A common application of distillation in the separation sciences is the purification and recovery of solvents especially from HPLC and GPC usage. There is a range of equipment supplied for recycling of solvents and useful sources of information can be found on the internet, for example the web pages for B/R Instruments and Recycling Sciences are included in the Further Reading.

The applications of distillation in analysis are widespread, with the technique being used to characterize materials and as a means of preparing samples prior to analysis. Standard apparatus and methods are described for many specific applications. Reference to the general texts and the standards detailed in the Further Reading will provide a source of information for future applications.

See also: **II/Distillation:** Energy Management; Historical Development; Laboratory Scale Distillation; Multicomponent Distillation; Vapour-Liquid Equilibrium: Theory.

Further Reading

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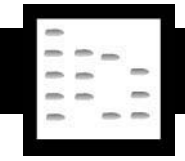
ANION EXCHANGERS FOR WATER TREATMENT: ION EXCHANGE

See **III / WATER TREATMENT / Anion Exchangers: Ion Exchange**

ANTIBIOTICS



High-Speed Countercurrent Chromatography

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Introduction

Development of antibiotics requires considerable research effort in isolation and purification of the desired compound from a complex matrix such as fermentation broth and crude extract. The purification of antibiotics by liquid–liquid partition dates back to the 1950s when the countercurrent distribution method (CCD) was used for separation of various natural products such as peptide antibiotics, aminoglycoside antibiotics and penicillin. However, CCD had serious drawbacks such as bulky fragile apparatus, long separation times and excessive dilution of samples. In the early 1970s an efficient continuous countercurrent separation method called countercurrent chromatography was introduced followed by the advent of high speed countercurrent chromatography (HSCCC) a decade later. Because of its high partition efficiency and speedy separation,

HSCCC has been widely used for separation and purification of natural products including a number of antibiotics as listed in **Table 1**. Being support-free chromatographic systems, HSCCC and CCD share important advantages over other chromatographic systems by eliminating complications arising from a solid support such as sample loss and decomposition.

Selection of Two-Phase Solvent System

Among the purification of natural products, the isolation of antibiotics is one of the most difficult tasks since the crude sample often contains, in addition to numerous impurities, a set of closely related components that tend to exhibit similar partition behaviour in a given solvent system. Consequently, successful separation necessitates a painstaking search for a suitable solvent system, which often requires days, weeks and even months of hard trial. Once a suitable solvent system is found, however, the separation is usually completed within several hours.

HSCCC utilizes two immiscible solvent phases, one as a stationary phase and the other as a mobile phase. Solutes are subjected to a continuous partition process between these two phases along the column space free of a solid support, hence the separation is almost entirely governed by the difference between their partition coefficients.

Table 1 Separation of antibiotics by HSCCC

Sample	*Amount*	*Solvent system*	*Mobile phase*
Daunorubicin derivatives		Chloroform/ethylene chloride/hexane/methanol/water (1:1:1:3.5:1)	UP
Gramicidins A, B, and C	100 mg	Benzene/chloroform/methanol/water (15:15:23:7)	UP
Siderochelin A	400 mg	Chloroform/methanol/water (7:13:8)	UP
Efrotomycin	670 mg	Carbon tetrachloride/chloroform/methanol/water (5:5:6:4)	UP
Pentalenolactone	50 mg	Chloroform/methanol/water (1:1:1)	UP
Bu 2313B	200 mg	*n*-Hexane/dichloromethane/methanol/water (5:1:1:1)	LP
A 201E	350 mg	Carbon tetrachloride/chloroform/methanol/water (2:5:5:5)	UP
Tirandamycin A and B	134 mg	*n*-Hexane/ethyl acetate/methanol/water (70:30:15:6)	UP
Actinomycin complex	83 mg	Ether/hexane/methanol/water (5:1:4:5)	UP
Benzanthrins A and B (quinone antibiotics)	620 mg	Carbon tetrachloride/chloroform/methanol/water (4:1:4:1)	UP
Coloradocin	400 mg	Chloroform/methanol/water (1:1:1)	UP
Candicidin (polyene macrolide antibiotics)	100 mg	Chloroform/methanol/water (4:4:3)	?
2-Norerythromycins (macrolide antibiotics)	500 mg	*n*-Heptane/benzene/acetone/isopropanol/0.01 mol L^{-1} citrate buffer (pH 6.3) (5:10:2:3:5)	UP
Niddamycins (macrolide antibiotics)	200 mg	Carbon tetrachloride/methanol/0.01 mol L^{-1} potassium phosphate buffer (pH 7) (2:3:2)	UP
Tiacumicins (macrolide antibiotics)	200 mg	Carbon tetrachloride/chloroform/methanol/water (7:3:7:3)	UP
Coloradocin (macrolide antibiotics)	400 mg	Chloroform/methanol/water (1:1:1)	UP
Sporaviridin complex	100 mg	*n*-Butanol/diethylether/water (10:4:12)	LP
Dunaimycin (macrolide antibiotics)		*n*-Hexane/ethyl acetate/methanol/water (8:2:10:5)/(70:30:15:6)	UP
Bacitracin complex	50 mg	Chloroform/ethanol/water (5:4:3)	LP
Bacitracin complex	50 mg	Chloroform/ethanol/methanol/water (5:3:3:4)	LP
Mycinamicins	Analytical works	*n*-Hexane/ethyl acetate/methanol/8%aq. ammonia (1:1:1:1)	LP
Colistins	Analytical works	*n*-Butanol/0.04 mol L^{-1} TFA (1:1) containing 1% glycerol	LP
Pristinamycins (macrolide antibiotics)	1 mg	Chloroform/ethyl acetate/methanol/water (3:1:3:2)	UP
Pristinamycins (macrolide antibiotics)	1 mg	Chloroform/ethyl acetate/methanol/water (2.4:1.6:3:2)	UP
Ivermectin	25 mg	*n*-Hexane/ethyl acetate/methanol/water (19:1:10:10)	LP
Colistin	20 mg	*n*-Butanol/0.04 mol L^{-1} TFA (1:1)	LP

