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Original Research Article

Development of Stability Indicating UV-Method for The Determination of Artesunate in Bulk and Pharmaceutical Dosage Form

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Abstract: Artesunate is a natural product in Artemisia apiacea, Acronychiapubescens, and Artemisia carvifolia. It is indicated for the initial treatment of severe malaria. The World Health Organization recommends Artesunate as a first-line treatment for malaria. The main aim of our study was to develop and validate a simple, accurate, cost-effective UV- Visible Spectrophotometric method for estimating Artesunate in bulk and pharmaceutical dosage forms. Degradation studies for Artesunate using the UV Spectroscopic method currently not available. The study has extended towards investigating stress degradation behavior by exposing it to various forced degradation conditions. A solvent was used to develop a method of Artesunate, methanol, and water in the ratio of 1:9. The λ_{max} was found to be at 248nm. The linearity range was fixed as 10 µg/ml-50µg/ml with an R^2 of 0.9997. The % recovery was 98-102%, indicating the method's accuracy. The developed method is validated as per ICH Q2 guidelines. The LOD and LOQ were 0.435µg/ml and 1.31µg/ml respectively. The % RSD was found to be well within the limits. The drug showed less stability in oxidative and thermal conditions.

Keywords: Artesunate, Stability, Validation, ICH Guidelines, UV- Visible Spectrophotometry, Degradation.

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1. INTRODUCTION

1.1 UV/Visible Spectrophotometry

Molecular absorption spectroscopy in the UV and Visible spectral regions is widely used to determine many inorganic, organic, and biological species quantitatively. Spectroscopy is based on the interaction between light and matter. When the matter absorbs the light, it undergoes excitation and de-excitation, producing a spectrum. ThePrinciple of UV/Visible Spectroscopy is based on chemical compounds' absorption of ultraviolet or visible light. [1-4] It is important to note that the difference in the energies of the ground state and the excited state of the electron is always equal to the amount of ultraviolet radiation or visible radiation absorbed by it. UV-Visible Spectroscopy is governed by Beer-Lambert's Law. [5-7]

1.2 Beer-Lambert's Law

As per the Beer-Lambert law, the greater the number of absorbing molecules (that can absorb light of a specific wavelength), the greater the extent of radiation absorption.

$$A = \varepsilon b c$$

The law states that the concentration is the direct measurement of the amount of an absorbing species present and is independent of wavelength. [8-11]Artesunate is the first-line treatment for children or adults with severe malaria, commonly used with another antimalarial drug. In May 2020, Artesunate was approved for medical use in the United States. Before this approval, intravenous (IV) Artesunate was only available through the Expanded Access program of the U.S. Food and Drug Administration (FDA), which allowed the Center for Disease Control and Prevention (CDC) to provide IV artesunate to people in the U.S. with severe malaria.

1.3 Drug Profile

Artesunate is an artemisinin derivative that is the hemisuccinate ester of the lactol resulting from reducing the lactone carbonyl group of artemisinin. It is used, generally as sodium salt, to treat malaria. It has a role as an antimalarial, a ferroptosis inducer, and an antineoplastic agent. It is an artemisinin derivative, a sesquiterpenoid, a dicarboxylic acid monoester, a cyclic acetal, a semisynthetic derivative, and a hemisuccinate. [12] Chemical structure and properties of the drug are depicted in Table and Figure 1.

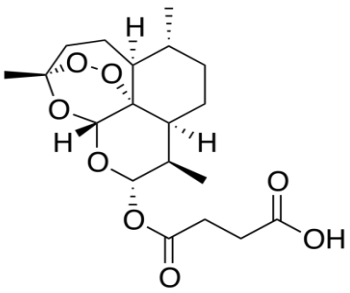

Fig 1: Structure of Artesunate

Table 1. Drug Profile of Artesunate showing different properties of the drug

Generic name	Artesunate
Brand name	Ridsunate (100mg) Norsunate (200mg)
Chemical name	4-oxo-4-[[(1R,4S,5R,8S,9R,10R,12R,13R)-1,5,9-trimethyl-11,14,15,16-tetraoxatetracyclo[10.3.1.04,13.08,13] hexadecan-10-yl] oxy] butanoic acid
Drug class	Anti-malarial
Colour and texture	White and powder form
Molecular weight	384.43g
Empirical formula	$C_{19}H_{28}O_8$

