J IRAN CHEM SOC
DOI 10.1007/s13738-016-0951-6

ORIGINAL PAPER

Chiral separation of ketoprofen on an achiral NH_2 column by HPLC using vancomycin as chiral mobile phase additive

Dehbiya Gherdaoui[1,2] · Hafsa Bekdouche[1] · Said Zerkout[1] · Rachid Fegas[1,2] · Michel Righezza[2]

Received: 24 January 2016 / Accepted: 28 July 2016

Abstract A high-performance liquid chromatographic method for chiral separation of ketoprofen racemate was developed. (R)- and (S)-ketoprofen enantiomers were separated on a LiChrosorb NH_2 column (250 mm × 4.6 mm, i.d 5 µm) at 20 °C, using 2-propanol/potassium dihydrogen phosphate buffer (pH 6.0, 0.05 M) (50:50 v/v). Containing vancomycin as the mobile phase, at a flow rate of 0.8 ml min^{-1} and detection wavelength of UV, the detector was set at 310 nm. Under these conditions, ketoprofen enantiomers could be separated with a selectivity factor (α) of 2.172 and a resolution (Rs) of 4.78 using extremely low concentrations of the vancomycin chiral additive.

Keywords Ketoprofen · Chiral separation · Vancomycin · Chiral mobile phase additive

Introduction

Ketoprofen, (±)-(R, S)-2-(3-benzoyl phenyl)-propionic acid as shown in Fig. 1, is a nonsteroidal anti-inflammatory drug (NSAID) that has analgesic, anti-inflammatory and antipyretic properties. It is widely used clinically for treatment of arthritis and rheumatic diseases. However, (S)-ketoprofen and (R)-ketoprofen display significantly different pharmacological activities. The pharmacological activity has been contributed mainly to the S-form, while R-form is either inactive or reduced active form compared to S-form [1–4].

The literatures are rich with analytical methods for the determination of ketoprofen enantiomers. Some of the direct approaches for the analysis of ketoprofen enantiomers by HPLC and CE are either using chiral stationary phases (CSP) or chiral mobile phase additives (CMPA) [5–10].

Macrocyclic antibiotics among them glycol-peptides (namely, vancomycin, teicoplanin and ristocetin A) have been frequently employed in HPLC, capillary electrophoresis and capillary electrochromatography. These compounds have a great variety of functional groups, cavities and numerous stereogenic centers, which offer many interaction possibilities including, hydrogen bonding, electrostatic and π–π interactions, inclusion, steric interactions, dipole stacking or combinations thereof [11–23].

Vancomycin as shown in Fig. 2 has been the most commonly used glycopeptide in the enantioseparation. Vancomycin was introduced by Armstrong et al. to resolve over 100 racemates including nonsteroidal anti-inflammatory drugs, antineoplastic drugs, pesticides and numerous *N*-derivatized amino acids [19, 24–32].

The low concentration of antibiotics in the run buffer is one of the advantages of the glycopeptides as a chiral selector (CMPA). It is usually sufficient to use only 1–4 mM solution; when they are added to the buffer fortunately they do not absorb in UV region [9, 32–36].

The aim of this work is to present more extensive results of the enantiomers ketoprofen separation using vancomycin as CMPA, by studying the effect of its concentration on selectivity and resolution of ketoprofen.

We have also compared the results obtained with those obtained when ketoprofen was separated by C8 column,

✉ Dehbiya Gherdaoui
ghardaouidah@yahoo.fr

[1] Laboratory to Research of Bioactive Products and Enhancement of Biomass, Department of Chemistry, Ecole Normale Superieur Vieux Kouba, 16050 Algiers, Algeria

[2] CNRS, iSm2 UMR 7313, Aix Marseille Université, 13397 Marseille, France

Published online: 03 August 2016

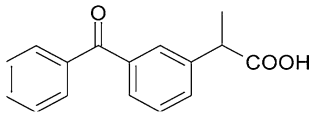

Fig. 1 Chemical structure of ketoprofen

using acetonitrile–triethylamine acetate (TEAA) buffer (pH 5.2, 20 mM) (35:65, v/v) containing vancomycin as the mobile phase [9] and column based on vancomycin immobilized by reductive amination to aldehyde functionalized silica, using mobile phase composed of a mixture of 40 % methanol in ammonium nitrate buffer (50 mM) at pH 5.0 [10].

