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Versatile two-phase solvent system for alkaloid separation by high-speed counter-current chromatography

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Abstract

In order to find a versatile high speed counter-current chromatography solvent system that can be used as a general prefractionation system for most alkaloids, the crude extracts of five Chinese traditional medicinal herbs, *Cortex phellodendri*, *Semen strychni*, green tea, *Sophora flavescens* ait, and *Datura mete* L. were resolved. All separations were performed only with a two-phase system composed of $CHCl_3$–CH_3OH–water (4:3:2). The water had different acidities controlled by adding NaH_2PO_4 or HCl to each sample. The fractionated components were identified by thin-layer chromatography, which confirmed this solvent system was versatile and very useful for the separation of alkaloids.

Keywords: Pharmaceutical analysis; Counter-current chromatography; Alkaloids

1. Introduction

High-speed counter-current chromatography (HSCCC) is a liquid–liquid partitioning chromatography method in which the stationary phase is immobilized by a centrifugal force. When the mobile phase is pumped through sample components are partitioned between the mobile and stationary phase and separated on the basis of differences in their partition coefficients [1].

With the advantage of eliminating the use of a solid support matrix, HSCCC is very suitable for separation of active components from traditional Chinese medicinal herbs and other natural products. In the past, there were many successful studies for the separation of various components such as alkaloids, flavonoids, lignans tannins, terpenes and saponins [2]. But those methods have been not used widely by the researchers in phytochemistry or pharmacy because the selection of a two–phase solvent system is difficult for them. The selection of a solvent system is the most important step in performing HSCCC. Selecting a solvent system for HSCCC means simultaneously choosing the column and the eluent. It is very useful to develop a fast and simple selection method with a two-phase solvent system as a general prefractionation step which is able to separate a broad range of compounds.

The alkaloids are weakly basic compounds. Most of them may be solved in acid solutions. If adding a base to the aqueous phase to raise the pH to about

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9.5~11, the alkaloids can be extracted with chloroform. This fact shows that the solubility of alkaloids in a two-phase solvent system of HSCCC can be changed by controlling the pH. The $CHCl_3$–CH_3OH–water solvent system provides reasonably short settling times and has been used widely for HSCCC [3]. In this paper an extensive search for a HSCCC solvent system for the separation of alkaloids from the crude plant extracts is described.

Cortex phellodendri, *Semen strychni*, green tea, *Sophora flavescens* ait, and *Datura mete* L. are Chinese traditional medicinal herbs. Their main active components are alkaloids [4]. The crude extracts were resolved by HSCCC.

2. Experimental

2.1. Apparatus

The HSCCC experiments were performed using a multilayer coil planet centrifuge constructed at the Beijing Institute of New Technology Application, China. The apparatus has a pair of column holders symmetrically on the rotary frame at a distance of 8 cm from the central axis of the centrifuge (β=0.5–0.75). The multilayer coil was prepared by winding a 1.6 mm I.D. polytetrafluoroethylene (PTFE) tube directly onto the holder hub with a total capacity of 260 ml. The system was equipped with a metering pump (Model NS-1007, Beijing Institute of New Technology Application), a UV detector (Model 8823A-UV, Beijing Institute of New Technology Application), a recorder and an injection valve.

2.2. Reagents

All organic solvents and chemical reagents are analytical-reagent grade (Beijing Chemical Factory). Silica gel plates were from Qingdao Ocean Chemical Factory (China). *Cortex phellodendri*, *Semen strychni*, green tea, *Sophora flavescens* ait, and *Datura mete* L. were purchased from the Kunming medical market of China.

2.3. Extraction of crude alkaloids

The preparations of samples of crude alkaloids from the herbs were extracted with ethanol. Then, the ethanol extracts were acidified with dilute hydrochloric acid and precipitated impurities were removed by filtration. The aqueous phases were extracted with chloroform. Finally, by adding a base to the aqueous phase to raise the pH to about 9.5~11, the alkaloids were extracted again with chloroform. The chloroform solutions were evaporated to obtain the crude alkaloids [4].

2.4. Preparation of two-phase solvent system and sample solution

In the present studies, the two-phase solvent system was composed of chloroform–methanol–water (4:3:2, v/v/v) in which the water had different acidities, controlled by NaH_2PO_4 buffer solution or HCl. Each solvent mixture was thoroughly equilibrated in a separating funnel at room temperature and the two phases were separated before use.

