



UTILITY OF BROMATE-BROMIDE MIXTURE IN THE ESTIMATION OF DIHYDROARTEMISININ IN PHARMACEUTICALS

Emmanuel Attih^{1*}, Cyril Usifoh², Victor Anah¹ and Timma Uwa³

¹Department of Pharmaceutical and Medicinal Chemistry University of Uyo, Uyo Nigeria.

²Department of Pharmaceutical and Medicinal Chemistry, University of Benin, Benin city, Nigeria.

³Department of Pharmaceutics and Pharmaceutical Technology, University of Uyo, Uyo, Nigeria.

***Author for Correspondence: Dr. Emmanuel Attih**

Department of Pharmaceutical and Medicinal Chemistry University of Uyo, Uyo Nigeria.

Article Received on 31/03/2016

Article Revised on 20/04/2016

Article Accepted on 11/05/2016

ABSTRACT

Two titrimetric, one spectrophotometric and one kinetic methods were developed and validated for the estimation of Dihydroartemisinin (DHA) in pharmaceuticals. The methods were based on the oxidative bromination of DHA, by active bromine generated *in situ* by the action of acid on Bromate-Bromide mixture. The first method was the direct titration of the Bromate-Bromide mixture with DHA in acid medium using methyl orange as indicator. In the second titrimetric method the excess bromine generated after the oxidative bromination of DHA by Bromate-Bromide mixture was determined iodometrically. In the spectrophotometric method a measured excess of Bromate-Bromide mixture was reacted with DHA in acidic medium, the residual Bromine was determined by a fixed amount of methyl orange resulting in a chromogen whose absorbance was measured at 520nm. In the Kinetic method, the time taken for the liberated bromine to bleach the methyl orange when different concentration of DHA was reacted with the Bromate-Bromide mixture at 4-5°C was measured and plotted against the drug concentration. The two titrimetric methods were stoichiometric in the ratio of 1:1 (DHA : kBrO_3) and applicable within the range of 5.0-20mg/ml and 5.0-30mg/ml, respectively. In the spectrophotometric method Beer's law was obeyed, with a linear regression equation of ($A = 0.0113C + 0.0009$, $r = 0.9998$, $n=10$) and applicable in the range of 0.5-40mg/ml. the molar absorptivity and Sandell sensitivity of $2.10 \times 10^4 \text{ L}/\mu\text{g}/\text{cm}$ and $1.35 \times 10^{-2} \mu\text{g}/\text{cm}^2$ respectively. The limits of detection and quantification were 0.75 $\mu\text{g}/\text{ml}$ and 1.72 $\mu\text{g}/\text{ml}$ respectively. In the kinetic method the time taken for the bleaching of the methyl orange was proportional to the DHA concentration in the range of 5-60mg/ml with the regression equation of $T = 0.0188C + 0.006$ ($r = 0.9979$, $n=5$). The methods were evaluated statistically via relative error (accuracy) and relative standard deviation (precision) the intra and inter day variations were < 3%. The method were compared statistically with a pharmacopoeial method and successfully used to assay DHA in tablets and paediatric powders. The accuracy and applicability were confirmed by recovery studies via standard addition method with the results showing no interference from pharmaceutical excipients.

KEYWORDS: Bromate-Bromide mixture, dihydroartemisinin, kinetic, malaria, spectrophotometric titrimetric.

INTRODUCTION

Though curable and treatable malaria remain a major source of concern in terms of morbidity and mortality in the world. A situation where 3.3 billion people are at risk of malaria and 106 countries marked an endemic, with between 655,000 – 1.2million death recorded every hour calls for greater and closer look at the menace.^{[1],[2],[3]} The integrated approach of vector control and the use of Artemisinin Combination therapy (ACT) as recommended by WHO and authorities of endemic recorded some major success in the fight against malaria.^[4] The successes recorded so far are threatened and could be in Jeopardy following the emergence of multidrug, resistant malaria parasites reported in

Southeast Asia.^{[5],[6]} The emergence of this multidrug resistant malaria parasite may not be unconnected with the widespread production and distribution of counterfeit/substandard/fake artemisinin derivatives in Southeast Asia.^{[7],[8],[9],[10],[11]} It also been reported that counterfeit/substandard/fake artemisinin derivatives are distributed in sub Saharan Africa.^{[12],[13]}

WHO defines counterfeit drugs as medicines which are deliberately and fraudulently mislabeled with respect to identify and/or with fake packaging, with wrong ingredient, without active ingredient or with insufficient active ingredient.^{[14],[15]} The menace of counterfeit/fake though largely unreported cannot be over emphasized.

Apart from the problem of resistance pharmaceutical excipients used in the production of the substandard drugs are cheap, substandard and potentially dangerous substances such as metamizole, Safrole, melamine^[16] and diethylene glycol. These excipients are known carcinogens and some could cause serious renal failure.

