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# Effect of pH and Gastrointestinal Enzymes on Stability of Psoralen, Bakuchicin and Bakuchiol using Simultaneous TLC Densitometric Method and Standardization of commercial formulations containing *Psoralea cordyfollia* Linn.

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## ABSTRACT

*Psoralea corylifolia* is used for treatmet of skin diseases such as psoriasis, vitiligo. Psoralen is responsible for its effectiveness against psoriasis. Bakuchicin and Bakuchiol are DNA polymerase and topoisomerase II inhibitors. To study the effect of pH and gastrointestinal (GI) enzymes on Psoralen, Bakuchicin and Bakuchiol from *Psoralea corylifolia* Linn using a simple, sensitive, accurate and robust high performance thin layer chromatographic (HPTLC) method. The method was performed on silica gel 60 $F_{254}$with n- Hexane : Ethyl acetate ( 7.5 : 2.5 v/v) as the mobile phase. Densitometric scanning at 285 nm for Psoralen, Bakuchicin and Bakuchiol was used. The method was validated as per the guidelines of International Conference on Harmonization (ICH). In addition the applicability of the method was tested for the standardization of both mono and polyherbal formulations containing the above markers. The $R_f$ values of 0.37, 0.48 and 0.63 were obtained for Psoralen, Bakuchicin and Bakuchiol respectively. The linearity range of 20-120 ng spot$^{-1}$, 20-120 ng spot$^{-1}$ and 80-280 ng spot$^{-1}$ with good correlation coefficients of $r^2$ = 0.998, 0.998 and 0.999 were obtained for Psoralen, Bakuchicin and Bakuchiol respectively. The method was applied for the *in vitro* stability studies of above markers in simulated gastric and intestinal fluids to study the effect of pH and GI enzymes. Psoralen was found to be most stable in the simulated physiological fluids whereas other two compounds showed instability. The method was found to be precise, robust and suitable for the routine quality control analysis of plant extracts and polyherbal formulations.

**Keywords:** *Psoralea corylifolia* Linn, Leguminoceae, HPTLC, Enzymatic stability

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**Abbreviations :**

*P.corylifolia*: *Psoralea corylifolia*
SGF : Simulated Gastric Fluid
SIF : Simulated Intestinal Fluid
HPTLC : High performance thin layer chromatography
ICH : International Conference on Harmonization
GI : Gastro Intestinal

## 1. INTRODUCTION

*Psoralea corylifolia* Linn. (Fam: leguminoceae) is the most popular herb used in Indian traditional medicines since ancient time. It has been officially listed in Chinese Pharmacopoeia[1]. The different parts of the plant such as seeds, seed oil, roots and leaves are used for therapeutic effects [2]. The plant is widely exploited since ages for its effect against several skin diseases, such as psoriasis, leukoderma, and leprosy[3]. It also possess anthelmintic, laxative, diuretic, aphrodiasic, antipyretic, analgesic, anti-staphylococeal and antifungal activity [4,5,6].

*Psoralea corylifolia* Linn contains wide variety of phytochemicals such as Psoralen, Angelicin, Bakuchiol, Psoralidin, Isopsoralen [7,8,9] etc. The flavonoids present in the seed are Corylifolin, Bakuchicin, Psoralidin, Bavachin, Corylifolinin, Bavachinin Corylin, Corylidin [10,11,12,13] Psoralen (Fig.1a) bakuchicin (Fig 1b) and bakuchiol (Fig 1c) are considered to be the main constituents responsible for pharmacological activities of the plant.

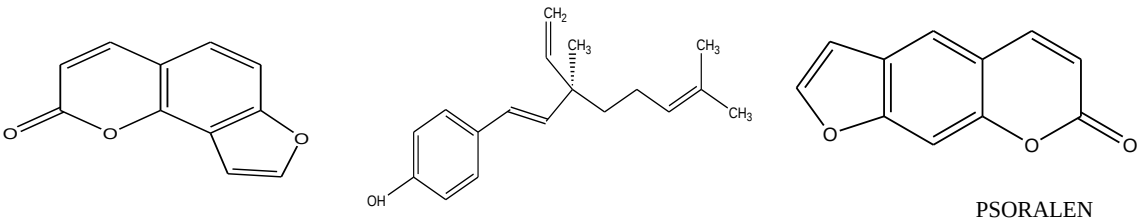


Fig. 1. Structure of Bakuchicin, Bakuchiol and Psoralen

Psoralen is responsible for inducing pigmentation of skin. It is used to treat skin diseases such as psoriasis and vitiligo, renal weakness and other kidney dysfunctions [14]. Estimation of Psoralen from natural herbs and herbal products is done by different analytical methods like HPLC [14,15,16], TLC [17], HPTLC [18], Micellar Electrokinetic Capillary Chromatography[19], LC- Electrospray ionization mass spectrometry[20].

Bakuchicin, a furanocoumarin compound, exhibits antibacterial, antioxidant activity[21] and topoisomerase II inhibitor activity[22]. It is estimated by different analytical methods such as HPLC [16] and HPLC-MS[23].

