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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.

Keywords: Tissue-smashing based ultra-rapid extraction; High-throughput extraction; herbal medicines; quality control; ultra-rapid.

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 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 Figure 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*) was 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.

100 *2.3. Apparatus*

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

104 Chromatographic analyses were carried out on a Shimadzu HPLC system consisting of a pump
105 (LC-20AB), an auto-sampler (SIL-20A), UV/VIS detector (SPD-20A) and automatic column
106 temperature control oven (CTO-20AC). Separation was performed on an Amethyst C18-P column
107 (5 μm, 4.6×250 mm). Shimadzu Labsolutions software were used for the chromatographic
108 analysis.

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

111 *2.4. Analysis*

112 For HPLC analysis of *S. miltiorrhiza* sample, the mobile phase consisted of 0.1% formic acid
113 water (A) and acetonitrile (B) using a gradient elution of 27-30% B at 0-8 min, 30-70% B at 8-15
114 min, 70-85% B at 15-30 min and 85-100% B at 30-40 min. The detection wavelength was set at
115 286 nm for salvianolic acid B and 270 nm for cryptotanshinone and tanshinone IIA. The
116 chromatographic conditions for American ginseng were using 0.025% phosphoric acid water (A)
117 and acetonitrile (B) with a gradient elution of 19-20% B at 0-25 min, 20-40% B at 25-60 min and
118 40-100% B at 60-70 min. The wavelength was set at 203 nm for ginsenosides analysis. The
119 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.

2.6. Reference extraction procedures

UAE: Accurately weighed 0.5 g powder of *S. miltiorrhiza*, *P. quinquefolius* and *L. japonica*. The powders were then mixed with 40 ml of methanol and placed into a 150 ml conical flask. UAE was conducted on a KH-300DB digital ultrasonic cleaner (Kunshan Ultrasonic Instrument Co., Ltd. Kunshan, Jiangsu, China) with frequency set at 100 Hz for 40 min.

HRE: 0.5 g herbal powder of *S. miltiorrhiza*, *P. quinquefolius* and *L. japonica* were weighed accurately to a 100 ml round-bottom flask with 40 ml of methanol, and then the system was extracted for 120 min at 75 °C.

All of the obtained extracts were cooled to room temperature and the loss of the solvent was replenished with methanol. After centrifugation at 13,000 rpm for 10 min, the supernatant was filtered through 0.22 μm filters and then injected in to HPLC system for analysis.

3. Results and discussion

3.1. Optimization of the TSURE process

Sequential investigations of a number of main variables potentially affecting the TSURE procedure were conducted to obtain an efficient extraction. The univariate method was used to optimize the five major parameters including time, extraction volt, solid-liquid ratio, extraction solvent and number of extraction cycles. The *S. miltiorrhiza* sample from Shandong-4 was used to test extraction efficiencies. The results were graphically summarized in Figure 2.

3.1.1. Optimization of the extraction solvents

The extraction solvents in this study tested were methanol and ethanol. As shown in Figure 2, methanol offered better extraction efficiencies than ethanol, especially for water-soluble salvianolic acid B, probably because of the stronger cell wall breaking effect of methanol [22]. Addition of water as the solvents causes possible blistering under intensive stir. Thus water was not considered in this work.

3.1.2. Optimization of the extraction time

Extraction time plays a vital role in the TSURE method. To identify the optimal time, we evaluated TSURE method in a range from 0.5 to 5 min at normal temperature. Results indicated

159 that extraction time more than 2 min did not show a significant increase in the extraction efficiency
160 for salvianolic acid B, cryptotanshinone, and tanshinone IIA from *S. miltiorrhiza*. When the
161 extraction time increased to 5 min, the extraction efficiency of salvianolic acid B decreased
162 probably because of thermal degradation of the compound at an extraction temperature higher than
163 85 ºC [23]. Therefore, the optimal extraction time in this study was chosen to be 2 min.

