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Development and Validation of HPTLC Method for Estimation of Glycyrrhizic Acid in Herbal Formulation

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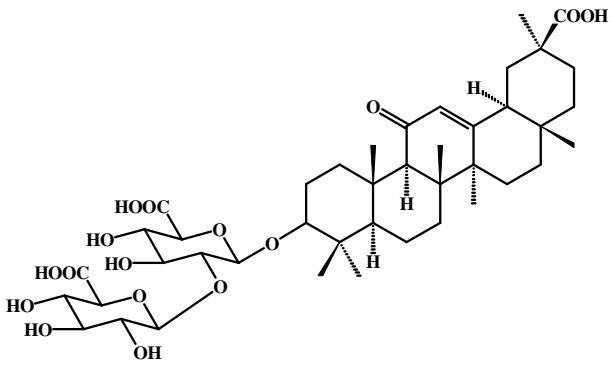
A simple, accurate, selective, precise and economical high performance thin layer chromatographic method was developed on silica using solvent system chloroform:glacial acetic acid:methanol:water in the ratio of 60:32:12:8 at 254 nm wavelength in absorbance mode for the analysis of glycyrrhizic acid in crude drugs as well as in herbal dosage form. The solvent was found to give well defined, compact and sharp peak of glycyrrhizic acid at R_f 0.28 ± 0.02. Method was validated for accuracy, precision and specificity. It shows good linearity in the range of 100-500 ng/spot (r^2 = 0.9908) with slope and intercept 2802.03 and 31.06, respectively. The method found to be precise (RSD % < 3) and accurate (recovery 99-102 %). The proposed method was applied for quantitative estimation of glycyrrhizic acid in a solid herbal formulation consisting of powdered roots of *Glycyrrhiza glabra*, which showed the presence of glycyrrhizic acid in formulation in the range of 0.030-0.055 % w/w. The proposed method found simple and economic to be used in general laboratory conditions for quality control of crude drugs and herbal formulations.

Key Words: High performance thin layer chromatography, Validation, Glycyrrhizic acid.

INTRODUCTION

Glycyrrhiza glabra has been used medicinally in traditional system of medicine from thousands of year for treatment of number of diseases as a single drug as well as in compound formulations. It is commonly known as licorice, Yashtimadhu in Ayurvedic system of medicine[1]. The most important and well-known bioactive component of licorice root are the triterpene glycosides, mainly glycyrrhizic acid (GL) and its aglycone, 18 β-glycyrrhetinic acid (GLA), a pentacyclic triterpene belonging to the β-amyrin series[2]. Glycyrrhizic acid is the main and sweet component widely used antiinflammatory agent[3] and metabolized to glycyrrhetic acid, which inhibits 11 β-hydroxysteroid dehydrogenase and other enzymes involved in the metabolism of corticosteroids[4]. It has been reported to possess hepatoprotective[5], cytotoxic[6], antiulcer[7] and antiviral[8,9] activities. Literature survey reveals that, most of the quantification works on glycyrrhizic acid has been done by HPLC[10-16]. Only one TLC[17] work has been reported for the estimation of this important molecule with narrow range of linearity and also lacking proper validation. Since, the HPTLC analysis are simple, cost effective and less time consuming as compared to HPLC and other analytical methods, it was thought worthwhile to develop a HPTLC method for the determination of glycyrrhizic acid in crude drug and herbal formulation.



Glycyrrhizic acid

EXPERIMENTAL

Glycyrrhizic acid reference standard was obtained from Sami Labs Ltd., Bangalore as a gift sample and all the chemicals and reagents used were of analytical grade and were purchased from Merck, India.

TLC instrumentation and conditions: HPTLC analysis was performed on silica gel 60 F_{254} plates of 10 cm × 10 cm size. The standard, sample and blank solutions were applied in the form of bands of width 4 mm with automated sample applicator using Camag 100 microlitre syringe (Hamilton, Switzerland). A constant application speed of 120 nL/s was

employed and the space between two bands was 5.8 cm. The mobile phase was composed of chloroform:glacial acetic acid: methanol:water in the ratio of 60:32:12:8. Linear ascending development was carried out in twin trough glass chamber saturated with mobile phase. The optimised chamber saturation time for mobile phase was 20 min at room temperature and the chromatogram developed up to the length of 80 cm. The developed plates were dried in current of air with the help of air dryer. Densitometric scanning was performed on Camag TLC scanner III in the wavelength of 254 nm operated by WINCATS software. The source of radiation utilized was deuterium and tungsten lamps.

Preparation of standard solutions and calibration plot: Different volumes of standard of glycyrrhizic acid (1 mg/mL), spotted 0.1, 0.2, 0.4 and 0.5 µL were spotted in triplicate on a TLC plate to obtain concentrations of 100, 200, 400 and 500 ng per spot of glycyrrhizic acid. Plate was developed and scanned as discussed above to get calibration plot and regression equation.

