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Evaluation of Stability and TLC Fingerprinting of the Artemether Component in Artemether-Lumefantrine Combination Suspension Formulations Available in Nigeria Pharmaceutical Market

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: 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 necessary for use. The study aimed to review the first-

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order derivative spectrophotometric method for simultaneous estimation of artemether and its derivatives in pure and combined formulations, and their stability profiles, then develop a simple, precise, and fast technique for their rapid physicochemical analysis.

Methodology: 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 dichloromethane (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.

Results: 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 - 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).

Conclusion: The devised method can be applied to the routine quality control analysis of artemether-lumefantrine suspension in addition to existing analytical techniques.

Keywords: Artemether; assay; fingerprint; lumefantrine; malaria; quality control; stability.

1. INTRODUCTION

World Health Organization (WHO) reported in 2021 that malaria affected an estimated 247 million individuals and 619,000 mortalities worldwide, with a disproportionately high incidence in African countries, accounting for 95% of cases and 96% of deaths, with children accounting for 80% [1]. WHO recommends the use of artemisinin-based combination therapy (ACT) as the first-line treatment for *Plasmodium falciparum* malaria [2]. This is due to the recorded safety of ACT formulations, owing to their distinct structure and antimalarial mechanism [3]. Nigeria used ACTs instead of chloroquine as the first-line treatment for uncomplicated malaria by WHO recommendations [4]. 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 [5,6] Since ACT is usually administered as tablets, children have challenges swallowing oral pills [7]. 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 [8]. 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 (Fig. 1A) [9].

Artemisinin and its derivatives, including artemether, artesunate, dihydroartemisinin, and arteether, are more effective than other medications in eliminating plasmodia from human blood [3]. Apart from its significant antimalarial activities, *A. annua* has also been related to significant anticancer qualities [10]. 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 [11].

Artemether(3R,5aS,6R,8aS,9R,10S,12R,12aR)-10-methoxy-3,6,9-trimethyldecahydro-12H-3,12-epoxy[1,2]dioxepino[4,3-i]isochromene) (Fig. 1A) is a crystalline powder that is white to slightly yellow in color, with a melting point between 86° - 90°C and a specific rotation between +166° and +173°. According to the National Center for Biotechnology Information [12], 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 [13]. Identification, impurity assay, particle size, microbiological limits, and residual solvents are among the tests and acceptance standards used to regulate the quality of artemether in preparations [14,15].

Lumefantrine ((Z)-2-(dibutylamino)-1-(2,7-dichloro-9-(4-chlorobenzylidene)-9H-fluoren-4-yl)ethan-1-ol) (Fig. 1B), a yellow, crystalline powder, has a melting point between 128℃ - 132℃, stable at room temperature, soluble in dichloromethane (DCM), dimethyl sulfoxide (DMSO), chloroform, and ethyl acetate; sparingly

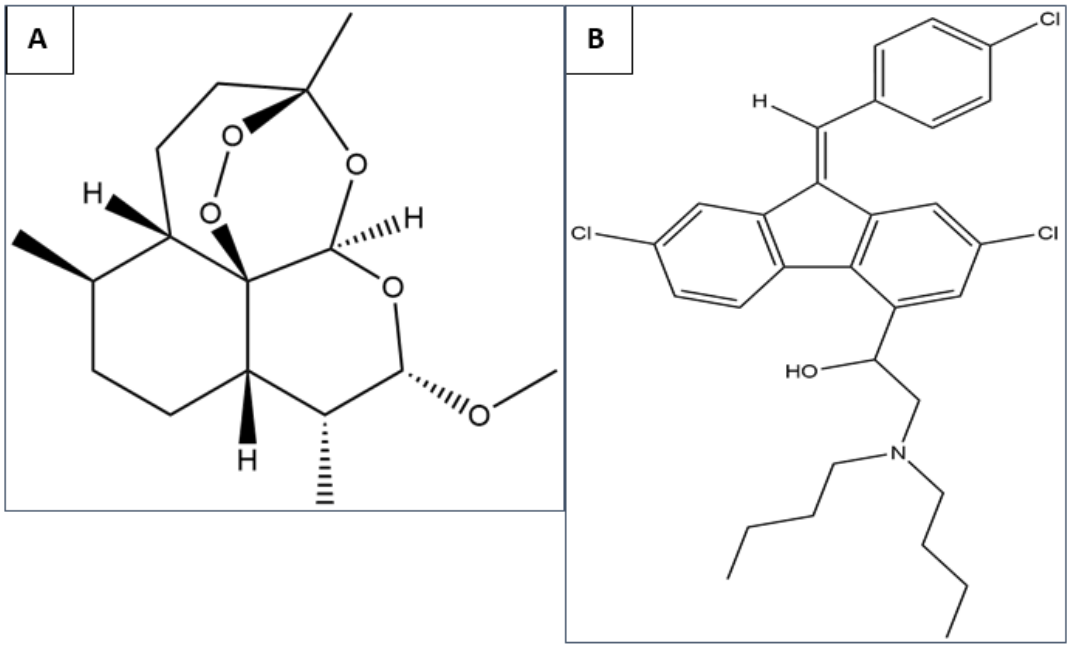

Fig. 1. Structure of artemether (A), and Lumefantrine (B)
(ChemDraw Image)

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 [16].

