ORIGINAL ARTICLE

Estimation of psoralen from herbal formulations containing *Psoralea corylifolia* using the RP-HPLC-DAD method and its application to a pharmacokinetic study

Sunita Shailajan, Sasikumar Menon[1], Ashish Singh[1], Mandar Mhatre[1], Neelam Sayed, Harshvardhan Joshi, Bhavesh Tiwari

Herbal Research Lab, Ramnarain Ruia College, Matunga, [1]Institute for Advanced Training and Research in Interdisciplinary Science, Sion Koliwada, Sion, Mumbai, Maharashtra, India

Background: *Psoralea corylifolia* L. (Fabaceae, seeds) is used in many formulations for the treatment of a wide range of ailments. **Aims and Objective:** To develop a reverse phase high-performance liquid chromatography-photodiode array detector (RP-HPLC-DAD) method for quantitation of psoralen from *P. corylifolia* and its related formulations. **Materials and Methods:** Separation and detection of psoralen from various herbal formulations was achieved on reversed phase Cosmosil C_{18} column using acetonitrile: Distilled water (40: 60, v/v; flow rate – 1.0 mL/minute) and the PDA detector (247 nm). The method was validated as per the norms of the International Conference on Harmonisation (ICH) guidelines and applied to study the pharmacokinetics of an oil-based Ayurvedic preparation, in terms of bioavailable psoralen. **Results:** The HPLC method showed a linear detector response from 20.0 to 5000.0 ng/mL (r^2=0.9998) for psoralen. The content of psoralen in *P. corylifolia* and its marketed formulations was determined, which showed remarkable variations as per the nature and complexity of the formulation. The absorption and elimination profile of psoralen, from an oil-based Ayurvedic preparation, was developed on its topical application in rabbits. Psoralen was detected in the plasma 0.25 hours post application of *Bakuchi Taila* with 0.46% bioavailability. **Conclusion:** The method was found to be sensitive, accurate and reproducible. Therefore, it can be recommended for marker-based standardisation and quality assurance of *P. corylifolia* and its formulations. It can also be applied to study the pharmacokinetic profile of the traditional preparations of *P. corylifolia*.

Key words: Formulations, *Psoralea corylifolia*, psoralen, pharmacokinetics, RP-HPLC-DAD

INTRODUCTION

Psoralea corylifolia L. (Fabaceae); a medicinally important plant, indigenous to tropical and subtropical regions of the world, is reported in the Indian Pharmaceutical Codex, the Chinese, British and American Pharmacopoeias and in different traditional systems of medicines, such as, Ayurveda, Unani and Siddha.[1] Seeds of *P. corylifoila* have been used as a remedy to treat a wide variety of diseases like tuberculosis, psoriasis, leprosy, cancer, gynecological bleeding, vitiligo and so on.[2] These therapeutic effects may be attributed to the phytochemical constituents of *P. corylifoila* like psoralen, isopsoralen, psoracorylifols (A–E), corylifols (A–C), psoralidin, bavachalcone, bavachin and so on;[3] among them, psoralen [Figure 1] and isopsoralen have specifically been reported to possess antitumor, antibacterial and antiviral activities.[4]

Access this article online	
Quick Response Code:	Website: www.greenpharmacy.info
	DOI: 10.4103/0973-8258.104935

Among the various analytical tools; the use of HPLC in the marker-based standardisation of traditional herbs and their complex preparations is well-supported by the recent reports.[5-8] Although, there exist a number of reports for quantification of psoralen using HPLC from varied matrices,[9-11] most of these methods have issues like the use of no organic phase, varied flow rate, expensive columns and gradient elution, along with incomplete validation as per international norms, which make them cumbersome for implementation in quality control (QC) laboratories. Neither has psoralen been estimated from traditional preparations available in the Indian market nor has the pharmacokinetics of some traditional preparation been reported using bioavailable psoralen.