Bakuchiol a meroterpenoid phenol present in the seeds of *Psoralea corylifolia* is yellow coloured liquid. It exhibits different biological activities such as antitumor[24], antimicrobial[25], antibacterial [26,27], hepatoprotective and DNA polymerase inhibitor activity[22]. HPLC [14, 16], GC[28] and GC-MS[29] methods have been reported for the estimation of Bakuchiol.

Recently, the number of commercial herbal formulations of Bakuchi are available for various treatments such as psoriasis, vitiligo, leprosy etc. The major problem with any medicines is their oral bioavailability and stability. In the gastrointestinal tract, drug molecules are exposed to acidic and alkaline pH and digestive juice containing enzymes such as pepsin and pancreatin. This may sometimes lead to degradation of such compounds, hence interfere with the absorption thereby bioavailability. There is no data available to provide insight into the stability of psoralen, bakuchicin and bakuchiol in gastrointestinal system. Moreover the herbal formulations are needed to be standardized to ensure their safety and efficacy. Hence the present study aimed to establish the effect of pH and enzymes on psoralen, bakuchicin and bakuchiol using simulated physiological fluids at 37°C and to standardize the herbal formulations of Bakuchi with respect to the above marker components. Although a HPLC method is reported for the simultaneous estimation of the above components, it is either tedious or time consuming method. Nowadays HPTLC is becoming a routine analytical technique because of its advantages of low operating cost, high sample throughput, simplicity, and speed, the need for minimum sample clean up, reproducibility, accuracy, reliability, and robustness. The literature survey revealed that there is no method available yet to simultaneously estimate psoralen, bakuchicin and bakuchiol by HPTLC. In light of these all observations it was decided to develop a validated HPTLC method for simultaneous estimation of psoralen, bakuchicin and bakuchiol. The method was applied to study the effect of pH and GI enzymes on these three components in simulated gastro- intestinal fluids. In addition the method was successfully applied for the standardization of mono and polyherbal formulations containing *Psoralea corylifolia* Linn.

## 2. EXPERIMENTAL

### 2.1 Materials and reagents

Crude plant powder of Psoralen, Bakuchicin and Bakuchiol was obtained from the Ayurvedic Aaushadhalaya, local market of Pune, India. The formulation containing *Psoralea corylifolia* was procured from the local market and designated as MF1 and MF2.

Reference standards Psoralen (>96%), Bakuchicin (>96%), and Bakuchiol (>95%) obtained from Natural Remedies pvt ltd, Bangalore, India were used. All chemicals and reagents used were of analytical grade.

### 2.2 Preparation of standard solutions

Standard stock solution of Psoralen, Bakuchicin and Bakuchiol (1000µg/mL) was prepared by dissolving 1 mg each of accurately weighed markers in methanol and making up the volume to 1 mL with methanol. Sample solutions were prepared by dilution of the stock solution with methanol to reach a concentration range of 10 - 120 µg/mL, 20 - 130 µg/mL, and 40-320 µg/mL for Psoralen, Bakuchicin and Bakuchiol respectively. The complete dissolution of the markers was ensured by ultrasonication for 15 minutes. One microlitre of each sample solution was applied on HPTLC plates to obtain the final concentration of 10-120 ng spot$^{-1}$, 20 - 130 ng spot$^{-1}$ 40-320 ng spot$^{-1}$ for Psoralen, Bakuchcin and Bakuchiol respectively.

### 2.3 Chromatographic conditions:

Standard and sample solutions were applied to the plates by means of a Camag (Muttenz, Switzerland) Linomat V automated spray-on band applicator equipped with a 100.00-µL syringe (Hamilton) and operated with the settings of band length 6 mm, application rate 10 s µL−1, distance between bands 12 mm, distance from the plate edge 10 mm, and distance from the bottom of the plate 10 mm. Plates were developed to 8 cm beyond the origin with n-Hexane: Ethyl acetate ( 7.5: 2.5 v/v) as mobile phase in twin trough glass chambers after saturation of the chamber with mobile phase vapour for 30 min (the optimum chamber-saturation time) at room temperature. After development, mobile phase was evaporated from the plate by use of an air-dryer for 10 min. Densitometric scanning was then performed in absorbance mode at 285 nm for Psoralen, Bakuchicin and Bakuchiol using the deuterium lamp as the source of radiation. A Camag model III TLC scanner linked with CATS (V 3.5, Camag) integration software was used. The slit dimensions were 5 mm × 0.45 mm and the scanning speed 10 mm s$^{-1}$. Calibration was performed by triplicate application of 1.0–10.0 µL of mixed stock standard solution, resulting in 20–120 ng per band for Psoralen, 30–130 ng per band for Bakuchicin and 120-320 ng per band for Bakuchiol. Peak area and concentration data were treated by linear least-squares regression analysis. The amounts of Psoralen, Bakuchicin and Bakuchiol were determined from the

respective calibration plots obtained by plotting the concentration of standard against peak area.

### 2.4 Validation of the method

The proposed method was validated for specificity, linearity, precision, accuracy and robustness following the ICH guidelines [30, 31, 32].

