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QUANTITATIVE ESTIMATION OF PIPERINE FROM PHARMACEUTICAL DOSAGE FORM BY HPTLC

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A simple, precise, accurate and rapid high performance thin layer chromatographic method has been developed and validated for the estimation of Piperine in capsule dosage forms. The stationary phase used was precoated silica gel 60F 254. The mobile phase containing Toluene and Methanol in proportion of 80:10 v/v was used to separate the spot of Piperine. The detection of spot was carried out 332nm. The Rf value was found to be 0.49± 0.01. The method was validated in terms of Linearity, Accuracy and Precision. The linearity curve found to be linear in between 10-45 ng/spot. The Limit of Detection (LOD) and Limit of Quantification (LOQ) were found to be 1.23ng and 9ng. The proposed method can be used to determine the drug content from marketed formulations.

Keywords : HPTLC, Piperine, Validation, Residual Standard Deviation (R.S.D)

INTRODUCTION

The plant Piper longum is universally known and widely cultivated all over warm part of India. The main active constituent of Piper longum are Piperine (4-5%) and volatile oil (1%) and other minor constituents are piperlonguminine, piplartine, a waxy alkaloid N-isobutyldecatrans-2- trans–4-dienamide, Piperidine alkaloids: pipernonaline, piperundecalidine, sesamin and a lignin derivative terpendoids resin dihydrostigmasterol.[1-2]

The objective of the present work was to develop an accurate, specific and reproducible method for the estimation of Piperine from pharmaceutical dosage forms.

MATERIALS AND METHODS

A Camag HPTLC system comprising of Linomate V automatic sample applicator, Hamilton Syringe, Camag TLC Scanner-3, Camag Win CAT software, Camag Twin trough chamber and stationary phase precoated silica gel 60F 254 were used. Referance compund Piperine was purchased from Subhod Trading Company, Pune Maharashtra. The capsule containing Piperine extract were purchase from the local market. All chemicals were used are of AR grade.

Chromatographic Conditions

Stationary phase	:	HPTLC precoated, silica gel 60, F254 (Merck)
Thickness	:	0.2 mmMode of application
	:	BandBand width
	:	6mmSeparation technique
	:	AscendingTemperature
	:	25 ± 3°Saturation time
	:	25min. (10min. chamber alone + 15min.with plate)
Migration distance	:	70 mm
Measurement mode	:	Absorbance /
ReflectanceSlit dimension	:	5.0 x
0.45mmScanning mode	:	Single levelScanning
wavelength	:	332nmDetection TLC
	:	UV-Densitometric scanning (CAMAG 3 Scanner)

Preparation of Sample and STD stock solution

20 capsules were weighed and the average weight was determined. The capsule granules are subjected to fine powder with the help of mortal and pestle. The 1000 mg of powder sample was refluxed with 40mL of methanol and concentrated to 10mL and final volume was adjusted to 20mL by methanol to make the concentration of 50mg/ml.

Standard Piperine solution (5mg/µL)

It was prepared by dissolving 10mg of Piperine in 10ml methanol under ultrasonicator, which yields a solution of concentration 1mg/ml, which was further diluted with methanol to yield a concentration of 0.005mg/ml.

Preparation of Mobile Phase

Mobile Phase was prepared by mixing Toluene and Methanol in the proportion of 80:10 v/v.

Calibration Curve

Aliquot of Standard solution of Piperine was applied in duplicates 2µL, 3µL, 4µL, 5µL, 6µL over the silica gel 60F 254 plate. The plate was developed and analyzed as described earlier.

RESULTS AND DISCUSSION

Quantitative Estimation

In the chromatogram of the drugs extracted from the

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capsule, many well resolved spots were observed, out of these spots one spot was matches with the Rf value of shown by Standard Piperine and having the same ëmax (332nm). The drug content as per label claim was found to be 98.24 with % Residual Standard Deviation (R.S.D) is 1.26. The low %R.S.D values as shown in (**Table-1**) indicated the suitability of this method for routine analysis of Piperine in Pharmaceutical dosage form.

VALIDATION

Calibration Curves

Calibration graph was found to be linear over the concentration range 10-45ng/spots. Linearity was evaluated by determining seven standard working solutions in duplicate. The peak area and concentration was subjected to least square linear regression analysis to calculate the calibration equation Y=327.29+153.11X and regression coefficient (r2) was 0.999.[3-4]

TABLE 1: Analysis of Piperine.

