Journal of Chromatography, 593 (1992) 363–371
Elsevier Science Publishers B.V., Amsterdam

CHROMSYMP. 2471

Separation of pristinamycins by high-speed counter-current chromatography

I. Selection of solvent system and preliminary preparative studies

S. Drogue, M.-C. Rolet, D. Thiébaut* and R. Rosset

Laboratoire de Chimie Analytique, Ecole Supérieure de Physique et de Chimie Industrielles de la Ville de Paris, 10 Rue Vauquelin, 75005 Paris (France)

ABSTRACT

Partition coefficient determination and direct application of high-speed counter-current chromatography allowed the selection of suitable chloroform–ethyl acetate–methanol–water solvent systems for the separation of macrolide antibiotics. Sample loading limits were found to be 10 ml and 200 mg for a total volume capacity of 108 ml. This allows scale-up to higher volume counter-current chromatographic apparatus and direct competition with semi-preparative high-performance liquid chromatography for crude extract samples.

INTRODUCTION

Pristinamycins are macrolide antibiotics mainly acting on Gram-positive *Staphylococcus* and *Streptococcus*. They consist of two families: pristinamycins I with acid–base properties and pristinamycins II without such properties. Fig. 1 shows the molecular structure of the four components that we investigated, *i.e.*, pristinamycins IA and B and pristinamycins IIA and B. As pristinamycins are obtained from a fermentation broth, the direct preparative separation of the crude extract by high-performance liquid chromatography (HPLC) is not possible without damage to the stationary phase and a tedious crystallization pretreatment is necessary to obtain the purified samples that can be injected on to the columns.

Counter-current chromatography (CCC) uses a liquid stationary phase retained in the apparatus by a centrifuged force field. The planetary motion of the column promotes solute mass transfer between the stationary and mobile phases. Consequently, efficient separations can be obtained and there is no risk of stationary phase degradation. The principles and examples of applications of the technique can be found in special issues of *Journal of Chromatography* and *Journal of Liquid Chromatography* devoted to CCC [1–5].

This paper described how suitable solvent systems for the separation of pristinamycins were selected and modified until satisfaction resolution was obtained on our analytical unit. Then, sample loading limits were studied for comparison with semi-preparative HPLC. Preliminary promising results are presented.

EXPERIMENTAL

Apparatus

High-speed counter-current chromatography (HSCCC). The HSCCC system was a Model CPHV 2000 (SFCC, Neuilly-Plaisance, France)

0021-9673/92/$05.00

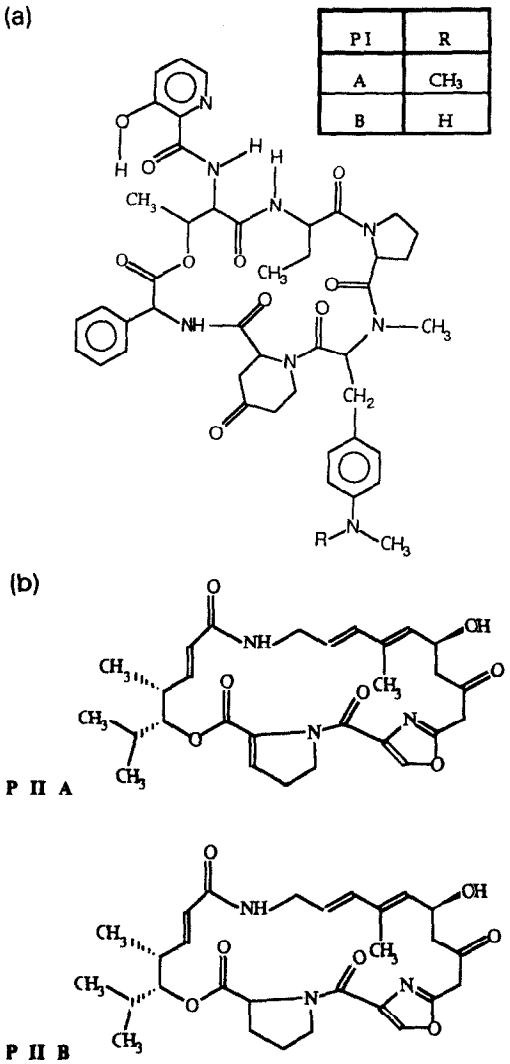

Fig. 1. Structures of (a) pristinamycins IA and B and (b) pristinamycins IIA and B.

