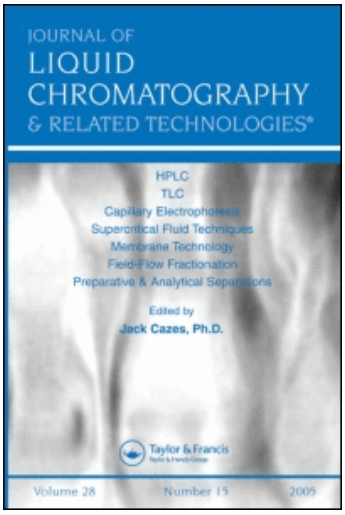

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Rapid and Simple Method for Quality Control of Raw Materials of Herbs by HSCCC

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Rapid and Simple Method for Quality Control of Raw Materials of Herbs by HSCCC

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Abstract: The component of the traditional Chinese medicine (TCM) can be influenced by soils, climates, and growth stages. The quality of TCM mostly depends on the quality of the raw materials of the used herbs. A portable instrument and simple analysis method are urgently needed to be used for quality control of the raw herbs. In this study, analytical CCC (Model HS06) was applied to analyze three herbs including Fructus Arctii (*Arctium lappa* L.), Cortex Magnoliae Officinalis (*Magnolia officinalis* Rehd. et Wils.), and Fructus Psoraleae (*Psoralea corylitolia* L.), which were collected from different growth locations. The results showed that analytical CCC gave similar resolution as preparative CCC. In this analytical CCC operation, the crude sample is loaded directly without any pre-treatment, and only 1–10 mg sample is used for each injection. The separation time is often shorter than 1 hour and can compete with that of HPLC. HSCCC chromatograms can be used as the comparison patterns for the content control of the major bioactive compounds. The results demonstrated that it is feasible to control the raw herb's quality by analytical CCC.

Keywords: Countercurrent chromatography, Quality control, TCM

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INTRODUCTION

Secondary metabolites, depending on growing circumstances, usually are active constituents of traditional Chinese medicine (TCM). As a result, a TCM may have variable chemical components and contents according to the different soils and climates that they had been grown in, as well as growth stages when being harvested.[1] The quality of TCM is mostly dependent on the quality of the raw materials of the used herbs. In the laboratory or factory, the instruments such as HPLC, GC, and TLC were often used to control the herb's quality. But the technical conditions of the producing area or purchasing market are hard and limited. Hence, a portable instrument and a simple analysis method are urgently needed to be used for quality control of the raw herbs.

High-speed countercurrent chromatography (HSCCC) is a kind of liquid-liquid partition chromatography without any solid matrix, which eliminates irreversible adsorption of samples on solid support.[2] This technique has the maximum capacity with an excellent sample recovery and a wide range of selection of solvent systems as compared to HPLC. Furthermore, it permits introduction of the crude sample directly into the column. Therefore, HSCCC has recently been widely used in separation and purification of a variety of natural products.[3] Inexpensive instruments and commonly used chemicals make HSCCC more effective than HPLC. Gu et al. reports that HSCCC can be used in the development of fingerprinting of TCM.[4]

In this study, an analytical CCC (Figure 1) was applied to the analysis of three herbs, including Fructus Arctii (*Arctium lappa* L.), Cortex Magnoliae Officinalis (*Magnolia officinalis* Rehd. *et* Wils.), and Fructus Psoraleae (*Psoralea corylitolia* L.), which were collected from different growth locations. The feasibility of analytical CCC used as a method for controlling the raw herb's quality is discussed in our present report.

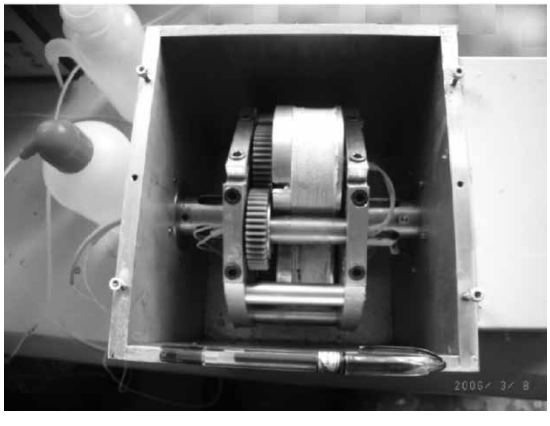

Figure 1. A photograph of the rotor of 36 mL Model HS06 of Beijing U.E. Biotech.

