DEVELOPMENT AND VALIDATION OF HPTLC METHOD FOR DETERMINATION OF 6-GINGEROL IN SOFT GELATIN CAPSULE CONTAINING GINGER OLEORESIN

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Abstract
A sensitive and reliable HPTLC method has been developed for quantitative estimation of 6-gingerol in the soft gelatin capsule containing Ginger oleoresin. Chromatography was performed on silica gel 60 F254 percolated TLC plate using n-hexane: ether (4.0: 6.0, v/v) as solvent system and densitometric determination was carried out by TLC scanner (CAMAG) at 254 nm in reflectance/absorbance mode. The Rf value of 6-gingerol was found to be 0.27 ± 0.01. Linearity was found to be in the concentration range of 200 ng to 1400 ng. The linear regression data for the calibration plots showed a good linear relationship with r²=0.997 for 6-gingerol. The accuracy of the method was checked by conducting recovery studies at three different levels, using the standard addition method. The average recovery of 6-gingerol was found to be 99.96%. The proposed HPTLC method provide a good resolution of 6-gingerol and can be used for quantification of 6-gingerol present in soft gelatin capsule. The method is rapid, simple and precise.

Key words: HPTLC, 6-gingerol, Soft gelatin capsule, Ginger oleoresin, Zingiber officinale.

Introduction
Adraka (Ginger or Sunthi) consists of fresh or dried rhizomes of Zingiber officinale, Family Zingiberaceae. The plant is universally known and widely cultivated all over the warm parts of India.1 Ginger and ginger oleoresin is used in many Ayurvedic preparations. The drug undertaken for the study is ginger oleoresin. The oleoresin is obtained by method of percolation of the powdered rhizome of Zingiber officinale. It contains pungent, as well as non pungent principles of gingers. Fresh sample of gingerin contains gingerols mainly 6-gingerols, shogaols, and zingerone.2-6 Zingiber officinale reported as an anti-platelet aggregator, stomachic, anti-emetic, anti-inflammatory and effective in motion sickness.7-12

As the literature survey13-19 clearly reveals that there is no proper analytical method available for the quantitative estimation of 6-gingerol in the soft gelatin capsule containing Ginger oleoresin. The proposed method overcomes the problems regarding sample preparation of Ayurvedic formulations which are commonly faced by the analyst.

So, the present study was designed for the development and validation of simple HPTLC method for the determination of 6-gingerol in soft gelatin capsule developed by Baidyanath Life Sciences Pvt. Ltd, Nagpur, India. The proposed method was validated as per ICH guidelines and its applicability for quality control purpose.20

Materials and Method
Composition of soft gelatin capsule:

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Ingredients</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Allium sativum oil</td>
<td>5 mg</td>
</tr>
<tr>
<td>2</td>
<td>Zingiber officinale oleoresin</td>
<td>5 mg</td>
</tr>
</tbody>
</table>
Drugs and Chemicals:
Reference standard 6-gingerol (>95%) is purchased from Natural Remedies Pvt ltd Bangalore. All chemicals were Analytical grade or HPLC grade and purchased from Merck Chemicals, India. Stationary phase was pre-coated silica gel aluminium plate 60 F254 was obtained from Merck, Germany.

Preparation of standard stock solution:
Accurately weighed 1mg of 6-gingerol was dissolved in 2 ml methanol and was sonicated and diluted with methanol up to 10 ml (100 ng/µl).

Preparation of test solution of soft gelatin capsule:
The contents of 20 capsules were mixed. An accurately weighed quantity of capsule contents (1.0811 g) was dissolved in chloroform (20 ml) and was sonicated for 3 min. It was filtered and evaporated on water bath (60°C) till dried. Methanol (10 ml) was added and filtered through Whatman filter paper no.1. It was evaporated on water bath till dried. The solution was reconstituted to 10 ml (108.11µg/µl) with methanol and filtered by whatman filter paper No.1. The filtrate was used for estimation of 6-gingerol.

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 60 F254 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 n-hexane: ether (4.0: 6.0 v/v). Linear ascending development was carried out in a 20 cm × 10 cm twin trough glass chamber (Camag, 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 CAMAG software. The source of radiation used was deuterium lamp emitting a continuous UV spectrum between 190 and 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 6-gingerol was computed from peak areas.

Results and Discussion
Mobile phase development:
The mixtures of several mobile phases were tried. The solvent system n-hexane: ether (4.0: 6.0 v/v) was selected for estimation of 6-gingerol, which gave good resolution. Figure 1 is showing chromatographic separation of 6-gingerol at Rf 0.27. Figure 2 is showing chromatographic separation of 6-gingerol in soft gelatin capsule. The absorption spectrum of 6-gingerol is shown in Figure 3. The wavelength 254 nm was used for quantification of sample.
Figure 1: HPTLC chromatogram of standard 6-gingerol
Figure 2: HPTLC chromatogram of soft gelatin capsule

Figure 3: overlain spectrum of standard 6-gingerol and soft gelatin capsule
Validation of Method:

The method was validated for linearity, specificity, accuracy, recovery, intra-day and inter-day precision, ruggedness, robustness, LOD and LOQ in accordance with ICH guidelines.

