Development of validated HPTLC method for quantification of Jatamansone in Jatamansi oil

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In the present study an attempt has been made to develop a new, simple, sensitive, precise and robust high-performance thin layer chromatographic (HPTLC) method for the quantitative estimation of Jatamansone in a steam distilled extract of rhizomes of Nardostachys jatamansi (Family: Valerianaceae). The rhizomas covered with fibres are considered bitter tonic, antispasmodic, hepatoprotective, emmenagogue and stomachic and prescribed in the treatment of hysteria, epilepsy, chest pain, convulsions and palpitation of the heart. They also possess neurotrophic effects which improves cognition. It is also considered as an alternative drug in the treatment of Alzheimer’s disease, parkinson’s and related disorders. A steam-distilled volatile oil obtained from the rhizomes contained 72 components. Various sesquiterpenes, lignans, and neolignans have been reported to be present in the roots of the plant. The oil is composed of eight monoterpenes (2.0%) and 25 sesquiterpenes (66.0%). Various sesquiterpenes (such as Jatamansic acid and Jatamansone) have been reported to be present in the roots of the plant.

Jatamansone has been analyzed by gas chromatography so far but not with HPTLC. Recently HPTLC is widely employed for the quantification of many components. The methodology has been applied for the first time for estimation of Jatamansone in Nardostachys jatamansi. HPTLC technique has an advantage that it provides visualization of the separated components of the sample. It also provides on line identification of analyte by in-situ spectrum scanning and post chromatographic derivatization with anisaldehyde-sulphuric acid and Rf comparison with the standard. It requires very little sample clean-up since the layer is disposable. Several samples can be run simultaneously using small quantities of mobile phase (eco-friendly) thus reducing the time and cost per analysis. The present work describes a simple, sensitive, specific and reproducible HPTLC method for the quantification of Jatamansone in Nardostachys jatamansi. 

EXPERIMENTAL

Chemicals

All chemicals including solvents were of analytical grade from E. Merck, India. The HPTLC plates Silica 60F254 (20 cm·20 cm) were purchased from E. Merck (Darmstadt, Germany).

Plant material:

Rhizomes of Nardostachys jatamansi (Valerianaceae) were purchased from a recognized and licensed ayurvedic store in Mumbai (India) and were identified and authenticated by the Department of Pharmacognosy, Bharati Vidyapeeth’s college of Pharmacy, Navi Mumbai. The collected rhizomes were cleaned, air dried and powdered.

Extraction of oil:

The air dried powdered rhizomes of Nardostachys jatamansi was subjected to steam distillation. After 6hours, the oil was collected. The amount of oil collected was rich in sesquiterpenes.

Preparation Of Standard Solution

Stock solution of Jatamansone 1mg/10ml was prepared using methanol and different amounts (250ng - 1750mcg) of these were loaded on a TLC plate.
silica gel HPTLC 60F254 (10 · 10 cm) plate of 0.20 mm layer thickness. Samples and standards were applied to the plate as 6 mm wide bands with an automatic TLC sampler (ATS 4) under a flow of N2 gas, 8mm from the bottom and 8mm from the side and the space between two spots was 5mm of the plate. The linear ascending development was carried out in a CAMAG twin trough chamber (10 cm · 10 cm) which was presaturated with 15 mL mobile phase Petroleum ether: acetone (3:1 v/v) for 60 min at room temperature (25 _C ± 2 _C) and 50% ± 5 relative humidity. The length of the chromatogram run was 9 cm. Subsequent to the development; TLC plates were dried in current air with the help of an air dryer in a wooden chamber with adequate ventilation. The flow of air in the laboratory was maintained unidirectional (laminar flow, towards exhaust). The post chromatographic derivatization was carried out by air drying for 10 min. Quantitative evaluation of the plate was performed in absorption-reflection mode at 285 nm², using slit width 6 · 0.4 mm and data resolution 100 lm/step and scanning speed 20 mms/1 with a computerized CAMAG TLC Scanner-3, winCATS software version 1.2.3. Quantification of Jatamansone in N. jatamansi was performed by the external standard method, using Jatamansone as standard. Each sample analysis was carried out in triplicate.

CALIBRATION CURVE

Stock solution of Jatamansone (1 mg/ 10 mL) was prepared in methanol and different amounts (250ng – 1.75mcg) of these were loaded on a TLC plate, using ATS4 for preparing six point calibration graph. Spots corresponding to 250, 500, 750, 1000, 1250, 1500ng/spot were spotted on precoated TLC plate, using semiautomatic spotter under nitrogen stream. The TLC plate was developed, dried by hot air and photometrically analyzed as described earlier. The calibration curve was prepared by plotting peak area versus concentration (ng/spot) corresponding to each spot.

