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# Development and Validation of HPTLC Method for Determination of Vasicine in Polyherbal Cough Syrup

**Research article**

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## Abstract

A new and simple HPTLC method was developed and validated for the quantitative estimation of biologically active compound vasicine in polyherbal Cough Syrup. TLC aluminium plates precoated with silica gel 60F-254 (0.2 mm thickness) were used. The linear ascending development was carried out in twin trough glass chamber saturated with mobile phase Ethyl acetate: methanol: ammonia (8.0 : 2.0 : 0.2 v/v/v) and densitometric determination was carried out by TLC scanner (CAMAG) at 254 nm in reflectance/absorbance mode. The Rf value of vasicine was found to be 0.54 ± 0.03. Linearity was found to be in the concentration range of 200 ng to 1600 ng. The linear regression data for the calibration plots showed a good linear relationship with $r^2$=0.99 for vasicine. According to the ICH guideline the method was validate for accuracy, precision, recovery, robustness and ruggedness. The vasicine content quantified from polyherbal formulation (Cough Syrup) was found well within limits. The proposed method is accurate, precise, reproducible, and can be adopted for routine analysis of vasicine from polyherbal cough syrup by HPTLC.

**Key words**: HPTLC, vasicine, polyherbal cough syrup, *Adhatoda vasica*

## Introduction

*Vasaka* (Syn: *Adulsa*) consists of fresh & dried leaves of plant *Adhatoda vasica* Nees Fam: Acanthaceae. The plant is distributed all over the India & plains of India and in lower Himalayan range (1). Dried leaves of *vasaka* are used in many *Ayurvedic* preparations for treatment of affections of respiratory tract either in decoction or powder form. It mainly acts as expectorant & bronchodilator & helps in liquefying the sputum so that is brought up more easily (2). The polyherbal syrup containing *vasaka* is used to treat cough and cold. The drug mainly contains bioactive pyrralazoquinazoline alkaloids vasicine and vasicinone along with other alkaloids as vasicol, adhatonine, vasicinone, vasicinol, vasicinolone etc. in minor quantities (3-6). The chief alkaloid vasicine is reported in all parts of the plant, the highest being in inflorescence (7). Vasicine reported the expectorant, diuretic, antispasmodic, asthman, bronchitis cough activity (8-11).

In the present study, HPTLC method was developed and validated for the quantitative estimation of biologically

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active compound vasicine in polyherbal Cough Syrup developed by Baidyanath Life Sciences Pvt.Ltd, Nagpur, India

As the literature survey (12-18) clearly reveals that there is no proper analytical method available for the quantitative estimation of vasicine in polyherbal cough syrups. The proposed method overcomes the problems regarding sample preparation of *Ayurvedic* syrup formulations which are commonly faced by the analyst.

Thus the present study aims to develop a rapid, efficient and reproducible method of analysis for vasicine in polyherbal cough syrup by HPTLC. The proposed method was optimized and validated as per the International Conference on Harmonization (ICH) guidelines (19).

## Materials and Methods

### Composition of polyherbal cough syrup:

| Sr. No. | Ingredients | Quantity (Each 10 ml contains) |
|---|---|---|
| 1 | *Sunth* | 300 mg |
| 2 | *Baheda* | 100 mg |
| 3 | *Pipali* | 300 mg |
| 4 | *Vasaka* | 450 mg |
| 5 | *Tulsi* | 250 mg |
| 6 | *Lisora* | 150 mg |
| 7 | *Mulethi* | 150 mg |
| 8 | *Haridra* | 150 mg |
| 9 | *Kankol* | 50 mg |
| 10 | *Kumari* | 150 mg |

### Drugs and Chemicals:

Reference standard vasicine (>97%) is purchased from Spic pharmaceuticals, India. Analytical grade of Chloroform, Ethyl acetate, Ammonia, and Methanol were purchased from Merck Chemicals, India. Stationary phase was pre-coated silica gel aluminium plate 60 $F_{254}$ was obtained from Merck, Germany.

