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Development and Validation of Stability-Indicating GC-MS Method for Simultaneous Determination of Lumefantrine and Artemether

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Abstract

A sensitive, accurate and precise stability-indicating gas chromatography mass spectrometry (GC-MS) method was developed for simultaneous determination of lumefantrine and artemether in pure form and pharmaceutical formulation.In this study, comprehensive stress testing of lumefantrine and artemether have been carried out according to ICH guidelines Q1A (R2). Lumefantrine and artemether were subjected to forced degradation studies under hydrolytic (acid and base), oxidative, photolysis, and thermal stress conditions. The chromatographic separation was performed usinga ZB5 column with (30 m ×0.53 mm, 1.50 µm), and helium as a carrier gas. The proposed method showed well separation between the drugs and their degradation products and had a good accuracy. The method was linear with 12-120 ng mL^{-1} and 4-40 ng mL^{-1} for lumefantrine and artemether respectively. The method showed to be linear (r^2 > 0.999), precise (RSD <0.53%), accurate (recovery of 99.61% for lumefantrine and 99.57% for artemether), specific and robust. LOD and LOQ values were 0.750 ng mL^{-1} and 2.500 ng mL^{-1} for lumefantrine and 0.590 ng mL^{-1} and 1.966 ng mL^{-1} for artemether, respectively. The method was validated according to ICH guidelines and applied for determination of the cited drugs in their pharmaceutical formulation.

Keywords: Lumefantrine, Artemether, Stability indicating method, Pharmaceutical formulation.

1. Introduction

Artemether, (1R,4S,5R,8S,9R,10S,12R,13R)–10–methoxy- 1,5,9-trimethyl-11,14,15,16 tetraoxatetracyclo [10.3.1.$0^{4,13}$.$0^{8,13}$] hexadecane, is semi-synthetic anti-malarial agent.[1]Artemether is freely soluble in acetone, soluble in tetrahydrofuran, methanol and ethanol, and practically insoluble in water. It has a molecular formula of $C_{16}H_{26}O5$, and molecular weight of 298.37 g/mol as shown in **Fig 1.** Lumefantrine, (Z)-2-(dibutylamino)-1-[2,7-dichloro-9-(4-chlorobenzylidene)-9H-fluoren-4-yl]ethanol, is an aromatic fluorine derivative that is used in combination with artemether for the treatment of Malaria.[2,3-4]

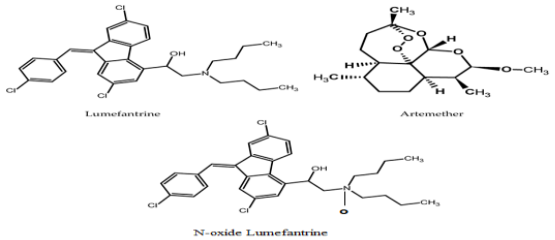

Figure 1: chemical structure of lumefantrine and the related impurities.

Lumefantrine is soluble in tetrahydrofuran, and dichloromethane, and practically insoluble in water. It has a molecular formula of $C_{30}H_{32}C_{l3}NO$, and molecular weight of 528.9 g/mol as shown in **Fig 1**.[2] Lumefantrine is official in European Pharmacopoeia,[3] International Pharmacopoeia,[5] and world malaria report.[6] There are different analytical techniques applied for the determination of lumefantrine in the pharmaceutical formulation such as spectrophotometric determination,[7-10] potentiometric,[11] HPLC chromatographic method,[11-13] GC coupled to flame ionization detector,[14] and there are another available instrumental techniques for the determination of lumefantrine and artemether as a combination in the pharmaceutical formulation, the available techniques include a colorimetric method,[15] spectrophotometric determination,[16-18] microemulsion electrokinetic chromatography,[19] HPLC chromatographic method.[1,20-21] The aim of the present work was to develop and validate a new GC- MS method for determination of lumefantrine and artemether in pure form and its pharmaceutical formulation. This study describes forced degradation

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of lumefantrine and artemether as prescribed under international council for harmonization (ICH) guidelines to quantify the percentage of drug degradation.[22]The developed method was validated in accordance with ICH guidelines Q2 (R1).[23]

2. EXPERIMENTAL

2.1. Apparatus

The GC Perkin Elmer Clarus 500 model (Perkin Elmer Technologies, USA), Mass detector (Perkin Elmer Technologies, USA).The control of the GC system and data processing were performed using PerkinElmer TurboMass™ GC/MS software.

