ELSEVIER

Journal of Chromatography A, 732 (1996) 245–253

JOURNAL OF CHROMATOGRAPHY A

Reversed-phase chiral ion-pair chromatography at a column temperature below 0°C using three generations of Hypercarb as solid-phase

Anders Karlsson*, Christine Charron

Analytical Chemistry, Astra Hässle AB, S-431 83 Mölndal, Sweden

Received 14 November 1995; accepted 24 November 1995

Abstract

Enantiomeric separations using chiral ion-pair chromatography have been investigated with porous graphitic carbon, Hypercarb®, as the solid-phase. The enantiomers of several aminoalcohols were successfully separated as diastereomeric ion pairs with N-benzyloxycarbonyl-glycyl-L-proline (L-ZGP) or N-benzyloxycarbonyl-glycylglycyl-L-proline (L-ZGGP) dissolved in polar solvents e.g., methanol as the mobile phase. The influence of the solute structure as well as the counter ion structure on the chiral recognition were examined. The position of substituents in the aromatic ring, type of alkyl group attached to the nitrogen and the number of methylene groups between the asymmetrical carbon atom and the nitrogen atom were studied. The present study shows that a column temperature below 0°C improved the enantioselective resolution. A stable and robust chromatographic system with a short equilibration time is presented. The chiral ion-pair chromatographic system could be used for the determination of enantiomeric impurities of less than 0.1% and is possible even if the enantiomer in low concentration is eluted after the main enantiomer.

Keywords: Enantiomer separation; Column temperature; Temperature effects; Amino alcohols

1. Introduction

Chromatographic techniques are the most common way to simultaneously separate enantiomers and determine enantiomeric purity in pharmaceutical formulations, as well as assay enantiomers in biological samples. The enantiomers can be derivatized with an optical pure reagent to form diastereoisomers that can be separated using achiral gas or liquid chromatographic systems [1,2]. Optical isomers can also be separated in the direct mode by using a chiral selector immobilized on the stationary phase [3,4] or dissolved in the mobile phase [5]. In the latter case enantiomeric separation is obtained due to differences in formation-constant of the diastereomeric complexes in the mobile phase and/or in the adsorption to the stationary phase [6]. Macromolecules [7], counter ions [8] and esters [9] are some of the chiral selectors used as mobile phase additives.

Chiral counter ions have previously been used for the enantiomeric separation of acids and amines using silica-based materials in the straight phase mode [8,10]. N-Derivatized peptides, e.g., N-benzyloxycarbonylglycyl-L-proline (L-ZGP) was suc-

*Corresponding author.

0021-9673/96/$15.00
SSDI 0021-9673(95)01291-5

cessfully used for the enantiomeric separation of amines using LiChrosorb DIOL as the solid-phase [11].

Hypercarb is a new liquid chromatographic support with unique adsorption properties. This support, that consists of porous graphitic carbon allowing $\pi-\pi$ electron interaction with the solutes, has previously been used for the separation of position isomers [12] as well as for the separation of diastereoisomers [13]. The unique adsorption properties of porous graphitic carbon were also used for separation of racemic amines using chiral counter ions dissolved in non-polar or polar mobile phase solvents [14,15]. The enantiomers of several racemic amines and acids were baseline separated using N-derivatized peptides or quinine as the chiral counter ion [16].

The influence of the three generations of Hypercarb columns on enantioselective retention of aminoalcohols, using N-derivatized peptides as the chiral selector dissolved in polar mobile phase solvents, was examined in this study. The effect of solute structure, i.e., position of substituents in the aromatic ring, different alkyl groups at the nitrogen essential for the electrostatic attraction and the distance between the asymmetrical carbon atom and the nitrogen atom on enantioselective recognition were evaluated together with the influence of the counter-ion structure. A stable chromatographic system was obtained after 15 column volumes of mobile phase had passed the column.

The effect of the column temperature on enantioselective resolution was studied. Several baseline separations will be shown using chiral ion-pair chromatography at a column temperature below 0°C.

