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Stability Study of Artesunate under Stress Condition Using High Performance Liquid Chromatography Method

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

The stability of Artesunate in solid state and Liquid towards direct sunlight showed first-order reaction kinetic rate, the reaction rate of artesunate was pH dependent. The rate of photothermal 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℃ 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 degradants.

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Keywords: Stability of artesunate; under stress condition; HPLC.

1. INTRODUCTION

Pharmaceutical intended to be used in tropics like antimalarial compounds are required to maintain their stability under most severe storage conditions. Understanding of the stability characteristics of drug substances and drug products is a critical responsibility of pharmacist in formulation development determining appropriate storage condition for drug substance or product required knowledge of the conditions that induce degradation mechanism. Antimalarial compounds are weak acids or weak bases; hence their solubility is a function of pH. These compounds also show different photo reactivity in solution as well as in solid state. The formulation process can change crystal modification and photo stability of drugs. Artesunate is in the class of medications known as artemesinins, which are derivatives from the "quinghaosu" or sweet wormwood plant (Artemisia annua) and it is recommended by the World Health Organization (WHO) in preference to quin-idine for the treatment of severe malaria and has been used worldwide for many year [1].

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 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 [2,3]. 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 the pharmaceutical product may deteriorate with time and along the chain of the drug supply system under the prevalent condition of storage.

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.

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 [4,5].

Artesunate (ART) is a readily available anti-malarial in combination therapy, the standard method used to determine the authenticity of ART tablets involves high performance liquid chromatography (HPLC). In many countries, resources to purchase and maintain such equipment are expensive and not always available. Primary aromatic amine was treated with sodium nitrite and hydrochloric acid for diazotization reaction followed by coupling with Artesunate at pH 4, 6, and 8 medium to form a yellow colored azo dye compound which exhibits maximum absorption (λ max) at 420 nm. These

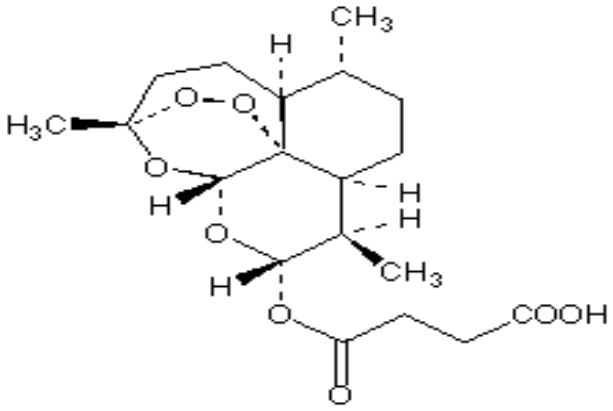

Fig. 1. Chemical structure of artesunate

experiments were repeated twice for Artesunate tablets. The colorimetric method can be used to obtain a rapid visual assessment of tablet authenticity. The method can also be used to quantify the drug content of tablets, when used in conjunction with a spectrophotometer.

The specificity and simplicity of this colorimetric method (Artesunate - benzene diazonium salt test) will certainly be useful in detecting counterfeit Artesunate. Simple UV method has become necessary for the assay of this drug because, UV unlike HPLC is simple, rapid and readily available in malaria endemic areas of the world. This will also help to checkmate influx of fake and adulterated products into the drug market and reduce the burden of malaria. In order to assay Artesunate by UV method, it is necessary to involve it in a reaction process that would break the endo peroxide ring and introduce a least one double bond in the molecule. There is also a novel method is based on the oxidation of FeII to FeIII by artesunate in acid medium and the subsequent formation of Ferric-thiocyanate complex (blood red) chromogen which absorbs, UV-VIS light maximally at 480 nm [6].

The main procedure used to determine the authenticity of artesunate tablets included high-performance liquid chromatography (HPLC). As artesunate is not stable in gas chromatography analysis, this technique is not convenient for characterizing the intact structure of this sesquiterpene endoperoxide. In many countries, resources to purchase and maintain HPLC are not always available, therefore, they are particularly vulnerable to the growing problem of counterfeit artesunate as well as other drugs. Artesunate do not possess reactive groups like Antimalarial such as Chloroquine and Sulfadoxine. tablets. HPLC method has been developed for quantification of Artesunate in combined tablet formulation. The validation procedure confirms that this is an appropriate method for their quantification in the plant material and formulation. It is also used in routine quality control of the raw materials as well as formulations containing any or all of these compounds. From the structure of artesunate Fig. 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 242 nm. HPLC analysis of this mixture revealed the presence of two prominent peaks. The international pharmacopoeia prescribes titration or HPLC for the assay of artesunate. Some workers have developed some methods for assay of artesunate [7,8].

