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Stability indicating HPTLC method for active principle psoralen and its application to accelerated stability testing of marketed formulation

Mrinalini Damle* & Sayali Sheth

Department of Quality Assurance, AISSMS College of Pharmacy, Kennedy road, Near RTO, Pune 411 001, Maharashtra, India

*E-mail: damle_mc@aissmscop.com

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Psoralen is a phytoconstituent found in the plant. *Psoralea corylifolia* is also known as Bawchi/Bakuchi in India. A stability indicating HPTLC method for estimation of psoralen has been developed. The effects of accelerated conditions on the psoralen content from marketed formulation (seed powder) were studied. The powder of dried seeds and standard psoralen were applied to silica gel 60 F_{254} aluminium-supported precoated TLC plates using optimised mobile phase containing toluene: ethyl acetate 9: 1 (v/v). Densitometric scanning was conducted at λmax= 246 nm. A compact peak for psoralen (Rf= 0.62±0.03) was observed with linearity ranging from 40 ng-200 ng/band with good correlation coefficient of r^2= 0.991. The standard marker was subjected to degradation studies as per ICH Q1A (R2) guidelines and found susceptible to degradation in all conditions like hydrolysis, photolytic, oxidation and thermal. The marketed formulation (seed powder) was exposed to accelerated conditions for 3 months. The method was found to be reproducible, selective and reliable for estimation of stability of standard psoralen in marketed formulations.

Keywords: Bawchi, HPTLC, Psoralen, Seed powder, Stability

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Psoralen is an active principle found in the plant *Psoralea corylifolia* Linn. (Family: Fabaceae) also known as Bawchi / Babchi / Bakuchi that is used frequently in the treatment of severe skin disorders like psoriasis, eczema, vitiligo and leprosy[1,2]. Psoralen is administered in the PUVA therapy (psoralen+ UV A) orally or applied on the skin for sensitising the skin before exposure to the UV radiation[3] so that more radiation is absorbed effectively to further prevent the growth of harmful cells. Not limited to only psoriasis, psoralen is also reported be used as antihelminitic, antibacterial, antifungal and in type II diabetes[4]. Besides this there were methods reported on the simultaneous estimation of psoralen, bakuchiol and bakuchicin by HPLC and HPTLC in oil and seed powder and other marketed formulations[5-7]. But no method was reported on forced degradation studies of psoralen by HPTLC[8,9]. Hence a simple and reliable HPTLC method was developed and applied to estimate the stability of the marker and its marketed formulation (seed powder)[10].

*Corresponding author

Materials and Methods

Materials and reagents

Reference standard psoralen (95% pure) was procured from Yucca Enterprises, Mumbai (India). The seed powder which is commercially available as Bawchi churna was obtained as gift sample from Green Pharmacy, Pune (India). Bakuchi tailam was purchased from local pharmacy. The chemicals toluene, ethyl acetate, methanol and acetone (AR grade) were purchased from Loba Chemie Pvt. Ltd.

Instruments and equipment

CAMAG HPTLC system (Muttenz, Switzerland) comprising of Camag Linomat-5 applicator, WinCat software version 1.3.0 and Camag TLC scanner 3.

Preparation of standard stock solution

200 mg of reference standard was dissolved in 100 mL of methanol and diluted further to get concentration of 100 µg/mL.

Preparation of sample

A) *Seed powder*

2 g of seed powder was weighed and transferred to 100 mL volumetric flask containing methanol. This solution was sonicated for 30 min and filtered using

Whatmann filter paper. The filtrate was made up with methanol upto 100 mL.

B) *Oil*

2 mL of oil was transferred to a 100 mL volumetric flask and dissolved in acetone and volume was made up to 100 mL with acetone.

Experimental conditions

Stationary phase

Aluminium TLC plate precoated with silica gel 60 F_{254}.

Mobile phase

Toluene: Ethyl acetate (9:1 v/v)

Plates were developed in CAMAG 20x10 twin trough chamber and saturation time was 15 min.

