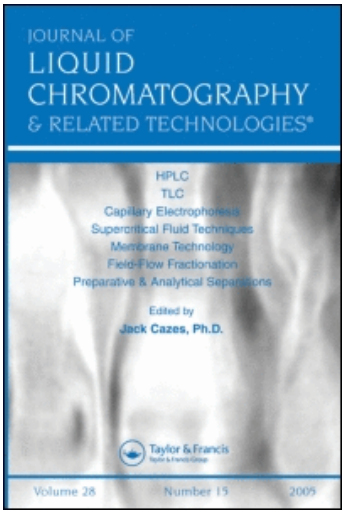

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Extraction and Analysis of Liquiritin from Licorice

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Abstract: The extraction conditions of liquiritin from licorice were investigated. By changing the different extraction solvents, methods, and extraction times, the optimum extraction condition was methanol as an extraction solvent, and 120 min dipping time under room temperature. The extracts of licorice were separated and determined by reversed-phase high performance liquid chromatography with an acetonitrile/water (20:80, v/v, containing 1% acetic acid) as the mobile phase. Under the optimum extraction condition, 0.085 mg/g of liquiritin was extracted from Chinese licorice and the recovery was around 81.04%.

Keywords: Extraction, Glycyrrhizic acid, HPLC, Licorice, Liquiritin

INTRODUCTION

Licorice, the root of the *glycyrrhiza* plant species, has been used medicinally for more than 4000 years.[1] It is a Chinese herb commonly used as an expectorant and to arrest coughing, reduce fever, comfort the stomach, alleviate urgency, and potentiate the effects of various other herbs.[2] Liquiritin (LQ) (Figure 1) has antiviral properties[3,4] and antioxidative[5] properties, and is the most prevalent flavonoid in licorice.[6] It has anti-inflammatory, anti-ulcer, anti-hepatotoxic, and antivirus activities.[7–10] In many countries, it is used as a major therapeutic agent to treat allergic dermatitis and chronic viral hepatitis.[11]

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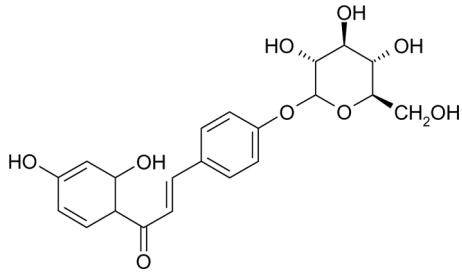

Figure 1. Molecular structure of liquiritin (LQ).

Reversed-phase preparative high performance liquid chromatography (HPLC) has been applied to the purification of LQ from licorice and there have been some papers on the extraction of LQ with complex methods.[12] Hence, in this study, a simple and convenient extraction process to extract LQ from licorice by liquid-liquid extraction followed with RP-HPLC analysis was established. By changing extraction solvents, process, and dipping times, the optimum extraction condition was established, and 0.085 mg/g LQ was successfully extracted from 1.0 g Chinese licorice.

EXPERIMENTAL

Chemicals

Licorice was purchased from a local market. Liquiritin was obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Methanol, acetonitrile, chloroform, and n-hexane (HPLC Grade) were purchased from Duksan Pure Chemical Co., Ltd. (Korea). Water was twice distilled and filtered (FH-0.45 μm, Advantec MFS, Inc., Japan) using a decompressing pump (Division of Millipore, Waters, USA).

HPLC Analysis

The HPLC system in this study is comprised of a M930 solvent delivery pump (Young Lin Co., Korea), a UV detector (M 720 Absorbance Detector, Young-In Scientific Co., Korea), and an integrated data system (Autochrowin. Ver. 1.42, Young Lin Co., Korea). The injection valves with 25 μL and 20 μL sample loops were used. The mobile phase was acetonitrile/water (containing 1% acetic acid, vol. %) with 1.0 mL/min

as the flow rate and UV wavelength was set at 252 nm. All the solvents must be filtered by a Disposable Syringe Filter Unit (0.2 μm) for further HPLC analysis.

Sample Preparation

The licorice roots were oven dried, sliced, and crushed into powder for use in the extraction experiments. The standards of LQ were dissolved in methanol to yield a final concentration of 0.2 mg/mL. All experiments were carried out at ambient room temperature.

RESULTS AND DISCUSSION

Effect of Different Extraction Solvents

As Table 1 shows, 50.0 mL of each extraction solvent was mixed with 1.0 g licorice powder using the same 60.0 min dipping time under room temperature. The extracted amount of LQ by methanol was higher than others. Hence, methanol was used in the subsequent experiments.

Influence of Different Extraction Methods

In order to obtain the optimum extraction conditions, dipping and ultrasonic methods were established. Two mixtures of 50.0 mL methanol and 1.0 g licorice were prepared. One mixture was extracted by the dipping method with 20.0, 30.0, 60.0, 120.0, and 240.0 min. The other mixture used the ultrasonic method with 2.0, 5.0, 10.0, 20.0, and 30.0 min.

Table 1. Extracted amounts of LQ with different solvents

Extraction solvents \ Compound (mg/g)	LQ
Chloroform	*
n-Hexane	*
Water	0.050
Methanol:Water (25:75)	0.062
Methanol:Water (50:50)	0.068
Methanol:Water (75:25)	0.079
Methanol	0.085

*not detected.

