Indian Journal of Advances in Chemical Science

Article

Validated Spectrophotometric Method for the Determination of Lumefantrine in Pharmaceutical Formulation by Charge-Transfer Complexation with Picric Acid

Ukpe Ajima[1]*, Johnson O. Onah[1], Bilma W. Obidah[1], Paul N. Olotu[2]

[1]Department of Pharmaceutical Chemistry, University of Jos, PMB 2084, Jos, Nigeria, [2]Department of Pharmacognosy and Traditional Medicine, University of Jos, PMB 2084, Jos, Nigeria

ABSTRACT

Lumefantrine is an aryl amino alcohol derivative widely used in combination with artemether for the therapy of malaria. A simple and accurate spectrophotometric method has been developed and validated for the quantitative determination of lumefantrine in finished pharmaceutical products. The proposed method is based on the charge-transfer (CT) reaction of lumefantrine (as n-electron donor) with picric acid (2, 4, 6-trinitrophenol) as π-acceptor to give a highly colored CT complex. The different variables affecting the reaction such as reagent concentration, dilution solvent, and reaction time were carefully optimized to achieve the highest sensitivity. The lumefantrine-picric acid CT complex was quantified spectrophotometrically at 420 nm, and Beer's law was obeyed over the concentration range of 20–100 µg/mL with correlation coefficient (r^2) of 0.9981. The limit of detection and quantification were found to be 0.884 µg/mL and 1.507 µg/mL, respectively, for the method. The proposed method was also found to give comparable results with the official method as no statistically significant differences were found in the percentage drug content for five brands assayed by both methods. The developed method can, therefore, be successfully applied to analyze lumefantrine content in both raw materials and finished pharmaceutical products.

Key words: Lumefantrine, Spectrophotometry, Picric acid, Charge transfer complex, Method development.

1. INTRODUCTION

Malaria is a parasitic disease caused by protozoa belonging to the Plasmodium species and is a leading cause of morbidity and mortality globally. The latest WHO estimates show that there were approximately 219 million cases of malaria in 2017 and an estimated 435,000 deaths [1]. The Plasmodium parasite has developed resistance to almost all of the previously effective medications such as chloroquine, sulfadoxine and this led the WHO to recommend the use of artemisinin-based combination treatments as first-line therapy for malaria [2]. Aside from the emergence of resistance, another major challenge facing malaria control programs, especially in developing countries is the high prevalence of substandard and counterfeit drugs. A recent study reported that as much as 40% of the artemisinin-containing antimalarial medicines circulating in some Asian and sub-Saharan African countries are counterfeit [3]. This has been linked to high demand for these drugs which provides an economic incentive for unscrupulous individuals involved in counterfeiting and adulterating these essential drugs. There is, therefore, the need to continuously monitor the quality of the antimalarial drugs available in the market.

Lumefantrine (or benflumetol) is an aryl amino alcohol class antimalarial drug widely used in endemic areas for the treatment of malaria [4]. It is commercially available as a fixed-dose combination with artemether [5] and has been shown to be highly effective in the treatment of resistant malaria. Chemically, lumefantrine is a racemic fluorene derivative with the chemical name (1RS)-2-(dibutylamino)-1-{(9Z)-2,7-dichloro-9-[(4-chlorophenyl)methylidene]-9H-fluoren-4-yl}ethanol. Both the dextrorotatory and laevorotatory forms have been demonstrated to have similar antimalarial action against the Plasmodium parasite. The drug is highly plasma protein bound (≈99%) [6] and its mechanism of antimalarial action is still a subject of investigation [7].

Lumefantrine monograph is official in USP Salmous [8] and also in the International Pharmacopoeia [9] which specifies a non-aqueous titrimetric method for the assay of the drug. A review of existing literature reveals that several unofficial assay methods have been developed and deployed for the determination of the drug. Some of these include ultraviolet (UV) spectrophotometry [10-15]. Another group [16] developed a colorimetric assay method in which a charge transfer (CT) complex was formed between lumefantrine and chloranilic acid with measurements carried out at 520 nm. High-performance liquid chromatography (HPLC)-UV methods have also been developed for the drug [17-24]. Several reverse phase-HPLC methods have also been used for the simultaneous estimation of lumefantrine and artemether [25-29]. Several liquid chromatographic tandem-mass spectrometry methods have also been developed as alternatives to HPLC-UV for the determination of lumefantrine [30-37]. Other methods that have been used for lumefantrine assay include: High-performance thin-layer chromatography [38], potentiometry [39], microemulsion electrokinetic chromatography [40], and gas chromatography [41].

