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Direct liquid chromatographic enantioseparation of sotalol and other β-blockers using an α_1-acid glycoprotein-based chiral stationary phase

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Abstract

The behaviour of an α_1-acid glycoprotein-based chiral stationary phase (Chiral AGP) towards changes in pH and organic modifier in the mobile phase was investigated in order to deduce suitable conditions for the liquid chromatographic enantioseparation of a series of β-adrenoreceptor blocking drugs. The effects of the pH of the mobile phase on retention, selectivity and resolution were studied. Methanol was the only non-ionic modifier tested in the mobile phase while different aliphatic carboxylic acids (C_4 to C_8) and alkanesulfonic acids (C_6 to C_8) were used as ionic modifiers. The influence of the nature and concentration of these modifiers on retention and enantioselectivity was investigated. Under these conditions, enantiomeric separations could be obtained for more than 70% of the β-blocking agents examined. The use of heptanoic acid as an ionic additive in the mobile phase has permitted the resolution of sotalol enantiomers. An enantioselective assay for sotalol was then developed and validated.

Keywords: Enantiomer separation; Chiral stationary phases, LC; Mobile phase composition; Sotalol; Beta-blockers; Carboxylic acids

1. Introduction

It is well known that the enantiomers of chiral drugs can present differences in pharmacological activity or efficacy. Enantioselective analytical techniques have therefore become more and more important in the field of drug analysis. Enantiomeric separations are often performed nowadays by liquid chromatography (LC). The use of chiral stationary phases (CSPs) is by far the most popular way to perform enantioselective LC assays.

Among the numerous CSPs currently available, those based on the utilisation of a protein as the chiral selector have become increasingly popular due to their wide range of applicability [1]. The Chiral-AGP stationary phase is obtained by immobilizing α_1-acid glycoprotein on silica [2]. This CSP was used for the enantioselective determination of a series of drugs in bulk form or in biological fluids [3–16].

β-Adrenoreceptor blocking agents are well known to be strongly affected by chirality. The chiral separation of β-blocking drugs can be performed by means of different chromatographic techniques, with gas chromatography (GC), thin-layer chromatography (TLC) or LC being the most frequently used

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techniques [17]. In LC, numerous CSPs were used for the separation of the enantiomers of β-blocking drugs. Some CSPs consisted of immobilized proteins such as bovine serum albumin (BSA) [18], α_1-acid glycoprotein [2,6,8,10–16], cellulobiohydrolase [13,19,20] and ovomucoid [13,21,22]. Other ones were based on the use of cyclodextrins [13,15,23,24] or cellulose derivatives, especially cellulose Tris(3,5-dimethylphenylcarbamate) [13,15,25–27], as chiral selectors. Some Pirkle-type columns were also reported to be suitable for achieving chiral separation of β-blockers [28,29]. More recently, the enantioseparation of β-blocking drugs was also achieved in capillary electrophoresis (CE) by adding derivatized cyclodextrins as chiral selectors to the running buffer [30].

In the case of sotalol, enantiomeric separations have been achieved in LC using different CSPs [10,31–33] or after derivatization with a chiral reagent [34,35]. The separation of sotalol enantiomers could be achieved on another chiral column based on the immobilization of α_1-acid glycoprotein (Enantiopac) by the addition of hexanoic acid to a phosphate buffer [10,32].

This paper deals with the enantioseparation of eleven β-blockers by LC using a Chiral-AGP column. The influence of different parameters on retention and enantioselectivity has been investigated. In accordance with earlier observations [12,14,16,36–41], the effects of the mobile phase pH, the concentration of methanol and the addition of some ionic organic modifiers, aliphatic carboxylic acids or alkane sulfonic acids, have been studied in order to deduce suitable conditions for the resolution of the racemic β-blocking drugs examined.

The enantioselective method developed for sotalol has been validated: linearity, limits of detection and of quantitation as well as intra-day and inter-day reproducibilities have been determined.

2. Experimental

2.1. Apparatus

The LC equipment consisted of a Model L-6200 A pump, a Model AS-2000 A autosampler equipped with a 100-μl loop, a L-5025 programmable column oven and a L-4250 UV–Vis detector, all from Merck-Hitachi (Merck, Darmstadt, Germany).

The data were collected on an IBM-compatible computer (PC-AT; CPU type 80386) and the results were printed on a HP deskjet 500 (Hewlett-Packard, USA) and on a Model BD 9 recorder (Kipp & Zonen, Delft, Netherlands). The whole chromatographic system was controlled by the computer using Merck-Hitachi D-6000 HPLC Manager software.

