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Research Article

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Sensitive methods for the spectrophotometric determinations of some antimalarial drugs

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ABSTRACT

Two new methods are proposed for the determination of dihydroartemisinin (DHA), artemisinin (ART), and artesunate (ARTS) in pure form and in pharmaceutical formulations. Method A is based on the formation of blue colored chromogen when the drugs react with Folin-Ciocalteu's (F-C) phenol reagent in alkaline medium. The colored species have absorption maxima at 722, 721 & 741 nm and second method uses bromate-bromide mixture and crystal violet (CV) as reagents, the absorbances of which could be measured at 596 nm. First method obeys Beer's law in the concentration range of 10.00– 120.00, 10.00-80.00 and 10.00-80.00 µg mL^{-1} for FC-DHA, FC-ART and FC-ARTS systems respectively and second method obeys Beer's law in the concentration range of 2.00-20.00, 2.00-22.00 & 2.00-30.00 µg mL^{-1} for DHA, ART, & ARTS respectively . The apparent molar absorptivities for the first method are calculated to be 0.11 x 10^4, 0.23 x 10^4 and 0.1 x 10^4 $lmol^{-1}cm^{-1}$ and for the second method 5.92 x 10^4, 3.98 x 10^4 & 1.37 x 10^5 for DHA, ART & ARTS respectively. The proposed methods are successfully applied to the determination of ARTS in pharmaceutical formulations.

Key words: Spectrophotometry, antimalarials, F-C's reagent, bromate-bromide mixtutre.

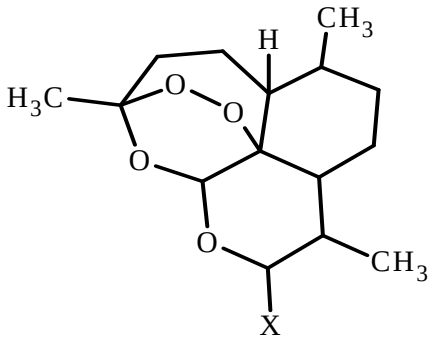
INTRODUCTION

Malaria is the most deadly vector-borne disease in the world. Approximately 300 million people worldwide are affected by malaria and between 1 and 1.5 million people die from it every year. The first symptoms of malaria are very similar to regular influenza e.g. fever, headache and muscular pain. Malaria has traditionally been treated with quinolines such as chloroquine, quinine, mefloquine etc. Unfortunately, most *plasmodium falciparum* strains are resistant to chloroquine [1].

Artemisinin is a drug used to treat multi-drug resistant strains of *falciparum* malaria. Artemisinin is a sesquiterpene lactone which is formed both in vivo and differentiated in vitro cultures, by *Artemisia annua* and is equally distributed throughout the plant. Artemisinin appears to be sequestered in glandular trichomes which happen in stems, leaves, and inflorescences. Dihydroartemisinin [2-6], the reduced lactol derivative of artemisinin [2, 7-9] with retention of the endoperoxide bridge is an antimalarial drug which possesses bioactivity with less toxicity [10, 11]. Its dimmers have both antmalarial and antitumour activities. It is more potent than artemisinin and is active by virtue of the endoperoxide. Artesunate, is also an antimalarial drug and a derivative of artemsinin with retention of the endoperoxide bridge which possesses bioactivity with less toxicity. Artesunate and its active metabolite dihydroartemisinin are potent blood schizonticide which acts by increasing the oxidant stress on the intra-erythrocytic stages of the parasite [12]. Their activity against strains of the parasite that had become resistant to

conventional chloroquine therapy and the ability due to its lipophilic structure, to cross the blood brain barrier, it was particularly effective for the deadly cerebral malaria.

Several analytical techniques [13-19] have been used for the determination of DHA, ART & ARTS, which rely upon sophisticated and expensive instrumentation and also expensive chemicals. Here we report two simple spectrophotometric methods for the determination of antimalarials. Method A is based on the reduction of F-C's reagent by the drugs in alkaline medium. Method B involves the oxidation of the drugs by liberated bromine and the residual oxidant is determined by using crystal violet.



