



DETERMINATION OF ARTEMISININ IN *ARTEMISIA ABROTANUM* AND *ARTEMISIA PALLENS* BY LC/MS METHOD

J.SURESH^{1*}, K. MRUTHUNJAYA¹, N. PARAMAKRISHNAN¹, M. N. NAGANANDHINI¹

JSS College of Pharmacy, JSS University, S.S. Nagara, Mysore Email: sureshjoghee@yahoo.com

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ABSTRACT

A simple, selective, rapid and precise reverse phase LC/MS method has been developed for the standardization of *Artemisia abrotanum* and *Artemisia pallens* (Family: Asteraceae) using Artemisinin as an analytical marker. The method was carried out on a Princeton SPHER C₁₈ (150 x 4.6 mm i.d. 5 μ) column with a mobile phase consisting of Acetonitrile: Water (60:40 v/v) at a flow rate of 0.5 ml/min. Detection was carried out at 283 nm. The calibration curve was linear in the range of 10 μ g/ml to 140 μ g/ml of Artemisinin and the correlation coefficient was 0.9992, indicating good linear dependence of peak area on concentration. The developed method was validated in terms of accuracy, precision, linearity, limit of detection and limit of quantitation. The proposed method can be used for the standardization of Artemisinin in the ethanolic extracts *Artemisia abrotanum* and *Artemisia pallens* extract

Keywords: Standardization; *Artemisia abrotanum*; Artemisinin, RP-LC/MS

INTRODUCTION

Genus *Artemisia* (Asteraceae) is popularly known as Sage Brush or Wormwood is bitter aromatics. *Artemisia* is the largest genus comprising 400 species widely distributed in South Africa and South America, and 34 species are found in India (Fulzele, 2002). This genus is named in honor of Artemis the Greek goddess of chastity. Some of them are source of volatile oil. Almost all species consists of sesquiterpene lactones (Slokar, 1992).

Artemisia species invariably found as small fragrant shrubs or herbs and most yield essential oils. Some of these oils found used medicine as vermifuges, stimulants and in perfumery etc., where as the leaves of some species are used as culinary herbs. The plants themselves are popular among gardeners as cultivated ornamentals. Some *Artemisia* species are used as stomachic, stimulant, flavoring, antihelminthic, antibacterial, anti-inflammatory, antispasmodic, carminative etc (Srisilam, 2000).

Artemisia abrotanum was traditionally considered as an antiseptic, astringent, emmenagogue, expectorant, febrifuge, stomachic, stimulant, and tonic, antiinflammatory, vermifuge, spasmolytic and used for treating upper respiratory tract disease. Infusions make a bitter tonic which strengthens and supports digestive functions by increasing secretions in the stomach and intestines. It has also been used against cancer, cough, fever and tumors (Stenimetz, 1957; Bjork *et al.*, 2002a; Bjork *et al.*, 2002b). The plant has been screened for various pharmacological activities such as antiinflammatory, expectorant and spasmolytic (Bergendorff and sterner, 1995; Bjork, 2001). *Artemisia pallens* has been widely used in Indian folk medicine for the treatment of diabetes mellitus. This plant is accredited with anthelmintic, antipyretic and tonic properties. It is also considered as a good fodder. The oil possesses antispasmodic, antibacterial, antifungal

and stimulant properties (Asolkar, 1981). The plant has been screened for the following pharmacological activities, antimicrobial, antidiabetic, antinociceptive and wound healing activity

Ramezani *et al.*, (2004) have reported the methanol extracts of aerial parts of four *Artemisia* species shows antimicrobial activity against two gram positive bacteria, *B.subtilis* and *S. aureus*. Bergendorff *et al.*, (1995) have reported the Spasmolytic flavonols extracted from aerial parts of *Artemisia abrotanum* L. (Asteraceae), has show dose dependent relaxing effect on the carbacholine induced contraction of guinea pig trachea. Because of its widespread use in various geographic regions and to detect its adulteration with other materials, it is important to standardize the different parts of

Artemisia abrotanum. Rasal *et al.*, (2004) have reported the wound healing activity of alcoholic and aqueous extracts of *Artemisia pallens*. They have also found that the wound healing activity due to the presence of its active principle sesquiterpene.

No method of standardization of this potentially bioactive plant has been reported so far. We have therefore, developed an LC/MS method for the standardization of its extract using Artemisinin (Figure 1) as marker compound. The method was validated as per the ICH guidelines (ICH, Q2A, 1994 and ICH, Q2B, 1996).

EXPERIMENTAL

Plant material

Artemisia abrotanum and *Artemisia pallens* were collected from in and around Ootacamund, a famous hill station in southern India, belonging to the district Nilgiris of Tamil Nadu state. The plant was identified and authenticated by Medicinal Plants Survey and Collection Unit, Ootacamund, Tamil Nadu, India.

Extraction procedure

The dried plant material was powdered and passed through sieve no. 20 and extracted separately using methanol by soxhlation. The extracts were concentrated to dryness under reduced pressure and controlled temperature. All extracts were preserved in refrigerated condition till further use.

