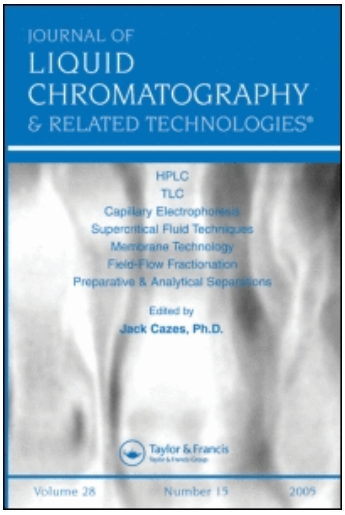

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Semi-Preparative Separation of Taraxeryl-Acetate and Coumarins from *Artemisia Dalailamae* Kraschen by High-Speed Countercurrent Chromatography

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SEMI-PREPARATIVE SEPARATION OF TARAXERYL-ACETATE AND COUMARINS FROM *ARTEMISIA DALAILAMAE* KRASCHEN BY HIGH-SPEED COUNTERCURRENT CHROMATOGRAPHY

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ABSTRACT

High-speed countercurrent chromatography (HSCCC) has been successfully applied to the semi-preparative separation of taraxeryl-acetate, isofraxidin and scopoletin from *Artemisia dalailamae* Kraschen. The separations were performed with a two-phase solvent system composed of chloroform-methanol-water (2:2:1, v/v/v). The main three pure fractions were analyzed by MS, IR, NMR etc. for structure determination. The results indicate that the method is suitable for semi-preparative separations of these compounds.

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INTRODUCTION

Countercurrent chromatography (CCC) is a special liquid-liquid partition chromatography without using solid support matrix. As a result, CCC eliminates adsorptive loss entirely and denaturation of samples. Samples can be recovered quantitatively [1]. CCC is very suitable for preparative separation of natural products.

In the past thirty years, a great advance has been made in CCC. More than ten sets of CCC instrumentation have come out successively. In the early of 1980s, especially, an epochmaking development in CCC technique was brought forth by the discovery of a unique hydrodynamic phenomenon which led to the development of high-speed countercurrent chromatography (HSCCC)[2,3]. The new method is characterized by high partition efficiency and large retention capability of the stationary phase under a high flow-rate of the mobile phase, yielding efficient separation in a few hours or less than one hour. Now, HSCCC has developed into analytical and preparative instrumental approaches.

HSCCC is now finding increasing use in separation problems, especially in the field of natural products, such as alkaloids[4, 5], flavonoids [4, 6], flavonol glycosides[7] and plant hormones[8, 9].

This paper presents the results of semi-preparative separations of a crude chloroform extract from *Artemisia dalailamae* Kraschen and two semi-pure fractions of this extract by HSCCC.

EXPERIMENTAL

Apparatus

The multilayer coil planet centrifuge used in the present study was produced by the Beijing Institute of New Technology Application, Beijing, China. The apparatus holds a pair of column holders symmetrically on the rotary frame at a distance of 8 cm from the central axis of the centrifuge. The separation column was prepared by winding a long piece of PTFE tubing, about 110 mm in length, 1.6 mm i.d. and 0.3 mm wall thickness , directly onto one of the holder hubs of 6 cm diameter to form a multilayer

coil. The β value, which is the ratio of the rotational radius to the revolutional radius, ranges from 0.4 at the internal terminal to 0.7 at the external terminal. The total capacity of the multilayer coil was measured as 250 ml approximately. The revolution speed of the apparatus is adjustable with a speed controller in the range from 0-1000 rpm; 800 rpm was used in the present separations.

The solvent was pumped into the column with a constant-flow pump (Model PB-1A, Beijing Orient Scientific Instrument Factory, Beijing, China). A UV detector (Model ZW-1, Beijing Institute of New Technology Application, Beijing, China) was used to monitor the effluent at 260 nm. A chromatographic data system (C-R2AX; Shimadzu Kyoto, Japan) was used to record the chromatogram.

Selection and Preparation of Two-phase Solvent System

The samples used in the present experiments were of medium polarity, so a chloroform-water system was selected as the basic dinary solvent system with methanol as assistant. A series of chloroform-methanol-water solvent systems in different proportions were prepared , and the respective settling time and two-phase volume ratio were determined. A two-phase solvent system composed of chloroform-methanol-water at a volume ratio of 2:1:1 was chosen and used in the present experiments. The solvent mixture was thoroughly equilibrated in a separatory funnel at room temperature and the two phases were separated shortly before use.

Preparation of Sample Solutions

A crude alcoholic extract from *A. dalailamae* Kraschen was dissolved in warm distilled water and the residue removed by filtration. The aqueous solution was then extracted successively with petroleum benzine, chloroform and n-butanol. The chloroform extract was evaporated to dryness and then dissolved in the lower chloroform phase of the two-phase solvent system at a concentration of about 500 mg/ml. Two semi-pure fractions of isofraxidin and scopoletin obtained from the separation of this chloroform extract by

classical silica gel column chromatography followed by crystallization, were also dissolved in the lower chloroform phase respectively. The three sample solutions were used in the subsequent experiments.

