

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

The stability of Artesunate in solid form towards direct sunlight first order reaction kinetic rate 0.260 min^{-1} , the reaction rate of artesunate was pH dependent. The rate of photo thermal decomposition in aqueous medium was found to be increased with pH increased. The hydrolysis of artesunate in alkaline medium were very low compared to acid medium hydrolysis. All the investigated pharmaceutical excipients were found to increase the thermal instability of artesunate at 70°C in aqueous medium. The tendency of which to increase the thermal stability was in the following order: methyl paraben, Propyl paraben, talcum powder, sodium benzoate, providing and aerosol respectively. Artesunate was affected when exposed to UV radiation in solid and liquid form, artesunate is a photo labile 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 used.

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.

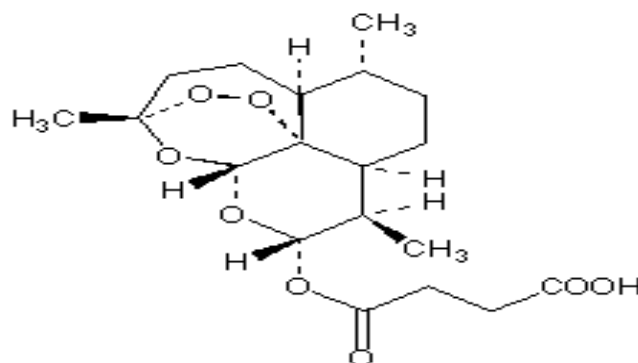


Figure1 : Chemical structure of artesunate

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-oil, hydrogen succinate

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 ^(3, 4).

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), ⁽⁵⁾. Artesunate was thermally labile drug, lack of UV or florescent chromophore for absorption and lack of functional group for derivatization ⁽⁶⁾.

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 accidents 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 C18, Waters Spherisorb®5. 0µm ODS2 4.6 mm x 150mm.

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.

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 equal volume of acetonitrile and 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), flow rate 1.5ml/min., detection wavelength 216 nm and injection volume 20 μ l. ⁽⁷⁾.

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 30 min, 1h, 1.5 h, 2.0 h, 2.5 h, 3.0 h, 3.5 h, 4.0 h, 4.5 h 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 thermostated 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 hour, 6 h, 7 h, and 15 h and 16 h.

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 1h,2h,3h,4h,5h,6h,7h and 13h 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 relaxed for 2 hours, the solutions were cooled and adjust to pH 7 and extracted with chloroform, evaporated, dissolved 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 :⁽⁸⁾.

5 Grams of artesunate raw material was placed in stability chamber at 40 °C and 75% RH for 4 months, 0.260 mmole 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 ^(9,10). 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

Table1: effect of solvents on artesunate spectrum

Solvent	Dielectric constant	Density g/ml	λ max	Absorbance
Acetone	21.00	0.786	205.90	3.24
Acetonitrile	37.00	0.786	216.00	1.13
Acetic acid	6.20	1.49	203.10	0.697
1, propanol	20.00	-	209.00	0.674
Ethanol	30.00	0.789	202.50	0.924
Methanol	33.00	0.791	203.10	0.881
Dichloromethane	9.10	1.326	206.00	0.100
Toluene	2.40	0.879	216.9	2.785
Ethyl acetate	6.00	0.894	201.10	0.433

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	.0046
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

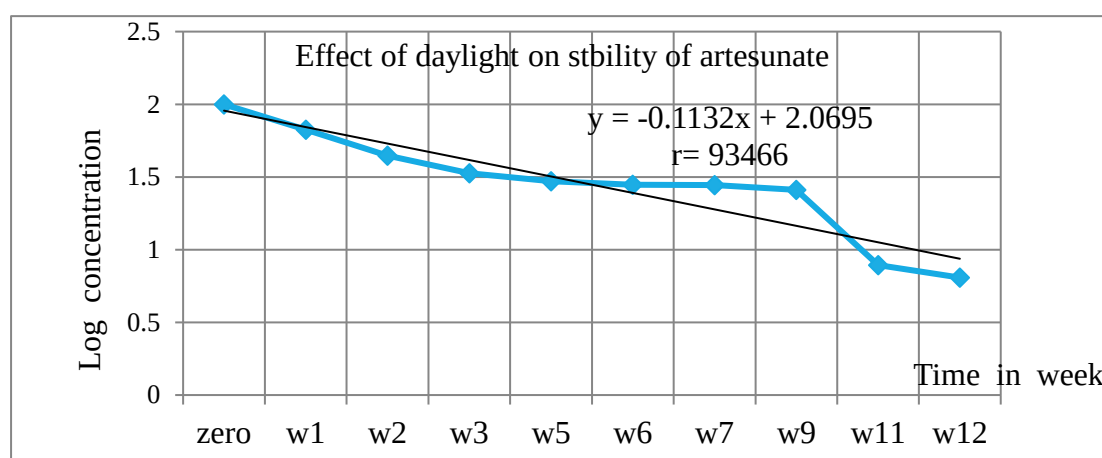


Figure 2: effect of day light on instability of artesunate

Figure 2 indicating that artesunate degradation at direct sunlight showed first reaction kinetic rate.