2. Experimental

2.1. Chemicals

Acetonitrile (p.a.), 2-propanol (p.a.) and NaOH were obtained from Merck (Darmstadt, Germany). N-Benzyloxycarbonyl-glycyl-L-proline (L-ZGP) was from Nova Biochem (Läufelfingen, Switzerland) and N-benzyloxycarbonyl-glycylglycyl-L-proline (L-ZGGP) was supplied by Sigma (St. Louis, MO, USA). Methanol (p.a.) was purchased from Fisons (Loughborough, UK). The Hypercarb columns (I, II and III) were supplied by Shandon (Astmoor, UK). (*R,S*)-H 170/40, (*R,S*)-H 170/31, (*R,S*)-H 177/19, (*R,S*)-H 106/59, (*R,S*)-H 9/64, (*R,S*)-H 138/03, (*R,S*)-H 105/29, (*R,S*)-H 117/78, (*R,S*)-H 128/80, (*R,S*)-H 56/56, (*R,S*)-H 84/79, (*R,S*)-H 48/47, (*R,S*)-H 52/13, (*R*)-, (*R,S*)-metoprolol, (*S*)-alprenolol, (*R,S*)-alprenolol and (*R,S*)-atenolol (for structures see Fig. 1) were all from Astra Hässle (Mölndal, Sweden).

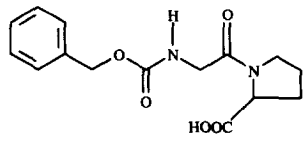
2.2. Apparatus

The chromatographic system consisted of a Model 250 binary LC pump- (Perkin Elmer, Norwalk, CT, USA), an AS 3000 injector and a Linear 200/UV-Vis detector (Spectra-Physics, San Jose, CA, USA). The experiments were carried out using different Hypercarb columns made of polished stainless steel and packed with porous graphitic carbon [100×4.6 mm I.D., 7 (I and II) or 5 μm (III)]. The solute solutions, injected twice, and the mobile phases were all freshly prepared. All the solutes were detected at 272 nm and the mobile phase flow-rate was 1 ml min^{-1}, unless otherwise stated. The temperature of the column and solvent reservoir was controlled by means of a water–ethanol bath, GRANT type LTD6 (Cambridge, UK).

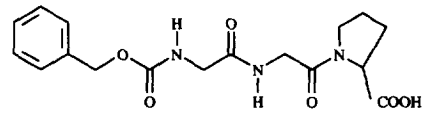
3. Results and discussion

3.1. Enantioselective retention on the three Hypercarb columns

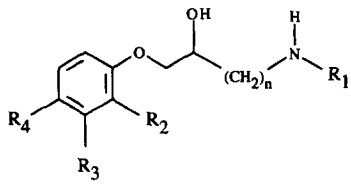
The three generations of Hypercarb columns were tested with respect to enantiomeric separation of some aminoalcohols using chiral ion-pair chromatography, structures in Fig. 1. L-ZGP or L-ZGGP (see Fig. 1) were the two chiral counter ions used. The Hypercarb columns differ in particle size, Hypercarb I and II (7 μm) and Hypercarb III (5 μm). The difference between Hypercarb I and II is that the distribution in particle size is smaller for Hypercarb II (information obtained from Shandon). Previous works showed that the Hypercarb I column was superior to Hypercarb II regarding enantioseparation of alprenolol using mobile phases with the counter ion, i.e., L-ZGP dissolved in non-polar solvents, e.g., dichloromethane [17].

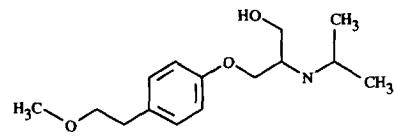


L-ZGP



L-ZGGP





H 84/79 (16)

Number	Name	n	R_1	R_2	R_3	R_4
1	H 9/64	1	$CH(CH_3)_2$	H	H	H
2	H 106/59	1	$CH(CH_3)_2$	$CH_2CH_2OCH_3$	H	H
3	H 177/19	1	$CH(CH_3)_2$	H	$CH_2CH_2OCH_3$	H
4	metoprolol	1	$CH(CH_3)_2$	H	H	$CH_2CH_2OCH_3$
5	H 170/31	2	$CH(CH_3)_2$	H	H	$CH_2CH_2OCH_3$
6	H 170/40	3	$CH(CH_3)_2$	H	H	$CH_2CH_2OCH_3$
7	H 128/80	1	$CH(CH_3)_2$	H	H	C(O)H
8	H 48/47	1	$CH(CH_3)_2$	H	H	NO_2
9	H 52/13	1	$CH(CH_3)_2$	H	H	OCH_3
10	H 138/03	1	$CH(CH_3)_2$	H	H	$CH_2CH_2NHC(O)NHC(CH_3)_3$
11	atenolol	1	$CH(CH_3)_2$	H	H	$CH_2C(O)NH_2$
12	H 117/78	1	$CH_2CH_2CH_3$	H	H	$CH_2CH_2OCH_3$
13	H 105/29	1	$C(CH_3)_3$	H	H	$CH_2CH_2OCH_3$
14	alprenolol	1	$CH(CH_3)_2$	H	CH_2CHCH_2	H
15	H 56/56	1	$CH(CH_3)_2$	H	$CH_2CH_2CH_3$	H

Fig. 1. Solutes and counter-ion structures.

