Application of HPTLC for GI stability evaluation of Psoralen, Bakuchiol and Bakuchicin in simulated physiological fluids

DR. SATHIYANARAYANAN L.

ASSOCIATE PROFESSOR

DEPARTMENT OF PHARMACEUTICAL CHEMISTRY

POONA COLLEGE OF PHARMACY,
BHARATI VIDYAPEETH DEEMED UNIVERSITY, PUNE,
MAHARASHTRA, INDIA

Overview

- Introduction
- Scope of the work
- Objectives
- Experimental
- Results and discussion
- Summary and Conclusion
- References

NATURAL PRODUCTS AS MEDICINES

- Attracts researchers worldwide
- Medicinal Plants – Rich source of natural products
- Phytoconstituents – Play vital role in the treatment of various ailments.
- Poly herbal formulations

POLYHERBAL FORMULATIONS

- **Advantages:**
  - Enjoying renaissance among the customers throughout the world.
  - Herbal drug preparation itself as a whole is regarded as the active substance.
  - The profile of the constituents in the final product has implication in efficacy and safety.
  - Synergistic and additive effects and at the same time having less side effects
- **Disadvantages:**
  - Lack of standard quality control profiles.
  - complex nature
  - inherent variability of the chemical constituents
  - difficult to establish quality control parameters.
  - the reproducibility of the total configuration of constituents is difficult
- Hence herbal formulations are needed to be standardized and their safety and efficacy should be ensured.

POLYHERBAL STANDARDIZATION

- Standardization is an important aspect for establishing the quality and efficacy of Polyherbal formulations.
- It ensures reproducible pharmaceutical quality of herbal products.
- General approaches used for standardization:
  - Thin-layer chromatography (TLC)/gas-liquid chromatography (GLC)/high performance liquid chromatography (HPLC) fingerprint profiles.
  - Fingerprint profiles with marker compounds.
  - Quantification of active principles.

STABILITY IN GASTROINTESTINAL FLUIDS

- The major problem with any herbal medicines is their oral bioavailability and stability.
- In the gastrointestinal tract, drug molecules are exposed to
  - Acidic and alkaline pH
  - Digestive juice containing enzymes such as pepsin and pancreatin.
- Exposure leads to degradation of herbal compounds.
- Leads to interfere with the absorption thereby reduction in bioavailability.

PSORALEA CORYLIFOLIA **Linn.**

Herb :	Babchi
Biological name :	*Psoralea corylifolia* Linn.
Family:	*Fabaceae*
Description :	Erect , Herbaceous plant possess broad elliptic leaves. Flowers are yellow, purple or bluish in colour. Bears pods containing single seed.
Active ingredients :	Seeds yield essential oil, **psoralen,** resin, isopsoralen, psoralidin and furanocoumarin **Bakuchicin , Bakuchiol** .
Main uses :	Anthelmentic, antibacterial, antifungal, diuretic, laxative, Antitumor, Antidiabetic & to treat skin problems .

EXISTING ANALYTICAL METHODS

- **HPLC Method For Simulteneous Estimation Of Psoralen , Bakuchicin & Bakuchiol in Psoralea Corylifolia.**

  A reverse phase UV (295 nm). Mobile phase :potassium dihydrogen orthophosphate and ortho-phosphoric acid in water and acetonitrile flow-rate of 1.5 mL/min. The linearity range of 0-500 ng/mL

- **Analysis of Bakuchiol, Psoralen, Angelicin in crude drugs & commercial concentrated products of Fructus Psoraleae by HPLC.**

  Gradient mobile phase : 20 % aqueous acetonitrile to 100% acetonitrile. The lower limit of detection for bakuchiol, psoralen and angelicin was 175, 37.5 and 43.7 ng/mL respectively, with a relative standard deviation of less than 2%.

- **Determination of Psoralen and Plumbagin from its polyherbal oil Formulations by an HPTLC densitometric method .**

  Mobile phase:Toluene : ethyl acetate (7.5: 2.5 v/v)

  Psoralen (R_f: 0.37) and plumbagin (R_f : 0.77)

- **Thin Layer Chromatographic Analysis Of Psoralen in Babchi (Psoralea corylilifolia) Oil .**

  Aluminium TLC plates precoated with silica gel 60F254

  Mobile Phase: *n* hexane–acetone–formic acid (2:1:0.025 *v*/*v*) -mobile phase.

  (*R*F value 0.32 ± 0.02) at 250 nm. range 20–200 ng per spot.

HPLC, GC, GC-MS for Bakuchiol, and HPLC, LC-MS for Bakuchicin also were reported.

