Determination of Dihydroartemisinin In Bulk And Pharmaceutical Formulations By Redox Titrations And UV – Spectrophotometry Using Potassium Permanganate As Oxidimetric Reagent

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ABSTRACT

Two new methods are proposed for the determination of dihydroartemisinin (DHA) in bulk and pharmaceutical formulations. Method A Titrimetry, based on the redox reaction of DHA and potassium permanganate in acid medium. Method B, UV-Spectrophotometry based on the redox reaction of DHA and potassium permanganate in acid medium giving a product which absorbs UV light at 520nm. In both methods the amount of potassium permanganate used is proportional to the amount of DHA. Experimental conditions for good linearity, sensitivity, specificity accuracy and precision were optimized. In method A (titrimetry) the calculations are based on a 2:5 (DHA: $KMnO_4$) reaction stoichiometry) and this method is applicable over the practical range of 5.0 – 20mgml^{-1}. Method B obeys Beer's law. The calibration curve generated is linear with correlation coefficient of (r) of 0.9998 (n = 10). The molar absoptivity is 2.27 x10^{3} Lmol cm^{-1} and sandell sensitivity of 0.126μgcm^{-2} The limit of detection (LOD) and limit of quantification (LOQ) were determined as per the current ICH guidelines and found to be 0.88 and 2.66μgml^{-1} respectively. Accuracy and precision of both method were determined using intra and inter day variations at three different concentration level of DHA the relative standard deviation (RSD %) were <2.00 and <2.5 respectively. The two methods were used to assay DHA in 4 brands of tablets formulation procured locally in Uyo. South-South, Nigeria at three different concentration level, t and F value of <2.40 and <3.0 at 95% confidence level and 5 degree of freedom. These values are lower than tabulated t and F values. The methods were statistically compared with an official method with congruent results. There was no interference from common pharmaceutical excipients. Recovery study was also performed via standard addition procedure with excellent recoveries.

Keywords: Alaxin, codisin, cotecxin, dihydroartemisinin redox reactions

INTRODUCTION

The scourge of malaria within the tropics is real and malaria remains the foremost cause of mortality and morbidity in the developing countries of the world. Current projections suggest that if global warning remains unchecked, it could re-establish itself in Europe and North America[1].Global warning has impacted upon malaria disease burden making it difficult to predict future disease patterns.[2a,2b.] Despite international efforts to "roll back malaria' the 2008 World Malaria Report revealed the disease still affects approximately 3 billion people in 109 countries, 45 within the WHO African region[3]. However the most critical problem facing the treatment of malaria is the development of resistance to classical quinolne antimalaria compounds such as chloroquin and antifolates such as fansidar (sulphadoxime pyrimethamine. Consequently, to overcome the problem of resistance, the World Health Organization (WHO) and health authorities in malaria endemic countries recommend the use of Artemisinin combination therapy (ACT), that is the combination of an Artemisinin and its derivatives any other anti-malaria drug. Artemisinin and its derivatives induce more rapid reduction of parasite faster than any other know drug [4,5]. This novel quality of Artemisinin and its derivative is in serious jeopardy due to the activity of counterfeiters and drug fakers. Their activities have resulted in the delivery of sub therapeutic doses of these Artemisinin antimalarials to patients thereby risking the development of resistance by the malaria parasites. In fact recent reports show that there is reduced artemisinins sensitivity of *P.falciparum* parasite in Cambodia, Thailand, Myanmar and Vietnam,[6-8].

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The problem of counterfeit or poor quality antimalarials is well established in Africa and the presence of counterfeit arteninisinin has been reported in Africa[9] just as in South-East Asia[10, 11.] If these fake and counterfeit arteminisinins are allowed to follow genuine drugs into African drug market it will precipitate a very serious public health problem. Therefore, the aim of this work is to develop very simple environment friendly and inexpensive method of assay for Artemisinin and its derivatives.