164 *3.1.3. Optimization of the extraction volt*

165 TSURE method uses a high speed motor to deliver energy to the tissue crushing head in
166 contact with the samples and solvents. Larger the extraction volt is, higher speed the head achieves.
167 Extraction volt was evaluated in a range from 90-110 V with 2-min extraction time. The extraction
168 efficiencies at 100 V were better than those at 90 V and 110 V for all the three target compounds.
169 Similarly, the extraction volt higher than 100 V resulted in an obvious decrease of the yield of
170 salvianolic acid B because of its thermal instability in the case of increased temperature to 90 ºC
171 induced by high-speed stir, indicating the difficulty to control temperature at high volt.

172 *3.1.4. Optimization of the solid-liquid ratio*

173 Another major step in the development of the TSURE method was to select a suitable
174 solid-liquid ratio. In this study, ratios varying from 1:40 to 1:120 were investigated. As shown in
175 Figure 2, a solid-liquid ratio at 1:80 was observed to achieve acceptable efficiency for the TSURE
176 procedure and no significant improvement was indicated with a higher ratio at 1:100 and 1:120.

177 *3.1.5. Optimization of the extraction cycles*

Selection of a suitable extraction cycle is critical in this study. Data are shown in Figure 2. Extraction cycle of the TSURE method is usually conducted once. With a purpose to increase the extraction yield of the three analytes, the TSURE method was performed for a total of 1, 2 or 3 cycles. As seen in Figure 2, no significant difference was observed with multiple extraction cycles than once. To save the time and solvents, the optimal extraction cycle was once.

On the basis of these results, the optimized conditions for TSURE method were established using methanol as the solvent, a solid/liquid ratio at 1:80, an extraction volt at 100 V, and a single extraction for 2 min.

3.2. Comparison of the TSURE approach with the reference extraction procedure

The performance of TSURE was compared with HRE and UAE in terms of extraction efficiency and extraction time. They were conducted at the optimal conditions according to the experimental section from the 2010th Chinese Pharmacopoeia [24]. The concentrations of the three target compounds extracted by the three methods were compared in Table 1. As shown in Table 1, in reference to the students' *t* test, the total yield obtained between the TSURE and HRE showed no significant difference with a *p*-value 0.12. The efficiency of the UAE was significantly lower than that of TSURE method with a *p*-value < 0.05. Similar conclusion was also observed from the results obtained in other two herbal materials, *P. quinquefolius*, and *L. japonica*. This is probably because the TSURE method combines the process of smashing, vibration and stir together to extract the bioactive ingredients in herbal materials. However, in UAE procedure, the smashing and stirring process were not contained. At the late stage of UAE, there was equilibrium of extraction for the solvent that was unchanged. The UAE is thus usually considered as a moderate extraction

technique.

The advantages of TSURE method over HRE are consumption of less time, low energy required and normal temperature operation. First, the extraction time was shortened from 120 min with HRE to 2 min with TSURE method, providing an ultra-rapid alternation for qualification and quantification of *S. miltiorrhiza*. Second, TSURE was operated at normal temperature. Though stir process will generate some heat, the temperature remains under 50 °C. For compounds like volatile oil or semivolatile organics, TSURE method may be a good choice. We demonstrate here that the extraction of salvianolic acid B, cryptotanshinone and tanshinone IIA in *S. miltiorrhiza* using TSURE occupied higher or equal extraction efficiency as compared to the conventional methods with a high-speed extraction process. Supplementary Figure S2 showed no significant difference of the chemical components in *S. miltiorrhiza* after TSURE, UAE and HRE, indicating the stability of ingredients during TSURE process.

3.3. Method validation

All compounds determined by HPLC were identified by comparison of retention times with those of the reference compounds. In addition, spiking the samples with the standard compounds further confirmed the identities of these peaks. Typical chromatograms obtained from *S. miltiorrhiza*, *P. quinquefolius*, and *L. japonica* extracted by TSURE were shown in Figure 3. To validate the novel TSURE-HPLC method, the linearity, repeatability, recovery and detection limits were investigated under the optimal condition.