No HPTLC method has been reported for simultaneous determination of the three markers

SCOPE OF THE WORK

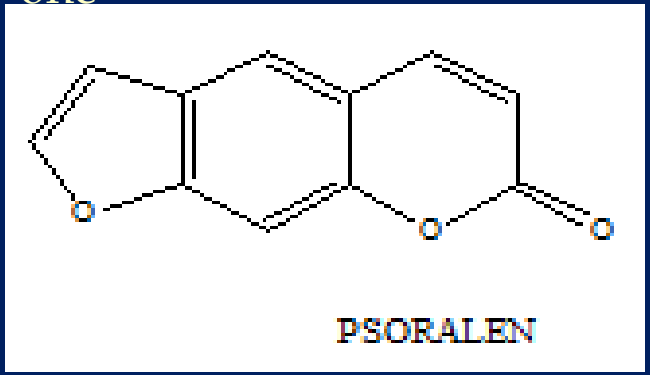
- Need for standardization of herbal formulations containing Babchi to ensure safety and efficacy.
- Need to establish the stability of selected phytoconstituents in the GI environment
- Need to develop simple, accurate robust analytical method for the simultaneous estimation of the said markers

OBJECTIVES

- To develop validated HPTLC method for simultaneous determination of Psoralen, Bakuchicin and Bakuchiol.
- To validate the developed HPTLC method as per ICH guidelines Q2 (R1).
- To apply developed method for standardization of polyherbal formulations.
- To carry out stability studies of Psoralen, Bakuchicin and Bakuchiol in simulated gastro-intestinal fluids.

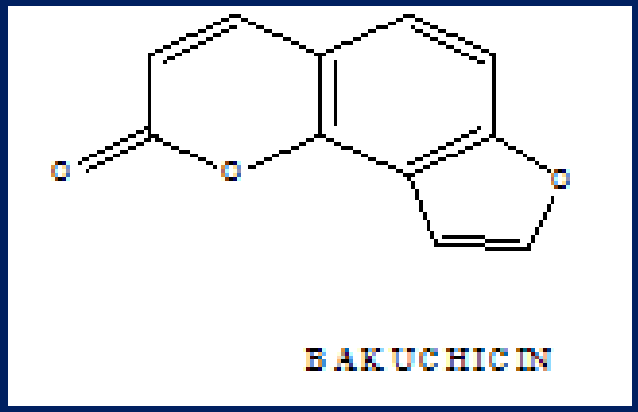
PSORALEN

- Chemical name : 7H-furo[3,2-g]chromen-7-one
- Molecular formula : $C_{11}H_6O_3$
- CAS Number : 66- 97- 7
- Molecular weight :186.16 g/mol
- Appearance : colourless, odourless, tasteless
- Solubility : Very soluble in chloroform, soluble in alcohol;
- Melting point : 162- 165 °C
- Category : Topical Pigmentation agent – Psoriasis
- Storage : Sample should be kept at $2^o \pm 8^o$ C



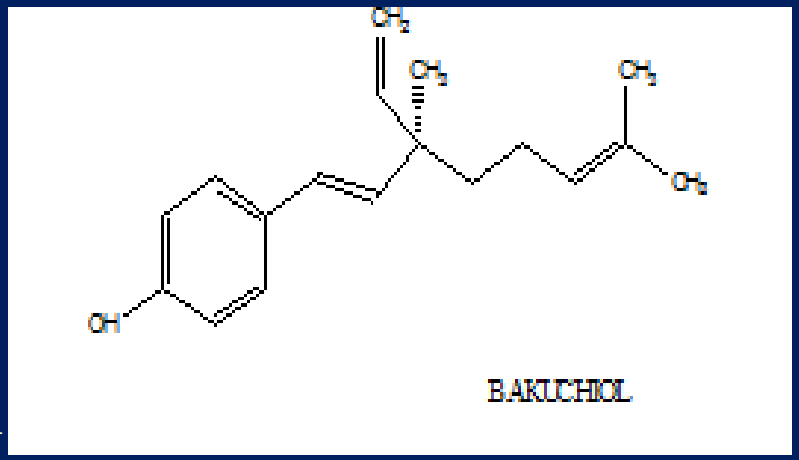
BAKUCHICIN

- Chemical name : 8- Oxo-8H-furo [2, 3- f] [1] Benzopyran
- CAS Number :523-50-2
- Molecular formula :$C_{11}H_6O_3$
- Molecular weight :186
- Appearance : Off white powder
- Solubility : Soluble in methanol and dimethylsulfoxide
- Melting point : 138- 140°C
- Category : Topoisomerase II inhibitor, Antibacterial, Antioxidant
- Storage : Sample should be kept at 2^o -8^o **C.**



