

## Extraction and separation of glabridin from licorice by reversed phase high performance liquid chromatography

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**Abstract:** The extraction and separation of glabridin from licorice root by HPLC was performed in this work. First, by investigating the different extraction solvents, extraction methods and extraction times, a one-hour ultrasonic extraction procedure with ethyl acetate as the extraction solvent was optimized. Then the ethyl acetate extraction was applied to RP-HPLC for separation of glabridin. The column efficiencies and resolutions were experimentally investigated with different mobile phase compositions. Baseline separation of glabridin was obtained under the mobile phase composition of 50/50 vol.% (ACN/water). The retention time of glabridin was 20.3 min. The peak of glabridin was collected from the HPLC elution for several times and identified by LC/MS. Under the optimum extraction and HPLC separation methods, 1.26 g of glabridin per kg licorice root could be extracted.

**Key words:** glabridin, licorice, extraction, separation, mobile phase optimization

### 1. Introduction

The consumption of *Glycyrrhiza glabra*, the licorice plant, can be traced for the past 6000 years. The licorice root has long been employed as a flavoring and sweetening agent, as well as a demulcent and expectorant in western countries.<sup>1</sup> The genus *Glycyrrhiza* consists of approximately 30 species, in which six species produce a sweet saponin glycyrrhizic acid (glycyrrhizin). Among the plants of this genus, three species (*G. glabra*, *G. uralensis* and *G. inflata*) are generally recognized as licorice and used as flavorings, sweeteners and herbal medicine. These medicinal plants had been used for lengthening one's

life span, improving health, detoxification and cures for injury and swelling in China and Japan (described in first Chinese dispensatory and traditional Chinese medicine modified in Japan).<sup>2,3</sup> At present, glycyrrhizin and its aglycone, glycyrrhetic acid, the main components in licorice root, are clinically used in treatments of hyperlipaemia, atherosclerosis, viral diseases, and allergic inflammation such as chronic hepatitis and atopic dermatitis, while minor components of licorice, which are mostly flavonoids, have some biological action supplementing the efficacy of licorice.<sup>4,5</sup>

Glabridin play an important role in the suppression of LDL in human body and the controlling of

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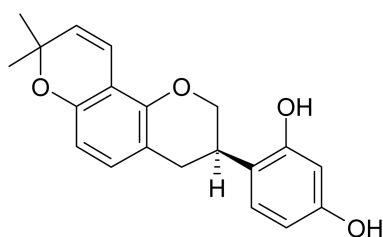


Fig. 1. Molecular structure of glabridin.

cholesterol, which is the cause of various geriatric diseases. Licorice root is a resource that can get easily. So, development of a separation procedure for glabridin will be of commercial value hereafter.

Glabridin (Fig. 1) is a main pyranoisoflavan in European licorice, *G. glabra* var. *typica* (Spanish and Italian licorice) and *G. glabra* var. *glandulifera* (Russian licorice), but is not the constituent of Chinese and Kirghiz *G. glabra*. Glabridin has been reported to exhibit multiple pharmacological activities such as cytotoxic activity, antimicrobial activity against *Helicobacter pylori*, methicillin-resistant *Staphylococcus aureus* and others, estrogenic and antiproliferative activity against human breast cancer cells, effect on adenosine 3',5'-cyclic monophosphate phosphodiesterase, melanogenesis, inflammation, low-density lipoprotein (LDL) oxidation, inhibition of human cytochrome P450s 3A4, 2B6 and 2C9 activities and protection of mitochondrial functions from oxidative stresses.<sup>3</sup> It has been demonstrated that flavonoids, flavanol, flavone and flavonol derivatives isolated from the licorice root can successfully inhibit LDL oxidation. The root extracts of *Glycyrrhiza glabra* exhibited antioxidant activity and two antioxidant flavonoid compounds were isolated from it, i.e. glabrene and glabridin.<sup>3-7</sup> From the in vivo experiments, LDL from humans and atherosclerotic apolipoprotein E-deficient mice (E<sup>0</sup> mice), which had been fed licorice extract or glabridin (the major flavonoid component isolated from licorice root), were more resistant to oxidation than LDL from placebo-fed humans and mice. It has been suggested that oxidative modification of LDL plays an important role in the development of human atherosclerosis. Thus, protecting LDL from oxidation by such compounds

as flavonoids may be an effective strategy to retard or prevent the progression of the disease.<sup>7</sup> Otherwise, certain flavonoids isolated from licorice roots show effects on arachidonic acid metabolism, as well used as antiplatelet agents, antitumorigenic, antimicrobial, antiviral, antiinflammatory and also antioxidants. Due to the function of skin whitening and antioxidizing of glabridin, the concern to its commercialization is being increased. Recently, many cosmetic companies use an usefulness licorice root extract as the raw material of the cosmeceuticals.<sup>8,9</sup>

The purpose of this research is to establish the extraction process of the glabridin from licorice root. So, we try to offer a basis analytical index for commercial process and to find an optimized separation procedure of glabridin by HPLC.

