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Tissue-smashing based ultra-rapid extraction of chemical constituents in herbal medicines

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ABSTRACT

Sample extraction is the first challenge in analysis of herbal medicines (HMs). Numerous methods have been developed to improve extraction efficiency, use less solvent and short time. In this work, a tissue-smashing based ultra-rapid extraction (TSURE) method has been proposed through the designed particle crushing, drastic stir, and dynamic molecular permeation at normal temperature. Factors in TSURE like extraction time, volts, and solvents were optimized for extraction efficiency of salvianolic acid B, cryptotanshinone, and tanshinone IIA from *Salvia miltiorrhiza*. The TSURE method was validated in terms of repeatability (RSD < 2.2%) and extraction recoveries (93–106% with RSD < 5.0%). TSURE showed a comparable extraction efficiency to conventional heat reflux extraction (HRE) and better than ultrasonic assisted extraction (UAE). The extraction time was about 2.0–3.0 min for TSURE, 60 times faster than the performance of HRE and 20 times faster than UAE. Microscopic analysis showed that the Krummbein diameter of plant particles after extraction were about 600–1200 μm for HRE and UAE, and decreased to 50–80 μm for TSURE. Subsequently, the developed TSURE was applied to high-throughput extraction of 19 *S. miltiorrhiza* samples collected in different regions of China. Besides, application of TSURE to other herbal medicines was also investigated, including *Panax quinquefolius* and *Lonicera japonica*. TSURE method provided an ultra-rapid and promising alternation for extraction of ingredients in herbal medicines, and can be extended to pharmaceutics, foods and cosmetics.

1. Introduction

Sample preparation is the first challenging step in analysis and quality control of botanicals and herbal medicines (HMs) [1]. Efficient sample extraction strategy can improve extraction efficiency and enrich the target analytes [2,3]. As stated in the previous studies, some conventional and simple methods, such as ultrasonic assisted extraction (UAE), heating under reflux extraction (HRE), are commonly used [4,5]. Methanol and ethanol are most widely referred as the solvents [6,7]. These methods, however, are usually time-consuming, solvent-consuming, and may have low extraction efficiencies [8,9].

In recent years, many ultra-pressure or ultra-temperature extraction methods have been introduced for extraction of analytes of interest present in plant materials, such as pressurized liquid extraction (PLE), microwave-assisted extraction (MAE), and supercritical fluid extraction (SFE) [10–12]. The newer methods use relatively less solvent, take shorter time and are more efficient [5]. Various sample preparation techniques are summarized and compared for the extraction of plant materials [13]. Each has advantages and limitations depending on the projected use of results and the properties of analytes [14]. Undoubtedly, methods that are simple, rapid and environmentally friendly will be preferred [15].

Tissue-smashing based ultra-rapid extraction (TSURE) was first introduced as a new extraction technique in 1993 [16]. The operating process of TSURE method is similar to juice squeezing [17]. The TSURE enables ultra-rapid extraction of target ingredients at normal temperature through the designed particle crushing, drastic stir, and dynamic molecular permeation [18]. An ultra-rapid extraction process provided by TSURE is meaningful for sample analysis, such as the qualification and quantification of ingredients from herbal medicines. In TSURE process, plant particles were crushed into smaller ones with the help of high-speed shear force and mixing power. In addition, under the partial negative pressure permeation, soluble balance between solid materials and solvents

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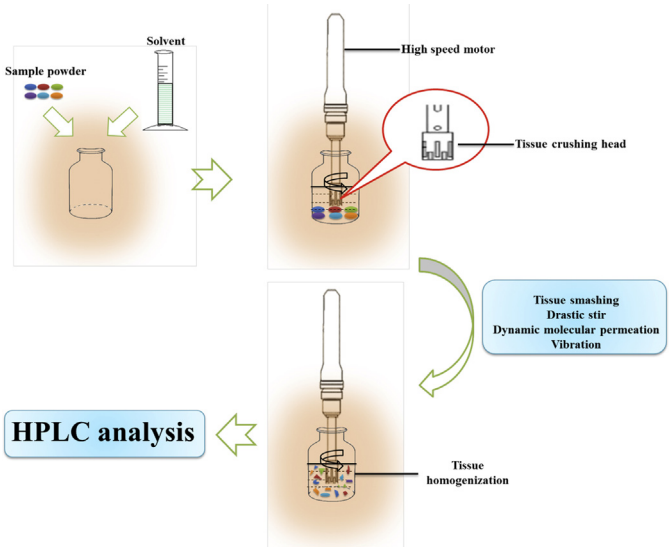

Fig. 1. Summary diagram of the TSURE method. Accurately weighted samples were added into an extraction bottle and mixed with solvent. Then the tissue crushing head was lifted under solvent surface. After several minutes, a homogeneous mixture contains analytes of interest was obtained.

can be rapidly achieved [19]. The main advantages of TSURE are its versatility, ultra high speed, flexibility and low cost [20]. Summary diagram of the TSURE method was shown in Fig. 1.