SAMPLE	LABEL CLAIM (mg EXTRACT/CAPSULE)	% OF DRUG FOUND	% R.S.D
Piperine	200	98.24	1.26

*R.S.D→ Residual Standard Deviation

TABLE 2: Results and statistical data for recovery study of piperine

S. NO.	Sample Applied (µg)	Piperine Present in applied sample (µg)	Standard piperine added (ng)	Total added conc. (µg)	Found conc. (ng)	% Recovery	Mean %	% RSD
1	50	0.215	50	2.93	2.84	96.97	98.10	1.009
2	100	0.385	100	3.95	3.90	98.86		
3	150	0.583	150	5.98	5.88	98.47		

TABLE - 3 Results and statistical data for precision study piperine

Track	Amount of sample applied (µg)	Amount of piperine estimated (µg)	Mean	% R.S.D.
1	500	18.69	18.19	0.82
2	500	17.86		
3	500	19.96		
4	500	19.46		
5	500	17.28		
6	500	15.91		

TABLE 4: RESULTS AND STATISTICAL DATA OF LOD & LOQ BY SIGNAL TO NOISE RATIO

SAMPLE	LIMIT OF DETECTION (ng)	LIMIT OF QUANTIFICATION (ng)
Piperine	1.230	9

TABLE- 5 RESULTS AND STATISTICAL DATA OF DIFFERENT ANALYST STUDY

ANALYTES	AMOUNT OF SAMPLE APPLIED (µg)	AMOUNT OF PIPERINE ESTIMATED (µg)	MEAN	% RSD
I	250	12.12	11.99	1.53
	250	11.86		
II	250	10.66	11.03	1.60
	250	11.26		

TABLE 6: RESULTS FOR THE DIFFERENT PARAMETERS OF RUGGEDNESS & ROBUSTNESS

PARAMETER	INITIAL CONDITION	CHANGE IN CONDITION	EFFECT
Mobil phase composition	Toluene: Methanol (80:10)	Toluene: Methanol (82:12)	No proper resolution
Development Distance	7 cm	6 cm and 8 cm	No effect on RF
Saturation Time	25 min	Without saturation	Band get curved and tailing
Temperature	25±3 ºC	40±3 ºC	No effect on RF and shape of Curve
Extraction Time	20 min	30 min	No change in conc.
Sample application	Individual application	Over spotting	Very little change in conc.

