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QUANTITATIVE ANALYSIS OF GLYCYRRHIZIC ACID BY HPTLC IN HERBAL FORMULATION

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ABSTRACT

Many of the traditional herbal formulations contain extracts of Glycyrrhiza glabra, which contains glycyrrhizic acid as an active constituent. An attempt has been made to develop a simple, precise, rapid and cost-effective high-performance thin-layer chromatographic (HPTLC) method for estimation of glycyrrhizic acid in an herbal formulation. Precoated silica gel 60 F254 plates with Toluene: Ethyl acetate: Chloroform: Glacial acetic acid, 11:6:3:0.5, as mobile phase were used in chromatographic determinations. The plates were scanned and estimation was done at wavelength of maximum absorption 260 nm for glycyrrhizic acid. The R_f value was found to be 0.37, under these experimental conditions linearity was observed between 0.8-2.6µg/spot and average recovery was found to be 96.75% for Gycyrrhizic acid.

Keywords: *HPTLC, Gycyrrhizic acid, Validation; Herbal formulation*

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INTRODUCTION

Herbal medicines are the oldest remedies known to mankind; these generally contain more than one herb in the combination. Development of methods for analysis of plant products possesses difficulty, due to their unknown chemical profile, more so in case of multi components herbal formulations. Liquorice, the roots of Glycyrrhizia glabra, has been used as a medicinal herb for over 4000 years. The active components of this plant have extensive therapeutic usage throughout the world and are subjected to enormous works in recent years. Liquorice (*Glycyrrhiza glabra*) is also known as "sweet root". The word "Glycyrrhiza "is made from two Greek words: Glykys, meaning "sweet" and Rhiza, meaning "root" [1]. Licorice root has been used in both Eastern and Western medicine to treat a variety of illnesses ranging from the common cold to liver disease also found in numerous traditional formulas. The main active constituent of *Glycyrrhiza glabra* is a triterpenoid saponin called Glycyrrhizic acid which is approximately 50 times sweeter than sugar [2]. Roots and rhizomes of Glycyrrhiza glabra which are harvested in autumn are used in estrogenic demulcent, pectoral, antioxidant, antispasmodic, anti-inflammatory, antibacterial and expectorant drugs [3]. In the present study, an attempt has been made to develop a simple, rapid and accurate HPTLC method for the estimation of Glycyrrhizic acid in an herbal formulation and validate the method to quantify Glycyrrhizic acid in its herbal formulation.

MATERIALS AND METHODS:

All the solvents and reagents of analytical grade purchased from M/s. Qualigens India Ltd. were used. The solvents were redistilled before use. Glycyrrhizic acid standard was purchased from sigma chemicals. Precoated silica gel plates (TLC plates, silica gel aluminium sheets with 60 F254) were purchased from M/s. Merck India Ltd.

Ayurvedic Formulation:

Herbolax Capsules manufactured by Himalaya Health Care were procured from the local market.

Instrumentation and chromatographic conditions [4 – 8]**:**

The samples were spotted in the form of bands with 8 mm size on precoated Silica gel plates using Linomet V (Camag) applicator. The plates were washed with Methanol and activated for 10 min. prior to application. The application rate was set at 150 nL/ sec. The monochromator band width was set 20 nm, each track was scanned thrice and base line correction was used.

Mobile phase Toluene: Ethyl acetate: Chloroform: Glacial acetic acid, 11:6:3:0.5 was used for development of sample. The plates were allowed to dry at room temperature (25 ± 20) at relative humidity of 60% ±5. The optimized chamber saturation time was 35 min. at room temperature; dried plates were scanned and quantified at 260 nm.

Calibration curve for Glycyrrhizic acid:

A stock solution of Glycyrrhizic acid (220 µg/ml) was prepared in Methanol. Aliquot of above solution (2, 4, 6, 8, 10 and 12 µL) were applied with the band width of 8 mm, on TLC plate (10X20 cm) silica gel 60 F254 Merck. The plate was developed as above procedure. After developing the plates were dried and standard calibration curve of Glycyrrhizic acid was plotted at 260nm. Peak areas for each band were recorded. Calibration curve was obtained by plotting peak area vs concentration of Glycyrrhizic acid.

Preparation of Test sample:

Sample was taken from 20 capsules and 100 ml of 1N HCl solution was added in a round bottom flask. This mixture was refluxed for an hour then cooled and filtered. Filtrate is taken in a separating funnel and

extracted with Chloroform [9]. Chloroform extract is dried in vacuum and residue was dissolved in Methanol to get concentration of 1.5 mg/ml. 10 µL of sample solution was applied on same plate in triplicate and the plate was developed as above procedure. After developing the plate was dried and peak areas for each band were recorded. Spectrum of Glycyrrhizic acid in test sample was confirmed by overlaying the spectra of standard calibration curve.

