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Short communication

Supercritical fluid extraction of sinomenine from *Sinomenium acutum (Thumb) Rehd et Wils*

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Abstract

Supercritical carbon dioxide, with and without a methanol modifier, was used to extract sinomenine from *Sinomenium acutum (Thumb) Rehd et Wils*. Sinomenine determinations were carried out using high-performance liquid chromatography (HPLC). The results show that the yield obtained after 2.5 h extraction with methanol-modified supercritical carbon dioxide was the highest (7.47 mg/g), while that obtained with only supercritical carbon dioxide was the lowest (0.17 mg/g). The recovery obtained with supercritical carbon dioxide, with and without a methanol modifier, could not be increased greatly by the method of the alkalinization of sample. Higher recoveries were obtained than extraction using methanol in Soxhlet extractor.

Keywords: Supercritical fluid extraction; Sinomenine; *Sinomenium acutum (Thumb) Rehd et Wils*

1. Introduction

The vine stem of *Sinomenium acutum (Thumb) Rehd et Wils* has been used in traditional Chinese medicine for expelling wind-dampness (a treatment used in traditional Chinese medicine for wandering arthralgia due to retention of wind and wetness in the channels, muscles, and joints by the application of antirheumatic drugs), promoting diuresis and alleviating pain for treating various rheumatic diseases [1]. Its component, sinomenine (7,8-didehydro-4-hydroxy-3,7-dimethoxy-17-methylmorphinan-6-one; Fig. 1) has showed some actions of immunosuppression [2], anti-inflammation [3], arthritis amelioration [4], and block of tissue remodeling [5], protection against hepatitis induced by lipopolysaccharide [6]. Regulation of hepatobiliary excretion [7].

Extraction, using organic solvents, of sinomenine from the vine of *S. acutum (Thumb) Rehd et Wils* has been reported with subsequent analysis by high-performance liquid chromatography [8], thin-layer chromatography [9] and capillary electrophoresis [10]. No report has been done on the use of supercritical fluid extraction (SFE) for extraction of sinomenine from the herbal medicine. The purpose of this study, therefore, was to develop an off-line method for SFE of sinomenine from the medicinal plant with analysis by HPLC and compare SFE with classical Soxhlet extraction.

2. Experimental

2.1. Materials

S. acutum (Thumb) Rehd et Wils was purchased from a drugstore (Ningbo, China) and was ground into powder using herbal pulverizer. Part of the powder was wetted with ammonia water (10%) and then was dried at 60 °C. Sinomenine standard was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China).

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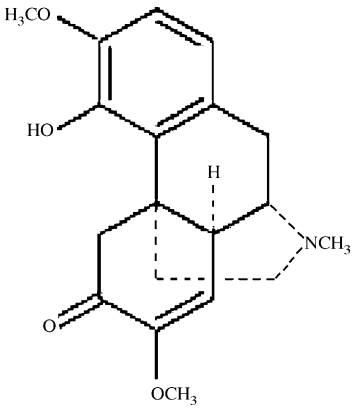

Fig. 1. Structure of sinomenine.

2.2. Procedure for supercritical fluid extraction

A supercritical fluid extractor SFE-ed SFE-2 (Applied Separation, USA) was used. The Sped-ed SFE is a screening system extractor that allows a modularity of extraction but requires more handling of the controls. It is capable of pressure up to 680 bar and temperature up to 240 °C, static and dynamic extraction with flow from 0 to 10 L/min (gaseous fluid) and extraction vessels from 5 mL to 1 L. A metering valve is used to vary flow. Collection is at room temperature and atmospheric pressure. The extracted analytes are collected in glass vial (50 mL) with a rubber plug at the top. A metal extension to the metering valve is used to pierce the rubber plug and allow collection directly in the collection solvent (methanol). A hypodermic needle connected to a flow meter also pierces the plug for the mensuration of flow.

