**Research Article**

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# SIMULTANEOUS ESTIMATION OF CURCUMIN AND PIPERINE IN HERBAL COUGH SYRUP USING HPTLC

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**Abstract**

Many of the traditional herbal formulations contain extracts of Piper longum and Curcuma longa, piperine and curcmin respectively, being active constituents of these two herbs. An attempt has been made to develop a simple, precise, rapid, and cost-effective high-performance thin-layer chromatographic (HPTLC) method for simultaneous estimation of these in a herbal cough formulation (Virak of Syrup). Pre-coated silica gel 60F-254 plates with chloroform: ethanol: ethyla cetate: acetic acid (9.5:0.5:1.0:0.2,v/v/v/v) as mobile phase were used in chromatographic determinations. The plates were scanned and the compounds were quantified at 360nm. The respective RF values of curcumin and piperine were 0.58 and 0.69. Under these experimental conditions linearity was observed between 200- 800ng/spot for curcumin and between 30- 90ng/spot for piperine and average recovery was 99.06% for curcumin and 95.13% for piperine.

***Keywords***: *HPTLC, curcumin, piperine, herbal cough syrup.*



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## 1. Introduction

Herbal medicines are the oldest remedies known to mankind; these generally contain more than one herb in its combination. However, one of the impediments in the acceptance of the Ayurvedic formulations is the lack of standard quality control profiles [1,2]. Curcuma longa (Zingiberaceae), commonly called Haldi, is a well- known plant drug in Ayurvedic medicine. Curcumin, demethoxycurcumin, and bis-demethoxycurcumin, three major pharmacologically important curcuminoids, have been isolated from Curcuma longa [3,4]. It has been used for the treatment of various diseases and disorders like cold, cough, bronchitis, asthma and dyspnea [5]. Piperine is an alkaloid found naturally in the plants belonging to the piperaceae family, such as Piper nigrum L, commonly known as black pepper and Piper longum L, commonly known as long pepper. Piperine is widely used in various herbal cough syrups for its potent anti-tussive and bronchodilator properties [6]. Ansari et al. [7] reported stability indicating HPTLC determination of curcumin in bulk drug and pharmaceutical formulations. Chauhan et al. [8] reported HPLC method to determine piperine in different piper species. Suther et al. [9] reported HPTLC method for identification of different piper species and their mixture. Kulkarni et al. [10] developed HPTLC method for the determination of piperine from Piper nigrum. Shanmugasundaram et al. [11] reported quantitative estimation of piperine in cough syrup by HPTLC. No articles related to simultaneous estimation of curcumin and piperine in herbal cough syrup was found. So the present study is focused to develop a rapid, efficient and reproducible method for the analysis of curcumin and piperine in herbal cough syrup by HPTLC.

## 2. Experimental

### 2.1. Material

Curcumin was purchased from Natural Remedies Banglore, India, and piperine was purchased from Sigma Aldrich Banglore, India. All chemicals and reagents used were of analytical grade and were purchased from Merck Chemicals, India.

### 2.2. HPTLC instrumentation

The sample were spotted in the form of bands of width 8 mm with a Camag microliter syringe on precoated silica gel aluminium plate 60F-254 (20cm × 10cm with 0.2mm

thickness, E. Merck, Germany) using a Camag Linomat V (Switzerland). A constant application rate of 150 nl/s was employed and space between two bands was 14.4mm. The slit dimension was kept at 6mm × 0.90 mm. and 20mm/s scanning speed was employed. The mobile phase consisted of chloroform: ethanol: glacial acetic acid: ethyl acetate (9.5:0.5:0.1:1.0,v/v). Linear ascending development was carried out in twin trough glass chamber saturated with the mobile phase. The chamber saturation time for mobile phase was 15 min. at 200c. The length of chromatogram run was 80mm. Subsequent to the development; TLC plates were dried at Camag hot plate. Densitometric scanning was performed on Camag TLC scanner III in the reflectance- absorbance mode at 360 nm and operated by WINCAT software (V 3.15, Camag). The source of radiation utilized was deuterium lamp emiting a continuous UV spectrum between 190nm and 400nm. Video densitometry of the chromatoplate was carried out with the help of CAMAG Reprostar 3 with cabinet cover and mounted digital camera. Concentrations of the compound chromatographed were determined from the intensity of diffusely reflected light. Evaluation was via peak areas with linear regression.

2.3. Calibration curves of standard curcumin and piperine

A stock solution of curcumin (200µg/ml) and piperine (1000µg/ml) were prepared in methanol. 1ml stock solution of standard piperine was transferred in 50ml volumetric flask and made to volume with methanol so that (20ng/µl) solution was obtained. Different volume of stock solution of curcumin and piperine 1, 1.5, 2, 2.5, 3, 3.5, 4µl were spotted on the TLC plate to obtained concentration of 200, 300, 400, 500, 600, 700, 800ng/spot of curcumin and 20, 30, 40, 50, 60, 70, 80ng/spot of piperine respectively. The calibration graph was plotted by using data of peak areas against corresponding concentrations.