Generally speaking, the two-phase solvent system should satisfy the following requirements:

1. *Retention of the stationary phase*. Since the system eliminates the solid support, the retention of the stationary phase in the separation column entirely depends upon the hydrodynamic interaction between the two solvent phases in the rotating column under a centrifugal force field. While the hydrodynamic motion of the two phases is highly complex, the retention of the stationary phase may be predicted by the following simple procedure to measure the settling time of the two phases under gravity: Place 2 mL of each phase of the equilibrated two-phase solvent system into a 5 mL capacity graduated cyclinder (alternatively, a 13 mm o.d. and 100 mm long glass test tube equipped with a plastic cap may also be used) which is then sealed with a stopper. Gently invert the cylinder five times to mix the contents and immediately place it on flat surface to measure the time required for the mixture to settle into two layers. This settling time should be considerably less than 30 s for stable retention of the stationary phase.

2. *Partition coefficient* (K). The partition coefficient is the key parameter for HSCCC. It is usually expressed by the analyte concentration in the stationary phase divided by that of the mobile phase. For a successful separation, the K value of an analyte should be close to 1. If $K \ll 1$, the analyte will elute close to the solvent front resulting in loss of peak resolution. On the other hand, if $K \gg 1$, the analyte will remain in the separation column for a long period of time, producing an excessively broad peak. In order to separate two components, the ratio between their partition coefficients, which is called separation factor (α), should be 1.5 or greater for a standard semipreparative multilayer coil HSCCC equipment providing a moderate partition efficiency of about 800 theoretical plates.

HSCCC Separation of Antibiotics

As mentioned earlier, HSCCC has been successfully applied to the separation of a variety of antibiotics (**Table 1**). The list includes peptide antibiotics, which are strongly adsorbed on the silica gel used as the stationary phase in column chromatography. Sample loading capacity of HSCCC widely varies from 1 mg to 10 g, depending on the tube diameter and the length of the multilayer coil used as the separation column. Two-phase solvent systems may be selected according to the hydrophobicity of the analytes, i.e. *n*-butanol solvent systems for hydrophilic groups, chloroform systems for moderately hydrophobic groups, and *n*-hexane systems for the most hydrophobic groups. Below, we describe the HSCCC separation of selected antibiotics including sporaviridins, bacitracins, colistins and ivermectins, especially focusing on the procedures for optimization of two-phase solvent systems.

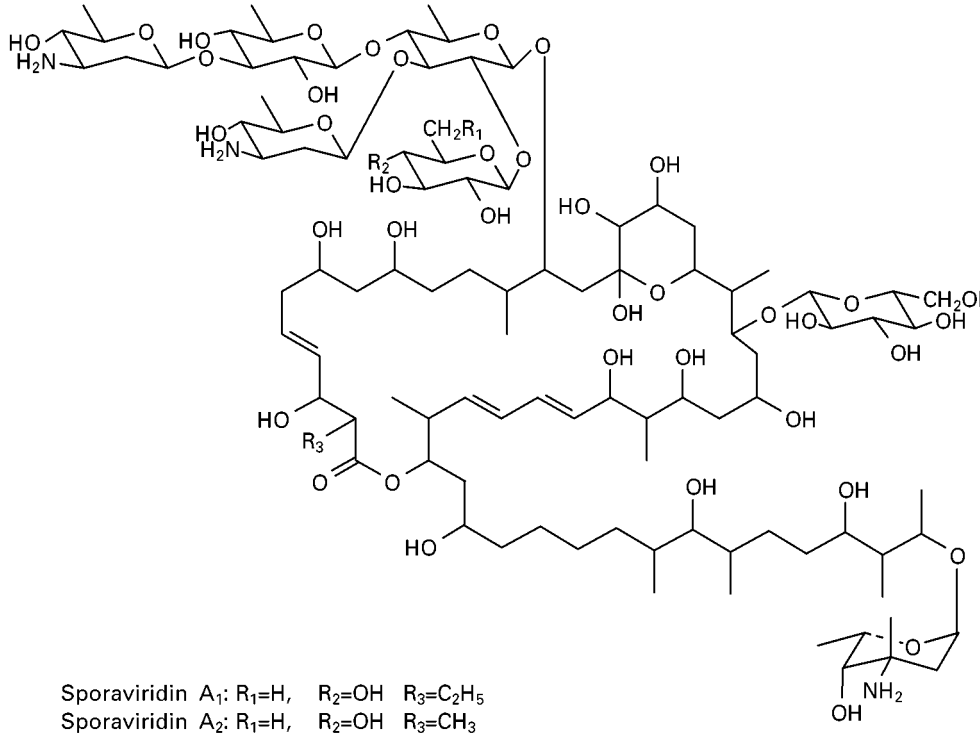
The apparatus used in the following separations was a HSCCC-1A prototype multilayer coil planet centrifuge (Shimadzu Corporation, Kyoto, Japan) with a 10 cm orbital radius which produces a type-J synchronous planetary motion at 800 rpm. The multilayer coil was prepared by winding about 160 m of PTFE (polytetrafluoroethylene) tubing onto the column holder. Unless otherwise indicated, all separations were performed under the following conditions: speed of revolution: 800 rpm; stationary phase: organic phase; flow rate: 3 mL min^{-1}; elution mode: head to tail.

Sporaviridins

Sporaviridins (SVD, **Figure 1**) are basic water-soluble antibiotics produced by *Kutzneria viridogrisea* (formerly) *Streptosporangium viridogriseum*) and they are active against Gram-positive bacteria, acid-fast bacteria and trichophyton. As shown in **Figure 2**, they consist of six components each having a 34-membered lactone ring and seven monosaccharide units, one pentasaccharide (viridopentaose) and two monosaccharides.

