Asian J. Pharm. Res. Vol 2, Issue 2, 76-80, 2012.

e-ISSN 2231 – 363X
Print ISSN 2231 – 3621

Asian Journal of PHARMACEUTICAL RESEARCH
Journal homepage: - www.ajprjournal.com

VALIDATED DERIVATIZATION SPECTROFLUOROMETRIC METHOD FOR ARTESUNATE AND ARTEMETHER IN TABLET DOSAGE FORM

R.K. Sangeetha[*1], T. Sudhakaran[2], M.S. Prakash[3]

[1,2]College of pharmacy, Sri Ramakrishna institute of paramedical sciences, Coimbatore – 641 044, Tamil Nadu, India.
[3]K.M College of pharmacy, Uthangudi, Madurai - 625 107, Tamil Nadu, India.

ABSTRACT

A validated derivatization spectrofluorometric method was developed for the determination of artesunate and artemether in tablet dosage form. Artesunate and artemether are semi synthetic drugs obtained as a derivative of artemisinin. Both of the drugs did not have native fluorescence with a fused sesquiterpene lactone ring system varying in the substitution at C_{10}.The drug artemether was treated with acetic anhydride and sulphuric acid and artesunate was treated with acetic acid and sulphuric acid in fixed ratio and heated to convert the compounds into a fluorescent moiety. The peroxide linkage present in the fused lactone ring system is converted into a unsaturated group for artemether and artesunate. The excitation wavelength was found to be at 438 nm and 303 nm whereas the emission wavelength was found at 508 nm and 609 nm for artesunate and artemether respectively. The method was validated according to the ICH guidelines for validation of analytical procedures. The linearity was found in the concentration range of 100 to 500 ng/ml for artesunate and between 100 to 800 ng/ml for artemether. The assay values were found between 98.2 to 100.67 % for both the compounds with a %RSD of 0.022842 and 0.4740 respectively. The % RSD of intraday and interday assay was found to be less than 1. Hence the reported method was found to be precise and accurate for the quantitative determination of artesunate and artemether in tablet dosage form. The interference studies was done with the excipients present in the formulations and found to have no interferences. The solution of artesunate was found to be stable for 2 hours and for artemether was found to be stable for 1 hour.

Key words: Spectrofluorimetry, Artemether, Artesunate, Derivatization, Validation.

INTRODUCTION

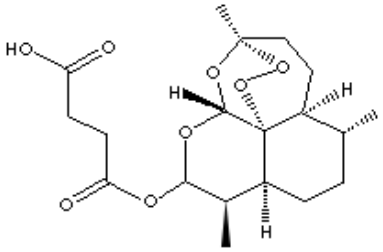
Artesunate and artemether are semi synthetic drugs derived from the natural anti malarial drug artemisinin which is traditional Chinese medicine. Chemically artemether is (3R,5AS,6R,8aS,9R,10S,12R, 12aR) Decahydro - 10-methoxy- 3, 6, 9- trimethyl- 3,12-epoxy-12H pyrano [4,3-j]-1,2-benzodioxepin and artesunate is(3R,5aS,6R,8aS,9R,10S,12R,12aR)–Decahydro-3,6,9-trimethyl-3,12-epoxy12H-pyrano[4,3-j]-1,2benzodioxepin-10-ol hydrogen. The artesunate has a hydrogen succinate at C_{10} position and artemether has methoxy at C_{10} position. They both have similar peroxide linkage in the fused heptane ring. The compounds do not have any native fluorescence and absorption in the UV visible region due to the lack of unsaturation group. The fluorescence was developed by treating them with acid and heat .Artesunate was treated with acetic acid and sulphuric acid (2:1) and heated at 100^{o} C developing greenish brown fluorescence. Artemether was treated with acetic anhydride and sulphuric acid (5:1) developing a greenish yellow fluorescence. The method was validated according to ICH guidelines. The literature survey reveals that few methods such as Colorimetry [1], Differential pulse polorography [2], HPLC with post column alkali and pre column acid derivatization [3] and UV detection [4] were reported for Artemether. Methods like HPLC [5,6], UV Spectophotometry with simulated intestinal fluid [7] and LC-MS method [8] were reported for artesunate. The presence of peroxide bridge in the structure of the compound offers the advantage of the use of acids to

Corresponding Author :- **R.K. Sangeetha** Email:- geetkaran@gmail.com

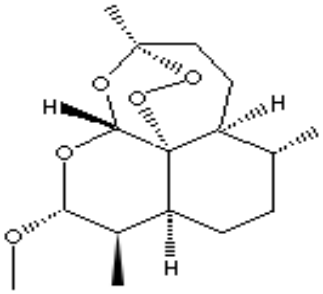
Asian J. Pharm. Res. Vol 2, Issue 2, 76-80, 2012.

derivatize both the drugs in a unsaturated compound.

