

Stability Study of Artesunate under stress condition using high performance liquid chromatography method.

¹Elsadig.H.R. Rajab*¹ and Ahmed E.M.Saeed²

¹Amipharma Laboratories Ltd, Khartoum, Sudan.

² Department of Chemistry, Collage of Science, Sudan University of Science and Technology.

* Corresponding author; email: ehrrajab@hotmail.com

The stability of Artesunate in solid form and Liquid towards direct sunlight showed first-order reaction kinetic rate, the reaction rate of artesunate was pH dependent. The rate of photo thermal decomposition in aqueous medium was found to speed up the reaction while the pH decreased. The hydrolysis of artesunate in the alkaline medium was very low compared to acid medium hydrolysis. All the investigated pharmaceutical excipients were found to accelerated the thermal instability of artesunate at 70 0C in an aqueous medium. The tendency of which to accelerated the thermal stability was in the following order: povidone, aerosil, propyl paraben, methyl paraben, sodium benzoate, maze starch and talcum powder. Artesunate was affected when exposed to UV radiation in solid and liquid form, artesunate is a photolabile drug. The results obtained from the accelerating stability study of artesunate for four months showed that no change in chemical and physical properties of artesunate. Official HPLC method was used it's not enough to determinate decomposed products of artesunate decomposed.

Keywords: stability of artesunate under stress condition, HPLC

Introduction:

The stability of drugs is of continued importance for the drug industry. Temperature, relative humidity, pH of the solution, oxygen and light are among many factors affecting on the rate of degradation of a drug either chemically or physically. A formulated drug product must have a shelf-life sufficient to cover the time taken for transportation from the site of manufacture to the storage site until the drug administration process for clinical use is completed [1,2].

The harsh conditions in Sudan like the longest day sunlight and subtropical conditions prevailing in Sudan, pointed the necessity of investigating the photo and thermal stability of artesunate as raw material and finished products.

The quality of pharmaceutical product may deteriorate with time and along the chain of the drug supply system under the prevalent condition of storage.

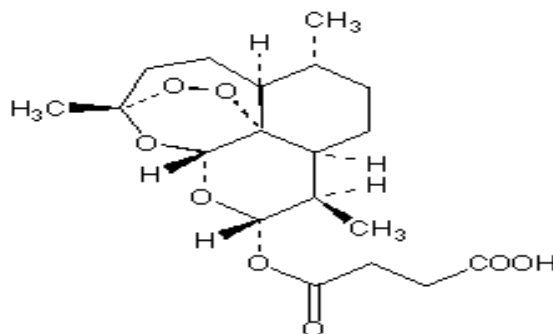


Figure1 : Chemical structure of artesunate

Chemically artesunate is (3R, 5aS, 6R, 8aS, 9R, 10S, 12R, 12aR) -Decahydro-3, 6, 9-trimethyl-3, 12-epoxy-12H-pyrano (4, 3-j) -1, 2-benzodioxepin-10-ol, hydrogen succinate

Literature survey revealed that very few methods have been reported for the estimation of Artesunate in bulk and pharmaceutical dosage form as it is very less detectable for PDA detector because artesunate lacks an intensive chromophore for UV absorption. A simple, rapid, precise and accurate UV-vis spectrophotometric method developed, [3,4] has been used for screening UV_{max} of artesunate.

To date, neither pharmacopeial monographs nor methods have been reported in scientific literature to determine AS and MQ in fixed-dose combination tablets. Analytical methods to determine AS and MQ in fixed-dose combination tablets are needed to evaluate their quality in endemic areas, as well as to detect counterfeit drug products. One method in which to determine AS in tablets is described in the International Pharmacopoeia, whereas a method to determine MQ in tablets is described in the Brazilian Pharmacopoeia. Methods for the single determination of AS or MQ in tablets were also found in prior scientific literature. In addition, the simultaneous assay of AS and MQ in plasma and a method presenting a single extraction procedure and two HPLC systems to determine AS and MQ in human plasma have been published in the literature. [5]

A new spectrophotometric method is developed for the determination of artesunate (Artesunate) in pharmaceutical preparation and bulk drug. The method involved heat-catalyzed derivatization of the drug with acetic anhydride and concentrated sulphuric acid producing a yellow colored product peaking at 420.6 nm [6].

Artesunate and its active metabolite Dihydroartemisinin are potent blood schizonticides, active against the ring stage of the parasite. Artesunate is ideal for the treatment of severe malaria, including cerebral malaria [7,8]

Artesunate is defined as a semi-synthetic sesquiterpene belonging to the amorphane subclass of cadinane sesquiterpenes. It is the most water-soluble derivative of artemisinin, which is extracted from the leaves of *Artemisia annua* L. (qinghao), [9]. (Artesunate was thermally labile drug, lack of UV or florescent chromophore for absorption and lack of functional group for derivatization [10].

From the structure of artesunate figure: 1, it can be seen that there are two functional groups are labile to be decomposed, this labile groups are O-O endoperoxide bond when

it comes in contact with high ion concentration it has been cleavage of reactive oxygen species, the other function group is methoxy acetate as ester(nucleophilic reactant of the C=O in ester.).