The pH of the mobile phase buffers was adjusted using a Model 632 pH meter from Metrohm (Herisau, Switzerland). The chiral LC column was a Chiral-AGP column (100×4 mm I.D.) from Chrom-Tech (Norsborg, Sweden) and the guard column was a LiChroCart (4×4 mm I.D.) from Merck.

2.2. Chemicals and reagents

Acebutolol hydrochloride, atenolol, metoprolol tartrate, oxprenolol hydrochloride and pindolol were purchased from Sigma (St. Louis, MO, USA). Bevantolol hydrochloride was obtained from Parke-Davis (Bornem, Belgium). Celiprolol hydrochloride was provided by Rorer (Brussels, Belgium), betaxolol hydrochloride was provided by Synthelabo (Brussels, Belgium) and metipranolol by Dr. Mann Pharma (Berlin, Germany). Propranolol and sotalol hydrochlorides were kindly provided by the SMB Department of Research and Development (Brussels, Belgium). All β-blocking drugs were in the racemic form and used without further purification. Sodium monohydrogenphosphate, sodium dihydrogenphosphate, sodium hydroxide and phosphoric acid were of analytical-reagent quality from Merck. Butyric, pentanoic, hexanoic, heptanoic and octanoic acids were purchased from Sigma. Hexane-, heptane- and octanesulfonic acids (sodium salts) were also obtained from Sigma. Methanol was of HPLC grade from Janssen Chimica (Geel, Belgium).

The analytical column was packed with a CSP consisting of an α_1-acid glycoprotein immobilized on silica (particle size, 5 μm) from ChromTech, whereas the guard column was filled with LiChrospher 100 DIOL (particle size, 5 μm) from Merck. The water used in all experiments was of Milli-Q quality from Millipore (Bedford, MA, USA).

2.3. Chromatographic systems

To study the influence of the pH of the mobile phase and of the methanol concentration, the mobile phases consisted of mixtures of 10 m*M* phosphate buffer and methanol.

For the study of the influence of ionic organic modifiers, the mobile phases consisted of 10 m*M* phosphate buffers (pH 7.0) to which the modifier was added. The mobile phases were adjusted to pH 7.0 with a 10% sodium hydroxide solution, if necessary.

The mobile phase used for validation of the method developed for the enantioseparation of sotalol consisted of a 10 m*M* phosphate buffer, pH 7.0, containing heptanoic acid (15 m*M*). Before use, all mobile phases were filtered through a 0.22-μm filter and degassed for 15 min in a Sonicor SC-100-22TH ultrasonic bath (Copiague, NY, USA).

The flow-rate was 0.9 ml/min. UV detection was performed at 220 nm in all experiments, except for the validation of the enantioselective assay of sotalol (230 nm).

2.4. Standard solutions

For studies on the influence of the mobile phase pH, methanol concentration and the nature and concentration of ionic modifiers, stock solutions were prepared by dissolving 30 mg of each β-blocker in 50 ml of methanol. These solutions were then diluted daily with water to obtain a final concentration of 60 μg/ml.

For the validation of the enantioselective assay of sotalol, the stock solution was prepared by dissolving 12.5 mg of sotalol hydrochloride in 25 ml of water. This solution was then diluted with water to give a final concentration of 2.5 μg/ml for each sotalol enantiomer. Solutions were then prepared for calibration with final concentrations ranging from 25 to 500 ng/ml for each sotalol enantiomer.

3. Results and discussion

3.1. Influence of the pH of the mobile phase

The influence of the pH of the mobile phase was studied using all β-blockers (see Fig. 1) as model compounds.

In the pH range studied, all β-blockers are essentially present in the cationic form. Indeed, the pK_a values of these compounds are approximately nine.

The influence of the pH of the mobile phase on the retention of different β-blockers, except oxprenolol, is shown in Table 1. An increase in the mobile phase's pH causes a strong increase in the capacity ratios of both enantiomers of each β-blocker tested. This increase in retention is much more pronounced on Chiral-AGP than on octadecylsilica stationary phases, the first eluting enantiomers often being 30–50 times more retained at pH 7.0 than at pH 4.5. This could be explained by conformational changes in the immobilized protein, induced by the pH changes in the mobile phase [14,16,41].