In the international pharmacopoeias, dihydroartemisinin is assayed by HPLC and Uv-Vis spectrophotometry". A careful search of the literature show that many methods have been developed by several workers for the assay of artemisinin and its derivates. These include HPCL[12], LCMS[13], capillary electrophoresis[14]. Thin layer chromatography[15, 16,] .These methods are good and quite sensitive but most of the equipment are expensive and can hardly be procured by majority of the health institutions available here. However, a few method have recently been developed by Newton, *et al* 2003 [17] and Green M. D. *et al* 2001 [18]. In this method only 1% of the surface of the tablet is used for the colourimetric method, just as this method is cheap and good. It is not specific as other drugs like doxycycline, tetracycline also give the yellow colour when reacted with the TR Red salt. That apart, sophisticated counterfeiters can coat the outer surface of tablet with the active ingredient only, in which case only 1% of the tablet is genuine. The method developed here is simple, inexpensive, specific, precise and accurate. A simple redox reaction between dihydroartemisinin and potassium permanganate is performed and the result measured using uv spectrophotometer.

MATERIALS AND METHODS

Apparatus

All absorbance measurements were recorded using Heλ losβ model of uv spectrophotometer, from, Thermo Electron Coperation,USA.

Reagent and Chemicals

All reagents and chemicals used for this work were analytical grade. Stock solutions were prepared using distilled water.

Potassium permanganate (0.02m) (Merck Germany) was prepared by dissolving 3.2g in sufficient distilled water to produce 1,000ml and heating the water bath for 1 hour, then cooled and filtered using glass wool. The resulting solution was then standardized using analar grade oxalic acid.

Sulphuric acid (2M) was prepared by appropriate dilution of concentrated sulphric acid (sigma) Sp. Gr 1.18).

Dihydroantemisinin: Reference drug was a kind gift from the Directorate of pharmaceutical service, University of Uyo teaching hospital and used as given.

The standard dihydroartemisinin solution was prepared in absolute ethanol. For titrimetry a concentration of 1mg/ml was prepared, for spectrophotometric method appropriate dilutions were made to obtain a concentration of 100μg/ml.

Procedure

Titrimetry:

Different 10ml aliquot of standard dihydroartemisinin containing 1.0 – 10 mg were accurately transferred into 100ml beaker using a burette. The content of the beaker was acidified using 8ml of 2M sulphuric acid and mixed thoroughly. Then the total volume in the beaker was adjusted to 20ml by the addition of 2ml of absolute ethanol and shaken to mix well and then titrated against 0.02M KMnO4 solution to a first permanent pink colour. This process was repeated until three consecutive determinations agreed within 0.1ml. The amount of DHA was determined using the equation.

Amount of DHA (mg) = $VM_w S/n$

Where V = ml of KMnO4 reacted

Mw = relative molecular wt of DHA

S = Strength of $KMnO_4$, moles / L

n = number of moles of KMno4 reacting per mole of DHA.

Spectrophotometry

Different aliquots 0.5 – 5.0ml of DHA standard solution containing 10μg/ml were accurately transferred into a series of 10ml calibrated volumetric flask using a micro burette. The volume in the flask was made up to 5ml using absolute ethanol. Then 1ml of 2M H_2SO_4 was added and shaken gently to mix well. This was followed by the addition of 2ml of 0.02M $KMnO_4$. The content of the flask was made up to the mark and swirl gently to mix and the absorbance was then measured at 520nm. A calibration curve was then generated by plotting the measured absorbance vs concentration from where the concentration of the unknown was read. The unknown concentration could also be calculated from the regression equation of the Beer's law data.

Procedure for Tablets:

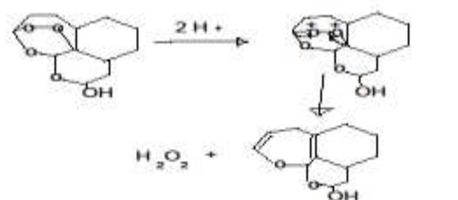
Twenty tablets of DHA (local brand) were weighed singly to determine the average weight of each tablet. The 20 tablets were then pulverized into

very fine powder. Then an amount of the powder equivalent to 100mg was carefully measured and transferred into a 100ml beaker and 20ml of absolute ethanol was added and sonicated for 20 minutes and transferred into a 100ml standard volumetric flask. Then 40ml of ethanol was added and shaken vigorously to extract the drug into the solution. Finally the content was made up to the 100ml mark and shaken well and then filtered using a Whatman filter paper No.42. The first 10ml portion of the filtrate was discarded. The resulting drug solution was 100mg/ml from where suitable aliquot was used for the titrimetric method. For the spectrophotometric method the drug solution was diluted step wise to obtain a concentration of 100µg/ml from where a suitable aliquot was then analysed.

