

Assay of Dihydroartemisinin by Iodometric Titration

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ABSTRACT

The iodometric titration method is developed for the determination of dihydroartemisinin in tablets and in bulk powders. The method is based on the redox reaction between the drug and potassium iodide in acid condition. Iodine is generated in situ by this reaction. The liberated iodine is then titrated with standardised sodium thiosulphate reagent with boiled starch as indicator. It was found that the percentage content of active ingredient per tablet was 98% which falls within the International pharmacopoeia standard (90-110%). This titrimetric method is based on a 1:1 reaction stoichiometry of dihydroartemisinin: iodine. This method is applicable over the range of 5-70mg and was used to assay other popular brands of dihydroartemisinin like Alaxin, Cotecxin and Santexin tablets sold in drug outlets in Uyo, Nigeria. The proposed method was found to be very useful when applied to these tablet preparations with mean recoveries of 98% to 104%. The method was evaluated for precision, accuracy and recovery studies using standard addition technique, with the relative error of < 2%, RSD < 3% and a coefficient of variation < 3%. The results of this method was statistically compared with the reference method by applying the students t-test and F-test at 95% confidence level ($t = 2.77$ and $F = 6.33$) the calculated t and F values did not exceed the tabulated values at 4 degrees of freedom showing no significant difference between the proposed method and the reference method in terms of precision and accuracy.

Keywords: Alaxin, Cotectxin, Dihydroartemisinin, Iodometric titration, redox reaction, santecxin.

INTRODUCTION

Malaria, a typical tropical disease remains and could continue to remain the most important source of concern in terms of morbidity and mortality. Though endemic within the tropics, it is a source of very great concern as it has extended to over 40% of the world's population (Roberts *et al.*, 2001). Over 20 million people live in sub-Saharan Africa, and WHO had estimated that in 1998, there were 273 million cases of malaria worldwide, and 1 million deaths were due to it. Burke *et al.* (2003) stated that approximately 300-500 million people were infested annually and 1.2-2.7 million lives were lost common; malaria is not confined to the tropical zone of the world, and 'imported'

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malaria is an increasing serious problem. Effective treatment of malaria has been compromised by the prevalence of multidrug resistant malaria parasites. (Coker *et al.*, 2001; WHO 2006).

Artemisinin derivatives are the fastest active antimalaria drugs (Meshnicet *al.*, 1996). Dihydroartemisinin (DHA) is a derivative of Artemisinin extracted from *Artemisia annua* (qinghao), a Chinese plant used for over 2000 years for the treatment of fevers (Sanjeevet *al.*, 2004, Woodrow *et al.*, 2004). Three derivatives that are actually more active than Artemisinin are artesunate, artemether and arteether. Their mechanism of action against malaria parasite is inextricably linked to the single endoperoxide bond in their molecule. (Liu *et al.*, 1979; Li Y *et al.*, 1981; Lin *et al.*, 1987, Klayman *et al.*, 1985; China Co-operative Research Group, 1982). Dihydroartemisinin a reduced lactol derivative is the main active blood schizonticidal metabolite of the semi-synthetic artemisinin derivative (Na-Bangchanget *al.*, 1999). All of them are readily metabolised to the biologically active metabolite dihydroartemisinin (Robert *et al.*, 2001). Dihydroartemisinin is assayed by HPLC and UV-Vis Spectrophotometry (International Pharmacopoeia, 2003). Many analytical methods have also been developed for assay of artemisinin derivatives like those of Gabriels and Plaizier-Vercammen (2004); Naiket *al.*, (2005), Van Quekelbergheet *al.*, (2008) etc. These methods are very good but the equipment used are very expensive, only few government hospitals and research institute can afford them. Since the report of the distribution of fake artesunate in South East Asia (Newton *et al.*,