equipped with three identical columns connected in series and arranged symmetrically around the central axis of the centrifuge. The columns were prepared from 1.6 mm I.D. polytetrafluorocthylene (PTFE) tubing wound on to a holder to give a total capacity of 108 ml and the connections between the columns were made of 0.8 mm I.D. PTFE tubing. The columns underwent synchronous planetary motion and revolved around their own axis, avoiding twisting of the column flow tubes. The maximum revolution speed attainable was 2000 rpm. The β value (ratio of the rotational radius to the revolution radius) ranged from 0.5 at the internal terminal to 0.85 at the external terminal.

Apart from the HSCCC system, the complete HSCCC apparatus consisted of a Shimadzu Model LC 5 A reciprocating HPLC pump (Touzart et Matignon, Vitry sur Seine, France) for the mobile organic phase and a preparative pump (LC-XPS pump, Pye Unicam, Philips Industries, Bobigny, France) for the stationary aqueous phase. The columns were connected to the pumps by 0.8 mm I.D. PTFE tubing via a three-way valve. Samples were injected into the column via a Rheodyne Model 7125 injection valve equipped with different loops of variable volume. Each solvent system was thoroughly equilibrated in a separating funnel at room temperature and the two phases were separated shortly before use. After filling the columns with the stationary phase, injections of the samples dissolved either in the mobile or the stationary phase were carried out into the mobile phase according to a special injection procedure.

A Sedex 45 evaporative light-scattering detector (ELSD) (Sédéré, Vitry sur Seine, France) manufactured for HPLC was used without modification. The basic principles of the ELSD were described previously [6]. This detector allows the detection of samples whose UV absorption is poor and also the use of high-UV cut-off solvents, and the baseline obtained is very stable even with slight bleeding of the stationary phase during the separation.

HPLC. Using the procedure developed by Rhône-Poulenc Rorer (Centre de Recherche de Vitry-Alfortville, France), HPLC analyses of sample solutions for the determination of partition coefficients were performed on a Pecosphere 5 CR C_8 (5 μm) column (150 × 4.6 mm I.D.) (Perkin Elmer, Saint Quentin en Yvelines, France), thermostated at 35°C in a Crocosil oven, with 0.1 *M* phosphate buffer (pH 2.9)–acetonitrile (67.5:32.5, v/v) as mobile phase pumped by a Shimadzu Model LC 5 A reciprocating HPLC pump (Touzart et Matignon) at a flow-rate of 1 ml/min. The injection solvent was water–acetonitrile (60:40, v/v). Injections of the sample vials by a Rheodyne Model 7125 injection valve equipped with a 20-μl sample loop were automatically carried out by an Kontron 360 automatic injector (Kontron Instruments, Montigny-Le-Bre-

tonneux, France), the column effluent was monitored at 220 nm by a Model 2550 UV detector (Varian, Orsay, France) and all chromatographic data were stored in a CR4A Shimadzu data system (Touzart et Matignon).

Reagents

All organic solvents were of HPLC grade except methanol, which was of analytical-reagent grade. Alcohols were purchased from Prolabo (Paris, France) and other organic solvents from Rathburn (Chromoptic, Montpellier, France). Each phase was filtered before use through on-line filters (2 μm) after the pumps. Water was doubly distilled. Nitrogen (L'Air liquide, Paris, France) supplied the nebulizer of the ELSD system.

Pure pristinamycins IA and IB were provided by Rhône-Poulenc Rorer.

RESULTS AND DISCUSSION

Selection of solvent systems

In CCC, there are three main criteria in choosing a solvent system. First, the solvent systems must be composed of two immiscible phases, second, their selectivity towards samples of interest has to be sufficient to lead to separations with good resolution and third, the stationary phase retention must be at least of 50% of the total column volume when applied on a CCC unit.

The main criterion is the second one. The selectivity of different solvent systems can be evaluated by determination of the partition coefficients for each component. All partition coefficients are expressed as the ratio of the concentration in the organic phase to that in the aqueous phase, whichever phase is the mobile phase, whereas the ratios of concentration in the stationary phase to that in the mobile phase were used to predict possible separations. The selectivity (α) of the solvent system is the ratio of the partition coefficients of the solutes. Different methods are commonly used for the determination of partition coefficients [7–9]. As crude pristinamycins consisted of four main components, HPLC, which allowed the simultaneous determination of the four partition coefficients and did not require the use of pure samples, was chosen to measure their concentrations.