Experimental

Materials and instruments

The analysis was performed by using a chromatographic system from Waters Alliance e2695 HPLC system with 2998 PDA detector. The HPLC system was equipped with Empower 3 software.

Sartorius semi-micro analytical balance bp121s, Digital pH meter mettler toledo and ultrasonic cleaner, NH_2 column (250 mm × 4.6 mm) LiChrosorb with different diameter particle manufacture by Merck KGaA.

Chemical compounds

Racemate ketoprofen and S-ketoprofen from Sigma Aldrich, all the reagents used in this work (methanol, 2-propanol, potassium hydroxide KOH, potassium dihydrogen phosphate KH_2PO_4), were of analytical-reagent grade purchased from Sigma Aldrich.

Procedure

0.05 M phosphate buffer was prepared by dissolving potassium dihydrogen phosphate and adjusted to the desired pH using 0.1 M potassium hydroxide. Buffer solutions were filtered with 0.22-μm membrane filter.

The vancomycin stock solutions were prepared by dissolving vancomycin in a buffer solution followed by ultrasonic degasification. The vancomycin solutions were stored at +4 °C when not in use.

The mobile phase was prepared by mixing the buffer solution pH 6.0 with 2-propanol (50/50).

These conditions were obtained in previous work, when we studied the effect of organic modifier, pH buffer and stationary phases on the retention time of vancomycin. The analyte ketoprofen was prepared by dissolving ketoprofen in methanol. The flow rate was 0.8 mL/min and the injection volume was 10 μL.

First, the ketoprofen racemate was injected by using the mobile phase without dissolving vancomycin in it to determine the retention time of ketoprofen.

Ketoprofen racemate was injected in the second step by using the same conditions with dissolving vancomycin in the buffer solution.

S-ketoprofen was injected in the same conditions to determine the retention time of the enantiomer S and to identify the two peaks.

Results and discussion

Effect of diameter particle of column

Two chiral chromatographic columns were used with the same adsorbent material NH_2 and the same dimensions (250 mm × 4.6 mm). These two columns have different

