
A HPTLC Determination and Fingerprinting of Bacoside A in *Bacopa monnieri* and its Formulation

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A simple sensitive HPTLC method developed for the analysis of bacoside A in the plant *Bacopa monnieri* and in its commercial monoherbal capsule formulation. The stationary phase was pre-coated silica gel G60F₂₅₄ (20x11cm, aluminum sheet). The mobile phase used was chloroform:methanol:water (18:9:0.6). The plate was scanned and quantified at 540nm 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 formulations containing *Bacopa monnieri* saponins.

Bacopa monnieri, Scrophulariaceae, (also sometimes referred to as *Bacopa monniera* or *Herpestis monniera*) is a medicinal plant used for centuries in the Ayurvedic system of medicine¹. More recently, it has gained popularity in western countries as a brain tonic or capable of improving mental ability and memory². Although *Brahmi* is an important herbal drug in Ayurvedic medicine, some confusion exists regarding the botanical identification of it because *Centella asiatica* is sometimes known as *Brahm*³. *Bacopa monnieri* has been used in Ayurvedic medicine as a specific agent to develop and improve memory and concentration. It contains different types of saponins. The steroidal tetracyclic triterpenoid, bacoside A, is regarded as the major active principle having pharmacological actions³⁻⁵. To minimize batch variations and to add scientific validity to herbal formulations, it is necessary that like modern drugs, herbal drugs should also be analyzed and proper quality control techniques should be developed to verify the quality and quantity of the herbs added in the formulation, particularly when it is a polyherbal drug. HPTLC is fast emerging as one of the major tools by which the quality control of herbs in formulations can be maintained and identification of various chemical markers of the herbs can be easily done⁶. Various methods like spectrophotometry^{7,8},

spectrofluorimetry⁹, HPLC¹⁰ have been reported for the estimation of bacoside A. The present report describes a simple reproducible sensitive and rapid method of HPTLC analysis for the qualitative and quantitative estimation of the phytochemical marker namely bacoside A from *Bacopa monnieri* and from a marketed monoherbal formulation, Memory plus capsules. The formulation contains *Bacopa monnieri* extract (125 mg) and is indicated to control stress, anxiety, nervousness and enhancement of memory.

The whole plant of *Bacopa monnieri* (Linn) was procured from M/s. Ganesh Stores, Coimbatore and authenticated by Botanical Survey of India, Coimbatore-641003. A voucher specimen is deposited in the herbarium of the College of pharmacy, Sri Ramakrishna Institute of Paramedical Science, College of Pharmacy, Coimbatore. Standard bacoside A was procured from M/s. Natural Reminder Pvt. Ltd., Bangalore.

Instruments used for the estimation were Camag Linomat IV automatic sample applicator, Camag TLC Scanner 3 and CATS 4 software for interpretation of the data. Standard solution of pure bacoside A (1 mg/100 ml) in methanol was prepared to yield stock solution of 100 µg/ml concentration. From this various concentrations of 30, 60, 90, 120, 150, and 180 µg/ml was prepared by diluting with methanol. Two microlitres of standard solution of bacoside A was applied on pre-coated TLC silica gel G60F₂₅₄ plates

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(E. Merck) using a Camag Linomat IV automatic sample applicator. The plate was developed with chloroform: methanol:water (18:9:06 v/v), in a twin-trough chamber to a distance of 9.3 cm. After removal from the chamber, the plate was dried in air for 15 min, was scanned and quantified at 540 nm using a Camag TLC scanner 3. Data of peak area of each band was recorded. Standard curve for bacoside A in the range of 30–180 $\mu\text{g/ml}$ was generated by plotting the peak area against concentration of bacoside A.

About 25 g of shade dried whole plant of *Bacopa monnieri* was ground to make fine powder to pass through No. 60 mesh sieve and 10 g from it was accurately weighed and exhaustively extracted with 90% methanol. The extract was concentrated and successively partitioned with petroleum ether, chloroform, diethyl ether and finally with n-butanol. The extracts were filtered, pooled and transferred to a 100 ml volumetric flask and volume was made up to 100 ml with methanol. Five microlitres from the above sample solution was spotted in triplicate along with 100 μl of standard solution (120 $\mu\text{g/ml}$) on pre-coated silica gel G60F₂₅₄ TLC Plate. The plate was developed and scanned as mentioned above. Peak areas were recorded and the amount of bacoside A present in plant (raw material) was estimated using the calibration curve for bacoside A.

Twenty capsules were decapped and ground to fine powder. A weight equivalent to 10 mg of bacoside A was transferred to a conical flask and extracted with n-butanol (x25 ml.). The extracts were filtered through Whatman filter paper No. 41 and the residue was washed with 10 ml of methanol. The extracts and washings were pooled and transferred to a 100 ml volumetric flask and volume was made up to 100 ml with methanol. Five microlitres from the above sample solution was spotted in triplicate along with 10 μl standard solution (120 $\mu\text{g/ml}$) on pre-coated silica gel G60 F₂₅₄ TLC plate. The plate was developed and scanned as mentioned above. Peak areas were recorded and the amount of bacoside A present in the formulation was estimated using the calibration curve for bacoside A. Results

of analysis of *Bacopa monnieri* extract and formulation are tabulated in Table 1.