### Preparation of standard stock solution:

Accurately weighed 1mg of vasicine was dissolved in 2 ml methanol and was sonicated and diluted with methanol up to 10 ml (100ng/µl).

### Preparation of test solution of polyherbal cough syrup:

Polyherbal cough syrup 50 ml was diluted with 50ml of distilled water. The solution was acidified with dilute Hydrochloric acid. The resulting solution was extracted in a separating funnel with chloroform (3X25 ml).The chloroform layer was rejected. The aqueous solution was basified with dilute ammonia solution and the resulting solution was again extracted with chloroform (3X25ml). All chloroform layers were combined and evaporated. The solution was reconstituted to 5 ml (12882000ng/µl) with methanol and filtered by whatman filter paper No.1.The filtrate was used for estimation of vasicine.

### Chromatographic condition:

The samples were spotted in the form of bands, width 6 mm with a Camag 100 microlitre sample (Hamilton, Bonaduz, Switzerland) syringe on silica gel pre-coated aluminum plate $60F_{254}$ plates, (20cm × 10 cm with 250 µm thickness; (E. Merck, Darmstadt, Germany) using a Camag Linomat V (Switzerland) sample applicator. The plates were prewashed with methanol and activated at 110˚C for 5 min prior to chromatography. A constant application rate 0.1µl/s was used and the space between two bands was 10 mm. The slit dimension was kept at 6 mm × 0.45 mm and the scanning speed was 20 mm/s. The monochromatic bandwidth was set at 20 nm, each track was scanned three times and baseline correction was used. The mobile phase was Ethyl acetate: methanol: ammonia (8.0: 2.0: 0.2 v/v/v). Linear ascending development was carried out in a 20 cm × 10 cm twin trough glass

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chamber (Camag, Muttenz, Switzerland) saturated with the mobile phase. The optimized chamber saturation time for the mobile phase was 30 min at room temperature (25˚C ± 2) at relative humidity 60 % ± 5. The length of each chromatogram run was 8 cm. Following the development, the TLC plates were dried in a current of air with the help of an air dryer. Densitometric scanning was performed using a Camag TLC scanner III in the reflectance/absorbance mode at 254 nm and operated by CATS IV CAMAG software. The source of radiation used was deuterium lamp emitting a continuous UV spectrum between 190and 400 nm. Concentrations of the compounds were determined from the intensity of the diffused light. Evaluation was by peak areas with linear regression. The amount of vasicine was computed from peak areas.

A standard solution of vasicine in methanol (100ng/µl) was applied in 2, 4, 6, 8, 10, 12, 14 and 16 µl, on the TLC plate to prepare linear calibration curve.

## Results and Discussion

### Mobile phase development:

The mixtures of several mobile phases were tried. The solvent system Ethyl acetate: methanol: ammonia (8.0: 2.0: 0.2 v/v/v) was selected for estimation of vasicine, which gave good resolution. Figure 1 is showing chromatographic separation of vasicine at Rf 0.54. Figure 2 is showing chromatographic separation of vasicine in polyherbal cough syrup. The absorption spectrum of vasicine is shown in Figure 3. The wavelength 254 nm was used for quantification of sample.

