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Analytical Methods

Development of sample preparation method for isoliquiritigenin, liquiritin, and glycyrrhizic acid analysis in licorice by ionic liquids-ultrasound based extraction and high-performance liquid chromatography detection

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ABSTRACT

An ionic liquid-based ultrasonic-assisted extraction (ILUAE) method had been used for the effective extraction of isoliquiritigenin (IQ), liquiritin (LQ) and glycyrrhizic acid (GA) from licorice. The ionic liquids with different cations and anions were investigated in this work and 0.5 M 1-butyl-3-methylimidazolium bromide solution was selected as solvent. In addition, the technical parameters including soaking time, solid–liquid ratio, ultrasonic power and time were optimized. Compared with the conventional solvent extraction, the proposed approach exhibited higher efficiency, which indicated the ILUAE was an efficient, rapid and simple sample preparation technique. There was no degradation of the target analytes had been observed at the optimum conditions which was evidenced by the stability studies performed with standard of IQ, LQ and GA. The proposed method also showed high reproducibility and was environmental friendly.

1. Introduction

Licorice, the roots and stolons of the *Glycyrrhiza* plant species, such as *Glycyrrhiza uralensis*, *Glycyrrhiza inflata* and *Glycyrrhiza globra*, is a traditional herbal medicine with many health benefits such as antiallergic, antiinflammatory, immunomodulating, antiulcerous, antidotal, gastroprotective, antioxidant and antiviral properties (Gupta et al., 2008; Kondratenko et al., 2001). Licorice has been in medical used for more than 4000 years in China (Aoki et al., 2005). The main active components in the licorice are isliquiritigenin (IQ), liquirtin (LQ) and glycyrrhizic acid (GA). IQ is a chalcone derivative contained in licorice. Pharmacology studies indicated that IQ has many pharmacological effects such as antioxidant (Vaya, Belinky, & Aviram, 1997), cytoprotective (Kim et al., 2004b), antiflammatory and analgesic (Morteza-Semnani, Saeedi, & Hamidian, 2004), antiplatelet aggregative (Tawata, Aida, & Noguchi, 1992), antiangiogenic (Kobayashi, Miyamoto, Kimura, & Kimura, 1995), chemopreventive (Hsua, Kuob, & Lin, 2005) and antitumor activities (Baba et al., 2002; Yamamoto et al., 1991). LQ, a major flavonoid isolated from licorice, is glycosidic form of liquiritigenin (Kitagawa et al.,1998). LQ posses various biological effects such as antiallergic, antitussive (Taniguchi et al., 2000), immunomodulating (Ohtake et al., 2000), antiviral (Hatano, Yasubara, & Miyamoto, 1988) and antioxidant properties (Vaya et al., 1997). GA, one another of the bioactive components of liquorice (Kiso et al., 1984), has been shown to be active against a variety of viruses including herpes simplex type 1 (HSV-1), human cytomegalovirus (HCMV), varicella-zoster virus (VZV), hepatitis A, B, and C (HAV, HBV, and HCV) viruses, influenza virus, and human immunodeficiency virus-1 (HIV-1) (Baba & Shigeta,1987; Pompei, Flore, Marccialis, Pani, & Loddo, 1979). GA is also used as edulcorator in food industry (Kim et al., 2004a). GA, a healthy sweetener of high sweetness but low calorific value, can be added to food, beverages and confectionery industry. Moreover, GA is a good component in cosmetics and tobacco industries. The traditional extraction methods include heat-reflux extraction (HRE) and Soxhlet extraction (SE), which have many drawbacks such as time-consuming, using of a large amount of toxic and hazardous organic solvents and bad environment conditions. In order to overcome these abovementioned problems, an alternative extraction technique with unique advantages such as simple, rapid, high efficiency, environmental friendly and low cost is necessary. And the solvents with unique properties including high stability, safe and ease of handling should be selected.

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In recent years, the development and use of environmentally friendly methods has become increasingly popular. Ultrasound-assisted extraction (UAE) is an extraction technique that offers high reproducibility, short extraction times, simple manipulation, and low solvent consumption, temperature and energy input (Jiao & Zuo, 2009; Wang & Zuo, 2011; Zuo et al., 2004).

Ionic liquids are composed of organic cations and inorganic or organic anions. Ionic liquids are liquid near room temperature or by convention below 100 °C (Wilkes & Zamorotko, 1992). Ionic liquids have been proposed as greener alternatives or replacement to conventional volatile organic solvents (Hoffmann, Nüchter, Ondruschka, & Wasserscheid, 2003; Huddleston et al., 2001; Welton, 1999) owning to their unique chemical and physical properties such as negligible vapour pressure, good thermal stability, wide liquidus range, good dissolving and extracting ability, designable structures and among others (Anderson, Ding, Ellern, & Armstrong, 2005; van Rantwijk & Sheldon, 2007; Welton, 1999). Ionic liquids are promising solvents in the preparation of various useful substances from plant samples such as alkaloids (Ma et al., 2010), lignans (Ma et al., 2011), and polyphenolic compounds (Du, Xiao, Luo, & Li, 2009), and ionic liquid-based silica as sorbent used for extracting liquiritin and glycyrrhizic acid (Tian, Bi, & Row, 2009). So far, to our best knowledge, the simultaneous extraction of flavonoid glycosides and saponin using ionic liquid has not been reported in the literature. The present paper aimed to develop a rapid, greener and effective ionic liquid-based ultrasonic-assisted extraction (ILUAE) method for simultaneous extraction of the main active components IQ, LQ and GA from licorice.

