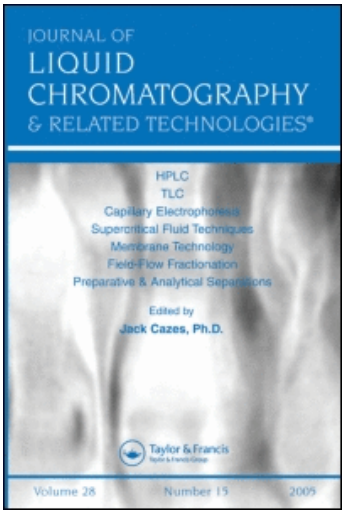

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Microwave Assisted Extraction of Major Active Ingredients in *Panax quinquefolium* L

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Microwave Assisted Extraction of Major Active Ingredients in *Panax quinquefolium* L.

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ABSTRACT

A detailed procedure to extract ginsenoside R_{b1}, ginsenoside R_c, and ginsenoside R_d from *Panax quinquefolium* L. was developed by using an obturated microwave assisted extraction (MAE) device with a pressure controlling unit. The compounds were analyzed by HPLC. The major parameters that influence the extraction efficiency were optimized. Ethanol, 50%, 1 : 40 solid/liquid ratio, 100–140 mesh particle sample size, and 3 min extraction time were chosen. MAE was 60 times more efficient in operation time than that of the Soxhlet extraction, and 20 times more efficient than that of the ultrasonic extraction. The solvent consumption

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also was greatly reduced. MAE was proven to be a rapid and reliable sample preparation method for ginsenosides.

Key Words: Microwave assisted extraction (MAE); *Panax quinquefolium* L; Ginsenosides; HPLC.

INTRODUCTION

Panax quinquefolium L. is one of the most commonly used Chinese herbals. Its major active ingredients have been found to be neutral ginsenosides and classified as ginsenosides R_{b1}, R_{b2}, R_c, R_d, R_e, R_{g1}, R_{b1} and R_c count for 70–80% of the total ginsenosides of the ginseng from British Columbia, Canada.[1] High-performance liquid chromatography has been considered as a powerful tool for the separation and determination of various components of ginsenosides. However, sample pre-treatments of ginsenosides usually were required to remove the complicated matrix interference. Soxhlet extraction, as one of the most commonly used sample pre-treatment methods, was used to extract the ginsenosides from *P. quinquefolium* L., but it was time-consuming and labor intensive.[2]

Microwave assisted extraction (MAE) is a solvent extraction method using microwave energy to create localized super heat and raise solvent temperature, and significantly increases the diffusion and extraction rates.[3] Therefore, MAE greatly improves extraction yield and selectivity, and reduces the extraction time and solvent consumption.[4] It has wide application for the extraction of major active ingredients of Chinese herbal medicines, such as extraction of glycyrrhizic acid from licorice root,[5] taxanes from taxus biomass,[6] felodipine tablets,[7] and tanshinones from *Salvia miltiorrhiza* Bunge.[8,9] In this paper, the influence of MAE parameters on extraction yields of ginsenoside R_{b1}, ginsenoside R_c, and ginsenoside R_d from *P. quinquefolium* L. was studied. The results of MAE were compared with Soxhlet extraction and ultrasonic extraction.

EXPERIMENTAL

Apparatus

A MSP-100 Microwave sample preparation system with pressure control unit (Beijing Rayme Sci & Tech Co. Ltd., Beijing, China) was used. The output power of the microwave oven is 850 W. The HPLC 1100 system (Hewlett-Packard, USA) consisted of a pre-clean up kit (Agilent, USA), an

ODS-C_{18} Column (150 mm × 4.6 mm i.d.) and an UV-detector. The separation was monitored at 203 nm.

Reagents and Materials

Ethanol and KH_2PO_4 were analytical reagent grade. Acetonitrile used in the HPLC analysis was chromatographic reagent grade. Ginsenoside R_{b1}, ginsenoside R_c, and ginsenoside R_d standards were purchased from the China Institute for Drugs and Biological Products Identification, Beijing.

Extraction Procedures

The root of *P. quinquefolium* L. was ground and sieved into different sizes. MAE: 0.5 g of the pieces of *P. quinquefolium* L. (100–140 mesh) was mixed with 20 mL appropriate solvent, then irradiated at the desired microwave power and irradiating time. Ultrasonic extraction: 0.7 g of the sample was placed in a 50 mL conical flask, followed by the addition of 40 mL solvent and processed for 60 min. Soxhlet extraction: 1.5 g sample was extracted with 40 mL appropriate solvent for at least 4 hr by the Soxhlet method.

