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STUDIES BY RP-HPLC METHOD FOR THE QUANTIFICATION OF GLABRIDIN IN POLYHERBAL FORMULATION

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ABSTRACT

A method for analysing glabridin in crude pharmaceuticals and polyherbal formulations has been developed and validated. It is straightforward, economical, selective, precise, and robust. Phase-reversal chromatography is carried out on a C18 column using a gradient elution technique with acetonitrile and water as the mobile phases at a flow rate of 1 mL/min.Glabridin is detected at 230 nm, and with a retention time of 14.9+0.02 min, a strong peak is observed. The calibration plot's linear regression analysis results revealed a strong linear association between response and concentration in the 1–500 mg/mL range; the linear regression equation is y 5 26.683x – 142.17, and the regression coefficient is 0.9992. In compliance with the recommendations of the International Conference on Harmonisation, the method is validated for accuracy, precision, repeatability, robustness, and detection and quantification limitations. The procedure is exact, repeatable, selective, and accurate for the analysis of glabridin, according to statistical analysis. Licorice (Glycyrrhiza glabra Linn.) and various herbal formulations including licorice as an ingredient can be subjected to quality control and standardisation through the application of the high-performance liquid chromatography method that has been developed, verified, and proposed for glabridin measurement.

Key Words-: *Glycyrrhiza glabra, Harmonisation, Glabridin*

INTRODUCTION

For almost 4,000 years, people have been consuming licorice, sometimes referred to as "sweet root," which is the root of the leguminous Glycyrrhiza plant species. [4-6[(3R)-8,8-dimethyl-3,4-dihydro-2H-pyrano][6, 5-

f]chromen-3-yl] Glabridin[Benzene-1,3-diol] A significant polyphenolic flavonoid unique to Glycyrrhiza glabra L. is shown in Figure 1. Recent investigations have demonstrated the anticancer efficacy of glabridin by blocking the focal adhesion kinase/Rho signalling pathway, which in turn prevents the migration, invasion, and angiogenesis of human non-small cell lung cancer A549 cells and MDA-MB-231 human breast adenocarcinoma cells (1, 2). It has also been observed that glabridin exhibits a number of pharmacological properties, including antioxidant (3), anti-Helicobacter pylori (4), antifungal (5), estrogenic, anti-proliferative (6, 7) and antinephritic activity (8). Furthermore, glabridin suppresses inflammation (10), melanogenesis (9), and serotonin reuptake (10).

In India and many other nations, the Unani medical method is widely used. Various herbal formulations, including solid (Qurs, Habbs, Safoof), semi-solid (Khamira, Itrifal, Majoon), and liquid (Sharbat) preparations, are employed in the Unani system (11, 12). The tablet form Qurs-e-Gul is listed in the National Formulary of Unani Medicines (NFUM). It is frequently given in the Unani medical system as a deobstruent and to treat cardiac-related issues and jaundice. Glycyrrhiza glabra (28.57% w/w), Pistacia lentiseus (4.7% w/w), Bamboosa bambo Druce (4.76% w/w), Nardostachys jatamansi (14.28% w/w), Rosa damascene (47.6% w/w), and excepients are its five constituents (13).

Glabridin has been quantified using a number of analytical techniques, including capillary electrophoresis (17), liquid chromatography–mass spectrometry (LC–MS) (18), and high-performance liquid chromatography (HPLC) (14–16). The isocratic HPLC technique that was previously reported was found to have a relatively narrow linearity range (10–100 mg/mL) and to have a poor recovery rate of 92%. Additionally, it was discovered that the isocratic approach that was previously published was unsuitable for separating the primary ingredients in polyherbal formulations from their immediate contaminants. The development and validation of a gradient reversed-phase (RP)-HPLC method was deemed worthwhile in order to quantify glabridin in both crude drug and polyherbal formulations. This method is capable of effectively separating glabridin in multicomponent formulations with improved validation parameters. Comparatively speaking, the recently developed and verified HPLC method is easier to use and more effective for quantifying glaciridin in polyherbal mixtures than previously documented techniques.

METHODOLOGY

Reagents and chemicals

Glabridin (98%) was obtained as a gift sample from Sami Labs (Bangalore, India). HPLC-grade acetonitrile and methanol were purchased from Merck (India). Milli-Q water used throughout

the experiment was prepared using a Millipore water purification system.

