ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 14, Issue 6, 2021

Online - 2455-3891
Print - 0974-2441

Research Article

DEVELOPMENT AND VALIDATION OF ULTRAVIOLET–VISIBLE SPECTROPHOTOMETRIC METHOD FOR ESTIMATION OF GLYCYRRHIZIN IN METHANOLIC EXTRACT OF *GLYCYRRHIZA GLABRA* L

IMRAN HYDER[1], ARIF NASEER[1]*, ADIL AHMAD[2]

[1]Department of Pharmaceutics, Adarsh Vijendra Institute of Pharmaceutical Sciences, Shobhit University, Saharanpur, Uttar Pradesh, India. [2]Department of Pharmacognosy and Phytochemistry, Jamia Hamdard, New Delhi, India. Email: arif.naseer@shovhituniversity.ac.in

Received: 30 November 2020, Revised and Accepted: 26 April 2021

ABSTRACT

Objective: The pure form of glycyrrhizin was bought from Yucca Enterprises, Mumbai-37, India. The pure form of drug used in the treatment of acne vulgaris disease. Pharmacognostical studies of *Glycyrrhiza glabra* L. indicated surprising antibacterial action against *Propionibacterium acnes.*

Methods: A simple, rapid, accurate, precise, and economic spectrophotometric technique for estimation of glycyrrhizin in methanolic extract of *G. glabra* L. have been developed. Glycyrrhizin exhibit absorbance most at 254 nm when phosphate buffer (pH-6.8) methanol is used as solvent in 70:30 proportion, so absorbance was once measured at the identical wavelengths for the determination of glycyrrhizin. Glycyrrhizin obeys Beer Lambert's law in the concentration range of 4–24 μg/ml.

Results: This method was validated according to International Council for Harmonization guidelines and can be adopted for the general analysis of glycyrrhizin in hydroalcoholic extract of *G. glabra.* The approach is simple, rapid, safe, accurate, affordable, and beneficial for the standardization of licorice products.

Conclusion: The were results applied in the routine analysis and quality control of pharmaceutical dosage forms containing glycyrrhizin.

Keywords: Glycyrrhizin, *Glycyrrhiza glabra*, Methanolic extract, Validation.

 DOI: http://dx.doi.org/10.22159/ajpcr.2021v14i6.40401. Journal homepage: https://innovareacademics.in/journals/index.php/ajpcr