#### 2.4.1 Linearity, LOD and LOQ

Linearity was determined by construction of calibration plots and linear least-squares regression analysis as described above. The limit of detection (LOD) and limit of quantification (LOQ) were determined by diluting known concentrations of standard stock solution until the average responses were approximately three (For LOD) or ten times (for LOQ) the responses of the blank. Six replicate determinations were performed using methanol alone as blank.

#### 2.4.2 Precision

Precision study was carried out for the repeatability of sample application and measurement and the result was expressed as % RSD of peak areas. Variability of the method was studied by analyzing aliquots of standard solutions of Psoralen (20, 40, 60, 80, 100, 120 ng/spot), Bakuchicin (30, 50, 70, 90,110,130 ng/spot) and Bakuchiol (120,160,200,240,280,320 ng/spot) on the same day (intra-day precision) and on different days (inter-day precision), and the results were expressed as % RSD.

#### 2.4.3. Robustness

Robustness of the method was checked by making intentional changes in the parameters. Small change in the mobile phase composition was tried (Formic acid ± 0.01 ml). The amount of mobile phase and temperature were varied in the range of ± 5%. The plates were prewashed with methanol and activated at 60°C ± 5 for 5, 10, 15 min respectively prior to chromatography. Time from spotting to chromatography and from chromatography to scanning was varied from 0, 30, 60 and 90 min. Robustness was done at three different concentration levels 40, 80, 120 ng $spot^{-1}$ for psoralen , 50, 90, 130 ng $spot^{-1}$ for bakuchicin and 160, 240, 320 ng $spot^{-1}$ for bakuchiol respectively.

#### 2.4.5. Specificity

The identity of the spot of the markers in sample was confirmed by comparing the $R_f$ and spectra of the spot with that of the standard.

#### 2.4.6.Accuracy

The accuracy of the method was tested by performing recovery studies at three levels (80, 100, and 120% standard addition). The amount of markers present in the 250 mg of the crude extracts was determined from the regression equation. Known amount of the standard was added at three levels and recovery was found. The percent recovery as well as the average percent recovery was calculated.

#### 2.4.7. Stability in sample solution

Solutions of two different concentrations of 100 and 200 ng/spot for Psoralen, Bakuchicin and Bakuchiol and were prepared from sample solution, stored at room temperature for 0.5, 1.0, 2.0, 4.0 and 24 hr and analysed.

#### 2.4.8. Spot Stability

Two-dimensional chromatography using the same solvent system was applied to find out any decomposition occurring during spotting and development.

### 2.5. Stability studies of Psoralen, Bakuchicin and Bakuchiol in simulated physiological fluids

Degradation of psoralen, bakuchicin and bakuchiol was investigated in both simulated gastric (SGF) and intestinal fluid (SIF). 0.2 ml of sample solution each of psoralen, bakuchicin and 0.4 ml of bakuchiol was added to SGF and SIF and diluted to 1ml with SGF and SIF. SGF and SIF were prepared as per USP procedure [33]. The solution was incubated and maintained at 37°C for up to 72 hrs. Samples were taken at specified time intervals and analyzed by HPTLC.

#### 2.5.1. Effect of pH on Psoralen, Bakuchicin and Bakuchiol

Effect of pH on Psoralen, Bakuchicin and Bakuchiol was studied at pH 1.2 and 6.8 at 37°c. The same procedure was followed to prepare sample solutions as mentioned above excluding the gastric and intestinal enzymes.

### 2.6. Estimation of Psoralen, Bakuchicin and Bakuchiol in crude powder

500 mg of the powder of plant materials was extracted separately with (4x25) ml of methanol by ultrasonication for 45 min. The pooled extracts of samples were concentrated transferred to 10 mL volumetric flask and the volume was made up with methanol. The extract was centrifuged at 5000 rpm for 10 min and the supernatant was filtered through a 0.45 µm filter membrane before chromatographic analysis and analysed for drug content. The analysis was repeated six times.

### 2.7. Analysis of commercial formulations

To determine the content of Psoralen, Bakuchcin and Bakuchiol in tablets of mono and polyherbal formulation, the contents of twenty tablets of both formulations were weighed, their mean weight determined and were finely powdered. The weight of powder equivalent to tablet content was transferred into a 50 mL volumetric flask containing 25 mL methanol, sonicated for 45 min and diluted to 50 mL with methanol. The resulting solution was centrifuged at 3000 rpm for 15 min and supernatant was analyzed for the said markers. 10 µL of the filtered solution was spotted on the HPTLC plate followed by development and scanning.

## 3. RESULTS AND DISCUSSION

### 3.1. Optimization of chromatographic conditions

Initially, mobile phase was selected on the basis of previous reports of psoralen [11] consisting of toluene, ethyl acetate, and methanol. After several trails, replacement of toluene with n-hexane was found to be suitable for the movement of all three markers. Finally mobile phase consisting of n-Hexane: Ethyl acetate (7.5: 2.5 v/v) was found to give desirable $R_f$ value. The optimized mobile phase can able to give symmetrical, well-resolved reproducible peaks with good shape and baseline separation. The $R_f$ values obtained were 0.37, 0.48 and 0.63 for Psoralen, Bakuchicin and Bakuchiol respectively (Fig.2). The identities of the bands from the sample extracts and commercial formulations were confirmed by overlapping the densitograms of standard with that of samples (Fig. 3).