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	0.030629
Propyl paraben	0.05%	4.8	0.0274
Sodium benzoate	0.1%	7.4	0.02239
Talcum powder	0.1%	9.3	0.02303
Areosil	0.05%	4.5	0.01404
Povidone	0.1%	3.5	0.02233
Control	0.1%	3.80	0.0262

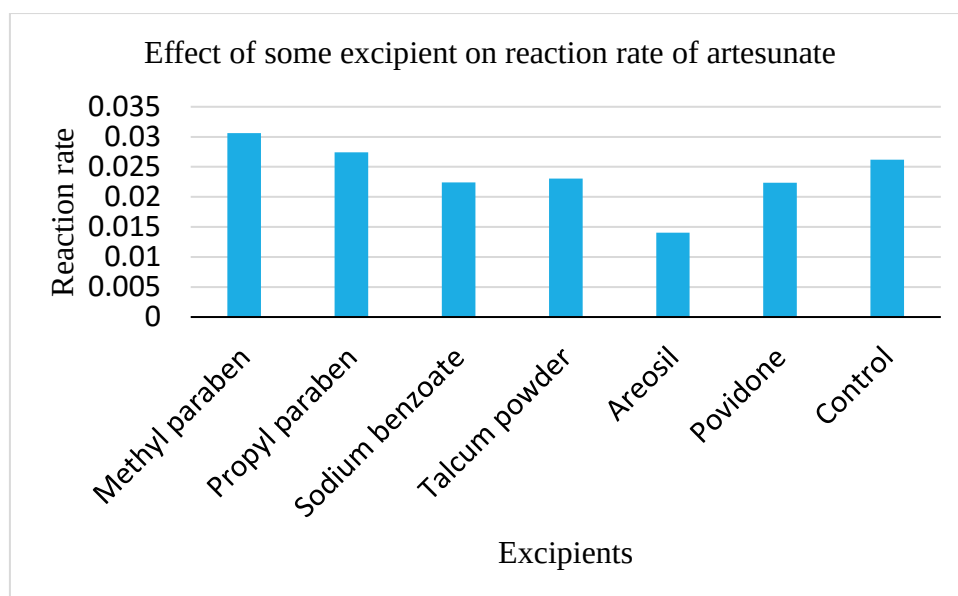


Figure3: the effect of some excipient on reaction rate of artesunate at 70 °C

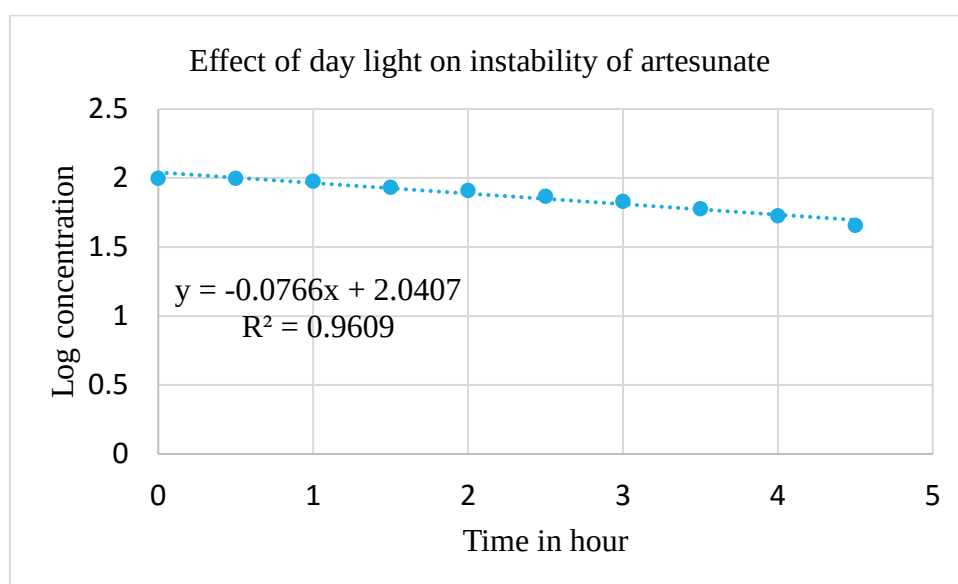


Figure 4: effect of daylight on instability of artesunate in liquid form

Table 4: the effect of UV radiation in liquid form

Time in hour	Concentration %	Log C
Zero time	99.85	1.993
1.0h	103.41	2.014
2.0h	104.30	2.018
3.0h	120.05	2.079
4.0h	115.00	2.060
5.0h	116.78	2.067
6.0h	57.65	1.760
7.0h	51.70	1.713
13.0h	30.3	1.481

Table 5: effect of UV radiation in solid form

Time in hour	Concentration %	Log C
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

