Academic Sciences Asian Journal of Pharmaceutical and Clinical Research

Vol 5, Issue 4, 2012 ISSN - 0974-2441

Research Article

# A VALIDATED RP-HPLC METHOD FOR THE ESTIMATION OF DIOSGENIN FROM POLYHERBAL FORMULATION CONTAINING *TRIBULUS TERRESTRIS* LINN.

**PANKAJ K. GUPTA*[1, 2], DHEERAJ H. NAGORE[1], VINOD V. KUBER[1] AND SURESH PUROHIT[3]**

**[1] R&D, Tulip Lab Pvt. Ltd., MIDC Ranjangaon, Pune, India,[2] Research Scholar, Jodhpur National University, Jodhpur, India,[3] Department of Pharmacology, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India. Email:** pkg2010@gmail.com

*Received:23 july 2012, Revised and Accepted:31 August 2012*

**ABSTRACT**

In present study a simple, rapid and specific high-performance-liquid-chromatographic (HPLC) method has been developed and validated for estimation of bioactive marker diosgenin (DSG) from polyherbal formulation containing gokhru (*Tribulus terrestris* Linn.) extract. Separation of DSG was achieved on reverse-phase 250mm×4.6mm, 5µ, symmetry-C8 column with mobile phase of water and methanol. Scanning wavelength was optimized and selected as 205nm. The linear regression analysis of calibration plots showed good linear relationship with $r^2$=0.9996 in concentration ranges of 25-75µg/mL. The method was validated for its calibration curve, specificity, precision and robustness. The recovery was found in range of 98.06-99.50%. The developed HPLC method can be used to quantify DSG for quality control and standardization of polyherbal formulations containing gokhru. The analytical data were compared by statistical evaluation and the method was found to be repeatable and selective.

**Keywords:** Diosgenin, HPLC, *Tribulus terrestris*, Quantitation

## INTRODUCTION

Traditionally herbal formulations have been used by the human society to prevent and treat various diseases. Herbal medicines are in great demand in developing as well as developed countries for the primary health care because of their wide range of medicinal activities. Gokhru / Gokshur (*Tribulus terrestris* Linn.) is one of the important plant reported to be used in many diseases, especially in treatment of sexual dysfunction. Plenty of polyherbal formulations containing gokhru extract are very commonly used in such type of dysfunctions. Gokhru is described to have aphrodisiac properties; hence, it has been used to treat impotency as well as to improve the erection[1, 2].

Gokhru, a member of the Zygophyllaceae family is an annual herb found in many areas of the world. It has been used as traditional medicine in many areas, such as India, China and Turkey[3]. Gokhru has long been used in medicine in India to treat sexual problems in both men and women[4]. Not only that but gokhru is also reported to have other medicinal properties include diuretic properties, increased endothelial nitric oxide production, direct smooth muscle relaxant effects etc[5].

A large amount of potential active components have been identified in gokhru, including steroidal saponins such as diosgenin, it is obtained by hydrolysis of crude saponins isolated from gokhru. Alkaloids and flavonoids with the many other saponins are also present like ruscogenin, yamogenin, epismilagenin, neotigogenin, gitogenin, neogitogenin, desgalactotigonin, F-gitonin, desglucolanatigonin, gitonin, and tigogenin[6].

Indeed, current research indicates that the steroidal saponins, particularly the dominant saponin like diosgenin and protodioscin, are responsible for the pharmacological activities of gokhru[7,8,9]. Diosgenin is often used as a raw precursor for the production of steroidal drugs and hormones such as testosterone, gluco-corticoids and progesterone[10].

Standardization of herbal formulations in terms of quality of raw materials, manufacturing practices, and composition is important to ensure quality and optimum levels of active principles for their bio-potency. Recently, the concept of marker-based standardization of herbal drugs is gaining momentum. Identification of unique compounds in herbs as markers and development of analytical methodologies for monitoring them are the key steps involved in marker-based standardization.

The present research aimed at developing simple, accurate, sensitive and more convenient, less time consuming HPLC method for routine analysis of the DSG from polyherbal formulation. The developed analytical method was validated as per ICH guidelines.

