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Research Article

DEVELOPMENT AND VALIDATION OF CHROMATOGRAPHIC METHOD FOR ESTIMATION OF ARTESUNATE IN API AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, specific, precise and accurate chromatographic method for estimation of Artesunate in API and Pharmaceutical dosage form was developed by Kromasil C18 column having150 mm length, 4.6 mm internal diameter, 5µ particle size. Peak was observed inmobile phase consist of Acetonitrile: 0.1 M Sodium Acetate buffer (Adjusted with OPA, pH 3) in the proportion of 60:40 v/v. The flow rate was 1ml/min. The estimation was carried out at 224 nm. The retention time was found to be 5.3 minfor Artesunate. Linearity was found in range of 1000-6000 mcg/ml (Artesunate).The method was validated as per ICH guideline Q2R1. All validation parameters were found to be within accepted range specified in ICH guideline Q2R1.

INTRODUCTION

Artesunate is an antimalarial agent. It is a water-soluble hemisuccinate derivative ofartemisinin. Artemisinin is a sesquiterpene lactone isolated from Artemisia annua, an herb that has traditionally been used in China for the treatment of malaria. 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. It is also active against chloroquine and mefloquine resistant strains of P. falciparum.

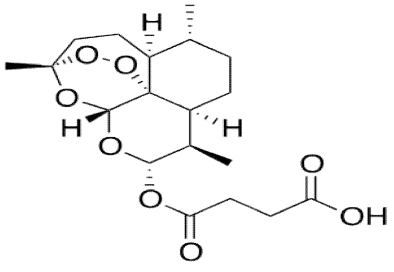

Fig 1 Structure of Artesunate

MATERIALS AND METHOD[4]

Instruments

Shimadzu Model SPD-20A instrument, series RP-HPLC system with UV detector. Kromasil Column C_{18}, 150 X 4.6 mm, 5µm (particle size). Sonicator of Soltec-sonica ultrasonic cleaner (Spincotech Pvt. Ltd.) and analytical balance was of Uni bloc-Shimadzu

Chemicals

Artesunate standard was supplied by Ipca Laboratories Limited Ratlam, MP.

Acetonitrile, Methanol and Distilled water of HPLC grade was purchased from Merck (India) Ltd. Mumbai. Artesunate injection (containing 60mg) was procured from local market. Acetic acid, Sodium acetate and Ortho-phosphoric acid (OPA) of AR-grade was purchased from Merck (India) Ltd. Mumbai.

HPLC condition

A chromatographic separation of drug was achieved using kromasil, 150 X 4.6, 5µm (particle size) C_{18} column with mobile phase having composition of Acetonitrile: 0.1M Sodium Acetate Buffer (60:40 v/v) (pH- 3). Drug was

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monitored at detection wavelength of 224 nm, the flow rate was 1 ml/min, and injection volume was 20 µl. Retention time of Artesunate was about 5.3 minute.

Preparation of Mobile Phase

0.1M Sodium acetate was prepared bydissolving 34.0 ml Sodium acetate in 250ml of HPLC grade distilled water and 15.0ml of Acetic acid in 250ml of HPLC grade distilled water and mix both the solution and mark up to 1000ml with distilled water to get 1 M Sodium acetate buffer (pH-3, adjusted with OPA). Filter the buffer through 0.45µm cellulose filter. Acetonitrile and Buffer was sonicate for 20 minutes.

Preparation of Standard Solution

Accurately weighed quantity of Artesunate (100 mg) was transferred to 10 ml volumetric flask, dissolved and diluted up to the mark with diluent to give a stock solution of 10, 000 mcg/ml. An aliquot (6 ml) of the solution was transferred to a 10 ml volumetric flask and diluted up to the mark with diluent to give working standard solution of 6000mcg/ml. From this solution (10,000 mcg/ml) 1 ml was taken and diluted up to 10 ml with diluent (1000 mcg/ml).

Preparation of Sample Solution

For the analysis of Artesunate, 2ml was taken from test solution and diluted up to 10 ml with diluent. The solution was filtered through 0.45µ Millipore filter. The resulting Solution will be of concentration 2000 mcg/ml.

Method Validation

Specificity

Specificity of an analytical method is its ability to measure the analyte accurately and specifically in the presence of component that may be expected to be present in the matrix. Chromatogram of standard, test and blank is done.

Linearity and Range (n=5)

Linearity response to determine by analyzing different concentrations for calibration curve in range of 1000-6000 mcg/ml for Artesunate. Peak area were measure at each level. Peak area were plotted against concentration and equation of straight line and correlation co-efficient was determine.

