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Short communication

Comparison of HPLC, UV spectrophotometry and potentiometric titration methods for the determination of lumefantrine in pharmaceutical products

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ABSTRACT

This paper describes the development and evaluation of a HPLC, UV spectrophotometry and potentiometric titration methods to quantify lumefantrine in raw materials and tablets. HPLC analyses were carried out using a Symmetry C_{18} column and a mobile phase composed of methanol and 0.05% trifluoroacetic acid (80:20), with a flow rate of 1.0 ml/min and UV detection at 335 nm. For the spectrophotometric analyses, methanol was used as solvent and the wavelength of 335 nm was selected for the detection. Non-aqueous titration of lumefantrine was carried out using perchloric acid as titrant and glacial acetic acid/acetic anhydride as solvent. The end point was potentiometrically determined. The three evaluated methods showed to be adequate to quantify lumefantrine in raw materials, while HPLC and UV methods presented the most reliable results for the analyses of tablets.

1. Introduction

The incidence of malaria worldwide is estimated to be 300–500 million clinical cases each year, with about 90% of these occurring in Africa, mostly caused by *P. falciparum*. One of the greatest difficulties facing malaria control worldwide is the spread and intensification of parasite resistance to antimalarial drugs. The limited number of such drugs has lead to increasing difficulties in the development of antimalarial drug policies and adequate disease management [1]. The search for new antimalarial drugs involving novel molecular targets and treatment regimens, which should provide both high efficacy and safety for use, has become an urgent subject of study [2].

Lumefantrine (previously called benflumetol) was synthesized originally in the 1970s by the Academy of Military Medical Sciences in Beijing, China [3]. It is a racemic fluorene derivative, named 2-dibutylamino-1-[2,7-dichloro-9-(4-chlorobenzylidene)-9H-fluoren-4-yl]-ethanol (Fig. 1). It conforms structurally, physicochemically and in mode of action to the aryl amino alcohol group of antimalarial agents including quinine, mefloquine and halofantrine [4].

Nowadays, lumefantrine is commercially available in a coformulated product with artemether, in a fixed dose combination (artemether 20 mg + lumefantrine 120 mg) [5]. Many studies have proved that this association is highly effective in the treatment of several types of malaria, mainly multi-drug-resistant *falciparum* malaria, resulting in high cure rates and stronger prevention against reinfection [6–8]. This drug association is the first line therapy for uncomplicated malaria recommended by World Health Organization (WHO), mainly in countries where malaria is resistant to conventional treatments [9].

Previous studies have reported the determination of lumefantrine, all of them employing high performance liquid chromatography with UV detection at 335 nm and focusing mainly in its quantitation in plasma or blood [3,10–12]. However, methods for lumefantrine determination in pharmaceutical products, such as raw material and tablets, have not been reported to date, and lumefantrine monographs are not available in official pharmacopoeias. The increasing utilization of this antimalarial drug as first line treatment for malaria in many countries demands the development of new and alternative methods to successfully determine lumefantrine in raw material and pharmaceutical dosage forms.

The purpose of this study was to develop and validate analytical methods to quantify lumefantrine in raw materials and tablets, using HPLC, UV spectrophotometry and potentiometric titration. The results obtained by these methods were statistically compared, using analysis of variance (ANOVA). In addition, the reliability and feasibility of them were evaluated, focusing on routine quality control analysis.

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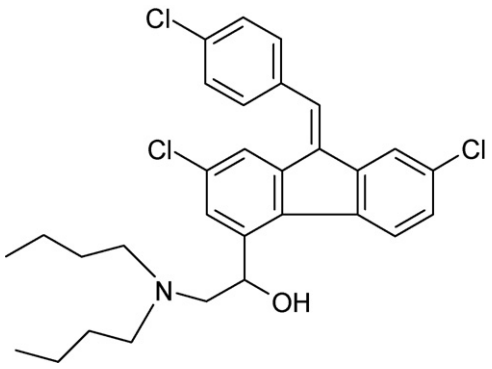

Fig. 1. Chemical structure of lumefantrine.

2. Experimental

2.1. Reagents and materials

Lumefantrine reference standard and raw material were purchased from Dafra Pharma (Turnhout, Belgium). Coartem® tablets (artemether 20 mg + lumefantrine 120 mg) (Novartis, Basel, Switzerland) were kindly donated by Brazilian Health Ministry. Water was purified using a Millipore system (Bedford, MA, USA). Methanol, trifluoroacetic acid, dichloromethane (HPLC grade) and perchloric acid, glacial acetic acid and acetic anhydride (analytical grade) were obtained from Tedia (Fairfield, OH, USA).

