ORIGINAL ARTICLE

Determination of bacoside A by HPTLC in *Bacopa monnieri* extract

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A simple sensitive HPTLC method developed for the determination of bacoside A in the plant *Bacopa monnieri* extracts. The stationary phase was precoated silica gel GF254. The mobile phase used was dichloromethane: methanol: water (4.5: 1.0: 0.1 v/v/v). The plate was scanned and quantified at 225 nm for bacoside A. The method was validated in terms of linearity, accuracy and specificity. The proposed HPTLC method provides a faster and cost effective qualitative control for routine analysis of bacoside A in extracts containing *Bacopa monnieri* saponins.

Key words: Analysis, bacoside A, HPTLC

INTRODUCTION

Bacopa monnieri (Family: Scrophulariaceae) is a medicinal herb, found throughout the Indian subcontinent in wet, damp and marshy areas.[1] It is used in traditional Indian medicine, the Ayurveda, for the treatment of anxiety, and in improving intellect and memory for several centuries.[2,3] In addition to memory boosting activity, it is also claimed to be useful in the treatment of cardiac, respiratory and neuropharmacological disorders like insomnia, insanity, depression, psychosis, epilepsy and stress.[4,5] It was reported to possess anti-inflammatory, analgesic, antipyretic, sedative, free radical scavenging and anti-lipid peroxidative activities.[6,7] The pharmacological properties of *Bacopa monnieri* were studied extensively and the activities were attributed mainly due to the presence of characteristic saponins called as "bacosides".[8]

There are few methods like spectrophotometric, HPTLC method and HPLC method[9-15] reported in the literature for quantification of bacosides in plant extracts. As the number of aged people suffering from cognitive problems increases, the memory boosters have gained immense importance and there is an urgent need to develop sensitive and reliable quality control techniques to establish the authenticity and purity of memory boosting drugs. In the present study, a simple HPTLC method was developed and validated for separation and quantification of bacoside A in *Bacopa monnieri* plant extracts.

MATERIALS AND METHODS

Plant Material

The authenticated sample of whole plant material of *Bacopa monnieri* Linn. Penn. (voucher specimen no. NISCAIR/RHMD/Consult/07-08/882/66/4) and reference standard of bacoside A were provided by M/s Natural Remedies Pvt. Ltd. Bangalore, India.

Preparation of the Plant Extract Solution

Coarse powder of the dried material of *Bacopa monneira* (Linn.), was extracted to exhaustion with methanol using a soxhlet apparatus. The methanol extract thus obtained was dried separately under reduced pressure at a room temperature not exceeding 40°C. About 100 mg of extract is dissolved in 10 ml of methanol, sonicated and filtered through membrane filter.

Preparation of Standard Solution

The reference standard stock solution (42 mg/ml) of bacoside A was prepared in methanol. From stock solution further dilutions were done to get the lower concentrations.

Chromatographic Conditions

The following chromatographic conditions were used to quantify the bacoside A: Stationary phase: Silica gel GF254 (E. Merck) precoated TLC plates
Mobile Phase: Dichloromethane: Methanol: Water (4.5: 1.0: 0.1 v/v/v)
Sample volume: 2 µl

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Temperature: Ambient room temperature
Migration Distance: 8 cm
Detection wavelength: 225 nm

Procedure

Before spotting, the plates were pre-washed with methanol. Standard and sample solutions were applied to the plates as sharp bands by means of Camag Linomat V sample applicator. The spots were dried in a current of air. The mobile phase (20 ml) was poured into a twin trough glass chamber whole assembly was left to equilibrate for 30 min and the plate was placed in the chamber. The plate was then developed until the solvent front had travelled at a distance of 80 mm above the base of the plate. The plate was then removed from the chamber and dried in a current of air. Detection and quantification was performed with Camag TLC Scanner 3 at a wavelength of 225 nm.

Assay

Standard and sample solutions were spotted on an HPTLC plate (E. Merck). The percentage of bacoside A present in *Bacopa monnieri* extract was calculated by comparison of the areas measured for the sample and standard solution. [Fig. 1] represent the chromatograms of standard bacoside A.

Linearity

Linearity was performed by applying standard solution at different concentrations ranging from 8.4 to 50.4 µg/spot on 20 × 20 cm HPTLC plates, precoated with silica gel GF254 (E. Merck) in the form of sharp 6 mm bands; the distance between two adjacent bands was 11.6 mm. The plates were developed in a solvent system of dichloromethane: methanol: water (4.5: 1.0: 0.1 v/v/v), up to a distance of 80 mm, at room temperature. The plates were dried in air.

