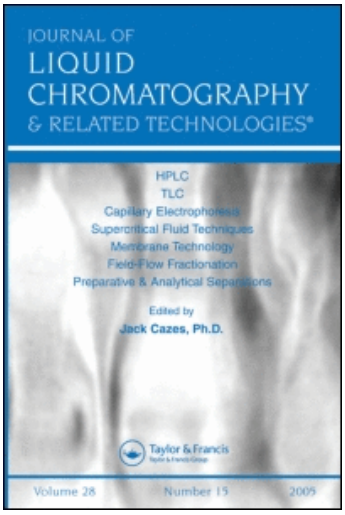

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COMPARATIVE STUDY OF THE CHIRAL RESOLUTION OF β-BLOCKERS ON CELLULOSE TRIS (3,5-DIMETHYL-PHENYLCARBAMATE) PHASES IN NORMAL AND REVERSED PHASE MODES

Martin G. Schmid[a]; Orsolya Gecse[b]; Zoltan Szabo[b]; Ferenc Kilár[c]; Gerald Gübitz[a]; Imran Ali[d]; Hassan Y. Aboul-Enein[d]
[a] Institute of Pharmaceutical Chemistry, Karl-Franzens University of Graz, Graz, Austria [b] Department of Analytical Chemistry, University of Pecs, Pecs, Hungary [c] Department of Analytical Chemistry, University of Pécs, Pécs, Hungary [d] Pharmaceutical Analysis Laboratory, Biological and Medical Research Department (MBC-03), King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia

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Martin G. Schmid,[1] Orsolya Gecse,[1,†] Zoltan Szabo,[1,†] Ferenc Kilár,[2] Gerald Gübitz,[1] Imran Ali,[3] and Hassan Y. Aboul-Enein[3,*]

[1] Institute of Pharmaceutical Chemistry, Karl-Franzens University of Graz, Universitätsplatz 1, A-8010 Graz, Austria
[2] Department of Analytical Chemistry, University of Pécs, Ifûsàg ùtca 6, H-7624 Pècs, Hungary
[3] Pharmaceutical Analysis Laboratory, Biological and Medical Research Department (MBC-03), King Faisal Specialist Hospital and Research Centre, P.O. Box 3354, Riyadh 11211, Saudi Arabia

ABSTRACT

The chiral separation of β-blockers on cellulose tris (3,5-dimethylphenylcarbamate) columns is described. The separation power of a Chiracel OD normal phase column, a Chiracel OD-RH

*Corresponding author. E-mail: enein@kfshrc.edu.sa
†Present address: Department of Analytical Chemistry, University of Pecs, Ifusag utica 6, H-7624 Pecs, Hungary.

reversed phase column, and a Chiracel ODRH reversed phase narrow-bore column were compared. Hexane–2-propanol–diethylamine mixtures were used as mobile phase in the normal phase mode and sodium perchlorate buffer containing different amounts of acetonitrile in the reversed phase mode. Sixteen μ-blockers were resolved under normal phase conditions and eleven under reversed phase conditions. The values of α and Rs on normal phase column ranged from 4.46 to 1.10 and 10.04 to 0.97, respectively. The values of α and Rs on reversed phase columns varied from 4.04 to 1.04 and 3.98 to 0.40 (on normal bore column) and 4.36 to 1.06 and 2.26 to 0.40 (on narrow bore column).

INTRODUCTION

Beta-adrenergic blockers (β-bockers) are used in the treatment of hypertension, angina pectoris, cardiac arrythmias, and glaucoma.(1) It is well established that the enantiomers of β-blockers show qualitatively and quantatively different pharmacological activities. The S (-) enantiomers exhibit an about 50–500 fold higher β-blocking activity compared to the R (+) enantiomers.(2-5) The pharmacokinetics and the metabolic pathway are also different for the enantiomers. (3,6,7) However, despite these facts, with a few exceptions most of the β-blockers are still administered as racemates (Fig. 1). Therefore, the development of methods for the separation of the enantiomers on analytical and preparative scale is of great interest. Numerous publications focus on the chiral separation of β-blockers by HPLC and, more recently, also by CE.

