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# Simple Spectrometric Determination of Artesunate and Dihydroartemisinin in Pharmaceuticals using Potassium Iodate and Indigo Carmine as Reagents

Emmanuel Attih*, Aniekan Ebong, Ekarika Johnson and Emmanuel Etim

Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmacy, University of Uyo.

## ABSTRACT

The distribution of counterfeit artemisinin derivatives in sub-Saharan has reached a dangerous level. Based on this, a simple sensitive and reproducible method is developed for the assay of artesunate (ART) and dihydroartemisinin (DHA) in tablets. The method is based on the reaction of the drug with a known excess of the antioxidant (potassium iodate) in acid medium and thereafter determination of residual antioxidant by reacting with a fixed amount of indigo carmine n and measuring the absorbance at 610 nm. Absorbance is proportional to increasing drug concentration. The method obeys Beer's law at the range of 0.5 – 5.0 and 0.5 – 6.0 for ART and DHA respectively. The correlation coefficient of the curve generated by least square method was 0.9989 and 0.9996 for ART and DHA respectively. The molar absorptivities were $2.0 \times 10^4$ and $2.1 \times 10^4$ L/mol/cm and Sandell sensitivity of 0.0192 and 0.013 µg/cm$^2$ respectively for ART and DHA. Limit of detection and quantification were 0.48 and 1.58, and 1.48 and 1.58 µg/mL respectively for ART and DHA. The intraday and inter day accuracy (R.E.%) and precision (RSD%) were ≥ 1.0 ≤ 2.6 in all cases. The method was statistically compared with an official method via student t-test and Varian Ratio test F-tests and used successfully to evaluate ART and DHA tablet procured in Uyo metropolis. The accuracy of the method was further tested by performing recovering study via standard addition method with excellent necrosis raging ≥ 99.9 ≤ 103 and standard deviation of between 1.07 – 1.86 meaning that the excipients have no effect on the performance of the method.

Key words: Artesunate, Dihydroartemisinin, Indigo carmine, Redox reaction, Potassium iodate

## INTRODUCTION

We may soon encounter untreatable malaria if the current trend of manufacture and distribution of counterfeit/fake artemisinin antimalarial is not put under control. There has been reports of massive manufacturing and distribution of counterfeit and fake artemisinin derivative in southeast Asia especially in the Greater Mekong area (Rozendaal 2001, Newton *et al.,* 2001, Newton *et al.,* 2003, Newton *et al.,* 2006). Unfortunately, these counterfeit antimalarial are massively imported into malaria-endemic sub Saharan African countries (Atemkang *et al.,* 2007, Nayyar *et al.,* 2012). These substandard drugs can directly be linked to the emergence of multidrug resistant plasmodium species in Southeast Asia especially, in the Grarter Mekong region (WHO, 2014). Originally malaria was usually endemic in the tropical region of the world, but with the twin factors of global warming and international travel, malaria has spread to other parts of the world and could be reestablish in Europe and America (Robert *et al.,* 2001, Duew *et al.,* 2006, McMichael *et al.,* 2006). Bearing this in mind, if the emergence of multidrug resistant species of *Plasmodium* is not checked then that may precipitate serious public health crises. The World Health Organization defined counterfeit drugs as those which are "deliberately mislabeled with respect to identity and/or source. Counterfeiting can apply to both branded and generic product with counterfeit products including drugs with the correct ingredients or with the wrong ingredients, without active ingredients, with insufficient active ingredients or with fake packaging". The producers of counterfeit/fake drugs especially antimalarial are very sophisticated and can produce drug with holograms that have manufacturing and expiry dates looking absolutely genuine which can fool drug quality control government agents even and pharmacist in the field (Ambroise-Thomas, 2012). Apart from the danger of multidrug resistance a real and potent danger is the use of substandard pharmaceutical excipients. Some of these pharmaceutical are quite dangerous and are known carcinogen and example of these are safrole and melannine (Ambroise-Thomas, 2012). Due to the high schizonticidal property of the artemisinin antimalarial the WHO and malarial endemic countries adopted the use ACT (Artemisinin Combination Therapy) as first line treatment of malaria. This means the combination of artemisinin derivative and another drug for the treatment of malarial. The effectiveness of these artemisinin derivatives has made them serious candidate for massive faking and counterfeiting.