RESULTS AND DISCUSSION

For more than a century potassium permanganate has been used as a valuable oxidising agent in redox titrimetry[19] [20] [21].

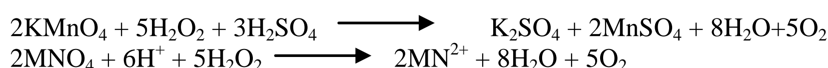
Quite recently several drugs have been assayed using potassium permanganate as the oxidimetric agent. Some of the drugs include Pantoprazole[22], Pipazethate HCl and dextromethorphan HBr [23] Cefixidine[24] Tramadol HCl [25]. Dihydroartemisinin contain the endoperoxide bond which is inextricably linked to its schizonticidal activity against blood schizonts of plasmodium. Under acid conditions as shown in the scheme 1 below it is likely that hydrogen peroxide is generated due to the protonation of the two oxygen centres of the endrperoxide bond.



Scheme 1: H_2O_2 Generated *in situ* by the cleavage of the endoperoxide bond

Reaction Scheme 1

In this proposed method the determination of percentage weight of hydrogen peroxide generated in situ depends upon mutual oxidation – reduction of potassium permanganate and the hydrogen peroxide. This two oxidizing agent mutually reduce each other with evolution of oxygen as shown in the following equation.



$$2KMnO_4 + 5H_2O_2 + 3H_2SO_4 \longrightarrow K_2SO_4 + 2MnSO_4 + 8H_2O + 5O_2$$

$$2MNO_4 + 6H^+ + 5H_2O_2 \longrightarrow 2MN^{2+} + 8H_2O + 5O_2$$

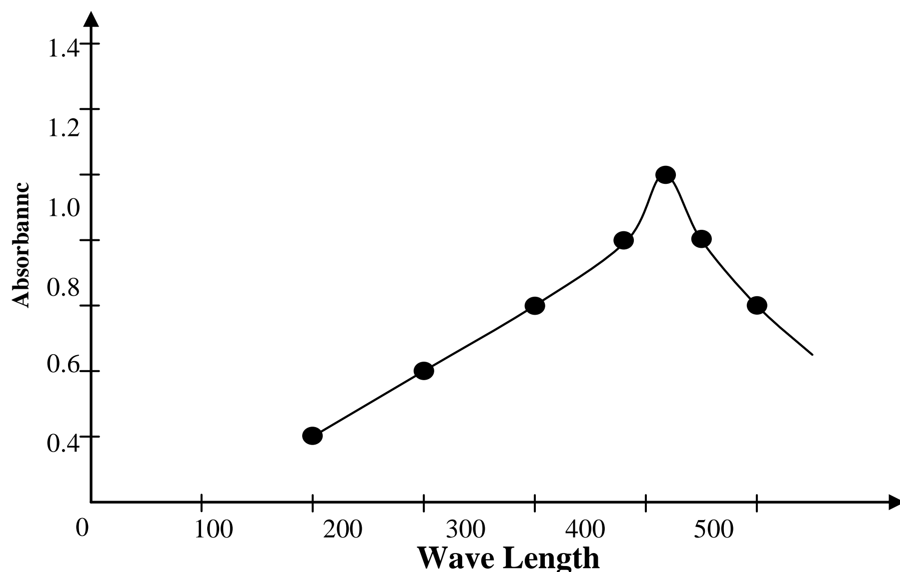


Fig 1 Absorption Spectra of the oxidation product of 10µg/m of DHA and $KMno_4$
5"O" comes from $5H_2O_2$
$5H_2O_2 = 50 = 10H$ ($5H_2O_2 = 2MnO_4 = 10e$)

Based on the quantitative equation the titrimetric and spectrophotometric determination were carried out in acid conditions. The amount of potassium permanganate consumed was found to be proportional to the amount of H_2O_2 in the solution in this case dihychoartemisinin.

Method Development
The experimental variables of this reaction were carefully tested and optimized for maximum accuracy and precision. The optimum conditions for these methods were obtained by varying the parameters one at a time while keeping others constant and observing the effect produced on the absorbance.

