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An HPLC method for simultaneous estimation of psoralen, bakuchicin and bakuchiol in *Psoralea corylifolia*

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Abstract

Objective: To develop an HPLC method for estimation of psoralen, bakuchicin and bakuchiol in *Psoralea corylifolia*. Materials and methods: An gradient, reverse phase (RP) HPLC procedure using a mixture of Phosphate buffer and acetonitrile as mobile phase, C18 column as stationary phase and UV detector. Results: The developed method shows high resolution, accuracy and reproducibility. Conclusions: The method developed is accurate, precise and specific.

Key Words: *Psoralea corylifolia*, psoralen, bakuchicin, bakuchiol, HPLC

1. Introduction

Psoralea corylifolia Linn (Fam. Leguminosae) popularly known as babchi is a common herbaceous weed which grows throughout the plains of India. In Ayurveda, the seed have been recomended as anthelmintic, laxative, diuretic and aphrodisiac. They are considered useful in leucoderma, ulcers, scabies, leprosy vitiated conditions of pitta and dermatitis [1-2]. The *anti-staphylococeal* activity of the petroleum ether extract of the seeds has been established [3]. The seeds also reported to exhibit anti-inflammatory, antipyretic, analgesic [4] and antifungal activities [3]. Besides these reports, there is no mention in the literature about the simultaneous estimation of psoralen, bakuchicin and bakuchiol in the seeds of *Psoralea corylifolia*. Hence the present investigation was planned to estimate these compounds simultaneously by HPLC.

2. Materials and method

2.1 Plant material

Psoralea corylifolia seeds were collected from Natural Remedies Pvt. Ltd. garden in November 1999 and taxonomically identified by NISCOM,

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New Delhi. Voucher specimen were deposited at the Pharmacognosy Department of Natural Remedies Pvt. Ltd., Bangalore

2.2 Chemicals

Acetonitrile and methanol were obtained from Ranbaxy (HPLC grade), whereas petroleum ether, orthophosphoric acid, potassium hydroxide and potassium di-hydrogen phosphate were obtained from Ranbaxy (AR grade).

2.3 Extraction of Plant Material

2 g of powdered *Psoralea corlifolia* seeds was accurately weighed into a 250 ml beaker. The contents were extracted with 75 ml of methanol for 10-15 minutes on a boiling water bath. After cooling the methanolic extract was filtered through suitable filter paper (Whatmann 41 grade). The mark obtained was further extracted with 3x75 ml methanol and filtered. The combined methanolic layer was concentrated to exactly 100 ml. 10 ml of this solution was further diluted to 25 ml with methanol.

2.4 Equipments

Shimadzu integrated liquid chromatographic system LC/2010 comprising of system controller unit, degassing unit, low pressure gradient unit, 4 solvents, pump unit, mixer, Auto sampler, Column oven, Uv-vis detector and class VP ver 6.0 work station was used for analysis.

Column: ODS-C18 (Phenomenex) Luna 5 µ C18(2) 250 x 4.6 mm column was used.

2.5 Experimental conditions

The gradient analysis was performed (Refer Table 1) at a flow rate 1.5 ml/min using mobile phase prepared by dissolving 0.136 g potassium dihydrogen orthophosphate and 0.5 ml orthophosphoric acid dissolved in 1000 ml water, (solvent A) and Acetonitrile (solvent B).

Table 1.
Gradient conditions for isolation of psoralen, bakuchicin and bakuchiol.

Time	Flow	% of Solvent (A)	% of Solvent (B)
0.01	1.5	80	20
18.00	1.5	50	50
25.0	1.5	20	80
32.0	1.5	20	80
35.0	1.5	50	50
40.0	1.5	80	20
45.01	1.5	80	Stop

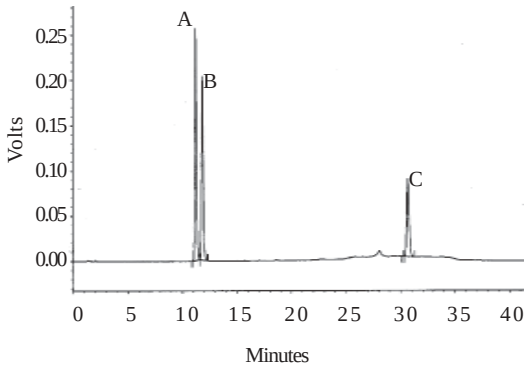
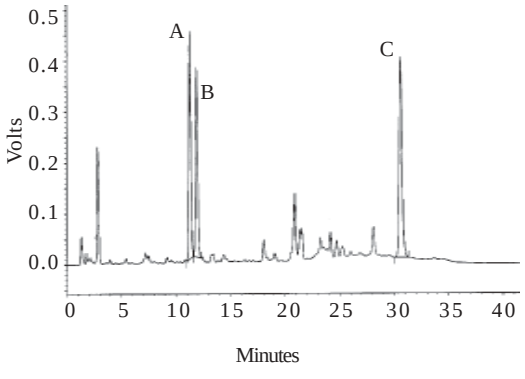


