test results revealed the presence of the artemether compound in the reference standard as well as the six brands of suspensions (A, B, C, D, E, and F) utilized in the study. Artemether melting point was obtained between 86 and 89 °C; within the International Pharmacopeia specified range. The chromatograms of Artemether and derivatives showed Rf values of 0.25 (impurity A), 0.3 (artenimol impurity B), 0.35 (impurity C), 0.4 (α-artemether: impurity D), and 0.55 (artemether). The devised method can be applied to the quality control analysis of artemether-lumefantrine suspension in addition to existing analytical techniques.

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Keywords: Artemether, Lumefantrine, TLC, Fingerprint, Stability, Malaria, ACT

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Remove TLC & ACT.

Introduction

The World Health Organization (WHO) reported in 2021 that malaria was a threat to nearly half of the world's population, with an estimated 247 million infections and 619,000 mortalities worldwide, with a disproportionately high incidence in African countries, accounting for 95% of cases and 96% of deaths, with children under the age of five accounting for almost 80% (WHO, 2023). WHO recommends the use of artemisinin-based combination therapy (ACT) as the first-line treatment for *Plasmodium falciparum* malaria (WHO, 2019). This is due to the recorded safety of ACT formulations, owing to their distinct structure and antimalarial mechanism (Nosten & White, 2007). Nigeria used ACTs instead of chloroquine as the first-line treatment for uncomplicated malaria by WHO recommendations (WHO, 2005). All African countries where malaria is endemic have adopted ACT because it is safer, more effective, and reduces the risk of developing antimalarial resistance compared to previous monotherapy (Dondorp, 2008; Price &

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Douglas, 2009). Since ACT is usually administered as tablets, children have challenges swallowing oral pills (Batchelor & Marriott, 2015). Although tablets could be carefully divided and used for children, the procedure may cause a loss of active components and result in either an under-dose or an overdose (Bassat *et al.* 2015). The most popular antimalarial medications are those that include artemisinin or its derivatives. The Chinese herb *Artemisia annua* L. yielded artemisinin. It has a unique peroxy group-containing sesquiterpene lactone (Figure 1A) (Wang *et al*., 2019).

Artemisinin and its derivatives, including artemether, artesunate, dihydroartemisinin, and arteether, are more effective than other medications in eliminating plasmodia from human blood (Nosten & White, 2007). Apart from its significant antimalarial activities, *A. annua* has also been related to significant anticancer qualities (Ehrhardt & Meyer, 2009). Exciting results from leukemia and other cancer cell research have been reported. The active ingredients appear to be selectively toxic to some types of breast cancer and prostate cancer cells (Das, 2015).

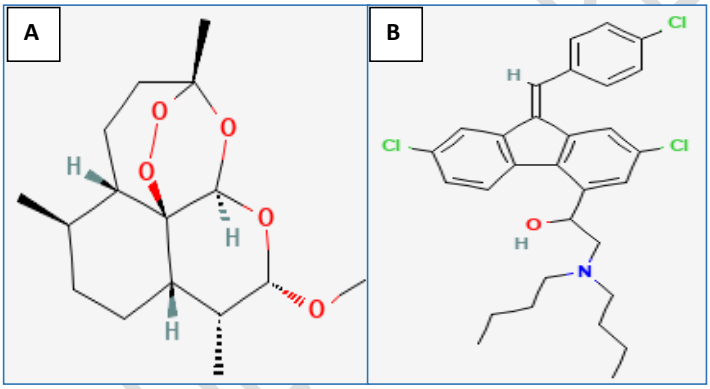

Figure 1: Structure of artemether (A), and Lumefantrine (B).

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Artemether is a crystalline powder that is white to slightly yellow in color, with a melting point between 86° and 90°C and a specific rotation between +166° and +173°. According to the National Center for Biotechnology Information (2021), artemether is nearly insoluble in water, easily soluble in acetone, and soluble in methanol and ethanol. With two known polymorphs (A and B), eight asymmetric centers, and optically active. In pharmaceutical formulations, Polymorph A is a stable species at room temperature (Shrikant, & Dorota 2009). Identification, impurity assay, particle size, microbiological limits, and residual solvents are among the tests

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and acceptance standards used to regulate the quality of artemether in preparations (Belew *et al.*, 2019; Kumar *et al*., 2013).