2.2. Instruments and analytical conditions

The HPLC analyses were carried out on an Agilent 1200 system (Palo Alto, CA, USA), composed of a quaternary pump, autosampler, diode array detector (DAD) and HP ChemStation software. The column used was a Symmetry C_{18} (250 mm × 4.6 mm i.d.; 5 μm particle size) from Waters (Milford, MA, USA), maintained at 30 °C. DAD detection was performed at 335 nm and UV spectra from 200 to 400 nm were recorded on-line for peak identification. The mobile phase consisted of methanol and 0.05% trifluoroacetic acid (80:20), at a flow rate of 1.0 ml/min. The injection volume was 20 μl.

Ultraviolet spectrophotometric analyses were carried out on a Shimadzu UV 160A (Shimadzu, Kyoto, Japan) spectrophotometer, in a 1 cm quartz cuvette. The wavelength of 335 nm was selected for the quantitation of lumefantrine and the measurements were obtained against methanol as a blank.

Lumefantrine non-aqueous titration was carried out on a Titrator DL53 (Mettler Toledo, Columbus, OH, USA), equipped with a combination glass electrode DG113 (Mettler Toledo, Columbus, OH, USA), containing 1 M LiCl in ethanol as the inner solution. 0.1 M perchloric acid volumetric solution was prepared in glacial acetic acid/acetic anhydride, and standardized with potassium biphthalate, previously dried at 120 °C for 2 h. The end point was determined potentiometrically.

2.3. Preparation of standard and sample solutions

Lumefantrine standard solution: Approximately 15 mg of lumefantrine reference standard were accurately weighed and transferred to a 50 ml volumetric flask. Dichloromethane (1 ml) was added to ensure complete solubilization and the solution was diluted to volume with methanol. An aliquot of 4 ml of the obtained solution was transferred to a 50 ml volumetric flask. The volume was adjusted with either methanol for spectrophotometric analysis or mobile phase for chromatographic analysis, resulting in solutions of 24 μg/ml.

Lumefantrine sample solutions: Approximately 30 mg of lumefantrine raw material, or a portion of the powdered tablets equivalent to about 30 mg of lumefantrine, were accurately weighed and transferred to a 100 ml volumetric flask. Dichloromethane (2 ml) was added and the solution was diluted to volume with methanol. An aliquot of 4 ml of this solution was transferred to a 50 ml volumetric flask. The volume was adjusted with either methanol for spectrophotometric analysis or mobile phase for chromatographic analysis, to obtain a solution at 24 μg/ml of lumefantrine.

2.4. Validation

The optimized spectrophotometric and chromatographic methods were completely validated according to the procedures described in ICH guidelines Q2(R1) for the validation of analytical methods [13].

Linearity: Standard solutions containing 300 μg/ml of lumefantrine in methanol were prepared, in triplicate. Aliquots of these solutions were diluted in mobile phase (for HPLC analysis) or methanol (for UV analysis), to five different concentrations, corresponding to 12, 18, 24, 30 and 36 μg/ml of lumefantrine. Calibration curves with concentration versus peak area or absorbance were plotted for each method and the obtained data were subjected to regression analysis using the least squares method.

Precision: The intra-day precision was evaluated by analyzing six tablet samples ($n = 6$), at 100% of the test concentration (24 μg/ml), using the UV and the HPLC methods. Similarly, the inter-day precision was evaluated in three consecutive days ($n = 18$). Lumefantrine contents and the relative standard deviations (R.S.D.) were calculated.

Accuracy: Lumefantrine reference standard was accurately weighed and added to a mixture of the tablet excipients, at three different concentrations (18, 24 and 30 μg/ml). At each concentration, samples were prepared in triplicate and the recovery percentage was determined by UV and HPLC methods.

Specificity: A solution containing a mixture of the tablet excipients was prepared using the sample preparation procedure and injected onto the chromatograph, to evaluate possible interfering peaks. For the spectrophotometric analysis, the UV spectrum of this solution was recorded in the range of 200–400 nm, to evaluate the presence of possible interfering bands at 335 nm. In addition, spectral purities of lumefantrine peaks in chromatograms obtained with sample solutions were evaluated using the UV spectra recorded by the diode array detector.

Detection and quantitation limits: Diluted lumefantrine standard solutions were prepared and analyzed by both chromatographic and spectrophotometric methods, at decreasing concentrations, in the range of 0.01–0.40 μg/ml. For the HPLC method, the limit of detection (LOD) was defined as the concentration for which a signal-to-noise ratio of 3 was obtained and, for the limit of quantitation (LOQ), a signal-to-noise ratio of 10 was considered. To determine the limits of detection and quantitation in the UV method, the absorbance values, UV spectra and relative standard deviation of the measured values of the diluted solutions were evaluated.

