

Optimal Extraction of Glycyrrhetic Acid From Licorice Root

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Abstract: Licorice is a self-propelling plant that has many applications in pharmaceutical and food industries. *Glycyrrhiza glabra* is a kind of licorice whose main active constituent is a triterpenoid saponin called glycyrrhizin. If either bound of glycyrrhizin is broken, it would lose its sweet taste and convert to glycyrrhetic acid which is used as an anti-inflammatory agent. In order to produce glycyrrhetic acid, glycyrrhizin without isolation is hydrolyzed in an acidic medium to release glycyrrhetic acid. After filtration, glycyrrhetic acid is extracted from resulting cake by ethyl acetate. The extract is then evaporated and the resulting solution is acetylated to produce glycyrrhetic acid acetate. The ester is then purified by hexane and hydrolyzed with sodium hydroxide to produce glycyrrhetic acid. The glycyrrhetic acid produced in this way is dissolved in methanol and decolorized by activated carbon. Finally, water is added to solution to precipitate white glycyrrhetic acid, which is purified by crystallization in aqueous ethanol to yield the final purified product.

Key words: Licorice, *glycyrrhiza glabra*, glycyrrhizin, glycyrrhetic acid, enoxolone, triterpenoid saponin

INTRODUCTION

Licorice is a self-propelling plant, which is approximately 1.5 m in height with light, spreading pinnate foliage and typical papilionaceous flowers containing at least 14 different species. *Glycyrrhiza glabra* is a kind of licorice as the most biologically active herbs. It has been known as an herb known since several thousand years ago in both western and eastern countries. The active components of this plant have extensive therapeutic usage throughout the world and is a subject to enormous works in the recent years^[1]. Roots and rhizomes of *glycyrrhiza glabra* which are harvested in autumn are active pharmaceutical parts of plant and are used in estrogenic, demulcent, pectoral, antioxidant, antispasmodic, anti-inflammatory, anti bacterial and expectorant drugs^[1]. In some countries, licorice root is used as a sweetener in food and tobacco products. The main active constituent of *glycyrrhiza glabra* is a triterpenoid saponin called glycyrrhizinic acid or glycyrrhizin which is in the form of a mixture of calcium and potassium salts. Chemical structure of glycyrrhizinic acid is shown in Fig. 1. Glycyrrhizin is extremely sweet (50 times sweeter than sucrose) but is safely used for diabetics^[2]. The amount of glycyrrhizin in licorice, considerably depends on harvesting conditions (i.e. licorice on salty soil has more glycyrrhizin). In the roots of

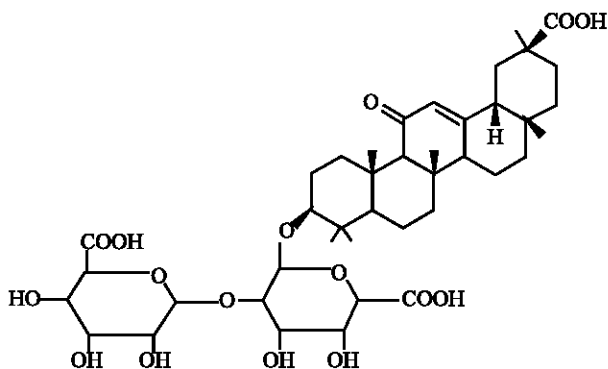


Fig. 1: Glycyrrhizic Acid

the plant it is varied between 6 to 13% wt. Glycyrrhizin and its salts have pharmaceutical properties; They inhibit liver cell injury caused by many chemicals and is successfully used in the treatment of chronic hepatitis and cirrhosis in Japan^[1]. They also inhibit the growth of some DNA and RNA viruses, inactivating herpes simplex virus particles irreversibly^[1]. Upon hydrolysis, ether bounds of glycyrrhizin are broken and it loses sweet taste by converting to glycyrrhetic acid plus two molecules of glucuronic acid (Fig. 2). Glycyrrhetic acid is a pentacyclic triterpenoid derivative of the beta amyryn type with two natural optical isomers: 18 β -Glycyrrhetic

Table 1: Specifications of glycyrrhizin, glycyrrhetic acid and glycyrrhetic acid acetate