Accuracy (n=3)

The accuracy of the method was determined at 0%, 80%, 100%, & 120% by calculating recoveries of Artesunate by the standard addition method. Known amount of standard solutions of Artesunate were added to pre-quantified sample solution of Artesunate. Each solutions was injected in triplicated and the percentage recovery was calculated by measuring the peak areas and fitting these value into the regression equation of the respective calibration curves.

Precision

The repeatability of the proposed method was determined by measuring the corresponding responses 6 times of Artesunate The intra-day and inter-day precisions of the proposed method was determined by measuring the corresponding responses 3 times on the same day and on 3different days over a period of 1 week for 3 different concentration of Artesunate

LOD and LOQ

The LOD is estimated from the set of 3 calibration curves used to determine method linearity.
LOD was calculated as,

LOD =3.3 × (SD/Slope)

SD = Standard deviation of the Y- intercepts of the 5 calibration curves.

Slope = Mean slope of the 5 calibration curves.

The LOQ was estimated from the set of 5 calibration curves used to determine method linearity.

LOQ was calculated as,

LOQ =10 × (SD/Slope)

SD = Standard deviation of the Y- intercepts of the 5 calibration curves.

Slope = Mean slope of the 5 calibration curves.

Robustness

The Robustness Study was performed by altering the method parameters like changing flow rate. The change in the response of Artesunate was noted.

Flow rate was changed from 1ml/min to 0.8ml/min and 1.2ml/min.

Assay

For the analysis of Artesunate (60mg/6ml), 2ml was taken from test solution and diluted up to 10 ml with diluent. The solution was filtered through 0.45µ Millipore filter. The resulting Solution will be of concentration 2000 mcg/ml.

RESULTS AND DISCUSSION

The analytical method was found to be specific as there was no interference of any excipients or impurities which can be shown from figure 2, 3 and 4.Overlay of linearity was shown in figure 5 in the range of 1000-6000 mcg/ml respectively and regression coefficient was found to be 0.9997 which is shown in figure 6and calibration data are shown in table 1 and regression data is shown in table 2.The %RSD for repeatability was found to be 1.281 as mentioned in table 3. The %RSD for intraday precision was found to be 0.7639-0.9930 of Artesunate mentioned in table 4. The %RSD for interday precision was found to be 0.9259-1.2009 mentioned in table 5. The % recovery for Accuracy of Artesunate was found to be in range of 98.44-101.83 mentioned in table 6. The % RSD for robustness was found to be 1.050-1.218 for Artesunate as mentioned in table 7. The % assay was found to be 99.56-102.89% as mentioned in table 8.

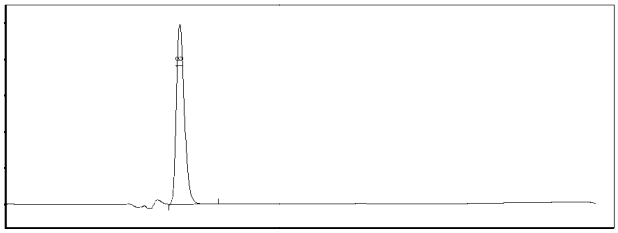

Figure 2 Chromatogram of blank

Figure 3 Chromatogram of standard solution of Artesunate

Figure 4 Chromatogram of Test Solution of Artesunate

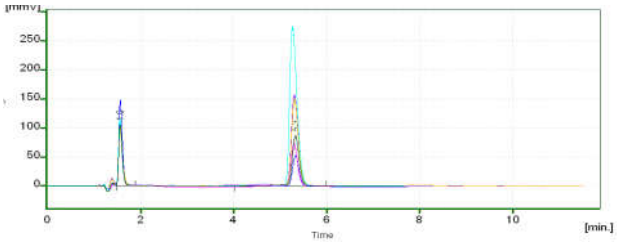

Figure 5 HPLC Overlay Spectra of Artesunate

Table 1 Calibration data for Artesunate at 224 nm

Conc. (mcg/ml)	Mean Response ± SD	% RSD
1000	507.71±6.0532	1.1916
2000	608.43±4.0799	06704
3000	719.50±6.854	0.9526
4000	821.64±5.951	0.7241
5000	925.66±11.057	1.1937
6000	1023.74±12.588	1.2296

