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ESTIMATION OF RUTIN IN POLYHERBAL TRANSDERMAL GEL USING HPTLC

Resa P. Parmar[1]*, Niranjan S. Kanaki[2]

[1]Lecturer, B. K. Modi Government Pharmacy College, Rajkot.

[2]Associate Professor, K. B. Institute of Pharmaceutical Education & Research, Gandhinagar.

Abstract:

Rutin, the most abundant biological flavonoid vitamin P, act as a scavenger of various oxidizing species. It is widely present in multivitamin preparations and more than 70 herbal remedies. 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 60F_{254} as the stationary phase. The solvent system consisted of ethyl acetate, formic acid, glacial acetic acid and water. 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 0.48. 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 99.73%, 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.*

Corresponding author:

Ms. Resa P. Parmar,

B. K. Modi Government Pharmacy College,
Government Polytechnic Campus, near aji dam,
Bhavnagar road, Rajkot, Gujarat- 360003
Mobile: 9723617300
Email: resapparmar@gmail.com

QR code

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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 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 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. [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 gel prepared for the treatment of arthritis.

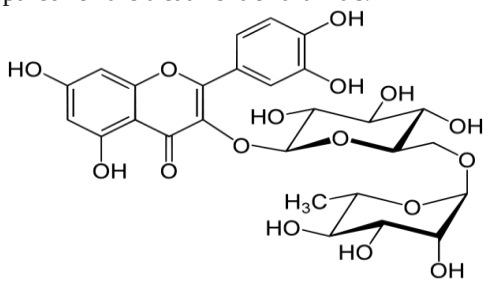

Fig. 1: Chemical structure of Rutin

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 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 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 ± 2^0C 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 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).

