

# Removal of Lipid-soluble Substances from *Sophora flavescens* Ait. by Supercritical Carbon Dioxide Prior to the Extraction of Matrine-type Alkaloids



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**Abstract:** *Sophora flavescens* Ait. is a traditional Chinese medicine that has been used for the treatment of various diseases for centuries. Matrine-type alkaloids are the primary active substances that can be used in medicine and pesticide. In this paper, supercritical carbon dioxide (SC-CO<sub>2</sub>) was used to remove lipid-soluble substances so that a cleaner sample of *S. flavescens* was available for extraction of matrine-type alkaloids. The recovery of lipid-soluble substances (R<sub>1</sub>) and the recovery of matrine-type alkaloids (R<sub>2</sub>) were investigated. Parameters of supercritical fluid extraction (SFE), such as pressure, temperature, time and their effects were evaluated. Furthermore, a comparison between this method and the direct solvent extraction method was made, and an average of 72 % higher yield of matrine-type alkaloids was obtained by the former method. The high yield of matrine-type alkaloids was obtained at pressure 12–18 MPa, temperature 60–75 °C for 2 h.

**Key words:** supercritical fluid extraction; matrine-type alkaloids; lipid-soluble substances

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## 超临界二氧化碳去除苦参中脂溶性物质的研究

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**摘 要:** 苦参是一种用于治疗多种疾病的传统中药材, 苦参碱类生物碱是其主要的活性物质, 既有药用价值又有杀虫效果。作者利用超临界二氧化碳(SC-CO<sub>2</sub>)去除苦参中的脂溶性物质, 建立了节能环保的后续苦参碱提取工艺。检测了脂溶性物质(R<sub>1</sub>)和苦参总碱(R<sub>2</sub>)的得率; 评价了超临界流体萃取(SFE)过程的参数, 如压力、温度、时间对R<sub>1</sub>和R<sub>2</sub>的影响, 并与直接溶剂萃取进行对照。结果显示, 使用本法所得苦参总碱的平均产率比直接用溶剂萃取高72%, 在压力为12~18 MPa、温度60~75 °C、萃取时间2 h下苦参碱得率较高。

**关键词:** 超临界流体萃取; 苦参碱; 脂溶性物质

*Sophora flavescens* Ait. (family of Leguminosae), a typical traditional Chinese medicine, is the root of perennial deciduous shrub and is widely distributed in China. Bitter in flavor and cold in nature, it has effects on heart, liver, large intestine and bladder channels. There have been more than 20 types of alkaloids obtained from *S. flavescens*, among which the matrine-type alkaloids<sup>[1]</sup>, particularly matrine and oxymatrine<sup>[2]</sup> have great effects on anti-hepatitis, anti-arrhythmia, anti-tumor, and so on<sup>[3-6]</sup>. The research of an effective extraction method of retrieving matrine-type alkaloids from *S. flavescens* is therefore crucial. SFE, as an alternative

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technique to conventional liquid-solid extraction method, is a more environmentally friendly extraction process. SFE has long been applied comprehensively on active elements recovery from herbal plants and natural products for a variety of uses<sup>[7]</sup>. In our preliminary experiments, SFE with and without modifiers were applied to the extraction of matrine-type alkaloids from *S. flavescens*, but no ideal results were obtained. The yield of matrine-type alkaloids was lower than that through the traditional solvent extraction. In addition, it was found that lipid-soluble substances greatly interfered the extraction of matrine-type alkaloids from *S. flavescens*. The aim of this work was to investigate a more efficient method to extract lipid-soluble substances from *S. flavescens* by SC-CO<sub>2</sub> prior to the extraction of matrine-type alkaloids by the conventional solvent extraction method.

## 1 Experimental

### 1.1 Raw materials

The slices of *S. flavescens* (purchased from Jianlian Chinese Traditional Medicine Chain Store, Ji'nan) were used in this work. The slices were dried in a vacuum oven to a constant weight at 80 °C (24 h), and were then ground and sieved to produce samples with average particle sizes below 0.3 mm. The samples were sealed in polyethylene bag for later experiments.

