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Supercritical carbon dioxide extraction of glycyrrhizic acid from licorice plant root using binary entrainer: Experimental optimization via response surface methodology

Ali Hedayati, S.M. Ghoreishi*

Department of Chemical Engineering, Isfahan University of Technology, Isfahan, 84156-83111, Iran

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ABSTRACT

In this study, the extraction of Glycyrrhizic acid (GA) from *Glycyrrhiza glabra* (licorice) root was investigated by Soxhlet extraction and modified supercritical CO_2 with methanol and water as co-solvents and 30 min of static time. Design of experiment was carried out with response surface methodology (RSM) using Mini Tab software 17. The operating temperature (45–85 °C), pressure (10–34 MPa), dynamic extraction time (40–120 min), CO_2 flow rate (0.8–2 ml/min) and methanol concentration in water (0–100% as the binary co-solvent) were considered as the range of operating variables. The high performance liquid chromatography (HPLC) was used to identify and quantitatively determine the amount of extracted GA. Response surface analysis verified that R^2 and modified R^2 of the model were 98.05% and 94.51%, respectively. The RSM modeling predicted the optimal operating conditions to be the pressure of 29.6 MPa, temperature of 68 °C, CO_2 flow rate of 2 ml/min, dynamic extraction time of 108 min, methanol concentration of 46.5% in water (v/v) in which the maximum recovery of 54.4% was obtained. The accuracy of the modeling optimal GA recovery was validated with triplicate experiments giving the average extraction recovery of 54 ± 1%.

1. Introduction

Glycyrrhiza glabra a leguminous shrub having a height of 70–200 cm occurs mainly in sub-tropical regions where it grows wild and also under cultivation [1]. There are several species of *G. glabra* (licorice), including *G. glabra* L. var. *typica* Reg. et Herd., *G.glabra* L. var. *pallida* Boiss., *G. glabra* L. var. *glandulifera* Reg. et Herd. and *G. glabra* L. var. *violacea* Boiss. (Persian or Turkish liquorice) [2].

Licorice root and rhizomes are extensively used in herbal medicine for their demulcent, antacid, antiulcer [3], anti-inflammatory, expectorant, tonic, diuretic, laxative and sedative properties [4,5].They also possess antipyretic [6], antimicrobial, antiheroes [7], antiviral, anti-allergic, antioxidant and anti-cancerous activities [8]. It is widely used worldwide in food confectionery and pharmaceutical products, such as cough syrups, herbal supplements, chewing gums, drinks and candy. In the traditional system of medicine, *G. glabra* is recommended for the treatment of epilepsy [3]. Studies have shown that the extract has estrogenic activity and may help regulate the estrogen-progesterone ratio [9,10] and growth-inhibitory effect on breast cancer cells [11,12]. Many of the phenolic compounds isolated from licorice root may also help to protect low density lipoprotein (LDL) and red blood cells from oxidative damage [13–15]. Licorice root extract has also been shown to be beneficial for the liver. It has been used in Japan for more than twenty years as a treatment for chronic hepatitis, and studies with licorice root have shown a significant reduction of serum amino-transferase and a significant improvement in liver histology [16–18]. It has also been found that the licorice juice cause differences in the salivary pharmacokinetics of paracetamol if consumed with paracetamol [19].

Chemical constituents of the root include several bioactive compounds, such as glycyrrhizin (about 16%), different sugars (up to 18%), flavonoids, saponins, sterols, starches, amino acids, gums and essential oils. Kitagawa [20] reported the detail structure of 33 constituents in licorice root and their sweetness. Glycyrrhizin is a pentacyclic triterpenoid glycoside. The glycoside usually occurs in a combined calcium or potassium salt form of Glycyrrhizic acid (GA) which is a weak acid containing three carboxyl and five hydroxyl groups. Molecular structure of GA is shown in Fig. 1. Moreover, GA is a polar compound.

* Corresponding author. Tel.: +98 31 33915604.
E-mail address: ghoreshi@cc.iut.ac.ir (S.M. Ghoreishi).

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Nomenclature and units

A_0	constant
A_i	Coefficient of linear parameters
A_{ii}	Coefficient of quadratic parameters
A_{ij}	Coefficient of interaction parameters
k	Number of variables
P(MPa)	extraction pressure
Q(ml/min)	CO_2 flow rate
R (%)	Recovery
t (min)	Dynamic extraction time
T (°C)	Extraction temperature
X_i	Real values
$X_{i,c,p}$	Real values at the center point
Y	yield
Z_i	Coded value of the independent variable

Greek letters

ζ	Step change in variable X_i
ε	Residual associated with experiments

Glycyrrhizic acid the most studied active constituent in licorice is a sweet tasting material. It is 50 times sweeter than sugar and is widely used as a sweetening additive in the food industry [21,22]. GA has anti-inflammatory, anti-ulcer, anti-oxidant, anti-hepatotoxic and anti-virus activities [23–26]. There have been reports that GA has cancer chemo protective function [27] and that it has been used clinically in patient with acids [28]. GA is also used as an additive in some foods and toothpaste. Therefore GA extraction from licorice root and its characterization is considered to be an important research area.