*Corresponding Author: Email: emmanuelattih@uniuyo.edu.ng, Phone 07036671935

The fight against counterfeit/fake artemisinin derivative require highly technical laboratory with state of the art analytical equipment since counterfeits are sophisticated These equipment are hardly procured by malaria endemic countries because the cost is prohibitive. Those previously donated by donor countries and organization are hardly functional due to epileptic power supply in these countries. This research work is to develop very simple, sensitive, cost effective method for the assay of artemisinin derivatives dihydroartemisinin and Artesunate (DHA and ART) in tablets current in sale in Uyo, metropolis in Akwa Ibom State Nigeria

Dihydroartemisinin and Artesunate are officially assayed by HPLC and UV-Vis spectrophotometer and titrimetry. A careful search of the literature shows that some workers have used several methods for the assay of DHA and ARTs these methods include HPLC, HTPLC, UV-vis spectrophotometry (Attih, *et al.,* 2015, 2016; Adefoke and Soye, 2011). These methods are quite sensitive in the present circumstances. However most of the methods have some obvious short coming such an high pH control, solvent extraction with organic solvent that could be hazardous to the environment or the analyst, some even require serious heating. To the best of our knowledge no method so far has been developed using potassium iodate for the assay of DHA and ACT in tablets. This method could be used in routine laboratories and in the field for the assay of DHA and ART in tablets even at the point of entry of drug.

**Redox – Reaction based on**

Spectrophotometric determination of Artesunate and Dihydroartemisinin based on Redox reaction with potassium iodate

**Materials and Method**

**Apparatus**: All spectral measurements were carried out using UV-VIS spectrophotometer Heλlos β model from Thermo Electron Corporation, USA. With 1cm matched quartz cell.

**Chemicals and Reagents**

**Potassium iodate**: 5% solution was prepared by dissolving 12.5g of the chemical (BDH England) in 20 mL of distilled water and shaken to dissolve completely. This was then diluted to make up to 250 mL is a 250 mL capacity volumetric flask.

**Indigo carmine**: 2000 µg/mL solution was prepared by dissolving 22.2 mg of the dye (90%) in enough distilled water to make up to 100 mL in a 100 mL volumetric flask.

**Hydrochloric Acid (2M)**

**Sulphuric Acid (5M)** Solution was prepared by diluting 272 mL of the concentrated sulphuric acid (BDH England) (Sp.gr. 1.84) with 728 mL of water slowly.

**Standard Artesunate Solution (200 µg/mL)**: Stock standard solution of Artesunate was prepared by carefully weighing out 100 mg of pure Artesunate (donated by the Director of Pharmaceutical Services, University of Uyo Teaching Hospital) and transferred into a 100 mL capacity volumetric flask and diluted with distilled to the 100 mL mark. The resulting solution with 1 mg/mL standard drug solution was further diluted appropriately to obtain 200 µg/mL using distilled water.

**Standard Dihydroartemisinin Solution (100 µg/mL)**: Pure DHA powder was donated by the Director of Pharmaceutical Service University of Uyo Teaching Hospital. Standard Stock solution was prepared by measuring 100 mL of the pure DHA powder and carefully transferred into a 100 mL capacity volumetric flask and dissolved in enough 98% ethanol to make up to the 100 mL mark of the volumetric flask. The resulting DHA solution with a concentration of 1mg/mL was further diluted to give a working concentration of 100 µg/mL for the analytical work.

**General Procedure (Artesunate)**

Aliquots of pure ARTS solution (0.5 to 4.0 mL) with the concentration of 200 µg/mL were accurately transferred to a series of 10 mL capacity calibrated volumetric flask. Distilled water was used to adjust the volume in the flasks to 4 mL. One (1 mL) of 5M sulphuric acid was added to each of the flask and 2 mL of the 5% potassium iodate was added and swirled gently to mix well. The resulting mixture was allowed to stand for about 15 minutes with gently shaking from time to time within the 15 minutes. Finally 0.5 mL of the indigo carmine (200 µg/mL) was added to the flask and diluted to the 10 mL mark of the 10 mL calibrated flask. The content of the flask was mixed well and the absorbance of the resulting solution was measured at 610 nm against reagent blank prepared exactly as the analyte solution but without the ART solution. A standard curve was generated by plotting absorbance vs concentration of ART and the concentration of the unknown was read from the standard curve or deduced from the regression equation derived from Beer's law data (Attih *et al*., 2016)