X=OH (Dihydroartemisinin)

X=O (artemisinine)

X=OOC -(CH $_2$)$_2$-COOH (artesunate)

EXPERIMENTAL SECTION

Apparatus

A UV-2550 Visible spectrophotometer (Shimadzu, Japan) with 1cm quartz cell was used for all the measurements.

Reagents and Materials

All solutions were prepared with distilled water. NaOH (1M), Folin-Ciocalteu's reagent (20 %) (2.0 Normal, Loba chemie Ltd., India) were prepared and diluted appropriately to get the working concentration. For method B, 0.1 g of $KBrO_3$ (Spectrochem Private Ltd., Mumbai, India) and 1.0 g of KBr (Spectrochem Private Ltd., Mumbai, India) was dissolved in distilled water and the solution was made up to the mark in a 100 ml standard flask. This was diluted to get 250 µg ml^{-1} $KBrO_3$ solution. Crystal violet (0.06 M) was prepared by dissolving accurately weighed amount in distilled water and made up to the mark in a 100 ml standard flask.

Standard Drug Solution

A 1000 µg ml^{-1} of DHA, ART, ARTS were prepared by dissolving an accurately weighed amount of the drug in ethanol and made up to the mark in a 100 ml calibrated flask. The drug solutions were diluted appropriately to get the required working concentration.

Method A

Determination of DHA

Different aliquots containing (10.00-120.00 µg ml^{-1}) DHA were transferred into a series of 10.0 ml calibrated flasks using a micro burette. To each flask NaOH (0.1 M, 3.0 ml) was added and shaken well, then added 2.0 ml of F-C's reagent (5 %), kept for five minutes and diluted up to the mark and absorbance of each solution was measured at 722 nm.

Determination of ART

Different aliquots containing (10.00-80.00 µg ml^{-1}) of ART were transferred into a series of 10.0 mL calibrated flasks using a micro burette. To each flask 3.0 ml of NaOH (0.1 M) was added followed by 3.0 ml of F-C's (5 %)

reagent, shaken well and kept for five minutes, diluted to the mark with ethanol and the absorbance was measured at 721 nm.

Determination of ARTS

Different aliquots containing (10.00-80.00 µg ml^{-1}) of ARTS were transferred into a series of 10.0 ml calibrated flasks using a micro burette. To each flask 2.0 ml of NaOH (1 M) was added followed by 3.0 ml of F-C's (20 %) reagent, shaken well and kept for five minutes diluted to the mark with ethanol and the absorbance was measured at 721 nm.

Method B

Determination of DHA, ART & ARTS

Accurately measured volumes of the drug solutions equivalent to 2.00-20.00, 2.00-22.00 & 2.00-30.00 µg ml^{-1} of DHA, ART & ARTS respectively, were transferred into a series of 10 ml standard flasks. Then, a volume of 1 ml of 2 M HCl was added to each flask followed by 1.0 ml of bromate- bromide mixture. The reaction mixture was shaken well and kept aside for 10 min. Then, 2.0 ml of CV was added to each flask, diluted up to the mark with distilled water and absorbance of each solution was measured at 596 nm.

Analysis of Tablet

An amount (Falcigo, Aurochem Ltd., India) equivalent to labeled amount of artesunate was ground into a fine powder and dissolved in ethanol, filtered into a 100 ml standard flask and a convenient aliquot was then subjected to analysis using the proposed procedures.