BAKUCHIOL

- Chemical name : 4-(3,7-Dimethyl-3-vinyl-octa-1,6-dienyl)phenol
- CAS Number : [10309-37-2]
- Molecular formula :$C_{18}H_{24}O$
- Molecular weight :256.39
- Appearance : pale yellow liquid



- Solubility : Soluble in dimethylsulfoxide and 100 % ethanol
- Category : Antitumor, Antibacterial, DNA Polymerase inhibitor, Antioxidative
- Storage : Sample should be kept at -20° C and protected from light

EXPERIMENTAL

Instrumentation:

Spotting device	:	Linomat V Automatic sample spotter; CAMAG
Syringe	:	CAMAG microlitre syringe;
HPTLC Chamber	:	Glass twin trough chamber (20×10×4cm3);
Densitometer	:	HPTLC Scanner 3 is controlled by WinCats
HPTLC plates	:	20×10 cm2, 0.2mm thickness precoated with silica gel 60 F254; (E. Merck, Darmstadt, Germany; #1.05548);

Method development

Validation of method

Analysis of pharmaceutical formulations

Stability study in simulated gastro-intestinal fluid

RESULT AND DISCUSSION

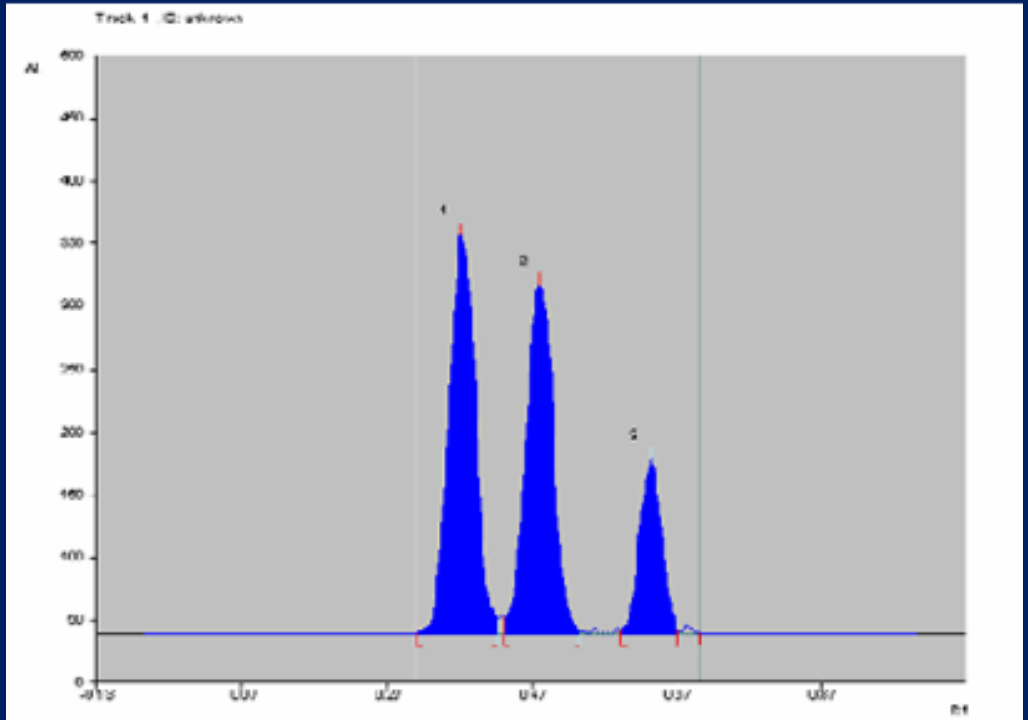
CHROMATOGRAPHIC CONDITIONS

- Mobile phase:

  n-Hexane: Ethyl acetate

  (7.5:2.5 v/v)

