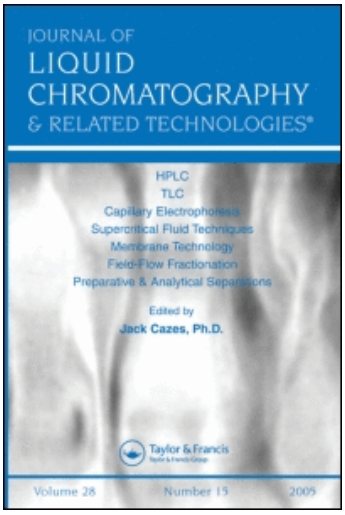


Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Use of a Macrocyclic Antibiotic as the Chiral Selector for Enantiomeric Separations by TLC

D. W. Armstrong[a]; Y. Zhou[a]
[a] Department of Chemistry, University of Missouri-Rolla, Rolla, Missouri

To cite this Article Armstrong, D. W. and Zhou, Y.(1994) 'Use of a Macrocyclic Antibiotic as the Chiral Selector for Enantiomeric Separations by TLC', Journal of Liquid Chromatography & Related Technologies, 17: 8, 1695 — 1707
To link to this Article: DOI: 10.1080/10826079408013451
URL: http://dx.doi.org/10.1080/10826079408013451

JOURNAL OF LIQUID CHROMATOGRAPHY, 17(8), 1695-1707 (1994)

USE OF A MACROCYCLIC ANTIBIOTIC AS THE CHIRAL SELECTOR FOR ENANTIOMERIC SEPARATIONS BY TLC

DANIEL W. ARMSTRONG* AND YIWEN ZHOU
University of Missouri-Rolla
Department of Chemistry
341 Schrenk Hall
Rolla, Missouri 65401

ABSTRACT

The macrocyclic antibiotic, vancomycin, was used as a chiral mobile phase additive for the thin layer chromatographic (TLC) resolution of 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC) derivatized amino acids, racemic drugs and dansyl-amino acids. Excellent separations were achieved for most of these compounds in the reversed phase mode. Both the nature of the stationary phase and the composition of the mobile phase strongly influenced enantiomeric resolution. The best results were obtained using diphenyl stationary phases. Acetonitrile was the organic modifier that produced the most effective separations with the shortest development times. It is highly likely that macrocyclic antibiotics will play a major role in future enantiomeric separations.

*To whom correspondence should be sent.

INTRODUCTION

Enantiomeric separations in thin layer chromatography (TLC) are accomplished using either chiral stationary phases (1-8) or chiral mobile phase additives, CMAs (9-15). In contrast to HPLC, in which many chiral stationary phases are available, most TLC enantiomeric separations utilize CMAs. This is because only ligand-exchange-type stationary phases are available commercially for TLC. The most widely utilized CMAs are cyclodextrins and their derivatives (10-13,15). However, a few other classes of additives have been used successfully as well such as chiral ion interaction agents (14) and ligand exchange types (9). In this work we report on a member of a new class of chiral selectors (i.e., macrocyclic antibiotics). Vancomycin is an antibacterial compound that inhibits mucopeptide biosynthesis. Previously we found that it was useful in the HPLC separation of enantiomers when immobilized on 5 μ silica gel (16). Recently we have used other macrocycles in HPLC and capillary electrophoresis (16,17). To our knowledge this is the first report on their use as chiral mobile phase additives in chromatography.

EXPERIMENTAL SECTION

Materials. All racemic analytes were obtained from Aldrich (Milwaukee, WI) or Sigma (St. Louis, MO). Chiral macrocyclic antibiotics such as vancomycin were obtained from Advanced Separation Technologies, Inc. (Whippany, NJ). All dansyl amino acids were purchased from Sigma. 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC) derivatizing kit was from Waters (Bedford, MA). Chemically bonded Diphenyl-F reversed phase plates (5 x 20 cm, 250 μ layer thickness) were from Whatman Chemical Separation Division, Inc. (Clifton, NJ).

