DEVELOPMENT OF HPTLC METHOD FOR ESTIMATION OF RUTIN IN POLYHERBAL TRANSDERMAL GEL

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ABSTRACT

Rutin, the most abundant biological flavonoid vitamin P, act as a scavenger of various oxidizing species. In the present study an attempt has been made to develop a simple, precise, rapid, selective and cost-effective high-performance thin-layer chromatographic (HPTLC) method for estimation of rutin from polyherbal transdermal gel prepared for the treatment of arthritis. The method employed TLC aluminium plates precoated with silica gel 60F254 as the stationary phase. The solvent system consisted of ethyl acetate, formic acid, glacial acetic acid. Densitometric analysis was carried out in the absorbance mode at 275nm. This system was found to give compact spots for rutin at Rf value of 0.54. Response was a linear function of the amount applied to the plate in the ranges 1-6 µg. The % of rutin from transdermal gel was found to be 100.94%, which was well within the limit. The developed HPTLC method would be an important tool in the quality control method for polyherbal formulations.

KEYWORDS: Rutin, HPTLC, Polyherbal Transdermal Gel.

INTRODUCTION

Rutin (5, 7, 3’, 4’ tetra hydroxy flavonol-3β-D–rhamnoglucoside) is used in pharmaceutical formulations for its vitamin P activity. It is the most abundant bioactive flavonoids. It is a yellow crystalline powder on hydrolysis yields quercetin, rhamnose and glucose. It was shown to act as a scavenger of various oxidizing species, like superoxide anions, hydroxyl and peroxy radicals. As a result of these biological effects, its several pharmacological activities were widely exploited, including, anti inflammatory, antiallergic, antitumor,
antibacterial, antiviral and antiprotozoal. Rutin has shown regulatory activity of hormones, such as transport, metabolism and action of thyroid hormones. Therefore, rutin was widely present in multivitamin preparations and more than 70 herbal remedies. Rutin is abundantly found in black wheat and also in apple peels, garlic, tomatoes and black tea. Phytochemical evaluation is one of the tools for the quality assessment, which includes preliminary phytochemical screening; chemo profiling and marker compound analysis using modern analytical techniques. In the last two decades High Performance Thin Layer Chromatography (HPTLC) has emerged as an important tool for the qualitative, semi-quantitative and quantitative phytochemical analysis of herbal drugs and formulations. This includes TLC fingerprint profiles and estimation of chemical markers and biomarkers. The major advantage of HPTLC is that several samples can be analyzed simultaneously using a small quantity of mobile phase. Various methods for the determination of rutin in drugs and extract have been reviewed. Simultaneous estimation of rutin and quercetin by UV spectrophotometric method, High Pressure Liquid Chromatography (HPLC) and HPTLC is reported. Rutin is simultaneously estimated with gallic acid by UV spectrophotometric method and HPTLC method. Rutin and ascorbic acid are simultaneously estimated by Reverse Phase High Pressure Liquid Chromatography (RP-HPLC) method. In the present study an attempt has been made to develop a simple, precise, rapid, selective and cost-effective high-performance thin-layer chromatographic (HPTLC) method for estimation of rutin from polyherbal transdermal gel prepared for the treatment of arthritis.

**Fig. 1: Chemical structure of Rutin.**

**MATERIALS AND METHODS**
Rutin standard was procured from Yucca Enterprises, Mumbai. Silica gel 60F254 TLC plates (20×10 cm, layer thickness 0.2 mm, E. Merck, Germany) were used as a stationary phase. All chemicals and reagents were of analytical grade and obtained from SD Fine Chem. Ltd. India. Prepared formulation was used for analysis.
Preparation of transdermal gel formulation
Weighed amount of carbopol 934P (0.5%w/w) and HPMC K15M (1%w/w) were placed in known amount of distilled water containing sodium benzoate (0.2%w/w) separately. After complete dispersion, the polymer solutions were kept in dark for 24 hours for complete swelling. Polymer solutions were mixed by stirring on magnetic stirrer. Accurately weighed amount of drugs were dissolved in a specified quantity of suitable solvent. Methyl salicylate (10%w/w), propylene glycol (10%w/w) and menthol (1%w/w) were added to it. The drug solution was added slowly to the aqueous dispersion of polymer with the help of high speed stirrer (500 rpm) taking precaution that air did not entrap. Triethanolamine was added to neutralize the carbopol 934P and to form the gel. The pH was adjusted 6.8.

Apparatus
The HPTLC system (Camag, Muttenz, Switzerland) consisted of Linomat V autosprayer connected to a nitrogen cylinder, a twin trough chamber (10 × 10 cm), a derivatization chamber and a plate heater. Precoated silica gel 60 F254 TLC plates (10 × 10 cm, layer thickness 0.2 mm, E. Merck KGaA, Darmstadt, Germany) were used as stationary phase. TLC plates were prewashed twice with 10 ml of methanol and activated at 80°C for 5 min prior to sample application. Analysis was carried out using a TLC scanner III with win CATS software. (V 1.4.6, Camag).

