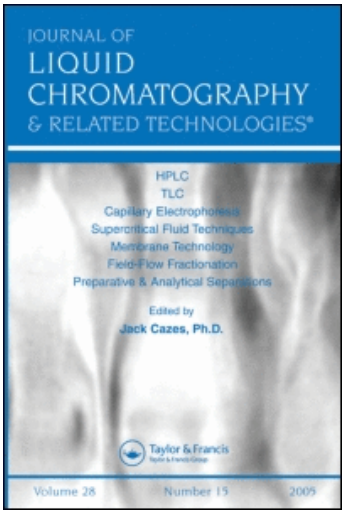

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

VERSATILE TWO-PHASE SOLVENT SYSTEM FOR ANTHRAQUINONE PREFRACTIONATION BY HIGH SPEED COUNTERCURRENT CHROMATOGRAPHY

L. M. Yua[a]; X. X. Chen[a]; P. Ai[a]; M. Zi[a]; P. Wu[a]; Z. Y. Li[a]
[a] Department of Chemistry, Yunnan Normal University, Kunming, P. R. China

Online publication date: 30 November 2001

J. LIQ. CHROM. & REL. TECHNOL., 24(19), 2961–2970 (2001)

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L. M. Yua,* X. X. Chen, P. Ai, M. Zi, P. Wu, and Z. Y. Li

Department of Chemistry, Yunnan Normal University,
Kunming 650092, P. R. China

ABSTRACT

In order to find a high-speed countercurrent chromatography solvent system that can be used as a general prefractionation step for anthraquinone, the crude extracts of five traditional Chinese medicines, Radix rubiae, Rhizoma polygoni cuspidati, Semen cassiae, Radix et rhizoma rhei, and Aloe were separated with a two-phase system composed of $CHCl_3$:CH_3OH:H_2O = 4:x:2 in which x was between 3 and 4 for different samples. The fractionated components were identified on TLC. This solvent system gave a good separation in most of the anthraquinones.

INTRODUCTION

High-speed countercurrent chromatography (HSCCC) is a high-efficiency, speedy separation method. The method provides excellent means for separation

*Corresponding author.

without complications arising from the use of solid support.(1) It is very suitable for separations of active components from the traditional Chinese medicinal herbs and other natural products.

In the past, some HSCCC scientists have successfully separated various components, such as alkaloids, flavonoids, anthraquinones, lignans tannins, terpenes, saponins et al.(2) But those methods have been not used widely by the researcher of phytochemistry or pharmacy because the selections of two–phase solvent systems are 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. This paper describes a simple and fast reproducible method that can be used as a general prefractionation step for anthraquinone compounds.

Anthraquinones compounds have a wide polarity range. In general, they were extracted from plants with methanol or ethanol, because most anthraquinones have a good solubility in alcohol.(3) The $CHCl_3/CH_3OH/H_2O$ solvent system contains the methanol and has been used widely for HSCCC.(4) Radix rubiae, Rhizoma polygoni cuspidati, Semen cassiae, Radix et rhizoma rhei, and Aloe are traditional Chinese medicines, whose main active components are anthraquinones.(3) Therefore, the $CHCl_3/CH_3OH/H_2O$ solvent system was used for separation of anthraquinones in this study.

EXPERIMENTAL

Apparatus

The present studies were performed with a multilayer coil planet centrifuge constructed at the Beijing Institute of New Technology Application, Berjing, 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 multilayer coil separation column was prepared by winding a 1.6 mm I.D. polytetrafluoroethylene (PTFE) tube directly onto the holder hub to form multiple coiled layers with a total capacity of 260 mL.

The system was equipped with a metering pump (Model NS-1007, Beijing Institute of New Technology Application, China), a UV detector (Model 8823A-UV, Beijing Institute of New Technology Application, China), a recorder, and a sample injection valve.

Reagents and Materials

All organic solvents and chemical reagents were of analytical grade and purchased from Beijing Chemical Factory, China.