The aims 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 reaction kinetic rates of artesunate thermal decomposition in an aqueous medium.

2. 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 250 mm. 3.0 µm.

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.

2.1 Method Applied and Instrument Used

Samples were analyzed by HPLC consist of SYKAM, pump S11211, solvent delivery system Autosampler S 5200, sample injector loop reading 20µl and detector, UV visible S3200.The UV detector was set at 216 nm. The mobile phase is a mixture of 44 volume of acetonitrile 56 buffer pH 3 (1.36 grams of potassium dihydrogen phosphate was dissolved in1000 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 1.5 ml/min., and 20 µl injection volume [9,10].

2.2 Effect of pH on UV Spectrum of Artesunate

Accurate weight of 100 mg (equivalent to 0.26 m mole) of artesunate was dissolved with methanol in 100 ml volumetric flask 1 ml 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 400 nm.

2.3 Effect of Daylight on Instability of Artesunate Solid Form

A Thin solid film of artesunate powder was evenly spread on (20x20x0.3 cm) 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, 100 mg of degraded artesunate was dissolved in acetonitrile and analyzed by HPLC.

2.4 Effect of Daylight on Instability of Artesunate in Liquid Form

Accurate weight of 100 mg (equivalent to 0.26 m 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.

2.5 Effect of Some Pharmaceutical Excipients in the Reaction Rate of Thermal Decomposition of Artesunate at 70℃

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

2.6 Effect of UV Radiation on Artesunate Raw Material

2.6.1 Solid form at 254 nm

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.

2.6.2 Liquid form at 254 nm

Accurately weighed of 100mg (equivalent to 0.26 m 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.

2.7 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 reflexed 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.

2.8 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 100 ml 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.

2.9 Accelerating Stability Study of Artesunate in Solid Form

5 Grams of artesunate raw material was placed in stability chamber at 40℃ and 75% RH for 4

months, 0.260 m mole was dissolved in acetonitrile and analyzed by HPLC interval monthly for four months.

3. RESULTS AND DISCUSSION

A number of drugs undergo decomposition in solution upon the addition of acids or bases. Dependent on the pKa most drugs are salts of either weak acids or bases in nature. Therefore, in aqueous solution, drug molecules dissociate partially or completely. This dissociation usually has an effect on the drug efficacy or therapeutic effect. Obviously, there are often general acid-base catalysis (from buffer systems) or specific [H+] and [OH-] catalysis in aqueous solution. Although buffer salts are commonly used in pharmaceutical liquids to regulate the pH of the solution, some catalyse the degradation [11]. UV-Vis spectroscopy is based upon measuring the electronic transitions within the UV-Vis range. Generally, Molecules containing π-electrons or non-bonding electrons (n-electrons) can absorb the energy in the form of ultraviolet or visible light to excite these electrons to higher anti-bonding molecular orbitals. For organics, this is stuff with a high degree of conjugation such as aromatic compounds. Include also ligands from metal ion solutions. So, when pH changes, particularly in the case of metal ion solutions, the ligands are directly affected which can result in a shift in the UV-Vis absorption. Artesunate showed maximum absorption at wavelength 216 nm pH 4, which render to use for analysis Table 1.