***Corresponding author:**
E-mail: descar84@yahoo.com

ISSN NO: 2320-0898 (p); 2320-0928 (e)
DOI: 10.22607/IJACS.2019.702002

Received: 12th January, 2019;
Revised: 30th March, 2019;
Accepted: 17th April, 2019

The aforementioned methods each have their unique strengths and advantages, but many of them are generally complex in nature and may require the use of expensive instruments.

The ever increasing use of lumefantrine (in combination with artemether) as first-line treatment for malaria in malaria-endemic regions demands the development of new and alternative methods which are simple and easy to carry out to enable the successful assay of the drug. The aim of the present work is, therefore, to develop a validated, rapid, reliable, and sensitive analytical method for the quantification of lumefantrine in pure form and also in the combined pharmaceutical dosage form.

2. MATERIALS AND METHODS

2.1. Materials

Analytically pure drug sample of lumefantrine was obtained from Emzor Pharmaceuticals, Lagos, Nigeria. Five different brands of marketed tablet formulation (Artemether 80 mg and lumefantrine 480 mg) were procured from local pharmacy stores in Jos, Nigeria. All solvents and chemicals were of analytical grade and used without further purification.

2.2. Instruments and Software

Lumefantrine quantification was performed using double beam UV–visible spectrophotometer (Shimadzu Corporation, model UV 1650) having two matched quartz cells with 1 cm optical pathlength in the wavelength range from 200 to 800 nm. Other equipment used included electronic analytical balance (Mettler Toledo, UK). UV Probe Software version 2.35 (Shimadzu 1650, Japan) was used for method development and validation of the spectrophotometric method. The rest of the calculations were performed with the help of Microsoft Excel 2010 software (Microsoft Corporation, USA).

2.3. Preparation of Lumefantrine Standard Solution

An accurately weighed 100 mg of standard lumefantrine was transferred into a 100 mL volumetric flask, dissolved in 25 mL dimethylformamide, and the volume was made up to mark with dimethylformamide to obtain a 1000 µg/mL stock solution. This stock solution was subsequently diluted to obtain a working standard solution and was used for optimization experiments.

2.4. Preparation of Picric Acid Solution

A 100 mg of the crystals of picric acid was weighed accurately and dissolved in dimethylformamide which was made up to the 100 mL marks of the volumetric flask to give a concentration of 100 mg/100 mL (0.1% w/v). This solution was further diluted to give a concentration of 0.01% w/v.

2.5. General Procedure

Different aliquots (1.0, 2.0, 3.0, 4.0, and 5.0 mL) of the standard lumefantrine solution (200 µg/mL) in dimethylformamide were transferred to a series of pre-calibrated 10 mL-volumetric flasks followed by addition of 2.0 mL of picric acid solution (0.01% w/v) to each of the flasks in order. The flasks were shaken and the contents allowed to react for 10 min before their volumes were made up to 10 mL with dimethylformamide to give final drug concentration in the range of 20–100 µg/mL. The above solutions were all prepared in triplicates. The absorbance of each solution was measured at 420 nm against a reagent blank.

2.6. Optimization of Reaction Conditions

The optimal conditions for the CT reaction between lumefantrine and picric acid were determined using the single factor test method in which a single analytical condition is varied while keeping others constant. The conditions studied include investigating the effect of varying concentration of picric acid, solvent polarity, and reaction time.

2.7. Stability of the CT Complex

The stability of the complex was monitored at 420 nm over a 24 h period.

2.8. Stoichiometry of the Reaction

To establish the mole ratio of the reaction between lumefantrine and picric acid, Job's method of continuous variation of equimolar solutions was employed. The solutions equivalent to 4.365×10^{-5} M were prepared by dissolving the calculated amounts of lumefantrine in DMF. A solution of picric acid of similar concentration (4.365×10^{-5} M) was also prepared in DMF. A series of solutions were then prepared in which the total volume of lumefantrine and picric acid was maintained at 5 mL. The drug and the reagent were mixed in various proportions (0:5, 1:4, 2:3, 3:2, 4:1, and 5:0), and the absorbance of the resultant charge-transfer complex was measured at 420 nm. The absorbance was then plotted against the mole fraction of the drug ([drug]/([drug] + [reagent]).

2.9. Method Validation

The method was validated with respect to linearity, sensitivity, precision, and accuracy in accordance with ICH Q2 (R1) guidelines [42].

2.9.1. Linearity

The linear relationship between concentration and absorbance for the drug was evaluated over the concentration range of 20–100 µg/mL. The linearity range for lumefantrine was replicated 5 times.

2.9.2. Sensitivity

The sensitivity of the method was measured in terms of limit of detection (LOD) and limit of quantification (LOQ). LOD and LOQ of the developed method were calculated from the standard deviation of the response (σ) and slope of the calibration curve (S) of each drug using the formula, limit of detection =3.3*σ/S; Limit of quantitation=10*σ/S.