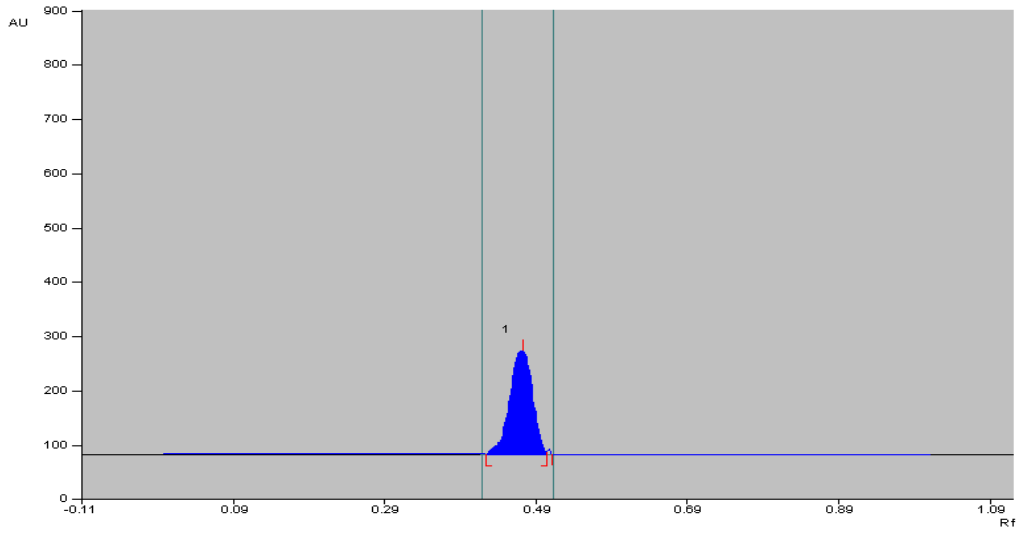

Fig. 2: HPTLC chromatogram of standard rutin

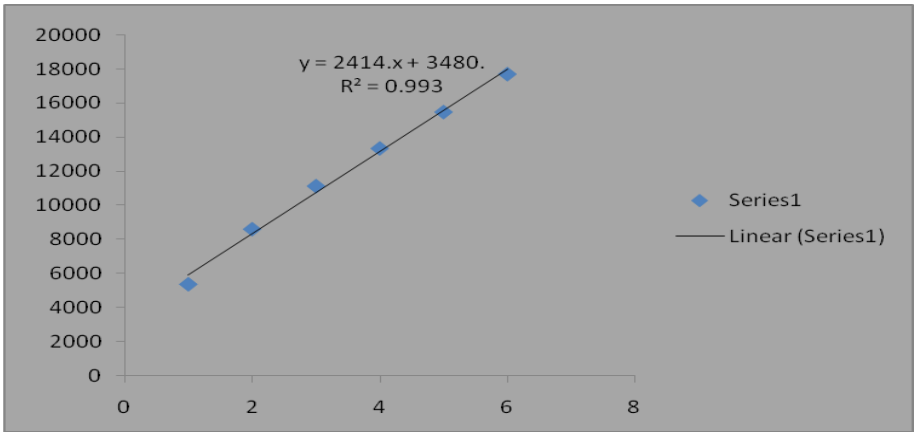

Fig.3: Calibration curve of rutin

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 gel

Rutin extracted from polyherbal transdermal gel formulation showed well isolated peaks at Rf 0.48 (Fig. 4). The percentage of rutin from transdermal gel was found to be 99.73, 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.

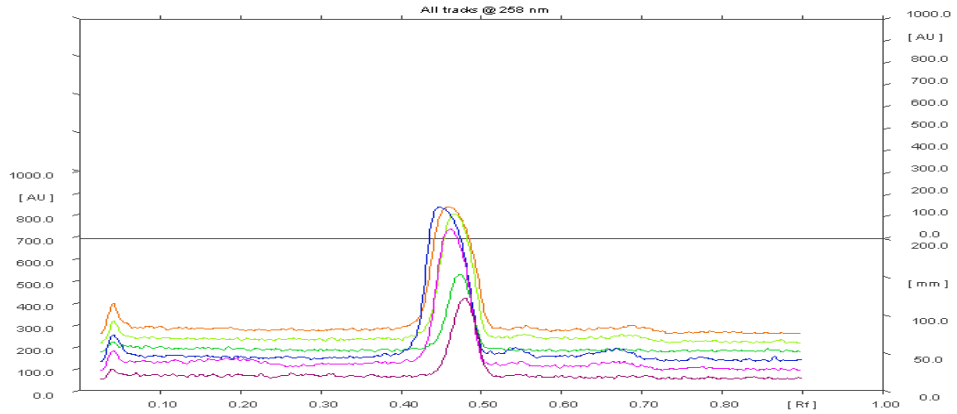

Fig. 4: HPTLC chromatogram of rutin in transdermal gel

Table 2: Analysis of polyherbal transdermal gel by HPTLC

Drug	Rf	% Drug found
Rutin	0.48	99.73

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.

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REFERENCES:

1.Erlund T, Kosonen GA, Alfihan JM, Mäenpää J, Perttunen K, et al. (2000) Pharmacokinetics of quercetin from quercetin aglycone and rutin in healthy volunteers. Eur J Clin Pharmacol 56: 545-553.

2.Ravishankar M N, Shrivastava N, Jayathirthaa M G, Padh H and Rajani M, *J. Chromatography*., **2000**; 744: 257-262.
3.Chaudhri S. P., Bangar J. V., Akuskar G. K., Ratnaparkhi M. P. Development and validation of UV spectrophotometric method for simultaneous estimation of rutin and quercetin in niosome formulation. Der Pharmacia Lettre, 2014;6 (3):271-276.
4.Subramanian et al. Development and validation of HPLC method for the simultaneous estimation of quercetin and rutin in *Aganosma dichotoma*. Int J Pharm Pharm Sci, Vol 6, Suppl 2, 606-608 International Journal of Pharmacy and Pharmaceutical Sciences, 2014; 6 (2): 606-608
5.Pawar N P, Salunkhe V R, Development and validation of HPTLC method for simultaneous estimation of rutin and quercetin in hydroalcoholic extract of triphala churna. International Journal of PharmTech Research, 2012; 4 (4): 1457-1463.
6.Pawar N P, Salunkhe V R, Development and validation of UV spectrophotometric method for simultaneous estimation of rutin and gallic acid in hydroalcoholic extract of triphala churna. International Journal of PharmTech Research, 2013; 5 (2): 724-729.
7Archana. D., Rajasekaran. A, Arivukkarasu. R. HPTLC Method for estimation of Gallic acid and Rutin in Haritaki -An Ayurvedic Formulation. Int.J. PharmTech Res.2011; 3 (2):986-999
8.Habib HII, Omar SK, Mohamed HS, Estimation of Rutin and Ascorbic Acid in Some Libyan Herbal Plants by RP-HPLC. Med Aromat Plants,2016;5(4): 255.