EXPERIMENTAL

Apparatus

The analytical CCC instrument employed in the present study is a Model HS06 (Prof. Zhang's new patent, 2006 200007213) fabricated by Beijing U.E. Biotech., Beijing, China. It is equipped with two multilayer coil separation columns connected in series (I.D.: 1.2 mm, total volume: 36 mL). The β value of the column varied from 0.4 at the internal terminal to 0.8 at the external terminal ($\beta = r/R$, where r is the rotation radius or the distance from the coil to the holder shaft, and R is the revolution radius or the distances between the holder axis and central axis of the centrifuge). The revolution speed is adjustable from 0 to 2000 rpm with a speed controller, and 1800 rpm was used in the present studies. The system was also equipped with one NS-1007 constant flow pump, a Model 8823A-UV monitor operating at 254 nm, a Yakogawa 3057 recorder, and a manual injection valve with a 1 mL sample loop.

Materials

Organic solvents including *n*-hexane, *n*-butanol, chloroform, ethanol, ethyl acetate, and methanol were all of analytical grade and were purchased from Guangcheng Chemical Factory, Tianjin, China. Reverse osmosis Milli-Q water (Millipore, USA) was used for all solutions and dilutions. The herbs were collected from Heibai, Shandong, Anhui, and Guangdong province, China.

Solvent System for HSCCC

The following three solvent systems were used: Solvent system A: ethyl acetate–*n*-butanol–ethanol–water (5:0.5:1:5, v/v).[5] Solvent system B: *n*-hexane–ethyl acetate–methanol–water (1:0.4:1:0.4, v/v).[6] Solvent system C: *n*-hexane–ethyl acetate–methanol–water (1:0.7:1:0.8, v/v).[7] Each mixture was equilibrated thoroughly in a funnel at room temperature. The upper phase and lower phase were separated before use.

Sample Preparation

Each powered dried sample (10 g) was extracted with 50 mL of 95% ethanol or chloroform using an ultrasound bath (25 MHZ, 250 W) for 20 min. The extracts were filtered through filter paper for removal of the particles. The residues were re-extracted with the same solvent, and the extracts were

pooled and concentrated under vacuum at 70°C to yield the final crude samples. A 10 mg amount of crude sample was dissolved in 1 mL of upper phase before loading on the HSCCC separation column.

HSCCC Separation

The multilayer coiled column was first entirely filled with the upper phase. The lower aqueous phase was then pumped into the head end of the column at a suitable flow rate of 2 mL/min for Model HS06, while the apparatus was rotated at an optimum speed of 1800 rpm. After hydrodynamic equilibrium was reached, as indicated by a clear mobile phase eluting at the tail outlet, the sample solution was injected through the sample port. The effluent from the tail end of the column was continuously monitored with a UV detector at 254 nm and the chromatogram was recorded.

RESULTS AND DISCUSSION

Fructus Arctii (Niubangzi in Chinese), the dried fruits of *Arctium lappa* L. (Compositae), is one of the most popular traditional Chinese medicines officially listed in the Chinese Pharmacopoeia (2005 edition). Arctiin, an active compound of Fructus Arctiin, was a "marker compound" used for the quality control of this herb and its products. Figure 2 shows the analytical CCC separation of the crude ethanol extract (10 mg) of Fructus Arctii and the standard arctiin (1 mg) using the above solvent system A. The upper