Optimisation of method

The method was optimised by mobile phase trials and chromatographic conditions with reference to Indian Pharmacopoeia 2018[11] which reported composition of toluene: ethyl acetate (7:3 v/v) and saturation time was kept 20 mins. The scanning was done at 246 nm since it is the wavelength of maximum absorption as shown in the spectrum (Fig. 1). The peaks obtained were broader and tailing was observed and Rf was higher as compared to the mobile phase ratio of 9:1 v/v and saturation time of 15 min which gave well resolved peaks. The oil of bakuchi (bakuchi tailam) was found to be highly soluble in acetone but showed no specific peak for psoralen as compared to reference standard.

Forced degradation studies

The forced degradation conditions were optimised for degradation in range of 10-20% (Table 1).

1) *Hydrolysis at different pH*

- *Acidic pH*

For the acidic conditions 1 mL of 0.1N HCl was added to 1 mL 200 µg/mL of standard psoralen and this mixture was kept for 1 h at room temperature and volume was made up to 10 mL with methanol to get concentration of 20 ppm. Band of 4 µL of this solution was applied to TLC plate. The psoralen gave percent recovery of 78% in HCl which shows its stability towards acidic environments (Fig. 2).

- *Basic pH*

For the basic conditions 1ml of 0.1N NaOH was added to 1 mL 200 µg/mL standard psoralen and this mixture was kept for 15 min at room temperature and volume was made up to 10 mL with methanol to get concentration of 20 ppm. Band of 4 µL of this solution was applied to TLC plate. The basic conditions are favourable for psoralen and drug shows longer shelf life if base might be used in formulation as per the percent recovery of 85.49% as compared to the standard psoralen peaks without exposure to conditions at 246 nm wavelength (Fig. 2).

Table 1 — Results of forced degradation studies of standard psoralen

Sr no.	Stress conditions	% Recovery
1	0.1N HCl for 1 h at RT	78.48
2	0.1N NaOH for 15 min at RT	85.49
3	UV light 200 watts hour/ m^2	87.78
4	Fluorescence 1.2 million lux hours	25.0
5	Oxidation by 30% v\v H2O2	73.64
6	Thermal degradation at 60ºC	
	2 h	76.97
	4 h	76.31

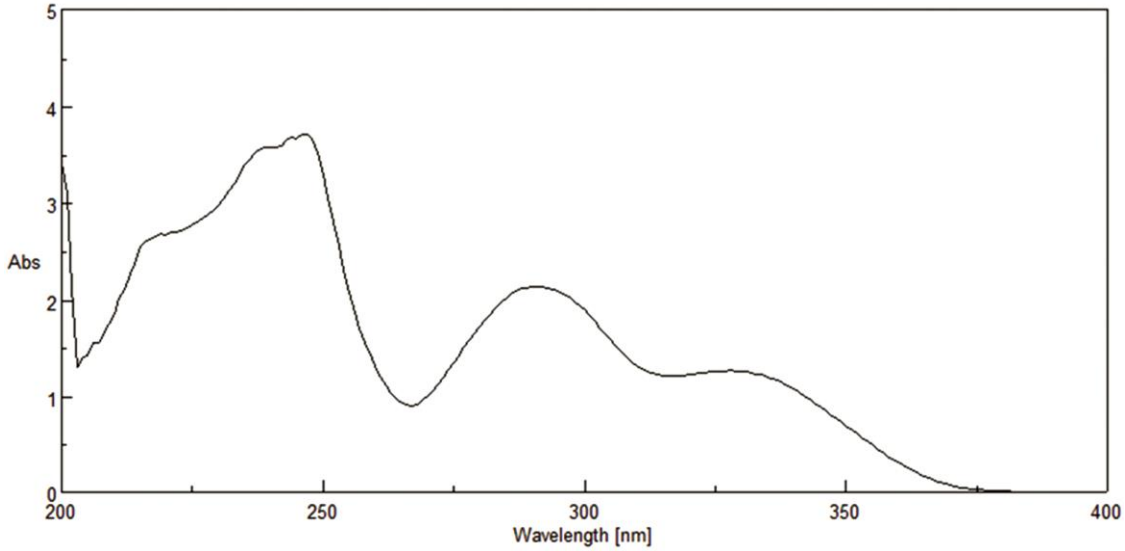

Fig. 1 — UV spectrum of Psoralen standard showing highest absorbance at 246 nm.