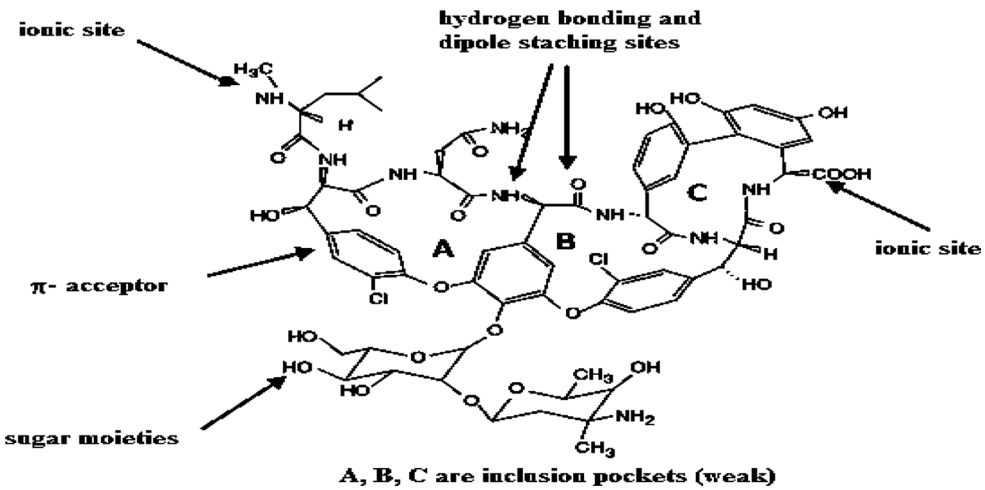

Fig. 2 Chemical structure of vancomycin

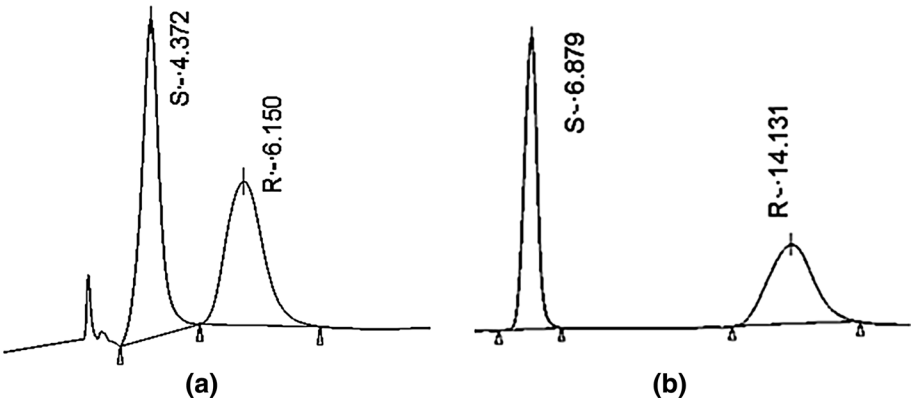

Fig. 3 Chromatograms of the ketoprofen in 2 mM of vancomycin, pH 6.0 phosphate buffer/2-propanol (50/50, v/v); diameter column 10 μm (**a**) and diameter column 5 μm (**b**)

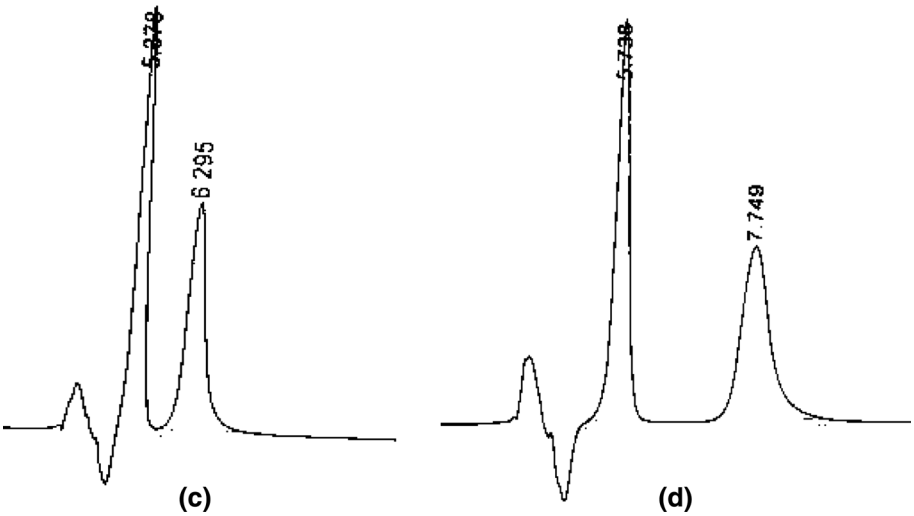

Fig. 4 Chromatograms of the ketoprofen with 5 μm diameter, pH 6.0 phosphate buffer/2-propanol (50/50, v/v); 1 mM of vancomycin (**c**) and 1.5 mM of vancomycin (**d**)

particle size: one column, with a particle size of 5 μm, was used for analytical purposes (measurement of additive concentrations) and the other, with a particle size of 10 μm, was used to study the effect of particle to the separation Fig. 3.

For the same mobile phase and vancomycin concentration, Fig. 3 shows a better separation when we use a column with particle size of 5 μm then using another with particle size of 10 μm (that is to say with much lower peak widths). Using columns with small particle size allows to achieve good separation with increased efficiency and thus increased resolution.

Effect of chiral selector concentration

The concentration of the chiral selector is an important variable controlling the chiral recognition.