A known quantity of grounded sample (2 g) was placed in the extraction vessel and the void volume was filled with celite. Before the extraction was started, the extraction vessel was preheated in the oven for 10 min. The extraction conditions were as follows: extraction time, static extraction for 5 min and then dynamic extraction for 1 h; temperature, 40 °C; pressure 600 bar; flow-rate of carbon dioxide (gaseous fluid) 0.5 L/min. The extract was collected in a glass vial containing 5 mL of methanol, and then quantitatively transferred to a 25 mL volumetric flask and made up to the mark with methanol. For methanol-modified supercritical carbon dioxide the above procedure was repeated except that the temperature was 60 °C, pressure 300 bar and, in addition to the flow of carbon dioxide (0.5 L/min gaseous fluid), a flow of methanol (0.4 mL/min) was used. This solution was further diluted 10 times prior to analysis.

2.3. Solvent extraction

A 2-g amount of grounded sample was extracted in a 50-mL of Soxhlet extractor for 8 h with methanol at a syphon rate of 1 cycle/4 min.

2.4. Analysis of extracts

An HPLC system (Knauer, Berlin, Germany) equipped with a Knauer pump (model K-501) was used for the analysis of extracts. Sample and standard were injected (20 μL) onto a Inertsil SIL separation column (250 mm × 4.6 mm i.d.; GL Sciences Inc., Tokyo, Japan). The mobile phase was methanol: diethylamine (100:0.25, v/v) at a flow-rate of 1 mL/min. An UV–vis detector (Knauer, model K2501) was used at a wavelength of 264 nm. A calibration graph was produced for sinomenine over the concentration range of 2.6–106 μg/mL and showed a linear response with a correlation coefficient of 0.9996 ($n = 6$).

3. Result and discussion

Supercritical carbon dioxide and methanol-modified supercritical carbon dioxide were used to extract sinomenine from the powdered vine stem of *S. acutum*, in order to evaluate the feasibility of SFE and to identify the concentration of sinomenine. The HPLC chromatograms of the extract using methanol-modified supercritical carbon dioxide and methanol in Soxhlet extractor are showed in Fig. 2(a and b), respectively. It is possible to identify the sinomenine peak, which appears at a retention time of approximately 6.5 min.

It was reported that alkaloids could exist in the form of combination with organic acids. The yield of extraction of some alkaloids could be enhanced by alkalinizing sample and making them free [11]. Fig. 3 shows the effects of alkalinized sample with and without NH_4OH (10%) on the yield of sinomenine at pressure of 600 bar using supercritical carbon dioxide and methanol-modified supercritical carbon dioxide. It can be seen that the yield of sinomenine for the alkalinized samples is a little higher than that without alkalization with supercritical carbon dioxide only, while the yield for both alkalinized and non-alkalinized samples are similar with methanol-modified supercritical carbon dioxide. It means that the extraction yield of sinomenine obtained with methanol-modified supercritical carbon dioxide could not be improved by the method of alkalinizing sample. The yields obtained with methanol-modified supercritical carbon dioxide were much higher than that with supercritical carbon dioxide only.

The effect of extraction pressure on the recovery of sinomenine utilizing methanol-modified supercritical carbon dioxide is showed in Fig. 4. It is noticed that the recovery among 300–600 bar are similar (7.5–8.1 mg/g, within 2 h). It is possible to extract over 95% of sinomenine within 1 h (all yields being based on the recovery obtained after 2 h). The results for extraction of sinomenine from powdered vine stem of *S. acutum* using CO_2 with and without methanol modifier, as well as using methanol in Soxhlet extractor are summarized in Table 1. It shows that extraction with methanol-modified supercritical CO_2 produces about 15% greater yields than Soxhlet extraction with methanol.

Table 1
Extraction yield of sinomenine from *Sinomenium acutum (Thumb) Rehd et Wils*

Sample	Extraction condition	mg/g yield (w/w), mean ± SD ($n = 5$)
Alkalinized powder	Temperature, 40 °C; pressure, 600 bar; flow-rate, 0.5 L/min CO_2; dynamic extraction time, 1 h	0.43 ± 0.04
Non-alkalinized powder	Temperature, 40 °C; pressure, 600 bar; flow-rate, 0.5 L/min CO_2; dynamic extraction time, 1 h	0.16 ± 0.02
Non-alkalinized powder	Temperature, 60 °C; pressure, 300 bar; flow-rate, 0.5 L/min CO_2 and 0.4 mL/min methanol; dynamic extraction time, 1 h	7.30 ± 0.60
Alkalinized powder	Temperature, 60 °C; pressure, 300 bar; flow-rate, 0.5 L/min CO_2 and 0.4 mL/min methanol; dynamic extraction time, 1 h	7.34 ± 0.75
Powder	Soxhlet extractor; extraction solvent, methanol; extraction time, 8 h	6.36 ± 0.67