The purpose of the presented study was to evaluate the usefulness of the three Hypercarb columns when the chiral counter ion was dissolved in polar mobile phase solvents.

At room temperature only minor differences in chromatographic performance were obtained using the three different Hypercarb columns. At this column temperature the obtained separation factors were low, e.g., less than 1.10 for the enantiomers of metoprolol. However, at column temperatures below 0°C all the three Hypercarb columns gave base-line separation for the two enantiomers of metoprolol (Fig. 2). Throughout the rest of this study Hypercarb II or III are the chromatographic columns used as these two columns gave the highest column efficiencies (Fig. 2).

3.2. Influence of column temperature and mobile phase on enantioresolution

In most chiral chromatographic systems the selectivity factors and capacity factors increased, while the column efficiency decreased with decreasing column temperature. A change in column temperature could, therefore, improve or impair the enantioselective resolution due to the relative change in the chromatographic parameters given above. Several authors have described interesting results regarding enantioseparation and column temperature [18–22]. Increased separation factors were obtained at increased column temperature when using an immobilized protein, CBH, as the chiral selector [18]. In this study [18] the capacity factor of the first

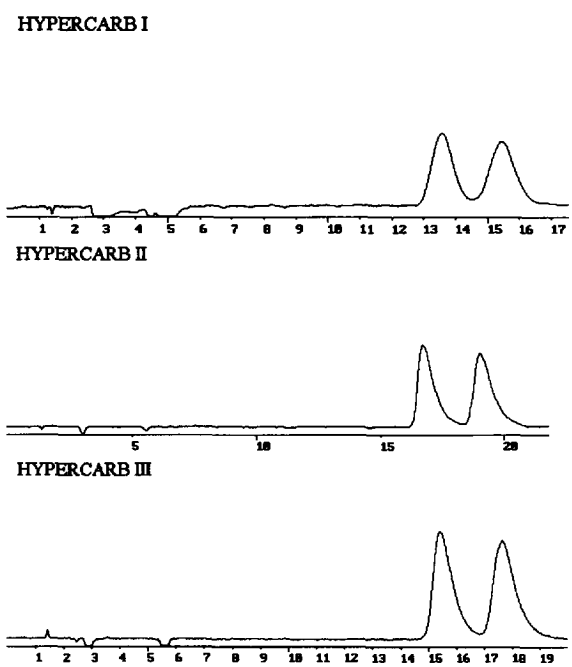


Fig. 2. Influence of the solid phase on enantioselectivity. Solid phase, Hypercarb I, II and III; mobile phase, 5 m*M* L-ZGP and 3 m*M* NaOH in methanol; column temperature: −5°C; solute: (*R,S*)-metoprolol; first eluted enantiomer, (*R*)-metoprolol.

eluted enantiomer of propranolol decreased whereas the capacity factor of the other enantiomer increased with increasing column temperature. Another study showed that the separation factor increased with increasing column temperature using chemically bonded β-cyclodextrin as the stationary phase [19], but in this case the capacity factors decreased for both the enantiomers. Another interesting study was performed using supercritical fluid chromatography [20]. In this study two structurally related racemates behaved differently. The enantioselectivity for one of the compound increased while the enantioselectivity for the other compound decreased with an increase in column temperature [20]. Further, examples of reversed retention order of the enantiomers in normal-phase [21], as well as in reversed-phase chromatography [22] when changing the column temperature have been observed.

In the present study, the influence of column temperature on enantioselective retention for the enantiomers of metoprolol and alprenolol is given as van 't Hoff plots (Fig. 3). By using the van 't Hoff plots the isoenantioselective temperatures, where the selectivity factor of the respective racemate is 1.0, of metoprolol and alprenolol were calculated. The isoenantioselective temperature (T_{iso}) was found to be 47°C for both metoprolol and alprenolol. The plots could also be used to estimate differences in $\Delta\Delta G^\circ$ for the respective racemate at different temperatures. The calculated data at −5°C were $\Delta\Delta G^\circ_{-5}$ (metoprolol) = 297 J mol^{-1} and $\Delta\Delta G^\circ_{-5}$ (alprenolol) = 543 J mol^{-1}. From the value of the partial molar excess thermodynamic parameters $\Delta\Delta H^\circ$ and $\Delta\Delta S^\circ$ it was found that the enantioselectivity arises mainly from enthalpic considerations.