### 2.4. Method validation

#### 2.4.1. Precision

Repeatability of sample application and measurement of peak area were carried out using six replicates of the same spot (600 ng/spot of curcumin and 60ng/spot of piperine).

#### 2.4.2. Limit of detection and limit of quantification

Limits of detection and limits of quantification were determined by calculation of the signal-to-noise ratio. Signal-to-noise ratios of approximately 3:1 and 10:1 were used for estimating the detection limit and quantification limit respectively.

#### 2.4.3. Robustness of the method

By introducing small changes in the mobile phase composition, the effects on the results were examined. Mobile phases having different composition of chloroform: ethanol: glacial acetic acid: ethyl acetate (9.5: 0.5: 0.1: 1.0 and 9.0:1.5: 0.2: 0.5v/vv/v) was tried.

#### 2.4.4. Recovery studies

The analyzed samples were spiked with extra 80, 100 and 120% of the standard curcumin and piperine the mixtures were reanalyzed by the proposed method. The experiment was conducted in triplicate. This was done to check for the recovery of the drug at different levels in the formulations.

### 2.5. Analysis of Curcumin and piperine in Herbal cough syrup

To determine the content of curcumin and piperine in syrup 15ml of syrup was diluted with distilled water and extracted with 50ml of dichloromethane. Aqueous layer (upper) was re-extracted with dichloromethane twice (each with 20ml). All extracts were combined. Potassium sulphate was added and filtered. Filtrate was evaporated to dryness and the residue obtained was dissolved in 5ml methanol. 10µl (1800µg/spot) of the solution was applied on TLC plate followed by development and scanning as described in section 2.2.

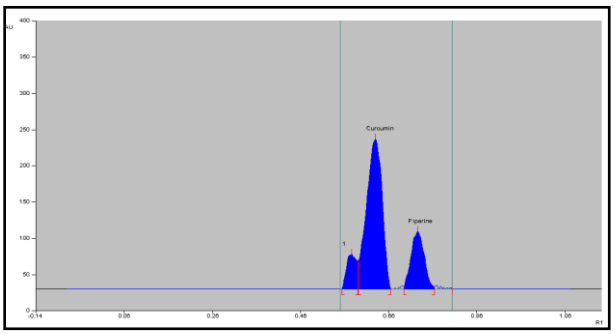


Fig.1. A typical HPTLC chromatogram of curcumin and piperine (Rf = 0.63 and 0.72).

## 3. Results and discussion

### 3.1. Development of the optimum mobile phase

The standard solution and the test solution were spotted on HPTLC plates and different combination solvents have been tried to get good separation and stable peak. The mobile phase chloroform: ethanol: glacial acetic acid: ethyl acetate (9.5: 0.5: 0.1: 0.2 v/v) was selected for estimation of the drugs by HPTLC method, which gave good resolution with Rf value of 0.63 for Curcumin and 0.72 for piperine (Fig. 1). Well-defined spots were obtained when the chamber was saturated with the mobile phase for 20 min at room temperature.

### 3.2. Calibration curves

The present HPTLC method for estimation of Curcumin and piperine simultaneously showed a good correlation coefficient (r = 0.9997) in the concentration range of 200- 800ng/spot for Curcumin and r = 0.99525 in the concentration range of 20- 90ng/spot for piperine with respect to the peak area.

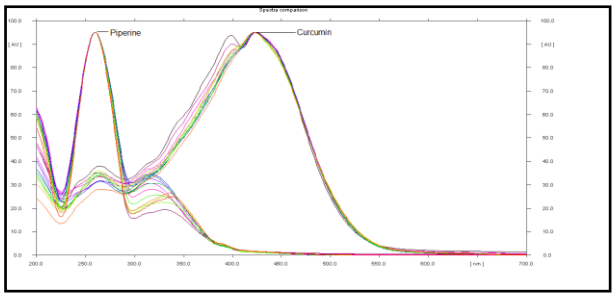


Fig 2: Shows overlap spectra of curcumin and piperine simultaneously at 200nm -700nm 3.3. Validation of the method

### 3.3.1. Precision

The repeatability of sample application and measurement of peak area were expressed in terms of %RSD and results are depicted in Table 1.

