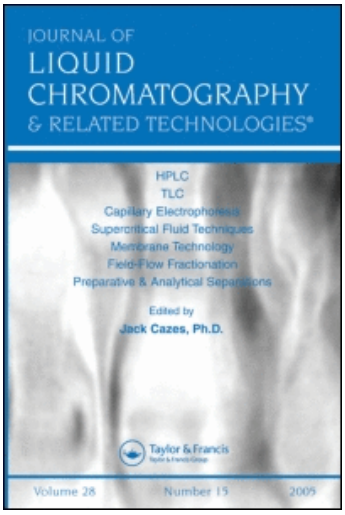

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Evaluation of a New Macrocyclic Antibiotic as a Chiral Selector for Use in Capillary Electrophoresis

V. Scott Sharp[a]; Donald S. Risley[a]; Sharon McCarthy[a]; Bret E. Huff[a]; Mark A. Strege[a]
[a] Pharmaceutical Sciences Division Lilly Corporate Center, Lilly Research Laboratories, Indianapolis, IN

To cite this Article Sharp, V. Scott , Risley, Donald S. , McCarthy, Sharon , Huff, Bret E. and Strege, Mark A.(1997) 'Evaluation of a New Macrocyclic Antibiotic as a Chiral Selector for Use in Capillary Electrophoresis', Journal of Liquid Chromatography & Related Technologies, 20: 6, 887 — 898
To link to this Article: DOI: 10.1080/10826079708013660
URL: http://dx.doi.org/10.1080/10826079708013660

J. LIQ. CHROM. & REL. TECHNOL., 20(6), 887–898 (1997)

EVALUATION OF A NEW MACROCYCLIC ANTIBIOTIC AS A CHIRAL SELECTOR FOR USE IN CAPILLARY ELECTROPHORESIS

V. Scott Sharp, Donald S. Risley, Sharon McCarthy,
Bret E. Huff, Mark A. Strege

Lilly Research Laboratories
Pharmaceutical Sciences Division
Lilly Corporate Center
Indianapolis, IN 46285

ABSTRACT

A new macrocyclic antibiotic, LY307599, has been evaluated as a chiral selector for the separation of the enantiomers of flurbiprofen using capillary electrophoresis (CE). The effect of varying separation buffer parameters such as buffer strength, pH, LY307599 concentration and methanol concentration were assessed. Using the optimized CE conditions, the separation of flurbiprofen enantiomers can be achieved using LY307599 as a chiral selector.

INTRODUCTION

Several approaches have been used to obtain chiral separations with capillary electrophoresis. These include the use of micelle-forming surfactants in micellar electrokinetic capillary chromatography (MECC),[1-7] immobilized chiral selectors in capillary gel electrophoresis[8-11] and various additives in free solution capillary electrophoresis (FSCE), also referred to as capillary zone electrophoresis (CZE).[12-39]

Free solution capillary electrophoresis is often the method of choice due to its capacity for the separation of a diverse array of analytes.[12] Within the realm of FSCE, specific additives have been successfully used to separate enantiomers including the use of native and modified cyclodextrins,[13-19] crown ethers,[20-23] carbohydrates,[24] proteins,[25-29] and enantioselective metal complexes.[30-31] More recently, macrocyclic antibiotics have been used as chiral selectors.[32-40] Within this group, the ansamycins rifamycin B and rifamycin SV have shown broad chiral selectivity for positive and negatively charged analytes respectively.[35] Rifamycin B has also been shown to be selective in the separation of racemic amino alcohols.[33] The glycopeptides ristocetin A, teicoplanin, vancomycin and A82846B have also shown promising chiral selectivity for many amino acid derivitives and carboxylic acids.[32,34, 36-40] Macrocyclic antibiotics such as A82846B provide multiple π-π electron interaction sites, hydrogen bonding locations and inclusion cavities for potential enantiospecific interactions. Despite similarity of these sites among macrocyclics, each displays varying success in the separation of different chiral analytes. In a recent report, a comparison of A82846B and vancomycin illustrated that a relatively small alteration in structure can significantly improve the resolution of flurbiprofen and dansyl valine enantiomers.[39] In this report we examined LY307599, a A82846B derivitive, for its applicability as a chiral selector using CE. Flurbiprofen, a non-steroidal antiinflammatory drug, was selected as the test analyte for evaluating LY307599 as a chiral selector in CE because the enantiomers have been successfully separated in other reports with vancomycin and A82846B.[32,39]

