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Original Research Article

Simultaneous Estimation of Boswellic Acid, Curcumin and Diosgenin in a Polyherbal Transdermal Patch by HPTLC

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Abstract

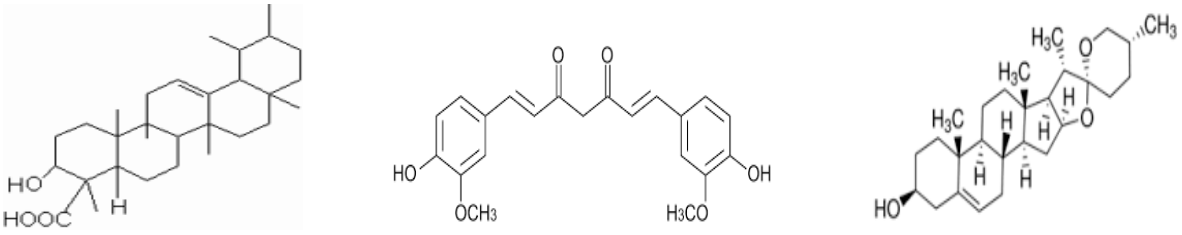
Background: Boswellic acid, curcumin and diosgenin are important phytoconstituents present in *Boswellia serrata*, *Curcuma longa* and *Trigonella foenum-graecum*, respectively. Traditionally, these plants are used in the treatment of arthritis. In the present study an attempt has been made to develop a simple, precise, rapid, selective and cost-effective high-performance thin-layer chromatographic (HPTLC) method for simultaneous estimation of boswellic acid, curcumin and diosgenin from polyherbal transdermal patch prepared for the treatment of arthritis. ***Material and method:*** The method employed TLC aluminium plates precoated with silica gel $60F_{254}$ as the stationary phase. The solvent system consisted of hexane and acetone. Densitometric analysis was carried out in the absorbance mode at 423nm for curcumin whereas for boswellic acid and diosgenin, plates were scanned at 540nm after derivatization. ***Result:*** This system was found to give compact spots for boswellic acid (Rf value of 0.54), curcumin (Rf value of 0.30), and diosgenin (Rf value of 0.64). Response was a linear function of the amount applied to the plate in the ranges 1-6 µg for boswellic acid, curcumin and diosgenin. The % of boswellic acid, curcumin and diosgenin from transdermal patch was found to be 99.46%, 99.47% and 98.85% respectively, which was well within the limit. ***Conclusion:*** The developed HPTLC method would be an important tool in the quality control method for polyherbal formulations.

Keywords: Boswellic acid, Curcumin, Diosgenin, HPTLC, Polyherbal transdermal patch.

INTRODUCTION

In recent years, due to fast and busy lifestyle, mental tension, low physical activity, many diseases and disorders are increasing. One of the most common musculoskeletal disease and disorder is Rheumatoid Arthritis (RA) [1]. RA is both an extravascular immune complex disease and a disorder of cell-mediated immunity leads to chronic inflammation, granuloma formation and joint destruction. Diverse and complex factors are involved in the etiopathogenesis of RA such as genetic background, rheumatic factor (circulating antibodies), immune complexes, compliment activation, lymphocytes, arachidonic acid metabolites, free oxygen radicals etc [2,3]. In modern allopathic system, many medicines are prescribed for this disorder, like analgesics, non-steroidal anti-inflammatory drugs (NSAIDs), disease-modifying anti-rheumatic drugs (DMARDs) and corticosteroids, but they have many side effects. Therefore, to avoid their side effects, now days, people are much inclined to use herbs-based medicines rather than modern allopathic [1,2]. Herbal medicines have been widely used all over the world since ancient times and have been recognized by physicians and patients for their better therapeutic value as they have fewer adverse effects as compared with modern medicines. Phytoconstituents, agents derived from plants, can modify the expression of pro-inflammatory signals clearly and therefore they have potential against arthritis. These include flavonoids, terpenes, quinones, catechins, alkaloids, anthocyanins and anthoxanthins, all of which are known to have anti-inflammatory effects [4]. Boswellic acid, a constituent of *Boswellia serrata* (Family- Burseraceae) showed anti-inflammatory, anti-rheumatic and anti-pyretic activities with no ulcerogenic effect and well tolerated in as high a dose as 2 gm/kg (p.o.) in mice. It improves blood supply to joints and restores integrity of vessels obliterated by spasm of internal damage [5]. Curcumin, a constituent of *Curcuma longa* (Family-Zingiberaceae) has been shown to be an effective anti-inflammatory agent with no analgesic and anti-pyretic activity. It is well tolerated in as high a dose as 2 gm/kg (p.o) in mice [6]. It has been reported to inhibit both lipoxygenase and cyclooxygenase as well as a potent scavenger of oxygen free radicals [7]. Diosgenin, a steroidal sapogenin constituent of *Trigonella foenum-graceum*

