Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2018, 10(2):11-19

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Sensitve Titrimetric and Spectrophotometric Determination of Artesunate in Pharmaceuticals using Chloramine T as Oxidimetric Reagent

Attih EE[1*], Johnson EC[1], Ambe DA[2], Umoh A[1] and Akpan A[1]

[1]*Department of Pharmaceutical and Medicinal Chemistry, University of Uyo, Nigeria*
[2]*Department of Pharmacognosy and Natural Medicine, University of Uyo, Nigeria*

ABSTRACT

Artesunate (ART) is a frontline drug for the treatment of malaria, mostly in combination with other antimalarial, such as amodiaquin. There are reported cases of widespread manufacture of ART and other artemisininderivative derivatives in South-East Asia and sub-Saharan Africa. If this trend is not checked there is likely going to be public health crises as this may likely lead to the emergence of multidrug resistant plasmodium parasites. Bearing this in mind three simple methods are developed based on the use of chloramine T as the oxidometric reagent. In method 1 choramine T is titrated directly with the drug while in the second titrimetric and the spectrophotometric methods known excess of chloramine T is determined after the redox reaction with increasing amount of ART. The three methods are stoichiometricand qualitative. In the trimetric method A (direct) and the indirect iodometric method B, the amount of the ART determined was proportional to the concentration of the chloramine T. in the spectrophotometric method Beer's law was obeyed in the range of 0.5-50 µg as shown in the calibration graph generated. The correlation coefficient of 0.9998 was obtained based on least square method. The molar absorptivity and Sandell sensitivity were 2.6 × 10^4 L/mol/cmand 0.0168 µg/ml respectively. The limit of detection and quantification were determined as per current ICH guidelines and found to be 0.0756 – 0.36 respectively. The methods were statistically compared with an official method via student T test and varianceratio test (F test) with calculated values lower than the tabulated values in all cases showing good accuracy and precision. The proposed methods were then used to determine locally purchased ART tablets in Uyo, Nigeria. The workability and accuracy of the methods were further confirmed byrecoverystudies via standard addition method. The results show that pharmaceutical excipients had no effect on the methods.

Keywords: Titrimetric and spectrophotometric determination; Oxidimetric reagent; Chloramine T

INTRODUCTION

Malaria remains the most deadly parasitic disease in the world. Though malaria is endemic in the tropical region of the world, it is a great source of concern as it has extended for over 40% of the world population [1]. The twin factor of international travels and global warming is helping the spreading of the disease to other regions of the world. Current projections suggest that if global warming remains unchecked it could re-established itself in Europe and North America [2,3]. The fight against malaria is well coordinated with the use of long lasting insecticide nets and the introduction and use of Artemisinin combination therapy (ACT). Unfortunately the novel achievement recorded so far is in jeopardy courtesy of activity of counterfeiters of artemisinin derivatives. The manufacture and the distribution of counterfeit/fake artemisininderivatives was first reported in the South-East Asia [4-7] this situation has spread to sub Saharan Africa [8,9]. The manufacture and distribution of counterfeit artemisinin products could be the cause of the multi-drug resistant malaria parasites in the South-East Asia [10,11]. The reason for the emergence is most likely due to the fact that sub therapeutic artemisinin derivative is administered to unsuspecting patients since these fake medicines are cheap. The trade in counterfeit drug is worldwide affecting developing and

developed countries though its affect developing countries most. This is because these malaria endemic countries do not have strong Drug control/Regulatory system. They do not have in place tight penal system. Those apprehended are often released with little or no punishment at all. Counterfeiters are in the business to make money. They are in this unfortunate trade because of the huge profit involved. They do not pay taxes to government as these drugs hardly pass through the authorized route. If this situation is allowed to continue we may soon encounter a serious public health crisis because there could be the emergence of untreatable malaria. These counterfeiters are very sophisticated. They produce anti-malarials that look absolutely genuine which can fool any professional or government agent in the field. They produce antimalarial blister packs, bearing manufacturing dates; expiry dates looking absolutely genuine [12]. An added danger in this process is the use of substandard and dangerous chemicals as excipients. Some of these chemicals include diethylene glycol, safrole, and melamine causing irreversible organ damage to the heart, kidney and the liver. Some of the chemicals are known carcinogens. To check this trend sophisticated LaboratoryEquipment such as HPLC, GC-MS, LC-MS are needed. Those malaria endemic countries are poor and can hardly procure same. Those supplied by donor agencies can hardly be maintained because of epileptic power situation. To solve this problem at this particular point simple sensitive and reproducible method which is affordable is needed, hence the development of titrimetric and spectrophotometric methods for the assay of Artesunate. Some methods have been developed for the assay of artesunate [13-19]. A careful search of the literature reveals that no method for assay of Artesunate has been developed using redox reaction between ART and Chloramine T with some dyes. The reagents and chemicals are very affordable and can be of no hazard to the environment and the analyst.