Instrumentation and general conditions

Chromatographic experiments were conducted on a YL9100 HPLC system (South Korea) that comprised quaternary YL9110 pumps, a variable wavelength programmable YL9120 ultraviolet (UV)-visible detector, YL9130 column oven and a system controller. The instrument was controlled by use of YL-Clarity software installed with the equipment. Samples were injected by using a rheodyne injector fitted with a 20-mL fixed loop. Standard and sample solutions were filtered through a 0.22-mm syringe filter before injection. The separation was achieved by using a C18 reversed-phase column (Merck Lichrocart 250-4, Lichrosphere 100 RP-18e, 5 mm, Sorbent lot number L57020637). The mobile phase consisted of acetonitrile and water in gradient elution method from 50 to 80% in 20 min. The flow rate was kept at 1.0 mL/ min. All the analyses were performed at room temperature and detection was carried out at a wavelength of 230 nm using a UV-visible detector.

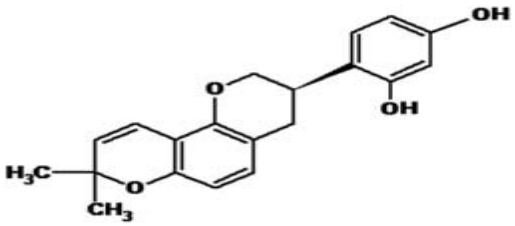

Figure 1. Chemical structure of glabridin

Preparation of sample solutions

Preparation of sample for crude One gram of the powdered crude drug was extracted with 25 mL of 30% aqueous ethanol by soaking overnight and then refluxing on a water bath for 45 min. It was filtered and evaporated to dryness under reduced pressure. The obtained residue was then reconstituted in HPLC-grade methanol and the volume was adjusted to 25 mL, which was filtered through a 0.22-mm syringe filter before injecting into the HPLC column.

Preparation of sample for polyherbal formulation twenty tablets were randomly selected from the formulation and average weight was determined. The tablets were triturated to get a uniform fine powder. Two grams, accurately weighed, of the powdered sample was extracted with 25 mL

of 30% aqueous ethanol, as discussed previously. The obtained residue was then reconstituted in HPLC-grade methanol and volume was adjusted to 25 mL, which was filtered through a 0.22-mm syringe filter before injecting into the HPLC column.

Validation methodology

Method validation was carried out to confirm the suitability of the proposed analytical method for its intended use. The proposed method was validated as per International Conference on Harmonization (ICH) (19)

guidelines for different parameters such as linearity, accuracy, precision, limit of detection (LOD) and limit of quantification (LOQ) and robustness, similar to the previously reported laboratory methods (20–23).

Calibration curve for glabridin

A stock solution of glabridin with a known concentration of 1,000 mg/mL was prepared in methanol and different aliquots were made to get six different desired concentrations from 1 to 500 mg/mL, which were injected (20 mL each) by rheodyne injector and chromatographed as per the previously mentioned protocol. The stock solution was kept in dark for storage to avoid possible degradation that may result from exposure to light.

Accuracy as recovery

The accuracy of the method was determined by recovery studies using the standard addition method. Preanalyzed samples were spiked with standard glabridin at three different concentration levels, i.e., 50, 100 and 150%, and the mixtures were reanalyzed by the proposed method. Data obtained was analyzed for percent recovery.

Precision

The precision of the method was carried out by performing repeatability and intermediate precision. In repeatability, six different injections of the same standard sample (three concentrations) were injected and calculated in the assay. The percent relative standard deviation (%RSD) of the area and retention time (Rt) were calculated. In intermediate precision, intra-day, inter-day, and inter-system precisions were performed. Intra-day and inter-day precisions were performed by preparing and applying three different concentrations of standard in triplicate six times a day and on six different days, respectively. Inter-system precision was performed by repeating the same procedure in a different HPLC system. Assay for each analysis was calculated and %RSD was determined.

LOD and LOQ

The LOD and LOQ were determined based on the basis of signal-to-noise ratio. The concentration of the sample with a signal-to-noise ratio of three was fixed as the LOD. The concentration of the sample with a signal-to-noise ratio of ten was fixed as the LOQ.