INTRODUCTION

Glycyrrhiza glabra (also known as Liquorice in English, Mulethi in Hindi and called Yashtimadhu in Ayurveda) is a huge herb used in Indian medications, home fixes, individuals' drugs, and Ayurveda. Liquorice (British English) or licorice (American English) is the establishment of *G. glabra* from which a sweet flavor can be eliminated. Licorice is an herb utilized in nourishment and pharmaceutical for a huge number of years in the customary medication framework. It is herbaceous suffering, creating to 1 m in height, with pinnate leaves around 7–15 cm long, with 9–17 flyers. The blossoms are 0.8–1.1 cm long, purple to pale whitish blue, made in a free inflorescence. The natural product is a oval case, 2–2 cm long, containing a few seeds [1]. The helper metabolites of this plant have for a long while been evaluated for their use in facilitating respiratory pains, (e.g. bronchitis, hypersensitivities, colds, tuberculosis, and sore throats), their demulcent effect (alleviating and covering specialist), assuaging stomach consumption side effect including acid reflux coming about because of reflux or some other reason, and treating gastritis, provocative issue, liver issues, and skin maladies [2]. *G. glabra* containing glycyrrhizin, triterpene glycoside, glabric acid, flavanones, and isoflavones indicated surprising antibacterial action against *Propionibacterium acnes* [3]. When veered from extracts of *G. glabra* and *Calendula officinalis* by agar circle dissipating framework (because of the closeness of alkaloids, flavonoids, glycosides, and terpenoids) suggesting them as dependable phytoconstituents for the antiacne action [4]. *G. glabra* is a plant utilized in herbalism and conventional medicine over the world for its ethnopharmacological value. It is found to contain significant phytoconstituents, which are viably utilized as mitigating, hostile to bacterial, hostile to parasitic, hostile to diabetic, antiviral, against ulcer, antitussive, against oxidant, skin brightening, and against the diuretic agent. Its attaches were additionally shown to have upper, hypotensive hepatoprotective, spasmolytic, and memory fortifying activity. Licorice pulls are utilized for their demulcent property [5]. This plant species is accounted for in the writing for the organic activities, for example, calming and expectorant, controls hacking and has hormonal effects. Restoratively, it is used inside for Addison's affliction asthma, bronchitis, peptic ulcer, joint irritation, negatively defenseless complaints, and steroid treatment. Since liquorice separate is utilized in auto-insusceptible ailment and has remedial advantages in immunodeficiency conditions like AIDS. Externally, liquorices are utilized for dermatitis, herpes, and shingles. Components of the licorice root have both estrogenic and against the estrogenic activity. It is subsequently, a critical herb for treating hormone-related female issues. It is used as an imperativeness tonic, particularly for the spleen and stomach, and the root is added to various formulae. Roots of *G. glabra* being tonic, a demulcent purgative emollient is utilized in genitourinary diseases. It is additionally valuable in gout, asthma, sore throat, tonsillitis, tooting, sexual debility, epilepsy, hyperplasia, fever, hacks, skin sicknesses, swellings, acidness, leukorrhea, dying, jaundice, hiccough, raspiness, and vitiated conditions Licorice is a significant fixing in therapeutic oils for epilepsy, loss of motion, stiffness, and hemorrhagic diseases. It is additionally utilized in the treatment of the runs, fevers, and fever with daze and anuria [6]. Since the beginning of recorded history, individuals have used licorice (predominantly the species *G. glabra* L., Leguminosae) as a cure. Customs from different land areas and unmistakable periods have recorded their wide use [7].

G. glabra as having significant medicinal properties including the healing of ulcers and wounds and extinguishing thirst. In addition, licorice has shown anti-inflammatory, antiarthritic, against arrhythmic, antibacterial, antiviral, and expectorant action. It is currently realized that glycyrrhizic acid and its aglycone glycyrrhetinic acid present in the root separate are liable for these biological activities [8-11].

METHODS

Instruments

Absorbance estimations were made on Systronics AU-2701 ultraviolet (UV)/Visible spectrophotometer with a couple of coordinated with quartz cells of 1 cm width, the Elder computerized balance utilized for gauging, and Ultra sonicator of Prama instruments was utilized sonicating the medication and test arrangement.

Materials

The pure form of glycyrrhizin was bought from Yucca Enterprises, Mumbai-37, India. The extract arranged by a persistent Soxhlet extraction process and was dried using evaporating dish and heating mental and used for analysis. Every one of the synthetics and reagents was of insightful evaluation.

Selection of common solvent

After assessing the solubility of marker and extract in various solvents Phosphate Buffer (pH-6.8):ethanol in 70:30 extents has been chosen as a very common solvent for spectrophotometric assessment.

Selection of wavelength

A representative spectrum of glycyrrhizin in the selected solvent is shown in Fig. 1. The dilution was obtained to the concentration of 5 µg/ml and was scanned in the UV range (200–400 nm) in a 10 mm cell against solvent blank. The study of the spectrum revealed that glycyrrhizin shows a well-defined λ max at 254 nm (Fig. 1). Thus, 254 nm wavelength was selected for spectrophotometric evaluation.

