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## HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHIC METHOD FOR QUANTIFICATION OF PIPERINE IN AN AYURVEDIC FORMULATION

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### **Abstract:**

*A validated HPTLC method for the estimation of piperine in an ayurvedic formulation is described. Separation was achieved on pre-coated silica gel plate 60f<sub>254</sub> using Ethyl acetate: Chloroform: Hexane (60: 10: 30 v/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> 0.45 from other components. The linearity for piperine was found to be in the concentration range of 10-50µg/ml. The percentage w/w content of piperine was 1.53%. The average percentage recovery of piperine in formulation was observed with in acceptable limit. The proposed method was found to be accurate, precise and reproducible and can be adopted for routine analysis of piperine in herbal formulation.*

**Keywords:** validated HPTLC method, piperine, Quantitation

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## INTRODUCTION

Herbal formulations show the number of problems when quality aspect is considered. This is because of nature of the herbal ingredients and different secondary metabolites present therein. Mainly, variation in the chemical profile of the herbal due to intrinsic and extrinsic factors (growing, harvesting, storage and drying processes) [1-3]. Chromatographic fingerprint have been suggested to check for authenticity or provide quality control of herbal medicine [4]. Chromatography has the advantage of separating a complicated system into relatively simple sub-systems and then presenting the chemical patterns of herbal medicine in the form of a chromatogram. The World Health Organization (WHO) accepts fingerprint chromatography as an identification and quality evaluation technique for medicinal herbs since 1991 [5]. Fingerprints can be a unique identification utility for herbs and their different species. The Govt. of India has also adopted the “fingerprint” approach for botanicals because it supports the traditional concept and is easy to practice at different levels of sophistication [6-7]. The Ajmodadi churna (AC) is well known ayurvedic formulation official in Ayurvedic Formulary of India, traditionally used for abdominal pain, carminative, antispasmodic, and helps in all painful conditions like sciatica and stiffness [8]. As the literature survey there is no proper analytical method available for the quantitative estimation of piperine in Ajmodadi churna. The present study focused to develop a rapid, efficient and reproducible method for the analysis of piperine in Ajmodadi churna by HPTLC.

## MATERIALS AND METHODS

All the chemicals used were of analytical grade (CDH Chemicals Ltd.). Piperine standard was purchased from sigma chemicals. Precoated silica gel plates

(TLC plates, silica gel aluminium sheets with 60 F254) were purchased from M/s. Merck India Ltd.

### Ayurvedic Formulation:

Ajmodadi churna manufactured by Himalaya Health Care procured from the local market.

### Instrumentation and chromatographic conditions<sup>9-13</sup>:

#### 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 60f<sub>254</sub> pre-washed with Methanol

Mobile phase: Ethyl acetate: Chloroform: Hexane (60: 10: 30 v/v/v)

Distance between bands: 7mm

Separation technique: Ascending development

Scanning mode: Absorbance

Lamp: Deuterium

Wavelength: 330nm

The optimized chamber saturation time was 35 min. at room temperature.

#### Preparation of Test sample:

Powdered Ajmodadi churna was reflux with 60 ml Methanol for 1hr. then filtered the extract and re-refluxed the marc left with 40 ml of Methanol for 1 hour. After the filtration filtrates were combined. Concentrated the methanolic extract under vacuum till the semisolid mass was obtained. The residue was dissolved in 75 ml Methanol and filtered through sintered glass funnel by vacuum filtration. The filtrate was centrifuged at 2000 rpm for 20 minutes, the supernatant was collected in 100 ml volumetric flask and volume was made up with Methanol [14, 15].

#### Estimation method:

The sample was spotted on the plate with the 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.

#### VALIDATION:

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

#### Linearity:

The linearity of the method was assessed by performing 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  $\mu\text{g/ml}$  of piperine. A calibration curve was constructed for the sample by plotting peak areas against concentrations.