Methods. 0.6 M sodium chloride was added to all mobile phases. Sodium chloride was used to stabilize the plate binder. Acetonitrile was used as the organic modifier. Occasionally 1% triethylammonium acetate buffer (pH = 4.1) was also used as indicated in the Tables and Figures. Chiral selectors were first dissolved in the sodium chloride solution and placed in an ultrasonic bath for about 15 minutes, then the organic modifier was added to complete the mobile phase mixture.

The plates were developed at room temperature (22°C) in 6 (i.d.) x 23 cm cylindrical glass chambers. It took approximately 1-3 hrs to completely develop a 5 x 20 cm TLC plate. All compounds were fluorescent. Spot visualization was done by using a fixed-dual wavelength (254 nm and 365 nm) ultraviolet hand lamp.

The AQC derivatives were obtained according to reference 18. Approximately 100 pmol of each compound was dissolved in 60 μL of sodium borate buffer (0.2 M. pH 8.8) in a vial; vortexed for several seconds and then 20 μL of AQC solution was added (3 mg per 1 lL of acetonitrile). The vial was heated in an oven for 10 minutes at 50°C. The resulting solutions were used in TLC without further purification.

RESULTS AND DISCUSSION

Recently we proposed the use of macrocyclic antibiotics as a new class of chiral selectors for enantiomeric separations (16,17). In this work it is shown that vancomycin is an effective chiral mobile phase additive for the TLC resolution of several enantiomeric compounds.

Vancomycin consists of three fused macrocycles (Figure 1). This amphoteric molecule contains five aromatic rings as well as peptide and carbohydrate moieties. It has a molecular weight of 1,449 and an

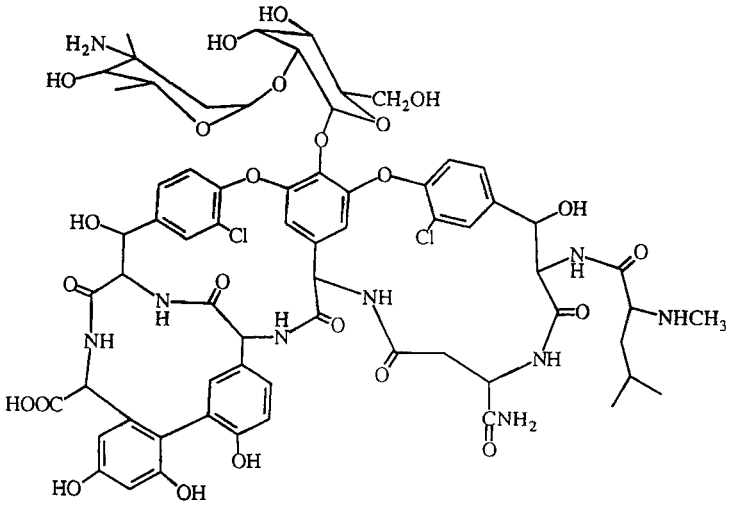
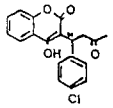
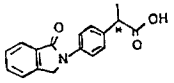
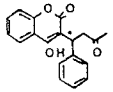
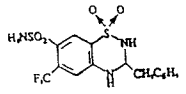
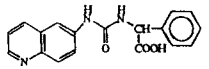
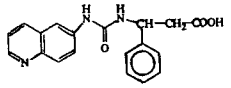
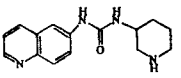


Figure 1. Molecular structure of vancomycin.

isoelectric point of ~ 5 (16). Vancomycin is produced by the bacteria (*Streptomyces orientalis*). It is soluble in water, partially soluble in methanol and hydro-organic solvent mixtures, and insoluble in higher alcohols and nonpolar organic solvents (16).

