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Gas chromatographic method for the determination of lumefantrine in antimalarial finished pharmaceutical products

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ABSTRACT

A simple method has been developed and validated for quantitative determination of lumefantrine in antimalarial finished pharmaceutical products using gas chromatography coupled to flame ionization detector. Lumefantrine was silylated with N,O—bis(trimethyl-silyl)trifluoro-acetamide at 70°C for 30 minutes, and chromatographic separation was conducted on a fused silica capillary (HP-5, 30 m length × 0.32 mm i.d., 0.25 μm film thickness) column. Evaluation of the method within analytical quality-by-design principles, including a central composite face-centered design for the sample derivatization process and Plackett—Burman robustness verification of the chromatographic conditions, indicated that the method has acceptable specificity toward excipients and degradants, accuracy [mean recovery = 99.5%, relative standard deviation (RSD) = 1.0%], linearity (=0.9986), precision (intraday = 96.1% of the label claim, RSD = 0.9%; interday = 96.3% label claim, RSD = 0.9%), and high sensitivity with detection limits of 0.01 μg/mL. The developed method was successfully applied to analyze the lumefantrine content of marketed fixed-dose combination antimalarial finished pharmaceutical products.

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1. Introduction

There were an estimated 216 million episodes of malaria in 2010, of which approximately 81%, or 174 million cases, were in the African Region. An estimated 655,000 malaria deaths were recorded in 2010, of which 91% were in Africa. Moreover, about 60% of the cases of malaria worldwide and >80% of malaria deaths occur in Africa, south of the Sahara. Approximately 86% of malaria deaths globally were of children under 5 years of age; most of these are caused by infection with *Plasmodium falciparum* and *Plasmodium vivax* [1–7].

The World Health Organization has recommended that all antimalarials should consist of a combination of an artemisinin derivative with a codrug such as lumefantrine, amodiaquine, or mefloquine [8]. A combination consisting of β-artemether and lumefantrine has been proved to be highly efficacious in children and adults, even against multidrug-resistant strains of *P. falciparum* [9]. Therefore, lumefantrine-containing combinations are incorporated in the World Health Organization essential drug list for the treatment of malaria in endemic areas of the tropical climate.

Lumefantrine, also called benflumetol, was first synthesized in the 1970s by the Academy of Military Medical Sciences, Beijing, China, and registered in China for the treatment of malaria in 1987. It is a racemic aromatic fluorene derivative, named (Z)-2-(dibutylamino)-1-[2,7-dichloro-9-(4-chlorobenzylidene)-9*H*-fluoren-4-yl]ethanol (Fig. 1) [10]. Structurally, physicochemically, and pharmacologically, lumefantrine belongs to the aryl amino alcohol group of antimalarial agents [11].

Worldwide malaria control programs are facing one of the greatest health care challenges due to the increasing problem of resistance in many parts of the world and the limited number of antimalarial medicines available. This has led to increasing difficulties in developing antimalarial treatment policies and providing prompt and effective treatment to all in need [5]. This increase in resistance can partially be attributed to substandard antimalarial drugs, resulting in treatment failure and ultimately increased morbidity and mortality [6,7]. Rapid identification of these substandard antimalarial medicines combined with regulatory measures is of paramount importance to combat this problem [6]. Therefore, appropriate analytical methods are required to evaluate the quality.

Many methods have already been reported for the determination of lumefantrine in finished pharmaceutical products (FPPs) [12–14]. High-performance liquid chromatography (HPLC) methods are also reported for the simultaneous determination of lumefantrine and β-artemether in artemisinin-based antimalarial fixed-dose combination (FDC) products [15,16]. Microemulsion electrokinetic chromatography was developed as an alternative method to liquid chromatography for the determination of lumefantrine [17]. However, at this moment, there is no gas chromatographic (GC) assay method available, despite GC being a suitable technique in poor resource economies due to its ease of operation and maintenance, lower use costs, and high separation efficiency [18]. Analysis of poorly soluble and weakly basic drugs by reverse-phase liquid chromatography remains a problem [19,20]. Lumefantrine is a nitrogen-containing basic compound [10], which can form asymmetrical peaks that can compromise separation and quantitation when analyzed using reverse-phase HPLC [21]. Moreover, selectivity issues are prominent in HPLC methods for the simultaneous analysis of β-artemether and lumefantrine in FPPs as a result of the presence of multiple related impurities and excipients, especially in pediatric formulations [16].

