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RESEARCH ARTICLE

Quantitative Estimation of Tecomin in Tecomella Undulata Bark Using HPTLC Method

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ABSTRACT

A new, simple, sensitive, selective, precise and robust high-performance thin-layer chromatographic (HPTLC) method for analysis of tecomin has been developed and validated for the determination of tecomin in aqueous extract of tecomella undulata bark. The analyte was extracted with methanol and applied on TLC aluminium plates along with standard using Linomat IV spray on sample applicator (CAMAG). Analysis of tecomin was performed on pre-coated TLC aluminium plates with silica gel as the stationary phase and prewashed with methanol. Linear ascending development was carried out in twin trough glass chamber saturated with mobile phase consisting of toluene: acetone: formic acid (2.5: 0.5: 0.2 v/v/v). Spectrodensitometric scanning was performed by TLC scanner III (CAMAG) in absorbance mode at the wavelength of 221 nm. The system was found to give compact spots for tecomin (Rf value of 0.65). The method was validated for precision, specificity and recovery. Statistical analysis of the data showed that the method is reproducible and selective for the estimation of tecomin.

KEYWORDS: tecomella undulata, tecomin, HPTLC

1. INTRODUCTION

The drug consists of heartwood, stem bark, leaves and seeds of *Tecomella undulata* (Sm.) Seem syn. *Tecomella undulata* (Roxb.) of faimly bignoniaceae, commonly known as Rohida, is a well known plant in the ayurvedic system of medicine.[5-7] It is usually a shurb, found in small patches, but when cultivated it may grow as high as 12 meters with a girth up to 2.4 meters. The species has been identified as an important for environmental conservation in arid zones as a stabilizer of shifting sand dunes, providing shelter for wild life. It is also a very useful species for afforestation of the drier tracts due to its drought and fire resistant properties.[8-9]

The bark of *Tecomella undulata* is strongly astringent and specified for diseases of liver and spleen, internal tumors and diseases of abdomen incl. ascitis. Charka prescribed powdered bark, its decoction and extract in clarified butter in jaundice, enlarged spleen, anemia, intestinal warms and urinary disorders. The paste of root was given in leucorrhoea.[10-11]

2. MATERIAL AND METHOD:

MATERIAL:

The bark of *Tecomella undulata* was collected from the fields of Nohar, Hanumangarh (Rajasthan), in the month of November 2009 at morning time. The bark was identified by Dr. HB Singh (Scientist Incharge), NISCAIR (National Institute of Science Communication and Information Resources), New Delhi. (Ref. No. NISCAIR/RHMD/Consult/2009-10/1326/128) CAMAG HPTLC system (Switzerland) comprising of Hamilton 100 ml HPTLC syringe, Camag Linomat IV semiautomatic sample applicator, CAMAG twin trough chamber (20x20 cm), CAMAG TLC scanner III, Camag CATS IV integration software. Silica gel- G60F254, 20 X 20cm TLC plate was used as stationary phase. Tecomin reference standard was purchased from Clearsynth Labs (P) Ltd. Mumbai. Methanol, ethyl acetate, toluene, acetone, formic acid, chloroform and n-butanol used were of analytical grade. The solvent was run for 80 mm, band length 6 mm, slit dimension 6.00 x 0.30 mm and detection wavelength 221 nm were configured as standard parameters for the present study.

Method:

A) PREPARATION OF SAMPLE SOLUTION:

Accurately weighted 10 mg of aqueous extract of *Tecomella undulata* was dissolved in 10 ml distilled water (1000 µg/ml) and passed through 0.45 Millipore filters.

B) PREPARATION OF TECOMIN SOLUTION:

The standard solution was prepared by dissolving 10 mg tecomin in 10 ml purified water (1000µg/ml). The working standard of 200 µg/ml was prepared from standard solution by diluting with purified water. Different concentrations of 10, 20, 30, 40, 50 µg/ml were prepared from standard solutions.