Polysaccharide based chiral HPLC phases have been found to be an excellent tool for the chiral separation of β-blockers.(8-26) To date, only a few papers deal with the use of cellulose based phases for the separation of β-blockers in the reversed phase mode.(8,11,12,14,15,17,26-31) This paper deals with comparative studies on the chiral separation of β-blockers on a Chiralcel OD normal phase column, a Chiralcel OD-RH reversed phase column, and a newly developed Chiralcel OD-RH narrow-bore reversed phase column.

EXPERIMENTAL

Chemicals and Materials

All reagents were of analytical grade. 2-Propanol, hexane, and acetonitrile (gradient grade) were obtained from Merck (Darmstadt, Germany). Sodium perchlorate, perchloric acid, and diethylamine were from Fluka (Buchs, Switzer-

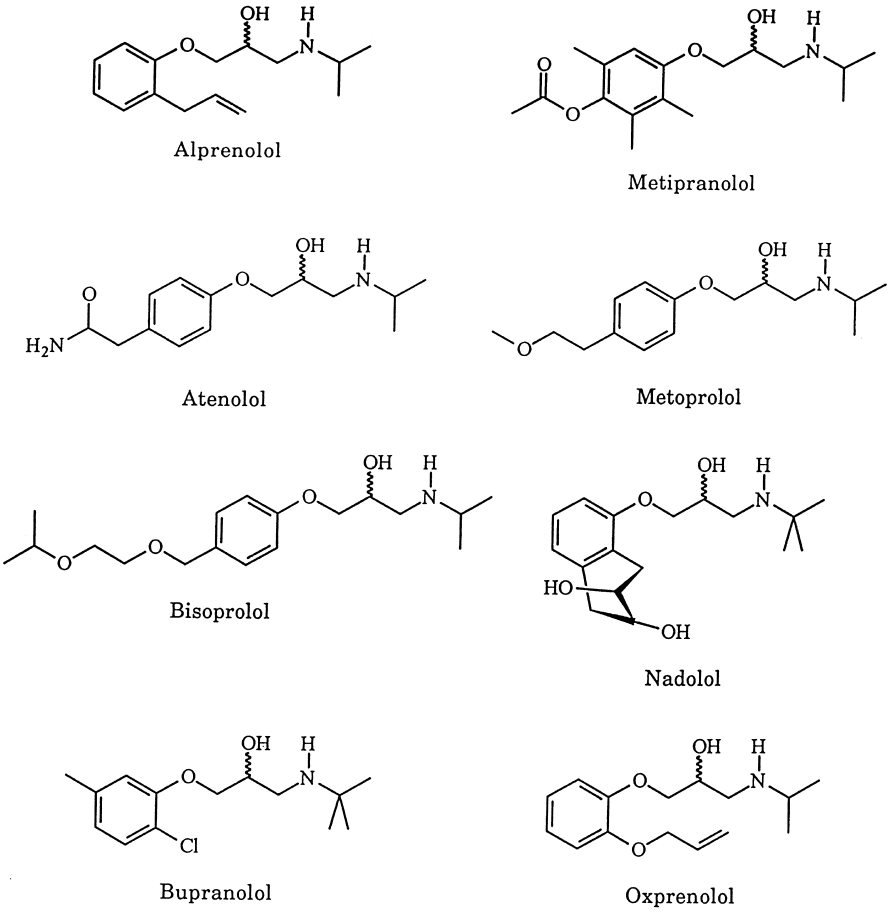


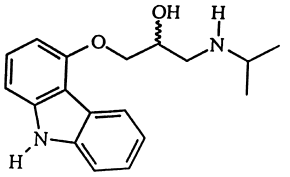
Figure 1. Chemical structures of the analytes.

(continued)

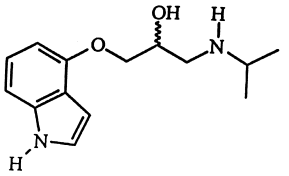
land). β-Blockers were purchased from Sigma (St. Louis, MO, USA) or were generous gifts from other departments.

