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Simultaneous Determination and Quantitation of Artemeter and Lumefantrine in Antimalarial Tablet Formulation using High Performance Liquid Chromatography with UV Detection

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Abstract

Artemether-lumefantrine (AL) combination therapy is now the most used anti-malarial treatment in the world. In Ghana, it has been used as a first line treatment for uncomplicated Plasmodium falciparum malaria since 2004. In this paper, a reliable High Performance Liquid Chromatography method method was developed and validated for the simultaneous determination of AL in commercial fixed-dose combination tablets in the Kintampo-North Municipality. The method employed a Jasco HPLC system equipped with C18 reverse phase column and a mobile phase of acidified methanol and triethylamine buffer (85:15) pH 2.7 as the mobile phase. The flow rate was 1ml/min and detection were by means of a UV detector set to 222nm. The isocratic mode of elution was employed. The retention time of lumefantrine was 5.22 ± 0.19 minutes and artemether, 4.19 ± 0.22 minutes. The method was validated by evaluation of different parameters such as accuracy, precision, linearity, ruggedness and robustness. The percentage recovery for artemether and lumefantrine ranged between 99.18-100.19 and 99.96-100.07 respectively. Six brands of artemether-lumefantrine fixed-dose combination tablets (two local and four foreign) from selected chemical shops and pharmaceutical shops in the Kintampo- North Municipality were analyzed. Of the six brands of artemether-lumefantrine fixed-dose combination tablets analyzed, all passed with respect to their artemether and lumefantrine content using the developed HPLC method. The percent recovery for the local brands ranges from 93.5 to 99.2% and from 91.3 to 97.2% for artemether and lumefantrine respectively. For the foreign brands, 92.05 to 105.0% and 95.8- 99.9% for artemether and lumefantrine respectively, which complies with the International Pharmacopoeia range of 90110. The optimized and validated RP-HPLC method is simple, sensitive, precise, accurate and reproducible. The developed method has been validated as per ICH guidelines and meets all the acceptance criteria given. Hence it can be used in routine analysis for the simultaneous determination of artemether and lumefantrine in bulk as well as in pharmaceutical preparations.

Keywords: Artemether; Lumefantrine; High; Performance; Liquid; Chromatography

Abbreviations: ACT: Artemisinin-based Combination Therapy; AM: Artemether; API: Active Pharmaceutical Ingredient; BP: British Pharmacopoeia; HPLC: High Performance Liquid Chromatography; ICH: International Conference on Harmonization ; IP: International Pharmacopoeia; LU: Lumefantrine; LOD: Limit of Detection; LOQ: Limit of Quantitation; ODS: Octadecylsilane; RP-HPLC: Reverse Phase HPLC; RSD: Relative Standard Deviation; SALMOUS: Standards for Articles Legally Marketed Outside the U.S; SD: Standard Deviation; USP: United States Pharmacopoeia; UV: Ultra-Violet; WHO: World Health Organizations

Introduction

Malaria continues to be one of the major public health problems in Africa, Asia and Latin America. About 219 million cases of malaria and an estimated 660 000 deaths were recorded, out of these, 90 % occurred in Africa [1]. In Ghana malaria accounts for more than 60% of under-five hospital admissions, and 8% of

under-five mortality and 9.2% of maternal deaths. The use of antimalarial medications is the only practical means of preventing mortality and lowering morbidity brought on by the disease in many malaria-endemic locations, particularly in the African region [2]. Several routinely prescribed antimalarial medications, including chloroquine and sulfadoxine-pyrimethamine, no longer work on Plasmodium falciparum. The WHO has advised that all antimalarial medications should combine an artemisinin derivative with a co-drug as a result [3]. These treatments incorporate two active substances with various modes of action [1]. It is quite concerning because ACT-resistant P. falciparum has just been found along the Thailand/Cambodia border [4], There is evidence that drug-resistant falciparum malaria has migrated from Asia into Africa [5].

Artemisinin derivatives are incredibly important antimalarial medications, and because of their quick action and lack of adverse effects, they are in high demand in endemic areas, making them vulnerable to forgery and counterfeiting [6]. Checking the quality of antimalarials is necessary to prevent the emergence of resistance. For reliable and accurate results, High performance liquid chromatography is a very powerful tool for the quantification of these medications. There are currently HPLC methods for the analysis of lumefantrine and the assay of artemether in finished pharmaceutical products (FPP) [7] as well as for lumefantrine analysis [8]. However, only a few HPLC methods were reported for the quantitative determination of Artemether and lumefantrine in fixed combination anti-malarial products [8-11]. Hence this study seeks to add to the existing methods, a method that is rapid, economical, precise and accurate for the assay of artemether and lumefantrine.