RESULTS AND DISCUSSION

Method A

The mixed acids in the F-C reagent are the final chromogen and involve the following chemical species: $3H_2O.P_2O_5$ .13 WO_3 .5MoO_3 .10H_2O and $3H_2O.P_2O_5$ .14 WO_3 .4 MoO_3 .10H_2O. The F-C reagent is used in the determination of many phenolic compounds [20] and a large number of substances of pharmaceutical interest [21-23]. The proposed method is based on the formation of a blue colored chromogen, following the reduction of phosphor-molybdo tungstate mixed acid of the F-C's reagent [24] by antimalarials, in the presence of sodium hydroxide. Drug probably effects reduction of 1, 2 or 3 oxygen atoms from tungstate and / or molybdate in the F-C's reagent, there by producing one more possible reduced species which has characteristic intense blue color. The effect of different variables such as nature and strength of alkali, optimum volumes of NaOH and F-C reagent, reaction time were studied and optimized for attainment of maximum color and stability of colored species. Condition under which reaction of drugs with F-C's reagent fulfils the essential requirements was investigated. All conditions studied were optimized at room temperature.

Selection of reaction medium

To find a suitable medium for the reaction, different aqueous bases were used, such as sodium hydroxide, sodium carbonate or bicarbonate, sodium acetate. The best results were obtained when sodium hydroxide was used. In order to determine the optimum concentration of sodium hydroxide for the determination of DHA and ART, different volumes of 0.1 M sodium hydroxide solution (0.5, 1.0. 2.0, 2.5. 3.0 ml) were used to constant concentration of the drugs. It is evident that 3.0 ml of 0.1 M sodium hydroxide solution is found optimum. Larger volumes had no effect on the absorbance of the colored species. For ARTS 2.0 ml of 1 M NaOH is found to be optimum. Maximum colour developed within 5 minutes and it was stable up to 1hr. Absorption spectra of the colored complexes was measured against a reagent blank in the range 400-800 nm (Fig. 1).

Method B

The proposed spectrophotometric method is indirect and is based on the determination of the residual bromine (*insitu* generated) after allowing the reaction between drugs and a measured amount of bromine to be complete. The surplus bromine was determined by reacting it with a fixed amount of crystal violet. The method relies on the bleaching action of bromine on the dye, the decolouration being caused by the oxidative destruction of the dye. Drugs when added in increasing amounts to a fixed amount of *insitu* generated bromine, consumes the latter proportionately and there occurs a concomitant fall in the amount of bromine. When a fixed amount of CV is added to decreasing amounts of bromine, a concomitant increase in the concentration of dye results. Consequently, a

proportional increase in the absorbance at a wavelength of 596 nm (Fig. 2) is observed with increasing concentrations of DHA, ART & ARTS.

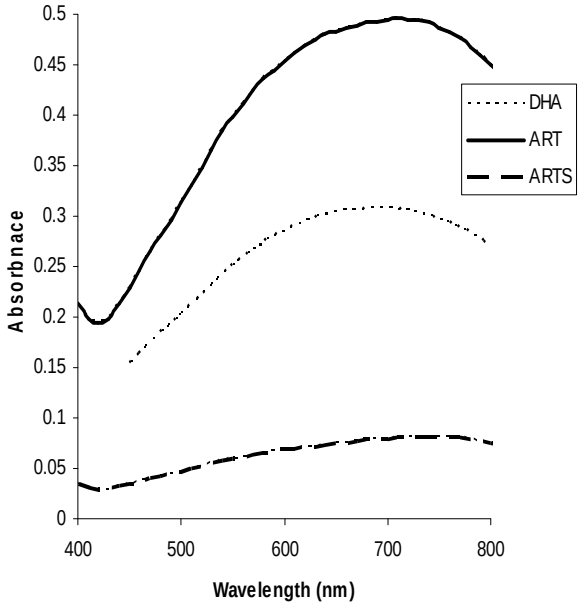


Figure 1: Absorption spectra of DHA-FC, ART-FC & ART-FC system for method A.