Hence, in the current study, a simple RP-HPLC-DAD method was developed and validated, as per the ICH guidelines, to evaluate psoralen from some herbal and

Address for correspondence: Dr. Sunita Shailajan, Associate Professor in Botany, Ramnarain Ruia College, Matunga, Mumbai - 400 019, India. E-mail: sunitashailajan@gmail.com
Received: 07-06-2012; **Accepted:** 26-07-2012

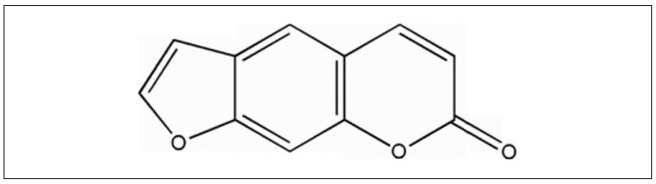

Figure 1: Structure of psoralen

Ayurvedic formulations of different matrices, and it was applied to study the pharmacokinetics of *Bakuchi Taila,* an oil-based Ayurvedic preparation, after its topical application on rabbits.

MATERIALS AND METHODS

Materials and Reagents

Fruits of *P. corylifolia* were procured from Bharat Trading, Mumbai (India), authenticated by a Taxonomist and the voucher specimens were deposited in the Herbal Research Laboratory, Mumbai. The material was dried in an oven maintained at 40°C for seven days. It was then powdered, sieved through a mesh (B.S.S. - 85) and preserved in an airtight container. *Mahamanjishthadi Kashaya* [Nagarjun Pharmaceutical (P) Ltd. (Batch No. 132)], *Mahamanjishthadi Kadha* [Sandu Brothers Pvt. Ltd. (Batch No. 172)], *Bakuchi Taila* [Shree Shanker Ayurvedic Pharmacy (Batch No. 105)], Tablet Purim [The Himalaya Drug Company (Batch No. A108005)], *Bakuchi Ghan Vati* [Shree Shanker Ayurvedic Pharmacy (Batch No. 104)] and *Bavchi Lep Goli* [Dhoot Papeshwar, No batch No.] were procured from the local market of Mumbai (India). Reference standard psoralen (95% pure) was procured from Natural Remedies (Bangalore, India). HPLC-grade methanol, acetonitrile and water were procured from Merck [Darmstadt, Germany (Batch Nos. SB0SF60135, SC0SF66211 and SH8SF80905, respectively)].

Sample Preparation

P. corylifolia fruit powder was extracted in methanol (0.1:10, w/v) and was kept standing overnight. The organic layer was separated, diluted twice (plant extract: mobile phase, 1: 9) and filtered through 0.45µ nylon membrane prior to the chromatographic analysis. A similar procedure was applied for Tablet Purim (TP), *Bakuchi Ghan Vati* (BGV) and *Bavchi Lep Goli* (BLG) by mixing the sample and methanol in the ratio 1: 10 (w/v), where BGV and BLG were diluted twice in the mobile phase, while TP was diluted only once. A sample of *Mahamanjishthadi Kashaya* (MKS), *Mahamanjishthadi Kadha* (MKD) and *Bakuchi Taila* (BT) was obtained by mixing the sample and methanol in the ratio 1: 9, v/v. After separating the upper organic layer and filtering through a 0.45µ nylon membrane filter, BT was diluted twice in the mobile phase (1: 9), while MKS and MKD were directly injected into the chromatographic system.

Chromatographic System

Chromatography was performed at room temperature with a Jasco HPLC PU-980 pump equipped with AS-1555-10 Auto Sampler and Jasco UV 970 detector. Chromatograms were recorded by means of the Borwin chromatogram software, version 1.21. Separation was achieved on Cosmosil C_{18} column with a dimension of 150×4.6 mm and particle size of 5 µm. The mobile phase used was a mixture of acetonitrile and distilled water in the volume ratio of 40:60 (v/v), delivered at flow rate of 1.0 mL/minute. The spectral data from the photodiode array detector were collected over the range of 265-320 nm and the chromatograms were plotted at 247 nm. Peaks were assigned according to their retention time as well as based on the ultraviolet (UV) spectra, for both the standard and sample, under an optimized chromatographic condition. A chromatographic profile of the standard compound psoralen (1000.0 ng/mL) is displayed in Figure 2.

Preparation of Standard and Quality Control Samples

Stock solution (1000.0 ng/mL), linearity samples (20.0-5000.0 ng/mL) and quality control samples (60.0, 500.0 and 4250.0 ng/mL) of psoralen were prepared in methanol.

Method Validation

The HPLC method was validated as per the ICH guidelines.[12] The validation parameters addressed were sensitivity, system suitability, specificity, linearity, precision, accuracy, assay, recovery, ruggedness and stability.

Estimation of Psoralen from *P. corylifolia* and its Formulations

Samples of *P. corylifolia* and its formulations (TP, BGV, BLG, MKS, MKD and BT) in methanol were injected into the HPLC system and analysed as per the optimized chromatographic conditions. The psoralen content in each was determined using the regression equation.