Calibration curves for salvianolic acid B, cryptotanshinone and tanshinone IIA were

219 constructed using the areas of the chromatographic peaks determined at six increasing
220 concentrations. As shown in Table 2, a good linearity was observed with $r^2 > 0.999$ within test
221 ranges for the three analytes. Limits of detection and quantification (LODs and LOQs) were
222 calculated at 6 times based on the signal-to-noise ratio of 3 and 10. The LODs and LOQs were 0.32
223 ng and 1.60 ng for salvianolic acid B, 0.03 ng and 0.06 ng for cryptotanshinone, 0.02 ng and 0.10
224 ng for tanshinone IIA. As consistent with publications, tanshinones are better than salvianolic acids
225 in the sensitivity detected by UV [25]. The repeatability was estimated by five repetitive samples
226 obtained by TSURE. As Table 3 shows, the RSDs of the analytes were in the range of 1.6-2.2%,
227 indicating that the repeatability of the method is acceptable.

228 Recovery test of the optimized TSURE procedure was examined by analyzing spiked *S.*
229 *miltiorrhiza* samples with certificated low, medium and high concentrations for salvianolic acid B,
230 cryptotanshinone and tanshinone IIA. As presented in Table 3, excellent recoveries in a range of
231 93-106% were observed with a relative standard deviation (RSD) of 0.30-4.81% for the
232 Shandong-4 samples, suggesting that the proposed TSURE method is reliable.

233 *3.4. Application of the TSURE method to 19 S. miltiorrhiza samples and other herbs*

234 Figure 4 summarized the results of extraction yields of salvianolic acid B, cryptotanshinone
235 and tanshinone IIA using TSURE in 19 *S. miltiorrhiza* samples cultivated in different regions of
236 China. To test the universality of TSURE, the present method was applied to extraction of two
237 different botanical materials, roots of *P. quinquefolius* and flowers of *L. japonica*. As the data
238 shows, the concentrations of the constituents varied remarkably among the 19 *Salvia* samples. The
239 concentration ranged from 6.1 mg/g to 47.1 mg/g for salvianolic acid B, 0.1 mg/g to 2.0 mg/g for

cryptotanshinone, and 0.7 mg/g to 2.6 mg/g for tanshinone IIA. Quality control is of great importance since the chemical content of herbal materials differs, depending on the geographic information of the locations and even the season of its collection, and the subsequent processing. As shown in Figure 4, 4 *Salvia* samples collected from Gansu Province possess the lowest yield of target compounds comparing with other regions. This may because the dry climate in Gansu is unfavorable for the compounds enrichment in *S. miltiorrhiza*.

For *P. quinquefolius*, we focused on four major bioactive ginsenosides, Rg1, Re, Rb1 and Rc. As shown in Supplementary Figure S 3(A), TSURE method showed no significant differences in extraction efficiency compared with UAE (p= 0.78), but a little lower than HRE in the extraction efficiency of ginsenoside Rb1 (p= 0.002). The possible reason is that in HRE, the use of heat may cause an increase in the solubility of materials and rate of mass transfer. Moreover, ginsenosides are relatively not easily dissolved in organic solvents such as methanol. As shown in Supplementary Figure S 3(B), for *L. japonica*, no significant differences were observed in extraction efficiency for chlorogenic acid, 3,5- dicaffeoylquinic acid, and 4,5-dicaffeoylquinic acid between TSURE and UAE (p= 0.46). HRE showed higher extraction efficiency than TSURE for chlorogenic acid (p= 0.0003).

3.5. Mechanism of TSURE

3.5.1. Tissue-smashing effect during TSURE

To further investigate the mechanism of TSURE, microstructure analysis was used. Figure 5 indicated that the effects of different extraction methods on herbal particle sizes of *S. miltiorrhiza*.