Artesunate



Artemether



Instruments and reagents used

Shimadzu UV-Visible spectrophotometer 1201, Jasco 750 FP spectrofluorimeter and shimadzu AY220 – Analytical single pan balance were used to do the work. The solvents and acids used are of analytical grade procured from s.d.fine chemicals Ltd., Mumbai. The pure drug was procured commercially. The tablets were procured from the local market.

Preparation of standard stock solutions

10 mg of each of artesunate and artemether pure drug was accurately weighed and transferred to 10 ml volumetric flask individually. To the drug artesunate acetic acid and sulphuric acid (2:1) was added by placing the flask in a ice bath. The artemether solution was prepared by adding acetic anhydride and sulphuric acid (5:1) by placing in ice bath. Both the solutions were brought to the room temperature and kept in the boiling water bath (100^{o}C) for 5 minutes with shaking at regular intervals. Then the solutions were brought to the room temperature and made up to the volume with methanol cautiously.

Preparation of test stock solutions

20 tablets of each of artesunate and artemether was weighed separately and made into a fine powder .Tablet powder of 10 mg equivalent of the each drug was weighed accurately and transferred to a 10 ml volumetric flask. The fluorescence was developed in a same manner as for the standard drug for both the drugs. The volume was made with methanol and the solution was filtered through a whatmann filter paper.

Validation

Specificity

The method developed was checked for its specificity by analyzing the excipients such as starch, lactose, talc and magnesium separate separately in the similar procedure used for the pure drug and no specific absorption peak and fluorescence found at the wavelength used for both the drugs.

Linearity and range

From the standard stock solution further dilutions were done to give a concentration range of 100 -500 ng/ml for artesunate and 100-800 ng/ml for artemether. Linearity of both the drugs were found to obey the *Beer-Lambert's law* within the above mentioned range above which there was decrease in the fluorescence intensity. The values are reported in table no 1.

Accuracy

The test stock solution was further diluted to give a concentration of 300 ng/ml and emission spectra was obtained by fixing the excitation wavelength at 438 nm for artemether and 303 nm for artesunate. The assay was repeated 5 times and the average is reported in the table no 1.

Precision

Intraday and interday assay studies were performed .The intraday assay studies was done within the same day using 300ng/ml test solution. The interday studies were done using the same concentration for three successive days. The % RSD calculated was found to be below 1 and the recovery values were within the limits of 99.05 and 101.45 %.

Limit of detection and limit of quantitation

The limit of detection and limit of quantitation was done experimentally and found to be 50 ng/ml and 100 ng/ml for artesunate and 75 ng/ml and 100 ng/ml for artemether respectively.

Stability

The stability was checked for both the drugs for every 15 minutes under similar experimental conditions and the fluorescence was found to be stable for 2 hours for artesunate and one hour for artemether.

RESULTS AND DISCUSSIONS

Artesunate and artemether have no reported spectrofluorimetric method for its analysis in pharmaceutical formulations. The literature survey reveals the development of stability studies, UV spectroscopic method and post column derivatisation HPLC method. The present study was validated according to ICH guidelines for validation of analytical procedures. So the

Asian J. Pharm. Res. Vol 2, Issue 2, 76-80, 2012.

