Development And Validation Of Hptlc Method For Determination Of Diosgenin In Polyherbal Tablet

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Abstract

Aim: The present study was designed with an objective of developing a validated, simple, accurate and precise HPTLC method for the determination of Diosgenin from Polyherbal Tablet containing *Tribulus terrestris*.

Method: High performance thin layer chromatography (HPTLC) method was developed and validated for analysis of Diosgenin from polyherbal Tablet. Chromatographic separation was achieved on precoated silica gel HPTLC aluminum plate 60 F_{254} using Toluene: Acetone (8.5:1.5) as mobile phase. Detection was performed at 430 nm wavelength by TLC scanner (CAMAG).

Results: The R_f value of Diosgenin was found to be 0.41 ± 0.02. The linear regression analysis data for calibration plots showed good linear relationship ($r^2 = 0.997$) in the concentration range of 240 - 1440 ng/spot. The accuracy of the method was checked by conducting recovery studies at three different levels, using standard addition method. The average recovery of diosgenin was found to be 98.48 %. Precision (% RSD < 2%) of the developed HPTLC method was in accordance with the ICH guidelines.

Conclusion: The developed procedure can be selectively used in the assay and quality control of Diosgenin from various *Ayurvedic* Polyherbal Tablet formulations without interference from other herbal ingredients and excipients.

Key words: HPTLC, Diosgenin, Polyherbal Tablet, *Tribulus terrestris.*

Introduction

Gokhru consist of dried, ripe entire fruits of *Tribulus terrestris,* Family Zygophyllaceae. The plant is distributed throughout India [1]. Fruits are used as diuretic, tonic and also in the treatment of calculous affections and painful micturition. They are also used as aphrodisiac, impotence and kidney diseases [2-5]. Gokhru is a common ingredient of *Ayurvedic* preparations. The drug contains traces of alkaloids, harmine and Harman. Additionally, they contain saponins, which on hydrolysis yield, steroidal sapogenin like diosgenin, gitogenin, chlorogenin and ruscogenin [6-9].

Several analytical methods [10-14] have been reported for the estimation of diosgenin from the *Ayurvedic* Polyherbal formulations. However in the polyherbal formulations containing minor amount of drug, interference of other herbal ingredients and excipients may occur.

The proposed method overcomes this problems regarding sample preparation of *Ayurvedic* formulations which are commonly faced by the analyst.
So, the present study was designed for the development and validation of simple HPTLC method for the determination of diosgenin in Polyherbal Tablet developed by Siddhayu Ayurvedic Research Foundation Pvt. Ltd, Nagpur, India.

The proposed method was validated as per ICH guidelines and its applicability for quality control purpose [15].

Materials and Method

Composition of Polyherbal Tablet:

Sr.No.	Name of Ingredients	Qty in each tablet
1.	Kokilaksha (*Hygrophila spinosa*)	64 mg
2.	Vanyakahu (*Lactuca serriola*)	32 mg
3.	Kapikachchhu (*Mucuna pruriens*)	32 mg
4.	Swarnavang Bhasma (Mosaic gold)	32 mg
5.	Salab mishri (*Orchis mascula*)	130 mg
6.	Vidarikand (*Pueraria tuberosa*)	64 mg
7.	Gokshur (*Tribulus terrestris*)	64 mg
8.	Jivanti (*Leptadenia reticulata*)	64 mg
9.	Shailyam (*Parmelia perlata*)	32 mg

Drugs and Chemicals:

Reference standard Diosgenin (>95%) is purchased from Sigma Aldrich. All chemicals were Analytical grade or HPLC grade and purchased from Merck Chemicals, India. Stationary phase was pre-coated silica gel aluminium plate 60 F_{254} was obtained from Merck, Germany.

Preparation of standard stock solution:

Accurately weighed 1.2 mg of Diosgenin was dissolved in 2 ml methanol and was sonicated and diluted with methanol up to 10 ml (120 ng/μl).

Preparation of test solution of polyherbal Tablet:

Accurately weighed 5 g of crushed tablet powder was refluxed with 25 ml methanol for 30 min. The solution was filtered and evaporated on water bath. The remaining mark was dried and treated with petroleum ether (40˚- 60˚). The solution was sonicated and boiled on water bath. Petroleum ether was discarded. This procedure was repeated twice. The remaining mark was dried on water bath and treated with dil. HCl. The solution was sonicated, boiled and filtered. The solution was cooled and neutralised with 10 % $NaHCO_3$ solution. The extraction was done with chloroform (3 × 25 ml) in separating funnel. All chloroform layers were combined and washed with water. The chloroform layer was filtered through anhydrous sodium sulphate. The filtrate was evaporated and volume was made upto 10 ml with methanol.