Lumefantrine (Figure 1B), a yellow, crystalline powder, has a melting point between 128°C and 132°C, stable at room temperature, soluble in dichloromethane (DCM), dimethyl sulfoxide (DMSO), chloroform, and ethyl acetate; sparingly soluble to a lesser extent in ethanol and methanol, and insoluble in water. It only has one polymorphic shape and a single chiral center. Identification, impurities assay, particle size, microbiological limits, and residual solvents, are used to regulate the quality of lumefantrine (Ezzet *et al*., 2000).

Because t-butyl peroxide is a well-known generator of free radicals, the antimalarial action of artemisinins has been attributed to their ability to produce free radicals (Woodrow et al., 2005). Free radicals produced by the iron and artemisinin interaction have been suggested to mediate artemisinin's antimalarial properties (Jian et al., 2010). It appears that the endoperoxide bridge, which generates singlet oxygen and free radicals that are highly cytotoxic to plasmodia, is required for artemether's antimalarial activity (WHO, 2010). Hormonal contraceptive effectiveness may be diminished by ACT (D'Arcy, 1986). Also, CYP3A4 inducers may have reduced artemether concentrations and anti-malarial efficacy (Byakika-Kibwika et al., 2010). CYP3A4 inhibitors, such as grapefruit juice and ketoconazole, can cause elevated levels of artemether (Lefevre *et al*., 2002). Artemether-lumefantrine oral suspension consist of 7.9 mg of β-artemether and 47.4 mg of lumefantrine (Elizabeth *et al*., 2008).

One of the most commonly used chromatographic methods is the TLC technique (Ettre and Kalasz, 2001). The presence of a gas phase in TLC sets it apart from all other chromatographic methods and can have a substantial impact on separating the components of pharmaceutical mixtures (Coskun, 2016). Many factors might affect the stability of pharmaceutical products, including interactions between active ingredients and excipients, dosage form, packaging system, temperature, and moisture conditions encountered during shipment, storage, and handling (Singh & Bakshi, 2000). Microbiological changes, such as bacterial growth and changes in preservative efficacy, can also affect the stability of a pharmaceutical product (Carstensen et al., 2000; Singh, 2000). While multiple UV Spectrophotometric approaches for quantifying artemether in various biological fluids have been developed, their utility in regular analysis may be limited due to the high heating conditions required. The study aimed to design and evaluate a precise, and fast ratio first-order derivative spectrophotometric method for assessing artemether in both pure and

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combined formulations, and then determine the stability in selected artemether-lumefantrine oral suspensions.

Materials and Method

List of materials (Chemical reagents) used in the study

Distilled water, NaOH, dichloromethane (DCM), Artemether powder, dihydroartemisinin powder, petroleum ether, ethyl acetate, acetic acid, acetonitrile, artemether/lumefantrine suspensions, etc. All reagents were of analytical standards.

Drug samples and drug reference standard

May and Baker Pharmaceutical Limited produced the artemether powder reference standards. Five distinct brands of artemether/lumefantrine suspensions were gathered from Lagos pharmacies, with the expiration dates noted. Before starting the research, the samples were carefully preserved.

Preparation of solvent and solutions

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As a solvent, an equal proportion of distilled water and acetonitrile was used. A 500 mL volumetric flask was filled with a mixture of 150 mL of distilled water and 150 mL of acetonitrile.

To make solution 1, 100 mL of water and 4 mL of NaOH were mixed with 100 mg of Artemether powder. The samples were extracted with 60 mL of dichloromethane (DCM), which was then evaporated until absolutely dry. The clear supernatant was used after centrifuging the residue for 15 minutes in 20 mL of solvent. 50 mL of the solvent was mixed with 5 mg of dihydroartemisinin and 5 mg of artemether, respectively making the stock solution (2). The stock solution was diluted further with the solvent. From 2.0 mL to 20 mL of solution (3); from 3.0 mL to 20 mL of solution (4), from 5.0 mL to 20 mL of solution (5); from 1.0 mL to 2 mL of solution (6), from 3.0 mL to 4 mL of solution (7), respectively.