### 1.2 Solvents

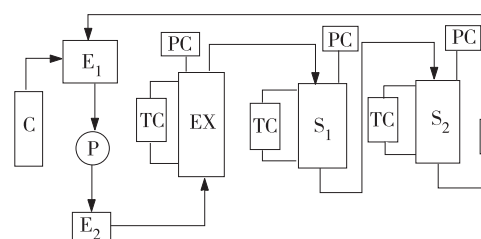
Carbon dioxide of 99.99 % purity used in this work was supplied by air separation workshop of Jinan Fertilizer Factory. The carrier gas helium for GC-MS system (99.999 % purity) was from Jinan Huayang Gas Company. All other solvents and reagents were purchased from Shanghai Chemical Reagents Company. The alcohol used in GC-MS analysis was chromatographic grade. All other solvents and reagents used in analytical determinations and extractions were analytic grade.

### 1.3 Instruments and equipments

Vacuum oven (Model ZhB101-1) was bought from Zhangdian Laboratory Instrument Factory, Shandong Province. SFE equipment (model HA231-50-015) was supplied by Hua'an SFE Equipment Company Ltd., Jiangsu Province. Electron analysis balance (model JY1002) was from Shanghai Precise Instrument Company. Thermostat water bath (model HH. SY21-Ni, Beijing Changfeng Instrument and Apparatus Corporation, Ltd.) was used. GC-MS system (THERMO, TRACE-DSQ) was purchased from USA, equipped with a fused silica capillary column TR-5MS(30 m × 0.25 mm × 0.25 μm; THERMO, USA).

### 1.4 Supercritical fluid extraction apparatus and procedure

The removal of lipid-soluble substances from *S. flavescens* was carried out in a supercritical extraction set. The experimental flow diagram was shown in Fig. 1, presenting a circulating system. The set of apparatus mainly consists of a CO<sub>2</sub> cylinder, a cooling system, a heater, a high-pressure pump, a flowmeter, one extractor and two separators all coupled with thermostat water bath. CO<sub>2</sub> coming out of cylinder was in gas form and was condensed through E<sub>1</sub> into liquid form prior to be pumped by high-pressure pump to the downstream. Before delivered to the extractor, liquid CO<sub>2</sub> was heated by E<sub>2</sub>, a thermostat water bath to evaporate. Extraction experiments were performed within a tubular extractor: 1 L of capacity, internal diameter (*D*) 5.5 cm, height/diameter ratio (*H/D*) 7.6, pressure limit of 50 MPa. The SFE extract was separated from solvent within two tubular separators: 0.5 L of capacity, internal *D* 4.5 cm, *H/D* 7.0, pressure limit of 30 MPa. The pressure in



C. CO<sub>2</sub> cylinder; E<sub>1</sub>. cooling system; P. high-pressure pump; E<sub>2</sub>. heater; EX. extractor; S<sub>1</sub>, S<sub>2</sub>. separators; F. flowmeter; TC. temperature control; PC. pressure control

**Fig. 1 Experimental diagram of supercritical fluid extraction**

separator 1 could be set at a higher value than in separator 2 to accomplish a two-grade separation. A flowmeter was connected to the downstream of the separator 2 to determine the flow rate of supercritical fluid in the circulating system.

### 1.5 Two-step extraction method

The two-step extraction method was applied in this experiment. The first step was to remove lipid-soluble substances from the sample through SFE method. SFE experiments were performed at a temperature in the range of 35–75 °C, a pressure in the range of 12–35 MPa and a residence time in the range of 2–8 h. The second step was to extract matrine-type alkaloids from the SFE treated sample by traditional organic solvent extraction. Detailed processes were shown as following.

