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SIMULTANEOUS ESTIMATION OF BOSWELLIC ACID AND DIOSGENIN IN POLYHERBAL TRANSDERMAL GEL USING HPTLC

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Abstract:

Background: Boswellic acid and diosgenin are important phytoconstituents present in Boswellia serrata and Trigonella foenum-graecum, respectively. Boswellic acid is a pentacyclic triterpenoid whereas diosgenin is a steroidal sapogenin. 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 and diosgenin from polyherbal transdermal gel 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 540nm after derivatization for boswellic acid and diosgenin.

Result: This system was found to give compact spots for boswellic acid (Rf value of 0.54) 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 and diosgenin. The % of boswellic acid and diosgenin from transdermal gel was found to be 99.46% 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, Diosgenin, HPTLC, Polyherbal transdermal gel.*

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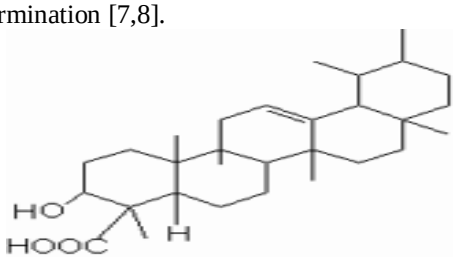
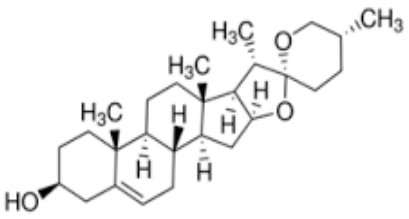
INTRODUCTION:

In recent years, due to fast and busy life style, 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]. 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 [6]. 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 chromatographic (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.[7] In the present study an attempt has been made to develop a simple, precise, rapid, selective and cost-effective HPTLC method for simultaneous estimation of boswellic acid and diosgenin from polyherbal transdermal gel prepared for the treatment of arthritis. Literature survey showed no HPTLC method present for the simultaneous estimation of boswellic acid and diosgenin in the polyherbal formulation. However, individual analytical methods have so far been reported for its determination [7,8].



Boswellic acid



Diosgenin

Fig.1: Chemical structure of boswellic acid and diosgenin

MATERIALS AND METHODS:

Boswellic acid and diosgenin standards were 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:
Amount of 10 mg each of boswellic acid 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 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 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). Plate was derivatised by anisaldehyde sulphuric acid reagent. Scanning was performed using a Camag TLC scanner 3 at 540nm after derivatization The Rf, peak areas and absorption spectra were recorded.

Calibration Curves of Boswellic acid and Diosgenin:
A stock solution of boswellic acid 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 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 540nm after derivatization for boswellic acid and diosgenin. The peak areas were recorded. Calibration curves were prepared by plotting peak areas versus concentration.

Estimation of boswellic acid 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 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 and diosgenin in polyherbal transdermal gel formulation. The mobile phase, Hexane: Acetone (7:3), gave good resolution with Rf 0.54 for boswellic acid and Rf 0.64 for diosgenin. Under the chromatographic condition employed, standard compounds, boswellic acid and diosgenin have shown sharp peaks and good separation (Figure 2).

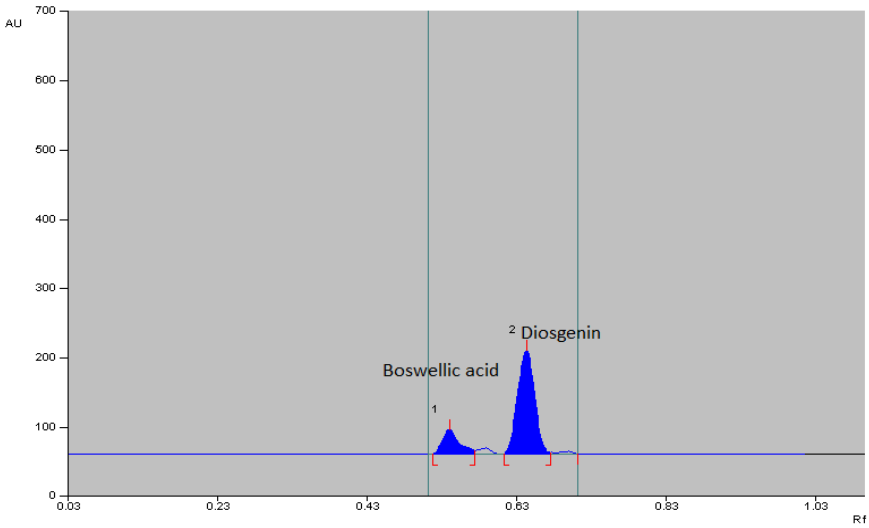


Fig. 2: HPTLC chromatogram of standard boswellic acid and diosgenin

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

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

Calibration Curves:

The relationship between the concentration and peak response was linear within the range of 1to 6 µg per spot for boswellic acid and diosgenin with correlation coefficient of 0.993 and 0.998 respectively. The value of slope and intercept were 380.6 and 614.3, 671.9 and 2107 for boswellic acid and diosgenin, respectively (Table 1). No significant difference was observed in the slopes of standard curves.

