

# QUANTITATIVE ESTIMATION OF PIPERINE IN HERBAL COUGH SYRUP BY HPTLC METHOD

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## ABSTRACT

A validated HPTLC method for the estimation of piperine in herbal cough syrup is described. Separation was achieved on pre-coated silica gel plate 60F<sub>254</sub> using Ethyl acetate : Hexane (60 : 40 v/v) as mobile phase. Quantitation was carried out by the use of densitometer in absorbance mode at 330nm. The method gave good separation of piperine at R<sub>f</sub> 0.37 from other compounds. The linearity for piperine was found to be in the concentration range of 10-50µg/ml. The percentage w/w content of piperine was found to be 1.526. The average percentage recovery of piperine in sample was found to be 98.15%. The proposed method is accurate, precise and reproducible, and can be adopted for routine analysis of piperine in herbal cough syrup.

**Key words:** Piperine, Herbal cough syrup, HPTLC.

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## INTRODUCTION

Piperine is an alkaloid found naturally in plants belonging to the *Piperaceae* family, such as *Piper nigrum* L<sup>1</sup>, commonly known as black pepper and *Piper longum* L, commonly known as long pepper. Piperine is the trans-trans stereoisomer of 1-piperoylpiperidine. It is also known as (E, E)-1- piperoylpiperidine and (E, E)-1- [5-(1, 3-benzodioxol-5-yl)-1-oxo-2, 4-pentdienyl] piperidine. Piperine is widely used in various herbal cough syrups for its potent anti-tussive and bronchodilator properties<sup>2</sup>. As the literature survey<sup>5-13</sup> clearly reveals that there is no proper analytical method available for the quantitative estimation of piperine in herbal cough syrups, the present study focused to develop a rapid, efficient and reproducible method for the analysis of piperine in herbal cough syrup by HPTLC<sup>3-4</sup>.

## EXPERIMENTAL

### Instruments used:

Application mode: CAMAG Linomat IV Sample applicator

Scanner mode: CAMAG TLC Scanner III

Development mode: CAMAG Twin trough chamber

### Chromatographic conditions:

Stationary phase: Pre-coated Silica gel plate 60 F<sub>254</sub> pre-washed with Methanol

Mobile phase: Ethyl acetate: Hexane (60:40 v/v)

Distance between bands: 7mm

Separation technique: Ascending development

Scanning mode: Absorbance

Lamp: Deuterium

Wavelength: 330nm

**Preparation of Standard Stock Solution:**

An accurately weighed quantity (50 mg) of piperine was dissolved in diluent [chloroform] taken in 50ml volumetric flask. Then the volume is made up to 50ml with diluent to obtain a stock solution having 1 mg/ml concentration of piperine.

**Preparation of Standard Solution:**

The standard stock solution of piperine was diluted to prepare working standard with concentration of 15µg/ml.

**Preparation of Sample Solution:**

For the extraction of piperine from cough syrup, accurately weighed 10gms of sample was taken, diluted with distilled water and extracted with dichloromethane till the aqueous layer gave negative test for alkaloids. The extracts were pooled together; sodium sulphate was added, filtered and dried to remove the solvent. Accurately weighed quantity of residue was dissolved in acetonitrile and used as the sample solution. Dilution was made to get a concentration of piperine similar to that of working standard, mixed well using ultrasonicator, centrifuged and filtered through Whatman filter paper no.1. The filtrate was used for estimation.

**Estimation method:**

The sample was spotted on the chromplate with help of Linomat IV spotting system. The chromatograms were recorded. The peak area for piperine was noted down by scanning the chromatogram. The amount of drug present was calculated by comparing the peak area values of sample with that of standard. The results were tabulated in Table I.

**VALIDATION:**

To validate the developed method parameters like linearity, range, system repeatability test, accuracy in terms of recovery, precision in terms of percentage relative standard deviation were studied.