The SDV complex is soluble only in polar solvents such as water, methanol and *n*-butanol, and is extracted with *n*-butanol from the fermentation broth. Therefore, a two-phase solvent system containing *n*-butanol as a major organic solvent was mainly examined. We found that the SVD sample was entirely partitioned into the upper organic phase in a *n*-butanol/water binary two-phase solvent system (**Table 2**). This result indicated that the hydrophobicity of the *n*-butanol phase must be decreased to obtain a suitable partition coefficient. A nonpolar solvent such as *n*-hexane or diethyl ether was added to the *n*-butanol solvent system as a modifier. Initially, the volume of *n*-butanol was fixed at 10 mL while that of the diethyl ether was varied, and a two-phase system composed of diethyl ether/*n*-butanol/water (10:4:10) was selected. Next, the volumes of *n*-butanol and diethyl ether were fixed while that of water was varied from 11 to 14. At a solvent ratio of 10:4:12, almost evenly dispersed partition coefficients among the six components were obtained as shown in **Table 2**. Therefore, this solvent system was selected for the separation of the SVD components.

The preparative HSCCC separation of six components from the SVD complex was performed. In this experiment the retention of the stationary phase, elution time, and elution volume were 75%, 3.5 h and



Sporaviridin A_1: R_1=H, R_2=OH R_3=C_2H_5
Sporaviridin A_2: R_1=H, R_2=OH R_3=CH_3
Sporaviridin B_1: R_1=H, R_2=NH_2, R_3=C_2H_5
Sporaviridin B_2: R_1=H, R_2=NH_2, R_3=CH_3
Sporaviridin C_1: R_1=OH, R_2=OH, R_3=C_2H_5
Sporaviridin C_2: R_1=OH, R_2=OH, R_3=CH_3

Figure 1 Structures of sporaviridins. (Reproduced with permission from Oka H *et al.* (1998).)

500 mL, respectively. The six components were eluted in an increasing order of their partition coefficients yielding high purity of components A_1 (1.4 mg), A_2 (0.6 mg), B_1 (0.7 mg), B_2 (0.5 mg), C_1 (1.1 mg), and C_2 (1.4 mg) from 15 mg of the SVD complex. HPLC analyses of the purified components are illustrated in **Figure 3**.

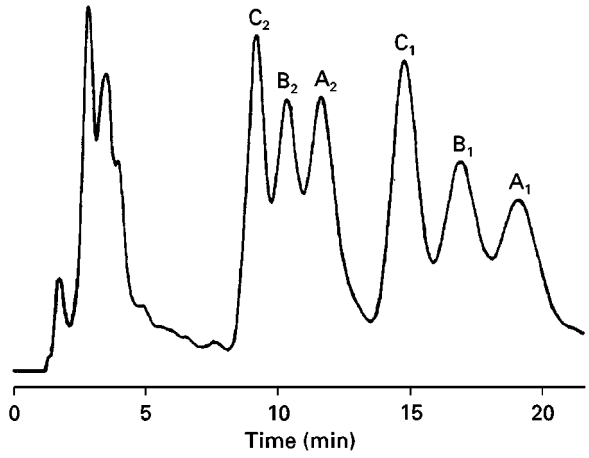


Figure 2 HPLC separation of sporaviridins. Column, Cosmosil 5C18 (5 μm, 4.6 × 150 mm); mobile phase, methanol/1mol L^{-1} ammonium chloride (74 : 26); flow rate, 1 mL min^{-1}; detection, 232 nm. (Reproduced with permission from Oka H *et al.* (1998).)

Bacitracins

Bacitracins (BCs) are peptide antibiotics produced by *Bacillus subtilis* and *Bacillus licheniformis*. They exhibit an inhibitory activity against Gram-positive bacteria and are most commonly used as animal feed

Table 2 Partition coefficients of SVD components with *n*-butanol systems

n-Butanol/ diethyl ether/water	*Partition coefficients* (UL^{-1})					
	C2	*B2*	*A2*	*C1*	*B1*	*A1*
10 : 0 : 10	2.96	6.41	6.65	4.87	8.81	9.09
10 : 3 : 10	0.96	2.17	2.78	1.84	3.34	4.19
10 : 4 : 10	0.50	1.12	1.59	1.04	1.85	2.91
10 : 5 : 10	0.38	0.78	1.11	0.74	1.25	2.12
10 : 6 : 10	0.39	0.90	1.19	0.81	1.39	1.70
10 : 7 : 10	0.24	0.63	1.10	0.59	1.08	1.82
10 : 4 : 11	0.31	0.89	1.24	0.70	1.50	2.00
10 : 4 : 12	0.38	1.09	1.41	0.80	1.85	2.32
10 : 4 : 13	0.37	1.05	1.51	0.73	1.58	2.10
10 : 4 : 14	0.29	1.09	1.17	0.57	1.22	1.74