Artesunate (ART) is a readily available anti-malarial in combination therapy. The assay method has posed a challenge because it does not have a readily recognizable absorption chromophore needed for UV spectroscopy. A simple and rapid assay method involving two reaction steps has been developed for assay of ART in pharmaceutical formulations. The method involves basic reaction of ethanolic solution of ART with 0.1N sodium hydroxide and then neutralization and acidification of this reaction mixture with 0.1M solution of acetic acid in 20% ethanol. This gave a good UV spectrum for ART with λ_{max} at 242nm. HPLC analysis of this mixture revealed the presence of two prominent peaks. The peaks were further identified with HPLC-mass spectrometer to possibly be those of glycal and furanose acetal. The method was validated for stability, linearity, accuracy, intra- and inter-day precision and was used to assay ART in commercial tablets. The method is simple and suitable for the assay of ART in pharmaceutical formulations. [11]

Artesunate and Dihydroartemisinin are assayed using HPLC, titrimetry and uv spectrophotometry [12]. A careful search of the literature suggests that most other method developed for the assay of artesunate and DHA are few [13,14]

The aim of this study is to investigate the stability of artesunate in solid and liquid forms towards photo and thermal reactions and direct exposure to Ultraviolet radiation and the effects of some excipients on the rates of artesunate thermal reaction in aqueous medium.

Materials and methods:

HPLC was performed using a Sykam HPLC system consisting of pump S1122 solvent delivery system, S5200 sample injector and S3210 UV/VIS detector, column used was ODS 4.6 mm x 250mm. 3.0 μ m

UV/VIS Spectrophotometer double beam: UV-1800 Shimadzu Japan. Stability chamber Model No: NEC-2281210. Ultraviolet radiation 254 NM, model M01 4492 England. and Water bath thermostatic controller MPH, D3006 Germany.

All the chemical reagents and solvents used in the current experiments are analar grade, directly used without further purification and acetonitrile, methanol, ethanol and Potassium dihydrogen phosphate are HPLC grade. All the pharmaceutical excipients used are British pharmacopeia grade. Artesunate raw material purity 99.86% batch number 7006ASRI India. Artesunate raw material was kindly delivered by Amipharma laboratory Batch No: ASE0115013 has manufacturing date:10-2015 and Expiry date:09-2019. Vital India.

HPLC was applied to determine the assay of artesunate, thermal decomposition and hydrolysis in alkaline, acidic medium.

Method applied:

The mobile phase is a mixture of 44 volume of acetonitrile 56 buffer pH 3 (1.36 grams of potassium dihydrogen phosphate was dissolved in 1000 ml of water and adjust to pH 3.0 with phosphoric acid. using a stainless-steel column (250 cm × 4.6 mm) packed with particles of silica gel 3 µm, the surface of which has been modified with chemically bonded octadecylsilyl groups the flow rate 1.5ml/min., detection wavelength 216 nm and 20 µl injection volume [15].

The effect of solvents on the UV spectrum of artesunate:

Accurate weight of 100mg (equivalent to 0.26 m mole) of Artesunate was dissolved in methanol, ethanol, acetonitrile, acetic acid, ethyl acetate, acetone, toluene, and 1-butanol separately. All the above solvents were completed to 100ml volumetric flask, and scanned between 200-400 nm.

Effect of pH on UV spectrum of artesunate:

Accurate weight of 100mg (equivalent to 0.26 m mole) of artesunate was dissolved with methanol in 100ml volumetric flask 1ml of the solution was dissolved in 10 ml volumetric flask, completed to the mark with buffer solution ranging from pH 2 to pH 12. The solutions were scanned under UV from 200 to 400nm.

Effect of daylight on instability of artesunate solid form:

A Thin solid film of artesunate powder was evenly spread on (20x20x0.3cm) covered with another glass plate of the same type and sealed together with a gum tape. The artesunate directly exposed to sunlight over a period of 11 weeks. Every week the powder was scraped off and collected in a dry amber glass container, 100mg of degraded artesunate was dissolved in acetonitrile and analyzed by HPLC.

Effect of daylight on instability of artesunate in liquid form:

Accurate weight of 100 mg (equivalent to 0.26m mole) of artesunate was dissolved in ethanol/water 30:70 V/V. the solution was directly exposed to sunlight for 5 hours. Aliquots were withdrawn at 0.5, 1.1.5,2,2.5,3,3.5 and 4.0 hours' respectively and Analyzed by HPLC.

Effect of some pharmaceutical excipients in the reaction rate of thermal decomposition of artesunate at 70 °C:

The required excipients were weighted and transferred into 100ml volumetric flask contains 70:30 ethanol/water plus 100 mg of artesunate, the volume was completed to the mark and heated in water-bath thrmostated at 70 °C. Aliquots were withdrawn at 0,5,10,15,20,25.30, minutes into small beaker, cooled in water-bath and analyzed by HPLC.

