Optimization of Smashing Tissue Extraction Technology of *Schisandra chinensis* Fruits by Orthogonal Test

TANG Yun[1], LIU Yan-ze[2, 3], HAN Ling[4], ZHAO Yu-qing[1, 5*]

1. Shenyang Pharmaceutical University, Shenyang 110016, China
2. Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences, Beijing 100193, China
3. Bio-organic and Natural Product Laboratory, McLean Hospital/Harvard Medical School, MA 02478, USA
4. Liaoning Benxi Third Pharmaceuticals Co., Ltd., Benxi 117004, China
5. Key Laboratory of Structure-Based Drug Design & Discovery, Ministry of Education, Shenyang Pharmaceutical University, Shenyang 110016, China

Abstract: **Objective** To optimize the extract technology of active lignins from the fruits of *Schisandra chinensis*. **Methods** The content of schizandrin, gomisin A, and deoxyschizandrin were selected as standards to evaluate the efficiency of smashing tissue extraction (STE). Solid-liquid ratio, extracting times, ethanol concentration, and extracting time were investigated through orthogonal test. **Results** The optimized conditions for STE were ten times amount of 80% EtOH, extracting for three times, and 2 min for each time. **Conclusion** STE could obtain relatively higher yield, simplicity of operation, and benefit for environment protection. It could be better choice for the extraction of *S. chinensis*.

Key words: deoxyschizandrin; gomisin A; orthogonal test; *Schisandra chinensis*; schizandrin; smashing tissue extraction
DOI: 10.3969/j.issn.1674-6384.2012.03.014

Introduction

Extraction is a very fundamental and necessary step in full utilization of active ingredient from crude drug, whatever for manufacturing formulated patent medicines, such as tablet, pill, paste, capsule, instant powder, and tincture, etc, or preparing any single herbal extract for pharmacological, phytochemical, and analytical researches. To make clinical decoction, herbal tea, and herbal wine also needs the extraction process. Traditionally to finish an extraction procedure needs hours to days, e.g., boiling extraction, refluxing extraction, Soxhlet extraction, soaking extraction, and so on. Even for ultrasonic extraction, 30 min once for several times is usually required. Recently developed extraction technology called smashing tissue extraction (STE) significantly improved the efficiency by shortening the time to seconds to minutes, and achieved quite success in several herbal cases (Han *et al*, 2011; Dai, Tan, and Zhou, 2006; Li and Liu, 2011; Sun *et al*, 2011; Liu *et al*, 2011; Tang, Liu, and Zhao, 2012). Another remarkable advantage is that STE is operated under room temperature. So, further investigations on STE for different herbal medicines containing different types of active compounds are very interesting and attractable.

Schisandra chinensis (Turcz.) Baill, an important traditional Chinese medicine and edible product, has been used for 2000 years and has the effects of convergence and astringency, benefiting vital energy and promoting the production of body fluid, invigorating the kidney, and calming down the mood (Dai, Tan, and Zhou, 2006). It was reported that lignanoids had apparent effect on tranquilizing nerve centre. Therefore, in *Chinese Pharmacopoeia 2010* (Pharmacopeia Committee of P. R. China, 2010), HPLC was established to determine the content of schisandrin which was the marker component of *S. chinensis* fruits.

STE could finish the extraction within seconds or minutes, which is just one tenth to one hundredth of

* Corresponding author: Zhao YQ Address: College of Chinese Materia Medica, Shenyang Pharmaceutical University, No.103, Wenhua Road, Shenhe District, Shenyang 110016, China Tel: +86-24-2398 6523 Fax: +86-24-2398 6522 E-mail: zyq4885@126.com
Received: October 26, 2011; Revised: January 18, 2012; Accepted: March 2, 2012
Fund: E&T modern center for Natural Products of Liaoning Province of China (2008402021)
Online time: July 18, 2012 Online website: http://www.cnki.net/kcms/detail/12.11410.R.20120718.1733.003.html

ordinary methods, such as refluxing extraction. STE not only avoids the decomposition of heat-sensitive ingredients, but also supplies relatively higher yield, simplicity of operation, and benefit to environment protection (Liu, 2007).

For the first time this study reports the utilization of STE to optimize the extracting technology of *S. chinensis* fruits by orthogonal test. The data presented here provide the reference for manufacture.

Materials and methods

Chemicals and reagents

Schizandrin reference substance (110857-200406) and deoxyschizandrin reference substance (0764-200107) were obtained from National Institute for Food and Drug Control; Gomisin A reference substance was prepared in the laboratory (purity > 99%); *Schisandra chinensis* (Turcz.) Baill was purchased from Liaoning Benxi Third Pharmaceuticals and identified by Prof. SUN Qi-shi in Shenyang Pharmaceutical University; Methanol was of chromatographic grade, water was ultrapure water, and other reagents were of analytical grade.