FIGURE 1. Densitogram of piperine

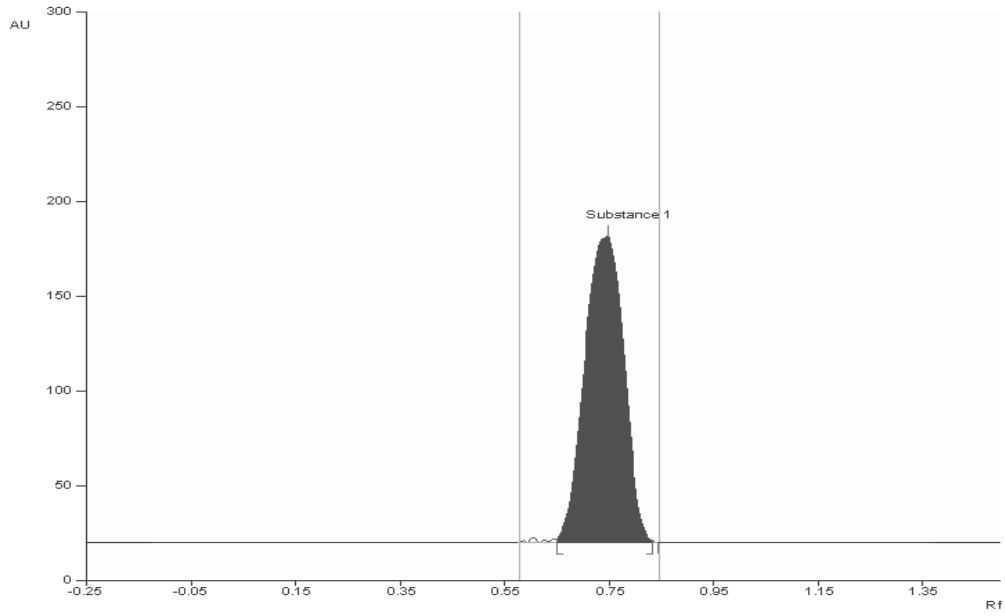
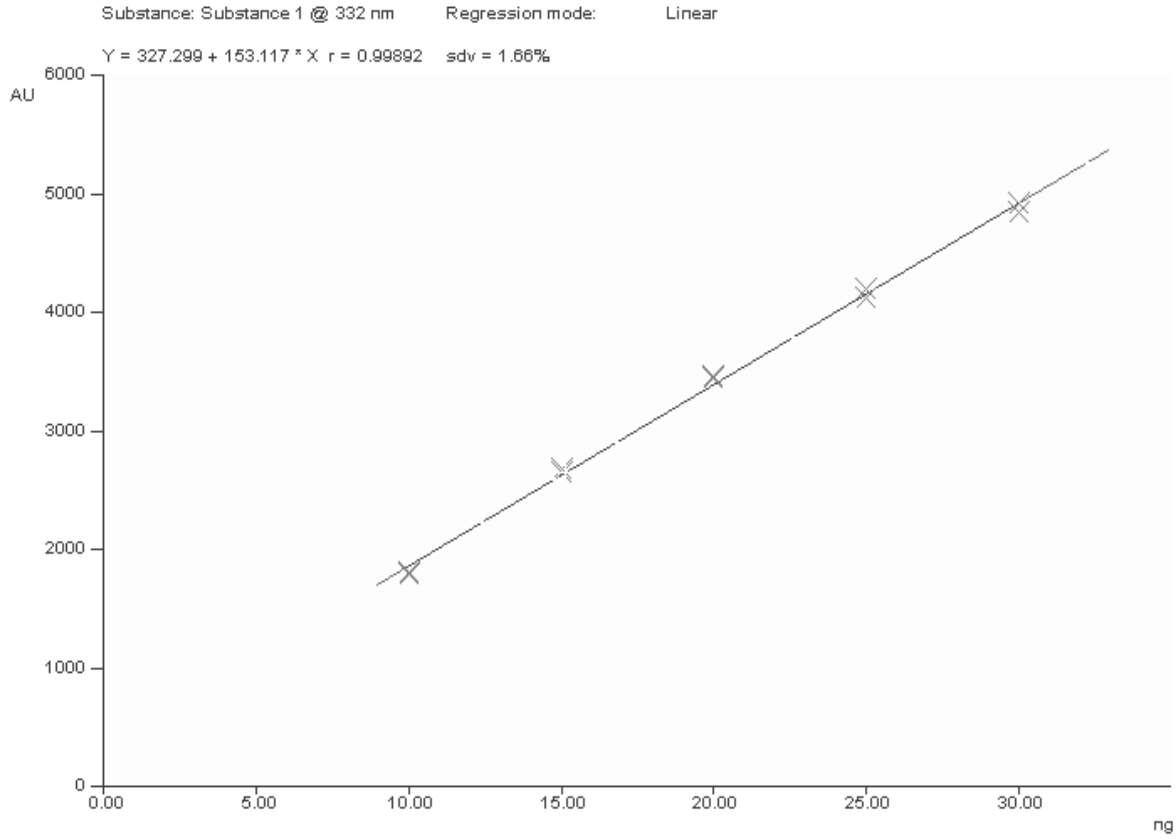


FIGURE 2. Calibration graph for piperine



Accuracy (Recovery Studies)

To study accuracy of the developed method, recovery studies were carried out using standard addition method at three different level and the % recoveries were calculated. The average % recovery was 98.10% with % R.S.D 1.009 (Table 2). The result revealed that there was no interference of excipients.[3-5]

Precision

The repeatability of sample application and measurement of peak area were expressed in terms of % R.S.D. Precision studies were carried out by using the sample solution. Six spots of 10µL of sample solution were applied on the plate & the plate was scanned 332nm after development. The amount of Piperine present in per track were calculated by using regression equations Y=327.29+153.11X. The results were revealed that the % R.S.D. was found to be < 2% i.e 0.82 (**Table-3**).

Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD is the amount of applied sample producing the peak area which is equal to the sum of the mean blank area (15 noise peak) and three times of its standard deviation.
LOQ is the amount of applied sample producing the peak area which is equal to the sum of the mean blank area (15 noise peaks) and ten times of its standard deviation.[5, 6]
The LOD and LOQ were found to be 1.23ng and 9ng (**Table-4**).

Ruggedness and Robustness

The study of ruggedness & Robustness was carried out by keeping all the parameters constant except for the time, day and analysts.[5-6] the results were shown in (**Table-5-6**)

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