

Phytochemical standardization of Vasicine in cough syrup by high performance liquid chromatography-densitometry

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Abstract: The highly oriented modern detection techniques provide a precise and definite tool for investigation in natural medicines. Current study directed the standardization of eminent biomarker Vasicine in a natural cough syrup. A highly accurate and precise method of High-performance thin layer chromatography (HPTLC) has been developed to certify the quantity of vasicine inside the syrup. Ethyl acetate, chloroform, ethanol and ammonia (6:3:1: 1 v/v) were mobile phase for the study. The TLC plate silica gel G60F₂₅₄ was used with CAMAG Scanner III and CAMAG Linomate 5. The detected *R_f* value was 0.51 in both sample and reference standard at 254 nm. International conference of Harmonization (ICH) guidelines were followed for the validation of the developed method. Linearity was achieved in the range of 200µg to 1600µg with co-efficient correlation $r^2=0.9995$. Accuracy was found in between 98.9 to 101.4% however precision was good at both inter and intra-day. As per the standardization of ICH, the developed method was found to be reproducible and showed sharp similar peak with high resolution.

Keywords: Poly herbal syrup, biomarker, HPTLC, vasicine.

INTRODUCTION

Traditional medicine is considered to be the most ancient technique of healing (Humber, 2002). It has postulated that more than two third of American are using alternate therapy for the treatment. More than 1500 herbs are sold for the purpose of traditional medicine and dietary supplements (Organization, 2001). Now the pharmaceutical marketing is moving towards the natural and herbal remedies after the discoveries and development of natural medicines (Seidl, 2002). The global herbal market stands at \$60 billion annually and is expected to increase at an average annual growth rate of 6.4% (Ansari and Inamdar, 2010). As per WHO, the natural herbal medicine gained a market value of \$ 43 billion per year (Aschwanden, 2001).

Natural products have supreme importance due to therapeutics applicability and are a supreme reservoir due to its structure and chemical assortment. Recent pharmacopeia reveals that 120 different chemical substances works as lifesaving medications (Goswami *et al.*, 2002).

Approximately 80% of the total world population seeks herbal medicine as their source of primary health care as a consequence of limited access to modern medicine and poverty, particularly, in the developing countries (Bodeker

et al., 2005, Mukherjee, 2002, Farnsworth *et al.*, 1985, Drugs, 1994). Standardization of herbal medicine is important for definite qualitative and quantitative values, quality, precision, safety and reproducibility within the technical standards (Folashade *et al.*, 2012). The herbal medicinal have lot of variation due to several factors including genotype, chemo type, ecotype, drying and storage conditions etc. As per the need, WHO raised the concern on quantification of biomarkers inside the herbs for quantification of the chemical constituents and fingerprint profiles. Modern detection techniques have made many questions unveil with the help of technologies including mass spectrometry (MS), high performance liquid chromatography (HPLC), gas chromatography (GC) and high performance thin-layer chromatography (HPTLC) etc.

The intent to design present study was to evaluate the essential biomarker from polyherbal cough syrup which is prescribed for the treatment of cough in both children and adult with the particular dose. The in-vitro anti-oxidant and reducing activity has been determined (Rehman *et al.*, 2016) with the good coverage of clinical efficacy in each quartile (Rehman *et al.*, 2017a, Rehman *et al.*, 2017b, Khanum *et al.*, 2017). The syrup contains *Cordia latifolia*, *Piper longum*, *Adhatoda vasica*, *Hyssopus officinalis*, *Glycyrrhiza glabra*, *Viola odorata* and *Alpinia galangal* (Rehman *et al.*, 2017b). The biomarker vasicine is the important biomarker in syrup due to its bronchodilator and expectorant activity and produce strong anti-tussive

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effects. It also helps in other respiratory disorders including asthma, common cough, whooping cough and cold (Rayees *et al.*, 2014, Jaradat, 2005).By means of HPTLC, enough work has not published yet for poly herbal syrup. Therefore, this study is designed towards the essential biomarker estimation to quantify the presence as per standard guidelines and practice.

MATERIALS AND METHODS

Composition of syrup

Each 5ml contains

| Name of active ingredient | Herb: Thick extract ratio |
|---|---------------------------|
| Thick aqueous extract of <i>Adhatoda vasica</i> Nees. | 5 : 1 |
| Thick aqueous extract of <i>Glycyrrhiza glabra</i> L. | 4 : 1 |
| Thick aqueous extract of <i>Piper longum</i> L. | 4 : 1 |
| Thick aqueous extract of <i>Hyssopus officinalis</i> L. | 4 : 1 |
| Thick aqueous extract of <i>Alpinia galanga</i> (L.) Wild | 4 : 1 |
| Thick aqueous extract of <i>Viola odorata</i> L. | 4 : 1 |
| Thick aqueous extract of <i>Mentha piperita</i> L. | 4 : 1 |

Chemicals

Ethyl acetate, chloroform, ethanol and ammonia from Merck Pakistan were used as a mobile phase. Vasicine as a reference standard from Sigma-Aldrich GmbH (Germany) were used while the remaining reagents and chemicals used in the experiment were of high analytical grade.