HPLC-Conditions

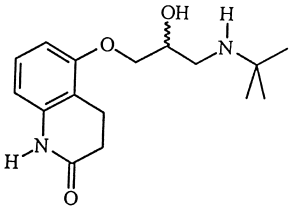
HPLC was performed using a HP 1090 system (Hewlett-Packard, Palo Alto, California, USA) equipped with a diode array UV-detector. Samples were injected by an autosampler, the amount was 8 μL. Detection was performed at 208 nm. A Chiracel OD (25 x 0.46 cm, particle size, 10 μm) column (flow rate



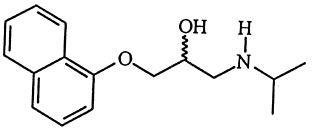
Carazolol



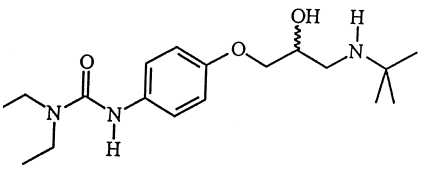
Pindolol



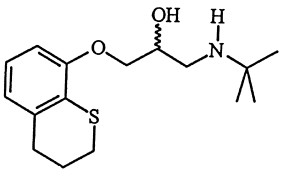
Carteolol



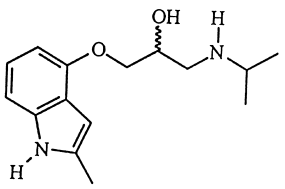
Propranolol



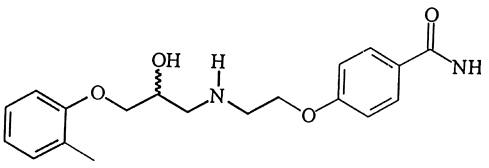
Celiprolol



Tertatolol



Mepindolol



Tolamolol

Figure 1. Continued.

0.5 mL/min.), a OD-RH (15 x 0.46 cm, particle size, 5 μm) column (flow rate 0.4-0.6 mL/min.), and an OD-RH (15 x 0.2 cm, particle size, 5 μm) narrow bore column (flow rate 0.2 mL/min.) were used (Daicel Chem. Ind., LTD, Tokyo, Japan). As mobile phase, mixtures of hexane–2-propanol–diethylamine (in various proportions) were used for the OD-column and a mobile phase of an aqueous sodium perchlorate solution adjusted with perchloric acid to pH 2 / acetonitrile (in various ratios) was used for the OD-RH columns. All separations were carried out at room temperature.

RESULTS AND DISCUSSION

The OD column was operated in the normal phase mode using 2-propanol–hexane–diethylamine in different compositions (Table 1). Diethylamine was found to improve the peak shape.(11) The separation data for this column are given in Table 1. Figure 2 shows the separation of mepindolol and bupranolol on the OD-column using 2-propanol–hexane–diethylamine as a mobile phase. Although the normal phase column was found to be slightly superior to the reversed phase columns regarding resolution power, the latter are

Table 1. Separation Data for ß-Blockers on a Chiralcel OD (25 × 0.46 cm) Column with 0.4 - 0.6 mL/min. Flow Rate (H: n-Hexane P: 2-Propanol D: Diethylamine)

		Mobile Phase						
	Flow	H	P	D	k'1	k'2	α	Rs
Alprenolol	0.40	15	85	0.40	0.25	0.48	1.92	2.62
Atenolol	0.40	20	80	0.40	0.30	0.52	1.73	1.76
Bisoprolol	0.40	50	50	0.40	0.17	0.38	2.23	2.76
Bupranolol	0.60	80	20	0.40	0.28	0.56	2.00	3.52
Carazolol	0.40	50	50	0.40	1.05	1.27	1.21	1.63
Carteolol	0.40	20	80	0.40	0.15	0.29	1.93	2.07
Celiprolol	0.60	80	20	0.40	2.27	3.05	1.34	2.14
Mepindolol	0.40	20	80	0.40	0.39	1.74	4.46	10.04
Metipranolol	0.60	90	10	0.40	1.04	1.27	1.22	1.54
Metoprolol	0.40	20	80	0.40	0.15	0.42	2.80	3.56
Nadolol	0.60	80	20	0.40	4.63	5.16	1.10	0.97
Oxprenolol	0.40	15	85	0.40	0.49	1.31	2.67	7.16
Pindolol	0.40	20	80	0.40	0.24	1.31	5.46	9.50
Propranolol	0.40	20	80	0.40	0.59	0.81	1.37	2.13
Tertatolol	0.40	15	85	0.40	0.48	1.46	3.04	7.89
Tolamolol	0.40	15	85	0.40	1.34	2.47	1.84	3.78