Sample particles of 200 g were dynamically extracted in the extractor at desired temperature and pressure for a desired residence time, using CO<sub>2</sub> as supercritical fluid at a constant flow rate 35 kg/h. Simultaneously, the continuously collected extract was conveyed by supercritical CO<sub>2</sub> sequentially to two separators at the same temperature of 45 °C, but at different pressures of 10 and 4 MPa respectively. In the separators, the extract was gradually separated from CO<sub>2</sub> solvent. The extraction experiment was terminated after the designed residence time was up. The system was then decompressed, the extracts were discharged through the outlet valves at the bottoms of two separators into two 50 mL glass flasks, and the residue was discharged to a flat tray. The residue was first placed under ambient atmosphere for the evaporation of CO<sub>2</sub>, and was then dried in the vacuum oven at 80 °C until a constant weight.

The SFE treated sample was extracted in a sealed glass vessel of 2 L with agitation, using nontoxic alcohol (95 % with the addition of hydrochloric acid) as solvent. The molar number of HCl compared with total solvent volume is 1 mol/L. Total ratio of solvent volume (mL) to sample weight (g) was 8 for the entire three extractions performed. The ratio was 3 for the first two extractions and was 2 for the third extraction. Each extraction was carried out for 2 h at a temperature of 60 °C, provided by a thermostat water bath. Shortly after the termination of the first 2 h extraction, the extract solution was vacuum filtered by a self-made vacuum apparatus and the filtrate was collected and combined in the 2 L plugged glass bottle to be ready for the second 2 h extraction. The same procedure was repeated to be ready for the third 2 h extraction. After all three extractions were done, the extract solution was vacuum evaporated in a 1000 mL distillation flask kept in thermostat water bath at 60 °C with a vacuum pressure of 366.6 kPa provided by a self-made vacuum evaporation system. After most of the solvent had been evaporated, the extract solution was condensed to the total matrine-type dark brown alkaloids paste. The paste was then transferred onto a 10 cm × 10 cm tray and dried at 80 °C in the vacuum oven mentioned above. Twenty-four hours later, the paste was turned into powder with a constant weight. The content of matrine-type alkaloids was determined by GC-MS method. Every experiment was performed three times and average results were taken in the analysis.

### 1.6 Comparison with the conventional extraction

In order to compare the recovery of matrine-type alkaloids through our two-step extraction method with conventional solvent extraction method, i. e. to measure the influence of lipid-soluble substances removal by SFE, sample particles of 200 g without SFE treatment were extracted in the absolutely same manner as the second step of our two-step extraction method. The extract solution was also vacuum evaporated to the paste form and dried to the constant-weight alkaloids powder. GC-MS method was applied to determine the content of the obtained matrine-type alkaloids. The chemical structures of major matrine-type alkaloids of *S. flavescens* were shown in Fig. 2. The extraction experiment was

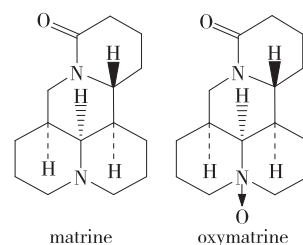


Fig. 2 Chemical structures of major matrine-type alkaloids of *S. flavescens*

also carried out 3 times and average result was taken for future analysis.

### 1.7 Recoveries analysis

The recovery of SFE extract, lipid substances ( $R_1$ ), and the recovery of matrine-type alkaloids ( $R_2$ ) with two-step extraction method were investigated.  $R_1$  was evaluated by determining the dry weight differences between the original and spent substrate (% , mass fraction, dry basis).  $R_2$  was correlated to the grams ratio of obtained total matrine-type alkaloids to 200 g of original dry substrate (% , mass fraction, dry basis).