The influence of vancomycin concentration content in the mobile phase on the chiral separation was investigated by varying the vancomycin concentration in the range of 0–2 mM Fig. 4. Figure 5 shows the effect of vancomycin concentration on factor retention, selectivity and resolution of ketoprofen enantiomers. The chiral resolution and selectivity increased with the increasing vancomycin concentration in the mobile phase, and ketoprofen was separated with a good selectivity of 2.17 at low concentration of 2 mM and short time retention on achiral NH_2 column and mobile phase consisting 2-propanol/buffer pH 6.0 (50/50).

Comparative study

Among the most mentioned studies in the chiral separation of ketoprofen with the present work the study of Guo [9], and Bouchair [10]. Table 1 shows a comparison of the enantioselectivity and resolution in this work with values reported in the literature. Good resolution was obtained in the use of column NH_2 with vancomycin as CMPA and the chiral Column vancomycin immobilized on diol with selectivity of 2.17 and 2.02, respectively. The use of the NH_2 column enables to obtain better resolution than the use of C8 column by low concentration of the chiral selector.

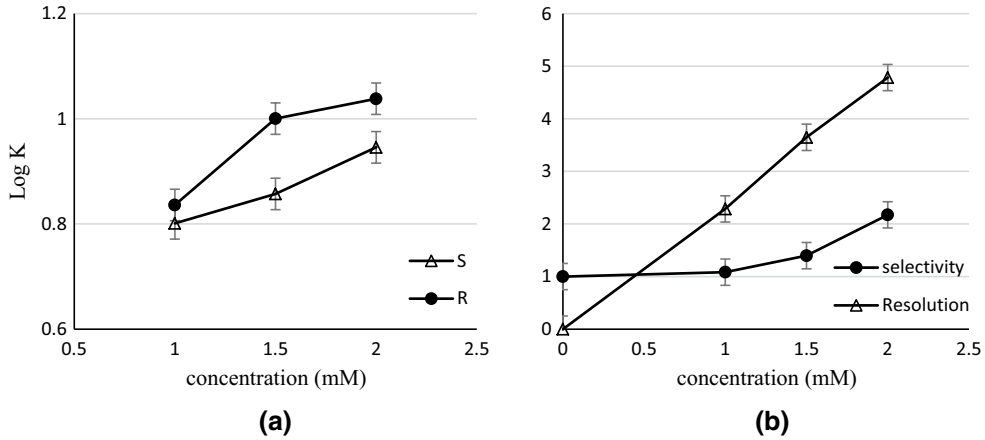

Fig. 5 Vancomycin concentration effect on retention factor (**a**), selectivity and resolution of the ketoprofen enantiomers (**b**)

Table 1 Comparison of the enantioselectivity with resolution of ketoprofen enantiomers

Chromatography condition	Selectivity	Resolution	References
Column, Hypersil BDS C8 column Mobile phase, acetonitrile–20 mM TEAA buffer (35:65, v/v, pH 5.2) containing 3.0 mM vancomycin	1.20	1.95	Guo [9]
Column vancomycin immobilized on diol Mobile phase: 50 mM ammonium nitrate buffer: Methanol (60:40, v/v), pH 5.0	2.02	3.62	Bouchair [10]
Column LiChrosorb NH_2 Mobile phase: 2-propanol/potassium dihydrogen phosphate buffer (pH 6.0, 0.05 M) (50:50 v/v) containing 2.0 mM vancomycin	2.17	4.78	

Conclusion

In this study, the macrocyclic antibiotic vancomycin was used as chiral selector (CMPA) to the enantioseparations of ketoprofen with NH_2 column. The chromatographic conditions that were determined could afford good resolution.

The effects of vancomycin additive concentration were also investigated. It has been found that a low concentration of vancomycin can give high enantioselectivity.

It was indicated that the vancomycin concentration in the mobile phase has a significant effect on the resolution of the enantiomers; column NH_2 with vancomycin as CMPA and column vancomycin immobilized on diol showed similar enantioseparation of ketoprofen. However, optimum enantioseparation was achieved at lower vancomycin concentration with NH_2 column than that of C8 column.