2.9.3. Precision

The precision of the developed method was evaluated by performing intraday and inter-day precision studies. Intraday precision was carried out by performing three replicates (at morning, afternoon, and evening) at three different concentrations (50, 100, and 150 µg/mL for lumefantrine) on the same day, and percent relative standard deviation (%RSD) was calculated. Interday precision study was assessed by analysis of the mentioned concentrations of the drug on three different days in triplicate, and % RSD was calculated.

2.9.4. Accuracy

To ascertain the accuracy of the proposed method, recovery studies were carried out using a standard addition method by adding a known amount of standard (5, 10, and 15 µg/mL) to the marketed tablet (10 µg/mL) at 50, 100, and 150% level. The mean percentage recovery was calculated. Recovery studies were performed in triplicate.

2.10. Analysis of Marketed Formulation

For the analysis of lumefantrine in the tablets, an amount of powdered drug equivalent to 0.10 g for each brand was weighed and dissolved in some DMF in a 100 mL volumetric flask. The solution was sonicated for 30 min, and the volume was later made up to mark with the same solvent. The solution was filtered through Whatman filter paper No. 42. Then, 1 mL filtrate was taken and transferred to another 10 mL volumetric flasks and further processed using the optimized CT complexation procedure as outlined above. The content of lumefantrine in the brands was also analyzed using the official assay method which

is non-aqueous titration with perchloric acid [9]. Sample solutions were prepared and analyzed in triplicate.

2.11. Statistical Analysis

Statistical analysis was performed using the student's t test to compare the assay results for different brands containing lumefantrine using the newly developed method and the standard non-aqueous titrimetric method. $p \leq 0.05$ was considered significant.

3. RESULTS AND DISCUSSION

The interaction between CT reagents and basic drugs in certain solvents leads to the formation of colored products which has been attributed to the dissociation of ion-pair salt into radical anions [43]. Lumefantrine usually shows maximum absorption at 234 nm which is in the UV region. On its reaction with the CT reagent (picric acid), it shows a red (bathochromic) shift. The nitrogen atom present in the tertiary amine of lumefantrine serves as n-electron donor, donating electrons to the picric acid which is a pi-acceptor and this result in the formation of the CT complex. The dissociation of this CT complex leads to the formation of the intensely yellow colored radical ions which gives maximum absorbance at 420 nm. The proposed mechanism for the formation of the CT complex is illustrated in Figure 1. Picric acid has been used for the spectrophotometric determination of drugs containing n-electron donors [44].

3.1. Optimization of Reaction Conditions

3.1.1. Effect of reagent volume

It was found that increasing the reagent concentration increases the absorbance of the colored complex. However, 2 mL of 0.01% picric acid was found to be sufficient for the attainment of maximum and reproducible color intensity (Figure 2).

3.1.2. Effect of solvent

Different solvents such as chloroform, methanol, dichloromethane, acetonitrile, and dimethylformamide were tried for the CT reaction in the present study. Dimethylformamide was found to be best suited as it leads to the formation of stable complex and also gives maximum color intensity (Figure 3); hence, it was also used as the diluting solvent in the study.

3.1.3. Effect of reaction time

The optimum reaction time was determined by following the absorbance of the color developed with respect to different time intervals (Figure 4). In this study, complete color development was attained after 10 min, and the complex remains stable for 10 h at room temperature.

3.2. Stoichiometry of the Reaction

The composition of the complex formed was determined by employing Job's method of continuous variation [45]. A mole ratio of 1:1 was obtained from the reaction of equimolar solutions of lumefantrine and picric acid. The stoichiometric ratio of drug**:** reagent is illustrated in Figure 5.

3.3. Method Validation

3.3.1. Linearity

Using the optimized experimental conditions for the reaction, calibration graphs (Figure 6) were obtained by plotting the measured absorbance versus varying concentration of lumefantrine and linear regression analysis by least squares method was carried out to obtain the values of correlation coefficients. The proposed method obeys Beer's law and linear plots with low intercept, and good correlation coefficients were obtained in the concentration ranges of 20–100 µg/mL. Other statistical parameters, namely, intercept (b), slope (a), molar absorptivity, and Sandell's sensitivity values were calculated as well and are given in Table 1.