Effect of Acid: Ten ml (10ml) each of three different types of acid. Sulphuric acid phosphoric acid and ethanoic acid (H_2SO_4, H_3PO_4 and CH_3COOH). Were used for this redox reaction. Of the three, sulphuric acid was found to be the most suitable, even though phosphoric acid gave some promise, but need very high concentration to obtain optimum reaction. All other variables were kept constant while the volumes of the acid were changed. The molar concentration of the acid used were also measured. It was found that 1ml of 2M sulphuric acid was most suitable for this redox reactions. Hydrochloric acid and Nitric acid were not used for obvious reasons. Hydrochloric acid in this case liberate chlorine which interferes with the redox reaction and Nitric acid itself is an oxidizing agent.

Effect of Concentration of Potassium Permanganate ($KMnO_4$)
The linear relationship between the decrease absorbance and the volume of $KMnO_4$ used was confirmed by varying the volume of $KMnO_4$ used each time, and keeping other variables constant. The decrease in absorbance was proportional to the volume. The volume that gave the optimum absorbance was 2ml of 0.02M $KMnO_4$. Further addition of small amount of 0.02M $KMnO_4$ gave no further absorbance in the spectrophotometric method. For the titrimetric method 0.02M $KMnO_4$ was found to be suitable.

Method Validation
Linear range and suitability
Of the range investigated the amount of 0.02M $KMnO_4$ consumed was found to be proportional to the amount of H_2O_2 present in the solution.

The percentage content was found to be 110% $\pm$ 1.12. The spectrophotometric method, had a calibration curve which was linear within the range o f 2.5 to 50µg/ml^{-1} of DHA.

The calibration curve generated by plotting the measured absorbance values against the concentration of DHA using the least square method had the calibration equation of A = 0.95C (where C is the concentration of the drug in µg/ml). With a regression coefficient 0.9998 (n=10). The molar absorptivity and Sandell sensitivity were calculated/determined based on the ICH guidelines and found to be 2.25 x 10^3 l Mol^{-1} $cm^{-1}$and 0.12µg/cm^2 respectively.

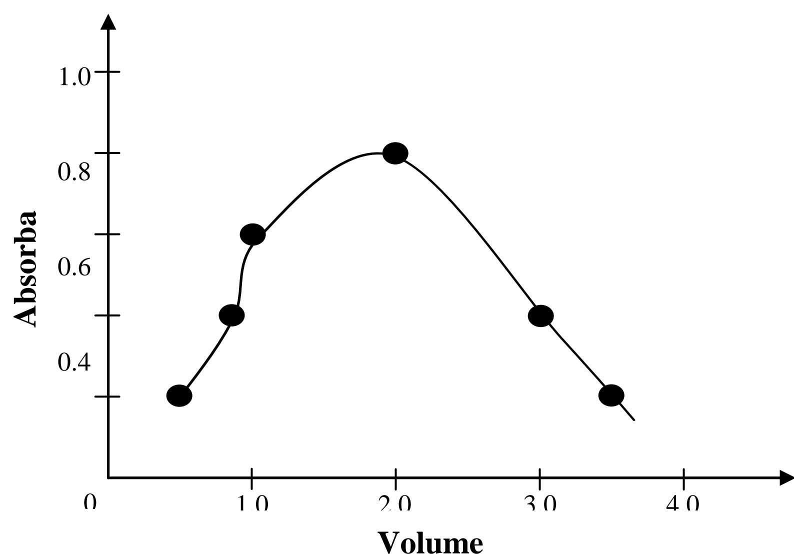


Fig. 2: Effect of volume of 0.0 2M $KMnO_4$ on the Absorbance of the product of the redox reaction of $KMnO_4$ and DHA