EXPERIMENTAL

The CE analysis was conducted using an automated Beckman P/ACE 2000 Instrument (Fullerton, CA) and the data collected on an in-house Hewlett Packard Model 1000 minicomputer (Palo Alto, CA). The 50 μm internal diameter 37 cm eCAP bare silica capillary (30 cm effective length) was obtained from Beckman Instruments, Inc. (Fullerton, CA). The absorbance wavelength was set at 254 nm. Samples were injected using a 5.0 second low pressure interval (0.5psi). The capillary temperature was controlled at 25 °C.

Sodium borate, 0.1 N and 5.0 N sodium hydroxide, 5.0 N hydrochloric acid and acetone were purchased from EM Science (Gibbstown, NJ). Potassium phosphate was obtained from Mallinckrodt (Paris, KY). Methanol was obtained from Curtin Matheson Scientific, Inc. (Houston, TX). The water used for buffer and sample preparation was deionized and filtered

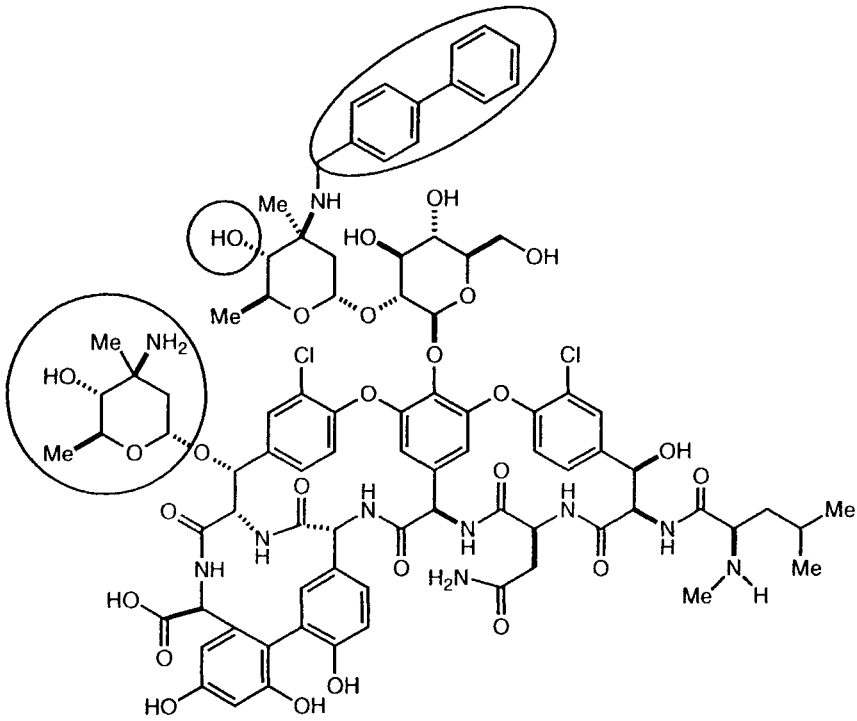

Figure 1. The structure of LY307599 (Molecular weight = 1731).

through a Millipore Milli-Q™ water purification system (New Bedford, MA). Run buffers and sample solutions were filtered through Acrodisc® Filters (0.2 μm) from Gelman Sciences (Ann Arbor, MI). Buffer pH was measured using a Brinkmann Model 691 pH meter (Westbury, NY). Flurbiprofen was obtained from Sigma-Aldrich Company (St. Louis, MO). Hydrochloride and trifluoroacetate salts of LY307599 were synthesized at Eli Lilly and Company (Indianapolis, IN).