Economically, the genuine drug manufacturers are disadvantaged as patients go for cheaper antimalarials. Since counterfeit medicines do not pass through traditional route of import government of endemic countries are losing revenue in form of taxes. Counterfeiters are sophisticated; they produce tablets looking absolute genuine making their detection particularly difficult. Their packaging is always a perfect copy and their quality cannot be assessed readily by lay persons or even experts of pharmaceutical industry without the aid of a quality testing laboratories.^[16] Officially dihydroartemisinin is assayed using HPLC and uv-vis spectrophotometry.^[18] Methods have been developed using HPLC.^[19] LC/MS.^[20] GC/MS.^[21] These equipment are very costly and are hardly affordable by primary health care unit in Africa. Some other simple methods have also been developed^{[22],[23],[24]}, which are sensitive, affordable but some have some obvious shortcomings. These methods are based on the reaction of the bromate/bromide mixture with dihydroartemisinin in acid condition. This reaction results in the generation of bromine *in situ* the reaction is quantitative which also provide analytical window for quantitative determination of DHA via the four proposed method.

EXPERIMENTAL

Apparatus

All spectral measurements were recorded using Heylos B. model of uv-vis spectrophotometer from Thermo Electron Corporation, USA. Equipped with 1cm quartz cell to match.

Chemicals and Reagents

All chemicals and reagent used were analytical grade. Bromate/Bromide mixture (0.05M KBrO₃ – 0.5M KBr). This was prepared by weighing out 0.835grams of KBrO₃ (British drug House) and 6grams of KBr (British Drug House) and transferred to a 1 litre volumetric flask. This resulting solution was used for the titrimetric and the kinetic determinations. Appropriate dilution was made to obtain the working concentration for the spectrophotometric method.

Hydrochloric Acid (5M) solution was by appropriately dissolving 443ml prepared of the concentrated Acid (BDH) Sp.gr. 1.18 litre of distilled water. Sulphuric Acid (2M) solution was prepared by diluting the concentrated acid (Merck, Germany) Sp. gr. 1.84) in enough distilled water to give 2M Solution.

Potassium Iodide 10%: was prepared by dissolving 10g of the chemical (Merck Darmstadt Germany) in enough distilled water to make up to 100ml.

Potassium bromide 5%: was prepared by dissolving 5g of the chemical (BDH England) in enough distilled water to make up a 100ml of the solution.

Potassium bromate 1%: was prepared by dissolving 1g of the chemical (BDH England) in enough distilled water to make up 100ml of solution.

Methyl orange: 400ug/ml: This was prepared by dissolving 47.06mg of the dye (Merck Darmstadt Germany) in distilled water and made up to the 100ml mark of the 100ml capacity volumetric flask.

Starch solution 1%: starch indicator was prepared by dissolving 10g of the chemical (Merck Darmstadt Germany) in about 10ml of water and made into slurry/paste in a 100ml capacity beaker. Then boiling water was poured to make up to 100ml. and cooled to room temperature.

Sodium thiosulphate (0.05M): was prepared by dissolving 12.41g of the chemical (BDH. England) in 1 liter of boiled and cooled distilled water. Standard solution of Dihydroartemisinin (DHA). Pharmaceutical grade DHA was donated by the directorate of Pharmaceutical Services, University of Uyo, Teaching Hospital for the research work. A stock solution containing 1mg/ml was prepared by dissolving 100mg of the pure drug powder in 100ml of Absolute ethanol (BDH), used for the titrimetric methods A and B. The resulting solution of (1mg/ml) was further diluted stepwise to obtain working concentrations of 100µg/ml and 20µg/ml for method C and D respectively using the same ethanol.]

Pharmaceutical excipients

(1) Lactose (10mg), starch (50mg), magnesium stearate (20mg), talc (10mg), sodium citrate (20mg), sodium alginate (10mg), acacia (20mg) and sucrose (10mg)

METHODS

- Titrimetric Method (Direct)
- Different 10ml aliquot of the pure drug containing 5-20mg were accurately measured and transferred into 100ml titration flask. The content of the flask was acidified using 5ml of 2M sulphuric acid.

Then 5ml of 5% potassium bromide was added and titrated against 1% potassium bromate using two drops of methyl orange as indicator to a colorless end point. A blank titration was carried out replacing the drug with 10ml of absolute ethanol. The amount of the drug in the aliquot was determined using the following formula.

$$\text{Amount of drug (mg)} = \frac{VMwt.R}{n}$$

Where V = Volume of potassium bromate consumed
Mwt = relative molecular wt of the drug
R = number of moles of bromate reacting.

N = the molarity of the bromate solution reacting with the drug.