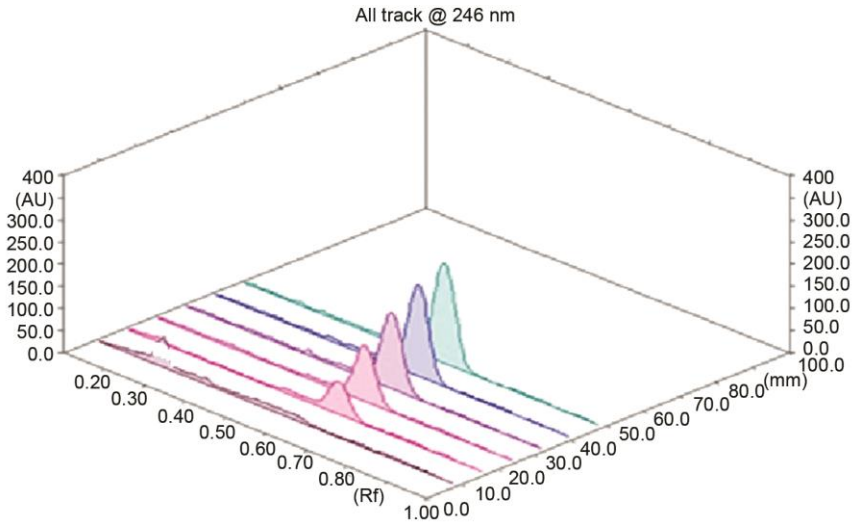

Fig. 2 — Three dimensional display of the linearity range of reference standard psoralen (40-200 ng/band) The linearity was tested in the range of 40-200 ng amount spotted as bands.

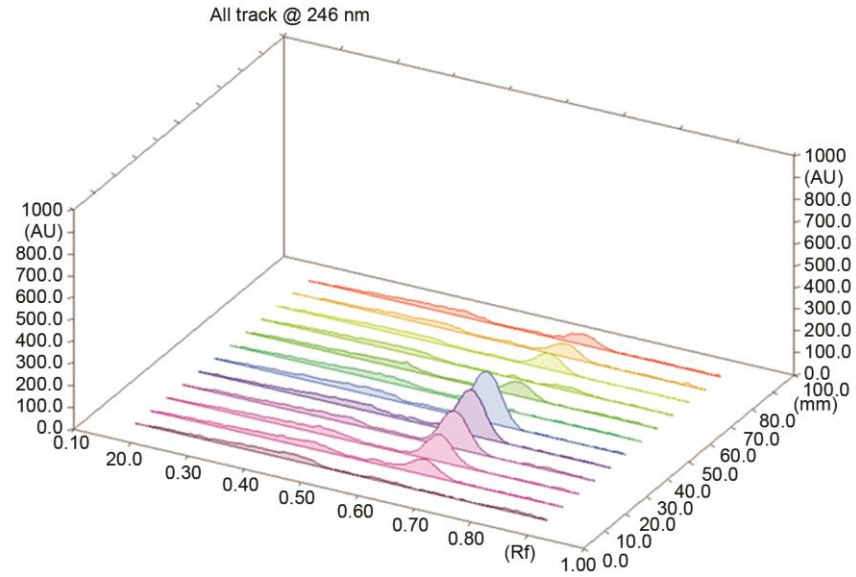

Fig. 3 — Three dimensional densitogram of linearity (track 2-6) and stress samples (acid, base, UV and fluorescence) at track 8, 10, 11,12 (80 ng/band), respectively.