Apparatus

CAMAG Scanner III with fixed HPTLC plate of 20 x10 cm, CAMAG Linomate 5 or Equivalent by CAMAG, Muttenz, Switzerland. HPTLC silica gel G60F₂₅₄ pre coated with 0.2 mm thickness BY Merck and installed software linked with win CATS by CAMAG, 100µl syringe with the settings band length 6 mm, distance between band 20 mm.

Sample preparation

Total 20 tablets were dissolved in 50 ml of water. The resultant solution was then transferred into a 250ml separating funnel and 25 ml of chloroform and 3 ml of hydrous ammonia was added to the funnel. It was shaken carefully for 3 minutes. Once the layers were separated, the lower layer i.e. the chloroform layer was filtered with the aid of a filter paper with anhydrous sodium sulphate (about 10 g) in a round bottom flask of 500ml capacity.

Alkaloid extract and 25ml of chloroform was again added to the top water layer and was repeated four times using 25 ml portions (5 times in total). The chloroform extract was then transferred into the same round bottomed flask. Filtration was carried out using sodium sulphate and was washed out with 25 ml of chloroform and was added to the combined extract. Water bath under vacuum was employed to dry out the combined chloroform extract. The sample solution was then obtained by dissolving the dry residue in 5ml of ethanol.

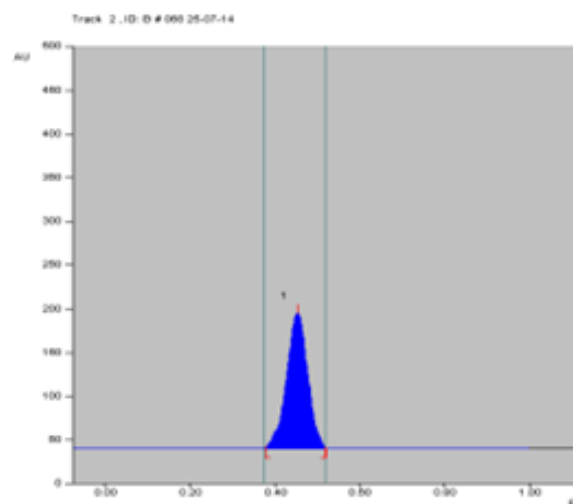


Fig. 1: Vasicine Standard

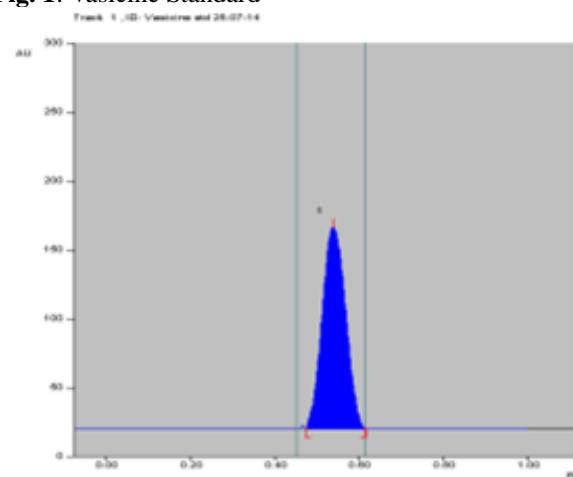


Fig. 2: Vasicine Sample

Preparation standard solution of Vasicine

1.4 mg of Vasicine was weighed and dissolved in small volume of methanol. The solution was then transferred into a 10ml volumetric flask. The remaining volume to the mark was adjusted using methanol.

Application procedure

Using CAMAG Linomat 5 (automated spray on applicator equipped with a 100µl syringe and operated with the settings band length 6 mm, distance from the bottom of the plate 15 mm, distance from the plate side

Table 1: Regression Equation

| Drug | Regression Equation | Correlation coefficient (r^2) |
|----------|---------------------|-----------------------------------|
| Vasicine | $Y=315.41x+97.38$ | 0.9995 |

Table 2: Accuracy of Vasicine

| Drug | RSD % | Recovery % |
|----------|-------|------------|
| Vasicine | 0.64 | 100.46 |
| | 0.33 | 98.9 |
| | 0.01 | 101.4 |

Table 3: Inter and Intra-day precision for Vasicine

| Drug | Inter-day | | Intra-day | |
|----------|-----------|------------|-----------|------------|
| | RSD% | Recovery % | RSD% | Recovery % |
| Vasicine | 0.36 | 100 | 0.08 | 99.9 |
| | 0.35 | 99.98 | 0.23 | 100 |
| | 0.003 | 99.6 | 0.36 | 100.1 |

edge 20 mm and distance between band 20 mm) 2 spots (in the form of band) of each i.e. sample and standard solution were applied on the same plate.