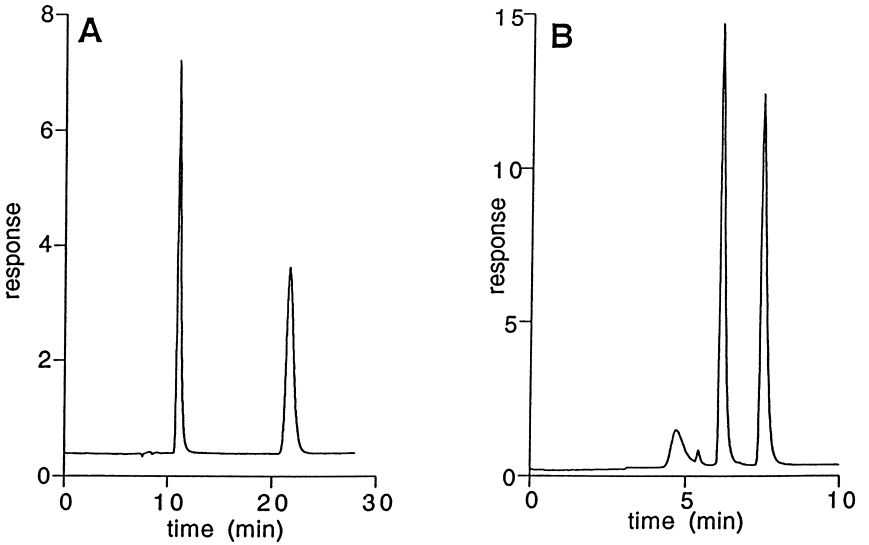


Figure 2. Chiral separation of mepindolol (A) and bupranolol (B) on the Chiralcel-OD column (25 x 0.46 cm). Mobile phase: A: n-hexane/2-propanol/diethylamine (20:80:0.4, v/v/v), flow rate: 0.4 mL/min. B: n-hexane/2-propanol/diethylamine (80:20:0.4, v/v/v), flow rate: 0.6 mL/min.

advantageous regarding handling, choice of mobile phases, solubility problems of samples, and compatibility with biological samples. However, as can be seen from Table 2, remarkable resolutions are obtained for a broad spectrum of compounds also in the reversed phase mode simply by using a 0.5 M sodium perchlorate solution adjusted to pH 2 with perchloric acid with different amounts of acetonitrile as mobile phases. Figure 3 shows the resolution of pindolol and oxprenolol on the OD-RH column. Figures 4 and 5 show chromatograms of the chiral separations of alprenolol and propranolol on the OD-RH (15 x 0.46 cm) and OD-RH (15 x 0.2 cm) columns, respectively. In Table 3 the separation data for the narrow-bore column are given.

With decreasing amounts of acetonitrile the resolutions generally improved, however, connected of course with an increase in retention times. Figure 6 shows the dependence of selectivity (resolution) on acetonitrile content using oxprenolol as a model compound. The elution order was checked for some β-blockers by injecting the authentic enantiomers in the cases when one of the enantiomers was available. The elution order was found to be R (+) before S (-) for metoprolol, propranolol, and atenolol both in normal phase and reversed phase mode.

Table 2. Separation Data for β-Blockers on a Chiralcel OD-RH (15 × 0.46 cm) Column with 0.5 mL/min. Flow Rate (PS: Perchlorate solution A: Acetonitrile)

	Mobile Phase					
	PS	A	k'1	k'2	α	Rs
Alprenolol	75	25	6.65	8.03	1.21	2.17
Bisoprolol	80	20	6.12	7.47	1.22	1.48
Carazolol	70	30	20.31	22.81	1.12	1.32
Mepindolol	50	50	0.48	1.94	4.04	3.98
Metipranolol	80	20	11.47	12.33	1.08	0.60
Metoprolol	80	20	2.7	3.29	1.22	1.91
Oxprenolol	70	30	1.94	2.47	1.27	2.34
Pindolol	50	50	0.38	0.82	2.36	3.05
Propranolol	60	40	1.87	2.92	1.56	2.48
Tertatolol	70	40	5.49	5.74	1.04	0.40
Tolamolol	80	20	11.28	13.85	1.23	1.69

