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Original Article

DEVELOPMENT AND VALIDATION OF A HPTLC METHOD FOR SIMULTANEOUS DENSITOMETRIC ANALYSIS OF GLYCYRRHETIC ACID AND SOLASODINE IN HERBAL DRUG FORMULATION

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ABSTRACT

Objective: To develop and validate a simple, precise, selective, and accurate high-performance thin layer chromatographic method for simultaneous densitometric analysis of glycyrrhetic acid and solasodine in the polyherbal formulation.

Methods: The method was developed using HPTLC silica gel GF_{254}precoated aluminium plate as the stationary phase and Chloroform: Methanol (9:1 v/v) as the mobile phase. Quantization of glycyrrhetic acid was achieved by determining the area under the curve at 267 nm using CAMAG TLC Scanner and CATS 3 software. Since the structure of solasodine lacks conjugated double bond, it does not give any fluorescence either in the absorbance mode or reflectance mode hence solasodine was derivatized using 0.5% anisaldehyde sulphuric acids which gave a bluish spot as seen on TLC plate. These spots were scanned at 546 nm wavelength using CAMAG TLC Scanner and CATS 3 software.

Results: The retention factor for glycyrrhetic acid and solasodine were found to be 0.52±0.01, 0.40±0.01% w/w respectively. The developed HPTLC method was validated using parameters described in International Conference on Harmonization (ICH) guideline. The proposed method showed good linearity in the range of 400-2000 ng $spot^{-1}$ for glycyrrhetic acid as well as for solasodine. The content of glycyrrhetic acid and solasodine in marketed polyherbal formulation were found to be 0.67%±0.8 and 0.10±0.35%w/w respectively.

Conclusion: The developed method can be used as quality control tool for the routine analysis of glycyrrhetic acid and solasodine in polyherbal formulation.

Keywords: Glycyrrhetic acid, Solasodine, HPTLC, Simultaneous estimation

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INTRODUCTION

Glycyrrhetic acid is an aglycone of glycyrrhizin, a triterpenoid compound isolated from *Glycyrrhizaglabra*. It possesses various activities like anti-inflammatory, analgesic, antiasthmatic etc [1, 2]. Solasodine is a steroidal glycoalkaloid, isolated from *Solanum xanthocarpum* possesses anti-asthmatic and mucolytic activities [3, 4], hence they forms one of the most important constituents of almost each and every polyherbal formulation used for the management of asthmatic conditions. Extensive literature survey revealed that few HPTLC [5-7] and HPLC [8, 9] methods are available for estimation of Glycyrrhetic acid individually and in combination with other marker compounds in the same plant. HPTLC [10, 11] and HPLC [12, 13] methods have also been reported for estimation of Glycyrrhetic acid individually and in combination with other marker compounds. Even though both of these compounds are most commonly used together, there were no reports found for simultaneous estimation of glycyrrhetic acid and solasodine. Hence the objective of the work was to develop and validate a simple, accurate and reproducible method for the simultaneous HPTLC analysis of glycyrrhetic acid and solasodine in polyherbal formulations.

MATERIALS AND METHODS

Solvents and chemicals

Standard of glycyrrhetic acid and solasodine were purchased from Natural Remedies Private limited, Bangalore, India. Polyherbal formulation (Askon syrup) used in the study was purchased from the local market of Ahmedabad. All chemicals and reagents used were of analytical reagent grade and purchased from Merck specialities Pvt. Ltd. (Mumbai, India). Double distilled water was used in the present work.