Thin-Layer Chromatographic Fingerprinting

Preparation of the mobile phase

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Petroleum ether, ethyl acetate, and acetic acid make up the mobile phase. In the TLC glass tank, 40 mL of petroleum ether, 10 mL of ethyl acetate, and 5 mL of acetic acid were measured and combined.

Spotting, development, and visualization of TLC plate

Apply 20 µl of solutions (1), (3), (4), (5), (6), and (7) individually to the plate. After applying, the areas were allowed to dry in a cold air current for about 15 minutes. Develop along a 12-cm path. The plate was allowed to thoroughly dry in the open air or cold air circulation after removing it from the chromatographic chamber. A spray was used to immerse the plate with sulfuric acid. Dry in the oven at 140°C for 10 minutes. The chromatogram was observed in daylight.

Results

Physicochemical parameters

Tests for Artemether reference standard and powders for suspension identification: All five brands and the standard Artemether powder yielded the anticipated yellow color. It was discovered that the artemether reference standard's melting point ranged from 86 to 89 °C.

TLC analysis

The findings of the Thin Layer Chromatography study indicated the presence of dihydroartemisinin and α-artemether as well as artemether in samples A, B, C, D, E, and F. Figures 2A through 4F, respectively, show the Thin Layer Chromatography analysis findings for samples A, B, C, D, E, and F as well as the reference standard powder artemether.

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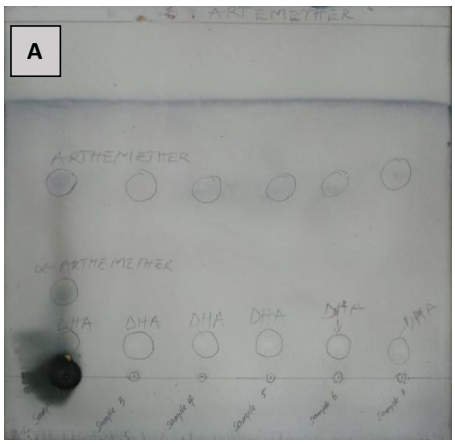