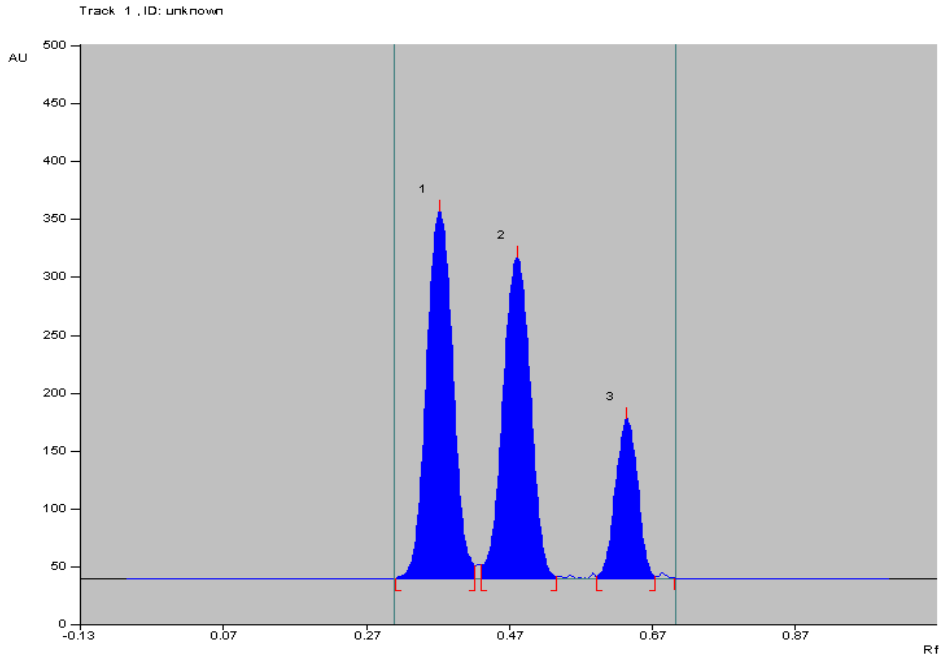


Fig. 2: Densitogram of standard Psoralen, Bakuchicin and Bakuchiol

Peak 1: Psoralen Peak 2: Bakuchicin Peak 3: Bakuchiol

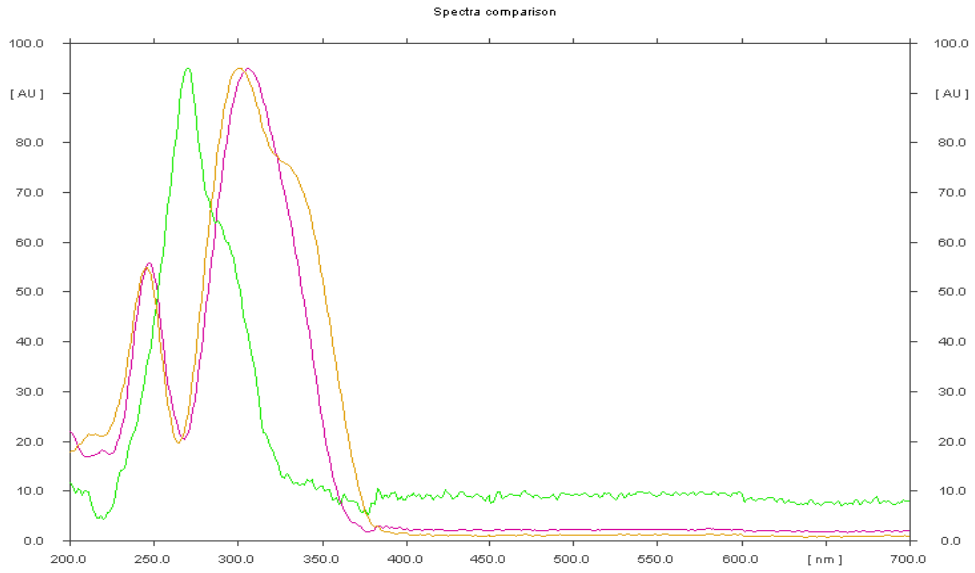


Fig 3: Spectral overlay of Psoralen, Bakuchicin and Bakuchiol

### 3.2. Validation of the method

The linear regression data (**Table 1**) showed a good linear relationship over a concentration range of 20-120 ng spot$^{-1}$ ($r^2$ = 0.998) for Psoralen , 30-130 ng spot$^{-1}$ ($r^2$ = 0.998) for Bakuchicin and 120-320 ng spot$^{-1}$ for Bakuchiol (($r^2$ = 0.999). The signal: noise ratios of 3:1 and 10:1 were considered as LOD and LOQ respectively. The limit of detection (LOD) and the limit of quantitation (LOQ) were found to be 10 ng spot$^{-1}$ and 20 ng spot$^{-1}$ for Psoralen, 20 ng spot$^{-1}$ and 30 ng spot$^{-1}$ Bakuchicin , 80 ng spot$^{-1}$ and 120 ng spot$^{-1}$ for Bakuchiol respectively. The repeatability of the sample application and measurement of peak area were expressed as % RSD and found to be 1.28 and 1.21, 1.62 and 1.67, 1.45 and 1.75 for psoralen**,** bakuchicin and bakuchiol respectively. The results of intermediate precision experiments are shown in (**Table 2).** The developed method was found to be precise as the RSD values for repeatability and intermediate precision studies were < 2%, respectively as recommended by ICH guidelines.