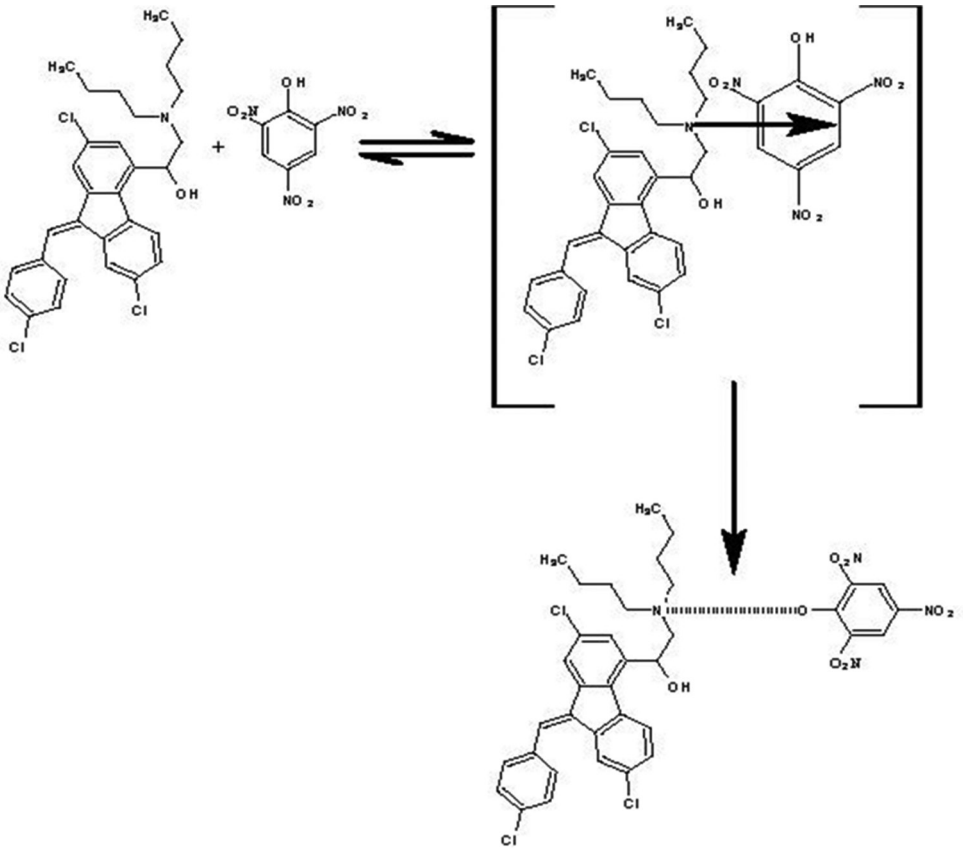

Figure 1: Possible reaction pathway for the formation of charge-transfer complex between lumefantrine and picric acid.

3.3.2. Sensitivity

LOD and LOQ were determined based on the standard deviation of response (intercept) and slope of the calibration curve, according to ICH guideline (ICH, 1996). LOD and LOQ of the developed method were found to be 0.884 and 1.507 µg/mL indicating the sensitivity of the proposed method (Table 1).

3.3.3. Precision

The experiment was repeated 3 times within a day (intraday precision), and the average %RSD values of the results were calculated. Similarly, the experiment was repeated on 3 different days (interday precision) and the average %RSD values were calculated. The interday variation ranged between 0.19 and 1.90% while the intraday variation ranged from 0.29 to 1.88% for the method. The developed method was found to be highly precise as the %RSD values for intraday and interday precision were all <2% [42].

3.3.4. Accuracy

The accuracy of the method was evaluated by applying the standard addition method where good mean recoveries were obtained ranging from 99.96 to 100.49% for the method. %RSD values were found to be <2% confirming the accuracy of the proposed method.

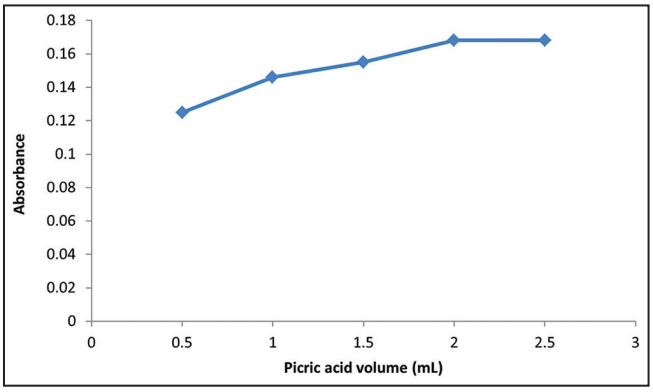

Figure 2: Effect of reagent (picric acid) volume on lumefantrine-picric acid charge transfer complex formation.

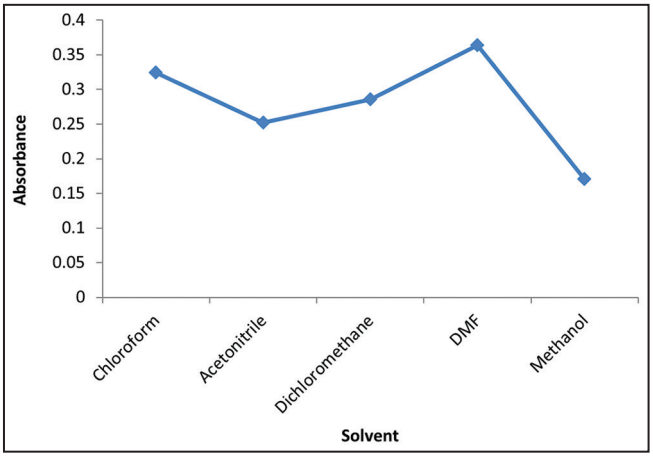

Figure 3: Effect of solvent on lumefantrine picric acid charge transfer complex formation.

