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Research Article

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR ESTIMATION OF DIOSGENIN IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

Diosgenin is a steroidal sapogenin possessing estrogenin and antitumour properties. The pharmacological property of a steroidal saponin has been described including their hypocholesterolemic, antidiabetic and antioxidant activities. A simple, accurate and sensitive HPLC method was developed for estimation of diosgenin.The chromatographic separation was carried out on RP C_{18} ods Hypersil column (150 × 4.6 mm, 5µm) using mobile phase acetonitrile: water in the ratio of 90:10 v/v at flow rate 1 ml/min with UV detection at 203 nm. Diosgenin was well resolved on the stationary phase, and the retention time was 10.5 minutes. The calibration curve was linear (r = 0.997) in the concentration range 2-10 µg/ml. The method was statistically validated for precision, accuracy, LOD, LOQ, robustness and recovery. The RP-HPLC method was successfully applied for identification of diosgenin in marketed herbal formulation containing *Trigonella foenum graecum*. The proposed method may be extended to study the degradation of diosgenin under different stress conditions, as per the recommendations of ICH guidelines.

Keywords: Diosgenin, HPLC, Steroidal sapogenin, *Trigonella foenum graecum*, Validation.

INTRODUCTION

Herbal medicines are in great demand in the developed as well as developing countries for primary healthcare because of their wide biological activities, higher safety margins and lesser costs. Herbal medicines has been enjoying renaissance among the customers throughout the world. The quality control of herbal medicine that is, the profile of the constituents in the final product has implications in efficacy and safety. Due to the complex nature and inherent variability of the chemical constituents of the plant based drugs, it is difficult to establish quality control parameters and modern analytical techniques like HPLC and HPTLC are expected to help in circumventing this problem [1]. For past few decades, compounds from natural sources have been gaining importance because of the vast chemical diversity that they offer. This has led to phenomenal increase in the demand for herbal medicines in the last two decades and a need has been felt for ensuring the quality, safety and efficacy of herbal drugs [2]. Diosgenin is a steroidal sapogenin possessing estrogenic and antitumor properties. Steroidal sapogenins are secondary metabolites whose biosynthetic precursors are sterols, particularly cholesterol [3].

Fenugreek (*Trigonella foenum graecum* L.; family Leguminoceae) is one such plant whose seeds and leaves are used not only as food but also as an ingredient in traditional medicine. Its leaves are consumed widely in India as a green leafy vegetable and are a rich source of calcium, iron, h-carotene and other vitamins. In Ayurvedic and Unani systems of medicine, fenugreek is used for the treatment of epilepsy, paralysis, gout, dropsy, chronic cough and piles, immunomodulatory effects [4].

The literature survey records a variety of therapeutic actions of *Trigonella foenum-graecum* including hypocholesterolaemia, hypoglycaemia, antibacterial, antiviral, anti-inflammatory, antipyretic activity [5]. Several analytical methods have been reported for the determination of diosgenin based on HPLC [6], HPTLC, TLC [7-8], but there are very few analytical methods were reported on estimation of diosgenin in fenugreek and its dosage form.

The objective of the present work was to developed an accurate, repeatable and robust method for the determination of diosgenin. The proposed method was validated in compliance with ICH guidelines [9].

MATERIALS AND METHODS

Instrumentation

Absorption spectra was recorded with a shimandzu UV-1700 spectrophotometer with 10 mm matched quartz cells attached to a PC with UV Probe software Version 2.10 (Shimandzu, Kyoto, Japan). The chromatographic system (Shimandzu, Kyoto, Japan) consisted of Shimandzu LC-20 at prominence solvent delivery module, a manual Rheodyne injector with a 20 µL fixed loop and a SPD-20A Prominence UV- visible detector. The separation was performed on a Phenomenex C_{18} column (150 × 4.6 mm, 5 µm particle size, Phenomenex Torrance, USA) preceded by an ODS guard column (10µm, 10 mm×5mm) at an ambient temperature. Chromatographic data recorded and processed using a Spinchrom Chromatographic Station CFR Version 2.4.0.193 (Spinchrom Pvt. Ltd., Chennai, India).

Materials

Diosgenin was purchased from Total Herb Solution Pvt. Ltd., Mumbai (India). Divya Madhunashini Vati (Divya Pharmacy, Haridwar) were procured from the local market. Methanol, acetonitrile and water of HPLC grade were purchased from Qualigens, Mumbai, India. All the other solvents and reagents used were of analytical grade.