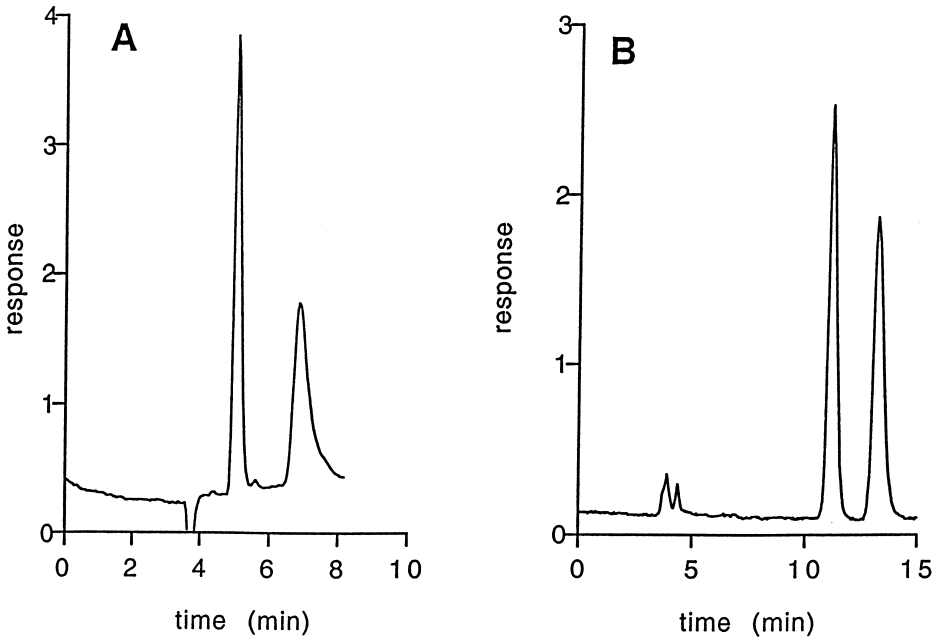


Figure 3. Chiral separation of pindolol (A) and oxprenolol (B) on the Chiralcel-OD-RH column (15 x 0.46 cm). Mobile phase: A: 0.5 M Perchlorate solution, pH 2/acetonitrile (50 : 50, v/v), flow rate: 0.5 mL/min. B: 0.5 M Perchlorate solution, pH 2/acetonitrile (70 : 30, v/v), flow rate: 0.5 mL/min.