Chemicals and reagents

Acetonitrile LC/MS grade was procured from E.merck (India) Ltd, Mumbai. Water LC/MS grade was obtained from a Milli-QRO (0.2 μ) water purification system. All other chemicals used were of analytical grade.

Apparatus and chromatographic conditions

Chromatographic separation was performed on a Shimadzu® liquid chromatographic system equipped with a LC-10AT-vp solvent delivery system (pump), SPD M-10AVP photo diode array detector and Rheodyne 7725i injector with 20 μ l loop volume. Class-VP 6.01 data station was applied for data collecting and processing (Shimadzu, Japan). A Princeton SPHER C₁₈ (150 x 4.6 mm i.d. 5 μ) was used for the separation. Mobile phase of a mixture of Acetonitrile : Water (60:40 v/v) was delivered at a flow rate of 0.5 ml/min with detection at 283 nm. The mobile phase was filtered through a 0.2 μ membrane filter and degassed. The injection volume was 10 μ l. Analysis was performed at ambient temperature.

Preparation of artemisinin standard stock solution

Accurately transfer 10 mg of artemisinin working standard into a 10 ml volumetric flask and dissolve in methanol. Make the final volume with methanol to give 1.0mg/ml solution of artemisinin. Label and store the solution in a refrigerator below 2-8°C.

Preparation of sample solution

Artemisinin extracts 10 mg was accurately transferred into a 10 ml volumetric flask and dissolve in methanol. Final volume was made up with methanol to give 1.0mg/ml solution of artemisinin.

RESULTS AND DISCUSSION

Chromatography

Standardization of Artemisinin in *Artemisia abrotanum* and *Artemisia pallens* by RP-LC/MS method was carried out using the optimized chromatographic conditions. Typical chromatogram of Artemisinin (Figure 2, 3) and ethanolic extract of *Artemisia abrotanum* and *Artemisia pallens* were shown in Figures 4 and 5. Detection was done at 283 nm. The peak area ratios of standard and sample solutions were calculated. The assay procedure was repeated six times and the mean peak area and mean peak area ratio of standard were calculated. The results are given in Table 1. The low relative standard deviation (RSD) values are indicative of the high accuracy and precision of the method.

Method validation

Accuracy and precision

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out six times and the percentage recovery and standard deviation of the percentage recovery were calculated and is presented in Table 2. From the data obtained, added recoveries of standard drugs were found to be accurate.

The precision of the method was demonstrated by inter-day and intra-day variation studies. In the intra day studies, six repeated injections of validation samples (50, 100 and 150 µg/ml) were made and the peak area and percentage co-efficient of variation (CV) were calculated and is presented in Table 3. In the inter day variation studies, six repeated injections of validation samples (50, 100 and 150 µg/ml)

were made for three consecutive days and peak area and percentage CV were calculated and is presented in Table 3. From the data obtained, the developed RP-LC/MS method was found to be precise.

Linearity and Range

The linearity of the method was determined at five concentration levels ranging from 10 to 150 µg/ml. The calibration curve was constructed by plotting peak area against concentration of drugs. The slope and intercept value for calibration curve was $y = 12557$ ($R^2 = 0.9992$). The results show an excellent correlation between peak area and concentration of Artemisinin within the concentration range indicated above. The calibration curve is shown in Figure 6.

Limit of Detection and Limit of Quantification

The Limit of Detection (LOD) (the smallest concentration of the analyte that gives a measurable response, signal to noise ratio of 3) and Limit of Quantification (LOQ) (the smallest concentration of the analyte, which gives response that can be accurately quantified, signal to noise ratio of 10) of the developed method were determined by injecting progressively low concentrations of the standard solutions using the developed RP-LC/MS method. The LOD and LOQ were found to be 50,100 and 150 ng/ml, respectively (Table 4).

Ruggedness and Robustness

The ruggedness of the method was determined by carrying out the experiment on different instruments like Shimadzu LC/MS (LC-10AT), Agilent LC/MS and Waters Breeze LC/MS by different operators using different columns of similar type like Hypersil C₁₈, Phenomenex LUNA C₁₈ and Hichrom C₁₈. Robustness of the method was determined by making slight changes in the chromatographic conditions. It was observed that there are no marked changes in the chromatograms thus demonstrating that the developed RP-LC/MS method is rugged and robust.

System suitability studies

The column efficiency and peak asymmetry were calculated for the standard solutions. The values obtained demonstrated the suitability of the system for the analysis of Artemisinin in ethanolic extracts of *Artemisia abrotanum* and *Artemisia pallens*. System suitability parameters may fall within $\pm 3\%$ standard deviation range during routine performance of the method (Table 4).