A variety of different reversed phase TLC stationary phases were used in an attempt to achieve good enantiomeric separation and resolution. Diphenyl-type stationary phases gave the best results in terms of low streaking and good spot integrity during development (see Experimental). The separation results are collected in Table I. The racemates resolved are of three types: underivatized pharmaceuticals, AQC-amino acids and dansyl amino acids. When enantiomerically pure standards were available, the retention order was determined (Table I). The mobile phase generally consisted of between 17 and 40 volume percent acetonitrile plus 0.6 M $NaCl_{(aq)}$ (Table I, footnote a). The salt in the water helped to stabilize the

Table I Reversed-Phase TLC Enantiomeric Separation Data Using a Vancomycin Chiral Selector on Diphenyl-F Plate

Compound	R_{f1}	R_{f2}	α	R_s	Conc.(M)	Mobile Phase[a]
1. Coumachlor 	0.14	0.20	1.4	2.5	0.05	4/6/0
2. Indoprofen 	0.58	0.63	1.1	1.6	0.05	4/6/0
3. Warfarin 	0.04	0.06	1.5	1.2	0.04	2/10/0
4. Bendroflume-thiazide 	0.02	0.06	3.0	1.8	0.05	0.5/8.5/1
5. AQC[b]-α-amino phenylacetic acid 	0.13(L)	0.16(D)	1.2	1.9	0.025	2/10/0
6. AQC[b]-3-amino-3-phenylpropinic acid 	0.12 0.11	0.18 0.19	1.5 1.7	2.5 2.2	0.04 0.025	2/10/0 2/10/0
7. AQC[b]-3-aminopiperidine dihydrochloride 	0.24	0.28	1.2	1.7	0.025	2/10/0

(continued)

Table I (continued)

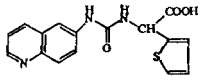
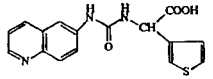
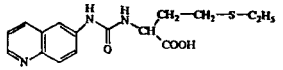
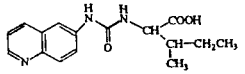
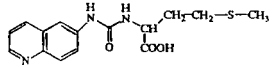
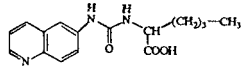
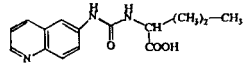
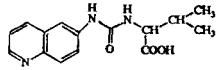
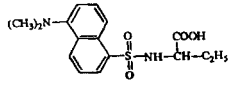
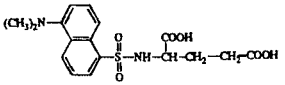
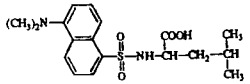
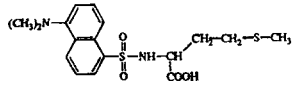
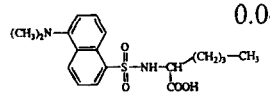
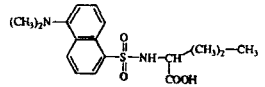
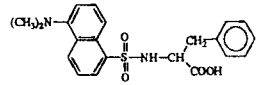
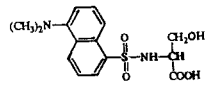
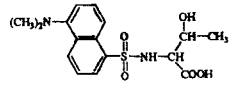
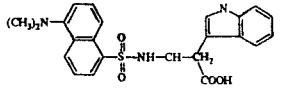
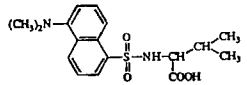
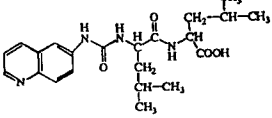
8. AQC[b]-α-amino-2-thiopheneacetic acid	0.16 0.16	0.18 0.19	1.1 1.2	1.2 1.6	0.025 0.04	2/10/0 2/10/0
						