Robustness of the method

Robustness of the method was performed by introducing very small changes in the analytical methodology at a single concentration level (100 mg/mL). Robustness of the proposed method was determined in two different ways, i.e., by making deliberate changes in the flow rate and by changing the detection wavelength of the analysis. In the present study, the robustness was evaluated by using the Box-Behnken response surface design (24). The design simultaneously evaluated the effects of the three important parameters on peak area: flow rate of the mobile phase, detection wave length and temperature of the column oven. Design Expert version 7.0.0.1

(Stat-Ease, Minneapolis, MN) was used to evaluate the results.

Three-dimensional graphs represented peak area dependence on flow rate of the mobile phase and detection wavelength temperature of the column and flow rate of the mobile phase and temperature of the column and detection wavelength. Effects of the selected factors were evaluated over a range of conditions by determining the maximum area response of the glabridin peaks.

RESULTS AND DISCUSSION

Method development

In order to create an HPLC method suited for glabridin analysis in crude drug and polyherbal formulations, a range of mobile phases were explored. The acetonitrile-phosphate buffer (pH 3.5 adjusted with orthophosphoric acid), 80:20 (% v/v), methanol–water, 50:50 (% v/v), acetonitrile–phosphate buffer (pH 3.5 with orthophosphoric acid), 60:40 (% v/v), acetonitrile–water, 50:50 (% v/v), and acetonitrile–water, 60:40 (% v/v) were among the investigated mobile phases. At a retention period of 9.1+0.02 minutes, a strong peak of glabridin was seen when acetonitrile and water were mixed 60:40 (% v/v). Unfortunately, due to inadequate separation from the immediate contaminants, this mobile phase proved ineffective in effectively separating the chemical in the polyherbal formulation. In order to achieve good separation from the contaminants, a strong peak at the Rt of 14.9+0.02 min was obtained by creating a gradient elution system with an acetonitrile concentration of 50 to 80% within 20 minutes (Figure 2). Additionally, YL Clarity software was used to optimise chromatographic settings and determine system appropriateness parameters such as theoretical plates (7,739), tailing factor (0.982), and asymmetry (0.958) of glabridin. These calculations demonstrated the applicability of the suggested approach.

Validation of analytical method

Linearity

A stock solution containing 1,000 mg/mL was made, and aliquots ranging from 1 to 500 mg/mL were taken from it in order to evaluate the linearity of the procedure. For storage, the stock solution was maintained in the dark. With an excellent regression coefficient value of 0.9992, the linearity of the calibration for glabridin was evaluated in the range of 1–500 mg/mL; the regression equation is y ¼ 26.683x – 142.17. It was discovered that the slope+SD and intercept+SD were, respectively, 25.13633+0.52 and 141.11+1.2.

Accuracy

The accuracy of the method was determined by recovery studies. The preanalyzed samples were spiked with standard at three different concentration levels, i.e., 50, 100 and 150%. The mixtures were reanalyzed by the

proposed method and found to be within the limit of 97.39–103.25%, which is better than the method reported by Shanker et al. (14). The values of recovery percent and %RSD are listed in Table I.