Artemether absorption improves in the presence of food. It has a high protein binding percentage (95.4%). Peak artemether concentrations are measured 2 hours after oral administration. In the human body, the hepatic enzymes CYP3A4/5 convert it to dihydroartemisinin (an active metabolite that is 47-78% bound to protein). Both the parent drug and the active metabolite are eliminated in 1-2 hours [17]. Lumefantrine binds to protein 99.7% of the time, is hepatically metabolized to desbutyl-lumefantrine by the same enzyme (CYP3A4), has an elimination half-life of 72-144 hours, and is thought to clear any residual parasites that remain after the combination treatment [18].

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 [19]. Free radicals produced by the iron and artemisinin interaction have been suggested to mediate artemisinin's antimalarial properties [20]. 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 [21]. Hormonal contraceptive effectiveness may be diminished by ACT [22]. Also, CYP3A4 inducers may have reduced artemether concentrations and anti-malarial efficacy [23]. CYP3A4 inhibitors, such as grapefruit juice and ketoconazole, can cause elevated levels of artemether [24]. Artemether-lumefantrine oral suspension consist of 7.9 mg of β-artemether and 47.4 mg of lumefantrine [25].

One of the most commonly used chromatographic methods is the TLC technique [26]. 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 [27]. 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 [28]. Microbiological changes, such as bacterial growth and changes in preservative efficacy, can also affect the stability of a pharmaceutical product [29,30]. 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 combined formulations, and then determine the stability in selected artemether-lumefantrine oral suspensions.

2. MATERIALS AND METHODS

2.1 Chemical Reagents

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

2.2 Drug Samples and 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.

2.3 Preparation of Solvent and Solutions

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 [31]. 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 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, and from 3.0 mL to 4 mL of solution 7, respectively.

2.4 Thin-Layer Chromatographic Fingerprinting

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. About 20 µl of solutions 1, 2, 3, 4, 5, 6, and 7 were respectively introduced to the TLC plate and allowed to dry in a cold air current for about 15 minutes, thereby developing 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, then dried in the oven at 140°C for 5 minutes. The chromatogram was observed in daylight.

3. RESULTS AND DISCUSSION

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 - 89 °C. The TLC results indicated the presence of dihydroartemisinin and α-artemether as well as artemether in samples A, B, C, D, E, and F. Fig. 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.

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 finished pharmaceutical products. 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 [32,33]. Pharmacokinetic studies make extensive use of quantitative or qualitative drug and metabolite related research [34].