A considerable increase of the selectivity factors,

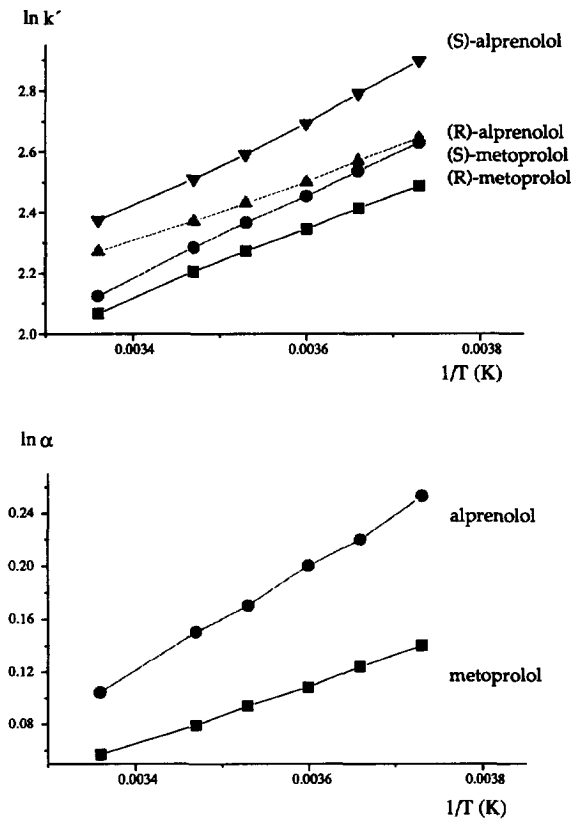


Fig. 3. Effects of column temperature on the enantioselective retention. Solid phase, Hypercarb II; mobile phase, 5 m*M* L-ZGP and 3 m*M* NaOH in methanol; flow-rate, 1 ml min^{-1}.

1.06 to 1.16 for metoprolol and 1.1 to 1.3 for alprenolol, was obtained by decreasing the column temperature from 25 to −5°C. However, the capacity factors increased only moderate, approximately by 50%, by the same change in column temperature. Since only a moderate decrease of the column efficiencies was obtained in the studied temperature range (Fig. 4) the decrease in the column temperature increased the enantioselective resolutions. Although a rather low column temperature (−5°C) was used, the performance of the chromatographic peaks was good (Fig. 2). In fact, complete enantiomeric resolution of several of the aminoalcohols was obtained at sub-zero column temperature within acceptable retention times when using L-ZGGP as the chiral counter ion (Fig. 5).

3.3. Influence of counter ion and solute structure on enantioselectivity

Enantioselective retention was obtained for several aminoalcohols when using L-ZGP or L-ZGGP as the chiral counter ion dissolved in methanol Tables 1–4. The influence of position in the aromatic ring, i.e. no substituent or substituent in *ortho-*, *meta-* or *para-*position, is given in Table 1. The enantioselectivity decreased in order *ortho* > *meta* > *para* > no substituent, as does the retention. The influence of different substituents in *para* position is also given in

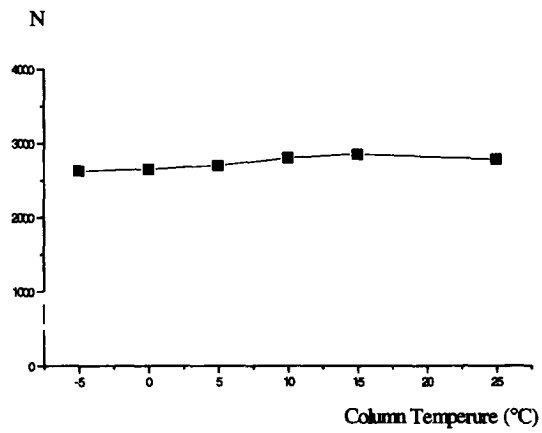


Fig. 4. Effect of column temperature on the column efficiency. Solid phase, Hypercarb II; mobile phase, 5 m*M* L-ZGP and 3 m*M* NaOH in methanol; solute, (*R*)-metoprolol; flow-rate, 1 ml min^{-1}.