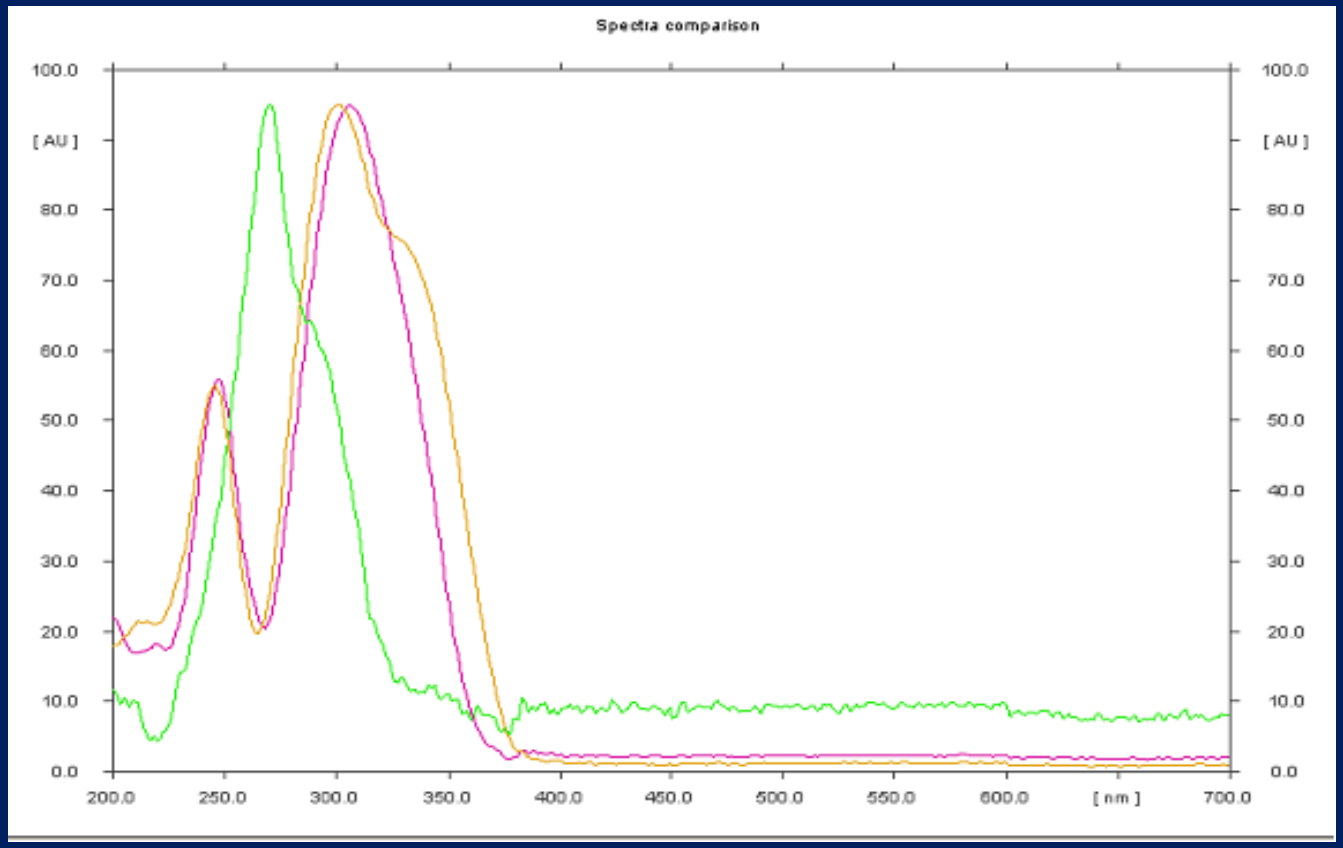
- R_f :
  - Bakuchicin: 0.48,
  - Bakuchiol: 0.63
  - Psoralen: 0.37
- Run distance: 8 cm
- Band width: 6 mm
- Scanning wavelength: 252 nm
- Saturation time: 20 min