The effect of the pH of the mobile phase on enantioselectivity is also presented in Table 1. The increase of the mobile phase pH improves the enantioselectivity for some β-blockers, such as bevantolol, pindolol or metipranolol, but it should be noted that no enantiomeric separation was observed for acebutolol, betaxolol or sotalol when this parameter was modified. Under these conditions (methanol concentration=15%), atenolol and celiprolol were not enantioseparated, however, a slight resolution was obtained for these two compounds when the methanol concentration was less than 15% (see Table 2).

3.2. Influence of methanol concentration

The influence of methanol concentration was investigated on all β-blockers, except oxprenolol.

The effect of methanol concentration on retention and enantioselectivity is shown in Table 2. An increase in methanol concentration causes a decrease in the retention of both enantiomers for each β-blocker tested. The decrease in retention was found to be particularly pronounced in the lower concentration range of methanol, as generally observed in reversed-phase high-performance liquid chromatography (HPLC).

An increase in methanol concentration also leads to a decrease in enantioselectivity for all β-blockers tested (Table 2). However, the decrease in enantioselectivity is more important for metoprolol than it is

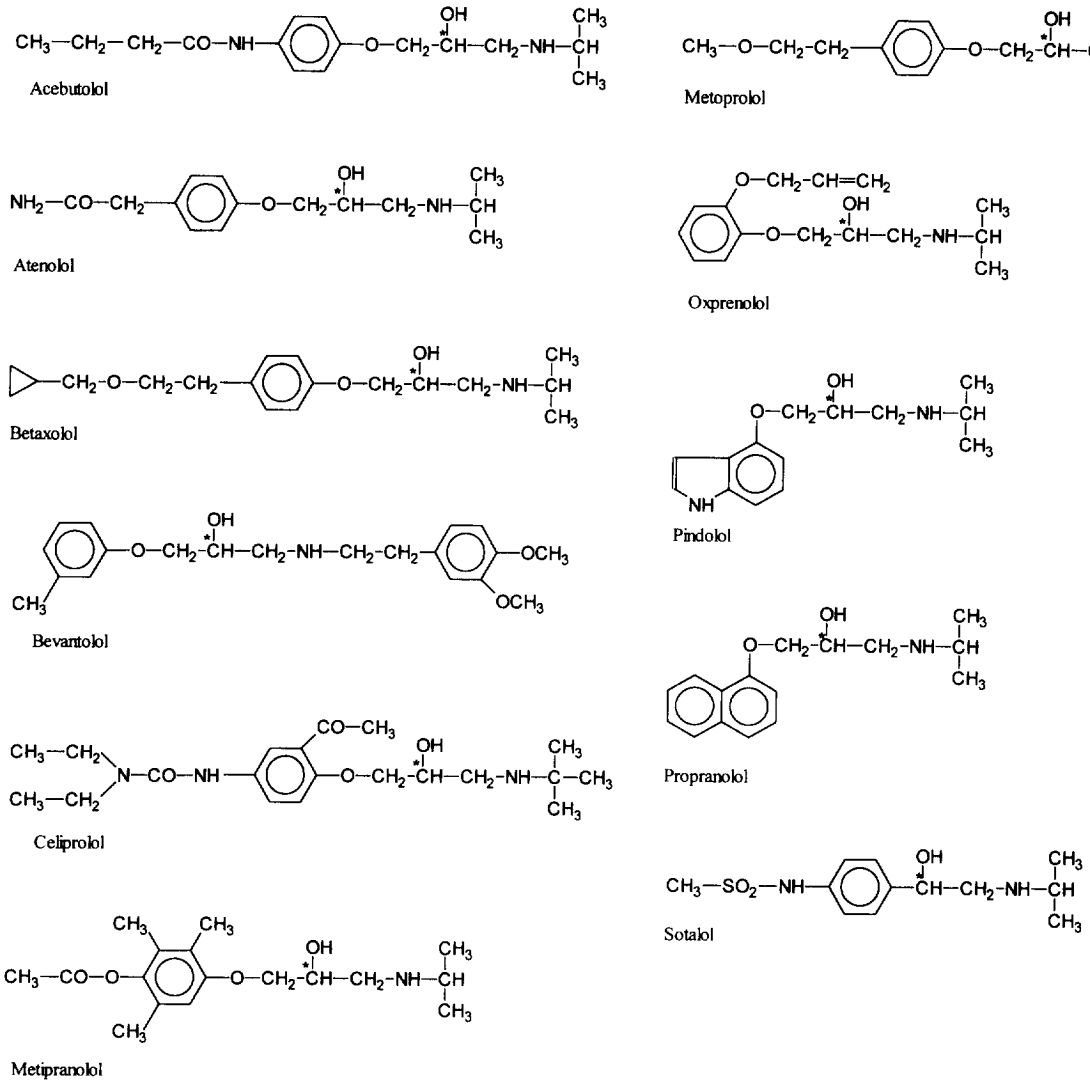

Fig. 1. Chemical structures of the β-blockers that were examined.

for other β-blockers, such as metipranolol, pindolol or propranolol. The effect of methanol concentration on enantioselectivity therefore seems to vary with the compound examined, which could be explained by the presence on the CSP of different kinds of binding sites, chiral and non-chiral, for which methanol can compete with the analytes [2,38].