This paper reports a GC coupled to flame ionization detector (GC-FID) method for the quantitative determination of lumefantrine in antimalarial FPPs using silylation with *N*,*O*-bis(trimethyl-silyl)trifluoro-acetamide (BSTFA).

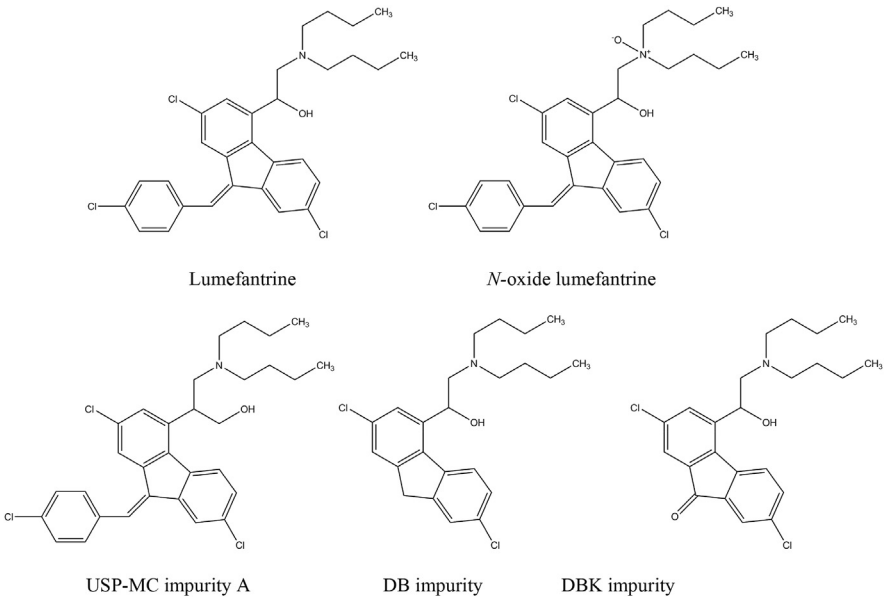

Fig. 1 – Chemical structure of lumefantrine and the related impurities. DB = desbenzyl; DBK = desbenzylketo.

2. Materials and methods

2.1. Materials and reagents

Lumefantrine active pharmaceutical ingredient (API) and Co-Artesiane FPP powder for oral suspension were obtained from Dafra Pharma International (B-2300 Turnhout, Belgium). The standards of desbenzylketo (DBK) impurity, N-oxide lumefantrine, and desbenzyl (DB) impurity were prepared in house at the Laboratory of Drug Quality and Registration (DruQuaR) of Ghent University, Ghent, Belgium [14]. Coartem and Artemine samples were collected in Ethiopia. United States Pharmacopoeia Medicines Compendium (USP-MC) standard of impurity A was purchased from US Pharmacopoeia (Basel, Switzerland). Analytical solutions were prepared using unstabilized HPLC-grade tetrahydrofuran (THF; Fisher Scientific, Leicestershire, UK), and derivatization was performed using extra pure BSTFA (Fisher Scientific).

2.2. Gas chromatography

An Agilent 7820 GC system (Agilent Technologies, Waldbronn, Germany) was used to perform the analysis with a liquid autosampler. Samples were introduced in a split/splitless injection port, and detection was performed by means of FID. An HP-5 (30 m length × 0.32 mm i.d., 0.25 μm film thickness) column (Agilent Technologies) was used for separation. The output signal was recorded and processed using EZChrom Elite software (Agilent Technologies, Santa Clara, CA, USA).

The column oven was programmed with an initial column oven temperature of 80°C for 1 minute, and increased to 325°C at a rate of 10°C/min, holding at 325°C for 9.5 minutes. The total run time was 35 minutes. The injector and detector temperatures were kept at 300°C and 340°C, respectively. Helium (Air Products and Chemicals, Allentown, PA, USA) was used as a carrier gas with a head pressure of 106.7 kPa resulting in an initial column flow of 3.2 mL/min and an average velocity of 50 cm/s. Helium was also used as a makeup gas for the FID detector. The makeup gas flow rate was 25 mL/min, while for hydrogen and air the flow was 30 mL/min and 400 mL/min, respectively. The split ratio was set at 10:1, and a 4 mm i.d. deactivated open-glass tube liner, packed with fused silica wool, was employed. Samples were injected by the instrument's autosampler with an injection volume of 1.0 μL, and THF was used to rinse the syringe between injections.