Flurbiprofen samples were prepared at 1.0 mg/mL in 100 mM sodium borate buffer, unless otherwise noted. The 100 mM borate buffer was adjusted with 5.0 N NaOH or 5.0 N HCl to obtain the desired pH. The stability of the macrocyclic was not assessed, therefore all solutions of LY307599 were prepared fresh daily. LY307599 was first dissolved in methanol followed by the addition of the 100 mM borate to achieve the desired run buffer composition. Acetone was used as a neutral marker for electroosmotic flow (EOF).

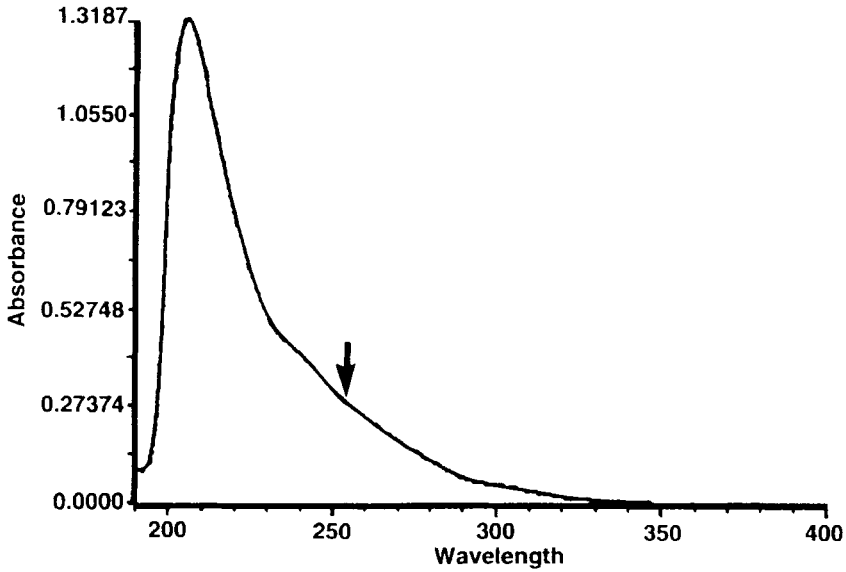

Figure 2. The UV spectrum of LY307599.

RESULTS AND DISCUSSION

The structure of LY307599 is shown in Figure 1. LY307599 is structurally similar to vancomycin and A82846B which have been previously reported.[32,39] The macrocyclic antibiotic LY307599 differs from vancomycin in three ways: The disaccharide amino sugar is epimeric at C-4 (epi-vancosamine), contains a biphenyl moiety on the amine at C5, and an additional epi-vancosamine at amino acid 6. The only difference between LY307599 and A82846B is the addition of the biphenyl moiety. The macrocyclic antibiotic LY307599 absorbs strongly in the lower UV range as shown in Figure 2.

Fortunately, the compound does not absorb greatly at 254 nm, allowing for the detection of flurbiprofen (Figure 3) at this wavelength. LY307599 presented unique challenges compared to A82846B and vancomycin due to its limited solubility in aqueous buffers, especially below pH 8.0, thus requiring lengthy development and parameter optimization to achieve enantioseparation.

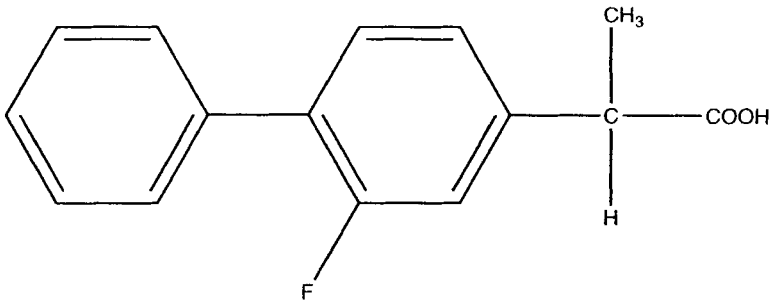

Figure 3. The structure of flurbiprofen.

