Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 4 [8] July 2015: 90-99

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD
Global Impact Factor 0.533
Universal Impact Factor 0.9804

ORIGINAL ARTICLE **OPEN ACCESS**

Sensitive UV-Spectrophotometric Determination Of Dihydroartemisinin and Artesunate in Pharmaceuticals Using Ferric-Hydroxamate Complex Formation

[1] **ATTIH, EMMANUEL E., USIFOH C. O.[2] AND OLADIMEJI, H. O.[1]**

1. Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmacy, University of Uyo, Nigeria.
2. Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Corresponding author's email: emmanuelattih@uniuyo.edu.ng

ABSTRACT

A simple and sensitive spectrophotometric method is developed for the determination of Dihydroartemisinin (DHA) and Artesunate (ARTS) in bulk and tablet formulations. The method is based on the reaction of the proposed drugs with hydroxylamine hydrochloride in alcoholic medium leading to the opening of the lactone ring and the final interaction of the product with ferric chloride to form the wine red ferric-hydroxamate complex which is determined spectrophotometrically at λmax 525. Beer's law was obeyed in the range of 2.0-80µg/ml and 2.0-90µg/ml, with regression coefficient of 0.9998 and 0.9996 for DHA and ARTS respectively, when absorbance was plotted against increasing concentrations of DHA and ARTS. The molar absorptivities were $1.7x10^3 Lmol^{-1}Cm^{-1}$ and $1.36x10^3 Lmol^{-1}Cm^{-1}$ and the corresponding Sandell sensitivities of $1.6x10^{-2}µg/cm^2$ and $2.8x10^{-2}µg/cm^2$ respectively. The limit of detection (LOD) and limit of quantification (LOQ) determined as per the current ICH guidelines were found to be 0.30µg/ml and 0.36µg/ml, 0.96µg/ml and 1.20µg/ml for DHA and ARTS respectively. The intraday and interday accuracy expressed as relative error (RE %) were ≤ 3.75% and the corresponding precision expressed as relative standard deviation (RSD %) < 2.0%. The validation method was used to assay DHA and ARTS in commercial tablets brands and the results showed good congruence with the reference methods. The accuracy and validity of the method was evaluated by performing recovery studies via standard addition method, the results showed excellent recoveries with no appreciable interference from excipients.

***Keywords**: Artesunate, assay, dihydroartemisinin, ferric-hydroxamate, malaria, spectrophotometry.*

Received 21.04.2015 Revised 10.06.2015 Accepted 22.06. 2015

INTRODUCTION

Malaria is the most deadly parasite disease in the world. Over 300 million new malaria infections and millions of death occur worldwide each year (1). Though endemic within the tropics it is the source of very great concern as it has extended to over 40% of the world's population (2). As international travels become more and more common, malaria is no longer confined to the tropical zones of the world alone as "imported malaria" is an increasing public health problem. Current projection suggest that if global warming remains unchecked, it could re-establish itself in Europe and North-America (3,4). That apart global warming has impacted upon malaria burden making it difficult to predict future disease pattern (5). However the most critically serious problem facing the treatment of malaria is the appearance of multi drug resistant species. This has been a great source of concern as they have developed resistance against quinoline antimalarials (e.g. chloroquine) and antifolates (e.g. Fansida-sulphadoxine-pyrimethamine). The increasing failure of the predominantly used and safe drugs, especially chloroquine (CQ) and sulphadoxine-pyrimethamine (SP), has been a serious obstacle towards global malaria control. Consequently, to overcome the resistance problem, the World Health Organization (WHO) and health authorities in malaria endemic countries recommend the use of a therapeutic combination (6). Artemisinin combination therapy (ACT) was recommended. ACT is the combination of Artemisinin derivative with other drugs. The efficiency of the ACT soon became the mainstay for the treatment of falciparum malaria; because of its efficiency and near absence of resistance. However, recent reports

show that some resistance have developed in South-East Asia, especially in two foci (Cambodia and Thailand), and (Myanmar and Vietnam) (7,8,9). The decreased sensitivity of *plasmodium falciparum* to the artemisinin is largely due to the prolonged administration of sub therapeutic doses of artemisinin derivative to patients. This most probably could have been as a result of production and distribution of fake artemisinin in South-East Asia (10,11). There are serious concerns that this may also become a major problem in Africa as has been demonstrated by a study conducted in Kenya and Democratic Republic of Congo (12). In spite of all these, Artemisinin derivatives remain the hope for the future treatment of falciparum malaria especially when in combination with other drugs. Artemisinin is a sesquiterpene lactone derived from *Artemisia annua*, Asteracceae, a vigorous weedy annual, native of China where it is known as Quighaousu. Artemisinin and its derivatives (Artemeter, Artesunate and Dihydroartemisinin) are targets for faking or counterfeiting bearing in mind the potent and rapid antimalarial activity. If counterfeit Artemisinin derivative follow genuine products into West Africa or any tropical country for that matter it will most likely precipitate very serious public health crises. Hence the reason for the attempt to develop assay methods for their detection at the point of procurement. Officially, Artesunate and Dihydroartemisinin are assayed using HPLC, titrimetry and uv-spectrophotometry (13). A careful search of the literature suggests that most other method developed for the assay of artesunate and DHA are few (14-15). All these methods were mostly developed for the assay of the drug in body fluids. That apart, most of these methods are simple sensitive and reproducible but some have some inherent short comings. The proposed method is accurate, precise, sensitive, reproducible, affordable and ecofriendly with no hazard posed to the analyst or the environment. The method is based on the reaction of the drugs with hydroxylamine hydrochloride in alcoholic medium leading to the opening of the lactone ring. The products of these now interacts with ferric chloride producing a wine red ferric hydroxamate - drug complex which is determined spectrophotometrically at λmax 525nm.