## MATERIALS AND METHODS

### Tablet formulation

A core tablet formulation containing 250 mg of *Epimedium brevicornum* Linn. extract IH (Jiaherba Phytochem, Xian, China), 100 mg of *Tribulus terrestris* Linn. extract IH (Amruta Herbals, Indore, India), 100 mg of *Withania somnifera* Linn. extract IH (Amruta Herbals, Indore, India), 100 mg of *Lepidium meyenii* Walp. extract IH (Phytoconcentrate, Ahmedabad, India), 100 mg of L-arginine BP (Amol Biotech Ltd, Shanghai, China) as actives; and microcrystalline cellulose BP (RanQ Remedies Pvt. Ltd., Sinner, India), crospovidone BP (Boai NKY Pharmaceutical Ltd., Jiaozuo, China), croscarmellose sodium BP (Aditya Chemicals, Ahmedabad, India), methyl hydroxybenzoate BP (Alta Lab Ltd., Khopoli, India), propyl hydroxybenzoate BP (Alta Lab Ltd., Khopoli, India), purified talc BP (Nilkanth, Jodhpur, India), magnesium stearate BP (Amithi Drugs, Ahmedabad, India) and colloidal anhydrous silica BP (Evonik Degussa Corp., New Jersey, USA) was developed and prepared in-house. The blue coloured ready mix film coating composition (Ideal Cures Pvt. Ltd., Thane, India) for the tablet formulation contains hypromellose BP, macrogol BP, purified talc BP, titanium dioxide BP and lake indigo carmine IH.

### Chemicals

HPLC grade solvents like methanol were obtained from Merck Ltd., Mumbai, India. Standard Diosgenin was purchased from Sigma Aldrich, India. All the chemicals used in the experiment were of analytical grade.

### Chromatographic conditions for HPLC

High performance liquid chromatography was performed with Waters 2695 Alliance system with a 2996 photodiode array detector (PDA). DSG was separated on a reverse-phase 250 mm × 4.6 mm, 5µ, Symmetry C8 column (Waters). The mobile phase was prepared from water (solvent A) and methanol (solvent B). The mobile phase was degassed and filtered through 0.45-µm filter before use. The proposed gradient program for the separation is mentioned in table 1.

**Table 1: Table shows gradient program for the mobile phase**

| Time (minute) | Solvent A (%) | Solvent B (%) |
|---|---|---|
| 0 | 15 | 85 |
| 20 | 15 | 85 |
| 21 | 02 | 98 |
| 25 | 02 | 98 |
| 26 | 15 | 85 |
| *30* | 15 | 85 |

The mobile phase flow rate was kept at 1 mL $min^{-1}$. Before the first injection, the column was saturated for 30 minutes with the initial mobile phase. The column temperature was maintained at 30°C±2°C. The injection volume was kept 5 µL. The PDA was set at 205 nm to acquire the chromatogram. The DSG was identified by comparing the retention time and spectra obtained from sample and standard solutions. The present work was performed in an air-conditioned room maintained at 25°C±3°C.

**Preparation of calibration curve**

The stock solution of the standard DSG was diluted to obtain seven different concentrations (5-100 µg $mL^{-1}$) for the preparation of calibration curve and these were injected into the system. These samples were analyzed by the method as described under chromatographic conditions. The peak areas were recorded and calibration curve was prepared by plotting average peak area against concentration of DSG. The data of peak areas against concentration was treated by linear least regression analysis.

**Preparation of test solution**

20 Tablets of polyherbal formulation were weighed and crushed in to powder form. The powder of the formulation equivalent to 1 gm of gokhru extract i.e. 10 tablets equivalent powder was accurately weighed. To this 90 mL 3 N hydrochloric acid was added and it was kept on reflux for one and half hour on boiling water bath at 100°C. This mixture was allowed to cool at room temperature and diluted up to the mark with water. The mixture was extracted with diethyl ether (75 mL x 3). Ether layer was separated and allowed to evaporate collectively. The residue was dissolved in 100 mL of methanol. This resulting solution was used as test solution.

**Validation of HPLC method**

International Conference on Harmonization (ICH) guidelines (CPMP/ICH/381/95; CPMP/ICH/281/95) were followed for the validation of the analytical procedure. The proposed HPLC method was validated in terms of precision, specificity, linearity, accuracy, solution stability and robustness of the sample application on the in-house tablet formulation as per the guidelines[11].