**General Procedure (DHA)**

Different aliquots of standard stock solution of DHA (0.5 to 4.0 mL) with a concentration of 100 µg/mL were carefully measured and transferred into a series

of 10 mL capacity calibrated volumetric flask. Ethanol (98%) was used to adjust the content of the flask to 4.0 mL. The content of the flask was acidified using 1 mL of 5M sulphuric acid and shaken to mix well and allowed to stand for 15 minutes. The solution in the flask was swirled gently from time to time during the 15 minutes. Finally 0.5 mL of the indigo carmine (200 µg/mL) was added to the flask and diluted using ethanol to the 10 mL mark of the calibrated volumetric flask. The content of the flask was mixed well and the absorbance was measured at 610 nm against reagent blank prepared similarly but without the DHA. A standard curve was generated by plotting absorbance against DHA concentration from where the concentration of the unknown is determined or deduced from the regression equation derived using Beer's law data (Attih *et al*., 2016)
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**Procedure for Tablets**

Twenty tablets of ART (or DHA) were randomly selected, weighed and crushed using mortar and pestle into a fine powder. A portion of the powder equivalent to 100 mg was weighed and transferred to a 100 mL capacity volumetric flask containing 40 mL of distilled water (Ethanol 98% in case of DHA).The mixture in the flask was shaken vigorously to extract the drug. The content of the flask was made up to the 100 mL mark and shaken vigorously and filtered using Whatman filter paper No. 42. The first 10 mL of the filtrated was discarded. The resulting mixture with a concentration of 1mg/mL was diluted appropriately to obtain the working concentration of 200 µg/mL ART and 100 µg/mL of DHA from where a suitable aliquot was analyzed (Attih *et al*., 2015).

**Procedure for Placebo Blank**

Pharmaceutical excipients were used to prepare the placebo blank. The blank with the composition of methyl cellulose (5 mg), talc (5 mg), sodium alginate (5 mg), sodium citrate (5 mg) magnesium stearate (5 mg), lactose (5 mg) maize starch was added to bring the mixture to 100 mg. This mixture was thoroughly mixed and homogenized. A solution was prepared as described in the procedure for tablet assay above. A suitable aliquot was prepared and analyzed as per the general procedure as discussed above (Attih *et al*., 2015)

**Procedure for Assay of the Drug in Synthetic Mixture**

To prepare a synthetic mixture of the drugs, 100mg of ART (or DHA) powder with 100 mg of the placebo blank powder as prepared above and homogenized. Then 100 mg of the resulting synthetic mixture was carefully measured and transferred into 100 mL capacity volumetric flask containing 20 mL of water in the case of ART and ethanol in the case of DHA. The mixture was sonicated for 10 minutes and further shaking vigorously for 20 munities to extract the drugs. Subsequently the resultant solution was made up to the 100 mL mark with distilled water (ethanol in case of DHA) and filtered using Whatman filter paper No. 42. The first 10 mL of the filtrate was discarded and the resulting solution with the concentration of 1 mg/mL was diluted appropriately to 200 µg/ mL and 100 µg/ mL for the spectrophotometric analysis. (Attih *et al*., 2015)

**Results and Discussions**

Hydrogen peroxide is liberated *in situ* by the action of acid in ART (or DHA); this is because the endoperoxide bond of the drug is cleaved. The Potassium iodate reacts with the hydrogen peroxide. This redox reaction leads to the liberation of iodine which destructively bleaches the dye (indigo carmine). The proposed method is based on the oxidation of the drugs (ART or DHA) by a measured excess of potassium iodate. Increasing concentration of the drugs results in decreasing amount of iodine available for the oxidation of the dye leading to increasing absorbance of the chromogen formed which absorbs maximally at 610 nm. In this case the increase in concentration of the drug in proportion to the absorbance.

