**Research Article**

# DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHOD FOR ESTIMATION OF GLYCYRRHETINIC ACID IN HYDRO-ALCOHOLIC EXTRACT OF *GLYCYRRHIZA GLABRA*

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**ABSTRACT**
A simple, rapid, accurate, precise, and economic spectrophotometric method for estimation of Glycyrrhetinic acid in hydro-alcoholic extract of *Glycyrrhiza glabra L.* have been developed. Glycyrrhetinic acid show absorbance maximum at 254 nm when Phosphate Buffer (pH-6.8) : Ethanol are used as solvent in 70 : 30 proportion, so absorbance was measured at the same wave lengths for the estimati1on of Glycyrrhetinic acid. Glycyrrhetinic acid obeys the Beer Lambert's law in the concentration range of 5-35µg/mL. A method was validated according to ICH guidelines and can be adopted for the routine analysis of Glycyrrhetinic acid in hydro-alcoholic extract of *Glycyrrhiza glabra*. The method is simple, rapid, safe, accurate, economical and useful for standardization of the licorice products.

**Keywords:** Glycyrrhetinic acid, *Glycyrrhiza glabra*, hydro-alcoholic extract, validation.

## INTRODUCTION
Licorice, the root of *Glycyrrhiza glabra* L. belongs to Leguminosae family has been used medically for over 2000 years. Historically[1], the dried rhizome and root of this plant were employed medicinally by the Egyptian, Chinese, Greek, Indian, and Roman civilizations as an expectorant and carminative. In modern medicine, licorice extracts are often used as a flavoring agent to mask bitter taste in preparations, and as an expectorant in cough and cold preparations. Licorice extracts have been used for more than 60 years in Japan[2] to treat chronic hepatitis, and also have therapeutic benefit against other viruses, including human immunodeficiency virus (HIV), cytomegalovirus (CMV), and *Herpes simplex*.

It is one of the oldest and most frequently employed folk medicine in China and Europe, are widely used to treat diseases of the respiratory tract, gastrointestinal and cardiovascular system, etc.[3]
*Glycyrrhiza glabra* as having important medicinal properties, including healing of ulcers and wounds and quenching thirst. Also licorice has shown anti-inflammatory, antiarthritic, anti-arrhythmic, anti-bacterial, antiviral and expectorant activity. It is now known that glycyrrhizic acid and its aglycone glycyrrhetinic acid present in the root extract are responsible for these biological activities.[4-8]
Standardization of licorice products on the basis of active components, which are responsible for its biological activities, is

useful to avoid the side effects. Glycyyhizin and its aglicone glycyrrhetinic acid, the active components of licorice have been determined quantitavely for standardization of the licorice products.

In this study, a simple, rapid, safe, accurate and economical UV method with 254nm λ max have been used for quantitative and qualitative analysis of glycyrrhetinic acid in *Glycyrrhiza glabra* extract, which can be used for standardization of licorice products.

## MATERIALS AND METHODS
### Instruments
Absorbance measurements was made on Systronics AU-2701 UV/Visible spectrophotometer with a pair of matched quartz cells of 1 cm width, Elder digital balance used for weighing, and Ultra sonicator of Prama instruments was used sonicating the drug and sample solution.

### Materials
Pure glycyrrhetinic acid was purchased from Yucca Enterprises, Mumbai-37. The extract prepared by continuous hot extraction method and was dried using evapourating dish and heating mental and used for analysis. All the chemicals and reagents were of analytical grade.

### Selection of common solvent
After assessing the solubility of marker and extract in different solvents Phosphate Buffer (pH-6.8) : Ethanol in 70: 30 proportions has been selected as common solvent for spectrophotometric evaluation.

### Selection of wavelength
A representative spectrum of glycyrrhetinic acid in selected solvent is shown in figure 1. The dilution was obtained to the concentration of 5μg/ml and was scanned in UV range (200-400nm) in 10 mm cell against solvent blank. The study of spectrum revealed that Glycyrrhetinic acid show a well defined λmax at 254 nm (Figure 1). Thus 254nm wavelength was selected for spectrophotometric evaluation.