Effect of UV radiation on artesunate raw material.**a) Solid form at 254nm**

5.0 grams of artesunate raw material were exposed to UV radiation 254 nm; 100 mg of artesunate exposed sample was dissolved in 100 ml volumetric flask, completed with acetonitrile and analyzed at, zero time, 1, 6, 7, 15 and 16 hours.

b) Liquid form at 254nm

Accurately weighed of 100mg (equivalent to 0.26m mole) of artesunate was dissolved in 100 ml volumetric flask contain 30:70 V/V ethanol/water, solution directly exposed to UV radiation and analyzed by HPLC at 1,2,3,4,5, 7 and 13 hours respectively.

Acidic and basic hydrolysis of artesunate raw material.

Accurate weighed of 100 mg (equivalent to 0.26m mole) of artesunate test sample was dissolved in 50 ml of 2 M HCL or 2 M NaOH, and refluxed for 2 hours, the solutions were cooled and adjust to pH 7 and (extract with 15x2 of chloroform, combine the extract and filtrate, evaporate using a rotary evaporator dissolve the residue in ethanol, dried and analyzed by HPLC).

Effect of pH on artesunate exposed to daylight:

Accurate weight of 100mg (equivalent to 0.26 m mole) of artesunate was dissolved in 70 ml of acetonitrile, the volume was completed to 100ml volumetric flask. Pipette out 1ml into 25 volumetric flasks completed with buffer (pH 2 to 12). All the flasks exposed to sunlight over 12 hours, and the samples analyzed by HPLC at 216 nm.

Accelerating stability study of artesunate in solid form: [16]

5 Grams of artesunate raw material was placed in stability chamber at 40 °C and 75% RH for 4 months, 0.260 m mole was dissolved in acetonitrile and analyzed by HPLC interval monthly for four months.

Results and discussion

Effect of solvents on UV spectrum of artesunate

When absorption spectra are recorded in solvents of different polarity, it is found that the positions, intensities and shapes of the absorption bands are usually modified, the spectral shifts are attributed to specific solute-solute and solute-solvent interaction in the form of hydrogen bonding or bulk solvent properties. Apart from these interactions, there are several other factors that may influence the spectra such as acid-base chemistry or charge-transfer interactions. The magnitude of the spectral shifts in various solvents (with different polarities) mainly depends on the strength of the intermolecular hydrogen bond(s) between the substituent groups of the spectrally active molecule and the –OH or NH groups of the solvent molecules and whether the intermolecular hydrogen bonds are present or not. For the molecular systems without intermolecular hydrogen bond, the spectral shifts are sensitive to the solvent polarity. Thus, in many molecules the bands (π - π^*) are shifted bathochromically when the solvent polarity increases. These changes were attributed to hydrogen-bonding interaction between the solute molecule and the solvent molecule. On the other hand, the spectral shifts in the molecular systems with intermolecular hydrogen bonds are very small. In the absence of the intermolecular hydrogen bonds in all the intermolecular hydrogen bonded systems, the solvent shifts are well interpreted by the solvent polarity function [16,17]. Artesunate due to its chemical structure is lack of chromophore so the effect of polar solvents is different due to the polarity of solvents

Table: 1

Artesunate is the most widely used artemisinin derivative throughout the world for the treatment of severe malaria infections and multi-drug resistant infections caused by *P. falciparum*, yet more than one-third of the antimalarial drugs tested over the past decade

in Southeast Asia and sub-Saharan Africa were either adulterated or of poor quality, containing the incorrect chemical formulation. Therefore, identification of authentic artesunate is essential to prevent needless fatalities. A colorimetric test has been developed by the (Central for disease control and prevention) CDC to determine the authenticity of artesunate in tablets. The test is based on the reaction of an alkali decomposition product of artesunate with a diazonium salt, fast red TR (FRTR), that results in an appearance of a yellow color in the presence of artesunate. The colored reaction product can be measured by spectrophotometry at 420 nm. The test is specific to artesunate when conducted at pH 4, paper microfluidics has great potential to generate inexpensive, easy-to-use, rapid and disposable diagnostic devices, based on calorimetric assay, which allows for a semi-quantitative analysis of the artesunate tablets by comparing the developed yellow color on the paper test to a color-coded key chart that comes with the kit. [18].

Table1: effect of solvents on artesunate spectrum

Solvent	Dielectric constant	UV cutoff	λ max	Absorbance
Acetone	21.00	329	205.90	3.24
Acetonitrile	37.00	190	216.00	1.13
Acetic acid	6.20	210	203.10	0.697
1, propanol	20.00	-	209.00	0.674
Ethanol	30.00	205	202.50	0.924
Methanol	33.00	205	203.10	0.881
Dichloromethane	9.10	232	206.00	0.100
Toluene	2.40	285	216.9	2.785
Ethyl acetate	6.00	256	201.10	0.433

General solvents with no aromatic ring have lower cutoff wavelength than aromatic solvents. The behavior of artesunate was differed according to the polar and non-polar solvents, due to the fact that UV-chromophoric system in artesunate is absent, so acetonitrile was the best one for analysis Table 1.

