**RESEARCH ARTICLE**

# Estimation of Active Components in Gokshura Tablet and Pushyanug Churna Formulation using High-performance Thin Layer Chromatography Method

Priyanka Rathore,*[1] Kratika Daniel,[2] Vivek Daniel,[3] Charanjeet Singh,[4] Yashwant,[4] Anil K. Gupta[5]

[1]*Institute of Pharmaceutical Sciences, SAGE University, Indore, Madhya Pradesh, India*
[2]*Oriental College of Pharmacy and Research, Oriental University, Indore, Madhya Pradesh, India*
[3]*N.T.V.S,Institute of Pharmacy, Nandurbar, Maharashtra, India*
[4]*Biyani Institute of Pharmaceutical Sciences, Jaipur Rajasthan, India*
[5]*Jaipur College of Pharmacy, Jaipur, Rajasthan, India*

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**ABSTRACT**

Gokshura tablet is an ayurvedic formulation with gokhru **(***Tribulus terrestris)* as best fixing recommended for building vitality levels. It enhances life, sexual want and drive.

Pushyanug churna is an ayurvedic polyherbal formulation, hence this seems essential to explain the material institutionalization, various bioactive markers, blends exhibit in the polyherbal ayurveda compositions such as pushyanug churna. Point of the exhibit effort has been to create what's more, approve a high-performance thin layer chromatography (HPTLC) strategy for assurance of diosgenin present in gokshura tablet. Mangiferin and chlorogenic acid are present in pushyanug churna.

Diosgenin, a biomarker chemical found in gokshura tablets, and mangiferin, a biomarker compound found in pushyanug churna, were standardized using recently developed easy and accurate HPTLC procedures. Pre-coated silica gel 60-F254 was employed at the stationary phase and a mixture of toluene, ethyl acetate, and formic acid (in the proportions 5:4:1) was employed for the mobile phase in the development methodology for diosgenin. In the mobile phase, mangiferin, ethyl acetate and methanol were added in a ratio of 40:60 v/v were utilized. In the chlorogenic acid mobile phase, ethyl acetate:formic acid:acetic acid:water (10:1.1:1.1:2.6 v/v). It was determined that the $R_f$ value of the marker chemical was 0.77 (diosgenin) in gokhsura tablet and 0.23 mangiferin, 0.75 chlorogenic acid in pushyanug churna. For bioactive marker chemicals found in in-house and commercially available formulations, the developed HPTLC approach has shown to be straightforward, sensitive, specific, and dependable.

**Keywords:** Gokshura tablet, Pushyanug churna, Polyherbal, Bioactive marker.

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**Conflict of interest:** None

## INTRODUCTION

Public attention in herbal medicine has increased rapidly in recent years.[1-3] The world health organization (WHO) estimates that between 65 and 80% of people in underdeveloped nations rely on plants to meet their most basic healthcare requirements because of extreme poverty and limited expertise in advanced medical technologies.[4] To facilitate worldwide harmonization, The WHO have produced certain guiding principles to assist the affiliated nations in instituting countrywide policies concerning plant-based pharmaceuticals and studying their projected safety, effectiveness, and quality.[5-7]

Herbal formulation gokshura, gokharu (*Tribulus terrestris*) plant contains alkaloids, gums, tannins, sugars, sterols, basic oil, peroxidase, diastase and glucoside. Gokshura is utilized inside to treat, diabetic mellitus, dysuria, bronchial asthma, cough, hemorrhoids, rheumatic joint inflammation, stomach agony, arthralgia and heart afflictions. The entire plant of gokshura is a great love potion and has rejuvenating powers. Moreover, improves sexual want and drive, which enhances sexual execution. Non-hormonal bio-stimulator expands the level of common endogenous testosterone.[8] The gokharu contains diosgenin as a chief constituent.