Densitogram of standard Psoralen, Bakuchicin and Bakuchiol

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

SPECTRAL OVERLAY



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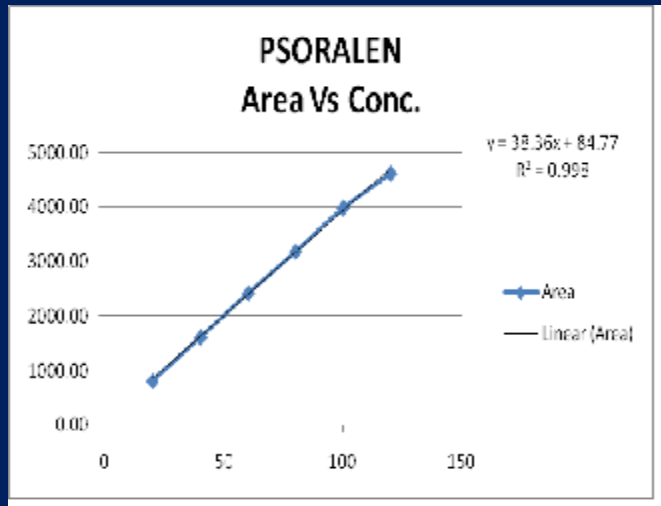
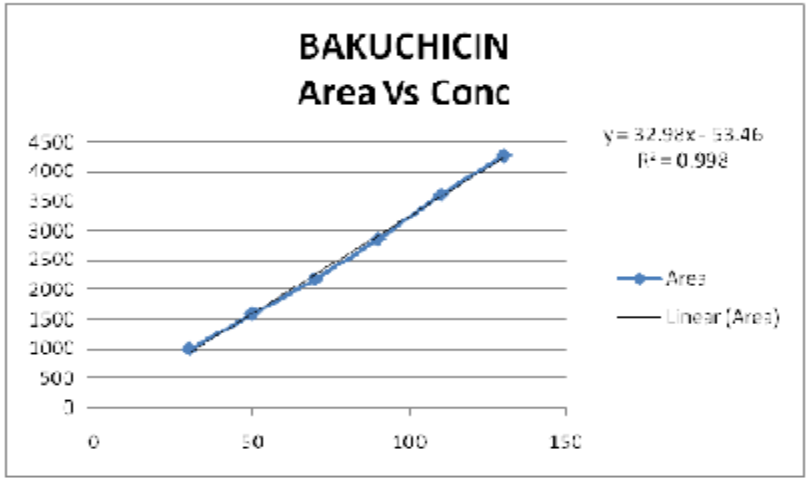
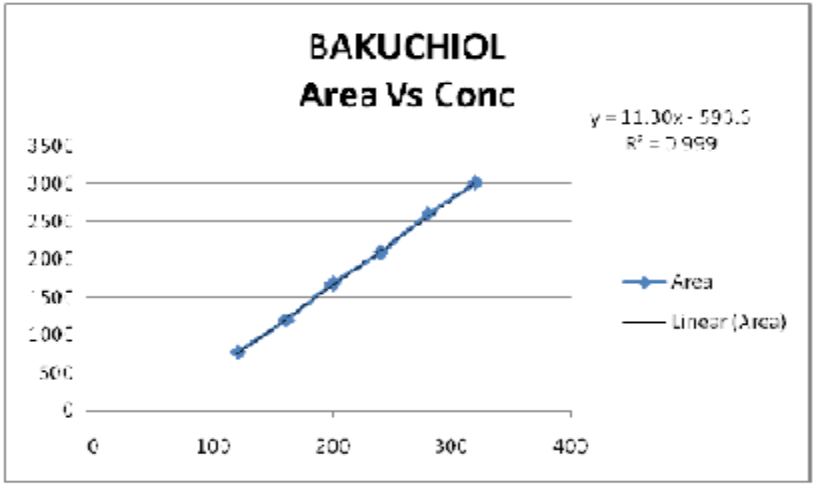
VALIDATION OF METHOD







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 1: Linear regression data from calibration curves

PRECISION

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

LOD AND LOQ

- The signal to noise ratios of 3 : 1 and 10 : 1 were considered as LOD and LOQ respectively
- The LOD and LOQ were found to be

	LOD	LOQ
• Psoralen :	10 ng/spot	20 ng/ spot
• Bakuchicn	20 ng/ spot	30 ng/ spot
• Bakuchiol :	80 ng/spot	120 ng /spot

SPECIFICITY

The peak purity of Psoralen, bakuchicin and bakuchiol was assessed by comparing their respective spectra at peak start, apex and peak end positions of the spot i.e., r (S, M) = 0.9986 and r (M, E) =0.9983. Good correlation (r=0.9989) was also obtained between standard and sample spectra of psoralen, bakuchicin and bakuchiol.

ROBUSTNESS

PARAMETER	SD of peak area			% RSD [a]		
	Psoralen	Bakuchicin	Bakuchiol	Psoralen	Bakuchicin	Bakuchiol
Mobile phase composition (± 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