EXPERIMENTAL

Apparatus

All spectral determination were made using spectrophotometer model Hey los β. From thermo electron corporation, USA. With 1cm matched quartz cells

Reagents and Chemicals

All chemicals used were of analytical grade and solutions were prepared and diluted by using bidistilled water.

Solvents

Absolute ethanol (Sigma, Germany)

Reagents

Hydroxylamine hydrochloride (Sigma, Germany)

Sodium hydroxide (Sigma, Germany) was prepared by dissolving 40g of the sodium hydroxide pellet in 100ml of bidistilled water in a 1000ml volumetric flask and made up to the mark (1M).

Ferric Chloride (B.D.H, England) was prepared by dissolving 5 grams of the chemical in 20ml of bidistilled water and made up to the mark of a 100ml volumetric flask with bidistilled water.

Hydrochloric acid (B.D.H, England), 2M was prepared by adding 111.0ml of concentrated Acid (Sp. gr 1.18) to 889ml of bidistilled water.

Excipients

Glucose, lactose, magnesium stearate, sodium alginate, talc, starch (Chinese Pharmaceutical and Chemical Company, Uyo, Nig Ltd).

Pure Drugs

Pure drugs ARTS and DHA were received as a kind gift from the Director of Pharmaceutical Services, University Teaching Hospital, Uyo and was used as given. Ferric Chloride (B.D.H, England) was prepared by dissolving 5 grams of the chemical in 20ml of bidistilled water and made up to the mark of a 100ml volumetric flask with bidistilled water.

Recommended Procedure

Different aliquots of 0.5-5.0ml of standard solution of 100µg/ml of dihydroartemisinin in ethanol was carefully transferred into a series of 10ml calibrated volumetric flask. Using a micro burette the volume in the flask was adjusted to 5ml using absolute ethanol, followed by the addition of 2ml of 0.2M hydroxylamine Hydrochloride. The mixture was swirl gently to mix well. Then 2ml of 1M Sodium hydroxide was added mix well and allowed to stand in the boiling water bath for 15 minutes. The resulting solution was brought out from the water bath and allowed to cool. Finally 2 drops of 2M hydrochloride acid was added followed by 2 drops of ferric chloride shaken and finally made up to the mark by adding distilled water. A standard calibration curve was generated by plotting the absorbance

versus the concentration of the drug at 525nm against a reagent blank prepared exactly but with the omission of the drug. The concentration of the unknown was read from the standard calibration curve or evaluated from the regression equation deduced using Beer's law data.
Exactly the same procedure was carried out for Artesunate.

