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Microwave-assisted extraction and high-speed counter-current chromatography purification of ferulic acid from *Radix Angelicae sinensis*

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Abstract

High-speed counter-current chromatography (HSCCC) was successfully applied to purify ferulic acid from the extracts of *Radix Angelicae sinensis*, which were extracted by microwave-assisted extraction (MAE). A solvent system consisted of *n*-hexane–ethyl acetate–methanol–water (3:7:5:5, v/v) was employed. The purity of ferulic acid was over 98% assayed by HPLC. The optimal extraction conditions can be concluded: 850 W of microwave power, 9 min of irradiation time, 90% of ethanol concentration, 6:1 ratio of liquid/solid, 140 min of herbal material soak time and particle sample size 250 ± 9.9 μm, the results showed that microwave-assisted extraction was a promising method for extracting ferulic acid.

Keywords: High-speed counter-current chromatography; Ferulic acid; *Radix Angelicae sinensis*; Microwave-assisted extraction

1. Introduction

Radix Angelicae sinensis (Chinese name Danggui), one of the most commonly used traditional Chinese medicines, is used to enrich blood, activate blood circulation, regulate menstruation and amenorrhoea, relieve pain and relax bowels and so on [1]. In recent years, this herb is also regarded as a female tonic, dietary supplements and one of the cosmetic ingredients sold in many counties and regions, such as China, Europe, USA and Japan.

Over 70 compounds had been isolated and identified from *R. A. sinensis* [2,3]. Pharmacological research showed that bioactivity of ferulic acid (Fig. 1) was related to the medicinal functions of *R. A. sinensis*, so ferulic acid was the main maker of *R. A. sinensis* [4]. Traditional method of extraction of ferulic acid was to reflux *R. A. sinensis* for 4–5 h in 70% ethanol. However, ferulic acid is heat sensitive and its structure would be destroyed during long time heating in ethanol, so that it is not a good process for extraction of ferulic acid. Though the ferulic acid could be chemically synthesized, the reaction time was long (3 weeks), much solution was needed, the yield was low and the product was the mixture of *trans*- and *cis*-ferulic acid [5,6]. So the natural one from extraction was better than that one synthesized, and the extraction process was much more environment-friendly than synthetic process. In this paper, a new microwave-assisted extraction method was adopted, which only irradiates a few minutes in solvent. It is a more effective and economical process than traditional one [7].

The conventional method of purifying ferulic acid was to utilize column chromatography, which required several steps, and resulted in low recoveries of the target products. High-speed counter-current chromatography, being as a support free liquid–liquid partition chromatography, eliminates irreversible adsorption of sample onto the solid support. This method has been successfully applied to the separation and purification of different kinds of natural products [8–10]. However, no report has been published on the use of HSCCC for purification of ferulic acid from the extracts of *R. A. sinensis*. This paper describes HSCCC successful separation and purification of ferulic acid extracted from *R. A. sinensis*.

2. Experimental

2.1. Instrumentation

An Agilent/HP 1100 series HPLC–DAD system consisting of a vacuum degasser, quandary pump, thermostated column com-

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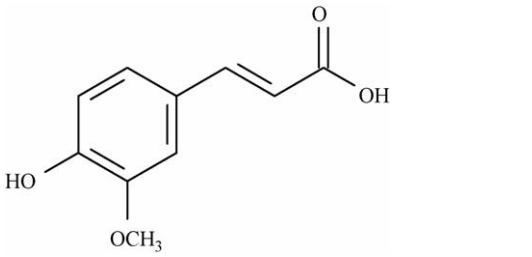

Fig. 1. Structural formula of the ferulic acid, molecular weight 194.