3.3. Optimisation of enantiomeric separations for β-blocking agents

On the basis of these observations, many enantiomeric separations of β-blocking drugs could be achieved.

The initial pH of the mobile phase should be high enough to provide suitable retention of the compound being tested. The enantiomeric resolution can then be optimized by a further increase of the pH.

The methanol concentration also plays an important role, especially for regulating retention at the optimum pH.

By optimizing these two parameters, 70% of the β-blockers tested could be at least partially enantioseparated. The corresponding chromatographic

Table 1
Influence of the pH of the mobile phase on retention, enantioselectivity and resolution

		pH Value			
		4.5	5.5	6.5	7.0
Acebutolol	k_1'	0.22	1.5	4.1	5.1
	α	–	–	–	–
	R_s	–	–	–	–
Atenolol	k_1'	ND	0.83	2.3	2.7
	α	ND	–	–	–
	R_s	ND			
Betaxolol	k_1'	0.39	2.7	9.4	13.8
	α	–	–	–	–
	R_s	–	–	–	–
Bevantolol	k_1'	1.1	4.7	26.0	53.6
	α	–	1.09	1.17	1.39
	R_s	–	<0.7	0.95	2.8
Celiprolol	k_1'	0.22	1.8	4.9	7.0
	α	–	–	–	–
	R_s	–	–	–	–
Metipranolol	k_1'	0.74	4.8	18.6	37.7
	α	1.12	1.27	1.34	1.34
	R_s	<0.7	1.5	2.3	2.5
Metoprolol	k_1'	0.04	1.26	3.50	4.61
	α	–	–	1.11	1.15
	R_s	–	–	<0.7	<0.7
Pindolol	k_1'	0.56	3.3	12.7	18.6
	α	–	1.08	1.12	1.16
	R_s	–	<0.7	<0.7	1.3
Propranolol	k_1'	3.4	25.4	retention	retention
	α	1.08	1.13	times >75 min	times >75 min
	R_s	<0.7	0.97		
Sotalol	k_1'	ND	1.1	2.5	2.7
	α	ND	–	–	–
	R_s	ND	–	–	–

Chromatographic conditions: 10 m*M* phosphate buffer–methanol (85:15, v/v). k_1' = Capacity ratio of the first eluting enantiomer; α = enantioselectivity; R_s = resolution; ND = not determined; – = no visible enantioselectivity or resolution.

conditions are given in Table 3 and some examples of enantiomeric separations are shown in Fig. 2. No chiral resolution was observed for acebutolol, betaxolol and sotalol under these conditions.

3.4. Influence of the nature and concentration of ionic organic modifiers

The influence of the nature and concentration of anionic modifiers such as aliphatic carboxylic acids and alkanesulfonic acids was investigated using sotalol as a model compound (since the enantiomers of this compound could not be separated by changing only the pH and methanol concentration in the mobile phase).

Because of the low retention of sotalol on Chiral-AGP, these experiments were performed with a 10 m*M* phosphate buffer (pH 7.0) as the mobile phase, to which anionic modifiers were added.

No chiral separation was achieved for sotalol by the addition of alkanesulfonic acids (5–30 m*M*). However, it should be noted that an increase in the concentration of alkanesulfonic acids caused the retention of sotalol enantiomers to decrease while the

Table 2
Influence of methanol concentration on retention, enantioselectivity and resolution