Apparatus

JHBE—50S Herbal Blitzkrieg Extractor (Henan Jinnai Sci-Tech Development Co., Ltd., China); BS—124S Precise Electronic Balance (Sartorius Corporation); RE—52A Rotary Evaporator (Shanghai Yarong Shenghua Instrument Factory); Germany Knauer high performance liquid chromatogram (UV Detector K-2501 and attemperator); Eurochrom for windows chromatography work station; Eurospher 100-5 C_{18} (250 mm × 4 mm, 5 μm) chromatographic column with precolum.

Chromatographic conditions and system suitability

The mobile phase was methanol-water (73:27); The flow rate was 1.0 mL/min; The detection wavelength was 250 nm; The column temperature was 30 ℃. Mixed reference substances solution and sample solution were injected to HPLC, respectively, under the chromatographic conditions described above, the resolution > 1.5, number of theoretical plates > 3500, tailing factor between 0.95 — 1.05, and HPLC chromatograms are shown in Fig. 1.

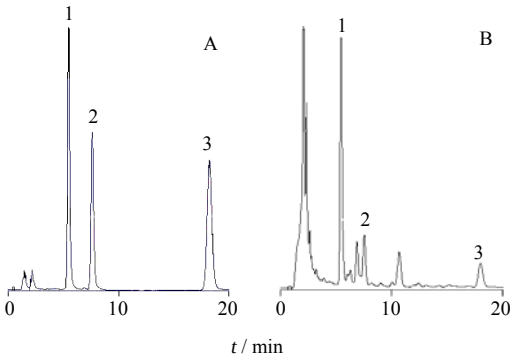

Fig. 1 HPLC chromatograms of mixed reference substances (A) and sample (B)

1: schizandrin 2: gomisin A 3: deoxyschizandrin

Preparation of standard solution

Schizandrin reference substance (5.2 mg), gomisin A reference substance (5.0 mg), and deoxyschizandrin reference substance (5.2 mg) were respectively dissolved in methanol and adjusted to 5 mL in 5 mL volumetric flasks; Gomisin A (1 mL) and deoxyschizandrin (1 mL) solutions were dissolved in methanol and adjusted to 5 mL in a 5 mL volumetric flask. The reference solution was filtrated separately with 0.45 μm filter before injection to HPLC, separately.

Sample preparation

The $L_9(3^4)$ orthogonal test was adopted (Table 1). Nine samples of *S. chinensis* fruits were extracted by STE according to the orthogonal design. Ethanol solution obtained by filtration was concentrated to dry under reduced pressure. The residue was dissolved in methanol and diluted to 100 mL in a volumetric flask, and the sample solution was filtrated with 0.45 μm filter before injecting to HPLC.

Linear correlation

Reference solutions of schizandrin (2, 4, 6, 8, 10, and 13 μL) were determined by HPLC, respectively,

Table 1 Factors and levels of $L_9(3^4)$ orthogonal test

Levels	Factors			
	Solid-liquid ratio	Extracting times	Extracting time / min	Ethanol concentration / %
1	6	1	1	75
2	8	2	2	80
3	10	3	3	85

and the standard curve of peak area as vertical coordinate and the corresponding content as abscissa were prepared. A good linear correlation (peak area *vs* injected amount) was observed in the range of 2.08—13.52 μg of schizandrin. The equation of calibration curve was $Y = 33.507X + 2.8493$ ($R^2 = 0.9991$); Reference solutions of gomisin A (3, 6, 9, 12, 15, and 18 μL) were determined by HPLC, separately, and the standard curve of peak area as vertical coordinate and the corresponding content as abscissa were prepared. A good linear correlation of gomisin A (peak area *vs* injected amount) was observed in the range of 0.6—3.6 μg. The equation of calibration curve was $Y = 26.038X - 0.078$ ($R^2 = 0.9993$); Reference solutions of deoxyschizandrin (2, 4, 6, 8, 10, and 12 μL) were determined by HPLC, respectively, and the standard curve of peak area as vertical coordinate and the corresponding content as abscissa was prepared. A good linear correlation of deoxyschizandrin (peak area *vs* injected amount) was observed in the range of 0.416—2.496 μg. The equation of calibration curve was $Y = 41.885X - 2.0494$ ($R^2 = 0.9992$).