Instrumentation and chromatographic conditions

The sample solutions were spotted on precoated silica gel aluminium plate 60F254 (20 cm × 10 cm) with 250 µm thickness (E. Merck, Darmstadt, Germany) in the form of bands of 6 mm width with a Hamilton syringe (100 µL) using a Camag Linomat V (Switzerland) sample applicator. The plates were prewashed with methanol, activated in an oven at 105 °C for 20 min, and then allowed to cool to attain room temperature before sample application. The slit dimension was kept at 5 mm × 0.45 mm and 10 mm/s scanning speed was employed. Plates were then developed, at a constant temperature with 20 ml mobile phase consisting of Chloroform: Methanol (9:1 v/v). Linear ascending development was carried out in 20 cm x 10 cm twin trough glass chamber (Camag, Muttenz, Switzerland) previously saturated with the mobile phase. The optimized chamber saturation time for mobile phase was 25 min at room temperature (25±2 °C) at a relative humidity of 60±5%. The optimum wavelength for detection and quantification of glycyrrhetic acid was 267 nm. Since solasodine lacks any conjugated double bond it does not give any fluorescence either in the absorbance mode or reflectance mode so it required ion-pair complexation of the solasodine followed by in situ colour development in an acidic solvent front for achieving a chromophore which could be scanned in the visible range of the scanner. Densitometric scanning was performed within 10 min after derivatization process using Camag TLC scanner III with winCATS software version 1.3.4.

Preparation of standard solutions

Preparation of standard solution of glycyrrhetic acid

A stock solution of glycyrrhetic acid (1 mg ml-1) was prepared by dissolving 10 mg of accurately weighed glycyrrhetic acid in chloroform and volume was made up to 10 ml with chloroform in a volumetric flask. From this, 1.0 ml of stock solution was transferred to 10 ml volumetric flask and the volume was adjusted to 10 ml with chloroform to obtain a standard solution containing 100µg ml-1 of glycyrrhetic acid.

Preparation of standard solution of solasodine

A stock solution of solasodine (1 mg ml^{-1}) was prepared by dissolving 100 mg of accurately weighed solasodine in methanol and

the volume was made up to 100 ml with methanol. From this 1.0 ml of stock solution was transferred to 10 ml volumetric flask and the volume was adjusted to 10 ml with methanol to obtain a standard solution containing 100µg ml^{-1} of solasodine.

Selection of detection wavelength

After chromatographic development and derivatization process, bands were scanned over the range of 400-2000 ng $spot^{-1}$ and the spectra were overlain. The one marker of the ingredients of the formulation is glycyrrhetic acid which does not require any derivatization as they have distinct absorption maxima under UV. Hence, solasodine was derivatized with 0.5% anisaldehyde sulphuric acid which gave a bluish spot as seen from the chromatogram. These spots were scanned at 546 nm wavelength, at which the best detector response was obtained.

Construction of calibration plots

Linearity was evaluated in the range of 400-2000ng $spot^{-1}$ for glycyrrhetic acid and solasodine. For the calibration curve accurately measured a standard stock solution of glycyrrhetic acid and solasodine (400, 800, 1200, 1600, 2000ng) were spotted on pre-coated TLC plate under nitrogen stream using Linomat V spotter. Then chromatographed and scanned as described above.

Sample preparation for glycyrrhetic acid and solasodine

40 ml of syrup was extracted with 60 ml chloroform for 20 min. Then the solution was filtered and the filtrate was discarded. Then the marc was refluxed for 1 h with 90 ml of 0.5 M H_2SO_4. Then the solution was cooled and filtered. The unfiltered mixture was shaken with chloroform (2x20 ml) to extract glycyrrhetic acid. The filtrate was basified with ammonia and extracted with chloroform (2x20 ml) to extract solasodine. Both the Chloroform extracts were combined. Volume was made up to 100 ml with chloroform.

Assay validation

The proposed method was optimized and validated as per the International Conference on Harmonization (ICH) guidelines [14]. All measurements were performed in triplicates.

Precision studies

In order to judge the quality of the proposed HPTLC method, precision was determined. The precision of the proposed HPTLC method was verified by Intra-day and inter-day precision studies. Intra-day precision was performed by analysis of single concentration in six replicates of mixed standard solutions of glycyrrhetic acid (1200ng $band^{-1}$), solasodine (1200ng $band^{-1}$) which were prepared on the same day. Intermediate precision was performed by repeating analysis on three consecutive days. The peak areas were recorded and percentage relative standard deviation (% RSD) was calculated.

