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QUANTIFICATION OF PIPERINE BY HPTLC METHOD

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Abstract:

In present study HPTLC method has been carried out for determination of piperine of different manufactures. These days' numbers of Pharmaceutical companies are manufacturing Tribhuvankirti Rasa. In this study five samples out of which one is lab prepared (self-prepared) Tribhuvankirti Rasa and four are market preparations. The alcoholic extract of Tribhuvankirti Rasa and standard piperine samples were applied on TLC aluminium plate pre coated with silica gel 60 F 254 and developed using toluene: Ethyl acetate (7: 3) v/v as a mobile phase, using UV detector at wavelength of 254 nm. Content of marker compound in the samples were found similar.

Key Words: *Tribhuvankirti Rasa, Piperine, HPTLC*

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INTRODUCTION:

Tribhuvankirti Rasa is an Ayurvedic Shastrokta medicine which is mentioned in Yogaratnakara [1]. Tribhuvankirti Rasa is one of celebrated and most popular drug compound for the management of *Jwara* amongst Ayurvedic physicians today. It can be said that the mercurial preparation having approbation/appreciation in complete world owing to its potential of curing *Sannipata Jwara* (highly regarded as life threatening) is called Tribhuvankirti Rasa[2].

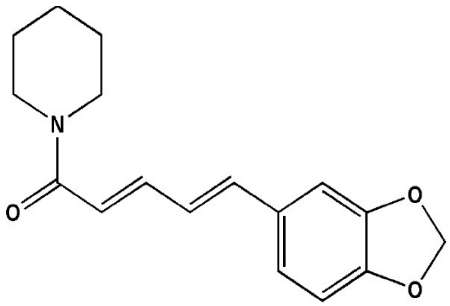

Fig 1: Chemical structure of piperine

Composition of Tribhuvankirti rasa

Table 1: Composition of Tribhuvankirti Rasa

Sr.no	Sanskrit name	Scientific name	Parts used	Quantity
1	Hingula-suddha	Cinnabar	-	1 part
2	Visa (Vatsanabha)-suddha	*Aconitum ferox* Wall.	Root	1 part
3	Sunthi	*Zingiber officinale* Rosc.	Rhizome	1 part
4	Marica	*Piper nigrum* Linn.	Fruit	1 part
5	Pippali	*Piper longum* Linn.	Fruit	1 part
6	Tankan-suddha	Borex	-	1 part
7	Magadhisipha (pippali)	*Piper longum* Linn.	Root	1 part
8	Tulsi- svarasa	*Ocimum Sanctum* Linn	Leaf	Q.S.(for bhavana 3 times)
9	Ardraka- svarasa	*Zingiber officinale* Rosc.	Rhizome	Q.S. (for bhavana 3 times)
10	Dhatura- svarasa	*Datura metal* Linn.	Leaf	Q.S. (for bhavana 3 times)

MATERIALS AND METHODS:

Materials

All ingredients of formulation was procured from the Local Market, Vadodara and also identified and authenticated by the Botanists of M.S.University, Gujarat and coded as SD1 for study. And four market samples are as SD2, SD3, SD4, and SD5.

Chemicals: Analytical grade; Toluene, Ethyl acetate, Ethanol. TLC Aluminium pre coated plate with Silica gel 60 F254 (10X10 cm; 0.2 mm thick). Reference standard- Piperine procured from Aldrich (Lot No.08214 PE-027, CAS 94-62-2, P 459007).

Methods

Preparation of Tribhuvankirti Rasa Tablet

All the drugs were mixed with hingula and triturate well. Prepare the Bhavana (levigating media) of each Surasa patra (Tulsi patra) -svarasa, Ardraka – svarasa, Hema patra (Dhatura patra) – svarasa. Then give the Bhavana to powder mixture one by one of each Surasa patra (Tulsi patra) -svarasa, Ardraka – svarasa, Hema patra (Dhatura patra) – svarasa, three times each. Make the tablet of 1 Rati (125 mg).

Development of TLC Profile

Equipment's used

CAMAG Linomat V applicator, CAMAG Twin Trough Chamber (size 20x10 cm) with SS lid, CAMAG Dipping Chamber, TLC Aluminium pre-coated plate with Silica gel 60 GF254 (size 10X10 cm; 0.2 mm thick) E. Merck, visualization in Camag UV detector, scan by TLC scanner version IV

Sample Preparation

Extract about 1 gm, accurately weighed powdered drug with 100 ml of ethanol consecutively three times in a Soxhlet apparatus. Filter and concentrate the combined extract under vacuum and make up the volume to 10 ml with ethanol in a volumetric flask.