Analysis of glycyrrhizic acid in formulation: The Yashtimadhu tablets were analyzed by the newly proposed method for the glycyrrhizic acid content. For the analysis, 10 tablets were selected randomly, powdered and weighed accurately around 1 g was refluxed using 20 mL of methanol for 0.5 h and filtered. The procedure was repeated for 2 times again using fresh methanol to ensure complete extraction. The extracts were pooled and evaporated to dryness in rotvapor below 45 ºC. The residue left was reconstituted in methanol. Further, 2 µL of the sample was applied in triplicate on TLC plate for the quantification using proposed method.

Validation: The proposed method was validated as per ICH guidelines[18,19] for precision, accuracy, LOD and LOQ, which are similar to the methods reported by laboratory[20-26].

Precision: The precision of the method was checked for intermediate precision. The interday precision was determined by analyzing the same concentration of standard glycyrrhizic acid solution for 6 times on the same day while intraday precision was determined by analyzing the corresponding standard daily for 6 days over a period of 1 week.

Accuracy as recovery: The accuracy of the method was determined by doing recovery studies. The pre analyzed samples were spiked with standard at three different concentration levels *i.e.*, 50, 100 and 150 % and the mixtures were reanalyzed by the proposed method.

Limit of quantification and limit of detection: The limit of quantification (LOQ) and limit of detection (LOD) was determined by signal-to-noise ratio. The concentration of sample giving signal to noise ratio of 3 was fixed as the LOD. Whereas the concentration of the standard giving signal to noise ratio of 10 was fixed as LOQ.

RESULTS AND DISCUSSION

A novel HPTLC analytical method was developed for the determination of glycyrrhizic acid in herbal formulation. The mobile phase employed was consisting of chloroform:glacial acetic acid:methanol:water in the ratio of 60:32:12:8. The detection was done at 254 nm using scanner III (Camag), which showed sharp, compact and well resolved peak of glycyrrhizic acid at R_f value 0.28 ± 0.02 (Fig. 1). The method was validated as per ICH guidelines in terms of linearity, accuracy, precision, LOD and LOQ. The results of the validation were given in Table-1. The method was found to be linear in the range of 100-500 ng/spot (r^2 = 0.9908) with slope 2802.03 ± 55.3 and intercept 31.06 ± 0.51.

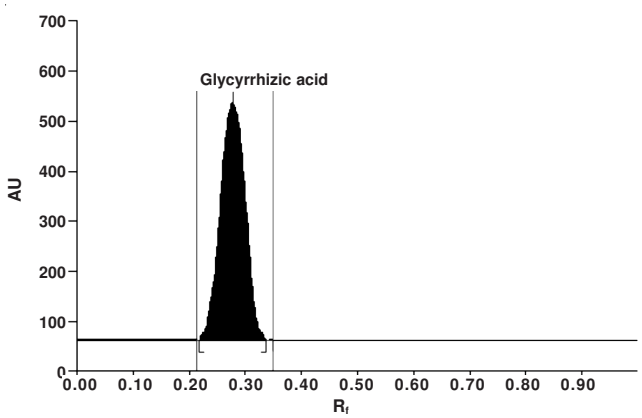


Fig. 1. HPTLC chromatogram of standard glycyrrhizic acid

TABLE-1
SUMMARY OF VALIDATION PARAMETERS

Parameters	Range
Linearity (ng/spot)	100-500
Regression equation	Y = 2802.03x + 31.06
Regression coefficient	0.9908
Intercept ± SD	31.06 ± 0.51
Slope ± SD	2802.03 ± 55.3
LOD (ng/spot)	19.4
LOQ (ng/spot)	60.2
Precision (RSD %)	
Interday precision	1.62
Intraday precision	0.81

The percentage RSD of inter day and intraday precision was calculated and found to be 1.62 and 0.81, respectively. These values indicate the method is precise.

The recovery experiment was conducted in triplicate and found to be within the limit of 99.4-100.9 %. The result recovery study (Table-2) indicates the proposed method is accurate to estimate glycyrrhizic acid in different type of formulations.

TABLE-2
RECOVERY STUDIES

Drug added to the sample (%)	Theoretical content (ng)	Amount recovered	Recovery (%)	RSD (%)
0	70	69.6	99.4	1.10
50	105	104.3	99.3	0.88
100	140	141.3	100.9	0.30
150	175	176.6	100.9	1.20

Limit of detection and limit of quantification of the proposed method were found to be 19.4 and 60.2 ng/spot, respectively.

The Yashtimadhu tablets were analyzed by the proposed method. A clear, well defined spot was obtained at the R_f = 0.28 ± 0.02 without any interference and the content of glycyrrhizic acid was found to be in the range of 0.030-0.055 % w/w.

Conclusion

The HPTLC method developed for the determination of glycyrrhizic acid is simple, economic, accurate, precise, rapid, sensitive and selective. It can be used for routine quality control and analysis of several formulations of traditional system of medicine containing glycyrrhiza as an ingredient.

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ERRATUM

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Assay of Lercanidipine Hydrochloride with Azocaramine-G, Fe(III)/$K_3[Fe(CN)_6]$ and Folin Ciocalteu Reagent

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