**Precision**

The precision of the system was carried out by six replicate injections from the same vial of standard at the analytical concentration and was expressed in terms of percent relative standard deviation, % RSD (Acceptance criterion: %RSD should not be more than 2.0%). Six different samples of the polyherbal formulation were analyzed for method precision. The percent assay of DSG and percent RSD was calculated (Acceptance criterion: %RSD should not be more than 2.0%). The intermediate precision was carried out on two different systems for six different samples by two different analysts. The DSG content and percent RSD was calculated (Acceptance criterion: %RSD should not be more than 2.0%).

**Specificity**

The specificity of the method was ascertained by analyzing diluent, standard, drug samples of equivalent concentration and placebo (the placebo for gokhru extract was prepared by using the same composition mentioned under tablet formulation, except the gokhru extract) to examine the interference of diluent and placebo with analyte peak. The specificity of the method was studied by assessment of peak purity of DSG using Waters empower software and diode array detector (Acceptance criterion: peak purity should pass).

**Accuracy**

The accuracy of the method was determined from recovery studies. A known but varying amount of analyte was spiked to placebo at 80%, 100% and 120% recovery levels of the standard in triplicate. The samples were analyzed according to the proposed method. The percentage recovery was calculated against respective level (Acceptance criterion: % recovery should be in the range of 95%-105%).

**Solution stability**

The sample solution was prepared as per the proposed method and subjected to stability study at room temperature for 24 hrs. The sample solution was analyzed at initial and at different time intervals up to 24 hrs. The change in peak area response of DSG in sample solution with respect to time was calculated as absolute percent difference against initial response.

**Robustness**

The robustness of the method was determined by slight deviation in the method parameters. The parameters selected were deviation in the wavelength, column temperature, flow rate and mobile phase gradient. The retention time of DSG was determined and %RSD with system suitability parameters was observed.

**RESULTS AND DISCUSSION**

**Chromatographic study**

The composition of solvent systems in HPLC was optimized by testing different solvent compositions of varying polarity and the best results were obtained by using present method which produces highly symmetrical peaks showing good resolution between DSG and other peaks as shown in figure 1 and figure 2. The scanning wavelength selected was 205 nm for DSG provides comparable results. Peak purity was assessed by comparison of overlay spectra of DSG standard and test peak at the start, apex and end was found satisfactory as shown in figure 3. DSG was resolved at retention time around 14 minutes on HPLC.