Technology for the extraction of GA from licorice has been the subject of recent reports. Among the different existing techniques, single pot extraction (SPE) in batch mode is the most widely practical in herbal industry [29,30]. Solvent extraction is not affordable due to the high consumption of energy and solvent, also complete solvent recovery is not possible which is very important in food and drugs application. In addition, organic solvents particularly chlorinated ones that are used in traditional separation methods are very harmful to humans, environment and the ozone layer so they are rejected in the view of green chemistry [31]. Thus it is necessary to develop an effective and optimal extraction method. Application of various novel techniques such as microwave assisted extraction [32], microwave assisted micellar extraction [33], ultrasound assisted extraction [34] and multi stage countercurrent extraction [35] for the extraction of GA from licorice root has been already reported in literatures. Disadvantages of these methods could be eliminated by replacing the toxic liquid solvent with supercritical CO_2 (SC-CO_2), because CO_2 is inexpensive, nontoxic, nonflammable with low critical temperature and pressure and environmentally benign. Attentions to the supercritical technology are growing due to the unique properties of supercritical fluids, such as high selectivity, liquid-like densities, gas-like viscosities and low surface tension. The low polarity is the only major drawback of CO_2 that leads to low extraction recovery of GA. Thus co-solvents can be used to change the polarity of SC-CO_2 and increase its solvation power to desired analyte [36]. The enormous advantages of supercritical fluids have led to their wide utilization in different industries [37–39].

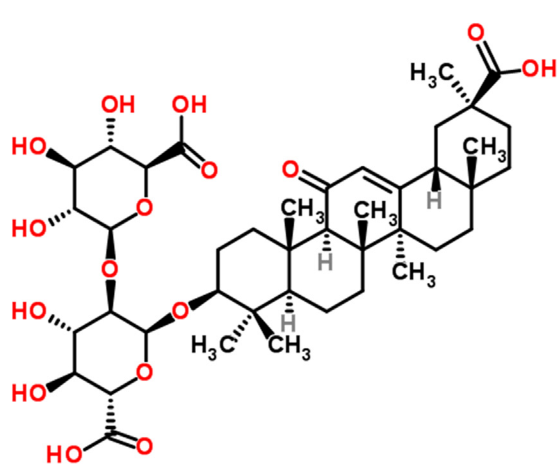

Fig. 1. Molecular structure of Glycyrrhizic acid.

The main objective of this research was GA extraction from Persian licorice by modified SC-CO_2 in periodic static-dynamic procedure for pharmaceutical application. The optimization was carried out by response surface methodology (RSM). The RSM is useful for modeling, problem analysis and optimization when a response (i.e., extraction recovery) is influenced by several variables such as pressure, temperature, flow rate of SC-CO_2, co-solvent concentration (type of polar modifier) and dynamic time. In current work the other effective variable (static time) was fixed at optimum value of 30 min that was obtained by experiment. Furthermore, a binary entrainer (methanol–water) was used to enhance the recovery of GA.

2. Experimental

2.1. Materials

Persian Licorice root was purchased from agricultural research center. The samples were ground and screened with mesh size of 20–35 (0.841–0.507 mm). The primary samples were stored inside a sealed bag and were maintained in a cold and dry place for extraction experiments. Methanol (purity ≥ 99.9%, Merck), pure acetic acid (Merck) deionized water and industrial grade carbon dioxide (purity > 99%, Ardestan) were utilized for Soxhlet and supercritical fluid extraction and HPLC analysis. Glycyrrhizic acid ammonium salt (>95%, Sigma-Aldrich) was used in the HPLC analysis (Jasco HPLC equipped with a UV detector) to obtain a standard spectrum.

2.2. Soxhlet extraction

For Soxhlet extraction, 2 g of ground licorice root was weighted and set in a Soxhlet apparatus and then continuously extracted for 8 h using methanol as solvent. After extraction, the solvent was evaporated by rotary vacuum evaporator (30 °C), and the extract was dried at 70 °C to remove residual solvent to desired amount. Then the amount of GA was determined by HPLC method. The results showed that Soxhlet extraction provided a yield of 138 mg GA/g licorice root, which was considered as the total extractable GA content while calculating the GA recovery by SC-CO_2 extraction.

2.3. Supercritical fluid extraction: apparatus and procedure

To carry out the objectives of this study, the supercritical extraction system shown in Fig. 2 was used. To increase the purity of the CO_2, which is stored in a CO_2 cylinder (1), it was passed through a column of molecular sieve (Merck, Molecular Sieve 5A-K28751105148) and metal porous filter (Mott Metallurgical, 1003630-01-050) (2). Then, CO_2 is cooled down in a chiller and pump head cooler (3, 4) in the range of −10 to −5 °C. Then liquefied CO_2 is charged by a feed pump (reciprocating pump, Jasco, PU-1580, maximum pressure = 35 MPa) (5) through the needle valve (6) and is fed to the oven that controlled the temperature (298–523 ± 0.5 K)