Chromatographic condition:

The samples were spotted in the form of bands, width 6 mm with a Camag 100 microlitre sample (Hamilton, Bonaduz, Switzerland) syringe on silica gel pre-coated aluminum plate 60 F_{254} plates, (20 cm × 10 cm with 250 μm thickness; (E. Merck, Darmstadt, Germany) using a Camag Linomat V (Switzerland) sample applicator. The plates were prewashed with methanol and activated at 110˚C for 5 min prior to chromatography. A constant application rate 0.1μl/s was used and the space between two bands was 10 mm. The slit dimension was kept at 6 mm × 0.45 mm and the scanning speed was 20 mm/s. The monochromatic bandwidth was set at 20 nm, each track was scanned three times and baseline correction was used. The mobile phase was Toluene: Acetone (8.5:1.5). Linear ascending development was carried out in a 20 cm × 10 cm twin trough glass chamber (Camag, Switzerland) saturated with the mobile phase. The optimized chamber saturation time for the mobile phase was 30 min at room temperature (25˚C ± 2) at relative humidity 60 % ± 5. The length of each chromatogram run was 8 cm. Following the development, the TLC plates were dried in a current of air with the help of an air dryer. Densitometric scanning was performed using a Camag TLC scanner III in the reflectance/absorbance mode at 430 nm after derivatisation with Anisaldehyde Sulhuric acid regent. Concentrations of the compounds were determined from the intensity of the diffused light. Evaluation was by peak areas with linear regression. The amount of diosgenin was computed from peak areas.

Results and Discussion

Mobile phase development:

