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RESEARCH ARTICLE

VALIDATION OF REPORTED HPLC METHOD FOR THE SIMULTANEOUS DETERMINATION OF ARTEMETHER AND LUMEFANTRINE IN LOW INCOME LABORATORIES

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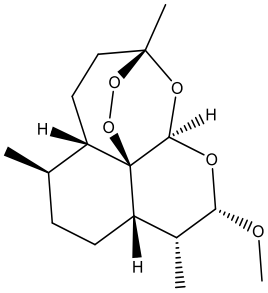
ABSTRACT

Objectives: Methods used in drug analysis should be simple, reproducible, precise, accurate and applicable in both developed and underdeveloped countries. This is exceptionally important in Africa where counterfeits of lifesaving drugs abound. It is therefore necessary that independent validation of published new analytical methods should be carried out to establish reproducibility and suitability in low income countries.**Methods:** Two sample preparation methods and one high pressure liquid chromatography (HPLC) method reported for the simultaneous determination of lumefantrine and artemeter in Coartem® tablets were used to analyse two batches of 30 Coartem® samples containing 24 tablets each. The analysis was carried out on a BLC-10 HPLC with a Dstar variable C18 (250 × 4.6mm, 5µm) column and UV detector (210 nm, 254 nm). The sample preparation methods and HPLC conditions were as reported. **Result:** Quantitative determinations of the individual drug content in the samples were not possible because no linear correlation was obtained for the plot of area versus concentration of the reference standards. Sample preparation methods are very important in obtaining good chromatograms. **Conclusion:** The methods as reported in those published work may not be effective methods for the simultaneous determination of artemether and lumefantrine in fixed dose combination tablets like Coartem® in low income laboratories.

Keywords: Artemether, lumenfanthrine, coartem, HPLC, assay, ACT.

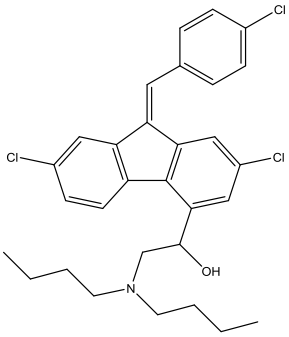
INTRODUCTION

Malaria is a global life-threatening disease.An estimated 3.3 billion people are at risk of malaria [1]. Malaria is one of the most important parasitic infections and the effective control of this disease poses a great challenge to the public health sector in developing countries [2]. It remains the most important tropical parasitic disease and one of the major public health challenges in the poorest countries of the world, particularly in sub-Saharan Africa, as the prospect of an effective vaccine remains uncertain. Nigeria has the highest malaria cases in the world. The country contributes 23% which is almost a quarter of the global malaria cases [3]. Amongst the most effective treatments are those which combine an artemisinin derivative with a longer acting component. The approved treatment of first choice in Nigeria is Artemether–Lumefantrine (Coartem®). Due to the effectiveness of this combination and its relatively high price, counterfeiters were enticed and presently, many counterfeits and substandard Coartem® are in the pharmacies and open drug markets.



(1R,4S,5R,8S,9R,10S,12R,13R)-10-Methoxy-1,5,9-trimethyl-11,14,15,16-tetraoxatetracyclo[10.3.1.04,13.08,13]hexadecane

Artemeter



2-(Dibutylamino)-1-[(9Z)-2,7-dichloro-9-(4-chlorobenzylidene)-9H-fluoren-4-yl]ethanol

Lumefantrine

Considering the level of counterfeiting and for quality control purposes, Coartem® like every other drug dosage form should be subjected to quatitative analysis to determine the percentage of the API for comparison with compedial standards.

Some methods have been reported for the determination of the presence of either artemether or lumefantrine in various pharmaceutical and biological matrices as seen in the International Pharmacopoeia [4]

However, few methods are reported for the simultaneous determination of artemether and lumefantrine in biological and formulation matrices. Cesar and coworkers reported a simultaneous determination of lumefantrine and artemeter using HPLC-UV[5]

The simultaneous quantitation of artemether-lumefantrine in pharmaceutical dosage forms using HPLC-UVwas also reported by Sridhar[6]

Arun and coworkers developed a reverse phase HPLC-UV programme for a simultaneous estimation of artemether and lumefantrine in tablet dosage forms. Their method was reported to be linear, precise, accurate, specific and robust for the estimation intended [7].