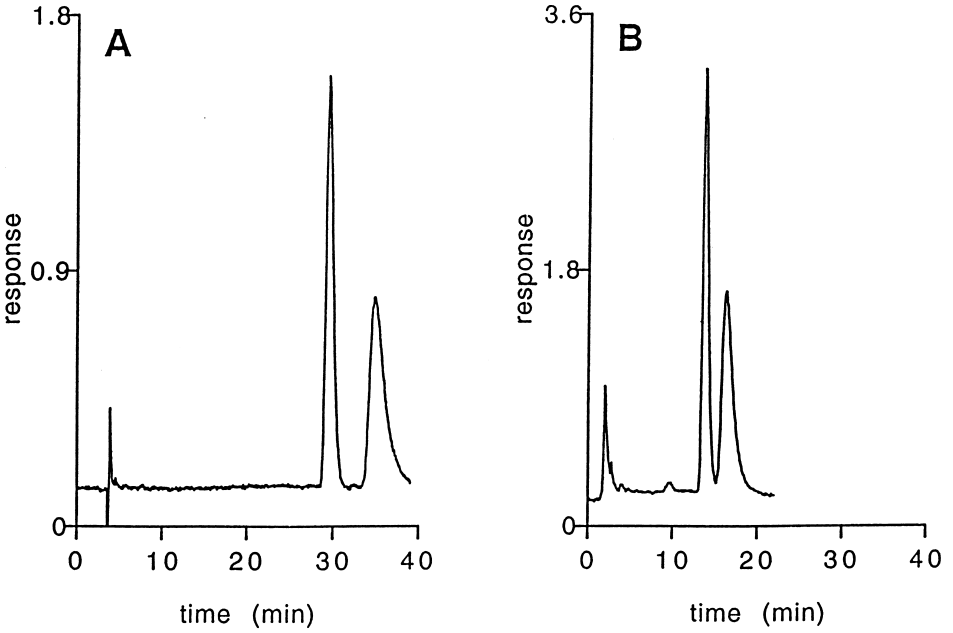


Figure 4. Separation of alprenolol on the [A] (15 x 0.46 cm) and [B] (15 x 0.2 cm) Chiracel-OD-RH column. Mobile phase: A: 0.5 M Perchlorate solution, pH 2/acetonitrile (70 : 30, v/v) flow rate: 0.5 mL/min. B: 0.5 M Perchlorate solution, pH 2/acetonitrile (70 : 30, v/v) flow rate: 0.5 mL/min.

OD and OD-RH phases are based on macroporous silica gel coated with semi-synthetic polymers consisting of D-glucose units in β-1,4-linkage derivatized with 3,5-dimethylphenylcarbamate groups. The chains are assumed to form a helical structure with cavities, which can include stereoselectively compounds containing aromatic moities.(32) The main interactions responsible for chiral recognition are supposed to be hydrogen bondings between the carbonyl group of the phenylcarbamate group of the selector, which is located outside of the cavity and the hydroxy group of the β-blocker.(8,11) The chiral recognition mechanism between β-blocker and the cellulose based chiral stationary phases had been discussed in details by Aboul-Enein.(11) Dipole-dipole interactions may also contribute to chiral recognition.(33-35) The π-π interactions between the dimethylphenylcarbamate of the chiral selectors and the aromatic ring system of the β-blocker are assumed to be further supporting forces in resolution.

The strength of these interactions depending on the individual structure of the β-blockers plays an important role in chiral recognition resulting in different degrees of resolution. The higher retention of nadolol may be explained on the

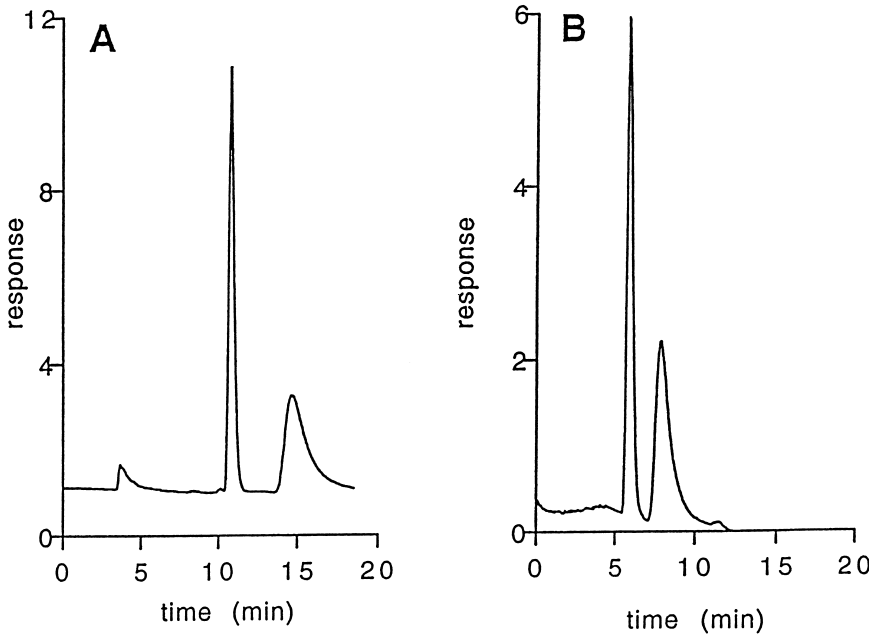


Figure 5. Separation of propranolol on the [A] (15 x 0.46 cm) and [B] (15 x 0.2 cm) Chiracel-OD-RH column. Mobile phase: A: 0.5 M Perchlorate solution, pH 2/acetonitrile (70 : 30, v/v) flow rate: 0.5 mL/min. B: 0.5 M Perchlorate solution, pH 2/acetonitrile (70 : 30, v/v) flow rate: 0.5 mL/min.