2.3. Solutions

2.3.1. Preparation of lumefantrine standard solution

Lumefantrine standard solution was prepared at 100 μg/mL concentration in THF. This standard solution (250.0 μL) was transferred into a microvial and evaporated to dryness under nitrogen to obtain the residue, providing the final concentration of 500 μg/mL after derivatization.

2.3.2. Preparation of lumefantrine test sample solution

Four samples of FDC tablets (Coartem and Artemine) containing 120 mg of lumefantrine per tablet and one sample of powder for oral suspension (Co-Artesiane) containing 1080 mg of lumefantrine per bottle were analyzed using the developed GC-FID method. For this, a homogeneous FPP powder weight equivalent to 10.0 mg lumefantrine was accurately weighed and transferred to a 10.0 mL volumetric flask. THF was added, shaken for 5 minutes and diluted to volume using the same solvent. The mixture was centrifuged (3 minutes at 1914 g), and a test sample solution was prepared at 100 μg/mL concentration (10 × dilution) in THF. This test sample solution (250.0 μL) was transferred into a microvial and evaporated to dryness under nitrogen to obtain the residue, providing the final concentration of 500 μg/mL after derivatization.

2.3.3. Preparation of lumefantrine impurity solutions

Four different lumefantrine impurity [DB derivative, lumefantrine USP-MC related impurity A (alcohol isomer), N-oxide-lumefantrine, and DBK derivative] solutions were prepared at 1 mg/mL concentration in THF [14]. Each of these lumefantrine impurity solutions (25.0 μL) was quantitatively transferred into a microvial and evaporated to dryness under liquid nitrogen to obtain the residue, providing the final concentration of 500 μg/mL after derivatization.

This solution can also be used as a system suitability solution as part of the control strategy of analytical quality-by-design approach.

2.4. Derivatization

The BSTFA derivatives of standards and sample solutions were prepared from the dry residues obtained as described above by reacting with 50.0 μL BSTFA solution in airtight glass vials at 70°C for 30 minutes in an oven. The resulting solutions were cooled and injected into GC without removing any excess of the derivatizing agent.

For optimization and robustness evaluation of the sample derivatization process, a central composite face-centered design with 11 runs including three center points was used for evaluating the influence of incubation time (minutes) and temperature (°C). A lumefantrine reference standard solution at 100% label claim (lc) was prepared and analyzed using the different experimental composite face-centered conditions indicated in the supplementary material online. Peak area for the main lumefantrine peak and the quantitative presence of other peaks (with a reporting threshold of 0.1% with reference to the main peak) were evaluated as responses.

2.5. Validation

Validation of the method was performed based on the International Conference on Harmonization guideline [22].

2.5.1. Linearity of calibration curve

From a stock solution containing 100 μg/mL lumefantrine in THF, different aliquots were transferred into a microvial and evaporated to dryness under liquid nitrogen to obtain the residue, providing the final concentrations of 400 μg/mL, 450 μg/mL, 500 (100% lc), 550 μg/mL, and 600 μg/mL after derivatization. Calibration curves for concentration versus peak area were plotted, and the obtained data were subjected to linear regression analysis.

2.5.2. Precision

For intraday precision, six sample solutions ($n = 6$) were prepared at 500 μg/mL lumefantrine concentration after

derivatization and analyzed using GC. Similarly, the inter-day precision was evaluated in 3 consecutive days ($n = 18$). Lumefantrine concentrations were determined and relative standard deviations (RSDs) calculated.

2.5.3. *Accuracy (recovery test)*

Accuracy was tested by recovery experiments where lumefantrine reference solutions were added to a placebo sample at three levels: 75%, 100%, and 125% lc. At each level, samples were prepared in duplicate and recovery percentage was calculated.

2.5.4. *Specificity*

Specificity of the method was evaluated by injecting lumefantrine reference standard solution and its impurity solutions (DB, USP-MC impurity A, *N*-oxide-lumefantrine, and DBK), both separately and mixed.

2.5.5. *Limit of detection and limit of quantitation*

Standard solutions of lumefantrine were prepared by serial dilutions, with concentrations ranging from 10 μg/mL to 0.05 μg/mL after derivatization, and injected onto the GC system. The limit of detection (LoD) was defined as the concentration for which a signal-to-noise ratio of 3 was obtained, and the limit of quantitation (LoQ) was considered to be the concentration at which the signal-to-noise ratio was 10.