Figure 5(A), 5(B) and 5(C) showed the microscopic graphs of samples after HRE, UAE, and TESRE respectively. In order to get a microscopic image without bubbles, the particles were soaked in cold water overnight. The Krummbein diameter of particles photographed in wet status after HRE, UAE and TSURE were offered as 600-1200 μm for HRE and UAE and 50-80 μm for TSURE, respectively. In this method, the rotating speed of tissue crushing head can achieve 15000-30000 r/min. With the ultra-high speed, sample tissues are crushed into smaller size in a moment.

3.5.2. Drastic stir

During the dissection between high-speed inner and stable outer edges, a strong vortex in the center of inner edge was developed to drive the stir of crushed samples, inducing a rapid concentration change to the whole system. With smaller particles, rapid exposure and transfer of extracted sample molecules to solvent environment will occur. In TSURE, an alternation of balance and unbalance between solvent and material particles was proceeded to achieve the final thorough smashing and fully balanced extraction.

3.5.3. Ultra-rapid dynamic molecular permeation

In working status, the whole instrument was an ultra-dynamic system. Due to the high-speed rolling between the inner and stable outer edges, a vortex negative pressure was developed and the dissection of samples occurred. Under this negative pressure, molecular permeation was observed between the inner and outer side of the outer edge, meaning that the smashed or extracted molecules were surrounded, dissociated and replaced by solvents. Finally, the molecules would be

separated from herbal materials to finish the extraction process.

3.5.4. Strong vibration effect

It is believed that ultrasonic wave can accelerate maceration process [26]. The destructive effect of vibration on the plant tissue and dispersion system had been well studied [27]. This instrument can produce a vibration equivalent to 1/60 of the ultrasonic wave with a high speed rotation [16]. Undoubtedly, the solubility equilibrium between the inner and outer smashed sample particles can be strongly accelerated with the vibration effect.

4. Conclusions

In this work, a simple tissue-smashing based ultra-rapid extraction method proved to be efficient and validated to extract chemical constituents from herbal materials. Comparing with the conventional methods, TSURE provides excellent acceleration of the extraction process and higher or equal extraction yields with the optimized conditions. The big noise generated by the instrument is a major drawback that should be overcome in the future research. In addition, this promising extraction method offers an alternative reference for the application of scale-up production in pharmaceutical, food and cosmetic industries.

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376 **Figure Legends**

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

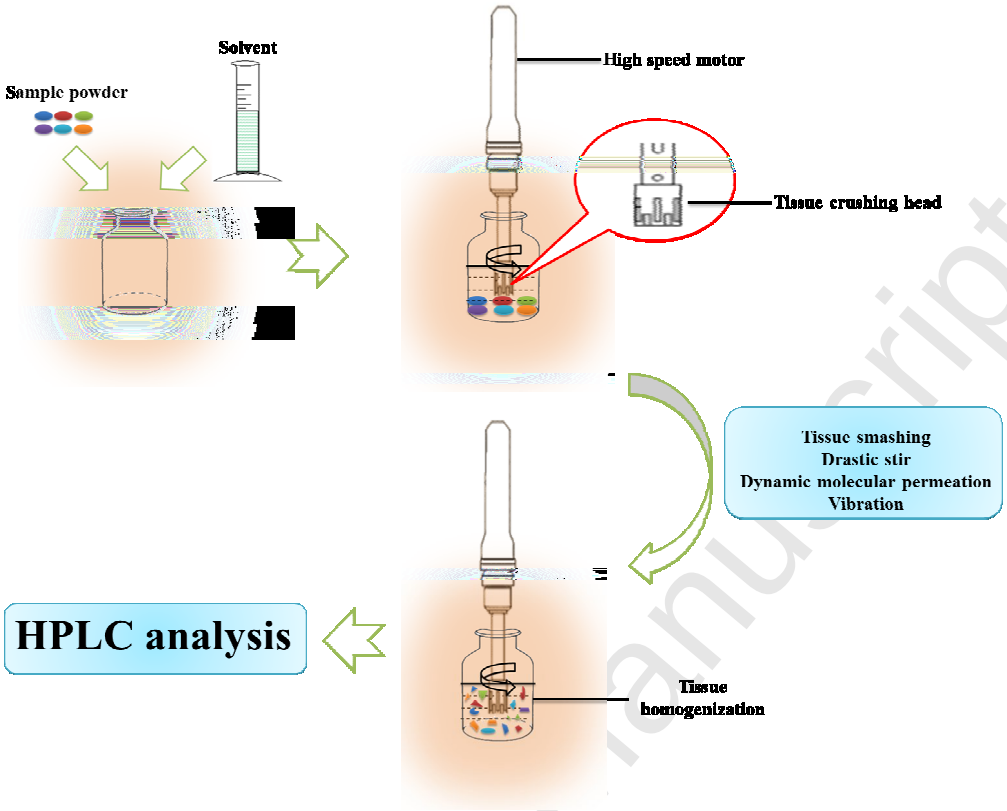
380 **Figure 2.** Extraction efficiencies of three target analytes in *S. miltiorrhiza* under different
381 conditions. The condition with an asterisk was chosen for the optimized TSURE procedure. The
382 error bars represent standard deviation of the triple analyses.