Taking into account the relative hydrophobicity of pristinamycins, several hydrophobic solvent systems based on heptane–water mixtures were investigated. The basic system heptane–water (1:1, v/v) was not selective enough so modifiers such as methanol and ethyl acetate were added in various proportions to modify the polarity of this system. Unfortunately, with heptane–methanol–water or heptane–ethyl acetate–water solvent systems, all the partition coefficient values were of the same magnitude and, considering the accuracy of their determination, no solvent system with sufficient selectivity could be found.

Based on the data for the Craig separation of these macromolecules [10], dichlorethane–water–methanol solvent systems were studied. The solvent system used with Craig's Machine, dichlorethane–water–methanol (40:7:30, v/v/v) gave partition coefficients and selectivity that were too low. Partition coefficients and selectivity were increased, by first modifying the proportion of water in the system, then changing the pH of the aqueous phase (pristinamycins I have acid–base properties) and then increasing the proportion of another components of the solvent system. In this investigation, two kinds of difficulties were encountered. Some proportions resulted in miscible solvent systems which were therefore not consistent with CCC and, second, neither the aqueous nor the organic phase was retained as the stationary phase in the CCC unit irrespective of the mobile phase flow-rate and the rotation speed.

As reported in numerous papers, most separations of antibiotics by counter-current chromatography use a two-phase solvent system based on chloroform–water systems [11–18]. Commonly, methanol, carbon tetrachloride, ethyl acetate or an aqueous buffer is added to modify the polarity of the solvent system and increase its selectivity. The first attempts were to add methanol, which is miscible in the two phases. All solvent system compositions are presented in Table I and on a ternary diagram (Fig. 2) that is very useful in CCC [19–23] to study the influence of each component of solvent systems on partition coefficient and in HPLC to study the polarity and selectivity of various solvents (Snyder selectivity diagram [24]). Ternary diagrams were used to determine ranges where solvent systems provided separations not too slowly and with good resolution. Either the partition coefficients

TABLE I

PARTITION COEFFICIENTS FOR CHLOROFORM–METHANOL–WATER SOLVENT SYSTEMS

No.	Solvent system composition (volumes)			P IA	P IB	P IIA	P IIB
	Chloroform	Methanol	Water				
1	3	1	3	∞	∞	∞	∞
2	1	1	1	∞	∞	∞	∞
3	2	5	4	99	86	31	22
4	1	2	1	23	4.9	3.9	–[a]
5	1	3	1	single phase			
6	1	5	1	single phase			

[a] Partition coefficient not available.

values were too high (solvent systems 1–4) or the solvent system was composed of a single phase (solvent systems 5 and 6). As methanol was mostly miscible with water, ethyl acetate which was mostly miscible with chloroform was added; hence both the aqueous and organic phase polarities were modified. The solvent system chloroform–ethyl acetate–methanol–water (3:1:3:2, v/v) was applied on the

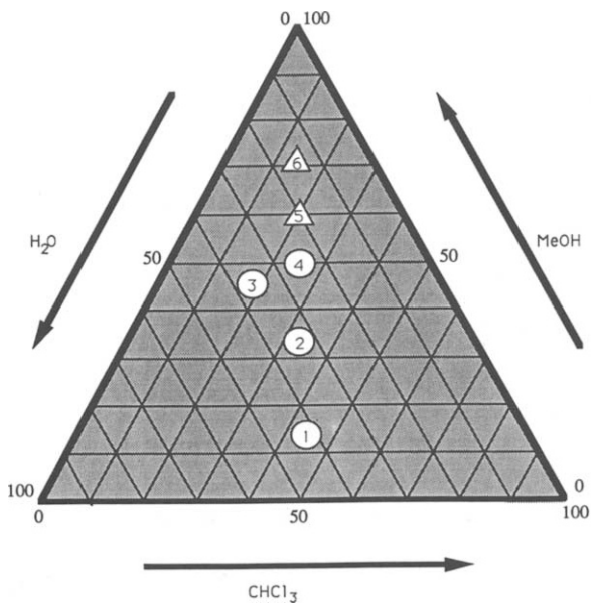

Fig. 2. Ternary diagram for chloroform–methanol–water solvent systems. Compositions of each components are expressed in volume percentage and numbers refer to Table I. △, Single-phase solvent system.