Accuracy

In order to evaluate the validity of the proposed method, accuracy was evaluated through the percentage recoveries of known amounts of a mixture of glycyrrhetic acid and solasodine added to solutions of marketed herbal formulation. Marketed herbal formulation was spiked with the known amount of standards, and the percent ratios between the recovered and expected concentrations were calculated. The analyzed samples were spiked with 80, 100 and 120 % of 0.512 mg glycyrrhetic acid and 4.05 mg of solasodine standard solutions. The percent ratios between the recovered and expected concentrations were estimated.

Specificity

The specificity of the proposed HPTLC method was estimated by analyzing the standard marker and sample. Peaks for glycyrrhetic acid and solasodine were confirmed by comparing the retention time. Excipients present in the herbal formulation did not interfere with the peaks of glycyrrhetic acid and solasodine. The method was found to be specific.

Robustness studies

The effects of small, deliberate variation of the analytical conditions on the peak areas of the drugs were examined. The robustness of the proposed chromatographic method was performed at a concentration of 1200 ng $band^{-1}$ for glycyrrhetic acid as well as for solasodine. The standard deviation of peak areas and % RSD were calculated for each variable parameter.

RESULTS AND DISCUSSION

HPTLC method optimization

For HPTLC method, Chromatographic conditions were optimized to achieve the best resolution and peak shape for glycyrrhetic acid and solasodine. Different mobile phases in different proportion were tried and the mobile phase containing Chloroform: Methanol (9:1 v/v) was selected as optimal for obtaining well-resolved peaks of glycyrrhetic acid (R_f = 0.52) and solasodine (R_f = 0.40) with acceptable system suitability parameters. The optimum wavelength for detection and quantification of glycyrrhetic acid was 267 nm and solasodine was derivatized with 0.5% anisaldehyde sulphuric acid which gave a bluish spot as seen from the chromatogram. These spots were scanned at 546 nm wavelength. The retention factors for glycyrrhetic acid and solasodine were found to be 0.52±0.01 and 0.40±0.01, respectively. Representative TLC chromatogram obtained from a mixed standard solution of glycyrrhetic acid and solasodine is shown in fig. 1 and 2.