Radix rubiae, Rhizoma polygoni cuspidati, Semen cassiae, Radix et rhizoma rhei, and Aloe were purchased from Kunming traditional Chinese medicine market of China.

Extraction of Crude Anthraquinones

Radix rubiae, Rhizoma polygoni cuspidati, Semen cassiae, Radix et rhizoma rhei, and Aloe were extracted with ethanol at a normal temperature, respectively. Then these extracts were filtered and the filtrates were evaporated to dryness under reduced pressure.(3)

HSCCC Procedure

The HSCCC experiment was performed with a two-phase solvent system composed of chloroform/methanol/water (4:x:2, *v/v/v*). The ratio of methanol was between 3 and 4 in different samples. The solvent mixture was thoroughly equilibrated in a separatory funnel at room temperature and the two phases were separated shortly before use. In each separation, the multilayer coil column was first entirely filled with the upper stationary phase. Then, the lower mobile phase was pumped into the inlet of the column at a flow of 2.0 $mL.min^{-1}$, while 20 mg crude anthraquinones extracts in 2.0 mL of a phase mixture consisting of equal volumes of each phase was introduced into the column through a sample injection valve, and the apparatus was rotated at 800 rpm. After running for 120 min, the centrifuge was stopped and distilled water was pumped into the column at 4 mL/min for 185 min. The effluent from the outlet of the column was continuously monitored with a UV detector at 254 nm. Top fractions of peaks were collected according to the chromatograms.

TLC Analysis

The silica gel G thin-layer chromatography (TLC) plates were purchased from Qingdao Ocean Chemical Factory, China. The TLC plate was stained with a 5% KOH methanol solution to detect the anthraquinones.(3)

RESULTS AND DISCUSSION

The anthraquinone compounds have a wide polarity range and good solubility in the methanol. The two-phase solvent system composed of

$CHCl_3$:CH_3OH:H_2O = 4:x:2 was selected for separations of crude anthraquinone extracts from Radix rubiae, Rhizoma polygoni cuspidati, Semen cassiae, Radix et rhizoma rhei, and Aloe because the $CHCl_3$/CH_3OH/H_2O solvent systems contain the methanol and provide nearly equal volumes of the upper and lower phase with reasonably short settling times. Here x was changed from 3 to 4 in different samples. Changing the ratio of methanol in the solvent system, permitted changing, simultaneously, the selectivity of upper and lower phase; as methanol can dissolve in chloroform and water and may change the polarity of two phases.

Figure 1A shows three chromatograms of crude anthraquinones extracted from Radix rubiae obtained by HSCCC. The separation was better when the ratio of solvent system was 4:4:2. The top fractions of each peak were collected and analyzed with TLC. Peaks 1 to 9 gave a positive color reaction and peaks1, 2, 4, 5, 7, 8, 9 only had a spot on the TLC. They were illustrated in Fig 1B.

Figure 2A was the chromatogram of crude anthraquinones extracted from Rhizoma polygoni cuspidati. When the ratio of methanol was 3.8, the separation was better. Most of the top fractions of peaks produced positive color reactions, but peak 11 give a negative reaction. Peaks 1, 2, 3, 6, 7, 10 had a single spot on the TLC. They were illustrated in Figure 2B.

The chromatograms of crude anthraquinones extracted from Semen cassiae are shown in Figure 3A. The separation was better when the ratio of methanol was 3.8. Each peak gave a single spot on the TLC, except peaks 11 and 12 (Figure 3B).

The crude anthraquinones extracted from Radix et rhizoma rhei also were separated by HSCCC (Figure 4A). The chromatogram of the 4:3.8:2 solvent system was better than the chromatograms of 4:3.5:2 and 4:4:2. The top fractions of each peak were collected and analyzed with TLC. All of them gave positive color reaction, and six of them produced a single spot on the TLC (Figure 4B).

Figure 5A shows the chromatogram of crude anthraquinones extracted from Aloe. The separation was better when the ratio of methanol was 3.6. The top fractions of each peak were collected and most of the peaks also gave a single monochromatic spot on the TLC (Figure 5B).