Standard solution

Dissolve 2.5 mg of piperine in ethanol in a 10 ml volumetric flask and make up the volume.

Chromatography

TLC Aluminium pre coated plate with Silica gel60 F254 (20x10 cm2; 0.2 mm thick) was used with Toluene: Ethyl acetate (7:3) V/V as mobile phase. Alcoholic extract of samples and Piperine standard solution applied on plate by using Linomat V applicator. CAMAG Twin Trough Glass Chamber (20x10 cm2) with SS lid was used for development of TLC plate. The Twin Trough Glass Chamber was saturated with mobile phase for 30 minutes. TLC plate was developed to 8 cm distance above the position of the sample application. The plate was removed from the chamber and air dried at room temperature. HPTLC finger print profile was snapped by CAMAG Reprostar IV, before deivatization under UV 254 nm, 366 nm and after derivatization (Fig. 2). The derivatized plate was scanned immediately using Camag TLC Scanner IV at wavelength 254nm. Wincats an integrated Software 4.02 was used for the detection as well as for the evaluation of data.

Estimation of piperine in the drug[3]

Apply 10µl of the test solution on the TLC plate. Develop the plate in the system to obtain the chromatogram and determine the area of peak. Calculate the amount of piperine present in the sample from the calibration curve of piperine.

$$\% \text{ Piperine} = \frac{T}{S} * \frac{Cs}{Ct} * 100$$

T = Area of Test
S = Area of Standard
Cs= Concentration of standard
Ct= Concentration of test

Table 2: Area and Rf Value of Test and Standard Sample

Sample	AUC (Area Under Curve)	Rf value
Standard Piperine	21177.80	0.37
SD1	16183.50	0.36
SD2	9390.25	0.35
SD3	7654.10	0.36
SD4	12348.20	0.35
SD5	16183.50	0.35