Method B (Iodometric titration)

Different 10ml aliquot containing 5-20mg of dihydroartemisinin was accurately measured and transferred into a 100ml iodine flask and acidified with 5ml of 2M sulphuric acid. Then 10ml of the (0.05M Bromate – 0.5 Bromide) mixture was added shaken gently to mix well and allowed to stand for 15min with gentle swirling occasionally. At the expiration of the 15 minutes 5ml of 10% potassium iodide was added. The liberated iodine was determined using sodium thiosulphate (0.05M) with the starch indicator added close to the end point. A blank determination was carried out without the DHA. The amount of DHA in each aliquot was then estimated from the amount of bromate reacted using the formular.

Amount of Drug (mg) = (B – S) Mwt.R.

Where

B = Volume of sodium thiosulphate consumed in the blank titration

S = Volume of sodium thiosulphate consumed in the titration of sample containing the drug.

Mwt. = Molecular weight of the drug.

R = Molarity of the Bromate-Bromide mixture

Method C (Spectrophotometric method)

Different aliquots (0.25, 0.5, 1.0, 1.5 – 4.5ml) containing 100µg/ml of Dihydroartemisinin were accurately transferred to 10ml calibrated volumetric flask using micro burette. The volume inside each flask was adjusted to 5ml using absolute ethanol. The contents of each flask was acidified using 2ml of 5M HCL; Then 1ml of the Bromate-Bromide mixture was added and shaken to mix well. The resulting solution was allowed to stand for 15 minutes with gentle swirling after every 5 minutes. Finally 1ml of methyl orange (50µg/ml) was added and the content of each flask was made up to the mark using absolute ethanol and the absorbance of the resulting solution was measured at 520nm. The calibration curve was generated by plotting the absorbance against the drug concentration from where the concentration of the unknown is read from. The unknown concentration could also be determined from the regression equation derived using the Beer's law data.

Method D (Kinetic Method)

Different aliquots (5, 10, 15, 20, 25) of containing 250µg/ml of the pure DHA were measured and accurately transferred into different 50ml calibrated volumetric flask each containing 25ml of methyl orange solution (40µg/ml in 1M sulphuric acid). This was diluted to the 50ml mark using absolute ethanol. Two sets of 5 clean test tubes each were selected. To the first set of 5 test tubes 5 ml of the drug/dye solutions were accurately transferred into. To the second set of 5 test tubes 5ml each of the Bromate – Bromide solution were accurately transferred into. Both tubes were placed in a

bath at between 4 – 5°C. the stop watch was started and the content of the two tubes were mixed into one tube noting the time of the mixing as (T_i) the content was stirred gently using the thermometer; until the colour of the methyl orange was discharged and the time noted as (T_f). (final time). The actual time required for the total bleaching of methyl orange is given by the equation.

$$T_a = T_f - T_i$$

A blank determination was performed simultaneously by mixing equal volume of the Bromate-Bromide mixture without the drug (DHA); noting the time required for the bleaching of the dye to be T_b . The corrected time was determined using the equation $T_c = T_a - T_b$. The calibration curve was generated by plotting the corrected time T_c against the DHA concentration. The concentration of the unknown was determined from the regression equation.

Methods for tablets

Twenty tablets of each of brands of DHA procured locally from pharmacies in Uyo South-South Nigeria were selected separately weighed and pulverised into five powder. An amount of the powder equivalent to 250mg was weighed accurately and transferred into a 250ml calibrated volumetric flask containing 50ml of absolute ethanol. The drug mixture was sonicated for 10 minutes and shaken vigorously. Another 100ml of absolute ethanol was further added and shaken vigorously for 20 minutes to extract the drug. Finally the amount in the flask was made up to the mark with absolute ethanol mixed well and filtered using whatman filter paper No. 42. The first 10ml of the filtrate was discarded, and a convenient aliquot from the resulting drug solution (1mg/ml) was assayed via the titrimetric methods (A & B), 1ml each of the resulting drug solution were further diluted to obtain the working concentrations of 100µg/ml and 250µg for the spectrophotometric and Kinetic methods respectively.

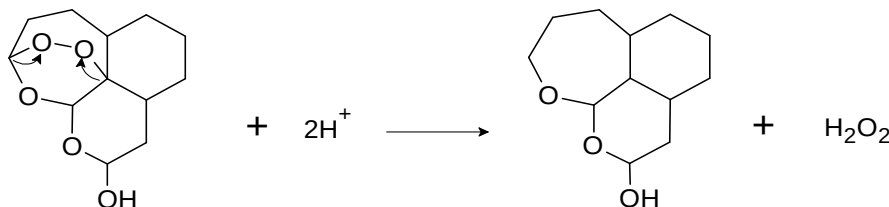
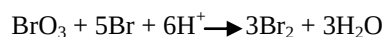
Procedure for DHA Paediatric Powders

The powders from 5 bottles of alaxin (DHA) and santecxin (paediatric powders) procured from local pharmacies in Uyo, South South Nigeria were separately pooled together and mixed appropriately and homogenised. A portion of the mixture (powder) equivalent to 100mg was measured accurately and transferred into a 100ml capacity volumetric flask containing 20ml of distilled and shaken vigorously. A further 50ml of distilled water was added and also shaken to extract the content finally. The volume in the flask was made upto 100ml mark and filtered using the whatman filter paper No. 42. The resulting solution was extracted using four 25ml portion of petroleum ether. The extracts were pooled together and fan dried. The resulting residue was transferred into a 100ml capacity volumetric flask and dissolved with absolute ethanol which also used to make up to the mark followed with shaking to mix well. The resulting solution was assayed

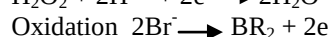
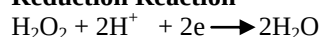
using the two titrimetric methods. Appropriate dilutions were done further to obtain working concentration for the spectrophotometric and Kinetic assays respectively.