Table 1. Effect of pH on Artesunate on UV Spectrum 200- 400 nm

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

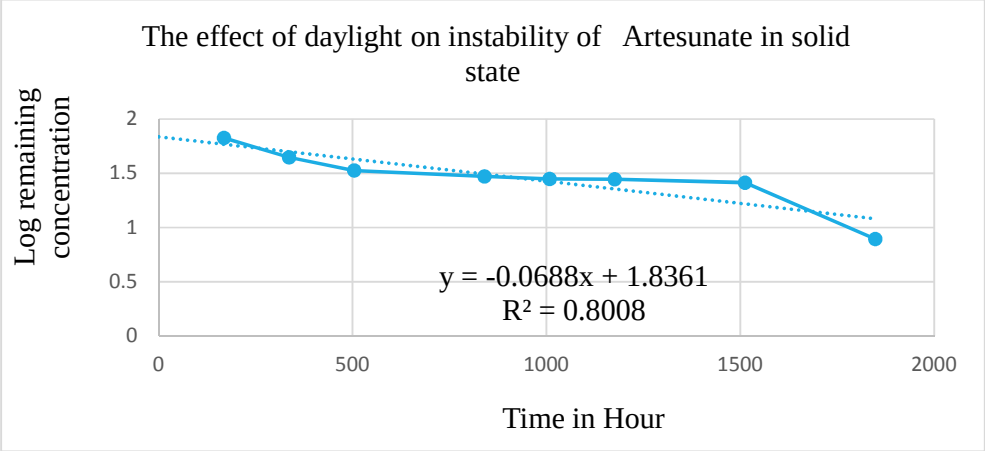

Fig. 2. Effect of day light on instability of artesunate in solid state

Table 2. the effect of excipients of reaction rate of artesunate at 70℃

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

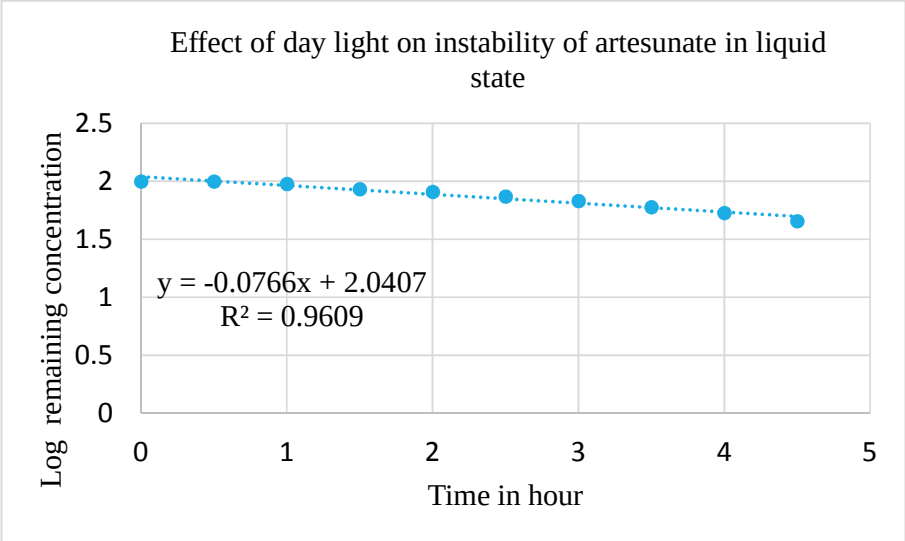

Fig. 3. Effect of daylight on instability of artesunate in liquid state

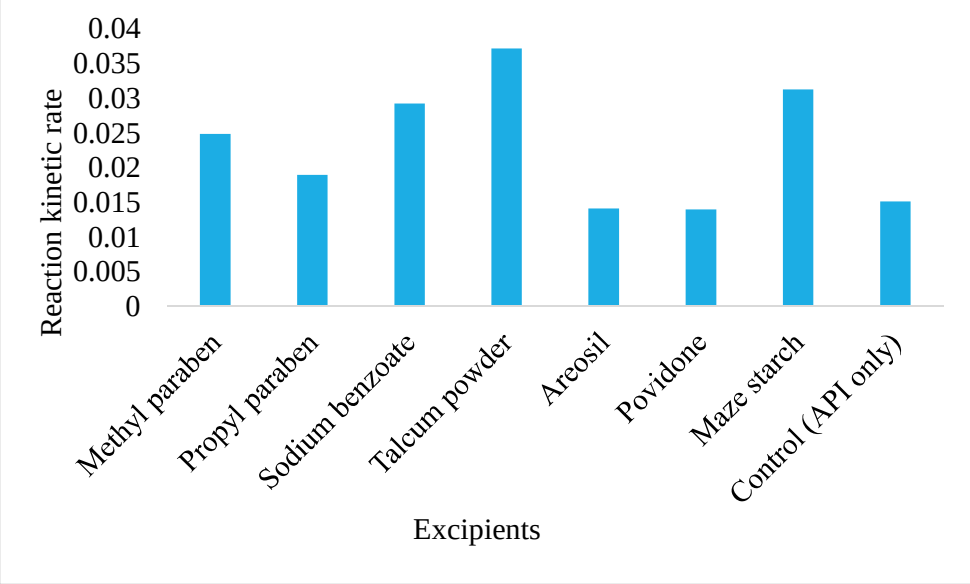

Fig. 4. The effect of some excipient on reaction rate of artesunate at 70 ^{0}C

The rate of reaction of artesunate over time interval was calculated when exposure to direct sunlight the rate of a reaction in solid state 0.0688, liquid state 0.0766 h^{-1}, in solid and liquid degradation, the reaction was first order kinetic rate.