2.5.6. *Robustness*

For robustness evaluation, a Plackett–Burman (fractional factorial) experimental design consisting of 11 runs, including three center points, was used to investigate four factors: injector temperature (°C), final column temperature (°C), temperature gradient (°C/min), and pressure (kPa) (Modde version 8; Umetrics Inc., San Jose, CA, USA). Two test solutions [lumefantrine reference standard solution at 100% lc and a solution containing a mixture of lumefantrine (at 100% lc) and its related impurities (at 1% lc each)] were prepared and analyzed using different experimental conditions by varying the different analytical parameters: injection temperature (290°C, 300°C, and 310°C), final column temperature (320°C, 325°C, and 330°C), temperature gradient (8°C/min, 10°C/min, and 12°C/min), and pressure (102 kPa, 107 kPa, and 112 kPa). Chromatographic resolution (between lumefantrine peak and two related impurities, *N*-oxide lumefantrine and USP-MC impurity A) (R_s), retention time (RT), peak asymmetry (A_s), peak area of lumefantrine, and LoD for the two lumefantrine impurities (*N*-oxide lumefantrine and USP-MC impurity A) were evaluated under each condition.

3. Results and discussion

3.1. *Method of development*

The analytical target profile was to develop a stability-indicating quantitative assay for lumefantrine in FPPs that can be used in poor resource economies. The GC-FID methodology is thus an appropriate technique. The quality target method profile includes that the method should be International Conference on Harmonization validatable within a good manufacturing practices (GMP) environment of a Quality Control laboratory, including compliance with general pharmacopeial chromatographic requirements such as minimal resolution and maximal asymmetry.

As lumefantrine has relatively high melting (128–132°C) and boiling (642.5°C) points at 760 mmHg [23,24], and a free alcohol functional group in its structure (Fig. 1) that affects the inherent volatility of the compound [25], direct GC analysis without derivatization was unsuccessful. Using silylation reactions [26,27], the nonvolatile and unstable (degrading at 200–300°C) lumefantrine molecule could, however, be successfully analyzed with GC. The widely available BSTFA was used as a derivatization reagent in our GC-FID method.

To develop the stability-indicating GC-FID assay for lumefantrine, different chromatographic factors were initially evaluated using a one-factor-at-a-time approach. These factors include injection port (temperatures ranging from 150°C to 400°C were tested) and oven program. In the final method, lumefantrine eluted at an RT of 26.0 minutes. RT and relative response factor, defined as the ratio of the response of the impurity and the API under identical chromatographic conditions [28], values for lumefantrine and its related impurities are presented in Table 1. All the lumefantrine-related impurities (DB, USP-MC impurity A, *N*-oxide-lumefantrine, and DBK) were eluting at different RTs without any interference with the lumefantrine main peak. The run time of analysis was 35 minutes. Moreover, relative response factor values were established to control lumefantrine-related impurities in the absence of reference impurity standards (due to high cost and stability of the standards, and difficulty in the isolation of these standards for usage). A typical chromatogram obtained for a mixture of lumefantrine API and its related impurities is presented in Fig. 2.

3.2. *Validation*

3.2.1. *Linearity*

A linear correlation was found between the peak areas and the concentrations of lumefantrine, in the assayed range

Table 1 – RT and RRF for lumefantrine and its related impurities using GC-FID analytics.

Compound	DB	DBK	*N*-oxide lumefantrine	Lumefantrine	USP-MC impurity A
RT (min)	20.14	20.54	23.78	26.04	26.25
RRF	0.97	0.56	0.57	1.00	0.76

DB = desbenzyl; DBK = desbenzylketo; GC-FID = gas chromatography coupled to flame ionization detector; RRF = relative response factor; RT = retention time.

