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HPTLC Estimation of Harmine from the Stem Bark of *Symplocos racemosa* Roxb.

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An attempt was made to quantify harmine in different samples of *Symplocos reacemosa.* Precoated silica gel G (aluminium backed) plates were used as stationary phase and toluene : ethyl acetate : methanol (6 : 2 : 2) was used as mobile phase. Detection and quantification were performed by densitometry at λ 324 nm. The linear range was 100 to 500 ng. The proposed HPTLC method was found to be reproducible, accurate and precise.

Key Words: HPTLC, Harmine, *Symplocos racemosa.*

INTRODUCTION

Symplocos reacemosa Roxb. (Symplocaceae), known as *Lodhra* in Ayurveda, is an ingredient of many *rasayanas.* The bark is astringent and therefore used to treat diarrhoea and dysentery. It is used as a tonic, antioxytocic, amoebicide and in conjunctivitis and ophthalmia[1, 2]. A decoction of bark is used to treat bleeding gums, mennorrhagia and other uterine disorders[3]. Symposide, a glycoside, shows antifibrinolytic activity, alcoholic extract reduces the frequency and intensity of contractions *in-vitro* in both pregnant and non-pregnant uteri. Antagonization of acetylcholine-induced contractions and prolongation of the quiescent period are also observed[4]. The therapeutic efficiency of *S. racemosa* is due to presence of alkaloids, of which harmine is the major one[5]. HPTLC techniques are more practical for the quantitative estimation of active principles as they are simple, reliable and cost-effective. In the present study, an accurate and rapid HPTLC method has been developed for the first time for quality control determination of harmine from *S. racemosa* stem bark.

EXPERIMENTAL

All the chemicals used experiments were of analytical grade. Harmine (98%) as chemical maker was purchased from Himedia, Mumbai, India. Two different samples of stem bark of *Symplocos racemosa* Roxb. (Symplocaceae) from different geographical sources were collected as follows:

Sample 1 (S1): Stem bark sample from Bangalore (Karnataka).
Sample 2 (S2): Stem bark sample from Baroda (Gujarat).
Sample 3 (S3): Stem bark sample from Chota Nagpur (Maharashtra).

The samples were authenticated in the by comparing with standard samples kept in the pharmacognosy laboratory and voucher specimens were preserved. The samples were powdered to 40 mesh and stored at 25°C in air-tight containers.

TLC conditions: Plate: Precoated silica gel $60F_{254}$ HPTLC plate (E. Merck) (0.2 mm thickness). Spotter: CAMAG Linomat 5. Developing chamber: CAMAG glass twin trough chamber. Scanner: CAMAG TLC scanner 3 and WINCATS 4.0 integration software.

Preparation of standard solution

Standard solution of harmine: A stock solution of harmine was prepared by dissolving 10 mg of accurately weighed harmine in methanol and making up the volume to 100 mL with methanol. From this stock solution standard solutions of 100 to 500 ng/μL were prepared by transferring aliquots (1 to 6 mL) of stock solution to 10 mL volumetric flasks and adjusting the volume with methanol.

Calibration curve for harmine

A 10 μL volume of each of the standard solution of harmine was applied in triplicate to a TLC plate. The plates was developed in a solvent system of toluene-ethyl acetate-methanol (6 : 2 : 2) (v/v) up to a distance of 8 cm. After development the plate was dried in air and scanned at 324 nm. The peak areas were recorded. Calibration curves of harmine were prepared by plotting peak areas *vs.* concentration.

Preparation of sample solutions

Accurately weighed 1 g amount of powder of stem bark of *S. racemosa* was extracted for 6 h with 1 mL of ammonium hydroxide and 10 mL of methanol and made up to 10 mL methanol in a volumetric flask.

Method validation

The method was validated for precision, repeatability and accuracy. Instrumental precision was checked by repeated scanning of the same spot of harmine (concentration: 300 ng) seven times and was expressed as coefficient of variance (CV). The repeatability of the method was tested by analyzing 300 ng/spot of standard solution of harmine after application on the TLC plants ($n = 5$) and was expressed as % CV and was calculated. The variability of the method was studied by analyzing aliquots of different concentrations of standard solution of harmine (100 ng/spot, 300 ng/spot and 500 ng/spot) on the same day (intra-day precision) and on different days (inter-day precision) and relative standard deviation (RSD) was calculated.

The accuracy of the method was tested by performing recovery studies at two levels by addition of 50% and 100% of harmine to one of the sample powders. To 2.5 g of stem bark powder (containing 5.05 mg of harmine) known amounts of standard harmine were added (2.5 mg and 5 mg) and extracted and estimated as described above. The percentage recovery as the average percentage recovery was calculated.

Estimation of harmine from *S. racemosa* stem bark

A 10 μL volume of sample solution was applied in triplicate on a precoated

silica gel G60 HPTLC plate (E. Merck) with the CAMAG Linomat V. Sample spotter. The plate was developed and scanned. The peak areas were recorded. The amount of harmine present in the sample was calculated using the calibration curve for harmine.