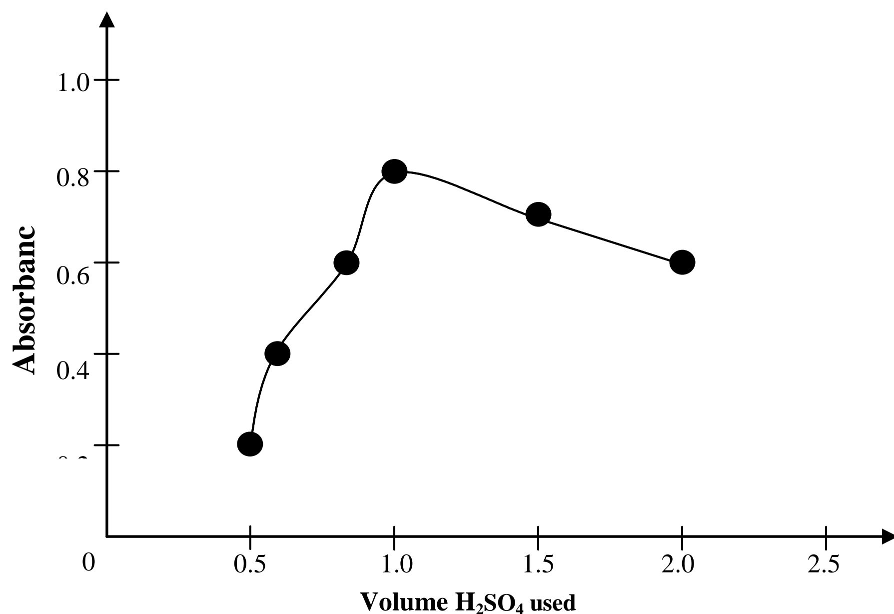


Fig. 2 Effect of the volume of 2M H_2SO_4 on the Absorbance of the product formed by the redox reaction of $KMnO_4$ with DHA

The limit of detection and limit of quantification were also calculated based on ICH guidelines using the following equation LOD = 3.3 SD/slope and LOQ = 10SD/slope where SD in the standard deviation of replicate determination values under the same conditions as the sample analysis in the absence of the analyte, while S is the slope of the calibration curve.

The LOD and the LOQ were found to be 0.88 and 2.66μgml^{-1} respectively.

Selectivity
The efficiency and the selectivity of these analytical methods were determined by a systemic study of additives and excipients usually present in tablet formulation (e.g. glucose, starch, lactose calcium carbonate, sodium alginate, magnesium stearate, talc, and methyl cellulose). Ten (10) milligrams each of this excipients were extracted with ethanol and the solution made as discussed under the procedure for tablet above. A suitable aliquot of the resulting solution was subjected to analysis by titrimetry and spectrophotometry using the recommended procedure. There was no measurable change in the absorbance. An error of not more than 1.8% in the absorbance was observed indicating that there was no reasonable interference from the pharmaceutical excipient tested. To further test for selectivity and specificity, a placebo blank was prepared similar to the one described above with these excipient in the synthetic mixture, 100µg of dihydroartemisinin was added and shaken vigorously to mix well and the resulting solution was then subjected to analysis using the procedure for tablet as describes above. Ten (10) ml of the resulting solution was then analysed using titrimetric method (n=5). The observe percentage recovery was 108% ± 0.82. This solution was diluted stepwise to obtain a concentration of 100µg/ml of DHA from this 5ml aliquot was analysed using the spectrophotometric method (n=6) the percentage recovery was observed to be 103% with a standard deviation of 0.58. This showed that the method was specific as it was only applicable to aliquots that contain the analyte.

Accuracy and Precision
To determine accuracy and precision of these proposed methods, solutions of DHA were prepared and analysed in replicates at 3 concentration levels. The precision was determined as the relative standard deviation and accuracy was determined as percentage relative error (Er%) of the suggested methods was calculated at 95% confidence level. The percentage relative error was determined from the equation.

$$Er\,\% = \left(\frac{Found - Taken}{Taken} \times \frac{100}{1} \right)$$

The intra day and inter day precision and accuracy results are as shown in table 1 below. The results for the accuracy and precision showed that both titrimetric and spectophotometric methods are repeatable and reproducible.

Table 1: Intra day and inter day Accuracy and Precision

	Intra day Accuracy & Precision				Inter day accuracy and precision		
Method	DHA taken	DHA Found	ER %	RSD%	DHA found	ER%	RSD %
Titrimetry	3.00 5.00 7.00	3.05 5.03 7.06	1.60 0.60 0.80	1.40 1.31 1.50	3.05 5.05 7.07	1.60 0.60 1.00	1.58 1.10 1.40
Spectrophotometry	3.00 5.00 7.00	31.03 51.60 71.80	3.00 2.40 2.50	1.30 1.10 1.21	31.00 51.20 71.80	3.00 2.40 2.50	1.60 1.20 2.00

ER relative Error. RSD – Relative Standard deviation for titrimetry, DHA taken is in milligrams and that of spectrophotometry is in µg/ml.