partment and diode array detection (DAD) (Agilent, Palo Alto, CA, USA) was used for acquiring chromatogram. The preparative HSCCC instrument (Model TBE-300, Shanghai Tauto Biological Company, China) was equipped with three preparative coils connected in series (diameter of polytetrafluoroethylene (PTFE) tube, 2.6 mm; total volume, 119 ml) and a 10 ml sample loop. The revolution speed of the instrument could be regulated with a speed controller in the range between 0 and 999 rpm, an optimum speed of 800 rpm was used in the experiment. The solvent was pumped into the column with the AKTA purifier pump P-900 (Amersham, USA), the flow rate is up to 10 ml/min and pressure up to 25 MPa, an optimum speed of 1.2 ml/min was used in the experiment. The continuous monitoring of the effluent was achieved with a monitor UV-900, a multi-wavelength UV–vis monitor for simultaneous monitoring of up to three wavelengths in the range 190–700 nm (Amersham, USA).

Microwave extractor (Model VIP 272, National Engineering Research Center for Chinese Traditional Medicine, Shanghai, China), ZX-4A vacuum pump and ZK 82J electrothermal vacuum desiccator (Shanghai Experimental Instrument Company, Shanghai, China) were used in the experiment.

2.2. Plant material and chemicals

Danggui (Gansu, China) was purchased from Shanghai Yanghetang Medicinal Materials company, authenticated by Shanghai Chinese Traditional Medicine Research Institute with the quality being compiled with that of Chinese Pharmacopoeia.

N-Hexane, ethyl acetate, ethanol and phosphoric acid were of analytical grade, methanol (Merk), acetonitrile (Merk) and pure water were of HPLC grade. Ferulic acid standard sample was purchased from National Institute for the Control of Pharmaceutical and Biological Products, Ministry of Health, Beijing, China.

2.3. Microwave-assisted extraction ferulic acid from R. A. sinensis

In the 2 l flask, 100 g shattered *R. A. sinensis* was mixed with ethanol at different concentrations. After irradiating the mixture with microwave adjusted to certain power level for a few minutes, the extract was filtered when it was still hot to obtain the extraction solution. The extraction solution was concentrated to dryness by evaporator under reduced pressure and dried in vacuum. The dry powder, whose content of ferulic acid was 1.75 mg/g (ferulic acid/dry powder, mg/g), was stored in the desiccator.

2.4. HSCCC separation and purification

A two-phase solvent system consisted of *n*-hexane–ethyl acetate–methanol–water (3:7:5:5, v/v) was used. Each solvent mixture was thoroughly equilibrated in a separating funnel at room temperature and the two phases were separated shortly before use.

The multiplayer coiled column was first entirely filled with the upper and lower organic phase of solvent system at a flow rate of 10 ml/min with pumps A and B separately but the same time. Then, the apparatus was rotated at 800 rpm and the lower phase of solvent was pumped through the column at a flow rate of 1.2 ml/min. After the upper phase emerged and the volume unchanged, the liquid–liquid equilibrium was established in the column. The dry powder, which contains 1.75 mg/g of ferulic acid, was dissolved in the lower phase solvent directly, then injected through the injection valve. The effluent was on-line monitored with UV detector at 316 nm and peak fractions were collected according to the chromatogram, respectively.

2.5. HPLC analysis and MASS and ^{1}H NMR identification

The fraction of ferulic acid from the preparative HSCCC separation was analyzed by HPLC (Fuji silysia C_{18} 5 μm column, 200 mm × 4.6 mm). Mobile phase was performed with 0.85% phosphoric acid:acetonitrile (83:17). The flow rate was 1.0 ml/min. Detection wavelength was 316 nm. Temperature was 35 °C. The pure ferulic acid obtained from the preparative HSCCC separation was characterized by electron impact-mass spectroscopy (EI-MS) (GC-TOFMS, Micromass, UK) and ^{1}H NMR (Bruka Advance 500 MHz spectrometer referenced to TMS), respectively.