C) CHROMATOGRAPHIC CONDITIONS:

Analysis was performed on 20 cm × 20 cm HPTLC silica gel-G60F254 plates. The plate cleaned by

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predevelopment to the top with methanol, and dried in an oven 105^{0}C for 5 min. Sample and standard zones were applied to the layer as bands by means of a CAMAG. Linomat-4 semiautomatic sample applicator equipped with a 100 µl syringe and operated with the settings band length 6 mm, application rate 150 µl/sec, distance between bands 8 mm, distance from the plate side edge 6.5 mm, and distance from the bottom of the plate 2 cm.

D) CALIBRATION CURVE OF TECOMIN:

Series of standard solution (10, 20, 30, 40 and 50 µl) of tecomin applied triplicate onto TLC plate to generate Calibration curve. The plate was developed in the mobile phase toluene: acetone: formic acid (2.5: 0.5:0.2 v/v/v) and dried in an oven 105^{0}C for 5 min. The standard zones were quantified by linear scanning at 251 nm by use of a TLC Scanner III CAMAG. Data of peak height and peak area of each spot was recorded. The calibration curve was prepared by plotting concentration (mg/spot) versus peak area (Figure 3).

3. METHOD VALIDATION

PRECISION:

Precision was carried out at three different concentration levels 10, 30, 50 µg/spot.

SPECIFICITY:

The specificity of method was ascertained by standard tecomin and samples (extracted from aq. extract of tecomella undulata bark). Spots of the diluent methanol, standard tecomin, extracted samples were spotted on TLC plate in duplicate and run. The spots for tecomin that eluted were confirmed with R_f value of standard tecomin.

RECOVERY STUDIES:

Recovery Study was performed by spiking 10, 20, 30 and 40 µg/spot of standard drug externally to the pre-spotted (10 µg/spot) samples. The experiment was conducted in triplicate and applied onto the plate in duplicate.

4. RESULTS:

Figure 1 showed the HPTLC chromatogram of aqueous extract of tecomella undulata bark extract and figure 2 showed the HPTLC chromatogram of tecomin. Tables 1-4 showed precision, specificity, recovery studies and concentration of tecomin in aqueous extract of tecomella undulata bark.