EXPERIMENTAL SECTION

Equipment

All spectral measurements were carried out using spectrophotometer Hey Loss (thermo Electron corporation USA) with 1 cm matched quartz cells.

Reagents and Chemicals

All reagents used were of analytical grade and solutions were prepared and diluted using distilled water.

Solvent:

Ethanol (Sigma, Germany).

Reagents:

Chloramine T (CAT): A 0.01 M solution of chloramine T was prepared by dissolving 2.82 g of the chemical (Sigma-Aldrich, Germany) in 100 mL of distilled water and diluting to 1.0 litre mark in 1.0 litre capacity volumetric flask. This was used for the titrimetric methods. The resulting solution was diluted to 160 µg/mL for the spectrophotometric method.

Potassium iodide (KI): A 10% solution of KI was prepared by dissolving 10 g of the chemical (BDH, England) in about 20 mL of distilled water and diluting to 100 mL mark in the 100 mL capacity volumetric flask.

Starch indicator: A 1% starch indicator solution was prepared by dissolving 1 g of the chemical in 10 mL of distilled water and made into slurry. This was made into starch mucilage by using boiling distilled water and making up to the 100 mL mark of the beaker

Sodium thiosulphate: A 0.02 solution of sodium thiosulphate prepared by dissolving about 5 g of the substance (BDH, England) in sufficient distilled water and diluting to 1 litre of distilled water. The resulting solution was standardized iodometrically.

Sulphuric acid (2 M): A 2 M solution of sulphuric acid was prepared by adding 28 ml of concentration (Sp.gr 1.82, BDH England) to 222 ml of distilled water and cooled before use.

Indigo carmine (200 µg): To obtain this, a 1000 mg/ml of carmine was prepared by dissolving of the dye (Merck, Germany, 90% dye content) in 20 ml of distilled and diluting to 100 mL mark with distilled water and filtered. The resulting solution was appropriately diluted with distilled water to obtain 200 µg/ml.

Hydrochloric acid (5M): This solution was prepared by diluting 111 ml of the concentrated acid (Aldrich, Germany, Sp.gr1.18) to 250 ml with distilled water.

General Procedures

Titrimetric method A:

Differently aliquots of ART 3-9 ml containing 1mg/ml were carefully and accurately transferred into 100 ml capacity titration flask. The content in the flasks were rounded up to 15ml using distilled water. Then 5 ml of 2 M sulphuric acid was added and shaken to mix well. The resulting solution was then titrated against 0.01 M chloramine T using 1 drop of methyl orange as indicator. A blank titration was performed exactly as above but without the drug. The amount of pure ART present in each aliquot was calculated using the formula:

$$Amount\ of\ ART(mg) = \frac{V_{mtw} \times C}{n}$$

Where, V = volume of CAT consumed (ml); mwt = molecular weight of the drug; C = molar concentration of CAT; N = is the number of moles of CAT reacting with each mole of the drug (ART).

Titrimetric method B:

Different aliquots of ART 5-9 ml containing1 mg/ml solution were accurately transferred into 100 ml capacity titrimetric flask. The content of each flask was rounded up to 15 ml using distilled water. The content of the flask was acidified using 5 ml of 2 M sulphuric acid and 10 ml of 0.01 M CAT was added. The resulting solution was allowed to stand for 20 minutes with gentle swirling after every 5 minutes within this period. Finally 5 ml of 10% potassium iodide was added and shaken gently and titrated against sodium thiosulphate using 1 ml of boiled starch added as indicator. The indicator was added when the iodine colour almost discharge. The titration was continued until the blue black colour was discharged completely. A blank titration was performed exactly as described here without the drug. The amount of ART per aliquot was evaluated from the equation:

$$Amount\ of\ ART\ (mg) = \frac{(T_b - S)M_w \times R}{2}$$

Where, T_b=volume of sodium thiosulphate consumed in the blank titration; S = volume of sodium thiosulphate consumed during determination of ART sample; mwt = molecular weight of ART; R = strength of chloramine T solution per mL used.

