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Stability indicating RP-HPLC method for Anti-Malarial drug

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ABSTRACT

Artesunate (1) (ART), also called as dihydroartemisinin-12-α-succinate, it is a semisynthetic peroxide-bridged sesquiterpene lactone compound derived from Artemisinin, the bioactive component of the Chinese medicinal herb called Artemisia annua. An accurate, simple and precise HPLC method was developed and validated for forced degradation studies of drug Artesunate .The column was used C18 column (150X4.6mmX5µm) column by isocratic elution. The mobile phase composition consisted of Acetonitrile Water and trifluoroacetic acid (TFA) in the ratio of 55:45:0.1 v/v. The analysis was performed at a 1ml/min flow rate. The analytical performance parameters such as linearity, precision, accuracy, specificity, limit of detection (LOD) and lower limit of quantification (LOQ) were determined according to International Conference for Harmonization ICH Q2 (R1) guidelines.

Keywords: Artesunate, HPLC, Stability, Malaria, Validation

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INTRODUCTION

Artesunate

Artesunate is an anti-malarial drug. [1] It is also known as a dihydroartemisinin-12-α-succinate[2] WHO recommends these drugs as frontline drugs for treatment of malaria[3]. It is chemically known as 4-oxo-4 [(1R,4(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.[3] The chemical formula of Artesunate is $C_{19}H_{28}O_8$ having molecular weight of 382.42. It is a white crystalline powder that is slightly soluble in water.[4]It has relatively low oral bioavailability of approximately 40%.[4] It is freely soluble in acetone and ethanol and very soluble in dichloromethane [5].It is obtained by the reduction of Artemisinin ,a sesquiterpene lactone endoperoxide Synthesis of Artesunate from Artemisinin can be achieved by reducing Artemisinin to DHA using NABH4 in MEOH. Acylation of DHA to Artesunate can be accomplished by treating di isobutyl aluminum hydride (DIBAL) with succinic anhydride in the presence of a base with similar antimalarial activity.[6]

Artesunate is mainly used in the treatment of plasmodium falciparum.[7] The mechanism of action Artemisinin and it's derivative act may be due to reactive oxygen group (ROS) generated by endoperoxidase moiety, cell cycle arrest[8] .Just like Artemisinin the determination of Artesunate is challenging ,[9] because it does not have a distinct chromophore and presents a peroxide bridge which absorbs at lower wavelengths[10]

The main objective of the current research was to develop and validate more accurate sensitive, simple and cost-effective reverse phase HPLC method for stability studies of Artesunate.

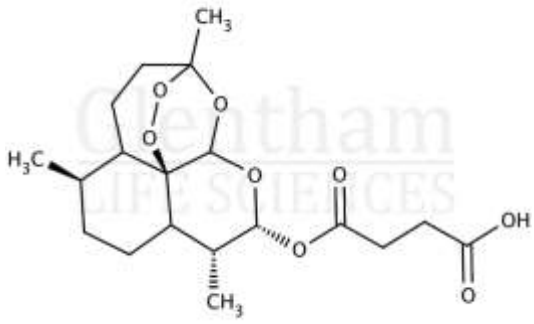

Figure 1: Chemical structure of Artesunate

MATERIALS AND METHOD

Material

Artesunate was gifted by SKM PHARMA Pvt. ltd HPLC grade Acetonitrile was obtained from Merk Life Sciences Pvt .ltd, HPLC grade water from Thermo Fisher Scientific, India and HPLC

graded Trifluoracetic acid (TFA) from Fisher Scientific India. All used ingredient were of analytical grades

Instrumentation

The HPLC system YOUNG LIN-HPLC (ACME9000) equipped with UV/VIS Detector (730D) by these components were connected to a multi-instrument data collecting and data processing system (software Autochrome3000), analytical balance of Shimadzu Model -ATX224. By using syringe filter of 25mm/0.2µm by using membrane filter of 4.5µm.by using ultrasonic cleaner for ultrasonication, and hypersil BDS column C18 with a dimension of 250mm, 4.6mm.5m were also employed.