9. AQC[b]-α-amino-3-thiopheneacetic acid	0.17	0.22	1.3	2.6	0.025	2/10/0
						
10. AQC[b]-ethionine	0.14	0.17	1.2	1.4	0.025	2/10/0
						
11. AQC[b]-alloisoleucine	0.14(L)	0.21(D)	1.5	3.1	0.025	2/10/0
						
12. AQC[b]-methionine	0.19(L)	0.23(D)	1.2	1.5	0.025	2/10/0
						
13. AQC[b]-norleucine	0.13(L)	0.16(D)	1.2	1.6	0.025	2/10/0
						
14. AQC[b]-norvaline	0.21(L)	0.25(D)	1.2	2.3	0.025	2/10/0
						
15. AQC[b]-valine	0.23(L)	0.27(D)	1.2	2.0	0.025	2/10/0
						
16. Dansyl-α-amino-n-butyric acid	0.09(L) 0.09(L)	0.15(D) 0.21(D)	1.7 2.3	2.7 4.1	0.04 0.08	2/10/0 2/10/0
						

Table I (continued)

17. Dansyl-glutamic acid	0.21(L)	0.23(D)	1.1	1.4	0.04	2/10/0
						
18. Dansyl-leucine	0.03(L)	0.09(D)	3.0	3.0	0.04	2/10/0
						
19. Dansyl-methionine	0.05(L)	0.12(D)	2.4	3.4	0.04	2/10/0
						
20. Dansyl-norleucine	0.03(L)	0.07(D)	2.3	1.6	0.04	2/10/0
	0.04(L)	0.16(D)	4.0	4.9	0.08	2/10/0
						
21. Dansyl-norvaline	0.05(L)	0.12(D)	2.4	3.3	0.04	2/10/0
						
22. Dansyl-phenylalanine	0.03(L)	0.05(D)	1.7	1.5	0.04	2/10/0
						
23. Dansyl-serine	0.15(L)	0.20(D)	1.3	1.8	0.04	2/10/0
	0.16(L)	0.24(D)	1.5	2.8	0.08	2/10/0
						
24. Dansyl-threonine	0.13(L)	0.17(D)	1.3	2.5	0.05	2/10/0
						
25. Dansyl-tryptophan	0.01(L)	0.03(D)	3.0	1.3	0.04	2/10/0
						

(continued)

Table I (continued)

26. Dansyl-valine 	0.06(L)	0.10(D)	1.7	1.5	0.04	2/10/0
27. AQC[b]-leu-leu	R_{f1}(D-L)	R_{f3}(L-D)		α_1(DLLD)		R_{S1}(DLLD)
(0.04M, 2/10/0)[a] 	0.03	0.10		3.3		5.0
	R_{f2}(L-L)	R_{f4}(D-D)		α_2(LDLD)		R_{S2}(LDLD)
	0.04	0.24		6.0		11.6
(0.02M, 1.5/4.5/0)[a]	R_{f1}(D-L)	R_{f3}(L-D)		α_1(DLLD)		R_{S1}(DLLD)
	0.17	0.22		1.3		4.5
	R_{f2}(L-L)	R_{f4}(D-D)		α_2(LDLD)		R_{S2}(LDLD)
	0.18	0.42		2.3		18.5

[a] mobile phase compositions listed indicate the volume ratios of acetonitrile/0.6 M NaCl/1% triethylammonium acetate buffer (pH = 4.1).
[b] AQC stands for 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate, a fluorescent-tagging-agent (see Experimental section).

binder on the TLC plate. Most of the racemates were better than baseline resolved. Interestingly, the retention order of all AQC-amino acids and dansyl amino acids (for which standards were available) was the same. The D-enantiomer always had a greater R_f value than the L-enantiomer (Table I).

Figure 2 shows the TLC separation of indoprofen and coumachlor using vancomycin as the chiral mobile phase additive. Figure 3 shows analogous separations of five racemic AQC-amino acids. Figure 4 shows the complete resolution of all four stereoisomers (two pairs of enantiomers) of the dipeptide leucyl-leucine. In this case the relative retention of each isomer is known.