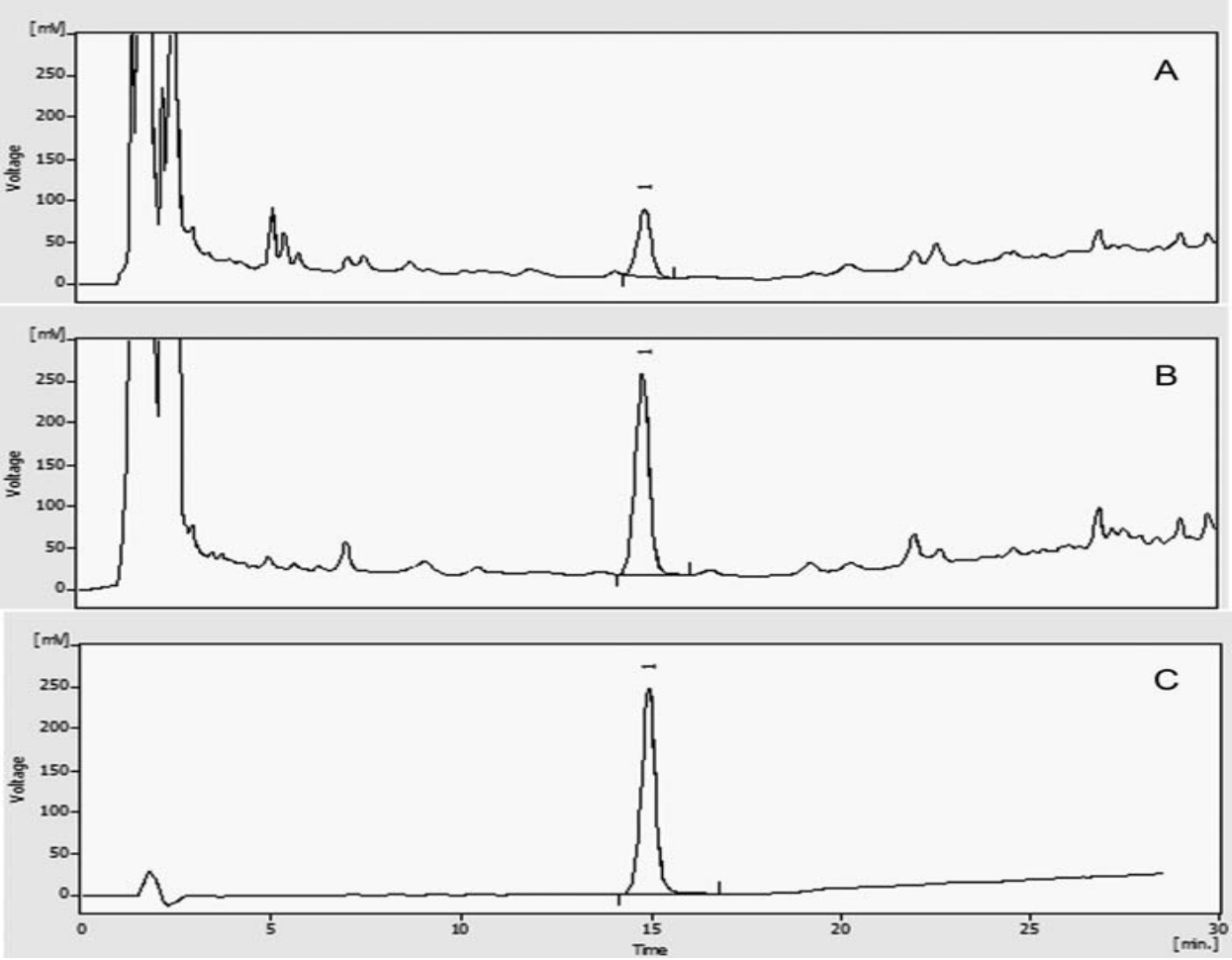

Figure 2. HPLC chromatogram of glabridin at 230 nm: standard (A); Glycyrrhiza glabra (B); Qurs-e-Gul tablet formulation (C).

