QUANTIFICATION OF (-) EPICATECHIN BY HPTLC METHOD IN CASSIA FISTULA CRUDE DRUG, LAB EXTRACT AND COMMERCIAL EXTRACT

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ABSTRACT
A simple sensitive HPTLC method developed for the quantification of (-) epicatechin in the plant Cassia fistula crude drug, lab extract and commercial extract. The stationary phase was precoated aluminium silica gel G F 254 Plates. The mobile phase was chloroform: acetone: formic acid (75:16.5:8.5). The plate was scanned and quantified at 364 nm for (-) epicatechin. The amount of (-) epicatechin was estimated by comparing the peak area of standard and the same were present in the crude drug, lab extract and commercial extract. The content of (-) epicatechin were found to be 2.63% w/w, 5.85% w/w & 3.75% w/w for Cassia fistula crude drug, lab extract and commercial extract respectively. The method was validated in terms of linearity, accuracy and specificity. This estimation technique is very much useful for the estimation of (-) epicatechin present in the various medicinal plants.

Keywords: Cassia fistula, (-) epicatechin, Estimation, HPTLC

INTRODUCTION

Cassia fistula belonging to family Caesalpinaceae is native to India, Amazon and Sri Lanka, and is now widely cultivated worldwide. It is having moderate laxative, liver disorder, Jaundice, anti diabetic and lowers cholesterol activity. Cassia fistula leaf contains (-) epiafzelechin, (-) epiafzelechin-3-O-glucoside, (-) epicatechin, procyanidin B2, rhein, rhein glucoside, sennoside A & B, chrysophanol, physcion [1, 2, 3, 4, 5, 6]. High Performance Thin Layer Chromatography (HPTLC) is emerging as a versatile, high throughput & cost-effective technology that is uniquely suited to assessing the identity and quality of botanical materials [7, 8].

The aim of the present work is to develop a method for estimation of (-) epicatechin by HPTLC Technique simultaneously.

MATERIALS AND METHODS

Plant materials

Cassia fistula leaf material was collected at Ooty and authenticated by Dr. S. Rajan, Field Botanist, Medicinal Plant Collection and Survey Unit, Department of Ayush, Emerald, Ooty. Commercial extract of Cassia fistula was obtained from Amsar Pvt. Ltd., Indore, (M.P.). The marker compound was obtained from Natural Remedies Pvt. Ltd., Bangalore, India.

Preparation of the plant extract

Coarse powder of the dried material of Cassia fistula leaf extraction was carried out by maceration method (for 7 days) by using ethanol 90% as a solvent.

Method development of HPTLC

Standard preparation

5 mg of (-) Epicatechin was dissolved in 5 ml of methanol (1mg/ml concentration).

Sample Preparation (extracts)

Crude drug preparation: 1000 mg of powdered Cassia fistula crude drug was dissolved in 10 ml of methanol and slightly warmed on water bath and filtered through Whatman filter paper, and the same solution was used for HPTLC analysis (100 mg/ml concentration).

Extraction preparation: 1000 mg of lab extract was dissolved in 10 ml of methanol and slightly warmed on water bath and filtered through Whatman filter paper, and the same solution was used for HPTLC analysis (100 mg/ml concentration). The same procedure was followed for the preparation of commercial extract.

Chromatographic Condition

Stationary phase: Precoated Silica Gel G F 254 Plates (Merck)

Mobile phase: Chloroform: Acetone: Formic acid (75:16.5:8.5)

Saturation: 40 mins

Development chamber: CAMAG twin trough development chamber

Applicator: CAMAG Linomat IV applicator

Scanner: CAMAG Scanner III CATS (4.06), Switzerland

Mode of scanning: Absorption (deuterium)

Detection wavelength: 364 nm

Volume applied (Standard): 8 µl

Volume applied (Sample): 10 µl each sample

Procedure

Before spotting, the plates were pre-washed with methanol. Standard and samples solutions were applied to the plates as sharp bands by means of CAMAG Linomat IV applicator. The spots were dried in a current of air. The mobile phase (20 ml) was poured into a twin trough glass development chamber was left to equilibrate for 30 minits and the plate was placed in the chamber. The plate was then developed until the solvent front had travelled at a distance of 75 mm above the base of the plate. The plate was then removed from the chamber and dried in a current of air. Detection and quantification was performed with CAMAG Scanner III at a wavelength of 364 nm[9, 10, 11].

