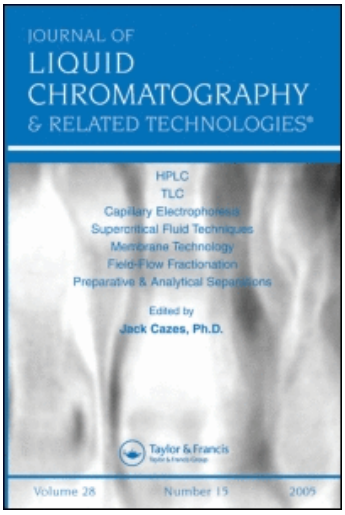

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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Online publication date: 22 March 2000

To cite this Article Weiss, E. , Ziffer, H. and Ito, Y.(2000) 'USE OF COUNTERCURRENT CHROMATOGRAPHY (CCC) TO SEPARATE MIXTURES OF ARTEMISININ, ARTEMISITENE, AND ARTEANNUIN B', Journal of Liquid Chromatography & Related Technologies, 23: 6, 909 — 913
To link to this Article: DOI: 10.1081/JLC-100101497
URL: http://dx.doi.org/10.1081/JLC-100101497

J. LIQ. CHROM. & REL. TECHNOL., 23(6), 909–913 (2000)

USE OF COUNTERCURRENT CHROMATOGRAPHY (CCC) TO SEPARATE MIXTURES OF ARTEMISININ, ARTEMISITENE, AND ARTEANNUIN B

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ABSTRACT

A series of solvent systems was developed for CCC to separate mixtures containing variable amounts of artemisinin, artemisitene, and arteannuin B. To purify multigram quantities of artemisinin by CCC it was necessary to maximize the amount of a mixture that could be dissolved in a fixed quantity of the solvent mixture.

INTRODUCTION

Malaria is one of the most formidable health problems affecting some 300-500 million people worldwide. The World Health Organization (WHO) estimates that 2-3 million people, primarily children under the age of 5, die each year of malaria.[1] In recent years, the appearance of drug resistant strains of the

disease has rendered useless chloroquine and mefloquine, the two most commonly used drugs to treat the disease.

The lead compound in the search for new antimalarial drugs is artemisinin, which was first isolated by Chinese investigators from the Chinese qinghao plant (*Artemisia annua* L).[2] Since its discovery in 1979, several groups have prepared a host of derivatives in an effort to increase its antimalarial activity. Clinical testing by WHO has shown that artemisinin in combination with either chloroquine or mefloquine promotes a rapid cure in individuals infected with drug resistant strains of *Plasmodium falciparum*. In our own search for more active synthetic artemisinin derivatives we needed to purify multigram quantities of artemisinin, **1**, from partially purified extracts of *A. annua* L. Since artemistene, **2**, was also present in our extracts and has not been employed as a synthetic intermediate, we wanted to isolate **2** for other synthetic efforts.

Our extracts contained three compounds, **1**, **2**, and arteannuin B, **3** in addition to minor impurities (Figure 1). We required multigram quantities of **1** for our synthetic efforts. Although the mixtures could be analyzed by thin layer chromatography on silica gel, their R_f values were too similar to allow multigram quantities of **1** to be purified by column chromatography on the same support. Acton *et al*.[3] had separated **1** and **2** from **3** by column chromatography on silica gel. They were unable to remove the 5-10% of **2** present in the mixture of **1** and **2** by crystallization or column chromatography, however, they were able to separate **2** from approximately 0.5 g of the mixture of **1** and **2** by CCC (Ito's multilayer coil separator-extractor).[4] We decided to investigate the use of CCC alone to purify multigram quantities of **1** and to isolate **2**.

A determination of the partition coefficients for **1**, **2**, and **3** (Table 1) shows that, whereas, the solvent system employed by Acton *et al*. provided good sep-

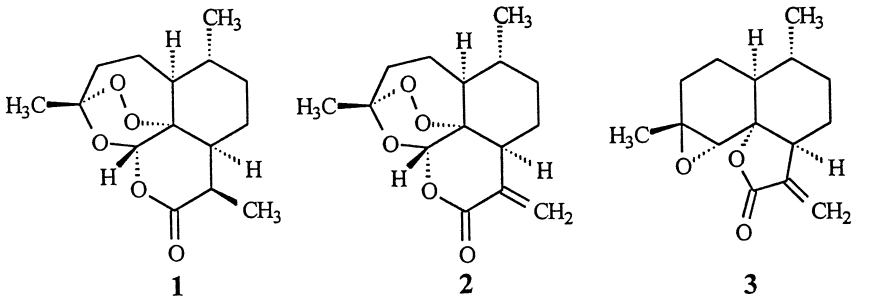

Figure 1. Structures of compounds in crude extracts.

Table 1

Partition Coefficients for 1,2, and 3 in Solvent Mixtures

Compound	Aceton et al.	First Solvent System 8:2:5:5	Modified Solvent System 6:4:5:4
1	0.81	1.68	1.50
2	0.14	0.97	0.88
3	0.11	0.68	0.58

arations of **1** and **2**, it could not be employed for purifying mixtures containing significant quantities of **3**. We describe here, two new solvent systems: the first was employed to separate each component from a mixture of **1**, **2**, and **3**, the second was utilized to purify multigram quantities of **1** from 9 g of a mixture of **1**, **2**, and **3**.