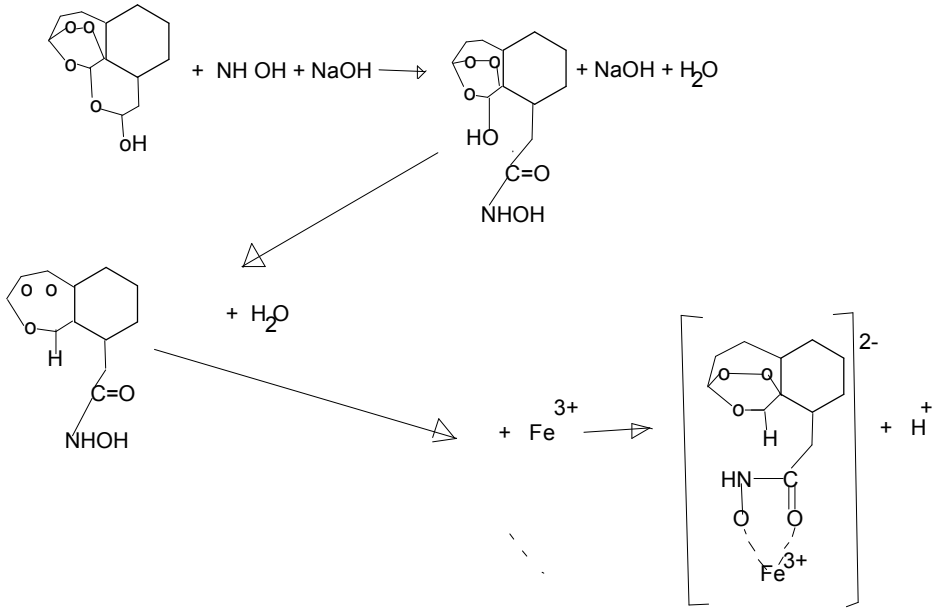
Procedure for Tablets

Twenty tablets of the drugs randomly chosen and accurately weighed singly to determine the average weight of each tablet and the whole tablets were pulvirised into a fine powder. An amount of the powder equivalent to 100mg was accurately measured into a 100ml volumetric flask 20ml of absolute ethanol was added and sonicated for 10 minutes. The mixture in the flask was shaken vigorously as different volumes of absolute ethanol was added to extract the drug. The mixture in the flask was made up to the mark with absolute ethanol. The resulting drug mixture was filtered using Whatman filter paper No. 42. The first 10ml portion of the filtrate was discarded. The resulting drug extract solution with the concentration of 10mg/ml was diluted stepwise to obtain a working concentration of 100µg/ml from where and appropriate aliquot was subjected to analysis as described in the general procedure above.
different brands of Artesunate and Dihydroartemisinin tablets were separately analyzed by the method described under this procedure for tablets.

RESULTS AND DISCUSSION

The main aim of this work was to develop a simple, accurate but sensitive method of determination of DHA and ARTS in bulk pharmaceutical and tablet formulations.
The principle behind this determination is based on the formulation of ferric hydroxamate complex with DHA and ARTs resulting in a red-violet colour. The intensity of the colour is proportional to the concentration of the complex and therefore to the concentration of the studied drugs, hence the spectrophotometric determination at λ_{max} 525nm. Carboxylic acid derivatives e.g. esters, acid anhydride, amide, imides and lactones give hydroxamic acid with an alkaline hydroxylamine reagent (18). The mechanism of this reaction leading to the formation of the complex is a typical nucleophilic reaction of which the C=O bond is attacked leading to the formation of the hydroxamic acid which then forms a complex with ferric ions.
The studied drugs are lactones. The lactone ring opens in alkaline medium when warmed; leading to the formation of the hydroxamic acid and the ultimate interaction with Ferric ions to give the Ferric hydroxamate complex (fig. 1).

Figure 1: **Mechanism for the formation of Ferric hydroxamate complex**



Ferric-Hydroxamate complex

(Reddish - Violet complex)

Scheme for proposed reaction of DHA and hydroxylamine / ferric chloride

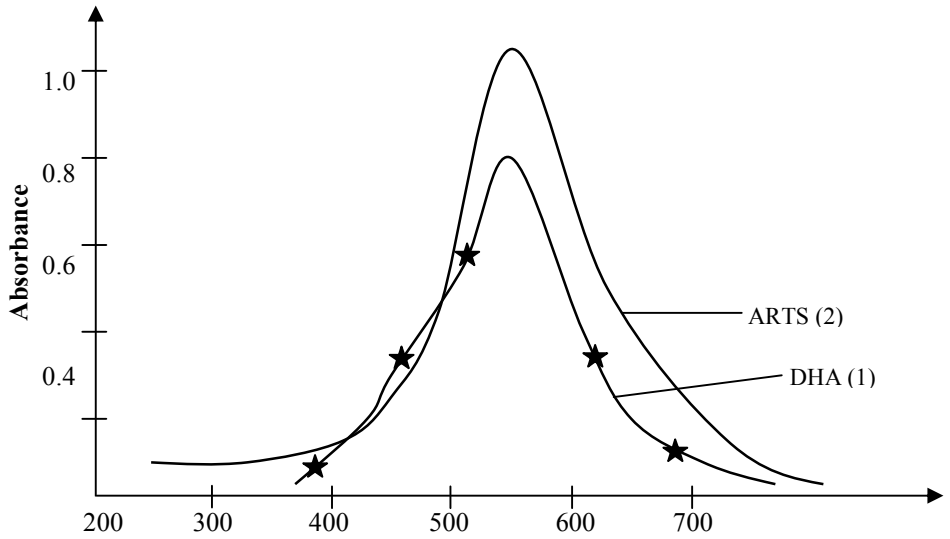


Fig II: **Absorption Spectra of (1) 20µg/ml DHA and (2) 20µg/ml reacted with 2ml of 0.2M NaOH, 2ml of 0.2M NaOH and a drop each of 0.2M FeCl and 2MHCl**

Optimization of Reaction Variables

All experimental variables were measured and fully optimized. Usually the other variables are kept constant while varying the studied parameter to see its effect on the absorbance of the complex.

Effect of Hydroxylamine Hydrochloride

The absorbance of the complex was observed as the volume and concentration of hydroxylamine hydrochloride was increased and all other variables kept constant. It was observed that there was a general increase in the intensity and ultimately the absorbance of the complex reaching a maximum of 2ml of 0.2M hydroxylamine. Further addition resulted in some erratic results. The complex was quickly formed but became very unstable. The reason for this could be that in the formation of the hydroxamic acid excess hydroxylamine results in the system which reacts irreversibly with Fe^{3+} reducing same to Fe^{2+} (ferrous) iron (18).

$$2NH_2OH + 2Fe^{3+} \longrightarrow 2Fe^{2+} + N_2O + 2H^+ + 2H_2O$$

Generally 2ml of 0.2M hydroxylamine hydrochloride was found suitable for a total 10ml volume reaction.