Application to Pharmacokinetics

The developed HPLC method was further applied, to evaluate the pharmacokinetics of BT. New Zealand Albino strain rabbits (female) weighing 1.3 to 1.7 kg were used to evaluate the safety and pharmacokinetics of BT. The study was approved by the Institutional Animal Ethics Committee (CPSEA/315). The animals were obtained from Haffkine Biopharmaceuticals, Mumbai, and were maintained at 26±2°C, in a 12-hour light-dark cycle and ambient humidity. They were housed in polypropylene cages, fed with a standard pellet diet (Amrut Laboratory Animal Feed, India) and water was provided *ad libitum*.

Safety Evaluation of *Bakuchi Taila*

In order to evaluate the safety of BT, a skin irritation study was conducted on three female healthy rabbits, as per the Organisation for Economic Co-operation and Development

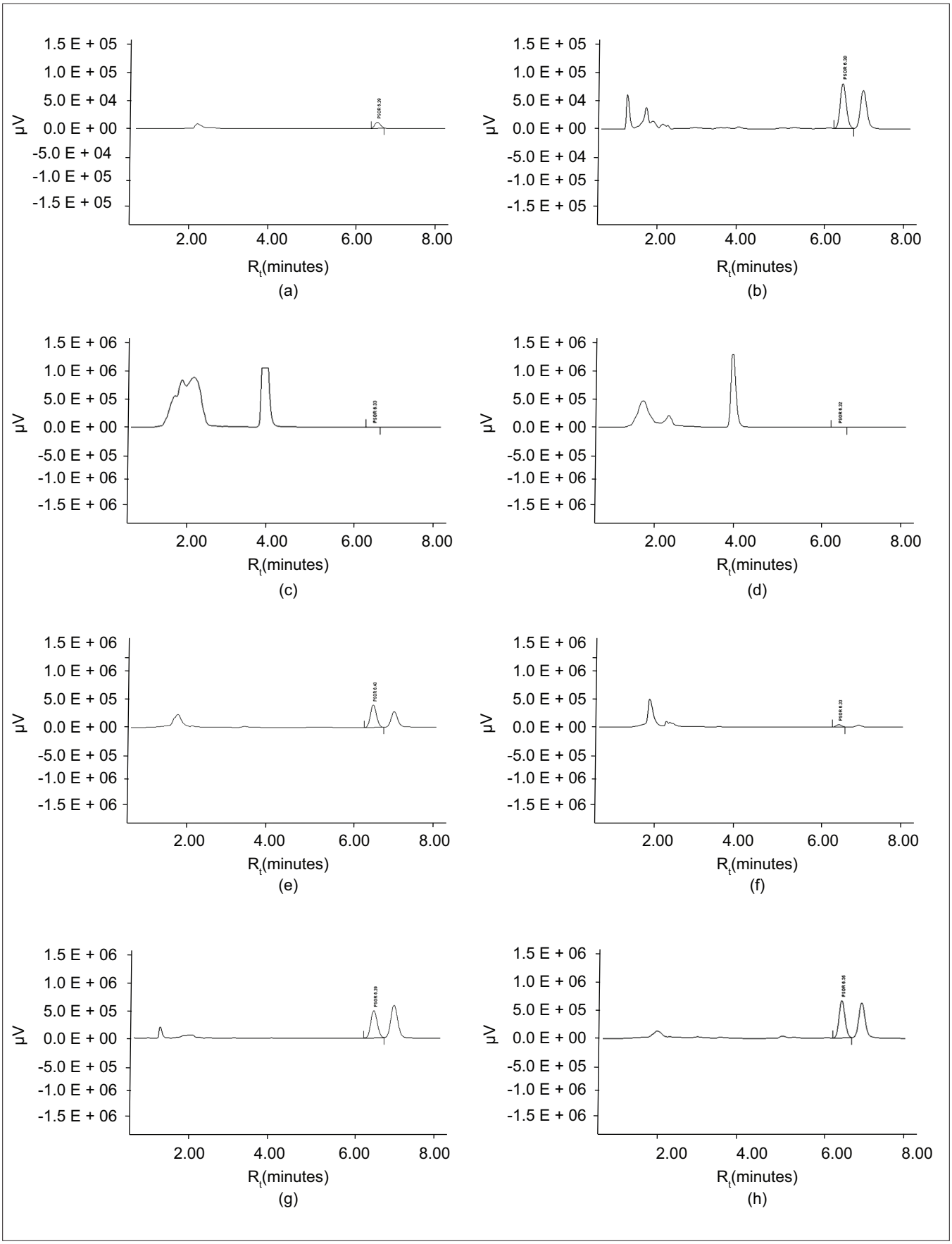

Figure 2: HPLC chromatograms of (a) Psoralen 1000 ng/mL; (b) *Psoralea corylifolia*; (c) MKS; (d) MKD; (e) BGV; (f) PT; (g) BT and (h) BLG