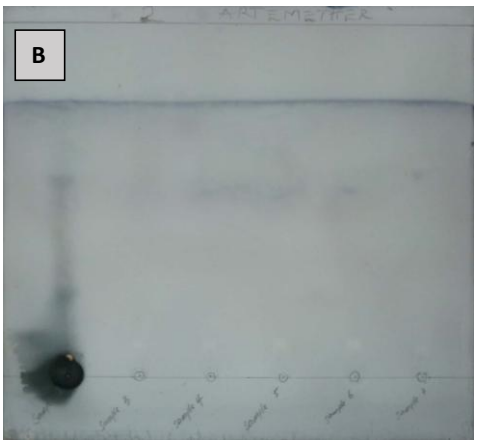

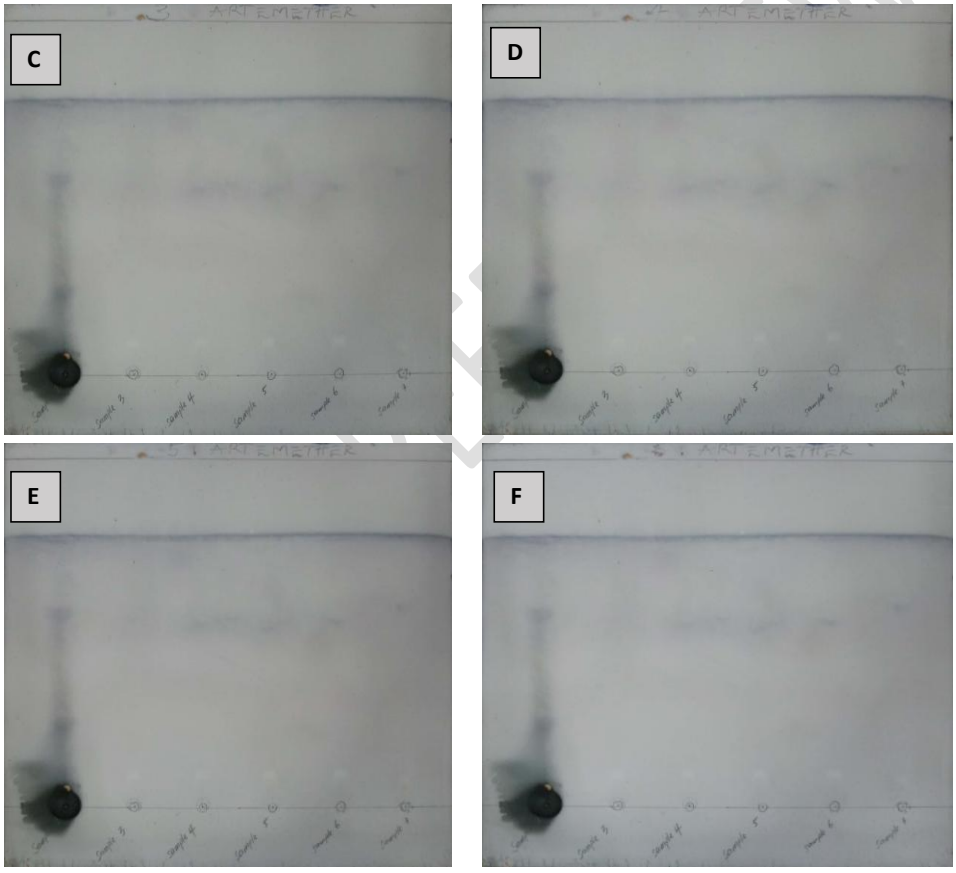

Figure 2: TLC chromatogram for sample A - F

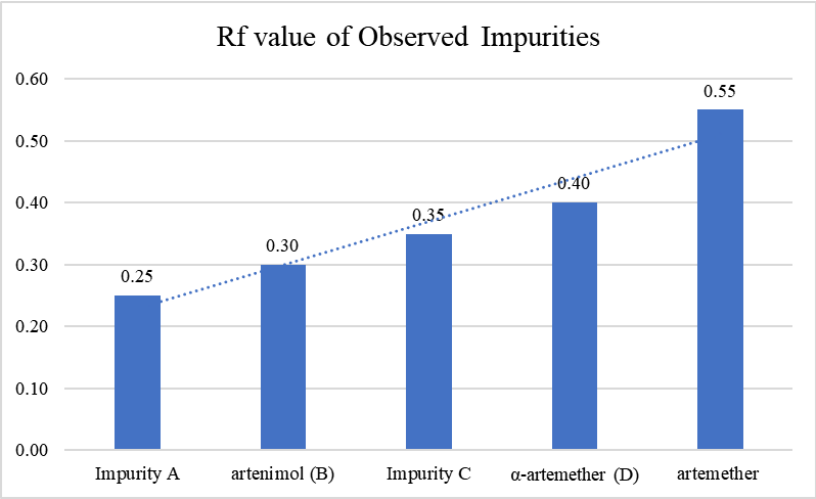

Figure 3. The Rf value of the observed impurities from the test samples

Discussion

Changes in the appearance, consistency, homogeneity of content, clarity (solution), moisture content, particle size and shape, pH, and package integrity of a pharmaceutical product may have an impact on its stability. Abrasion, impact, vibration, and temperature fluctuations such as freezing, thawing, or shearing can all induce physical changes. Chemical reactions such as solvolysis, oxidation, reduction, and racemization in pharmaceutical products can result in the formation of a degradation product, a decrease in the potency of the active pharmaceutical ingredient (API), a loss of excipient activity such as antioxidants and antimicrobial preservative action, and so on (Carstensen et al., 2000). Unrecognized foreign materials could be present in completed goods. As a result, it is necessary to develop an effective method for detecting and identifying foreign components in dosage forms using readily available analytical techniques (Pifferi et al., 1999; Misiuk, 2010). Pharmacokinetic studies make extensive use of quantitative or qualitative drug and metabolite research (Shah *et al*., 2013).

The color test results revealed that artemether was present in both the reference standard and the six powder brands for suspension analysis (A, B, C, D, E, and F). Artemether RS has a melting point between 86 and 89 °C, which is within the specified range of 86 to 90 °C (IP, 2019). This proves that the sample was artemether, and the sample's high melting point verifies its purity. A TLC fingerprinting examination of Artemether and associated compounds yielded the following Rf values: impurity A was 0.25, impurity B (artenimol) was 0.3, impurity C was 0.35, impurity

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D (α-artemether) was 0.4, and artemether was 0.55, (Figure 3), which were in consonant with official standards (IP, 2019).