Selection of solvent (Diluents)

Based upon the solubility and chemical nature of Artesunate the HPLC grade Acetonitrile Water and Trifluoroacetic acid (55:45:1) were selected as a diluent for preparation of standard and stock solution

Preparation of mobile phase

Prepare homogeneous mixture of 550ml HPLC Grade Acetonitrile ,450ml Water and 1ml of HPLC Grade Trifluoroacetic acid .Mix it .shake it well , sonicate it for 2min ,filter the mobile phase through 0.2µm membrane filter paper , sonicate for 10 min before use

Preparation of stock solution

Weighed accurately 50 mg of Artesunate standard, and transferred it into 10 ml of volumetric flask and add diluent up to the mark and make up volume with diluent and shake well, sonicate for 10 min and filter it through 0.2µm syringe

Preparation of standard solution

Pipette out 4ml of stock solution and transfer it in 20ml of volumetric flask, dilute it up to mark with diluent .mix it ,shake well , sonicate for 10 min and filter it through 0.2µm syringe

Forced degradation studies

Artesunate was produced by putting the sample under different stressful conditions. The examination into force degradation provides information on the circumstances under which the medicine is unstable.

Influence of Acid, Alkaline and Neutral Hydrolysis,

Accurately weighed 250mg of ART std and transferred to 50ml of volumetric flask, dilute it up to the mark with diluent. Hydrochloric acid (0.1N, 20ml) and sodium hydroxide (0.1N, 20ml) were added to separate flasks containing drug samples and mixed properly for both degradation respectively and Heat at 60°C for 2 hr. The samples were neutralized with base or acid as

appropriate and diluted up to the marks with diluent to obtain stock solutions. Make up the volume 100 ml with the help of diluents shake well and filter the solution through 0.2µm syringe filter and inject it to HPLC.

Influence of peroxide degradation

Accurately weighed 250 mg of ART std and transferred it to 50 ml of volumetric flask, dilute it up to the mark with diluent. Take 4ml of stock and 4 ml of 3% H_2O_2 solution .Heat at 60°C for 2 hr. then cool it and make up volume up to 20 ml with diluent .Shake well and filter with 0.2µ syringe filter. And inject it to HPLC.

Influence of Heat and Light:

In thermal degradation ART placed onto a glass plate and stored in an oven at 105°C for 6 hours in oven then cool it and take 50mg of drug dilute it up to 10 ml, then take 4 ml of solution transfer it to 20 ml of volumetric flask and make up volume up to20 ml with diluent shake well and filter the solution through 0.2µm syringe filter and inject it to HPLC and in case of photolytic degradation ART was kept on petri dish in UV cabinet for 6-12 hr. take 50 mg of ART and transferred it to 10 ml of volumetric flask and make up volume with diluent . and take 4ml of solution and transferred it to 20ml volumetric flask and make up volume with diluent , shake well and filter the solution through 0.2µm syringe filter and inject it to HPLC.

Method Validation:

The technique validation characteristics, such as specificity, linearity, accuracy, precision, limit of detection, limit of quantitation, and robustness, were examined in accordance with ICH guideline Q2 (R1)

Specificity:

Specificity is defined as the capacity of an analytical method to measure an analyte precisely and specifically in the presence of components that may be anticipated to be present in the sample matrix is known as specificity. Artesunate solution chromatograms and samples that had been degraded were examined to determine the method's specificity and stability indicating qualities. Acidic, alkaline, oxidative, thermal, and photolytic stress conditions were used, and the degraded samples were compared to freshly made sample solutions.

Linearity

In a series of 10mL volumetric flasks, standard solutions (2,3,4,5,6 ml, respectively, From std solution of Artesunate, Aliquots were prepared in the concentration range of 500-1500µg/ml Under the previously mentioned operating chromatographic conditions, a 20-L aliquot of each solution was injected. Peak areas versus concentrations were plotted to create the calibration curve,

and the regression equation was computed. The average of three determinations was used for each response.