Preparation of standard stock solution and study of Beer-Lambert's law

A stock solution of glycyrrhizin standard (Yucca Enterprises, India) was prepared by dissolving an accurately weighed 1mg of glycyrrhizin in 10 ml of methanol in a volumetric flask. From this solution, various concentrations of the standard solution were prepared in 10 ml of methanol in the volumetric flask to obtain concentrations 4, 8, 12, 16, 20, and 24 µg/ml. UV-Spectrum of 24 µg/ml stock solution of glycyrrhizin standard was taken to determine its λ_{max} value. The absorbance of the resulting solutions was estimated at 254 nm. A calibration curve as concentration versus absorbance was developed to examine Beer-Lambert's Law and regression equation, as shown in Fig. 2.

Analysis of the herbal extract

Accurately weighed 10 mg of herbal hydroalcoholic extract of Licorice and was transferred to 10 mL volumetric flask and dissolved Phosphate Buffer (pH-6.8):methanol in 70:30 proportions and final volume was adjusted with the same solvent in 10 mL volumetric flask. The sample solution was then filtered through Whatman filter paper No.41. From the above solution, 0.1 mL of the solution was taken and diluted to 10 mL with Phosphate Buffer (pH-6.8):methanol in 70:30 proportions to get the final concentration containing 10 µg/mL of glycyrrhizin. The analysis procedure was repeated 6 times with extract. The results of extract analysis are reported in Table 1.

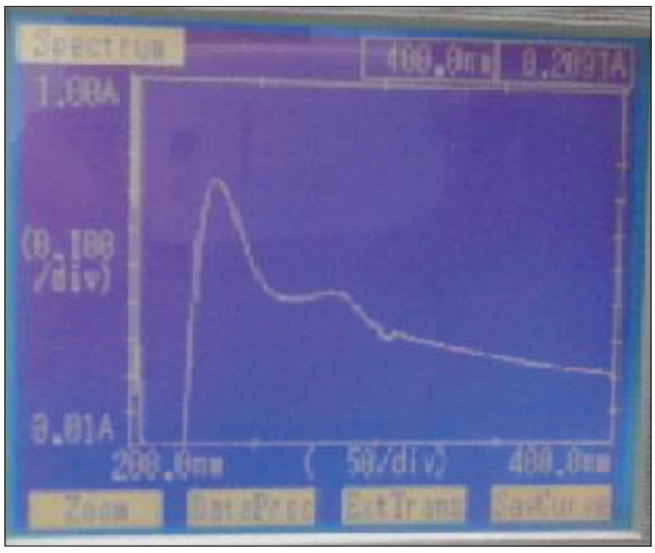


Fig. 1: Ultraviolet spectra of glycyrrhizin in methanol

Validation of the developed methods [12,13]

Linearity

For extract, appropriate dilutions of standard stock solutions were tested according to the developed methods. For method, Beer Lambert's concentration range was found to be 4–24 µg/mL. The linearity information for the strategy is introduced in Table 2.

Accuracy

To check the accuracy of the proposed method, recovery studies were carried out 80, 100, and 120% of the test concentration according to the International Council for Harmonisation (ICH) guidelines. The recovery study was performed 3 times at each level. The result of the recovery studies is reported in Table 1.

Precision

Interday and intraday precision

The interday and intraday precision was controlled by assay of the sample solution on the same day and different days at different time intervals, respectively (six replicates). The results of the same are presented in Table 3.

Robustness

The assessment of robustness should be considered during the improvement stage and depends on the kind of technique under investigation. 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 (LOD)

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 dependably distinguished.

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

Where σ = the standard deviation of the response
S = the slope of the calibration curve.