The sample solutions were prepared by dissolving 20 mg crude alkaloid extract in 2 ml of the above phase mixture consisting of equal volumes of each phase.

2.5. HSCCC procedure

The multilayer coiled column was first entirely filled with the upper phase at a flow-rate of 10 ml min^{-1}, the lower phase was pumped into the inlet of the column at a flow of 2.0 ml min^{-1} in the head-to-tail elution mode, while a 2.0 ml sample solution containing 20 mg crude alkaloids was introduced into the column through an injection valve and the apparatus was rotated at 800 rpm. The effluent from the outlet of the column was continuously monitored with a UV detector at 254 nm. Fractions of top peaks were collected according to the chromatograms.

2.6. TLC analysis

All fractions from HSCCC were spotted on silica gel plates and developed in saturated normal chambers (saturation time 30 min). Visual detection was done by iodic vapor or spraying Dragendorff reagent.

3. Results and discussion

As alkaloids are weakly basic, the acidity of the solvent may often change their solubilities. We have conducted a literature search for suitable solvent systems previously used for alkaloids and considered the solvent systems provide nearly equal volumes of the upper and lower phase with reasonably short settling times. The two-phase solvent system composed of $CHCl_3$–CH_3OH–water (4:3:2) was selected for separations of alkaloids coming from five medicinal herbs. The acidity of the water was changed for different alkaloids.

Fig. 1 shows three chromatograms of crude alkaloids extracted from *Cortex phellodendri* obtained by HSCCC. The aqueous phases were 0.3 mol/l HCl (pH=0.5), 0.05 mol/l HCl (pH=1.3) and 0.01 mol/l HCl (pH=2.0). The retentions of stationary phase were 65.1% (Fig. 1A), 66.9% (Fig. 1B) and 68.2% (Fig. 1C). When the acidity was 0.05 mol/l HCl, the separation was best. The top fractions of each peak were collected and analyzed with TLC. Peaks 4–8 gave single monochromatic spots on TLC. Peak 1 and peak 2 contained some impurity. Peak 3 had two alkaloids. Dragendorff reagent was monochromatic color reagent. TLC is shown in Fig. 2.

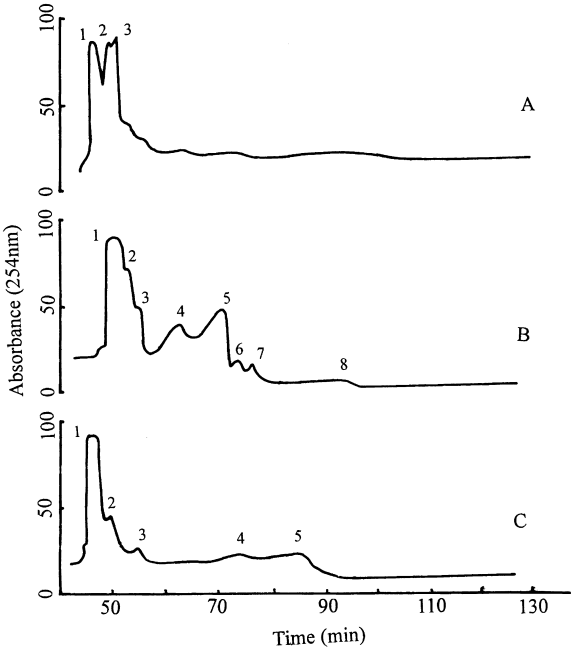

Fig. 1. Chromatograms of crude alkaloid extract from *Cortex phellodendri* obtained by HSCCC. Experimental conditions: apparatus: HSCCC centrifuge; column: Ito multilayer coil, 1.6 mm I.D., 260 ml capacity; solvent system: $CHCl_3$–CH_3OH–water (4:3:2), in which the acidities of water were 0.3 mol/l (A), 0.05 mol/l (B) and 0.01 mol/l (C) HCl; mobile phase: lower phase; sample: 20 mg crude alkaloid extract from *Cortex phellodendri* dissolved in 2 ml solvent; flow-rate: 2 ml/min; revolution speed: 800 rpm; retention of stationary phase: 65.1% (A), 66.9% (B) and 68.2% (C); detection: 254 nm.