2.2. Materials and reagents

Lumefantrine 99% and artemether 99%were kindly provided by Egyptian International Center for Import. Malari-Co tablet was purchased from (Multi-Apex Company) the Egyptian market. Each Tablet contains 20 mg of artemether and 120 mg lumefantrine. Hydrogen peroxide (30%), prepared as 3% H_2O_2 aqueous solution. Hydrochloric acid (37%), prepared as 0.1N and 1N HCL aqueous solutions. Sodium hydroxide pellets (99.0%) prepared as 0.1N and 1N NaOH aqueous solutions, all this reagents purchased from (Sigma-Aldrich (Steinheim, Germany)). Tetrahydrofuran and N, O- bis(trimethyl-silyl) trifluoro - acetamide (BSTFA) (Fisher Scientific-UK), deionized water and helium gas.

2.3. Standard solutions

Standard stock solution (12 µg mL^{-1}) of lumefantrine and (4 µg mL^{-1}) of artemether were separately in tetrahydrofuran. Lumefantrine and artemether working solutions in the desired concentrationrange was prepared by appropriate dilution ofstandard stock solution with tetrahydrofuran and derivatization was performed using extra pure BSTFA. The quality control (QC) samples were prepared from another prepared lumefantrine and artemether stock solutions. The stock solutions were prepared once a month, kept at 2–8 °C in a refrigerator and brought to room temperature before use.

2.4. Procedure

2.4.1. Chromatographic condition

A Zebron ZB5 column (30m, 0.53 mm, 1.50 µm, Phenomenex, USA) was used for the chromatographic separation.Helium was applied as a carrier gas, a flow rate of 1.5 mL min^{-1} and an injection volume of 2 µL.The oven temperature program was as follows: initial temperature maintained at 100 °C for 1 min, raised to 200 °C at a rate of 25 °C /min, hold 1 min, then raised to 350 °C at a rate of 10 °C/min, hold 3 min, and the injector temperature was maintained at 300 °C. Tetrahydrofuran was used as diluent. The mass spectrometry ionization modes were electron ionization (standard) positive/negative chemical ionization (optional); compounds leaving the GC column are fragmented by electron impact. SIM was used as acquisition mode in order to increase the detector sensitivity of the measurement. The charged fragments are detected, and the subsequent spectrum obtained can be used for identify the molecule. Fragmentation patterns are reproducible, and can be used to produce quantitative measurements.

2.1. 2.4.2. Construction of the calibration graph

Aliquots of 2 µL of analytes standard solutions at six different concentrations (12–120 ng mL^{-1}) of lumefantrine and (4–40 ng mL^{-1}) of artemether were injected into GC-MS system. The procedure was carried out in triplicate for each concentration. The analyte peak area obtained was plotted against the corresponding concentration of the analyte (expressed as ng mL^{-1}).

2.2. Derivatization

Derivatization of standards and sample solutions were prepared from the dry residues by reacting with 50 µL BSTFA solution at 70°C for 30 min in airtight glass vial; the obtained solutions were cooled before injected into GC.

2.3. 2.4.3. Application to pharmaceutical formulation

Five Malari-Co® tablets (20mg of artemether and 120 mg of lumefantrine) were weighed and then finely powdered. Appropriate weight of powder equivalent to one tablet was accurately weighed, transferred to 100- mL volumetric flask and the volume was made up to 50 mL with tetrahydrofuran. The solution was shaken vigorously for 20 min then mixed for 30 min and filtrated. The volume was completed with tetrahydrofuran to produce a stock solution labeled to contain 0.2 mg mL^{-1}artemether and 1.2 mg mL^{-1} of lumefantrine. This stock solution was diluted to obtain a test sample solution containing 2 ng mL^{-1} of artemether and 12 ng mL^{-1} of lumefantrine, and then 250 µL of test sample solution in microvial was evaporated to dryness using nitrogen followed by derivatization using BSTFA.

2.4. 2.4.4. Procedure for stability studies

- ***Acid degradation***

Accurately weights of 4 mg of artemether or 20 mg of lumefantrine were transferred to two 100-ml volumetric flask. To each flask, 5ml 0.1N HCL (or 5ml 1N HCL) and 45ml tetrahydrofuran were added. Each flask was kept at 50°c for 5 hrs on reflux chamber. The mixtures was neutralized with 5ml 0.1N NaOH (or 5ml 1N NaOH) and the volumes were made up to 100-ml with tetrahydrofuran. Finally after derivatization the acidic degradation product was diluted with tetrahydrofuran to obtain 40 ngmL^{-1} of artemether or 100 ng mL^{-1} of lumefantrine and analyzed using the proposed procedure.