### 1.8 GC-MS analysis

Alkaloid fractions obtained by two different methods were analyzed in a GC-MS system. The carrier gas was helium (1.2 mL/min); a split ratio of 1/100 was used. The temperatures of the injector and detector were 250 and 260 °C, respectively. The column was heated to 110 °C and maintained for 1 min. Temperature was then ramped up to 260 °C at a rate of 10 °C/min and maintained for 10 min. One microliter of the samples was injected (1.000 0 g of matrine-type alkaloids powder diluted in 100 mL anhydrous ethanol). Identification of substances was based on the comparison of substance mass spectrum with GC-MS system data bank (NIST MS library). Total matrine-type alkaloids were quantified by the ratio of the total peak area integration of all matrine-type alkaloids to all components that could be recognized in sample.

## 2 Results and discussions

### 2.1 Comparison of results

Samples of *S. flavescens*, 200 g per share, were treated through two-step extraction method, through which the highest recovery rate of matrine-type alkaloids reached 1.41 % at 20 MPa, 75 °C with residence time of 3 h at a constant SC-CO<sub>2</sub> flow rate of 35 kg/h. This result was a 72 % gain compared to the recovery rate of 0.82 % obtained through the conventional solvent extraction method. Detailed comparisons were listed in Table 1. The results show that the extract weight of 6.54 g from solvent extraction with two-step extraction method is greatly less than that of 15.62 g with solvent extraction method. However, an amazingly higher volume of matrine-type alkaloids content of 2.82 g was found, compared to that of 1.64 g through solvent extraction method. These results demonstrate that the removal of lipid substances by SFE is efficient, and that SFE has an important effect on eliminating the interfering components for matrine-type alkaloids extraction. SFE supplied a cleaner material for the second step extraction, and as a result, the recovery of matrine-type alkaloids was remarkably enhanced.

**Table 1 Results comparison of two extraction methods**

treatment methods	extract weight/g	matrine-type alkaloids content/g	matrine-type alkaloids recovery/%
solvent extraction	15.62 ± 0.05	1.64 ± 0.03	0.82 ± 0.02
two-step extraction <sup>1)</sup>	6.54 ± 0.05 <sup>a,b</sup>	2.82 ± 0.03 <sup>b</sup>	1.41 ± 0.02 <sup>b</sup>

1) a. obtained by solvent extraction of the second-step; b. the optimal results obtained under all experimental conditions

### 2.2 Analysis of the influence of process parameters

**2.2.1** The effect of pressure on  $R_1$  and  $R_2$  In order to evaluate the effect of pressure on  $R_1$  and  $R_2$ , SFE experiments were carried out at a pressure range of 12–35 MPa at two different temperatures of 40 and 60 °C for two different residence times of 3 and 5 h, respectively. Flow rate of SC-CO<sub>2</sub> was fixed at 35 kg/h. The results are shown in Fig. 3. In Fig. 3(a),  $R_1$  increases with the increase of pressure at different temperatures and residence times. The highest  $R_1$  is 2.15 %, which is consistent with the prediction that solubility of supercritical CO<sub>2</sub> will be enhanced by the increase of CO<sub>2</sub> density as a result of the increase of pressure at a constant temperature. Fig. 3(b) indicates the trend of  $R_2$  with the increase of pressure at the same temperature and residence time. When temperature is 40 °C with 3 h residence time,  $R_2$  increases with pressure, the same

as  $R_1$ , and reaches a highest value of 1.36%. However, when temperature is 60 °C with 5 h residence time,  $R_2$  decreases with the increase of pressure. These observations have shown that the increase of lipid substances recovery does not directly lead to the increase of matrine-type alkaloids recovery in the SFE residue. The two trends lines cross at 21.5 MPa. Below 21.5 MPa,  $R_2$  at 60 °C with 5-hour residence time is higher than that at 40 °C with 3 h residence time. Over 21.5 MPa,  $R_2$  is lower at 60 °C with 5 h residence time. Therefore, both pressure and temperature affect  $R_2$ . In order to get a high  $R_2$  of over 1.00%, either a pressure range of 12–18 MPa at 60 °C, for 5 h, or a pressure range of 23–35 MPa at 40 °C for 3 h could be chosen.