2003), a few simple methods were developed. These methods were quite simple, precise and good but had some flaws. An example is the colorimetric field method for the assay of artesunate in tablets by Green *et al.*, (2000). The method is simple, precise and affordable. Scraping the surface of a tablet for the assay was a good idea as only 1% of the tablet is needed but this cannot be full proof as sophisticated drug adulterators and fakers can coat the outer part of the tablet with a thin layer of active ingredient only (In this case could be 1% of active ingredient). The second method by Green *et al.*, 2001, for authentication of artemether, artesunate, and dihydroartemisinin antimalarial tablets using a simple colorimetric method specified the use of the dye Fast Red TR salt. The resultant yellow colour is not specific because drugs like doxycycline, tetracycline and amodiaquine also produce the yellow colour. The rapid parasiticidal effect of dihydroartemisinin which is the central metabolite of all artemisinins makes it a good candidate for faking and adulteration by unscrupulous drug vendors. The limitation of these attempts to develop simple assay methods for Artemisinin has necessitated the search for other simple but accurate methods. DHA as a typical peroxide liberates iodine from iodides. The molecular iodine is generated in situ by the cleavage of the endoperoxide linkage of the dihydroartemisinin. The iodine liberated is titrated against standardised sodium thiosulphate reagent using boiled starch solution as indicator with the discharge of the blue colouration as the end point.

EXPERIMENTAL

Reagents

All chemicals/reagents used were analytical grade and all solutions were freshly prepared in distilled water.

- (1) Sodium thiosulphate (0.05M). The solution was prepared by dissolving 7.910g (BDH) of the chemical in water and diluted to 1 litre of water.
- (2) Potassium iodide (10%) (BDH) chemical was prepared by dissolving 10g of the 100mls of H₂O.
- (3) Absolute Ethanol
- (4) Starch indicator (1%). About 1g of soluble starch (BDH England) was made into a slurry with 20ml of cold water and mixed with 80ml of boiled water.
- (5) Dihydroartemisinin was obtained from Pharmacy division of the University of Uyo Teaching hospital.

METHOD A

60mg of DHA was weighed into a 100ml iodine flask and 20ml of absolute ethanol was added and shaken properly to dissolve completely. Then 2.5ml of potassium iodide (8g/L) was added and this was acidified with 2.5ml of sulphuric acid (100g/L). The resulting mixture was shaken and corked properly and

placed in a dark cupboard for 30 min. At 5 min interval the content of the flask was swirled gently. At the expiration of 30 minutes the resulting solution was titrated with 0.05M solution of sodium thiosulphate (freshly prepared and standardized). In the course of titration, 4 drops of 1% starch indicator was added near the end point. The end point was at the point the blue-black colouration disappeared. Two more titrations were carried out and the results recorded. The same procedure was repeated daily for 3 more days.

METHOD B

20 tablets each of Cotexin, Alaxin and Santecxin were crushed separately, and 60mg were weighed out and dissolved in 20mls of absolute ethanol and filtered into three 100ml iodine flask, respectively and the whole procedure repeated exactly as in method A above.

A blank determination was carried out without DHA as a correction for any iodate that may be present in the Potassium iodide. This volume was subtracted from the analysis volume obtained.

RESULTS

Table 1: Results of titration of sodium thiosulphate with the liberated iodine after the reaction of DHA with potassium iodide in acid condition

TITRATIONS			
	1 ST	2 ND	3 RD
Final volume of sodium thiosulphate	41.50ml	42.30ml	41.30ml
Initial volume of sodium thiosulphate	0.00ml	1.00ml	0.00ml
Volume of sodium thiosulphate used	41.50ml	41.40 l	41.30ml

Average volume of sodium thiosulphate used = 41.40ml

Table 2:Result of the titration of sodium thiosulphate with the liberated iodine after the reaction of Cotecxin with potassium iodide under acid condition

TITRATIONS			
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	1 ST	2 ND	3 RD
Final volume of sodium thiosulphate	40.60ml	41.60ml	40.60ml
Initial volume of sodium thiosulphate	0.00ml	1.00ml	0.60ml
Volume of sodium thiosulphate used	40.60ml	40.60ml	40.00 l

Average volume of sodium thiosulphate used = 40.60ml

Table 3:Result of titration of sodium thiosulphate with the liberated iodine after the reaction of Alaxin with potassium iodide under acid condition

TITRATIONS			
	1 ST	2 ND	3 RD
Final volume of sodium thiosulphate	40.50ml	40.00ml	38.50ml
Initial volume of sodium thiosulphate	2.00ml	1.50ml	0.00ml
Volume of sodium thiosulphate used	38.50ml	38.50ml	38.50ml