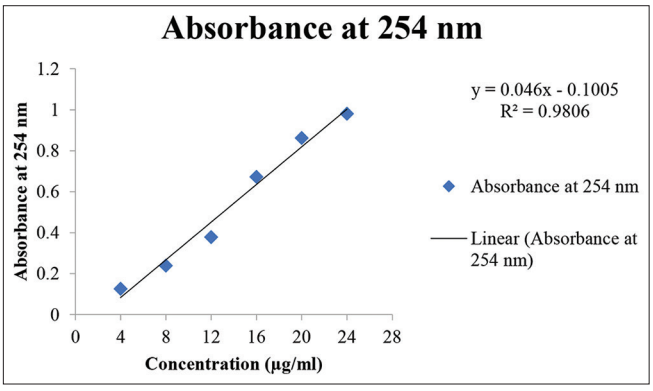
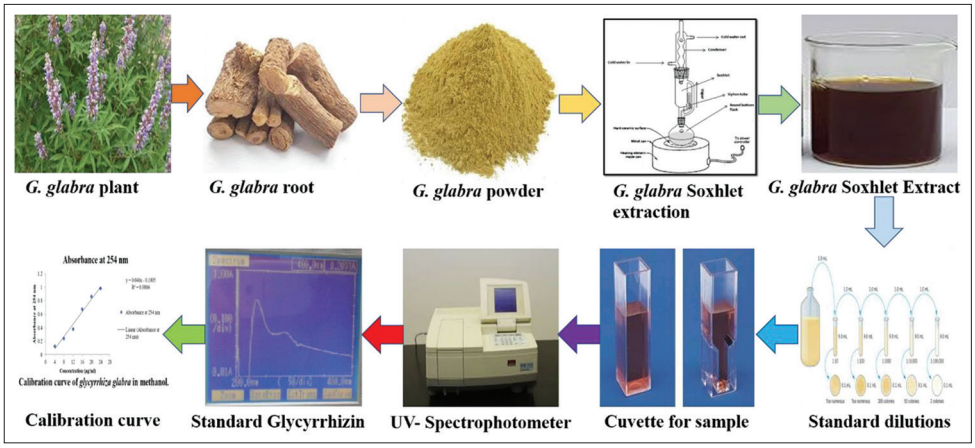


Fig. 2: Calibration curve of *Glycyrrhiza glabra* in methanol



Graphical representation of *Glycyrrhiza glabra* L.

Table 1: Recovery study

The concentration of the drug added to the extract (%)	glycyrrhizin % Recovery±SD*	%RSD
90	100.4±2.453	1.1256
100	100.3±1.482	1.0967
110	100.6±1.634	0.8752

(*): Average of six determinations

Table 2: Validation parameters

Parameters	Glycyrrhizin
Detection wavelength	254 nm
Linearity range	4–24 µg/mL
Regression equation	Y=0.046, X=0.1005
Correlation coefficient	0.9806
Slope	0.046
Intercept	0.1005
LOD	3.228 µg/mL
LOQ	9.782 µg/mL

LOD: Limit of detection, LOQ: Limit of quantification

Table 3: Interday and intraday precision

Parameter	Inter-day precision		Intraday precision	
	%Amount found±SD*	%RSD	%Amount found±SD*	%RSD
Glycyrrhizin	102.562±0.834	0.7456	101.453±1.562	0.9456

(*): Average of six determinations

Table 4: Analysis of the extract

Formulation	Drug	Amount found±SD*	Percentage found±SD*
Extract	Glycyrrhizin	9.743±0.0981	51.971±0.418

(*): Average of three determinations

Table 5: Robustness

Formulation	Drug	Amount found±SD*	Percentage found±SD*
Sample 1	Glycyrrhizin	9.231±0.0841	100.764±0.1982
Sample 2	Glycyrrhizin	9.862±0.0678	101.455±1.662

Limit of quantitation (LOQ)

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 evaluated with satisfactory exactness and accuracy.

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

Where ϭ = the standard deviation of the response

S = the slope of the calibration curve.

RESULTS AND DISCUSSION

The linearity range for the alcoholic extract of licorice was found to be 4–24 µg/ml at the selected wavelength. The coefficient of correlation for glycyrrhizin at 254 nm is 0.9806. And it shows a very good regression value at its respective wavelength and the results of the recovery study reveal that any small change in the glycyrrhizin concentration in extracts in the solution could be accurately controlled by the proposed techniques.

The percentage estimation of glycyrrhizin from the extract by the method is 51.973% with a standard deviation of <2 (Tables 1-5).