Besides the above investigation, there was an article published on the separation of anthraquinones by the $CHCl_3$/CH_3OH/H_2O solvent system.(5)

CONCLUSIONS

From the above comprehensive studies, we know that $CHCl_3$/CH_3OH/H_2O was an excellent solvent system for HSCCC. The anthraquinones have a wide polarity range. Most of them have a good solubility in methanol. Methanol may change, simultaneously, the selectivity of the upper phase and lower phase by changing the ratio of methanol in the $CHCl_3$/CH_3OH/H_2O solvent system.

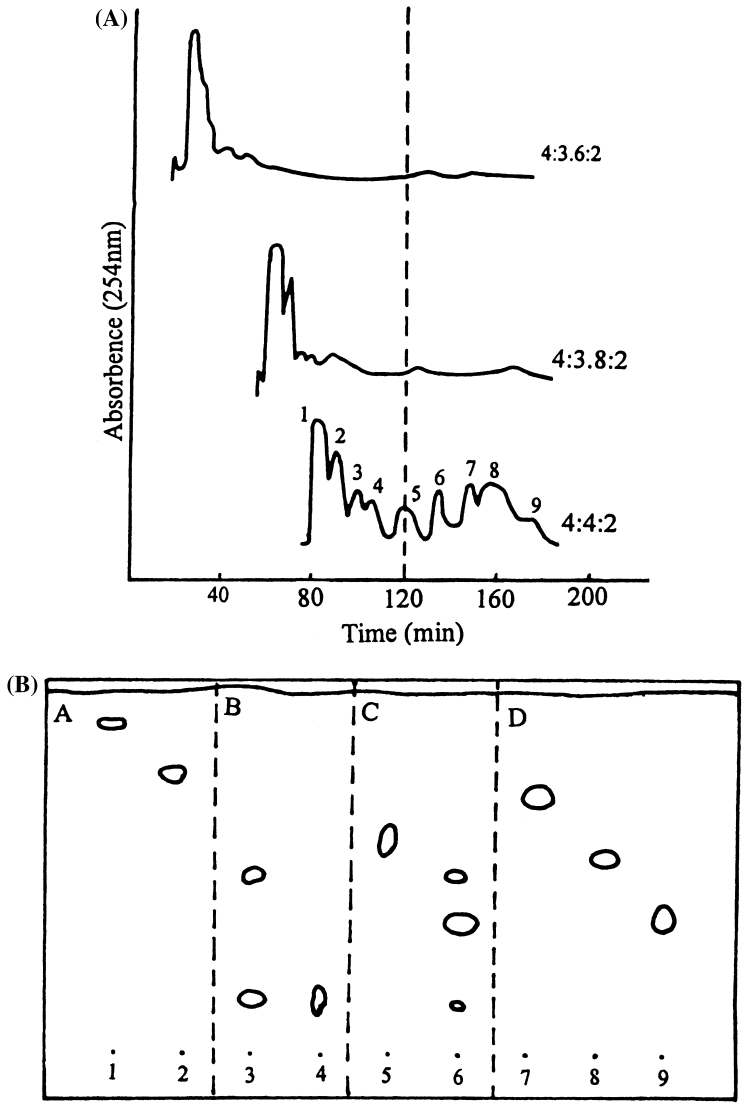

Figure 1. (A) HSCCC separation of crude anthraquinones from Radix rubiae. (B) TLC analysis of HSCCC fractions from Radix rubiae developed with solvent system composed of A: chloroform-methanol (1.9:0.1, v/v); B: chloroform-methanol (9.3:0.7, v/v); C: chloroform-methanol (1.9:0.6, v/v); D: chloroform-methanol (1:1, v/v).