Table 1: Estimation of Artemisinin content

Extract	Amount of Artemisinin [% w/w]	RSD (%) (n=6)
<i>Artemisia abrotanum</i>	0.0115	2.73
<i>Artemisia pallens</i>	0.1031	4.91

Table 2: Results of recovery analysis of Quercetin in HMF

Extract	Amount of Artemisinin present in (ng) A	Amount of Artemisinin Added to A (ng) B	Total Artemisinin Taken (A+B) (ng) C	Total Artemisinin found (ng) D	% Recovery (D/C) x 100 (Mean)
<i>Artemisia abrotanum</i>	500.0	250.0	750.0	741.0	98.80

Table 3: Results of intra and inter-day variability during validation

Concentration (µg/ml)	Intra-day (n =6)		Inter-day (n =6)	
	Accuracy (%)	Precision (CV)	Accuracy (%)	Precision (CV)
50.0	97.05	5.01	96.71	5.19
100.0	98.65	1.64	98.87	1.03
150.0	99.56	0.36	98.23	1.24

Table 4: System suitability studies

S. No	Parameters	Artemisinin
1	Theoretical Plate	3500
2	Asymmetric factor	0.71
3	LOD (ng/ml)	50
4	LOQ (ng/ml)	150
5.	Tailing factor	1.2

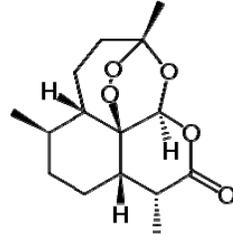


Fig. 1: Structure of Artemisinin

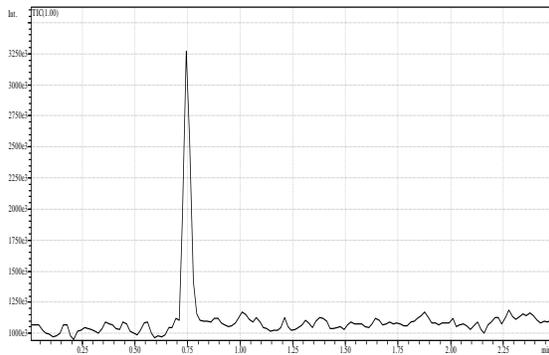


Fig. 2: Standard chromatogram of Artemisinin

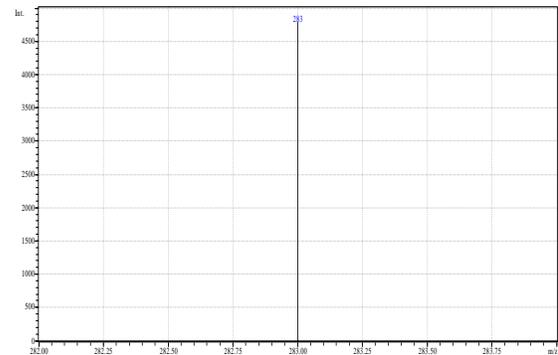


Fig. 3: Chromatogram of Artemisinin Standard at Sim (+) Mode

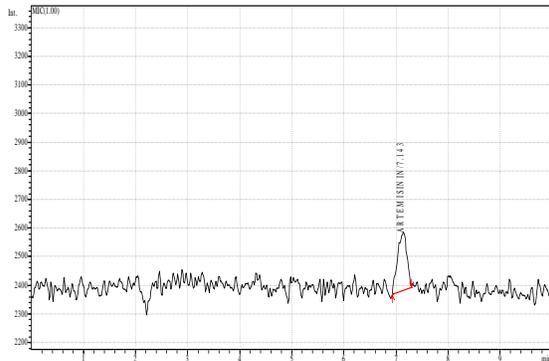
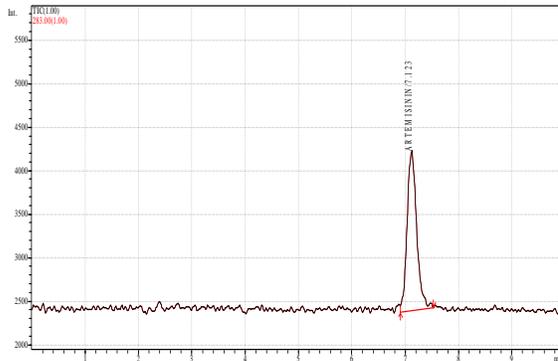
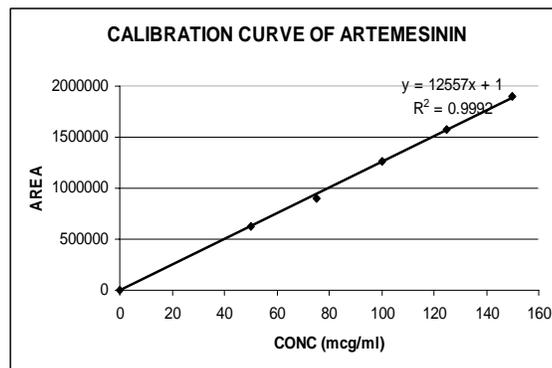
Fig. 4: Typical chromatogram of *Artemisia abrotanum*Fig. 5: Typical chromatogram of *Artemisia pallens*

Fig. 6: Calibration curve of Artemisinin

CONCLUSION

The proposed RP- LC/MS method for standardization of Artemisinin in ethanolic extract *Artemisia abrotanum* and *Artemisia pallens* is simple, rapid, accurate, precise, linear, rugged and robust. Hence the present RP-LC/MS method is suitable for the standardization of Artemisinin in *Artemisia abrotanum* and *Artemisia pallens*.

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