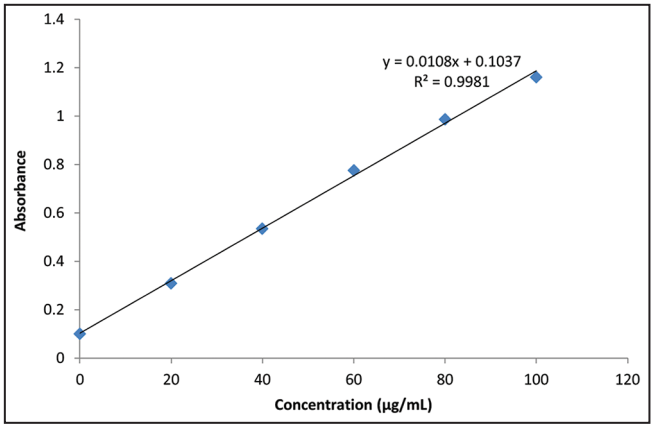

Figure 6: Calibration curve for lumefantrine picric acid charge-transfer complex.

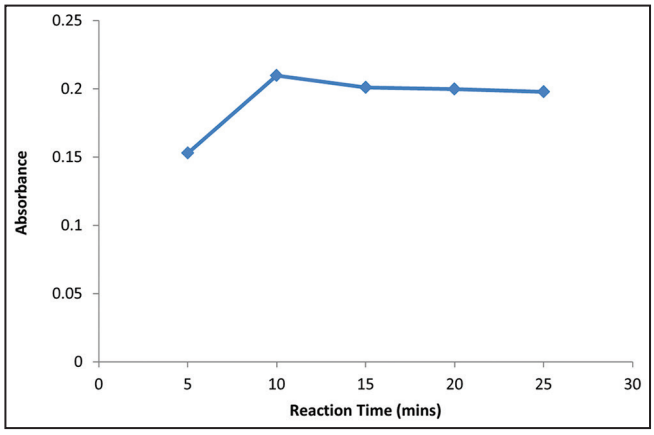

Figure 4: Effect of reaction time on lumefantrine picric acid charge transfer complex formation.

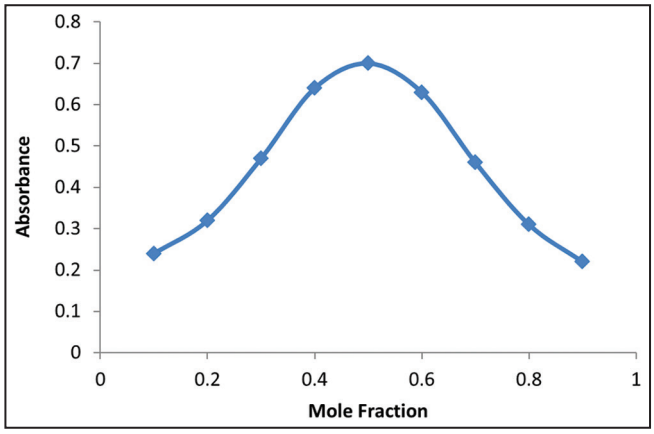

Figure 5: Continuous variation plots for lumefantrine picric acid charge-transfer complex.

Table 1: Summary of analytical parameters for the proposed method.

Parameter	Result
Absorption maximum	420 nm
Regression equation	Y_{abs}=0.0108x+0.1037
Correlation coefficient (r^2)	0.9981
Beers law limit	20–100 µg/mL
Molar absorptivity	8.63×10^4 L/mol/cm
Sandell's sensitivity	1.632 µg/cm^2
Limit of detection	0.884 µg/mL
Limit of quantification	1.507 µg/mL

Table 2: Percentage content (%) of lumefantrine±SD in tablets using proposed method compared to the International Pharmacopoeia method.