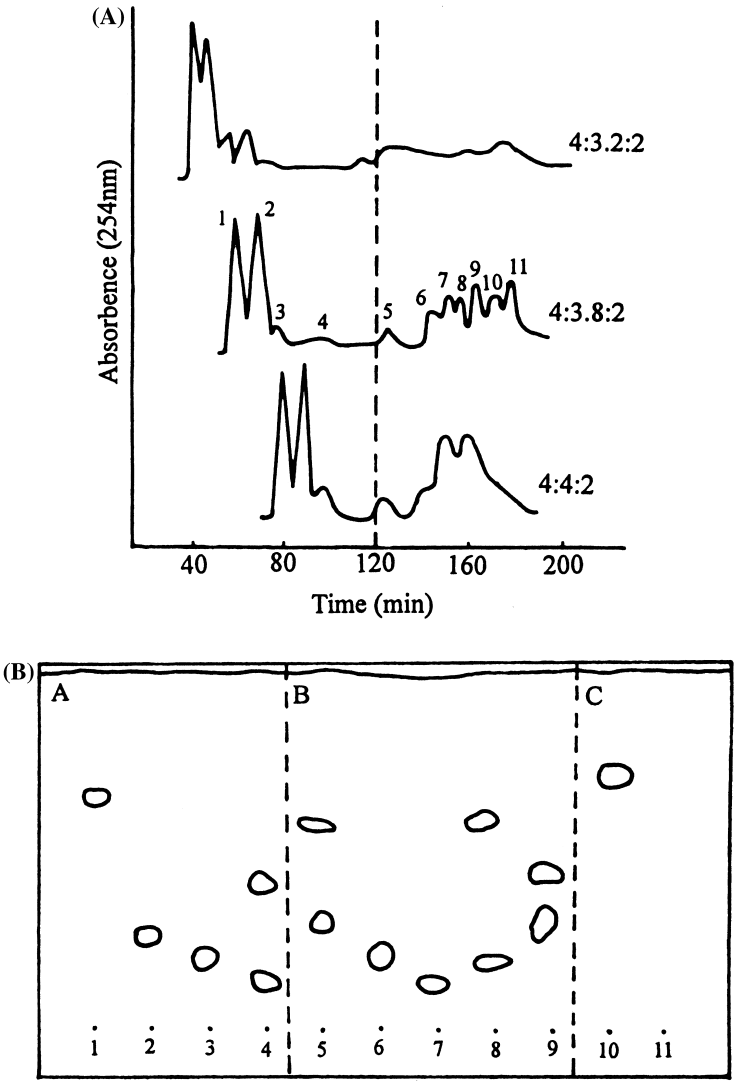

Figure 2. (A). HSCCC separation of crude anthraquinones from Rhizoma polygoni cuspidati. (B) TLC analysis of HSCCC fractions from Rhizoma polygoni cuspidati developed with solvent system composed of A: ethyl acetate-methanol (2.4:0.1, v/v); B: ethyl acetate -methanol (2.3:0.2, v/v); C: ethyl acetate-methanol (1.9:0.6, v/v).