Materials and Methods

Reagents and materials

Methanol, ethanol, Triflouroacetic acid, Hydrochloric acid, trielthylamine and acetonitrile used were HPLC grade from BDH, Europe. Sodium Phosphate was obtained from Sigma Aldrich, and Hydrochloric acid from Elitech Solutions, France. Artemether and lumefantrine standards were obtained as generous gift from Pharmanova, Accra, Ghana. AL tablets were sampled from selected chemical shops and three pharmaceutical shops in Kintampo.

Instrumental and analytical condition

The HPLC analyses were carried out on a JASCO HPLC system equipped with a stationary phase consist of an Ultracarb 3µ C_18, (20) 200*3.2mm and a UV detector. The injection volume was 20 µL. The separation of artemether and lumefantrine was evaluated in different proportions of different solvents or different proportions of the same solvents or same proportion with different conditions such as the flow rate and, for each condition, the retention times were noted. The optimized condition was achieved using a mobile phase comprising acidified Methanol and triethylamine buffer (85:15).

Preparation of standard solutions

Artemether–lumefantrine standard solution

4mg lumefantrine and 24mg artemether were accurately weighed and transferred into a 25mL volumetric flask, sonicated and diluted to volume with the mobile phase to give a solution of 160µg/mL of artemether and 960µg/mL of lumefantrine. The solution was then filtered using a sintered glass filter. 20µL of this solution was injected into the column and the chromatographs recorded.

Preparation and Analysis of Tablet Formulations

Twenty Tablets of artemether and lumefantrine were weighed and finely powdered. A quantity equivalent to 4mg of artemether and 24mg of lumefantrine was transferred into 25 mL volumetric flask and appropriate amount of diluent was added. The contents were sonicated to dissolve completely and the volume was made up to the mark with diluent and filtered through sintered glass filter. 1 mL of stock solution was transferred to a 10 Ml volumetric flask and made volume up to the mark with diluents to get final concentration of 16µg/mL and 96µg/mL for artemether and lumefantrine, respectively. 20µL of this solution was injected into the column and the chromatograph recorded. To calculate the artemether and lumefantrine content in the tablets, their respective peak areas were inserted into the linearity equation from the linearity graph to determine their contents.

Validation Linearity

Aliquot portions of standard stock solution 0.2, 0.4, 0.6, 0.8 and 1.0 mL were taken in separate 10 mL volumetric flasks. The volume was adjusted to the mark with diluent to obtain concentrations of 3.2, 6.4, 9.6, 12.8, 16.0µg/mL and 19.2, 38.4, 57.6, 76.8, 96.0, 115.2 µg/mL for Artemether and Lumefantrine, respectively. Calibration curve was plotted over a concentration range of 3.2-16µg/mL for artemether and 19.2-115.2µg/mL for lumefantrine. Calibration curve was constructed by plotting peak area v/s concentration, the graph must be linear and the regression equation was calculated.

Precision

One set of three different concentrations of mixed standard solutions of artemether and lumefantrine were prepared. All the solutions were analyzed in triplicates, in order to record any intraday variations in the results. For inter-day variations study, three different concentrations of the mixed standard solutions in linearity range were analyzed on three consecutive days. The peak areas were recorded and the Relative Standard deviation (RSD) was calculated for both series of analyses.

Robustness

In the robustness study, the influence of small, deliberate variations of the analytical parameters on retention time of the drugs was examined. The following factors were selected:

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1. Flow rate of the mobile phase (2.7±0.02ml/min)

2. Wavelength at which the drugs were recorded (222±1nm).

Accuracy

The accuracy of the method was determined by calculating the recovery of the analyte of interest by the standard addition method: Known amounts of working standard of artemether (1.6µg) and lumefantrine (9.6µg) were added to solutions of various concentrations like: 3.2µg, 6.4µg and 9.6µg of artemether and 19.2µg, 38.4µg and 57.6µg of lumefantrine. Each sample was prepared in triplicate and injected. Sensitivity

Limit of detection (LOD) and quantification (LOQ) were estimated from the signal-to-noise ratio. The LOD and LOQ were calculated by the use of the equations:

LOD = 3 σ/s.