**Linearity and range:**

The linearity of the method was assessed by performing single measurement at several analyte concentrations. A minimum of 5 concentrations were recommended for linearity studies. Varying quantities of standard stock solution was diluted with diluent to give a concentration of 10-50 µg/ml of piperine. A calibration curve was constructed for the sample by plotting peak areas against concentration.

There exists a linear relationship in the range of 10-50 µg/ml of piperine. From the constructed curve Coefficient of Variance was calculated. The results were tabulated in Table II.

**System Repeatability:**

The intra and inter day variations of the method were performed using five replicate injections of three different concentrations, which were prepared and analyzed on the same day and on three different days over a period of one week. The intra and inter day variation in the peak area of the standard solution and amount were calculated in terms of percentage relative standard deviation. The results were tabulated in Table III.

**Accuracy:**

Accuracy of the developed method can be assessed by performing recovery studies. To ensure the reliability of the method, recovery studies were carried out by mixing a known quantity of standard drug with the pre-analyzed sample formulation and the contents were reanalyzed by the proposed method. The results were tabulated in Table IV.

**RESULTS AND DISCUSSION**

The solvent system of the mobile phase having Ethyl acetate: Hexane (60: 40 v/v) gave dense, compact and well separated spots of the drug from the mixture at the wavelength of 270nm.

The assay values were found to be within the standard acceptable limits (Table I) and so the method can be adopted for estimation of piperine in syrup formulation. The statistical validation was also done. (Table I)

Linearity studies were carried out and there exists linearity in the concentration range of 10-50  $\mu\text{g/ml}$  for piperine. (Table II)

Lower percentage relative standard deviation of measurements in the intra and inter day repeatability studies indicates the precision of the developed method. (Table III).

The good average recovery values obtained in recovery studies indicate that the proposed method is accurate for estimation of drug in syrup formulation. (Table IV).

Thus the developed method was found to be accurate, precise, suitable and cost effective for the estimation of piperine in syrup formulation.

**TABLE-I**  
**QUANTITATIVE ESTIMATION**

S.NO	SAMPLE	% w/w content of piperine*	S. D	%R. S. D	S. E
1.	Syrup sample	1.536	0.1705	0.0111	0.0762

\* Mean of five values

**S.D** – Standard deviation **R.S.D** – Relative standard deviation **S.E** – Standard error

**TABLE -II**  
**LINEARITY**

PARAMETER	PIPERINE
$R_f$	0.37
Linearity Range	10 to 20 $\mu\text{g/ml}$
Regression (r)	0.9988

**TABLE-III**  
**SYSTEM REPEATABILITY AND PRECISION**

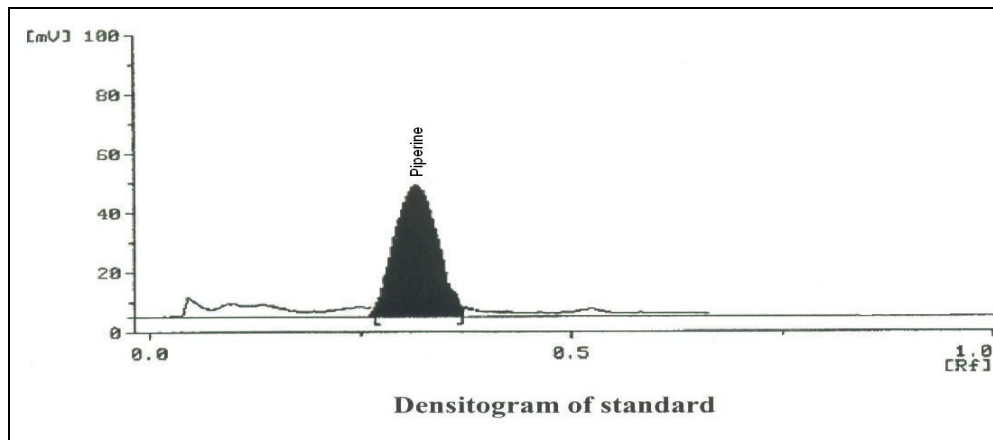
Drug	Concentration $\mu\text{g/ml}$	Intra-day measured concentration*		Inter-day measured concentration*	
		Mean ( $\mu\text{g/ml}$ )	%R. S. D	Mean ( $\mu\text{g/ml}$ )	%R. S. D
Piperine	10	9.38	0.124	10.21	0.0291
	30	28.26	0.0989	29.05	0.0572
	50	49.24	0.1020	50.42	0.1246