Spectrophotometric Method

Different aliquots (0.5-5.0 mL) with concentrations of 50 µg/mL were carefully transferred into a series of calibrated 10 mL capacity volumetric flask using micro-burette. The content of each flask was rounded up to 5 mL using distilled water. The content of flask was acidified with 2 ml of 2 M H_2SO_4 followed by 1 M of 600 µg/ml of chloramine T for 15 minutes with gentle swirling occasionally within this 15minutes interval. At the expiration of15 minutes 1 mL of 200 µg/ml of indigo carmine was added and the volume in each flask was rounded up to the 10 ml mark of the volumetric flask and swirled gently to mix well. The absorbance of each reaction solution of each flask was measure at 610 nm. A calibration graph was generated from which the unknown concentration was generated or evaluated from regression equation derived from the Beer´s law data (Table 1).

Assay of Tablet

Twenty (20) tablets of artesunate purchased locally within Uyo metropolis, South-South Nigeria, were weighed and powdered using ceramic mortar and pestle. An amount of the powder equivalent to 100 mg was transferred to a 100 ml capacity volumetric flask containing 20 ml of distilled water. The content in the flask was sonicated and shaken for 5 minutes. The content in the flask was further diluted with 50 ml of distilled water and shaken vigorously to extract the drug. Finally, the solution in each was rounded up to the 100 ml capacity volumetric flask and filtered using Whatmann filter paper number 42. The first 10 ml of the filtrate was discarded. The resulting solution with concentration of 1 µg/ml was obtained and used for the titrimetric methods. 1 ml of the solution was approximately diluted to obtain a working concentration of 50 µg/ml for the spectrophotometric.

Assay of Placebo Blank

A placebo blank of pharmaceutical excipients was prepared using the composition. Talc (5 mg), sodium citrate (5 mg), lactose (5 mg), Acacia (5 mg), methyl cellulose (5 µg), Magnesium stearate (5 mg), sodium alginate (5 mg), maize starch was added to bulk up the mixture to 100 mg. The resulting mixture was mixed thoroughly and homogenised and a solution prepared as described in the procedure for assay for tablet above. A suitable aliquot was analysed using the method described.

Assay of ART in Synthetic Mixture

One hundred (100 mg) of pure ART powder and 100 mg of the placebo blank powder as prepared abovewere mixed together and homogenised. Then 100 mg of the resulting synthetic mixture was measured and transferred into a 100 ml capacity volumetric flask containing 20 ml of distilled water and sonicated for 10 minutes and shaken vigorously. There after 50 ml of distilled water was added and also shaken vigorously. Finally the resulting solution was made up to the 100 ml mark with distilled water and filtered using whatman filter paper number 42. The first 10 ml of the filtrate was discarded. The resulting solution with a concentration of 1 mg/ml was obtained from where suitable aliquots analysed using the titrimetric methods. One (1 ml) of the synthetic mixture solution was further diluted using distilled water to a working concentration of 50 µg/ml for the spectrophotometric method.

RESULTS AND DISCUSSION

Chloramine T is a very versatile oxidizing agent. It has been used for the array of many pharmaceuticals. A careful search of the literature reveals that though chloramine T has been used for the determination of many pharmaceuticals to the best of our knowledge no mention has been made of its use for the determination of artesunate.

In acid medium, it behaves like a hypo-chloride releasing active chlorine (electrophilic chlorine).

The mechanism of the reaction is not very clear but the principle is based on the determination of the excess chloramine T left after the reaction with artesunate.