(OECD) guideline No. 404.[13] Approximately 24 hours before the test, fur was removed by closely clipping (one inch^2) the right and left area on the dorsal side of each rabbit. BT was applied neat on the patch at the right side (Test), while the patch on left side was selected as the control (without application of BT). Observations for irritation in terms of erythema and oedema according to the Draize test[14] were recorded 24, 48 and 72 hours after topical application of BT in order to check the skin irritation potential (if any).

Dermal Absorption Study of *Bakuchi Taila*

For the dermal absorption study of BT, 3.0 ml of oil was applied on the right ear pinna and changes in plasma pattern, with time, were monitored using HPLC. The blood samples were collected from the marginal veins of the ear, 0, 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75, 2.0, 4.0, 6.0 and 12.0 hours post application. The blood samples were collected in heparinised Eppendorf tubes. Plasma was separated after centrifuging the content at 4000 ×g for 10 minutes and stored at −20°C until further analysis.

Plasma Extraction

The plasma samples (200.0 µL) were taken in separate Eppendorf tubes and deproteinised by acetonitrile (2000.0 µL). Each tube was vortexed for 60 seconds and then centrifuged at 4000 ×g for 10 minutes. The upper organic layer (180.0 µL) was removed carefully and evaporated under nitrogen at 50°C, using a low vacuum evaporator. The residue obtained was reconstituted in 50.0 µL of acetonitrile: Distilled water (40:60, v/v) and injected into the HPLC system for the analysis of psoralen.

Method Validation for Psoralen in Plasma

In order to evaluate the content of psoralen in the plasma samples the HPLC method was validated according to the guidelines of the US Food and Drug Administration (FDA).[15] The validation parameters addressed were linearity, sensitivity, precision, accuracy, recovery and stability.

Evaluation of Pharmacokinetic Parameters

From each of the plasma samples collected, a concentration of psoralen was estimated using the linear regression equation. The actual dose of psoralen was calculated from the total volume of BT applied to the rabbit skin. Pharmacokinetic parameters were estimated by using the plasma concentration – time curve. Relevant pharmacokinetic parameters (C_{max}, T_{max}, AUC_{0-12}, $AUC_{0-\infty}$, $t_{1/2}$ and K_e) were estimated using the WinNonlin software Version 9.1 (non-compartment model).

Statistical Evaluation

All estimates are expressed as Mean±SD, *n*=3. Statistical analysis was performed using Microsoft Excel 2007.

RESULTS AND DISCUSSION

Development and Validation of the High-Performance Liquid Chromatography Method for Estimation of Psoralen

In the development of the HPLC method for evaluation of psoralen from *P. corylifolia* L. and various herbal formulations, different chromatographic conditions were tried, to improve the separation of psoralen in suitable time and peak separations. Psoralen was detected and accurately quantified by using RP-HPLC-DAD, with acetonitrile and distilled water (40:60, v/v) as the mobile phase. The Retention time (R_t) of psoralen was 6.3 min under optimized chromatographic conditions. The specificity of the intended method was established by comparing the HPLC retention times and absorption spectra of the target peaks from the analyzed samples with the reference compound. The limit of detection (LOD) and limit of quantification (LOQ) was found to be 5.0 ng/mL and 20.0 ng/mL, respectively, for psoralen, suggesting the sensitivity of the method. The percent coefficient of variations during the system suitability study was observed to be 0.24 and 1.39% for retention time and response, respectively, which were not more than 2% as per the acceptance criteria.[16]

The response for psoralen was found to be linear in the range of 20.0-5000.0 ng/mL with a coefficient of determination of 0.9998. This resulted in a regression equation y=68234x+49114. Within the batch, precision for the quality control samples of psoralen was 86.77-100.29%, which was within the acceptance limit of 85-115%. Also, the between-batch precision for the quality control samples of psoralen was 86.83-100.67%, which was within the acceptance limit of 85-115%.[16]

Mean recovery for the quality control samples of psoralen was found to be within the acceptance limit of >95% (99.14-101.53%).[16] Psoralen was found to be stable for 12 hours in the auto sampler and for 10 days at room temperature (RT) [Table 1]. The method was found rugged, as the values obtained were within the acceptance limits [Table 2].