Many methods are available in the literature about the assay ofArtesunate. Many analysts have reported UV spectrophotometric estimation in combinations. Forced degradation study utilizing the UV spectrophotometric method has yet to be reported in bulk and pharmaceutical formulations for Artesunate; subsequently, the present study aimed to develop a cost-effective UV spectrophotometric method encompassing all the parameters by ICH guidelines. [13-20]

1.4 Method Development

Method development is choosing a precise test approach to ascertain a formulation's composition.

1.5 Method Validation

Method validation is a quantitative evaluation approach to ensure that the analytical test system is suitable for its intended use and can produce accurate and relevant analytical data.

1.6 Stability Studies

Maintaining a formulation's physical, chemical, microbiological, therapeutic, and toxicological characteristics in a container or closed system for its life is referred to as "stability testing" of pharmaceutical preparations.

1.7 Aim and Objective

The study's main aim was to develop a cost-effective, less time-consuming method for estimating Artesunate with the help of a UV Visible Spectrophotometer, which adheres to the specifications as per ICH guidelines.

2. MATERIALS AND METHODS

Artesunate was supplied by Chandra Laboratories, Hyderabad, Telangana, and India and was used without further purification. The assay was done using tablets of 200mg strength.

2.1 Instrumentation

Table 2: Instruments Used

S. No.	Instrument	Manufacturer
1	Digital balance	Enertech
2	Sonicator	Enertech
3	Photostability Chamber	Thermo Lab
4	Hot air oven	Bio-Technics India
5	UV- Visible Spectrometer	P.G. Instruments Ltd

2.2 Diluent preparation

Methanol and water in a ratio of 1:9 were used.

2.3 Standard solution preparation

Took 10mg of the drug, dissolved it in 1ml of methanol, and 9ml of water was added to adjust the volume to 10ml to obtain a concentration of 1000µg/ml'. From this solution, the standard solutions required for linearity study were prepared with the same diluent.

2.3.1 Test Preparation

Weighed 10 tablets and powdered them. The powered tablets equivalent to 100mg of Artesunate were weighed and taken into a 100-volumetric flask. Then, 10ml of methanol was added and shaken well to dissolve it, and 90ml of water was added to adjust the volume to 100ml.

2.3.2 Selection of Absorption

The standard solution was scanned for Artesunate in the 400 to 200 nm spectrum mode. λ_{max} of artesunate was found to be 248 nm.

2.4 Validation of The Method

2.4.1 Linearity

Standard solutions of 10µg/ml – 50µg/ml were scanned for Artesunate in the 200-400nm, and the results are reported in Table 3.

2.4.2 Accuracy

Accuracy was ascertained by spiking the standard at three levels (50%,100%, and 150%) to the 10µg/ml sample formulation.

2.4.3 Precision

Six replicates of three different concentrations were scanned within the entire linearity range, and % RSD was reported for repeatability (intraday) and intermediate precision (inter-day)

2.4.4 Robustness

Three replicates of 30µgml concentration were scanned by changing the $\lambda_{max} \pm 5\%$.

2.4.5 Rugg*edness*

Different analysts did three replicates with concentrations of 30µg/ml.

2.5 Forced degradation studies

2.5.1 Acidic degradation studies

10mg of bulk drug was accurately weighed and transferred in a 10ml clean volumetric flask. The drug was solubilized by adding 80:20 ml of NaOH and acetonitrile, and then the rest volume was made up using 0.1N HCl. This solution was refluxed for 6h at 50°C in a water bath. At 0h, 5ml of this solution was taken and diluted to 5ml with diluent; continuous samplings were done hourly, subsequent absorbance was measured, and percentage degradation was calculated.

2.5.2 Alkali degradation studies

10mg of bulk drug was accurately weighed and transferred in a 10ml clean volumetric flask. The drug was solubilized by adding the solvents to 80:20%v/v of NaOH and acetonitrile. The rest volume was made up using 0.1N NaOH solution. This was refluxed for 6h at 50°C in a water bath. At 0h, 5ml of this solution was taken and diluted to 5ml with diluent; continuous samplings were done hourly, subsequent absorbance was measured, and percentage degradation was calculated.