Fig. 3 shows the chromatograms of crude alkaloids extracted from *Semen strychni*. The aqueous phases were 0.02 mol/l HCl (pH 1.7), 0.008 mol/l HCl (pH 2.1), 0.001 mol/l HCl (pH 3) and the retentions of stationary phase were 64.2% (Fig. 3A), 65.4% (Fig. 3B) and 67.1% (Fig. 3C), respectively. When the acidity of the water was 0.008 mol/l HCl, the separation was best. The top fractions of peaks 1–8 produced single monochromatic spots on the TLC with the Dragendorff reagent (Fig. 4).

The chromatograms of crude alkaloids extracted from green tea are shown in Fig. 5. The aqueous phases were 2.5×10^{-2} mol/l NaH_2PO_4 and their pH values were adjusted to 4.1, 5.6 and 6.0 with 6 mol/l HCl or NaOH, retentions of stationary phase were 65.5% (Fig. 5A), 66.9% (Fig. 5B) and 67.7% (Fig. 5C), respectively. The separation was best when the acidity of water was pH 5.6. Each peak gave a single monochromatic spot on the TLC when the visual detections were done by iodic vapor (Fig. 6).

The crude alkaloids extracted from *Sophora*

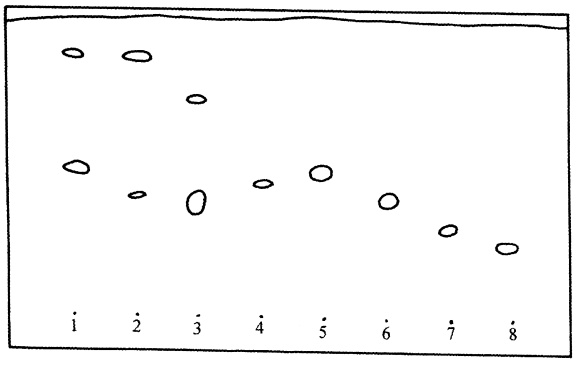

Fig. 2. TLC analysis of HSCCC top fractions from *Cortex phellodendri* in Fig. 1B. The analyses were made on a silica gel G TLC plate developed with chloroform–methanol–aqueous ammonia (4:1:0.04, v/v/v) and stained with Dragendorff reagent to detect the alkaloids.

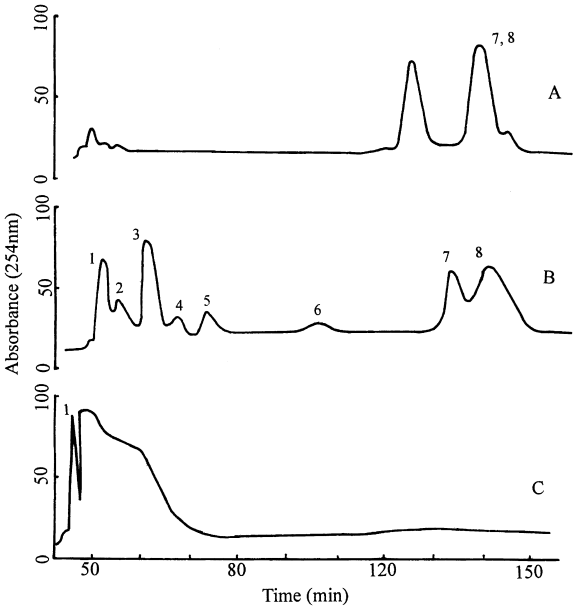

Fig. 3. Chromatograms of crude alkaloid extract from *Semen strychni* obtained by HSCCC. Experimental conditions: apparatus: HSCCC centrifuge; column: Ito multilayer coil, 1.6 mm I.D., 260 ml capacity; solvent system: $CHCl_3$–CH_3OH–water (4:3:2), in which the acidities of water were 0.02 mol/l (A), 0.008 mol/l (B) and 0.001 mol/l (C) HCl; mobile phase: lower phase; sample: 20 mg crude alkaloid extract from *Semen strychni* dissolved in 2 ml solvent; flow-rate: 2 ml/min; revolution speed: 800 rpm; retention of stationary phase: 64.2% (A), 65.4% (B) and 67.1% (C); detection: 254 nm.