The detector response for bacoside A was measured for each band at wavelength of 225 nm, using Camag TLC Scanner and winCAT software. The peak areas of bacoside A were recorded for each concentration. The linearity curve of bacoside A was obtained by plotting a graph of peak area of bacoside A *vs* applied concentration of bacoside A (µg).

Method Validation

The method was validated[16] for precision, repeatability and accuracy. The precision was checked by repeated scanning of the same spot of bacoside A (25.2 µg) three times each and was expressed as relative standard deviation (% RSD). The repeatability of the method was confirmed by analyzing 16.8 µg and 25.2 µg /spot of standard bacoside A solution (n = 3) and was expressed as % RSD. The precision of the method was studied by analyzing aliquots of standard solution of bacoside A (16.8 µg and 25.2 µg /spot) on the same day (intra-day precision) and on different days (inter-day precision) and the results were expressed as % RSD.

To study the accuracy, the recovery experiment was performed by the method of standard addition. The recovery of the added amount of standard was analyzed at three different levels, each being analyzed in a manner similar to that of described for the assay. Each level of additions was repeated three times on three different days and the recovery of the added amount of standard was calculated.

Limit of detection and limit of quantitation was also calculated by the proposed method.

RESULTS AND DISCUSSION

The method described utilizes silica gel GF254 HPTLC plates as stationary phase and dichloromethane: methanol: water (4.5: 1.0: 0.1 v/v/v) as mobile phase which gives good separation of bacoside A (Rf = 0.78). The results of method validation parameters are shown in Table 1. The identity of the band of bacoside A in the sample extract was confirmed by overlaying the UV absorption spectra of sample with that of reference standard which showed λmax at 225 nm

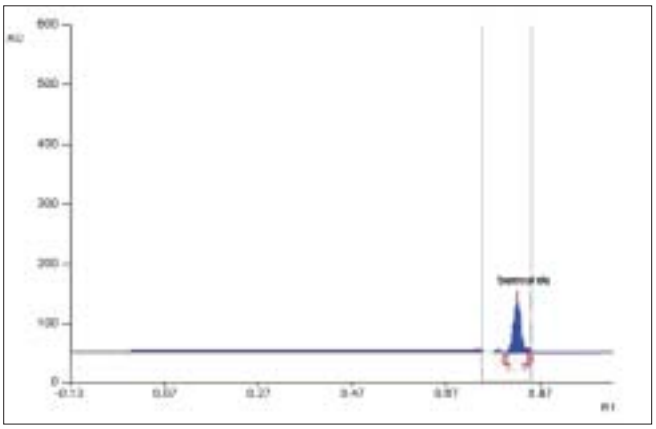

Figure 1: Typical chromatogram of standard bacoside A

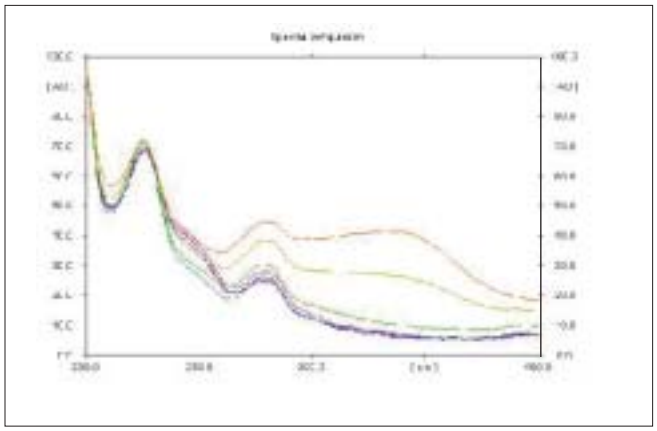

Figure 2: Overlaying UV absorption spectra of different bands of standard bacoside A and sample

[Fig. 2]. The calibration curve was linear in the range of 8.4 μg to 50.4 μg/spot and the correlation coefficient was determined. The correlation coefficient was found to be 0.9989. The limit of quantification was found to be 9.9 μg and the limit of detection was 3 μg /spot. The method was validated in terms of precision and reproducible expressed as % RSD which were found to be less than 2%. The recovery values obtained were 98.39 to 100.40 %, showing accuracy of the method. The average percentage recovery was found to be 99.46 %.

In conclusion the developed HPTLC method was simple accurate, precise, economic and can be utilised for the routine analysis and quantitative determination of bacoside A from *Bacopa monnieri*.

Table 1: Validation parameters for quantification of bacoside A by HPTLC

Parameters	Results
Precision (% RSD)	< 2%
Linearity	8.4 – 50.4 μg/spot
Limit of Detection	3 μg/spot
Limit of Quantification	9.9 μg/spot
Accuracy	98.39 – 100.40 %
Assay	41.5 % w/w

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