Another method for a simultaneous determination of artemether and lumefantrine in pure and pharmaceutical dosage forms using HPLC with UV detection was reported by Sunil and coworkers [8].

In this study, two of the reported sample preparation methods and HPLC conditions[5-7] were used to determine the lumefantrine and artemeter in Coartem®. The purpose of this study was to determine the adequacy of the sample preparation method adopted, the claims of linearity, simplicity and precision of the HPLC method and their adaptability in a low income country.

MATERIALS AND METHOD

Arun's method

Preparation of Standard solutions

The standard solutions were prepared according to Arun and co-workers7. Briefly, approximately 8 mg of Artemether and 50 mg of lumefantrine reference standards (Pauco Pharmaceutical Industry Ltd, Nigeria) were accurately weighed using an analytical balance(OHAUS Corp. Model: PA 201,USA) and transferred to a 50 mL volumetric flask, 10 mL of acetonitrile was added to ensure complete solublization. The volume was adjusted with the mobile phase. Further dilutions were made to get a final concentration of 0.16 mg/ml of artemether and 1mg/ml of lumefantrine

Preparation of samples of Coartem®

The samples were prepared according to Arun and co-workers. Briefly, the samples were properly labeled (as in C-1 to C-30) to aid easy identification. Then 20 tablets of Coartem® was weighed and powdered from the each pack and a quantity of the powder equivalent to 80 mg of artemether and 480 mg of lumefantrine was transferred into a 100 ml volumetric flask. Then 25 ml of acetonitrile was added and the mixture vortexing for 3 minutes, followed by dilution with the mobile phase to get the final concentration equivalent to 1000 µg/ml of artemether and lumefantrine. The analyses was carried out using BLC-10 High Performance Liquid Chromatography (Buck Scientific Instrument, USA) with a Carbon 18 Dstar C18 (250 mm × 4.6 mm, 5 µm) column and UV detector variableat 254 nm wavelength. Every other condition was as reported in the article.

Cesar's Method

Preparation of standard solutions

The standards were prepared according to Cesar and co-workers[7]. Briefly, approximately 40 mg of Artemether and 30 mg of Lumefantrine reference standards (Pauco Pharmaceutical Industry Ltd, Nigeria) were accurately weighed and transferred to a 100 mL volumetric flask, 2 ml of chloroform (BDH, Germany) was added to ensure complete solublization. The volume was adjusted with acetonitrile.

Further dilutions were made to get a final concentration of 0.4 mg/ml of artemether and 0.3 mg/ml of lumefantrine. HPLC analysis was carried out with Arun's method!

Preparation of Artemether stock solution

Approximately 175 mg of artemether reference standard was accurately weighed and transferred to a 100 ml volumetric flask. Then 2 ml of chloroform was added to ensure complete solublization and the volume was made up with acetonitrile to a final concentration of 1750 µg/ml of artemether.

Preparation of artemether work solution

A 10 ml of artemether stock solution was transferred to a 50 ml volumetric flask and the volume was adjusted with the mobile phase to a final concentration of 350 µg/ml of artemether

Calibration curve standard preparation

Standard solutions containing 750 µg/ml of lumefantrine were prepared in triplicate. Aliquots of these solutions were diluted in mobile phase to five different concentrations, corresponding to 150, 225, 300, 375, 450 µg/ml of lumefantrine.

Standard solutions of artemether containing 250 µg/ml of artemether were prepared in triplicate. An aliquot of these sample solutions were diluted in mobile phase and 10 ml of artemether stock solution, corresponding to 350 µg/ml of artemether was added using the standard addition procedure. Therefore, the final concentrations were 375, 387.5, 400, 412.5, and 425 µg/ml of arthemeter. Finally, calibration curves for concentration versus peak area were plotted for each compound.