It appears that two factors contribute to the effectiveness of this technique in resolving enantiomeric compounds. One is the obvious

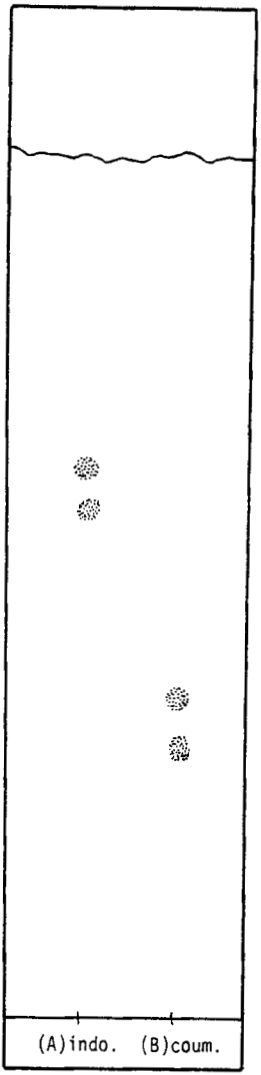


Figure 2. TLC chromatogram showing the separation of (A) indoprofen, and (B) coumachlor. The mobile phase consisted of 0.05 M vancomycin in 4:6 (by volume) acetonitrile: 0.6 M $NaCl_{(aq)}$. Diphenyl-F TLC plates (5 x 20 cm) were used. Spots were detected using a 365 nm UV hand lamp (see Experimental).

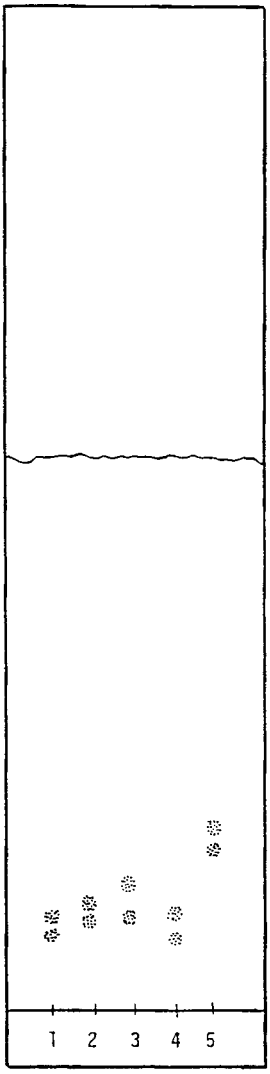


Figure 3. TLC chromatogram showing the separation of racemic: (1) AQC-ethionine, (2) AQC-α-amino-2-thiopheneacetic acid, (3) AQC-α-amino-3-thiopheneacetic acid, (4) AQC-α-amino phenylacetic acid, and (5) AQC-3-aminopiperidine dihydrochloride. The mobile phase consisted of 0.025 M vancomycin in 1:5 (by volume) acetonitrile: 0.6 M $NaCl_{(aq)}$. Other experimental conditions were the same as indicated in Figure 2.