Method for Placebo Blank and Synthetic Mixture

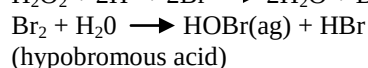
A placebo blank was prepared using pharmaceutical excipients usually co-formulated with the drugs into tablets and powders. This blank was prepared containing Lactose (10mg) Starch (50mg) Sodium Alginate (20mg) Sodium citrate (20mg) talc (10mg) magnesium stearate (20mg), Acacia (10mg) sucrose (10mg) mixed together and homogenized. Then 100mg of this mixture was dissolved in 100ml of ethanol and shaken appropriately and filtered. The resulting solutions were treated and analyzed as described under the procedure for tablets and pediatric powder. A synthetic mixture was prepared by mixing 15mg of DHA with 15mg of the homogenized placebo blank mixture. The resulting mixture (placebo blank and Drug) was prepared into solution as described in the "procedure for tablet". The resulting solution of the synthetic mixture was analysed using the methods described above.



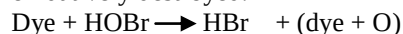
Reduction Reaction



Overall



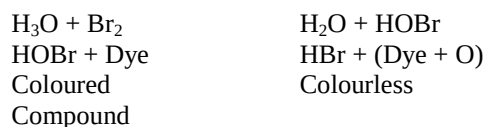
The hypobromous acid is very reactive and readily gives up its oxygen to the dye (methyl orange) to form a colourless compound. The dye (methyl orange) is oxidatively destroyed.



Coloured colourless

2.

+



The two proposed mechanisms are possible in acid environment with free bromine. The active free bromine generated *in situ* is the driving force for the reaction between DHA and bromate-bromide mixture. Due to the

RESULTS AND DISCUSSION

The proposed methods are based on the utility of bromate-bromide mixture as a powerful oxidizing agent. In acid condition the bromate-bromide mixture generates free bromine *in situ*. This reagent is used because of the instability of bromine solution due to its volatility.



Potassium bromate has very high purity and its solution is very stable but it is seldom used in direct volumetric work. The addition of the bromide to the bromate results in the generation of excess bromine *in situ*. These proposed methods for the determination of Dihydroartemisinin are based on oxidative bromination reactions by excess bromine generated *in situ* due to the action of acid on the bromate-bromide mixture. In the first method (Titrimetric Method A) excess bromine is generated which results in the oxidation of the drug and the oxidative destruction of the dye methyl orange. The reaction is quantitative as the amount of bromine reacting with drug is stoichiometric; the ratio of which was found to be 1:1 (DHA: KBrO_3) two mechanisms for the reaction are likely

versatility of bromate-bromide mixture as an oxidizing agent many pharmaceuticals have been assayed by its use.^[22]

Method B (Iodometric Titrations)

The active bromine generated *in situ* was allowed to completely react with DHA. The residual bromine quantitatively displaced iodine from the potassium iodide in acid medium. The liberated iodine in solution was then determined iodometrically using standardized sodium thiosulphate. The reaction stoichiometry was also found to be 1:1 (DHA: KBrO_3).

Spectrophotometric Method

In this method varying amount of DHA were allowed to react with a fixed amount of bromate-bromide mixture in acid medium for some specific time. After this the unreacted bromine was reacted with a specific amount of the dye methyl orange. The absorbance of the coloured chromogen generated was measured at 520nm. The absorbance observed varied linearly with the DHA concentration resulting in the calibration graph used in the quantification of DHA.

Kinetic Method

The active bromine generated *in situ* oxidatively destroyed the dye (methyl orange) used. This redox reaction is time dependent; hence the Kinetic method developed. In this method varying amounts of the drug-dye solutions were made to react with fixed amount of the bromate-bromide mixture in acid solution. As the amount of the drug-dye solution increase the active bromine available for the oxidative destruction of the methyl orange decreases hence the time for the oxidative destruction reaction increase. A corrected time was determined using the blank experiment. It was observed that the concentration of DHA varied proportionally with the corrected time which formed the basis for the determination of DHA. The oxidative destruction of the dye methyl orange by bromine generated from the bromate-bromide mixture has also been used in the determination of some pharmaceuticals.^[25]