Table 3. Separation Data for β-Blockers on a Chiralcel OD-RH (15 × 0.2 cm) Narrow Bore Column with 0.2 mL/min. Flow Rate (PS: Perchlorate solution A: Acetonitrile)

	Mobile Phase					
	PS	A	k'1	k'2	α	Rs
Alprenolol	75	25	5.18	6.31	1.22	1.50
Bisoprolol	80	20	4.84	5.91	1.22	1.15
Carazolol	75	25	39.59	45.05	1.14	1.00
Mepindolol	55	45	0.56	2.44	4.36	2.26
Metipranolol	85	15	26.63	28.32	1.06	0.50
Metoprolol	80	20	2.58	3.47	1.34	1.30
Oxprenolol	75	25	2.98	3.94	1.32	1.96
Pindolol	65	35	0.86	2.62	3.04	3.00
Propranolol	60	40	1.72	2.65	1.54	2.00
Tertatolol	70	40	5.75	6.06	1.05	0.40
Tolamolol	80	20	9.15	11.35	1.24	1.32

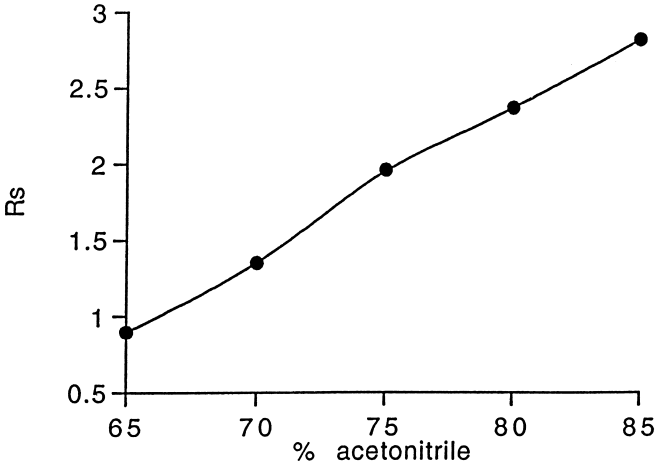


Figure 6. Influence of acetonitrile content on selectivity. Column: Chiracel OD-RH (15 x 0.2 cm) (flow rate.2 mL/min.); mobile phase: 0.5 M Perchlorate solution, pH 2 with different amounts of acetonitrile.

basis of the greater hydrogen bonding, as it contains four functional groups (3 –OH and one –NH-) which can take part in the hydrogen bondings with the chiral selectors, while the other β-blockers have 2-3 such types of functional groups which are capable to form hydrogen bondings. The higher retention time of carazolol observed in the reversed phase mode in comparison to other β–blockers may be due to stronger π-π interactions which is due to the presence of carbazole group, which possesses high π electron density compared to others which have only only aromatic groups with lower π electron densities e.g., phenyl, naphthalene, and indole groups. Besides, the well known hydrogen, π-π and dipole-dipole induced interactions, steric effect also plays an important role in the chiral resolution of various racemic analytes.(35) Therefore, hydrogen, π-π, dipole-dipole induced bondings and steric effect are the main forces responsible for the chiral resolution of studied β–blockers on cellulose based chiral stationary phaes.

CONCLUSION

Out of sixteen β-blockers studied, only 15 could be resolved on normal phase mode, while nine and seven have been resolved on reversed phase normal bore and reversed phase narrow bore columns, respectively. However, the partial resolution of nadolol occurred in normal phase mode. The partial resolution of metipranolol and tertatolol has been observed on reversed phase narrow bore col-

umn. Therefore, the normal phase mode is better than the reveresed phase for the enantiomeric resolution of the studied β-blockers under the reported chromatographic conditions.