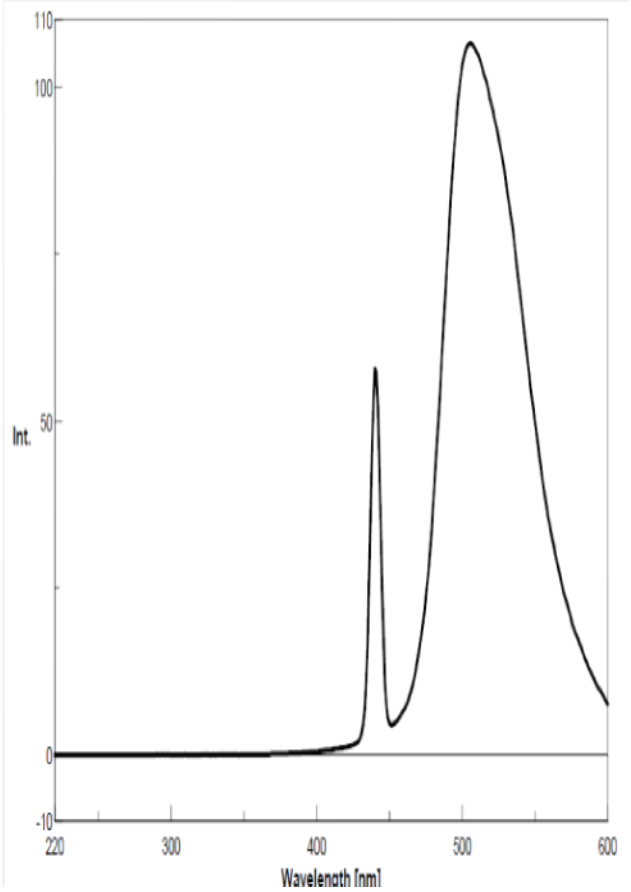
reported method can be used for the routine analysis artesunate and artemether in tablet dosage form.

Both the drugs as such do not have an absorption in the UV-Visible region due to the absence of unsaturation in the structure. The present method involves the derivatization of the artesunate and artemether in a fluorescent compound by treating with acetic acid and sulphuric acid in the ratio of 2:1 for artesunate and acetic anhydride and sulphuric acid in the ratio of 5:1 and heating at 100^{o}C for 5 minutes under controlled temperature conditions.

Table 1. Validation parameters

Parameters	Artesunate	Artemether
Linearity Range	100-500 ng/ml	100-800 ng/ml
Correlation coefficient(r^2)	0.99885	0.99961
Regression equation Slope(b) y-intercept(a)	y=-0.5+0.607*X 0.607 -0.5	y=0.964+0.407*X 0.40702 0.96429
Assay values(% RSD)	0.022842	0.4740
Accuracy studies	98.2 to 100.67 %	99.62-100.33%
Precision Intraday (% RSD) (n=6) Interday(% RSD) (n=6)	 0.3176 0.2520	 0.3086 0.7621
LOD &LOQ	50 ng/ml and 100 ng/ml	75 ng/ml and 100 ng/ml
Stability of fluorescence	2 hours	1 hour

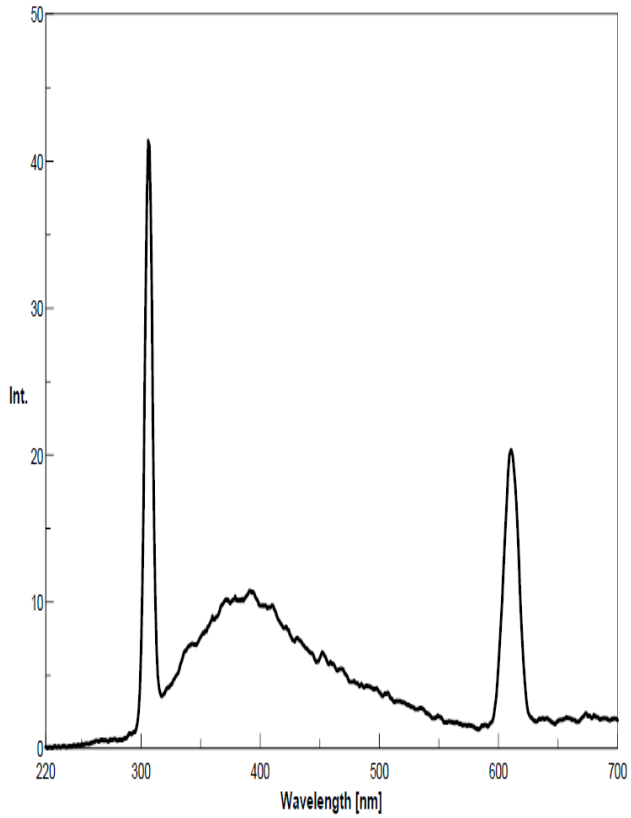
Figure 1. UV Spectrum of Artemether



Date	7/18/2012 11:08AM
File name	artemether 10 mcg
Model	FP-750
Serial No.	A9960557
Measurement Mode	Emission Spectrum
Band width(Ex)	5 nm
Band width(Em)	5 nm
Response	Medium
Sensitivity	Medium
Measurement range	220 - 600 nm
Data pitch	1nm
Excitation Wavelength	438.0 nm
Scanning speed	4000 nm/min
Sample ID	40
No. of cycle	1
Sample name	
Operator	College of Pharmacy
Comment	

Asian J. Pharm. Res. Vol 2, Issue 2, 76-80, 2012.