The result shows that the studied of pharmaceutical excipients were found to fasting the rate of artesunate thermal decomposition at 70℃ 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 2.

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 [12].

Table 3. The effect of UV radiation 254 nm in liquid form

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

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 Fig. 5, to some extent degraded in solid form seems to be slower Fig. 6, compared to the degradation in a liquid state Fig. 5. therefor artesunate is photolabile.

Table 4. Effect of UV radiation 254 nm in solid state

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

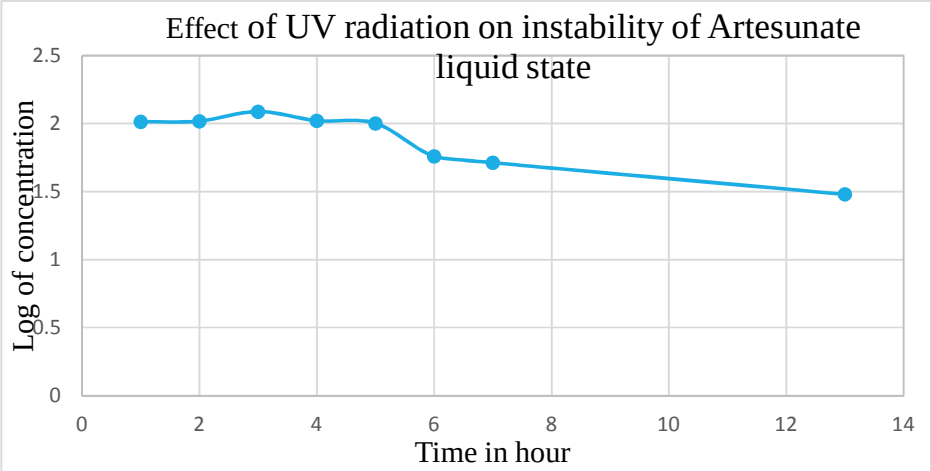

Fig. 5. Effect of UV radiation on instability of artesunate liquid state

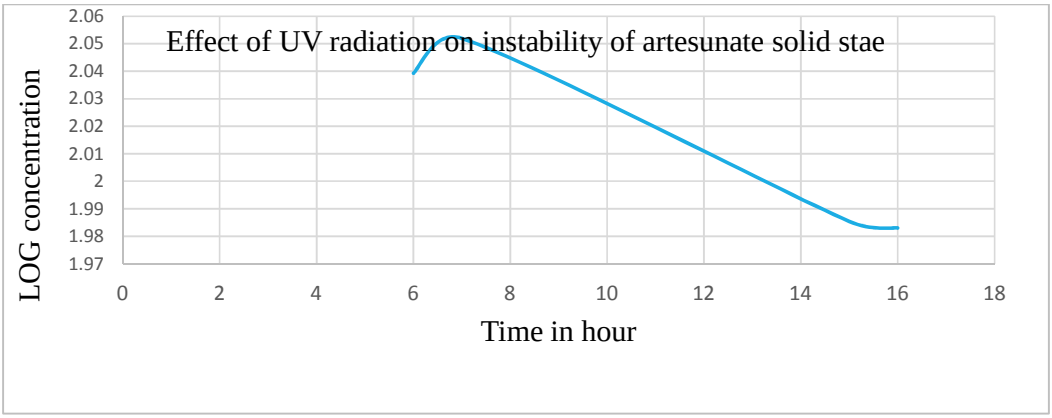

Fig. 6. Effect of UV radiation on instability of artesunate solid state

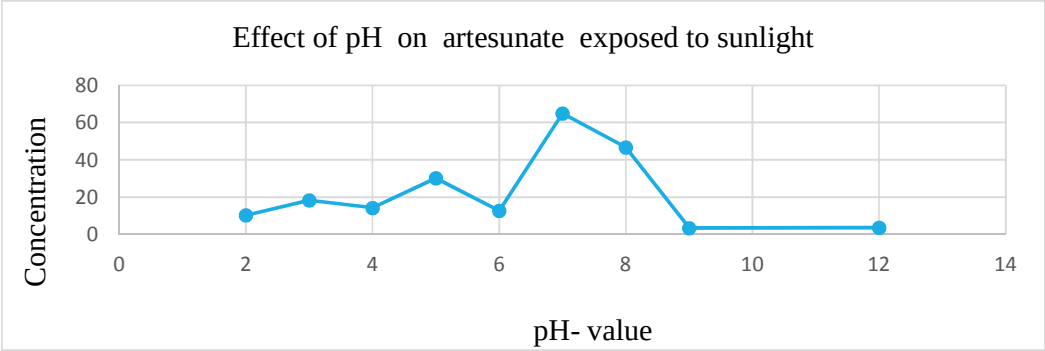

Fig. 7. Effect of pH on artesunate under sunlight

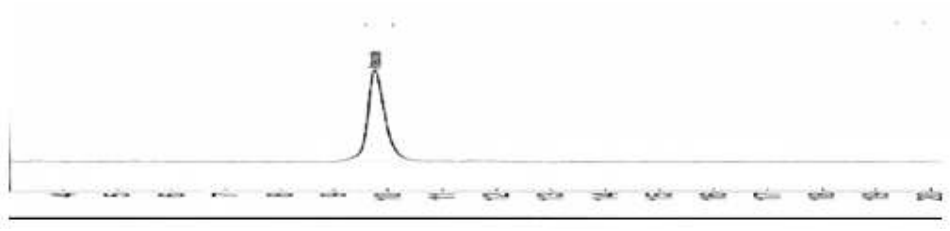

Fig. 8. HPLC chromatogram of pure artesunate

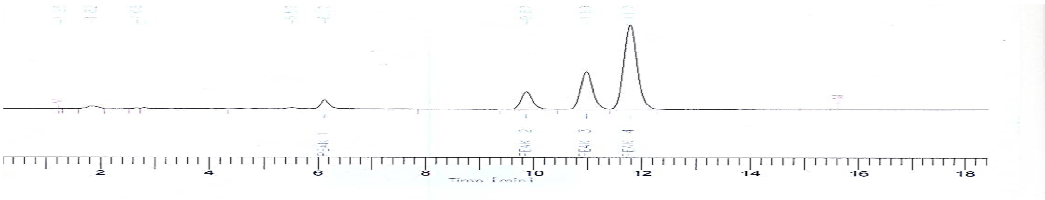

Fig. 9. Hydrolysis of artesunate in alkaline medium

From the observation, Fig. 7 artesunate at acidic PH 2,4 the degradation was increased, the drug was most stable at PH 7 to 8. The stability of artesunate is pH dependent.

Table 5. 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

We have established that artesunate stability varies as a function of pH and temperature.