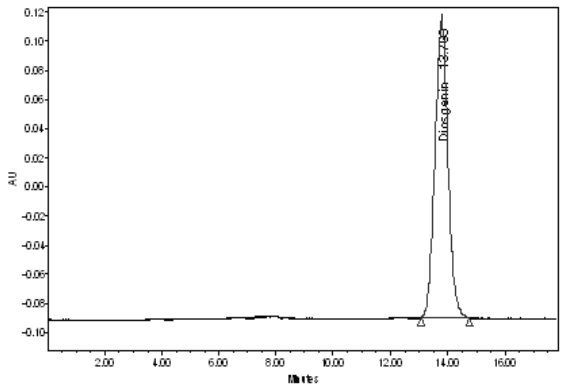


**Fig 1: It shows HPLC chromatogram of standard DSG**

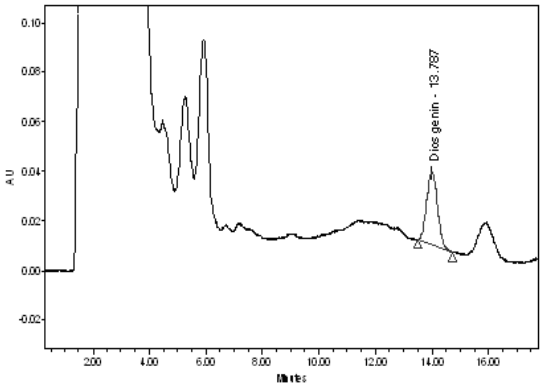


**Fig 2: It shows HPLC chromatogram of DSG from polyherbal formulation**

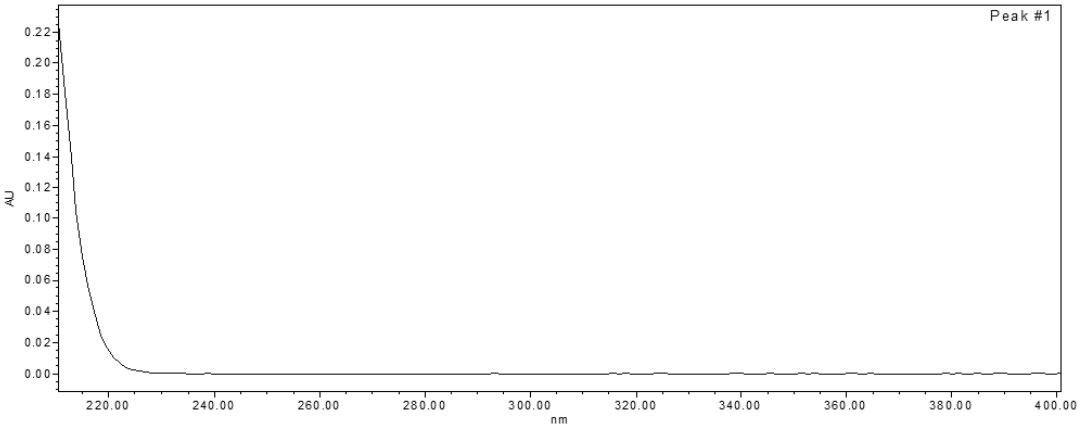


**Fig 3: It shows HPLC spectra of standard DSG**

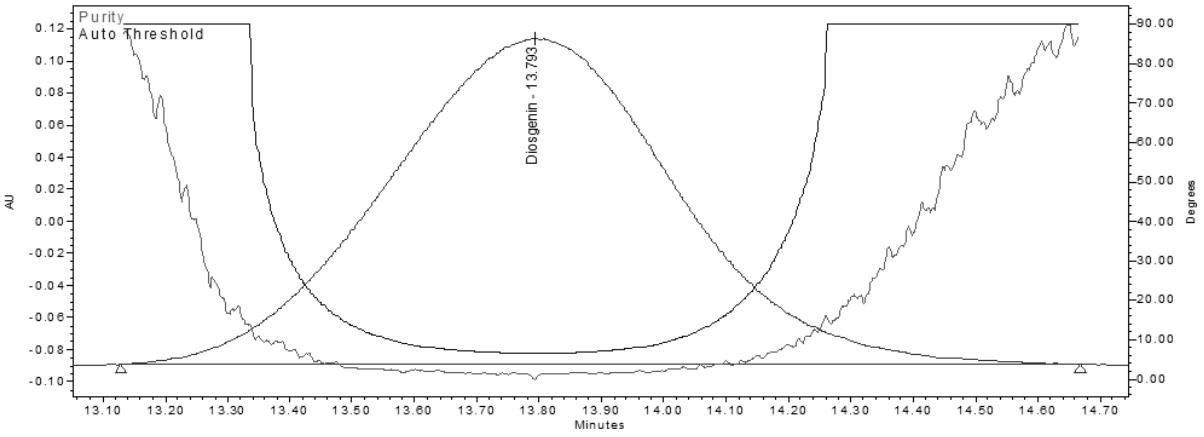


**Fig 4: It shows purity spectra of DSG from formulation**

**Calibration curve**

The calibration curve of peak area against concentration was linear in the range 25-75 µg $mL^{-1}$ for DSG. The calibration line was represented by linear equation y = 12266x - 6397.2, where *y* is response and *x* is amount. For this equation the correlation coefficient, $r^2$, was $r^2$=0.9996 (Table 2).

