REFERENCES
1. The Merck Index, 10th Ed., Merck sharp and Dohme research Labs., USA, 1983, 378.

Determination of Glycyrrhizin in Glycyrrhiza glabra and its extract by HPTLC

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A simple reproducible HPTLC method for the determination of glycyrrhizin in Glycyrrhiza glabra and its extract was developed and is described. The sensitivity was found to be linear in the range of 0.2 to 1.0 μg. The proposed method being precise, sensitive and reproducible can be used for detection, monitoring and quantification of glycyrrhizin in g. glabra and its extract.

GLYCYRRHIZA glabra Linn, commonly known as Mulethi, is a highly reputed ayurvedic plant and is used in herbal preparations as a tonic, expectorant, demulcent, mild laxative and for allaying cough and catarrhal affections1, 2.

Not many methods for quantitative estimation of glycyrrhizin have been reported in the literature. Some of these methods are gravimetric and colorimetric3, 4 which are not very precise. A HPLC method5, 6 has also been reported for the estimation of glycyrrhhetic acids, aglycone of glycyrrhizin which involves critical steps such as hydrolysis. The method presented in this paper is quick, simple, accurate and provides a clear resolution and separation of peaks.

Dried and powdered roots (1 g) were extracted with water (35 ml x 3). The extracts were filtered, pooled and dried over a steam water bath to make the final volume to 100 ml. In case of G. glabra extract, around 400 mg of dried powder extract was accurately weighed and dissolved in 100 ml distilled water. Two and 5 μl of these test samples were applied on an aluminium TLC plate precoated with Silica gel 60 F 254 (E. Merck) alongwith 2, 5, 7 and 10 μl of standard glycyrrhizin (concentration 0.10 mg/ml) from about 1 cm edge of TLC plate using a band width of 6 mm and 5 mm distance between tracks using a sample applicator Linomat IV (M/s Camag, Switzerland).

The chromatogram was developed in n-Butanol:Acetic acid:Water 5:1:4, (upper layer) upto 80 mm. The plate was

Table-1 : Estimation of Glycyrrhizin in G. glabra and its extract

<table>
<thead>
<tr>
<th>Name of Sample</th>
<th>% of Glycyrrhizin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Crude G. glabra</td>
<td>9.054</td>
</tr>
<tr>
<td>2. G. glabra extract DP</td>
<td>17.48</td>
</tr>
</tbody>
</table>

DP = dried powder
Each value is the average of three replicates

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Table-2 - Method Validation and Recovery

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Sample</th>
<th>Amount of sample taken (mg)</th>
<th>Amount of Glycyrrhizin present in A (mg)</th>
<th>Amount of Glycyrrhizin added to A (mg)</th>
<th>Total Glycyrrhizin taken B+C (mg)</th>
<th>Total Glycyrrhizin found (mg)</th>
<th>% recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Crude G. glabra</td>
<td>1010</td>
<td>91.04</td>
<td>4.37</td>
<td>95.41</td>
<td>95.23</td>
<td>99.81</td>
</tr>
<tr>
<td>2.</td>
<td>Crude G. glabra</td>
<td>1050</td>
<td>95.08</td>
<td>8.74</td>
<td>103.82</td>
<td>102.88</td>
<td>99.08</td>
</tr>
<tr>
<td>3.</td>
<td>Crude G. Glabra</td>
<td>1020</td>
<td>92.74</td>
<td>17.48</td>
<td>110.22</td>
<td>109.72</td>
<td>99.55</td>
</tr>
<tr>
<td>4.</td>
<td>G. glabra Extract</td>
<td>395</td>
<td>69.10</td>
<td>8.50</td>
<td>77.66</td>
<td>77.00</td>
<td>99.22</td>
</tr>
<tr>
<td>5.</td>
<td>G. glabra Extract</td>
<td>420</td>
<td>71.40</td>
<td>17.00</td>
<td>88.40</td>
<td>87.06</td>
<td>98.48</td>
</tr>
<tr>
<td>6.</td>
<td>G. glabra Extract</td>
<td>400</td>
<td>71.88</td>
<td>20.00</td>
<td>91.88</td>
<td>91.04</td>
<td>99.08</td>
</tr>
</tbody>
</table>

Note: Average percent recovery = 99.20

Air dried and scanned at 250 nm in absorbance mode using M/s Camag TLC Scanner II. The amount of glycyrrhizin was determined using the calibration curve plotted between concentration and area of standard glycyrrhizin which is reported in Table-1.

For method validation and to know the percent recovery, a known amount of standard glycyrrhizin was added to the crude drug as well as to its extract. The samples were processed and analysed as per the procedure mentioned above. The results are mentioned in Table-2.

Using the proposed method, the RF of glycyrrhizin was about 0.25. The calibration curve was linear in the range of 0.2 to 1.0 ug. The method allows reliable quantification of glycyrrhizin from other constituents of G. glabra. Further, recovery values were also found satisfactory which showed the reliability and suitability of the method. The proposed HPTLC method is rapid, simple and accurate for quantitative monitoring of glycyrrhizin in G. glabra and its extract.

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