2.5. Analysis of lumefantrine raw material and tablets

Samples of Coartem® tablets and lumefantrine raw material, employed for manufacturing pharmaceutical formulations, were analyzed by the validated HPLC and UV methods and also by potentiometric titration. Before the analysis, the tablets were weighed and finely powdered. The sample solutions for the HPLC and UV analyses were prepared as described in Section 2.3. For the

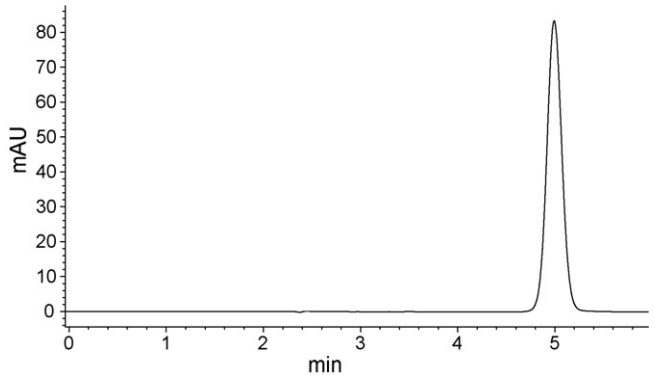

Fig. 2. Chromatogram obtained for a lumefantrine sample solution at 24 μg/ml, using Symmetry C_{18} column (250 mm × 4.6 mm i.d.; 5 μm particle size) at 30 °C and mobile phase composed of methanol and 0.05% trifluoroacetic acid (80:20), at a flow rate of 1.0 ml/min. Detection was performed at 335 nm.

potentiometric titration, 200 mg of lumefantrine were accurately weighed in a titration vessel, followed by the addition of 10 ml of acetic anhydride and 40 ml of glacial acetic acid. Samples were submitted to mechanical agitation until complete solubilization, before automatic titration.

The lumefantrine contents were determined using the three methods and the obtained results were statistically compared using ANOVA test and Tukey's multiple comparison test, applied at 0.05 significance level.

3. Results and discussion

During the chromatographic method development, methanol showed to be a more adequate organic solvent than acetonitrile, regarding the lumefantrine retention. Acidification of the mobile phase with trifluoroacetic acid was necessary in order to assure that lumefantrine was completely in the ionized form and thus obtaining a satisfactory peak shape. Hence, using a mobile phase pH of 2.8 and a C_{18} column with about 19% hydrocarbon load, an adequate peak symmetry (tailing factor = 1.04) and short run time (6 min) were achieved, as demonstrated in the chromatogram of Fig. 2.

After the evaluation of the lumefantrine UV spectrum, in the range of 200–400 nm (Fig. 3), the wavelength of 335 nm was selected for detection, due to the adequate molar absorptivity of lumefantrine in this region and the higher selectivity of this wavelength regarding possible interfering compounds or solvents in the samples.

The non-aqueous potentiometric titration of lumefantrine showed a well-defined inflexion on the titration curve, precisely indicating the end point. The potentiometric determination was less subjective and more precise than the use of visual indicators, therefore this method was chosen for the analyses.

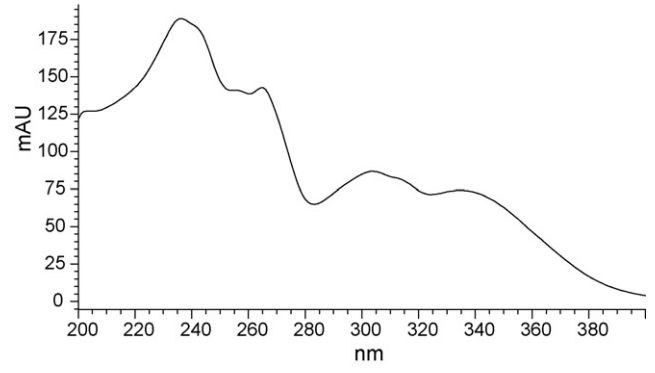

Fig. 3. UV spectrum of lumefantrine sample solution at 24 μg/ml, in methanol.

Table 1
Overview of the linearity data obtained for lumefantrine by the chromatographic and spectrophotometric methods

Regression parameters	HPLC	UV
Regression coefficient (r^2)	0.9999	0.9999
Slope ± standard error	34.06 ± 0.10	0.0291 ± 0.0001
Intercept ± standard error	1.70 ± 2.60	−0.0008 ± 0.0019
Relative standard error (%)	0.40	0.35
Concentration range (μg/ml)	12–36	12–36
Number of points	5	5

3.1. Validation

A linear relationship was found between the lumefantrine concentrations and the response of both HPLC and UV methods. The regression analysis data are presented in Table 1. High regression coefficients (r^2) values were obtained (0.9999 for both methods). A random pattern of the regression residues was found and no significant deviation of linearity was detected in the assayed range.

The precision data obtained for the evaluated methods are demonstrated in Table 2. All methods presented R.S.D. values lower than 2.0%, assuring a good precision. Potentiometric titration presented more precise results, compared to the instrumental methods.