Compound	IUPAC Name	Registry number	Mole weight	Chemical Formula	Chemical analysis(%)
Glycyrrhizin	Glucopyranosiduronic acid	1405-86-3	822.93	C ₄₂ H ₆₂ O ₁₆	C 61.3 H 7.59 O 31.11
Glycyrrhetic acid (Enoxolone)	3 β -hydroxy-11-oxoolean- 12-en-30-oic acid	471-53-4	470.68	C ₃₀ H ₄₆ O ₄	C 76.55 H 9.85 O 13.6
Glycyrrhetic acid (Acetoxolone)	3-O-acetyl-18 β -glycyrrhetic acid	6277-14-1	512.72	C ₃₂ H ₄₈ O ₅	C 74.96 H 9.44 O 15.6

Table 2: Solubility of glycyrrhizin and glycyrrhetic acid

Component	Solubility
Glycyrrhizin	- Freely soluble in hot water and alcohol - Practically insoluble in ether
Glycyrrhetic acid (Enoxolone)	-Freely soluble in chloroform and dioxan - Soluble in alcohol, pyridine and acetic acid -Practically insoluble in water and ether

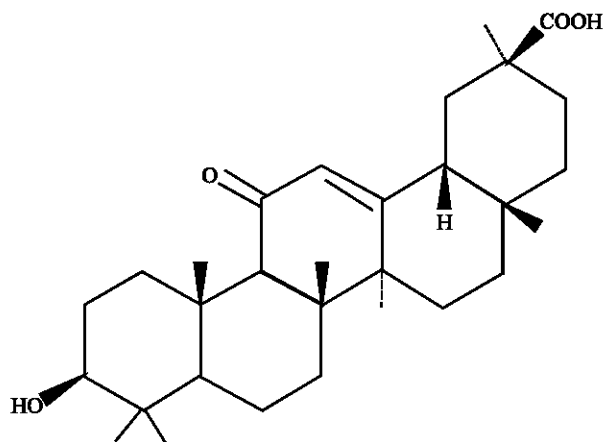


Fig. 2: Glycyrrhetic acid

acid and 18 α – glycyrrhetic acid. The first isomer is a white crystalline powder referred to as enoxolone in Pharmacopeia^[3]. It has anti-inflammatory properties like cortisone with no side-effects. The glycyrrhetic acid has expectorant and antipyretic property and is considerably applied as a flavoring agent and used to mask the taste of bitter drugs such as aloe, quinine, etc. Properties and analysis of glycyrrhizic acid, glycyrrhetic acid and glycyrrhetic acid acetate are shown in Table 1 and 2^[4]. Since 2001, glycyrrhetic acid is specified in Pharmacopeia^[3] as an individual monograph. According to the mentioned standards, the amount of total impurities of glycyrrhetic acid must be less than 7% wt and each impurity must not exceed 2% wt. of the glycyrrhetic acid^[3]. Although there are many methods to produce glycyrrhetic acid but most of these methods fail to produce pure glycyrrhetic acid according to these standards. Therefore the object of this study is to present an optimal method of extraction and purification of glycyrrhetic acid from licorice root.

MATERIALS AND METHODS

There are three general methods to produce glycyrrhetic acid:

Production of glycyrrhetic acid from a salt of glycyrrhizin:

In this method, glycyrrhizin is extracted from licorice root and then purified. It is then converted to glycyrrhetic acid. Different forms of this method are available as described below: - Concentrated aqueous extract of licorice root is treated with H₂SO₄ to precipitate brown sediment, which is then washed with water and is extracted by alcohol to isolate glycyrrhizin. Potassium salt of glycyrrhizin is precipitated by adding KOH solution to the extract which is filtered and crystallized twice in acetic acid. The salt is hydrolyzed with H₂SO₄ to give crude glycyrrhetic acid that is acetylated by acetic anhydride. The resulting cake is then purified by CHCl₃ and CH₃OH, then is hydrolyzed by NaOH solution to obtain white glycyrrhetic acid. Two times crystallization and acetylating are among the disadvantages of this process^[5].