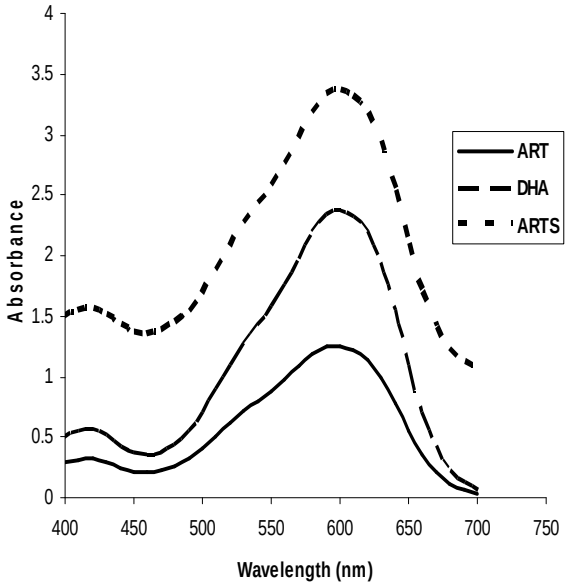


Figure 2: Absorption spectra of DHA-CV, ART-CV & ARTS-CV systems for method B.

Analytical Data

A linear correlation was found between absorbance and concentration of drug. Optical parameters such as molar absorptivity, Beer's law limit values were calculated (Tables 1a & 1b). Limit of detection (LOD) and limit of quantification (LOQ) were calculated according to ICH guidelines [25] as LOD =3.3 × σ/S and LOQ = 10 × σ/S,

where σ is standard deviation of y-intercept of regression lines (standard deviation of response) and S is slope of calibration curve. Linearity was evaluated by calculation of regression. Sensitivity of proposed methods is determined by calculating Sandell's sensitivity (μg/cm^2/0.001 Abs unit), which can be defined as smallest weight of substance that can be detected in column of unit cross section.

Table 1a- Analytical parameters (Using F-C's Reagent)

	Dihydroartemisinin	**Artemisinine**	**Artesunate**
λmax(nm)	722	721	741
Beer's Law Limit (μg mL^{-1})	10.00-120.00	10.00-80.00	10.00-80.00
Molar Absorptivity (Lmol^{-1}cm^{-1})	0.11×10^4	0.23×10^4	0.10×10^4
Limit of Detection** (μg mL^{-1})	1.50	0.97	0.33
Limit of Quantification** (μg mL^{-1})	4.54	2.94	1.00
Regression Equation*	Y=a+Bx	Y=a+bX	Y=a+bX
Slope (b)	0.002	0.006	0.001
Intercept (a)	0.013	0.011	0.009
Correlation Coefficient (r)	0.9965	0.9988	0.9865
Sandell's Sensitivity (μg cm^{-2})	0.250	0.122	0.384
Y is the absorbance and X is the concentration in μg mL^{-1}* * Calculated using ICH guidelines.*			

Table 1b- Analytical parameters (Using bromate-bromide mixture).

	Dihydroartemisinin	**Artemisinine**	**Artesunate**
λmax(nm)	796	796	796
Beer's Law Limit (μg mL^{-1})	2.00-20.00	2.00-22.00	2.00-30.00
Molar Absorptivity (Lmol^{-1}cm^{-1})	5.92×10^4	3.98×10^4	1.37×10^5
Limit of Detection** (μg mL^{-1})	0.28	0.35	0.28
Limit of Quantification** (μg mL^{-1})	0.84	1.07	0.87
Regression Equation*	Y=a+bX	Y=a+bX	Y=a+bX
Slope (b)	0.1177	0.1213	0.1142
Intercept (a)	0.2957	0.1860	0.5622
Correlation Coefficient(r)	0.9946	0.9936	0.9955
Sandell's Sensitivity (μg cm^{-2})	0.004	0.007	0.002
Y is the absorbance and X is the concentration in μg mL^{-1}.* * Calculated using ICH guidelines.*			

Table 2a- Evaluation of accuracy and precision (Using F-C'S reagent).