The developed method was validated for specificity, reproducibility and accuracy. The method is found to be specific for bacoside A, since it resolved the peak of bacoside A (Rf value=0.51) in presence of other recipients in the formulation (fig. 1) and also that of the *Bacopa monnieri* extract (Rf value=0.50) (fig. 2). The Rf value of standard bacoside A was found to be 0.51 (fig. 3). The specificity was confirmed by overlaying the spectra of standard bacoside A

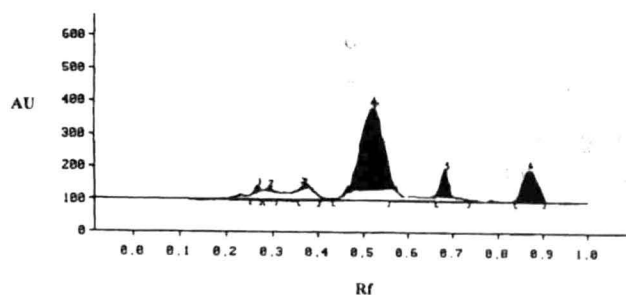


Fig. 1: TLC chromatogram of formulation at 540 nm. Solvent system: Chloroform:methanol:water (18:9:0.6 v/v). Peak for is bacoside A (Rf=0.51).

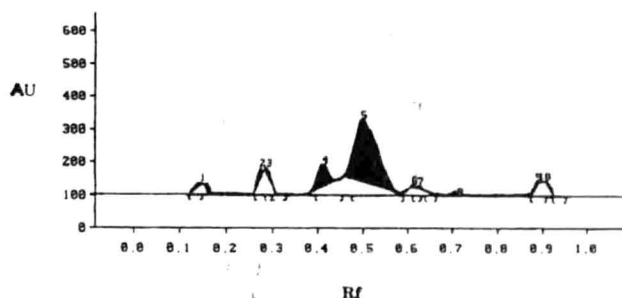


Fig. 2: TLC chromatogram of *Bacopa monnieri* extract at 540 nm. Solvent system: Chloroform:methanol:water (18:9:0.6 v/v). Peak for is bacoside A (Rf=0.50).

TABLE 1: ESTIMATION OF BACOSIDE A FROM BACOPA MONNIERI AND ITS FORMULATION

Sample	Amount of <i>Bacopa monnieri</i> extract	Labelled amount of bacoside A	Amount of bacoside A found (mg)*	Percentage of drug found
<i>Bacopa monnieri</i> plant	100 mg	2.5–3.0 mg	2.62±0.13	26.2±1.34
Formulation	125 mg/cap	3.75 mg	3.64±0.13	36.4±1.27

*Each value is a mean \pm standard deviation of three determinations.

TABLE 2: METHOD VALIDATION AND RECOVERY STUDIES OF BACOSIDE A IN *BACOPA MONNIERI* AND FORMULATION

Sample	Amount of sample taken (mg) a	Amount of Bacoside A present (mg) b	Amount of Bacoside A added (mg) c	Total Bacoside A taken (mg) d=b+c	Total Bacoside A found (mg) e	Percentage of recovery e/d x 100
<i>B.monniери-1</i>	1000	26.0	1	27.0	26.3±0.06	97.4
<i>B.monniери-1</i>	1000	26.0	2	28.0	27.6±0.07	98.6
<i>B.monniери-1</i>	1000	26.0	3	29.0	28.4±0.06	97.9
Formulation-1	1000	26.0	1	24.0	24.3±0.06	101
Formulation-2	1000	26.0	2	25.0	24.9±0.07	99.6
Formulation-3	1000	26.0	3	26.0	25.1±0.07	99.1

Average percentage recoveries of *Bacopa monniери* and Formulation are 97.97 and 99.98 respectively. The values are average of three determinations, observation ± standard deviation.

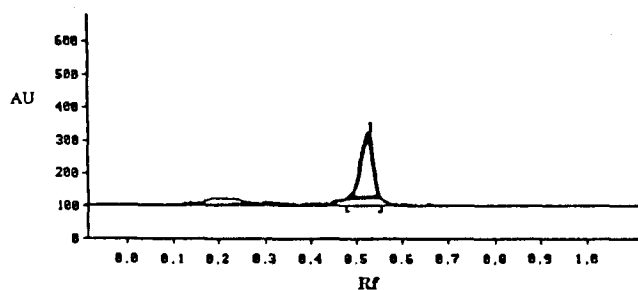


Fig. 2: TLC chromatogram of standard bacoside A at 540 nm.