**Table 2: Tablet shows method validation parameters for quantitation of DSG**

| Sr. No. | Parameters | Diosgenin |
|---|---|---|
| 1. | Specificity | Specific |
| 2. | Linearity (correlation coefficient ) | 0.9996 |
| 3. | Range (µg $mL^{-1}$) | 25-75 |
| 4. | System precision (% RSD) (n=6) | 0.23 |
| 5. | Method precision (% RSD) (n=6) | 0.33 |
| 6. | Intermediate precision (% RSD) (n=6) | 0.59 |
| 7. | Solution stability | Stable |
| *8.* | Regression equation | (y = 12266x - 6397.2) |

**Precision**

The repeatability of sample application and measurement of peak area were expressed in terms of %RSD. The %RSD values as depicted in table 2 shows that proposed method provides acceptable system, method and intermediate precision of DSG.

**Specificity**

Peak purity analysis for DSG peak indicated that the peak was homogeneous and there was no co-eluting peaks, no any interference from diluent, thus indicating the specificity of the method as mentioned in figure 4.

**Recovery**

The proposed method when used for estimation of DSG from polyherbal formulation after spiking with 80%, 100% and 120% of additional standard DSG afforded average recovery of 99.06% (range 98.61% - 99.50%) as depicted in table 3.

**Table 3:Table shows recovery data of dsg**

| Name of standard | Amount of Standard added (mg) | Amount of Standard recovered (mg) | % recovery | Average recovery (%) |
|---|---|---|---|---|
| Diosgenin (DSG) | 4.0 | 3.98 | 99.50 | 99.06 |
| | 5.0 | 4.95 | 99.08 | |
| | 6.0 | 5.91 | 98.61 | |

**Solution stability**

The absolute percentage difference for the area of analyte in sample solution to that of initial response does not exceed an acceptable limit upto 24 hours of preparation at room temperature indicating the stability of the sample solution.

**Robustness**

All the system suitability parameters were found to be within acceptable limit indicating the robustness of the method.

**CONCLUSION**

The proposed HPLC method is simple, rapid, specific and accurate techniques to quantify the DSG in the presence of other constituents from the formulation without compromising the accuracy. Method

validation proves that method is selective and reproducible for the estimation of diosgenin from polyherbal formulations.

**REFERENCES**

1. Devi DJ, Ramesh CU. Different chemo types of gokhru (*Tirbulus terrestris*): a herb used for improving physique and physical performance. Int J Green Pharmacy 2008; 3: 158–161.
2. Kalamegam G, Adaikan PG, Prasad RNV. Sexual effects of puncturevine (*Tribulus terrestris*) extract (protodioscin): an evaluation using a rat model. J Altern Complem Med 2003; 9: 257–265.
3. Bedir E, Khan IA. New steroid glycosides from the fruit of *Tribulus terrestris*. J Nat Prod 2000; 12: 1699–1701.
4. Chakraborty B, Chakraborty K. Some central effects of *Tribulus terrestris* Linn. Indian J Physiology Allied Science 1977; 31: 189–192.
5. Arcasoy HB, Erenmemisoglu A, Tekol Y, Kurucu S, Kartal M. Effect of *Tribulus terrestris* saponin mixture on some smooth muscle preparations: a preliminary study. Boll Chim Farm 1998; 137: 473–475.
6. Bourke CA. Staggers in sheep associated with the ingestion of *Tributes terrestris*, Aust Vet J 1984; 61: 360–363.
7. Ganzera M., Bedir E, Khan IA. Determination of steroidal saponins in *Tribulus terrestris* by reversed-phase high-performance liquid chromatography and evaporative light scattering detection. J Pharm Sci 2001; 90: 1752–1758.
8. Wu G, Jiamg S, Jiang F, Shu D, Wu H, Jiang S. Steroidal glycosides from *Tribulus terrestris*. Phytochemistry 1996; 42: 1677–1682.
9. Xu YX, Chen HS, Liang HQ, Gu ZB, Liu WY, Leung WN, et al. Three new saponins from *Tribulus terrestris*. Planta Med 2000; 66: 545–550.
10. Mehrafarin A, Qaderi A, Rezazadeh S, Naghdi BH, Noormohammadi G, Bioengineering of important secondary metabolites and metabolic pathways in fenugreek (*Trigonella foenum-graecum* L.). J Med Plants 2010; 9: 1–18.
11. ICH Q2B. Harmonized tripartite guideline, validation of analytical procedures: methodology, IFPMA, In: Proceeding of the International Conference on Harmonization (ICH), Geneva 1996.