383 **Figure 3.** Representative HPLC chromatogram of *S. miltiorrhiza* (A), *P. quinquefolius* (B), and *L.*
384 *japonica* (C) extracted using TSURE (peak 1, salvianolic acid B; peak 2, cryptotanshinone; peak
385 3, tanshinone IIA; peak 4, ginsenoside Rg1; peak 5, ginsenoside Re; peak 6, ginsenoside Rb1;
386 peak 7, ginsenoside Rc; peak 8, chlorogenic acid; peak 9, 3,5-dicaffeoylquinic acid; peak 10,
387 4,5-dicaffeoylquinic acid).

388 **Figure 4.** Extraction efficiencies of three target analytes in 19 individual *S. miltiorrhiza* samples
389 collected from different regions of China by TSURE method. The error bars represent standard
390 deviation of triple analyses.

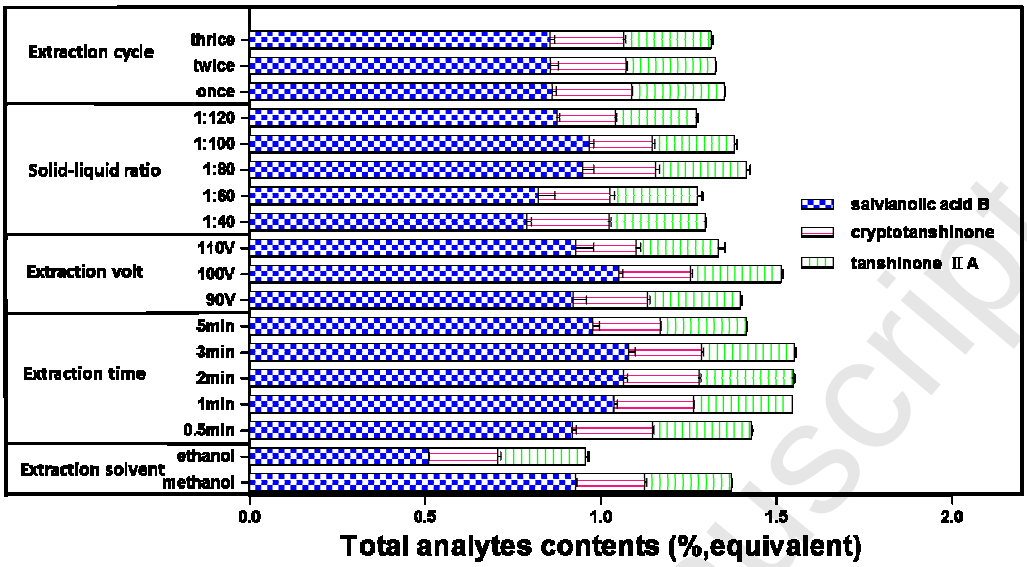
391 **Figure 5.** Micrographs of *S. miltiorrhiza* particles after different extraction methods: (A) HRE, (B)
392 UAE, (C) TSURE. The images were obtained using a Nikon Eclipse 50i microscope system.

