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POST-MARKET SURVEILLANCE (PMS) OF ARTEMETHER-LUMEFANTRINE FIXED-DOSE COMBINATIONS MARKETED IN BENIN

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ABSTRACT: In Benin, several studies still confirm the circulation of low-quality anti-malarial drugs, despite efforts to improve the supply chain. It is, therefore, necessary to carry out post-market quality surveillance of the same to ensure that only quality and efficacious medicines are sold to the general populace. The aim of this work was to evaluate the post-market quality of fixed-dose combinations of artemether-lumefantrine marketed in Benin. This descriptive and analytical study was executed in four stages over fourteen months, from December 2019 to January 2021. Thirty samples of artemether-lumefantrine (80/480 mg) fixed-dose combination tablets were collected from sixteen pharmacies in Cotonou and Porto Novo. The various quality control activities and tests conducted were: visual identification, pharmacopoeial tests (weight uniformity and disintegration tests), identification tests, *in-vitro* comparative dissolution tests and active ingredients content determination by HPLC−UV. The results of our analyses showed that none of the samples passed the visual identification test; all the samples passed the weight uniformity test in accordance with the specifications of the European pharmacopeia (Eur Ph). Regarding the identification tests of the active substances, we noted a clear overlap between the spectra of the reference and all the samples. However, one sample had a low content of the active ingredients (acceptance criteria of 90-110% for both lumefantrine and artemether). Also, two samples did not pass the disintegration test). The dissolution profiles of lumefantrine in these 3 samples, compared to that of Coartem® Princeps, were different ($f2A = 19.59$ $f2B = 9.91$ $f2C = 41.42$; <50).This study confirms the circulation of substandard drugs and the nee*d* for post-market surveillance of other pharmaceuticals sold in Benin in order to guarantee that only drugs that meet the quality standards are sold to consumers.

INTRODUCTION: In 1989, Benin opted for a drug cost recovery system through its subscription to the Bamako Initiative [1]. In the same year, the Central Purchasing of Essential Medicines and Medical Consumables (CAME), currently known as So BAPS SA (Beninese Society for the Supply of Health Products) was created and has gradually improved the supply chain of good quality generic drugs and at a lower cost [1].

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Despite these efforts, several studies in Benin have hinted at the circulation of substandard drugs in the market [2]. In developing countries, the most common counterfeit drugs are those that are used for the treatment of common diseases such as malaria (26.6%), inflammations (25.5%), infectious diseases (15.0%), *etc.* [3]. Counterfeit antimalarials are common in countries with weak drug regulatory

systems and where the distribution channels are difficult to track. Due to the relative high costs of these products, those who trade in them make significant profits [4]. In 2018, a study carried out by Mazu *et al.,* showed the circulation of substandard antimalarial drugs in both the informal and formal sectors with respective non-compliance rates of 16.67% and 7.14% [5]. Once antimalarials are on the market, post-marketing authorization quality surveillance is necessary to ensure that patients consume only quality and efficacious drugs. The *in-vitro* dissolution test is one of the methods recommended to demonstrate the quality and bioequivalence of certain generics to their innovator drugs [4]. Indeed, with the advent of many generics today, it is important to supplement quality control testing with comparative dissolution testing to see if these generics are equivalent to their innovator drugs. This work aimed to assess the post-market quality surveillance of antimalarial drugs recommended by the National Malaria Control Program (PNLP) to manage uncomplicated malaria.

MATERIAL AND METHODS:

Chemicals and Reagents: HPLC grade methanol and ammonium were purchased from VWR (Belgium); hydrochloric acid (37%) was supplied by Surechem Product Ltd (England). Benzalkonium chloride was obtained from Sigma-Aldrich (Germany). Artemether-lumefantrine reference chemicals were from Fourrts Laboratories Pvt. Ltd. Working Standard (India); Ultrapure water produced with PURELAB Chorus 1 Complete water purification system (Velolia, France). Different samples of artemether-lumefantrine tablets 80 mg/ 480 mg were randomly purchased in retail. They were submitted to visual inspection and instrumental analyses.