Accuracy (% Recovery):

Accuracy of the method was determined by calculating percentage recovery of Artesunate by the standard addition method. Known amount of standard solutions of Artesunate were added to a pre-analyzed sample solution of Artesunate. Each solution was injected in triplicate and the percentage recovery was calculated by measuring the peak areas and fitting these values into the regression equation of the calibration curve.

Precision

Artesunate solution (5000µg/mL) was repeatedly injected to test the repeatability, and the chromatogram was recorded each time. By measuring the equivalent responses three times on the same day and three other days over the course of a week for three different concentrations ART the intra-day and inter-day precisions of the devised approach were determined. Relative standard deviation was used to describe the outcomes.

Limit of Detection and Limit of Quantification

The calibration curve's slope (S) and standard deviation of response (σ) were used to calculate the calibration curve's limit of detection (LOD) and limit of quantitation (LOQ).

Robustness

Robustness of the method was determined by carrying out the analysis under condition during which mobile phase ratio, wavelength and flow rate was altered Variation in mobile phase ratio, wavelength and flow rate were seemed to have greater impact on resolution and hence it should be accurately controlled.

System-Suitability Test:

System suitability tests were done to ensure that the system's resolution and repeatability were sufficient for the required analysis. Retention duration, tailing factor, and theoretical chromatographic peak plates as %RSD of peak area for replicate injections were the parameters employed in this assay.

RESULTS AND DISCUSSION

Selection of Column and Mobile Phase:

Artesunate can be analyzed using reverse phase liquid chromatography (RP-HPLC), in accordance with the published literature and current knowledge of the molecule.

Table 1: Optimized chromatographic condition

Parameter	Chromatographic condition
Instrument	YOUNG LIN-HPLC(ACME9000)
Column	C18(Hypersil BDS)(4.6 x 250mm,5µm
Detector	UV/VIS Detector (730D)
Diluents	ACN:H_2O:TFA (55:45:0.1)
Mobile phase	ACN:H_2O:TFA (55:45:0.1)
Flow rate	1.0ml/min
Detection wavelength	228nm
Temperature	Ambient temperature
Run time	7 min
Injection volume	20µl
Retention time (Rt)	3.5min

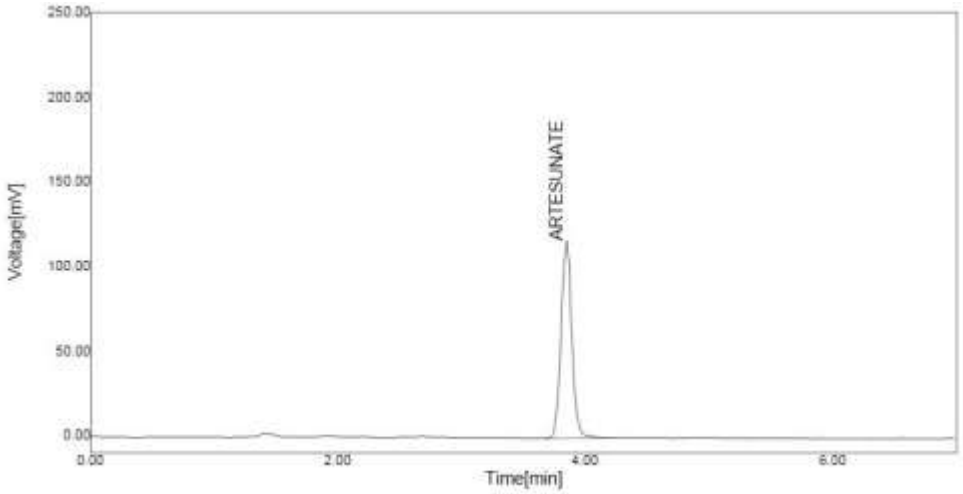

Figure 2: Chromatogram of Artesunate

Method Validation

Specificity

There is no interference from the blank at the retention time of Artesunate peak in standard solution and test solution, Retention time for Artesunate in standard solution and test solution are matching with each other