Solvent system: Chloroform:methanol:water (18:9:0.6 v/v). Peak for is bacoside A (Rf=0.51).

with the spectra of samples recorded on TLC scanner in UV range. Linearity range was found to be in the range of 30–180 µg/ml. The correlation coefficient (r) was 0.999 indicating good linearity between concentration and area. Precision of the instrument was checked by repeated scanning of the same spot (1000 ng/spot) of bacoside A seven times and the % CV was found to be 0.046. Reproducibility of the method was checked by analyzing a standard solution of bacoside A (120 µg/ml) after application (5 µl) on a TLC plate (n=6) and the % CV for peak area was found out to be 0.64. Accuracy of the method was evaluated by carrying out a recovery study. A varying known concentration of the standard bacoside A i.e. 1.0, 2.0 and 3.0 mg was added to about 1 g of finely powdered test samples separately in which the contents of bacoside A were estimated previously by the proposed method. The samples were extracted and analyzed separately as per the procedure mentioned above,

the results are compiled in Table 2. The % recovery for *Bacopa monniери* and the formulation were found to be in the range of 97–100. Hence this developed HPTLC method is quick and reliable for quantitative monitoring of bacoside A in raw material, processed powder and in herbal preparations containing *Bacopa monniери*.

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REFERENCES

- Sivaragan, V.V., and Balachandran, I., Eds, In; Ayurvedic Drugs and Their Plant Sources, Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi, 1994, 98.
- Asolkar, L.V., Kakkar, K.K., and Chakre, O.J., In; Glossary of Indian Medicinal Plants With Active Principles, Part I, CSIR Publications, New Delhi, 1992, 112.
- Anonymous, In; Indian Herbal Pharmacopoeia, Vol. I, Indian Drug Manufacturers Association, Worli, Mumbai, 1998, 30.
- Chaterjee, N., Rastogi, R.P., and Dhar, M.L., Indian J. Chem., 1965, 3, 24.
- Basu, N., Rastogi, R.P., and Dhar, M.L., Indian J. Chem., 1967, 5, 84.
- Sane, R.T., Patel, P.H., and Phadke, M, Indian Drugs, 1998, 35, 1, 37.

7. Pal, R., and Sarin, J.P.S., *Indian J. Pharma. Sci.*, 1992, 17, 54.
8. Singh, H.K., Rastogi, R.P., Srimal, R.C., and Dhawan, B.N., *Phytotherapy Research.*, 1998, 2, 70.
9. Gupta, A.P., Mathur, S., Gupta, M.M., Sushil, K.W., *J. Med. Aromatic. Plant. Sci.*, 1998, 20, 1052.
10. Renukappa, T., Roos, G., Klaiber, I., Vogler, B., Kraus, W., *J. Chromatogr. A*, 1999, 1, 847.

Simultaneous Spectrophotometric Determination of Amoxicillin Trihydrate and Metronidazole in Dental Films

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A simple, accurate and reproducible method for simultaneous estimation of amoxicillin and metronidazole in combined dosage form has been developed. The method involves analysis by multicomponent mode. Amoxicillin and metronidazole have absorption maxima at 272 nm and 320 nm, respectively, in alkaline borate buffer (pH 8.1). The results of analysis were validated statistically.

Amoxicillin is an amino-penicillin with spectrum of activity similar to that of ampicillin¹. Metronidazole is the prototype nitroimidazole introduced in 1959 and has broad-spectrum bactericidal activity against protozoa and many anaerobic bacteria². The combination of amoxicillin and metronidazole has been successfully used in the treatment of advanced periodontitis; especially with *A. actinomycetemcomitans* associated infections³. The rationale for the use of this combination is that metronidazole is very active against anaerobic microorganisms and is known to act synergistically with penicillin. Furthermore both drugs are bactericidal, which may be essential for the elimination of subgingivally occurring microorganisms. The combination of both the drugs covers a wide range of microflora, which is important for the successful treatment.

The IP suggests a titrimetric method with potentiometric determination of end point for amoxicillin and metronidazole^{4,5}. No spectrophotometric method is available for simultaneous estimation of these drugs in pharmaceutical

formulations. However an HPLC method for simultaneous quantification of amoxicillin and metronidazole in plasma has been reported⁶. This paper presents a simple, accurate, economical and reproducible method for the simultaneous analysis of amoxicillin and metronidazole in dental film formulation.

A Shimadzu UV spectrophotometer 1601 model with spectral bandwidth of 2 nm and wavelength accuracy of ± 0.5 nm was used. Ten mm matched quartz cells were employed for this work. Alkaline borate buffer of pH 8.1 (ABB) was used for the preparation of solutions. The buffer was prepared by placing 50 ml of 0.2 M boric acid and 50 ml of 0.2 M potassium chloride solution in a 200 ml volumetric flask, pH was adjusted to 8.1 with 0.2 M sodium hydroxide and water was added to make up the volume. Standard stock solutions of amoxicillin trihydrate and metronidazole of 400 $\mu\text{g/ml}$ and 100 $\mu\text{g/ml}$, respectively, were prepared in ABB. Film samples containing 5 mg each of amoxicillin and metronidazole were placed in 10 ml acetone to dissolve the polymer poly(lactide-co-glycolide) (PLGA). After the polymer had dissolved, volume was made up to 100 ml with ABB. Aliquots of solution were diluted to get a final concentration

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