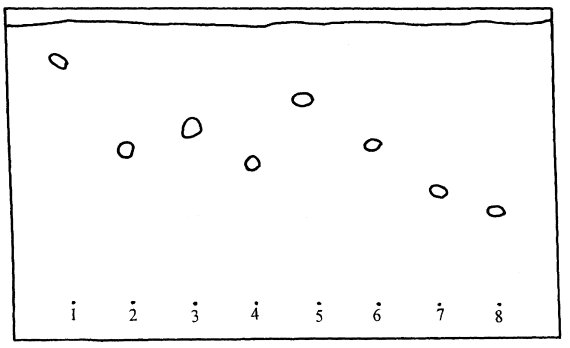

Fig. 4. TLC analysis of HSCCC top fractions from *Semen strychni* in Fig. 3B. The analyses were made on a silica gel G TLC plate developed with chloroform–methanol–aqueous ammonia (5:0.15:0.03, v/v/v) and stained with Dragendorff reagent to detect the alkaloids.

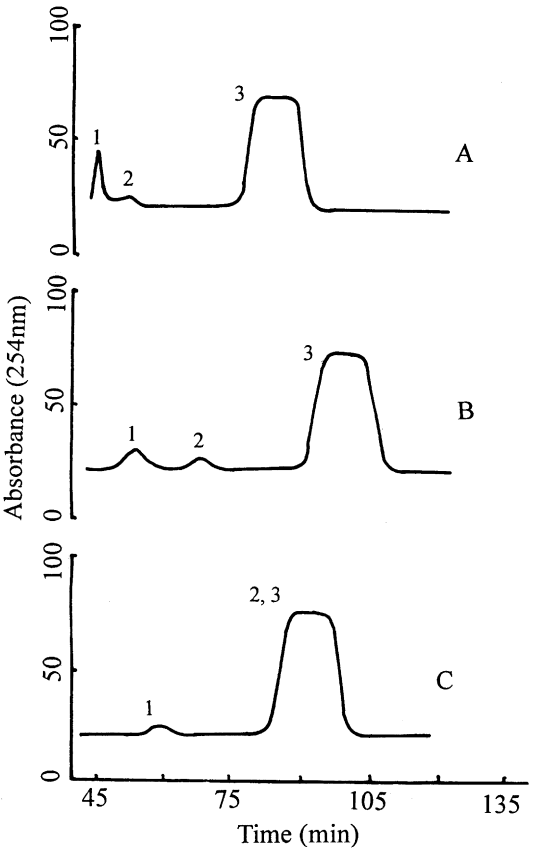

Fig. 5. Chromatograms of crude alkaloid extract from green tea obtained by HSCCC. Experimental conditions: apparatus: HSCCC centrifuge; column: Ito multilayer coil, 1.6 mm I.D., 260 ml capacity; solvent system: $CHCl_3$–CH_3OH–water (4:3:2), in which the water contained 2.5×10^{-2} mol/l NaH_2PO_4 and the pH was adjusted with 6 mol/l HCl or NaOH to 4.1 (A), 5.6 (B) or 6.0 (C); mobile phase: lower phase; sample: 20 mg crude alkaloid extract from green tea dissolved in 2 ml solvent; flow-rate: 2 ml/min; revolution speed: 800 rpm; retention of stationary phase: 65.5% (A), 66.9% (B) and 67.7% (C); detection: 254 nm.

flavescens ait also were separated by HSCCC (Fig. 7). Each aqueous phase contained 2.5×10^{-2} mol/l NaH_2PO_4 and their pH values were adjusted to 5.9, 5.4 and 5.1 with 6 mol/l HCl or NaOH and retentions of stationary phase were 70.4% (Fig. 7A), 71.5% (Fig. 7B) and 73.8% (Fig. 7C). The chromatogram of pH 5.4 was better than pH 5.9 or pH 5.1. The top fractions of each peak of Fig. 7B were collected and analyzed by TLC. Most of them produced a single monochromatic spot on TLC. Fraction of peak 1 gave a monochromatic color

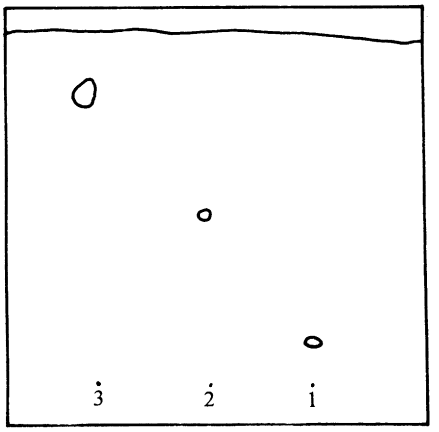

Fig. 6. TLC analysis of HSCCC top fractions from green tea in Fig. 5B. The analyses were made on a silica gel G TLC plate developed with chloroform–methanol (100:6, v/v) and stained with iodic vapor.