- Ammonium salt of glycyrrhizin is refluxed in H₂SO₄ and CH₃COOH for 90 min. After cooling and filtration, the cake is washed and the resulting glycyrrhetic acid acetate is extracted by CHCl₃. The extract is evaporated and the residue crystals are purified by a mixture of cyclohexane ethanol and butanol. Pure glycyrrhetic acid acetate is refluxed in NaOH solution and ethanol to break acetyl bound. The mixture is cooled and brought to pH=4 by HCl to precipitate glycyrrhetic acid. The resulting glycyrrhetic acid is decolorized in alcohol. After filtration, water is added to filtrate to precipitate white glycyrrhetic acid. This method is time consuming and has low efficiency (about 11%)^[6,7].
- Glycyrrhizin is extracted by acetone containing 3% HNO₃ from the licorice root. Ammonia solution is added to the extract to precipitate ammonium glycyrrhizate which is crystallized in acetic acid and dissolved in water. Lead acetate solution 10% is

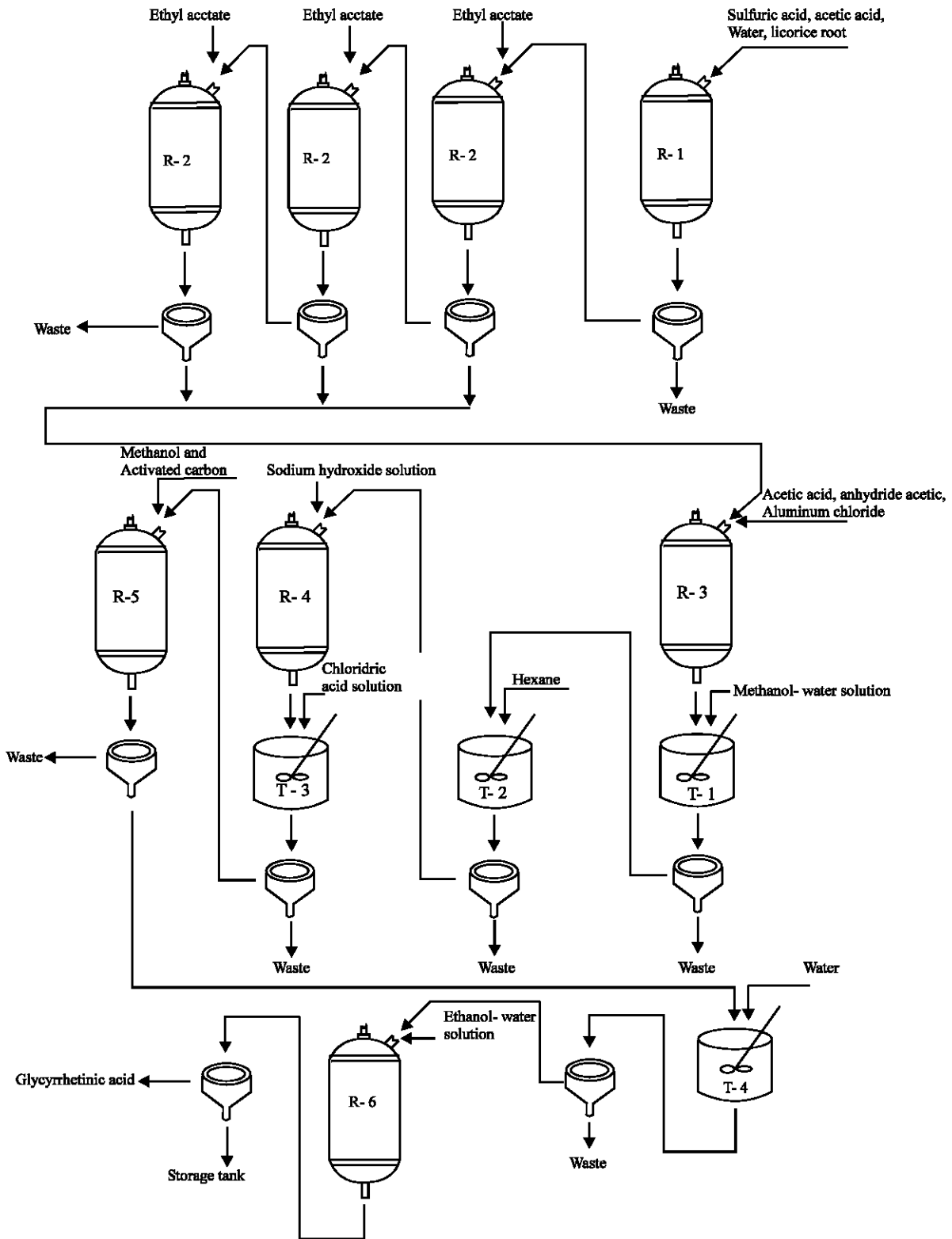


Fig. 4: Block diagram of glycyrrhetic acid extraction and purification process

added to the aqueous solution to precipitate lead glycyrrhizate, followed by the filtration and elutriation in alcohol. H₂S is passed through the resulting suspension to precipitate lead sulfide and release glycyrrhetic acid. Lead sulfide is separated by filtration and alcohol extract is evaporated. Residue crystals are crystallized in acetic acid to produce white glycyrrhetic acid. Since the application of H₂S is very tedious, this method could not be used safely^[8].

Production of glycyrrhetic acid by the enzymatic reactions: In this method glycyrrhetic acid is produced by hydrolysis of glycyrrhizin or its salts with aeromonas species or their cultured preparations in the presence of nitrogen, phosphate and potassium source. Efficiency of the method is about 91% and resulting glycyrrhetic acid has an acceptable purity. However this method is very expensive^[9,10].

Production of glycyrrhetic acid by hydrolysis of licorice root: In this method, glycyrrhizin is hydrolyzed directly, without isolation from licorice root. This method can be applied in different ways:

