

Simultaneous Determination of Diosgenin and Guggulsterone-Z in Gokshuradi guggulu tablet by High-Performance Thin-Layer Chromatography

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ABSTRACT :

The objective of this work was to develop and validate a high performance thin layer chromatography method for simultaneous determination of Guggulsterone-Z and Diosgenin in combined dosage form. The stationary phase used was silica gel G60F254. The mobile phase used was mixture of ethyl acetate: methanol: water (4:1:0.5 v/v/v). The detection of spots was carried out at 254 nm using absorbance reflectance mode. The method was validated in terms of linearity, accuracy, precision and specificity. The calibration curve was found to be linear between 200 to 800 ng/spot for guggulsterone Z and 50 to 350 ng/spot for diosgenin. The limit of detection and limit of quantification for the Guggulsterone-Z were found to be 5.05 and 15.08 ng/spot, respectively and for diosgenin 9.23 and 24.15 ng/spot, respectively. The proposed method can be successfully used to determine the drug content of marketed formulation.

Keywords: Diosgenin, HPTLC, simultaneous estimation, Guggulsterone-Z.

INTRODUCTION:

Guggulsterone-z is a steroidal compound present in dried gum of *Commiphora weightii* belonging to family Burseraceae. Diosgenin is steroidal saponin present as glycoside dioscin in root and tubers of the plant *Dioscorea deltoidea* belongs to family Dioscoreaceae. Both drugs traditionally used in the treatment of hyperlipidemia and antiinflammatory in rheumatism. [1,2]. Effective formulations are present in market for both plant extracts individually and also in combination. HPTLC is a more effective technique for the simultaneous determination in single samples in routine analysis. Literature survey showed fewer HPTLC methods present for the simultaneous estimation of Guggulsterone Z and diosgenin in marketed formulation. However, individual analytical methods have so far been reported for their determination [3,4]. Most of these methods are not precisely validated. The aim of the present investigation is to develop a HPTLC method for the simultaneous determination of these to important phytoconstituents. We have developed a method using ethyl acetate: methanol: water (4:1:0.5 v/v/v/v) as mobile phase on silica gel 60F254 HPTLC plates (0.2 mm; Merck). Quantitative estimation was accomplished by densitometry scanning with UV detector at 254 nm. Identification and quality evaluation of crude drugs is fundamental requirement of industry and other organizations dealing with Ayurvedic and herbal

products. Unfortunately, this requirement is often not possible to meet with the usual pharmacognostic test such as macro and microscopic evaluation, ash and extractive value etc. Directive on analytical control of crude drugs must take account of the fact that the material to be examined has complex and inconsistent composition. Therefore, the analytical limits are not as precise as those for single chemical entity. Adequate standard using chemical, instrumental and physicochemical methods is necessary [5].

MATERIAL AND METHODS:

Guggulsterone and diosgenin standard were supplied by Natural Remedies Bangalore, India. Pills containing *Commiphora weightii* (250 mg) and *Dioscorea deltoidea* (250 mg) were procured from the local pharmacy. All the chemicals and reagents used were of analytical grade. HPTLC system consists of Camag HPTLC (Germany) with linomat V automatic sample applicator, TLC scanner with CATS-4 software, Camag twin trough glass chamber, Hamilton syringe (100 µl) and ultrasonicator version software were used. Merck HPTLC plates coated with silica gel 60 F254 (0.2 mm thickness) on aluminium sheets were used as the stationary phase. Accurately weighed amount of standard guggulsterone (10 mg) and Diosgenin (5 mg) were transferred to 100 ml volumetric flask separately, and dissolved into and diluted up to the mark with methanol. The

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resulting solutions were sonicated for 15 min and filtered through Whatman filter paper No. 41. An aliquots portion of standard stock solution of Diosgenin (100 µg/ml) and guggulosterone (50 µg/ml) were applied as band ranging from 2-8 µl and 1-8 µl, respectively on TLC plate with Linomat V. The plates were placed in twin trough glass chamber previously saturated for 10 min with developing mobile phase. The plates were developed by normal vertical developing tank at ambient temperature for a distance of 80 mm. After development the plates were air dried and evaluated densitometrically in reflectance mode at 254 nm. Calibration curve was constructed by plotting peak areas versus concentration (ng/ml) and regression equation was calculated. Selection of the wavelength for densitometric evaluation was done by taking the spectrum of peak obtained from the standard diosgenin and guggulosterone (fig. 1). Wavelength of 254 nm was chosen as a common wavelength to match the concentration ratio of the drugs present in the formulation. The mobile phase of ethyl acetate: methanol: water (4:1:0.5 v/v/v) was selected because it gave highest resolution, minimum tailing and Rf values of 0.32 and 0.62 for Diosgenin and guggulosterone, respectively (fig. 2). Twenty marketed pills were accurately weighed and finely powdered. An accurately weighed amount equivalent to 200 mg guggulosterone and Diosgenin was transferred into 50 ml volumetric flask, along with 50 ml of methanol. The resulting solution was sonicated for 45 min, filtered through Whatman filter paper No. 41 and transferred into 250 ml volumetric flask; finally volume was made to mark with methanol. Further, the stock solution was diluted with methanol to get concentration of 1000 µg/ml. Two microliters of this solution was applied on TLC plate with Linomat V. the plates was developed in twin trough glass chamber under operating conditions described above and calculations were performed using peak area. System suitability tests are used to verify the reproducibility of the chromatographic system. To ascertain ef-