CPHV 2000 unit with the organic phase as mobil phase pumped from head to tail and promising re sults for the separation of pristinamycins II wer obtained. Hence this solvent system had to be im proved and adapted to the separation of pristina mycins I.

Separation of pristinamycins I and II using chloro form–ethyl acetate–methanol–water solvent system

Using the analytical HSCCC CPHV 2000 uni modified solvent systems were tried directly withou wasting any time in calculating low-accuracy part tion coefficients.

The separation of pristinamycins IIA and B wa first improved. Ethyl acetate was the component c the solvent system with the greatest influence o selectivity. Its proportion was modified until a sep aration with sufficient analytical resolution to allo scale-up to preparative separation was obtainec All the results in terms of solvent systems, resolu tion, selectivity and stationary phase retention fo CPHV 2000 are presented in Table II. Fig. 3 show chromatograms of the separation of pristinamycin IIA and B with increasing proportion of ethyl ace tate, the total volume of chloroform and ethyl ace tate being constant. If too much ethyl acetate wa added (Table II, solvent system 5), there was n longer any retention of the aqueous stationar phase. The best resolution between pristinamycin IIA and B was obtained when chloroform and ethy acetate were in equal proportions (Table II, solver system 4, *i.e.*, R_s = 2.21 against R_s = 1.2 with th first solvent system).

TABLE II

IMPROVEMENT OF THE PRISTINAMYCINS IIA AND IIB SEPARATION

Chloroform–ethyl acetate–methanol–water solvent system compositions in volumes, resolution between pristinamycins IIA and B, solvent system selectivity and stationary phase retention obtained on CPHV 2000. Aqueous stationary phase. Mobile phase flow-rate, 2 ml/min.

No.	Solvent system composition (volumes)				R_s (P IIA/P IIB)	α (P IIB/P IIA)	Capacity factor, k'		Stationary phase retention (%)
	Chloroform	Ethyl acetate	Methanol	Water			P IIA	P IIB	
1	3	1	3	2	1.2	1.5	0.52	0.8	81
2	2.5	1.5	3	2	1.87	1.67	0.30	0.61	80
3	2.4	1.6	3	2	2.0	1.7	0.83	1.4	78
4	2	2	3	2	2.21	1.76	0.61	1.06	77
5	1.5	2.5	3	2					0

Solvent system 4 in Table II, which offered the best resolution between pristinamycins IIA and B, was not at all selective towards pristinamycins IA and B. Through the protonable nitrogen and hydroxyl groups, pristinamycins I present acid–base properties so formic acid was added to solvent system 4 to control the aqueous phase pH and thus increase selectivity. Table III summarizes the results. Formic acid was added until there was less stationary phase retention (which induced a decrease in resolution), then the proportions of chloroform and ethyl acetate were changed (their total volume remaining constant) and the proportion of formic acid increased again, etc. As the proportions of methanol and water were maintained constant, the more interesting solvent systems, that is solvent systems 1 and 6–16 in Table III, were plotted on a ternary diagram (Fig. 4). For limited component proportions, general trends of resolution, solutes retention and stationary phase retention could be highlighted.

When the proportion of formic acid was increased (solvent systems 1–7 and 8 against 9), solute solubility in the stationary phase and retention increased, stationary phase retention tended to decrease until it became zero and resolution increased as long as there was no bleeding of the stationary phase. With a significant proportion of formic acid (solvent systems 7–16), the same trend as before was observed with increasing amount of ethyl acetate. Solvent system 12 was not selective; it showed the need for ethyl acetate to obtain sufficient selectivity, which was already observed for ethyl acetate to obtain sufficient selectivity, which was already observed for the optimization of the separation of pristinamycins IIA and B. The effect of an increasing proportion of chloroform on separation was mainly to lower the solute retention and to give excellent stationary phase retention (solvent systems 7, 8 and 10). As far as resolution was concerned, no obvious influence could be seen. The best solvent system for the separation of pristinamycins IA and B seemed to be solvent system 8, which combined rapidity (the separation lasted less than 30 min) and a resolution close to 2, which would allow easy semi-preparative scale-up studies that are now in progress.

