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Short Communication

Determination of three active principles in licorice extract by reversed-phase high-performance liquid chromatography

TUNG-HU TSAI
National Research Institute of Chinese Medicine, Taipei (Taiwan)
and
CHIEH-FU CHEN*
Institute and Department of Pharmacology, National Yang-Ming Medical College, Shih-Pai, Taipei 11221 (Taiwan)
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ABSTRACT

An improved high-performance liquid chromatographic technique with a photodiode-array detection system for the determination of the active principles of licorice extract such as glycyrrhizin or glycyrrhizic acid (GL), 18α-glycyrrhetinic acid (18α-GA) and 18β-glycyrrhetinic acid (18β-GA) is presented. A reversed-phase column (LiChrospher RP-18, 5 μm) was eluted with a linear gradient of methanol–perchlorate buffer (pH 7.5–7.7) from 1:9 to 10:0 in 120 min. It was found that 58.53 ± 9.14 mg of GL, 5.94 ± 1.60 μg of 18α-GA and 95.25 ± 27.20 μg of 18β-GA were contained in the aqueous extract of 1 g of licorice. The method can determine these components in one analytical step without any pretreatment of the tested sample. Hence this simple method can be used to determine GL and stereoisomers of glycyrrhetinic acid in any licorice extract and traditional Chinese prescriptions.

INTRODUCTION

Licorice or Glycyrrhizae Radix (Chinese name: Gancao), the root of *Glycyrrhiza uralensis* Fisch. (Leguminosae), is one of the most commonly used herbal medicines in traditional Chinese prescriptions. It is also used as a sweetener [1]. Glycyrrhizin or glycyrrhizic acid (GL), one of the active principles in licorice, is applied in cosmetic lotions [2] and as a vehicle in pharmaceutical preparations [3]. GL is hydrolysed to 18β-glycyrrhetinic acid (18β-GA) and two molecules of glucuronic acid after being absorbed from the gastrointestinal tract. Because of the similarity in chemical structure to steroids, GL and its hydrolysate produce mineralocorticoid-like effects [4]. They also inhibit the metabolic enzymes for adrenocorticosteroids [5]. Determination of the GL content in licorice has been reported [6–11]. However, the simultaneous determination of GL, 18α-glycyrrhetinic acid (18α-GA) and 18β-GA in licorice by

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high-performance liquid chromatography (HPLC) has not been described. In this work, an HPLC technique was developed to determine simultaneously the contents of GL, 18α-GA and 18β-GA in licorice extract in one analytical step.

EXPERIMENTAL

Materials and reagents

Licorice (*Glycyrrhiza uralensis* Fisch.) was purchased from a traditional Chinese pharmaceutical company in Taipei. GL, 18α-GA and 18β-GA were obtained from Sigma (St. Louis, MO, U.S.A.) and perchloric acid (70%), ammonia solution (32%) and methanol from E. Merck (Darmstadt, Germany).

Apparatus

The HPLC system (Waters Assoc., Milford, MA, U.S.A.) consisted of a Model U6K injector, two Model 510 pumps, a Model 441 detector, a Model 990 photodiode-array detector and a Model 740 integrator. Elution was carried out on a LiChrospher 100 RP-18 (5 μm) end-capped column (125 × 4 mm I.D.) (E. Merck) connected to a guard column (5 μm) (4 × 4 mm I.D.) (E. Merck). The detection wavelength was set at 254 nm. The mobile phase consisted of methanol (solvent A) and 0.1% perchloric acid (solvent B, adjusted to pH 7.5–7.7 with ammonia solution) and samples (10 μl) were eluted with a linear gradient of solvent A–solvent B from 1:9 to 10:0 in 120 min. The flow-rate was 1.0 ml/min.