Name of the drug	**Amount taken (μg mL^{-1})**	**Amount found (μg mL^{-1})**	**RE (%)**	**SD (μg mL^{-1})**	**RSD (%)**
Dihydroartemisinin	2.00	1.99	0.50	0.010	0.55
	4.00	4.00	-0.05	0.013	0.32
	6.00	5.99	0.06	0.010	0.18
	8.00	7.99	0.05	0.010	0.14
	12.00	11.99	0.06	0.020	0.13
	14.00	14.01	-0.07	0.154	1.07
Artemisinine	2.00	1.99	0.20	0.018	0.90
	4.00	3.99	0.20	0.013	0.32
	6.00	6.00	-0.02	0.014	0.23
	8.00	7.99	0.10	0.013	0.16
	10.00	10.00	-0.01	0.014	0.13
	12.00	11.99	0.05	0.023	0.19
Artesunate	2.00	1.99	0.05	0.009	0.45
	4.00	4.00	-0.01	0.006	0.14
	6.00	5.99	0.16	0.012	0.20
	8.00	7.99	0.07	0.018	0.22
	10.00	9.99	0.02	0.010	0.10
	12.00	11.99	0.05	0.012	0.10

**Mean value of five determinations.*
RE – Relative Error; SD - Standard Deviation; RSD-Relative Standard Deviation.

Table 2b- Evaluation of accuracy and precision (Using bromate-bromide mixture).

Name of the drug	Amount taken (µg mL^{-1})	Amount found (µg mL^{-1})	RE (%)	SD (µg mL^{-1})	RSD (%)
Dihydroartemisinine	20.00	19.80	1.00	0.29	1.51
	40.00	39.99	0.03	0.39	0.98
	60.00	59.68	0.53	0.32	0.55
	80.00	79.91	0.11	0.61	0.77
Artemisinine	10.00	9.85	1.50	0.15	1.62
	20.00	20.02	0.10	0.12	0.59
	30.00	29.96	0.13	0.22	0.83
	40.00	39.99	0.02	0.17	0.42
Artesunate	20.00	20.09	0.45	0.24	1.23
	40.00	40.01	0.04	0.11	0.29
	60.00	60.02	0.03	0.11	0.18
	80.00	79.97	0.03	0.06	0.08

**Mean value of five determinations.*
RE - Relative Error; SD - Standard Deviation; RSD - Relative Standard Deviation.

Accuracy and Precision

The accuracy of the method was established by analyzing the pure drugs at four levels within working limits and the precision was ascertained by calculating the relative standard deviation of five replicate determinations on the same solution containing the drugs at four levels and are presented in Table 2a & 2b. The relative error and relative standard deviation indicate the high accuracy and precision for the method.

Interference Study

In the pharmaceutical analysis, it is important to test the selectivity towards the excipients and fillers added to the pharmaceutical preparations. Several species which can occur in the real samples together with drug were investigated. The level of interference was considered acceptable.

Table 3- Results of assay of formulations by the proposed methods

Brand name	Falcigo[a]
Labeled amount (mg)	200
Reference value	
% Label claim ± SD	99.46± 0.03
1. F-C's reagent	
i. Amount found	199.5
ii. % Label claim ± SD	99.75±0.033
iii. t-test	t = 1.51
iv. F-test	F = 1.21
2. Bromate-bromide mixture	
i. Amount found	199.2
ii. % Label claim ± SD	99.60±0.035
iii. t-test	t = 1.71
iv. F-test	F = 1.36

**Mean of four determinations.*
Manufactured by Auro Chem Ltd., India.
Tabulated 't'-value at 95 % confidence level is 2.77.
Tabulated 'F'-value at 95 % confidence level is 3.69.

Application

The proposed methods were applied successfully to determine ARTS in tablets. The content of the tablet formulation was calculated by applying suitable dilution factor. The results were compared statistically with those of the tabulated value at 95 % confidence level. The calculated student's t-test (Table 3) did not exceed the tabulated value, indicating that there was no significant difference between the proposed methods and the tabulated value in respect to accuracy and precision.