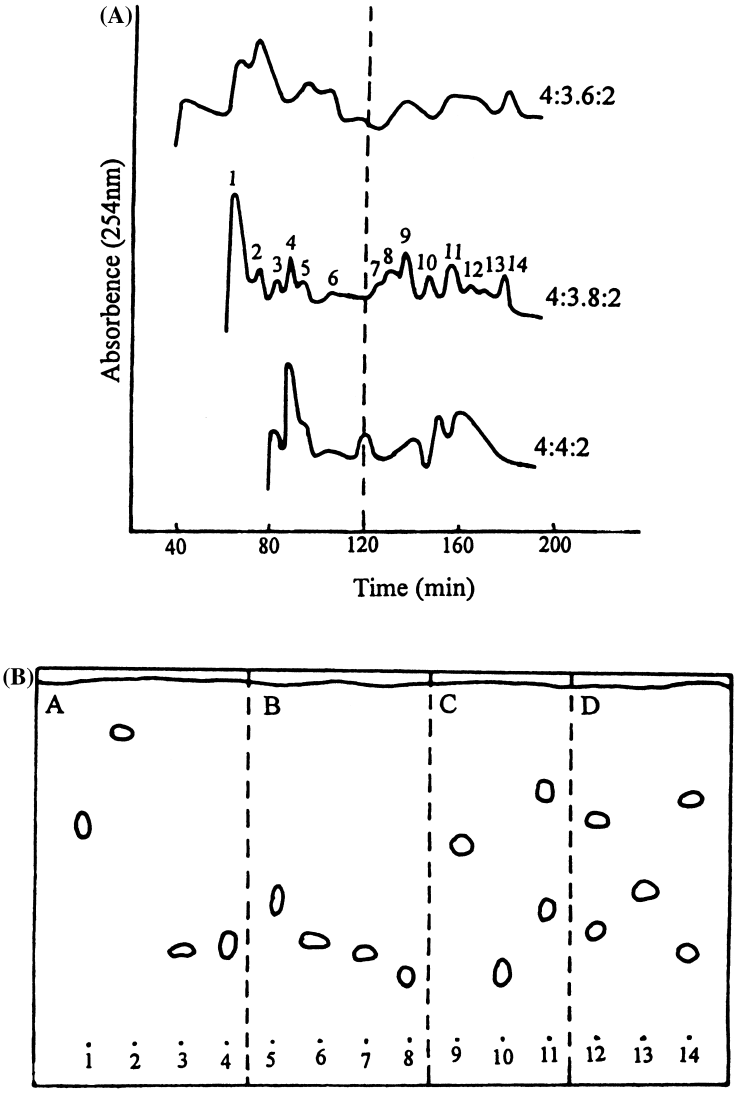

Figure 3. (A) HSCCC separation of crude anthraquinones from *Semen cassiae*. (B) TLC analysis of HSCCC fractions from *Semen cassiae* developed with solvent system composed of A: ethyl acetate-methanol (2.4:0.1, v/v); B: ethyl acetate-methanol (4.7:0.3, v/v); C: ethyl acetate-methanol (7:3, v/v); D: ethyl acetate-methanol (1:1, v/v).