		Concentration of methanol (%)					
		0	5	10	15	20	25
Acebutolol[a]	k_1'	22.9	11.0	6.9	5.1	4.1	ND
	α	–	–	–	–	–	ND
	R_s	–	–	–	–	–	ND
Atenolol[a]	k_1'	4.4	3.3	3.1	2.7	2.5	ND
	α	1.25	1.11	–	–	–	ND
	R_s	<0.7	<0.7	–	–	–	ND
Betaxolol[b]	k_1'	13.8	4.8	3.7	2.7	2.0	1.7
	α	–	–	–	–	–	–
	R_s	–	–	–	–	–	–
Bevantolol[a]	k_1'	retention	retention	retention	53.6	28.4	17.8
	α	times >75 min	times >60 min	times >60 min	1.39	1.33	1.22
	R_s				2.8	2.2	1.5
Celiprolol[a]	k_1'	43.4	19.2	10.7	7.0	5.0	ND
	α	1.95	–	–	–	–	ND
	R_s	<0.7	–	–	–	–	ND
Metipranolol[b]	k_1'	ND	11.1	7.4	4.8	3.0	2.3
	α	ND	1.35	1.34	1.27	1.23	1.17
	R_s	ND	1.9	2.0	1.5	1.3	0.81
Metoprolol[a]	k_1'	21.1	10.2	6.2	4.6	3.7	ND
	α	1.55	1.31	1.25	1.15	1.09	ND
	R_s	1.9	1.2	0.94	0.71	<0.7	ND
Pindolol[b]	k_1'	16.2	5.8	4.4	3.3	2.7	2.4
	α	1.12	1.10	1.10	1.08	–	–
	R_s	<0.7	<0.7	<0.7	<0.7	–	–
Propranolol[b]	k_1'	retention	72.9	40.7	25.4	12.3	8.1
	α	times >90 min	1.14	1.20	1.13	1.11	1.05
	R_s		1.2	1.6	0.97	0.71	<0.7
Sotalol[a]	k_1'	3.56	3.00	2.74	2.65	2.65	ND
	α	–	–	–		–	ND
	R_s	–	–	–	–	–	ND

Chromatographic conditions: ([a]) 10 m*M* phosphate buffer (pH 7.0)–methanol; ([b]) 10 m*M* phosphate buffer (pH 5.5)–methanol; k_1' = capacity ratio of the first eluting enantiomer; α = enantioselectivity; R_s = resolution; ND = not determined; – = no visible enantioselectivity or resolution.

Table 3
Chromatographic conditions for the enantioseparation of β-blockers on Chiral-AGP

β-Blocker	Selectivity	Resolution	Chromatographic conditions
Atenolol	1.25	0.70	Phosphate buffer, pH 7.0
Bevantolol	1.22	1.5	Phosphate buffer (pH 7.0)–methanol (75:25, v/v)
Celiprolol	1.05	<0.70	Phosphate buffer, pH 7.0
Metipranolol	1.34	1.7	Phosphate buffer (pH 6.5)–methanol (80:20, v/v)
Metoprolol	1.31	1.2	Phosphate buffer (pH 7.0)–methanol (95:5, v/v)
Oxprenolol	1.28	1.8	Phosphate buffer (pH 7.0)–methanol (70:30, v/v)
Pindolol	1.16	1.3	Phosphate buffer (pH 7.0)–methanol (85:15, v/v)
Propranolol	1.20	1.6	Phosphate buffer (pH 5.5)–methanol (90:10, v/v)