REFERENCES

1. Meyers, F.H.; Jawetz, E.; Goldheim, A. A. *Review of Medical Pharmacology*, 7th Ed.; Lange Medical Publications: Los Altos, 1980; 95.
2. Lee, E.J.D.; William, W.H.; Klin. Pharmcokin. **1990**, *18*, 339.
3. Nelson, W.; Burke, F.J. Org. Chem. **1978**, *43*, 3641.
4. Barret, A.M.; Cullun, V.A. J. Pharmacol. **1968**, *34*, 43.
5. Danilewicz, J.C.; Kamp, J.K. J. Med. Chem. **1973**, *16*, 168.
6. Smits, J.F.M.; Zoegers, H.H.M.; Struyker, B. Arch. Int. Pharmacodyn. **1989**, *298***,** 164.
7. Boyd, R.A.; Chin, S.K.; Don, P.O.; Williams, R.L.; Giacomini, K.M. Klin. Pharm. Ther. **1989**, *45*, 403.
8. Okamoto, Y.; Kawashima, M.; Aburatani, R.; Hatada, K.; Nishigama, T.; Masuda, M. Chem. Lett. **1986**, 1237.
9. *Application Guide*, 2nd Ed.; Daicel Chem. Ind., LTD: Tokyo, Japan.
10. Aboul-Enein, H.Y. Anal. Lett. **1993**, *26*, 271.
11. Aboul-Enein, H.Y.; Serignese, V. J. Liq. Chromatogr. & Rel. Technol. **1993**, *116*, 197.
12. Aboul-Enein, H.Y.; Islam, M.R. Anal. Lett. **1990**, *23*, 83.
13. Aboul-Enein, H.Y.; Islam, M.R. J. Chromatogr. **1990**, *511*, 109.
14. Aboul-Enein, H.Y.; Islam, M.R. Anal. Lett. **1990**, *23*, 973.
15. Aboul-Enein, H.Y.; Islam, M.R. Chirality **1989**, *1*, 301.
16. Aboul-Enein, H.Y.; Bakr, S.A. J. Liq. Chromatogr. & Rel. Technol. **1998**, *21*, 1137.
17. Aboul-Enein, H.Y.; Serignese, V. Biomed. Chromatogr. **1994**, *8*, 22.
18. Hartmann, C.; Krauss, D.; Spahn, H.; Mutschler, E. J. Chromatogr. B **1989**, *496*, 387.
19. Ching, M.S.; Lennard, M.S.; Gregory, A.; Tucker, G.T. J. Chromatogr. B **1998**, *497*, 313.
20. Lee, C.R.; Porziemsky, J.P.; Aubert, M.C.; Krstulovic, M.A. J. Chromatogr. **1991**, *539*, 55.
21. Okamoto, Y.; Aburatani, R.; Hatano, K.; Hatada, K. J. Liq. Chromatogr. & Rel. Technol. **1988**, *11*, 2147.
22. Krstulovic, A.M.; Fouchet, M.H.; Burke, J.T.; Gillet, G.; Durand, A. J. Chromatogr. **1988**, *452*, 477.
23. Lanchote, V.L.; Bonato, P.S.; Cerquiera, P.M.; Pereira, V.A.; Cesarino, E.J. J. Chromatogr. B **2000**, *738*, 27.

24. Szymura-Oleksiak, J.; Walczak, M.; Bojarski, J.; Aboul-Enein, H.Y. Chirality B **1999**, *11*, 267.
25. Ader, U.; Muschalek, V.; Schneider, M.P. Chirality **1993**, *5*, 554.
26. Vandenbosch, C.; Massart, D.L.; Lindner, W. J. Pharm. Biomed. Anal. **10**, *10*, 895.
27. Aboul-Enein, H.Y.; Abou-Basha, L.I.; Bakr, S.A. Chirality **1996**, *8*, 153.
28. Ishikawa, A.; Shibata, T. J. Liq. Chromatogr. & Rel. Technol. **1993**, *16*, 859.
29. Facklam, C.; Modler, A. J. Chromatogr. A **1994**, *664*, 203.
30. Erlandsson, P.; Marle, I.; Hansson, L.; Isaksson, R.; Pettersson, C.; Pettersson, G.J. J. Am. Chem. Soc. **1990**, *112*, 4573.
31. Aboul-Enein, H.Y.; Sirineges, V. Cellulose **1995**, *2,* 215.
32. Francotte, E.; Romain, M.; Lohmann, L.; Mueller, R. J. Chromatogr. **1985**, *347*, 25.
33. Wainer, I.W.; Alembik, M.C. J. Chromatogr. **1986**, *385*, 358.
34. Wainer, I.W.; Stiffin, R.M. Shibata, T. J. Chromatogr. **1987**, *411*, 139.
35. Okamoto, Y.; Yashima, E. Angew. Chem. Int. Ed **1998**, *37*, 1020.

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