In titrimetric method, chloramine T reacts directly with ART, methyl orange is used as the indicator. In the titrimetric method B, the excess chloramine T left after the redox reaction with ART is determined iodometrically. In the spectrophotometric method, the excess chloramine T is made up to react with fixed amount of indigo carmine. As the concentration of ART increases the amount of excess chloramine T left for the bleaching of indigo carmine decreases leading to increase in the absorbance of the coloured specie formed at 610 nm (Figure 1).

In acid condition oxidation, the endo-peroxide oxygen centre in the artesunate is protonated leading to the cleavage of the peroxide bond of artesunate leading to the generation of hydrogen peroxide *in situ*.

The hydrogen peroxide generated in situ reacts with the active chlorine also generated in situ from chloramine T producing hypo-chlorine acid which is very reactive and which readily gives up itsoxygen to highly coloured dye compound to form a colourless compound (bleaching).

Hydrochloric acid is also regenerated after bleaching the dye (Figure 2).

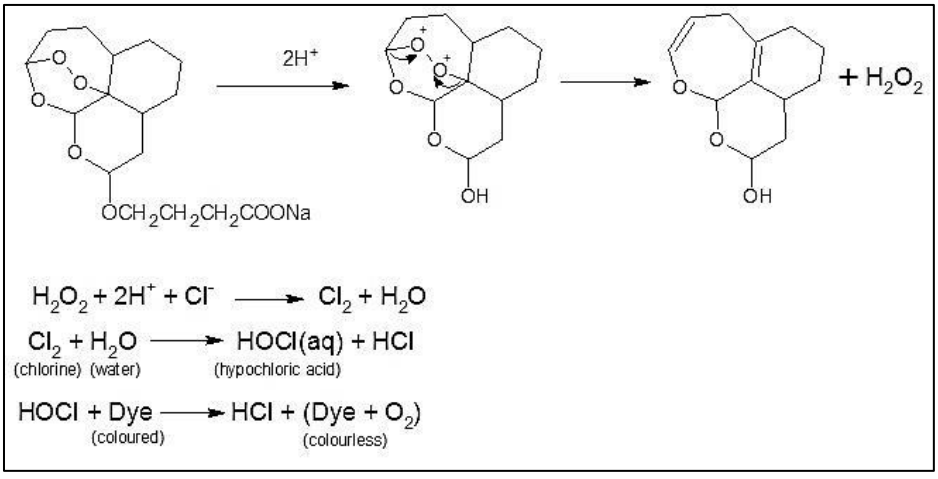

Figure 1: In the spectrophotometric method

Also,

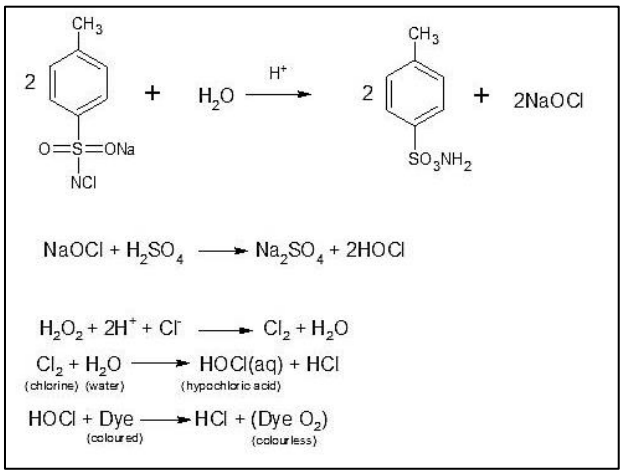

Figure 2: In acid condition oxidation

The stoichiometry of the titrimetric method A and the spectrophotometric method of [ART] to [CAT] and the [Dye] was found to be 1. While in the iodometric method the stoichiometry was:

$$H_2O_2 + 2H+ + 2I^- \longrightarrow I_2 + 2H_2O$$

$$H_2O_2 - I_2 = 2e$$

$$2NaS_2O_3 + I_2 \longrightarrow Na_2S_4O_6 + 2NaI$$

$$2S_2O_3^{2-} + I_2 = 2e - H_2O_2$$

Therefore the stoichiometry is [H_2O_2 : I_2]; the stoichiometry of iodine and thiosulphate $S_2O_3^{2-}$; hence the stoichiometry of H_2O_2 to thiosulphate is 1:2.