**Author for Correspondence: rathorepriyanka0211@gmail.com*

### Polyherbal Formulation of Pushyanug Churna

It is utilized as a part of the ayurvedic treatment of menorrhagia, metrorrhagia, leucorrhoea, menstrual confusion, excessive menstrual seeping of different etiology. It is prepared by more than 20 herbs. The pushyanug churna contains chlorogenic acid and mangiferin.[9] Innovative headways that occur into the disengagement process, decontamination, and auxiliary clarification of familiar blends have prepared it plausible to generate fitting methods in support of the investigation of value along with institutionalization of plant-oriented prescriptions.[10] The term high-performance thin-layer chromatography (HPTLC) refers to an improved version of the conventional technique of thin-layer chromatography (TLC). Automating the various processes, improving resolution, and enabling more precise quantitative measurements are all possible via various modifications to the standard TLC procedure. In light of its adaptability, dependability, excellent performance, and low cost. HPTLC is widely used to determine the identity and quality of plant components.[11,12]

According to the intracerebral brain hemorrhage (ICH) guidelines[13,14] accuracy, reliability, precision, and specificity were the four validation metrics established in the present investigation of diosgenin in the internal and commercial formulations of gokshura: tablet and mangiferin, chlorogenic acid in pushyanug churna.

## EXPERIMENTAL

### Standards and Chemicals

Merck Specialties Private Limited (Mumbai) provided the analytical level of the organic solvents that were purchased from them; diosgenin, mangiferin, and chlorogenic acid were all acquired from Yucca chemicals in Mumbai as identifying markers.

### Collection of Plant Materials

The medicinal plants that went into the making of gokhura have been procured from the regional marketplace in Mandsaur and Botany Scientist Dr. S.N. Mishra from K.N.K. College, Mandsaur acknowledges to the authenticity of materials.

### Preparation of Gokshura Churna

The gokshura churna followed the instructions provided in the Ayurvedic Formulary of India, Volume I. According to the Ayurvedic Pharmacopoeia of India, each little piece of plant material was correctly identified. Shade was provided as the herbs dried. These de-hydrated ingredients were mechanically pulverized and sieved through 100 meshes to get the desired consistency.

### Preparation of Test Sample

It has been thoroughly shaken after dissolving 100 mg of commercial sample into 10 mL of methanol at a concentration of 10 mg/mL. After 15 minutes, the liquid was processed through what man filter paper after being sonicated.

### Preparation of Diosgenin's Standard Stock Solution

Diosgenin's stock solution (10 mg/mL) has been made by dissolving 100 mg into enough petroleum ether/methanol to end up making 10 mL. After 15 minutes, the liquid was processed through what man filter paper after being sonicated.

### Preparation of sample Pushyanug Churna solution

The sample, which was 100 mg, has been dissolved into 10 mL of methanol at a concentration of 10 mg/mL, and the mixture was agitated well. After 15 minutes, the liquid was processed through what man filter paper after being sonicated.

### Preparation of Mangiferin's Standard Stock Solution

In a volumetric flask, 100 mg of the drug was dissolved into up to 10 mL of methanol (10 µg/mL) by vigorously shaking the liquid, followed by sonicating it for 15 minutes to achieve full dissolution and then filtering the solution *via* whatman filter paper.

### Preparation of Chlorogenic Acid Standard Stock Solution

In a volumetric flask, 100 mg of the drug was dissolved into up to 10 mL of methanol (10 µg/mL) by vigorously shaking the liquid, followed by sonicating it for 15 minutes to achieve full dissolution and then filtering the solution *via* Whatman filter paper.

**Table 1:** Preparation of Gokshura Churna

| *Herbs* | *Given code Part utilized* | *Quantity* |
|---|---|---|
| *Tribulus terrestris* | TT Fruit | 250 gm. |

**Table 2a:**Quantitative measurements parameters for method validation of Diosgenin

| *Method Property* | *Diosgenin* |
|---|---|
| $R_f$ | 0.77 |
| Specificity | Specific |
| Robustness | Robust |

**Table 2b:** Quantitative measurements parameters for method validation of Mangiferin and Chlorogenic acid

| *Method's Property* | *Mangiferin* | *Chlorogenic Acid* |
|---|---|---|
| $R_f$ | 0.23 | 0.77 |
| Specificity | Specific | Specific |
| Robustness | Robust | Robust |