Psoralen in Formulations of Complex Matrices

The content of psoralen in *P. corylifolia* L. and its formulations is depicted in Table 3. The content of psoralen was found to be altered in the complex matrices of the formulations. TP being a polyherbal combination in the form of tablet showed the maximum content of psoralen followed by BLG and BGV suggesting the interaction of psoralen with the constituents of other herbs. Among the three liquid preparations analysed, psoralen was found to be maximum in BT followed by MKD and MKS, as BT is the oil directly obtained from the seeds of *P. corylifolia*, while MKD and MKS are the mixtures of more than 40

plant extracts, as per their label claims. In the current study, the influence of the matrix effect was more evident in solid preparations, as the psoralen content was observed to be more in the solid preparations as compared to the liquid ones.

Table 1: Method validation parameters

Parameters	Results
Limit of detection (ng/mL)	5.0
Limit of quantification (ng/mL)	20.0
Linear range (ng/mL)	20.0–5000.0
Coefficient of determination (r^2)	0.9998
System suitability (% CV, *n*=5)	
Retention time (minutes)	0.21
Area	1.23
Precision (% CV, *n*=3)	
Within-batch	0.63–1.65
Between-batch	1.21–1.62
Recovery (%, *n*=7)	
Lower quality control sample	101.53
Middle quality control sample	99.49
Higher quality control sample	99.14
Stability	
Long-term stability	
Standard stock solution stability (for 10 days)	Stable at (4±1°C)
Short-term stability	
Bench top stability (for 6.00 hours)	Stable at (25±2°C)
Auto sampler stability (for 12.00 hours)	Stable at (4±1°C)

Table 2: Robustness/ruggedness of the method during method validation

Parameters	Results	
	% CV (*n*=3)	% difference
Change in columns		
Column 1	0.50–1.78	-0.41–4.25
Column 2	0.80–0.98	
Change in analysts		
Analyst 1	0.57–1.30	-4.46–(-0.69)
Analyst 2	0.47–1.38	
Change in days		
Day 1	0.42–1.04	1.45–4.74
Day 2	0.05–1.60	
Change in instruments		
Instrument 1	0.46–1.55	-0.42–0.86
Instrument 2	0.79–1.95	
Change in flow rates		
0.95 ml/minute	0.45–1.24	-2.17–1.01
1.00 ml/minute	0.72–1.69	
1.05 ml/minute	1.09–1.75	0.05–4.47
Change in injection volumes		
15 µL	0.09–1.31	47.24–48.86
20 µL	0.91–1.36	
25 µL	0.34–1.41	-50.00–(-45.88)
Change in mobile phase compositions		
39.5:60.5	0.54–1.03	0.11–1.05
40:60	0.03–1.10	
40.5:59.5	0.70–0.96	-1.31–1.33

Safety of *Bakuchi Taila* in Rabbits

The skin irritation test of BT on rabbits showed no signs of dermal irritation after its topical application. Its Primary Irritation Index (according to the Draize Test) was therefore calculated to be 0.00 [Table 4]. Thus, it was confirmed that BT had an adequate safety margin to be used on human skin.

Method Validation for Psoralen in Plasma

During plasma validation, the calibration curve for psoralen was linear in the range of 100.0-2000.0 ng/mL in the plasma. The linear regression equation for the calibration curve was y=13.75x-16.82 (r^2=0.999), where y was the peak area response at 247 nm and x was the concentration in ng/mL. Limit of detection and limit of quantitation for the developed method was found to be 60.0 ng/mL and 100.0 ng/mL, respectively. Thus, the method was found to be sensitive. Quality control samples of psoralen in plasma at three levels of 130.0, 460.0 and 1600.0 ng/mL were analysed for accuracy and precision. The relative standard deviation (RSD) values were less than 5%. Intra-day and inter-day accuracies for psoralen were 90.06-96.03% and 94.28-93.25%, respectively. These results indicated that the method had good precision and accuracy. Extraction recoveries of psoralen for quality control samples in rabbit plasma were found to be 93.68±0.86, 92.54±1.06 and 91.31±1.84%, respectively, with RSD of 0.92, 2.15 and 1.91%, respectively. Psoralen was found to be stable in plasma after 24 hours of storage at room temperature. Results of the stability evaluation after freeze–thaw cycles showed that psoralen was stable in the plasma for at least three freeze–thaw cycles.