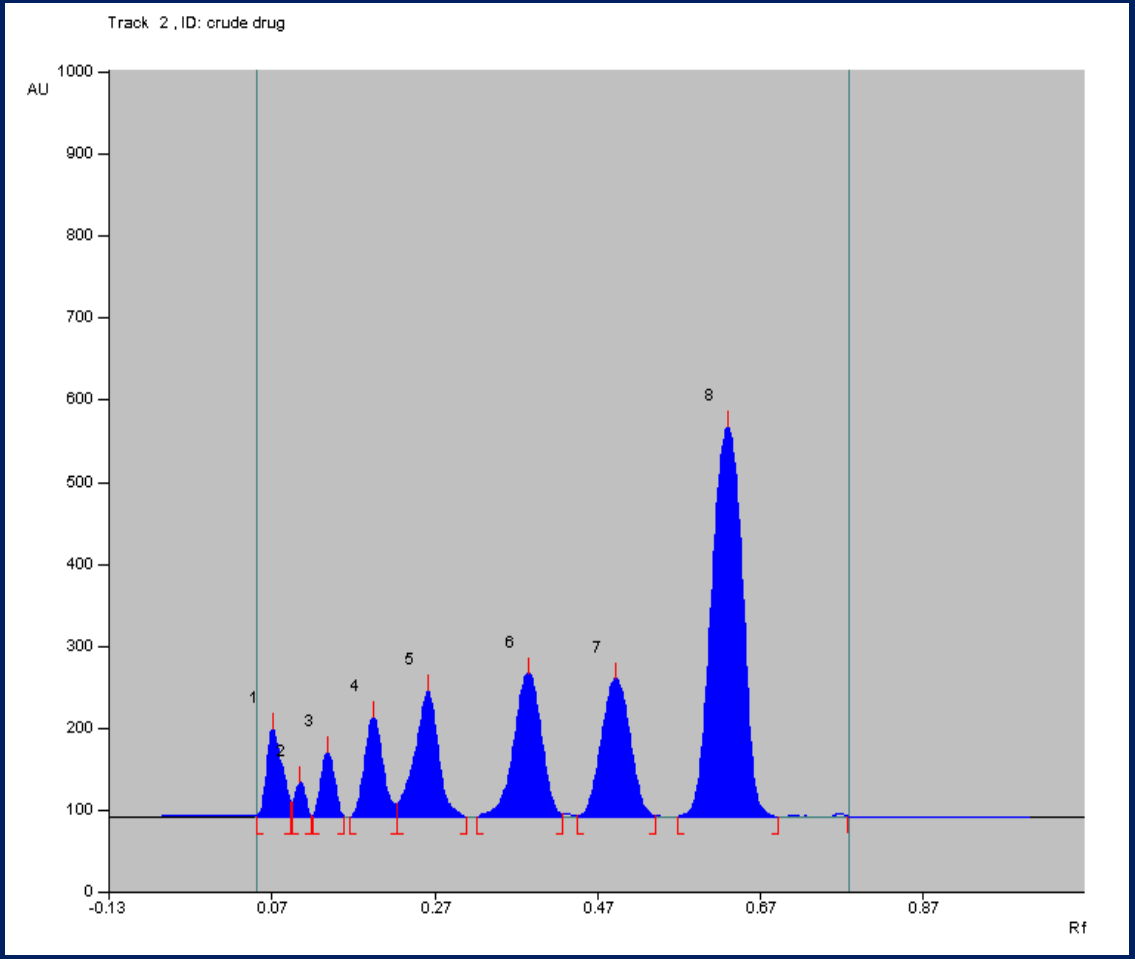
[a] n = 6, Average of three concentrations 40, 80, 120 ng spot^{-1} for Psoralen and 50, 90, 130 ng spot^{-1} for Bakuchicin and 160, 220, 340 ng spot^{-1} for Bakuchiol

RECOVERY STUDIES

Compound	Amount in sample (µg)	Amount added (µg)	Amount found* (µg)	Recovery (%)	Average (%)
PSORALEN	74.7	59.76	124.5 ± 4.50	99.29 ± 0.05	99.29
	74.7	74.7	138.4 ± 9.01	99.35 ± 0.10	
	74.7	89.64	153.84 ± 7.21	99.38 ± 0.28	
BAKUCHICIN	128	102.4	229.5 ± 5.50	99.34 ± 0.03	99.45
	128	128	243.66 ± 3.05	99.55 ± 0.04	
	128	153.6	270.6± 4.56	99.43± 0.08	
BAKUCHIOL	2225	1780	3995 ±6.89	99.68 ± 0.12	99.61
	2225	2225	4435± 4.08	99.65 ± 0.04	
	2225	2670	2486.66±6.11	99.48 ± 0.06	

ANALYSIS OF POLYHERBAL FORMULATION

Samples	Drug content * (% w/w)		
	PSORALEN	BAKUCHICIN	BAKUCHIOL
Crude extract	0.292 ± 0.0587	0.355 ± 0.0651	3.499 ± 0.9482
Marketed formulation 1	0.2 91 ± 0.012	0.462 ± 0.0693	7.73 ± 1.6476
Marketed formulation 2	0.124 ± 0.017	0.255 ± 0.013	4.330 ± 0.106



