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Development and Validation of HPTLC Method for Determination of Glycyrrhizin in Herbal Extract and in Herbal Gel

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ABSTRACT: A new, simple, sensitive, selective, precise and robust high-performance thin-layer chromatographic (HPTLC) method for analysis of glycyrrhizin has been developed and validated for the determination of glycyrrhizin in herbal extracts and in herbal gel. The analyte was extracted with ethanol and applied on TLC aluminium plates along with standard using Linomat 5 spray on sample applicator (CAMAG). Analysis of glycyrrhizin was performed on pre-coated TLC aluminium plates with silica gel as the stationary phase and prewashed with methanol. Linear ascending development was carried out in twin trough glass chamber saturated with mobile phase consisting of ethyl acetate-methanol-water-formic acid (15:2:1:1 v/v/v/v). Spectrodensitometric scanning was performed by TLC scanner III (CAMAG) in absorbance mode at the wavelength of 252nm. The system was found to give compact spots for glycyrrhizin (R_f value of 0.34 ± 0.04). The linear regression analysis data for the calibration plots showed good linear relationship with r^2=0.9981 in the concentration range 2-15 µl with respect to peak area. According to the International Conference on Harmonization (ICH) guidelines the method was validated for precision, recovery, stability in solution and robustness. The glycyrrhizin content quantified from herbal extracts and from the formulation was found well within limits. Statistical analysis of the data showed that the method is reproducible and selective for the estimation of glycyrrhizin.

Keywords: *Glycyrrhiza glabra,* Glycyrrhizin, HPTLC, Method development and validation, Monoammonium Glycyrrhizinate (MAG), Gel.

INTRODUCTION

Glycyrrhiza glabra is a vital herb in nature as it has wide therapeutic applications. Glycyrrhizin is main constituent that has anti-inflammatory, antimicrobial, anti ulcer activity. Glycyrrhizin is saponin glycoside and molecular formula is $C_{42}H_{62}0_{16.}$ Gel containing glycyrrhizin is effective for mouth ulcer and aphatae. The novel gel formulation contains carbopol, chitosan, PEG, glycerin.[1, 2]

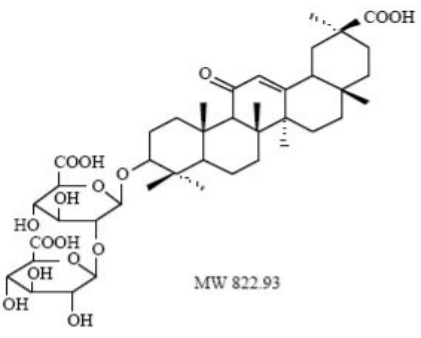

Figure 1. Chemical structure of Glycyrrhizin

Various analytical methods reported such as HPLC, HPTLC, and Capillary electrophoresis. The present paper describes precise, accurate, sensitive HPTLC method for determination of glycyrrhizin from the extract and formulation[3].

MATERIALS AND METHODS

Materials

A CAMAG TLC system comprising of a Linomat-5 applicator and CAMAG TLC III scanner. Stationary phase used was silica gel G60F254, 20x10 cm TLC plate. The Reference standard of Monoammonium Glycyrrhizinate (MAG) was obtained from Amsar Industries Ltd., Indore, India. Methanol, Glacial Acetic acid, Formic acid and Chloroform were used of AR Grade. The plates were developed in a CAMAG twin trough glass chamber (20 x 10 cm) by ascending method. Distance of solvent front 80mm, band length 6mm, slit dimension 5.00 x 0.45 mm and detection wavelength 252 nm were used for the present study.

Method

Preparation of Monoammonium Glycyrrhizinate (MAG) Solutions

The standard solution was prepared by dissolving 10 mg MAG in 10 ml methanol solution which gives 1000µg/ml. The working standard of 100 µg/ml was prepared from standard solution by diluting with methanol. Different concentrations of 2, 5, 10, 15, 20 µg/ml were prepared from standard solutions.

Chromatographic Conditions

Analysis was performed on 20 cm × 10 cm HPTLC silica gel G60F254 plates with fluorescent indicator. The plate cleaned by predevelopment to the top with methanol, and dried in an oven 105^0C for 5 min. Sample and standard zones were applied to the layer as bands by means of a CAMAG. Linomat 5 automated spray-on applicator equipped with a 100 µl syringe and operated with the settings band length 6 mm, application rate 4 µl/sec, distance between bands 4 mm, distance from the plate side edge 6.5 mm, and distance from the bottom of the plate 2 cm.