Preparation of standard solution

10 mg of diosgenin was weighed and dissolved in 5 ml methanol by means of sonicated for 15 min. The solution was diluted up to 10 ml with methanol (1 mg/ml). Pipette out 1 ml solution from stock solution and diluted up to 10 ml with methanol (100 µg/ml).

Chromatographic conditions

Chromatographic estimation was performed using an equilibrated Phenomenex RP-C18 column (particle size 5 µm; 150mm X 4.6 mm ID), a mobile phase consisting of acetonitrile: water: in the ratio of 90:10 v/v at flow rate of 1 ml/min with UV detection at 203 nm.

Calibration curve of Diosgenin

The content of diosgenin was determine using a calibration curve established with five dilution at concentration ranging from 2-10 µg/ml. Each concentration was measured in triplicate. The corresponding peak areas were plotted against the concentration of diosgenin injected peak identification was achieved by comparison of both Retention time (tR) and UV absorption spectrum for standard.

Application to pharmaceutical preparation (Divya Madhunashini Vati)

Weighed accurately 10 tablets and crushed in glass crucible to obtain fine powder. Dissolve average weight of tablet (522.4 mg) in 25 ml methanol. The content was sonicated for 15 min. The solution was filter through Whatman filter paper no.1. Pipette out 1.2 ml

solution from stock solution and diluted up to 10 ml with methanol. The solution was sonicated for 15 min. Pipette out 1 ml solution from stock solution and diluted up to 10 ml with methanol (250 µg/ml).

Method validation

The method was validated for linearity, accuracy, precision, robustness, LOD, LOQ and system suitability by the following procedures:

Linearity

Five different concentrations of diosgenin were analyzed and their calibration curve was constructed in the specified concentration range (2-10 µg/mL). The calibration plots were generated by replicate analysis (n = 3) at all concentration levels and the linear relationship was evaluated using the least square method within Microsoft Excel® program.

Precision

The precision was examined by performing the intra-day and inter-day assays of three replicate injections of the mixture of standard solutions at three concentration levels (10, 20 and 30 µg/mL). The intra-day assay precision was performed with the interval of 4 h in 1 day, while the inter-day assay precision was performed over 3 days.

Limit of detection and limit of quantification

The LOD and LOQ were determined by kD/S where k is constant (3.3 for LOD and 10 for LOQ), SD is the standard deviation of the analytical signal, and s is the slope of the concentration/response graph.

Robustness

The robustness of the method was evaluated by analyzing the system suitability standards and evaluating system suitability parameter data after varying, individually, the HPLC pump flow rate (± 0.1), wavelength (± 1) and mobile phase composition (± 2). Solution was injected 3 times for each change. Mean and SD were calculated for each peak. % RSDs was calculated for each component during each change.

Accuracy

The accuracy of the method was determined by calculating the recoveries of diosgenin by the method of standard addition. A known amount of standard (80%, 100% and 120%) was added to pre analyzed sample solution, and the amount of the standard was estimated by measuring the peak areas and by fitting these values to the straight-line equation of calibration curve.

RESULT AND DISCUSSION

A simple HPLC method was adopted for the determination of diosgenin in herbal formulation. To optimize the proposed HPLC method, all of the experimental conditions were investigated. For the choice of stationary phase, reversed-phase separation was preferred due to the drawbacks of the normal phase, e. g., hydration of silica with water that can cause peak tailing. To optimize the mobile phase, different systems were tried for chromatographic separation of the components. The best resolution was achieved using a mobile phase consisting of acetonitrile: water in the ratio of 90:10 v/v, which gave satisfactory result with shap well defined and resolved peak with minimum tailing as compaired to other mobile phase Figure 1. The absorption spectrum of diosgenin is shown in Figure 2. The wavelength 203 nm was used for quantification of sample.

The calibration curves (n=3) constructed for the markers were linear over the concentration range of 2-10 µg/ml Diosgenin. Peak areas of the markers were plotted versus the concentration and linear regression analysis performed on the resultant curve. The coefficients of determination 0.997 for diosgenin with % RSD values ranging from 0.5 to 2% across the concentration range studied were obtained following linear regression analysis Table 1.

The precision result of the solution at medium concentration is presented in Table 2, and it was shown that the RSD values of retention time were less than 1%, while the RSD values of peak area were less than 2 % both for intra-day assay and inter-day assay precision (Intra 4 h three injections, inter 3 days). The LOD and LOQ were found to be 0.52 and 1.577 µg/ml respectively for diosgenin.