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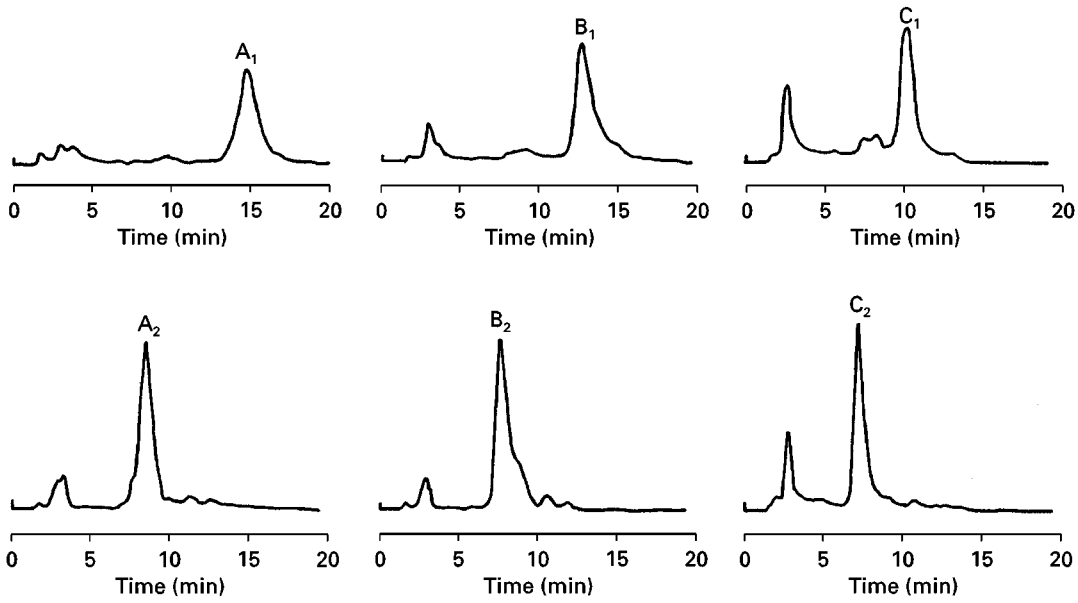


Figure 3 HPLC separation of sporaviridin components. For experimental conditions, see legend to Figure 2. (Reproduced with permission from Oka H *et al.* (1998) and Harada K-I *et al.* (1990).)

additives. Over 20 components are present in the bacitracin complex (**Figure 4**) among which BC-A and BC-B are the major antimicrobial components. BC-F is a degradation product and has nephrotoxicity. Only the structures of BC-A and -F have been determined (**Figure 5**).

We examined three groups of two-phase solvent systems containing *n*-butanol, ethyl acetate or chloroform as a major organic solvent, and ethanol and/or methanol as a modifier against water in each group. The *n*-butanol system produced suitable *K* values for peaks 13–18 whereas those for peaks 20–22 are too large. The ethyl acetate system represented by ethyl acetate/ethanol/water showed a long settling time, suggesting poor retention of the stationary phase in the column. The most promising results were obtained from the chloroform, ethanol and/or methanol, water systems as summarized in **Table 3**. Among all combinations for the solvent volume ratio, chloroform/ethanol/ methanol/water (5:3:3:4) and chloroform/ethanol/water (5:4:3) gave the most desirable *K* values.

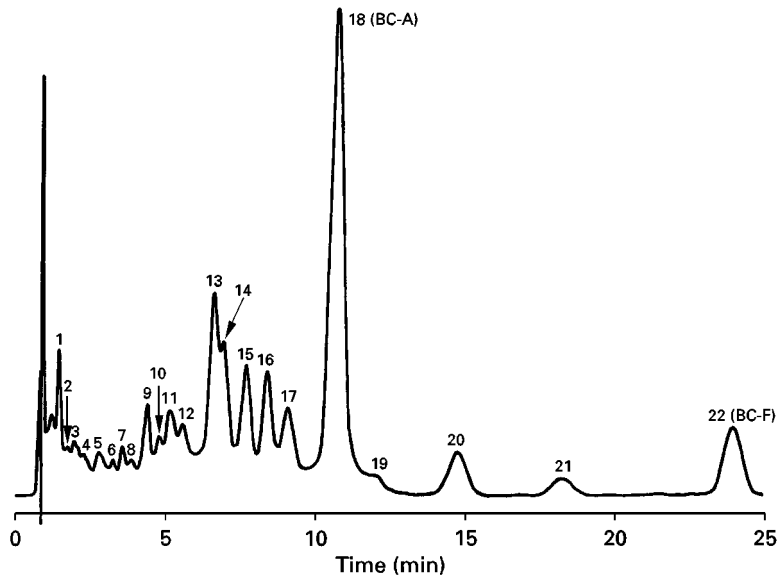


Figure 4 HPLC separation of bacitracins. Column Capcel Pak C_{18} (5 μm, 4.6 × 150 mm); mobile phase, methanol/0.04 mol L^{-1} sodium dihydrogen phosphate (6:4); flow rate, 1.3 mL min^{-1}; detection, 234 nm. (Reproduced with permission from Oka H *et al.* (1998) and Harada K-I *et al.* (1991).)

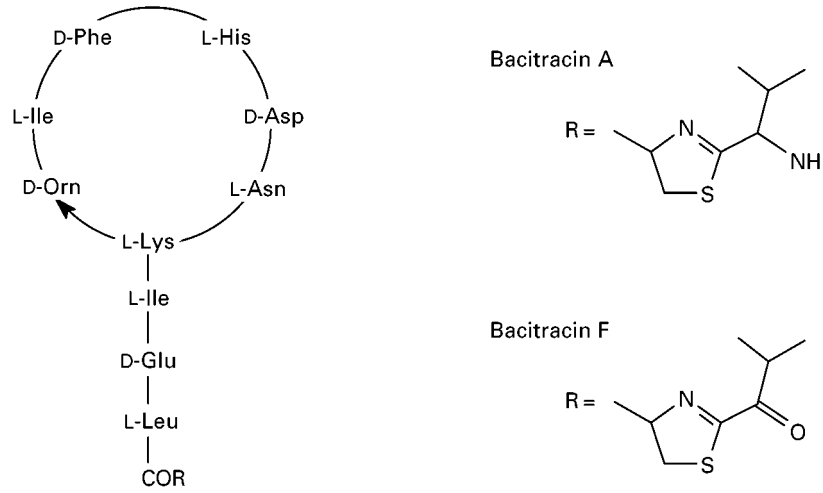


Figure 5 Structures of bacitracins A and F. (Reproduced with permission from Harada K-I *et al.* (1991) and Oka H *et al.* (1998).)

Figure 6 shows the countercurrent chromatogram of bacitracin components using the chloroform/ethanol/methanol/water (5:3:3:4) system. A 50 mg amount of the bacitracin complex was loaded into the HSCCC column. The retention of the stationary phase was 72.7% and the elution time was about 3 h. All components were eluted in an increasing order of their partition coefficients, yielding 5.5 mg of BC-A from peak 18 and 1.5 mg of BC-F from peak 22.