Development

The hydrochloride and acetate salts were used in this study due to their improved solubility over other salt forms and the free base. Solutions of the macrocyclic antibiotic in 50 mM phosphate buffer were achieved at pH 3.0 (4.0 mM, LY307599) and in 100 mM borate buffer above pH 9.0 (7.0 mM, LY307599). In order to dissolve 1.0 mM of LY307599 at pH 7.0, the addition of 60% methanol was necessary, thus direct comparison of LY307599 with previous data using vancomycin and A82846B in aqueous neutral pH conditions was not possible. A 1.0 mM solution of LY307599 in the pH 7.0 phosphate/methanol buffer provided the enantiomeric separation of flurbiprofen yielding a resolution of 1.5, but the high concentration of methanol required for solubility of the macrocyclic inhibited a reproducible system and resulted in a noisy baseline.

Due to the lack of solubility of LY307599 in neutral pH buffers, the separation of flurbiprofen enantiomers was attempted in both acidic and basic conditions. Using 4.0 mM of LY307599 in 50 mM pH 3.0 phosphate buffer, flurbiprofen enantiomers could not be separated. The EOF of the system was determined by injecting a 2% acetone solution and was found to be 5.1×10^{-5} cm^2/V s using a voltage of 30 kv.

More successful results were observed in the basic pH range of 9.0 to 10.0 using borate buffer and 4.0 mM LY307599 concentration. To maximize analyte interaction time in the capillary under basic conditions, the voltage of the system was reduced to 10 kv for analysis in this pH range. This also reduced the baseline noise of the system. Borate buffer was used at 100 mM

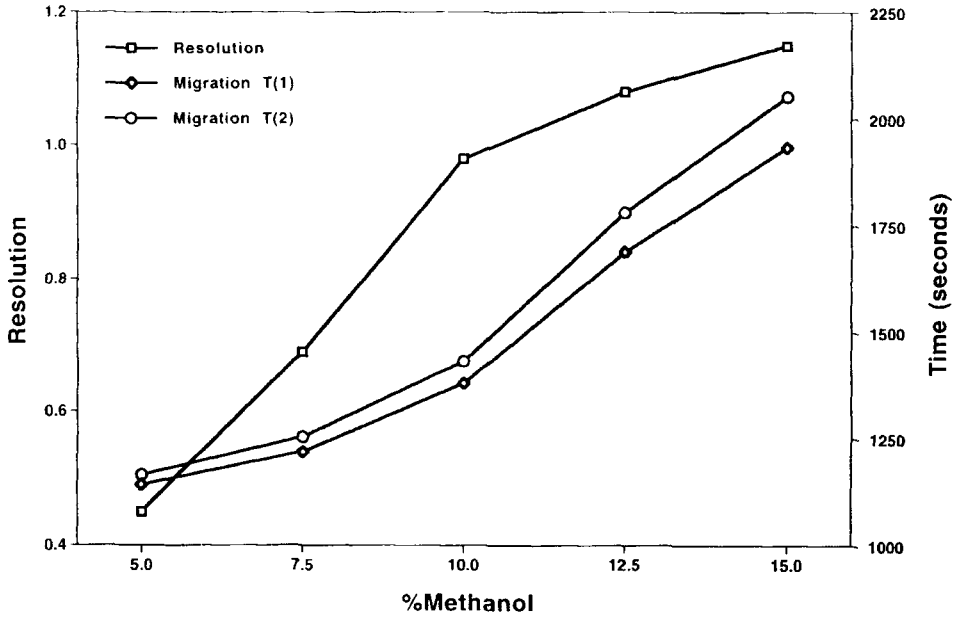

Figure 4. A plot of run buffer methanol content versus resolution and migration time. Run buffer is 3.0 mM LY307599 in methanol and 100mM borate at pH 9.2; Voltage is 10 kv; UV absorbance is 254 nm.

strength to further decrease EOF while maintaining a stable current of 60 μA. Early analysis revealed that the EOF was evidently still too fast to allow for sufficient interaction between flurbiprofen and 1.0 mM LY307599 in borate run buffer. Injections of flurbiprofen under these conditions resulted in a sharp analyte peak eluting in approximately 13.0 minutes.