METHOD DEVELOPMENT

Optimization of Reaction Conditions

In the proposed methods optimum reaction condition were studied and optimized. Under optimum experimental conditions all variables were kept constant while the particular variable that is studied is varied to observe its effect on the overall result of the experiment. Acid type, Volume and concentration of acid used

Three types of acid were used. Hydrochloric acid, sulphuric acid and Acetic acid were used. Hydrochloric acid and sulphuric acid gave the best results for the three proposed methods. The results observed for acetic acid was erratic. In the first titrimetric method sulphuric acid was used because it gave the best result. Though the stoichiometry of the reaction did not change. 2M sulphuric acid was found adequate for a 10ml aliquot of the reacting volume of titrimetric methods. Sulphuric acid was also used in the kinetic method while Hydrochloric acid was more suitable for the spectrophotometric method

REACTION TIME

In the titrimetric and spectrophotometric methods the reaction time for the complete oxidative bromination of the drug and the dye (methyl orange) was observed when other variables were kept constant. The time for the complete redox reaction under laboratory temperature was observed to be five (5) minutes. A reaction time of up to 10 minutes was allowed. This did not affect the stoichiometry of the reaction. In the kinetic method oxidative destruction of the dye was first under laboratory temperature a better result was obtained when the experiment was conducted at 5°C or lower, this afforded an opportunity for better evaluation of the experiment.

Method Validation

Titrimetry: The direct titrimetric and the iodometric titrimetric methods were applicable in the range of 5-20mg and 5-30mg/ml of DHA. Beyond these limits, the

results obtained were erratic and no longer proportionate. The correlation between the end points of the titrations and the drug concentration were evaluated by the least square method and found to be $r = 0.9927$ and $r = 0.9952$ respectively. In both cases the stoichiometry of the reactions observed to be between DHA and bromate-bromide was in the ratio of 1:1.

In the spectrophotometric method the calibration curve as generated by plotting absorbance Vs concentration of the drug was linear and obeyed Beer's law over the concentrations range of 0.5-40mg/ml. the molar absorptivity and Sandell sensitivity were $2.10 \times 10^4 \text{ l/}\mu\text{g/cm}$ and $1.35 \times 10^{-2} \mu\text{g/cm}^2$ respectively. The regression equation was in the form of a straight line equation $A = MC + b$.

Where A = absorbance, M being the slope and b the intercept.

$$A = 0.0113C + 0.0009 \quad (r = 0.9998, n = 10)$$

1

The Limit of Detection (LOD) and Limit of Quantification were evaluated according to the current ICH guidelines using the for equation.

$$\text{LOD } \frac{3.3\sigma}{s} \text{ and LOQ } = \frac{10\sigma}{s}$$

Where σ is the standard deviation of five blank determinations and 5 the slope of the calibration graphs.

LOD and LOQ were $0.75 \mu\text{g/}$ and $1.77 \mu\text{g/ ml}$ respectively.

In the Kinetic method a calibration graph was generated by plotting, the corrected time as a function of the DHA concentration. This was found to be linear having applicable range of 5 - 60 $\mu\text{g/ml}$. the regression equation as determined via the least square method was in the form of $T = mC + b$ where T was the corrected Time in seconds and m the slope while b is the intercept and C concentration of DHA.

$$T = 0.01888C + 0.006 \quad (r = 0.9979, n=5)$$

Limit of Detection (LOD) and Limit of Quantification calculated as per the ICH guideline (shown above) was found to be $0.37 \mu\text{g/ml}$ and $1.22 \mu\text{g/ml}$ respectively.

ACCURACY AND PRECISION

The accuracy and precision of the proposed methods were determine within a day (intraday) and 5 consecutive days (inter day) in the form of relative error (percent) (R. E. %) and relative standard deviation RSD% respectively. The pure DHA was prepared and analysed at three different concentration levels in seven replicates. The relative error (percent) RE% was calculated using the equation.

$$R. E. \% = \frac{\text{Amount found} - \text{Amount Added}}{\text{Amount Added}} \times 100$$

The result of accuracy and precision of the methods were generally < 3% and < 3.5 respectively and are recorded in table 1.

SENSITIVITY

The sensitivity of the methods were determined by the use of the placebo blank and the synthetic mixture as described earlier. The placebo blank result showed no effect on the absorbance hence there was no interference from common excipients. There were excellent recoveries of the added pure drug to the synthetic mixture confirming that common excipients such as talc, lactose, magnesium stearate, Acacia sucrose, sodium

citrate, sodium alginate had no major effect on the developed methods.

ROBUSTNESS AND RUGGEDNESS

Deliberate and minor variations were made in the volume of sulphuric acid, methyl orange, and contact time to test their effect on the proposed methods. The effect of these minor but deliberate changes is a test for the robustness of the methods. The results were not adversely affected by their changes. These methods were further tested for ruggedness by having the experiments performed by two other analysts using different but similar equipment. The results of accuracy R.E% and the precision RSD% showed no difference with the results obtained when the methods were developed.