[ART] : [$S_2O_3^{2-}$]: [I_2] = 1: 2: 1
H_2O_2] : [$S_2O_3^{2-}$] : [I_2]. Note H_2O_2 is from ART

Optimization-Parameters affecting these reactions were carefully studied and optimized for all the three methods. The variables studied includes, oxidant concentration, strength and type of acids, reaction time, concentration of dye and temperature.

Oxidant Concentration

The redox reaction of ART and chloramine T was instantaneous in acid medium, this was because active chlorine was released in situ which readily reacted with the hydrogen peroxide also generated in situ from ART. For method A, the active chlorine released in solution from chloramine T destroys the dye (methyl orange). This spectacular quantity of the oxidant chloramine T is the reason for its use in direct titration of some pharmaceuticals. These methods have also been reported by Basavania. An oxidant concentration of 0.01 M chloramine T was adequate for the direct titrimetric method. For method B, the concentration of the oxidant chloramine T in acid medium liberates active chlorine which displaced iodine quantitatively. As halogen, the amount of chlorine released is proportional to the iodine displaced as chlorine is more reactive than iodine. In a reaction, volume of up to 20 ml, 10 ml of 0.01 M of chloramine T was adequately able to affect the release of iodine from potassium iodide used in the iodometic determination. For method Cin acid medium, chloramine T, the oxidant, irreversible destroys indigo carmine. The oxidant changes the blue colour to light green colour in acid medium. Higher concentration of the oxidant completely destroys the blue colour of Indigo carmine. The artesunate is oxidized by a known excess of the oxidant chloramine T in acid medium. The residual oxidant now reacts with fixed amount of the dye (indigo carmine). As the amount of ART (the drug) increases the amount of chloramine T used for the redox reaction is increases, the amount of the oxidant left for the reaction with the fixed amount of the dye decreases leading to increasing absorbance of the coloured species in the λmax of the indigo carmine (610 nm). The increase in absorbance is proportional to increasing drug concentration. The upper limit of indigo carmine that can be destroyed by the oxidant iron first determined and found to be 10 µg/mL. The oxidant concentration of about 20 µg/mg of chloramine

T was adequate and could destroy the dye (colour) completely reducing the absorbance at 610 nm to almost zero. Different aliquots of ART were reacted with 1 mL of 160 µg/ml of chloramine T and the residual unreacted oxidant was then reacted with 1 mL of 100 µg/ml of oxidant solution in a total reaction volume of 10 ml.

Type and Strength of Acid

Sulphuric acid, hydrochloric acid, acetic acid were used. Sulphuric acid and hydrochloric gave excellent results for both titrimetric and spectrophotometric methods. Acetic acid also gave reasonable results. Nitric acid gave poor results may be because it is an oxidizing agent itself.

Contact Time

The contact time of these reactions is very important to this reaction as the oxidation-reduction reaction has to be completed between the oxidant and ART which was 15 minutes, at this time the oxidation period was completed. The contact time for the determination of excess oxidant was not very critical as a delay of effect up to 25 minutes had no effect on the overall results.

Dye Concentration

In a preliminary experiment, to determine the amount of the dye solution that could give maximum absorbance of the λmax of the dye indigo carmine (610 nm). It was discovered that the amount of indigo carmine was 10 µg/ml. Hence in a 10 mL reacting volume 1 ml of 100 µg/ml was found to be adequate.

Methods Validation

The three proposed methods were validated for linearity, sensitivity, accuracy and precision, selectivity and recovery. The ruggedness and the robustness were also evaluated.

Linearity and Sensitivity

In both the direct and indirect titrimetric methods a linear relationship exists between the concentration of oxidant and the drug ART. A linear range of 5-15 mL was observed in method A and 5 – 12 mL in titrimetric method B. the stoichiometry for method A was 1:1 [CAT:ART] while the for titrimetric method B was 1:2:1 = $[I_2]$: [ART]:$[S_2O_3^{2}]$:[CAT]. In the spectrophotometric method, Beers Law was obeyed in the range of 1.2 – 10 µg/ml. The calibration curve as determined via least square method show a correlation coefficient of 0.9989. The equation for the curve was express by the straight line equation: A = bC + a.

