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DEVELOPMENT OF A SIMPLE HPLC METHOD FOR THE QUANTITATION OF ARTEMISININ IN ARTEMISIA ANNUA HERB

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ABSTRACT
A high-performance liquid chromatographic (HPLC) method has been developed for the determination of Artemisinin in *Artemisia annua* Herb. The HPLC separation was carried out in isocratic mode using C18, sunfire column (4.6 x 250 mm, 5 ìm particle size) with a mobile phase composed of acetonitrile : water (85:15 v/v) at a flow rate of 1.0 ml/min. The detection was monitored at 216 nm. The method validation parameters showed good results for linearity, precision, accuracy, specificity, and in recovery studies. The calibration curve for Artemisinin was found linear from the range of 500 to 1200 ìg/ml. The interday and intraday studies (relative standard deviation) was obtained within the limits. The proposed HPLC method is precise, accurate and rapid for determination of Artemisinin in *Artemisia annua* Herb.

Key words: *Artemisinin; HPLC; Artemisia annua herb.*

INTRODUCTION

Artemisinin is chemically known as (3*R*,5a*S*,6*R*,8a*S*,9*R*,12*S*,12a*R*)-Octahydro-3,6,9-trimethyl-3,12-epoxy-12*H*-pyrano[4.3-*j*]-1,2-benzodioxepin-10(3*H*)-one. Artemisinin and Artemisia are official in Indian Pharmacopoeia 2007[1,2] and in International Pharmacopoeia[3]. It is used for the treatment of malarial infections. Artemisinin and its semisynthetic derivatives artemether and artesunate have been established as safe and effective antimalarials[4]. The toxicity of artemisinin drugs is much lower than that of quinine and its derivatives. Significant adverse effects or signs of toxicity have not been reported in human patients treated with therapeutic dosages. Several methods have been already reported by High Performance Liquid Chromatography using ELSD detector[5], High Performance Thin Layer Chromatography[6], Gas Chromatography - Mass Spectrometry[7] for the analysis of artemisinin and its analogues, but most of them are not widely adaptable to a large range of analogues.

The objective of the present work was to develop a simple and reproducible HPLC method for the determination of Artemisinin in *Artemisia annua* herb.

EXPERIMENTAL

Materials and Methods

All chemicals and reagents used were of HPLC grade. Acetonitrile was obtained from E. Merck, Mumbai and n-hexane was obtained from Qualigens Fine Chemicals, Mumbai.

Standard Artemisinin and Artemisia annua herb were obtained from CIMAP, Lucknow, India.

Instrument

The instrument used for the study was a Waters High Performance Liquid Chromatography, Millennium[32] Software system equipped with pump 600, inline degasser, 717 plus Autosampler, 486 Tunable Absorbance Detector. A Waters Sunfire C18 column (250 □ 4.6 mm), 5 □m particle size was used as the stationary phase.

Standard Preparation

10 mg of Artemisinin reference standard was dissolved in the mobile phase to produce 10 ml.

Sample Preparation

The collected *Artemisia annua* leaf were dried in shade, crushed into fine powder and the powder was passed through 80 mesh sieve, stored in airtight container at ambient condition. About 5 g of the powdered *Artemisia annua* was accurately weighed and refluxed with n-hexane for 6 hrs at 60±2° in an amber coloured round bottom flask. The contents obtained were cooled to room temperature and filtered through Whatman No. 41 and the filtrate was collected in 250-ml amber coloured volumetric flask. The marc was again refluxed with another 100 ml of n-hexane for 1 hour. The contents obtained were filtered and combined with the previous filtrate. The combined filtrate was evaporated to dryness on water-bath at 70°C. The residue was dissolved and diluted with mobile phase to get final solutions of 1000 □g/ml.

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Chromatographic Conditions

Column	:	Sunfire (W) C18 (250 □ 4.6 mm) x 5 □m particle size
Mobile Phase	:	Acetonitrile and Water in the ratio of (85:15, v/v).
Flow rate:	:	1.0 ml/min.
Detection wavelength	:	216 nm
Injection volume	:	20 □l
Pump mode	:	Isocratic
Run Time	:	10 minutes
Retention Time	:	4.68 minute

RESULT AND DISCUSSION

Method Development

System suitability test was carried out on freshly prepared solution of Reference standard of Artemisinin to check various parameters. System suitability results are as follows:

Retention Time	4.68
Tailing Factors	1.27
Theoretical Plate	1.001119 e + 003
Calibration Range	500 to 1200 □g/ml

Assay

Standard and sample solutions (20 □l) were separately injected on HPLC system. The amount of Artemisinin present per gram of *Artemisia annua* was calculated by comparison of the areas measured for the sample with the area measured for the standard solution of Artemisinin.

Validation of the Proposed Method

The developed method has been validated for the assay of Artemisinin as per ICH guidelines[8] by using following parameters.

Specificity and Selectivity

Specificity and selectivity were studied for the examination of the presence of interfering endogenous components. Reference solution containing Artemisinin was prepared along with blank. Result indicates that the retention time of Artemisinin about 4.68 and none of the impurities were interfering in its assay (Figure 1).

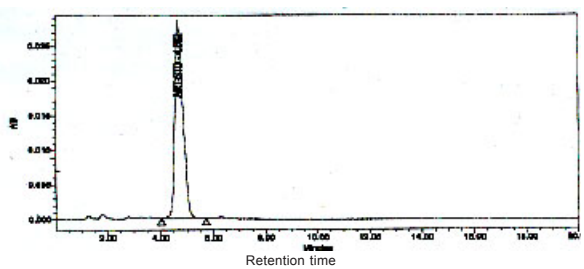

Figure 1 : Typical Chromatogram of standard Artemisinin

Linearity

Linearity was studied by preparing standard solutions at different concentration levels. The linearity range was found to be 500 to 1200 □g/ml.

Accuracy

Accuracy was determined by assay and recovery studies of Artemisinin. A known amount of standard Artemisinin was added into pre-analysed sample and subject them to the proposed HPLC method. The study was carried out at three different concentration levels and the % recovery was found in the range of 94.44 to 95.01 %.

Precision

Precision was studied to find out intra and interday variations in the test methods of Artemisinin in the concentration range 500 to 1200 □g/ml for three times on the same day and interday. Precision was determined by analysing corresponding standard daily for a period of three days. The % RSD in case of intraday and interday were found to be 0.565 and 0.318 respectively.

Stability

Stability of reagents, mobile phase, standard and sample solutions were studied for 48 hours and compared with the freshly prepared solutions and was found to be stable.

Limit of detection & Limit of quantization

Limit of detection and limit of quantization was calculated by the method which was based on the standard deviation (SD) of the response and the slope (S) of the calibration curve at levels approximating the LOD and LOQ, LOD = 3.3 (SD/S) and LOQ = 10 (SD/S), and found to be 0.396 □g/ml and 1.198 □g/ml respectively.

Robustness

This was done by small changing in the chromatographic conditions and found to be unaffected by small changing like □ 2% change in volume of organic solvents of mobile phase.

CONCLUSION

The proposed method can be suitably employed for the routine quality control analysis of Artemisinin from *Artemisia annua* Herbs.

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