Table 2: Effect of pH on Artesunate on UV Spectrum 200- 400nm

PH	λ max	Absorbance
2.0	202.00	0.330
3.0	202.00	.0.046
4.0	216.00	0.142
5.0	207.00	0.529
6.0	203.00	2.454
7.0	219.00	0.354
8.0	215.00	0.052
9.0	204.00	0.005
12	208.00	1.025

The stability of artesunate is pH dependent. This explains its rapid conversion to Dihydroartemisinin in the stomach as compared to its great stability in other components at a high pH and in the plasma. As a result, in the stomach at pH 1.2, artesunate is short-lived (at $t_{1/2}$ = 10.3 minutes), but at neutral pH its half-life is significantly longer (at $t_{1/2}$ = 7.3 hours in plasma) [19,20]. Artesunate was stable at neutral pH and showed a rapid conversion in acid pH, from the Table 2 at pH 4.0 artesunate showed less intense absorbance at 2016 nm, but at neutral pH the absorbance 0.354 at wavelength 219 nm.

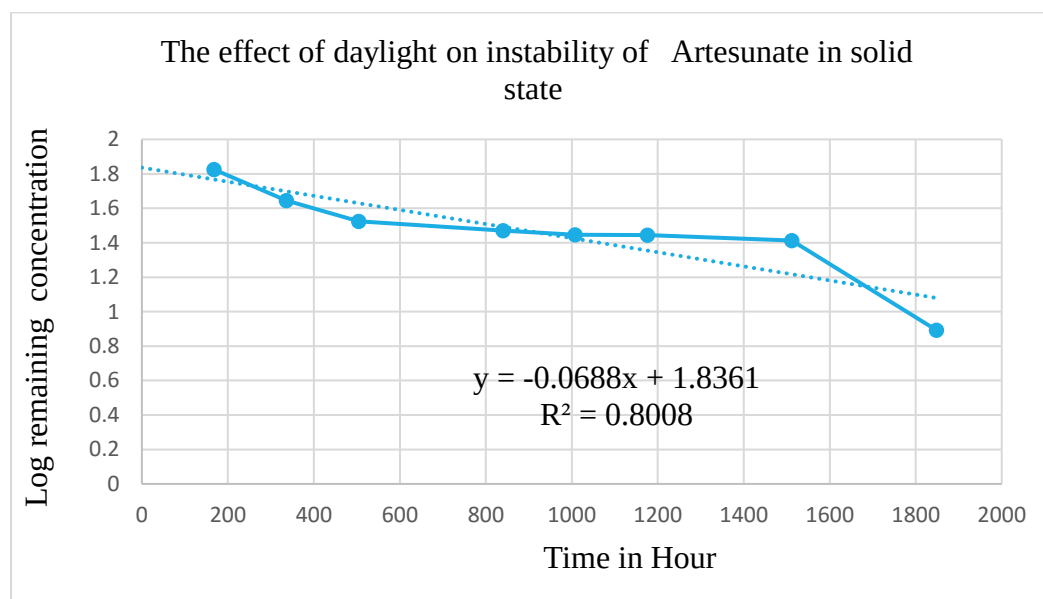


Figure 2: effect of day light on instability of artesunate in solid state

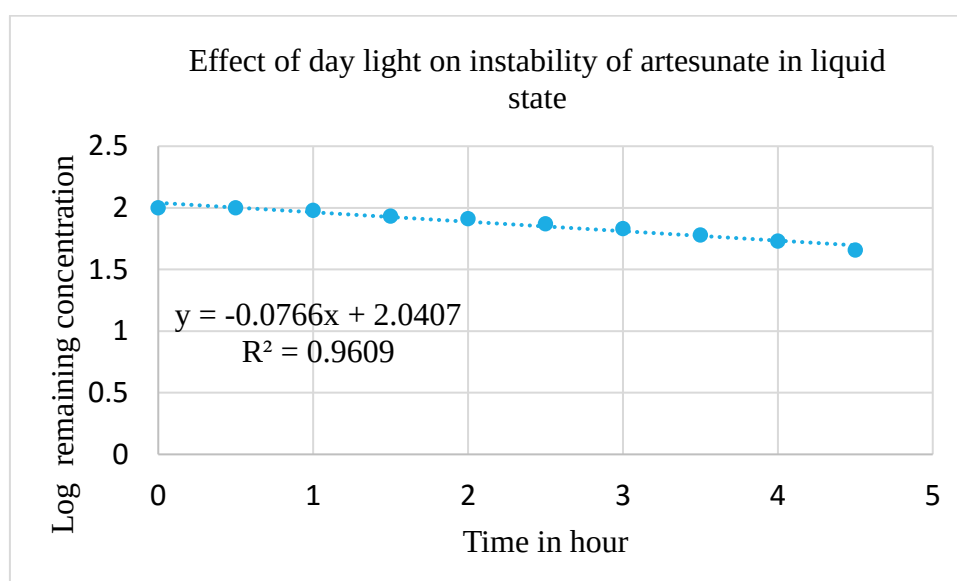


Figure 3: effect of daylight on instability of artesunate in liquid state

We calculate the average rate of a reaction over a time interval of daylight effect on instability reaction rate Figure 2 and Figure3 the concentration artesunate over that time period by the time interval was calculated, which indicate that the rate in solid state

=0.0688 hour⁻¹, the rate in liquid state = 0.0766 hour⁻¹. The negative slope improves the concentration was decreased and reaction kinetic rate was first order.