Ivermectins

Ivermectins B_1 are broad spectrum antiparasitic agents widely used for food-producing animals such as cattle and pigs. They are derived from avermectins B_1, the natural fermentation products of *Streptomyces avermitilis*. Avermectins B_1 have double bonds between carbon atoms at 22 and 23, whereas the ivermectins B_1 have single bonds in these positions (**Figure 7**). The ivermectins B_1 are a mixture of two major homologues, ivermectin B1a ($>80\%$) and ivermectin B1b ($\ll 20\%$), but a crude ivermectin complex also contains various minor compounds (**Figure 8**A).

Table 3 Partition coefficients of the bacitracin components

Chloroform ethanol/ methanol/ water	*Partition coefficients* (UL^{-1})					
Peaks	3, 14	17	18	20	21	22
5:2:3:4	7.20	2.46	4.17	0.64	0.65	0.48
5:2:1:4	∞	∞	33.27	1.62	1.38	0.75
5:3:3:4	3.35	1.40	2.37	0.57	0.47	0.45
5:3:0:3	11.1	3.20	5.34	0.32	0.35	0.27
5:4:0:2	3.19	1.05	2.00	0.25	0.26	0.21
5:4:0:3	5.49	1.46	2.20	0.16	0	0.16
5:4:0:4	6.10	2.04	2.68	0.14	0	0.10

(Reproduced with permission from Harada K-I *et al.* (1991) and Oka H *et al.* (1998).)

We selected a two-phase solvent system composed of *n*-hexane, ethyl acetate, methanol and water. This solvent system is conveniently used for the separation of components with a broad range of hydrophobicity by modifying the volume ratio between the four solvents. In the *n*-hexane/ethyl acetate/methanol/water (8:2:5:5) system first examined, the *K* values of the components corresponding to peaks 1–7 were 0, 0.46, 0.61, ∞, 1.86, 3.06, and 4.38, respectively.

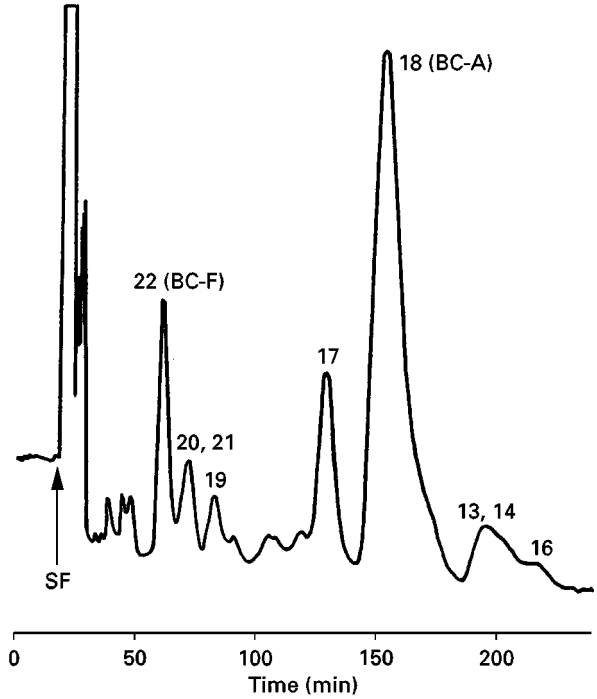
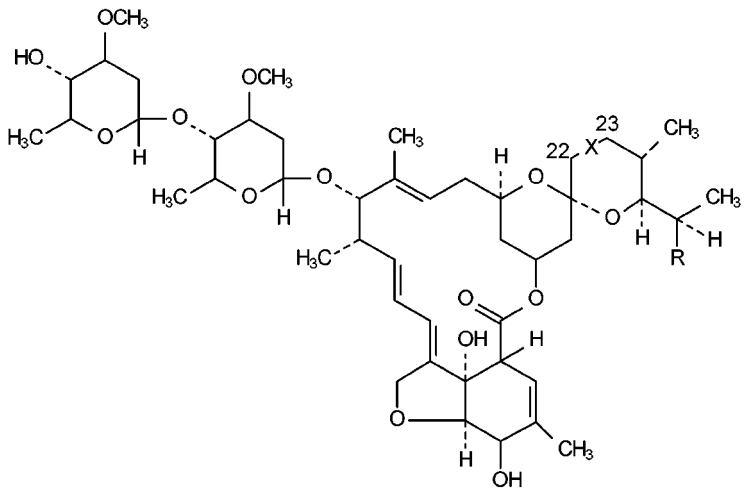


Figure 6 HSCCC separation of bacitracin components. Apparatus, HSCCC-1A; revolution, 800 rpm; solvent system, chloroform/ethanol/methanol/water (5:3:3:4); mobile phase, lower organic phase; flow rate, 3 mL min^{-1} detection, 254 nm. (Reproduced with permission from Harada K-I *et al.* (1991) and Oka H *et al.* (1998).)



	R	—C_{22}—X—C_{23}—
Ivermectin B1a	C_2H_5	—CH_2—CH_2—
Ivermectin B1b	CH_3	—CH_2—CH_2—
Avermectin B1a	C_2H_5	—CH=CH—
Avermectin B1b	CH_3	—CH=CH—

Figure 7 Structures of ivermectins and avermectins. (Reproduced with permission from Oka H *et al.* (1996, 1998).)

This indicates that the component corresponding to peak 6 (ivermectin B1a) is mostly partitioned in the upper organic phase (**Table 4**). Although the *n*-hexane/ethyl acetate/methanol/water (9:1:5:5) system somewhat improved the *K* value of peak 6, it was still too large and the α value between peaks 6 and 7 is smaller than 1.5. Finally a slightly less polar solvent mixture at the volume ratio of 19:1:10:10 yielded the best *K* value, as indicated in Table 4. The settling time of this solvent system was 7 s, promising excellent retention of the stationary phase. In addition, the volume ratio between the two phases is nearly 1, indicating that either phase can be used as the mobile phase without wasting the solvents. Therefore, the above solvent was selected for separation of ivermectin components.