Preliminary semi-preparative studies

The maximum volume and amount that can be injected on to the CPHV 2000 system without appreciable losses in efficiency and resolution were determined. A 1-mg amount of pristinamycin IA was injected while the sample volume was increased from 50 μl to 20 ml. For each injected volume, samples were diluted alternately with the mobile phase and the stationary phase. Figs. 5a and b and 6a and b show respectively the variation of efficiency *versus* injected volume and the ratio of injected volume to peak volume *versus* injected volume. Figs. 5a and 6a refer to mobile phase as sample diluent and Figs. 5b and 6b to stationary phase as sample diluent. As injection in mobile phase did not disturb the phase equilibrium and distribution, no stationary phase bleeding was observed but a dramatic decrease in

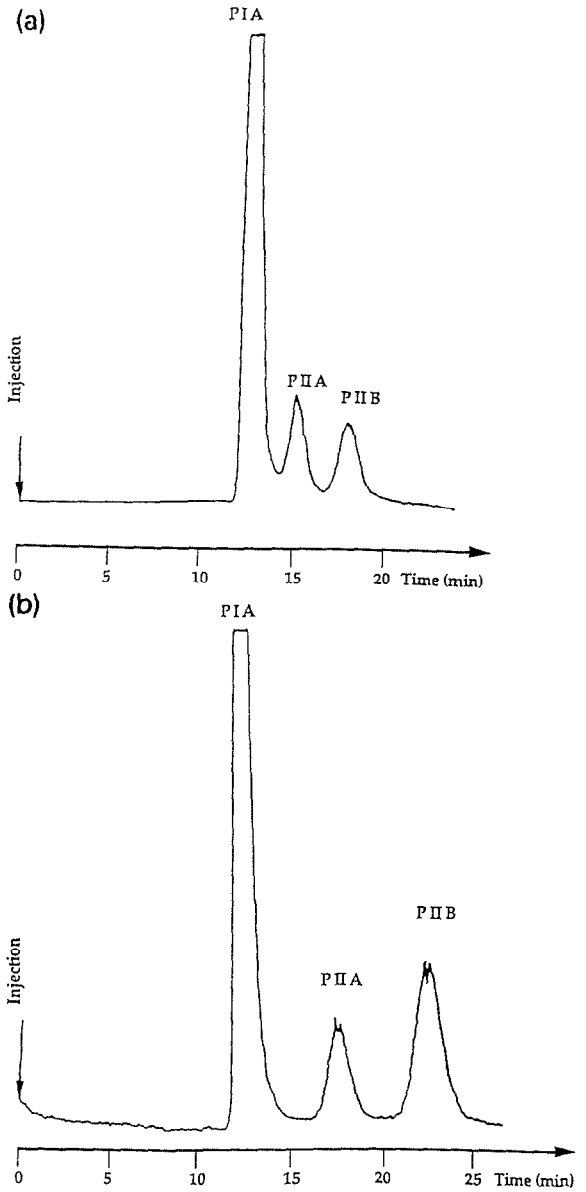

Fig. 3. Chromatograms for separation of pristinamycins IIA and B on CPHV 2000. Solvent systems: chloroform–ethyl acetate–methanol–water, (a) 3:1:3:2 and (b) 2.4:1.6:3:2 (v/v). Organic mobile phase flow-rate, 2 ml/min; injection, 50 μl; rotation speed, 1400 rpm; ELSD, 40°C; nitrogen pressure, 2 bar.

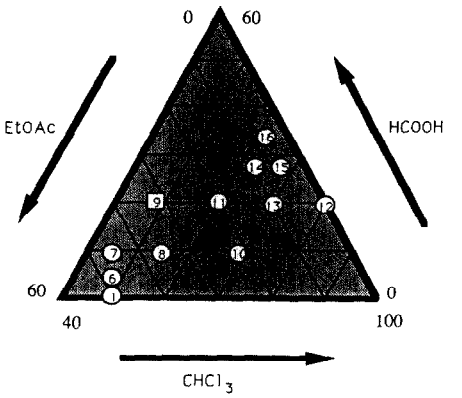

Fig. 4. Ternary diagram for chloroform–ethyl–acetate–methanol–water–formic acid solvent systems, all with 3 volumes of methanol and 2 volumes of water; compositions of the other three components are expressed in volume percentage and numbers refer to Table III. □, Interesting but no stationary phase retention.

peak efficiency occurred (1500 theoretical plates for a 50-μl sample loop against 20 theoretical plates for a 20-ml sample loop). In contrast, although injection in stationary phase disturbed the phase equilibrium and induced significant bleeding of the stationary phase for injected volumes over 5 ml, the peak efficiency increased slightly up to 2000 theoretical plates for 10 ml injected. A higher peak efficiency would have been obtained without taking into account that loss of stationary phase during separation induced artificially longer retention times. In additions with injections over 5 ml, peak compression occurred. This has already been observed [25–27] and could be of advantage for one-step preconcentration and separation of diluted samples.