LOQ = 10 σ/s

Where σ is the standard deviation of intercept of calibration plot and sis the average of the slope of the corresponding calibration plot.

Ruggedness

Sample solutions of artemether (16µg/mL) and lumefantrine (96µg/mL) were prepared and analyzed using slightly different operational and environmental conditions.

Results and Discussion

Chromatographic method development

Optimization of chromatographic mode, wavelength: Artemether and lumefantrine, both the API's are non-polar in nature, hence either reversed phase or ion-pair or non-aqueous chromatography was used. Detection wavelength was selected by scanning reference standards over a wide range of wavelength from 200nm to 400nm. A fixed concentration of AM-LU (10µg/ml) was analyzed at different wavelengths. From the responses, the λ max value was found to be 209nm and 335nm for artemether and lumefantrine, respectively. Using this data 222nm was selected as a detection wavelength at which the components showed well resolved peaks. The standard solution of artemether and lumefantrine was prepared and run through the system and different combinations of mobile phase and column were tried for isocratic mode to get well resolved, symmetric peaks. The method employed acidified methanol/triethylamine phosphate buffer (85:15), which is economical but have well resolved peaks.

Validation

Linearity: The method demonstrates linearity over a concentration range of 10-100ug/ml for lumefantrine and 3-20ug/ml for artemether. From the calibration curve the R2 value over this range was found to be 0.9991 and 0.9995 for artemether and lumefantrine respectively. This indicates a linear relationship between the concentrations of the two analytes.

Robustness: When some conditions of the mobile phase such as pH and column used for the method were varied, there was no statistically significant difference between results of the varied and old conditions of mobile and stationary phases using the student t-test. The flow rate and wavelength of detection were varied, with no significant difference in the peak areas. This indicates that the method is robust under varying condition of both flow rate and wavelength of detection (USP SALMOUS edition 2008).

Precision: Precision is reflected by percentage RSD values less than 2. These low values suggest high sensitivity of the developed method (USP SALMOUS edition 2008).

Sensitivity: With the developed method, the LOD and LOQ for LU were 338 and 1129ug/ml (0.333 and 1.129mg/L) respectively. AM had 90 and 300ug/ml (0.090 and 0.033mg/L as LOD and LOQ respectively.

Accuracy: The percent recoveries for Accuracy was within range of (97.6 -99.86) % for AM and 97.5-99.2 for LU which indicates that the method was accurate.

Assay of Tablets

According to the USP SALMOUS standard, lumefantrine tablets should contain not less than 90.0 percent and not more than 110.0% of the labeled amounts of artemether and of Lumefantrine. (USP SALMOUS edition 2009). Six commercial brands of (two local and four foreign) AM-LU tablets were analyzed for active substances using the developed method. Triplicate determinations were carried out. The respective contents of AM and LU were 93.5/91.3, 99.2/97.2, 96.62/97.0, 95.82/98.5, 105.2/99.9, and 92.05/95.8 percentage of the declared contents for Malar2DS, DANMETHER, Malafantrine, Coartem, Lonart and Artemos plus. The formulations complied with the (90-110%) of the label claim for the IP.

Application

Analysis of Marketed formulation

The validated method was used for the simultaneous estimation of artemether and lumefantrine in fixed dose combination tablets. Six brands were procured and analyzed with the proposed method and the results are presented in Table 5. The content (mg) and percentages of each of artemether and lumefantrine in the tablet sample was computed using peak areas and the regression equations from the calibration curves. The mean contents obtained for artemether and lumefantrine in the formulated products were very close to the labeled amount. The results show that the method is accurate in determining the content of the two active ingredients in fixed dose combination tablets.

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Conclusion

Considering the increasing use of ACT to treat malaria in endemic areas, the availability of simple and rapid analytical method is essential to evaluate the quality of formulations being used currently. From the present study it can be concluded that the optimized and validated RP-HPLC method is simple, sensitive, precise, accurate and reproducible. The developed method has been validated as per the ICH guidelines and it meets all the acceptance criteria given in ICH guidelines. Hence the method can be used in routine analysis for the simultaneous determination of Artemether and Lumefantrine in bulk as well as in pharmaceutical preparations.

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