Linearity

The linearity of the calibration curve was validated by the value of the correlation coefficient of the regression (r), which is y = - 4.127540x+0.6600. The linear correlation was found between the peak area and the concentration of Artesunate in the range of 5-15 mg/ml

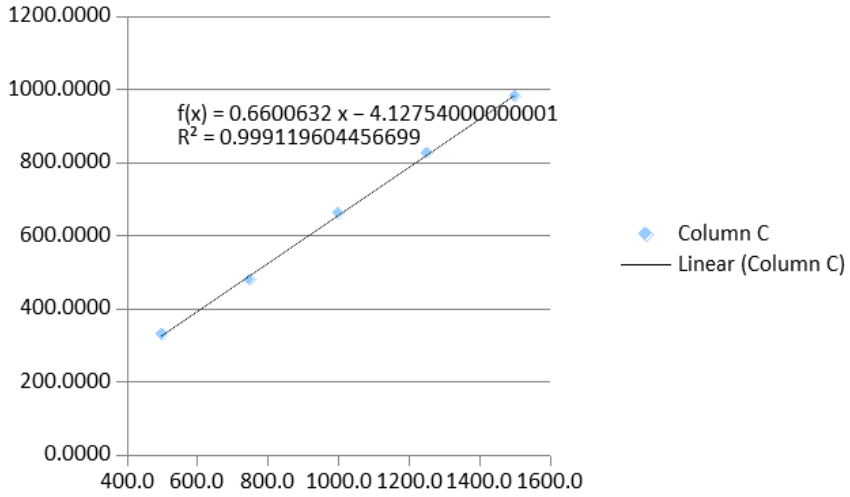

Figure 3: Calibration curve of Artesunate

Accuracy

Applying the traditional addition approach, the accuracy investigation was recoveries, which were determined to be within the range of 98.86- 101.12%.

Table 2: Result of % recovery study

Accuracy level	Mean recovery	S.D	%RSD
80%	100.48	0.449	0.44
100%	99.10	1.3172	1.33
120%	98.16	0.1274	0.13