Table 3: the effect of excipients of reaction rate of artesunate at 70 °C

Excipient	Concentration	pH value	Reaction rate min-1
Methyl paraben	0.05%	5.0	0.0248
Propyl paraben	0.05%	4.8	0.0189
Sodium benzoate	0.1%	7.4	0.0292
Talcum powder	0.1%	9.3	0.0371
Areosil	0.05%	4.5	0.01404
Povidone	0.1%	3.5	0.0139
Maze starch	0.1%	5.8	0.03124
Control (API only)	0.1%	3.80	0.01510

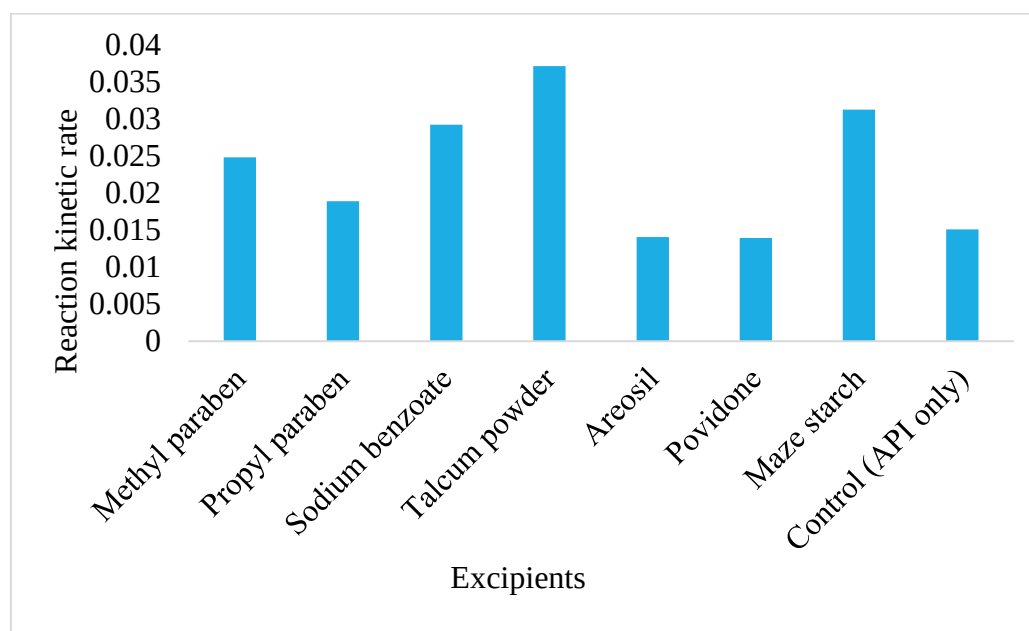


Figure4: the effect of some excipient on reaction rate of artesunate at 70 °C

The result shows that the studied of pharmaceutical excipients were found to fasting the rate of artesunate thermal decomposition at 70 0C in the water bath with thermostatic temperature, in the order of povidone, aerosil, propyl paraben, methyl paraben, sodium benzoate, maze starch and talcum powder. In this study, it was observed that at low pH the reaction was accelerated with respect to the pH value, so the reaction was pH dependent. table:3

Methylparaben and Propyl paraben are used together to control bacterial growth due to their broad antimicrobial spectrum with good stability and non-volatility also they possess a synergistic activity when used as combination. [21].

Table 4: the effect of UV radiation 254nm in liquid form

Time in hour	Concentration %	Log Concentration
Zero time	99.85	1.993
1.0 hour	103.41	2.014
2.0hour	104.30	2.018
3.0hour	102.05	2.088
4.0hour	105.1	2.0216
5.0hour	100.4	2.002
6.0hour	57.65	1.760
7.0hour	51.70	1.713
13.0hour	30.3	1.481