Brand	Label claim	Titrimetric method [9]	Developed method
Coartem®	480 mg	102.31±0.88	103.06±0.75
Gvither Plus®	480 mg	100.19±0.84	101.80±0.43
Kart-Lu®	480 mg	98.01±0.84	99.67±0.68
Larict Forte®	480 mg	100.68±1.04	101.90±0.54
Co-mal Forte®	480 mg	99.43±0.92	98.96±0.63

n (number of replicates) = 3, *indicates significant difference at $p<0.05$. SD: Standard deviation

3.4. Method Application in Dosage form Analysis

The proposed method was applied to the determination of lumefantrine in pharmaceutical dosage forms, and the results obtained are given in Table 2. The percentage content in the brands was also determined using the official non-aqueous titrimetric method and the statistical values of the t-test obtained at 95% confidence level, indicate that there were no significant differences found between the drug contents in the brands as assessed by both the new method and the official method. This suggests that the CT complexation method is reliable and accurate and can produce comparable results with the official methods for the assay of the drug. The high percentage recovery values also show that the method has the advantage of being potentially free from interference by excipients indicating that the method can be applied in the routine quality control of lumefantrine in bulk form and for marketed formulations in the combined dosage form.

4. CONCLUSION

A simple and rapid spectrophotometric method for the determination of lumefantrine by CT complexation with picric acid was successfully developed and validated as per the ICH guidelines. The procedure is accurate and precise and could find application in quality control of this essential antimalarial agent.

5. REFERENCES

1. WHO Global Malaria Program, (2018) ***World Malaria Report***, Available from: http://www.who.int. [Last accessed on 2018 Apr 12].
2. E. A. Ashley, N. J. White, (2005) Artemisinin-based combinations, ***Current Opinion in Infectious Diseases,* 18:** 531-536.
3. G. Nayyar, A. Attaran, J. P. Clark, M. J. Culzoni, F. M. Fernandez, J. E. Herrington, M. Kendall, P. N. Newton, J. G. Breman, (2015) Responding to the pandemic of falsified medicines, ***The American Journal of Tropical Medicine and Hygiene,* 92(6)**: 113-118.
4. C. Sisowath, J. Stromberg, A. Martensson, M. Msellem, C. Obondo, A. Bjorkman, J. P. Gil, (2005) *In vivo* selection of *Plasmodium falciparum* pfmdr1 86N coding alleles by artemether-lumefantrine (Coartem), ***Journal of Infectious Diseases,* 191(6)**: 1014-1017.
5. D. Blessborn, S. Römsing, A. Annerberg, D. Sundquist, A. Björkman, N. Lindegardh, Y. Bergqvist, (2007) Development and validation of an automated solid-phase extraction and chromatographic method for determination of lumefantrine in capillary blood on sampling paper, ***Journal of Pharmaceutical and Biomedical Analysis,* 45(2)**: 282-287.
6. D. Colussi, C. Parisot, F. Legay, G. Lefevre, (1999) Binding of artemether and lumefantrine to plasma proteins and erythrocytes, ***European Journal of Pharmaceutical Sciences,* 9:** 9-16.
7. H. P. Rang, M. M. Dale, J. M. Ritter, R. J. Flower, (2007) ***Rang and Dale's Pharmacology***, Edinburgh: Elsevier Churchill Livingstone, p536.
8. USP SALMOUS Standards. Available from: http://www.usp.org/pdf/EN/nonUSStandards/lumefantrine.pdf; http://www.usp.org/pdf/EN/nonUSStandards/lumefantrineArtemetherTablets.pdf. [Last accessed on 2018 Apr 08].
9. World Health Organization, (2008) ***International Pharmacopoeia***, Geneva: World Health Organization, Department of Essential Medicines and Pharmaceutical Policies.
10. R. Arun, A. A. Smith, (2010) Development of analytical method for lumefantrine by UV spectrophotometry, ***International Journal of Research in Pharmaceutical Science,* 1:** 321-324.
11. S. Sharma, M. C. Sharma, (2011) Simultaneous UV spectrophotometric method for the estimation of lumefantrine in pharmaceutical dosage forms, ***World Journal of Chemistry,* 6:**75-79.
12. D. Parashar, A. Kumar, R. M. Rayasa, (2013) Simultaneous estimation of artemether and lumefantrine in pharmaceutical dosage forms using derivative spectrophotometry, ***Asian Journal of Research in Chemistry,* 6:** 226.
13. T. M. Kalyankar, M. S. Attar, S. D. Deosarkar, (2013) Development UV–visible spectrophotometric method for simultaneous estimation of artemether and lumefantrine, ***Analytical Chemistry: An Indian Journal,* 12:** 83.
14. S. K. Baokar, A. Annadate, S. Undare, (2014) New spectrophotometric method development and validation of lumefantrine, ***PharmaTutor,* 2(5)**: 148-154.
15. S. R. Karajgi, A. R. Tanveer, N. V. Kalyane, (2016) Simultaneous determination of artemether and lumefantrine by area under curve UV spectrophotometric method, ***Journal of Pharmaceutical Science and Research,* 8(6)**: 506-511.
16. O. A. Adegoke, C. P. Babalola, P. O. K Isaac, O. A. Kotila, (2011) Spectrophotometric studies of the charge-transfer complexation between lumefantrine and chloranilic acid in acetonitrile, ***Acta Pharmaceutica Sciencia,* 53(1)**: 135-149.
17. S. M. Mansor, V. Navaratnam, N. Yahaya, N. K. Nair, W. H. Wernsdorfer, P. H. Degen, (1996) Determination of a new antimalarial drug, benflumetol, in blood plasma by high-performance liquid chromatography, ***Journal of Chromatography B: Biomedical Applications,* 682:** 321-325.
18. A. Annerberg, D. Blessborn, Y. Bergqvist, N. Day, N. J. White, (2005) Development and validation of a bioanalytical method using automated solid-phase extraction and LC-UV for the simultaneous determination of lumefantrine and its desbutyl metabolite in plasma, ***Journal of Pharmaceutical and Biomedical Analysis,* 37(5)**: 1081-1088.
19. S. S. Wega, K. Vandercruyssen, E. Wynendaele, M. D'Hondt, N. Bracke, L. Duchateau, C. Burvenich, K. Peremans, B. D. Spiegeleer, (2013) A rapid stability-indicating, fused-core HPLC method for simultaneous determination of β-artemether and lumefantrine in anti-malarial fixed dose combination products, ***Malaria Journal*, 12:** 145.
20. I. C. César, F. H. A. Nogueira, A. G. Pianetti, (2008) Simultaneous determination of artemether and lumefantrine in fixed dose combination tablets by HPLC with UV detection, ***Journal of Pharmaceutical and Biomedical Analysis,* 48(3)**: 951-954.
21. J. Sunil, M. S. Nath, M. U. Samba, (2010) HPLC method development and validation for simultaneous estimation of artemether and lumefantrine in pharmaceutical dosage forms, ***International Journal of Pharmaceutical Science,* 4:** 4-7.
22. R. Arun, A. A. Smith, (2011) Simultaneous HPLC-UV method