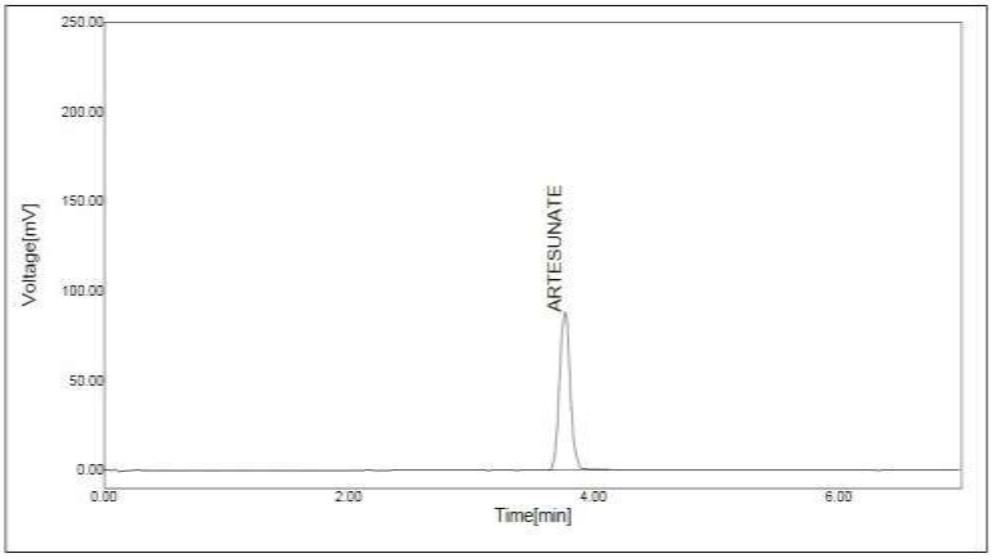

Figure 4: Chromatogram of Accuracy 80%

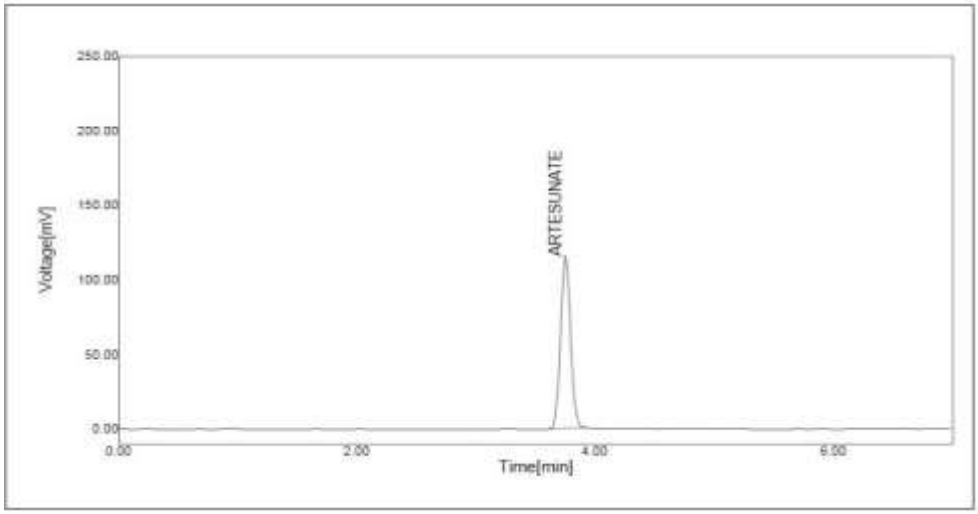

Figure 5: Chromatogram of Accuracy at 100%

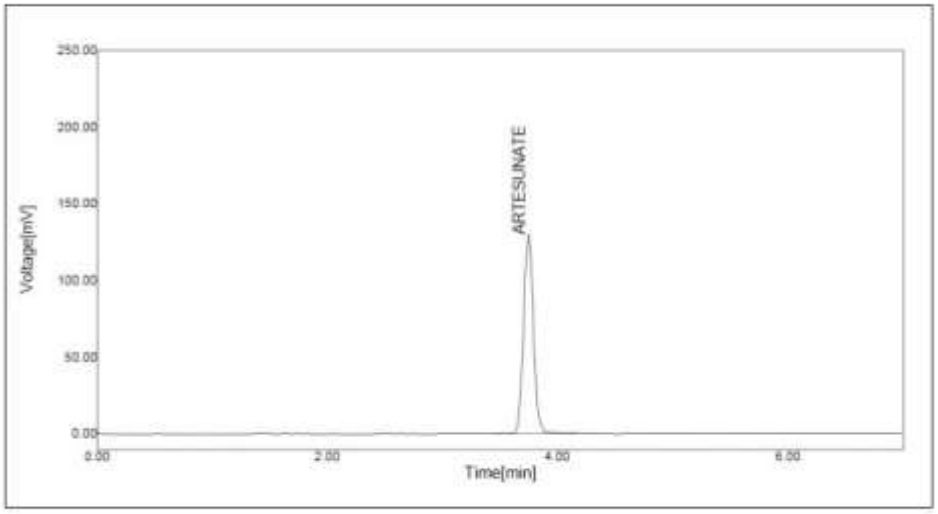

Figure 6: Chromatogram of Accuracy at 120%

Precision

Intraday precision

Table 3: Results for intraday precision

Name	Preparation	%Assay
Set-1	Prep-01	100.40
	Prep-02	98.78
Set-2	Prep-01	99.45
	Prep-02	99.35
Mean		99.5025
SD		0.669
% RSD (NMT 2.0)		0.67

Inter-day precision

Table 4: Results for interday precision

Name	Preparation	%Assay
Set-1	Prep-01	100.40
	Prep-02	98.78
Set-2	Prep-01	101.88
	Prep-02	99.26
Mean		100.08
SD		1.3790
% RSD (NMT 2.0)		1.38

Limit of detection and limit of quantification

The Limit of detection (LOD) for Artesunate was found to be 44.71µg/ml while the Limit of quantification (LOQ) was 135.49µg/ml.