Preliminary Visual Inspection Tests: This part consists of verifying the following information for each drug sample according to the WHO guidelines: differences in packaging, leaflet, labeling and physical appearance of the dosage forms characterized by specific size, shape and color in order to identify potential counterfeiting or deterioration[6].

Pharmacopoeial Tests: Different pharmacopoeial tests were conducted on the samples according to the specifications of the European Pharmacopoeia [7] particularly, weight uniformity and the disintegration tests.

Weight Uniformity Test: Ten tablets were selected at random and then weighed individually. The average weight was then calculated. Per the pharmacopoeial standard, the individual weight of not more than 2 of the 10 tablets should deviate from the average weight by a percentage higher than the standard stated by the same. None of the tablets should deviate by twice the acceptable percentage.

Disintegration Test: Six tablets randomly selected from each batch were put into six holding tubes attached to a carousel with a grid, at the rate of one tablet per tube; then, a disc was added to each of the six tubes. The six tubes containing the tablets and the disc are then immersed in a measuring beaker containing 800 mL of Milli-Q water. The beaker was placed in a water bath maintained at a temperature of 37 °C ± 0.5 °C. The disintegration time was noted for each tablet when there were no more tablet particles or the residue was just a soft mass with no palpable nucleus. The average time was compared to that defined by European Pharmacopeia [7]. For naked tablets, the maximum tolerable time is 15 min; for film-coated tablets, it is 30 min and at least 60 min for coated tablets.

Active Ingredients Identification by Thin Layer Chromatography: This test was intended to prove the presence of the active ingredients declared by the manufacturer. For each active ingredient, a reference solution was prepared from each drug's reference standard (SCRs) and a mixture of the two SCR. The test solutions were also prepared under the same conditions. The drugs were dissolved in a mixture of methanol: ortho phosphoric acid (0.1% in methanol; w/v).

The resulting solutions contained 1 mg/mL of artemether and 6 mg / mL of lumefantrine. The migration solution (mobile phase) was a mixture of toluene: ethyl acetate: anhydrous acetic acid: methanol (9: 2: 1: 1; v/v/v/v). The detection of lumefantrine was carried out in UV light at a wavelength of 254 nm while a revelation solution for artemether was used because this molecule does not absorb at this wavelength.

HPLC Method: The analyses by HPLC were carried out on a HITACHI VWR (VWR, Belgium) separation module coupled to HITACHI VWR N°5430 photodiode array (PDA) detector (VWR) applying a validated method for Artemisinin-based combination therapy (ACT) as described below **Table 1** [8]. The system is controlled with Chromaster System Manager Version 1.1 (VWR).

TABLE 1: CHROMATOGRAPHIC CONDITIONS FOR SAMPLE ASSAY

	Characteristics
Column	Chromolith® performance RP18 (100 x 4.6 mm I.D; dp 5 µm)
Mobile phase	Methanol: Ammonium formate buffer 10 Mm; pH 2.8 (82.5:17.5)
Rate	0.6 mL/min
Injection volume	10 µL
Temperature	25 °C
Detection wavelength	230 nm

Preparation of Control Solutions: This involved preparing independently two reference solutions containing lumefantrine at 1200 µg / mL and artemether at 200 µg / mL (control 1, control 2) in a 50 mL graduated flask.

Preparation of Samples for the Assay: Three solutions for each sample were prepared independently. After achieving weight uniformity, the weight of the sample to be taken was calculated so as to have a concentration of 200 µg / mL in artemether, and 1200 µg / mL in lumefantrine in a 50 mL graduated flask.