Separation of the drug was found to be similar when analyses were performed using different chromatographic system on different days. For robustness analysis, the standard deviation of peak areas was calculated for each parameter and % RSD was found to be less than 2%. The low values of %RSD as shown in (**Table 3)** indicate the method was robust. The accuracy of the method was checked by standard addition method. The results were presented in (**Table 4)**. The percentage recovery obtained was 99.39%, 99.68% and 99.85% for psoralen, bakuchicin and bakuchiol respectively. The average percentage value and low RSD value demonstrate the reliability and reproducibility of the proposed method for tablets.

**Table 1: Linear regression data for the calibration curves**

| PARAMETERS | PSORALEN | BAKUCHICIN | BAKUCHIOL |
|---|---|---|---|
| Linearity range | 20-120 ng/spot | 30-130 ng/spot | 120-320 ng/spot |
| $r^2$ | 0.998 | 0.998 | 0.999 |
| Slope ± S.D* | 38.36 ± 0.6881 | 32.99 ± o.7078 | 11.30 ± 0.1394 |
| Intercept ± S.D | 84.77 ± 53.59 | -53.70 ± 61.57 | -593.7 ± 32.12 |
| Confidence limit of slope[a] | 36.45 to 40.27 | 31.03 to 34.95 | 10.91 to 11.69 |
| Confidence limit of intercept* | -64.01 to 233.5 | -224.6 to 117.2 | -682.8 to -504.5 |
| Sy.x | 57.57 | 59.22 | 23.33 |

*p<0.001 - Slope significantly different from zero

[a]95% confidence limit.

Sy.x- Standard deviation of residuals from line.

**Table 2: Precision of the method**

| Drugs | Conc. µg/mL | Intraday Found Conc. ± SD | RSD (%) | Interday Found Conc. ± SD | RSD (%) |
|---|---|---|---|---|---|
| **PSORALEN** | 40 | 39.95 ± 0.087 | 0.217 | 40.18 ± 0.323 | 0.804 |
| | 80 | 80.75 ± 0.608 | 0.753 | 80.69 ± 0.512 | 0.634 |
| | 120 | 119.75 ± 1.581 | 1.320 | 120.47 ± 0.630 | 0.520 |
| **BAKUCHICIN** | 50 | 49.94 ± 0.264 | 0.528 | 49.60 ± 0.633 | 1.277 |
| | 90 | 90.48 ± 1.244 | 1.376 | 90.64 ± 1.184 | 1.307 |
| | 130 | 131.08 ± 1.926 | 1.469 | 130.92 ± 1.668 | 1.274 |
| **BAKUCHIOL** | 160 | 157.20 ± 1.263 | 0.804 | 156.08 ± 1.905 | 1.221 |
| | 240 | 232.00 ± 4.542 | 1.958 | 233.84 ± 3.522 | 1.506 |
| | 320 | 324.83 ± 5.360 | 1.650 | 325.49 ± 4.519 | 1.388 |

**Table 3: Robustness of the method**

| PARAMETER | SD of peak area | | | % RSD [a] | | |
|---|---|---|---|---|---|---|
| | Psoralen | Bakuchicin | Bakuchiol | Psoralen | Bakuchicin | Bakuchiol |
| **Mobile phase composition (formic acid ± 0.01 ml)** | 1.760 | 1.125 | 1.538 | 0.758 | 0.653 | 0.225 |
| **Amount of mobile phase (±5%)** | 1.556 | 1.378 | 1.265 | 0.164 | 0.835 | 0.325 |
| **Time from spotting to chromatography** | 1.558 | 1.621 | 1.823 | 0.435 | 0.651 | 0.539 |
| **Time from chromatography to scanning** | 1.643 | 1.059 | 1.238 | 0.139 | 0.589 | 0.325 |
| **Temperature (±5%)** | 1.258 | 1.151 | 1.089 | 0.248 | 0.543 | 0.158 |
| **Plate pretreatment** | 1.116 | 1.119 | 1.253 | 0.135 | 0.765 | 0.251 |

[a] n = 6, Average of three concentrations 40, 80, 120 ng spot$^{-1}$ for Psoralen and 50, 90, 130 ng spot$^{-1}$ for Bakuchicin and 160, 220, 340 ng spot$^{-1}$ for Bakuchiol