- ***Base degradation***

Accurately weights of 4 mg of artemether or 20 mg of lumefantrine were transferred to two 100-ml volumetric flask. To each flask, 5ml 0.1N NaOH (or 5ml 1N NaOH) and 45ml tetrahydrofuran were added. Each flask was kept at 50°c for 5 hrs on reflux chamber. The mixtures was neutralized with 5ml 0.1N HCL (or 5ml 1N HCL) and the volumes were

made up to 100-ml with tetrahydrofuran. Finally after derivatization the basic degradation product was diluted with tetrahydrofuran to obtain 40 ngmL^{-1} of artemether or 100 ng mL^{-1} of lumefantrine and analyzed using the proposed procedure.

- ***Oxidative degradation***

Accurately weights of 4 mg of artemether or 20 mg of lumefantrine were transferred to two 100-ml volumetric flask. To each flask, 5ml 3% H_2O_2 and 45ml tetrahydrofuran were added. Each flask was kept at 50°c for 10 hrs on reflux chamber. The volumes were made up to 100-ml with tetrahydrofuran. Finally after derivatization the oxidative degradation product was diluted with tetrahydrofuran to obtain 40 ngmL^{-1} of artemether or 100 ng mL^{-1} of lumefantrine and analyzed using the proposed procedure.

- ***Photo degradation***

Small quantity of artemether or lumefantrine powder was kept under UV chamber for 5 hrs; after that 4 mg of artemether or 20 mg of lumefantrine was weighed and transferred to 100 ml volumetric flask. The volume was made up to 100ml with diluent. Finally after derivatization the photolytic degradation product was diluted with tetrahydrofuran to obtain 40 ng mL^{-1} of artemether or 100 ng mL^{-1} of lumefantrine and analyzed using the proposed procedure.

- ***Thermal degradation***

Small quantity of artemether or lumefantrine powder was transferred to a Petri dish and kept at 50 °C in hot air oven for 10 hrs; after that 4 mg of artemether or 20 mg of lumefantrine was weighed and transferred to a 100 ml volumetric flask. The volume was made up to 100 ml with diluent. Finally after derivatization the thermal degradation product was diluted with tetrahydrofuran to obtain 40 ng mL^{-1} of artemether or 100 ng mL^{-1} of lumefantrine and analyzed using the proposed procedure.

1. Results and discussion

2.5. 3.1. Method development

Optimization of mass spectrometry parameters and chromatographic conditions was achieved to develop and validate a selective and rapid assay method for the determination of lumefantrine and artemether.

2.6. Optimization of experimental conditions

The chromatographic separation was optimized after taking intoaccount the resolution between the drugs and their degradation product. Helium was applied as a carrier gas. The column was performed by a flow rate of 1.5 mL min^{-1} and an injection volume of 2 µL.Tetrahydrofuran was the best choice to separate the intact drugs from their degradation product. The Chromatogram of standard solution of artemether and lumefantrine was shown in **Fig. 2**.

3.2. Forced degradation

Forced degradation of lumefantrine and artemether were carried out to confirm that during stability study or throughout the shelf life, any degradation product if found will not interfere with the lumefantrine and artemether. In addition, the forced degradation study would help to identify the type of degradation pathway (whether oxidative, alkali hydrolysis, acid hydrolysis, photolytic etc.) for each one. it was achieved according to forced degradation and stability indicating studies protocol.[24] Forced degradation was performed by degrading the sample with thermal heating at 50 °C, 0.1N NaOH, 1N NaOH, 0.1N HCL, 1N HCL, 3.0% H_2O_2, and photolytic degradation. From the forced degradation studies, after refluxing, the drug solution with 0.1N NaOH at 50°C for 5 hrs, the percentage degradation of artemether and lumefantrine was found to be 2.13 and 1.74%, respectively. The percentage of degradation using 3% H_2O_2 at 50°C solution was not noticeable but the percentage degradation of artemether was found to be 3.99 using 3%H2O2 at 50°C for 5 hrs. The percentage degradation of lumefantrine and artemether using 0.1N HCL at 50°C for 5 hrs was found to be 2.60 and 6.75%, respectively. However, in all other degradation methods, the percentage degradation exceeded 5%. N-oxide lumefantrine impurity (Lumefantrine N-oxide [$C_{30}H_{32}NO_2Cl_3$, MW 543.15]) was result of oxidative degradation of lumefantrine, and there are other degradation product
of lumefantrine and artemether was obtained under the present forced degradation and stability indicating studies as shown in **Fig 3** and **4**.