A 25 mg quantity of crude ivermectin was separated using the above solvent system at a flow rate of 2 mL min^{-1}. The retention of the stationary phase was 67.6% and the total separation time, 4.0 h. The HSCCC elution curve of the ivermectin components monitored at 245 nm is shown in **Figure 9**, where all components are separated into three peaks, A, B and C. HPLC analysis of each peak fraction and the column contents revealed that both HPLC and HSCCC systems elute all components in the same order: HPLC peaks 3, 5, and 6 correspond to HSCCC peaks A, B and C, respectively, while HPLC peak 7 was still retained in the HSCCC column. This separation yielded 18.7 mg of 99.0% pure ivermectin B1a (Figure 8B), 1.0 mg of 96.0% pure ivermectin

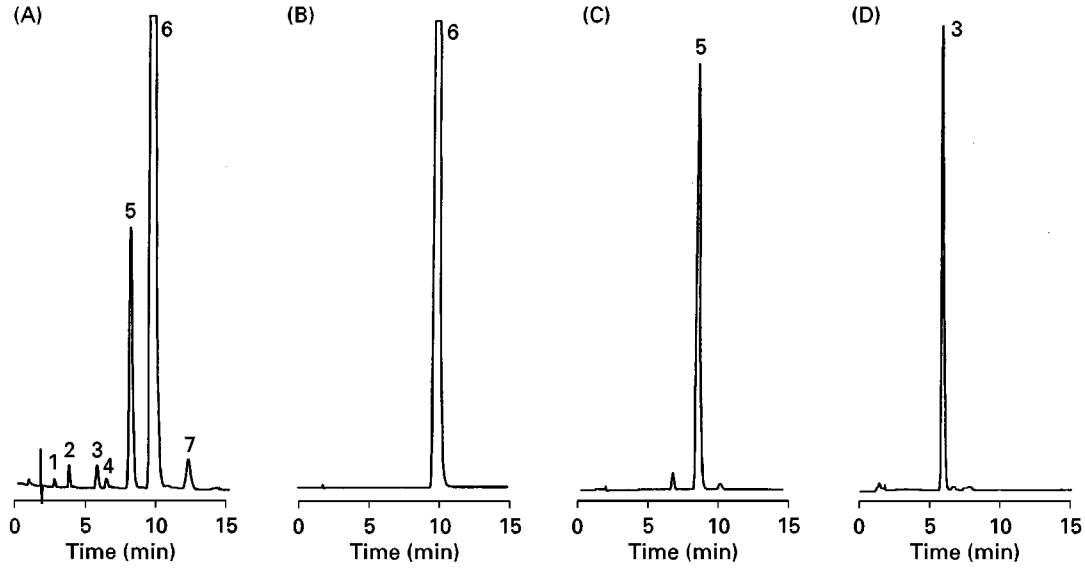


Figure 8 HPLC separation of ivermectin components. Column, TSK GEL-80 TsC_{18} (5 μm, 4.6 × 150 mm); mobile phase, methanol/water (9:1); flow rate, 1 mL min^{-1}; detection, 245 nm. (A) Crude ivermectin; (B) Fraction II (ivermectin B1a); (C) Fraction IV (ivermectin B1b); (D) Fraction VI (avermectin B1a). (Reproduced with permission from Oka H *et al.* (1998).)

Table 4 Partition coefficients of the ivermectin components (K = peak area of upper phase divided by peak area of lower phase.)

Solvent system	*Peak no.*						
	1	2	3	4	5	6	7
n-Hexane/ethyl acetate/methanol/ water (8:2:5:5)	0	0.46	0.61	∞	1.86	3.06	4.38
n-Hexane/ethyl acetate/methanol/ water (9:1:5:5)	0	0.15	0.33	∞	1.17	2.31	3.21
n-Hexane/ethyl acetate/methanol/ water (19:1:10:10)	0	0	0.18	0.48	0.79	1.36	2.83

(Reproduced with permission from Oka H *et al.* (1996, 1998).)

B1b (Figure 8C) and 0.3 mg of 98.0% pure avermectin B1a (precursor of ivermectin) (Figure 8D).

Colistin

Colistin (CL) is a peptide antibiotic produced by *Bacillus polimyxa* var. *Colistinus* that inhibits the growth of Gram-negative organisms. CL is a mixture of many components (**Figure 10**) where two main components are colistins A (CL-A) and B (CL-B). As shown in **Figure 11**, CLs-A and -B are linear-ring peptides that differ only in their *N*-terminal fatty acid. CL is used as a feed additive for domestic animals such as calf and pigs for preventing bacterial infection and/or improving feed conversion efficiency. CL is soluble in water, slightly soluble in alcohols, but insoluble in nonpolar solvents such as hexane and chloroform. From this property, we selected *n*-butanol and water as a basic solvent system. However, this combination was not suitable by itself, because the CL components were entirely partitioned into the aqueous phase. In order to partition the CL components partly into the *n*-butanol phase, various salts (NaCl and Na_2SO_4) or acids (HCl, H_2SO_4 and CF_3COOH or TFA) were added as a modifier. The desired effect was produced from TFA where the partition coefficients of CL components rose as the concentration of TFA in the solvent system was increased. This effect may be explained as follows: as shown in **Figure 11**, CLs-A and -B have five free amino groups in L-diamino-butyric acid (L-Dab), and these amino groups dissociate in the aqueous phase under neutral to acidic conditions. Since TFA forms an ion pair with these amino groups, the hydrophobicity of the CL components increases with the concentration of TFA resulting in their partition toward the organic phase. In order to determine the optimal concentration of TFA in the solvent system, K values of five components were measured at various TFA concentrations. As shown in **Figure 12**, the K value of each component increases with the TFA concentration, and at 40 mmol L^{-1} TFA concentration, K values of CL-A and CL-B reached 1.5 and 0.6, respectively. At this TFA concentration, the α values between the adjacent peaks are all greater than 1.5, promising a good separation for all components. The settling time of the solvent system was 28 s, which is within the acceptable range. Therefore, we selected a solvent system of *n*-butanol/40 mmol L^{-1} TFA aqueous solution (1:1) for the HSCCC separation.

