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Quantitative Determination of Bacoside by HPLC

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Accepted 21 June 1998
Received 2 February 1998

High pressure liquid chromatographic method for quantitative determination of Bacoside-A_3 the main active constituent of the saponin fraction having facilitatory effect on learning is described. A C_{18} column was used for the separation with acetonitrile:water (40:60) as mobile phase. the lowest quantitation limit was 26 µg/ml. The intra-day relative standard deviations and deviation from actual concentration values are less 6% and ±8% respectively, which showed good reproducibility and accuracy of the method.

BACOPA monniera L (Syn:*Herpestis monniera* L., HB & K), BRAHMI, is used as a reputed nervine tonic in the traditional system of medicine[1]. Its alcoholic extract was found to improve the performance of rats in several learning tests as manifested by better aquisition, consolidation and retention of newly acquired behavioural responses[2,3]. The activity has been localised in the saponin fraction designated as bacosides[4]. A traditional remedy developed by us from the plant *Bacopa monniera* containing bacosides as the active constituents is already marketed as *Memory Plus*. Earlier a U.V. spectrophotometeric method for quantitative determination of bacosides was developed[5]. In this communication, a HPLC method for the estimation of bacoside A_3[6], the main constituent of the bacosides is described.

Standard sample of bacoside A_3 was obtained from the Medicinal Chemistry Division of CDRI, acetonitrile and methanol both were of HPLC grade and were procured form E. Merck (India) Ltd., Bombay and used without further purification. All other reagents were of analytical grade and used without further purification. Aerial part of *Bacopa monniera* L. was collected from West Bangal, India. A voucher specimen is deposited in the Herbarium of CDRI. The air dried and powdered plant material was processed as reported earliar[7].

The HPLC system consisted of a Perkin Elmer 250 solvent delivery system, a Rheodyne (Cotati, CA, USA) model 7125 injector with a 20 µL loop and a Perkin Elmer model 235 diode array detector with a G.P. 100 printer plotter. Separation was carried out on a ODS E. Merck column (250 mm x 4 mm, ID, and 5 µm particle size). The column effluent was monitored at 215 nm. Chromatography was performed at 27+-3° at a flow rate of 1 ml/min of the mobile phase consisting acetonitrile:water (40:60). the mobile phase was filtered and degassed before use.

Stock standard solution containing 260 ug/ml of bacoside, A_3 was prepared by dissolving 2.6 mg of standard bacoside A_3 in 0.5 ml of methanol and then making up the volume upto 10 ml with mobile phase. Working standard solutions were prepared form the stock solution in the concentration range 26 to 260 µg/ml, using mobile phase. The sample (active fraction) solution containing 500 µg/ml was prepared by dissolving 5 mg of sample in 1 ml of

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CDRI Communication No. 5696

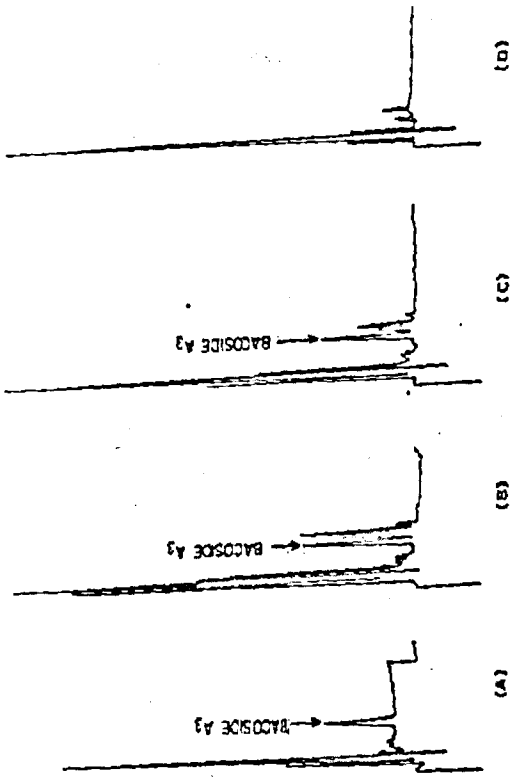

Fig. 1: HPLC chromatogrammes of (A) bacoside A_3 (B) bacoside sample (C) bacosides sample and bacoside A3 mixture (D) blank solution

methanol and making up the volume with mobile phase (10 ml).

Known amount of bacoside, A_3 was added to the preanalysed sample solution and the total bacoside A_3 content was determined using the calibration graph. The accuracy of the method was calculated on the basis of the difference in the mean calculated and added concentration and intra and the precision was obtained by calculating the inter day relative standard deviations (R.S.D.S.).

Effective resolution of bacoside A_3 in the processed[7] crude extract of *Bacopa monniera* was achieved by using acetonitrile:water 40:60 as mobile phase and a C_{18} column. No interfering peaks were detected. The retention time for bacoside A_3 in the processed *Bacopa monniera* extract was about 6 min (Fig. 1). Based on the signal to noise ratio of 3, the lowest quantitation limit was 26 ug/ml. Thus the method provided adequate detection limit for determining

Table 1: Analysis of capsules containing bacosides for bacoside A_3

B.No.	Amount of labelled bacosides (mg)	bacoside A_3 found (mg)
1	100.0	25.89
2	100.0	24.50
3	100.0	26.78

A_3 in the crude extract (Table-1).

The chromatogram peaks were integrated manually using the standard method

Peak area = Peak height x half height width.

External standardization by peak area was used for the determination of bacosides A_3. The calibration graph showed linearity in the range of 26-260 µg/ml with correlation coefficient of 0.9953. The intraday relative standard deviations are less than 6% indicating good reproducibility of the method. the deviation from actual concentration values are±8% which showed good accuracy of the method.

ACKNOWLEDGEMENTS

Authors thank Mrs M. Chaudhary for technical assistance.

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