Table 3: Assay results and method application

Sample	Concentration of psoralen in mg/ml or mg/g (Mean±S. D.)
P. corylifolia	0.478±0.0063
Mahamanjishthadi Kashaya	0.00030±0.00
Mahamanjishthadi Kadha	0.001±0.00
Bakuchi Taila	0.258±0.0032
Bakuchi Ghan Vati	2.914±0.0300
Bavchi Lep Goli	3.120±0.0374
Tablet Purim	4.095±0.0258

Table 4: Skin irritation studies of *Bakuchi Taila* showing primary irritation index

Rabbit ID No.	Total erythema+oedema						Average
	24 hours		48 hours		72 hours		
	Control	Test	Control	Test	Control	Test	
R-01	0	0	0	0	0	0	0
R-02	0	0	0	0	0	0	0
R-03	0	0	0	0	0	0	0
Mean	0	0	0	0	0	0	P. I. I.=0

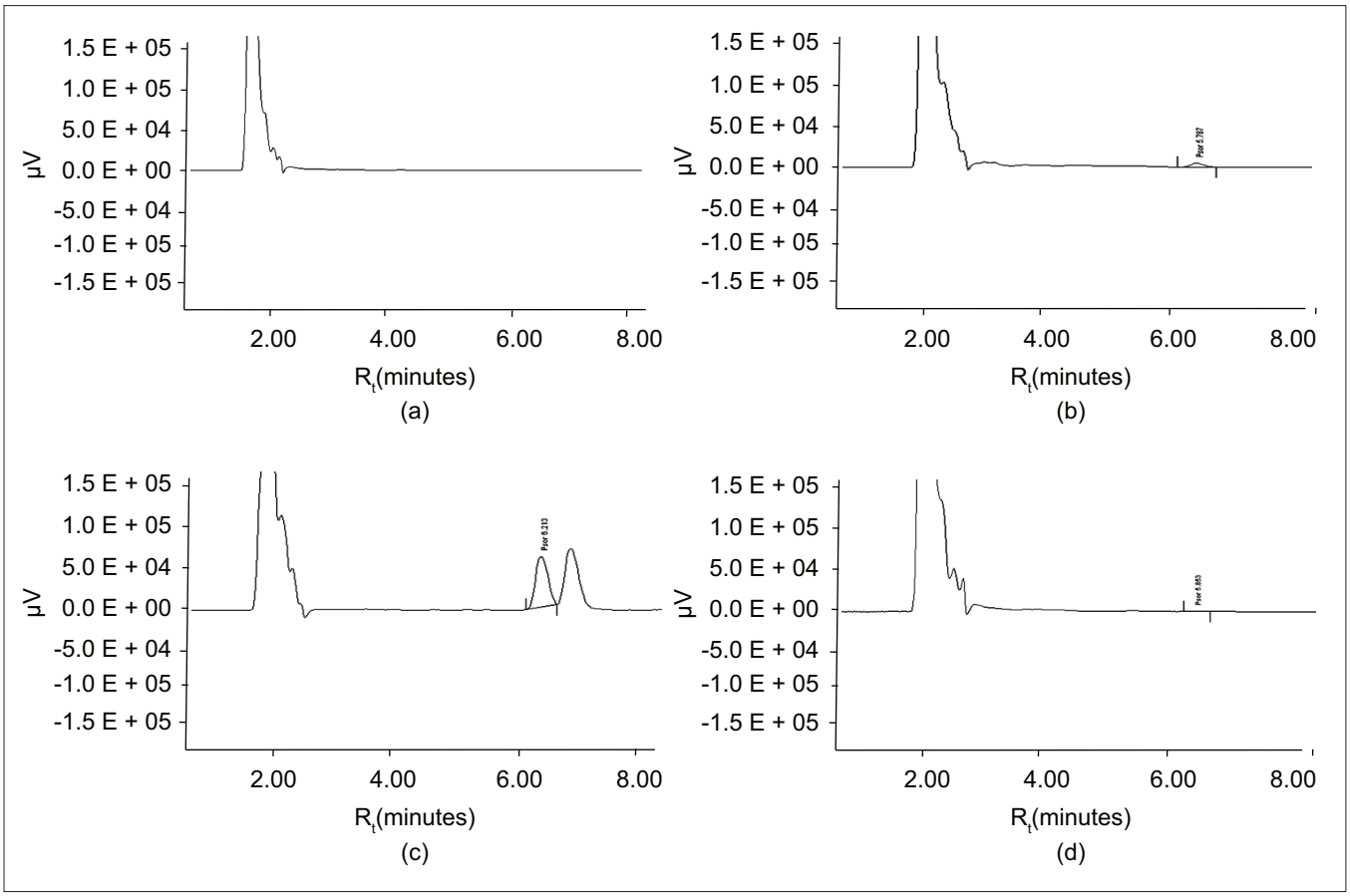

Figure 3: Representative HPLC chromatograms of (a) Blank plasma (b) Spiked psoralen 1000 ng/mL (c) Spiked BT (d) Psoralen in rabbit plasma after topical application of BT

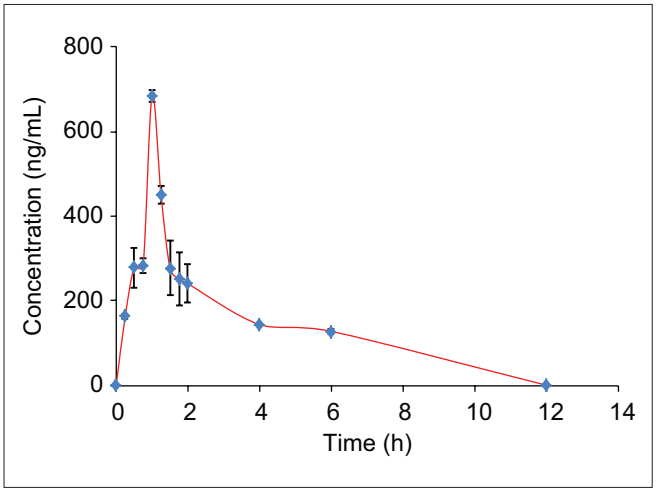

Figure 4: Mean plasma concentration–time profile of psoralen after topical application of BT on rabbits (*n*=3)

Dermal Absorption of Psoralen From *Bakuchi Taila* in Rabbits

Bakuchi Taila was applied on the rabbit's pinna and psoralen was quantified from rabbit plasma using the HPLC method. There has been a report on the quality control of BT[17] using thin-layer chromatography (TLC). However, the pharmacokinetic study of BT, using psoralen as a marker, has not been reported so far. In the current pharmacokinetic study a peak corresponding to psoralen was detected from rabbit plasma after application of BT, which was absent in the plasma before application of BT [Figure 3]. The mean plasma concentration-time profile (*n*=3) is shown in Figure 4, and the main pharmacokinetic parameters are summarized in Table 5. As per the concentration–time profile, the T_{max} of psoralen was at one hour.