## Preparation of standard stock solution and Study of Beer-Lambert's Law
The standard stock solutions of glycyrrhetinic acid was prepared by dissolving 10 mg of glycyrrhetinic acid in Phosphate Buffer (pH-6.8) : Ethanol in 70 : 30 proportion and final volume was adjusted with same solvent in 10mL of volumetric flask to get a solution containing 1000 μg/mL of glycyrrhetinic acid. Aliquots of working stock solutions of glycyrrhetinic acid was prepared with in the same solvent to get concentration in range of 5-35μg/ml of glycyrrhetinic acid. The absorbance of resulting solutions was measured at 254nm. A calibration curve as concentration vs. absorbance was constructed to study the Beer-Lambert's Law and regression equation, as shown in figure 2.

## Analysis of the herbal extract
Accurately weighed 20 mg of herbal hydroalcoholic extract of Licorice and was transferred to 10mL volumetric flask and dissolved Phosphate Buffer (pH-6.8): Ethanol in 70: 30 proportions and final volume was adjusted with same solvent in 10mL volumetric flask. The sample solution was then filtered through Whatman filter paper No.41. From the above solution 0.1mL of solution was taken and diluted to 10mL with Phosphate Buffer (pH-6.8): Ethanol in 70: 30 proportions to get final concentration containing 20μg/mL of glycyrrhetinic acid. Analysis procedure was repeated six times with extract. The results of extract analysis are reported in table 2.

## Validation of the developed methods[9, 10.]
### Linearity
For extract, appropriate dilutions of standard stock solutions were assayed as per the developed methods. For method the Beer-Lambert's concentration range was found to be 5-35 μg/mL. The linearity data for method is presented in table 1.
### Accuracy
To check the accuracy of the proposed method, recovery studies were carried out 80, 100 and 120% of the test concentration as per ICH guidelines. The recovery study was

performed three times at each level. The result of the recovery studies are reported in table 2.

## Precision
### Inteday and Intraday precision
The interday and intraday precision was determined by assay of the sample solution on the same day and on different days at different time intervals respectively (six replicates). The results of the same are presented in table 3.

## Robustness
The evaluation of robustness should be considered during the development phase and depends on the type of procedure under study. It should show the reliability of an analysis with respect to deliberate variations in method parameters.

If measurements are susceptible to variations in analytical conditions, the analytical conditions should be suitably controlled or a precautionary statement should be included in the procedure. One consequence of the evaluation of robustness should be that a series of system suitability parameters (e.g. resolution test) is established to ensure that the validity of the analytical procedure is maintained whenever used.

## Limit of detection
The detection limit is determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected.

$$DL = \frac{3.3\ \sigma}{S}$$

Where $\sigma$ = the standard deviation of the response
 S = the slope of the calibration curve

## Limit of quantitation
The quantitation limit is generally determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precision.

$$QL = \frac{10\ \sigma}{S}$$

Where $\sigma$ = the standard deviation of the response
 S = the slope of the calibration curve

## RESULT AND DISCUSSION
Linearity range for hydroalcoholic extract of licorice was found to be 5-35 µg/ at selected wavelength. The coefficient of correlation for glycyrrhetinic acid at 254 nm is 0.999. And it shows good regression value at its respective wavelength and the results of recovery study reveals that any small change in the glycyrrhetinic acids concentration in extracts in the solution could be accurately determined by the proposed methods. Percentage estimation of glycyrrhetinic acids from extract by method is 42.7033% with standard deviation less than two (table 1 & table 5).

The validity and reliability of proposed method was assessed by recovery study. Sample recovery for method was in good agreement with its respective claim (i.e. with assay purity), which suggest non interference by other components of extract in estimation (table 2).

Precision is determined by studying the repeatability and intermediate precision. Repeatability result indicates the precision under the same operating conditions over a short interval of time and interassay precision. Intermediate precision study expresses within laboratory variation in different days. In both intra and inter day precision study for both the methods % RSD are not more than 2.0% indicates good repeatability and intermediate precision (table                                3).

