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Ionic liquid-based ultrasound-assisted extraction and aqueous two-phase system for analysis of caffeoylquinic acids from Flos *Lonicerae Japonicae*

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ABSTRACT

In this work, an ionic liquid-based ultrasonic-assisted extraction (ILUAE) method was developed to extract caffeoylquinic acids (CQAs) from Flos *Lonicerae Japonicae* (FLJ). ILUAE parameters were optimized by response surface methodology, including IL concentration, ultrasonic time, and liquid–solid ratio. Optimized ILUAE approach gained the highest extraction yields of 28.53, 18.21, 3.84 mg/g for 3-*O*-caffeoylquinic acid (C1), 3,5-di-*O*-caffeoylquinic acid (C2), 3,4-di-*O*-caffeoylquinic acid (C3), respectively. C1–C3 are the three most abundant CQAs compounds in FLJ. The method showed comparable extraction yield and shorter extraction time compared with conventional extraction techniques. Subsequently, an aqueous two-phase system (ATPS) was applied in extraction solutions. Two trace CQAs, 5-*O*-caffeoylquinic acid (C4) and 4,5-di-*O*-caffeoylquinic acid (C5), were significantly enriched with signal to noise values increasing from less than 10 to higher than 1475. The results indicated that ILUAE and ATPS are efficient and environmentally-friendly sample extraction and enrichment techniques for CQAs from herbal medicines.

1. Introduction

Currently, the main concerns in phytotherapeutics are the efficacy, quality and safety of botanical medicines. For quality assurance of herbs, sample extraction is the crucial first step in analysis of constituents. The extraction of active components from herbal medicines is usually achieved by traditional methods such as soxhlet extraction, stirring extraction, or heat-reflux extraction (HRE) [1–3]. However, these extraction methods are usually time-consuming and require large volumes of volatile organic solvents. Therefore, development of environmentally-friendly and efficient extraction methods for herbal medicines is an ongoing effort in chemistry. Ultrasound-assisted extraction (UAE), with the main advantages of short extraction time, simple operation and satisfactory efficiency has become an alternative to traditional extraction techniques [4,5].

Ionic liquids (ILs) are gathering considerable attention as potential substitutes for traditional organic solvents due to their unique properties, including negligible vapor pressures, high thermal stability, and specific solvent abilities [6]. Therefore, UAE or microwave-assisted extraction (MAE) techniques coupled with ILs can improve the extraction efficiency of components from herbal medicines [7–12]. Aqueous two-phase system (ATPS) is usually formed by combing ILs with inorganic salts [13]. As an environmentally friendly separation and enrichment technique, ATPS has already been applied to purify and separate proteins [14], natural products [15], antibiotics [16] and metal ions [17].

Caffeoylquinic acids (CQAs) are the major bioactive components in Flos *Lonicerae Japonicae* (FLJ, "Jinyinhua" in Chinese) [18], and reported to have various biological activities, such as antioxidant, antiviral, and anti-inflammatory [19]. CQAs are also used as chemical markers for quality control of FLJ. In order to efficiently analyze the CQAs, Zhang et al. applied an IL-based UAE (ILUAE) method to extract 3-*O*-caffeoylquinic acid in FLJ. However, this method is not applied to other major CQAs [20]. In addition, some trace CQAs in FLJ are difficult to determined. Bonn et al. applied the solid-phase extraction method for enriching the galloyl-and caf-

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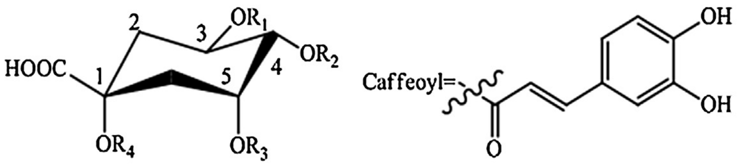
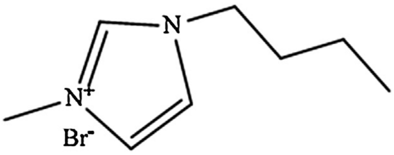


No.	Compounds	R1	R2	R3	R4
C1	3-O-caffeoylquinic acid	caffeoyl	H	H	H
C2	3, 5-di-O-caffeoylquinic acid	caffeoyl	H	caffeoyl	H
C3	3, 4-di-O-caffeoylquinic acid	caffeoyl	caffeoyl	H	H
C4	5-O-caffeoylquinic acid	H	H	caffeoyl	H
C5	4, 5-di-O-caffeoylquinic acid	H	caffeoyl	caffeoyl	H

A



B

Fig. 1. Chemical structures of CQAs (C1–C5) (A) and 1-butyl-3-methylimidazolium bromine [Bmim]Br (B).

feoylquinic acids from natural sources [21]. Solid-phase extraction (SPE) has good enrichment capability for a class of compounds with similar physical and chemical properties (such as acids). For analysis of some especial caffeoylquinic acids, ATPS may be a alternative enrichment technique.