Using the above solvent system, a 20 mg quantity of commercial CL was separated by HSCCC. The retention of the stationary phase was 45%. The

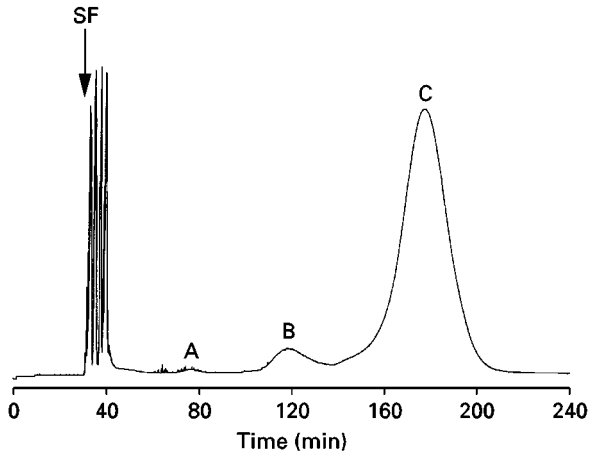


Figure 9 HSCCC separation of ivermectin components. Apparatus, HSCCC-1A; revolution, 800 rpm; solvent system, *n*-hexane/ethyl acetate/methanol/water (19:1:10:10) mobile phase, lower aqueous phase; flow rate, 2 mL min^{-1}; detection, 245 nm. (Reproduced with permission from Oka H *et al.* (1996, 1998).)

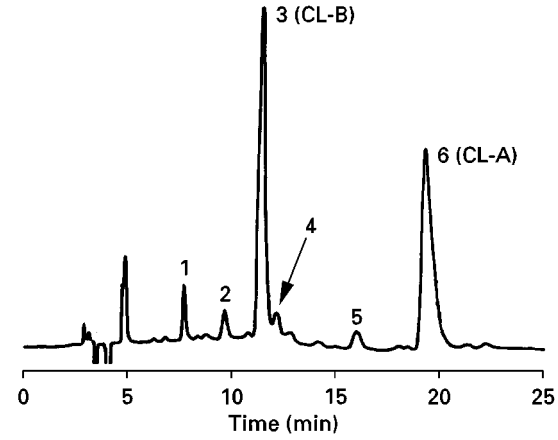


Figure 10 HPLC separation of commercial CL. Column, Chromatorex Ph (5 μm, 4.6 × 250 mm); mobile phase, acetonitrile/0.01 mol L^{-1} TFA aqueous solution (24:76); flow rate, 1.0 mL min^{-1}; detection, 210 nm. (Reproduced with permission from Ikai Y *et al.* (1998) and Oka H *et al.* (1998).)

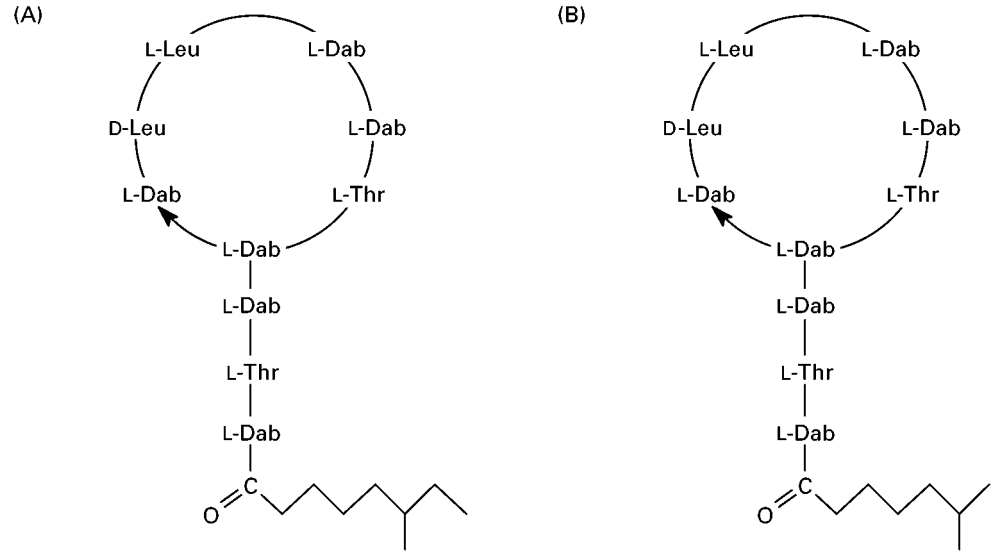


Figure 11 Structures of colistin components. (Reproduced with permission from Ikai Y *et al.* (1998) and Oka H *et al.* (1998).)

elution curve monitored at 220 nm is shown in **Figure 13**. According to the results of HPLC analysis and the elution curve, all collected fractions were combined into five large fractions as shown in **Figure 13**. The yields of CL-A and CL-B were 9 mg each and those of other minor components were 0.5–1.0 mg. HPLC analysis was performed for each fraction; as shown in **Figure 14**, the fractions of CLs-A and -B each produced a peak with a purity of over 90%.