Precision

The precision of the assay was determined by five injections of 20 μL No. 7 STE sample, repeatedly. RSD values of peak area for schizandrin, gomisin A, and deoxyschizandrin were 1.63%, 1.53%, and 0.92%, respectively. The results indicated that the standard curve had a high precision.

Stability

The stability of the assay was determined by six consecutive injections (every 2 h) of No. 7 STE sample. RSD values of peak area for schizandrin, gomisin A, and deoxyschizandrin were 0.55%, 0.50%, and 1.31%, respectively. The results showed that this method had an excellent stability within 10 h.

Repeatability

Five batches of *S. chinensis* fruits were extracted by using STE according to the same method as No. 7 STE sample. These samples were analyzed under the conditions described above. RSD values of peak areas for schizandrin, gomisin A, and deoxyschizandrin were 0.26%, 0.88%, and 0.80%, respectively, with a good repeatability.

Recovery

A certain amount of schizandrin, gomisin A, and deoxyschizandrin reference solution was mixed with the sample with known contents of schizandrin, gomisin A, and deoxyschizandrin at high, medium, and low concentration. Then, the mixed samples were analyzed under the conditions described above. The contents of schizandrin, gomisin A, and deoxy- schizandrin reference solution were calculated through one point external standard method. Average recovery was 99.06%, 101.5%, and 101.4% respectively, and RSD values were 1.81%, 2.06%, and 2.45%, respectively.

Results

The results of $L_9(3^4)$ orthogonal test were in Table 2. The optimum STE conditions for *S. chinensis* fruits were obtained as follows: solvent-to-feed ratio 10, 80% ethanol, three times, 2 min for each time.

Table 2 Results of $L_9(3^4)$ orthogonal test

No.	Solvent-to-feed ratio	Extracting times	Extracting time / min	Ethanol concentration / %	Content / ($mg \cdot g^{-1}$)
1	6	1	1	75	2.639
2	6	2	2	80	4.822
3	6	3	3	85	3.419
4	8	1	2	85	1.865
5	8	2	3	75	3.784
6	8	3	1	80	4.300
7	10	1	3	80	4.160
8	10	2	1	85	5.089
9	10	3	2	75	6.044
K_1	10.88	8.664	12.03	12.47	
K_2	9.949	13.70	12.73	13.28	
K_3	15.29	13.76	11.36	10.37	
R	5.344	5.099	1.368	2.909	

Verification test

S. chinensis fruits (10 g) were extracted at the optimum STE conditions. The contents of schizandrin, gomisin A, and deoxyschizandrin were 3.688, 1.138, and 0.6412 mg/g. And the total content was 5.467 mg/g.

Discussion

STE has been successfully applied to the extraction of active ingredients in *S. chinensis* fruits for the first time. The optimized conditions for STE extraction were 10 times amount of 80% EtOH, extracting for three times, 2 min for each time. These data demonstrate that STE could efficiently extract materials in a few minutes, which is impossible for other extraction methods; STE is running at room temperature, so it is more suitable for extracting heat-sensitive ingredients.

Overall, it proved that STE combining smash, soak, stir, and vibration together is an effective and practicable extraction technology.

References

Dai HF, Tan NH, Zhou J, 2006. Glycosides from *Schisandra chinensis*. *Chin J Nat Med* 4(1): 49-51.

Han B, Zhang LS, Liu XQ, Zou QP, 2011. Smashing tissue extraction technology of emulsion from *Armeniacae Amarum Semen*. *Drugs Clin* 26(2): 134-138.

Li JY, Liu YZ, 2011. Progress of smashing tissue extraction in research of Chinese materia medica. *Chin Tradit Herb Drugs* 42(10): 2145-2149.

Liu YZ, 2007. Principle and practice of smashing tissue extraction and herbal blitzkrieg extractor. *Chin J Nat Med* 5(6): 401.

Liu YZ, Gao WQ, Wang JW, Zhang Y, Zhao YQ, 2011. Smashing tissue extraction and HPLC determination of paclitaxel and 10-deacetylbaccatin from *Taxus x media*. *Chin Herb Med* 3(3): 235-238.

Pharmacopeia Committee of P. R. China, 2010. *Chinese Pharmacopoeia* (Vol. 1). Chemical Industry Publishing Company: Beijing.

Sun YL, Liu YZ, Xiao H, Wei YF, Zhao YQ, 2011. Smashing tissue extraction and GC analysis of active fatty acids from oil cake of perilla seeds. *Chin Herb Med* 3(1): 75-78.

Tang WZ, Liu YZ, Zhao YQ, 2012. A new homogenizing technology to obtain rosmarinic acid from perilla oil meal. *Chin Herb Med* 4(1): 70-73.