RESULTS AND DISCUSSION

Of the various solvent systems tried, that containing toluene-ethyl acetate-methanol (6 : 2 : 2 v/v) was found to be the most sutiable one. In this system, harmine was resolved ($R_f = 0.35$) (Fig. 1a) in the presence of other compounds in the sample extract (Fig. 1b). The identity of the bands of harmine in the sample extracts was confirmed by overlaying their UV absorption spectra with those of

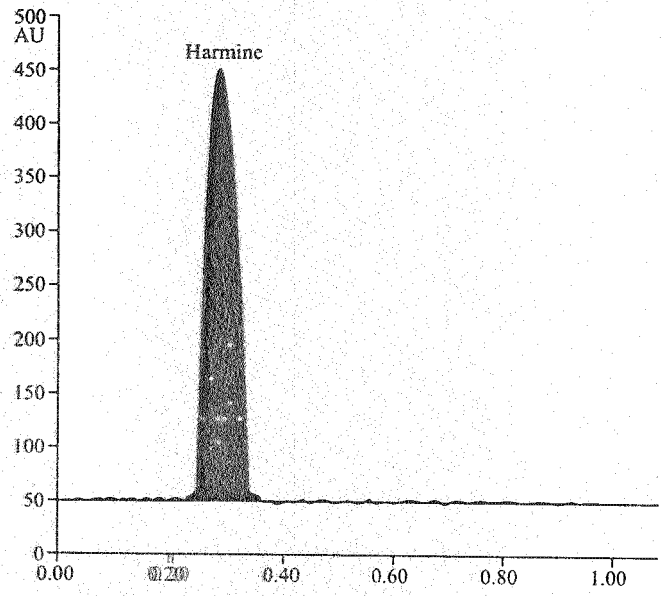

Fig. 1 (a)

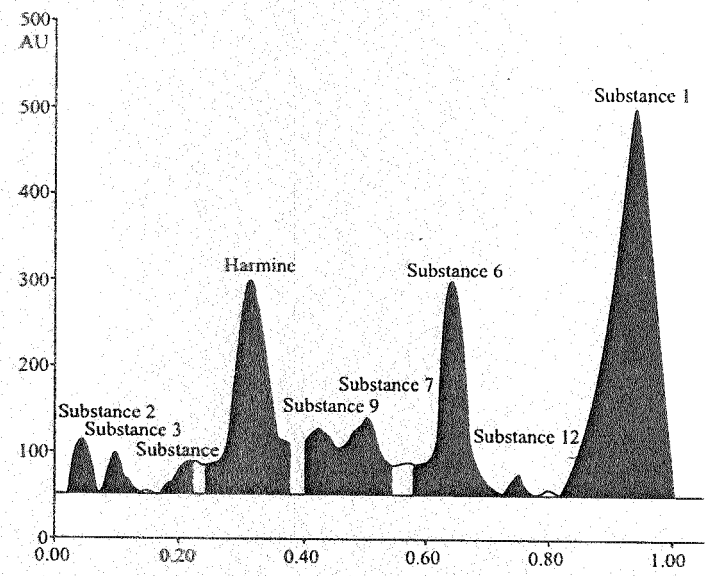

Fig. 1 (b)

the standard harmine using a CAMAG TLC Scanner 3. The purity of harmine band in the sample extracts was confirmed by comparing the absorption spectra at the start, middle and end position of the bands. Further, bands of harmine were detected by spraying with Dragendroff's reagent[6], after which the compounds appeared as violet bands.

Validation

The HPTLC method was validated in terms of precision, repeatability and accuracy (Table-1). The method is specific as it is well resolved harmine with an R_f value of 0.35, in the presence of the other components in the samples of stem bark. The relationship between the concentration of standard solution and the peak response was linear within the concentration range of 100–500 ng/spot with a correlation coefficient of 0.998.

The instrumental precision was studied by repeated scanning of the same spot seven times (% CV = 0.079). Repeatability of the method was tested by analyzing the standard solution (3000 ng/spot) five times (% CV = 0.966). Variability of the method was studied by analyzing aliquots of different concentrations on the same day (intra-day precision) and on different days (inter-day precision) and the RSD indicated that the method was precise. Accuracy of the methods was determined at two levels (50% and 100% addition) by adding a known amount of harmine to the powder of stem bark and the mixture was analyzed. The recoveries were found to be 97.59 and 98.15 with an average of 97.87% (Table-2).

TABLE-1
METHOD VALIDATION PARAMETERS FOR THE ESTIMATION OF HARMINE BY THE HPTLC METHOD

Serial No.	Parameters	Harmine
1.	Instrumental precision (% CV) (n = 7)	0.079
2.	Repeatability	0.966
3.	Limit of detection	30 ng
4.	Limit of quantification	100 ng
5.	Specificity	Specific
6.	Linearity (correlation coefficient)	0.998
7.	Range (ng/spot)	100–500

TABLE-2
RECOVERY STUDY OF HARMINE BY THE HPTLC METHOD (n = 3)

Serial No.	Amount present in sample (μg)	Amount added (μg)	Amount of harmine found in mixture (μg)	Recovery[a] (%)
1.	5.05	2.5	7.368 ± 0.051	97.59 ± 0.68
2.	5.05	5.0	9.864 ± 0.035	98.15 ± 0.35

[a]Mean ± SD

TABLE-3
HARMINE CONTENT IN DIFFERENT SAMPLES OF
S. RACEMOSA BY THE HPTLC METHOD

Sample	Content of harmine[b] (% w/w)
Sample 1 (Karnataka)	0.234 ± 0.0036
Sample 2 (Vadodara)	0.226 ± 0.0012
Sample 3 (Maharastra)	0.268 ± 0.0023

[b]Mean ± SD

Application of the method

The harmine content of two different samples of *S. racemosa* was found to be varying from 0.238 to 0.268 (w/w).

Conclusion

The described method allows the reliable quantification of harmine with good resolution from other constituents of *S. racemosa.* The proposed HPTLC method is rapid, simple, precise, low cost, and accurate and can be used for their quantification in plant material and also in routine quality control of herbal drugs.

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