2.5.3 Oxidative degradation studies

10mg of bulk drug was accurately weighed and transferred in a 10ml clean volumetric flask. The drug was solubilized by adding the solvents in the ratio of 80:20%v/v of NaOH and acetonitrile, and the rest volume was made up using H_2O_2. This solution was refluxed for 6h at 50°C in a water bath. At 0h, 5ml of this solution was taken and diluted to 5ml with diluent; continuous samplings were done hourly, subsequent absorbance was measured, and percentage degradation was calculated.

2.5.4 Photolytic degradation studies

10mg of bulk drug was accurately weighed and transferred in a 10ml clean volumetric flask. The volume was adjusted up to the mark with diluent. This solution was placed in the photo-stability chamber for 1 day. Sampling was conducted at 0hr with 0.1ml of solution diluted to 10ml with diluent, another after 24h, subsequent absorbance was measured, and percentage degradation was calculated.

2.5.5 Thermal degradation studies

A specific amount of bulk drug was weighed in a cleaned petri dish and placed into the oven at 50° C for 6hr; hourly, 10mg of bulk drug was transformed into 10µg/ml solution with diluents, and subsequent absorbance was measured, and percentage degradation was calculated. [21-23]

3. RESULTS AND DISCUSSION

3.1 Development of stability – indicating method

The developed method utilized UV spectrophotometric capabilities present in industrial setups for enumerating the stress degradation study along with Artesunate's qualitative and quantitative determination in pharmaceutical preparations. [24-30]

3.2 Validation of the developed stability-indicating method

The developed method was validated per ICH guidelines for determining Artesunate in pharmaceutical dosage forms.

3.3 Linearity and range

Artesunate concentration absorbance readings were recorded at 248nm for zero order using Methanol & Water in the ratio of 1:9 as blank. Fresh aliquots were made from a standard stock solution ranging from 10 to 50µg/ml. The drug showed linearity, and its correlation coefficient was 0.9997.

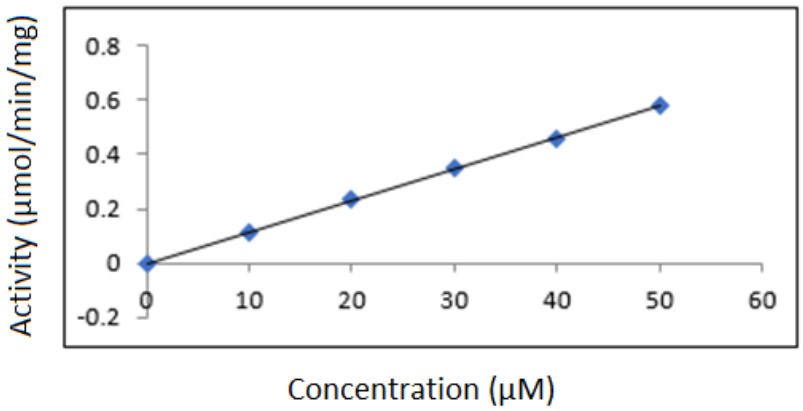

Fig 2: Calibration curve of Artesunate

Table 3. Linearity of Artesunate		
S. No	Artesunate	
	Conc	Abs
I	10	0.109
II	20	0.234
III	30	0.348
IV	40	0.456
V	50	0.578
Regression equation	y =0.0116x - 0.0014	
Slope	0.0116	
Intercept	0.0014	
R^2	0.9997	

3.4 Accuracy

At three levels of 50%, 100%, and 150% every three times, accuracy was done by calculating % recovery studies.

Table 4: Accuracy Study							
Pre-analyzed Conc (µg/ml)	% Sample Spiked	Sample (Tablet)	Standard (30 ppm)	Mean	SD	%RSD	% Recovery
30	50%	3	1.5	0.364333	0.001528	0.419	101.01
		3	1.5				
		3	1.5				
30	100%	3	3	0.398	0.001	0.251	101.51
		3	3				

		3	3				
30	150%	3	4.5	0.431	0.001	0.232	101.01
		3	4.5				
		3	4.5				

3.5 Precision

Intraday and Interday variation studies were performed to achieve precision, six solutions of 30µg/ml of Artesunate were prepared and analyzed three times daily, and the respective absorbances were recorded. Results were indicated as %RSD.