Where, A is the absorbance, C the drug concentration and b the slope and a, the intercept which was very negligible. The apparent molar absorptivity, Sandellsensitivity and other sensitivity parameters such as limit of detection (LOD), limit of quantification (LOQ), which were determined as per the International Committee on Harmonization (ICH) [20].

Accuracy and Precision

The inter day and intraday accuracy and precision were determined by conducting six replicated analysis at three concentration levels of the pure standard drug solution (Table 2). The relative standard error (%R.E.) and the accuracy were determined using the formula:

$$\%R.E. = \frac{[Amount\ found - Amount\ taken]}{Amount\ taken}$$

The relative standard deviation (RSD %) was used to determine the precision of the proposed methods. The values of accuracy and precision were >3.0% in all cases, showing high accuracy and precision of all the proposed methods (Table 3).

Table 1: Analytical and regression parameters of the spectrophotometric methods

S/No	Parameter	Value
1	Λmax, nm	610
2	Beers Law Limit (µg/mL)	0.5 – 5.0
3	Limit of detection (µg/mL)	0.0756
4	Limit of Quantification (µg/mL)	0.36
5	Molar Absorptivty (L/mol/cm)	2.60×10^4
6	Sandell Sensitivity (µg/cm^2)	0.0148
7	Regression Equation	A = 0.01 + 1.06
8	Slope	0.01
9	Intercept	1.06
10	Correlation	0.9998

Table 2: Evaluation of inter-day and intra-day accuracy and precision

Method	Amount of ART taken (mg)	Intra-Day Accuracy and Precision			Inter-Day Accuracy Precisioon		
		ART Found	RE %	RSD %	ART (mg) Found	RE%	RSD %
Titrimetric method (mg/mL)	2	2.03	1.5	0.67	2.05	2.5	1.12
	4	4.11	2.75	1.23	4.1	2.5	1.12
	6	6.13	2.17	1.06	6.15	2.5	1.12
Titrmetric Method B µg/mL	2	2.05	2.5	1.12	2.04	2	0.89
	4	4.09	2.25	1.01	4.08	2	0.9
	6	6.14	2.33	1.04	6.13	2.17	1.06
Spectrophotometric Method (µg/mL)	30	30.83	2.77	1.24	30.84	2.8	1.25
	60	61.51	2.51	1.13	61.53	2.55	1.14
	90	92.05	2.28	1.03	92.07	2.3	1.03

Table 3: Results of analysis of tablets using the proposed methods

Commercial ART analysed	Label claim (mg)	Reference	Found (% of label claim ± SD)		
			Method A tritrimetric	Method B Tritrimetric	Method C spectrophotometric
Artesunate (lever)	50	110.0±1.10	110.9 ± 1.20	111.0 ± 1.18	111.10 ± 1.30
			F=1.19	F=1.15	F=1.39
			t=1.25	t=1.20	t=1.44
Articin (Evans)	50	110.0±1.16	111.0 ± 1.15	111.0 ± 1.30	111.0 ± 1.30
			F=1.02	F=1.26	F=1.26
			t=1.29	t=1.41	t=1.28
Artesunate (Nero)	50	111.0±1.0	111.0 ± 0.96	110.9 ± 1.20	111.3 ± 1.31
			F=1.02	F=1.44	F=1.72
			t=1.65	t=1.17	t=1.76
Artemnate (Sanofi)	50	110.0±1.25	111.5 ± 1.10	111.2 ± 1.60	111.1 ± 1.26
			F=0.77	F=1.64	F=1.02
			t=2.10	t=1.32	t=1.39