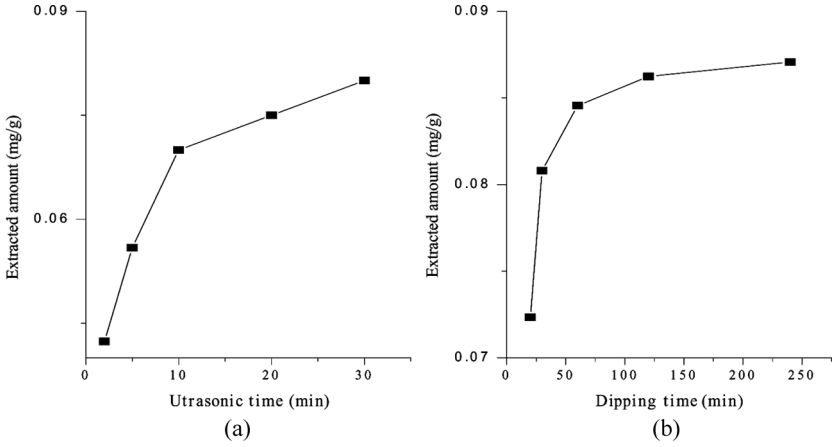

Figure 2. Effect of different ultrasonic and dipping times on the extracted amount of LQ.

However, comparing the results of the two methods in Figure 2, it was found that the amounts extracted of LQ via the dipping method were higher than ultrasonic method, while much more energy was required in the experiments. Thus, it was determined that the ultrasonic method was not appropriate for this approach.

Effect of Different Volumes of Methanol

As Figure 3 shows, the extracted amount of LQ increased as the volume of methanol was increased. However, beyond a volume of 70.0 mL, no further increase was observed. Therefore, the use of 70.0 mL of methanol was determined to be optimal for extraction in terms of the amount and type of solvent.

Method Validation

To ensure the specificity and selectivity of the method, concentrations of 0.2, 0.4, 0.5, 0.8, and 1.0 mg/mL were applied for standards solutions of LQ. Each concentration was injected 3 times in a column (C_{18}, 5 μm, 150 × 4.6 mm, RStech Corporation, Korea). The analyte peak area values were plotted against the corresponding concentrations of the analytes and the calibration curves were constructed by means of the least square method. The calibration curve of LQ showed good linearity ($r^2 = 0.998$) and the regression equations of LQ was $Y = 11531 \times -23.942$.

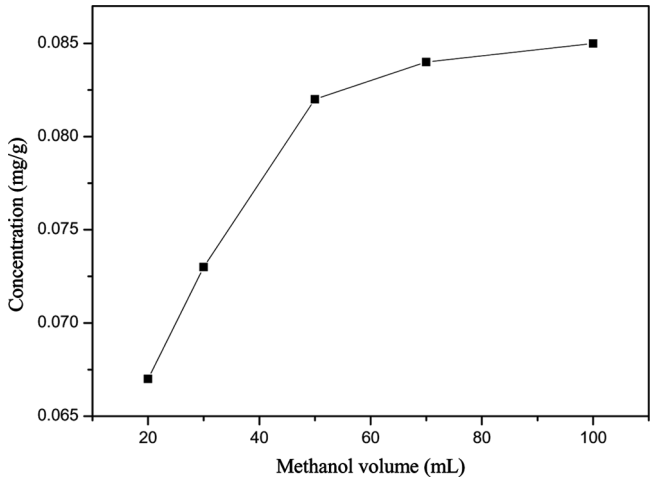

Figure 3. Effect of volumes of methanol on the extracted amount of LQ.

Assays of repeatability, calculated as the relative standard deviations (RSDs), were performed by injecting standard solutions of LQ 5 times in a 5 day period. The concentration of the standard solutions was 0.2 mg/mL and the injection volume was set at 10 μL.

The concentrations of LQ (0.15, 0.20, and 0.30 mg/g) were added to 3 mL of the extracts from licorice, respectively, to a final volume of 6 mL.

$$R = \frac{C_p - C_0}{C_m} \times 100\% \quad (1)$$

R: recovery rate, C_p: the total amount of the compound of final solvent, C_0: the amounts of the compound from licorice, C_m: the amount of the compound which was added. The measured concentration was compared with the theoretical concentration to calculate the recovery rate[13] by Equation (1)

Table 2. RSDs, Recovery rates and LODs of LQ from licorice

	RSD (%)		Recovery rate			
Compounds	Intra-day	Inter-day	Added (mg/g)	Recovery (%)	RSD (%)	LOD (ng/mL)
LQ	0.34	0.37	0.15	81.3%	0.38	365
	0.35	0.38	0.20	80.0%	0.37	
	0.33	0.38	0.30	81.0%	0.39	

The standard solution of LQ was diluted and injected until the limit of detection (LOD) was obtained at a signal/noise ratio of 3. The RSD of precision tests, the limit of detections (LOD) on standard solutions, and the recovery rates are presented in Table 2. Comparison with the real sample analysis verified that the values noted above were of acceptable precision and accuracy.

CONCLUSIONS

In this study, a simple and convenient method for the extraction of liquiritin from licorice is developed and validated. Methanol, as the extraction solvent and 120 min dipping time under room temperature, is the optimum condition to extract LQ. The extracted amount is 0.085 mg/g and recovery is around 81.04%.

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