RESULTS AND DISCUSSION

For the analysis of Jatamansi oil, Gas chromatography and UV-visible spectrophotometry have been used. But this analytical method helped in detection of chemical constituent and ?max for the same. In the spectrophotometry have been used. But this analytical method helped For the analysis of Jatamansi oil, Gas chromatography and UV-visible spectrophotometry have been used. But this analytical method helped in determining the amount of jatamansone present in the jatamansi oil. Jatamansone being highly soluble in methanol, methanol was selected for preparing its standard solution. Jatamansi oil also being highly soluble in methanol, it was used for preparing the dilutions too. Many dilutions of jatamansi oil was prepared and its concentration was made such that its peak area coincides the standard jatamansone and thereby quantifying the amount of jatamansone. The plates were prewashed with methanol to remove any adsorbed impurities and dried at 50C for 5min. Different mobile phases, viz toluene : ethylacetate; petroleum ether - diethyl ether, petroleum ether - ethyl acetate, and petroleum ether – acetone in different proportions were investigated. Of these mixture petroleum ether: acetone solution was selected as mobile phase because it gave a sharp peak for Jatamansone (Rf = 0.45).

It was observed that presaturation of chamber with mobile phase for 1hr ensures good separation and reproducibility. Scanning of jatamansone and jatamansi oil was performed at the wavelength of maximum absorbance of jatamansone 285nm. The method was validated for in terms of accuracy, specificity, linearity, limit of detection and limit of quantification. The peak areas for different concentration of jatamansone were found to be linearly dependent on the concentration in the range of 250-1500ng/spot (y = 9.7233x + 2.9643). Similarly jatamansi oil was found to be linear between the range 1.25-3.0mcg/spot. It was finally quantified that jatamansone present in jatamansi oil was found to be linear between the range 1.25-3.0mcg/spot. It was finally quantified that jatamansone present in jatamansi oil was found to be linear between the range 1.25-3.0mcg/spot. It was finally quantified that jatamansone present in jatamansi oil was found to be 25ng/ spot and 250ng / spot, respectively. The intra-day and inter-day precision of the method were found to be in terms of %CV and were 0.83 – 3.4% and 1.2 – 5.2%, respec-
The method was found to be accurate. The mean recovery was in the range 96.44 – 103.4%. A high degree of correlation (r=0.9980) was observed between the spectra of jatamansone scanned at the peak start, peak apex, and peak end positions, indicating the purity of the peak. The method validation parameters are as follows:

<table>
<thead>
<tr>
<th>S.no</th>
<th>Parameters</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Linearity</td>
<td>r = 0.9980</td>
</tr>
<tr>
<td>2.</td>
<td>Linearity range</td>
<td>250 – 1500 ng/spot</td>
</tr>
<tr>
<td>3.</td>
<td>Limit of detection</td>
<td>25ng/spot</td>
</tr>
<tr>
<td>4.</td>
<td>Limit of quantification</td>
<td>250ng/spot</td>
</tr>
<tr>
<td>5.</td>
<td>Precision (%CV)</td>
<td></td>
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<tr>
<td></td>
<td>Repeatability of measurements (area)</td>
<td>3.4</td>
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<tr>
<td></td>
<td>Inter-day</td>
<td>1.2 - 5.2%</td>
</tr>
<tr>
<td></td>
<td>Intra-day</td>
<td>0.83 - 3.4%</td>
</tr>
<tr>
<td>6.</td>
<td>Accuracy (%)</td>
<td>97.8 – 102.24%</td>
</tr>
<tr>
<td>7.</td>
<td>Specificity</td>
<td>Specific</td>
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</table>

**Analysis of jatamansi oil**

The extracted oil was analyzed by the developed HPTLC method. The average values obtained for Jatamansone is 20.32%.

**Identification of spots of jatamansone**

The 1st spot with 1.25mcg/spot of jatamansi oil was identical with standard jatamansone (Rf= 0.43) containing 250ng/spot and so on from the figure 1 and 2.

**CONCLUSIONS:**

The HPTLC method developed here for the quantification of Jatamansone in N. jatamansi is simple, rapid, cost-effective and easily adaptable for screening and quantitative determination than any other analytical technique.

**REFERENCES:**

5. Vidya S. Raoa,*, Anjali Raob, K. Sudhakar Karantha; Anti-convulsant and neurotoxicity profile of Nardostachys jatamansi in rats; *Journal of Ethnopharmacology* ; 102 (2005), 351-356.

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