### Calibration curve:

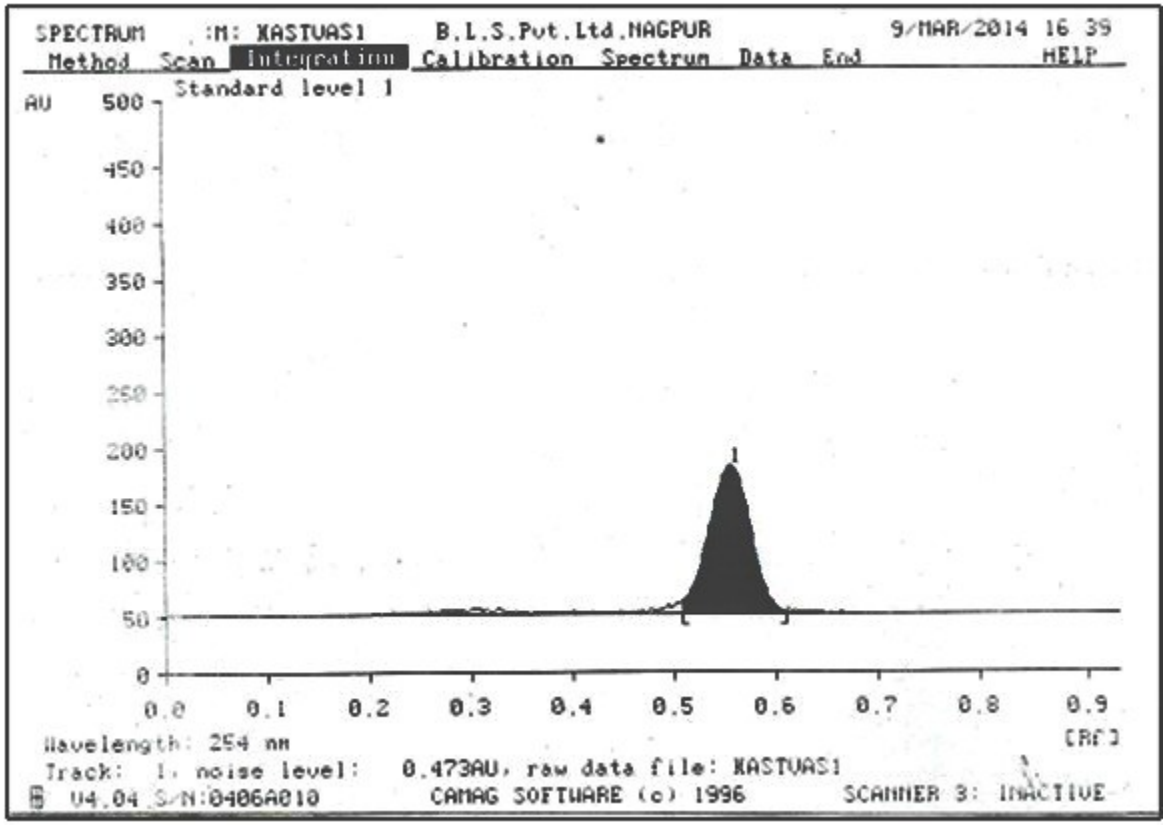


**Figure 1: Representative densitogram of standard Vasicine.**

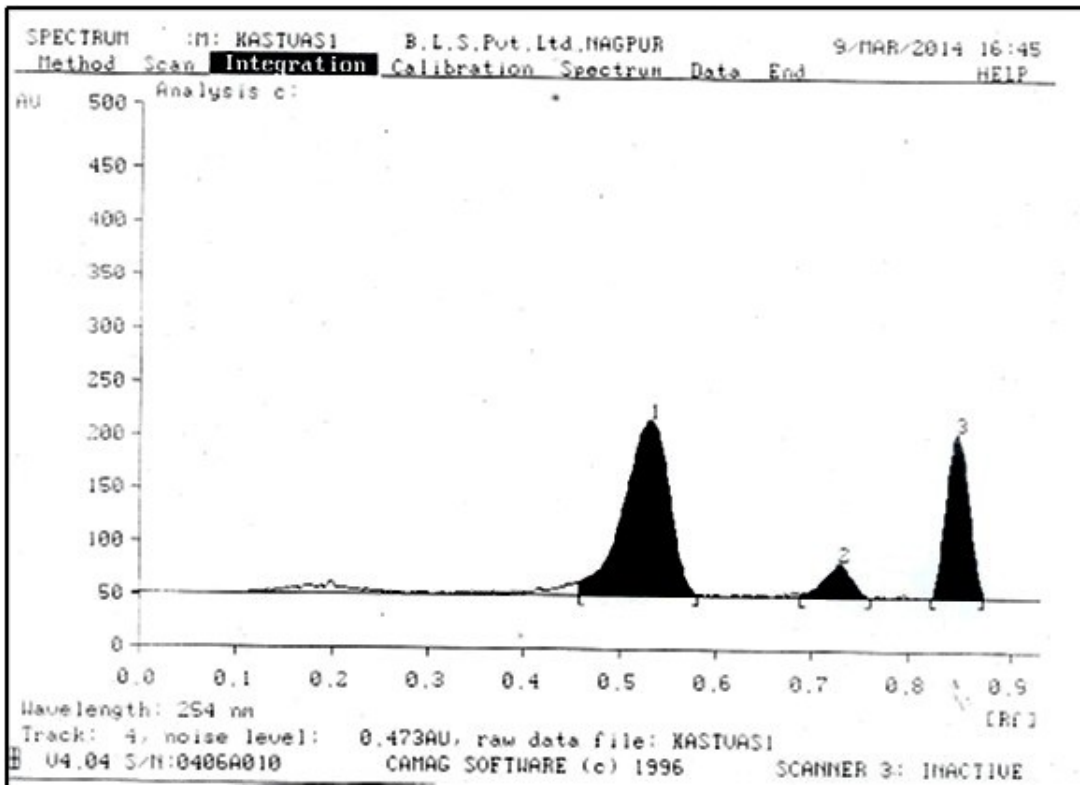


**Figure 2: Representative densitogram of Polyherbal Cough Syrup**

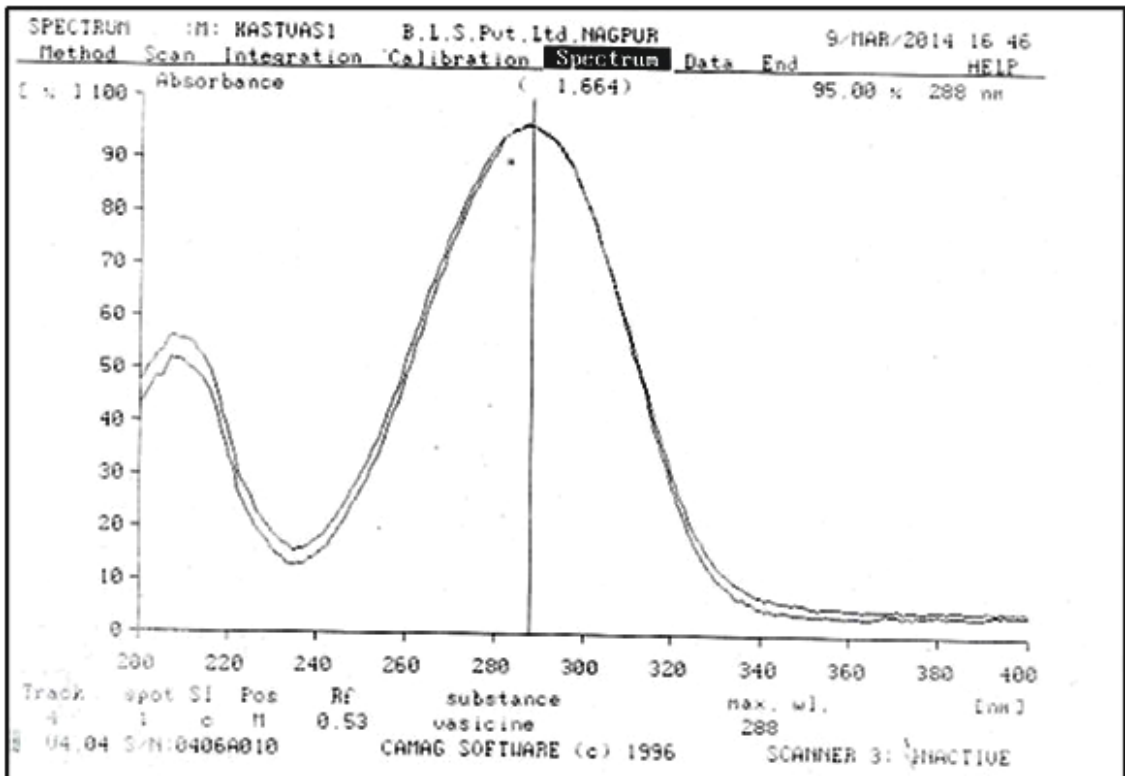


**Figure 3: UV Overlain Spectrum of Standard Vasicine and Polyherbal cough syrup.**

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**Method validation:**

**Specificity:**

The specificity of method was ascertained by standard vasicine and sample polyherbal cough syrup. The solutions of standard vasicine and sample polyherbal syrup were spotted on TLC plate in triplicate and run. The spot for vasicine in the samples was confirmed by comparing the Rf values and spectrum with standards. The validation parameters for the proposed method are shown in Table 1.

**Linearity:**

Calibration curve plots of vasicine peak area against concentration were linear in the range 200 -1600 ng/spot. The calibration line was represented by linear equation Y= 5.049x + 448.0 for vasicine. For this equation the correlation coefficient, $r^2$ was 0.99 for vasicine.