The addition of methanol and increase in LY307599 concentration in the run buffer significantly slowed EOF and resulted in flurbiprofen enantiomer separations. The EOF of the system using 100 mM sodium borate buffer (pH 9.2) was measured to be 3.0×10^{-4} cm^2/V s. The addition of 15% methanol to the run buffer reduced the EOF to 2.2×10^{-4} cm^2/V s in 100 mM borate buffer, while the EOF further decreased to 1.4×10^{-4} cm^2/V s with the addition of 4.0 mM of LY307599 to the borate buffer.

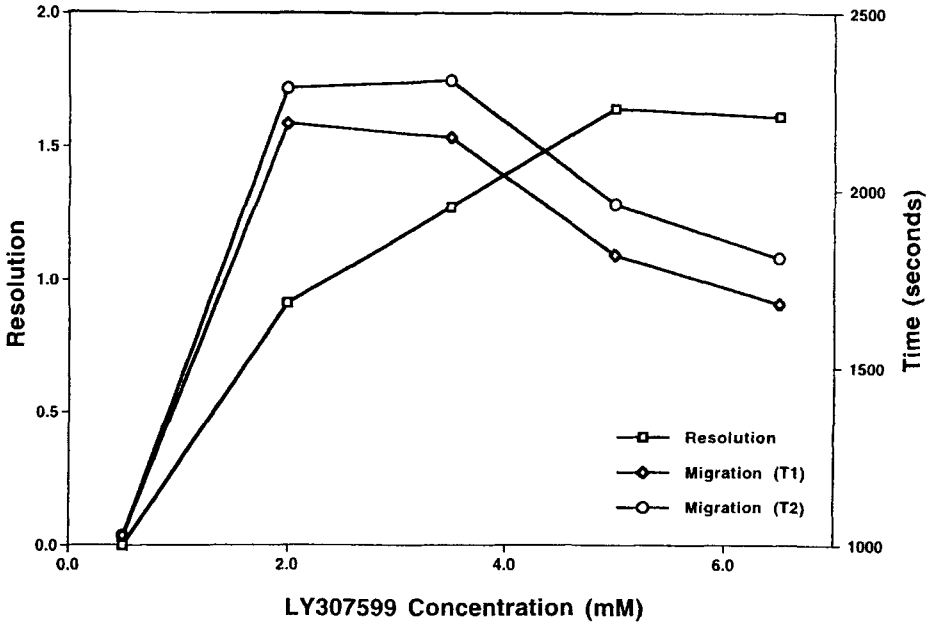

Figure 5. A plot of run buffer LY307599 concentration versus resolution and migration time. Run buffer is 15% methanol in 100mM borate at pH 9.2;. Voltage is 10 kv; UV absorbance is 254 nm.

Optimization

Using 10 mM borate buffer at pH 9.2 containing 3.0 mM LY307599, the effect of increasing methanol composition in the run buffer on flurbiprofen enantiomer separation was examined. Figure 4 shows the resulting effects on resolution and migration time. Resolution and migration both increased with increasing methanol content. Methanol content of 20% or greater in the run buffer resulted in lengthened migration times and degraded peak shapes. A run buffer containing 15% methanol was selected as optimum because of the satisfactory resolution of flurbiprofen enantiomers within a reasonable migration time.