RESULTS AND DISCUSSION

The result of the studies carried out on the sample preparation protocols and HPLC conditions as was reported by Arun and coworkers suggested that the result published was not reproducible and the method not reliable.

The result showed no chromatographic peak for the standards of artemether replicates collected at 254 nm (Table 1).This is likely due to following reasons. Firstly, the method reported using acetonitrile and 0.01M potassium dihydrogen orthophosphate buffer (70:30)] for their extraction but artemeter is not readily soluble in acetonitrile neither is it soluble in water. They reported using sonication probably to effect dissolution . He did not report sonicating the standard samples. Even when we vortexed, we didn't get good result. Our experience showed that the artemether is not readily soluble in acetonitrile even after vigorous vortexing. On filtering through Whatman no.1, the quantity of the standard dispersed remained almost the same as the merk meaning little dissolution was acheived.

Table 1: The Reference Standards.

Standards	Concentration(µg/ml)	Area
Artemether 1	160	0
Artemether 2	160	0
Lumefantrine 1	1000	0
Lumefantrine 2	1000	0

Artemether, is practically insoluble in water and highly soluble in dichloromethane & acetone. It dissolves freely in ethylacetate and dehydrated ethanol and it is soluble in chloform, methanol and ethanol. On the other hand, lumefantrine a yellow crystalline powder is practically insoluble in water and aqueous acids but dissolves freely in N, Ndimethylformamide, chloroform, and ethyl acetate. It is also soluble in dichloromethane and sparingly soluble in methanol and ethanol.

Considering the above solubility profiles of the two compounds, the choice of extracting and eluting solvent would be challenging. Using chloroform as the extraction solvent while using methanol-buffer as the eluting solvent for HPLC would have yielded better result but, lumefantrine is not readily soluble in methanol and precipitation may occur in-column posing problem with the estimation of lumefantrine. It appears Arun copied Cesar but the attempt to copy was shody. Cesar sonicated for 30 minutes even when he used chloroform to aid extraction while Arun sonicated for only 3 minutes. Cesar must have increased his sonication time following observations recorded during method developemnt. The reduction in sonication time in Arun's method would also impact on the solubility of the active ingredients.

In literature, the recommended wavelengths for the detection of artemether and lumefantrine are 200-220 nm and 330-350 nm respectively[9] while the USP recommended monitoring at 210 nm. The second problem with this work is that Arun monitored artemether a compound without chromophore at 254 nm giving preference to lumefantrintrine which is conjugated and could be detected even at 210 nm while Cesar monitored at 210 giving preference to artemether enhancing its on-column detection. This favored the detection of artemether than lumefantrine in this region because although lumefantrine is highly saturated with double bonds, 210 nm is far from the absorption max of lumefantrine and could have affected the signal but this would be better than monitoring artemether at 254 nm. However, even at the selected wavelength, the detection of artemether was still poor. Cesar also attempted battling this problem by means of artemether standard addition to the sample solutions to improve its detection

Comparing the Cesars's method to Arun's method, it is obvious that Cesar's would give a better extraction efficiency of samples

and better dissolution of standards than that of Arun because of inclusion of a solvent in which the drugs show adequate solubility. Also detailed description of work done which was lacking in Arun's work allows for easier replication and reproducibility.

This is because after preparing the samples using the Arun's method, on standing it was observed that the samples precipitated out because they were not soluble in the solvent. To manage this problem, the samples were centrifuged after which the supernatant was decanted into the respectively labeled test tubes for the assay.

When we used Cesar's method for sample preparation, no such problem was encountered because of the addition of chloroform to helped with the solubility of the samples. It was therefore not surprising that artemether was not detected in Arun's method because it would have precipitated on standing. Thus the Cesar employed a better sample preparation method than the former. However, the calibration curve obtained from Cesear's (Figs. 1&2) did not show linearity and therefore cannot be used for quantitation of the APIs in dosage form.

