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Original Article

Determination of 18 β-Glycyrrhetinic Acid in *Glycyrrhiza glabra* L. Extract by HPLC

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Abstract

A high performance liquid chromatography method was studied for determination of 18 β-glycyrrhetinic acid in *Glycyrrhiza glabra* L. (licorice) extract. The operating condition were C-18 reversed phase column (VP-ODS, (250×4.6 mm, 5 mm)) at room temperature, acetonitril/phosphoric acid (3/1) as mobile phase, at flow rate of 0.6ml/min (0-8min), 0.4ml/min (8-20min) and UV detection at 230 nm. The recoveries were %99.60-%102.81 with relative standard deviation between %0.01-%1.58. The relative standard deviation of the repeatability test was %2.96. The method is simple, rapid, safe, accurate, economical and useful for standardization of the licorice products.

Keywords: 18 β-Glycyrrhetinic acid; *Glycyrrhiza glabra*; High performance liquid chromatography (HPLC); Thin layer chromatography (TLC).

Introduction

Licorice, the root of *Glycyrrhiza glabra* L. has been used medically for over 2000 years. It was used during the time of Hammurabi (1). Indeed, Hippocrates, Theophrastus, Pliney the Elder and Galen have cited the extract of the root of *Glycyrrhiza glabra* as having important medicinal properties, including healing of ulcers and wounds and quenching thirst (1-4). Also licorice has shown anti-inflammatory, anti-arthritic, anti-arrhythmic, anti-bacterial, anti-viral and expectorant activity. A recent animal study indicates that licorice may be useful in treating lupus (4). It is now known that glycyrrhizic acid and its aglycone glycyrrhetinic acid present in the root extract are responsible for these biological activities (1-3).

Now, it is used extensively in the tobacco, food, confectionery, and pharmaceutical industry, throughout the world (2).

Beside these benefit effects, on prolonged use and with higher doses of licorice, some side effects may occur such as, mineralocorticoid effects, hypertension, inhibition of the rennin-angiotensin system, hypokalemia, myoglobinuria, lethargy, paraparesis hypertensive encephalopathy, quadriplegia (body paralysis)(4-5).

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. Different methods have been used for determination of these components such as, HPLC (6-16), gas chromatography (17), TLC densitometry (18); capillary electrophoresis (19). HPLC is widely used for this purpose. However, owing to complicated components in herbal

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drugs, the use of HPLC is restricted by its lengthy analysis time (about 50 min), poor resolution, the fact that the chromatographic column is easily contaminated and hard to clean (19) and using toxic solvents as mobile phase (20-21).

In this study, a simple, rapid, safe, accurate and economical high performance liquid chromatography (HPLC) method with 20 min analysis time and thin layer chromatography (TLC) method have been used for quantitative and qualitative analysis of 18β-glycyrrhetinic acid in *Glycyrrhiza glabra* (licorice) extract, which can be used for standardization of licorice products.

Experimental

Reagents and materials

Glycyrrhiza glabra extract was purchased from Shirin Daru Company (Shiraz-Iran), 18β-glycyrrhetinic acid standard from Rotichrom and other chemicals from Merck (Darmstadt, F.R.G.). Acetonitril used for the mobile phase was of HPLC grade.

Sample preparation

0.3 gr of licorice extract was dissolved in 20 ml methanol. The mixture was shaked for 30 minutes. The supernatant was centrifuged (5min at 700g) and decanted. The residue was taken up with 20 ml methanol and decanted after 30 minutes shaken two times subsequently. The supernatants were added together and evaporated to a concentrated solution (10 ml).Then it was filtered through a syringe filter (0.2 µm) and analyzed with HPLC.

Chromatographic analysis

Qualitative analysis: For TLC fingerprint up to 10 µl of the test solution and 5 µl of the standard solution (0.25 mg/ml) were applied manually on TLC aluminum sheet silica gel $60F_{254}$ (Merck) 5×8 cm. Samples were applied on two track with 1cm band length, 1 cm distance from lower edge, 1 cm distance between the sides and 1 cm track distance. Development in glass chamber, saturated for 20 minutes, with a mixture of 20 volumes of petroleum ether, 40 volume of benzene, 14 volume of ethyl acetate and 1 volume of acetic acid(6), and 7cm migration distance from the lower edge was carried out. The plate was allowed to dry for 10 minutes, then it was sprayed with anisaldehyde/sulphuric acid reagent and evaluated in visible.