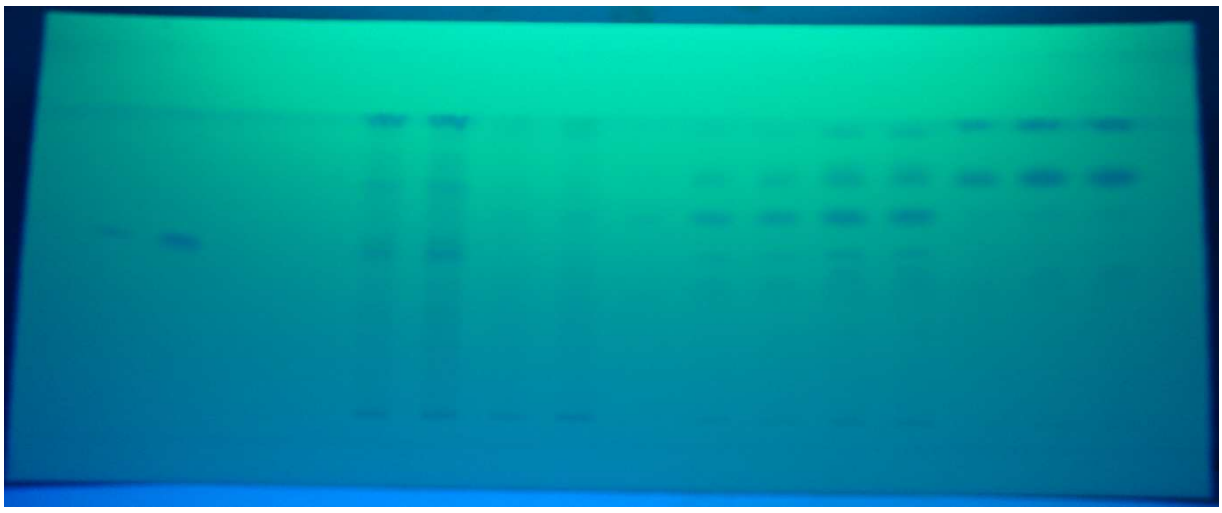

Fig. 1: TLC chromatogram of glycyrrhetic acid at 254 nm

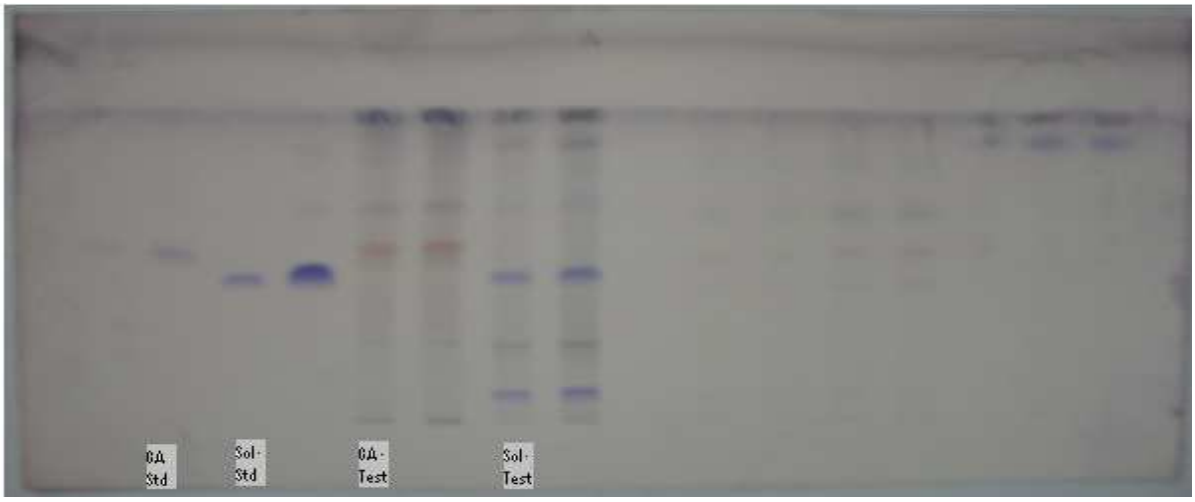

Fig. 2: TLC chromatogram of glycyrrhetic acid and solasodine after derivatization

Linearity, limit of detection and quantitation

The results were found to be linear in a range of 400-2000 ng spot^{-1} for glycyrrhetic acid and solasodine. The correlation coefficients (r) for the plots were 0.993 for glycyrrhetic acid and 0.992 for solasodine. The calibration plots obtained for glycyrrhetic acid and solasodine are shown in fig. 3 and fig. 4. The LOD and LOQ for glycyrrhetic acid and solasodine were found to be 22.97and 23.99 ng and 69.61and 72.72 ng, respectively.

Precision

The developed HPTLC method was found to be precise (table 1), with % RSD values for repeatability and intermediate precision studies below 2 % as recommended by ICH Q2 (R1) guideline.