Hence the maximum injection volume was 10 ml (10% of the column total capacity) in stationary phase sample diluent. The injected amount was then increased from 0.5 mg to 200 mg of a mixture of pristinamycins IA and B to study the variation of resolution. Each injection, consecutive or not, was performed in stationary phase and had to follow a specific procedure in order not to induce bleeding of the stationary phase and to obtain good reproducibility of solute retention times. It consisted in filling the apparatus while the injection valve was in the "inject" position. Then the injection valve was commuted to the "load" position and the stationary phase sample solution was loaded in the 10-ml sample loop. Before starting rotation and pumping the mobile phase, the injection valve was commuted to "inject" and 1 or 2 ml of pure stationary phase were pumped to prevent direct mixing between mobile

TABLE III

IMPROVEMENT OF THE SEPARATION OF PRISTINAMYCINS IA AND IB

Partial system compositions in chloroform (x), ethyl acetate (y) and formic acid (z) in volumes for typical solvent systems: chloroform–ethyl acetate–methanol–water–formic acid (x:y:3:2:z,v/v), resolution between pristinamycins IA and B, solvent system selectivity and stationary phase retention obtained on CPHV 2000. Aqueous stationary phase. Mobile phase flow-rate, 2 ml/min.

No.	Solvent system composition (volumes)			N_s	α	Retention time (min)		Capacity factor k'		Stationary phase retention (%)	Comments
	Chloroform	Ethyl acetate	Formic acid			P IA	P IB	P IA	P IB		
1	2	2	0.0019	0.55	1.54	16.98	18.05	0.13	0.20	72	
2	2	2	0.002	0.5	1.48	17.30	18.40	0.15	0.23	72	
3	2	2	0.005	0.6	1.44	15.20	16.35	0.21	0.31	77	
4	2	2	0.02	0.8	1.3	20.35	22.03	0.36	0.47	72	
5	2	2	0.04	1.1	1.43	20.28	22.53	0.35	0.50	73	
6	2	2	0.2	2.16	1.35	29.70	34.85	0.98	1.32	73	
	2	2	0.2	1.7	1.36	25.85	29.35	0.70	0.95	73–63	Slight bleeding of stationary phase
7	2	2	0.4	1.7	1.33	35.20	42.08	1.35	1.80	73–35	Bleeding of stationary phase
8	2.4	1.6	0.4	2.49	1.51	21.98	28.33	0.90	1.36	81	
	2.4	1.6	0.4	2.04	1.96	20.17	26.10	0.44	0.86	77–72	
9	2.4	1.6	1							0	No stationary phase retention
10	3	1	0.4	1.4	2.1	12.63	15.58	0.26	0.56	81	Weak resolution
11	3	1	1	2.7	1.58	26.48	36.63	1.94	3.07	80	
12	4	0	1	0	1	17.22	17.22	0.38	0.38	77	No selectivity
13	3.5	0.5	1	1.3	1.44	18.00	21.50	0.80	1.15	81	
14	3.5	0.5	1.5	2.5	1.32	31.80	39.60	2.18	2.96	80	
15	3.75	0.25	1.5	0	1	–[a]	–[a]	–[a]	–[a]	–[a]	No selectivity
16	3.75	0.25	2	0.65	1.24	15.40	16.40	0.43	0.52	80	Weak selectivity

[a] Data not available.