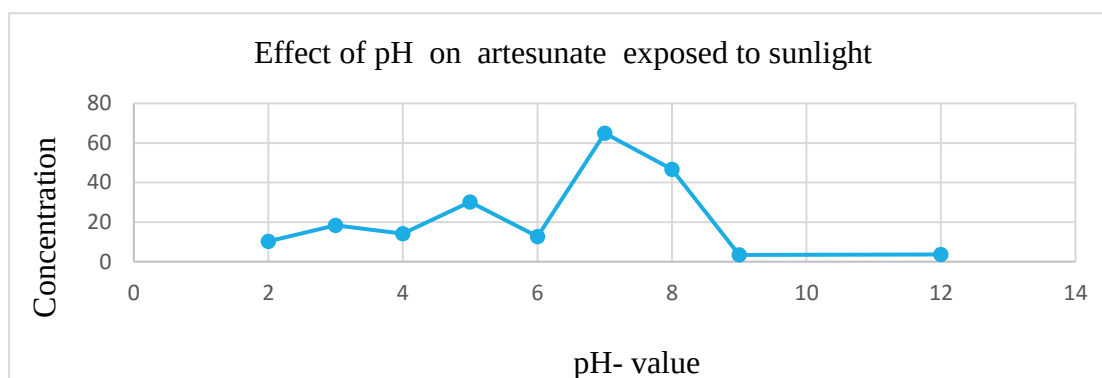


Figure 5: effect of pH on artesunate under sunlight

Table 6: hydrolysis of Artesunate in alkaline medium

Peak	Time (min)	Height (μ v)	Area (μ vs.)
Artesunate (standard)	10.945	578556.10	182529.84
1	6.118	31537.53	464844.79
2	9.865	62532.71	924233.14
3	10.971	134423.88	2106113.20
4	11.775	300938.19	5171807.49

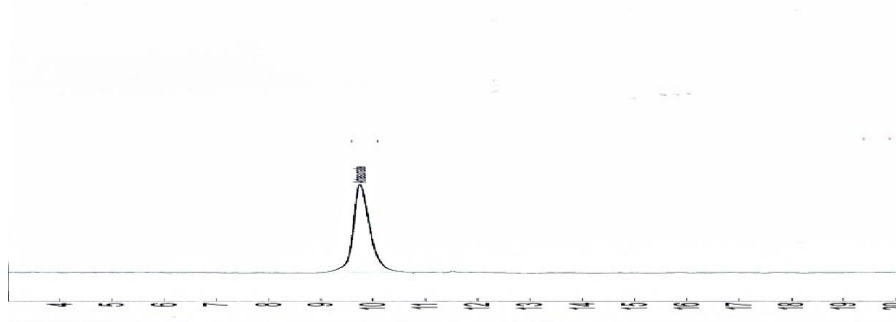


Figure 6: HPLC chromatogram of pure artesunate

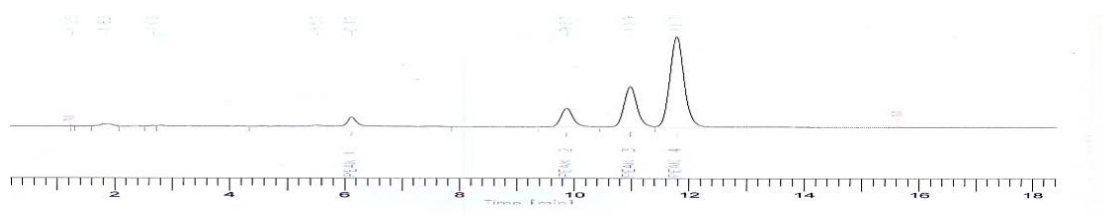


Figure 7: Hydrolysis of artesunate in alkaline medium

Table 7: hydrolysis of artesunate in acid medium

Component	Retention time	% degradation
Artesunate standard	10.88	0.0
Reflexes artesunate	10.79	55.55

Table 8: accelerating stability of artesunate ⁽¹¹⁾

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

Artesunate concededly was stable at accelerating stability chamber for four months
Table: which improve that stability of artesunate.

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 photo labile and thermally unstable drug. The hydrolysis in alkaline, acid media is time and pH dependent. 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) ^(5,3). Artesunate was stable at neutral PH and was a rapid conversion in acid pH.

The rate of photo thermal decomposition in aqueous medium was found to be increased with pH increased. The hydrolysis in alkaline medium were very low compared to acid medium hydrolysis.

From the structure of artesunate figure: 1, it can be seen that there's two functional groups are labile to be decomposed so artesunate stability render to photo and thermal decomposition.

The current photo and thermal stability studied on artesunate in solid state and aqueous medium experienced first order reaction kinetic rate

The result show that the studied of pharmaceutical excipients were found to increase the rate of artesunate thermal decomposition at 70 °C in water bath with thrmostated temperature, in the order of methyl paraben > propyl paraben > talcum powder > sodium benzoate > povidon > areosil SiO₂ with .In this study it was observed that some of excipients decrease the kinetic reaction rate and other increased, methyl parapen pH 5.0 and propyl parapen pH 4.8 both of them are ester so when hydrolyzed it generate the corresponding acid which lead to drop in pH value so degradation will increase. On the other hand, the excipients which is alkaline in nature (talcum powder) it has reaction rate 0.02303 min⁻¹ compared to acidic excipients table: 4.

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