- Licorice root powder is hydrolyzed in trichloroacetic acid for 18 hours at 95°C. The cooled suspension is neutralized with lime solution and filtered. Resulting cake is washed by water and released glycyrrhetic acid is extracted by ethanol. The extract is evaporated and residue crystals are crystallized twice in acetic acid to produce glycyrrhetic acid. However, purity of the resulting glycyrrhetic acid is not acceptable^[11].
- Extraction method employed in this study is detailed below and its block diagram is shown in Fig. 4: Licorice roots (*Glycyrrhiza glabra*) are dried and ground to mesh 100 micron. In order to hydrolyze glycyrrhizin, an acidic medium should be used because ether bounds are stable in alkaline medium and unstable in water and acidic medium and acid acts as catalyst^[12]. Therefore 100 g of this powder is hydrolyzed in R-1 with 375 mL acetic acid, 30 mL sulfuric acid and 600 mL water at 98°C for 3 h. A little of resulting glycyrrhetic acid is solved through the reaction and decreases efficiency so as the reaction is completed; medium pH is increased from 0.5 to 5 by adding 10 g dry sodium hydroxide then suspension is diluted by 1500 mL water to precipitate solute glycyrrhetic acid. In this stage, glycyrrhetic acid and some insoluble impurities are

precipitated and water-soluble impurities (i.e. sugar) are removed. The suspension is then filtered (by using client powder as filter aid). The resulting cake is washed by water to remove final traces of acids that might decompose some of the solid ingredients when it is dried. In the second stage, the dried solid is extracted three times in R-2, preferably hot, with an organic solvent to separate the desired component from the insoluble. The water- immiscible solvents are preferably used because the solvent recovery is easier. Therefore ethyl acetate or chloroform is used to bring glycyrrhetic acid in organic phase. The corresponding extract contains glycyrrhetic acid and some impurities. As shown later, glycyrrhetic acid has an alcoholic group and a carboxylic acid group, so the best method to separate it from other ingredients is to acetylate it or convert it to a salt according to acid group; but among these reactions, acetylating is faster and easier. By acetylating of glycyrrhetic acid, it converts to glycyrrhetic acid acetate which is called acetoxolone, (Fig. 3^[4]).