CONCLUSION

The proposed methods do not require any pretreatment of the drugs and tedious extraction procedure prior to their analysis. The newly developed methods are sensitive enough to enable quantification of the drug at low concentrations. These advantages encourage the application of the proposed methods in routine quality control analysis of DHA, ART & ARTS in pharmaceutical formulations.

Acknowledgements
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REFERENCES

[1] T Mukherjee, *Fitoterapia*, 62 (**1991**) 197.
[2]J–M Liu; M–Y Ni; J–F Fan; Y–Y Tu; Z–H Wu; Y–L Wu; W–S Zhou. *Acta Chim. Sinica*., **1979,** 37, 129.
[3]Y Li; P–L Yu; Y–X Chen; L–Q Li; YZ Gai; DS Wang; Y. –P .Zheng. *Acta Pharm. Sinic*., **1981,** 16, 429.
[4]D Jeremić; A Jokić; A Behbud; M Stefanović. *Tetrahedron Lett*., **1973**, 32, 3039.
[5]AJ Lin; DL Klayman; WK Milhous. *J. Med. Chem*., **1987**, 30, 2147.
[6]A Brossi; B Venugopalan; GL Dominguez; HJC Yeh; JL Flippen- Anderson; P Buchs; X–D Luo; W Milhous; W Peters. *J. Med. Chem*., **1988**, 31, 645.
[7]DL Klayman. *Science*, **1985**, 228, 1049.
[8]AR Butler; Y–L Wu. *Chem. Soc. Rev*., **1992**, 21, 85.
[9]HJ Woerdenbag; N Pras; WV Uden; TE Wallaart; AC Beekman; B Lugt. *Pharm. World Sci*., **1994**, 16, 169.
[10]SR Meshnick; TE Taylor; Kamchonwingpaisan. *Microbiol. Rev*., **1996**, 60, 301.
[11]RK Haynes; *Curr. Topics Med. Chem*., **2006**, 6, 509.
[12]PJ De Vries; TK Dien. *Drugs*, **1996**, 52, 818.
[13]H Naik, DJ Murry, LE Kirsch; Fleckenstein. *J. chromatogr. B: Analyst. Technol. Biomed. Life Sci*., **2006**, 816, 233.
[14]M Gabriels; JA Vercammen Plaizier. *J. Chromatogr. Sci*., **2004**,42, 341.
[15]M Rajanikanth; KP Madhusudhanan; RC Gupta. *Biomed. Chromatogr*., **2003**, 17, 440.
[16]K Na-Bangchang; K Congpuong; LN Hung; P Molunto; J Karbwang; *J. Chromatogr. B: Biomed. Sci. Appl*., **1998,** 708, 201.
[17]TV Sreevidya; B Narayana, *Indian. J. Chem.Tech*., **2008**, 15, 59.
[18]TV Sreevidya; B Narayana, *Eurasian J. Anal. Chem*., **2009**, 4, 119.
[19]C Okwelogu; B Silva; C Azubike; K Babatunde. *J. Chem. Pharm. Res*., **2011**, 3, 277.
[20]M Pesez; J Bartos. Colorimetric and fluorimetric analysis of organic compounds and drugs, Marcel Dekker, New York, **1974,** 83.
[21]AB Avadhanulu; ARR Pantulu. *East. Pharm*., **1993**, 36, 125.
[22]G Devala Rao; K Girish kumar; KPR Chowdary. *J. Indian Council Chemists*, **2000**, 17, 32.
[23]CSR Lakshmi; MN Reddy. *J. Inst. Chem*., (India). **1998**, 70, 152.
[24]O Folin; D Ciocalteu. *J. Biol. Chem*., **1927**, 73, 627.
[25]JN Miller & JC Miller. Statistics and Chemometrics for Analytical Chemistry, Prentice Hall, UK, **2005**.