Separation Procedure

The coiled column was first entirely filled with the upper aqueous phase as stationary phase, and then the apparatus was rotated at 800 rpm, while the lower chloroform phase was pumped into the coiled column at a flow-rate of 1.5 ml/min as mobile phase. When no upper stationary phase appeared from the outlet of the column and all of the system was in a state of equilibrium, the sample solution was injected through the sampling port. The effluent was continuously monitored by a UV detector at 260 nm, and collected with test tubes according to chromatograms. Three separations were performed in turn without the renewal of stationary phase.

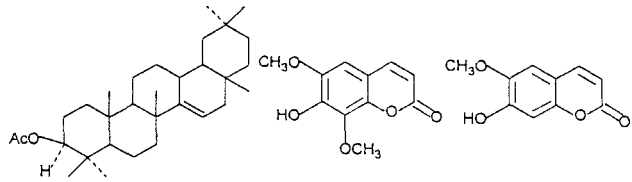
Structural Identification

The separations by HSCCC gave four pure compounds, and three of them were identified as taraxeryl-acetate, isofraxidin and scopoletin by MS, NMR, IR and element analysis. Their chemical structures were illustrated in Figure 1.

RESULTS AND DISCUSSION

Table 1 shows the systematic selection of the two-phase solvent system. A series of solvent systems composed of chloroform-methanol-water were prepared in different volume proportions. Their settling time and two-phase volume ratios were determined respectively.

Successful separation by HSCCC is mainly dependent on the selection of a suitable two-phase solvent system[10]. A two-phase solvent system with a settling time of less than 30 seconds is desirable for HSCCC apparatus[2,11,12]. At the same time, in order to avoid the excessive waste of solvent, the two-phase volume ratio should be close to 1[12]. According



(a) taraxeryl-acetate (b) isofraxidin (c) scopoletin

Figure 1. Structures of taraxeryl-acetate(a), isofraxidin(b) and scopoletin(c)

TABLE 1

Settling Time and Two-Phase Volume Ratios of a Series of Chloroform-Methanol-Water Solvent Systems.

No.	Volume proportion C-M-W[a]	Settling Time (seconds)	Volume Ratio UP/LP[b]
1	10:0:10	>30	1.0
2	10:1:9	>30	0.95
3	10:2:8	>30	0.94
4	10:3:7	>30	0.93
5	10:4:6	23	0.91
6	10:5:5	15	0.86
7	10:6:4	19	0.74
8	10:7:3	>30	0.52
9	10:8:2	--[c]	--[c]
10	11:5:5	21	0.82
11	9:5:5	15	0.98
12	8:5:5	15	1.0
13	7:5:5	>30	1.3

[a] C-M-W: Chloroform-Methanol-Water.
[b] UP/LP : Upper Phase/Lower Phase.
[c] -- : indicates solvent system exists in a single phase.

to these principles, the result of Table 1 shows that solvent systems of No. 5, 6, 7, 10, 11, 12 are more suitable to HSCCC and especially No.6,11,12, all with the shortest settling time of 15 seconds would produce much high and stable retention of the stationary phase in the coiled column of HSCCC. As the lower chloroform phase would be used as the mobile phase, the two-phase solvent system of No. 6, which is composed of chloroform-methanol-water at a 2:1:1 volume ratio, was selected for use in the present experiments.

Figure 2 shows the chromatogram of separation of the crude chloroform extract from *A. dalailamae* Kraschen by HSCCC. The sample size was 0.1 ml (or 50 mg). Three main peaks were eluted in one hour and are labeled as 1, 2, 3 in Figure 2. This separation gave 25.5 mg of 1, 4.4 mg of 2 and 5.0 mg of 3. Their chemical structures were then determined as taraxeryl-acetate, isofraxidin and scopoletin respectively.

Figure 3 and Figure 4 show the chromatograms of separations of the semi-pure fractions of isofraxidin and scopoletin by HSCCC.

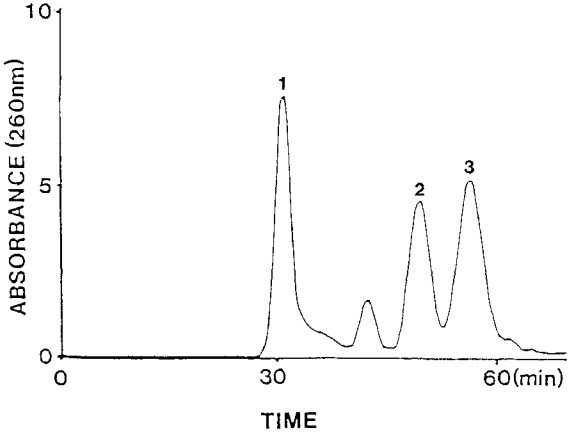


Figure 2. Chromatogram of separation of the chloroform extract from *A. dalailamae* Kraschen with chloroform-methanol-water (2:1:1), lower phase mobile, sample size 0.1ml (50 mg), flow-rate 1.5 ml/min. (1) taraxeryl-acetate, (2) isofraxidin and (3) scopoletin.