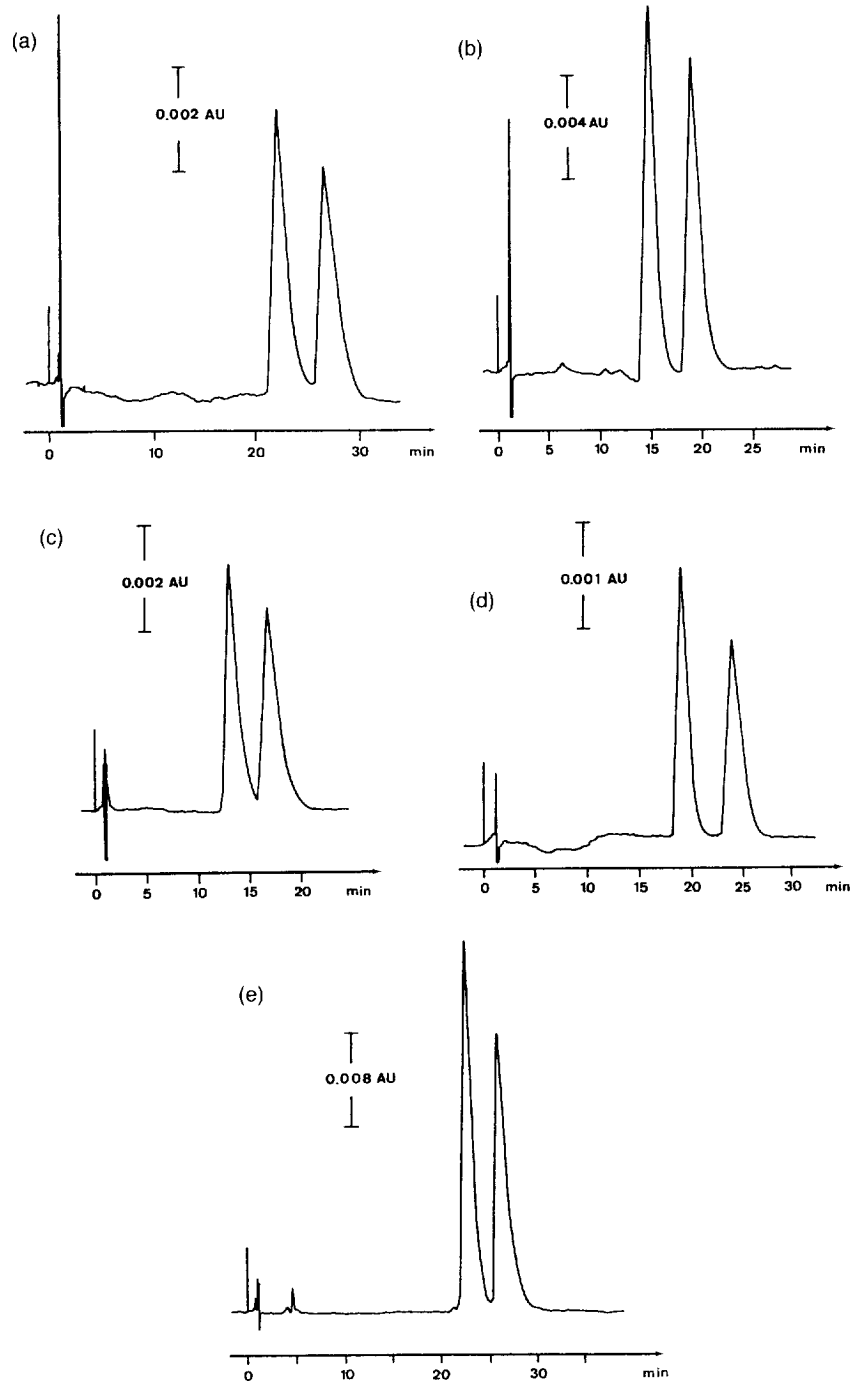

Fig. 2. Chiral separation of β-blocking drugs on Chiral-AGP. (a) Bevantolol, (b) metipranolol, (c) metoprolol, (d) oxprenolol and (e) pindolol. See Table 3 for chromatographic conditions.

length of the aliphatic chain did not seem to have any significant influence on retention.

On addition of aliphatic carboxylic acids (5–50 m*M*), an increase in the concentration of these acids was also found to cause a decrease in the capacity ratios of sotalol. As in the case of the alkanesulfonic acids, the length of the aliphatic chain had no significant influence on retention. These observations indicate that the effects of these anionic modifiers are widely different from those observed with the same kind of compounds in reversed-phase ion-pair chromatography.

The influence of the nature (chain length) and concentration of the aliphatic carboxylic acids on enantioselectivity is demonstrated in Fig. 3. The addition of octanoic acid did not lead to the enantioseparation of sotalol, while with the other aliphatic carboxylic acids that had shorter alkyl chains (C_4 to C_7) resolution of sotalol enantiomers was obtained. For butanoic and pentanoic acids, the highest concentration (50 m*M*) seems to be the most favourable for the enantioseparation. In the case of carboxylic acids with longer chain lengths, maximum selectivity is obtained in lower concentration ranges (20–50 m*M* for hexanoic acid and 10–30 m*M* for heptanoic acid).

The effects of aliphatic carboxylic acids on resolution are quite similar to those observed for enantioselectivity. It is interesting to note, however, that the highest resolution value was obtained by using heptanoic acid as the anionic modifier at a concentration of 15 m*M*. The separation of sotalol enantiomers under these chromatographic conditions is shown in Fig. 4. It should be noted that in a previous study [10] performed on the Enantiopac column, the addition of pentanoic acid did not give rise to any resolution of sotalol enantiomers. Moreover, the resolution values obtained with the Chiral-AGP column are higher (1.9 on the Enantiopac column with 50 m*M* hexanoic acid and 2.2 on the Chiral-AGP column with 15 m*M* heptanoic acid).