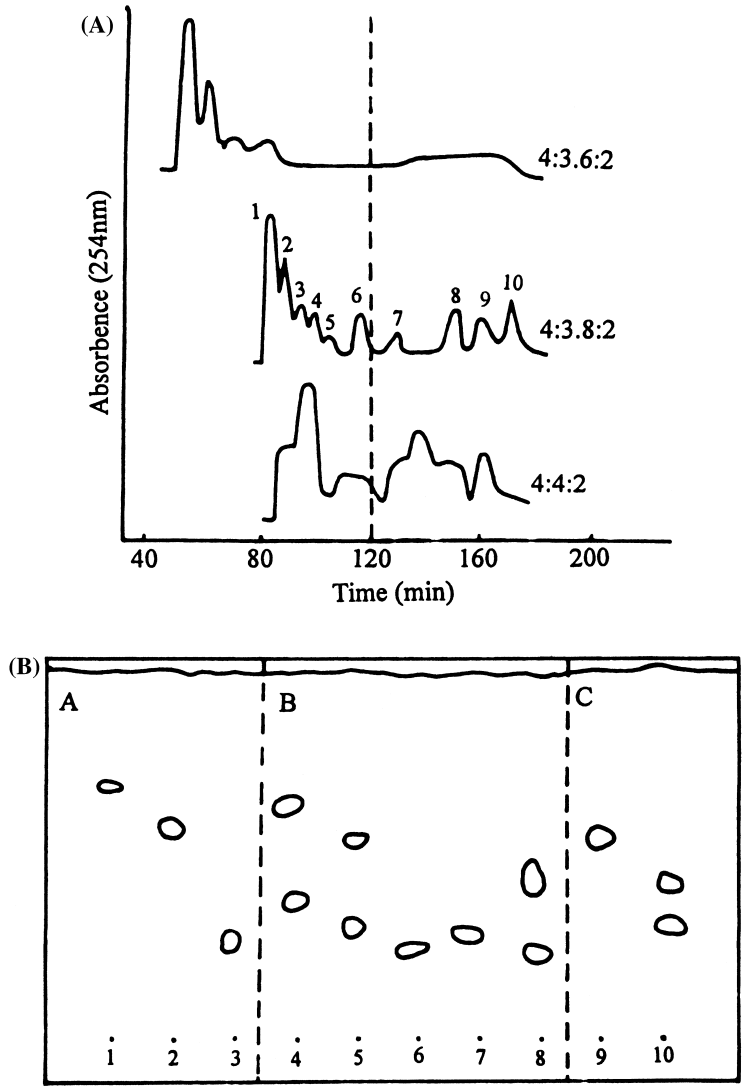

Figure 4. (A) HSCCC Separation of crude anthraquinones from *Radix et rhizome rhei*. (B) TLC analysis of HSCCC fractions from *Radix et rhizoma rhei* developed with solvent system composed of A: chloroform-methanol (1.6:0.03, v/v); B: chloroform-methanol (4.3:0.7, v/v); C: chloroform-methanol (3:1, v/v).

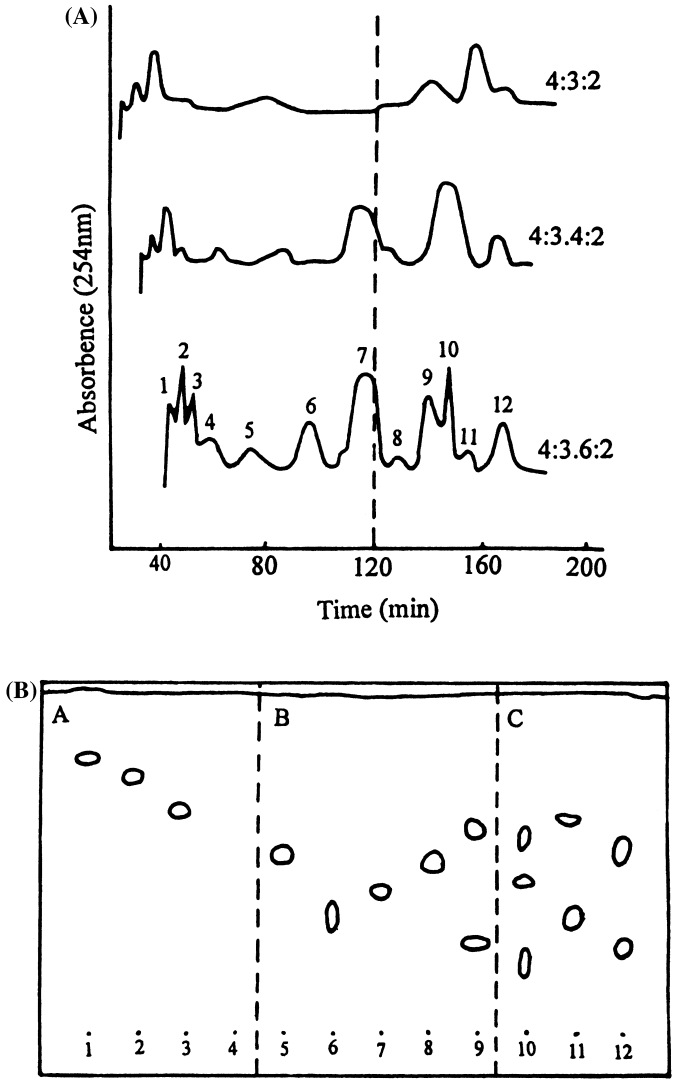

Figure 5. (A) HSCCC separation of crude anthraquinones from Aloe. (B) TLC analysis of HSCCC fractions from Aloe developed with solvent system composed of A: ethyl acetate-methanol (11:1, v/v); B: ethyl acetate-methanol (9:1, v/v); C: ethyl acetate-methanol (7:3, v/v).

Therefore, $CHCl_3$:CH_3OH:H_2O = 4:x:2 (x = 3 ~ 4) is a versatile high speed countercurrent chromatography solvent system for the anthraquinone separation.

ACKNOWLEDGMENTS

This work was supported by National Natural Science Foundation and Yunnan Province's Natural Science Foundation of China.

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Received April 28, 2001 Manuscript 5578
Accepted July 5, 2001