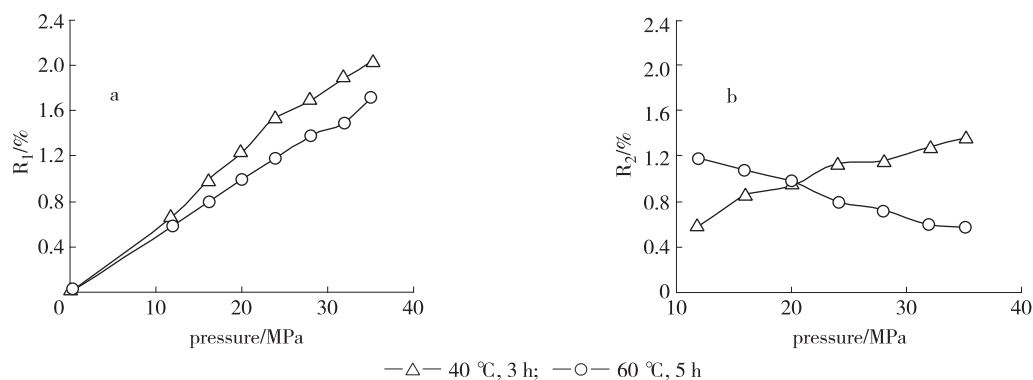


Fig. 3 Relationship between  $R_1$  (a),  $R_2$  (b) and pressure with different temperatures and residence times

**2.2.2** The effect of temperature on  $R_1$  and  $R_2$  Fig. 4 presents the influence of temperature in SFE experiments on  $R_1$  and  $R_2$ , respectively. SFE experiments were conducted at a temperature ranging from 35–75 °C, at two different pressures of 20 and 30 MPa, with residence time 3 and 5 h, and with a fixed SC-CO<sub>2</sub> velocity of 35 kg/h. Fig. 4 (a) shows that little change was observed on  $R_1$  with the increase of temperature under both experiment conditions.  $R_1$  is higher at 30 MPa with 5 h residence time, compared to that at 20 MPa with 3 h residence time. This result is consistent with the observation shown in Fig. 3 (a). Pressure is the decisive parameter in SFE experiments for  $R_1$ . Fig. 4 (b) shows  $R_2$  increases with the increase of temperature at 20 MPa with 3 h residence time, however, decreases with increase of temperature at 30 MPa with 5 h residence time. Most of the time,  $R_2$  at 20 MPa for 3 h is higher than that at 30 MPa for 5 h. No correlation could be made between  $R_1$  and  $R_2$  through two-step extraction method. Taking the value of 0.82%, achieved by conventional solvent extraction method, as a reference value, Fig. 4(b) shows that the temperature range of 60–75 °C at 20 MPa with residence time 3 h was considered to be suitable conditions for an optimal  $R_2$  range of 1.20%–1.41%, which was 50%–72% higher than the reference value 0.82%.