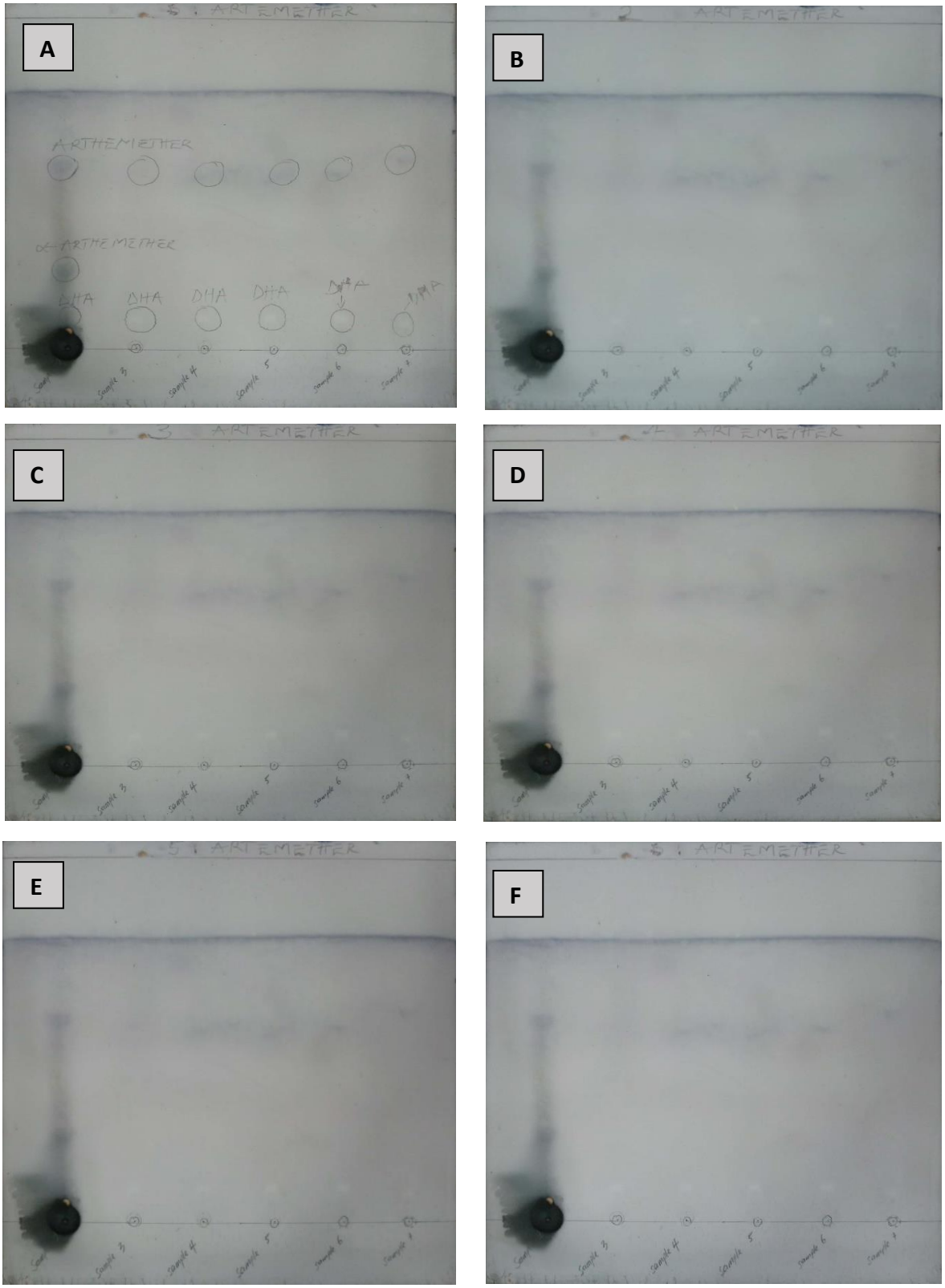

Fig. 2. TLC chromatograms for sample A – F

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 - 89 °C, which is within the specified range of 86 - 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 D (α-artemether) was 0.4, and artemether was 0.55, (Fig. 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, impurity B spots 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 main spot 5. Because of the presence of α-artemether, the spot corresponding to impurity D was not brighter than the spot produced by solution 4, and no other spot was brighter than the primary spot produced by solution 3 (Fig. 2A-F).

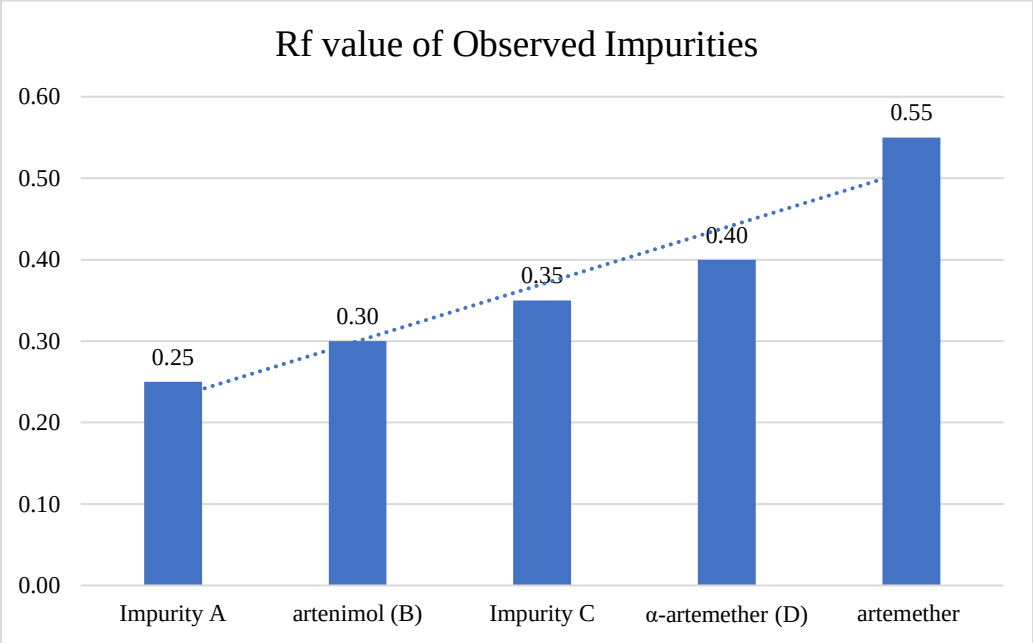

Fig. 3. The Rf value of the observed impurities from the test samples

4. CONCLUSION

The study has shown the possibility of conducting regular quality control analysis of artemether-lumefantrine suspension with a simple TLC method. The fixed-dose combination powder for suspension analysis was successfully conducted using this method. Hence routine quality control measures, exploring the use of TLC should be used in the determination of artemether and its derivatives either in single or combined dosage formulations and be incorporated into the manufacturing processes, ranging from raw materials to finished products, thereby ascertaining their stability and safety.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The study was approved by the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Madonna University Nigeria, Elele Campus, Nigeria.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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