Table 5: Results for (LOD) and (LOQ)

Parameter	Area
Correlation coefficient (r)	0.9996
STEYX	8.9432
SLOPE	0.6601
LOD (µg/ml)	44.71
LOQ (µg/ml)	135.49

Robustness

Table 6: Robustness of Artesunate

Robustness change in method parameter	Preparation	%Assay
Original method parameter	Test prep-1	100.40
Original method parameters	Test prep-2	98.78
Flow rate 0.90ml/min	Test prep	101.06
Flow rate 1.1ml/min	Test prep	102.28
Wavelength 226nm	Test prep	99.37
Wavelength241nm	Test prep	98.67
ACN:H2O:TFA, 52:48:0.1	Test prep	100.11
ACN:H2O:TFA, 58:42:0.1	Test prep	99.59
Mean		100.03
SD		1.2135
%RSD		1.21

System suitability

The % RSD of system-suitability test parameters was found satisfactory. The results are listed in Table 6

Table 7: System suitability parameter of Artesunate

Name	Area	RT(min)	TP(NLT2000)	TF(NMT2)
Standard Inj 01	677.2486	3.85	17187	098
Standard Inj 02	661.8599	3.80	16770	0.95
Standard Inj 03	657.3779	3.77	22328	1.07
Standard Inj04	654.6885	3.78	12587	1.08

Standard Inj05	648.7689	3.77	16488	0.99
Mean	659.7689	3.79		
SD	10.7494	0.0336		
%RSD	1.63	0.89		

Degradation Study:

Forced degradation study of Artesunate was carried out under various stress conditions as follows

Effect of Acid, Alkaline and Neutral Hydrolysis:

Effect of Oxidation: In oxidation stress condition, almost 8.25% of Artesunate was degraded and degradation peak appeared in chromatogram.

Effect of Heat:

Under dry thermal stress condition, Artesunate was degraded about 4.04% with degradation product. Artesunate was found to decompose 31.46% under acidic stress with a major degradation product at retention time of about 3.77 min and minor degradation product at retention time of about 3.85 min. Under basic stress, Artesunate decomposed about 29.95%.

Effect of light:

When Artesunate was exposed to sunlight or exposed to UV radiation in its powder form in its powder form and solution no degradation was seen

Table 8: Forced degradation studies of Artesunate

Types of Degradation	% Assay	% Degradation
Acid degradation	68.54	31.46
Base degradation	70.05	29.95
Peroxide degradation	91.75	8.25
Thermal degradation	95.96	4.04
Photolytic degradation	98.04	1.96