Figure 2. UV Spectrum of Artesunate



Date	7/18/2012 0:01PM
File name	ARTESUNATE10mcg em 180712(2)
Model	FP-750
Serial No.	A9960557
Measurement Mode	Emission Spectrum
Band width(Ex)	5 nm
Band width(Em)	5 nm
Response	Medium
Sensitivity	Medium
Measurement range	220 - 700 nm
Data pitch	1nm
Excitation Wavelength	303.0 nm
Scanning speed	4000 nm/min
Sample ID	54
No. of cycle	1
Sample name	
Operator	College of Pharmacy
Comment	

Various solvents like chloroform, acetonitrile, 0.1 M acetic acid and methanol were scanned between 400-200 nm for the absorbance spectra. Methanol was found to be the most suitable solvent as it gave a absorption spectrum at 438 nm for artemether and 303 nm for artesunate .The various experimental conditions such as ratio of acetic acid and sulphuric acid for artesunate, ratio of acetic anhydride and sulphuric acid for artemether, heating time and temperature were fixed.

The excitation wavelength was fixed at 438 nm and 303 nm for artesunate and artemether based on the absorption spectrum obtained. The emission wavelength was fixed at 508 nm and 609 nm for artemether and artesunate. The solution of artesunate was found to be stable for 2 hours and for artemether was found to be stable for 1 hour.

Acknowledgement

The authors thank Dr. T.K. Ravi M. Pharm., PhD., FAGE, for his valuable support during the course of the work.

REFERENCES

1. Green MD, Mount DL, Wirtz RA. Authentication of artemether, artesunate and dihydroartemisinin antimalarial tablets using a simple colorimetric method. *Trop Med Int Health*. 6(12), 2001, 980-2.
2. Debnath C, Haslinger E, Likussar W, Michelitsch A. Determination of the antimalaria drug artemether in pharmaceutical preparations by differential pulse polarography. *J Pharm Biomed Anal*. 41(2), 2006, 638-43.
3. Mohamed SS, Khalid SA, Ward SA, Wan TS, Tang HP, Zheng M, Haynes RK, Edwards G. Simultaneous determination of artemether and its major metabolite dihydroartemisinin in plasma by gas chromatography-mass spectrometry-selected ion monitoring. *J Chromatogr B Biomed Sci Appl*. 731(2), 1999, 251-60.
4. Atemnkeng MA, Marchand E, Plaizier-Vercammen J. Assay of artemether, methylparaben and propylparaben in a formulated paediatric antimalarial dry suspension. *J Pharm Biomed Anal*. 43(2), 2007, 727-32.
5. Thirupati Rao B, Murali Krishna P, Kishore Kumar R, Venkateshwarlu P. The forced degradation and solid indicating study and Validation of method for the determination of assay of Artesunate by HPLC. *Research Journal of Pharmaceutical, biological and chemical sciences*. 2(1), 2011, 111-117.
6. Lai CS, Nair NK, Mansor SM, Olliaro PL, Navaratnam V. An analytical method with a single extraction procedure and two separate high performance liquid chromatographic systems for the determination of artesunate, dihydroartemisinin

and mefloquine in human plasma for application in clinical pharmacological studies of the drug combination. *J Chromatogr B Analyt Technol Biomed Life Sci*. 857(2), 2007, 308-14.

7. Esimone CO, Omeje EO, Okoye FBC, Obonga WO, Onah BU. Evidence for the spectroscopic determination of Artesunate in dosage form. *J Vector Borne Dis*. 45, 2008, 281-286.
8. Carrie A. Morris, Luis Lopez-Lazaro, Donald Jung. Drug-Drug Interaction Analysis of Pyronaridine/Artesunate and Ritonavir in Healthy Volunteers**.** *Am J Trop Med Hyg*. 86(3), 2012, 489-95.