**Table 3:** Intraday and Interday precision

| *Amount (ng per spot)* | *Intraday precision (Mean. SD %RSD)* | *Interday Precision (Mean SD % RSD)* |
|---|---|---|
| *Diosgenin* | | |
| 2 | 98.00 0.11 0.12 | 98.02 0.11 0.10 |
| 5 | 97.02 0.41 0.43 | 97.03 0.83 0.80 |
| 8 | 98.020.14 0.14 | 99.10 0.71 0.70 |

**Table 4:** Intraday and Interday precision

| *Amount (ng per spot)* | *Intraday precision Mean. SD % RSD* | *Interday Precision Mean. SD %RSD* |
|---|---|---|
| *Marketed formulation* | | |
| 2 | 97.23 0.11 0.57 | 98.02 0.59 0.10 |
| 5 | 97.23 0.41 1.26 | 97.03 1.30 0.80 |
| 8 | 98.130.14 1.14 | 99.10 1.15 0.70 |

**Table 5:** Recovery study of (a) Diosgenin

| *S. no.* | *Level of recovery* | *80%* | | *100%* | | *120%* | |
|---|---|---|---|---|---|---|---|
| | | *Marketed* | *Inhouse* | *Marketed* | *Inhouse* | *Marketed* | *Inhouse* |
| | | 10 | 20 | 10 | 20 | 10 | 20 |
| 1. | Amount present (µg.) | 10 | 20 | 10 | 20 | 10 | 20 |
| | | 10 | 20 | 10 | 20 | 10 | 20 |
| | | 8 | 16 | 10 | 20 | 12 | 24 |
| 2. | Amount of Std. added | 8 | 16 | 10 | 20 | 12 | 24 |
| | | 8 | 16 | 10 | 20 | 12 | 24 |
| | | 7.6 | 15.6 | 9.80 | 19.70 | 11.15 | 23.90 |
| 3. | Amount recoverd | 7.65 | 15.8 | 9.75 | 19.75 | 11.09 | 23.70 |
| | | 7.71 | 15.9 | 9.71 | 19.8 | 11.20 | 23.75 |
| | | 95.0 | 97.5 | 98.0 | 98.5 | 92.91% | 99.58 |
| 4. | % Recovery | 95.62 | 98.75 | 97.5 | 98.75 | 92.41% | 98.75 |
| | | 96.37 | 99.37 | 97.1 | 99.00 | 93.33% | 98.95 |

**Table 6:** Result of Linearity of mangiferin and chlorogenic acid

| *Conc. µg/mL* | *Compound* | *5* | *10* | *15* | *20* | *25* |
|---|---|---|---|---|---|---|
| I | Mangiferin | 2590.5 | 4488.5 | 6230.1 | 8434.6 | 10372.3 |
| | Chlorogenic Acid | 2092.9 | 4183.8 | 6367.2 | 8134.5 | 10567.6 |
| II | Mangiferin | 2566.4 | 4470.4 | 6224.2 | 8430.7 | 10370.4 |
| | Chlorogenic Acid | 2089.8 | 4179.7 | 6371.3 | 8138.4 | 10571.2 |
| III | Mangiferin | 2561.5 | 4472.5 | 6221.3 | 8432.6 | 10369.4 |
| | Chlorogenic Acid | 2090.9 | 4182.9 | 6369.2 | 8141.4 | 10569.2 |
| Mean | Mangiferin | 2572.5 | 4477.1 | 6225.2 | 8432.6 | 10370.7 |
| | Chlorogenic Acid | 2091.2 | 4182.1 | 6369.2 | 8138.1 | 10569.3 |
| Standard deviation (SD) | Mangiferin | 15.52 | 9.899 | 4.484 | 1.950 | 1.473 |
| | Chlorogenic Acid | 1.571 | 2.154 | 2.050 | 3.459 | 1.803 |
| Relative standard deviation (RSD) | Mangiferin | 0.60 | 0.22 | 0.07 | 0.02 | 0.01 |
| | Chlorogenic Acid | 0.08 | 0.05 | 0.03 | 0.04 | 0.02 |