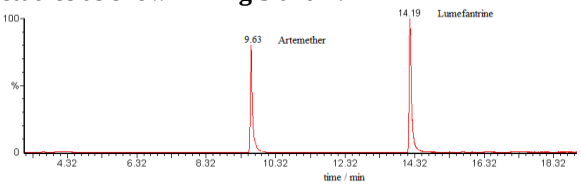


Figure 2: GC chromatogram of lumefantrine standard (120ng mL^{-1}) and artemether standard (40ng mL^{-1}).

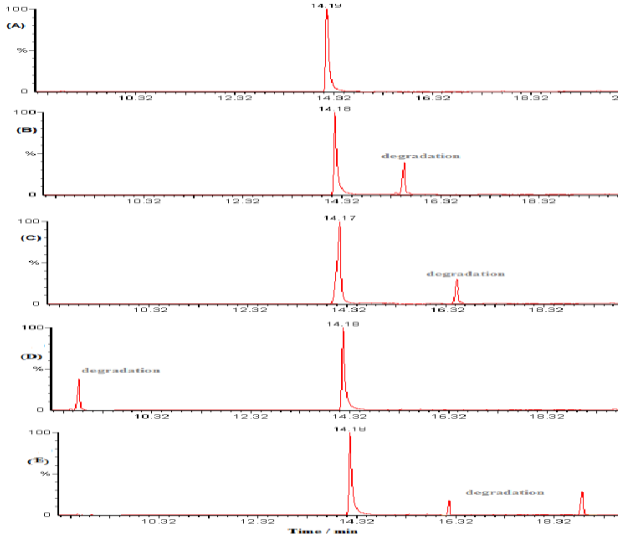
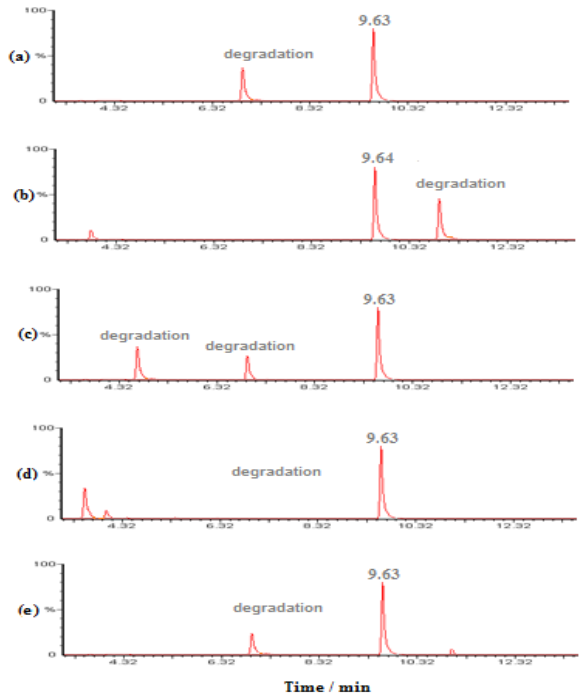


Figure 3: GC chromatograms of lumefantrine under different stress conditions: (A) standard lumefantrine, (B) basic hydrolysis (1.0 N NaOH), (C)

oxidative hydrolysis (30% H2O2), (D) thermal degradation, and (E) photo degradation, Respectively

Figure 4: GC chromatograms of Artemether under



different stress conditions: (a) basic hydrolysis (1.0 N NaOH), (b) oxidative hydrolysis (30% H2O2), (c) acidic hydrolysis (1.0 N HCL), (d) thermal degradation, and (e) photo degradation, Respectively

3.3. Method validation

3.3.1. Linearity and range

A linearity relationship was established by plotting the peak area values of the analyte versus the corresponding concentrations in ng mL^{-1}. The regression data results were summarized in **Table 1**.

3.3.2. Limit of detection and limit of quantification

LOD and LOQ were calculated according to ICH by comparing measured signals from samples with known low concentration of analyte with those of blank samples and establishing the minimum concentration at which the analyte can be reliable detected. The analytical parameters of the proposed methods are summarized in **Table 1**.

3.3.3. Accuracy and precision.

Accuracy and precision were evaluated at three different concentration levels within the same day to obtain repeatability (intra-day precision) and over three different days to obtain intermediate precision (inter-day precision). The accuracy and precision were calculated and expressed in terms of percent recovery and standard deviation, respectively. All values were within the acceptance variability limits as shown in **Table 1**.