There exists a linear relationship in the range of 10-50  $\mu\text{g/ml}$  of piperine. From the constructed curve coefficient of variance was calculated. The results were tabulated in Table I.

#### System Repeatability:

The intra and inter day variations of the method was 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 II.

#### Accuracy:

Accuracy of the developed method was 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 solution and the contents were reanalyzed by the proposed method. The results were tabulated in Table III.

## RESULTS AND DISCUSSIONS

The solvent system of the mobile phase having Ethyl acetate: Chloroform: Hexane (60: 10: 30 v/v/v) gave sharp, symmetric peak and improved spot characteristics for piperine. An assay value was found to be 1.53% and other statistical values of validation parameters were also found within the standard acceptable limits.

Linearity studies were carried out and there exists linearity in the concentration range of 10-50  $\mu\text{g/ml}$  for piperine (Table I). Lower percentage relative standard deviation of measurements in the intra and inter day repeatability studies indicates the precision of the developed method (Table II). The good average recovery values obtained in recovery study indicates that the proposed method is accurate for the estimation of piperine in herbal formulation (Table III). Thus the developed method was found to be accurate, precise, suitable and cost effective for the estimation of piperine in ayurvedic formulation; Ajmodadi churna.

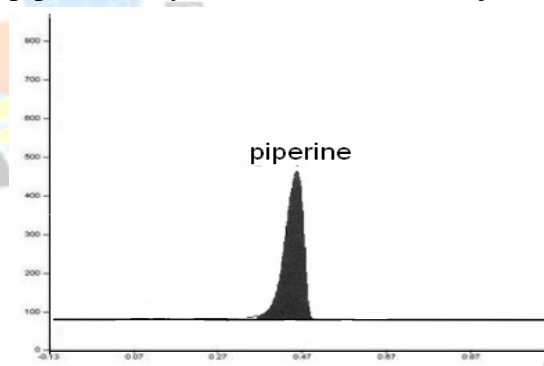
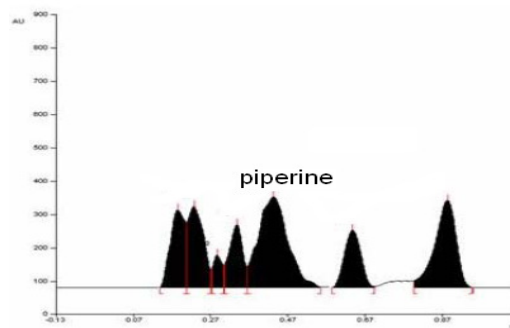


Fig. 1. Densitogram of standard piperine



**Fig. 2. HPTLC fingerprinting of Ajmodadi churna (peak showing piperine in sample formulation)**

1	15.2	80	99.75
2		100	98.15
3		120	98.18

**Table 1**  
**% drug content & linearity of piperine by HPTLC**

Parameters	Range
Linearity range	10-50 µg/ml
Correlation coefficient (r)	0.9997
% drug content	1.53%
R <sub>f</sub>	0.45

**Table-II**  
**System repeatability and precision**

Sample	Concentration µg/ml	Intra-day measured concentration		Inter-day measured concentration	
		Mean (µg/ml)	%R. S. D	Mean (µg/ml)	%R. S. D
10	9.36	0.123	10.22	0.029	1
30	28.36	0.098	29.05	0.057	4
50	49.23	0.102	50.41	0.124	5

**Table-III**  
**Recovery studies**

Level	Amount present µg/ml	% of standard Addition	% Recovery

**Conclusion:**

A simple, rapid and accurate HPTLC method for the estimation of piperine in Ajmodadi churna was developed which can be used as a valuable analytical tool in routine analysis to check the batch to batch variations. The method was showed excellent sensitivity, reproducibility, accuracy and repeatability, which is evidence by low percentage relative standard deviation. Hence it is suggested that the proposed HPTLC method can be effectively used for the routine analysis of piperine in Ajmodadi churna and its crude drugs.

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