The robustness study indicated that selected factor remained unaffected by small variation of these parameters. To ensure the insensitivity of the developed HPLC method to minor changes in the experimental conditions, it is important to demonstrate its robustness. None of the alterations caused a significant change in, retention time and. theoretical plates

A system suitability test was performed to evaluate the chromatographic parameter (retention time, capacity factor, theoretical plate number and tailing factor) before the validations run (Table 3). 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 percentage recovery of diosgenin was 96.67 in herbal formulation as shown in (Table 4).

Applicability of the developed method in formulation

The developed method was applied to the determination of diosgenin in the herbal formulation (Divya Madhunashini Vati) and the results are presented in Table 5**.** The simple, precise and accurate method was suitable for the routine analysis in pharmaceutical preparation.

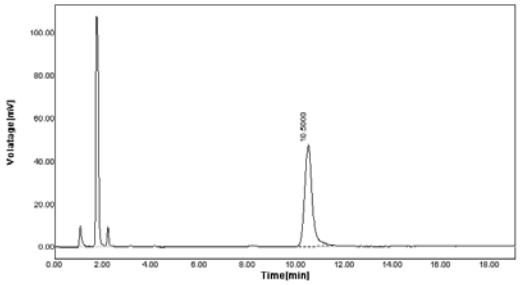

Fig. 1: Chromatogram of diosgenin (10 µG/ML): Peak (Rt 10.5 min), Mobile Phase:- Acetonitrile: Water (90:10, V/V), 203nm.

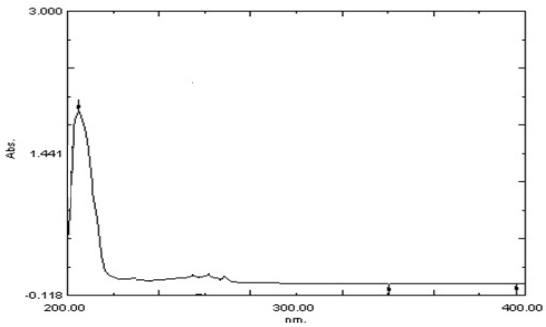

Fig. 2: Absorption spectra of diosgenin (10 µg/ml).

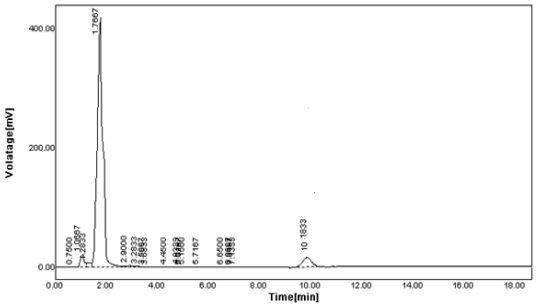

Fig. 3: Chromatogram of diosgenin in Tablet formulation.

Table 1: Regression analysis of calibration curve for diosgenin for the proposed HPLC method

Parameter	Diosgenin
Linearity range µg/ml	2-10
Slope	15.56
Intercept	2.454
Correlation coefficients r^2	0.997

Table 2: Summery of validation parameter for the proposed HPLC method.

Parameter	Diosgenin
Limit of detection	0.52
Limit of quantitation	1.577
Precision RSD%	
Intra day	0.18
Interday	0.27
Repetability(n=3)	0.31

Table 3: Result of system suitability parameter obtained for method.

Parameter	Diosgenin
Retention time (min.)	10.5
Capacity factor (K)	5.24
Therotical plate number (N)	6096.6
Tailing factor	1.0892

Table 4: Recovery study of diosgenin added to preanalysed sample using the proposed HPLC method. (n=3)

Amount Taken	Amount Added	Area	Amount Found	Amount Recover	% recovery
10	0	148.22	9.36		
10	8	267.50	17.03	7.33	96.6
10	10	297.5	18.93	9.63	96.3
10	12	328.12	20.92	11.62	96.83

Table 5: Assay results of herbal formulation for using proposed method

Herbal formulation	Component	Amount found by Proposed method (%w/w)
Tablet	Diosgenin	0.039

CONCLUSION

The developed HPLC technique is a precise, specific, accurate and robust for the determination of diosgenin. Statistical analysis proves that the method is reproducible and selective for the analysis of diosgenin. Since the proposed mobile phase effectively resolves diosgenin, the method can be used for qualitative as well as quantitative analysis of diosgenin in herbal formulations. From the above results, it can be concluded that the proposed method may be extended to study the degradation of diosgenin under different stress conditions, as per the recommendations of ICH guidelines.

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