2) *Photolytic degradation*

To check the stability of psoralen in photolytic conditions 20 mg of standard psoralen was weighed and exposed to UV light for 200 Watts hour/m^2 and fluorescent light for 1.2 million lux hours. 10 mg of this exposed sample was weighed and transferred to a 10 mL volumetric flask and methanol was added to get concentration of 1000 ppm. This solution was further diluted to get final concentration of 20 ppm. Bands of 4 µL of the solution was applied to TLC plate. The psoralen gave highest recovery in UV light of 87.78% and got most degraded in fluorescent light as the peaks were quite clearly visible in UV as compared to standard peak (Fig. 2; track no 4,5,11,12)

3) *Oxidation degradation studies*

To study the effect of oxidative conditions 1 mL of 30% v/v H_2O_2 was added to 1 mL 200 µg/mL standard psoralen and the mixture was kept for 4 h at room temperature and volume was made up to 10 mL with methanol to get concentration of 20 ppm. Band of 4 µL of this solution was applied to TLC plate. The oxidative conditions gave recovery of 73.64% which was fair enough for formulation to be stable for a longer time during such conditions (Fig. 3).

4) *Thermal degradation studies*

To study stability of psoralen in thermal conditions when the product may be exposed to humidity or heat the standard psoralen was weighed and exposed to heat in hot air oven for 2 h and 4 h at 60ºC. This sample was weighed and transferred to a 10 mL volumetric flask and methanol was added to get concentration of 1000 ppm. This solution was then further diluted to get final concentration of 20 ppm. Sample was spotted as bands of 4 µL of the solution on to TLC plate (Fig. 3).

Accelerated stability testing of marketed formulations

Details are presented in Table 2. The seed powder (bawchi churna) was exposed to accelerated conditions in stability chamber as per ICH Q1A (R2)[12] and maintained at 40ºC and 75% relative humidity for 3 months. The seed powder was assayed for degradation in percent content of psoralen at different time points of 1, 2 and 3 months in comparison to initial assay of powder without exposure (Fig. 4).

Method validation[12]

Details are presented in Table 3.

Linearity

Solution of 20 µg/mL was used to spot bands of 2,4,6,8 and 10 µL for the range 40-200 ng/band (Fig. 5 & 6).

Assay

1 mL of seed powder sample was diluted with 1 mL of methanol. 1 mL of this solution was taken and diluted to 10 mL with methanol to get concentration of 1000 µg/mL. Bands with

Table 2 — Results of accelerated stability studies of seed powder

Sr.no.	Time points	Rf	Percent content of psoralen
1	0 months (initial)	0.65±0.02	1.24%
2	1 month	0.64	0.71%
3	2 months	0.65	0.67%
4	3 months	0.66	0.62%

Table 3 — Validation of method

Sr no.	Parameter	Result
1	Linearity range	40-200 ng/band
2	Regression equation	y = 26.336x+383.68 r^2 = 0.991
3	Precision	
	Intraday	1.21 - 1.36%
	Interday	1.92 –1.96 %
4	Specificity	Purity of psoralen peak in the sample (correlation r [S, M] = 0.998, r [M, E] = 0.997.
5	Accuracy	98 to 102% recovery
6	Robustness	Robust
7	LOD	2.84 ng /band
8	LOQ	10.15 ng/ band