The mixtures of several mobile phases were tried. The solvent system Toluene: Acetone (8.5:1.5) was selected for estimation of diosgenin, which gave good resolution. Figure 1 is showing chromatographic separation of diosgenin at R_f 0.41. Figure 2 is showing chromatographic separation of diosgenin in polyherbal Tablet. The absorption spectrum of diosgenin is shown in Figure 3. The wavelength 430 nm was used for quantification of sample.
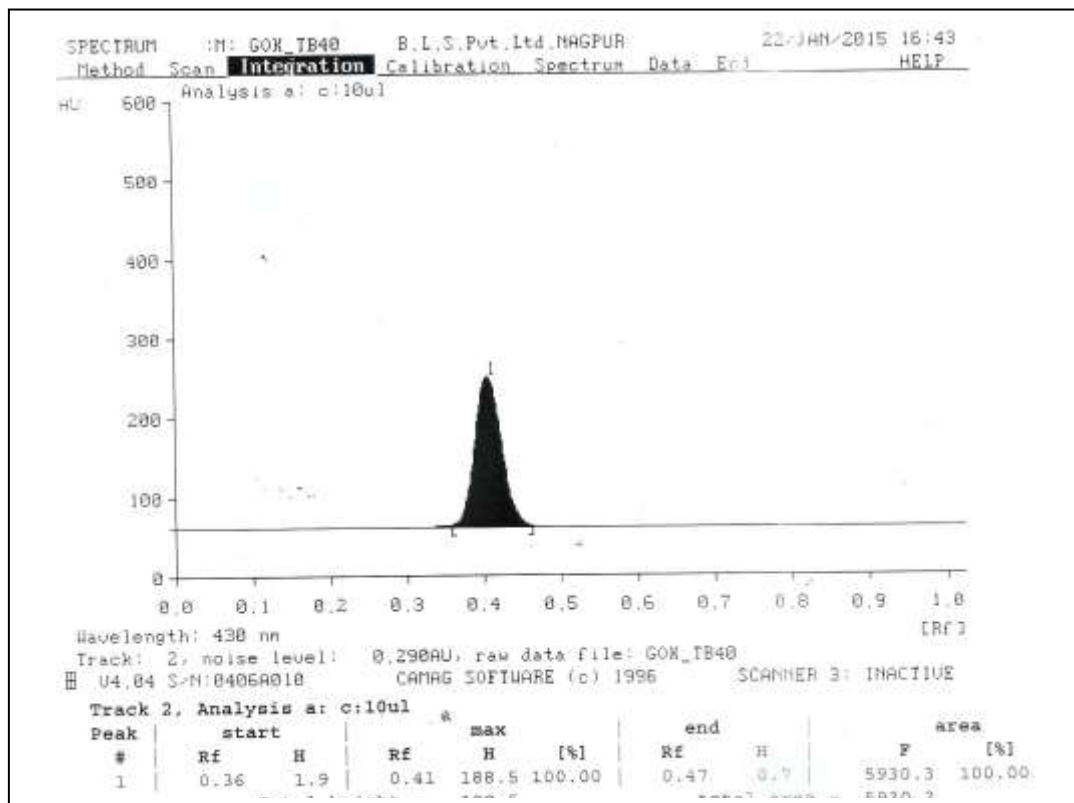
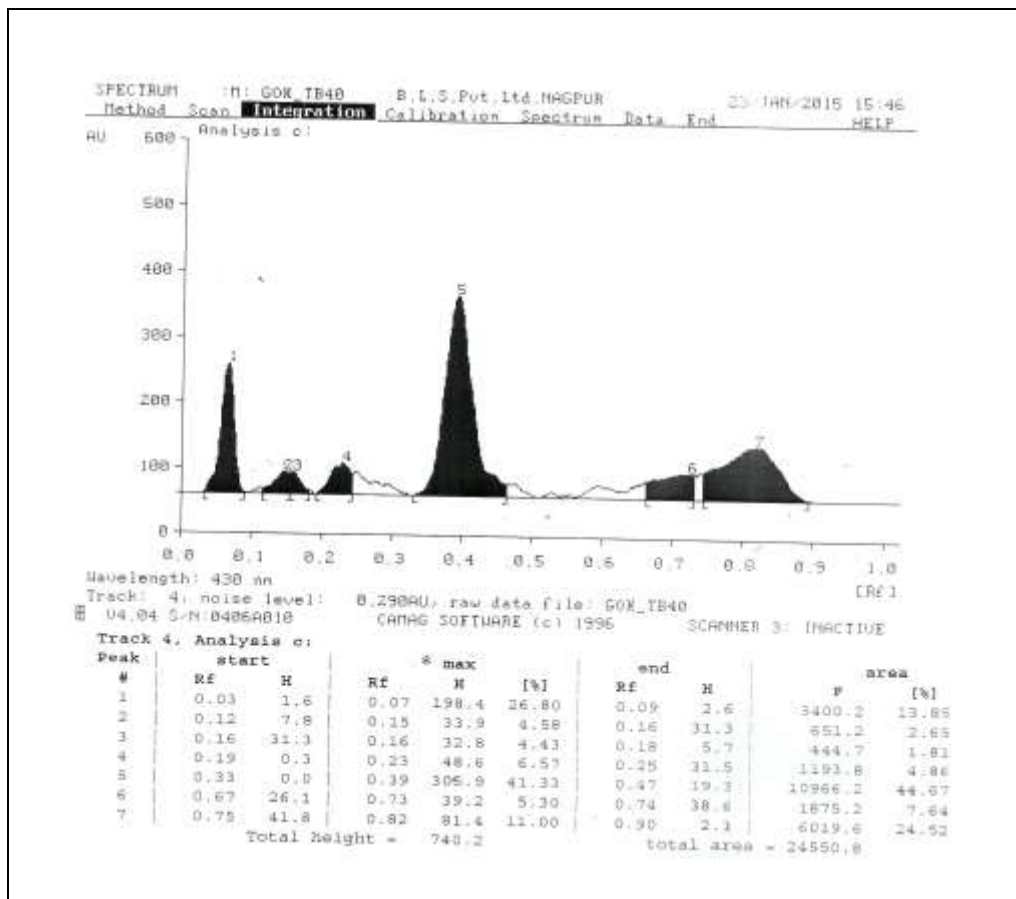**Figure 1: HPTLC chromatogram of standard diosgenin**