Method validation:

The developed method was validated in terms of Linearity, accuracy and precision as per ICH guidelines [10-11].

A) Accuracy:

The recovery study was performed by applying the known amount of the sample and the percentage recovery of that same amount calculated against the theoretical values. Preanalyzed samples were applied at three different concentration of the standard (80%, 100% and 120% w/w) containing Glycyrrhizic acid and analyzed with the instrument set up as same in case of the estimation of the sample. This was done to check the recovery of the drug at different levels in the formulations. The experiments were performed in triplicate.

B) Repeatability:

Repeatability (precision) was determined by repeated analysis of standard sample using the same equipment, same analytical procedures, and same laboratory and on the same plate. Repeatability of measurement was determined by spotting 10 µL of standard drug solution on TLC plate, after development spot was scanned six times without changing position. The % RSD was determined for Glycyrrhizic acid.

C) Linearity:

Linearity was determined by spotting various concentrations of standard and finding the regression.

Results and Discussions:

Optimization of mobile phase

Various proportions of Toluene, Ethyl acetate, Acetone, Chloroform were tried. The mobile phase containing Toluene: Ethyl acetate: Chloroform: Glacial acetic acid (11:6:3:0.5), gave sharp and symmetric peaks and improved spot characteristics for Glycyrrhizic acid. The spot at R_f 0.37 was identified as Glycyrrhizic acid with the help of the chromatogram of its standards (Fig 1, 2).

Calibration curves of standard Glycyrrhizic acid

Calibration graph was found to be linear and was evaluated by determining six standard spots containing 0.8-2.6µg/spot for Glycyrrhizic acid. Peak area and concentration was subjected to least square linear regression analysis to calculate the calibration equation and correlation coefficients.

Method validation

The regression data as shown in Table 1 describes a good linear relationship over concentrations 0.8-2.6µg/spot for Glycyrrhizic acid with the correlation coefficient 0.9996. Average recovery and the % RSD (for repeatability of the method) were found to be 96.75% and 1.0291 respectively for Glycyrrhizic acid (Table 1). The results shown meet the acceptance criterion for % RSD specified by the ICH which is a precision of less than 2-3 % RSD.

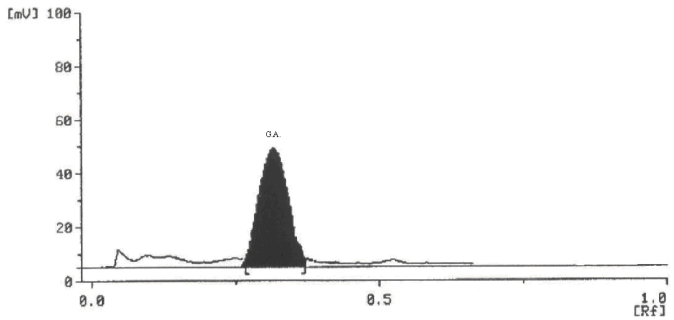

Fig. 1. Densitogram of Standard Glycyrrhizic acid (Peak showing Glycyrrhizic acid in standard solution)

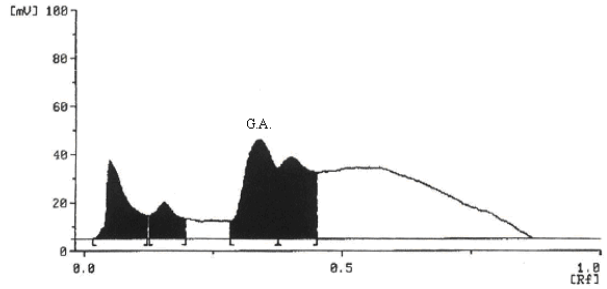

Fig. 2. Densitogram of Sample Formulation (Peak showing Glycyrrhizic acid in Sample Formulation)

Table 1
Method validation parameters for Glycyrrhizic acid by HPTLC.

Parameters	Range
Linearity range	0.8-2.6µg/spot
Correlation coefficient (r)	0.9996
Limit of detection (LOD)	114.36 ng
Limit of Quantification (LOQ)	670.69 ng
Average Recovery	96.75%
Repeatability (% RSD)	1.0291

Conclusion:

The developed HPTLC method has been shown to be selective, linear, precise and accurate. The results meet the guidelines of the International Conference on Harmonization (ICH) for validation of pharmaceutical assays of drug products. There was no interference in analysis of Glycyrrhizic acid from the other components present in the sample. The method was found to be simple, accurate and cost effective analytical method for routine analysis of Glycyrrhizic acid as an important marker in polyherbal formulation.

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