HPLC Analysis

The suspensions obtained by MAE, ultrasonic extraction, and Soxhlet extraction were centrifuged and filtered through a 0.34-μm membrane, and loaded on the ODS column for HPLC assay. The mobile phase was acetonitrile–KH_2PO_4 (30 : 70, v/v), flow-rate was 0.3 mL/min. The complete baseline separation was obtained with the retention time of 7.9, 10.5, and 22.2 min for ginsenoside R_{b1}, ginsenoside R_c, and ginsenoside R_d, respectively (Fig. 1). The detector responses were linear in the range of 0.5–5.0 μg with the calibration equations of $Y = 8671.7X + 1.2949$ ($r = 0.9997$, $n = 6$) for ginsenoside R_{b1}, $Y = 6352.7X + 5.9748$ ($r = 0.9996$, $n = 6$) for ginsenoside R_c, and $Y = 5265.3X + 6.124$ ($r = 0.9999$, $n = 6$) for ginsenoside R_d, respectively.

The extraction yield of ginsenosides was calculated by the following equation:

$$\text{Extraction yield (\%, w/w)} = \frac{\text{Mass of gensenoside in extracted solution}}{\text{Mass of panax quinquefolium L.sample}} \times 100\%$$

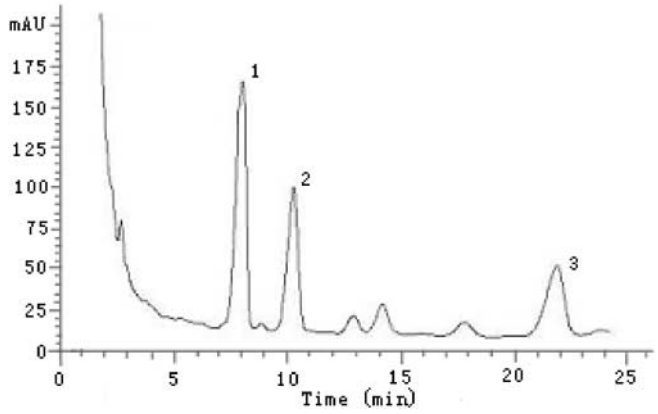

Figure 1. Separation of *P. quinquefolium* L. after MAE. 1, Ginsenoside R_{b1}; 2, ginsenoside R_c; 3, ginsenoside R_d. Detection at 203 nm.

RESULTS AND DISCUSSION

Effect of Ethanol on Ginsenosides Extraction

Ethanol is a non-toxic and water miscible solvent, and was used in ginsenosides extraction. In the MAE method, the water in the ethanol solution was easy to penetrate into the *P. quinquefolium* L. and enhanced the absorbtion of microwave energy. Therefore, the mass transfer of ginsenosides from the sample pieces to the solution was enhanced. This was confirmed by the result that the extraction yield of ginsenosides in water–ethanol solution is higher than that in pure ethanol (Fig. 2). The highest extraction yield of ginsenosides was obtained at 50% (v/v) ethanol to water solution. The 50% (v/v) ethanol aqueous solution was chosen as the extraction solvent.

Effect of MAE Time on Ginsenosides Extraction

The extraction yield of ginsenosides increased with longer MAE extraction time (Fig. 3). MAE reached the highest extraction yield at 3 min. No apparent improvement of extraction yield was observed when the MAE time was further increased up to 5 min. Long MAE times (>5 min) should

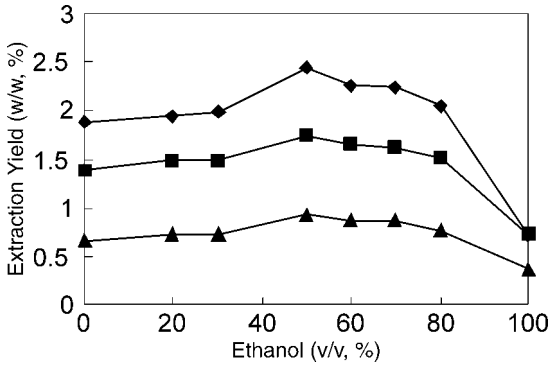

Figure 2. Effect of ethanol on extraction. Sample particle, 100–140 mesh; extraction time, 3 min; solid/liquid, 1 : 40. *Key*: ◆, Ginsenoside R_{b1}; ■, ginsenoside R_c; ▲, ginsenoside R_d.

be avoided since ginsenosides could be decomposed at high temperatures. Extraction time of 3 min was used in this study.