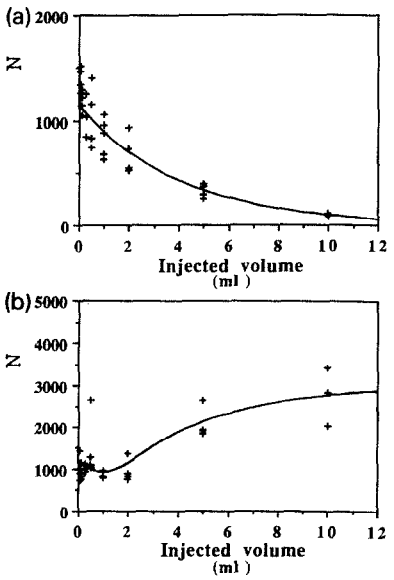

Fig. 5. Variation of the separation efficiency *versus* injected volume on CPHV 2000 for (a) mobile phase sample diluent and (b) stationary phase sample diluent . Solvent system: chloroform–ethyl acetate–methanol–water–formic acid (2.4:1.6:3:2:0.4, v/v). Organic mobile phase flow-rate, 2 ml/min; injection, pristinamycin IA 1 mg, 0.05–10 ml; rotation speed, 1400 rpm; ELSD, 40°C; nitrogen pressure, 2 bar.

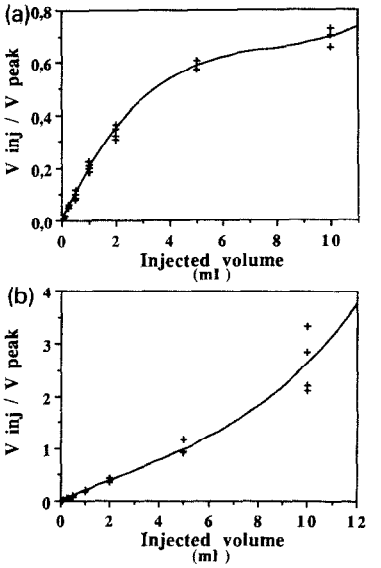

Fig. 6. Variaton of the ratio of injected volume to peak volume (V_{inj}/V_{peak}) *versus* injected volume on CPHV 2000 for (a) mobile phase sample diluent and (b) stationary phase sample diluent. Operating conditions as in Fig. 5.

phase and sample solution. Hence the first injection was made before the phases were equilibrated. For consecutive injections, mobile phase pumping was stopped but not the rotation and before being filled the sample loop was rinsed with 20 ml of pure stationary phase, the injection valve having been commuted to the "load" position. It ends as for the first injection: the injection valve in the "inject" position, a small volume of stationary phase was pumped and mobile phase pumping was resumed. Fig. 7 shows three consecutive chromatograms with

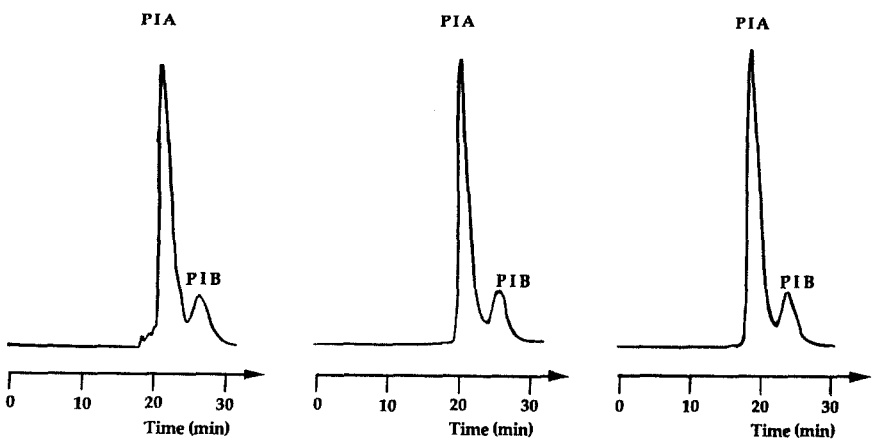

Fig. 7. Three consecutive chromatograms of the separation of pristinamycin IA following the special injection procedure. Operating conditions as in Fig. 5 except for sample size: 100 mg of pristinamycin IA dissolved in 10 ml of stationary phase.

100 mg injected in 10 ml of stationary phase. As a result of this injection procedure, mean retention times ± standard deviations were 21.9 ± 1.3 min for pristinamycin IA and 26.6 ± 1.2 min for pristinamycin IB and the stationary phase displaced corresponded to the 10 ml injected. Up to 200 mg of pristinamycins IA and B dissolved in stationary phase were injected (the sample solution corresponded to the solubility limit of the compounds) and the separation still had a mean efficiency blank (1000 theoretical plates) but no resolution.