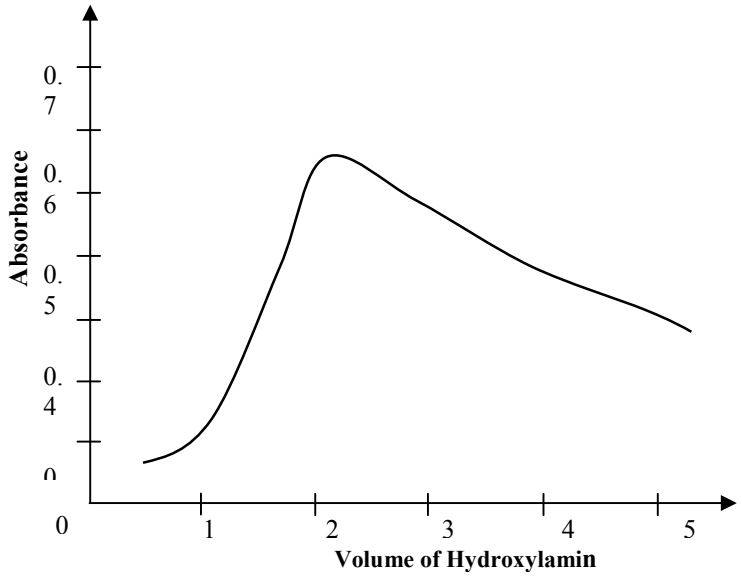


Fig 3: **Effect of Volume of Hydroxylamin on the Absorbance**

Effect Sodium Hydroxide

All other variables were kept constant and the volume and concentration of Sodium hydroxide varied and the absorbance of the complex measured. It was found that the intensity and hence the colour of this coloured complex was increasing up to a maximum of 2ml of 0.2M NaOH. Further addition reduced the stability of the complex. The formation of the complex between the hydroxamic acid and Ferric ion is formed under acidic medium. Therefore further increase in Sodium hydroxide will reduce the stability of the formed coloured complex.

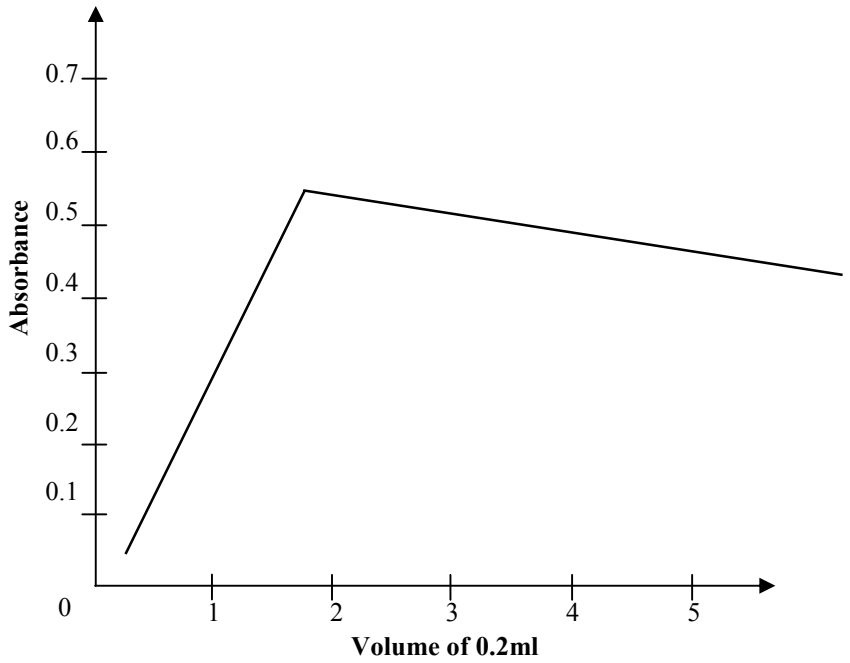


Fig 4: **Effect of Ferric Chloride on the Absorbance**

Effect of Ferric Chloride

The intensity and the absorbance were increased with the increase in the volume (drops) of 0.2M Ferric Chloride added. 2 drops of Ferric Chloride was found to be adequate. Further addition of the Ferric Chloride ion gave erratic results and instability of the complex formed.

Effect of Type of Acids

Different acids were used. These include Sulphuric Acid, Hydrochloride Acid, Acetic Acid, Phosphoric Acid, and Perchloric Acid. Hydrochloric Acid and Perchloric Acid gave the most stable coloured complex with the highest absorbance. Sulphuric acid and Phosphoric acid were also usable but form complexes that were not very stable.