393



393
394 **Figure 1**

395



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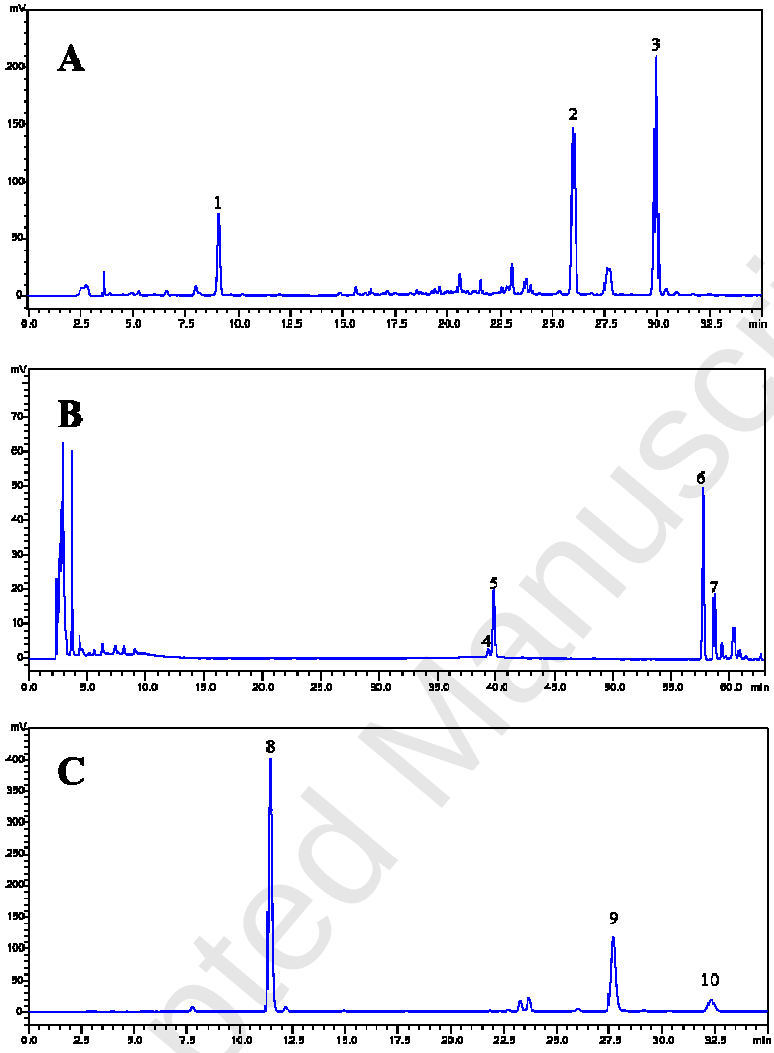
396 **Figure 2**