CONCLUSION

No direct transposition of solvent systems from Craig Machine separation of pristinamycins to CCC was possible because of the high stationary phase retention requirement of HSCCC. Therefore, an original solvent system was developed allowing the rapid separation of pristinamycins when the stationary phase was the aqueous phase. Injections in stationary phase permitted large amounts of purified sample to be separated without too much loss of resolution. Further investigations are being carried out direclty on the crude extract to scale-up the separation (300 to 1000 mL column volume) and to evaluate the yield of the process in terms of amount and purity. These preliminary results demonstrate that HSCCC can be a powerful tool for the fractionation of compounds.

ACKNOWLEDGEMENT

The authors thank Rhône-Poulenc Rorer for providing financial support for this work.

REFERENCES

1 *J. Liq. Chromatogr.*, 7, No. 2 (1984).
2 *J. Liq. Chromatogr.*, 8, No. 12 (1985).
3 *J. Liq. Chromatogr.*, 11, No. 1 (1988).
4 *J. Liq. Chromatogr.*, 13, No. 12 (1990).
5 *J. Chromatogr.*, 538, No. 1 (1991).
6 S. Drogue, M.-C. Rolet, D. Thiébaut and R. Rosset, *J. Chromatogr.*, 538 (1991) 91–97.
7 K. Hostettmann, M. Hostettmann-Kaldes and K. Nakanishi, *J. Chromatogr.*, 170 (1979) 355–361.
8 W. D. Conway and Y. Ito, *J. Liq. Chromatogr.*, 7 (1984) 275–289.
9 W. D. Conway and Y. Ito, *J. Liq. Chromatogr.*, 7 (1984) 291–302.
10 J. Preud'homme, P. Tarridec and A. Belloc, *Bull. Soc. Chim. Fr.*, (1968) 585–591.
11 G. M. Brill, J. B. McAlpine and J. E. Hochlowski, *J. Liq. Chromatogr.*, 8 (1985) 2259–2280.
12 D. G. Martin, C. Biles and R. E. Peltonen, *Am. Lab.*, 18 (1986) 21.
13 J. B. McAlpine, D. N. Whittern, J. E. Jochlowski and L. M. Ranfranz, poster presented at the *26th Interscience Conference on Antimicrobial Agents and Chemotherapy, September 29, 1986.*
14 J. E. Hochlowski, G. M. Brill, W. W. Andres and J. B. McAlpine, poster presented at the *26th Interscience Conference on Antimicrobial Agents and Chemotherapy, September 29, 1986.*
15 J. E. Hochlowski, S. J. Swanson, D. N. Whittern, A. M. Buko and J. B. McAlpine, poster presented at the *26th Interscience Conference on Antimicrobial Agents and Chemotherapy, September 29, 1986.*
16 H. Nakazawa, P. A. Andrews, N. R. Bachur and Y. Ito, *J. Chromatogr.*, 205 (1981) 482–485.
17 J. W. Lightbown, P. Newland, I. A. Sutherland and J. W. A. Dymond, *Proc. Anal. Div. Chem. Soc.*, 14 (1977) 34.
18 T. Y. Zhang, *J. Chromatogr.*, 315 (1984) 287–297.
19 A. Berthod, J. D. Duncan and D. W. Armstrong, *J. Liq. Chromatogr.*, 11 (1988) 1171–1185.
20 D. W. Armstrong, *J. Liq. Chromatogr.*, 11 (1988) 2433–2446.
21 W. D. Conway, presented at the *Pittsburgh Conference, New Orleans, 1988.*
22 A. Foucault and K. Nakanishi, *J. Liq. Chromatogr.*, 13 (1990) 3583–3602.
23 W. D. Conway, *Counter-Current Chromatography —Apparatus, Theory and Applications*, VCH, New York, 1990.
24 L. R. Snyder, *J. Chromatogr. Sci.*, 16 (1978) 223–234.
25 H. Oka, K.-I. Harada and M. Suzuki, H. Nakazawa and Y. Ito, *J. Chromatogr.*, 482 (1989) 197–205.
26 J. A. Glinski, G. O. Caviness and J. R. Mikell, *J. Liq. Chromatogr.*, 13 (1990) 3625–3635.
27 H. Oka, K.-I. Harada, M. Suzuki, H. Nakazawa and Y. Ito, *J. Chromatogr.*, 538 (1991) 213–218.