Table 5: Pharmacokinetic parameters during dermal absorption study of psoralen from *Bakuchi Taila*

Parameters	Values (Mean±SD, *n*=3)
C_{max} (ng/mL)	684.79±14.60
T_{max} (h)	1.00±0.000
λ (h^{-1})	0.17±0.039
$t_{1/2}$ (h)	4.32±0.96
AUC_{0-12} (ng/mL*h)	1265.81±47.85
$AUC_{0-\infty}$ (ng/mL*h)	13014.1±3396.76

A dose of 258.0 µg psoralen (from BT) was applied on the rabbit pinna, but up to 12 hours, 1.2 µg of psoralen was detected in the rabbit plasma, which showed its poor absorption from BT (0.46%). The detection of psoralen, however, proved that when BT was applied on the rabbit's

pinna, dermal absorption of the phytoconstituents took place, which probably facilitates biochemical changes. Thus, the proposed methodology was rapid, selective, required a simple sample preparation procedure and represented a good procedure for quantitation of psoralen from complex plant-based as well as biological matrices.

CONCLUSION

As single marker-based quantitative methods would be complementary approaches for the quality control and stability assessment of herbal preparations, the results of the current study could be used by industries for the characterisation of *P. corylifolia* and its formulations in order to check their uniformity. The developed RP-HPLC-DAD method, for the estimation of psoralen, could also be applied to various polyherbal formulations containing *Psoralea corylifolia* L.