Calculation of the Active Ingredient Content: The individual content of each of the solutions to be analyzed was calculated from the following formula:

% Active ingredient = Average sample area/(Average control test area) × Concentration control test/Concentration sample ×100

Comparative *In-vitro* Dissolution Test of Lumefantrine by HPLC-UV Analysis: This test was performed using a risk-based quality control approach. Only samples that did not pass the disintegration and active ingredient content tests were subjected to this test.

The dissolution medium was a mixture of hydrochloric acid 0.1 N (HCl) plus 95% benzalkonium chloride (in a beaker, 95 g of pure benzalkonium chloride were introduced, then 100 mL of Milli-Q water were added).

Preparation of Control Solutions: This involved preparing independently two reference solutions containing 600 µg / mL lumefantrine and 100 µg/mL artemether control 1, control 2.

Sample Preparation: Into each dissolution, a flask was introduced the tablet to be analyzed. 5 mL of the solution was taken after 10, 15, 20, 30, and 45 min of dissolution.

The samples were filtered directly using syringe filters with 0.45 µm pore diameter into sample vials and subjected to HPLC-UV analysis.

For the comparative dissolution tests, if the two products (sample and originator) demonstrated 85% dissolution at least in 15 min, the profiles were considered similar. If not, the value of the similarity factor f2 was calculated according to the formula below:

$$F_2 = 50. \text{Log } \{[1+ (1/n) \text{ n/ iml } (Rt-Tt)^2] -0.5 \times 100$$

Where n: number of sampling points, Rt: dissolution at time t of the reference and Tt: dissolution at time t of the test product. Two dissolution profiles are identical if $f_2 = 100$. These profiles are considered equivalent if f2 is greater than or equal to 50.

A sampling sheet was used to collect information on each sample. Data were entered using Epi Data 3.0 software. Processing and statistical analysis were performed using SPSS Statistics 19 and Microsoft Excel 2013 software.

RESULTS:

Preliminary Visual Inspection Tests: All samples were visually identified. From the results of the visual identification, it appears that all the samples showed at least one non-compliance with the preliminary visual inspection test.

This non-compliance relates to spelling errors noted in the instructions and on the primary and secondary packaging, the absence of sections on drugs interactions and the mode of administration in certain leaflets, and the absence of information relating to the names and addresses of the manufacturers on certain samples.

Pharmacopoeial Tests: All samples (100%) passed the weight uniformity test and twenty-eight (28/30) samples passed the tablet disintegration test.

Identification: Results of the TLC analysis showed that all the samples contained the declared active ingredients