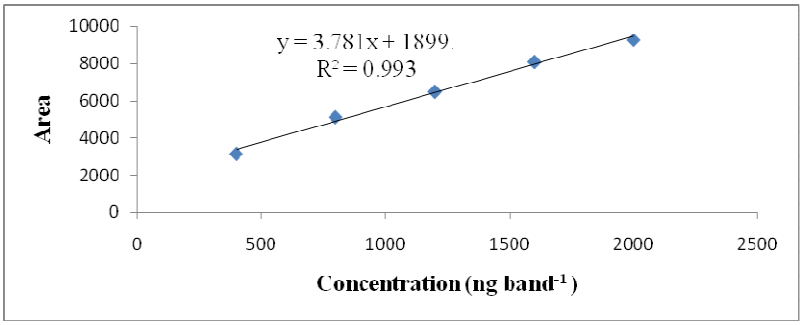

Fig. 3: Calibration curve for glycyrrhetic acid

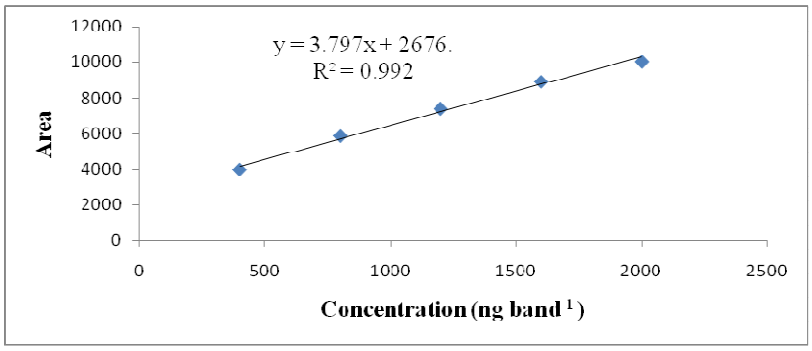

Fig. 4: Calibration curve for solasodine

Table 1: Intra and inter-day precision of the HPTLC method (n=6)

Marker compounds	Actual concentration	Intra-day variation, as RSD (%)	Inter-day variation, as RSD (%)
Glycyrrhetic acid	1200	0.41	0.73
Solasodine	1200	0.38	0.94

RSD = Relative standard deviation

Accuracy

Satisfactory recoveries for Glycyrrhetic acid and Solasodine were obtained (table 2), which indicate that the proposed chromatographic method is reliable for the simultaneous quantification of selected markers in this herbal formulation.

Analysis of marketed herbal formulation

The validity of the proposed method was applied to standardization for herbal dosage forms viz. Kofol OR AskonSyrup. The shape of the peaks was not altered by other substances present in the matrix. The percent content of both viz., Glycyrrhetic acid and Solasodine in marketed herbal formulation was found to be 0.67%±0.8 and 0.10±0.35 % respectively.

Robustness studies

Robustness of the method checked after deliberate alterations of the analytical parameters showed that areas of peaks of interest and retention factor remained unaffected by small changes of the operational parameters (% RSD<2). The summary of validation parameters of proposed method are given in table 3.

Table 2: Results of recovery studies (n=3)

Drug	Amount of sample (ng)	Amount of standard added(ng)	Amount of standard recovered±SD	% recovery±C. V. (%)
Glycyrrhetic acid	640	512	505.65±0.06	98.76±0.01%
	640	640	642.49±0.13	100.39±0.03%
	640	768	762.67±1.10	99.31±0.15%
Solasodine	506	405	402.4±1.9	99.36±0.47%
	506	506	500.4±0.51	98.89±0.10%
	506	607	609.7±1.96	100.44±0.33%

Table 3: Summary of validation parameters of proposed method

Parameters	Glycyrrhetic acid	Solasodine
Linearity range(ng spot-1)	400-2000	400-2000
Correlation coeficient	0.993	0.992
Precision		
Reproducibility (R. SD)	0.16	0.20
Instrumental (n=6)	0.41	0.38
Intraday (n= 6)	0.73	0.94
Inter day (n= 6)		
Accuracy (% Recovery)	99.49%	99.56%
Limit of detection	22.97ng	23.99ng
Limit of quantification	69.61ng	72.72ng
Specificity	Specific	Specific

LOD =Limit of detection.,LOQ =Limit of quantitation., RSD = Relativestandard deviation., n = Number of determinations

CONCLUSION

The validated HPTLC method employed proved to be simple, fast, accurate, precise and robust and thus can be useful in the quality control of glycyrrhetic acid and solasodine in the polyherbal formulation.

CONFLICT OF INTERESTS

Declare none

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