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Homogeneous ionic liquid microextraction of the active constituents from fruits of *Schisandra chinensis* and *Schisandra sphenanthera*

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ABSTRACT

Homogeneous ionic liquid microextraction (HILME) was developed for the extraction of schizandrin, schisantherin A and deoxyschizandrin from *Schisandra chinensis* and *Schisandra sphenanthera*. 1-Butyl-3-methylimidazolium tetrafluoroborate ($[C_4MIM][BF_4]$) aqueous solution was used as extraction solvent, and ammonium hexafluorophosphate ($[NH_4][PF_6]$) was used as ion-pairing agent. 1-Butyl-3-methylimidazolium hexafluorophosphate ($[C_4MIM][PF_6]$), which is barely soluble in water, was formed in situ, and was used as sample solution. High-performance liquid chromatography (HPLC) was employed for separation and determination of the analytes. The calibration curve showed good linear relationship ($r > 0.9998$). The recoveries were between 69.71% and 88.33% with RSDs lower than 4.86%. External standard method was adopted in the proposed method, and internal standard method was applied for the evaluation of the proposed method. The two methods were compared and the results indicated that the proposed method was acceptable and simple. The HILME is free of volatile organic solvents, and represents lower expenditures of sample, extraction time and solvent, compared with ultrasonic and Soxhlet extraction. There was no obvious difference in the extraction yields of active constitutions obtained by the three extraction methods.

1. Introduction

Both *Schisandra chinensis* (Turcz.) Baill. (*S. chinensis*) and *Schisandra sphenanthera* Rehd. Et Wils. (*S. sphenanthera*) are traditional Chinese medicines, and have been used for thousands of years. They are commonly used for the treatment of chronic cough and dyspnea, nocturnal emission, spermatorrhea, enuresis, frequent urination, protracted diarrhea, spontaneous sweating, night sweating, impairment of body fluids with thirst, shortness of breath and feeble pulse, diabetes and wasting-thirst caused by internal heat, palpitation and insomnia [1]. In recent decades, the medicines have also been used for the treatment of hepatitis [2]. The chemical constituents and contents of the bioactive constituents in the fruits of *S. chinensis* and *S. sphenanthera* are quite different [2]. Since 2000, they have been accepted as two different crude medicines in the Chinese Pharmacopoeia [1]. The fruits of *S. chinensis* and *S. sphenanthera* mainly contain lignans and volatile oils. Most of these lignans were found to have biological activities [2].

The extraction of active constituents from medicinal plants was traditionally performed by solvent extraction or maceration extraction. Unfortunately, these methods are usually time consuming, laborious, and a large amount of hazardous and volatile organic solvents are required, although the methods are often effective. As the analytical technique has rapidly developed, there has been a trend towards less (organic) solvent consumption, short extraction time and miniaturization in the analytical extraction.

Ionic liquids (ILs) consist of organic cations and organic or inorganic anions. The ILs emerge as possible "green" solvents [3,4] and have a wide utilization in synthesis [5], catalysis [6,7], separation [8] and electrochemistry [9] for their unique properties such as negligible vapor pressure, chemical and thermal stability, good solubility for both organic and inorganic molecules, and miscibility with water and organic solvents. In recent years, the ILs have attracted increasing interest and are used more and more as attractive alternatives to environmentally unfriendly solvents in sample preparation [10–15].

The benefit of using microwave-assisted extraction (MAE) to extract organic compounds directly from solid matrixes has already been demonstrated in recent years [16,17]. Compared with traditional and other modern extraction techniques, MAE is proposed as an efficient and alternative procedure for sample pretreatment. Furthermore, ILs can efficiently absorb and transfer microwave energy. As extraction solvents, ILs are very attractive in the microwave-assisted extraction of bioactive components from medicinal herbs.

The aim of the present study is to develop a effective, rapid and green homogeneous ionic liquid microextraction (HILME) method to extract three active lignans from *S. chinensis* and *S. sphenanthera*.

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To our best knowledge, there is no report about the application of proposed method in the extraction of active constituents from plant materials. For the comparison, the ultrasonic extraction (UE) and Soxhlet extraction (SE) were also applied. The UE is a standard method recommended in Chinese Pharmacopoeia [1]. The SE is widely accepted and applied for a long time. To evaluate the new extraction method, the SE is often adopted as a reference method.

2. Experimental

2.1. Chemicals and materials

Schizandrin, schisantherin A, deoxyschizandrin and diphenyl were obtained from Chinese Drug Biological Product Qualifying Institute (Beijing, China). The chemical structures of the analytes are shown in Fig. 1. Chromatographic grade acetonitrile and methanol were obtained from Fisher Scientific (Pittsburgh, Pennsylvania, USA). Ammonium hexafluorophosphate ($[NH_4][PF_6]$) and 1-alkyl-3-methylimidazolium ionic liquids (purity > 99%), including $[C_4MIM][BF_4]$, $[C_6MIM][BF_4]$ and $[C_8MIM][BF_4]$ were purchased from Cheng-jie Chemical Co. LTD (Shanghai, China). Water was purified with a distillator (Rong-hua Co. LTD, Jiangsu, China) and filtered through a 0.45 μm membrane.