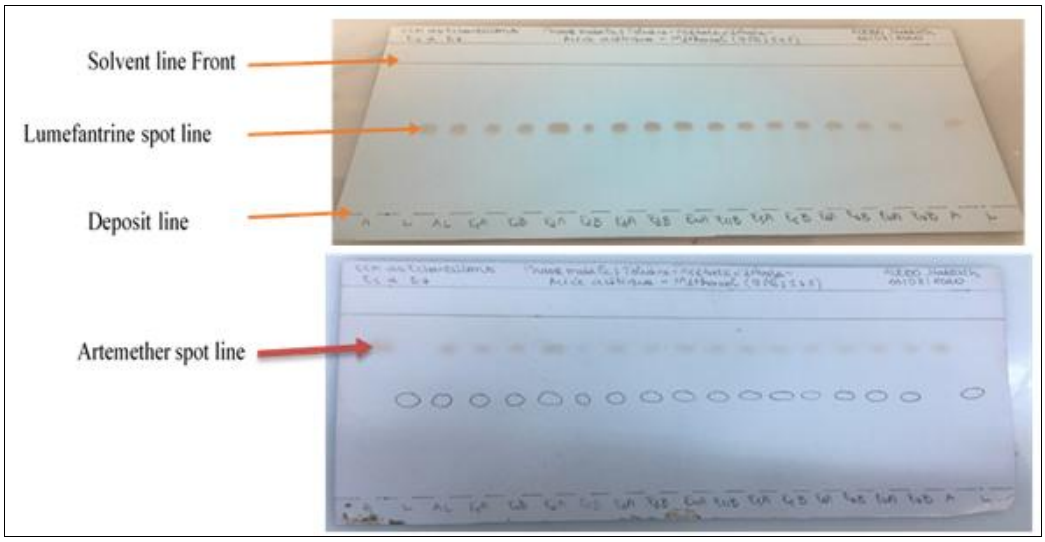

FIG. 1: TLC PLATE OF PURE REFERENCE COMPOUNDS AND SEVEN ARTEMETHER-LUMEFANTRINE SAMPLES OBSERVED AT UV AT 254nm

Active Ingredient Content Analysis: Almost all the samples contained the required quantity of the active ingredients (96.67%) except for one that did not meet the specifications of the European pharmacopeia 9 (Assay in %: 96.67; Specifications are set to 90.0% - 110.0% of the claimed nominal content (mg). This sample therefore *presents* a possible risk of under-dosing in the artemether and lumefantrine fixed-dose tablets.

Comparative *In-vitro* Dissolution of Lumefantrine Tablets: The dissolution test was carried out on the samples that did not comply with the disintegration test (Sample 9 (t=37 min 3S) and Sample 13 (t=23 min 21S) labelled E9 and E13 respectively; for naked tablets, the maximum tolerable time is 15 min) and on the sample which did not pass the identification test (Sample 23 (E23).

We compared their dissolution profiles to that of the innovator (COARTEM®; Sample 1 (E1). This comparison was only for lumefantrine since the low absorbance of artemether at the selected wavelength did not allow for the accurate determination of the same. The dissolution profiles of the samples were then compared to that of the innovator drug to determine the similarity there of. The results are shown in **Tables 2** and **3.**

TABLE 2: SUMMARY OF LUMEFANTRINE DISSOLUTION TEST

Sample (E)	Sampling time	Specification (Ph. Int)	APIcontent (%)	Compliance
1	45 min	Release of at least 60%	78.30	Conforms
9		of the API	51.30	Does not conform
13			11.50	Does not conform
23			64.60	Conforms

API: Active Pharmaceutical Ingredient

Two out of four samples did not comply with the requirements of this test. The innovator drug (Sample 1) had the best dissolution profile of 78.30% in 45 min.

TABLE 3: RESULTS OF THE COMPARATIVE IN VITRO DISSOLUTION TEST OF LUMEFANTRINE (N=3)

Sample (E)	Specification	Fit factor value	Compliance
9	$f2 \geq 50$	19.59	Does not conform
13		9.91	Does not conform
23		41.42	Does not conform

Fig. 2 shows the comparative release profiles of lumefantrine in 0.1 N HCl + 1% benzalkonium chloride medium from the tablets (480 mg) studied.

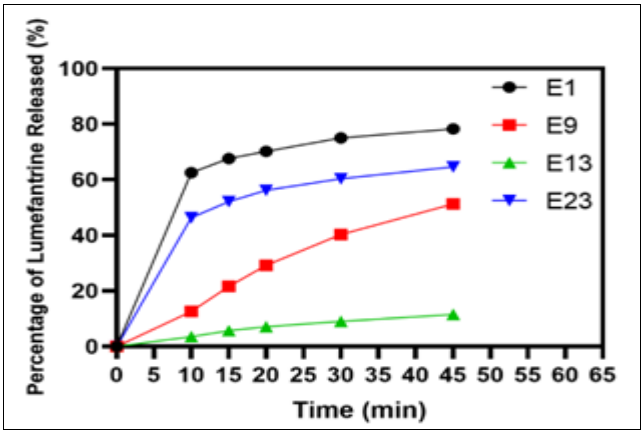

FIG. 2: COMPARATIVE RELEASE PROFILES OF LUMEFANTRINE IN 0.1 N HCL + 1% BENZALKONIUM CHLORIDE MEDIUM FROM THE TABLETS (9.91 <Ƒ2 <41.42)