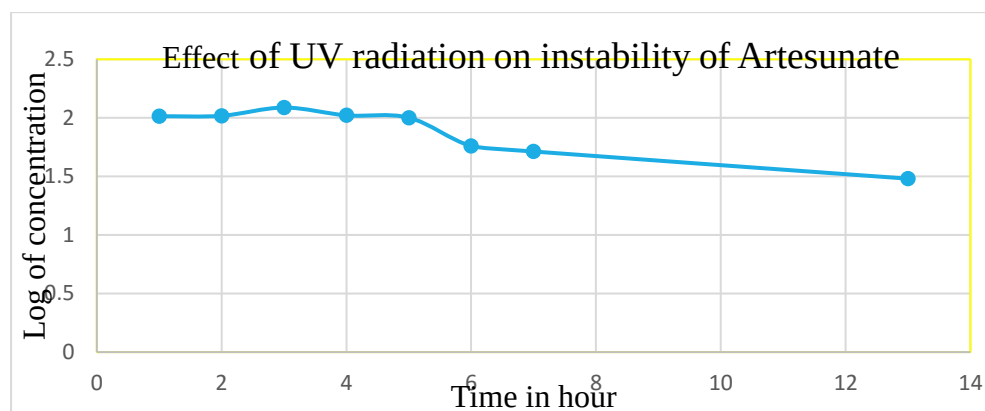


Figure5: Effect of UV radiation on instability of artesunate Liquid state

Table 5: effect of UV radiation 254 nm in solid form

Time in hour	Concentration %	Log Concentration
Zero time	99.85	1.9993
6.0h	109.49	2.0393
7.0h	112.73	2.0520
15.0h	96.71	1.9854
16.0h	96.18	1.9830

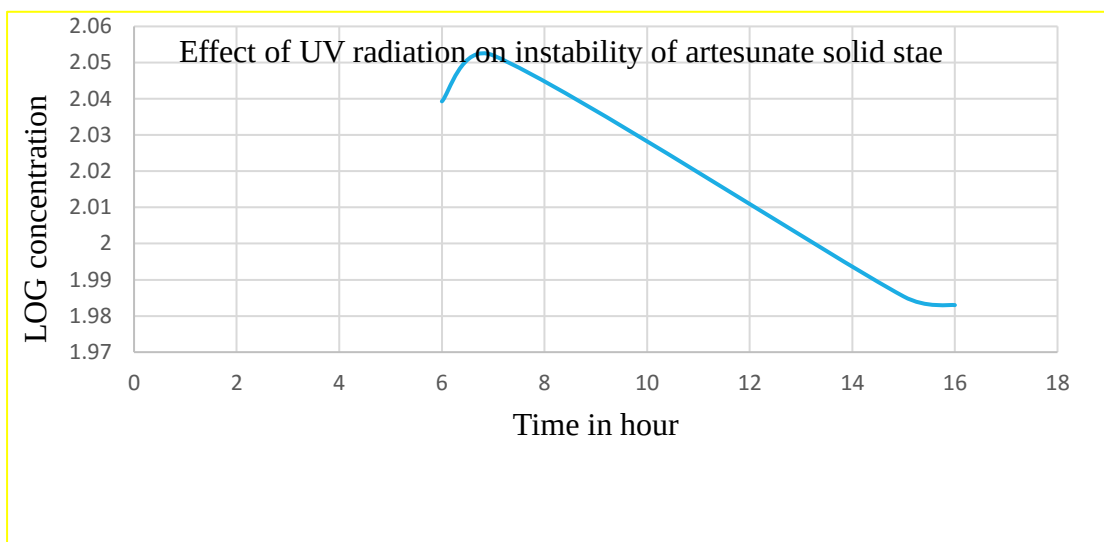


Figure 6: Effect of UV radiation on instability of artesunate solid state

Exposure of a drug to irradiation can influence the stability of the active ingredient, leading to changes in the physicochemical properties of the product. The influence of the selection of a protective packaging must be based on knowledge about the wavelength causing the instability. Artesunate showed significant degradation when exposure UV radiation in liquid form Figure 5, to some extent degraded in solid form Figure 6 was slower compared to the degradation in a liquid state, therefore artesunate is photolabile.

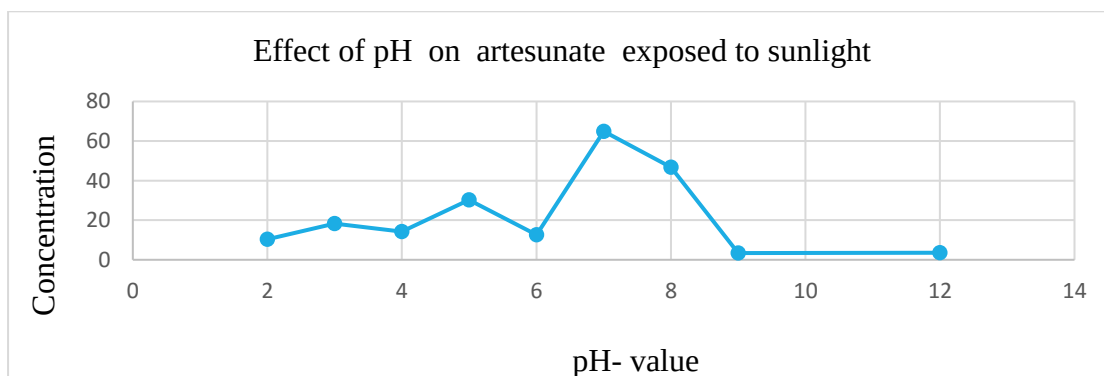


Figure 7: effect of pH on artesunate under sunlight

ART is the only soluble derivative in the market as sodium artesunate injection. Since ART has acidic characteristics owing to its carboxyl group, the influence of pH of the solution and buffers on the solubility rate can be expected. The effect of pH on artesunate under sunlight Figure 7 demonstrated the optimum pH of stability at neutral pH.