Conclusions

Because it is a support-free partition system, HSCCC has an important advantage over other chromatographic methods in that it eliminates various complications such as adsorptive loss and deactivation of samples as well as contamination from the solid support. As shown by our examples, HSCCC can isolate various components from a complex mixture of antibiotics by carefully selecting the two-phase solvent system to optimize the partition coefficient (K) of the target component(s). Compared with CCD and other countercurrent extraction methods, HSCCC can yield higher partition efficiencies in a shorter elution time. The HSCCC system can also be applied to microanalytical-scale separations without excessive dilution of samples. We believe that HSCCC is an

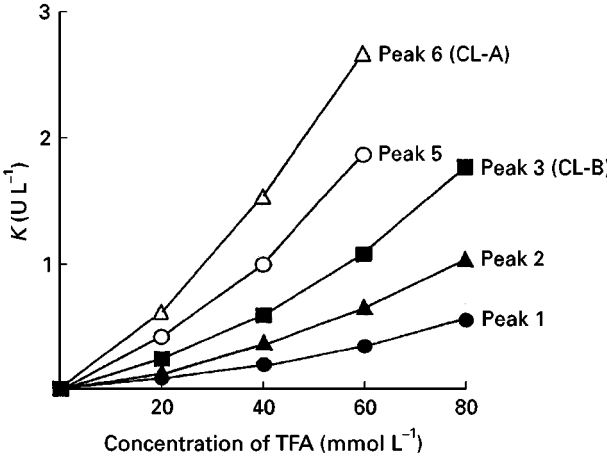


Figure 12 Effect of TFA concentration on the partition coefficients of CL components. (Reproduced with permission from Ikai Y *et al.* (1998) and Oka H *et al.* (1998).)

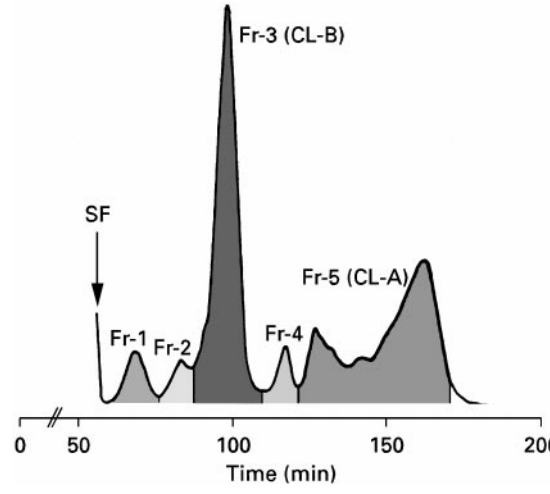


Figure 13 HSCCC separation of commerical CL. Apparatus, HSCCC-1A; revolution, 800 rpm; solvent system, *n*-butanol/0.04 mol L^{-1} TFA aqueous solution (1:1); mobile phase, lower aqueous phase; flow rate, 2.0 mL min^{-1}; detection, 220 nm. (Reproduced with permission from Ikai Y *et al.* (1998) and Oka H *et al.* (1998).)

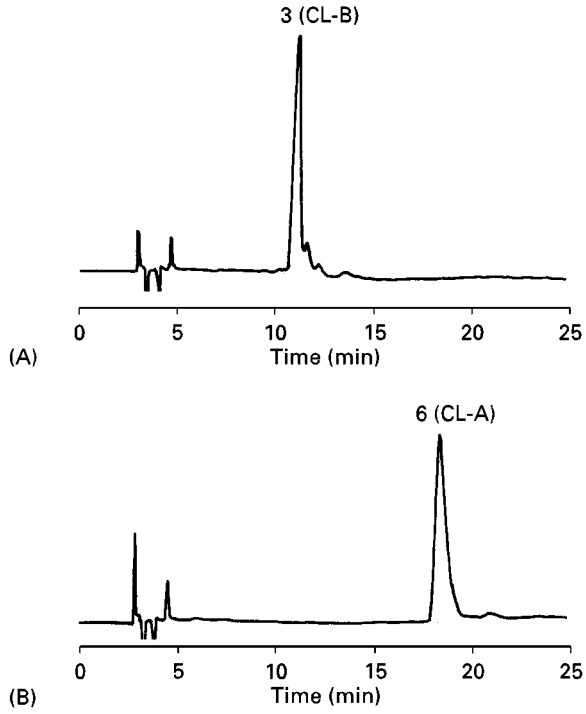


Figure 14 HPLC analysis of CL components of HSCCC fractions. (A) CL-A (Fraction 5); (B) CL-B (Fraction 3). (Reproduced with permission from Oka H *et al.* (1998).)

ideal method for separation and purification of antibiotics.

See also: **II/Chromatography:** Countercurrent Chromatography and High-Speed Countercurrent. Chromatography: Instrumentation. **Chromatography: Liquid:** Countercurrent Liquid Chromatography. **III / Antibiotics:** Liquid Chromatography. Supercritical Fluid Chromatography.

Further Reading

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Liquid Chromatography

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Introduction

High performance liquid chromatography (HPLC) has been widely used for the analysis of antibiotics because it is superior to conventional microbiological assays in terms of specificity, sensitivity and analysis time. In this article, HPLC conditions for the analysis of a variety of antibiotics are summarized. For analysis of biological samples, not only extraction methods but also derivatization methods are described, if necessary. Since it is not possible to list HPLC methods for all antibiotics in clinical use, only a few have been chosen from each class. Where a stereoisomer exists for the antibiotic of interest, the HPLC conditions that are able to resolve stereoisomers are described.

Aminoglycosides

Aminoglycosides are analysed by reversed-phase HPLC. However, derivatization is usually necessary owing to very poor UV or visible absorption. For detection of aminoglycosides without derivatization, electrochemical, refractive index or mass spectrometric detection may be used.

Amikacin

For determination of amikacin (**Figure 1**, structure 1), the serum sample is loaded onto the silica gel column, followed by addition of *o*-phthalaldehyde (a derivatizing reagent). The column is eluted with 95% ethanol (pH 10) and the eluent is heated at 50°C. After cooling, the mixture is injected onto an ODS