TABLE 1 - EVALUATION OF INTER-DAY AND INTRA-DAY ACCURACY AND PRECISION

Inter-Day Accuracy and Precision Intra-Day Accuracy and Precision

HODS	AMOUNT TAKEN (DHA)	AMOUNT OF DHA FOUND	RE%	RSD%	AMOUNT DHA FOUND	RE%	RSD%
Titrimetric A	5.0	5.12	2.40	0.98	5.14	2.80	1.14
	10.0	10.28	2.80	1.14	10.27	2.70	1.10
	15.0	15.39	2.60	1.06	15.40	2.67	1.09
Titrimetric B. (Iodometry)	5.0	5.09	1.80	0.74	5.11	2.20	0.90
	9.0	9.20	2.22	0.91	9.22	2.44	1.03
	13.0	13.30	2.31	0.94	13.32	2.50	1.01
Spectrophotometry	2.0	2.05	2.50	1.02	2.04	2.00	0.82
	4.0	4.11	2.75	1.12	4.11	2.75	1.12
	8.0	8.21	2.63	1.07	8.22	2.75	1.12
Kinetic Method	10	10.25	2.50	1.02	10.26	2.60	1.06
	20	20.36	1.80	0.73	20.38	1.90	0.78
	40	40.78	2.40	1.00	41.00	2.50	1.02

For the titrimetric methods the amount taken and found were in mg/ml. For the spechophotometric method and the Kinetic method the amount taken and found were in $\mu\text{g/ml}$. R.E% relative percentage error RSD% relative standard deviation percent.

TABLE 2 – RESULTS OF ANALYSIS OF TABLETS AND PURDERS BY THE PROPOSED METHODS'

Briand Name/Formulation	Label Claim (mg)	Titrimetric Method A	Titrimetric Method B	Spectrophotometric Method	Kinetic Method	Reference Method
Codisin tablets	60mg/tablet	111.0 $\pm$ 1.00 F = 1.25 t = 1.66	110.7 $\pm$ 1.68 F = 2.25 t = 0.92	111.1 $\pm$ 1.25 F = 1.25 t = 1.62	111.2 $\pm$ 0.75 F = 2.23 t = 2.36	110.0 $\pm$ 1.12
Alaxin tablets	60mg/tablet	110.9 $\pm$ 1.0 F = 1.25 t = 1.59	111.0 $\pm$ 0.75 F = 2.23 t = 1.32	111.3 $\pm$ 1.10 F = 1.04 t = 2.19	111.0 $\pm$ 0.93 F = 1.45 t = 1.82	110.0 $\pm$ 1.12
Cotecxin	60mg/tablet	111.2 $\pm$ 1.16 F = 1.05 t = 1.96	111.0 $\pm$ 0.70 F = 2.60 t = 1.99	111.8 $\pm$ 1.14 F = 1.02 t = 2.96	111.0 $\pm$ 0.76 F = 2.21 t = 2.02	110.0 $\pm$ 1.13
Santecxin suspension	60mg/tablet	111.5 $\pm$ 1.24 F = 1.27 t = 2.39	110.8 $\pm$ 1.80 F = 2.68 t = 1.59	111.7 $\pm$ 1.37 F = 1.55 t = 2.06	110.9 $\pm$ 0.85 F = 1.67 t = 1.75	110.0 $\pm$ 1.10
Santecxin suspension	180mg/80ml	111.3 $\pm$ 1.34 F = 2.48 t = 2.16	111.0 $\pm$ 1.45 F = 2.91 t = 1.57	111.8 $\pm$ 1.35 F = 2.52 t = 2.99	111.4 $\pm$ 1.46 F = 2.95 t = 1.17	110 $\pm$ 0.85

Mean of Seven Determinations

The value t (tabulate at 95% confiderum level and at degrees of freedom is = 2.44

The value of F (tabulated at 95% confidence level and at 6 degrees of freedom = 4.28