## 2. Experimental Section

### 2.1. Chemicals

The roots of *glycyrrhiza glabra* used in this study were provided from China. The standard chemicals of glabridin (the structure of glabridin was shown Fig. 1) were purchased from Wako pure chemical industries, Ltd (Japan). The standard sample solution of glabridin was prepared by dissolving 10 mg of glabridin in 10 ml of methanol. Methanol, ethanol, and ethyl acetate were obtained from Showa chemical Inc (Japan). Acetonitrile and water were obtained from Mallinckrodt chemical (U.S.A.). All other materials and solvents were of the highest purity or high-performance liquid chromatography grade.

### 2.2. High-performance liquid chromatography

The analytical HPLC system in this experiment was a Varian's manufacture equipped with the Prostar 230 ternary gradient pump with Prostar 310 UV-detector, and injection module from Prostar 410 autosampler. The data acquisition system was star LC workstation (Ver. 5.52) installed in a pc. The HPLC/MSD system was Agilent 1100 series that had quaternary pump, diode array detector and mass selective detector. Then, data acquisition system was

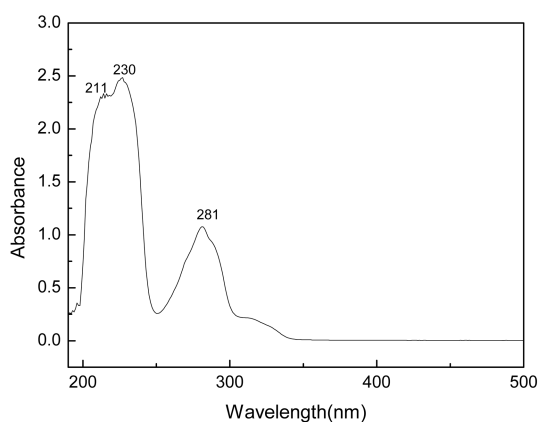


Fig. 2. UV/VIS spectrum of glabridin (standard).

Agilent LC/MSD chemstation rev.a.08.03(847). The RP-HPLC analytical column was Chrompack omnisphere 5  $c_{18}$  (Varian, U.S.A.), 250×4.6 mm. The flow rate of mobile phase was set at the 1.0 mL/min and the injection volume was 20  $\mu$ L. The UV wavelength was fixed at 230 nm. The experiment was performed at room temperature.

### 2.3. Extraction and isolation of glabridin

Powders of commercial *glycyrrhiza glabra* (20 g) were mixed with 100 mL of methanol, ethanol, acetone and ethyl acetate, respectively. The mixtures were put under room temperature for soaking extraction or under ultrasonic. The used solvents were filtered with ha-0.5  $\mu$ m membranes (division of millipore, Waters co.) before use. The extraction was filtered with PVdF 0.45  $\mu$ m (Waters co.) before being injected into the HPLC system.

## 3. Results and Discussions

It was so difficult to detect glabridin by HPLC, because We couldn't find a reference about the UV wavelength of absorption of glabridin. To find the UV wavelength of absorption of glabridin for HPLC detection, UV/VIS absorption spectrum was used to scan the diluted standard sample. The result can be seen in Fig. 2. Glabridin has three UV absorption peaks at 210, 230, 280 nm, respectively, and the strongest absorption occurred at 230 nm. Therefore,

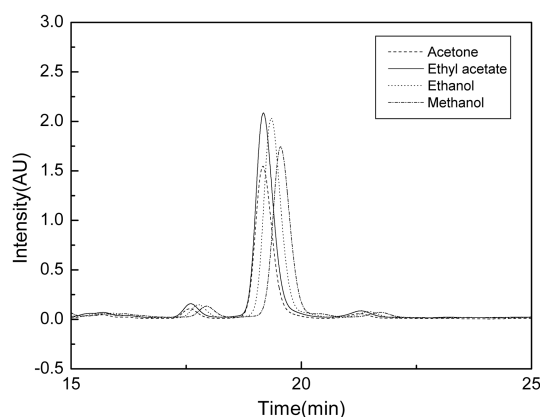


Fig. 3. Comparison of chromatogram.

all-through HPLC analytic wavelength was fixed at 230 nm.