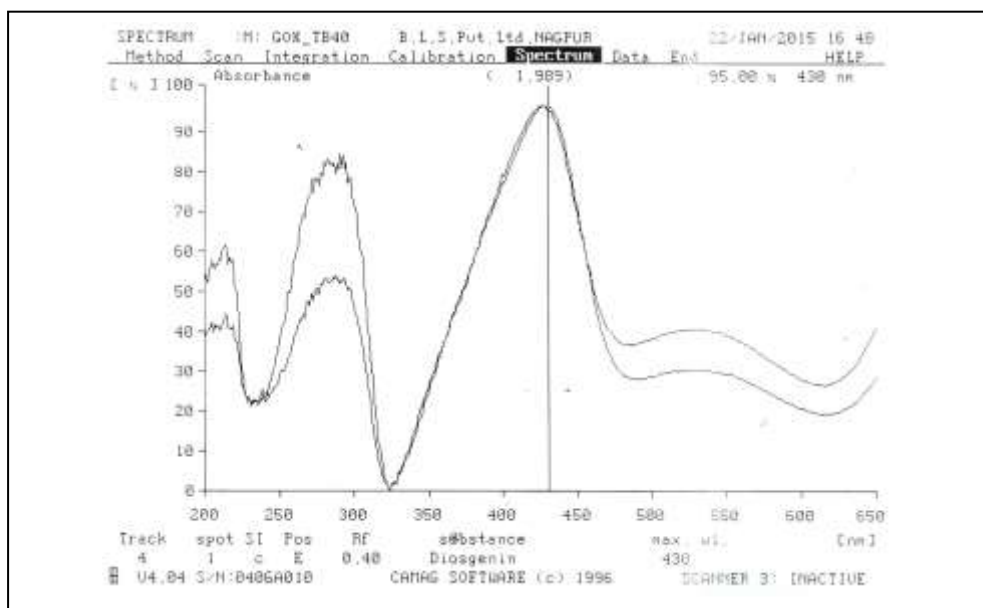**Figure 2: HPTLC chromatogram of polyherbal Tablet**

Figure 3: overlain spectrum of standard diosgenin and polyherbal Tablet

Validation of Method:

The method was validated for linearity, specificity, accuracy, recovery, intra-day and inter-day precision, ruggedness, robustness, LOD and LOQ in accordance with ICH guidelines.

Calibration curve:

A standard solution of diosgenin in methanol (120 ng/μl) was applied in 2, 4, 6, 8, 10, and 12 μl, on the TLC plate to prepare linear calibration curve.

Linearity:

Linearity was performed by applying standard solution at different concentrations ranging from 240 to 1440 ng/spot on HPTLC plates. Peak areas were recorded for each concentration. Linearity curve was obtained by plotting a graph of peak area vs. applied concentration. The calibration line was represented by linear equation Y= 4.769 x + 158.7 for diosgenin. For this equation the correlation coefficient, r^2 was 0.997 for diosgenin.

Specificity:

The specificity of method was ascertained by standard diosgenin and sample polyherbal Tablet. The solutions of standard diosgenin and polyherbal Tablet were spotted on TLC plate in triplicate and run. The spot for diosgenin in the samples was confirmed by comparing the R_f values and spectrum with standards. The validation parameters for the proposed method are shown in Table 1.

Recovery:

The accuracy was studied by the standard addition technique. Three different levels of standard were added to the previously analyzed samples, each level being repeated thrice. The results of recovery studies were expressed as percent recovery. The results are shown in Table 2.

Precision:

All the solutions were analyzed on the same day in order to record any intraday variations in the results. For Inter day variation study, three different concentrations of the standard solutions in linearity range were analyzed on three consecutive days. The repeatability of sample application and measurement of the peak area was expressed in terms of % RSD. The % RSD was found to be less than 2.0 in all cases indicate no significant variations in the analysis of diosgenin at the concentration of 960, 1200 and 1440 ng/spot.

Robustness:

The estimations were performed by introducing variations in the mobile phase distance development and saturation time in development chamber; the effects on the results were examined. Mobile phase distance was changed by ± 5 mm. The saturation time of mobile phase in the chamber was varied by ± 5 min. The % RSD was found to be

less than 1.0 in all cases indicates no significant variations in the analysis of diosgenin at the concentration of 1200 ng/spot.

Ruggedness:

The HPTLC method was evaluated by carrying out the analysis of standard solutions by two analysts using same operational and environmental conditions, the % RSD was found to be less than 1.0 in the analysis of diosgenin at the concentration of 1200 ng/spot.