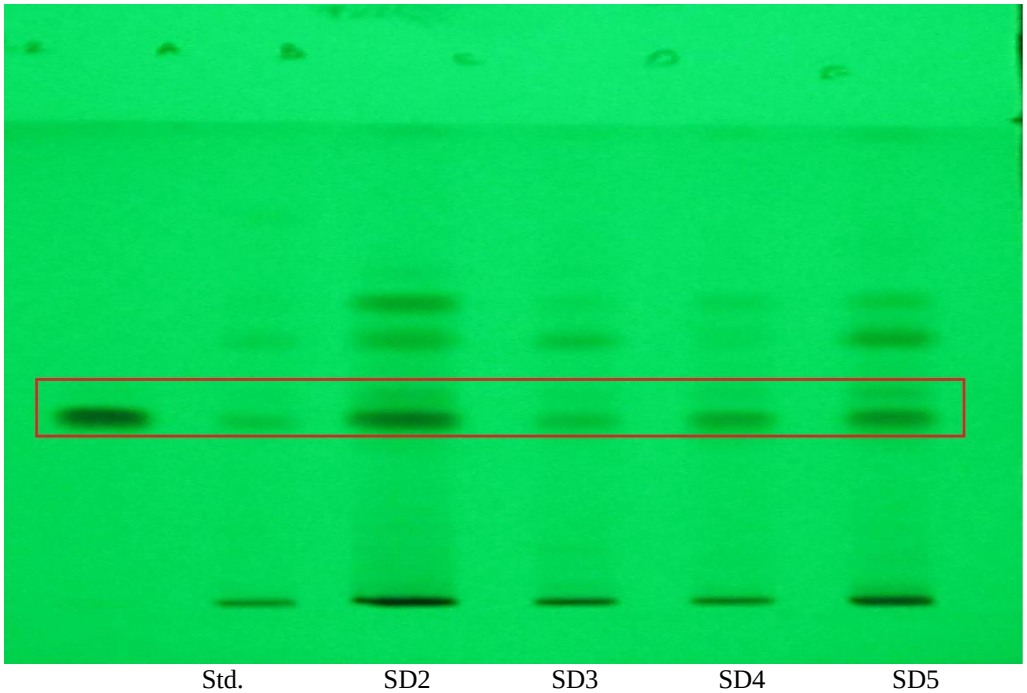

Fig 2: Finger Print at 254 nm

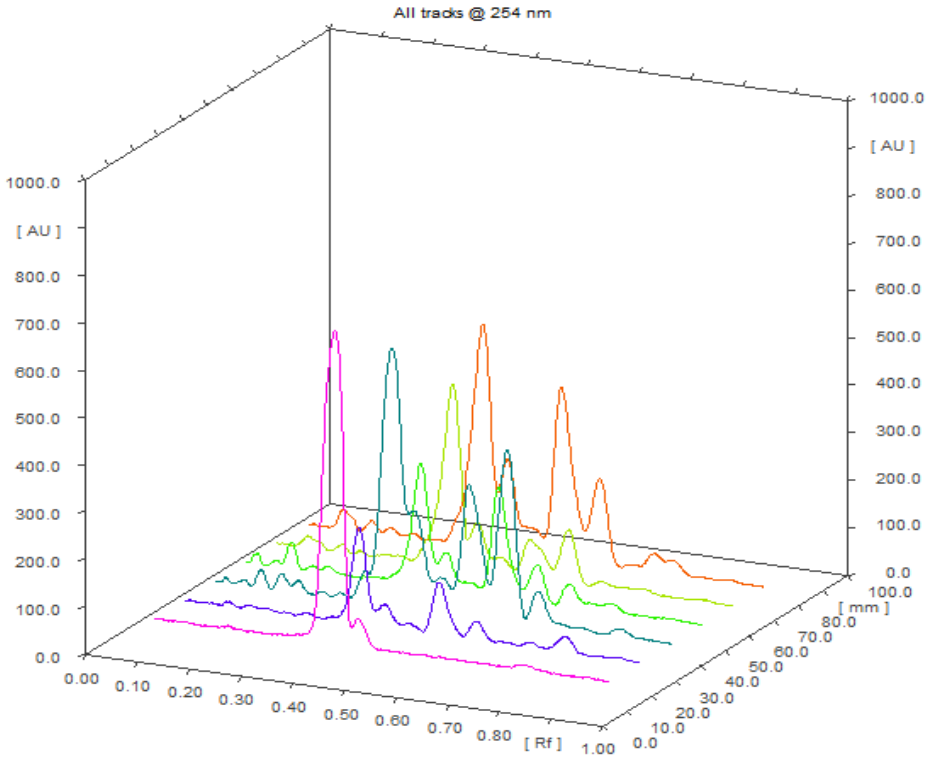

Fig 3: 3D Graph Representation

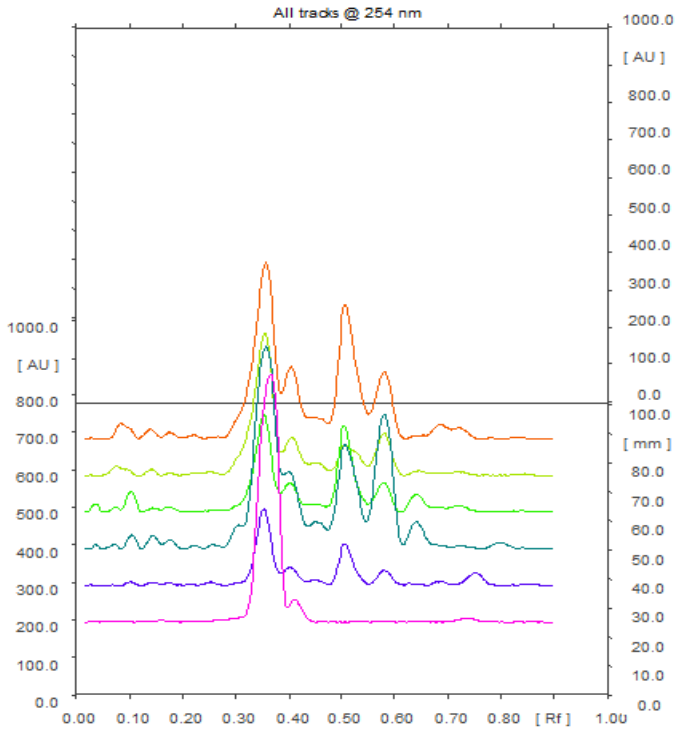

Fig 4: 2D Graph Representation

RESULTS AND DISCUSSION:

The quantity of piperine was found to be

Table 3: Quantity of Piperine

Sample	% of piperine
SD1	3.820 %
SD2	1.241 %
SD3	2.217%
SD4	1.807 %
SD5	3.820 %

When the plate was scanned at wavelength 254nm (Fig. 2). Quantities of Piperine found in samples were obtained automatically via graph and % Piperine found in samples was calculated

CONCLUSION:

The present work was carried out for quantification of piperine from Ayurvedic formulation-Tribhuvankirti Rasa. The developed and validated HPTLC methods are simple, precise, and accurate. Hence, the HPTLC methods may be considered as a tool for assistance for scientific organizations and manufacturers in developing standards.

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