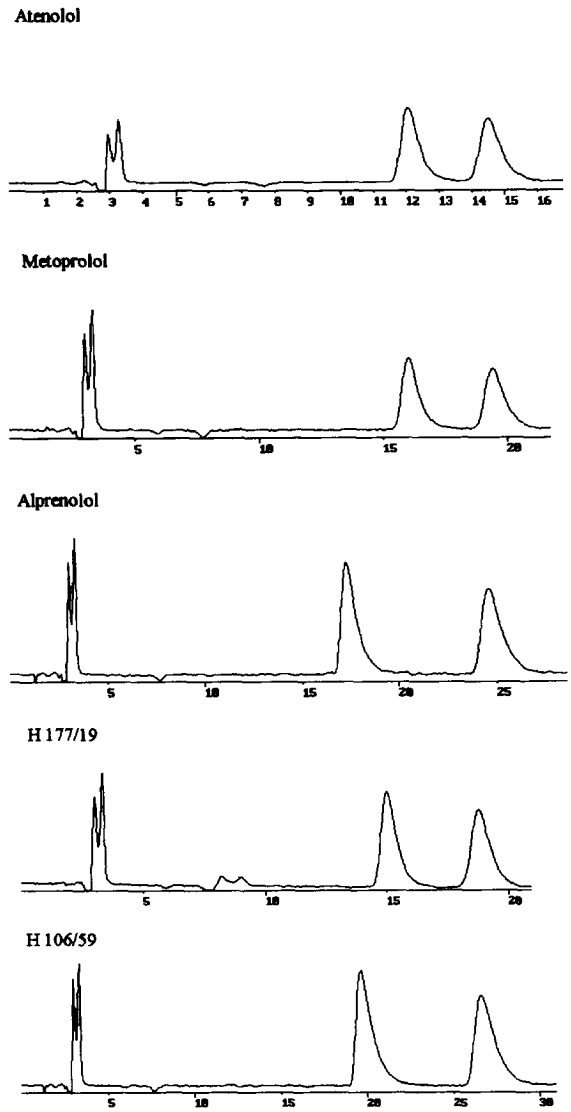
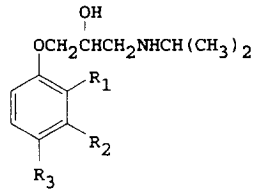


Fig. 5. Enantioselective separation. Solid phase, Hypercarb II; mobile phase, 5 m*M* L-ZGGP and 3 m*M* NaOH in methanol; column temperature, −5°C.

Table 1. The enantioselectivity was only slightly affected by the type of substituent. However, the retention increased for the two enantiomers which have a strong electron-withdrawing group attached to the aromatic ring, see solute 7 and 8. The change in enantioselective retention was probably caused by differences in the conformation of the diastereomeric ion pairs and also by differences in electron density of the aromatic ring in the solutes. The simultaneous

Table 1
Solute structure and enantioselective retention using L-ZGP or L-ZGGP as chiral counter ion



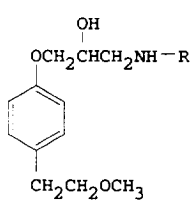
Number	R_1	R_2	R_3	L-ZGP		L-ZGGP	
				k'_1	α	k'_1	α
1	H	H	H	6.58	1.19	5.00	1.21
2	$CH_2CH_2OCH_3$	H	H	20.7	1.37	15.4	1.38
3	H	$CH_2CH_2OCH_3$	H	15.1	1.25	11.5	1.27
4	H	H	$CH_2CH_2OCH_3$	16.4	1.20	12.3	1.23
9	H	H	OCH_3	21.9	1.19	–	–
10	H	H	$(CH_3)_3CNHC(O)NHCH_2CH_2$	22.1	1.22	16.3	1.26
7	H	H	C(O)-H	37.6	1.21	–	–
8	H	H	NO_2	71.7	1.19	–	–
11	H	H	$CH_2C(O)NH_2$	11.0	1.20	9.0	1.23

Solid phase, Hypercarb; mobile phase, 5 m*M* L-ZGP or L-ZGGP and 3 m*M* NaOH in methanol–2-propanol (9:1); column temperature, – 5°C.

enantioselective separation of five of the solutes is given in Fig. 6.

The influence of the type of alkyl group attached to the nitrogen atom was also studied. A bulky alkyl group, *tert.*-butyl, decreased the enantioselective retention, probably due to steric hindrance. Surprisingly, a linear alkyl group at the nitrogen gave a lower enantioselectivity than the isopropyl, indicating differences in the conformation of the formed

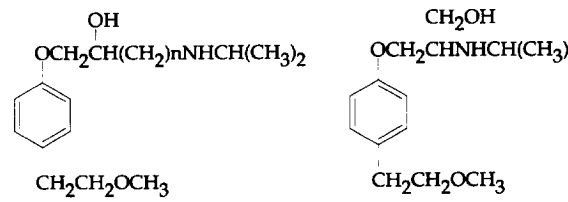
Table 2
Solute structure and enantioselective retention using L-ZGP or L-ZGGP as chiral counter ion



Number	R	L-ZGP		L-ZGGP	
		k'_1	α	k'_1	α
12	$CH_2CH_2CH_3$	24.5	1.08	18.4	1.15
4	$CH(CH_3)_2$	16.4	1.20	12.3	1.23
13	$C(CH_3)_3$	11.8	1.10	8.59	1.17

Solid phase, Hypercarb; mobile phase, 5 m*M* L-ZGP or L-ZGGP and 3 m*M* NaOH in methanol–2-propanol (9:1); column temperature, – 5°C.