Quantitative analysis: HPLC chromatograms were obtained using Shimadzu HPLC system with a 20 µl sample loop. The HPLC analysis was completed using a C-18 reversed phase column (VP-ODS, (250×4.6 mm, 5 mm)) and LC-10AD pump. The column effluent was monitored with a variable wavelength photodiode-array detector (SPD-10A), which has the ability to scan from 200-800 nm. The detector was connected to a computer and the data were analyzed by class VP software. Determination of 18β-glycyrrhetinic acid was done by using acetonitril / phosphoric acid (3/1 and PH=2.5) at flow rate of 0.6 ml/min (0-8 min), 0.4 ml/min (8-20 min). The detector wavelength was 230nm (22).

Standard curve preparation

Standard solutions were prepared by weighing a known amount of 18 β-glycyrrhetinic acid and creating standards by serial dilution, resulting in final concentrations of 1, 0.25, 0.0625, 0.0156, 0.0078 mg/ml. A blank solution was also prepared with methanol. Each set of standards and a blank was analyzed chromatographically 3 times. The peak area was recorded and standard curve was constructed by linear regression of mean peak area and concentration.

Accuracy and precision

In accordance with ICH guideline (23-24), the accuracy and precision of the method were studied by using 3 known concentration levels of 18 β-glycyrrhetinic acid (0.0625, 0.25 and 1 mg/ml) and 3 replicates each of the total analytical procedure, within day and between 3 days. These samples were considered as unknown samples and analyzed chromatographically using the proposed procedure.

Repeatability

In accordance with Q2B-ICH guideline (24), repeated analysis of a homogeneous sample was performed by the same analytical procedure and the same analyst, with the same equipment and in the same laboratory.

Results and discussion

Qualitative analysis: The TLC fingerprint (figure1) shows that licorice extract contained 18β-glycyrrhetinic acid. The R_f of 18β-glycyrrhetinic acid in both extract and standard samples was the same equal (R_f =0.12). In HPLC analysis, 18β-glycyrrhetinic acid is clearly observed at 10.3 ± 1 minutes in the chromatogram of standard, which matches with licorice extract.

Quantitative analysis

In HPLC analysis, the calibration function was determined by linear regression over the range 0.0078-1 mg/ml.The regression equation was Y=311.01X + 1.6184, where X is the concentration of standard samples (mg/ml), and the correlation factor was 0.9995 (figure2). The HPLC chromatograms of standard and extract are shown in figure 3.

The amount of 18β-glycyrrhetinic acid was determined in licorice extract and according to the analysis it is %0.022 (mg/100mg). The retention time of 18β-glycyrrhetinic acid was 10.3 ± 1 min which is desired and economical.

The results of studying the accuracy and precision are shown in table 1 and 2. The recoveries are %99.60 -%102.81 with relative standard deviations between %0.01 and %1.58, which are in acceptable ranges. Also, the relative standard deviation of repeatability test is acceptable (%2.96). The results of repeatability test are shown in table 3.

In conclusion, the method is a simple, rapid, safe, accurate and economical method for determination of 18 β-glycyrrhetinic acid in *Glycyrrhiza glabra* (licorice) extract. In This method just two safe solvents were used as mobile phase and it is preferred over the methods using toxic solvents (e.g.