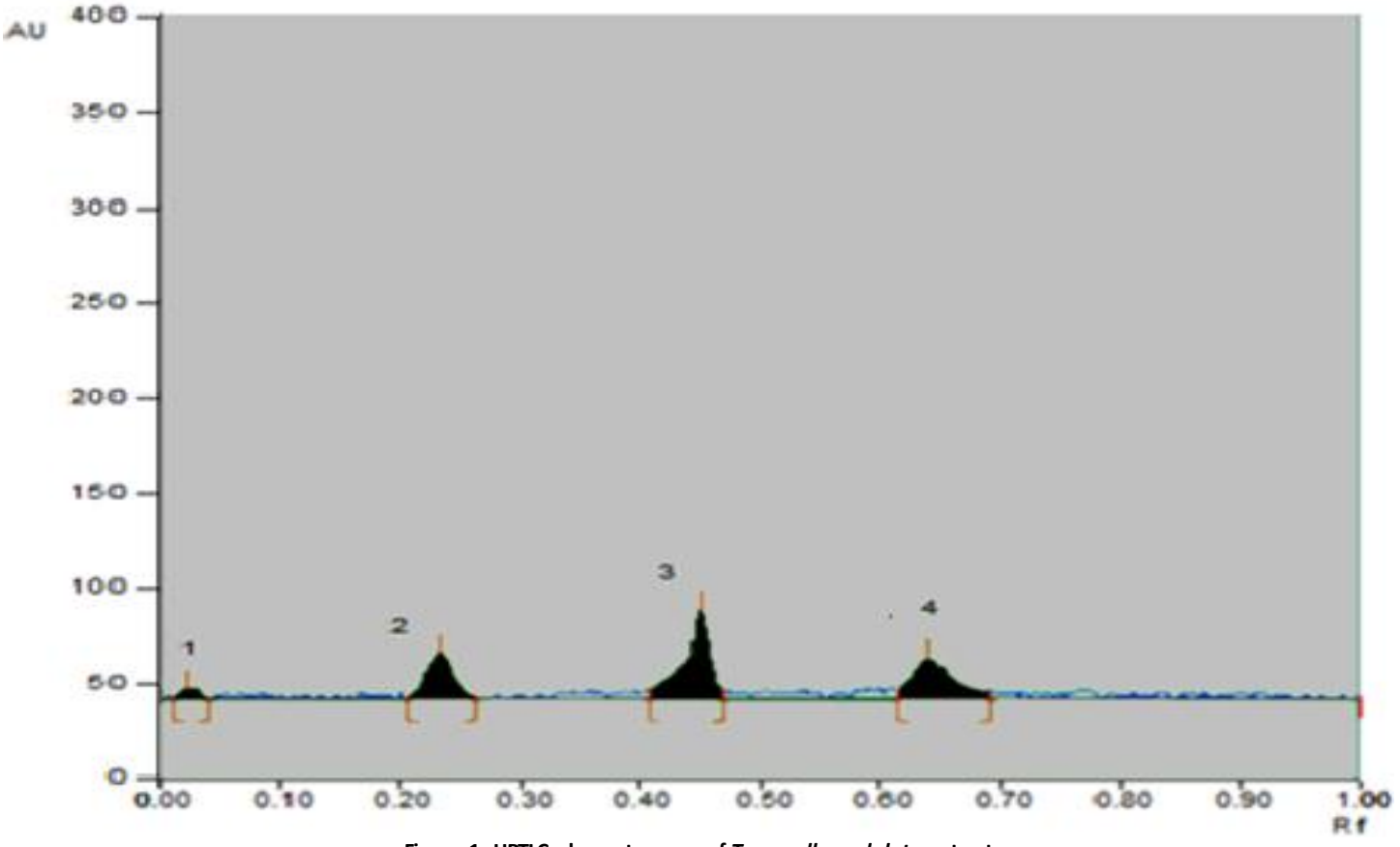

Figure 1: HPTLC chromatogram of *Tecomella undulata* extract

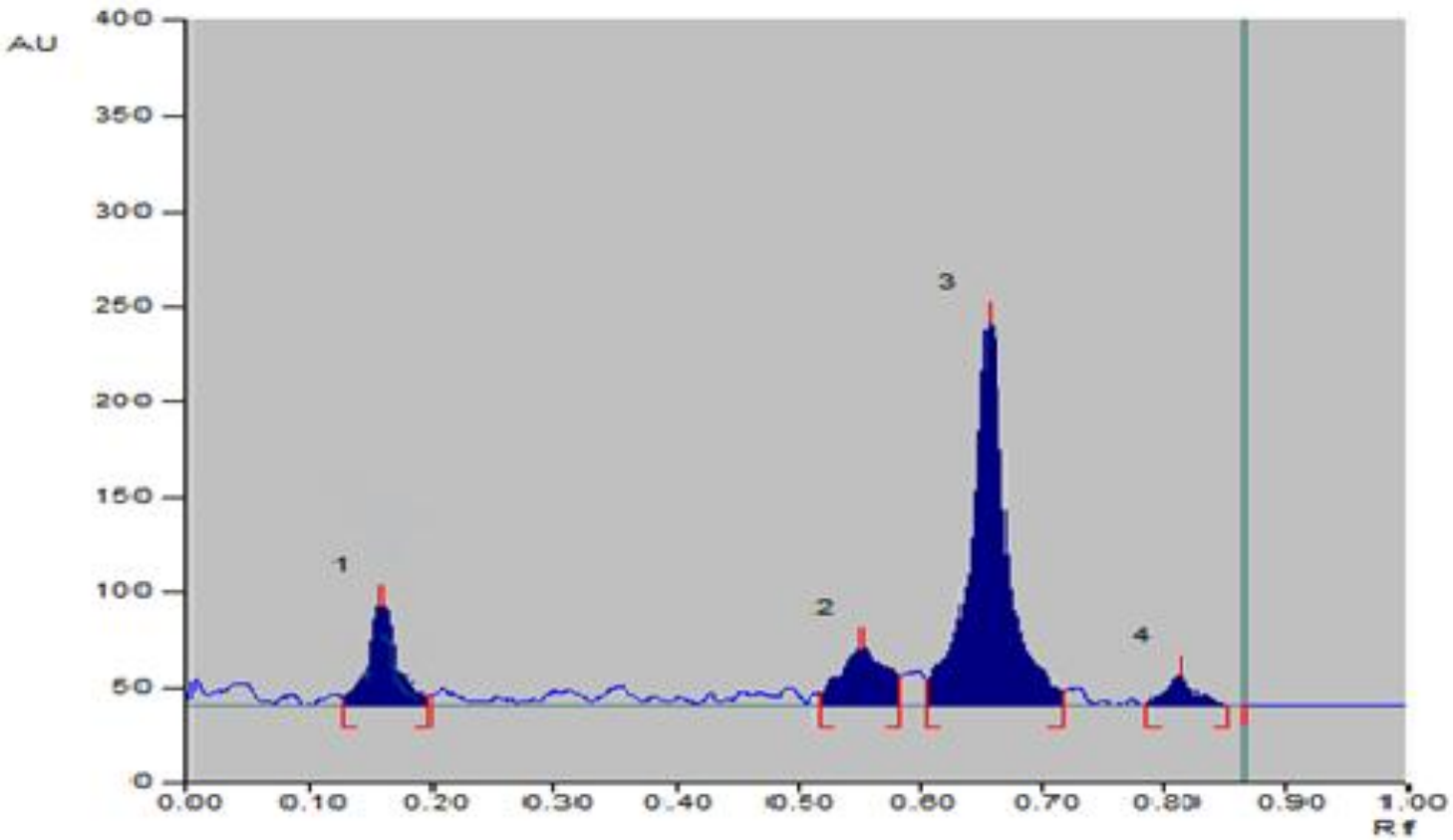

Figure 2: HPTLC chromatogram of Tecomin

Table 1: Comparative data of Standard and T. undulata bark extract

Track	Conc. of Tecomin (µg/spot)	Rf	Mean Peak
1	10	0.65	4337.4±70.84
2	20	0.65	5288.3±42.24
3	30	0.65	6111.0±110.76
4	40	0.65	6904.6±124.4
5	50	0.65	7677.1±145.44
6	Extract of *T. undulata*	0.65	4428.1±22.68

Table 2: Method validation parameters for calibration curve

S.N.	Parameters	Tecomin
1	Correlation-coefficient (r^2)	0.9983
2	Repeatability (% CV)	0.6352
4	Slope	829.57

Table 3 : Recovery study of marker compound by proposed HPTLC method

Marker	Conc. Taken (µg/spot)	Conc. Added (µg/spot)	Amount found Mean±SD (n=3)	Recovery	Avg. Recovery
Tecomin	10	0	9.99±0.43	99.90	99.10
	10	10	19.79±0.36	98.95	
	10	20	29.52±0.49	98.40	
	10	30	39.66±1.10	99.15	
	10	40	49.57±1.15	99.14	