In this work, an extraction method based on ILUAE was developed to extract CQAs from FLJ. The parameters of ILUAE were optimized by single-factor variation or response surface methodology, and the method was compared with conventional techniques. ATPS was subsequently employed to enrich extraction solutions. Consequently, two trace CQAs were significantly enriched in ATPS.

2. Materials and methods

2.1. Chemicals and materials

Dried flower buds were collected from Fengqiu, Henan province of China and authenticated by Prof. Ping Li from China Pharmaceutical University. Voucher specimens were deposited in the Department of Pharmacognosy, China Pharmaceutical University. Reference compounds consisting of five CQAs, including 3-*O*-caffeoylquinic acid (C1), 3,5-di-*O*-caffeoylquinic acid (C2), 3,4-di-*O*-caffeoylquinic acid (C3), 5-*O*-caffeoylquinic acid (C4), and 4,5-di-*O*-caffeoylquinic acid (C5) (see Fig. 1A) were previously isolated from species of *Lonicera* in the authors' laboratory. Their structures were elucidated based on spectroscopic methods (^{1}H and ^{13}C NMR and MS) with a purity of higher than 95% for each compound by HPLC analysis [22].

Sodium carbonate (Na_2CO_3), potassium hydroxide (KOH), dipotassium hydrogen orthophosphate (K_2HPO_4) and potassium orthophosphate (K_3PO_4), all above 99% pure, were obtained from Nanjing chemical reagent CO., Ltd., (Nanjing, China). 1-butyl-3-methylimidazolium bromine ([Bmim]Br) (>99%) was purchased from Lanzhou Greenchem ILS, LICP, CAS. (Lanzhou, China) (see Fig. 1B). HPLC grade of acetonitrile (ACN) was purchased from ROE (Newark, New Castle, DE, USA), HPLC-grade methanol and ethanol were obtained from Burdick & Jackson Honeywell Internation Inc., (Muskegon, MI, USA) while formic acid (>99%) was procured from ROE (Newark, New Castle, DE, USA). Deionized water (18 MΩ cm^{-1}) was prepared by a Millipore Milli-Q system (Millipore, Milford, MA, USA).

2.2. Preparation of standard solutions

All reference standards were dissolved in 1 mol/l aqueous solutions of [Bmim]Br to prepare mixed solutions and the concentrations of C1–C5 were 0.5 mg/ml, 0.4 mg/ml and 0.3 mg/ml, 0.28 mg/ml and 0.278 mg/ml, respectively. A series of standard solutions were used to construct calibration curves and they were stored at 4 °C until analysis.

2.3. Analytical methods

An Agilent Extend-C_{18} column was provided by Agilent Technologies (4.6 mm × 250 mm, 5 μm). The samples (5 μl) were analyzed on an Agilent 1100 Series HPLC system (Agilent Technologies, Palo Alto, CA, USA) at 25 °C. The mobile phase consisted of water containing 0.1% formic acid (A) and ACN (B). The separation was attained using a gradient program starting at 5% B, which was increased linearly up to 24% at a flow rate of 1 ml/min over 60 min, and the wavelength of detection was set at 340 nm. The data acquisition and analysis were performed by Agilent ChemStation software.

2.4. Ionic liquids based ultrasound assisted extraction

Dried flower buds were powdered into a homogeneous size by a disintegrator (HX-200A, Yongkang Hardware and Medical Instrument Co., Ltd., Sandong, China), sieved through a No. 60 mesh, and dried at 45 °C in the oven for 6 h. Powders (0.25 g) were mixed with 10 ml aqueous solutions of [Bmim]Br in a 25 ml flask, which was then sonicated at 40 kHz and 100 W (KQ2200E, Kunshan Ultrasonic Co., Ltd., Jiangsu, China). As is shown in Fig. 2, the extraction solutions were centrifuged at 13,000 × *g* for 10 min, and then injected into HPLC for analysis.

2.5. Experiment design to optimize extraction conditions of ILUAE

The extraction conditions of ILUAE were firstly optimized by single-factor variation, and then a series of experiments were designed by response surface methodology (RSM) to optimize the ILUAE method. The software design expert version 8.0.6 and Box-Benhnken design were employed for experimental designs,