Accuracy was investigated by means of a standard addition experiment. Both chromatographic and spectrophotometric methods exhibited mean recoveries (n = 9) close to 100% (Table 2), demonstrating an adequate accuracy.

In the evaluation of the specificity of the HPLC method, peak purities higher than 99.0% were obtained for lumefantrine in the chromatograms of sample solutions, demonstrating that other compounds did not co-elute with the main peak. The chromatogram obtained with the mixture of the tablet excipients showed no interfering peaks in the same retention time of lumefantrine. For the UV method, no absorption band was found at 335 nm, in the spectrum obtained with a mixture of the tablet excipients in methanol. Therefore, the method showed to be selective to quantify lumefantrine at this wavelength.

Considering the signal-to-noise ratio of 3 and 10, the LOD and LOQ obtained for the chromatographic method were 0.02 and 0.05 μg/ml, respectively. In the spectrophotometric analysis, at a concentration of 0.10 μg/ml of lumefantrine, it was possible to identify the absorption band at 335 nm and the corresponding absorbance value was 0.004. Hence, this concentration was settled as the detection limit. The quantitation limit of the UV method was 0.30 μg/ml, defined as the lower concentration that provided an adequate precision (R.S.D. < 2.0%) and absorbance value (0.010). According to the obtained results (Table 2), HPLC proved to be a more sensitive method, allowing the quantitation of lumefantrine in concentrations around five times lower than the UV method.

Table 2
Validation parameters of the evaluated methods for lumefantrine determination

Validation parameters	HPLC	UV	Potentiometric titration
Intra-day precision, n = 6 (R.S.D., %)	0.45	0.65	0.29
Inter-day precision, n = 18 (R.S.D., %)	0.67	0.62	0.46
Accuracy, n = 9 (mean recovery, %)	99.42	98.81	–
LOD (μg/ml)	0.02	0.10	–
LOQ (μg/ml)	0.05	0.30	–

Table 3
Lumefantrine contents in raw material and tablet samples obtained by HPLC, UV and potentiometric titration ($n = 12$)

Samples	Lumefantrine content (%) ± S.D.		
	HPLC	UV	Potentiometric titration
Raw material	99.47 ± 0.60	99.05 ± 0.82	99.82 ± 0.27
Tablet	97.94 ± 0.67	98.03 ± 0.73	101.41 ± 0.63

S.D.: standard deviation.

3.2. Analysis of lumefantrine raw material and tablets

The validated chromatographic and spectrophotometric methods and the potentiometric titration were applied to the analysis of lumefantrine in raw material and Coartem® tablets (Table 3). ANOVA test revealed statistically significant difference between the results obtained for both raw material and tablets samples, from the distinct methods, at a confidence level of 0.05. Turkey's multiple comparison test demonstrated that, for the raw material analysis, the means obtained by HPLC and UV, as well as those obtained by HPLC and titration, were statistically equivalents ($p > 0.05$); however, there was a significant difference between the values obtained by UV and titration ($p < 0.05$). For the analysis of the tablets, Turkey's test revealed statistic equivalence between HPLC and UV means ($p > 0.05$) and showed that the results of the titration method were statistically different from those obtained by HPLC and UV ($p < 0.05$).

A higher mean lumefantrine content was found when potentiometric titration was applied to the tablet analyses. The presence of excipients with basic characteristic in the tablet matrix, such as magnesium stearate, might interfere in the titration process, leading to higher values. Hence, potentiometric titration with perchloric acid did not show to be an adequate method to quantify lumefantrine in tablets.

Regarding the raw material analyses, potential interferences were not identified in any of the evaluated methods, although non-aqueous titration and spectrophotometric analysis could quantify degradation products or related substances that present similar chemical structure [14]. Chromatographic analysis showed to be the most sensitive and selective method, and might be applied successfully for lumefantrine trace analysis and quantitation in biologic matrices. We cannot discharge, however, the analyses time and cost. The spectrophotometric method and non-aqueous titration are clearly less expensive and require shorter analysis time, besides the easily of handling.

Since the use of lumefantrine as a potent antimalarial drug is widespread around the malaria endemic areas, the development and validation of simple and reliable methods are essential to assure the quality of the raw materials and pharmaceutical formulations marketed nowadays. The problem of counterfeit or substandard antimalarials is well established all over the world [15] and simple methods to identify and precisely quantify these drugs may be an important tool to avoid treatment inefficacy and development of resistance due to the exposition to subtherapeutic doses.

4. Conclusion

HPLC, UV spectrophotometry and potentiometric titration showed to be adequate methods to quantify lumefantrine in raw materials, while for the analyses of the tablets, the chromatographic and spectrophotometric methods presented the most reliable results. Since these methods are rapid and simple, they may be successfully applied to quality control analyses, with the aim of quantifying and identifying lumefantrine in pharmaceutical products.

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