(Family-Leguminosae), possesses anti-rheumatic and antiviral properties, suppresses inflammation, inhibits proliferation and induces apoptosis in a variety of tumour cells. It is often used as a raw precursor in the production of steroidal drugs and hormones such as, glucocorticoids, testosterone and progesterone [8]. Phytochemical evaluation is one of the tools for the quality assessment of crude drugs, 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. The major advantage of HPTLC is that multiple samples can be quantified simultaneously using a small quantity of mobile phase [9]. 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 simultaneous estimation of boswellic acid, curcumin and diosgenin from polyherbal transdermal patch prepared for the treatment of arthritis. Literature survey showed no HPTLC method present for the simultaneous estimation of boswellic acid, curcumin and diosgenin in the polyherbal formulation. However, individual analytical methods have so far been reported for its determination [9, 10].



Boswellic acid Curcumin Diosgenin

Fig.1: Chemical structure of boswellic acid, curcumin and diosgenin

MATERIALS AND METHODS

Boswellic acid, curcumin and diosgenin standards were 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 PVA 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 cm^2 was used. 800 mg Eudragit RL 100 and 200 mg of Eudragit RS 100 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 5%w/w menthol were added to the polymeric dispersion. Boswellic acids (60 mg/9cm^2), curcuminoids (120 mg/9cm^2) and diosgenin (20 mg/9cm^2) were accurately weighed, dissolved in ethanol 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 [11].

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

Amount of 10 mg each of boswellic acid, curcumin and diosgenin were weighed separately 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 9 cm^2 transdermal patch formulation was 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 n-hexane: acetone (7:3). 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 423 nm for Curcumin and 540nm after derivatization for Boswellic acid and Diosgenin. Plate was derivatised by anisaldehyde sulphuric acid reagent. The Rf, peak areas and absorption spectra were recorded.

Calibration Curves of Boswellic acid, Curcumin and Diosgenin

A stock solution of boswellic acid, curcumin and diosgenin (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 boswellic acid, curcumin and diosgenin, respectively. After development of the plate, peak area versus drug concentration data were treated by linear regression analysis. Plate was developed in the mobile phase n-hexane: Acetone (7:3) 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 423nm for curcumin and at 540nm after derivatization for boswellic acid and diosgenin. The peak areas were recorded. Calibration curves of boswellic acid, curcumin and Diosgenin were prepared by plotting peak areas versus concentration.

Estimation of boswellic acid, curcumin and diosgenin 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 twice once at 423 nm for analysis of curcumin and at 540nm after derivatization for boswellic acid and diosgenin analysis. The area of the peak that corresponds with the Rf of standards was recorded and the amount present in the sample solution was calculated from the regression equation obtained from the calibration curves.

RESULTS AND DISCUSSION

Development of the Optimum Mobile Phase

The TLC procedure was optimized with a view to quantify boswellic acid, curcumin and diosgenin in polyherbal transdermal patch formulation. The mobile phase, Hexane: Acetone (7:3), gave good resolution with Rf 0.54 for boswellic acid, Rf 0.30 for curcumin and Rf 0.64 for diosgenin. Under the chromatographic condition employed, standard compounds, boswellic acid, curcumin and diosgenin have shown sharp peaks and good separation (Figures 2 and 3).

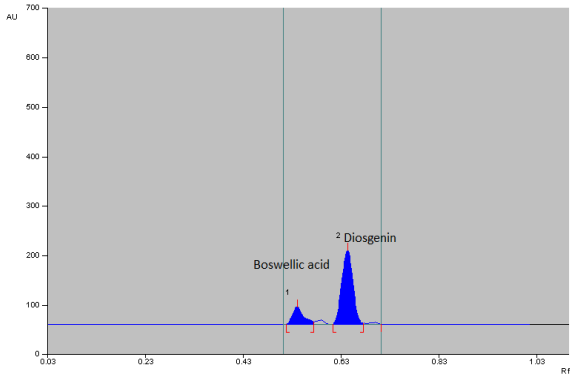


Fig. 2: HPTLC chromatogram of standard boswellic acid and diosgenin

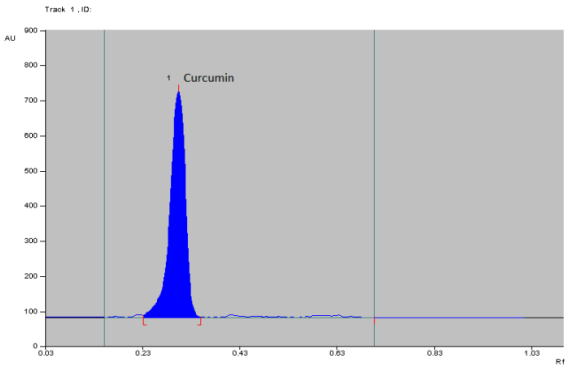


Fig. 3: HPTLC chromatogram of standard curcumin

Calibration Curves

The relationship between the concentration and peak response was linear within the range of 1to 6 µg per spot for boswellic acid, curcumin and diosgenin with correlation coefficient of 0.993, 0.994 and 0.998 respectively.