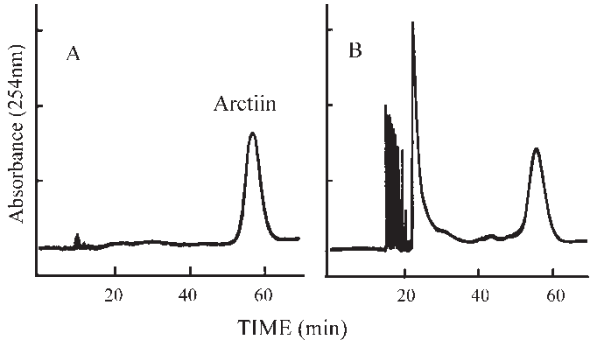

Figure 2. Chromatograms of the crude extract of Fructus Arctii and the standard arctiin by analytical CCC. Experimental conditions: column, multilayer coil of 1.2 mm i.d. PTFE tube with a total capacity of 36 mL; revolution speed, 1800 rpm; flow rate, 2.0 mL/min; detection, 254 nm; solvent system, ethyl acetate–*n*-butanol–ethanol–water (5:0.5:1:5, v/v); sample size, A:1 mg (standard), B:10 mg (crude sample); injection volume, 1 mL; retention of the stationary phase, 53%.

phase was used as the stationary phase while the lower phase was used as the mobile phase in the head to tail elution mode. As expected, the arctiin is well separated from other compounds. The analytical CCC gave similar resolution to the preparative CCC which we reported previously.[5] Table 1 shows the difference between analytical CCC and preparative CCC in separation of Fructus Arctii extract. In the analytical CCC operation, the crude sample is loaded directly, without any pre-treatment, and only 1–10 mg sample is used for each injection. The run time is also just 1/5 of the preparative CCC. However, the sample for preparative CCC often needs a pre-treatment in order to improve the yield of the target compound.

Cortex Magnoliae Officinalis, one of the most popular traditional Chinese medicines, has been used to treat a wide variety of clinical symptoms and diseases such as wind-stroke, cold damage, headache, cold and heat, blood impediment, and dead muscle. The major active constituents of Cortex Magnoliae Officinalis are considered to be honokiol and magnolol, which have been chosen as "marker compounds" for the chemical evaluation or standardization of Cortex Magnoliae Officinalis and its products in Chinese Pharmacopoeia (2005 edition). Figure 3 shows the separation of two crude ethanol extracts of Cortex Magnoliae Officinalis and the standard honokiol and magnolol using the above solvent system B. The two active compounds were eluted in the order of honokiol and magnolol. The chromatograms of two extracts of Cortex Magnoliae Officinalis are similar to the preparative CCC which we reported previously.[6] It is obvious that the contents of active compounds of the raw herbs from the two different growth locations are different (B and C).

Table 1. Difference between analytical HSCCC and preparative HSCCC in separation of *Fructus arctii* extract

Content of comparison	HS06 Analytical HSCCC	GS10 Preparative HSCCC[5]
Volume of separation column	36 ml	230 ml
Mass of crude sample	1–10 mg	300 mg
Sample pre-treatment	Load directly	Cleanup by AB-8 resin
Run time	60 min	300 ml
Volume crude sample	1 ml	15 ml
Weight of the apparatus	12 kg	30 kg
β Value	0.4–0.8	0.5–0.8
Revolutionary speed	1800 rpm	800 rpm
Retention of the stationary phase	53%	30%

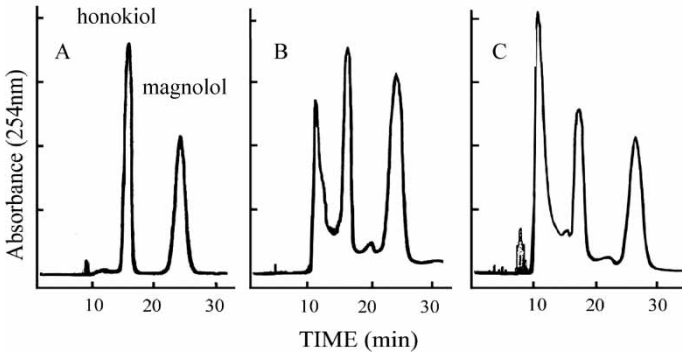

Figure 3. Chromatograms of the crude extracts of Cortex Magnoliae Officinalis and the standard honokiol and magnolol by analytical CCC. Experimental conditions: same as Figure 2, solvent system, *n*-hexane–ethyl acetate–methanol–water (1:0.4:1:0.4, v/v); sample size, A: honokiol and magnolol (0.6 + 0.4) mg, B & C: 10 mg (crude sample harvested from different locations); retention of the stationary phase, 73%.