Table 2:Result of analysis of tablet using these proposed methods

Tablets analysed	Label Claim (mg)/tablet	Found (percent tablet chain ± SD)		
		Reference method	Titration	Spectrophotometry
Alaxin	60	110.2%+1.20	109.4 ± 1.2 t = 1.63 F = 1.15	109.9 ± 1.40 t = 0.52 F = 1.56
Cotecxin	60	110.2% ±1.12	109±1.72 t = 1.73 F = 2.36	109.8 ± 1.20 t = 0.816 F = 1.44
Codisin	60	110.2% ± 1.12	108.95 ± 1.50 t = 2.04 F = 1.77	109.8% ±1.3 t = 0.75 F =1.35
Santecxin		110.2% + 1.12	109.96 ± 1.24 t = 0.59 F = 1.23	109.8 ± 0.90 t = 1.08 F = 1.55

* Mean of 6 determinations, calculation t and F values shown in the table. Tabulated t and f – values for 5 degrees of freedom at 95% = 2.57 and 5.05 respectively.

Table 3: Further assessment of Accuracy of the methods by recovery experiments (via standard addition method)

S/N	Brand (Tablet studied)	DHA in Tablet (mg)	Pine DHA added μg	Total fund	Pure DHA % ± SD	DHA Tablet μg/ml	Pure DHA added μgml^{+1}	Total DHA found μgml^{-1}	Pure DHA recovered pure ± SD
1.	ALAXIN	1.80	1.0	2.75	98.5 ±1.11	20.25	20.0	40.30	100 ±1.12
		1.80	2.0	3.78	99.5 ±1.10	20.25	40.0	60.38	100.2±1.13
		1.80	3.0	4.90	102.5 ±1.01	20.25	60.0	80.24	99.9 ±1.14
2.	CODSIN	2.08	1.0	3.07	99.7 ±1.13	44.20	20.0	64.60	101 ± 0.65
		2.08	2.0	4.10	100.5 ±0.86	44.20	40.0	85.00	101±1.13
		2.08	3.0	5.09	100.2 ±1.12	44.20	60.0	105.00	100.7 ± 1.65
3.	COTECXIN	1.50	1.0	2.49	99.6 ±0.91	42.80	20.0	62.70	99.8 ±1.46
		1.50	2.0	3.55	101.7±0.53	42.80	40.0	82.68	99.8 ±1.46
		1.50	3.0	4.70	104.4±1.12	42.80	60.0	104.4	101.1±0.16
4.	SAWTECXIN	1.20	1.0	2.32	105.4±1.61	31.50	20.0	53.08	103±0.84
		1.20	2.0	3.18	99±1.51	31.50	40.0	72.35	101.1 ±1.17
		1.20	3.0	4.28	101.9 ±1.10	31.50	60.0	93.20	102 ±1.16

For the accuracy = Mean value of 3 determination ± SD

Analytical Applications

The proposed methods were applied to DHA brands procured from Pharmacy shops in Uyo, South-South Nigeria. These brands include Alaxin, cotecxin, codisin and santecxin. The results obtained were statistically compared with the reference method in the international pharmacopoeia, using the t-test and f-test. The calculated t and f values were lower than the tabulate value at 95% confidence level and for 5 degrees of freedom.

CONCLUSION

The methods, one titrimetric and one spectrophotometric method were developed for the effective determination of dihydroartemisinin in bulk and tablet formulations based on very simple redox reactions between dihydroartemisinin and potassium permanganate. These methods were validated using the current ICH guidelines. The two methods are simple, precise, rapid and economical. The methods were devoid of solvent extraction leading to excessive loss of expensive organic solvents and unnecessary exposure to toxic effect of the chemicals to the analyst and the environment. Titrimetry as usual is the simplest and most straight forward and analytical method and will be very useful in small scale pharmacies who deal directly with most of the local populace. The two developed methods were used to determine tablets of different brands of dihydroartemisinin in Uyo, South, South Nigeria. The titrimetry method had a range of (5-20mg/ml) while spectophotometric method had a dynamic range of 5.0μg - 50μg/ml. The enormous advantages of these two developed methods cannot be over emphasized because of their simplicity while the titrimetry method is very affordable to the smallest licensed drug dealer, the spectrophotometric method is affordable to wholesale and distributors. Counterfeit and fake arteminisinin can be checked at this two point of the supply chain. With these two methods the spread and sale of counterfeit arteminisinin will be in check.

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