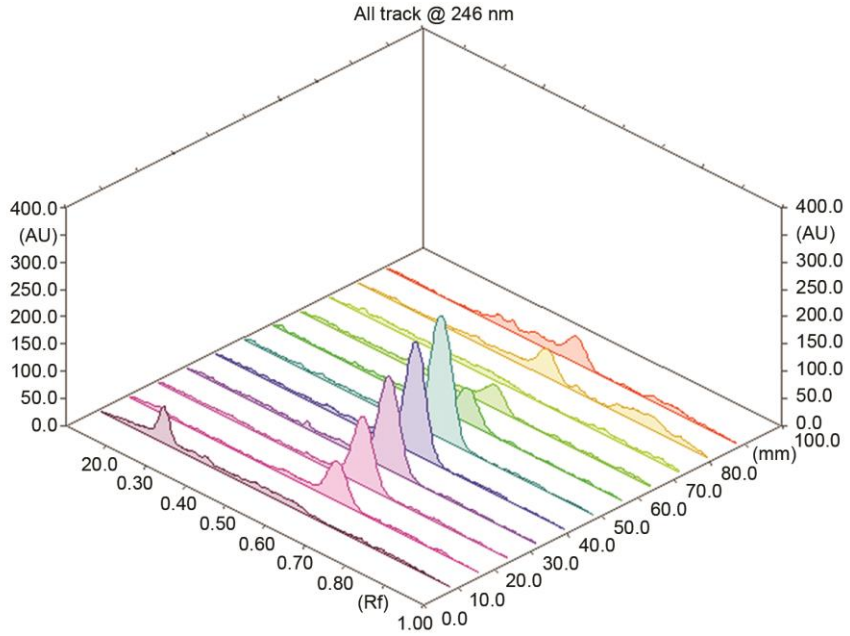

Fig. 4 — Three dimensional densitogram of linearity (track 2-6) and stress samples (thermal, oxidation) at track 7, 8, 10 (80 ng/band), respectively

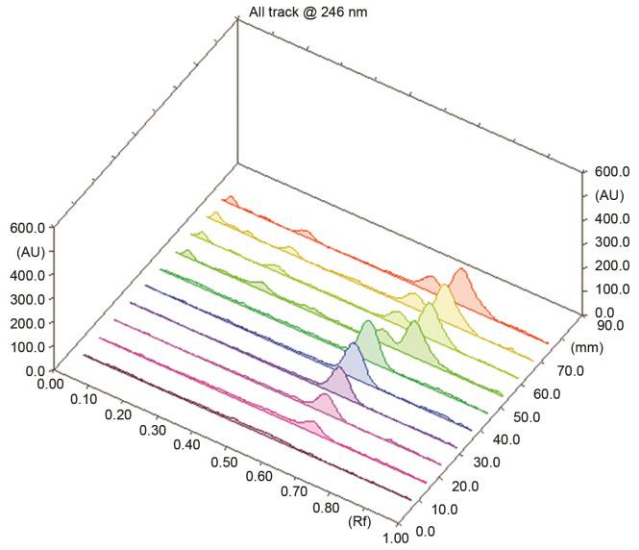

Fig. 5 — Three-dimensional display of standard linearity (track 2-6), initial assay (track 7,8) and after exposure to accelerated conditions (track 9,10)

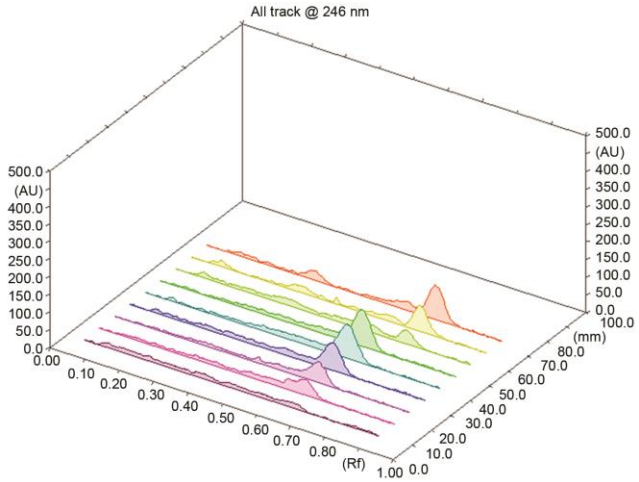

Fig. 6 — Three-dimensional display of linearity (track 2-6) with assay of bakuchi oil (no compact peak) (track 7-9)

concentration 10,000 ng/band with the reference standard (range 40-200 ng/band) were applied.