Analysis of the formulated transdermal gel:

Boswellic acid and diosgenin extracted from polyherbal transdermal gel formulation showed well isolated peaks at Rf 0.42 and 0.57, respectively (Figure 3). The percentage of boswellic acid and diosgenin from transdermal gel was found to be 99.46 and 98.85, which was well within the limits (Table 2). By considering Rf values of standard boswellic acid and diosgenin and peaks observed in sample, presence of these active chemical marker compounds was detected.

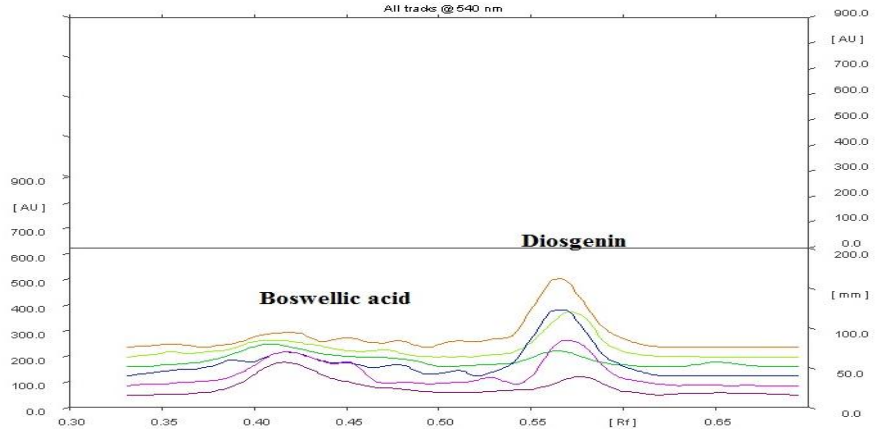


Fig.3: HPTLC chromatogram of boswellic acid and diosgenin in transdermal gel

Table 2: Analysis of polyherbal transdermal gel by HPTLC

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

CONCLUSION:

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

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REFERENCES

1.Srivastava C. Phytochemistry and medicobotany of some medicinal plants used in treatment of arthritis. Medicinal Plants. International Journal of Phytomedicines and Related Industries, 2009; 1(1).

2.Raut A. A., Joshi A. D., Antarkar D. S., Joshi V.R., Vaidya A. B. Antirheumatic formulation from Ayurveda. Ancient Science of Life XI (1, 2), 1991: 66-69.

3.Nuki, G., Ralston, S.H., and Lugmani, R. Diseases of the Connective Tissues, Joints and Bones. In: C. Haslett, R.E. Chilvers, A.A.J. Hunter, and A.N. Boon (Eds.), Davidson's Principles and Practice of Medicine, Churchill Livingstone, London, 1999: 802-837.

4.Khanna D., Sethi G., Ahn K. S., Pandey M. K., Kunnumakkara A. B., Sung B., Aggarwal A., Aggarwal B. B. Natural products as a gold mine for arthritis treatment. Current Opinion in Pharmacology. 2007; 7: 344–351.

5.Sharma M. L., Bani S., Singh G. B., Anti-arthritic activity of boswellic acids in bovine serum albumin (BSA)-induced arthritis, Int J Immunopharmacol. 1989; 11(6):647-52.

6.Omi Laila, Imtiyaz Murtaza, M. Z. Abdin, S. Ahmad, Nisar Ahmad Ganai, Majid Jehangir. Development and Validation of HPTLC Method for Simultaneous Estimation of Diosgenin and Quercetin in Fenugreek Seeds (Trigonella foenum-graceum). ISRN Chromatography, 2014:8.

7.Kshirsagar V. B., Deokate U. A., Bharkad V. B., Khadabadi S.S. HPTLC Method Development and Validation for the simultaneous Estimation of Diosgenin and Levodopa in marketed formulation. Asian Journal of Research Chem., 2008; 1(1): 36-39.

8.Vaykole A. M., Nirmal S. A., Jadhav R. S. & S. R. Pattan. Development and validation of HPTLC method to detect curcumin, piperine, and boswellic acid in polyherbal transdermal patch. Journal of Liquid Chromatography & Related Technologies, 2014; 37(3): 367-378.