3.3.4. Specificity

The specificity was evaluated from the GC-MS chromatogram. The GC-MS chromatogram of the samples shows that the method has sufficient specificity to resolve all related substances and the lumefantrine or artemether from each other. Furthermore, the mass detector also showed excellent mass purity for lumefantrine and artemether. Specificity of developed method was determined by chromatographic analysis of lumefantrine and artemether by applying different stress conditions, thermal heating at 50 °C, 0.1N NaOH, 1N NaOH, 0.1N HCL, 1N HCL, 3.0% H_2O_2, and photolytic degradation and the results were given in **Table 2**.

3.3.5. System suitability

System suitability test for a proposed chromatogram was applied, and the results detected that the proposed chromatographic conditions allow complete baseline separation between drugs and degradation products as shown in **Table 3**.

3.3.6. Robustness

Robustness was evaluated by changing the flow rate (1.5 ± 0.1ml min-1). The effect of GC oven temperature was studied at 100 ± 1°C (optimized temperature was 100°C). The measured response variances were the % RSDs as shown in **Table 1**. The degree of reproducibility of the results obtained as a result of small deliberate variations in the method parameters has proven that the method is robust.

3.3.7. Application to the finished product

Malari-Co® tablet can be determined using the proposed method. The obtained results showed absence of any interference from either excipients or additives. The results of the reported method,[21] were compared with that of the proposed method.The proposed method showed good accuracy and precision for assay of lumefantrine in Malari-Co® tablets and the values were listed in **Table 4**.

Table 1: Regression and validation data for estimation of lumefantrine and artemether by the proposed method.

Parameter	Lumefantrine	Artemether
Linearity range (ng mL^{-1})	12-120	4-40
LOD (ng mL^{-1})	0.75	0.59
LOQ (ng mL^{-1})	2.5	1.966
Regression parameter*	Y= a+ b C	Y= a+ b C
Correlation coefficient	0.9998	0.9999
Slope (b)	65260	4806
Intercept (a)	-59254	-1285

Accuracy (mean ± S.D)	99.616±0.451	99.57±0.484
Precision (% RSD)		
Repeatability	0.449	0.482
Intermediate precision	0.493	0.531
Robustness (%RSD)		
Flow Rate (±0.1mL)	0.458	0.529
GC oven temperature (±1 °C)	0.414	0.343

*Y= a + bC, where Y is the peak area and C is the concentration in ng mL^{-1}.

Table 2: Summary of the forced degradation study.

Degradation processes		% degradation	
		Lumefantrine	Artemether
Thermal degradation	Dry heat (50 ºC, 10 hrs)	9.32	5.17
Basic hydrolysis	1 N NaOH (5hrs reflux at 50 ºC)	8.79	9.66
Acidic hydrolysis	1 N HCl (5hrs reflux at 50 ºC)	8.94	18.12
Oxidative hydrolysis	3% H_2O_2(10hrs reflux at 50 ºC)	13..26	9.01
Photo degradation	Photolytic (UV, 5hrs)	6.44	5.01

Table 3: System suitability test for artemether and lumefantrine

Acceptance criteria	Results	
	Lumefantrine	Artemether
The %RSD for five replication injections of standard preparation for artemether and lumefantrine.	0.845	0.113
Resolution	1.12	
The Tailing factor	1.33	1.51
Theoretical Plates	2829	2944

Table 4: Results obtained after determination of Lumefantrine in Malari-Co tablet and comparison with the reported method.

Parameter	Proposed method		Reported method *	
	Lumefantrine	Artemether	Lumefantrine	Artemether
n[a]	5	5	5	5
%R	99.37	100.03	100.17	100.05
%RSD	0.845	0.113	1.087	0.766
SD	0.840	0.113	1.089	0.762
Variance	0.705	0.013	1.185	0.586
Student's t-test (2.306)[b]	1.306	0.983	____	------
F-value (6.388)[b]	1.681	1.601	____	-----

[a] Experiments number. [b] Tabulated values of "t "and "F" at (P = 0.05).

***Reported method**

HPLC method for determination of artemether and lumefantrine in combined dosage form was achieved using Gemini 5u C-18 column combined with a mixture of acetonitrile: buffer (35:65 v/v) mobile phase, PH adjusted with H_3PO_4 to be 2.5. The flow rate was 1.5 ml/min.[21]

2. Conclusion

This method is sensitive, and selective, and can be successfully applied for determination of lumefantrine and artemether in the pharmaceutical preparation.

Data availability

The readers can access the data represented in this research article by contacting with the author on this E-mail: ahmedabdrabou31@yahoo.com as the data presented is his own personal experiment and work.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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