The validity and reliability of the proposed method were evaluated by the recovery study. Sample recovery for the method was in acceptable concurrence with its respective claim (i.e. with assay purity), which suggests non-interference by other components of extract in estimation (Table 1).

Precision is determined by studying repeatability and intermediate precision. Repeatability result shows the precision under similar working conditions throughout a short time frame and inter-assay precision. The intermediate precision study expresses laboratory variation on different days. In both intra- and inter-day precision studies for both the methods % RSD are not more than 2.0% indicates good repeatability and intermediate precision (Table 3).

CONCLUSION

A very simple, accurate, precise, robust, and rapid UV spectrophotometric method was developed for the estimation of glycyrrhizin in bulk and its subsequent pharmaceutical formulation. The results reveal that the proposed method could be successfully applied in the routine analysis and quality control of pharmaceutical dosage forms containing glycyrrhizin.

AUTHORS' CONTRIBUTIONS

Md. Imran Hyder made substantial contribution to the conception, acquisition of data, and took part in the drafting of the article. Md. Arif Naseer and Adil Ahmad took part in revising it critically for important intellectual content, final approval of the version to be published.

AUTHORS' FUNDING

The authors received no specific funding.

CONFLICTS OF INTEREST

The authors declared that there are no conflicts of interest related to this study.

REFERENCES

1. Griffiths M, Huxley A. The New Royal Horticultural Society Dictionary of Gardening. Vol. 1. New York: Macmillan; 1994. p. 837-853.
2. Xu H, Fabricant DS, Piersen CE, Bolton JL, Pezzuto JM, Fong H, *et al*. A preliminary RAPD-PCR analysis of *Cimicifuga* species and other botanicals used for women's health. Phytomedicine 2002;9:757-62.
3. Nam C, Kim S, Sim Y, Chang I. Anti-acne effects of oriental herb extracts: A novel screening method to select anti-acne agents. Skin Pharmacol Physiol 2003;16:84-90.
4. Panichayupakaranant P, Tewtrakul S, Yuenyongsawad S. Antibacterial, anti-inflammatory and anti-allergic activities of standardised pomegranate rind extract. Food Chem 2010;123:400-3.
5. Kaur R, Kaur H, Dhindsa AS. *Glycyrrhiza glabra*: A phytopharmacological review. Int J Pharm Sci Res 2013;4:2470-7.
6. Ram S. A bibliometric assessment of liquorice (*Glycyrrhiza glabra*) research trends. Ann Lib Inf Stud 2015;62: 27-32.
7. Armanini D, Fiore C, Mattarello MJ, Bielenberg J, Palermo M. History of the endocrine effects of licorice. Exp Clin Endocrinol Diabetes 2002;110:257-61.
8. Baker ME. Endocrine activity of plant-derived compounds: An evolutionary perspective. Proc Soc Exp Biol Med 1995;208:131-8.
9. Norman HA, Pillai P, Baker ME. Licorice-derived compounds inhibit linoleic acid (C: 18: 2ω6) desaturation in soybean chloroplasts. FEBS Lett 1995;368:135-8.
10. Der Marderosian A, Beuther JA. The Review of Natural Products. St. Louis: Facts and Comparisons; 2001. p. 405-9.
11. Tanaka A, Horiuchi M, Umano K, Shibamoto T. Antioxidant and anti-inflammatory activities of water distillate and its dichloromethane extract from licorice root (*Glycyrrhiza uralensis*) and chemical composition of dichloromethane extract. J Sci Food Agric 2008;88:1158-65.
12. Folkers G. In: Lund W, editor. The Pharmaceutical Codex, Principles and Practice of Pharmaceutics. London: The Pharmaceutical Press; 1994. p. 1100.
13. Nash RA, Wachter AH. Pharmaceutical Process Validation. 3rd ed., Vol. 129. New York: Revised and Expanded, Marcel Dekkar Inc.; 2003. p. 760-92.