The extraction solvents of glabridin were ethanol, methanol, acetone, and ethyl acetate; typically these solvents were used in extraction process. Soaking extraction under room temperature and ultrasonic extraction method were applied. The extracted glabridin by different extraction solvents under 1 hour ultrasonic are: 1.26 g/kg licorice root by ethyl acetate, 1.21 g/kg licorice root by ethanol, 0.99 g/kg licorice root by methanol and 0.88 g/kg licorice root by acetone (the chromatograms can be found in Fig. 3). We replicated the experiments on various extraction conditions. Therefore, above quantitative results were optimized comparatively. The extraction efficiency

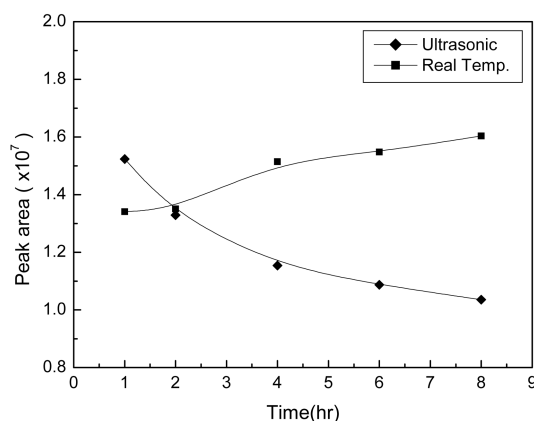


Fig. 4. Effect of extraction times on the extraction efficiency of glabridin by soaking and ultrasonic extraction methods.

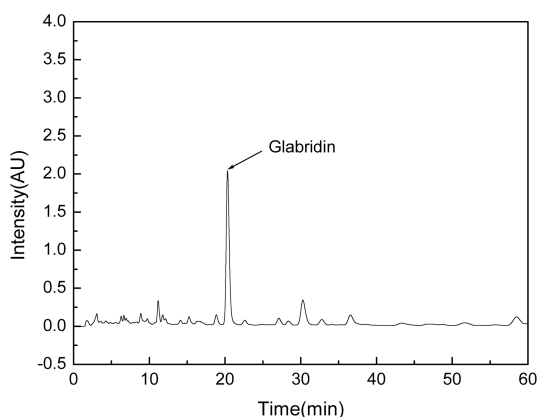


Fig. 5. Separation of glabridin in ethyl acetate extract by mobile phase composition. (Acetonitrile/water, 50/50 vol.%), flow rate 1.0 mL/min, Chrompack Omnispher 5 C<sub>18</sub>, 250×4.6 mm, UV wavelength 230 nm.

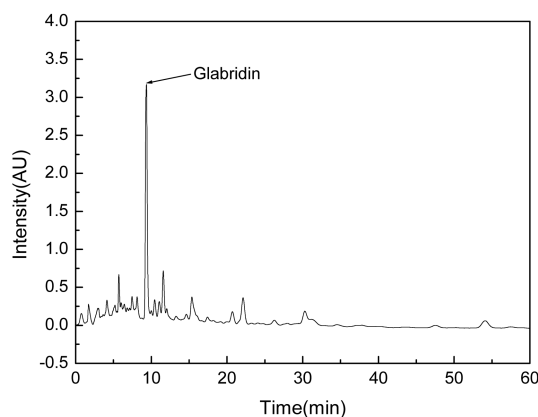


Fig. 6. Separation of glabridin in ethyl acetate extract by mobile phase composition. (Acetonitrile/water, 60/40 vol. %), other conditions are same with that of Fig. 5.

of glabridin by ethyl acetate was the highest. So ethyl acetate was chosen for the further experiments.

Effects of different extraction times, 1, 2, 4, 6 and 8 hour were investigated with ethyl acetate as the extraction solvent. From Fig. 4, when ultrasonic extraction was used, the extraction efficiency of glabridin decreased with the increase of extraction time. Quantitative analysis showed the extracted glabridin decreased from 1.29 g/kg to 0.88 g/kg of licorice root when the extraction time changed from 1 hour to 8 hours. In the case of the soaking extraction method under room temperature, the extraction efficiency increased with the increase of extraction time and best extraction result of 1.36 g/kg of licorice root could be obtained at the extraction time of 8 hours. Considering both the extraction efficiency and extraction time of the two extraction methods, the one-hour ultrasonic extraction method was chosen.