Using 15% methanol, the concentration of LY307599 in the run buffer was increased from 0.5 mM to 6.5 mM. In general, resolution increased with increasing LY307599 concentration as shown in Figure 5. Migration time

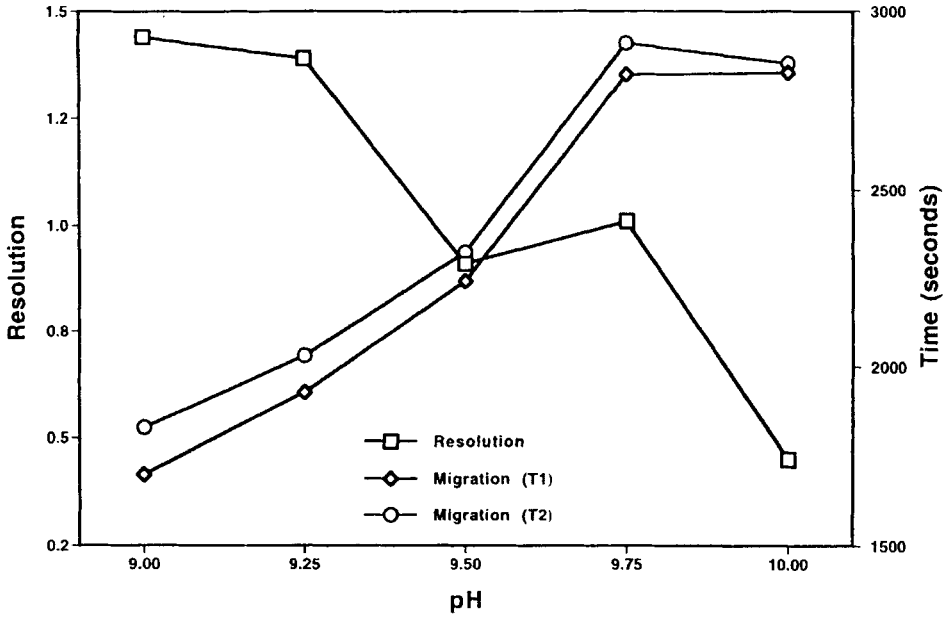

Figure 6. A plot of run buffer pH versus resolution and migration time. Run buffer is 15% methanol in 100 mM borate; Voltage is 10 kv; UV absorbance is 254 nm.

leveled off and began to decrease with increasing macrocyclic concentration. It was originally thought that this decrease in migration time might be due to methanol evaporation from sample vials during the lengthy analysis. The trial was repeated with each sample prepared immediately before analysis and the trend was reproduced. Further investigation is needed to study the effect of LY307599 interactions within this run buffer. Macrocyclic concentrations above 5.0 mM resulted in poor peak shapes and noisy baselines. An LY307599 concentration of 4.0 mM was chosen as optimum for resolution, peak shape and baseline noise.

Using 15% methanol and 4.0 mM LY307599, the pH of borate buffer was adjusted from 9.0 to 10.0 in increments of 0.25 pH units. Figure 6 shows the effect of increasing pH of the run buffer on the resolution and migration time. Resolution values decreased while migration times increased with increasing pH. It was originally thought that the higher pH run buffers would cause migration times to decrease as a result of a perceived higher EOF. However, a higher buffer ionic strengh at pH values greater than the pKa of borate may be