The chromatogram results showed that any spot corresponding to impurity A produced by solution (1) was not more intense than the primary spot produced by solution (7). In the chromatogram obtained with solution (6), the spots created by impurity B were not more intense than the spots induced by the presence of artenimol (a major impurity). The impurity C spot was not brighter than the solution's principal spot (5). Because of the presence of α-artemether, the spot corresponding to impurity D was no brighter than the spot produced by solution (4), and no other spot was brighter than the primary spot produced by solution (3) (Figure 2A-F).

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Conclusion

From the data obtained thin-layer chromatography is an easy, precise, sensitive, and affordable method. Artemether-lumefantrine suspension regular quality control analyses can be performed using this method in comparison to other analytical techniques. The fixed-dose combination powder for suspension analysis was successfully conducted using this method. Based on the aforementioned, routine quality control analysis, exploring the use of TLC for the determination of artemether and its related compounds in pure and combined formulations should be incorporated in the manufacturing processes, ranging from raw materials to finished pharmaceutical products. In the future, large sample sizes and cutting across products from all regions should be selected to ascertain the stability of these products, thereby building confidence among the end users.

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References

Bassat, Q., Ogutu, B., Djimde, A., Stricker, K., & Hamed, K. (2015). Tailoring a Pediatric Formulation of Artemether-Lumefantrine for Treatment of Plasmodium falciparum Malaria. *Antimicrobial agents and chemotherapy*, *59*(8), 4366–4374. https://doi.org/10.1128/AAC.00014-15

Batchelor, H. K., & Marriott, J. F. (2015). Formulations for children: problems and solutions. *British journal of clinical pharmacology*, *79*(3), 405–418.

Belew, S., Suleman, S., Mohammed, T., Mekonnen, Y., Duguma, M., Teshome, H., Bayisa, B., Wynendaele, E., D'Hondt, M., Duchateau, L., & De Spiegeleer, B. (2019). Quality of fixed-dose artemether/lumefantrine products in Jimma Zone, Ethiopia. *Malaria journal*, *18*(1), 236. https://doi.org/10.1186/s12936-019-2872-1

UNDER PEER REVIEW

Byakika-Kibwika, P., Lamorde, M., Mayanja-Kizza, H., Merry, C., Colebunders, B., & Van Geertruyden, J. P. (2010). Update on the efficacy, effectiveness, and safety of artemether-lumefantrine combination therapy for treatment of uncomplicated malaria. *Therapeutics and clinical risk management*, *6*, 11–20.

Carstensen JT (2000). Drug Stability, Principles and Practices, Marcel Dekker, New York.

Coskun O. (2016). Separation techniques: Chromatography. *Northern Clinics of Istanbul*, *3*(2), 156–160. https://doi.org/10.14744/nci.2016.32757

D'Arcy PF (1986). Drug interactions with oral contraceptives. *Drug Intell Clin Pharm.*; 20(5):353-62. doi: 10.1177/106002808602000504. PMID: 3519141.

Das A (2015). Anticancer effect of antimalarial artemisinin compounds. *Ann Med Health Sci* Res *5*(2): 93,

Dondorp, A.M. (2008). Clinical significance of sequestration in adults with severe malaria. *Journal of Clinical Biology*, 5(1-2); 56-57.

Ehrhardt, S., & Meyer, C. G. (2009). Artemether-lumefantrine in the treatment of uncomplicated Plasmodium falciparum malaria. *Therapeutics and clinical risk management*, *5*, 805–815. https://doi.org/10.2147/tcrm.s5375

Elizabeth A Juma, Charles O Obonyo, Willis S Akhwale, and Bernhards R Ogutu (2008). A randomized, open-label, comparative efficacy trial of artemether-lumefantrine suspension versus artemether-lumefantrine tablets for treatment of uncomplicated Plasmodium falciparum malaria in children in western Kenya. *Malar J.*; 7: 262. doi: 10.1186/1475-2875-7-262

Ettre L.S, Kalász H (2001). The story of thin-layer chromatography. LCGC 19:712–721

Ezzet F van Vugt M, Nosten F, Looareesuwan S, White NJ (2000) Pharmacokinetics and pharmacodynamics of lumefantrine (benflumetol) in acute falciparum malaria. *Antimicrob Agents Chemother 44: 697-704.*

Jian Li, Bing Zhou (2010). Biological Actions of Artemisinin: Insights from Medicinal Chemistry Studies. *Molecules.* 15, 1378-1397.