TABLE 3 - RESULTS OF RECOVERY STUDY VIA STANDARD ADDITION METHOD

S/ N	Formulat ion studied	TITRIMETRY A				TITRIMETRY B TOTAL				SPECTROPHOTOMETRIC METHOD				KINETIC METHD			
		Amou nt of drug (mg)	Amount of pure drug added (mg)	Total amount found (mg)	Recovery of pure DHA %	Amou nt of drug (mg)	Amou nt of pure drug added	Amoun t drug found mg	Recovery of pure drug % ± SD	Amou nt of drug µg/ml	Amount drug added µg/ml	Total found µg/ml	Recovery of pure drug %	Amoun t of drug µg/ml	Amoun t pure drug added	Total amoun t found	Recovery of pure drug % ± SD
1	Codisin	4.03	4.00	8.06	100.73± 1.5	5.03	5.0	10.00	101.0±1.00	40.20	20.00	60.52	101.64 ± 1.13	30.00	20.00	50.39	103.0 ± 2.08
		4.03	8.00	11.99	99.87 ± 2.0	5.03	10.0	15.40	103.5 ± 0.87	40.20	40.00	81.20	102.51 ± 1.76	30.00	40.00	71.02	102.5 ± 1.80
		4.03	12.00	16.05	100.1± 1.01	5.03	12.0	17.05	100.2 ± 1.10	40.20	60.00	102.30	103.5 ± 1.47	30.00	60.00	92.00	1.03.0 ± 2.35
2	Alaxin	5.08	4.00	9.10	100.5 ± 1.12	5.03	4.0	9.94	99.75 ± 1.20	41.00	20.00	61.08	103.40 ± 2.40	40.20	20.00	60.75	103.0 ± 1.94
		5.08	8.00	13.03	99.7 ± 1.12	5.03	8.0	14.30	104.40± 0.75	41.00	40.00	82.25	103.10 ± 2.20	40.20	40.00	81.20	103.5 ± 1.80
		5.08	12.00	17.10	100.2 ± 1.10	5.03	12.0	17.90	99.5 ± 1.60	41.00	60.00	102.06	101.64 ± 1.17	40.20	60.00	102.06	1.03.0 ± 2.35
3	Cotecxin	6.04	4.00	10.08	101.0 ± 2.05	6.05	4.0	10.00	98.75 ± 1.41	45.10	20.00	65.50	102.0 ± 1.41	41.00	20.00	61.60	103.0 ± 2.12
		6.04	8.00	14.06	100.2 ± 106	6.05	8.0	14.04	99.88 ± 1.13	45.10	40.00	86.10	102.50 ± 1.18	41.00	40.00	81.69	103.21± 1.22
		6.04	12.00	18.08	100.2 ± 2.11	6.05	12.0	18.00	99.60 ± 1.30	45.10	60.00	107.0	103.32 ± 2.20	41.00	60.00	102.9	103.10± 2.24
4	Santexin	6.95	4.00	10.96	100.2 ± 0.71	6.05	4.0	11.00	101.30± 1.18	50.10	20.00	70.40	101.50 ± 1.06	50.00	20.00	70.60	103.10± 2.12
		6.95	8.00	14.93	99.75 ± 1.25	6.05	8.0	15.07	105 ± 1.12	50.10	40.00	91.10	102.54 ± 1.76	50.00	40.00	91.00	103.20± 1.17
		6.95	12.00	18.99	100.33± 2.11	6.05	12.0	19.00	104 ± 1.50	50.10	60.00	112.10	101.60 ± 1.18	50.00	60.00	111.84	103.21±2.17
5	Santecxin suspensio n	6.95	4.00	11.05	102.5 ± 1.76	7.03	4.0	11.05	100.5 ± 1.0	50.10	20.00	70.45	101.80 ± 1.24	50.10	20.00	70.51	102.0±1.41
		6.95	8.00	15.09	101.8 ± 1.24	7.03	8.0	15.07	102.50± 1.15	50.10	40.00	91.08	102.50 ± 1.15	50.10	40.00	91.09	102.4±1.74
		6.95	12.00	19.25	103.25± 1.76	7.03	12.0	19.06	103.54± 2.40	50.10	60.00	112.20	103.54 ± 2.40	50.10	60.00	110.00	101.02±2.46

Mean value of 3 determinations

Mean of Seven Determinations

The value t (tabulate at 95% confidence level and at 6 degrees of freedom is = 2.44)

The value of F (tabulated at 95% confidence level and at 6 degrees of freedom = 4.28)

Application of the developed methods

The developed methods were successfully used to determine DHA in tablets and pediatric powders procured locally from pharmacies in Uyo South-South Nigeria. The results so obtained conformed to the label claim (table 2). The results were statistically compared with a reference method from the international pharmacopoeia via student's t-test as a measure of accuracy and variance ratio F-test as a measure of precision at 95% confidence level and at 6 degrees of freedom as shown in table 2. The calculated t – and F values obtain were below the critical (tabulated) values showing a good congruence between the proposed method and the reference methods in terms of precision and accuracy.

The accuracy and practicability of these proposed methods were further tested by performing recovery studies using the standard addition technique. A preanalyzed

tablet/paediatric powder was spiked with known amounts of the pure drug powder and analysed using the proposed methods. The recoveries of the added pure drug were excellent showing that common pharmaceutical excipients did not interfere with the propose methods as shown in table 3.

CONCLUSION

The methods developed as seen were very simple, reproducible and sensitive. The methods were devoid of exhaustive organic solvent extractions. The oxidinetric agent Bromate-Bromide mixture and all other chemicals used were eco-friendly and posed no hazard to the environment and the analyst. These methods as developed could emerge as method of choice especially in this region where sophisticated analytical equipment (e.g HPLC, CMS) are hardly available. The methods are therefore strongly recommended for use by regulatory and drug control agents in field stations and ports of entry for imported drugs.