Average total volume of sodium thiosulphate used = 38.50ml

Table 4:Result of titration of sodium thiosulphate with the liberated iodine after the reaction of Santecxin with potassium iodide under acid condition

TITRATIONS			
	1 ST	2 ND	3 RD
Final volume of sodium thiosulphate	42.60ml	44.60ml	45.60ml
Initial volume of sodium thiosulphate	0.00ml	2.00ml	3.00ml
Volume of sodium thiosulphate used	42.60ml	42.60ml	42.60ml

Average total volume of sodium thiosulphate used = 42.60ml

Determination of Percentage content of active Ingredient in 60mg of DHA (equivalent to 1 tablet)

1.422mg of DHA \equiv 1ml of 0.05M Na₂S₂O₃

xmg of DHA \equiv 1.30ml of 0.05M Na₂S₂O₃

x = 58.728mg

Percentage of Active ingredient in 60mg of DHA (1 tablet)

= $\frac{\text{Experimental wt}}{\text{theoreticalwt}} \times \frac{100}{1}$

= $\frac{58.73}{60} \times 100$

= 97.88%

The evaluation of percentage weight of Active ingredient for 1 tablet of Cotecxin, Alaxin and Santecxin gave 96.20%, 91.24% and 101.00% respectively.

DISCUSSION

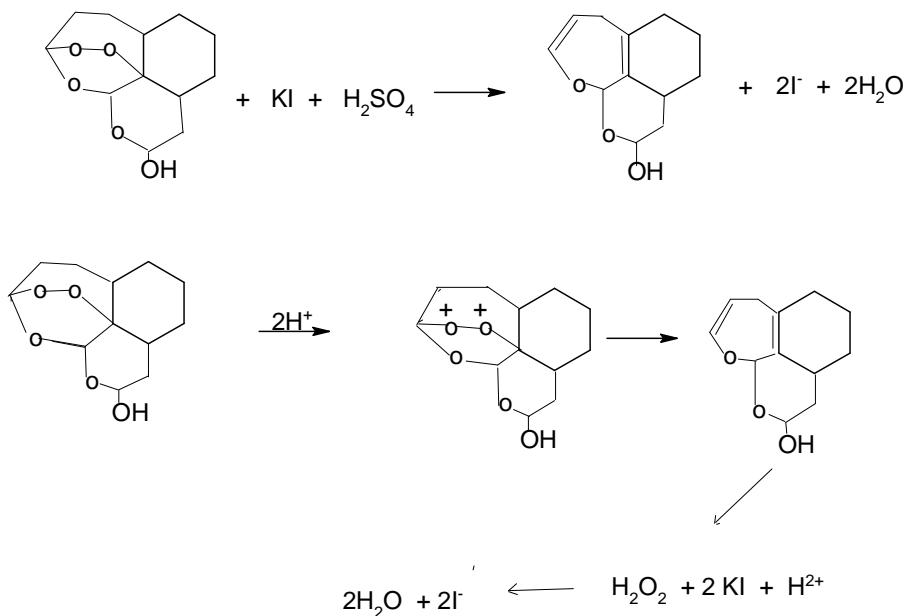
Dihydroartemisinin as a typical peroxide liberates iodine from potassium iodide in acid medium. It was observed that the amount of iodide liberated from the potassium iodide is proportional to the amount of sodium thiosulphate used during the titration. The amount of iodine generated in solution is proportional to the quantity of Dihydroartemisinin.

The stoichiometry of this reaction was calculated to be 1:1 (DHA:KI). The reaction

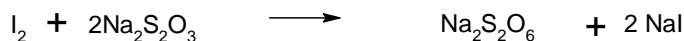
stoichiometry was not affected when 2.0-5.0ml of 0.5M sulphuric acid was used.

The mechanism of this reaction cannot immediately be ascertained, but it is likely that there is a protonation of oxygen centers

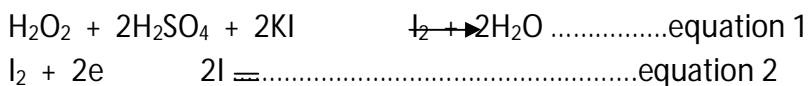
in the endoperoxide (O-O) leading to the generation of iodine from the potassium iodide.