Some other β-blocking drugs that had not been

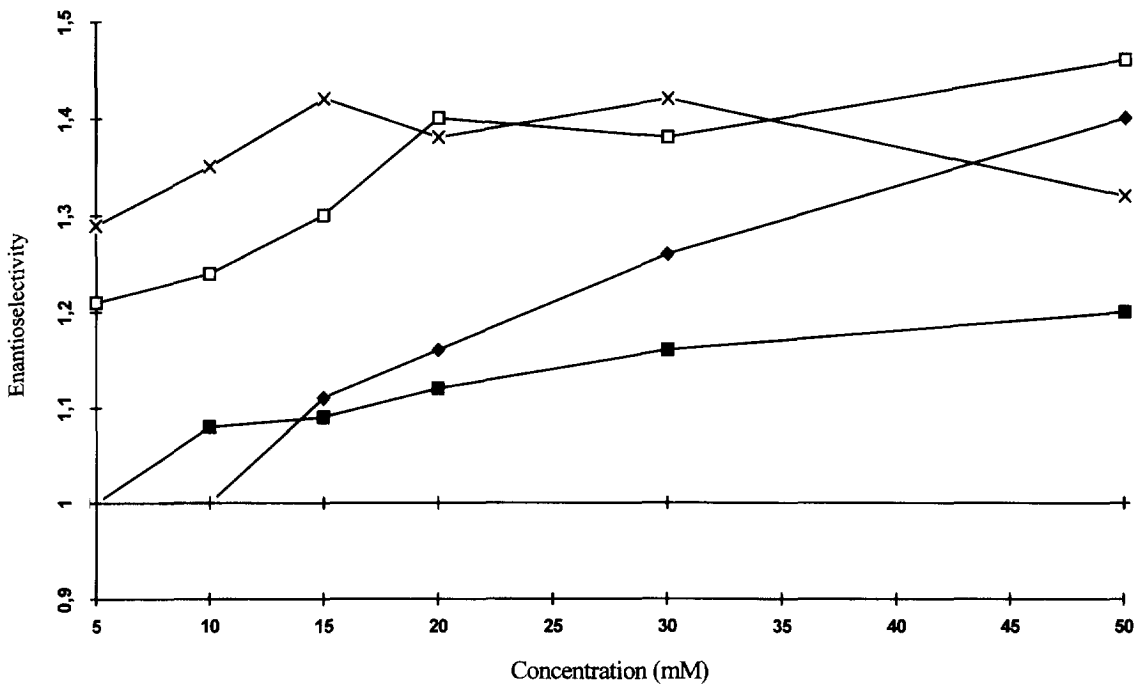

Fig. 3. Influence of the nature and concentration of aliphatic carboxylic acids on enantioselectivity in the case of sotalol. Chromatographic conditions: 10 m*M* phosphate buffer (pH 7.0) containing an aliphatic carboxylic acid. (♦) Butyric acid; (■) pentanoic acid; (□) hexanoic acid; (×) heptanoic acid; (+) octanoic acid.

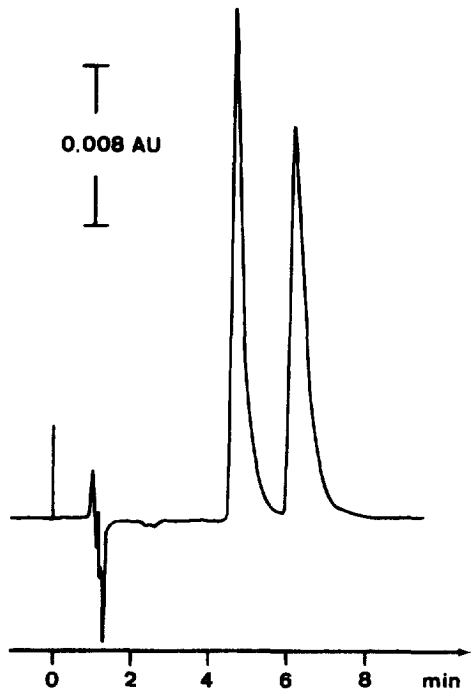

Fig. 4. Enantiomeric separation of sotalol. Chromatographic conditions: 10 m*M* phosphate buffer, pH 7.0, containing 15 m*M* heptanoic acid.

enantioseparated in previous experiments (see Tables 1 and 2) were tested under the same conditions, i.e., betaxolol and acebutolol, as well as atenolol, celiprolol and metoprolol.