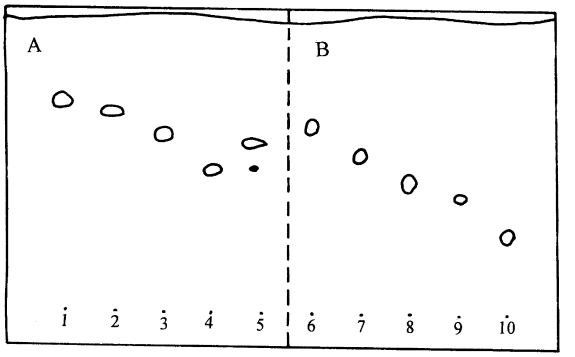

Fig. 8. TLC analysis of HSCCC top fractions from *Sophora flavescens* ait in Fig. 7B. The analyses were made on two silica gel G TLC plates developed with chloroform–methanol–aqueous ammonia (5:0.15:0.03, v/v/v) (A), chloroform–methanol–aqueous ammonia (80:3:1, v/v/v) (B) and stained with Dragendorff reagent to detect the alkaloids.

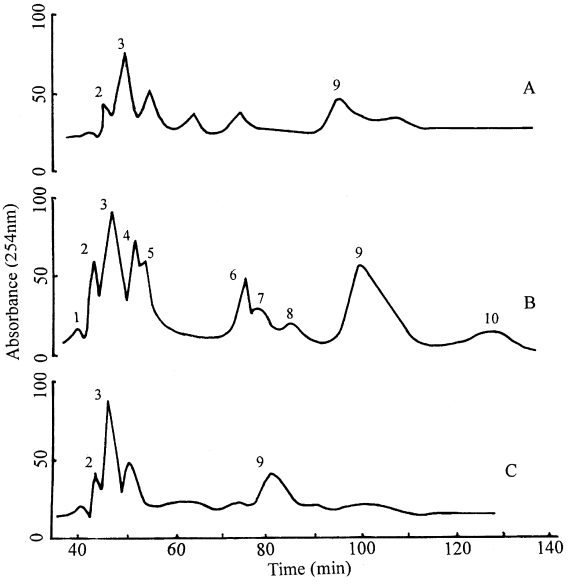

Fig. 7. Chromatograms of crude alkaloid extract from *Sophora flavescens* ait obtained by HSCCC. Experimental conditions: apparatus: HSCCC centrifuge; column: Ito multilayer coil, 1.6 mm I.D., 260 ml capacity; solvent system: $CHCl_3$–CH_3OH–water (4:3:2), in which the water contained 2.5×10^{-2} mol/l NaH_2PO_4 and the pH was adjusted with 6 mol/l HCl or NaOH to 5.1 (A), 5.4 (B) or 5.9 (C); mobile phase: lower phase; sample: 20 mg crude alkaloid extract from *Sophora flavescens* ait dissolved in 2 ml solvent; flow-rate: 2 ml/min; revolution speed: 800 rpm; retention of stationary phase: 70.4% (A), 71.5% (B) and 73.8% (C); detection: 254 nm.