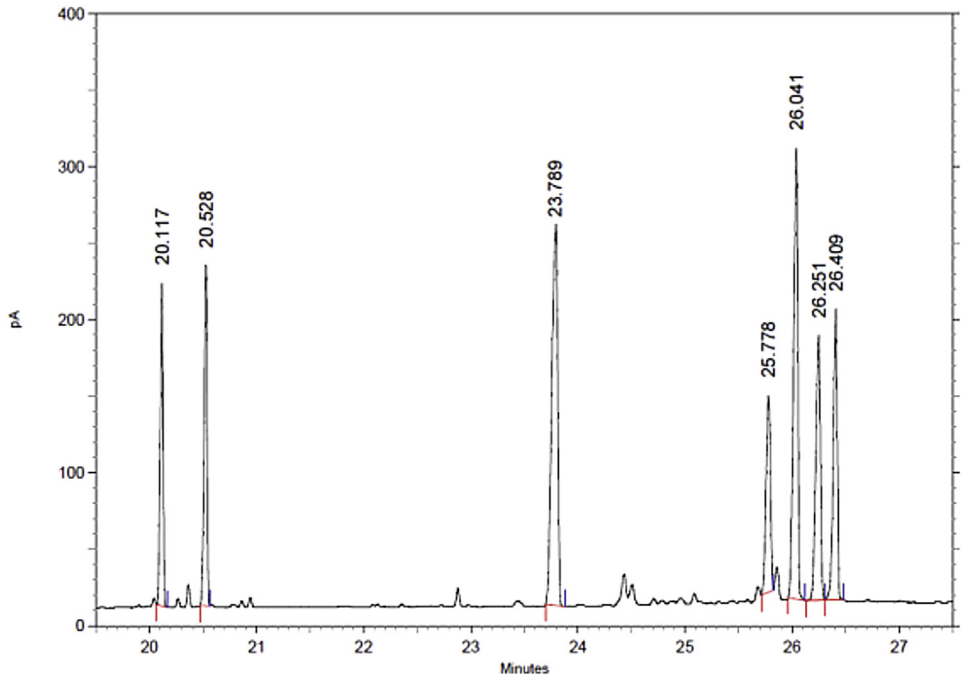

Fig. 2 – GC-FID chromatogram of BSTFA-derivatized mixture of lumefantrine API (RT 26.0 minutes) and its related impurities solution: DB (20.1 minutes), DBK (20.5 minutes), N-oxide lumefantrine (three peaks at RT 23.8 minutes, 25.8 minutes, and 26.4 minutes), and USP-MC impurity A (26.3 minutes). BSTFA = N,O-bis(trimethyl-silyl)trifluoro-acetamide; DB = desbenzyl; DBK = desbenzylketo; GC-FID = gas chromatography coupled to flame ionization detector; RT = retention time.

(80–120% lc). The regression analysis data are presented in Table 2, indicating the linearity of the calibration curve.

3.2.2. *Precision*

The mean content (±standard deviation) of lumefantrine in the intraday precision analysis ($n = 6$) was 96.1% lc ± 0.9% (RSD = 0.9%), while that of the interday precision analysis ($n = 18$) was 96.3% lc ± 0.8% (RSD = 0.9%). The intra- and interday precision %RSD values were lower than 2.0%, demonstrating appropriate precision of the method [29].

3.2.3. *Accuracy (recovery test)*

The recovery test was performed by analyzing a spiked placebo. Lumefantrine mean recovery was 99.5% (RSD = 1.0%), indicating the accuracy of the method.

3.2.4. *Specificity*

The chromatogram obtained for the mixture of lumefantrine API and its related impurities (Fig. 2) showed no related impurity peak interference with the main peak, proving that the method can be used for the quantification of lumefantrine in the presence of its related impurities, including its possible degradation products. Moreover, in the analyzed placebo samples, we did not observe any interfering peak from the excipients with the main peak.

Table 2 – Calibration curve for lumefantrine.

Regression parameters	Lumefantrine
Regression coefficient, R^2	0.9986
Slope ± standard error	13,085.77 ± 279.09
Intercept ± standard error	−735,783.40 ± 140,932.86
Relative standard error (%)	2.13
Concentration range (μg/mL)	400–600
F value	2198.44
Number of points	5

3.2.5. *Robustness of derivatization procedure*

The optimum derivatization conditions were set up to BSTFA solution in airtight glass vials at 70°C for 30 minutes in an oven. A composite face-centered design was applied to optimize the sample derivatization process.

Two factors, incubation time (from 20 minutes to 40 minutes) and incubation temperature (from 60°C to 80°C), that affect the yield of derivatization were considered. The factor levels are indicated in the supplementary information. Maximization of peak area of the derivatized analyte was the target of the optimization process. The coefficient plot for peak area (presented in the supplementary information) displays the regression coefficients with the 95% confidence interval (CI) for the linear and quadratic effects of incubation time (Time) and incubation temperature (Temp), and the product of the time and temperature. None of the regression coefficients differed significantly from zero. Therefore, the effect of both variables and their product on peak area is considered not significant at 95% CI. Optimal and most robust conditions were assigned to the midpoints (0 level), i.e., incubation temperature of 70°C and incubation time of 30 minutes.