Kumar SP, Jasrai YT, Pandya HA, George LB, Patel SK. **(**2013). Structural insights into the theoretical model of *Plasmodium falciparum* NADH dehydrogenase and its interaction with artemisinin and derivatives: towards global health therapeutics. OMICS **17:**231–241. http://dx.doi.org/10.1089/omi.2012.0129.

Lefevre G, Carpenter P, Souppart C, Schmidli H, McClean M, Stypinski D (2002). Pharmacokinetics and electrocardiographic pharmacodynamics of artemether-lumefantrine

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(Riamet®) with concomitant administration of ketoconazole on healthy subjects. *Br J Clin Pharmacol.*, 54(5): 485-92

Misiuk W. (2010). The role of assay methods in characterizing the quality of bulk pharmaceuticals. *Journal of Pharmacy & bioallied sciences*, *2*(2), 88–92. https://doi.org/10.4103/0975-7406.67007

National Center for Biotechnology Information (2021). PubChem Compound Summary for CID 68911, Artemether. Retrieved December 12, 2021 from https://pubchem.ncbi.nlm.nih.gov/compound/Artemether.

Nosten F, White NJ. (2007) Artemisinin-Based Combination Treatment of Falciparum Malaria. In: Breman JG, Alilio MS, White NJ, editors. Defining and Defeating the Intolerable Burden of Malaria III: Progress and Perspectives: Supplement to Volume 77(6) of *American Journal of Tropical Medicine and Hygiene*. Northbrook (IL): American Society of Tropical Medicine and Hygiene; 2007 Dec. Available from: https://www.ncbi.nlm.nih.gov/books/NBK1713/

Pifferi, G., Santoro, P., Pedrani, M., (1999). IL Farmaco, p 541.

Price R. N. and Douglas N. M. (2009). Artemisinin Combination Therapy for Malaria: 75 beyond Good Efficacy. Clinical Infectious Diseases. 49, (11): 1638-1640.

Shrikant Pagay, Dorota Matecka (2009). Center for Drug Evaluation and Research: Chemistry Review(S). NDA 22-268; Coartem (artemether/lumefantrine) Tablets, 20 mg/120 mg. Novartis Pharmaceuticals Corporation, Shrikant Pagay, Dorota Matecka. Division of Pre-Marketing Assessment II, Branch IV, ONDQA. https://www.accessdata.fda.gov/drugsatfda_docs/nda/2009/022268s000_ChemR.pdf

Singh S, and Bakshi M. (2000). Guidance on the conduct of stress test to determine inherent stability of drugs, *Pharm Technol Asia*, 2000, 24-36.

Singh S. (2000). Stability testing during product development in Jain NK Pharmaceutical product development, *CBS publisher and distributors, India*, 2000, 272-293.

Wang J, Xu C, Wong YK, Li Y, Liao F, Jiang T, Tu Y (2019). Artemisinin, the Magic Drug Discovered in Traditional Chinese Medicine. *Engineering*. 5 (1): 32–9. doi:10.1016/j.eng.2018.11.011

WHO, (2005). *Susceptibility of Plasmodium falciparum to Antimalarial Drugs. Report on Global Monitoring 1996–2004*, WHO/HTM/MAL/2005.1103, World Health Organization, Geneva, Switzerland, 2005.

WHO (2010). Guidelines for the Treatment of Malaria *Second edition* ISBN 978 92 4 154792 5 (NLM classification: WC 770)

UNDER PEER REVIEW

WHO, (2019). Treating malaria. Available at: https://www.who.int/activities/treating-malaria. Accessed 25th October, 2033

WHO, (2023). Malaria: Key facts. Available at: https://www.who.int/news-room/fact-sheets/detail/malaria. Accessed 25th November, 2033

Woodrow C.J, Haynes RK, Krishna S (2005). Artemisinins. *Postgraduate Medical Journal; 81:71*-78.

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