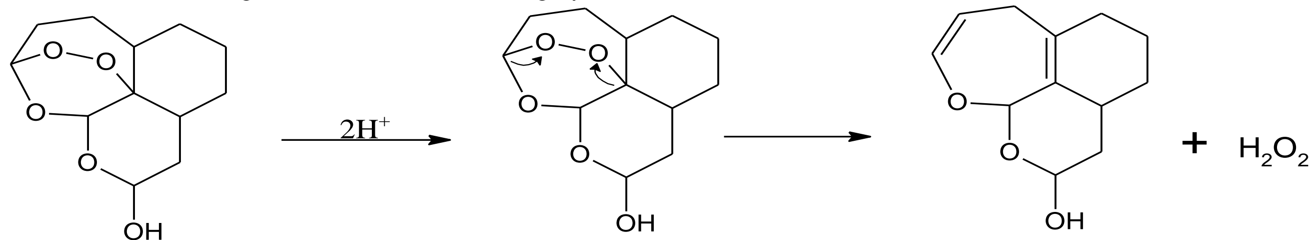
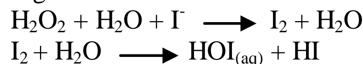
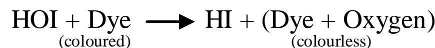



Figure 1: Reaction of acid with ART/DHA to release $H_2O_2$



$H_2O_2 + H_2O + I^- \longrightarrow I_2 + H_2O$

$I_2 + H_2O \longrightarrow HOI_{(aq)} + HI$



HOI + Dye (coloured) ⟶ HI + (Dye + Oxygen) (colourless)

**Optimization**
Experimental conditions responsible for the production of the coloured chromogen were carefully studied and optimized. This was done by varying values of a particular variable and keeping others constant and then observing its effect on the absorbance. Optimum condition for the redox reaction of potassium iodate and the drugs (ART or DHA) were maintained throughout the experiment.

**Effect of Acid**
Sulphuric acid, hydrochloric acid, ethanoic acid, and methanoic acid were used in the preliminary experiment. Sulphuric acid and hydrochloric acid were suitable. Ethanoic and methanoic acids gave results that were not qualitative. Nitric acid was not used because it is an oxidizing agent on its own. Sulphuric acid was found to be the most suitable for the formation of the yellow product. The value of the absorbance was not affected when 0.8 – 1.5 M of sulphuric acid was used. Beyond this higher concentration gave erratic and less sensitive results. Hence 1 mL of 2 M sulphuric acid gave optimum absorbance.

**Effect of Time**
Effect of time on the redox reaction of potassium iodate and the drugs (ART and DHA) was studied. The time taken for the complete yellow iodine colour was 15 minutes. At this time liberation of iodine was complete. The reaction of the liberated iodine and the dye indigo carmine was completed instantaneously. At this point, constant absorbance was observed. The reaction time was varied for 15 – 30 minutes and the colour was still stable.

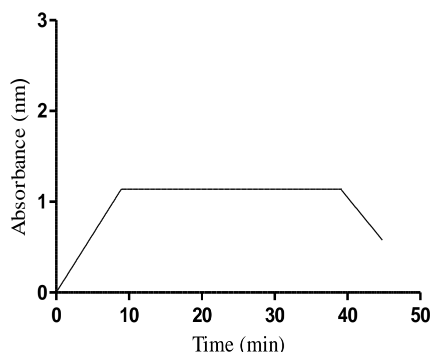



Figure 2: Effect of Time

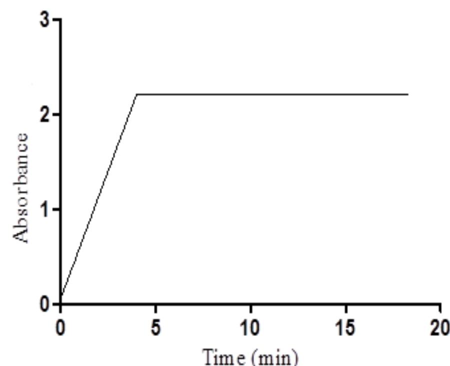



Figure 3: Effect of concentration

**Effect of Iodate Concentration**
The effect of iodate concentration was studied by keeping the concentration of the drugs (ART or DHA) constant and varying the concentration of iodate and the absorbance measured. The values of the absorbance remained fairly constant in the range of volume 1 – 3 mL in total reaction volume of 10 mL. Further increase in the volume of iodate showed erratic absorbance duet to instability of the formed chromogen, Hence 1 mL of 5 % $K1O_3$ in a total volume of 10 mL was adequate for this analysis.