for the estimation of artemether and lumefantrine in tablet dosage form, ***Journal of Pharmaceutical and Biomedical Analysis,*** **48:** 951-954.
23. I. F. Khalil, U. Abildrup, L. H. Alifrangis, D. Maiga, M. Alifrangis, L. Hoegberg, I. C. Bygbjerg, (2011) Measurement of lumefantrine and its metabolite in plasma by high performance liquid chromatography with ultraviolet detection, ***Journal of Pharmaceutical and Biomedical Analysis,*** **1:** 168-172.
24. N. S. Kotur, S. Sivasamy, S. Srinivasan, (2012) Analytical method development and validation for estimation of lumefantrine in pharmaceutical dosage forms by HPLC, ***Journal of Pharmaceutical Science and Research,*** **4:** 162-167.
25. S. R. Shah, M. Bapna, K. D. Brahmbhatt, R. A. Patel, C. M. Patel, (2013) Development and validation of analytical method for simultaneous estimation of artemether and lumefantrine in bulk and marketed fixed dose combination, ***International Journal of Pharmacy and Pharmaceutical Science,*** **4(3)**: 257-267.
26. M. Y. Zeng, Z. L. Lu, S. C. Yang, M. Zhang, J. L. Liao, S. L. Liu, X. H. Teng, (1996) Determination of benflumetol in human plasma by reversed-phase high-performance liquid chromatography with ultraviolet detection, ***Journal of Chromatograpy B: Biomedical Applications,*** **681:** 299-306.
27. B. Sridhar, R. K. Hanumantha, T. V. Srinivas, M. V. Seshu, K. Madhuri, J. V. Seshagiri, (2010) A validated reverse phase HPLC method for the simultaneous estimation of artemether and lumefantrine in pharmaceutical dosage forms, ***International Journal of Advances in Pharmaceutical Science,*** **1(1)**: 95-99.
28. R. Prasanna, D. Saravanan, J. Padmavathy, (2010) Method development and validation for the determination of lumefantrine in sold dosage form by RP-HPLC, ***International Journal of Pharmaceutical Research and Development,*** **8:** 84-90.
29. T. M. Kalyankar, R. B. Kakde, (2011) Reversed-phase liquid chromatographic method for simultaneous determination of artemether and lumefantrine in pharmaceutical preparation, ***International Journal of Chemical Technology and Research,*** **3:** 1722-1727.
30. E. M. Hodel, B. Zanolari, T. Mercier, J. Biollaz, J. Keiser, P. Olliaro, B. Genton, L. A. Decoster, (2009) A single LC-tandem mass spectrometry method for the simultaneous determination of 14 antimalarial drugs and their metabolites in human plasma, ***Journal of Chromatography B,*** **877(10)**: 867-886.
31. L. Wahajuddin, S. P. Singh, G. K. Jain, (2009) Determination of lumefantrine in rat plasma by liquid-liquid extraction using LC-MS/MS with electrospray ionization: Assay development, validation and application to a pharmacokinetic study, ***Journal of Chromatography B,*** **877(11-12)**: 1133-1139.
32. A. V. Silva, N. Mwebaza, M. Ntale, L. L. Gustafsson, A. Pohanka, (2015) A fast and sensitive method for quantifying lumefantrine and desbutyl-lumefantrine using LC–MS/MS, ***Journal of Chromatography B,*** **1004:** 60-66.
33. V. Munjal, N. Paliwal, B. K. Chaursia, B. Varshney, T. Ahmed, J. Paliwal, (2010) LC–tandem mass spectrometry method for quantification of lumefantrine in human plasma and its application to bioequivalence study, ***Chromatographia,*** **71(5/6)**: 505-510.