**Table 4: Recovery studies**

| Compound ( µg) | Amount added (%) | Total Amount (µg) | Amount found* (µg) | Recovery (%) | Average (%) |
|---|---|---|---|---|---|
| **PSORALEN 74.7** | 80 | 134.46 | 133.5 ± 4.50 | 99.29 ± 0.05 | 99.39 |
| | 100 | 149.4 | 148.2 ± 9.01 | 99.19 ± 0.10 | |
| | 120 | 164.34 | 163.84 ± 7.21 | 99.69 ± 0.28 | |
| **BAKUCHICIN 128** | 80 | 230.4 | 229.5 ± 5.50 | 99.60 ± 0.03 | 99.68 |
| | 100 | 256 | 255.66 ± 3.05 | 99.86 ± 0.04 | |
| | 120 | 281.6 | 280.4± 4.56 | 99.57± 0.08 | |
| **BAKUCHIOL 2225** | 80 | 4005 | 3995 ±6.89 | 99.75 ± 0.12 | 99.85 |
| | 100 | 4450 | 4446± 4.08 | 99.91 ± 0.04 | |
| | 120 | 4895 | 4889.66±6.11 | 99.89 ± 0.06 | |

*Mean ± Standard deviation (n=3)

### 3.3. Stability in sample solution

No additional peak was found in the chromatogram showed that the compounds are stable in sample solutions after storage of 24 hours.

### 3.4. Spot Stability

This was analysed by two dimensional chromatography. In case, if decomposition occurs during development, peak(s) of decomposition product(s) shall be obtained for the analyte both in the first and second direction of the run. No decomposition was observed ensure the spot stability.

### 3.5 Effect of pH (1.2) on marker components

The effect of pH on Psoralen in SGF at $P_H$ 1.2 was investigated at 37$^0$c. There was no change in the peak area observed for Psoralen. Psoralen was found to be most stable component where as slight degradation (4.18 %) of Bakuchiol was observed after 40 min. The Rf value of additional peak was found to be 0.62 [Fig. 7]. Bakuchicin was degraded completely in SGF at 37$^0$c within 3 min.

### 3.6. Effect of pH (6.8) on marker components

In SIF at 37$^0$c, Psoralen was found to be the most stable component whereas bakuchicin was degraded within 3 min. Bakuchiol was found to be stable up to 72 hrs.

### 3.7. Effect of pepsin enzyme on marker components at pH 1.2

The effect of pepsin enzyme on psoralen in SGF at pH 1.2 was investigated at 37$^0$c.There was no change in the peak area of psoralen and thus psoralen was considered to be most stable component. Bakuchicin was degraded completely within 3 minutes in SGF at pH 1.2. Slight degradation of bakuchiol (7.437 %) was observed in SGF at pH 1.2. The Rf value of additional peak was found to be 0.57 [ Fig. 8].

### 3.8. Effect of pancreatin enzyme on marker components at pH 6.8

There was no additional peak observed for psoralen in SGF at pH 6.8. There was no effect of pancreatin on psoralen in SGF at pH 6.8 and thus it was found to be stable. Bakuchicin was degraded within 3 min whereas Bakuchiol was found to be stable up to 72 hrs.

### 3.9. Analysis of the commercial formulations and Crude extract

Analysis of the amount of Psoralen, Bakuchicin and Bakuchiol studied application of the developed method in crude extracts and commercial tablets, using the regression equation mentioned above. The content of Psoralen, Bakuchicin and Bakuchiol with % R.S.D were found to be 0.292 ± 0.0587 , 0.355 ± 0.0651 and 3.499 ± 0.9482 in crude extract, 0.124 ± 0.017, 0.255 ± 0.013, 4.330 ± 0.106, in monoherbal formulation and 0.2 91 ± 0.012 , 0.462 ± 0.0693 and 7.73 ± 1.6476 in polyherbal formulation respectively (**Table 5**). The densitograms of crude extract, mono herbal and poly herbal formulations were shown in Fig. 4, 5 and 6 respectively.

**Table 5: Estimation of drug content in samples**

| Samples | Drug content * (% w/w) | | |
|---|---|---|---|
| | PSORALEN | BAKUCHICIN | BAKUCHIOL |
| **Crude extract** | 0.292 ± 0.0587 | 0.355 ± 0.0651 | 3.499 ± 0.9482 |
| **MF1** | 0.2 91 ± 0.012 | 0.462 ± 0.0693 | 7.73 ± 1.6476 |
| **MF 2** | 0.124 ± 0.017 | 0.255 ± 0.013 | 4.330 ± 0.106 |

*Mean ± Standard deviation (n=3)

**Table 6: Summary of the validation parameters**

| Parameter | Psoralen | Bakuchicin | Bakuchiol |
|---|---|---|---|
| Precision (%RSD) | 1.32 | 0.53 | 0.80 |
| Repeatability of Application (n= 7) | 1.28 | 1.62 | 1.45 |
| Repeatability of Measurement (n= 7) | 1.21 | 1.67 | 1.75 |
| Limit of Detection (ng) | 10 | 20 | 80 |
| Limit of Quantification( ng) | 20 | 30 | 120 |
| Specificity | Specific | Specific | Specific |
| Linearity (Correlation coefficient) | 0.998 | 0.998 | 0.999 |
| Range ( ng/spot) | 20-120 | 30-130 | 120-320 |
| Robustness | Robust | Robust | Robust |