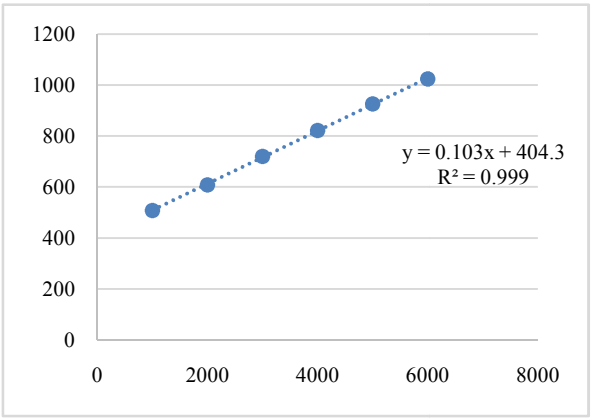

Figure 6 Calibration Curve of Artesunate for HPLC

Table 2 Data of regression analysis of Artesunate

Drug	Straight line equation of Calibration curve	Correlation Coefficient
Artesunate	Y = 0.1038x + 404.38	0.9997

Observation

A method is linear in a range of 1000-6000mcg/ml of Artesunate of standard concentration.

A correlation coefficient for Artesunate is 0.9997.The areas obtained were directly proportional to the concentration of analyte in the sample. The method can, therefore be termed as linear in the specified range.

Precision

Repeatability

The repeatability studies were carried out by measuring response for a single concentration for 6 times a day.

Table 3 Repeatability data for Artesunate

Conc of Artesunate (mcg/ml)	Absorbance (n=6)
3000	712.24
	716.99
	719.17
	724..26
	731.15
	737.03
Mean	723.47
SD	9.274
% RSD	1.281

Intraday precision

Intraday precision was performed by analyzing three different concentration within range, three times in a day (3*3 determination).

Table 4 Data of Intraday Precision for Artesunate

Artesunate		
Conc. (mcg/ml)	Mean response ±SD	% RSD
2000	620.34±6.160	0.9930
3000	718.78±5.826	0.8097
4000	823.30±6.296	0.7639

Interday precision

Interday precision was performed by analyzing three different concentrations within linearity range, on different days.

Table 5 Data for Interday Precision for Artesunate

Artesunate		
Conc. (mcg/ml)	Mean response ±SD	% RSD
2000	616.19±7.404	1.2009
3000	722.49±6.697	0.9259
4000	821.06±8.264	1.0060

Observation

Repeatability- The % RSD was found to be 1.281% for Artesunate. % RSD value was found to be less than 2.0 indicated that the method is precise.

Intraday precision- The %RSD was found to be 0.7639-0.9930% for Artesunate. % RSD value was found to be less than 2.0 indicated that the method is precise.

Interday precision- The % RSD was found to be 0.9259-1.2009% for Artesunate. % RSD value was found to be less than 2.0 indicated that the method is precise.

Table 6 Determination of Accuracy of Artesunate

Amount of % Artesunate present (mcg/ml)	Amount of std Artesunate added	Total amount of Artesunate present(mcg/ml)	Amount recoverd mean (mcg/ml)	SD n=3	% Recovery
2000	0	2000	1968.94	5.960	98.44
	80	3600	1999.48	3.455	99.97
	100	4000	2018.91	3.520	100.94
	120	4400	2036.60	3.757	101.83

Accuracy

Accuracy of the method was confirmed by recovery study from

marketed formulation at three levels (80%, 100%, and 120%) of standard addition

Robustness

Change in Flow Rate

Table 7 Data of Robustness for Artesunate

Drug	Flow rate	Mean ± SD	% RSD
Artesunate	0.8	715.48±8.720	1.218
	1.2	721.81±7.580	1.050

Assay

Table 8 Data of Assay for Artesunate

Amount of Artesunate(mcg/ml)	Mean ±SD	% RSD	% ASSAY
2000	618.0±5.670	0.917	99.56
	624.41±3.085	0.494	102.89
	628.77±3.065	0.486	101.66

Observation: The Assay for Artesunate was shown in table 6.21 .The percentage Assay was found to be 99.56-102.89%.

Summary of Validation parameter for RP-HPLC

Table 9 Summary of validation parameters for RP-HPLC

Parameter	Artesunate
Linearity range (n=5)	1000-6000mcg/ml
Accuracy(% Recovery)	98.44-101.83
LOD (mcg/ml)	0.033
LOQ (mcg/ml)	0.101
Repeatability (n=6)	1.281 % RSD
Intraday (n=3)	0.7639-0.9930 % RSD
Interday (n=3)	0.9259-1.2009 % RSD
Robustness(% RSD)	1.050-1.218
Assay (%)	99.56-102.89

CONCLUSION

The method was found to be simple, specific, accurate, economic and reproducible. Methods can be successfully applied for routine QC analysis. It reveals that RP-HPLC method was validated as per ICH guideline Q2 (R1) as all validation parameters were found within the range.

Acknowledgement

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