DISCUSSION: The fact that none of the samples passed the preliminary visual inspection tests could be explained by the fact that the tests took into account many more parameters. The study focused much more on packaging inscriptions and package leaflets as well as the condition of these packaging materials. It, therefore, emerges that the drugs sold in pharmacies present a multitude of non-conformities in their packaging. Yemoa *et al.*, in their work on antimalarials, gave some probable risks related to nonconformities to visual identification tests [10]. These include treatment failures, intoxication, drug misuse, degradation of the active ingredient, and others.

The lack of disintegration observed could have an impact on bioavailability because the drug will not be able to break down in the body at the time indicated, with the consequence of delaying the release of the active ingredient and its action in the body. Therefore, this serves as a clarion call to the regulatory authorities to be extra vigilant to guarantee that the drugs in use meet the quality standards. According to the requirements of the International Pharmacopoeia, Artemether-Lumefantrine tablets must contain not less than 90% and not more than 110% of the amounts of artemether and lumefantrine indicated on label [9]. Our study revealed a compliance rate of 96.67% with only a sample recording a lower content of the active ingredients (*i.e.*, artemether and lumefantrine). The study carried out by Mazu *et al.*, showed a non-compliance rate of 25% with also an outlier (a sample with lower than the recommended content for the active ingredients) [5]. The low content of the active ingredients may be due to a defect in the manufacturing process or maybe a deliberate attempt by the manufacturer to maximize profit. The main consequence of under-dosing on the patient is treatment failure. There is also a risk to public health because the use of substandard drugs promotes the resistance of parasites to the drugs. The lumefantrine dissolution test was performed on four samples, one of which was the innovator and the other three samples that did not comply with the disintegration test and the HPLC analysis. This is a risk-based quality control approach. A total of two samples did not meet the quality standards of this test.

It should be remembered that these two samples did not also pass the tablet disintegration test. This situation could be linked to the poor formulation of the said samples. In Burkina Faso, Yameogo *et al.*, also found a sample that did not pass both the disintegration and the dissolution tests [11]. In fact, disintegration is a parameter that considerably influences the stage of dissolution of the active ingredient from the dosage form. If a tablet takes a long time to disintegrate (beyond specification), there is a risk that the active substance's release rate will decrease. Defects in disintegration and dissolution may be the consequence of a poor choice of excipients in the formulation, a high concentration of binding agents, an insufficient amount of disintegrant, a compressive force that is too high or even, deterioration of the tablets during storage [12].

All samples released less than 85% lumefantrine after 15 min under test conditions. The lipophilic nature of lumefantrine could explain this situation [13]. However, it should be remembered that the purpose of combining two antimalarials is to limit the risk of parasitic resistance to artemisinin derivatives by combining a fast-acting antimalarial and a slow-acting antimalarial. It is therefore understandable why lumefantrine was released slowly. In this case, the calculation of the similarity factors is necessary to decide on the similarity or equivalence of the release profiles of the active ingredients.

All samples had values of $f\ 2 < 50$, which indicates a nonequivalence of the lumefantrine release profiles to that of the innovator drug. These results demonstrate the importance of controlling the market to ensure the quality of authorized medicine in our market.

CONCLUSION: The present study relates to the post-market surveillance of the artemether-lumefantrine (80/480 mg) antimalarial marketed in Benin. The non-conformities noted were: packaging irregularities, long disintegration time, low active ingredient content, and nonequivalence of lumefantrine release profiles to the innovator drug, COARTEM®.

This study confirms the reality of the circulation of substandard drugs and thus the need for post-market quality surveillance of other pharmaceuticals sold in Benin in order to guarantee that only quality and efficacious drugs are sold and consumed.

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CONFLICTS OF INTEREST: The authors declare no conflict of interest.

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