fectiveness of HPTLC method, system suitability tests were carried out on freshly prepared working standard solutions of Diosgenin and guggulosterone. This solution was spotted five times; parameters such as limit of detection (LOD) and limit of quantification (LOQ) were studied. The calibration curve was linear in concentration range of 100-800 ng/spot and 20-200 ng/spot with regression 0.995 and 0.9985, slope 3.908 and 2.694 for guggulosterone and Diosgenin respectively. The amounts of guggulosterone and diosgenin were found by the number of replicates of pharmaceutical preparation (n=5) performed by the inter-day assays. The relative standard deviation observed for both the drugs were very low. Recovery studies were carried out to study accuracy and precision of the method. These studies were carried out on plate at four levels i.e. multiple level recovery studies. Two microliters of pre-analyzed sample preparation of guggulosterone (311.19 ng) and diosgenin (70.69) was applied four times on TLC plate as band of 8 mm. These bands were spiked on the plate with guggulosterone and diosgenin standard stock solution and analysed by the method. LOD and LOQ are determine by standard ICH guidelines.

CONCLUSION:

This method was developed for the first time on HPTLC to estimate the two drugs in formulation, in order to analyze more samples at a time. The method is easy to perform, precise and accurate. There is significant difference between the RF values of the guggulsterone and diosgenin; therefore this analytical method can be utilizes for the simultaneous estimation of this substances.

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RESULTS:

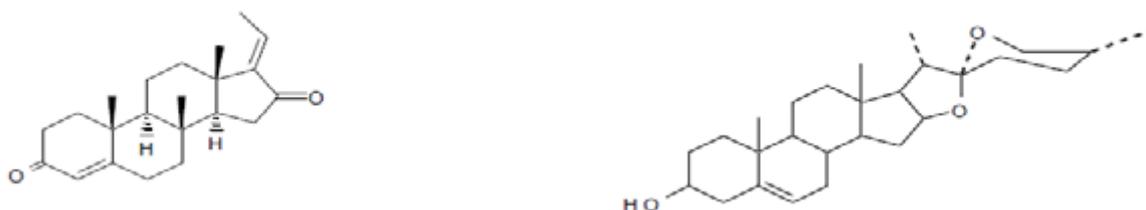


Fig. no 1: Structure of Guggulsterone and Diosgenin

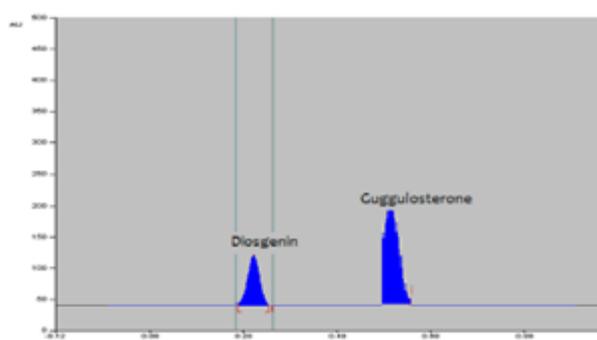


Fig. no 2: RF value of Diosgenin and Guggulsterone

Brand	Drug	Amount of drug added (ng)	Amount of standard added (ng)	Amount found (ng)	Recovery (%)
----	Diosgenin	200	100	299.13	99.74
		200	100	300.30	100.25
		200	200	400.76	100.39
		200	200	401.2	101.01
		200	300	499.87	99.67
				Mean	100.21
				R. S. D. (%)	1.386
	Guggulosterone	70.69	100	169.80	99.47
		70.69	100	171.60	100.5
		70.69	200	268.64	99.24
		70.69	200	269.80	99.67
		70.69	300	368.70	98.46
				Mean	99.46
				R. S. D. (%)	1.926

Table no. 1: Simultaneous Determination of Diosgenin and Guggulosterone-Z in Gokshuradi guggulu tablet

Parameters	Diosgenin	Guggulosterone
LOD	0.7	0.98
LOQ	2.34	3.35

Table No.2: Validation parameters

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