**Effect of the concentration of Indigo carmine**
The effect of the concentration of indigo carmine in the formation of the chromogen was studied in the range of (0.4 – 1.0 mL) of 200 µg/mL. The best absorbance was obtained when 0.5 mL of 200 µg/mL of indigo carmine was used. There was no change in absorbance up till when 1.0 mL of indigo carmine was used. Therefore 0.5 mL of 200 µg/mL was used for the method.

**Method Validation**
The proposed method was validated for linearity, accuracy and precision, selectivity robustness and ruggedness.

**Linearity and Selectivity**
There is a linear relationship between the drug (ART and DHA). The absorbance was found to be proportional to the drug concentration. Beer's law was obeyed in the range of 0.5-50 µg and 0.5 – 60 µg/mL respectively for ART and DHA. The stoichiometry was found to be 1:1 for drug and the oxidant. The calibration curve generated via the least square method showed a correlation coefficient of 0.9989. The equation for the curve was in the form of a linear equation of $A = bc + d$. Where A is the absorbance b is the gradient and d the intercept, which was very negligible.

The apparent molar absorptivity and Sandell sensitivity and other sensitivity parameters such as LOD and LOQ limit of detection and limit of quantification were determined as per the current ICA guidelines (International Committee on Harmonization). Using the formulae (Attih *et al.*, 2017)

$$\text{LOD} = \frac{3.3\sigma}{S}$$

$$\text{LOQ} = \frac{10\sigma}{S}$$

where σ is the standard deviation of five reagent blank and s the slope of the calibration curve.
All these values are recorded in Table 1

Table 1: Sensitivity and Regression parameters

| S/N | Parameter | Values | |
|---|---|---|---|
| | | ART | DHA |
| 1 | λmax (nm) | 610 | 610 |
| 2 | Linear range (µg/mL) | 0.5-5.0 | 0.5-6.0 |
| 3 | Molar absorptivity (mol/cm | $2.0 \times 10^4$ | $2.1 \times 10^4$ |
| 4 | Sandell Sensitivity (µg/cm$^2$) | 0.0192 | 0.013 |
| 5 | Limit of detection (µg/mL) | 0.48 | 0.50 |
| 6 | Limit of Quantification (µg/ml) | 1.48 | 1.58 |
| 7 | Intercept | 0.016 | 0.017 |
| 8 | Slope | 0.012 | 0.011 |
| 9 | Regression | 0.9989 | 0.9996 |

**Precision and Accuracy**

Intraday and interday precision and accuracy were evaluated by conducting 6 duplicate (n=6) determinations at three concentration levels of the pure standard drugs (ART or DHA) solution. The relative standard error (R.E.%) i.e. accuracy was determined using the formula (Attih *et al.*, 2017)

$$\%\,\text{R.E.}\,(\text{accuracy}) = \frac{Amount\ of\ drug\ found\ X\ Amount\ of\ drug\ taken}{SAmount\ of\ drug\ taken}$$

The relative standard deviation (RSD %) was used to determine the precision of the prepared method. All the values were low, in fact the values for both accuracy and precision were ≥ 3.0 % in all cases, indicating high accuracy and precision of the prepared method. The values are recorded in Table 2.