Effect of Solid/Liquid Ratio on Ginsenosides Extraction

The ratio of the amount of sample to the used solvent was defined as the solid/liquid ratio. Figure 3 showed the extraction yield of ginsenosides increasing with enhanced solid/liquid ratios. The solid/liquid ratio of

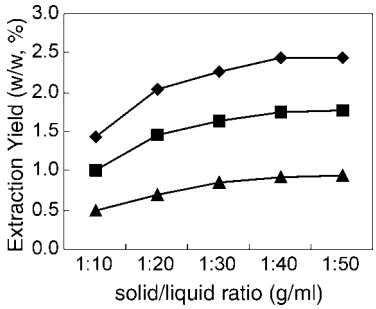

Figure 3. Effect of solid/liquid ratios on extraction. Sample particle, 100–140 mesh; 50% ethanol (v/v); extraction time, 3 min. *Key*: ◆, Ginsenoside R_{b1}, ■, ginsenoside R_c, ▲, ginsenoside R_d.

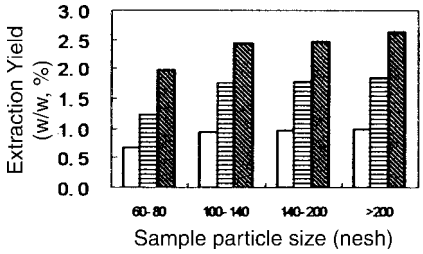

Figure 4. Effect of sample particle size on percentage extraction of ginsenosides. 50% ethanol (v/v); solid/liquid 1 : 40; extraction time, 3 min. *Key*: ▧, Ginsenoside R_{b1}; ▤, ginsenoside R_c; □, ginsenoside R_d.

1 : 40 (g/mL) was sufficient enough to reach the high extraction yield to meet the requirement in this experiment. Higher solid/liquid ratios would lead to further dilution of the sample concentration.

Effect of Sample Particle Size on Ginsenosides Extraction

Sample particle size also showed some effects on the ginsenosides extraction yield (Fig. 4). Smaller sample particle sizes had larger specific external areas. That caused solvents to easily penetrate into the sample particles. As a result, the extraction yield of ginsenosides was increased with the decreased sample particle size. The sample particle size of 100–400 mesh was chosen for MAE.

Reproducibility

The MAE and HPLC analysis procedures were repeated at the optimized conditions for six times. The RSDs were 7.2%, 4.0%, and 5.6% for ginsenoside R_{b1}, ginsenoside R_c, and ginsenoside R_d, respectively.

Comparison of MAE and the Conventional Extraction Methods

The extraction results of the MAE were compared with that of Soxhlet extraction and ultrasonic extraction by using the same solvent and sample particle size (Table 1). MAE can reach almost the same or even higher extraction yields of ginsenosides within 3 min than the Soxhlet Extraction

Table 1. Extraction yield of ginsenosides by MAE, Soxhlet extraction and ultrasonic extraction.

Method	Sample mass (g)	Ethanol (mL)	Extraction time (min)	Extraction yield (%, w/w) Ginsenoside Rb_1	Ginsenoside R_c	Ginsenoside R_d
MAE	0.5	20.0	3	2.44	1.74	0.93
Soxhlet extraction	0.7	40.0	180	2.51	1.68	0.87
Ultrasonic extraction	1.5	40.0	60	1.98	1.35	0.65

Solvent: 50%, sample particle size: 100–140 mesh.

in 3 hr and ultrasonic extraction can reach in 1 hr. MAE can reduce the extraction time to 60 times less than Soxhlet extraction and 20 times less than ultrasonic extraction.

CONCLUSIONS

MAE was a faster and more efficient extraction method than Soxhlet extraction and ultrasonic extraction. A good extraction yield for the ginsenoside R_{b1}, ginsenoside R_c, ginsenoside R_d from *P. quinquefolium* L. can be obtained within 3 min at 50% ethanol, solid/liquid ratio of 1 : 40, and sample particle size of 100–140 mesh. These results provided valuable data for the further study of large scale MAE of ginsenosides from *P. quinquefolium* L.

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