In this work, a TSURE method was developed and its potential in rapid extraction of constituents in HMs was systematically investigated. Factors in TSURE like extraction time, volts, and solvents were optimized for extraction efficiency of salvianolic acid B, cryptotanshinone, and tanshinone IIA from *Salvia miltiorrhiza*, one of the best-selling and most studied natural products [21]. The TSURE method was validated in terms of repeatability and extraction recoveries. TSURE was compared with two conventional methods HRE and UAE in extraction efficiencies and extraction time. Microscopic analysis was performed to test the plant particle sizes after extraction. Subsequently, the developed TSURE was applied to high-throughput extraction of 19 *S. miltiorrhiza* samples collected in different regions of China. Besides, the TSURE was also applied to the other two botanical materials, extraction of ginsenosides Rb1, Rc, Rg1 and Re from *Panax quinquefolius*, and extraction of chlorogenic acid, 3,5-dicaffeoylquinic acid and 4,5-dicaffeoylquinic acid from *Lonicera japonica*. This work demonstrates the potential of TSURE method for extraction of compounds of interest in herbal medicines and opens perspectives for similar studies on pharmaceutical, cosmetic and food industries.

2. Experimental

2.1. Plant materials

S. miltiorrhiza samples were collected from 19 different regions of China. Radix samples of American ginseng (*P. quinquefolius*) were purchased from Roland Ginseng, LLC (Wausau, WI, USA), and Flos samples of *L. Japonica* was obtained from Shandong Province, China. The botanical origins of the materials were identified by the authors. The sample specimens were deposited at room temperature in the stationary storage center with accession numbers named 2013S-1 to 2013S-19, 2013P-1 and 2013L-1 in State Key Laboratory of Nature Medicines, China Pharmaceutical University.

2.2. Chemicals and reagents

Acetonitrile was of HPLC grade from Merck (Darmstadt, Germany). Deionized water was further purified by a Milli-Q system (Millipore, Milford, MA, USA). Other chemicals were of analytical grade. All solvents and samples were filtered through 0.22 μm membranes before injecting into HPLC.

Reference compounds, including salvianolic acid B, cryptotanshinone, tanshinone IIA, chlorogenic acid, 3,5-dicaffeoylquinic acid and 4,5-dicaffeoylquinic acid were bought from Must Bio-Tech Co. Ltd. (Chengdu, China). The reference ginsenosides Rg1, Re, Rb1 and Rc were purchased from Jilin University (Changchun, China). Their structures shown in Supplementary Figure S1 were further elucidated in the authors' laboratory by ^{13}C NMR and MS data. The purity of each reference compound was determined to be higher than 95% by normalization of the peak areas detected by HPLC-UV.

2.3. Apparatus

TSURE experiments were performed on a JHBE-50S Herbal Blitzkrieg Extractor (Henan Jinnai Sci-Tech Development Ltd.). The extractor contains five major parts, including integrated volt controller, lifting controller, high speed motor, tissue crushing head and extraction bottle.

Chromatographic analyses were carried out on a Shimadzu HPLC system consisting of a pump (LC-20AB), an auto-sampler (SIL-20A), UV–vis detector (SPD-20A) and automatic column temperature control oven (CTO-20AC). Separation was performed on an Amethyst C18-P column (5 μm, 4.6 mm × 250 mm). Shimadzu Labsolutions software was used for the chromatographic analysis.

Microscopic test was performed by a Nikon Eclipse 50i microscope system and analyzed by NIS-elements F 3.0 version software.

2.4. Analysis

For HPLC analysis of *S. miltiorrhiza* sample, the mobile phase consisted of 0.1% formic acid water (A) and acetonitrile (B) using a gradient elution of 27–30% B at 0–8 min, 30–70% B at 8–15 min, 70–85% B at 15–30 min and 85–100% B at 30–40 min. The detection wavelength was set at 286 nm for salvianolic acid B and 270 nm for cryptotanshinone and tanshinone IIA. The chromatographic conditions for American ginseng were using 0.025% phosphoric acid water (A) and acetonitrile (B) with a gradient elution of 19–20% B at 0–25 min, 20–40% B at 25–60 min and 40–100% B at 60–70 min. The wavelength was set at 203 nm for ginsenosides analysis. The chromatographic conditions for *L. Japonica* were using 0.1% formic acid water (A) and acetonitrile (B) as the mobile phase in a linear gradient program of 10–20% B at 0–15 min, then 20% B isocratic elution for 15 min, 20–30% B at 30–40 min and 30–100% B at 40–50 min. The detector wavelength was set at 350 nm. All the sample volume injected was 10 μl and the flow rate was 1 ml/min with column temperature at 35 °C.

2.5. Tissue-smashing based extraction

All the dried samples of *S. miltiorrhiza*, *P. quinquefolius* and *L. japonica* were pulverized into powder through a 40 mesh sieve. Sample powder and solvent were mixed in an extraction bottle. The tissue crushing head was lifted under solvent surface. The designed extraction volt can be adjusted by twisting the integrated volt controller. Accurately weighed 0.5 g powder was extracted by TSURE method using solvents at different ratios to form a homogeneous solution. The loss of the solvent was supplemented. The sample solutions were then centrifuged at 13,000 rpm for 10 min and then filtrated through 0.22 μm filters before injecting into HPLC analysis.