Scholars Academic Journal of Pharmacy
Abbreviated Key Title: Sch Acad J Pharm
ISSN 2347-9531 (Print) | ISSN 2320-4206 (Online)
Journal homepage: http://saspublishers.com

OPEN ACCESS
Pharmacy

Analytical Method Development for Estimation of Rutin in Polyherbal Transdermal Patch by HPTLC

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DOI: 10.36347/sajp.2021.v10i12.006 | **Received:** 05.11.2021 | **Accepted:** 18.12.2021 | **Published:** 23.12.2021

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Abstract **Original Research Article**

Rutin, a natural plant pigment and the most abundant plant flavonoid, work 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 patch prepared for the treatment of arthritis. The method employed TLC aluminium plates precoated with silica patch 60F254 as the stationary phase and 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 patch 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 patch.

INTRODUCTION

Rutin (5, 7, 3', 4' tetra hydroxy flavonol-3β -D –rhamnoglucoside) is used for its vitamin P activity in various herbal formulations. It is the most abundant bioactive flavonoids. It is a yellow crystalline powder which on hydrolysis yields quercetin, rhamnose and glucose. It was shown to act as a scavenger of various oxidizing species, like superoxide anions, hydroxyl and peroxyl 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[1]. Rutin is abundantly found in buckwheat, 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. Over the past two decades, High-Performance Thin-Layer Chromatography (HPTLC) has emerged as an important analytical tool for the qualitative, semi-quantitative, and quantitative analysis of phytoconstituents in herbal drugs and their formulations. The major advantage of HPTLC is its ability to generate a characteristic chromatographic fingerprint of a plant extract or herbal formulation. This makes it particularly valuable for detecting adulteration, batch-to-batch variation, and consistency of phytochemical composition. Another advantage of HPTLC is that several samples can be analyzed simultaneously using a small quantity of mobile phase. [2] Various methods for the determination of rutin in drugs and extract have been reviewed. Simultaneous estimation of rutin and quercetin by UV spectrophotometric method [3], High Pressure Liquid Chromatography (HPLC) [4] and HPTLC [5] is reported. Rutin is simultaneously estimated with gallic acid by UV spectrophotometric method [6] and HPTLC method [7]. Rutin and ascorbic acid are simultaneously estimated by Reverse Phase High Pressure Liquid Chromatography (RP-HPLC) method [8]. 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 patch prepared for the treatment of arthritis.

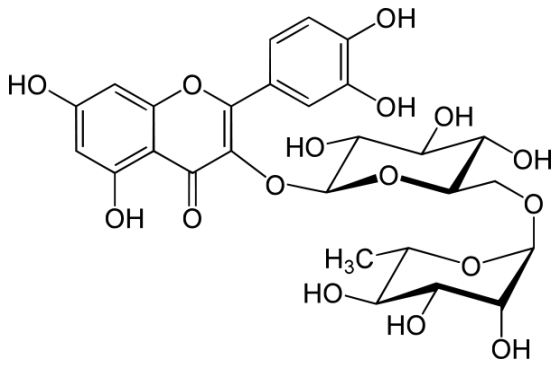

Fig. 1: Chemical structure of Rutin

MATERIAL AND METHODS

Rutin standard was procured from Yucca Enterprises, Mumbai. Silica patch 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 patch formulation

Backing membrane was prepared by casting 5% aqueous solution of Polyvinyl alcohol followed by drying at 60° C for 6 hrs. Drug loaded matrix type transdermal patches were prepared by solvent casting method. Petri-dish with total area of 38.465 cm2 was used. Eudragit RL100 800mg and Eudragit RS 100 800mg were accurately weighed and dissolved in dichloromethane: methanol (50:50). The polymeric dispersion was stirred for 2 min to remove entrapped air bubbles. PEG 400 and menthol were added to the polymeric dispersion. Accurately weighed amount of rutin was dissolved in methanol and added to polymeric dispersion. The solution was casted on the backing membrane placed in a petridish and dried at room temperature for 24 hrs. An inverted funnel was placed over the petridish to prevent fast evaporation of the solvent. After 24 hrs the dried patches were taken out, cut into small pieces (9 cm^2) and stored in desiccators for further studies.

Apparatus

The HPTLC system (Camag, Muttenz, Switzerland) consisted of Linomat V auto sprayer connected to a nitrogen cylinder, a twin trough chamber (10 × 10 cm), a derivatization chamber and a plate heater. Precoated silica gel 60 F_{254} 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 specified area of patch (1 cm^2) was dissolved in 10 ml methanol and kept in the ultrasonicator for 20 min for extraction of drugs from formulation. The solution was filtered through a 0.45 µm membrane and diluted suitably to analyze content of each drug.

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 ± 2^0C temperature and 40% relative humidity and allowed to travel up to 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 patch 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).

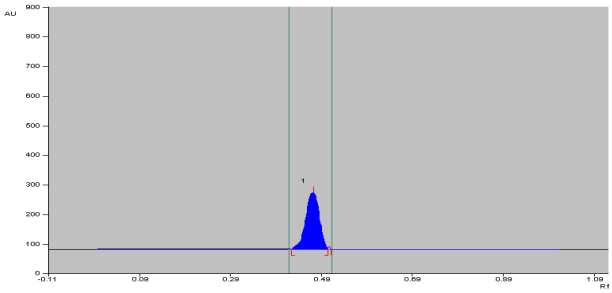

Fig. 2: HPTLC chromatogram of standard rutin

Calibration Curves

The relationship between the concentration and peak response was linear within the range of 1to 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)

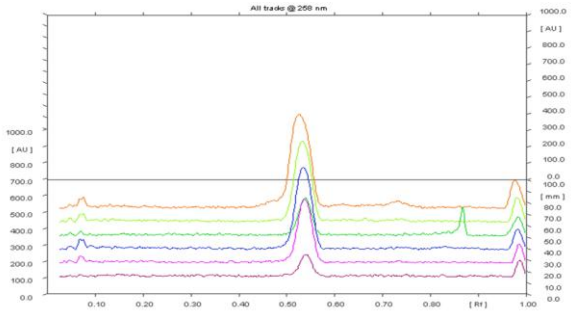

Fig.3 Calibration curve of rutin

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

Parameter	Rutin
Linearity range (µg/spot)	1-6
Slope	2414
Intercept	3480
Coefficient of correlation	0.993

Analysis of the formulated transdermal patch

Rutin extracted from polyherbal transdermal patch formulation showed well isolated peaks at Rf 0.54. (Fig. 4). The percentage of rutin from transdermal patch 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.

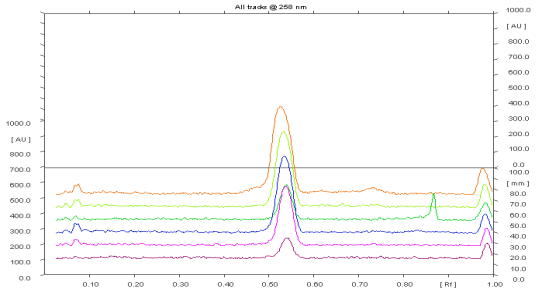

Fig. 4: HPTLC chromatogram of rutin transdermal patch

Table 2: Analysis of polyherbal transdermal patch by HPTLC

Drug	Rf	% Drug found
Rutin	0.54	100.94

CONCLUSION

This analytical method can be utilized for the estimation of rutin. The method can be used for its quantification in plant materials and 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.

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