Table 2: Evaluation of inter and intraday accuracy and precision

| | Amount of Drug taken | Inter and intraday accuracy and precision | | | Inter and inter day accuracy and precision | | |
|---|---|---|---|---|---|---|---|
| | | Amount found | RE% | RSD% | Amount found | RE% | RSD% |
| ART | 30 | 30.70 | 2.33 | 1.04 | 30.72 | 2.40 | 1.07 |
| | 60 | 61.48 | 2.47 | 1.10 | 61.47 | 2.45 | 1.10 |
| | 90 | 92.12 | 2.36 | 1.05 | 92.11 | 2.34 | 1.05 |
| DHA | 30 | 30.73 | 2.43 | 1.10 | 30.75 | 2.50 | 1.11 |
| | 60 | 61.50 | 2.50 | 1.12 | 61.49 | 2.48 | 1.11 |
| | 90 | 92.15 | 2.38 | 1.07 | 92.13 | 2.37 | 1.00 |

**SELECTIVITY**

The placebo blank and the synthetic mixture method as described earlier was the method used to determine the selectivity of the proposed method. In all cases the proposed method gave very good results and excellent recoveries ranging between ≥ 98.9 ≤ 120 with a standard deviation of 1.20-1.62. Showing that there was no interference from pharmaceutical excipients which are usually co-formulated with the drug.

**Robustness and Ruggedness**

The robustness of the proposed method was tested by observing two variables. The acid concentration and the reaction time were altered deliberately in small increment and observe their effect the overall results and the recoveries. It was discovered that deliberate

and minor increments of these variables had no adverse effect on the overall results. The ruggedness was determined by having the whole experimental process performed by two different analyst on two other spectrophotometers. The result showed the method was rugged and there was to large difference in the overall values obtained as per recoveries.

**Application of the Proposed Method to the Assay of Tablets**

To test the suitability and analytical applicability of the proposed method to the determination of ART and DHA in tablets, the proposed method was used to analyze tablets procured locally from community pharmacy shop in Uyo metropolis, in South-South Nigeria. The results obtained were statically compared to official pharmaceutical method (International pharmacopeia, 2005) via student's t-test (accuracy) and Varian's ratio F-test (precision) at 95% confidence level and at degrees of freedom of 4. The calculated values were lower than the tabulated value (t = 2.77 and F = 6.57). This showed there is some congruence of the proposed method with the official pharmaceutical method. These values are recorded in Table 3.

**Table 3:** The results of Analysis using the Proposed method compared with official pharmaceutical method

| S/N | Commercial tablets studied | Label claim | Reference method ± SD | Proposed method ± SD |
|---|---|---|---|---|
| 1 | ARTS Lever Artemisinin | 50 | 110.0 ± 1.20 | 110.8 ± 1.32<br>F = 1.21, t = 0.90 |
| 2 | Articin (Evans) | 50 | 110.0±1.18 | 111.0 ± 1.15<br>F = 1.05, t = 1.21 |
| 3 | DHA CONTECXIN | 60 | 110. ± 1.20 | 111.2 ± 0.06<br>F = 1.83, t = 1.49 |
| 4 | SAWTECXIN | 60 | 110.0 ± 1.22 | 111.4 ± 1.44<br>F = 1.39, t = 1.57 |

**Recovery Studies**

To further ascertain the practicability and accuracy of the proposed method, recovery studies were performed via standard addition method. In this method, a pre-analyzed tablet powder was spiked with pure ART or DHA powder at 3 different concentration level and the total was determined using the proposed method. The recoveries of the pure drug powder added (ART or DHA powder) were excellent in the range of ≥ 98 ≤ 104% with standard deviation of between 1.15 – 1.86. This values are recorded in Table. 4

Table 4: Results of Recovery Study via Standard Addition Method.

| Drug formation studied | Amount of tablet (µg/ml) | Amount of pure drug added | Total amount found | % Recovery of pure drug ± SD |
|---|---|---|---|---|
| ARTS | 40.20 | 20.0 | 60.6 | 102.0 ± 1.50 |
| Lever Artesunate | 40.20 | 40.00 | 81.00 | 102.0 ± 1.16 |
| | 40.20 | 60.00 | 100.62 | 100.7 ± 0.98 |
| Articin (Evans) | 41.00 | 20.00 | 60.98 | 99.9 ± 1.86 |
| | 41.00 | 40.00 | 81.60 | 101.5 ± 1.05 |
| | 41.00 | 60.00 | 101.50 | 101.0 ± 1.10 |
| DHA | 45.10 | 20.00 | 64.90 | 99.0 ± 1.20 |
| Cotecxin | 45.10 | 40.00 | 84.90 | 99.0 ± 1.15 |
| | 45.10 | 60.00 | 106.15 | 101.8 ± 1.07 |
| SAWTECXIN | 50.10 | 20.00 | 70.40 | 101.5 ± 1.12 |
| | 50.10 | 40.00 | 91.09 | 102.4 ± 1.00 |
| | 50.10 | 60.00 | 110.70 | 101.0 ± 1.18 |