2.2. Apparatus

A 1100 series liquid chromatograph (Agilent Technologies Inc., USA) equipped with photodiode-array detector (DAD) was employed. The chromatographic separation of the analytes was carried out on a Zorbax Eclipse SB-C18 column (3.5 μm, 4.6 mm × 150 mm, Agilent, USA). The flow rate of the mobile phase was maintained at 0.5 mL min^{-1}. The injection volume of the sample solution was 3 μL. The temperature of the column was controlled at 40 °C. The mobile phase consisted of acetonitrile (A), water (B) and methanol (C). Gradient program was as follows: 0–3 min, 40–30% B, 30% C; 3–12 min, 30–25% B, 30% C; 12–13 min, 25–10% B, 30% C. The absorbance was measured at a wavelength of 250 nm.

Microwave digestion system (CEM Corporation, USA) was used in the extraction step. High speed refrigerated centrifuge (Beckman Corporation, USA) was employed. The spectrophotometer (Shimazu, Japan) was used to evaluate in situ preparation of the reagent. KQ-100DE ultrasonic generator (Kunshan, Jiangsu, China) was used in the UE.

2.3. Sample preparation

Five kinds of samples (samples 1–5) cultivated in different areas were bought from normal drugstores (Changchun, China), among which samples 1–3 belong to *S. chinensis*, and samples 4–5 belong to *S. sphenanthera*. All the samples have been authenticated by professor Jingmin Zhang of the College of Pharmacology, Jilin university.

The samples were cleaned with water, and dried thoroughly in the cabinet drier at 50 °C for 24 h. The samples then were triturated with a pulverizer, passed through a 40 mesh stainless steel sieve and stored in a desiccator.

To evaluate the method of sample preparation, the thermal stability of the analytes at 50 °C was tested. 500 μg of schizandrin, schisantherin A and deoxyschizandrin were respectively added into a 2 mL volumetric flask. The flask was put in a thermostat at 50 °C for 24 h. Then the flask was allowed to cool down to room temperature and acetonitrile was added into the flask to the mark. The resulting solution was referred to as sample a. The sample b was prepared by directly dissolving the analytes. The concentration of the analytes in samples a and b were the same. The samples a and b were analyzed by HPLC in triplicate, respectively. The average peak areas of schizandrin, schisantherin A and deoxyschizandrin were 2538.3, 2215.7 and 2567.1 for sample a and 2524.8, 2235.6 and 2581.7 for sample b, respectively. The results show that analytes are stable under the conditions of sample preparation, and the method of sample preparation should be convincing.

2.4. Preparation of standard solutions

For each analyte, the standard stock solution was prepared by dissolving the analyte in acetonitrile, and stored at 4 °C. Working solutions were prepared by diluting the stock solutions with acetonitrile.

2.5. Preparation of spiked samples

The spiked samples were prepared by spiking the standard solutions into sample 1 powders. The concentrations of schizandrin, schisantherin A and deoxyschizandrin in the standard solution were 1645, 475.8, 649.5 μg mL^{-1}, respectively. The ratios of the schizandrin standard solution volume to the sample weight were 34, 17 and 1.2 μL mg^{-1}, respectively. The ratios of the schisantherin

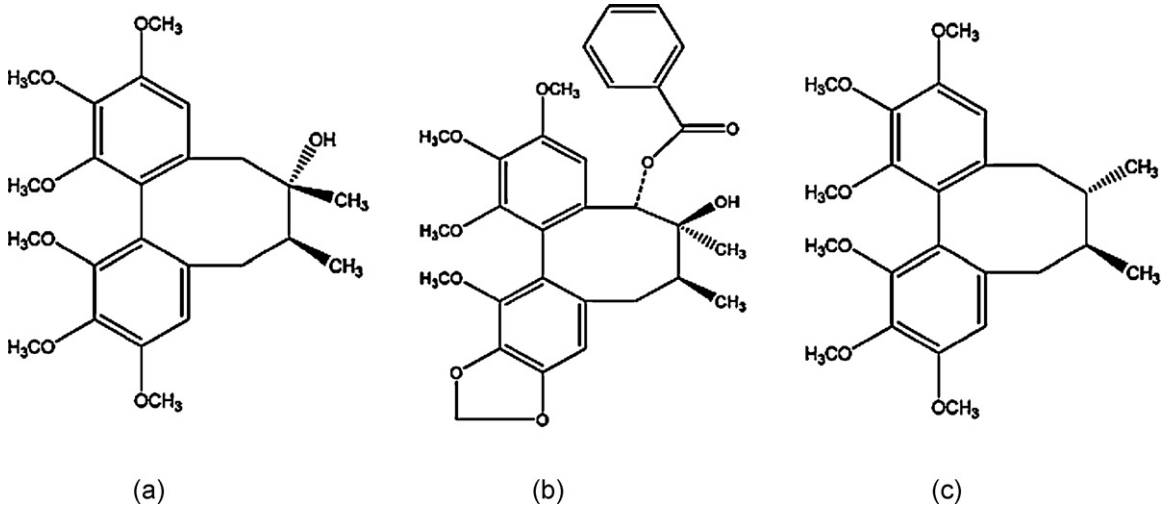

Fig. 1. Chemical structures of schizandrin (a), schisantherin A (b) and deoxyschizandrin (c).