EXPERIMENTAL

Apparatus

The high-speed CCC centrifuge used in this study is a prototype fabricated at the NIH machine shop. It is equipped with a set of three multilayer coiled columns connected in series. Each column was prepared by winding a single piece of 2.6 mm ID Tefzel tubing (Zeus Industrial Products, Raritan, NJ, USA) onto the holder hub making 9 coiled layers between a pair of flanges spaced 11.5 cm apart. The β values[5] range from 0.5 to 0.75, and the total column capacity measures approximately 1.6 L. The apparatus was operated at 600 rpm (ca. 40 times g at the holder axis) with a speed controller (Bodine Electric Company, Chicago, IL, USA).

Separation Procedure

For each separation the column was first filled entirely with the organic stationary phase followed by injection of the sample solution through the sample port. The column was then rotated at about 600 rpm while the aqueous mobile phase was passed through the column in a head to tail elution mode at a flow-rate of 6 mL/min. The effluent was collected into test tubes (6 mL/tube) using a fraction collector (Ultrorac, LKB Instruments, Stockholm, Sweden).

Use of Two-Phase Solvent Systems for Separating Mixtures

Two solvent systems were employed to separate mixtures of **1**, **2** and **3**. The first was a 8:2:5:4 mixture of hexane: ethyl acetate: ethanol: water. The partition coefficients are given in Table 1.

In order to dissolve larger quantities of a different mixture in the same volume we employed a 6:4:5:4 mixture of hexane: ethyl acetate: ethanol: water. The separation factors are given in Table 1 and differ somewhat from those of the first solvent system. However, we were able to dissolve a larger quantity of the mixture to be separated in 200 mL of the second solvent mixture.

Starting with 8.8 gm of a mixture we obtained 4.08 g of **1**, 0.62 gm of **2**, and 4.1 g of a mixture of **1**, **2**, and **3**. The latter material was rechromatographed using the same solvent system to yield 2.4 g **1**, 0.71 g. **2**, 0.67 g **3** and 0.32 g of a mixture of **1**, **2**, and **3**.

Analysis of CCC Fractions

The fractions were checked by thin layer on silica gel, employing 30% ethyl acetate and 70% hexane. The structures and purities of the purified materials were verified by examination of their ^{1}NMR spectra.

RESULTS AND DISCUSSION

The apparatus employed by Acton et al.[3] to separate 590 mg of a mixture of **1** and **2** was similar to that employed in our studies. However, the presence of **3** in our mixtures required a new solvent system capable of separating **2** and **3**. It was possible to separate the three components of the mixture by CCC without a preliminary separation of **1** and **2** from **3** by chromatography on silica gel. However in order to purify multigram quantities of **1** with the same equipment it was necessary to modify the solvent system as to increase the solubility of the crude extract in 200 mL of a mixture of upper and lower phases (100 mL of each phase). The modification consisted of decreasing the amount of hexane in the mixture and increasing the ethanol content. The modified system enabled us to dissolve 8.81 gm of a different mixture of **1**, **2**, and **3** in the same 200 mL mixture of upper and lower phases. Approximately half of the mixture we employed was not separated and had to be recycled using the same solvent system to complete purification of **1** and **2**. However, half of the mixture had been separated into its components during the first pass in the CCC purification. Since the same solvent system is used in each pass and the solvents are readily removed on a rotovap they can be recovered and reused for subsequent purifications. The separations are rapid and do not require the use of adsorbants.

ACKNOWLEDGMENTS

The authors thank Col. John Scovill at the Walter Reed Army Institute of Research, Washington D.C. for the mixtures of artemisinin, artemisitene and arteannuin B employed in this study. E. W. was a participant in the Colgate/NIH Study Group during the summer of 1998.

REFERENCES

1. WHO, Tenth Programme Report of UNDP/WORLD BANK/WHO Special Programme for Research and Training in Tropical Diseases, WHO, GENEVA, 1991, p.30.

2. (a) H. Ziffer, R. J. Highet, D. L. Klayman, "Artemisinin: An Endoperoxidic Antimalarial from *Artemisia annua* L.," **Progress in the Chemistry of Organic Natural Products, Vol. 72**, Springer, New York, 1997, pp. 123-214. (b) X.-D. Luo, C. C. Shen, "The Chemistry, Pharmacology, and Clinical Application of Qinghaosu (Artemisinin) and its Derivatives," Med. Res. Rev., **7**, 29-52 (1987).

3. N. Acton, D. L. Klayman, I. J. Rollman, J. F. Novotny, "Isolation of Artemisinin (Qinghaosu) and its Separation from Artemisitene Using the Ito Multilayer Coil Separator-Extractor and Isolation of Arteannuin B. ," **355**, 448-450 (1986).

4. **High-Speed Countercurrent Chromatography**, Y. Ito, W. D. Conway, eds.,Wiley & Sons, New York, 1996, pp. 121-175.

5. Y. Ito, "Experimental Observations of the Hydrodynamic Behavior of Solvent Systems in High-Speed Countercurrent Chromatography: Part II. Phase Distribution Diagrams for Helical and Spiral Columns," J. Chromatography, **301**, 387-403 (1984).

Received August 1, 1999 Author's Revisions August 31, 1999
Accepted September 14, 1999 Manuscript 5129