Preparation of licorice extract

Licorice (10 g) was cut into coarse pieces and refluxed at 50°C with 50 ml of the extraction solution (water, 0.05 *M* NaOH, 50% ethanol, 95% ethanol or methanol) for 30 min. This procedure was repeated twice. The two solvent filtrates were combined, condensed under reduced pressure, dried by lyophilization and stored at −20°C to maintain stability. The lyophilized samples were reconstituted with their respective extraction solutions before being subjected to HPLC. To minimize the effect of the solvent (0.05 *M* NaOH) on the analytical column, we restricted the injection volume to 10 μl.

Determination of GL, 18α-GA and 18β-GA

Calibration graphs for GL, 18α-GA and 18β-GA dissolved in methanol were constructed by HPLC of various amounts of these compounds (0.2, 0.5, 1, 2 and 5 μg for GL; 10, 20, 40 and 100 ng for 18α-GA or 18β-GA). The contents of GL, 18α-GA and 18β-GA in the crude extract of licorice were determined from the regression equation lines constructed for the three compounds.

RESULTS AND DISCUSSION

A typical elution profile of a mixture of GL, 18α-GA and 18β-GA is shown in Fig. 1. It should be mentioned the pH of the mobile phase is very important for good resolution. When the pH of solvent B was decreased, the retention time for the three compounds increased and the peaks for the stereoisomers of glycyrrhetinic acids overlapped. In addition, the peak for GL merged with those of other components of

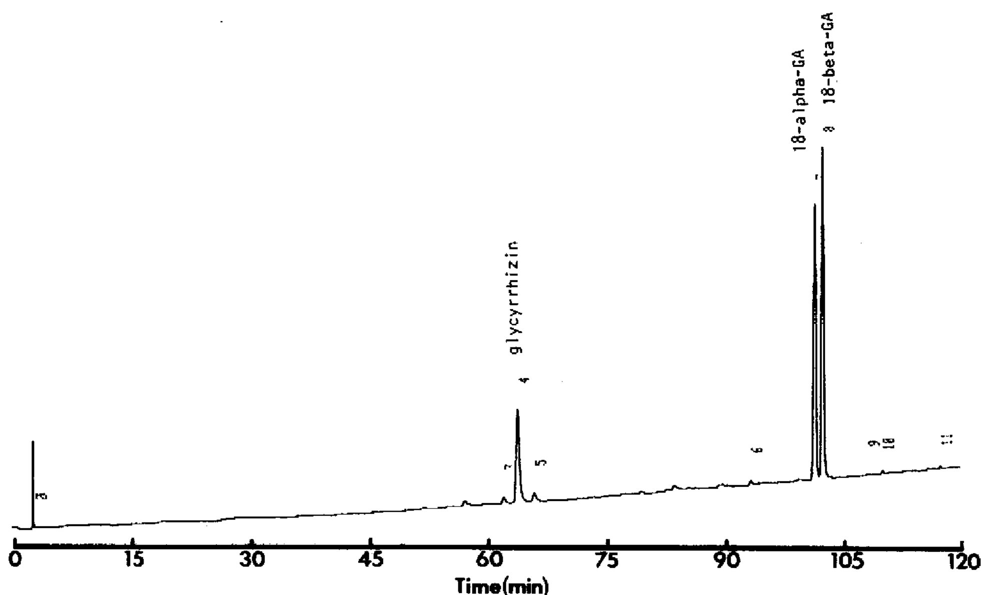

Fig. 1. Chromatogram of glycyrrhizin, 18α-GA and 18β-GA.

the licorice extract. The optimum pH for solvent B was 7.5–7.7. Under this condition, the retention tims for GL, 18α-GA and 18β-GA were 63.9, 101.4 and 102.5 min, respectively. Fig. 2 shows the chromatogram of a 50% ethanol extract of licorice. The peaks corresponding to GL, 18α-GA and 18β-GA were confirmed by the retention times and the spectra from photodiode-array detection. The content of each compound in the licorice extract was determined from the linear regression equation of the calibration graph for each compound. The equations for GL, 18α-GA and 18β-GA were $y = 6919 + 782152x$ ($r = 0.999$), $y = 103 + 1666x$ ($r = 0.999$) and $y = -530 + 1287x$ ($r = 0.999$), respectively, where x is amount of compound analyzed and y is response in peak area.