397



397

398 **Figure 3**

399

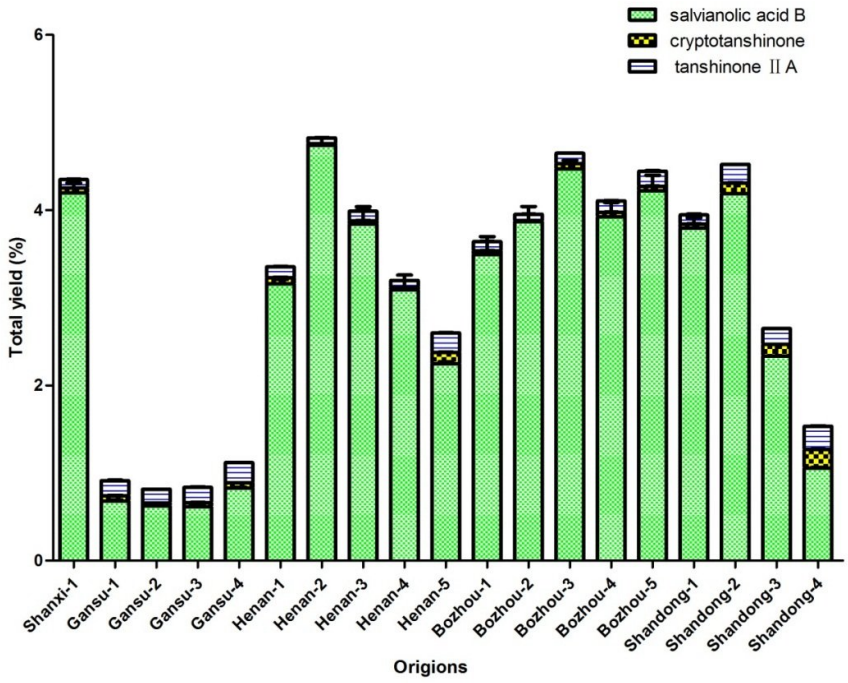
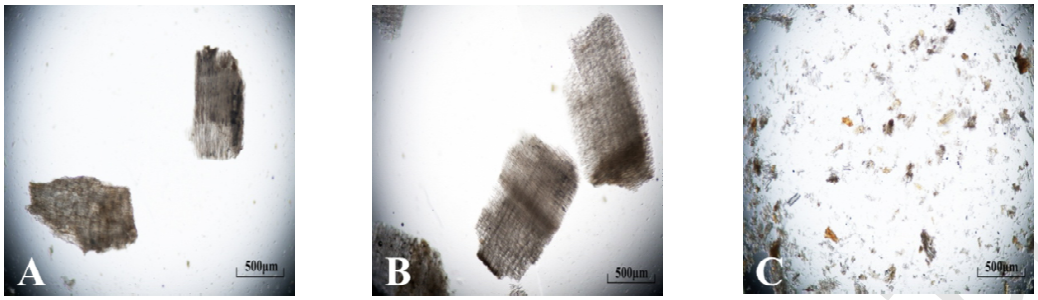


Figure 4



401

402 **Figure 5**

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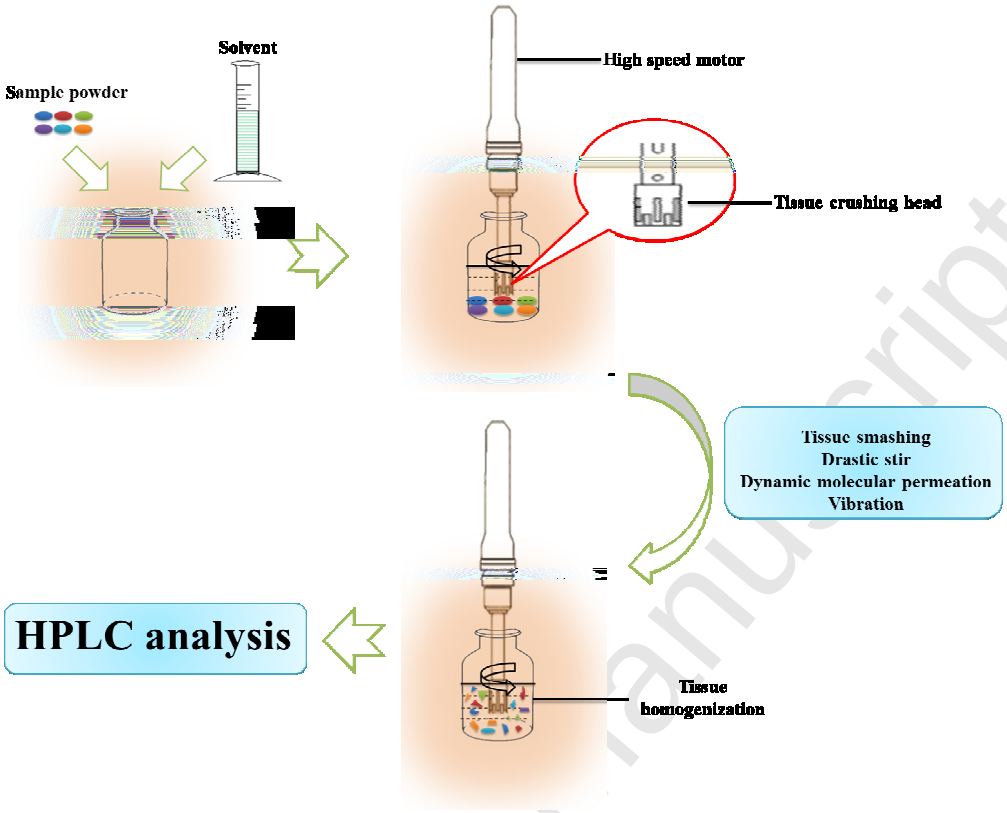
404