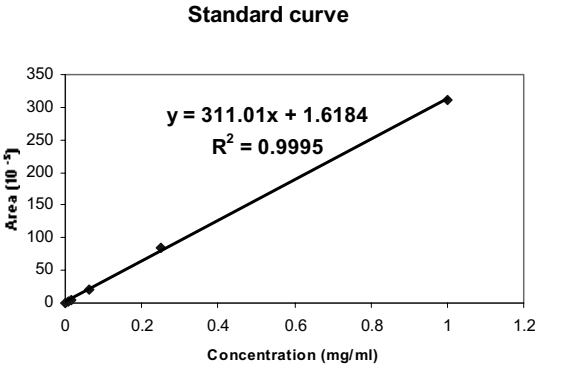

Figure 2. 18β-glycyrrhetinic acid standard curve

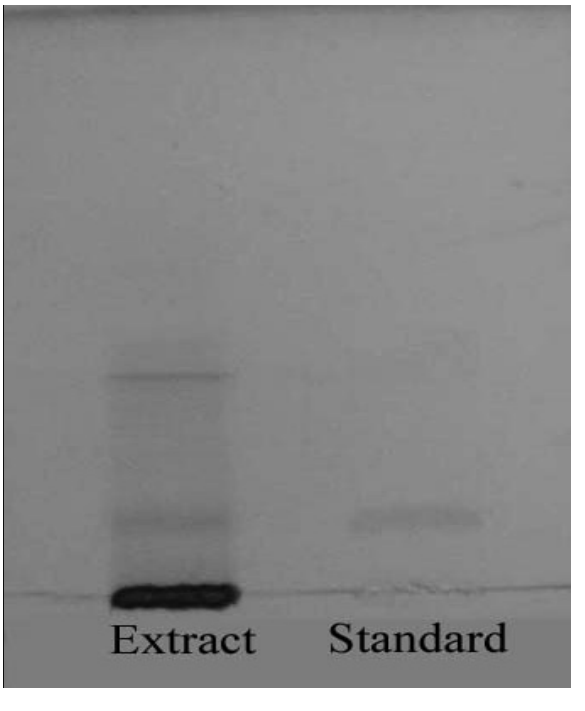

Figure 1. The TLC chromatogram of the licorice extract and 18β-glycyrrhetinic acid standard

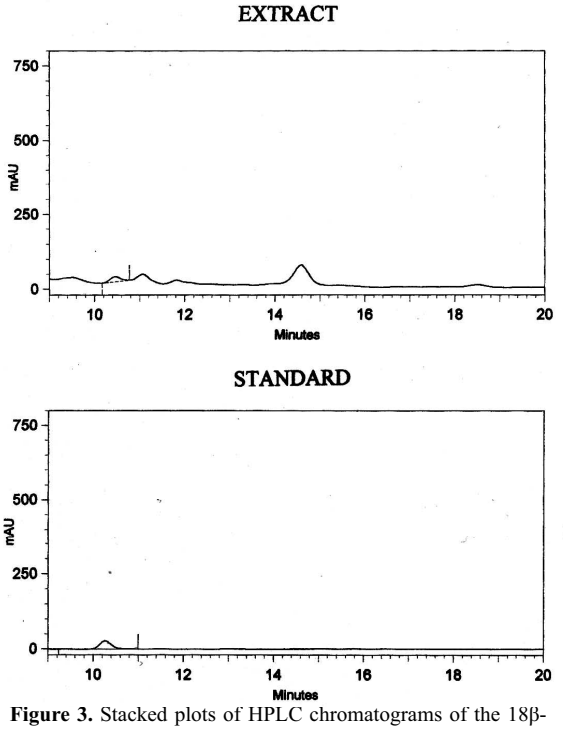

Figure 3. Stacked plots of HPLC chromatograms of the 18β-glycyrrhetinic acid in standard and extract samples

Table 1. Within-day accuracy and precision of the proposed HPLC method

expected concentration	mean determination	SD	Recovery (%)	RSD (%)	n
0.06250	0.06340	0.05	101.44	0.25	3
0.25000	0.25669	0.44	102.67	0.54	3
1.00000	0.99605	0.04	99.61	0.01	3

Table 2. Between-day accuracy and precision of the proposed HPLC method

expected concentration	mean determination	SD	Recovery (%)	RSD (%)	n
0.06250	0.06330	0.07	101.28	0.32	3
0.25000	0.25577	1.02	102.31	1.26	3
1.00000	0.99617	0.18	99.62	0.06	3
0.06250	0.06388	0.08	102.21	0.39	3
0.25000	0.25703	1.09	102.81	1.33	3
1.00000	0.99598	0.04	99.60	0.01	3
0.06250	0.06370	0.14	101.91	0.64	3
0.25000	0.25609	1.28	102.43	1.58	3
1.00000	0.99614	0.20	99.61	0.06	3

Table 3. Repeatability data

No.	Concentration Of18 β-glycyrrhetinic acid (mg/ml)
1	0.00672
2	0.00664
3	0.00640
4	0.00636
5	0.00668
6	0.00626
Mean	0.00651
SD	0.0002
RSD	2.9604
Percentage in extract	%0.022

Dioxin and THF) (20-21) or multi solvents system (25). Also the analysis time is only 20 min which is desired. This method can be used for determination of 18 β-glycyrrhetinic acid and also for standardization of licorice products.

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