The transformation of artesunate to dihydroartemisinin follows pseudo-first order kinetics at constant temperature; as a result, in the stomach at pH 1.2 artesunate is short-lived (t l/2 = 10.3 minutes), whilst at neutral pH its half-life is significantly longer (t l/2 = 7.3 hours in plasma) (unpublished data; manuscript in preparation). Esterases too play a role in the oxidation of artesunate [13].

Table 6. hydrolysis of artesunate in acid medium

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

Table 7. Accelerating stability of artesunate

Condition	Drug content	% Degradation
Initial	99.95	0
One month		
Ambient	100.0	-
40℃/75% RH	99.68	0.32
Three months		
Ambient	100.2	
40℃/75% RH	99.80	0.40
Four months		
Ambient	100.1	
40℃/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 >15º C above the ambient storage conditions [14].

Storage condition of 40℃ 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, Table 7.

4. CONCLUSION

Artesunate underwent degradation with time in acid, base, peroxide and UV radiation, an official method can be optimum to established the stress degradation (solid-liquid state). It is a practical scientific standardized guide for stress testing condition. Hyphen technique like HPLC-MS, GC-MS, and capillary electrophoresis can be useful for determination the degradation of artesunate.

COMPETING INTERESTS

Authors have declared that no competing interests exist

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