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Highlights

- Three chemical constituents from *Salvia Miltiorrhiza* were extracted by new designed TSURE
- Optimized TSURE offered good extraction efficiency that was comparable or even better than conventional methods
- TSURE method was extended to the quality control of *Salvia Miltiorrhiza*
- Application of TSURE to other herbal medicines was also explored and similar results were discovered
- Mechanisms of TSURE were discussed in detail for the first time

Table 1

Comparison of extraction efficiencies of target compounds in *S. miltiorrhiza* by TSURE and conventional extraction methods under the optimal conditions (mean ± S.D., n = 6).

Method	Extraction time(min)	Extraction volume (ml/g)	Salvianolic acid B (mg/g)	Cryptotan--shinone (mg/g)	Tanshinone IIA (mg/g)	Total yield (mg/g)	RSD (%)
TSURE	2	80	1.158±0.031	0.208±0.056	0.291±0.106	1.657±0.077	4.673
UAE	40	80	0.950±0.016	0.208±0.007	0.286±0.005	1.444±0.042	2.877
HRE	120	80	1.083±0.018	0.298±0.003	0.270±0.004	1.589±0.039	2.426

TSURE: tissue-smashing based ultra-rapid extraction;
UAE: ultrasonic assisted extraction;
HRE: heat reflux extraction

422 **Table 2**

423 Calibration curves, test range, and LODs for 3 analytes in *S. miltiorrhiza* by LC-UV.

No.	Analytes	Calibration curve	r^2	Linear range (μg)	LOQs (ng)	LODs (ng)
1	salvianolic acid B	y = 11962532x - 496388	0.9991	0.32-10.10	1.60	0.32
2	cryptotanshinone	y = 51551852x + 208610	0.9996	0.13-4.30	0.06	0.03
3	tanshinone IIA	y = 59718035x + 102353	0.9996	0.07-2.10	0.10	0.02

424 LOD: The limitation of detection (*S/N* > 3); LOQ: The limitation of quantification (*S/N* > 10)

425

Table 3

The precisions and recoveries of 3 target components in *S. miltiorrhiza* obtained by TSURE method.

Analytes	Repeatability (n=5, %)	Recovery (n=3)				
		O (mg)	S (mg)	F (mg)	Recovery (%)	RSD (%)
Salvianolic acid B	1.65	2.16	1.60	3.71	97.07	2.60
		2.16	2.15	4.36	102.43	0.30
		2.15	3.20	5.31	98.53	1.86
cryptotanshinone	1.90	0.50	0.25	0.74	93.47	1.99
		0.50	0.49	0.99	100.53	4.81
		0.57	0.73	1.29	99.33	2.13
tanshinone IIA	2.18	0.52	0.38	0.92	105.20	2.63
		0.65	0.64	1.27	96.00	3.71
		0.66	0.89	1.56	101.60	1.53

O: original contained; S: spiked; F: found