Moreover, no other peak was observed above the reporting threshold of 0.1%, indicating that the derivatization mixture

was stable and pure within the method operable design region, defined as 70 ± 10°C and 30 ± 10 minutes.

3.2.6. *Robustness of chromatography*

The Plackett–Burman design, a two-level fractional factorial design, was used to test the robustness of the chromatographic part of the method. This design is selected for robustness evaluation since it combines less experimentation with maximal information acquisition in the most efficient way.

Four factors, with deliberate small deviations from the method settings, were considered: injection temperature (from 290°C to 310°C), final column temperature (from 320°C to 330°C), temperature gradient (from 8°C/min to 12°C/min), and pressure (from 102 kPa to 112 kPa). The results of this design are given in the supplementary information.

The contour plots of these chromatographic factors for lumefantrine peak resolution (R_s) from *N*-oxide lumefantrine are presented in Fig. 3A–F, while R_s from lumefantrine-related compound A is presented in the supplementary information (Fig. S3A–F). All R_s results from both *N*-oxide lumefantrine and

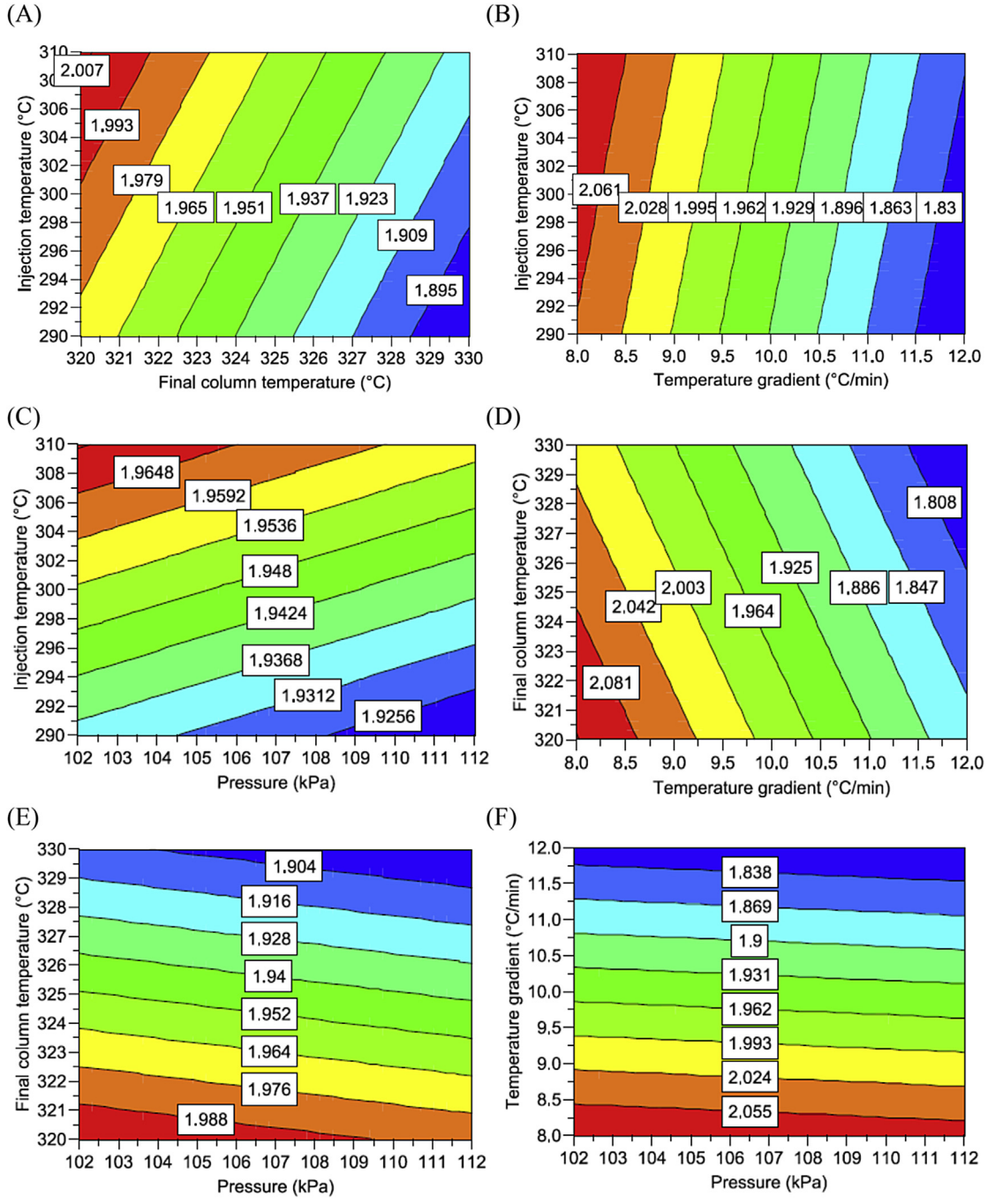

Fig. 3 – Contour plots of the different chromatographic factors for lumefantrine peak resolution (R_s) from *N*-oxide lumefantrine.

lumefantrine-related compound A were >1.5, revealing that the small deviations introduced in the four method parameters did not have a significant effect on the minimal R_s specification set in European Pharmacopoeia (Ph. Eur.)., which was defined as a critical method attribute [30,31]. The RT for DBK was 20.54 minutes, while that of lumefantrine main peak was 26.04 minutes, indicating a clear and noncritical separation of these two peaks.

The injection temperature, final column temperature, and pressure did not have a statistically significant effect on the RT of lumefantrine at 95% CI, although the RT was increasing from 24.6 minutes to 27.0 minutes with the decrease in the final column temperature from the (+) level (330°C) to the (−) level (320°C), and the same was true for pressure. An increase in temperature gradient from 8°C/min to 12°C/min led to the decrease in RT from 31.3 minutes to 22.6 minutes.

The effect of the deviations of the four chromatographic parameters from the method setting on peak area, peak asymmetry (A_s), and LoD was also evaluated (see supplementary information), and the effect of these four parameters was not significant at 95% CI. Moreover, all the results of the peak asymmetry A_s comply with the set specification in Ph. Eur. [30].