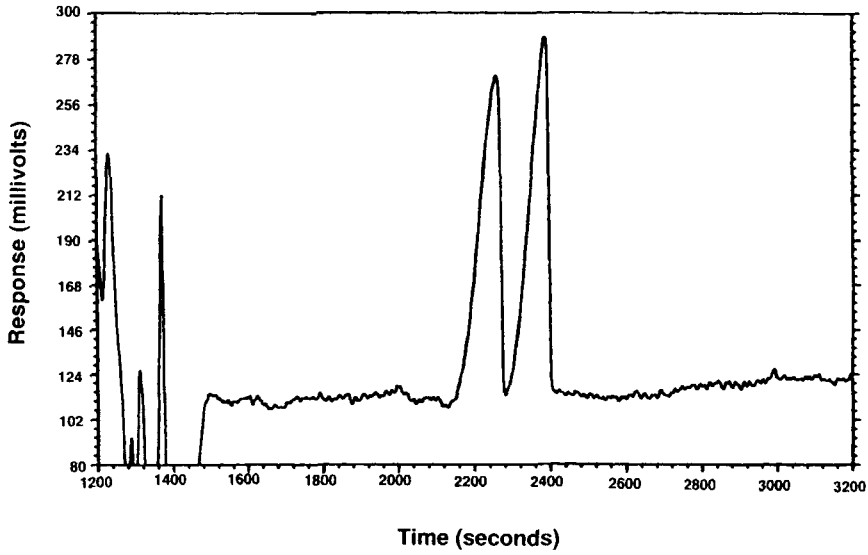

Figure 7. A sample electropherogram of the separation of flurbiprofen enantiomers Run buffer is 4.0 mM LY307599 in 15% methanol and 100 mM borate buffer at pH 9.2; Voltage is 10 kv; UV absorbance is 254 nm; Injection is 5 second low pressure interval (0.5 psi); Capillary temperature is 25°C; The sample is 1.0 mg/mL flurbiprofen.

the cause of the increased migration time. This effect would actually reduce EOF. Despite the increased time in the capillary at higher run buffer pH's, flurbiprofen is not as well resolved under these conditions. One plausible explanation for this is that at run buffer pH's approaching 10.0, the predominately negative character of both the analyte and LY307599 interfere with their stereospecific interaction.

An example electropherogram of the flurbiprofen enantiomer separation using the optimized run buffer conditions of 4.0 mM LY307599 dissolved in 15% methanol and 85% 100 mM borate is shown in Figure 7. A run buffer pH of 9.2 offered the best separation, without having to make a pH adjustment. It is worth noting that resolution values markedly increase with decreasing pH from 10.0 to 9.0. If it were not for the insolubility of LY307599 at and below pH 9.0, it is quite possible that this macrocyclic would serve as a much better chiral selector at a more neutral pH.

CONCLUSIONS

LY307599 has been shown to be another successful macrocyclic antibiotic chiral selector for the separation of enantiomers of flurbiprofen. The separation was shown to be affected by methanol content in the run buffer, run buffer pH and LY307599 concentration. The high EOF encountered at basic run buffer pH's was successfully countered with methanol. The parameters of run buffer pH, LY307599 concentration and methanol concentration were optimized to allow for adequate enantioseparation within a reasonable migration time.

ACKNOWLEDGMENTS

The authors would like to acknowledge Michael T. Quefeld for his help with the generation of the LY307599 UV absorbance spectrum.

REFERENCES

1. H. Nishi, T. Fukuyama, M. Matsuo, J. Microcolumn Sep., **1(5)**, 234 (1989).

2. S. Terabe, M. Shibata, Y. Miyashita, J. Chromatogr., **480**, 404 (1989).

3. R. O. Cole, M. J. Sepaniak, W. L. Hinze, J. High Res. Chromatogr., **13**, 570 (1990).

4. H. Nishi, T. Fukuyama, M. Matsuo, J. Chromatogr., **515**, 233 (1990).

5. H. Nishi, S. Terabe, T. Seiyaku, Electrophoresis, **11**, 691 (1990).

6. K. Otsuka, S. Terabe, J. Chromatogr., **515**, 221 (1990).

7. R. O. Cole, M. J. Sepaniak, LC-GC, **10**, 380 (1992).

8. A. Guttman, A. Paulus, A. S. Cohen, N. Grinberg, B. L. Karger, J. Chromatogr., **448**, 41 (1988).

9. S. Birnbaum, S. Nilsson, Anal. Chem., **64**, 2872 (1992).

10. I. Cruzado, G. Vigh, J. Chromatogr., **608**, 421 (1992).

11. Z. Aturki, S. Fanali, J. Chromatogr. A., **680**, 137 (1994).

12. Robert P. Oda, J. P. Landers, "Introduction to Capillary Electrophoresis," in **Handbook of Capillary Electrophoresis**, J. P. Landers, ed., CRC Press, Inc., Boca Raton, Florida, 1994, pp. 9-42.