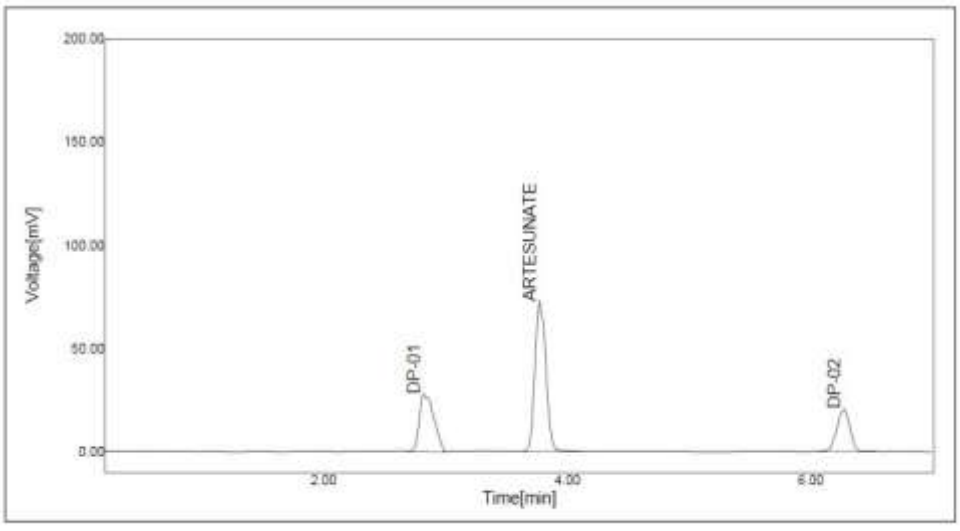

Figure 7: Acid degradation of Artesunate

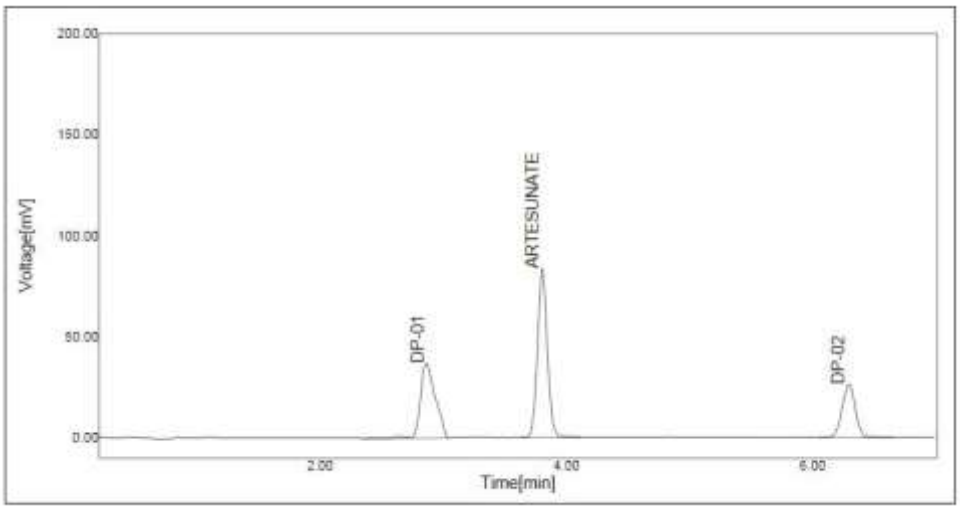

Figure 8: Base degradation of Artesunate

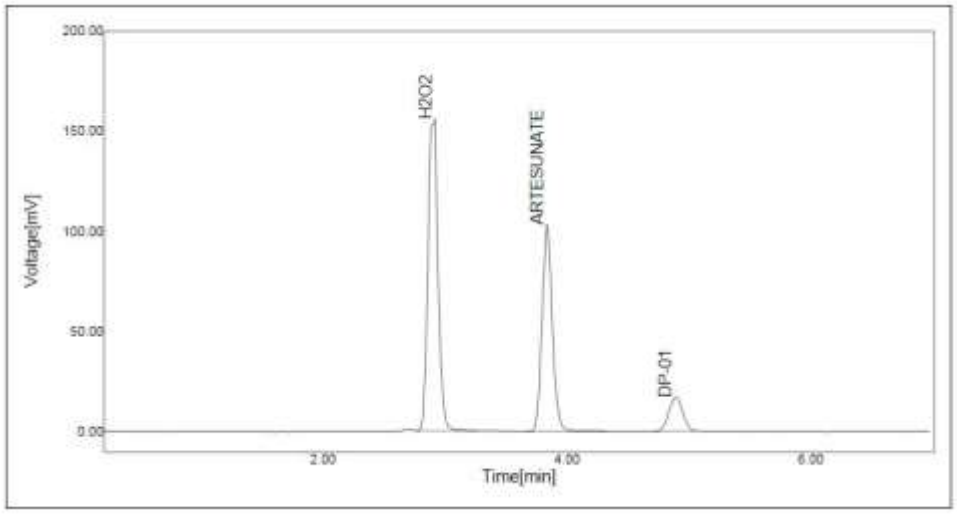

Figure 9: Peroxide Degradation of Artesunate

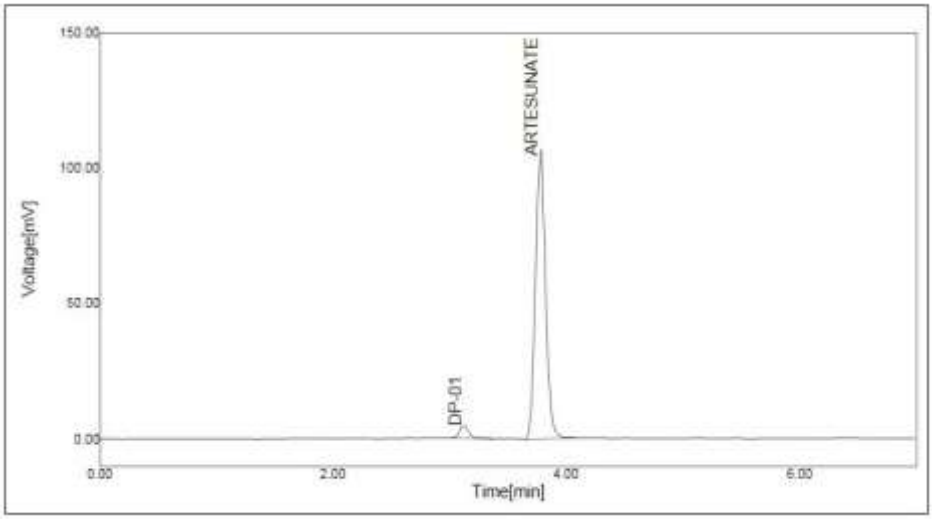

Figure 10: Thermal degradation of Artesunate

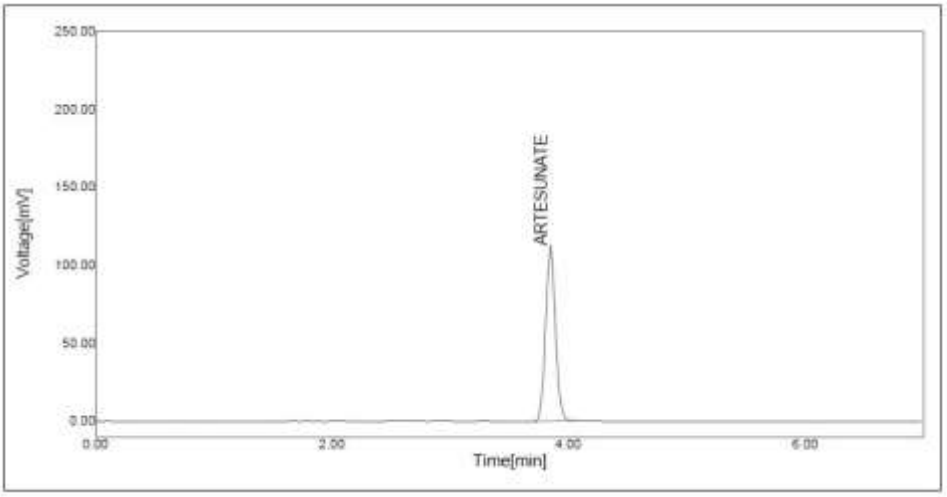

Figure 11: Photolytic degradation of Artesunate

CONCLUSION

A stability-indicating reverse phase liquid chromatographic method has been created and validated for determination of Artesunate in pharmaceutical dosage forms .This method was found to be simple ,accurate ,robust and reproducible and suitable for stability studies of Artesunate as there was no interference of any co-eluting impurities after stress studies. All the parameters are within limits and meets the acceptance criteria of ICH (Q2A) guidelines for method validation, hence it can be used in routine for stability studies of Artesunate in pharmaceutical dosage form.

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