### Table 1: Result of Validation parameters[9, 10]

| Parameters | Glycyrrhetinic acid |
|---|---|
| Detection wavelength | 254 nm |
| Linearity range | 5-35 µg/mL |
| Slope | 0.025 |
| Intercept | 0.005 |
| Correlation coefficient | 0.999 |
| Regression equation (y =a + bc) | y = 0.025x - 0.005 |
| Limit of detection | 0.514614 µg/mL |
| Limit of quantitation | 1.559437 µg/mL |

### Table 2: Recovery studies[9, 10]

| Concentration of the drug added to the extract | Glycyrrhetinic acid % Recovery ± SD* | %RSD |
|---|---|---|
| 80% | 100.5±1.1401 | 1.1345 |
| 100% | 100.226±0.8640 | 0.8618 |
| 120% | 100.388±0.8277 | 0.8245 |

*Average of six determinations

### Table 3: Interday and Intraday precision[9, 10]

| Parameter | Interday precision | | Intraday precision | |
|---|---|---|---|---|
| | %Amount Found ± SD* | % RSD | %Amount Found ± SD* | % RSD |
| Glycyrrhetinic acid | 101.4757±0.7051 | 0.6948 | 102.0218±0.9089 | 0.8909 |

*Average of six determinations

### Table 4: Result of analysis of extract

| Formulation | Drug | Amount found ± S.D* | % found ±S.D* |
|---|---|---|---|
| Extract | Glycyrrhetinic acid | 8.5406±0.003606 | 42.7033±0.393 |

*Average of three determinations

### Table 5: Robustness[9, 10]

| Formulation | Drug | Amount found ± S.D* | % found ± S.D* |
|---|---|---|---|
| Analyst 1 | Glycyrrhetinic acid | 8.706667 ± 0.061101 | 101.9437 ± 0.715413 |
| Analyst 2 | Glycyrrhetinic acid | 8.666667 ± 0.083267 | 101.4754 ± 0.974944 |
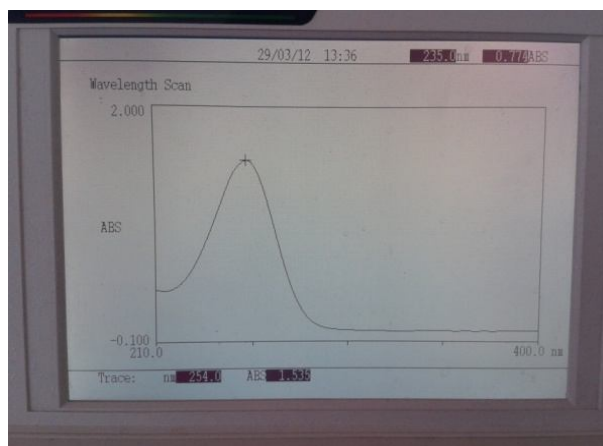


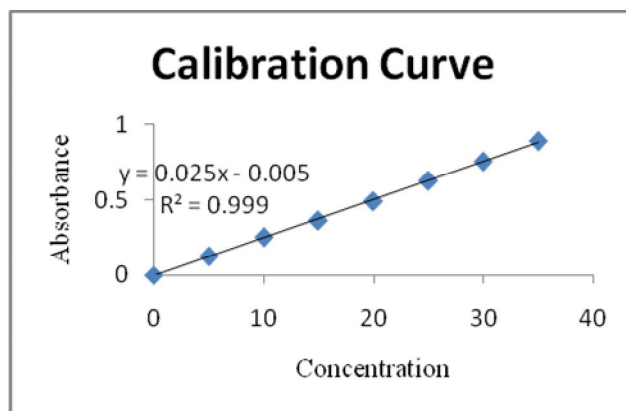**Fig. 1: UV Scan of Glycyrrhetinic acid**

**Fig. 2: Calibration curve of glycyrrhetinic acid**

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