Acid Type	Absorbance Difference	
	DHA	ART
Acetic	0.180	0.184
Hydrochloric	0.900	0.903
Sulphuric	0.615	0.620
Perchloric	0.899	0.901
Phosphoric	0.560	0.568

Effect of Temperature

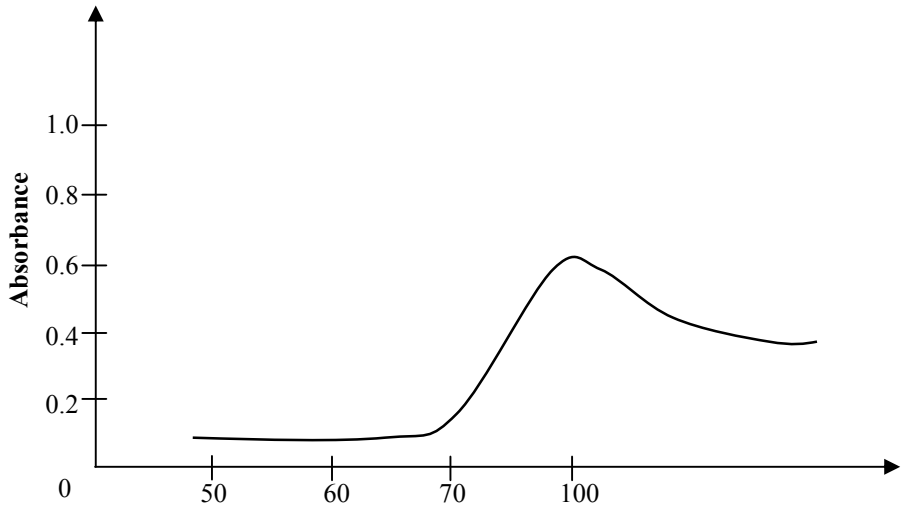


Fig 5: Effect of Temperature on the Absorbance of the coloured complex formed.

The effect of temperature on the formation and stability of colour was observed. It was observed that at lower temperatures the coloured complex was hardly formed. Stable colour complex were formed from 68^{o}C and peaked at 100^{o}C. Higher temperatures above hundred was not necessary as no better absorbance was observed.

Heating Time

A heating time of 15 minutes in a water bath maintained at 100^{o}C was optimal for the colour formation and stability. Higher heating time did not produce any better result, therefore 15 minutes was the heating time adequate reaction.

After cooling to room temperature the flask where placed in a dark cupboard for 30 minutes to allow for optimum formation of the coloured complex.

Effect of the Order of Addition

The most stable colour was formed with the order drug + hydroxylamine hydrochloride + sodium hydroxide + heat + ferric chloride + HCl.

Other order of addition did not give high colour intensity and hence low absorbance.

S/N	Order of Addition	Absorbance	
		DHA	ART
1	Drug + Hydroxylamine HCl + NaOH + Heat + Fe^{3+} + HCl	0.950	0.968
2	Drug + Hydroxylamine HCl + Heat + Fe^{3+} + NaOH + HCl	0.180	0.185
3	Drug + Hydroxylamine HCl + NaOH + Heat + HCl + Fe^{3+}	0.560	0.580
4	Hydroxylamine HCl + NaOH + Heat + Fe^{3+} + HCl + Drug	0.100	0.100
5	Hydroxylamine HCl + NaOH + Drug + Heat + Fe^{3+} + HCl	0.816	0.850

Stoichiometry of the Reaction

The stoichiometry of the Drugs hydroxamic acid and Fe^{3+} ion leading to the formation of the coloured complex was determined using the Job's method of continuous variation (19). This was done by keeping the sum of the molar concentration of both fixed, and the ratio of the concentrations of each two in this mixture was varied and the absorbance recorded at 525nm. The maximum absorbance was determined; this corresponds to the stoichiometric ratio of the reaction. It was found that the stoichiometric ratio was 1:1 for [Drug] : [Fe^{3+} - Hydroxamic Acid]. The colour produced in the addition of Fe^{3+} ion to the hydroxamic acid (fig. 6).

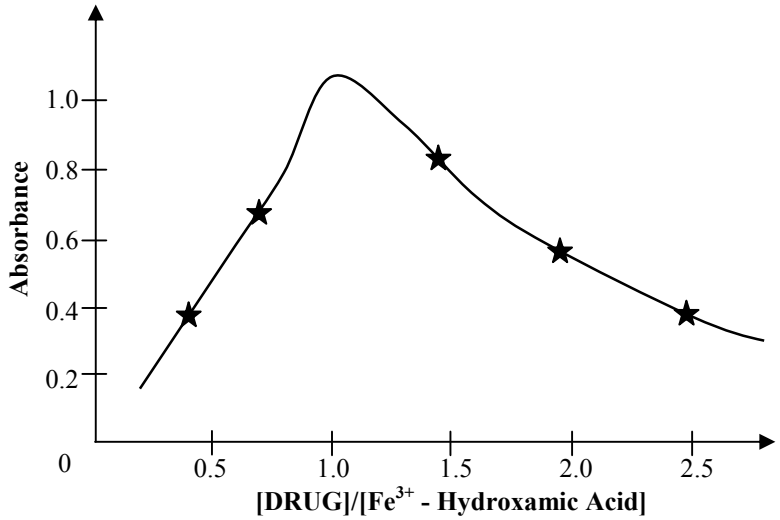


Fig 6 Job's plot for determination of the stoichiometry of the reactions leading to the formation drug / Ferric - complex