Preparation of Standard Solution
10 mg of rutin was weighed and transferred into 10 ml volumetric flask. These drugs were dissolved in 5 ml methanol by vigorous shaking and then volume was made up to mark with methanol to obtain a final concentration of 1mg/ml.

Preparation of Sample Solution
A 1g transdermal gel formulation was weighed and transferred into volumetric flask containing 10ml of methanol and kept in the ultrasonicator for 20 min for extraction of drugs from formulation.

HPTLC method and Chromatographic Condition
Chromatographic separation was performed on pre activated Merck TLC plates precoated with silica gel 60F254, 10×10. The samples and standards were applied onto the plates as a band with 8mm width using a Camag 100 microliter sample syringe (Hamilton, Switzerland) with a Camag Linomat 5 applicator 10 mm from the bottom of the plate at a delivery speed of
the syringe 10 s/ µl. The application parameters were identical for all the analysis. Linear ascending development was carried out in a twin trough glass chamber (10 × 10 cm) which was pre-saturated with the mobile phase. Plate was developed in the mobile phase ethyl acetate: glacial acetic acid: formic acid: water (100:11:11:25). The mobile phase was developed by a trial and error method. After development, the plate was removed and dried and spots were visualized in UV light (UV cabinet, Camag, Switzerland). Scanning was performed using a Camag TLC scanner 3 at 258 nm. The Rf, peak areas and absorption spectra were recorded.

**Calibration Curve of Rutin**

A stock solution of rutin (1mg/ml) was prepared in methanol. Different volumes of stock solution 1, 2, 3, 4, 5 and 6 µl were spotted on TLC plate to obtain concentrations of 1, 2, 3, 4, 5 and 6 µg per spot of rutin. After development of the plate, peak area versus drug concentration data were treated by linear regression analysis. Plate was developed in the mobile phase ethyl acetate: glacial acetic acid: formic acid: water (100:11:11:25) at 25 ± 2°C temperature and 40% relative humidity and allowed to travel up to a distance of 8 cm. After air drying of the plate, Scanning was performed at 258 nm. The peak areas were recorded. Calibration curve was prepared by plotting peak areas versus concentration.

**Estimation of rutin in the sample**

Exactly 10 µl of sample solution, prepared as mentioned above, was applied as bands and developed using optimized chromatographic conditions which was similar to the standards. The plate was scanned at 258 nm for analysis of rutin. The area of the peak that corresponds with the Rf of standard was recorded and the amount present in the sample solution was calculated from the regression equation obtained from the calibration curve.

**RESULTS AND DISCUSSION**

**Development of the Optimum Mobile Phase**

The TLC procedure was optimized with a view to quantify rutin in polyherbal transdermal gel formulation. The mobile phase, ethyl acetate: glacial acetic acid: formic acid: water (100:11:11:25), gave good resolution with Rf 0.48 for rutin. Under the chromatographic condition employed, standard compound, rutin has shown sharp peaks and good separation (Fig. 2).
Calibration Curves

The relationship between the concentration and peak response was linear within the range of 1 to 6 µg per spot for rutin with correlation coefficient of 0.993. The value of slope and intercept were 2414 and 3480 for rutin, respectively (Table 1). No significant difference was observed in the slopes of standard curves. (Figure 3).

Table 1: Linearity data for the estimation of rutin by HPTLC.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Rutin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity range (µg/spot)</td>
<td>1-6</td>
</tr>
<tr>
<td>Slope</td>
<td>2414</td>
</tr>
<tr>
<td>Intercept</td>
<td>3480</td>
</tr>
<tr>
<td>Coefficient of correlation</td>
<td>0.993</td>
</tr>
</tbody>
</table>

Analysis of the formulated transdermal gel

Rutin extracted from polyherbal transdermal gel formulation showed well isolated peaks at Rf 0.54. (Fig. 4). The percentage of rutin from transdermal gel was found to be 100.94, which
was well within the limits (Table 2). By considering Rf values of standard rutin and peaks observed in sample, presence of these active chemical marker compound was detected.

![HPTLC chromatogram of rutin transdermal gel.](image)

**Table 2 Analysis of polyherbal transdermal gel by HPTLC.**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Rf</th>
<th>% Drug found</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rutin</td>
<td>0.54</td>
<td>100.94</td>
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**CONCLUSION**

This analytical method can be utilized for the estimation of rutin. The method can be used for its quantification in plant materials and also in routine quality control of the raw materials as well as formulations containing this compound.

**ACKNOWLEDGEMENTS**

The authors are thankful to the Department of Pharmacognosy, K.B. Institute of Pharmaceutical Education & Research, Gandhinagar for providing HPTLC facility for this research work.

**REFERENCES**