Calibration Curve

2, 5, 10 and 15µl standard solution of Monoammonium Glycyrrhizinate was applied onto TLC plate to generate Calibration curve. The chromatograms were developed using said chromatographic conditions. The plate was dried in air and kept in hot air oven at 105 °C for 5 min. The standard zones were quantified by linear scanning at 254 nm (Fluorescent) by use of a TLC Scanner III CAMAG with a mercury source[4].

Analysis of glycyrrhizin in herbal extracts

The Glycyrrhiza powder 500mg was taken in conical flask and extracted twice with 10 ml of 70% ethanol, and sonicated for 10 min in ultrasonic bath. The solution was filtered through Whatman filter paper No.44 and the filtrate was used as further analysis.[5, 6]

Analysis of glycyrrhizin in herbal formulations

The weighted amount of gel equivalent to 10mg of glycyrrhizin was mixed with 70% ethanol with vigorous shaking and sonicated for 10 min in ultrasonic bath. The solution was filtered through Whatman filter paper No.44 and the filtrate was used as further analysis.

METHOD VALIDATION[7, 8]:

Precision

Repeatability:

Repeatability of sample application and measurement of peak area was carried out using the three replicates of same spot 1000 ng/spot. Repeatability is also termed intra-assay precision.

Intermediate precision:

The intra-day and inter-day variations for determination of glycyrrhizin were carried out at three different concentration levels 2, 5, 10 µl/spot.

Specificity

The specificity of method was ascertained by standard MAG and samples (extracted from powder and extracted from formulation). The spots of diluent ethanol, Placebo of formulation, standard MAG, extracted samples (extracted from powder and extracted from formulation) were spotted on TLC plate in duplicate and run. The spots for glycyrrhizin that eluted were confirmed with R_f value of MAG.[9,10]

Recovery Studies

Recovery Study was performed by spiking 20, 40 and 60 % of standard drug externally to the pre analyzed samples. The experiment was conducted in triplicate and applied onto the plate in duplicate. This was conducted to check the recovery of drugs at different levels of formulations.[11]

Robustness

The robustness of method can be performed by allowing variation in sample application time to scanning.

RESULT AND DISCUSSION

Mobile Phase Development

The mixtures of several mobile phases were tried to separate spot of glycyrrhizin from other spots and get stable peak. The solvent system used was ethyl acetate: Glacial acetic acid: Formic acid: water (15:2:1:1 v/v/v/v) was selected for estimation of glycyrrhizin, which gave good resolution. Good chromatogram in Figure 2 was attained with R_f value 0.34 ± 0.04. The wavelength of 252 nm was used for quantification of sample.

Table no.1. Comparative data of standard, extract and extract from formulation

Track	Rf	Height of Peak	Area	% Area
1	0.33	31.4	402	6.41
2	0.33	139.4	5532.7	62.97
3	0.33	215.5	8226.3	12
4	0.33	293.8	1179.5	10.39
5	0.33	208.6	7333.7	11.51
6	0.33	230.1	8708.1	29.64