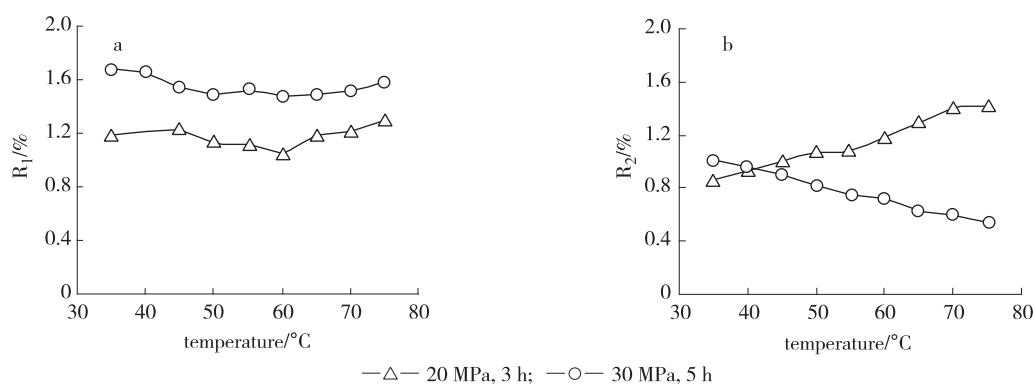
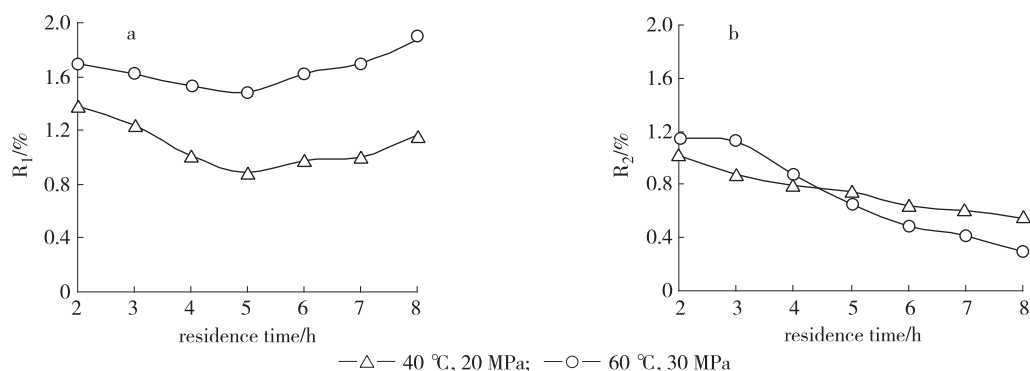


Fig. 4 Relationship between  $R_1$  (a),  $R_2$  (b) and temperature for different pressures and residence times

**2.2.3** The effect of residence time on  $R_1$  and  $R_2$  Fig. 5 presents the influence of residence time on SFE experiments on  $R_1$  and  $R_2$  respectively. To evaluate the effect of residence time, SFE experiments were performed for 2 to 8 h with the operating conditions of 20 MPa, 40 °C and 30 MPa, 60 °C with a fixed SC-CO<sub>2</sub> flow rate of 35 kg/h. Fig. 5 (a) shows two curves of  $R_1$  with the prolonging of SFE time at 20 MPa, 40 °C and 30 MPa, 60 °C. Two curves display the same trend;  $R_1$  initially decreases, but increases from the 5th hour. But residence time has a different effect on  $R_2$ . Fig. 5 (b) reveals that  $R_2$  decreases dramatically with residence time at the same temperatures and pressures as in Fig. 5 (a). For  $R_2$ , 2 h residence time is corresponded to the highest yields at 20 MPa, 40 °C and 30 MPa, 60 °C, respectively. From economic point of view, a shorter SFE time is charming if it is acceptable. In future experiments, shorter SFE extraction time will be attempted.



**Fig. 5 Relationship between  $R_1$  (a),  $R_2$  (b) and residence time for different temperatures and pressures**

### 3 Conclusions

In this work, supercritical CO<sub>2</sub> extraction was applied for the removal of lipid-soluble substances from *S. flavescens* Ait. and SFE was combined with conventional solvent extraction method to obtain matrine-type alkaloids. A wide range of SFE process variables were investigated, such as the pressures range of 12–35 MPa, the temperatures range of 35–75 °C and the residence time range of 2–8 h with a fixed CO<sub>2</sub> flow rate 35 kg/h. The results indicated that the best recovery of total matrine-type alkaloids obtained from SFE treated sample reached 1.41 %, which was 72 % higher than the value obtained by comparable solvent extraction method. The process parameters of SFE experiment were evaluated for  $R_1$  and  $R_2$ . No direct correlations have been found to explain the relationship between  $R_1$  and  $R_2$ . The trend of  $R_1$  was not consistent with  $R_2$  under equal SFE conditions. It will be investigated in later experiments. As for  $R_2$ , the suitable SFE operating parameters ranges are 12–18 MPa, 60–75 °C with residence time 2 h.

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