## CONCLUSION

A simple sensitive and reproducible method has been developed for the assay of ART and DHA in tablet formulation. The method is cost effective with no tight pH control redeem and exhaustive extraction with organic solvent that could be hazardous to the analyst and the environment. This method is recommended for use in routine quality control laboratory and in field stations by government agent at part of entry of drug to check the influx of Artemisinin pharmaceuticals.

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**REFERENCES**

Adegoke and Osoye, A. O. (2011) Derivitization of Artesunate and Dihydroartemisin for colorimetric Analysis using p-methyl aminobenzaldehyde, *European journal of Analytical Chemistry* 6(2): 104-113.

Ambriose, T. (2012). The tragedy caused by fake antimalarial drugs. *Mediterranean Journal of Hermatol infection disease* 4(1): e2012027.

Atemkeng, M.A., De Cock K.U. and Plaizer – Vercamen J (2007) Quality control of active ingredient in Artemisinin degrivaline antimalarial within Kenya and DRY Congo. *Journal of Tropical Medicine and International Health.* 112(1): 68-78.

Attih, E. E. and Amber (2015) Qualitative methods for the evaluation of Artesunate in Pharmaceuticals using N-Bromosuccinimide as oxidimetric reagent European Journal of Biomedical and Pharmaceutical sciences 4(65): 99-105.

Attih, E. E., Usifoh, C, Anah, V and Uwa, T. (2016) Utility of Bromate/Bromide mixture in estimation of dihydroartemisinin in pharmaceuticals. *European Journal of Biomedical and Pharmaceutical Sciences* 3(6): 91-98.

Attih, E. E., Oladimeji and Johnson, E. C. (2017), optimized and Validated methods for the evaluation of Artemisinin derivative in Pharmaceuticals using redox reactions. *Nigerian J. of Pharm. And Applied Science Res. (NIJOPHASR)* 6(2): 69-75.

Duew, M.G.B., Metcalfe, T., Discombe, M. J. and Ismail, F. M. D. (2006). Reactions of Artemisinin and artheether with acids implication for stability and mode of antimalarial action, *Journal of Med. Chem.* 49: 6065-6073.

IP (2005) International pharmacopeia. 3rd Edition, WHO, Geneva, Switzerland.

McMichael, A. J., Woodruff R.E. and Hales S. (2006) Climate Change and Human Health Present and Future Risks. *Lancet* 376(9513): 859-869.

Nayyar, G.M.L., Breman J.G. Newton P.N. and Herrughton, J. (2013). Poor Quality Anti-malarial drugs in Southeast Asia and sub-Saharan Africa. *The Lancet infection Disease* 12 (6): 488-496.

Newton, P.N McGready K., Fernandez, F., Green, M.D. Subjio, M. and Bruneton, C. (2006) Mamslaughbi by fake artesunate in south east Asia-will Africa be next? *Plus medicine* 3(6) :192.

Newton, P.N., Proux, P., Green M., Smithius, F and Rozendaal (2001) Fake artesunate antimalarial in southeast Asia. *Lancet* 373: 1948-1950.

Newton, P.N., Dorndorp, A., Green, M.D., Nayyar, M and White, N.J.(2003) counterfeit artesunate in South Asia. *Lancet* 362:167-169.

Robert, A., Bernot-Vical, Dechi-Cabanet., O., Maurier, O. (2001) From classical multi-malaria drug to new compounds based on mechanism of action of Artemisinin. *Journal of Pure and Applied Chemistry*, 73(7): 1173-1188.

Rozendaal, J. (2001). Fake Antimalarial Drug in Cambodia. *Lancet* 357(923)890

WHO (2014). World Health Organization Global Malaria Programme Status Report on Artemisinin Resistance;
http.www.who.int/malaria/areas/dm.resistance