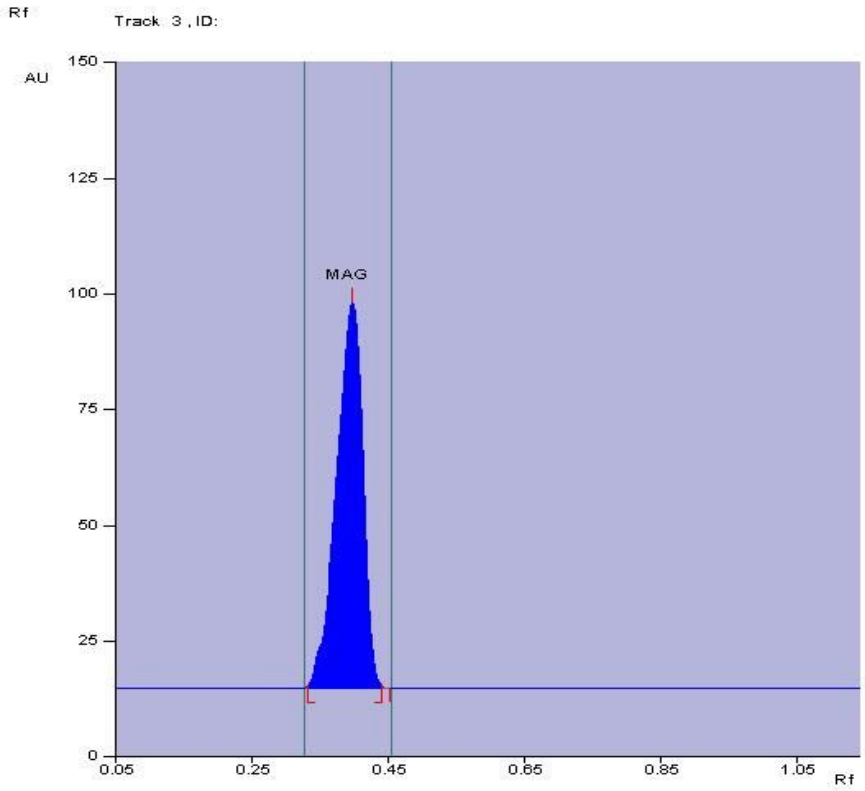

Figure 2. Chromatogram of representative sample

Calibration Curve, Linearity and Range

Linear regression data showed a good linear relationship over concentration range. The correlation coefficient r^2 was 0.998 shown in Table no.2.

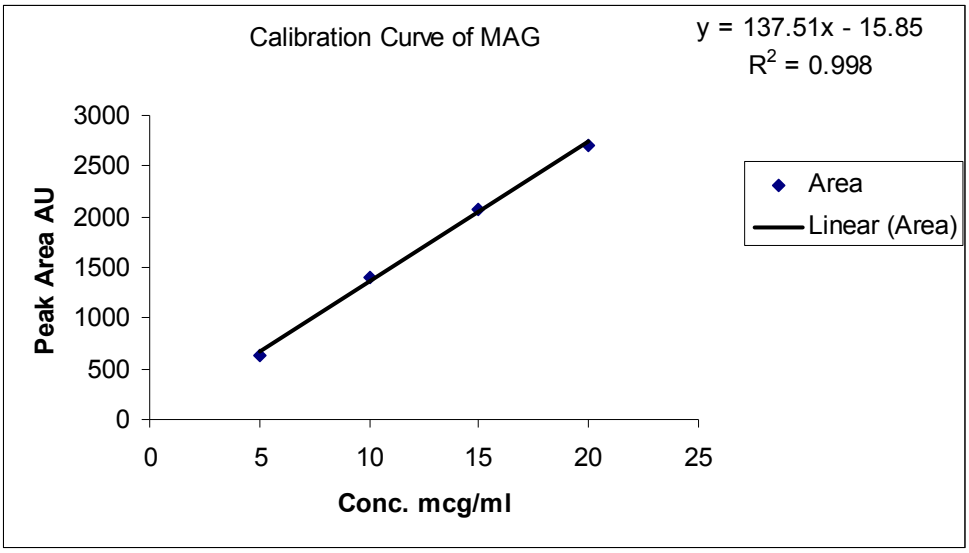

Figure 3. Calibration Curve of MAG

Table no.2. Linearity regression data for calibration curve

Parameters	**Value**
Linearity Range	200-1000ng/spot
Correlation of Coefficient (According to area)	0.999
Slope	141.97

Precision: The repeatability and intermediate precision were studied sepearatey and shown below in table 3 and 4 respectively.

Repeatability

It showed very low % RSD of peak area of drug.

Table no.3. Repeatability study

Sr. No.	Sample applied ng	Area AU	%RSD
1.	1000	255.6	0.056
2.	1000	251.6	0.064
3.	1000	258.2	0.056

Intermediate precision

Table no. 4. Intraday and Interday Precision

Sr. No.	Sample applied ng	Intraday % RSD	Interday RSD
1.	200	2.028	2.17
2.	400	1.98	1.9
3.	1000	1.54	1.6

Specificity

The mobile phase was optimized and it showed good result. Glycyrrhizin was found to be well separated from other constituents present in extracted sample. There was no interference of diluent, placebo and other constituent's peaks from extracted sample found at the R_f value of MAG peak, indicates specificity of the method.

Robustness of method

The time from sample application to scanning varied from 0, 20, 40, 60 mins. The standard deviation of peak areas was calculated for each parameter and % RSD was found to be less than 3 %.

Recovery studies

The proposed method when used for extraction and subsequent estimation of glycyrrhizin from gel after spiking with 20, 80, 120% standard MAG. The afforded recovery is listed below in Table no.5.

Estimation of glycyrrhizin in herbal extracts and in herbal gel

The optimized solvent system was used for the estimation of the glycyrrhizin from herbal extract and from herbal gel. There was no interference in analysis from other components present in extract. The resolution was good and components were observed at different R_f value. The total glycyrrhizin present in herbal extract was found to be 18.52% w/w, 17.92% w/w and 18.26% w/w. There was no interference from in analysis from other active components from extract and inactive components from formulation. The total glycyrrhizin present in formulations are 17.96%, w/w 17.85% w/w, and 18.1% w/w.