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

Linearity:
Linearity was performed by applying standard solution at different concentrations ranging from 200 to 1400 ng/spot on HPTLC plates. Peak areas were recorded for each concentration. Linearity curve was obtained by plotting a graph of peak area vs. applied concentration. The calibration line was represented by linear equation \( Y = 3.568x + 25.46 \) for 6-gingerol. For this equation the correlation coefficient, \( r^2 \) was 0.997 for 6-gingerol.

Specificity:
The specificity of method was ascertained by standard 6-gingerol and sample soft gelatin capsule. The solutions of standard 6-gingerol and soft gelatin capsule were spotted on TLC plate in triplicate and run. The spot for 6-gingerol 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.

Recovery:
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 results of recovery studies were expressed as percent recovery. The results are shown in Table 2.

Precision:
All the solutions were analyzed on the same day in order to record any intraday variations in the results. For Intera day variation study, three different concentrations of the standard solutions in linearity range were analyzed on three consecutive days. 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 6-gingerol at the concentration of 800, 1000 and 1200 ng/spot.

Robustness:
The estimations were performed by introducing variations in the mobile phase distance development and saturation time in development chamber; the effects on the results were examined. Mobile phase distance was changed by ±5 mm. 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 6-gingerol at the concentration of 1000 ng/spot.

Ruggedness:
The HPTLC method was evaluated by carrying out the analysis of standard solutions by two analysts using same operational and environmental conditions, the %RSD was found to be less than 1.0 in the analysis of 6-gingerol at the concentration of 1000 ng/spot.

Limit of detection (LOD) and Limit of quantification (LOQ):
The LOD and LOQ were calculated by using the values of slopes and intercepts of the calibration curve. The LOQ and LOD were calculated as 80.25 ng/spot and 243.19 ng/spot for 6-gingerol.
Table 1: Validation parameter for 6-gingerol by HPTLC

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Parameters</th>
<th>6-gingerol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Linearity range</td>
<td>200 ng-1400 ng</td>
</tr>
<tr>
<td>2</td>
<td>Correlation coefficient</td>
<td>0.997</td>
</tr>
<tr>
<td>3</td>
<td>Regression equation ( y = mx + c )</td>
<td>( Y = 3.568x + 25.46 )</td>
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<tr>
<td>4</td>
<td>Accuracy (mean recovery)</td>
<td>99.96%</td>
</tr>
<tr>
<td>5</td>
<td>Precision (RSD)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Interday</td>
<td>0.71</td>
</tr>
<tr>
<td></td>
<td>Intraday</td>
<td>0.65</td>
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<tr>
<td>6</td>
<td>Ruggedness/Robustness (RSD) between two experiments</td>
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<tr>
<td></td>
<td>Development distance</td>
<td>0.70</td>
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<td></td>
<td>Saturation time</td>
<td>0.83</td>
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<td>Analyst</td>
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<tr>
<td>7</td>
<td>Specificity</td>
<td>Specific</td>
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<tr>
<td>8</td>
<td>Limit of detection (LOD)</td>
<td>80.25 ng</td>
</tr>
<tr>
<td>9</td>
<td>Limit of quantification (LOQ)</td>
<td>243.19 ng</td>
</tr>
</tbody>
</table>

Table 2: Result and Statistical data for recovery study of 6-gingerol

| Sr. No. | Amount of Sample taken in µg/spot | Amount of 6-Gingerol present ng | Amount of 6-gingerol added ng | Total Amount of 6-gingerol ng | Total Recovery | % Recovery | Mean % Recovery |
|---------|-----------------------------------|---------------------------------|-------------------------------|------------------------------|----------------|------------|----------------|----------------|
| 1       | 1081.1                            | 1054.41                         | 200                           | 1254.41                      | 1232.20        | 98.23      | 99.96          |
| 2       | 1081.1                            | 1054.41                         | 250                           | 1304.41                      | 1309.88        | 100.42     |                |
| 3       | 1081.1                            | 1054.41                         | 300                           | 1354.41                      | 1371.34        | 101.25     |                |

Table 3: Estimation of 6-gingerol in soft gelatin capsule

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount taken (µg/spot)</th>
<th>Peak area</th>
<th>% R.S.D.</th>
<th>Amount found (ng)</th>
<th>% Amount found</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-Gingerol</td>
<td>1081.1</td>
<td>3787.6</td>
<td>0.87</td>
<td>1054.41</td>
<td>0.0975</td>
</tr>
</tbody>
</table>

Conclusion
The developed HPTLC technique is accurate, precise, specific and significant for routine analysis and quality control of soft gelatin capsule containing ginger oleoresin.

References