Table 4: Result of recovery study via standard addition method

	Titration Method A				Titration Method B				Spectrophotometric Method			
Drug formulation studied	Amt of ART in Tablet (mg/mL)	Amt of Pure ART added (mg/mL)	Total Amt found (mg/mL)	% Recovery of Pure ART+SD	Amt of ART in Tablet (mg/mL)	Amt of Pure ART added (mg/mL)	Total Amt found (mg/mL)	% Recovery of Pure ART+SD	Amt of ART in Tablet (µg/mL)	Amt of Pure ART added (µg/mL)	Total Amt found (µg/mL)	% Recovery of Pure ART+SD
Artesunate (Lever)	3.05	3	6.08	101.00 ± 1.17	3.05	3	6.09	101.33 ± 1.67	30	20	50.65	103.00 ± 2.30
	3.05	6.9	9.02	99.16 ± 1.16	3.05	6	9.04	99.80 ± 1.10	30	40	71	103.00 ± 1.77
	3.05	9	12.09	100.11 ± 2.03	3.05	9	12.1	99.40 ± 0.66	30	60	92.01	103.11 ± 2.37
Articin (Evans)	4.05	3	7.08	102.00 ± 2.50	4.05	3	7.12	103.00 ± 1.12	40.2	20	60.8	103.00 ± 2.12
	4.05	6	10.01	99.66 ± 1.25	4.05	6	10.1	102.00 ± 1.00	40.2	40	81.1	102.00 ± 1.59
	4.05	9	13.08	100.55 ± 2.51	4.05	9	13	100.66 ± 1.67	40.2	60	102	103.00 ± 2.12
Artesunate (Neros)	5.05	3	8.01	99.33 ± 1.13	5.05	3	8	99.00 ± 1.16	41	20	61.6	103.00 ± 2.12
	5.05	6	11.07	100.66 ± 2.11	5.05	6	10.95	99.66 ± 1.12	41	40	82.09	103.00 ± 1.92
	5.05	9	14	99.66 ± 1.16	5.05	9	14.19	101.72 ± 0.87	41	60	102.9	103.00 ± 2.24
Artesunate (Sanofi)	6.03	3	9.01	99.33 ± 1.15	6.03	3	9.01	99.33 ± 1.151	50	20	70.6	103.00 ± 2.12
	6.03	6	12.05	100.33 ± 1.20	6.03	6	12.05	101.20 ± 1.47	50	40	91	103.00 ± 1.76
	6.03	9	15.01	99.77 ± 1.10	6.03	9	15	99.60 ± 1.10	50	60	112.09	103.00 ± 2.46

Selectivity and Recovery
The selectivity of the methods was determined via the placebo blank and the synthetic mixture methods as described earlier. In all cases the proposed methods gave good results and excellent recoveries of between ≥ 98.5 ≤ 120.0 with standard deviation of 1.20 – 1.60 indicating that interference of pharmaceutical excipients formulated with the drugs had no influence on the proposed methods.

Robustness
Experimental variables such as acid concentration, reaction time were varied slightly. The acid concentration of upto 1.0 ml 2.5 M H_2SO_4 had no major influence on the resultsof the proposed methods.

Ruggedness
The proposed methods A and B were evaluated by different analyst and method C with two different analysts using two different UV-VIS spectrophotometer in all cases the results were not greatly affected.

Application of the Proposed Methods to Assay of Tablets of ART
The three methods as proposed were successfully used to evaluate four commercial brands of Artesunate procured locally in pharmacies within Uyo metropolis in South South, Nigeria. The results obtained compared statistically with official pharmacopoeial method (International Method, 2005) [21] via student T-test (accuracy) and variance ratio F-test (precision) at 95% confidence level and at four degrees of freedom. The calculated t and F values were lower than the tabulated values (t=2.77 and F=66.37) showing some congruence between the proposed methods and the official methods. The results are recorded in Table 3.

Recovery Studies
The practicability and accuracy of the proposed methods were further confirmed by performing recovery studies via standard addition methods. In this method, a pre-analysed tablet powder was spiked with pure ART at three different concentration levels and the total was determined using the proposed methods. The recoveries of the pure added ART powder were excellent (≥ 98 ≤ 104%) with standard deviation of between 1.15 – 1.87 as recorded in Table 4.

CONCLUSION

Three very simple, sensitive and reproducible methods for assay of ART in tablets have been developed and validated. The methods are cost effective with no tight pH control, tedious solvent extraction with organic solvents that are hazardous to the environment and the analyst these methods are recommended for field stations especially at the port of entry of the drug to check counterfeit especially in situations and countries where sophisticated analytical equipment are not available.

ACKNOWLEDGEMENT

The authors are grateful to the director of Pharmaceutical Services of the University of Uyo Teaching Hospital for providing pure artesunate used in this work. We are also grateful to TETFUND for financing this research work.

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