Another traditional Chinese medicine, Fructus Psoraleae, was analyzed by analytical CCC using the above solvent system C. The separation of three chloroform extracts of Fructus Psoraleae and the standard psoralen and isopsoralen are shown in Figure 4. The major feature of chromatograms is that all samples collected from different locations contain the same kinds of active components. However, the amount of each peak fraction varied greatly in different samples, which confirmed that location and climate had a great impact on the quality of the TCM. Comparing the standards, the approximate contents of active compounds can be estimated from the chromatogram.

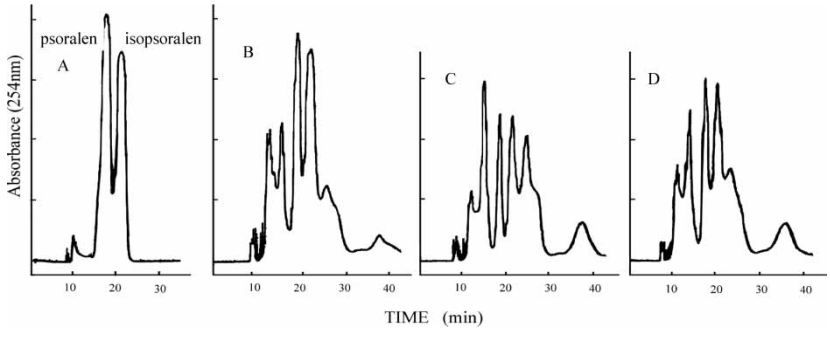

Figure 4. Chromatograms of the standard psoralen and isopsoralen (A) and the crude extracts of Fructus Psoraleae harvested from different locations (B-C) by analytical CCC. Experimental conditions: same as Figure 2, solvent system, *n*-hexane–ethyl acetate–methanol–water (1:0.7:1:0.8, v/v); sample size, A: psoralen and isopsoralen (1 + 1) mg, B: 10 mg, C: 10 mg, D: 10 mg; retention of the stationary phase, 75%.

Although HPLC is of high precision and is popularly used in quality control, it strictly requires complicated pretreatment of samples to remove residual solid particles and eliminate irreversible adsorptive loss of samples onto the solid support matrix. Furthermore, some samples with high viscosity and samples easily adsorbed onto the solid support matrix are not suitable for HPLC analysis. HPLC also needs to follow strict operation conditions. In contrast, HSCCC is a kind of liquid-liquid partition chromatography without any solid matrix, which eliminates irreversible adsorption of samples on solid support. This special structure of HSCCC makes it easy to analyze and separate the crude samples. The analytical CCC (HS06) model is a portable instrument, which need not strictly follow the standard operative conditions. In the previous studies, the analytical CCC had been used to support method development and solvent selection in preparative HSCCC with its short separation time and minimum solvent consumption.[3,8] The results clearly demonstrated that it is feasible to evaluate raw herb's quality by this method.

CONCLUSION

Three herbs, including Fructus Arctii, Cortex Magnoliae Officinalis, and Fructus Psoraleae were analyzed by analytical CCC, which produced a similar resolution to the preparative CCC. In the analytical CCC operation, the crude sample is loaded directly, without any pretreatment, and only 1–10 mg sample is used for each injection. This separation time is often shorter than 1 hour and can compete with that of HPLC. HSCCC chromatograms can be used as the comparison patterns for the content control of the major bioactive compounds. The results of our studies demonstrated that it is feasible to control the raw herb's quality by analytical CCC.

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