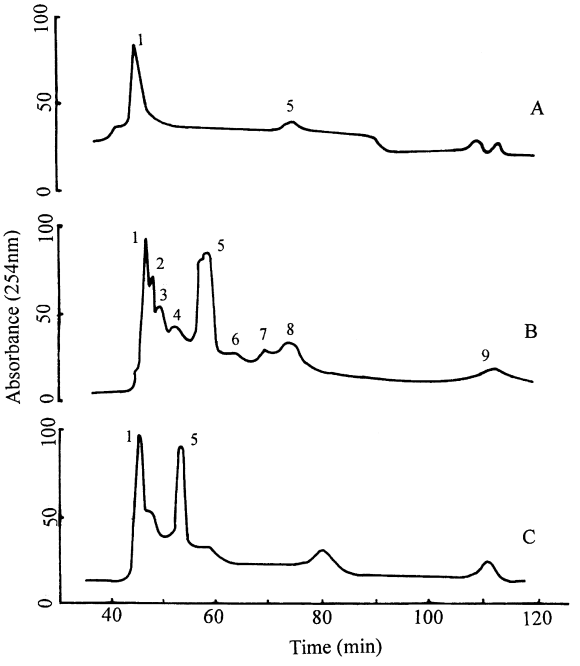

Fig. 9. Chromatograms of crude alkaloid extract from *Datura mete* L. obtained by HSCCC. Experimental conditions: apparatus: HSCCC centrifuge; column: Ito multilayer coil, 1.6 mm I.D., 260 ml capacity; solvent system: $CHCl_3$–CH_3OH–water (4:3:2), in which the water contained 2.5×10^{-2} mol/l NaH_2PO_4 and the pH was adjusted with 6 mol/l HCl or NaOH to 5.4 (A), 6.0 (B) or 6.2 (C); mobile phase: lower phase; sample: 20 mg crude alkaloid extract from *Datura mete* L. dissolved in 2 ml solvent; flow-rate: 2 ml/min; revolution speed: 800 rpm; retention of stationary phase: 70.4% (A), 72.3% (B) and 73.1% (C); detection: 254 nm.

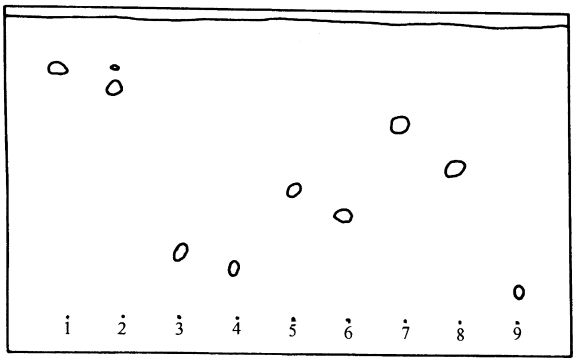

Fig. 10. TLC analysis of HSCCC top fractions from *Datura mete* L. in Fig. 9B. The analyses were made on a silica gel G TLC plate developed with chloroform–methanol–aqueous ammonia (5:0.33:0.03, v/v/v) and stained with Dragendorff reagent to detect the alkaloids.

reaction with iodic vapor and no color reaction with the Dragendorff reagent (Fig. 8).

Fig. 9 shows the chromatogram of crude alkaloids extracted from *Datura mete* L. Each aqueous phase contained 2.5×10^{-2} mol/l NaH_2PO_4 and their pH values were adjusted to 6.2, 6.0 and 5.4. In this case, the retentions of stationary phase were 70.4% (Fig. 9A), 72.3% (Fig. 9B) and 73.1% (Fig. 9C). The separation was best when acidity was pH 6.00. The top fractions of each peaks were collected and most of peak also gave a single monochromatic spot on the TLC with the Dragendorff reagent (Fig. 10).

Besides the above investigation, there are some other articles published on the separation of alkaloids by $CHCl_3$–CH_3OH–water solvent system [5–7].

4. Conclusions

The overall results show that when the pH of water in a solvent system is increased, the solubilities in the mobile phase of crude alkaloids from *Semen strychni*, *Sophora flavescens* ait and *Datura mete* L. are increased and elution becomes easy for those alkaloids. But for some crude alkaloids from *Cortex phellodendri* and green tea, elution is slower when the pH of water increases in the experimental pH range. The reason may be that those alkaloids have different types of molecular structure.

From the above comprehensive studies, we also know that $CHCl_3$–CH_3OH–water was an excellent solvent system for HSCCC. Most of the alkaloids are weakly basic compounds. Controlling the pH may change the solubility of some alkaloids in the two-phase solvent system of HSCCC. Therefore, $CHCl_3$–CH_3OH–water (4:3:2) in which water had different acidities controlled by NaH_2PO_4 or HCl, is a versatile HSCCC solvent system for the efficient separation of alkaloids from crude plant extracts.

Acknowledgements

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References

[1] Y. Ito, CRC Crit. Rev. Anal. Chem. 65 (1986) 17.
[2] L.M. Yuan, R.N. Fu, T.Y. Zhang, Chin. J. Pharm. Anal. 18 (1998) 60.
[3] Y. Ito, W.D. Conway, High-Speed Countercurrent Chromatography, Wiley, New York, 1996.
[4] Q.S. Lin, Phytochemistry, Science Press, Beijing, 1977.
[5] R.J. Petroski, Nat. Toxins 1 (1992) 84.
[6] F.J. Schmitz, F.S. DeGuzman, M.B. Hossain, D. Vander Helm, J. Org. Chem. 53 (1991) 804.
[7] S. Sakemi, H.H. Sun, C.W. Jefford, G. Bernardinelli, Tetrahedron Lett. 30 (1989) 2517.