\* Mean of five individual readings

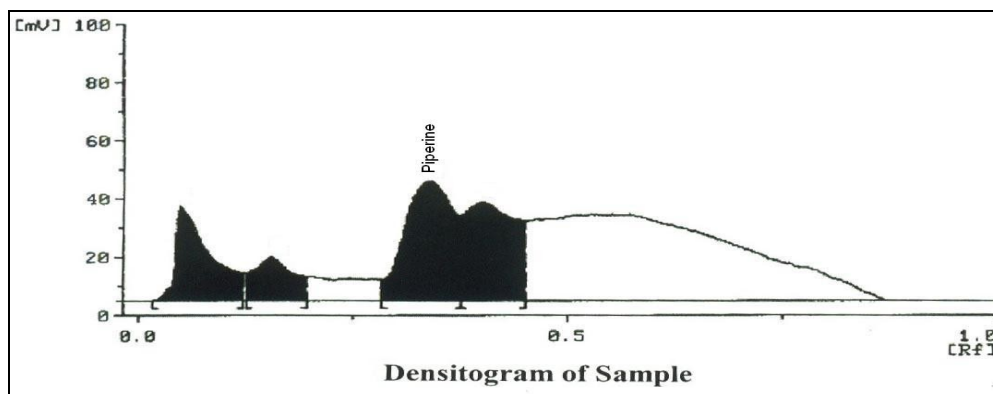
**TABLE-IV**  
**RECOVERY STUDIES**

S.No	Amount present $\mu\text{g/ml}^*$	Amount added $\mu\text{g/ml}^*$	Amount Recovered*	%Recovery*
1.	15.2	20	34.56	98.18
2.	15.2	40	54.17	98.13

- Mean of five individual readings

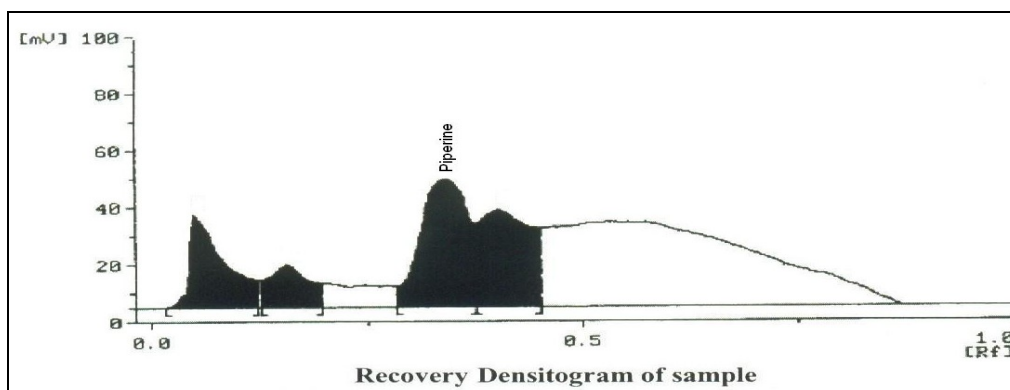


**DENSITOGAM OF STANDARD OF PIPERINE**  
Detection Wavelength – 330nm



**DENSITOGAM OF SAMPLE FORMULATION**

Detection Wavelength – 330nm



### DENSITOGAM OF RECOVERY STUDIES

Detection Wavelength – 330nm

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