DEVELOPMENT AND VALIDATION OF HPTLC METHOD FOR
ESTIMATION OF GALLIC ACID AND ANDROGRAPHOLIDE IN
POLYHERBAL FORMULATION

Dhara Shingala*, Rashmi Gaudani, Bhavesh Nayak and Trupti Ghodasara


ABSTRACT

A Gallic acid and Andrographolide possesses a wide range of biological activities and is used for the treatment of liver diseases. A simple HPTLC method has been developed for the quantification of Gallic acid and Andrographolide. Analysis of Gallic acid and Andrographolide were performed on TLC aluminium plates pre-coated with silica gel 60F-254 as stationary phase. Linear ascending development were carried out in twin trough glass chamber saturated with mobile phase consisting of Toluene: Acetone: Formic acid [9: 7: 1 v/v/v] for Gallic acid and Andrographolide at room temperature [25 ± 2ºC]. Spectrodensitometric scanning was performed by TLC scanner III (CAMAG) in absorbance mode at the wavelength of 254nm for Gallic acid and Andrographolide. The system was found to give compact spots for Gallic acid (R_f value of 0.45) and Andrographolide (R_f value of 0.51). The data for calibration plots showed good linear relationship with R^2 = 0.996 for Gallic acid and R^2 = 0.996 for Andrographolide in the concentration range of 200-1200 ng/spot and 100-600 ng/spot with respect to peak area. Gallic acid and Andrographolide were found to be 0.65% and 0.47% respectively in Polyherbal (Capsule) Formulation. The method was validated for accuracy, precision and recovery. The limits of detection and limits of quantification were determined. Statistical analysis of the data showed that the method is reproducible and selective for estimation of Gallic acid and Andrographolide.

Keywords: HPTLC, Gallic acid, Andrographolide, Herbal formulation.

INTRODUCTION

Herbal medicine involves the use of plants for medicinal purposes. Herbal medicines are in great demand in the developed as well as developing countries for primary healthcare because of their wide biological activities. However, one of the impediments in the acceptance of the Ayurvedic or Herbal formulations is the lack of standard quality control profiles. The quality control of herbal medicine that is, the profile of the constituents in the final product has implications in efficacy and safety. Due to the complex nature and inherent variability of the chemical constituents of the plant based drugs, it is difficult to
establish quality control parameters and modern analytical techniques like HPTLC and HPLC are expected to help in circumventing this problem\(^1\).

*Emblica officinalis* [Family Euphorbiaceae] and *Andrographis panniculata* [Family Acanthaceae] herbs are used in treatment of liver diseases. *Emblica officinalis* is a medium-sized deciduous tree with gray bark and reddish wood which successfully grows in variable agro-climatic and soil conditions \(^2\). It contains Gallic acid, phyllemblin, phyllembic acid, ellagic acid, chebulagic acid \(^3\). Gallic acid is responsible for hepatoprotective activity. It is used for liver ailments, digestive, stomachic, laxative, diuretic, antipyretic and tonic \(^4\). This plant has been well reported to exhibit hepatoprotective activity \(^5\).

*Andrographis panniculata* is an erect, branched, annual herb with dark green stem, growing up to 1m in height \(^6\). It contains Andrographolide, neoandrographolide and deoxyandrographolide \(^7\). Andrographolide is responsible for hepatoprotective activity. It is used for liver tonic, stomachic, cholagogue, blood purifier and anthelmintic \(^8\).

In the present paper, an accurate HPTLC method for estimation of Gallic acid and Andrographolide in herbal formulations is described. The proposed method was validated in compliance with ICH guidelines.

**MATERIAL AND METHODS**

Materials: All chemicals were collected from Rankem Pvt Ltd. Gallic acid and Andrographolide Markers were collected form Eucca Enterprise Ltd., Bombay. The herbal formulation was collected from Sunrise Pvt. Ltd., Santej, Gujarat, India.

Chromatographic Specifications

HPTLC was performed on 10 cm × 10 cm TLC aluminium plates coated with 200-µm layer thickness of silica gel 60F 254 (E. Merck, Germany). Samples were applied as 8 mm width bands using Camag 100 microlitre sample syringe (Hamilton, Switzerland) with a Camag Linomat V applicator (Camag, Switzerland). Linear ascending development with Toluene: Acetone: Formic acid [9: 7: 1 v/v/v] for Gallic acid and Andrographolide respectively as mobile phase were carried out in a twin trough glass chamber [Camag] (20 × 10 cm) previously saturated with mobile phase vapour for 30 mins (optimized chamber saturation time) at room temperature (25 ± 2°C). The
development distance was 80 mm. After development plates were air-dried. Scanning was performed using Camag TLC Scanner 3 at 254nm in the absorbance mode for Gallic acid and Andrographolide and operated by Win CATS Software. Concentrations of the compound chromatographed were determined from the intensity of the diffused light. Evaluation was by peak areas with linear regression. The amount of Gallic acid and Andrographolide was computed from peak areas.