The value of slope and intercept were 380.6 and 614.3, 3210 and 16964, 671.9 and 2107 for boswellic acid, curcumin and diosgenin, respectively (Table 1). No significant difference was observed in the slopes of standard curves.

Table 1: Linearity data for the estimation of boswellic acid, curcumin and diosgenin by HPTLC

Parameter	Boswellic acid	Curcumin	Diosgenin
Linearity range (µg/spot)	1-6	1-6	1-6
Slope	380.6	3210	671.9
Intercept	614.3	16964	2107
Coefficient of correlation	0.993	0.994	0.998

Analysis of the formulated transdermal patch

Boswellic acid, curcumin and diosgenin extracted from polyherbal transdermal patch formulation showed well isolated peaks at Rf 0.42, 0.21 and 0.57, respectively (Figure 4 and 5). The percentage of boswellic acid, curcumin and diosgenin from transdermal patch was found to be 99.46, 99.47 and 98.85, which was well within the limits (Table 2). By considering Rf values of standard boswellic acid, curcumin and diosgenin and peaks observed in sample, presence of these active chemical marker compounds was detected.

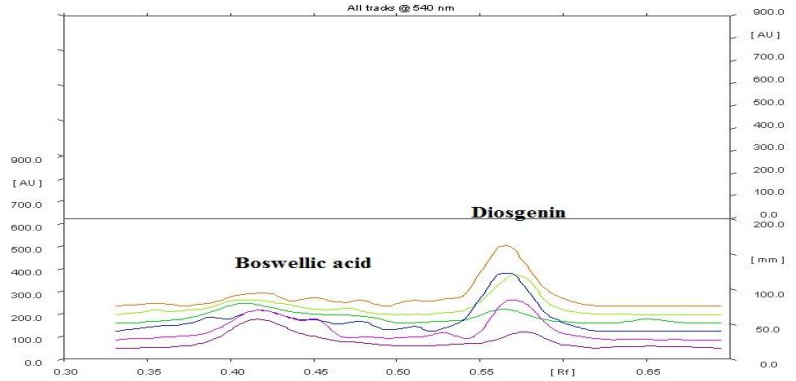


Fig.4: HPTLC chromatogram of boswellic acid and diosgenin in transdermal patch

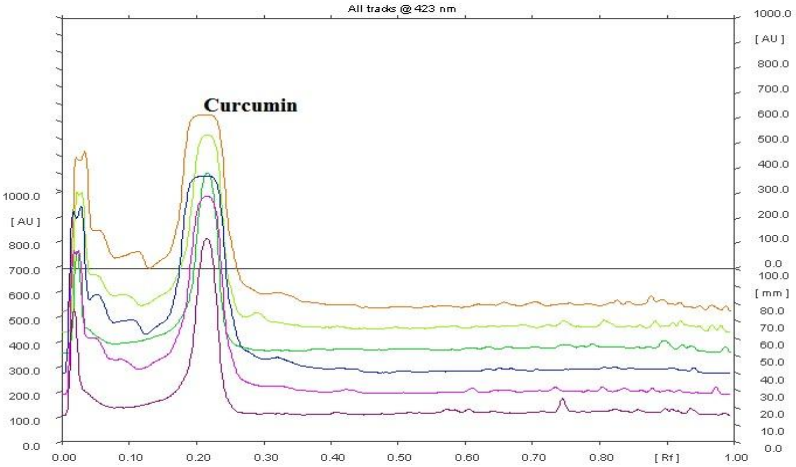


Fig.5: HPTLC chromatogram of curcumin in transdermal patch

Table 2: Analysis of polyherbal transdermal patch by HPTLC

Drugs	Rf	% Drug found
Boswellic acid	0.42	99.46
Curcumin	0.21	99.47
Diosgenin	0.57	98.85

CONCLUSION

There is significant difference between the Rf values of the boswellic acid, curcumin and diosgenin. Therefore, this analytical method can be utilized for the simultaneous estimation of boswellic acid, curcumin and diosgenin. The method can be used for their quantification in plant materials and in routine quality control of the raw materials as well as formulations containing any of these compounds.

Acknowledgements

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