Densitogram of crude drug

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

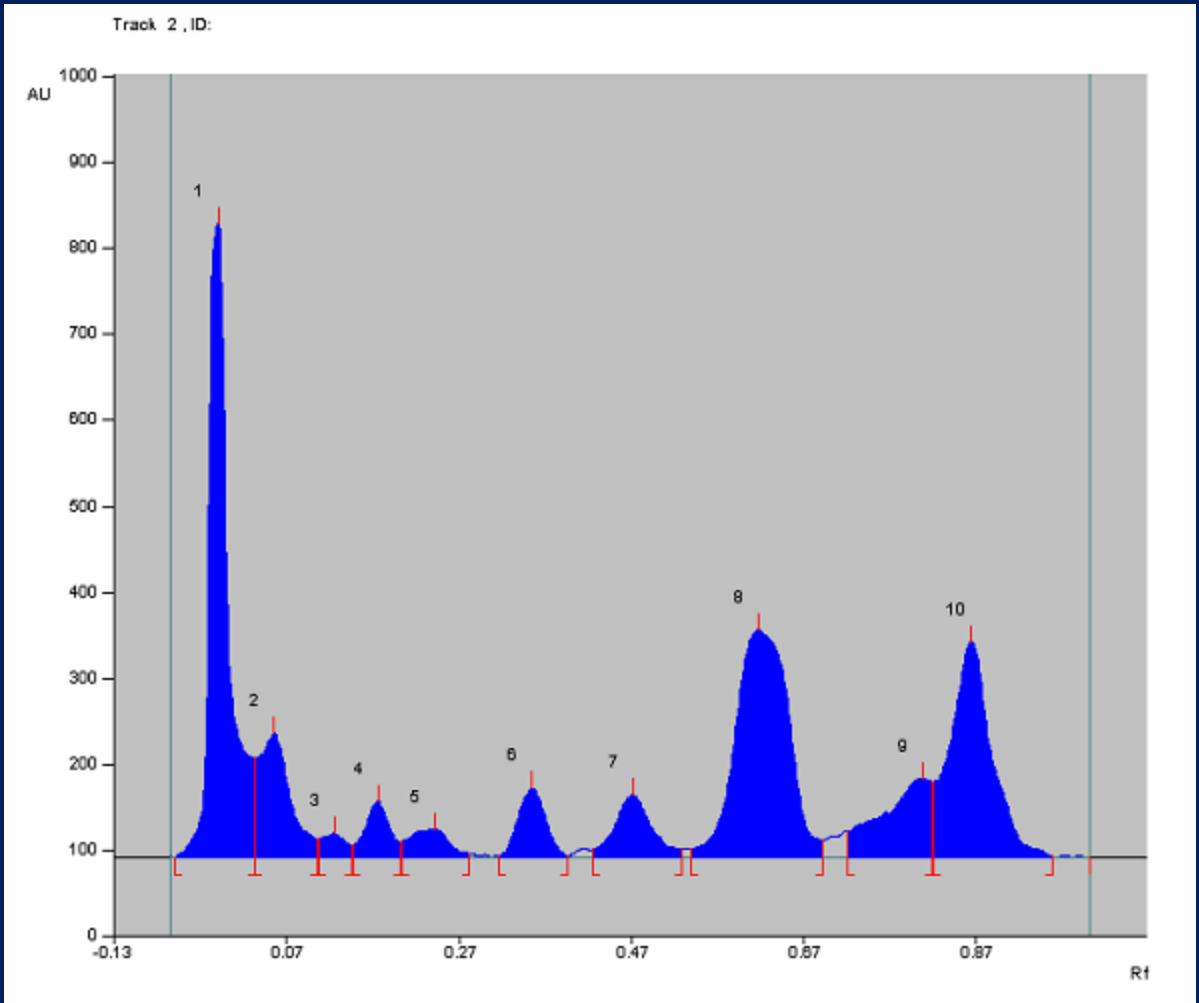


Fig.4: Densitogram of polyherbal formulation

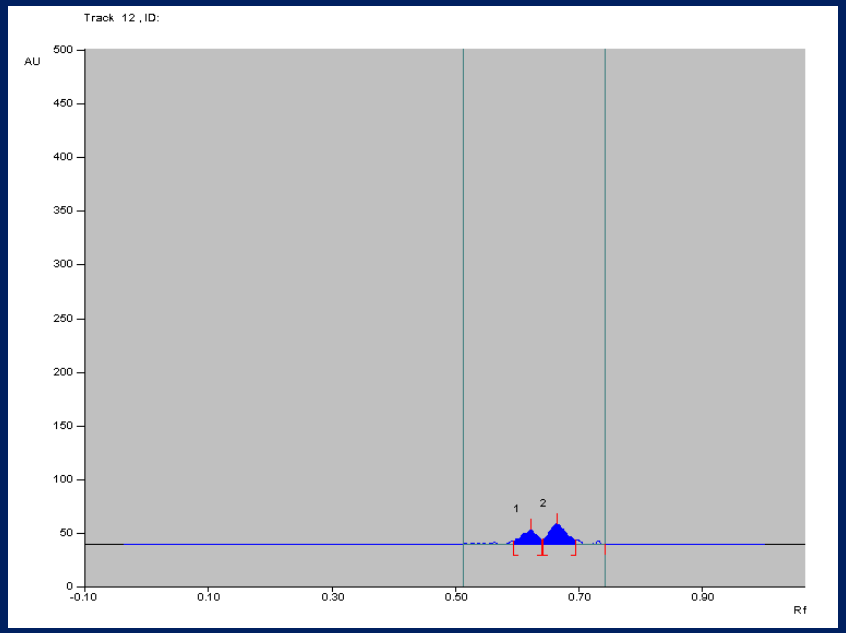
Peak 6: Psoralen

Peak 7: Bakuchicin

Peak 8: Bakuchiol

EFFECT OF PH AND GI ENZYMES ON THE MARKERS

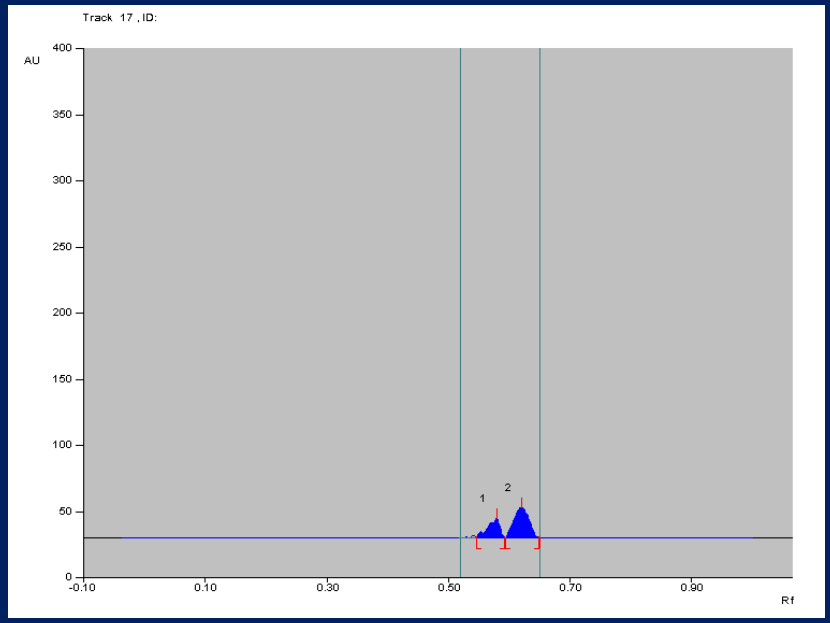
Name of the Marker	SGF		SIF	
	In presence of Enzyme	In absence of Enzyme	In presence of Enzyme	In absence of Enzyme
Psoralen	Stable	Stable	Stable	Stable
Bakuchicin	Completely degraded (3 min)	Completely degraded (3 min)	Completely degraded (3 min)	Completely degraded (3 min)
Bakuchiol	4.18 % after 40 min	7.437 % after 40 min	Stable (72 hrs)	Stable (72 hrs)



Densitogram of bakuchiol in simulated gastric fluid in absence of pepsin enzyme

Peak 1: degradation peak (**4.18 % after 40 min, Rf 0.61**)

Peak 2: standard bakuchiol



Densitogram of bakuchiol in simulated gastric fluid in presence of pepsin enzyme

Peak 1: degradation peak (**7.43 % after 40 min, Rf 0.58**)

Peak 2: standard bakuchiol

SUMMARY AND CONCLUSION

- 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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Sr. No.	Particulars	Numbers
1.	Patents Registered	41
2.	Research Publications	
	National Journals	315
	International Journals	429
3.	Papers Presented in National and International Conferences and Seminars	452
4.	Books Authored	44
5.	Invited Guest Lectures	250
6.	Workshops and Seminars conducted	34
7.	Collaborations with Industries	44
8.	Honors and Awards received	37

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