Therefore, the deviations from the target method setting for the four parameters, injection temperature, final column temperature, temperature gradient, and pressure, did not affect the chromatographic parameter specifications, revealing the robustness of the developed GC method.

3.2.7. *LoD and LoQ*

LoD and LoQ of lumefantrine were estimated based on the signal-to-noise ratio. According to the determined signal-to-noise ratio, the LoD and LoQ for lumefantrine were calculated to be 0.01 μg/mL and 0.04 μg/mL, respectively, indicating the sensitivity of the method.

Moreover, the new GC-FID method for the determination of lumefantrine in FDC products has more sensitivity (lower LoD and LoQ values) than the RP-C18 HPLC (LoD: 0.02 μg/mL and LoQ: 0.05 μg/mL) and the fused-core HPLC (LoD: 0.10 μg/mL and LoQ: 0.40 μg/mL) methods described in the literature [12,16].

3.3. *Analysis of marketed FDC products*

Table 3 gives the assay results of marketed samples obtained in Ethiopia. The lumefantrine content varied from 96.2% to 98.3% lc, within the 90–110% lc specifications [23]. The results were found to be comparable to the assay results obtained on the same samples using fused-core HPLC method, 97.9–101.5% lc [16].

Table 3 – Contents of lumefantrine in FDC products ($n = 6$ for each).

FDC samples	Batch/ lot no.	Lumefantrine mean content ±SD
Artemine tablets	7711	96.5 ± 0.4
	7976	96.6 ± 1.0
Coartem tablets	F2010	96.2 ± 0.6
	F2006	96.4 ± 0.9
Co-Artesiane powder for oral suspension	20460	98.3 ± 0.7

FDC = fixed-dose combination; SD = standard deviation.

4. Conclusion

A GC-FID method for lumefantrine assay in pharmaceutical preparations was developed and validated within an analytical quality-by-design approach. The method is linear, precise, and sensitive. It makes use of simple sample preparation procedures and is not solvent consuming. The RT of lumefantrine was 26.0 minutes, and there was no interference from its related synthesis and degradation impurities and excipients. The developed method was successfully applied to analyze lumefantrine content in different marketed antimalarial FPPs and can thus be applied to routine quality control of lumefantrine in pharmaceutical preparations.

Conflicts of interest

All the authors declare that there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jfda.2015.03.004

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