Table 3
Solute structure and enantioselective retention using L-ZGP or L-ZGGP as chiral counter ion

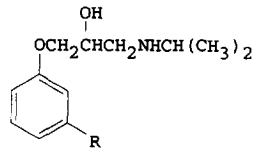


H 84/79

Number	n	L-ZGP		L-ZGGP	
		k'_1	α	k'_1	α
4	1	16.4	1.20	12.3	1.23
5	2	20.3	1.02	15.9	1.0
6	3	33.1	1.0	27.9	1.0
16	H 84/79	20.2	1.0	17.9	1.10

Solid phase, Hypercarb; mobile phase, 5 m*M* L-ZGP or L-ZGGP and 3 m*M* NaOH in methanol–2-propanol (9:1); column temperature, – 5°C.

Table 4
Solute structure and enantioselective retention using L-ZGP or L-ZGGP as chiral counter ion



Number	R	L-ZGP		L-ZGGP	
		k_1'	α	k_1'	α
14	CH_2CHCH_2	18.1	1.38	13.4	1.45
15	$CH_2CH_2CH_3$	22.3	1.43	–	–

Solid phase, Hypercarb; mobile phase, 5 m*M* L-ZGP or L-ZGGP and 3 m*M* NaOH in methanol–2-propanol (9:1); column temperature, −5°C.

diastereomeric ion pairs. The number of methylene groups between the asymmetrical carbon and the nitrogen also affected the chiral recognition (Table 3). The importance of the methylene groups was marked as a high separation factor was obtained only when the distance was one methylene group, i.e., $\alpha = 1.2$ for metoprolol.

The enantioselectivity of alprenolol and an analogue showed that the enantioselectivity was only slightly affected by a saturated or unsaturated sustituent in the *meta* position (Table 4).

The influence on enantioselective retention of two different chiral counter ions is shown in Tables 1–4. For all the solutes shorter retention times were found using L-ZGGP instead of L-ZGP. Furthermore, the separation factors were only slightly effected by using L-ZGP or L-ZGGP. However, for three solutes, 12, 13 and 16, increased separation factors were observed (Table 2 and Table 3). Most interesting, for H 84/79 with no methylene group between the asymmetrical carbon and the nitrogen, baseline sepa-

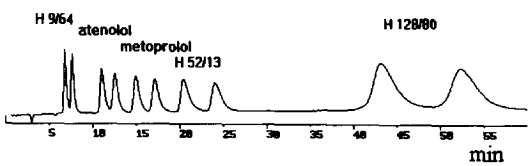


Fig. 6. Simultaneous enatioselective resolution of five amino-alcohols. Solid phase, Hypercarb III; mobile phase, 5 m*M* L-ZGP and 3 m*M* NaOH in methanol; column temperature, −5°C.