**Table 7:** Recovery studies of compounds

| *S. no.* | *Level of Recovery* | *80%* | *100%* | *120%* |
|---|---|---|---|---|
| | | 10 | 10 | 10 |
| 1. | Amounts Available (µg.) | 10 | 10 | 10 |
| | | 10 | 10 | 10 |
| | | 8 | 10 | 12 |
| 2. | Amount of Standard added | 8 | 10 | 12 |
| | | 8 | 10 | 12 |
| | | 7.94 | 9.94 | 11.59 |
| 3. | Amount recoverd | 7.88 | 9.97 | 11.61 |
| | | 7.90 | 9.91 | 11.60 |
| | | 99.25 | 99.04 | 96.58 |
| 4. | % Recovery | 98.05 | 99.07 | 96.75 |
| | | 98.75 | 99.01 | 96.66 |

## HPTLC Instrument Specification

TLC plates that are 20 cm by 10 cm For chromatography, 0.20 mm layers of pre-coated silicagel 60 F254 (Merck, Darmstadt, Germany) have been utilized. CamagLinomat V sample applicator with 100 ul syringe was employed to apply samples in 6mm broad bands with 75 mm in between. The application

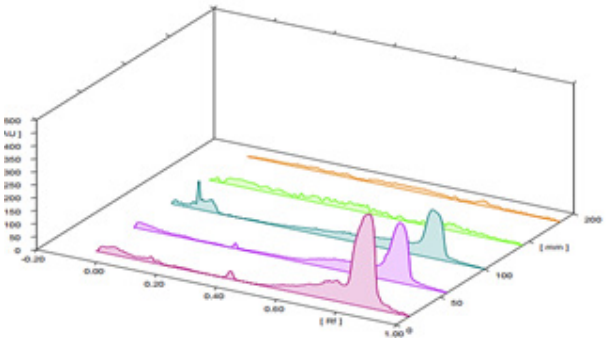

**Figure 1:** (a) Chromatograph of Diosgenin Rf-0.77.

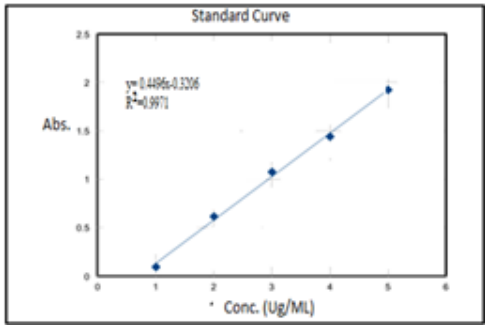


**Figure 2:** Calibration plot (a) Diosgenin.

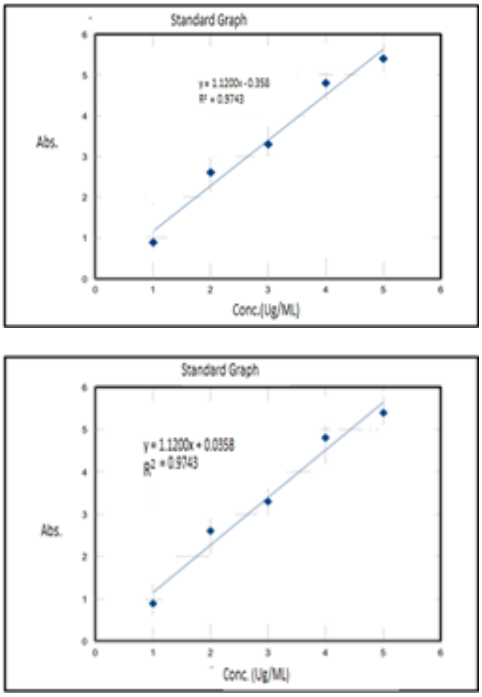


**Figure 3:** Calibration curve of mangiferin (standard) chlorogenic acid (standard).

rate was maintained at a steady 150 $nLs^{-1}$. Densitometric scanning was performed using a CAMAG TLC Scanner equipped with WINCAT software providing scanning and documentation purposes. The scanning speed was 100 nm/s, and the slit size was 6 mm by 0.45 mm. Under 254 nm, 366 nm, and daylight were the wavelengths of the radiation source.