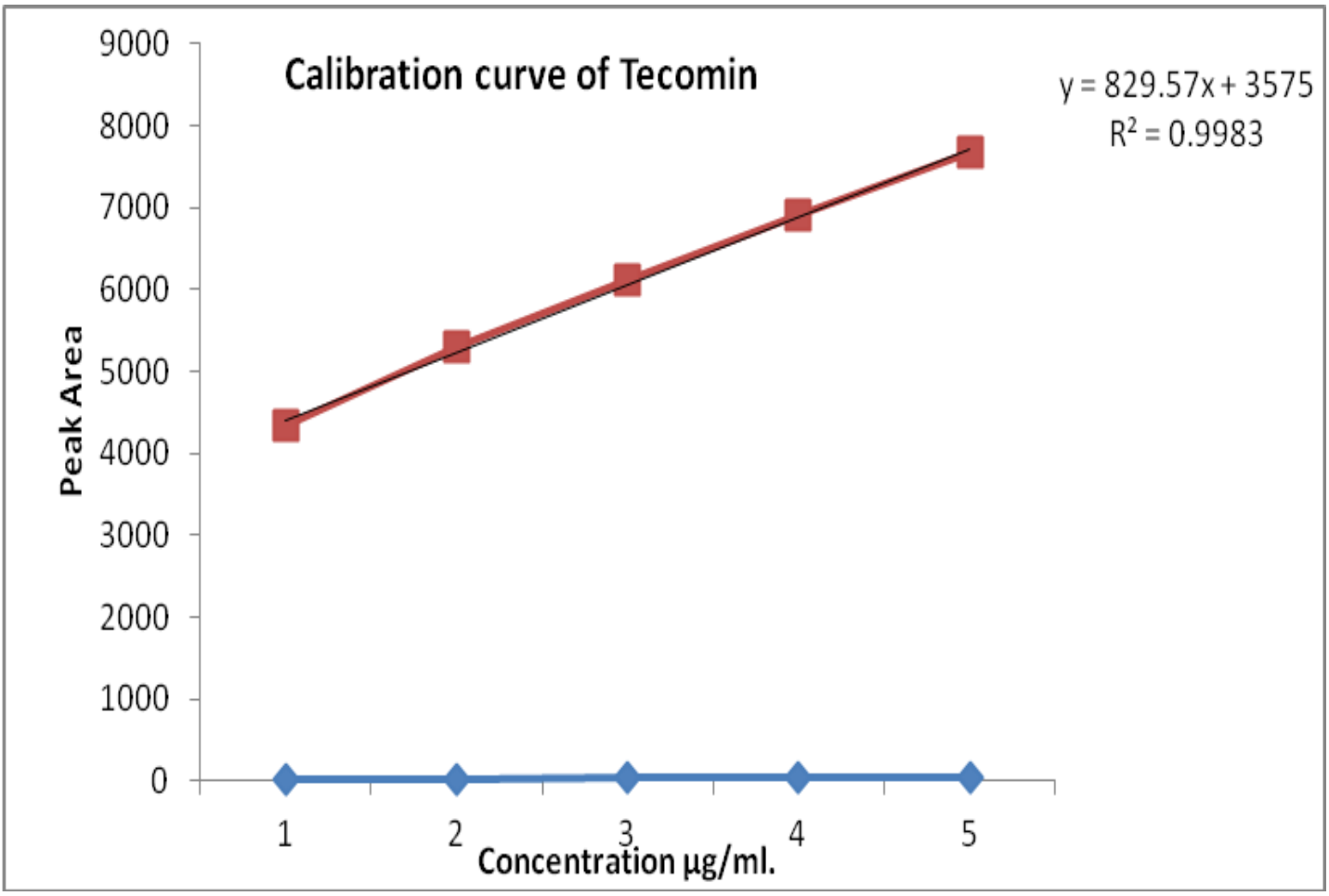

Figure 3: Calibration curve of Tecomin

Table 4: Content of Tecomin present in Extract

S.N.	Plant extract	Tecomin content (µg/ml)
1	Aqueous extract *Tecomella undulata*	1.10

5. CONCLUSION:

The developed HPTLC method is fast, simple, precise, specific and accurate. Statistical analysis proved that method is repeatable and selective for determination of tecomin.

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