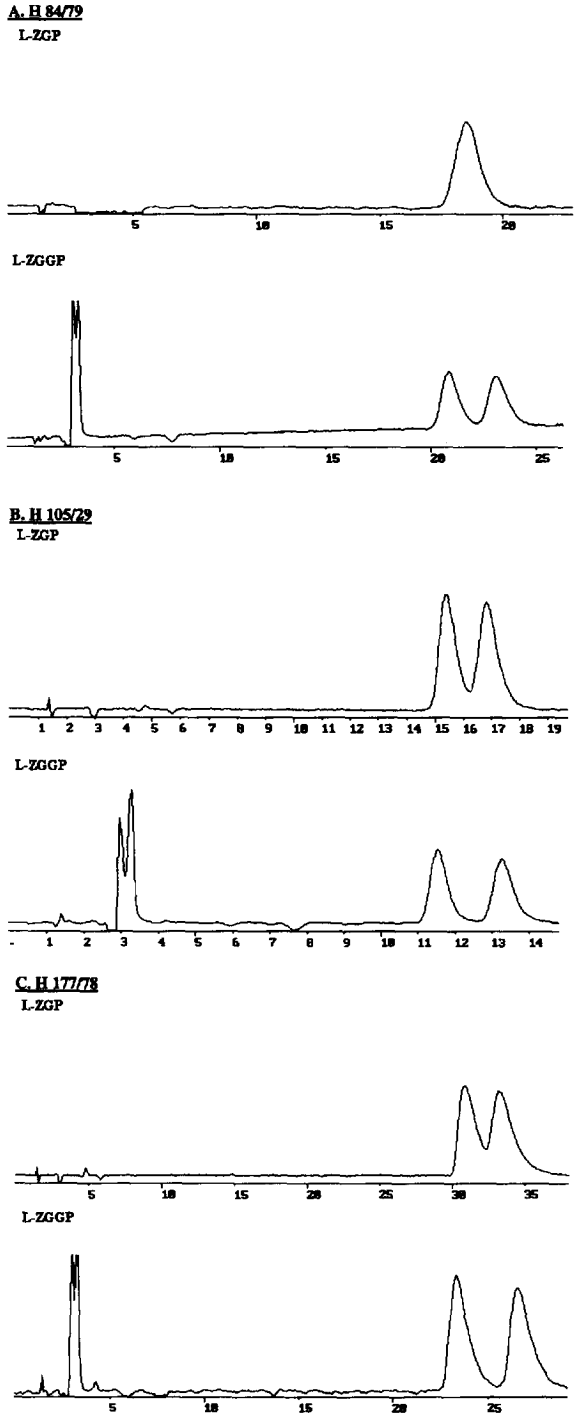


Fig. 7. Influence of counter-ion structure on the enantioselective retention. Solid phase, Hypercarb II; mobile phase, 5 m*M* L-ZGP or L-ZGGP and 3 m*M* NaOH in methanol–2-propanol (9:1); column temperature, −10°C.

ration was obtained using L-ZGGP but no enantioselectivity was observed using L-ZGP. Thus, the addition of an achiral group in the counter ion can highly affect the enantioselective separation (Fig. 7).

3.4. Enantioselectivity and combination of mobile phase solvents

Previous studies have shown that addition of other organic solvents than methanol to the mobile phase could affect the enantioselective retention [15]. The effect of addition of 2-propanol to the mobile phase was examined in this study (Table 5). An addition of 10 or 25% (v/v) of 2-propanol increased the chiral recognition for all solutes except for H 84/79. An explanation for this effect is probably that 2-propanol increased the ion-pair formation in the mobile phase due to higher ion-pair formation constants. Another positive effect of the addition of 2-propanol to the mobile phase is that the retention times for the solutes decreased by 25–45%.

3.5. Applications

The chiral chromatographic system was equilibrated within 15 min. The first injection was made directly after the break-through of the counter ion.

Fig. 8. Determination of enantiomeric purity of (*R*)-metoprolol. Solid phase, Hypercarb II; mobile phase, 5 m*M* L-ZGP and 3 m*M* NaOH in methanol; column temperature, 0°C; solute, 1% (*S*)-metoprolol in (*R*)-metoprolol.

From the break-through the amount of the counter ion adsorbed to the porous graphitic carbon surface could be calculated, less than 2 μmol of L-ZGP corresponding to 5 mg was adsorbed to approximately 1 g solid-phase material. Furthermore, the chromatographic system was extremely stable; no significant change in enantioselective retention or column efficiency was observed after 2500 column volumes of mobile phase passing the column.

The chiral ion-pair chromatographic system could be used for the determination of enantiomeric impurities even if the chiral impurity eluted after the main enantiomer exemplified by the injection of (*R*)-metoprolol (Fig. 8). The chromatographic method enabled the determination of less than 0.2% (*R*)-alprenolol in (*S*)-alprenolol (Fig. 9).

Table 5
Influence of mobile phase solvent on enantioseparation

Solute		Mobile phase solvent					
		Methanol (A)		Methanol–2-propanol (9:1, B)		Methanol–2-propanol (75:25, C)	
		k'	α	k'	α	k'	α
H 9/64	1	7.8		6.6		5.9	
	2	9.1	1.15	7.8	1.19	7.1	1.21
Atenolol	1	14.2		11.0		9.4	
	2	16.6	1.17	13.3	1.20	11.6	1.23
H 177/19	1	19.4		15.1		13.0	
	2	23.5	1.21	18.9	1.25	16.6	1.28
Metoprolol	1	21.0		16.4		14.0	
	2	24.4	1.16	19.6	1.19	17.1	1.22
H 84/79	–	32.6	1.00	25.4	1.00	20.4	1.00
H 106/59	1	26.5		20.7		17.9	
	2	34.6	1.31	28.3	1.37	25.0	1.40
Alprenolol	1	22.4		18.1		15.7	
	2	29.6	1.32	25.0	1.38	22.1	1.41

Solid phase, Hypercarb II; mobile phase, 5 m*M* L-ZGP and 3 m*M* NaOH in A, B, or C; flow-rate, 1 ml min^{-1}; column temperature, − 10°C.