Table 6: hydrolysis of Artesunate in alkaline medium

Peak	Time (min)	%Remaining concentration
Artesunate (unheated)	10.945	99.85
Deg1	6.118	5.44
Deg2	9.865	10.8
Artesunate (heated)	10.971	23.20
Deg 3	11.775	51.94

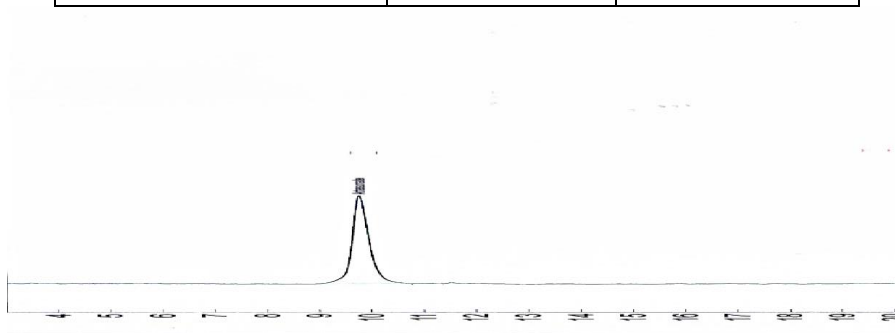


Figure 8: HPLC chromatogram of pure artesunate

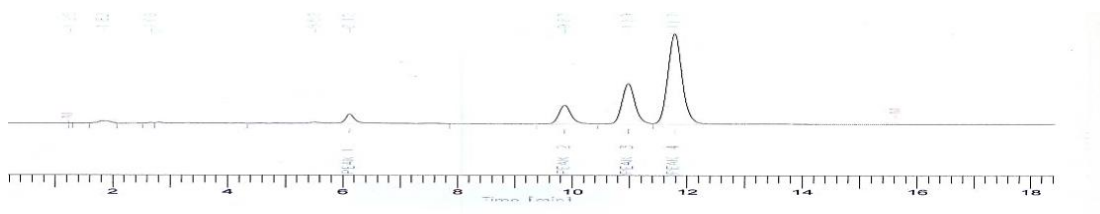


Figure 9: Hydrolysis of artesunate in alkaline medium

Table 7: hydrolysis of artesunate in acid medium

Component	Retention time	% Remaining concentration
Artesunate (unheated)	10.88	99.85
Reflexed artesunate	10.79	55.55

Artesunate having succinate ester groups in their molecular structure when subjected to hydrolysis in the presence of water. The degradation process by hydrolysis is accelerated by the presence of increased concentrations of hydrogen or hydroxyl ions. Hydrolysis of artesunate in acid or alkali medium showed different degradation products, hydrolysis in alkali media produce more than one degradants while in acid medium artesunate produces only on product, which improves the rapid conversion into Dihydroartemisinin its active metabolite.

Table 8: accelerating stability of artesunate [22]

Condition	Drug content	% Degradation
Initial	99.95	0
One month		
Ambient	100.0	-
40 °C/75% RH	99.68	0.32
Three months		
Ambient	100.2	
40 °C/75% RH	99.80	0.40
Four months		
Ambient	100.1	
40 °C/75% RH	99.45	0.65

According to ICH guidelines, the ambient study for drug product must be continued for a sufficient period of time beyond 12 months to cover the shelf life of the product. Intermediate storage condition data are required when a significant change occurs prior to completion of study under the accelerated storage condition. The accelerated storage condition must be >15° C above the ambient storage conditions.

Storage condition of 40 °C and relative humidity of 75% has been recommended for all the four zones for drug substances and drug products. Sudan at zone four so our conducted accelerating stability of artesunate for 4 months showed good observation for storage condition and retest of raw material each four or six month, Table8:

CONCLUSION

This study has demonstrated that artesunate was found to be unstable drugs towards acid, base and UV light into solid and liquid forms. The stability of artesunate is pH dependent. Artesunate was more stable at neutral pH and undergo rapidly converted. The studied pharmaceutical excipients were found to increase the rate of thermal degradation in aqueous media at 70 °C. Artesunate is a photolabile and thermally unstable drug. The hydrolysis in alkaline, acid media is time and pH dependent. The rate of photo thermal decomposition in aqueous medium was found to be increased with pH increased. The hydrolysis in the alkaline medium was very lowest speed compared to acid medium hydrolysis. The current photo and thermal stability studied on artesunate in solid state and aqueous medium experienced first-order reaction kinetic rate. Official method for determination or elucidate the degradant product of artesunate was not enough due to lowest intensity of the peak.