Purified glycyrrhetic acid acetate is a white powder with various pharmaceutical applications. Polarity difference between glycyrrhetic acid acetate and available relevant is more than polarity difference between glycyrrhetic acid and its relevant ingredients therefore it is purified easier than glycyrrhetic acid. To acetylating of glycyrrhetic acid, the extract is evaporated and the remainder is acetylated in R3 by 15 mL acetic anhydride, 12 mL acetic acid and 0.3 g aluminum chloride as a catalyst at 100°C for 2 hours. After cooling, 70 mL methanol-water solution 40% is added to suspension in T-1 to precipitate glycyrrhetic acid acetate. Adding this solution should be done quite slowly because the reaction of producing methyl acetate, in this stage, is highly exothermic. The suspension is filtered and resulting cake is washed with

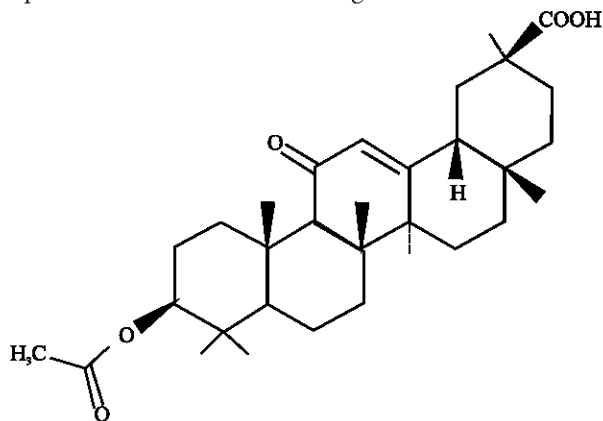


Fig. 3: Glycyrrhetic acid acetate

water in order to neutralization. The resulting cake, which contains glycyrrhetic acid acetate adherent some impurities, is mixed in T-2 two times by 35 mL. hexane for 30 min to remove impurities. Mixing is preferably done at room temperature because hot solvent dissolves much glycyrrhetic acid acetate and decreases efficiency. Glycyrrhetic acid acetate is hydrolyzed in R-4 by 50 mL sodium hydroxide solution 5% and 20 mL ethanol at 50°C for 3 h to break acetyl bound. Finally crude glycyrrhetic acid is precipitated in T-3 by adding cooled solution to 30 mL of 10% hydrochloric acid solution and then is filtered and resulting cake is washed with water in order to neutralization. Resulted glycyrrhetic acid is brown, therefore it is decolorized in R-5 by 100 mL methanol by 3 g. activated carbon at boiling temperature for 20 min then is hot filtered and water is added to clarified filtrate in T-4 to precipitate white glycyrrhetic acid. This product is dissolved in ethanol-water solution 47% weight by reflux in R-6 then the solution is cooled quite slowly at room temperature to precipitate white needle crystals of glycyrrhetic acid.

RESULT AND DISCUSSION

When water is added to clarified filtrate of final stage (decolorization stage); white glycyrrhetic acid is precipitated which has an impurity above the standard limit (2%). Crystallization is one of the common purification methods which can be used in this process. According to thin layer chromatography and high performance liquid chromatography tests, the unknown impurity has higher polarity as compared with glycyrrhetic acid. Therefore the solubility of this component in polar medium is more than glycyrrhetic acid. Glycyrrhetic acid is freely soluble in alcohol and is insoluble in water. Therefore the impurity of glycyrrhetic acid can be removed in alcohol –water solution. Impure glycyrrhetic acid is solved in alcohol – water solution at boiling temperature and crystallized at room temperature slowly. Crystallization should be done quite slowly to produce large and pure crystals. According to thin layer chromatography and high performance liquid chromatography tests, these crystals have acceptable purity.

CONCLUSIONS

The herbal properties of licorice root and glycyrrhetic acid have been studied. Also the others researches on extraction and purification of glycyrrhetic acid have been studied and a new method for purification of glycyrrhetic acid have been developed in this paper.

The resulting glycyrrhetic acid is a white needle crystal that has 99% purity. The total impurity of the crystals are less than 0.7% and each of its impurity is less than 0.2% and the other properties of the crystals are completely correspond to pharmacopeia standards^[3]. Studies can be conducted on shortening the process of extraction and purification of glycyrrhetic acid and increasing the efficiency of the process. Also the enzymatic reactions for production of glycyrrhetic acid should be more attention.

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