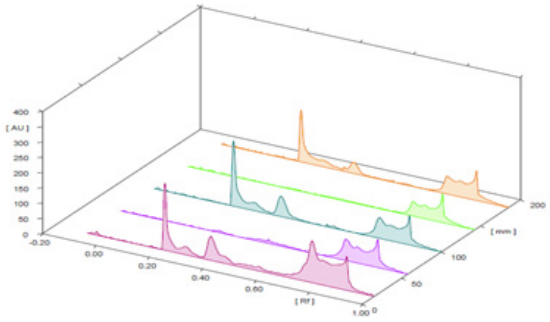

**Figure 1 (b):** Chromatograph of Mangiferin Rf-0.23 and Chlorogenic acid Rf- 0.75.

### Chromatographic Condition

A mobile phase consisting of toluene, ethyl acetate, and formic acid (in that order and at a volume ratio of 5:4:1) was used to get diosgenin. The chamber was pre-saturated with mobile phase vapors over 5 minutes at 252°C, and the solvent frontage (deployment distance) was approximately 7 cm when the plate was mounted. Following TLC plate development and air-drying, density scanning at 447 nm has been performed to detect diosgenin.

### Calibration Curves of Diosgeninin Herbal Preparation and their Analysis in the Preparation

The calibration curves has been constructed in order to ascertain linearity. TLC plates were sprayed with a 10 mg/mL liquid. Calibration plots were employed to determine the concentrations of diosgenin in both custom and commercially available preparations using densitometry scanning of every standard

### Calibration Curves of Mangiferin and Chlorogenic Acid in the Polyherbal Preparation and their Analysis in the Preparation

Calibration curves have been constructed to figure out the level of linearity. TLC plates were sprayed with a 10 mg/L liquid. Calibration plots were used to determine the concentrations of mangiferin and chlorogenic acid within commercially available preparations after densitometry scanning of every standard.

## METHOD VALIDATION

### Specificity

Phase specificity has sometimes been employed interchangeably. The word "specific" is often used to describe a technique that yields results for just one analyte..

### Linearity

Analytical techniques are said to be linear if their findings are proportionate to the concentration of the analyte in the sample across a certain range of concentrations. At some point in the analysis process, it will be necessary to make a judgment on a set of linear relationships. It is proven directly upon the drug substance by diluting a standard stock liquid of the medicine ingredient in accordance with the suggested approach. The ICH recommendations state that at least five concentrations should be used to prove linearity.

**Precision**

The precision of an analytical technique states the proximity of agreement (level of scatter) among a sequence of measurements attained from multiple sampling of a similar homogeneous sample.

**Accuracy**

The accuracy of the analysis procedure indicates a close match between the values obtained as exact conditional value or acknowledged reference value as well as the detected values. Accuracy estimation becomes very important because it deliberately strains the technique to remove drugs and impurities at superior and inferior levels. If an authenticated reference material or else control sample is not accessible, a known concentration by mass or volume may be added to the required, blank matrix.

**Robustness**

An analytical procedure's robustness is an assessment of its capability to be unaffected by minor but intentional changes to the technique parameters and offers a clue as to its dependability under typical conditions.