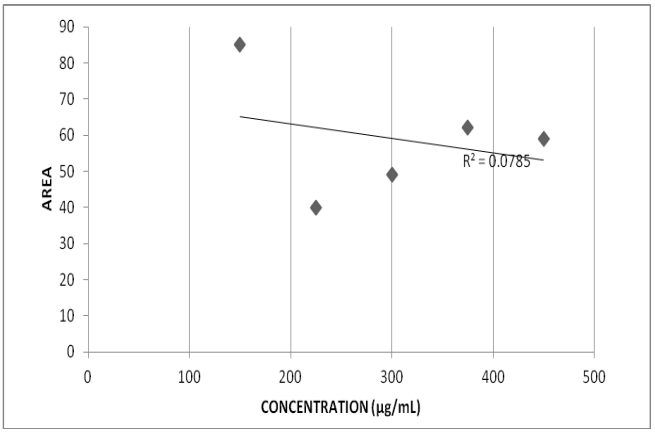


Figure 1: A graph of area versus concentration (µg/mL) of Lumefantrine reference standard values.

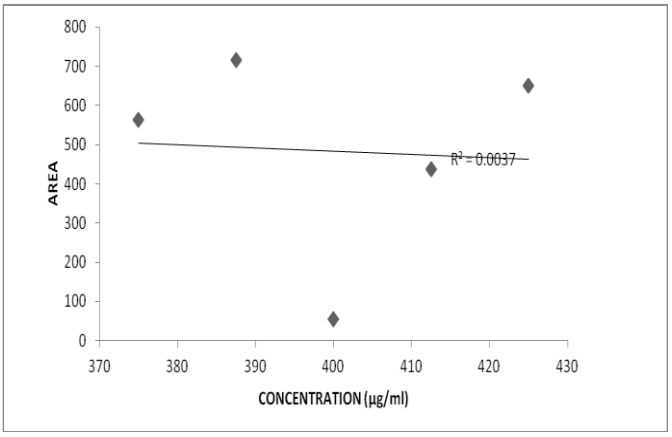


Figure 2: A graph of Area versus Concentration (µg/ml) of Artemether reference standard values.

The observed nonlinearity can be attributed to the preciptation of the compounds on injection because the eluting solvent used in the isocratic run still constituted 70:30 potassium dihydrogen orthophosphate buffer. Therefore, the solublization achieved by adding chloroform would have been lost in dilution in these solvent system in which APIs are not readily soluble in.In addition, because artemeter and lumefantrine are poorly soluble in acetonitrile, the elution of these drugs from the column might be non uniform resulting in the observed variation in peak areas and hence non-linear calibration curve.

To take care of this challenge, chloroform could have been used as the organic phase but chloroform is immissible with aqueous buffer. Methanol as the organic phase would have sufficed. Kalynkar and Kakde reported the simultaneous determination of lumefantrine and artemether using methanol as the extracting solvent as well as the HPLC organic phase[10]. The results were logical and predictable.

Absorbance detection is based on the principle that functional groups of a chemical compound can absorb light at one or more wavelengths in the UV or the visible light range. Majority of organic compounds have some absorbance in the UV-visible range and a high molar absorptivity (ε) of a compound allows low concentration of such compound to be measured [9]. But artemether is a compound with low molar absorptivity[5], thus working solutions of artemether was added to the samples as indicated by the Cesar's method, to increase the peak area of artemether in the chromatograms, thereby improving its detection.

Furthermore, Any procedure that requires mutiple and expensive equipment to carry out may not be suitable for laboratories in developing countries like Nigeria. Most school laboratories do not know what sonicator looks like. Also, since the desire of every analyst is to simplify analytical procedure, any procedure that requires less number of steps should be preferred to ones with extra steps.

CONCLUSION

The inefficiencies encountered using the methods employed in these experiments, hindered quantitative determination of the individual contents of the drugs: hence, the assay of the Coartem® samples for conformity with label claim and determination of degree of adulteration of this branded ACT. Therefore, the experiment proves that the methods employed cannot be used directly in low income countries.

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