3. Results and discussion

3.1. Microwave-assisted extraction ferulic acid from R. A. sinensis

Uniform design (v 4.0) was adopted for arranging the experiment, separate influential factor was considered, shown in Table 1. Total 26 experiments were conducted. Based on these experiments, the optimization parameters were 850 W of microwave power, 9 min of irradiation time, 90% of ethanol concentration, 6:1 ratio of liquid/solid, 140 min of herbal material soak time and particle sample size 250 ± 9.9 μm. In this way, 76.19% of ferulic acid was transferred from the *R. A. sinensis* into the extracts, which was higher than other methods. Table 2 shows the comparison of MAE with traditional reflux method and ultrasound assisted extraction method.

3.2. HSCCC separation

The sample used in HSCCC was the MAE extracts. As shown in Fig. 2, which was the HPLC analysis of standard fer-

Table 1
The parameters of MAE

Level	Ethanol concentration (%)	Ratio of liquid/solid	Factor irradiation time (min)	Soak time (min)	Particle sample size (μm)	Microwave power (w)
1	50	6:1	1	20	2000 ± 70.0	170
2	60	8:1	3	50	850 ± 29.0	340
3	70	10:1	5	80	355 ± 13.0	510
4	80	12:1	7	110	250 ± 9.9	680
5	90	14:1	9	140	180 ± 7.6	850

Table 2
Comparison of MAE (1) with traditional reflux (2) and ultrasound assisted extraction (3)

Extraction	Ratio of liquid/solid	Extraction time (min)	Extraction rate (%)	Content of ferulic acid (mg/g)
1	6	9	76.19	1.75
2	10	180	73.62	1.21
3	10	30	70.51	1.13

Extraction rate = quantity of ferulic acid in extracts/quantity of ferulic acid in raw material. Content of ferulic acid = quantity of ferulic acid/the extracts (dry powder).

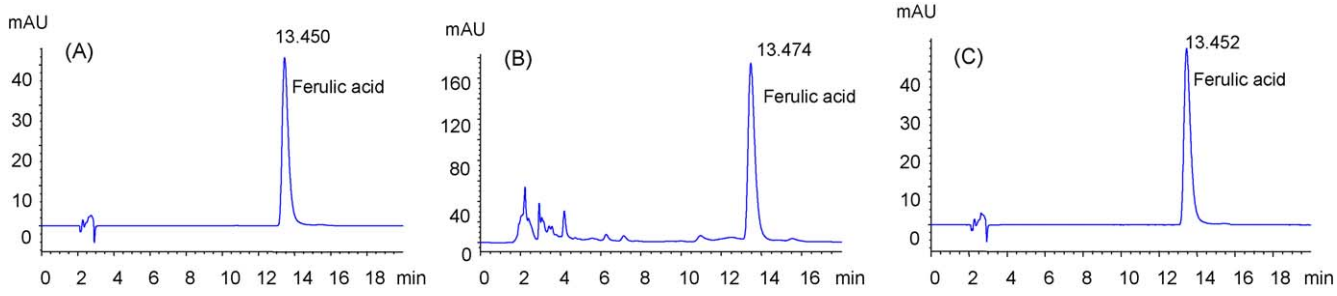

Fig. 2. Chromatogram of the standard of the ferulic acid (A), the MAE extracts from *Radix Angelicae sinensis* (B) and the peak 3 from HSCCC separation (C) by HPLC analysis, respectively.

ulic acid and the MAE extracts. Preliminary HSCCC studies were carried out with the two-phase solvent system consisted of *n*-hexane–ethyl acetate–methanol–water (30:10:32:10, 3:7:5:5, 4:6:5:5, 3:5:4:4 and 3:7:7:3, v/v). The results indicated that solvent system *n*-hexane–ethyl acetate–methanol–water at the volume ratio 30:10:32:10 and 4:6:5:5 could not give effective separation, solvent system *n*-hexane–ethyl acetate–methanol–water at the volume ratio 3:5:4:4 and 3:7:7:3 caused serious emulsification, while volume ratio 3:7:5:5 gave good results and fitted for separation demands. Fig. 3 shows the preparative HSCCC separation of 200 mg of MAE extracts.