ACKNOWLEDGMENT

We acknowledge the financial assistance from NMPB, Government of India (Project No. GO/MH-04/2009) for carrying out this work.

REFERENCES

1. Khushboo PS, Jadhav VM, Kadam VJ, Sathe NS. *Psoralea corylifolia* Linn.-"Kushtanashini". Pharmacogn Rev 2010;4:69-76.
2. Uikey SK, Yadav AS, Sharma AK, Rai AK, Raghuwanshi DK, Badkhane Y. The Botany, Chemistry, Pharmacological and Therapeutic Application of *Psoralea corylifolia* L. – A Review. Int J Phytomed 2010;2:100-7.
3. Feng L, Wang L, Jiang X. Pharmacokinetics, Tissue Distribution and Excretion of Coumarin Components from *Psoralea corylifolia* L. in Rats. Arch Pharmacal Res 2010;33:225-30.
4. Liu R, Li A, Sun A, Kong L. Preparative isolation and purification of psoralen and isopsoralen from *Psoralea corylifolia* by high-speed counter-current chromatography. J Chromatogr A 2004;1057:225-8.
5. Shailajan S, Menon S, Singh A, Mhatre M, Sayed N. A Validated RP-HPLC method for quantitation of trigonelline from herbal formulations containing *Trigonella foenum-graecum* (L.) seeds. Pharm Met 2011;2:157-60.
6. Shailajan S, Singh A, Tiwari B. Quality Control and Standardization of an Ayurvedic *Taila* formulation. Int J Biomed Res Anal 2010;1:78-81.
7. Champanerkar PA, Vaidya V, Shailajan S, Menon S. A sensitive, rapid and validated liquid chromatography-tandem mass spectrometry (LC-MS-MS) method for determination of mimosine in *Mimosa pudica* Linn. Nat Sci 2010;2:713-7.
8. Shailajan S, Menon SN, Singh A. Quantitative analysis of piperine from Ayurvedic polyherbal formulation using Reverse Phase High Performance Liquid Chromatography. Int J Pharma Bio Sci 2009;1:1-10.
9. Lin CF, Huang Yl, Chien MY, Sheu SJ, Chen CC. Analysis of Bakuchiol, Psoralen and Angelicin in Crude Drugs and Commercial Concentrated Products of *Fructus Psoraleae*. J Food Drug Anal 2007;15:433-7.
10. Dong NT, Bae K, Kim YH, Hwang GS, Heo OS, Kim SE, *et al*. Quantitative Determination of Psoralen and Angelicin from Some Medicinal Herbs by High Performance Liquid Chromatography. Arch Pharmacal Res 2003;26:516-20.
11. Novikova IY, Tulaganov AA. Using HPLC for the quality control of psoralen and related preparations. Pharm Chem J 2004;38:279-81.
12. International Conference on Harmonization (ICH). Validation of Analytical Procedures: Methodology. Q2 (R1). ICH harmonized tripartite guideline. 1998.
13. Organization of Economic Co-operation and Development (OECD). Guidelines for Testing of Chemicals. No. 404. Acute Dermal Irritation/Corrosion, Paris, France, 2004.
14. Draize JH, Woodard G, Calvery HO. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. J Pharmacol Exp Ther 1944;82: 377-90.
15. Food and Drug Administration (FDA) of the United States. Guidance for industry, Bioanalytical Method Validation, U.S. Department of Health and Human services, Centre for Drug Evaluation and Research (CDER) and Center for Veterinary Medicine (CVM), 2001.
16. Bansal S, DeStefano A. Key Elements of Bioanalytical Method Validation for Small Molecules. AAPS J 2007;9:E109-14.
17. Ali J, Akhtar N, Sultana Y, Baboota S, Ahmad S. Thin-Layer Chromatographic analysis of psoralen in babchi *(Psoralea corylifolia)* Oil. Acta Chromatogr 2008;20:277-82.

How to cite this article: Shailajan S, Menon S, Singh A, Mhatre M, Sayed N, Joshi H, *et al.* Estimation of psoralen from herbal formulations containing *Psoralea corylifolia* using the RP-HPLC-DAD method and its application to a pharmacokinetic study. Int J Green Pharm 2012;6:217-23.

Source of Support: NMPB, Government of India (Project No. GO/MH-04/2009), **Conflict of Interest:** None declared.