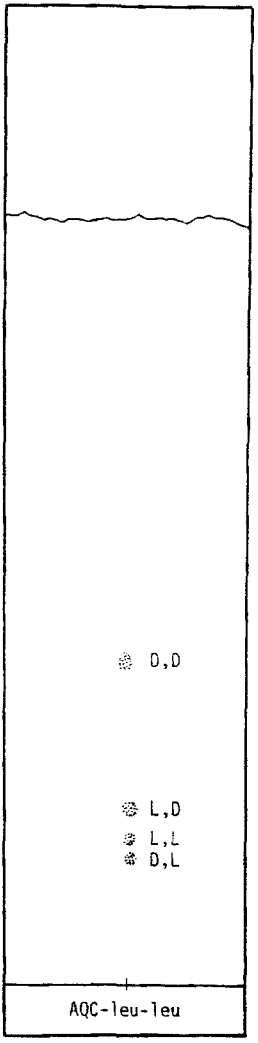


Figure 4. TLC chromatogram showing the separation of all four isomers (2 pairs of enantiomers) of AQC-leucyl-leucine. The stereochemistry of the compound represented by each spot is indicated. This was determined by developing pure standards in a separate experiment. The mobile phase consisted of 0.02 M vancomycin in 1:3 (by volume) acetonitrile: 0.6 M $NaCl_{(aq)}$. Other experimental conditions were the same as indicated in Figure 2.

enantioselectivity of the vancomycin CMA. However, the ability to maintain a relatively small spot size during development also is an important factor. In some cases TLC separations are inhibited by poor efficiency. That does not seem to be a limiting factor in these particular experiments, however.

Currently several other macrocyclic compounds are being evaluated as chiral selectors. Given the great structural variety of this class of compounds, it is likely not only that other analogous and effective chiral selectors will be found, but that they will have different enantioselectivities as well.

ACKNOWLEDGMENT

Support of this work by the Department of Energy, Office of Basic Sciences (grant DE FG02 88ER13819) is gratefully acknowledged.

REFERENCES

1. Yuasa, S.; Shimada, K.; Kameyama, M.; Yasui, M. and Adzuma, K., *J. Chromatogr. Sci.*, **18**, 311 (1980).
2. Wainer, I.W.; Brunner, C.A.; Doyle, T.D. *J. Chromatogr.*, **264**, 54 (1983).
3. Weinstein, S. *Tetrahedron Lett.*, **25**, 985 (1984).
4. Guenther, K.; Martens, J.; Schickedanz, M., *Angew. Chem. Int. Ed. Engl.*, **23**, 506 (1984).
5. Brinkman, U.A.T.; Kamminga, D., *J. Chromatogr.*, **330**, 375 (1985).
6. Alak, A.; Armstrong, D.W., *Anal. Chem.*, **58**, 582 (1986).
7. Gont, L.K.; Neuendorf, J., *J. Chromatogr.*, **391**, 343 (1987).
8. Martens, J.; Guenther, K. Schickedanz, M., *Arch. Pharm.*, **319**, 572 (1986).

9. Marchelli, R.; Virili, R.; Armani, E.; Dossena, A., *J. Chromatogr.*, **355**, 354 (1986).
10. Armstrong, D. W.; He, F.; Han, S., *J. Chromatogr.*, **448**, 345 (1988).
11. Armstrong, D. W.; Faulkner, Jr., J.R.; Han, S.M., *J. Chromatogr.*, **452**, 323 (1988).
12. Han, S.M.; Armstrong, D.W. in: "Planar Chromatography in the Life Sciences" Ed., Touchstone, J. C., John Wiley & Sons, N.Y. (1990) Ch. 7, pp 81-99.
13. Duncan, J.D.; Armstrong, D. W., *J. Planar Chromatogr.*, **3**, 65 (1990).
14. Duncan, J.D.; Armstrong, D.W.; Stalcup, A.M., *J. Liq. Chromatogr.*, **13**, 1091 (1990).
15. Duncan, J.D.; Armstrong, D.W., *J. Planar Chromatogr.*, **4**, 204 (1991).
16. Armstrong, D.W.; Tang, Y.; Chen, S.; Zhou, Y.; Bagwill, C.; Chen, J.-R., *Anal. Chem.* **66**, in press (1994).
17. Armstrong, D. W.; Rundlett, K.; Reid, G.L. III, *Anal. Chem.* submitted (1993).
18. Pawlowska, M.; Chen, S.; Armstrong, D.W., *J. Chromatogr.*, **641**, 257 (1993).

Received: January 6, 1994
Accepted: January 14, 1994