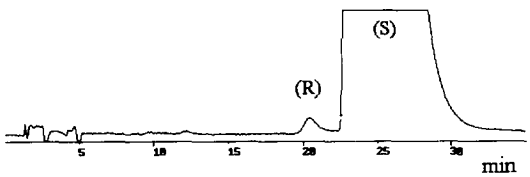


Fig. 9. Determination of the enantiomeric purity of (*S*)-alprenolol. Solid phase, Hypercarb II; mobile phase, 5 m*M* L-ZGP and 3 m*M* NaOH in methanol; column temperature, −10°C; solute, 0.18% (*R*)-alprenolol in (*S*)-alprenolol.

4. Conclusions

The enantiomers of some racemic aminoalcohols have been separated on porous graphitic carbon, using derivatized peptides, L-ZGP or L-ZGGP, as the chiral counter-ion. The study showed no significant differences between the three generations of Hypercarb columns when using polar solvents in the mobile phase at column temperatures below 0°C. The influence of substitution in the aromatic ring, groups attached to the nitrogen and distance between the asymmetrical carbon atom and the nitrogen of the samples on enantioselectivity was elucidated. Improved enantioselectivity by using L-ZGGP instead of L-ZGP as the chiral counter ion was observed for some of the racemates. The chromatographic system was equilibrated fast, within 15 min, and was stable for more than 2500 column volumes of mobile phase passing the column. The system could be used for determination of the enantiomeric purity even when the chiral impurity eluted after the main enantiomer. The enantiomers of several of the racemic aminoalcohols were baseline separated within 20 min using a column temperature below 0°C.

References

[1] R.W. Souter, Chromatographic Separations of Stereoisomers, CRC Press, Boca Raton, FL, 1985.
[2] S. Görög and M. Gazdag, J. Chromatogr. A, 659 (1994) 51.
[3] K. Balmér, P.-O. Lagerström, B.-A. Persson and G. Schill, J. Chromatogr., 592 (1992) 331.
[4] S.G. Allenmark, Chromatographic Enantioseparation: Methods and Application, Ellis Horwood, Chichester, 1989.
[5] C. Pettersson, in A.M. Krstulovic (Editor), Chiral Separations by HPLC: Application to Pharmaceutical Compounds, Ellis Horwood, Chichester, 1989, pp. 124.
[6] C. Pettersson, A. Karlsson and C. Gioeli, J. Chromatogr., 407 (1987) 217.
[7] C. Pettersson, T. Arvidsson, A.-L. Karlsson and I. Marle, J. Pharm. Biomed. Anal., 4 (1986) 221.
[8] C. Pettersson and G. Schill, J. Chromatogr., 204 (1981) 179.
[9] C. Pettersson, E. Heldin and H.W. Stuurman, J. Chromatogr., 528 (1989) 413.
[10] C. Pettersson, J. Chromatogr., 316 (1984) 553.
[11] C. Pettersson and M. Josefsson, Chromatographia, 12 (1986) 321.
[12] Q.H. Wan, P.N. Shaw, M.C. Davies and D.A. Barret, J. Chromatogr. A, 697 (1995) 219.
[13] W.C. Chan, R. Micklewright and D.A. Barrett, J. Chromatogr. A, 697 (1995) 213.
[14] A. Karlsson and C. Pettersson, J. Chromatogr., 543 (1991) 287.
[15] N.-H. Huynh, A. Karlsson and C. Pettersson., J. Chromatogr. A, 705 (1995) 275.
[16] A. Karlsson and C. Pettersson, Chirality, 4 (1992) 323.
[17] A. Karlsson, L. Svensson and C. Pettersson, in preparation.
[18] S. Jönsson, A. Schön, R. Isaksson, C. Pettersson and G. Pettersson, Chirality, 4 (1992) 505.
[19] K. Cabrera and D. Lubda, J. Chromatogr. B, 666 (1994) 433.
[20] R.J. Smith, D.R. Taylor and S.M. Wilkins, J. Chromatogr. A, 697 (1995) 591.
[21] W. Pirkle and P.G. Murray, J. High. Resolut. Chromatogr., 16 (1993) 285.
[22] A. Karlsson and A. Aspegren, in preparation.