**Preparation of Calibration Curve of Gallic acid**

Standard solution of Gallic acid was prepared in concentration 100 μg/ml and 2, 4, 6, 8, 10 and 12 μl from standard solution applied in triplicate on TLC plate to obtain final concentration 200-1200 ng/spot. The plate was then developed using the optimized mobile phase and the peak areas were plotted against the corresponding concentrations to obtain the calibration curves.

**Preparation of Calibration Curve of Andrographolide**

Standard solution of Andrographolide was prepared in concentration 400 μg/ml and 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 μl from standard solution applied in triplicate on TLC plate to obtain final concentration 100-600 ng/spot. The plate was then developed using the optimized mobile phase and the peak areas were plotted against the corresponding concentrations to obtain the calibration curves.

**Sample preparation:**

Solution was prepared by dissolving 5g of the extract of Polyherbal Formulation (Livogat capsule) in methanol and the volume was made up to 50 ml to get the concentration of 100 mg/ml.

**Method validation:**

The HPTLC methods developed for Gallic acid and Andrographolide were validated and parameters like precision, accuracy, recovery, LOD and LOQ were checked as per ICH guidelines [9].

**RESULTS AND DISCUSSION**

**Development of the Optimum Mobile Phase**

The HPTLC methods developed for Gallic acid and Andrographolide were optimized. The mobile phase Toluene: Acetone: Formic acid [9: 7: 1 v/v/v] was selected for
estimation of Gallic acid and Andrographolide, which gave a good resolution, dense, compact and well-separated spots as well as a well-defined peak at $R_f$ value of 0.45 and 0.51 for Gallic acid and Andrographolide respectively. The wavelength 254nm was used for quantification of sample. These optimized HPTLC methods were used for quantification of Gallic acid and Andrographolide from Polyherbal formulation.

Validation of HPTLC method
A. Linearity
A representative calibration curve of Gallic acid and Andrographolide were obtained by plotting peak area of Gallic acid and Andrographolide against the concentration range of 200-1200 ng/spot and 100-600 ng/spot respectively. The co-efficient of determination for Gallic acid and Andrographolide were found to 0.996 and 0.996 respectively [Figure 1, 2].

B. Accuracy [% Recovery]
The % Recovery of Gallic acid and Andrographolide given in Table 1 were found to be 100.01 and 100.16 which is satisfactory.

C. Limit of Detection
The minimum detectable limits were found to be 84.27 ng/spot and 62.91 ng/spot for Gallic acid and Andrographolide respectively [Table 2].

<table>
<thead>
<tr>
<th>Marker compound</th>
<th>Amount present in the sample (ng)</th>
<th>Amount found (ng)</th>
<th>Recovery (%)</th>
<th>Average Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gallic acid</td>
<td>960</td>
<td>961.54</td>
<td>100.16</td>
<td></td>
</tr>
<tr>
<td></td>
<td>960</td>
<td>960.25</td>
<td>100.03</td>
<td></td>
</tr>
<tr>
<td></td>
<td>960</td>
<td>958.66</td>
<td>99.86</td>
<td></td>
</tr>
<tr>
<td>Andrographolide</td>
<td>480</td>
<td>471.043</td>
<td>98.13</td>
<td>100.16</td>
</tr>
<tr>
<td></td>
<td>480</td>
<td>488.278</td>
<td>101.72</td>
<td></td>
</tr>
<tr>
<td></td>
<td>480</td>
<td>483.043</td>
<td>100.63</td>
<td></td>
</tr>
</tbody>
</table>
TABLE 2: RESULTS OF LOD AND LOQ

<table>
<thead>
<tr>
<th>Standards</th>
<th>LOD (ng/spot)</th>
<th>LOQ (ng/spot)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gallic acid</td>
<td>84.27</td>
<td>280.9</td>
</tr>
<tr>
<td>Andrographolide</td>
<td>62.91</td>
<td>209.7</td>
</tr>
</tbody>
</table>

TABLE 3: % CONTENT OF GALlic ACID AND ANDROGRAPHOLIDE IN POLYHERBAL FORMULATION

<table>
<thead>
<tr>
<th>Sample</th>
<th>Content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyherbal Formulation</td>
<td>Gallic acid</td>
</tr>
<tr>
<td></td>
<td>0.65</td>
</tr>
</tbody>
</table>

Figure 1
Calibration curve of Gallic acid standard

Figure 2
Calibration curve of Andrographolide standard
Figure 3
Chromatogram of Gallic acid standard

Figure 4
Chromatogram of Andrographolide standard

Figure 5
Chromatogram of Gallic acid in Polyherbal Formulation
CONCLUSION
The proposed HPTLC method was found to be rapid, simple and accurate for quantitative estimation of Gallic acid and Andrographolide in Polyherbal Formulation. Statistical analysis proves that the method is reproducible and selective for the analysis of Gallic acid and Andrographolide. The recovery values of Gallic acid and Andrographolide were found to be about 100.01 and 100.16 respectively. For Gallic acid and Andrographolide which shows the reliability and suitability of the method. The lowest detectable limit of Gallic acid and Andrographolide were found to be 84.27 ng/spot and 62.91 ng/spot respectively. Gallic acid and Andrographolide were found to be 0.65% and 0.47% respectively in Polyherbal Formulation.

REFERENCES


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