**Ruggedness**

The robustness of a technique is measured by how well it holds up under less-than-ideal implementation and is often tested by the original laboratory prior to collaboration with other labs.[15]

## RESULTS AND DISCUSSION

**Optimization of Mobile Phase**

The first stage in developing a successful chromatographic method is optimizing the solvent system to maximize extraction efficiency since the mobile phase plays a crucial role in the overall performance of the technique. Method which produces small, dense spots exhibiting high scores for determining diosgenin concentration. The optimal mobile phase compositions for TLC has been determined by experimenting with several mixes of variable polarity. For the optimal outcome, we found that diosgenin peaks were strong and clearly characterized at $R_f$ 0.77 when toluene:ethyl acetate:formic acid (5:4:1v/v) was employed. A preliminary saturation of the chamber with the mobile phase was performed for five minutes at room temperature prior to the development of the plate. In Figure 1, we see diosgenin shown as a 3D HPTLC overlay. Figure 1 (a) shows chromatograph of diosgenin (marker, in-house and marketed formulation), Figure 1 (b) shows chromatograph of mangiferin, chlorogenic acid and marketed formulation of pushyanug churna. Table 1 shown preparation of gokshura churna herbs and quantity.

Detection of diosgenin using a method that produces thick, compact spots exhibiting highly significant values. Various mixes of the mobile phase of varied polarities were tested to find the optimal composition for TLC. The best outcome was achieved by employing the combination of ethyl acetate and methanol (4:6 v/v)[19] produced strong and clearly defined mangiferin peaks with an $R_f$ value of 0.23. Despite the fact that solvent systems, chlorogenic acid peak of $R_f$ 0.75 were sharp and clearly outlined in ethyl acetate:formic acid: acetic acid:water (10:1.1:1.1:2.6 v/v). Table 3 shows Intraday and Inter-day precision value for in-house formulation gokhshura.

A preliminary saturation of the chamber with the mobile phase was performed for five minutes at room temperature prior to the development of the plate. Figure 2 displays calibration plot of diosgenin plotted by UV as an overlay of mangiferin and chlorogenic acid that was created using a 3D HPTLC technique. Figure 3 shown calibration plot of mangiferin and chlorogenic aicd plotted by UV. Table 4 shows intraday and inter-day precision value for pushyang churna

**Precision**

By repeatedly scanning, we were able to assess the accuracy of our instruments, which allowed us to modify the scanner's settings and ensure reliable measurements of peak area. Table 2 shows determined Rf value of diosgenin for gokhshura and Rf value of mangiferin and chlorogenic acid for pushyanug churna.

Toluene, ethyl acetate, and formic acid (5:4:1 v/v) were employed to create the mobile phase, while pre-coated silica gel60, F254 served as the stationary phase compound diosgenin was estimated after derivatization and $R_f$ of the diosgenin was found to be 0.77 and UV absorption at the wavelength of 447 nm. Table 5 shows recovery study value of diosgenin marketed and in-house.

**Linearity**

This method was developed using pre-coated silica gel60, F254 in the form of stationary phase as well as ethyal acetate:methanol (4:6 v/v) utilized in the form of a mobile phase, compound mangiferin was estimated after derivatization and $R_f$ of the mangiferin peaks was found to be 0.23 and UV absorption at the wavelength of 263 nm.

This method has been developed employing pre-coated silica gel60, F254 in the form a stationary phase, ethyl acetate:formic acid:acetic acid:water (10:1.1:1.1:2.6 v/v) employed as mobile phase, compound chlorogenic acid was estimated after derivatization and $R_f$ of the chlorogenic acid peaks was found to be 0.75 and UV absorption at the wavelength of 265 nm. Table 6 and table 7 shows linearity range of mangiferin and chlorogenic acid and recovery study value.

## CONCLUSION

The utilization of high-performance liquid chromatography (HPLC) and ultraviolet light (UV) has proven an effective method for establishing a chain of evidence between a plant's botanical identification and its chemical component profile. It provides the means for a flexible screening procedure and qualitative and quantitative determination.

The procedure is straightforward, cheap, and flexible for quantitative analysis of active components compared to analytical technique. This method was validated and found

to be in linearity, accuracy, precision, specificity, range, and robustness.

Conclusions drawn from the research facts and findings show that the HPTLC approach is straightforward, precise, specific, sensitive, and accurate regarding diosgenin's quantitative and qualitative evaluation throughout herbal preparations. Gohshura and mangiferin, chlorogenic acid in polyherbal preparation of Pushyanug Churna.

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