Limit of Detection (LOD), Limit of Quantification (LOQ)

The lowest amount of an analyte in a sample that can be detected but not necessarily quantitated is LOD whether LOQ is the lowest amount of analyte that can be quantitated in a sample with accuracy and precision. In the methods, LOD and LOQ were determined using the following equation:

$LOD = 3.3 \times \sigma / S$

$LOQ = 10 \times \sigma / S$

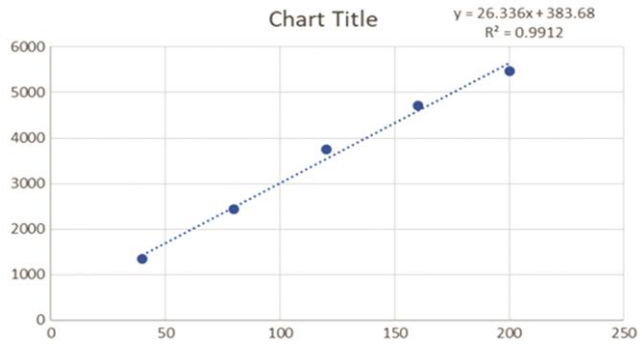

Fig. 7 — Calibration curve for linearity range of reference standard psorlen.

Where, "σ" is the standard deviation of y-intercept and "S" is the slope of calibration curve.

Precision

The method was studied as repeatability, and intraday and inter-day variation. The precision of the method was expressed as a percentage of relative standard deviation (% RSD).

Specificity

This study was performed by the peak purity of psoralen and was compared with the spectra of psoralen extracted from seed powder and psoralen reference standard at the peak start (S), peak maxima (M) and at the peak end (E) positions. The peaks from powder were more sharp and justifiable in reference to the oil of bakuchi (Fig. 7).

Accuracy

It was calculated by measurement of recovery. Pre-analyzed samples were spiked with standard at three different levels, 80, 100 and 120% of the amount originally present and after standard addition were reanalyzed. The recovery of experiment was carried out in sets of triplicates. The recovery of standard psoralen was between 98-102% as per stability studies (Table 3).

Robustness

The robustness of method was measured by making small but deliberate changes in the experimental conditions. Such as increase/decrease in saturation time, scanning at different wavelengths, time period between spotting development and scanning. The method was robust as the changes like saturation time of ±3 min and scanning at different wavelengths like 244 245, 247,248 nm showing good absorbance like the standard psoralen at 246 nm.

Results and Discussion

The mobile phase toluene: ethyl acetate in the ratio of 9:1 v/v showed better results with better peak shape and no interference of other components present in seed powder sample as compared to trail mobile phase ratio of 7.5:2.5 v/v. With the chromatographic conditions used for the experiment resulted in LOQ of 10.15 ng/band which shows that the method was sensitive enough to quantitatively detect psoralen in herbal formulation i.e., seed powder. The detection wavelength showed highest absorbance at 246 nm. Forced degradation studies were necessary to study the stability of psoralen in various conditions and accelerated condition studies proved that the potency decreases monthly when exposed to temperature above 40ºC and humidity 75% hence storage at room temperature is ideal for storage. The oil formulation contained less detectability of psoralen content which depicts that powder formulation i.e., the bawchi churna was pharmacologically more effective and stable as per the forced degradation studies. The accelerated studies testing (Table 2) (Fig. 5) showed that churna can be used for more than three months with still a adequate amount being retained after being exposed to the worst conditions considering the recovery of standard sample.

Conclusion

The developed stability indicating HPTLC method was found to be accurate, precise and reliable for quantitative estimation of psoralen from marketed formulations. It was found that psoralen was susceptible to degradation in all conditions with highest degradation in fluorescent light. The formulation should be formulated and protected accordingly. The method was validated as per ICH guidelines.

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Conflict of Interest

The authors declare that they had no coalition with any organization or entity with financial or non-financial interest in reference to matter and materials discussed in this manuscript.

Authors' Contributions

Data was conceived, collected by SS and analysis was designed by MD. The analytical work was performed by both authors.

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