Linearity

Linearity was performed by applying standard solution at different concentration range from 1 to 5 µg/spot on 20 x 20 cm HPTLC plates, precoated silica gel G F 254 Plates (Merck) in the form of sharp 7 mm bands; the distance between two adjacent band was 8 mm. The plates were developed in a solvent system of chloroform: acetone: formic acid (75:16.5:8.5), up to a height 75 mm, at room temperature [11]. The plates were dried in air. The detector response for (-) epicatechin was measured for each band at wavelength of 364 nm, using CAMAG TLC Scanner and winCat software. The peak area of (-) epicatechin were recorded for each concentration. The linearity curve of (-) epicatechin was obtained by plotting agraph of peak area of (-) epicatechin vs applied concentration of (-) epicatechin (µg).

Method validation

The method was validated for precision, repeatability and accuracy. The precision was checked by repeated scanning of same spot of (-) epicatechin (2 µg) three times each and was expressed as relative
standard deviation (% RSD). The repeatability of the method was confirmed by analyzing 1 µg, 2 µg and 5 µg of standard (-) epicatechin solution (n = 3) and was expressed as % RSD. The precision of the method was studied by analyzing aliquots of standard solution of (-) epicatechin (1 µg, 2 µg and 5 µg/spot) on the same day (intra-day precision) and on different days (inter-day precision) and the results were expressed as % RSD [13, 14].

To study the accuracy, the recovery experiment was performed by the method of standard addition. The recovery of the added amount of standard was analyzed at three different levels. Each level of addition was repeated three times on three different days and the recovery of the added amount of standard was calculated.

Limit of detection and limit of quantitation was also calculated by the proposed method.

RESULTS AND DISCUSSION

The amount of (-) epicatechin present in the crude drug, lab extract and commercial extract of Cassia fistula were estimated by using HPTLC technique by comparing with the peak area of standard and sample. The results are given in table 1. The results reveals that the Rf of the sample Cassia fistula crude drug, lab extract and commercial extract were matching with the standard Rf of marker compound (-) epicatechin and the amount of marker compound present in the samples were calculated. The content of (-) epicatechin was found to be 2.63 % w/w, 5.85% w/w & 3.75% w/w in Cassia fistula crude drug, lab extract and commercial extract respectively.

The calibration curve was linear in the range of 1 µg to 5 µg/spot and the correlation coefficient was determined. The correlation coefficient was found to be 0.9964. The limit of quantification was found to be 3µg and the limit of detection was 1 µg. The method was validated in terms of precision and reproducible expressed as % RSD which were found to be less than 2%. The recovery values obtained were 98.28 to 100.4%, showing accuracy of the method. The average percentage recovery was found to be 99.12%.

In conclusion the developed HPTLC method was simple accurate, precise, economic and can be utilised for the routine analysis and quantitative determination of (-) epicatechin in Cassia fistula.

Table No. 1. HPTLC quantification of (-) Epicatechin in Cassia fistula

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Sample</th>
<th>Standard Rf values</th>
<th>Sample Rf values</th>
<th>Amount of Marker Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cassia fistula crude material</td>
<td>0.04</td>
<td>0.04</td>
<td>2.63 %</td>
</tr>
<tr>
<td>2</td>
<td>Cassia fistula lab extract</td>
<td>0.04</td>
<td>0.04</td>
<td>5.85 %</td>
</tr>
<tr>
<td>3</td>
<td>Cassia fistula commercial extract</td>
<td>0.04</td>
<td>0.04</td>
<td>3.75 %</td>
</tr>
</tbody>
</table>

Table No. 2. Validation parameters for quantification of (-) epicatechin by HPTLC

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Precision (% RSD)</td>
<td>&lt; 2 %</td>
</tr>
<tr>
<td>Linearity</td>
<td>1 to 5 µg/spot</td>
</tr>
<tr>
<td>Limit of detection</td>
<td>1 µg/spot</td>
</tr>
<tr>
<td>Limit of quantification</td>
<td>3 µg/spot</td>
</tr>
<tr>
<td>Accuracy</td>
<td>98.28 to 100.4 %</td>
</tr>
</tbody>
</table>

Fig. 1: HPTLC Chromatogram of standard (-) Epicatechin
CONCLUSION

The developed HPTLC method was utilised for estimation of \((\cdot)\) epicatechin in\(\textit{ Cassia fistula}\) could be used as a valuable analytical tool in the routine analysis. \((\cdot)\) epicatechin can be used as one of the appropriate analytical markers present in the various medicinal plants.

REFERENCES