Scheme1 showing the liberation of iodine from KI in acid condition by Dihydroartemisinin.



Scheme 2 showing the reaction between the liberated iodine with sodium thiosulphate



This oxidation reaction was completed in 15mins and contact times up to about one

hour did not affect the stoichiometry of the reaction.

Statistical Analytical Data

This iodometric titration was found to be applicable over the range 5-70mg. Outside these limits, very inconsistent readings were obtained.

It was found that the titration end point was directly proportional to the amount of drug used. This relationship was evaluated by calculating correlation coefficient *r*, via the linear least square method and found to be - 0.9987; suggesting that the reaction between Dihydroartemisinin and potassium iodide proceeds stoichiometrically in the ratio of 1:1.

ACCURACY AND PRECISION OF THIS METHOD

The accuracy and precision of this method was evaluated. The drug DHA was determined at three different levels; each measurement was repeated five times as shown in table 3 below:

Table 5: Evaluation of accuracy and precision of the proposed method

Amount of DHA Taken	Amount found (mg)	Relative Error (%)	RSD (%)
7.00	7.11	1.57	2.86
10.00	10.12	1.20	2.38
15.00	14.86	0.93	2.57
20.00	20.24	1.20	2.38
25.00	24.86	0.56	2.57

RSD – Relative Standard Deviation, mean value of five determinations

This iodometric titration method is fairly accurate and precise as shown by the relative error (<2%) and the relative standard deviation (<3%). The reproducibility on a day-to-day basis was determined by analyzing the drug at five levels using average of three titrations for each product daily on three consecutive days. In terms of standard deviation, the day-to-day coefficient of variation was less than 3%.

Application of this Method

Three brands of commercially available tablets were analyzed for the percentage active ingredient in order to demonstrate the applicability of this proposed assay method. As shown in the table (20 below the result revealed a good agreement between the declared content and percentage active ingredient found). The results obtained by this proposed method were compared by the reference method

(International pharmacopoeia) by applying the students t-test and F-tests at 95% confidence level. The calculated t and F values did not exceed the tabulated values of (t + 2.77, F = 6.39) for four degrees of freedom, showing no significant difference between this proposed method and the reference method in terms of accuracy and precision.

This method was further subjected to test to ascertain its accuracy and validity by performing recovery studies via the standard addition technique. A known amount of the pure drug added to a tablet powder (pre-determined) was analyzed. The recoveries of the pure drug that was added was found to be quantitative (table 7) confirming that additives and excipients such as starch, lactose, etc had not interfered with the proposed method of determination.

Table 6: Result of the assay of tablets by the proposed method

Table Brand Name	Nominal Value/mg	Proposed Iodometric Titration Method
Alaxin	60	91.24 ± 0.85

		t = 1.72 F = 1.88
Santecxin	60	101.00 ± 1.00 t = 0.97 F = 1.13
Cotecxin	60	96.20 ± 1.60 t = 0.75 F = 1.70

Table 7: Result of the recovery study by the standard addition method

Preparation Studies	Amount of drug in table mg	Amount of pure drug added mg	Total amount found mg	Recovery of pure x drug %
Alaxin	5.03	5.0	10.10	102.25
	5.03	10.0	15.40	99.00
	5.03	12.0	17.05	103.00
Santecxin	5.95	4.0	9.94	99.75
	5.95	8.0	16.80	103.25
	5.95	12.0	17.90	98.00
Cotecxin	6.05	4.0	10.00	102.25
	6.05	8.0	14.04	102.25
	6.05	12.0	18.00	104.17

CONCLUSION

This iodometric titrimetric method can be used to assay Dihydroartemisinin in bulk or tablet formulation. This method seems to be the simplest method for the assay of Dihydroartemisinin with a practical range between 5mg-70mg. It is simple, reproducible, accurate, cheap and robust for use in all pharmaceutical outlets for the distribution of Dihydroartemisinin to check counterfeiting and adulteration as HPLC is very expensive and cannot easily be procured by small outlets. There is no interference from the excipients.

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