CONCLUSION

The developed HPTLC method is fast, simple, precise, specific and accurate. Statistical analysis proved that method is repeatable and selective for determination of Glycyrrhizin.

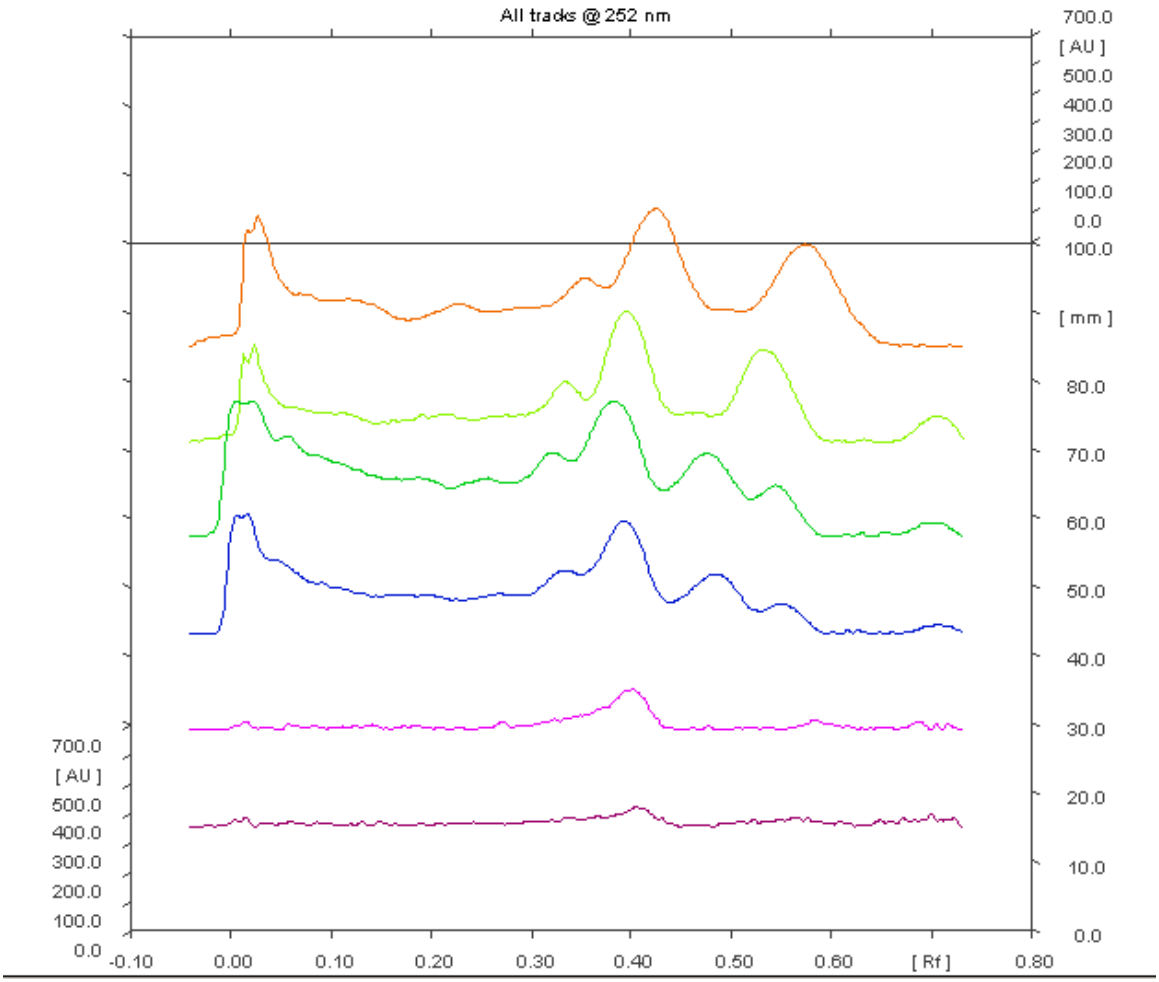

Figure 4. Comparision of Extracts peaks with Standard.

Table no. 5: Recovery studies

Sample	Amount added %	Amount recovered (mg)±SD	% Recovery	%RSD
Sample extracted from glycyrrhiza powder	20 40 60	19.12 39.2 58.9	95.60 98.00 98.16	0.169 0.298 0.275
Sample extracted from Gel	20 40 60	18.14 38.38 57.65	90.7 95.95 96.08	0.198 0.254 0.312

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