Table 5: Precision Studies (Intraday)

	10 AM Mean ± S. D	%RSD	1 PM Mean ± S. D	%RSD	5 PM Mean ± S. D	%RSD
LQC10ppm	0.108333 ± 0.000816	.753	0.108 ±0.000894	0.828	0.108167 ±0.000983	0.908
MQC30ppm	0.34733 ±0.000816	0.235	0.3475 ±0.000837	0.240	0.348167 ±0.000753	0.216
HQC50ppm	0.577833±0.001169	0.202	0.578±0.001095	0.189	0.577667±0.001033	0.178

Table 6: Precision Studies (Interday)

	10 AM Mean ± S. D	%RSD	1 PM Mean ± S. D	%RSD	5 PM Mean ± S. D	%RSD
LQC10ppm	0.1085 ±0.000837	0.771	0.107667 ± 0.000816	0.758	0.1075 ± 0.001049	0.975
MQC30ppm	0.347333 ±0.000816	0.235	0.347167 ± 0.000753	0.216	0.348 ± 0.000632	0.181
HQC50ppm	0.578167 ±0.000753	0.130	0.578 ± 0.000632	0.109	0.578667 ± 0.000816	0.141

3.6 Robustness

A robustness study was carried out for the drug with (±2nm) to the obtained maximum wavelength. Satisfactory results were obtained and are described below in Table 7.

Table 7: Robustness Studies

Conc(µg/mL)		248nm	
		246nm	250nm
30	Mean	0.347333	0.347667
30	SD	0.000577	0.000577
30	%RSD	0.166224	0.166064

3.7 Ruggedness

Two analysts carried out the ruggedness study of the drug using the same spectroscopic conditions as per ICH guidelines. The results were obtained well within the limits, as shown in Table 8.

Table 8: Ruggedness Study

Conc(µg/mL)		248nm	
		Analyst 1	Analyst 2
30	Mean	0.348333	0.348
30	SD	0.000577	0.001
30	%RSD	0.165746	0.287356

3.8 LOD & LOQ

To obtain the sensitivity of the drug, its LOD and LOQ values were found, which indicates the drug is sensitive, as shown in Table 9.

Table 9: LOD & LOQ values

Drug	LOD (µ g/ml)	LOQ (µg/ml)
Artesunate	0.435	1.318

3.9 Degradation Studies

The drug was made to go through various stress conditions, notably Acid, Base, UV light, Temperature, etc. The drug showed different amounts of degradation in different conditions shown in Table 10.

Table 10: Degradation results

Samples of various Stress Conditions	Time (hr)	Absorbance
Acid	1hr	0.301
	24hr	0.654
Alkali	1h	0.185
	24hr	0.379
Oxidation	1hr	0.655
	24hr	2.675
Thermal	1hr	0.187
	24hr	3.367
Light	1 hr	0.358
	24 hr	0.798

4. CONCLUSION

A simple spectroscopic method has been developed, validated, and checked for degradation studies in bulk and dosage forms. The method was found to be specific as there was no interference of any co-eluting impurities after the stress degradation study, along with qualitative and quantitative determination. Artesunate recovered 101% along with %RSD well within the specified limit. The proposed method under UV- Spectrophotometry was less expensive than other present methods like LC-MS, UPLC, and HPLC. It was more accurate, precise, and time-saving as it shows the drug's degradation results in less time under the proposed stress conditions.

5. AUTHORS CONTRIBUTION STATEMENT

All authors contributed to the research work done in this manuscript. Gangu Sreelatha planned the work, and J.S Swatantra, E. Ratna Sri executed it with the help of Gangu Sreelatha. Ch. Samatha, G. BhagyaLaxmi gave the facility, instruments, and chemicals for the research work. All authors wrote and revised the manuscript.

6. CONFLICT OF INTEREST

Conflict of interest declared none.

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