AKNOWLEDGMENTS

The authors are grateful to the Director of Pharmaceutical Services, University of Uyo Teaching Hospital for providing the pure drug (dihydroartemisinin) and Prof J N Eloff and The Phytomedicine Group of the University of Pretoria for providing the space and equipment for this work.

REFERENCES

1. World Health Organization, fact sheet on the World Malaria Report 2014.
2. World Health Organization (2012): WHO (2009-2012) Report, WHO/AFCO Malaria news letter December 4(2).
3. Nayar GML, Bremam I.G, Newton P.N. Herrieton , Poor-quality antimalaria drugs in South-East Asia and Subsalient Africa. *Lancet Infect Dis.*, 2013, 12: 488-96.
4. World Health Organization,WHO, World malaria report., 2008.
5. White N.J., Antimalaria drug Resistance, *J. Chin. Investigation.*, 2004; 113: 1084 – 1092.
6. Rosendaal J, Fake antimalaria circulating in Cambodia. *Bull Mekong Malaria.*, 2000; 7: 62-68.
7. Rosendaal J. Fake antimalaria drugs in Cambodia, *Lancet*, 2001; 357: (9259) 890.
8. Newton PN, Dondorp A. Green M. Mayxar M. White N. J. Counterfeit antimalarials in Southeast Asia, *Lancet.*, 2003; 362: 169.
9. Newton PN, White N.J. Rosendaal JA, Green MF Minder by Fake drugs: time for international action. *BMJ.*, 2002;324: 800-801.
10. Newton PN, Green M, Fernandez FM, Day NP, White NJ, Counterfeit anti-infective drugs. *Lancet infect Dis.*, 2006; 6: 602-613.
11. Dondorp am, Newton PN, Mayxay M. Van Damme W. and F.M Smithus et al. Fake antimalarials in Southeast Asia are a major impediment to malaria control. Multilateral won-sectional survey on the peralernue of Fake antimalarials. *Trop Med. Int. Health.*, 2004; 9:1246.
12. Atemkeng, M.A. DeCock K. and Plaizer-vercammen, . Quality control of active ingudients in ardimisinims-devirative antimalarials with Kenyan and DR Congo. *Trop Med. Int. Health*, 2007; 12: 68-74.
13. World Health Organization.WHO, Fake drug: A scourge of the system. *WHO Inf.*, 1995; 9: 127-129.
14. WHO counterfeit, fact sheets Revised November 14 2006.
<http://www.ww.Int/Medicines/services/counterfeit/impact/impact F>.
15. Amboise-Thomas P. The tragedy caused by Fake Antimalarial Drugs. *Meditter J. Hemetol. Infection. Dis* 4(1): e 2012, DoI 10.4084/MJHID., 2012; 027.
16. International Pharmacopoeia. 2003.Mnograph for Antimalarial drugs, Artemisinin and Derivatives. Vol.2 . 3rd Edition. WHO, CH 22 N Geneva 27 Switzerland.
17. Lim CK, Lord G, Current developments in LC-MS for pharmaceutical analysis. *Biol Pharm Bull.*, 2002; 25: 547-557.
18. Green M.D., Mount D.L, Wirtz RA, Authentication of Artemether, artiminate and dihydroactemisium antimalarial tablets using a simple colorimeric method. *Trop. Med. Int-Health.*, 2001; 980-982.
19. Na-Bangchang, K. Compunong, K, Hung LW, Molunto, P and Karborarg, J. 1998. Simple High Performance chromatographic method with electrochemical defection for simultaneous determination and dihydroartemisinin in Biological fluids. *Journal of chromatography.*, 1998; 708: 201-207.
20. Naik H., Murry D J.,Kirsch L E and Fleckenstein .Development and Validation of High performance liquid chromatography – mass spectroscopy for the determination of artesunate and dihydroartemisinin on human plasma. *Journal of Chromatogr.B. Analyt. Biomed. Life Sci.* 2005; 816:233.
21. Argawal, S. P, Ali, A, Duay and Ahuja S. Determiation of artemisini in bulk and pharmaceutical dosage form using HPLC. *Indian journal of pharmaceutical science.* 2009; 71: 98-100.
22. Gabriels M and Plaizer-Vercammen J. Development of a Reverse phased thin layer chromatography method for artemisinin and its derivatives. *Science*, 2004; 42(7) 341-7
23. Attih EE., Usifoh C., Ambe A and Eseyin O. A., Quantization titrimetric and spectrophotmetric determination of dihydroartemisium based on the redox reaction with cerium ammonium sulphate. *Journal of chemical and pharmaceutical research.*, 2015; 7(6): 732-738.
24. Basavaiah K. Application of Bromate-bromide mixture and methyl orange in titrimetric, spectrophotometric and kinetic assay methods for cyproheptadine in pharmaceuticals. *Indian journal of Chemical Technology.*, 2006; 360-366.