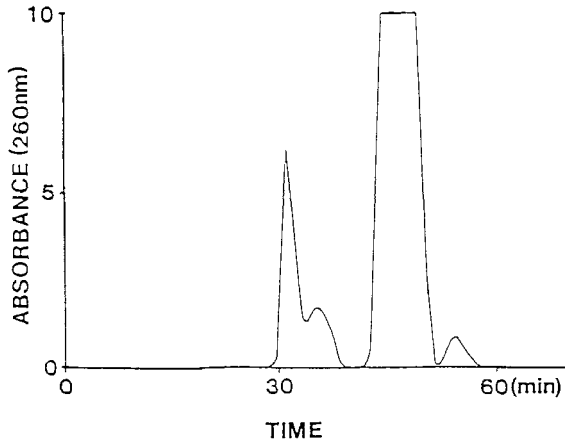


Figure 3. Chromatogram of separation of the semi-pure fraction of isofraxidin with chloroform-methanol-water (2:1:1), lower phase mobile, sample size 0.1ml, flow-rate 1.5 ml/min. The largest peak isofraxidin.

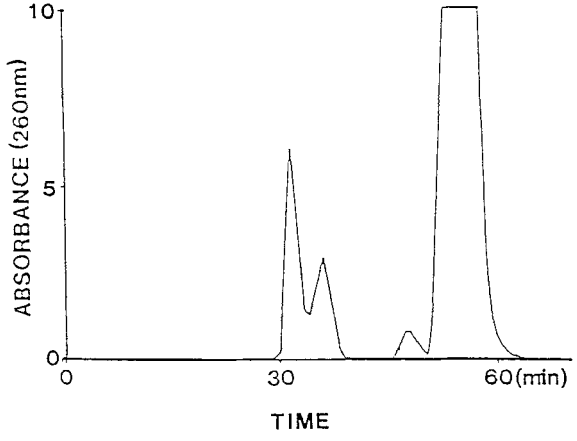


Figure 4. Chromatogram of separation of the semi-pure fraction of scopoletin with chloroform-methanol-water (2:1:1), lower phase mobile, sample size 0.1ml, flow-rate 1.5 ml/min. The largest peak is scopoletin.

The successful identifications of the three compounds show that both crude extract and semi-pure fractions have been successfully chromatographed. The results show that HSCCC has a good reproducibility and can be conduced without the renewal of stationary phase before every injection, when the range of polarity of a sample is not too wide.

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REFERENCES

1. Y. Ito, Countercurrent chromatography, Trends in Anal. Chem., 5(6), 142 (1986).

2. Y. Ito, Countercurrent chromatography, CRC Crit. Rev. Anal. Chem., 17(1), 65 (1986).

3. Y. Ito, Nature, 326, 419 (1987).

4. T. Y. Zhang, D. C. Cai and Y. Ito, Separations of flavonoids and alkaloids in medicinal herbs by high-speed countercurrent chromatography, J. Chromatogr., 435, 159 (1988).

5. D. G. Cai, M. J. Gu, G. P. Zhu, J. D. Zhang, T. Y. Zhang and Y. Ito, Semipreparative separation of alkaloids from *Cephalotaxus fortunei* Hook. F. by high-speed countercurrent chromatography, J. Liq. Chromatogr., 15, 2873, (1992).

6. T. Y. Zhang, X. Hua, R. Xiao and S. Kong, Separation of flavonoids in crude extract from sea buckthorn by countercurrent chromatography with two types of coil planet centrifuge, J. Liq. Chromatogr., 11, 233 (1988).

7. M. Vanhaelen and R. Varhaelen-Fastre, J. Liq. Chromatogr., 11,2969 (1988).

8. Y. Ito, J. Sandlin and W. G. Bower, J. Chromatogr., 244, 247 (1982).

9. N. B. Mandava and Y. Ito, J. Liq. Chromatogr., 7, 303 (1984).

10. Y. Ito and N. B. Mandava (Editors), Principle and Instrumentation of Countercurrent Chromatography: Theory and Practice, Marcel Dekker, New York, Ch. 3, p.79 (1988).

11. F. Oka and Y. Ito, J. Chromatogr., 538, 99 (1991).

12. Y. Ito and W. D. Conway, J. Chromatogr., 301, 405 (1984).

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