References

1. L. Igarza, A. Soraci, N. Auza, H. Zeballos, Vet. Res. Commun. **26**, 29 (2002)
2. Y. Liu, J. Xu, H. Wu, J. Biotechnol. **110**, 209 (2004)
3. F. Cabré, M.F. Fernàndez, L. Calvo, J. Clin. Pharmacol. **38**, 3 (1998)
4. A.L. Ong, A.H. Kamaruddin, S. Bhatia, Enzyme Microb. Technol. **39**(4), 924 (2006)
5. W.D. Armstrong et al., J. Chromatogr. A **689**, 285 (1995)
6. Q. Sun, S.V. Olesik, J. Chromatogr. B **745**, 159 (2000)
7. Z. Bosàkovà, J. Chromatogr. A **1088**, 94 (2005)
8. B. Kafková, Z. Bosáková, Chirality **18**, 531 (2006)
9. Z. Guo, H. Wang, Y. Zhang, J. Pharm. Biomed. Anal. **41**, 310 (2006)
10. N. Bouchair, M. Righezza, A. Hamdi, J. Iran. Chem. Soc. (2015). doi:10.1007/s13738-015-0635-7
11. T.J. Ward, D. Charles, Chirality **8**(1), 77 (1996)
12. D.W. Armstrong, U.B. Nair, Electrophoresis **18**, 2331 (1997)
13. H.Y. Aboul-Enein, I. Ali, Chromatographia (2000). doi:10.1007/BF02490991
14. A. Berthod, D.W. Armstrong, Anal. Chem. **72**, 1767 (2000)
15. T.J. Ward, J. Chromatogr. A **906**(1), 73 (2001)
16. I. D'Acquarica, F. Gasparrini, J. Adv. Chromatogr. **46**, 109 (2008)
17. T.L. Xiao, B. Zhang, F. Hui, J. Liq. Chromatogr. Relat. Technol **24**(17), 2673 (2001)
18. D.W. Armstrong, Y. Tang, S. Chen, C. Bagwill, Anal. Chem. **66**(9), 1473 (1994)
19. D.W. Armstong, J.-R. Chen, Chirality **6**, 496 (1994)
20. Y. Liu, A. Berthod, C.R. Mitchell, T.L. Xiao, B. Zhang, J. Chromatogr. A **978**(1), 185 (2002)
21. T.L. Xiao, D.W. Armstrong, Methods Mol. Biol. **243**, 113 (2004)

22. M.P. Gasper, A. Berthod, U.B. Nair, D.W. Armstrong, Anal. Chem. **68**, 2501 (1996)
23. I. Ilisz, A. Aranyi, J. Methods Mol. Biol. **970**, 137 (2013)
24. P.J. Patrick, H. Axelsen, Annu. Rev. Biophys. Biomol. Struct. **29**, 265 (2000)
25. H. Hashema, C. Tründelberg, J. Chromatogr. A **1218**, 6727 (2011)
26. C. Desiderio et al., J. Chromatogr. A **781**, 503 (1997)
27. H.Y. Aboul-Enein, Chirality **10**, 358 (1998)
28. E. Tesařová, K. Záruba, J. Chromatogr. A **844**, 137 (1999)
29. S. Fanali et al., J. Chromatogr. A **919**, 195 (2001)
30. I. Salma, E. Jourdan, F. Farhat, A. Villet, C. Grosset, A. Ravel, E. Peyrin, Anal. Chem. **74**, 5205 (2002)
31. W. Liu, F. Wang, J. Chromatogr. B **850**, 183 (2007)
32. A. Berthod et al., Talanta **43**, 1767 (1996)
33. J. Koidl, H. Hödl, J. Biochem. Biophys. Methods **70**, 1254 (2008)
34. M. Lämmerhofer, A. Gargano, J. Pharm. Biomed. Anal. **53**, 1091 (2010)
35. A.F. Prokhorovaa, E.N. Shapovalova, J. Chromatogr. A **1216**, 3674 (2009)
36. D.W. Armstrong, Y. Tang, S. Chen, Anal. Chem. **66**, 1473 (1994)