Limit of detection (LOD) and Limit of quantification (LOQ):

The LOD and LOQ were calculated by using the values of slopes and intercepts of the calibration curve. The LOD and LOQ were calculated as 96.66 ng/spot and 292.93 ng/spot for diosgenin

Table 1: Validation parameter for diosgenin by HPTLC

Sr. No	Parameters	diosgenin
1	Linearity range	240 ng -1440 ng
2	Correlation coefficient	0.997
3	Regression equation (y = mx+c)	Y = 4.769 x + 158.7
4	Accuracy (mean recovery)	98.48 %
5	Precision (RSD)	
	Interday	0.42
	Intraday	0.30
6	Ruggedness/Robustness (RSD) between two experiments	
	Development distance	0.31
	Saturation time	0.37
	Analyst	0.34
7	specificity	Specific
8	Limit of detection (LOD)	96.66 ng
9	Limit of quantification (LOQ)	292.93 ng

Table 2: Result and Statistical data for recovery study of diosgenin

Sr. No.	Amount of Sample taken in µg/spot	Amount of diosgenin present ng	Amount of diosgenin added ng	Total Amount of diosgenin ng	Total Recovery	% Recovery	Mean % Recovery
1	3000.0	2266.19	200	2466.19	2403.30	97.45	
2	3000.0	2266.19	250	2516.19	2494.04	99.12	98.48
3	3000.0	2266.19	300	2566.19	2537.19	98.87	

Table 3: Estimation of diosgenin in polyherbal Tablet

Component	Amount taken (µg/spot)	Peak area	% R.S.D.	Amount found (ng)	% Amount found
diosgenin	3000.0	10966.2	1.14	2266.19	0.0755

Conclusion

The proposed method for the estimation of diosgenin from Polyherbal tablet is accurate, linear, rugged, simple and rapid than the reported methods. The method therefore can be used for routine quality-control analysis and quantitative determination of diosgenin in polyherbal tablet formulations without interference from other herbal ingredients, excipients and auxiliary substances.

References

1. Indian Herbal Pharmacopoeia. Revised new Edn, Indian Drug Manufacturers Association, Mumbai, 2002, 459-466.
2. Rajpal V. Standardisation of Botnicals. Vol. I, Eastern publishers, New Delhi, 2002,

223-228.

3. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. Edn 33, Nirali Prakashan, Pune, 2005, 225-226.

4. The Useful Plants of India. Edn 3, Publications & Information Directorate, CSIR, New Delhi, 1994, 647-648.

5. Nadkarni KM. Indian Materia Medica. Edn 3, Vol. I, Popular Prakashan, Mumbai, 1976, 1229-1230.

6. Handa SS, Kapoor VK. Pharmacognosy. Edn 2, Vallabh Prakashan, Delhi, 1988, 229-230.

7. The Ayurvedic Pharmacopoeia of India. Edn 1, Part I, Vol. I, The Controller of Publications, Delhi, 1990, 40.

8. Dey AC. Indian Medicinal Plants used in Ayurvedic Preparations. Bishen Singh Mahendra Pal Singh, Dehra Dun, 1994, 52-53.

9. Tirtha SS. The Ayurveda Encyclopedia Natural Secrets to Healing, Prevention, & Longevity. Edn 1, Sri Satguru Publications, Delhi, 1998, 86.

10. Kshirsagar VB, Deokate UA, Bharkad VB, Khadabadi SS. HPTLC Method Development and Validation for the simultaneous Estimation of Diosgenin and Levodopa in marketed formulation. Asian Journal Research Chemistry 2008; 1(1): 36-39.

11. Parameswaran S, Koshti S. Quantitative Estimation of Piperine and Diosgenin in Ayurvedic formulation. British Journal of Pharmaceutical Research 2014; 4(8): 911-920.

12. Warke VB, Deshmukh TA, Patil VR. Development and Validation of RP-HPLC Method for Estimation of Diosgenin in Pharmaceutical dosage form. Asian Journal of Pharmaceutical & Clinical Research 2011; 4 (1): 126-128.

13. Ghodasara T, Gaudani R, Nayak B, Shingala D. HPTLC Method Development and Validation for the Simultaneous Estimation of Diosgenin and Gallic acid in marketed formulation. Pharma Science Monitor An International Journal of Pharmaceutical Science 2012; 3(4): 2664.

14. Jayaprakasm R, Ravit K, Athira K, Asha JB. Quantitative Determination Of Diosgenin In Polyherbal Formulation & Various Extracts of *Smilax china Linn* using standard marker by Validated Analytical Techniques. Universal Journal of Pharmacy 2013; 02(04): 83-90.

15. ICH Harmonized Tripartite Guideline. Validation of Analytical Procedures: Text and Methodology. Q2 (R1), November 2005.