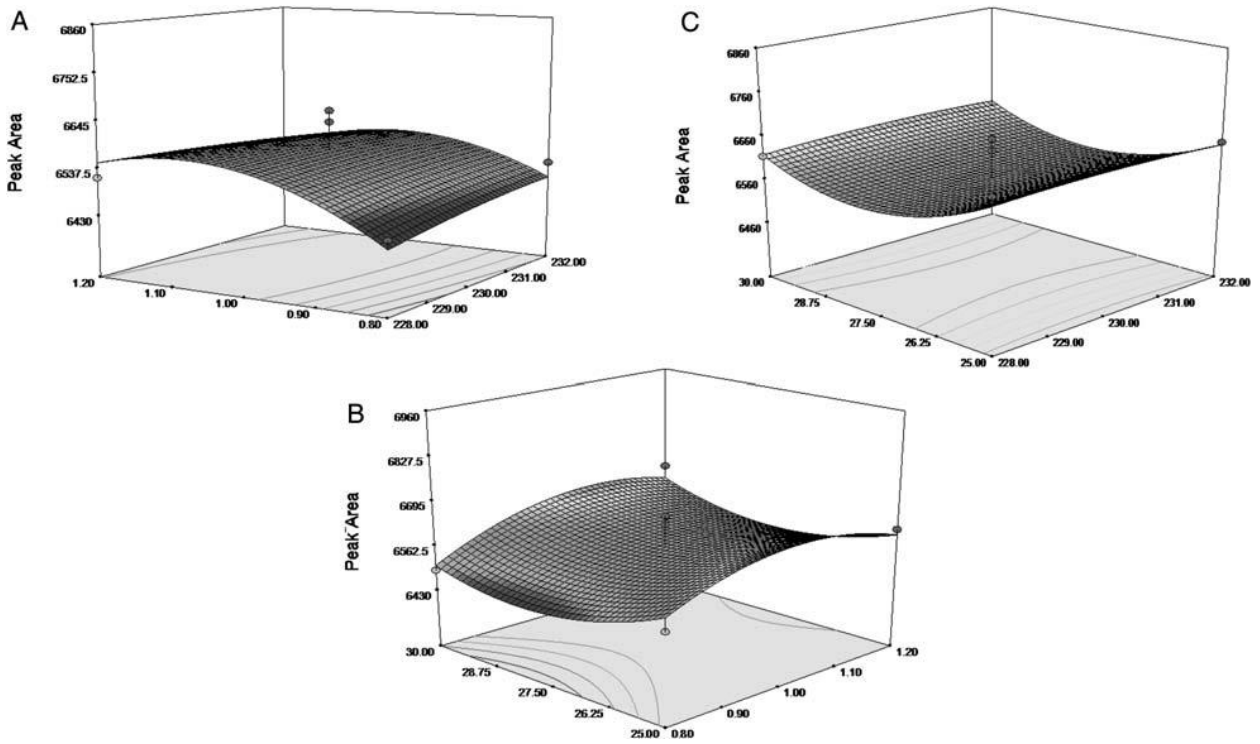

Figure 3. Three-dimensional graphs: Peak area ¼ f (flow rate of the mobile phase, detection wave length) (A); peak area ¼ f (temperature of the column, flow rate) (B); peak area ¼ f (temperature of the column, detection wave length) (C).

Precision

According to the ICH guidelines, repeatability and intermediate precision were used to determine the suggested method's precision. The assay was calculated using six distinct injections of the same standard sample (three concentrations) in order to test repeatability. Rt and the area's %RSD were computed. Three distinct sample concentrations were prepared and applied on three separate days, respectively, to achieve inter-day and intra-day precisions. To achieve inter-system precision, the same process was carried out again using a different system.

LOD and LOQ

The limits of quantification and detection were calculated by using the linearity curve method by using the formula LOD ¼ 3.3s/S and LOQ ¼ 10s/S, where s is the standard deviation of the response and S is the slope of the calibration plot. For the developed method, LOD was found to be 0.35 mg/mL and LOQ was calculated at 1 mg/mL. Once the LOD and LOQ were determined, six replicates of blank and standard solutions at the levels of LOD and LOQ were applied and the %RSD was calculated.

Robustness

The robustness was evaluated by using the Box-Behnken response surface design. The Design Expert software proposed the following polynomial equation for peak area: Peak area ¼ 6572.00 þ 0.76A þ 35.22B – 11.42C – 24.68AB þ 3.16AC þ 16.77BC – 7.38A2 – 73.39B2 – 69.63C2, where A is the detection wavelength (nm), B is

the flow rate (mL/min) and C is the temperature (8C). According to the equation, flow rate appeared to have more effect on the peak area than detection wavelength and temperature. As the flow rate increases, peak area also increases. The detection wavelength was also found to increase the peak area, as indicated by the positive coefficient value. However, the lower magnitude of the coefficient indicated that the effect of detection wavelength is less than the flow rate on peak area.

CONCLUSION

The development and validation of a straightforward, affordable, accurate, precise, repeatable, robust, and robust RP-HPLC–UV technique for glabridin analysis in crude drug and polyherbal formulation across a broad concentration range was accomplished. When glabridin was being eluted, there was no interference seen in either the standard or the samples.The validation data demonstrated the method's dependability and showed good precision and accuracy. The devised approach is substantially more sensitive than the current HPLC method described by Shanker et al. (14) for the detection of glabridin, with LOD and LOQ of 0.019 and 0.065 mg/mL. The low LOD (0.35 mg/mL) and LOQ (1 mg/mL) values reflect the good sensitivity of the method. For the regular examination of glabridin in crude medication and polyherbal formulation, the suggested approach proved to be quite effective.

This straightforward and proven analytical technique will support future studies on this significant anticancer moiety for measurement in various herbal preparations. Rather than using glycyrrhizin and glycyrrhetinic acid, which are less specific markers than glabridin, the method can be employed for quality control of glycyrriza root and many polyherbal products that contain it (25).

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