34. I. C. Cesar, J. A. D. Ribeiro, L. D. S. Teixeira, K. B. Bellorio, F. C. de Abreu, J. M. Moreira, P. R. Chellini, G. A. Pianetti, (2011) Liquid-chromatography–tandem mass spectrometry for the simultaneous quantitation of artemether and lumefantrine in human plasma: Application for a pharmacokinetic study, ***Journal of Pharmaceutical and Biomedical Analysis,*** **54:** 114-120.
35. P. Sethi, V. K. Dua, R. Jain, (2011) A LC–MS/MS method for the determination of lumefantrine and its metabolite desbutyl-lumefantrine in plasma from patients infected with *Plasmodium falciparum* malaria, ***Journal of Liquid Chromatography and Related Technology,*** **34(20)**: 2674-2688.
36. L. Huang, X. Li, F. Marzan, P. S. Lizak, F. T. Aweeka, (2012) Determination of lumefantrine in small-volume human plasma by LC–MS/MS: Using a deuterated lumefantrine to overcome matrix effect and ionization saturation, ***Bioanalysis,*** **4(2)**: 157-166.
37. K. Govender, L. Gibhard, L. Du Plessis, L. Wiesner, (2015) Development and validation of A LC–MS/MS method for the quantitation of lumefantrine in mouse whole blood and plasma, ***Journal of Chromatography B,*** **985:** 6-13.
38. P. K. Sajni, R. M. Singh, S. C. Mathur, G. N. Singh, C. L. Jain, R. K. Khar, A. A. Hafeez, (2010) A simple and sensitive HPTLC method for quantitative analysis of artemether and lumefantrine in tablets, ***Journal of Planar Chromatography,*** **23:** 119-122.
39. I. C. César, F. H. Nogueira, G. A. Pianetti, (2008) Comparison of HPLC, UV spectrophotometry and potentiometric titration methods for the determination of lumefantrine in pharmaceutical products, ***Journal of Pharmaceutical and Biomedical Analysis,*** **48(1)**: 223-226.
40. N. C. Amin, H. Fabre, M. D. Blanchin, J. Montels, M. Ake, (2013) Determination of artemether and lumefantrine in antimalarial fixed-dose combination tablets by microemulsion electrokinetic chromatography with short-end injection procedure, ***Malaria Journal,*** **12:** 202.
41. S. Suleman, Y. Verheust, A. Dumoulin, E. Wynendaele, M. D. Hondt, K. Vandercruyssen, L. Veryser, L. Duchateau, B. D. Spiegeleer, (2015) Gas chromatographic method for the determination of lumefantrine in antimalarial finished pharmaceutical products, ***Journal of Food and Drug Analysis,*** **23:** 552-559.
42. Validation of Analytical Procedures: Text and Methodology, (2005) ***International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, Q2(R1)***, p1-13.
43. M. S. Mahrous, M. Abdelsalam, A. S. Issa, M. Abdel-Hamid, (1986) Use of p-chloranilic acid for the colorimetric determination of some antimalarials, ***Talanta,*** **33:** 185-186.
44. K. Basavaiah, S. A. M. Abdulrahman, (2010) Use of charge transfer complexation reaction for the spectrophotometric determination of bupropion in pharmaceuticals and spiked human urine, ***Thai Journal of Pharmaceutical Sciences,*** **34(4)**: 134-145.
45. J. E. House, (2013) ***Inorganic Chemistry***. Oxford: Elsevier, p142.

****Bibliographical Sketch***

Ukpe Ajima is a Lecturer at the Department of Pharmaceutical Chemistry, University of Jos, Nigeria. He holds a Bachelor of Pharmacy degree and an M.Sc. in Pharmaceutical Chemistry. He joined the University of Jos in 2009 where his responsibilities include teaching Pharmaceutical Chemistry courses to undergraduate Pharmacy Students. His research is focused on Analytical method development and validation. He is a member of several national and international professional bodies including the Royal Society of Chemistry (RSC). He has 25 journal publications and five conference papers to his credit.