Figure 1. HPLC chromatogram of (A) psoralen, (B) bakuchicin, (C) bakuchiol



Figire 2. HPLC chromatogram of seeds of (A) psoralen, (B) bakuchicin, (C) bakuchiol in *P. corlifolia*

Table 2
Psoralen, bakuchicin and bakuchiol content in *Psoralea corylifolia*.

Sample	Psoralen	Bakuchicin	Bakuchiol
Sample 1	0.69	0.60	2.05
Sample 2	0.67	0.58	1.94
Sample 3	0.75	0.71	1.90

Values are in % w/w

2.6 Reference standard

The three compounds were isolated from *Psoralea corylifolia* by Phytochemistry laboratory, R&D, Natural Remedies Pvt. Ltd. and their identities were confirmed by comparing the UV, IR, ^{1}HNMR and ^{13}CNMR of the isolated with those reported in the literature [5-7].

2.7 Identification of psoralen, bakuchicin and bakuchiol peaks.

Separately 500 mcg/ml of methanolic solution of psoralen, bakuchicin and bakuchiol was prepared and 10µl of each solution was injected to identify the retention time.

2.8 Calibration curves

25 mg of each of psoralen, bakuchicin and bakuchiol were accurately weighed and added to a 50 ml volumetric flask, dissolved in HPLC grade methanol and the volume was made upto 50 ml with HPLC grade methanol to get 500 mcg/ml solution. Finally, appropriate dilutions were made to get 50, 100 and 200 mcg/ml solutions. 10 µl of each of these solutions was injected in triplicate and the average area was calculated for psoralen, bakuchicin and bakuchiol, calibration graphs were plotted and the regression co-efficients were calculated. (>0.99).

2.9 Estimation of psoralen, bakuchicin and bakuchiol in samples and recovery studies

The HPLC estimation was carried out by injecting 10 µl of the sample solution (refer 2.3). Percentage of psoralen, bakuchicin and bakuchiol were estimated using the area under the curve obtained from the sample by comparing the same with standard. The accuracy of estimation is validated using spike recovery studies. The percent recovery for psoralen, bakuchicin and bakuchiol were found to be 101.5, 102.0 and 100.4 respectively.

3. Results and discussion

An HPLC method for standardization of *Psoralea corylifolia* has been attempted using the three of its bioactive compounds psoralen, bakuchicin and bakuchiol as markers. The

Table 3.
Method validation parameters for simultaneous estimation of psoralen, bakuchicin and bakuchiol in *P.corylifolia*

Sl. No	Test Characteristics	Observed results
1.	Specificity	Very specific & no interference
2.	Linearity	Linear up to 500mcg/ml Co-relation coefficient $r^2 > 0.99$
3.	Range of quantification	0-500mcg/ml.
4.	Accuracy	± 2%
5.	Precision	Related standard deviation < 2%
6.	Repeatability & Reproducibility	Related standard deviation < 2%
7.	System suitability	Relative RT for psoralen 1.0, baukuchicin 1.05 and bakuchiol 2.25. The resolution between soralen and bakuchicin was 2.8

calibration curves for psoralen, bakuchicin and bakuchiol were found to be linear over the range of 0 to 500 mcg/ml (Table 3). The respective regression co-efficient for psoralen, bakuchicin and bakuchiol were found to be >0.99.

Mean relative retention time for psoralen, bakuchicin and bakuchiol under the conditions described above were 1, 1.05 and 2.25. Asymmetric factor is less than 1.2 and the resolution between psoralen and bakuchiol is 2.5.

This method provides simultaneous estimation of all the three compounds, where in a isocratic method estimation of bakuchiol is not possible as the peak will not elute if the resolution between psoralen and bakuchicin is > 2.

In conclusion, the assay method described herein is simple, precise and reproducible for quantifying psoralen, bakuchicin and bakuchiol, the three bioactive compounds in *Psoralea corylifolia*.

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