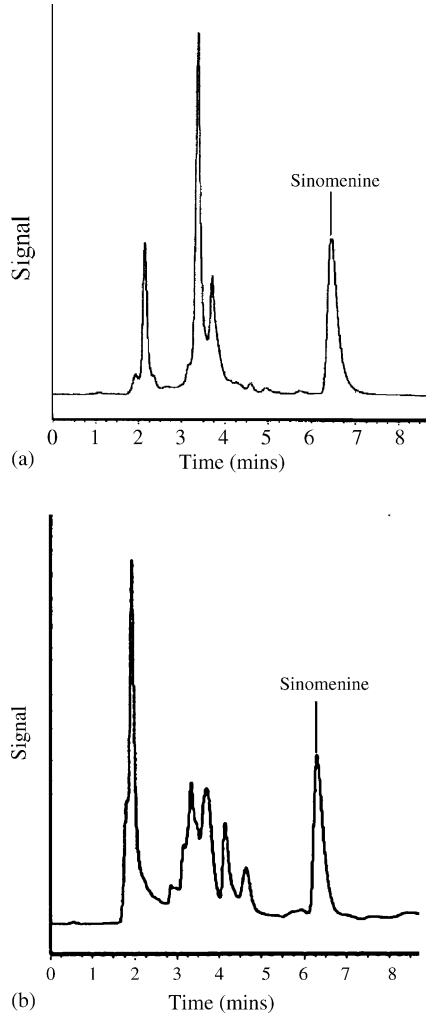

Fig. 2. (a) High-performance liquid chromatogram of extract obtained with methanol-modified supercritical carbon dioxide. (b) High-performance liquid chromatogram of extract obtained with methanol in Soxhlet extractor.

The study shows that the extraction of sinomenine with supercritical CO_2 only is not efficient for both of alkalinized and non-alkalinized samples. Higher yield can be obtained by the addition of a polar modifier, i.e. methanol in supercritical CO_2, which is also more efficient and rapid than using classical organic solvent method.

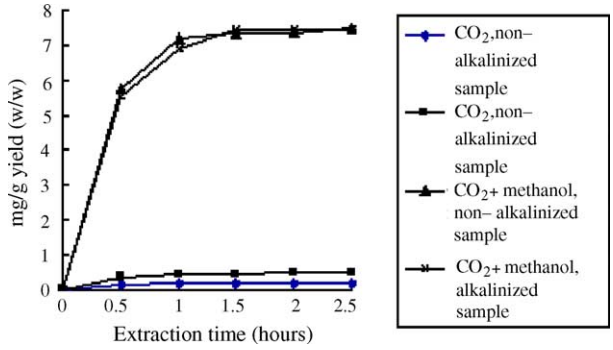

Fig. 3. Influence of alkalinized and non-alkalinized sample on the cumulative yield sinomenine extracted by CO_2 at 40 °C and 600 bar, and CO_2 + methanol at 60 °C and 600 bar.

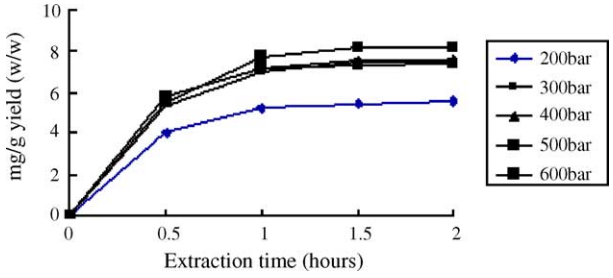

Fig. 4. Cumulative yield of sinomenine extracted from *Sinomenium acutum (Thumb) Rehd et Wils* using methanol-modified supercritical carbon dioxide over a range of pressure at 60 °C.

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