Validation of Proposed Method

Linearity and Sensitivity

Under optimum experimental conditions, standard calibration curves were generated by plotting the absorbance against the concentration of the studied drugs (ART and DHA) (figure 7). The calibration curves obeyed Beer's law and were described by the equation A = mc + b

Where A, is the absorbance, m = slope, c = molar concentration of the drug (DHA & ART) and b the intercept, obtained from regression analysis via least square method. The Beer's law range, the correlation coefficient for the studied drug were high, the slopes and intercepts of the calibration plots are recorded in table 1. Sensitivity parameters including molar absorptivity, Sandell sensitivity were also recorded in table 1. The limit of detection (LOD) and limit of qualification (LOQ) were determined as per the current ICH (20) guidelines. Using the formulae , LOD = 3.3 σ/s and LOQ = 10 σ/s

where σ represent the standard deviation of seven blank determinations and s representing the slope of the calibration curve. The results for both DHA and ART are recorded above in table 1 (A & B).

Precision and Accuracy

The precision and accuracy of this proposed method were evaluated by performing six replicate analysis of the calibration standard at three different concentration levels within same day (intra-day) and five consecutive days (inter-day). Percentage relative standard deviation (%RSD) represented the precision and percentage relative error (%Er) represented the accuracy of the method calculated using the formulae

$$\% \text{ Er} = \frac{\text{Amount Found - Amount Added}}{\text{Amount Added}} \times \frac{100}{1}$$

The intra-day and inter-day precision and accuracy is shown in table 2 as good results (A & B) showing the method is repeatable and reproducible.

Table 1: **Analytical Parameters and Optical Characteristics of the Proposed Methods**

Parameters	DHA	ART
Amax (nm)	525	525
Beer's Law Limit (µg/ml)	2.0-80	2.0-90
Molar Absorptivity ($Lmol^{-1}cm^{-1}$)	$1.7x10^3$	$1.36x10^3$
Sandell Sensitivity ($\mu g/cm^2$)	$1.6x10^{-2}$	$2.8x10^{-2}$
Regression Equation	A = 0.04c	A = 0.03c
Slope	0.03	0.02

Intercept	-	-
Correlation Coefficient	0.9998	0.9996
Limit of Detection (LOD) (µg/ml)	0.30	0.36
Limit of Qualification (LOQ) (µg/ml)	0.96	1.20

Table 2[A]: **Evaluation of Intra-day and Inter-day Accuracy and Precision for Dihydroartemisinin**

S/N	Amount of DHA Taken µg/ml	Intra-day Precision and Accuracy			Inter-day Precision and Accuracy		
		Amount of DHA Found	RE %	RSD %	Amount of DHA Found	RE %	RSD %
1	40	41.31	3.28	1.44	41.4	3.50	1.51
2	80	83.0	3.75	1.64	82.6	3.25	1.41
3	120	124.1	3.41	1.50	123.6	3.00	1.30

Table 2[B]: **Evaluation of Intra-day and Inter-day Accuracy and Precision for Artesunate**

S/N	Amount of ARTS Taken µg/ml	Intra-day Precision and Accuracy			Inter-day Precision and Accuracy		
		Amount of ARTS Found	RE %	RSD %	Amount of ARTS Found	RE %	RSD %
1	40	41.50	3.75	1.62	41.3	3.25	1.41
2	80	81.70	2.13	0.93	82.68	3.35	1.45
3	120	122.7	2.25	0.98	123.5	2.91	1.27

Table 3[A]: **Analysis of Dihydroartemisinin by Proposed Method**

S/N	Tablets Brand Analyzed	Label Claim (mg)	Reference Method	Results of the Proposed Method ±SD
1	Alaxin	60	110±0.96	110.98±1.0 F = 1.96, t = 1.60
2	Cotecxin	60	110±1.26	110.95±1.11 F = 1.28, t = 1.38
3	Santecxin	60	110±1.0	111.0±0.96 F = 1.02, t = 1.29
4	Codisin	60	110±1.11	111.1±1.1 F = 1.02, t = 2.41

Table 3[B]: **Analysis of Artesunate by Proposed Method**

S/N	Tablets Brand Analyzed	Label Claim (mg)	Reference Method	Results of the Proposed Method ±SD
1	Arsumax	50	100.50±0.63	99.80±1.0 F = 2.51, t = 1.32
2	Artesunate	50	100.50±0.63	99.50±0.68 F = 1.70, t = 2.41
3	Lever Artesunate	50	100.50±0.63	101.0±1.0 F = 2.51, t = 2.67
4	Articin	50	100.50±0.63	100.08±0.68 F = 2.51, t = 1.03

Mean of five determinations. The value of t (tabulated at 95% confidence level and 4 degree of freedom) is 2.77 .The value of F (tabulated at 95% confidence level and 4 degree of freedom) is 6.39.