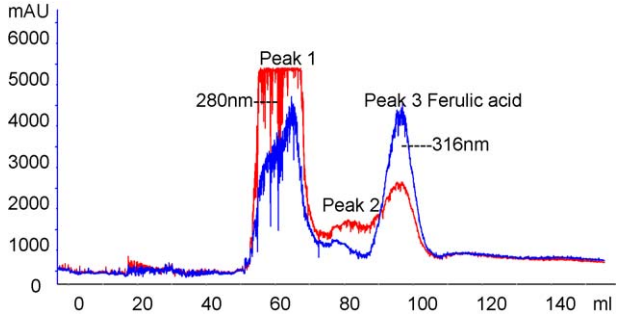

Fig. 3. Chromatogram of MAE extracts from *Radix Angelicae sinensis* by HSCCC, detection wave, 316 and 280 nm.

HPLC analysis of each peak fraction from this preparative HSCCC was compared with the ferulic acid standard sample and confirmed by their retention time and purity assay. Routine sample calculations were made by comparison of the peak area with those of the standard. The results revealed that peak 3 corresponds to ferulic acid, as shown in Fig. 2(C), and the purity of ferulic acid was over 98% (HPLC).

Fig. 4 is the electron impact-mass spectra of the purified peak 3 from the preparative HSCCC. The structural identification of

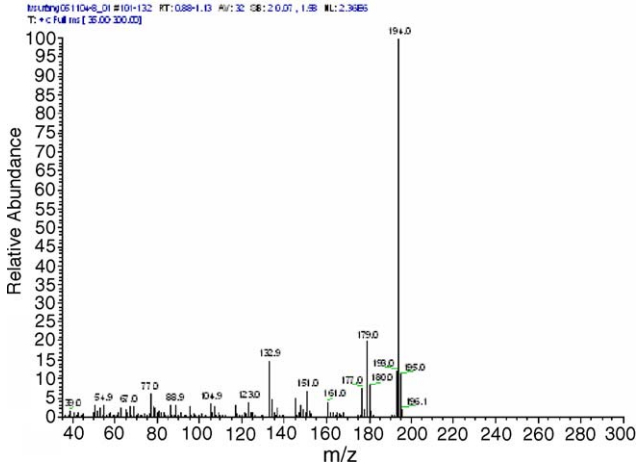

Fig. 4. EI-MS spectra purified peak 3 from preparative HSCCC.

peak 3 was carried out by EI-MS as follows: 194 (100%), 179 (23%), 177 (11%), 161 (4%), 151 (8%), 133 (16%), 105 (4%), 89 (3%) and 77 (7%). The molecular ion at *m/z* 194 corresponds to the formula $C_{10}O_4H_{10}$ of ferulic acid, other ionic peaks are the fragments of the molecule. ^{1}H NMR data gave the structural identification: ^{1}H NMR (d_6-DMSO) δ: 3.80 (s, 3H, CH_3), 6.36 (d, $J = 15.9$ Hz, 1H, CH), 6.77 (d, $J = 8.1$ Hz, 1H, ArH), 7.06–7.08 (dd, $J = 1.8$, $J = 8.1$, 1H, ArH), 7.27 (d, $J = 1.8$, 1H, ArH), 7.48 (d, $J = 15.9$Hz, 1H, CH), 9.57 (s, 1H, COOH). Therefore, all the results show that the compound in peak 3 is ferulic acid.

3.3. Conclusion

The overall results indicate that HSCCC is successfully used for separation and purification of ferulic acid from *R. A. sinensis* and microwave-assisted extraction can selectively extract the biological active substance from the traditional Chinese medicine. The present study indicates that HSCCC is a convenient and powerful tool for separation and purification of biologically active substance from other natural medicines. The extraction results also show MAE is a promising method in industrial extraction of natural medicines.

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