TLC development

The sample HPTLC plate was immersed in a CAMAG glass chamber having dimensions 20 x 10 cm. The glass chamber contained the solvent system ethyl acetate, chloroform, ethanol and ammonia (6:3:1:1). Sufficient time was given for the plate to develop or at least a distance of 8 to 9cm was travelled by the solvent system. The developed plate was dried using fume cupboard where it was placed for 10 minutes. Additional drying was achieved via hot air oven for 5 min at 105°C.

TLC scanning

The plate was scanned using densitometer. Linear scanning was performed at 254 nm. Scanning was performed via TLC Scanner III CAMAG with D₂&W absorption. The spot areas of the sample similar to that of the standard were then investigated.

The amount of Vasicine per 5ml of syrup was calculated by the following formula:

$$X = \frac{A_{SMP} \times W_{STD} \times \text{Sample dilution} \times P}{A_{STD} \times \text{Standard dilution} \times W_{SMP}} \times 100$$

W_{STD} – Standard weight, mg

W_{SMP} – Preparation weight, gm

A_{SMP} – Mean value of peak area of tested solution samples

A_{STD} – Mean value of peak area of standard solution samples

P – Actual vasicine content in standard sample, 98%

Validation

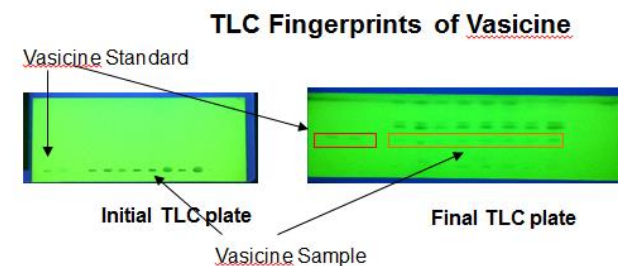
As per International conference of Harmonization (ICH) of technical requirement for pharmaceutical product registration for human use, the method had been validated

for specificity, selectivity, range, linearity, accuracy, recovery precision, robustness and ruggedness.

RESULTS

Development of Mobile Phase

For the development of mobile phase several mobile phase was tried and the solvent system with Ethyl acetate: chloroform: ethanol and ammonia in the ratio of (6:3:1:1 v/v/v/v) was selected for the estimation of Vasicine. The R_f value of standard was compare with the R_f of vasicine and was found to be 0.55, in both standard and poly herbal syrup as shown in fig. 1 and 2. The quantification of sample was carried out at wavelength 254nm.

**Fig. 3:** TLC figure print of Vasicine

Selectivity and specificity

The method was selected for the establishment of the peak of Vasicine in poly herbal formulation. The method demonstrates good resolution with free of interference from other excipients present in the formulation and thus the method is specific for this formulation as shown in fig. 4.

Range and linearity

The range of Vasicine against concentration was found linear in the range of 200 to 1600 ng/spot. As per equation the calibration line represented by liner equation as

$Y=315.41x+97.38$. By using this equation, the correction coefficient was found to be 0.9994 as shown below

Accuracy and recovery

As per standard addition technique, three different readings on different time along with different personals were taken as presented in table 2. The percentage recovery of herbal syrup was found to be 98.9 to 101.4 % as shown in table below

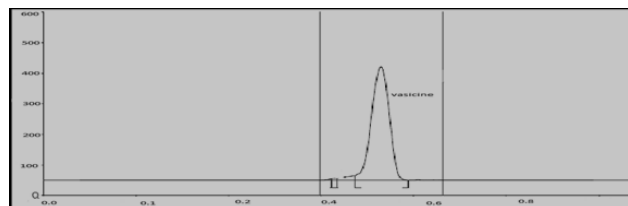


Fig. 4: Vasicine in dosage form

Precision

The precision was evaluated on the basis on intra and inter-day with 2 different samples of syrup as shown in table 3. The intra and inter day precision was found to be ≤ 1.4 (under acceptable range). The method was found to be accurate and precise at the conc. of 600, 800 and 1000 ng/spot.

Robustness and ruggedness

On the basis of mobile phase distance development, the results were analysed. The distance of mobile phase and saturation time of mobile phase were ± 5 mm and ± 5 min. As a result, the % RSD was 1.0 in all cases. It indicates that there are no significant variations in the analysis of Vasicine at the concentration of 600 ng/spot. For determination of ruggedness, performance was planned with change of analyst and % RSD was found to be less than 1.0 in the analysis of vasicine at the concentration of 400, 600 and 1000 ng/spot.

CONCLUSION

By quantitative estimation by HPTLC, the developed method was found to be highly accurate, precise, repeatable and reproducible. Quantitative estimation proved the presence of biomarker Vasicine in poly herbal cough syrup which shows its effectiveness for the labelled indication against cough.

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