**Precision:**

The repeatability of sample application and measurement of the peak area was expressed in terms of % RSD. The % RSD was found to be less than 2.0 in all cases indicate no significant variations in the analysis of vasicine at the concentration of 600, 800 and 1000 ng/spot.

**Robustness:**

The estimation was performed by introducing variations in the mobile phase distance development; the effects on the results were examined. Mobile phase development distance was changed by ± 5mm. The saturation time of mobile phase in the chamber was varied by ± 5 min. The % RSD was found to be less than 1.0 in all cases indicates no significant variations in the analysis of vasicine at the concentration of 600 ng/spot.

**Ruggedness:**

The estimation was performed by changing the analyst, the % RSD was found to be less than 1.0 in the analysis of vasicine at the concentration of 400 ng/spot.

**Accuracy:**

The accuracy was studied by the standard addition technique. Three different levels of standard were added to the previously analyzed samples, each level being repeated thrice. The percentage recovery of vasicine was 98.98% in polyherbal cough syrup.

**Table 1: Validation parameter for vasicine by HPTLC**

| Sr. No | Parameters | Vasicine |
|---|---|---|
| 1 | Linearity range | 200 ng-1600 ng |
| 2 | Correlation coefficients | 0.99 |
| 3 | Regression equation (y = mx+c) | Y = 5.049x + 448.0 |
| 4 | Accuracy(mean recovery) | 98.98% |
| 5 | Precision (RSD)<br>i. Interday<br>ii. Intraday | <br>0.27<br>0.47 |
| 6 | Ruggedness/Robustness(RSD) between two experiments<br>i. Development distance<br>ii. Saturation time<br>iii. Analyst | <br>0.44<br>0.47<br>0.47 |
| 7 | specificity | Specific |

**Table 2: Result and Statistical data for recovery study of vasicine**

| Sr. No | Vasicine in sample (ng) | STD added (ng) | Total amount | Actual amount | % recovery | Mean % |
|---|---|---|---|---|---|---|
| 1 | 103056.0 | 100 | 103156.0 | 100886.56 | 97.8 | |
| 2 | 103056.0 | 150 | 103206.0 | 102277.14 | 99.1 | 98.98 |
| 3 | 103056.0 | 200 | 103256.0 | 103307.62 | 100.05 | |

**Table 3: Estimation of vasicine in polyherbal cough syrup**

| Component | Amount taken (µg/spot) | Peak area Mean ± S.D. | % R.S.D. | Amount found (ng) | % Amount found |
|---|---|---|---|---|---|
| Vasicine | 103056 | 6624.2±18.2 | 0.26 | 1223.25 | 0.0011 |

**Conclusion:**

The newly developed method was found to be simple, specific, precise, rapid, and reproducible; can be used for quantification of vasicine in routine quality control of polyherbal cough syrup.

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