2. Experimental

2.1. Chemicals and plant materials

IQ, LQ and GA standards (98% purity) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Methanol of chromatographic grade was purchased from J & K Chemical Ltd. (Beijing, China). All ionic liquids: [C_4mim]Cl, [C_4mim]OH, [C_4mim]Ac, [C_4mim]NO_3, [C_4mim]HSO_4, [C_4mim]ClO_4, [C_2mim]Br, [C_4mim]Br, [C_6mim]Br, [C_8mim]Br, [C_{10}mim]Br, [C_{12}mim]Br, where C_2mim = 1-ethyl-3-methylimidazolium, C_4mim = 1-butyl-3-methylimidazolium, C_6mim = 1-hexyl-3-methylimidazolium, C_8mim = 1-octyl-3-methylimidazolium), C_{10}mim = 1-decyl-3-methylimidazolium,, C_{12}mim = 1-dodecyl-3-methylimidazolium) were gained from Chengjie Co. Ltd. (Shanghai, China) and used as received. All the other reagents obtained from Beijing Chemical Reagents Co. (Beijing, China) were of analytical grade. Deionized water was purified by a Milli-Q water purification system from Millipore (Bedford, MA, USA) was used throughout. All solutions and samples prepared for chromatographic analysis were filtered through a 0.45 μm nylon membrane (Guangfu Chemical Reagents Co. Tianjin, China) before injecting into HPLC.

Licorice was purchased from Sankeshu Medicinal Materials Market, Harbin City, Heilongjiang Province, China, and authenticated by Prof. Shao-quan Nie from the Key Laboratory of Forest Plant Ecology, Ministry of Education, Northeast Forestry University, China. Voucher specimens were deposited in the herbarium of this Key Laboratory. Dried licorice was powdered into a homogeneous size and then sieved (60–80 mesh) prior to extraction.

2.2. HPLC analysis and quantification

Chromatographic analyses were performed on a Waters HPLC system consisting of a pump (Model 1525), an auto-sampler (Model 717 plus), UV detector (Waters 2487 Dual λ absorbance detector) and automatic column temperature control box (Model 717). Kromasil-C_{18} column (5 μm, 4.6 × 250 mm, Akzol Nobel, Sweden).

For HPLC analysis, methanol–water–acetic acid (65:35:2, v/v/v) was used as the mobile phase with 10 μl injection volume, 1.0 ml/min flow rate, 25 °C column temperature. The UV detection wavelengths were 254 nm for IQ, GA and 275 nm for LQ. The elution time of each sample was 50 min, using isocratic elution, and the retention times of IQ, LQ and GA were 7.2, 9.0 and 31.9 min, respectively. For standard sample solution, various amounts of IQ, GA and LQ were dissolved in methanol to yield the stock solutions, respectively. Corresponding calibration curves for each compound are $Y_{IQ} = 5.446 \times 10^4 X + 2.337 \times 10^3$ ($r = 0.9997$), $Y_{LQ} = 1.402 \times 10^5 X + 8.085 \times 10^3$ ($r = 0.9998$) and $Y_{GA} = 3.748 \times 10^{5-} X + 5.125 \times 10^3$ ($r = 0.9998$). A good linearity was found for IQ, LQ and GA in the range of 0.00394–2.46, 0.00816–5.10 and 0.0168–10.5 mg/ml, respectively.

2.3. Ionic liquids based ultrasonic-assisted extraction

For the ultrasonic-assisted extraction experiments, an ultrasonic bath was used as an ultrasonic source. KQ-250DB ultrasonic bath (Kunshan, Jiangsu, China) with the maximum power of 250 W was used in the extraction step. The bath was a rectangular container (23.5 × 13.3 × 10.2 cm), to which 50 kHz transducers were annealed at the bottom. The bath power rating was 250 W on the scale of 20–100%. The temperature of which was controlled by the replacement between inlet and outlet water.

The preparation steps of ionic liquids aqueous solutions (0.5 M) were as follows: a certain mass of different ionic liquids (according to their molar mass) were accurately weighed and fully dissolved in deionized water, and then diluted the solution to 25 mL in a volumetric flask (25 mL) by deionized water, respectively. 0.5 g of dried sample powder was mixed with 5 mL of various ionic liquids aqueous solutions in a 25 mL flask. The flask was then partially immersed into the ultrasonic bath, which contains 2.5 L of water. The optimum anion, cation, concentration of selected ionic liquid, soaking time and solid–liquid ratio, ultrasonic power and time were systematically studied in this experiment. The extraction efficiency was expressed as the observed values of target analytes and the maximum amount in each curve was taken to be 100%.

2.4. Optimization ultrasonic-assisted extraction method by Response Surface Method (RSM)

To further study the interaction between the factors, we optimized the operating conditions by RSM and used the Central Composite Design software on data processing. Central Composite Design (CCD) with three factors was applied using Design-Expert 7.0 without any blocking. The bounds of the factors were 150–250 W ultrasonic power, 30–50 min ultrasonic time, and 8–12 ratio of liquid–solid. Specific protocols for experimental conditions were showed in Table 1a.

2.5. Stability, recovery and repeatability of ILUAE

Stability derived test was performed by IQ, GA and LQ standards dissolved in 0.5 M [C_4mim]Br by ultrasonic-assisted extraction (UAE) at the optimum conditions (200 W ultrasonic power, 40 min ultrasonic time and solid–liquid ratio 1:12).

The recoveries of IQ, GA and LQ were taken as the indicative markers for the stability of IQ, GA and LQ at the derived operating extraction conditions.

To determine the repeatability of the novel extraction method, five samples of the same weight (0.5 g) were processed under same