References:

- [1] Brazilian Journal of Pharmaceutical Science. Development and validation of an HPLC method for the simultaneous determination of artesunate and mefloquine hydrochloride in fixed-dose combination tablets .2013, vol 49. No 4.
- [2] Artesunate: Final text for revision of The International Pharmacopoeia (December 2009) World Health Organization (2009).
- [3] T.M. Kalyankar, R.B. Kakde, M.S. Attar, A.R. Kamble. Simultaneous spectrophotometric estimation of artesunate and mefloquine J. Chem., 2013 (2013), p.
- [4] Patidar Khushwant, Sarangdevot Y.S, Saraswat Nitin. Analytical Method Development and Validation of Artesunate in Bulk and Pharmaceutical Dosage Form by using RP-UPLC with Evaporative Light Scattering Detector. J Pharm Sci Bioscientific Res. 2016 6(1):111-119.
- [5] Naik, H., Murry, D. J., Kirsch, L. E. and Fleckenstein, L. (2005). Development and Validation of High Performance Liquid Chromatography-mass Spectroscopic Array for Determination of Artesunate and Dihydroartemisinin in Human Plasma. *J. Chromatograph. B. Analyt. Techno. Biomed Sci.*; 816:233.
- [6] Warhurst D.C. & Peters, W. (1984). Uptake of [3H] dihydroartemisinin by erythrocytes infected with Plasmodium falciparum in vitro. Transactions of the Royal Society of Tropical Medicine and Hygiene 78: 265-270.
- [7] Myra T. Koesdjojo, Yuanyuan Wu, Anukul Boonloed, Elizabeth M. Dun field, Vincent T. Remcho. Low-cost, high-speed identification of counterfeit antimalarial drugs on pape. ELSEVIER Talanta, 130(2014) P:122-127.
- [8] Chinyere Okwelogu, Boladale Silva, Chukwuemka Azubike and Keinde Babatunde. Development of a simple UV Assay method for Artesunate in Pharmaceutical Formulations. Journal of Chemical and Pharmaceutical research, 2011 3(3):277-285.
- [9] Mihaela Homocianu, Anton Airinei and Dana Ortansa Dorohoi. (2011). Solvent Effects on the Electronic Absorption and Fluorescence Spectra. Journal of Advanced Research in Physics 2 (1): 011105.
- [10] Batty, K. T., T. M. E. Davis, L. T. Thu, T. Q. Binh, T. K. Anh, and K. F. Ilett. (1996). Selective high-performance liquid chromatographic determination of artesunate and alpha- and beta-Dihydroartemisinin in patients with falciparum malaria. J. Chromatogr. B 677:345-350.
- [10] Lowery, T.H and Richardson, K.S. Mechanism and theory in organic chemistry, Harper Collins publisher 3rd Ed, (1987): 181-183.
- [11] International Conference on Harmonization (2003). Stability Data Package for Registration Applications in Climatic Zones III and IV; Stability Testing of New Drug Substances and Products; 68 (225): 65717-65718.
- [12] International Conference on Harmonization (2003). Stability Data Package for Registration Applications in Climatic Zones III and IV; Stability Testing of New Drug Substances and Products; 68 (225): 65717-65718.
- [13] Monograph for Antimalaria Drugs Artemisinin and Derivatives. (2009). International Pharmacopoeia Vol. 2. 3rd Edition. WHO CH 22nd Genera, Switzerland.
- [14] Na Banchang, K., Congpuong, K., Hung, L. N., Molunto, P. and Karbwang J.

(1998). Simple High Performance Liquid Chromatographic Method with Electrochemical Detection for Simultaneous Determination of Artesunate and Dihydroartemisinin in Biological Fluids. J. Chromatography. B. Biomed Science Appl. 708:201.

. [15] WHO. International pharmacopoeia, (2013).

[16] Mihaela Homocianu, Anton Airinei and Dana Ortansa Dorohoi. (2011). Solvent Effects on the Electronic Absorption and Fluorescence Spectra. Journal of Advanced Research in Physics 2 (1): 011105.

[17] Lowery, T.H and, K.S. Mechanism and theory in organic chemistry, Harper Collins publisher 3rd Ed, (1987) :181-183.

[18] Meena S.and S. M. Sandhya. A NOVEL METHOD FOR SPECTROPHOTOMETRIC DETERMINATION OF ARTESUNATE IN PURE FORM AND TABLETS. International Journal of pharmaceutical science and research .2012 vol 3(12) :5111-5115.

[19] World Health Organization, Antimalarial Drug Combination Therapy. Report of WHO Technical Consultation, WHO, Geneva, Switzerland, (2001).

and alpha- and beta-Dihydroartemisinin in patients with falciparum malaria. J. Chromatogr. B 677:345-350.

[20] Teja-Isavadharm P, Watt G, Eamsila C, Jongsakul K, Li Q, Keeratithakul G, Sirisopana N, Luesutthiviboon L, Brewer TG, Kyle DE. Comparative pharmacokinetics and effect kinetics of orally administered artesunate in healthy volunteers and patients with uncomplicated falciparum malaria. Am J Trop Med Hyg. 2001; 65:717–21

[21]. Japanese pharmacopoeia 2nd edition ,1984.

[22] International Conference on Harmonization (2003). Stability Data Package for Registration Applications in Climatic Zones III and IV; Stability Testing of New Drug Substances and Products; 68 (225): 65717-65718.