The extraction by ethyl acetate was applied to C<sub>18</sub> RP-HPLC for further isolation and preparation of glabridin. The HPLC conditions were as follows: UV wavelength 230 nm, flow rate 1.0 mL/min and the injection volume 20 μL. Different mobile phase systems were tested. When the mixture of water and methanol was used as the mobile phase, glabridin could not be separated from other peaks. The separation of glabridin was improved when methanol was changed

to acetonitrile. Figs. 5 and 6 illustrate the chromatograms of the ethyl acetate extraction under different mobile phase compositions. Optimum separation and retention time (20.3 min) could be obtained with the mobile phase of acetonitrile/water was 50/50 vol. % (ACN/water). Higher acetonitrile concentration (60/40 vol. % (ACN/water)) in the mobile phase resulted in shorter retention time but worse resolution. Otherwise by lower acetonitrile concentration (45/55 vol. % (ACN/water)) in the mobile phase, the retention time of glabridin was too long and this is not fit for fast analysis. Two kinds of detectors, i.e. UV and diode array detector (DAD) were applied. Comparison

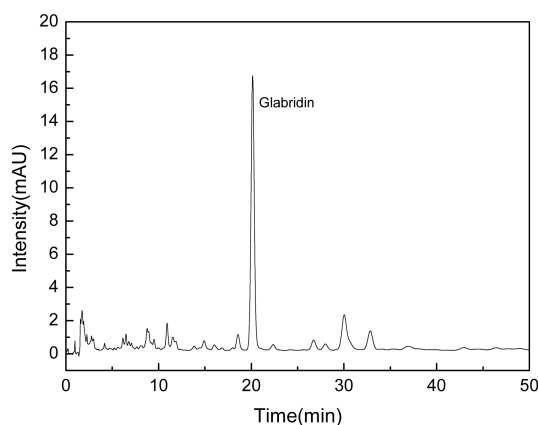


Fig. 7. Chromatogram of ethyl acetate extraction with DAD detector, other conditions are same with that of Fig. 5.

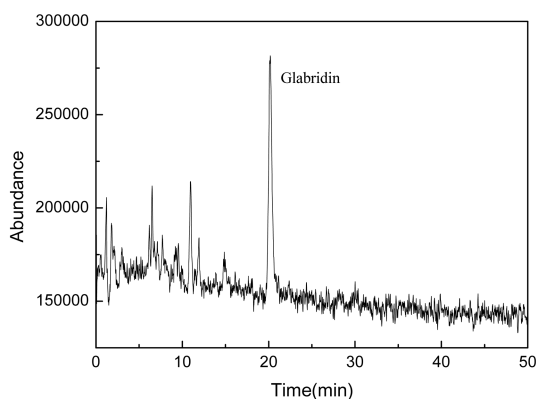


Fig. 8. Chromatogram of glabridin collected from HPLC elution by MSD detector.

of the chromatograms by UV detector (Fig. 5) and by DAD (Fig. 7) shows that DAD detector can obtain higher sensitivity. The quantitative experiments were done with DAD detector.

The peak of glabridin was collected from the HPLC elution for 100 times and concentrated. Glabridin was identified by MSD. The results can be seen in Fig 8. Therefore, in these experiments the column efficiencies and resolutions of glabridin were experimentally investigated with mobile phase composition.

#### 4. Conclusion

Glabridin, a known whitening and antioxidant reagent, is a main ingredient in the licorice root (*Glycyrrhiza glabra*). By optimizing the extraction solvents, the extraction methods and the extraction times, an optimum extraction method was developed. Finally, the ethyl acetate extraction by one-hour ultrasonic extraction method was applied to RP-HPLC for further purification and preparation. The preparative and analysis HPLC was carried out under an optimized condition with acetonitrile/water (50/50, vol. %) as the mobile phase, UV wavelength 230 nm and flow rate 1.0 mL/min. Quantitative

result showed that the optimized extraction methods, 1.26 g glabridin per kg licorice root, by ethyl acetate extraction solvent. Optimized separation time, 20.3 min, could be verifiable by the LC/MS with the ethyl acetate extracted sample. This work could provide useful procedure both for cosmetic and pharmaceutical industries.

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