Table I gives the contents of GL, 18α-GA and 18β-GA in different licorice extracts. It appears that 0.05 *M* NaOH is best for the extraction of GL and methanol is best for the extraction of 18α-GA and 18β-GA from licorice.

A number of techniques, such as thin-layer chromatography [6], three-dimensional HPLC [7], HPLC [8,9], spectrophotometry [10] and infrared spectrometry [11], have been used to determine the GL content in licorice or its preparations. However, there has been no report of the simultaneous determination of GL, 18α-GA and 18β-GA in licorice extract. The proposed reversed-phase HPLC technique provided an excellent separation of GL, 18α-GA and 18β-GA and can determine the contents of these three compounds in licorice extracts without any pretreatment of the tested samples. Hence, this technique should be useful for the quality control of licorice, for stability testing and for the pharmacokinetic study of the three compounds.

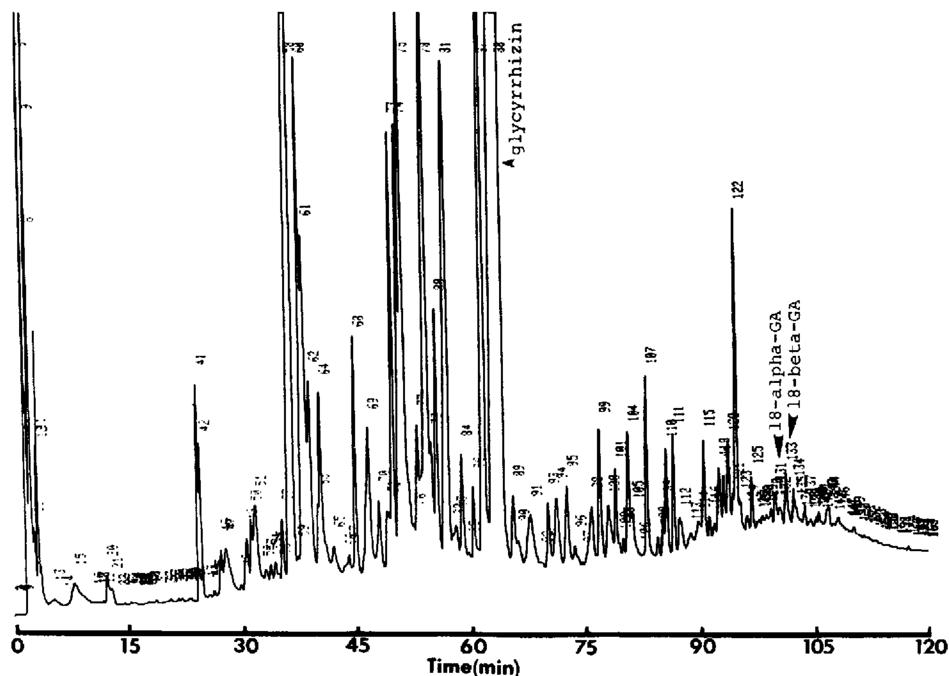

Fig. 2. Chromatogram of the 50% ethanol extract of licorice.

TABLE I

CONTENTS OF GL, 18α-GA AND 18β-GA IN DIFFERENT LICORICE EXTRACTS

Contents in 11 g of licorice. Data are expressed as mean ± standard error ($n = 4$).

Extracted solution	GL (mg)	18α-GA (μg)	18β-GA (μg)
0.05 *M* NaOH	83.25 ± 4.98	9.96 ± 2.03	76.14 ± 17.32
Water	58.35 ± 9.14	5.94 ± 1.60	95.25 ± 27.20
Ethanol (50%)	81.39 ± 4.72	119.61 ± 26.23	450.81 ± 38.72
Methanol	26.79 ± 2.61	550.56 ± 34.82	1273.47 ± 63.66
Ethanol (95%)	8.52 ± 0.90	479.94 ± 91.91	1118.07 ± 74.98

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