Table 1: Statistical data of method precision of Curcumin and Piperine

| S.No. | Application of sample (µl) | Quantity of Curcumin (ng) | Quantity of Piperine (ng) |
|---|---|---|---|
| 1 | 10 | 505.10 | 63.89 |
| 2 | 10 | 510.24 | 64.46 |
| 3 | 10 | 498.84 | 62.54 |
| 4 | 10 | 509.67 | 61.78 |
| 5 | 10 | 518.54 | 64.79 |
| 6 | 10 | 495.49 | 63.43 |
| Average | | 506.31 | 63.48167 |
| % RSD | | 1.65 | 1.81 |

### 3.3.2. Recovery studies

The proposed method when used for extraction and subsequent estimation of Curcumin and Piperine from syrup after spiking with 80, 100 and 120% of additional drug afforded recovery of 99.06±0.495%for Curcumin and 95.13±1.60% for Piperine as listed in Table 2, 3.

Table 2: Results and statistical data for recovery study of Curcumin

| S.No | Application of sample (µl) A | Amount present in Product A (ng) B | Amount added in B (ng) C | Amount present in (B+C) (ng) D | Total found curcumin (B+C) (ng) E | % Recovery F | Mean recovery (Avg. ± SD) | Total mean recovery |
|---|---|---|---|---|---|---|---|---|
| 1 | 5 | 250 | 200 | 450 | 445.05 | 98.90 | 98.96± 0.369 | 99.06± 0.495 |
| 2 | 5 | 250 | 200 | 450 | 447.12 | 99.36 | | |
| 3 | 5 | 250 | 200 | 450 | 443.84 | 98.63 | | |
| 4 | 5 | 250 | 250 | 500 | 498.65 | 99.73 | 99.13± 0.590 | |
| 5 | 5 | 250 | 250 | 500 | 492.75 | 98.55 | | |
| 6 | 5 | 250 | 250 | 500 | 495.55 | 99.11 | | |
| 7 | 5 | 250 | 300 | 550 | 544.56 | 99.01 | 99.09± 0.525 | |
| 8 | 5 | 250 | 300 | 550 | 548.08 | 99.65 | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 9 | 5 | 250 | 300 | 550 | 542.36 | 98.61 | | |

Table 3: Results and statistical data for recovery study of Piperine

| S.No | Application of sample (µl) A | Amount present in Product A (ng) B | Amount added in B (ng) C | Amount present in (B+C) (ng) D | Total found piperine (B+C) (ng) E | % Recovery F | Mean recovery (Avg. ± SD | Total mean recovery |
|---|---|---|---|---|---|---|---|---|
| 1 | 5 | 31.89 | 25 | 56.89 | 52.79 | 92.80 | 94.28±1.69 | 95.13±1.60 |
| 2 | 5 | 31.89 | 25 | 56.89 | 53.43 | 93.92 | | |
| 3 | 5 | 31.89 | 25 | 56.89 | 54.69 | 96.13 | | |
| 4 | 5 | 31.89 | 30 | 61.89 | 58.11 | 93.90 | 94.35±1.28 | |
| 5 | 5 | 31.89 | 30 | 61.89 | 57.78 | 93.36 | | |
| 6 | 5 | 31.89 | 30 | 61.89 | 59.29 | 95.80 | | |
| 7 | 5 | 31.89 | 40 | 71.89 | 69.56 | 96.76 | 96.75±1.85 | |
| 8 | 5 | 31.89 | 40 | 71.89 | 68.22 | 94.89 | | |
| 9 | 5 | 31.89 | 40 | 71.89 | 70.88 | 98.60 | | |

### 3.3.3. LOD and LOQ

Limits of detection and limits of quantification were determined by calculation of the signal-to-noise ratio. Signal-to-noise ratios of approximately 3:1 and 10:1 were used for estimating the detection limit and quantification limit respectively. LOD of Curcumin and Piperine was 20ng and 6ng respectively and LOQ of Curcumin and Piperine was 60ng and 14ng as listed in table 4, 5 respectively.

Table 4- Detection Limit (LOD) of Curcumin & Piperine- Based on Signal-to-Noise

| S. No. | Sample | Height | Respective concentration | Signal-to-Noise ratio |
|---|---|---|---|---|
| 1 | Blank | 4.8 | - | 14.6/ 4.8 = 3.0<br>16.0/ 4.8 = 3.3 |
| 2 | Curcumin | 14.6 | 20ng | |
| 3 | Piperine | 16.0 | 6ng | |

Table 5- Quantitation Limit (LOQ) of Curcumin and Piperine- Based on Signal-to-Noise

| S. No. | Sample | Height | Respective concentration | Signal-to-Noise ratio |
|---|---|---|---|---|
| 1 | Blank | 4.8 | - | 52.1/ 4.8 = 10.8<br>47.9/ 4.8 = 9.98 |
| 2 | Curcumin | 52.1 | 60ng | |
| 3 | Piperine | 47.9 | 14ng | |

## 4. Conclusion

The developed HPTLC technique is precise, specific and accurate. Statistical analysis proves that the method is repeatable and selective for the analysis of Curcumin and Piperine in syrup simultaneously. The method can be used to determine the purity of the drug available from various sources by detecting the related impurities.

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