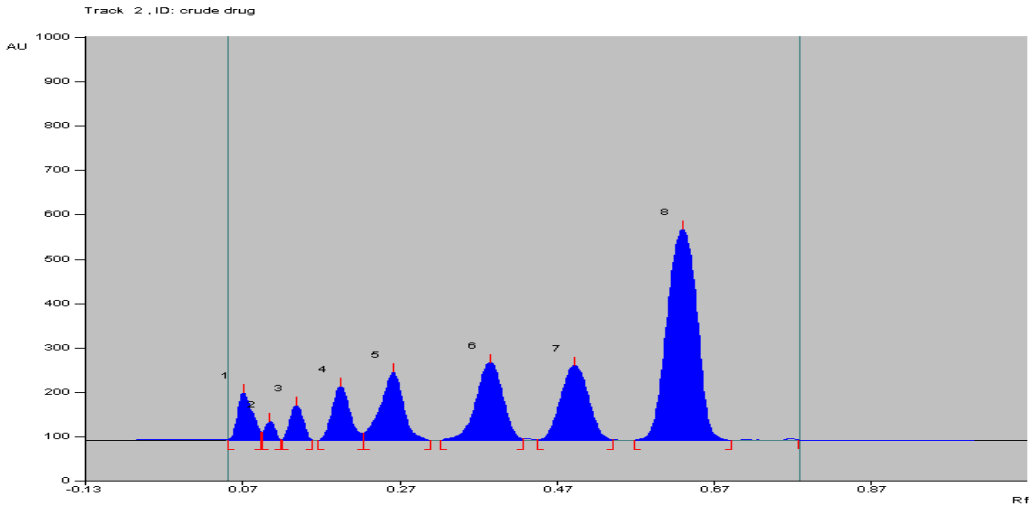


Fig. 4: Densitogram of crude drug

Peak 6: Psoralen Peak 7: Bakuchicin Peak 8: Bakuchiol

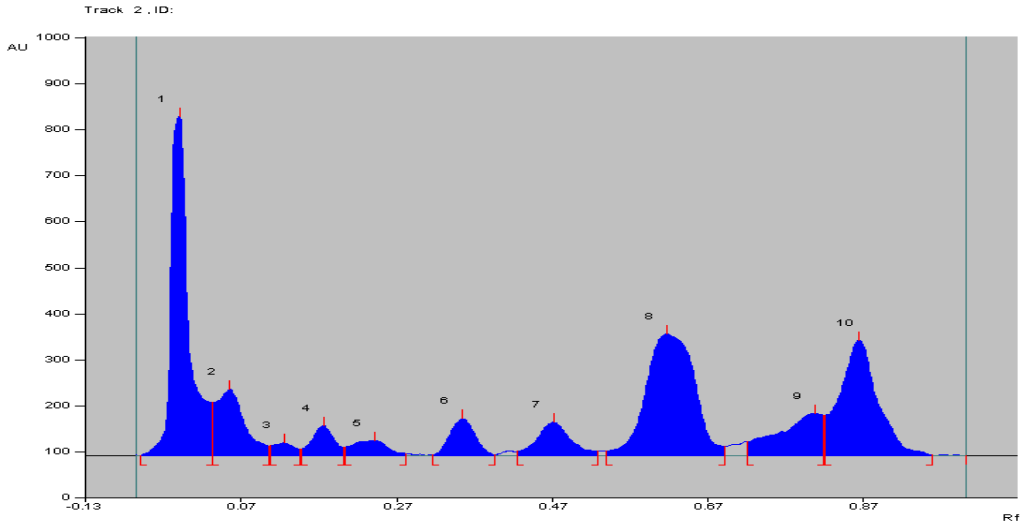


Fig. 5: Densitogram of polyherbal formulation

Peak 6: Psoralen Peak 7: Bakuchicin Peak 8: Bakuchiol

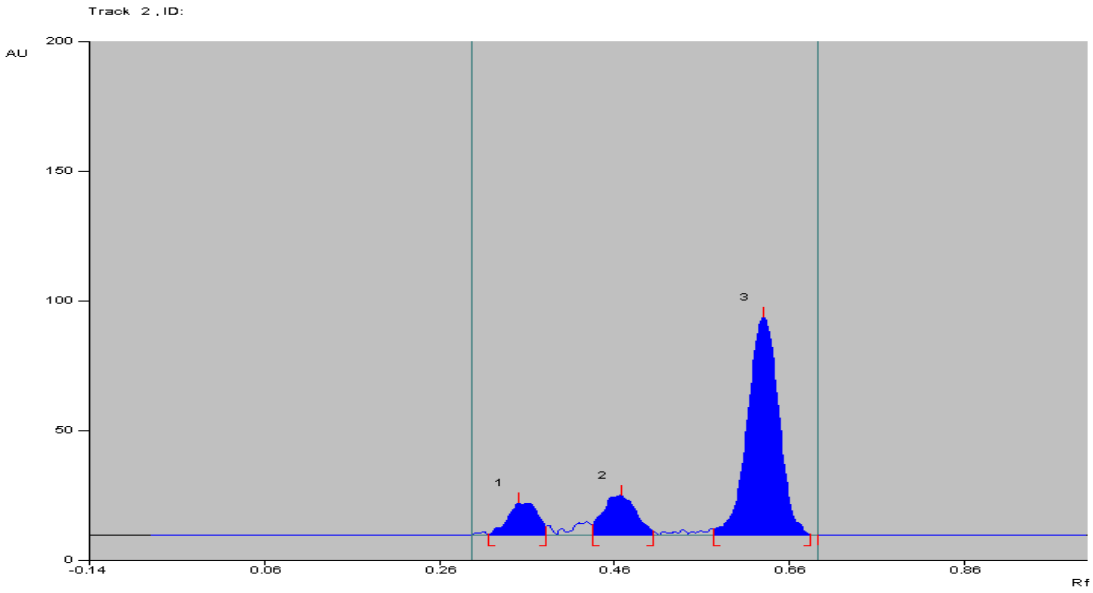


Fig 6: Densitogram of monoherbal formulation

Peak 1 : Psoralen Peak 2: bakuchicin Peak 3 : bakuchiol

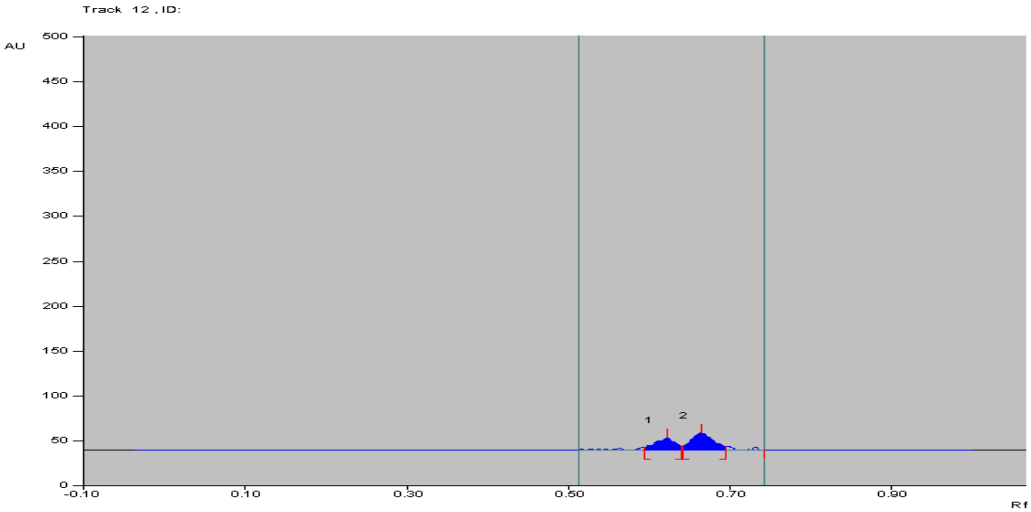


Fig: 7 Densitogram of stability of bakuchiol in simulated gastric fluid in absence of pepsin enzyme

Peak 1: degradation peak Peak 2: standard bakuchiol peak

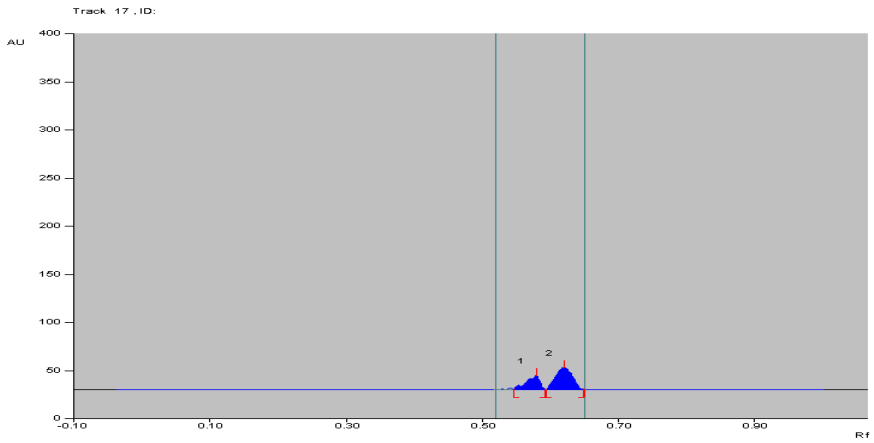


Fig: 8 Densitogram of stability of bakuchiol in simulated gastric fluid in presence of pepsin enzyme
Peak 1: degradation peak Peak 2: standard bakuchiol peak

## 4. CONCLUSIONS

We established a HPTLC method for the simultaneous estimation of the constituents Psoralen, Bakuchicin and Bakuchiol. The proposed method was found to be suitable for estimation of these markers in polyherbal formulations as it is proved to be precise, reproducible, reliable, accurate and robust. In addition the method was successfully applied for the *in vitro* stability studies of these compounds in physiological fluids. The study revealed that the DNA polymerase inhibitors bakuchicin and bakuchiol are not stable in the acidic pH which may lead to poor bioavailability.

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