Table 4: **Further Assessment of Accuracy by Recovery Experiment done via Standard Addition Method**

Tablet Studied	Amount of Drug (µg/ml)	Amount of Pure Drug Added (µg/ml)	Total Amount Found (µg/ml)	Recovery of Pure Drug %±D
Alaxin (DHA)	40.20 40.20 40.20	20.00 40.00 60.00	60.6 81.25 101.92	102±1.41 102.6±1.85 103.0±1.43
Cotecxin	41.00 41.00	20.00 40.00	61.55 82.50	103±1.63 103±1.88

	41.00	60.00	102.00	101.6±1.18
Santecxin	45.10	20.00	65.70	103±1.48
	45.10	40.00	86.20	102.7±1.94
	45.10	60.00	106.20	101.6±1.18
Codisin	50.10	20.00	70.50	101±1.63
	50.10	40.00	91.09	102.5±1.23
	50.10	60.00	111.00	102±1.06
Arsumax (ART)	30.00	20.00	50.48	102±1.01
	30.00	40.00	70.98	102±1.73
	30.00	60.00	92.01	103.4±1.37
Artesunate	40.20	20.00	60.62	102±1.48
	40.20	40.00	81.10	102±1.59
	40.20	60.00	102.09	103±1.80
Lever Artesunate	45.10	20.00	61.63	103.3±1.63
	45.10	40.00	86.20	103±1.38
	45.10	60.00	102.09	104 ±1.66
Articin	50.10	20.00	70.57	102.1±1.25
	50.10	40.00	91.10	102.5±1.25
	50.10	60.00	111.00	102.0±0.80

Robustness and Ruggedness

The robustness of the developed method was evaluated by making deliberate variations in some parameters (wavelength range, concentration of HCl and, heating time) each at a time and then investigating the effect on the performance of the proposed method. The methods performance remained unaffected by small deliberate variation of these parameters (table 5).

The method's ruggedness was evaluated by performing the same analysis as proposed by the method and expressed as RSD% by two analysts and in two different instruments and on three different days. There were no significant differences in the results between different analysis and different instruments used when compared statistically suggesting that the developed method was robust and rugged.

Effect of Interferences

The efficiency and selectivity of the proposed method was evaluated by adding 15mg of DHA (or ARTs) to a mixture of pharmaceutical excipients with the composition, talc (5mg) Lactose (10mg) starch (20mg) glucose (10mg) sodium alginate (30mg) and magnesium stearate (20mg) and analysed by the proposed methods. The percentage recovery was found to be 96.80±2.0% (n = 5) showing no series interference from excipients commonly used in dosage formulation.

Application of the Method for Tablet Formulation

Four bounds each of DHA and ARTs usually sold in pharmacies and local drug stores in Uyo, Nigeria were analysed using the proposed methods. The results as shown in table 3^A and 3^B revealed that there is a close agreement between the label claim and the results obtained. The proposed method were statistically compared with reference methods (b) for DHA and reference method (b) for ARTs in the international pharmacopoeia by applying the students t-test for accuracy and the variance ratio F-test for precision at 95% confidence level at 4 degrees of freedom. The t and F values obtained were below tabulated values showing that the proposed methods were accurate and precise compared with the reference methods.

Recovery study

The accuracy and applicability of the proposed method was further evaluated by performing recovery study via standard addition method. Pure DHA (or ARTs) was used to spike a preanalysed tablet powder at three different concentration levels and analysed by the proposed method and the percentage recovery of the added pure drug ranged between 101-104% with the standard deviation as shown in table 4. With the excellent recoveries it is agreed that inactive ingredients had no effect on the proposed method.

CONCLUSION

A novel method for the determination of dihydroartemisinin and artesunate in pharmaceuticals has been developed and validated. The proposed method was found to be simple, sensitive, robust, precise and accurate for the use in field stations and laboratories for routine analysis to check the counterfeit and fake artemisinin antimalarials being distributed or in circulation. The method has the advantages of the use of environment friendly reagents which pose no hazard to the analyst, no tedious extraction steps, no tight control of temperature and pH. Finally the methods wre executed with equipment which are inexpensive and easily affordable in most laboratories in this part of the world.

ACKNOWLEDGEMENTS

The authors are grateful to the Director of the Directorate of Pharmaceutical Service of the University of Uyo Teaching Hospital for providing pure Dihydroartemisinin and Artesunate powders used in this work. We are also grateful to Prof. Jacobus Eloff and the Phytomedicine Laboratory, Para clinical Sciences, Faculty of Veterinary Sciences, of the University of Pretoria, South Africa for providing the equipment and Lab Space where part of the work was executed.