13. S. Fanali, P. Bocek, Electrophoresis, **11**, 757 (1990).

14. M. Tanaka, S. Asano, M. Yoshinago, Y. Kawaguchi, T. Tetsumi, T. Shono, Fresnius J. Anal. Chem., **339**, 63 (1991).

15. T. Peterson, D. Trowbridge, J. Chromatogr., **603**, 298 (1992).

16. M. J. Sepaniak, R. O. Cole, B. K. Clark, J. Liq. Chromatogr., **15**, 1023 (1992).

17. C. Quang, M. G. Khaledi, J. High Res. Chromatogr., **17**, 99 (1994).

18. I. Bjornsdottir, S. H. Hansen, Chirality, **7**, 219 (1995).

19. B. Chankvetadze, G. Endresz, G. Blaschke, J. Cap. Elec., **2**, 235 (1995).

20. E. Hohne, G. J. Krauss, G. Gubitz, J. High Res. Chromatogr. Comm., **15**, 698 (1992).

21. R. Kuhn, F. Erni, T. Bereuter, J. Hausler, Anal. Chem., **64**, 2815 (1992).

22. R. Kuhn, F. Stoecklin, F. Erni, Chromatographia, **33**, 32 (1992).

23. Y. Walbroehl, J. Wagner, J. Chromatogr. A., **680**, 253 (1994).

24. A. D'Hulst, N. Verbeke, J. Chromatogr., **608**, 275 (1992).

25. G. E. Barker, P. Russo, R. A. Hartwick, Anal. Chem.. **64**, 3024 (1992).

26. S. Busch, J. C. Kraak, H. Poppe, J. Chromatogr., **635**, 119 (1993).

27. P. Sun, G. E. Barker, R. A. Hartwick, J. Chromatogr., **652**, 247 (1993).

28. P. Sun, N. Wu, G. Barker, R. A. Hartwick, J. Chromatogr., **648**, 475 (1993).

29. L. Valtcheva, J. Mohammad, G. Pettersson, S. Hjerten, J. Chromatogr., **638**, 263 (1993).

30. E. Gassmann, J. E. Kuo, R. N. Zare, Science, **230**, 813 (1985).

31. P. Gozel, E. Gassmann, H. Michelsen, R. N. Zare, Anal. Chem., **59**, 44 (1987).

32. D. W. Armstrong, K. L. Rundlett, J. R. Chen, Chirality, **6**, 496-509 (1994).

33. D. W. Armstrong, K. L. Rundlett, G. L. Reid, Anal. Chem., **66**, 1690-1695 (1994).

34. D. W. Armstrong, M. P. Gasper, K. L. Rundlett, J. Chromatogr. A, **689**, 285-304 (1995).

35. T. J. Ward, C. Dann III, A. Blaylock, J. Chromatogr. A., **715**, 337 (1995).

36. R. Vespalec, H. Corstjens, H. A. H. Billiet, J. Frank, K. Ch. A. M. Luyben, Anal. Chem., **67**, 3223 (1995).

37. T. J. Ward, C. Dann III, A. P. Brown, Chirality, **8**, 77 (1996).

38. K. L. Rundlett, M. P. Gasper, E. Y. Zhou, D. W. Armstrong, Chirality, **8**, 8 (1996).

39. A. Strege, B. E. Huff, D. S. Risley, LC•GC, **14**, 144-150 (1996).

40. K. Rundlett, D. Armstrong, Anal. Chem., **67**, 2088-2095 (1995).

Received August 18, 1996
Accepted September 30, 1996
Manuscript 4263