REFERENCES

1. Srivasta, M. Singh, H. and Naik, P. K. (2010). Molecular Modeling Evaluation of the Antimalaria Activity of Artemisinin Analogues: Molecular Docking and Rescoring Using Prime/MM-GBSA Approach. *Current Research Journal of Biological Science. ; 2(2):83-102*.
2. Robert, A., Benolt-Vical, F. O., Dechy-Cabmet, O., Maunier, B. (2001). From Classical Anti-malaria Drug to New Compound Based the Mechanism of Artemisinin. *Journal of Pure and Applied Chemistry. 73(7):1173-1188*.
3. Drew, M. G. B., Metcalfe, J., Dascombe, M. J. and Ismail F. M. D. (2006). Reactions of Artemisinin and Artemether with Acid: Implication for Stability and Mode of Antimalaria Action. *Journal of Medicinal Chemistry; 49: 6065-6073*.
4. McMichael, A. J., Woodruff, R. E., Hales, S. (2006). Climate Change and Human Health: Present and Future Risks. *Lancet. ; 367(9513) 859-869*.
5. World Health organization. (2008). The World Malaria Report, World Health Organization: Geneva, Switzerland .
6. Zakeri, S., Hemati, S., Piralmadi, S., Afsharpad, M., Raeisi, A. and Djadid, D. N. (2012). Molecular Assessment of Atpase Mutants Associated with Artemisinin Resistance among Unexposed and Exposed Plasmodium falciparum Classical Isolate to Artemisinin based Combination Therapy. *Malaria Journal. 11:373*.
7. WHO-GMP Update on Artemisinin - September (2011). (http://www.who.int/malaria/arupdate092011.pdf).
8. Noedl, H., Se, Y., Schaecher, K., Smith, B. L., Socheat, D., Fukuda, M. M. Artemisinin Resistance in Cambodia (ARC1) Study Consortium. Evidence of Artemisinin Resistance Malaria in Western Cambodia *N. Engl. J. Med.* 2008; 359:2619-2620.
9. Dondorp, A. M., Nosten, F., Yi, P., Dan, D., Phyo, A. A., Tarning, J., Lwin, K. M., Ariey, F., Hanpithalpong, W., Lee, S. J., Rongwald, P., Silamut, K., Imwong, M., Chotivanich, K., Lim, P., Herdman, T., An, S. S., Yeung, S., Singhasivanon, P., Day, N. P., Lindegurdh, N., Socheat, D., White, N. J. (2009). Artemisinin Resistance in *Plasmodium falciparium* Malaria N. *Engl. J. Med.* 361:455-467.
10. Rozendaal, J. (2001). Fake Antimalaria Drugs in Cambodia *Lancet.* 357, 890.
11. Newton, P., Prowy, S., G reen, M. D. Fake Artesunate in South East Asia *Lancet*. 2001;357, 1948-1950.
12. Atemkeng, M. A., De cook, K. U. and Raizier-Vercammen, J. (2007). Quality Control of Active Ingredients in Artemisinin Derivatives Antimalarials, within Kenya and DR Congo. *Journal of Tropical and International Health. ; 12(1) 68-74*.
13. Monograph for Antimalaria Drugs Artemisinin and Derivatives. (2009).International Pharmacopoeia Vol. 2. 3rd Edition. WHO CH 22nd Genera, Switzerland.
14. Na Banchang, K., Congpuong, K., Hung, L. N., Molunto, P. and Karbwang J. (1998).Simple High Performance Liquid Chromatographic Method with Electrochemical Detection for Simultaneous Determination of Artesunate and Dihydroartemisinin in Biological Fluids. *J. Chromatography. B. Biomed Science Appl. 708:201.*
15. Naik, H., Murry, D. J., Kirsch, L. E. and Fleckenstein, L. (2005). Development and Validation of High Performance Liquid Chromatography-mass Spectroscopic Array for Determination of Artesunate and Dihydroartemisinin in Human Plasma. *J. Chromatograph. B. Analyt. Techno. Biomed Sci.;* 816:233.
16. Van Quekelberhe, Goomro S. A., Cordonnier, J. A. and Jansea F. N. (2008). Optimization of an LC-MS Method for the Determination of Artesunate and Dihydroartemisinin Plasma Levels using Liquid-Liquid Extraction*. J. Anal. Toxicol.* ;32:133.
17. Teja-Isavadharm, P., Siriyanonda, D., Siripokasupkal, R., Apinan, R., Chanarat, N., Lim, A., Wannaying, S., Saunders, D., Fukuda, M. M., Miller, R. S., Weina, P. J. and Melendez, V.(2010). A Simplified Liquid Chromatography-mass Spectrometry Assay for Artesunate and Dihydroartemisinin, its Metabolite in Human Plasma. *J. Molecules.* 15:8747-8768.
18. Connors, K. A. (1982). A Textbook of Pharmaceutical Analysis, 3rd Edition. Wiley Inter-Science Publication, U.S.A. 1982: 529.
19. Job, P., Ann Chem. 16 (1936). 97 Advanced Physicochemical Experiments 2nd Edition. Oliver and Boyd, Edinburg; 1964.54.
20. ICH Guidelines, Q2(R1) (2005). Validation of Analytical Procedure: Text and Methodology. London.

CITATION OF THIS ARTICLE

ATTIH, Emmanuel E., Usifoh C. O. AND Oladimeji, H. O. Sensitive UV-Spectrophotometric Determination Of Dihydroartemisinin and Artesunate in Pharmaceuticals Using Ferric-Hydroxamate Complex Formation. Bull. Env. Pharmacol. Life Sci., Vol 4 [8] July 2015: 90-99