The influence of the nature of the five carboxylic acids on enantioselectivity for these β-blockers is shown in Fig. 5, with all of the carboxylic acids being added at the same concentration (10 m*M*). When octanoic acid was added to the mobile phase, no chiral separations were obtained. This was even the case for atenolol or metoprolol which were enantioseparated with mobile phases devoid of anionic modifiers. For these two compounds, enantioselectivity has a tendency to decrease as the length of the aliphatic chain increases. In the case of celiprolol, enantioselectivity is only slightly affected by the addition of carboxylic acids, from C_4 to C_7. On the other hand, the addition of aliphatic carboxylic acids has not led to the enantioseparation of acebutolol and betaxolol. A very favourable effect on enantioselectivity was only observed for sotalol. This could be related to the particular structure of this compound, which is somewhat different from that of the other β-blockers (Fig. 1).

3.5. Validation

The stereospecific assay of sotalol enantiomers was validated by studying linearity, limits of detection and quantitation and reproductibility. The linear regression analysis made by plotting the peak area (y) versus the analyte concentration (x) in ng/ml was studied at k different concentrations with n measurements at each concentration. The following equations were obtained (concentration range: 25–500 ng/ml; $k=6$, $n=3$):

first eluting enantiomer: $y = 29.21x - 132.44$

second eluting enantiomer: $y = 31.33x - 222.11$

The determination coefficients (r^2) of the regression lines obtained for both enantiomers indicate that the relationship between the response and the analyte concentration is linear in the concentration range studied ($r^2 = 0.9986$ for the first eluting enantiomer and 0.9970 for the second eluting enantiomer).

The limits of detection (LOD) and of quantitation (LOQ) for the two enantiomers were calculated from regression lines [42]. The LODs were estimated to be 7.8 and 11.5 ng/ml, respectively, for the first and the second eluting enantiomer. The LOQs were estimated to be 25.9 and 38.3 ng/ml, respectively.

Table 4 presents the results obtained for intra-day and inter-day reproducibilities. The intra-day reproducibility was investigated at three different analyte concentrations and inter-day reproducibility was studied at five different concentrations. In each case, relative standard deviations (R.S.D.s) were found to be quite acceptable.

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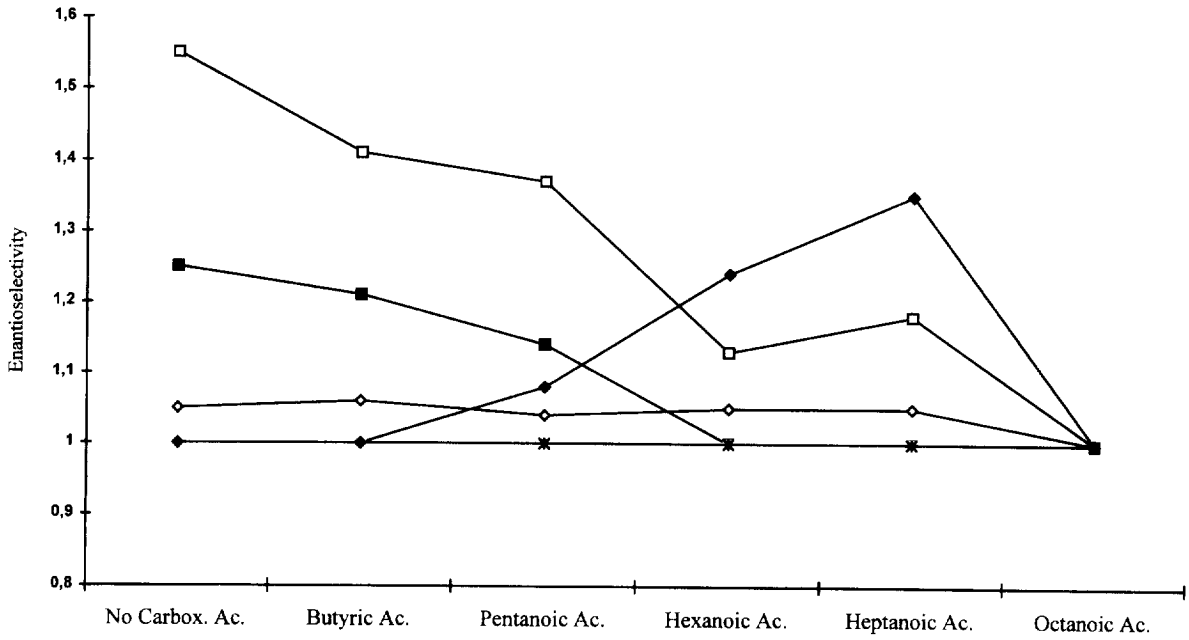

Fig. 5. Influence of the nature of aliphatic carboxylic acids on the enantiomeric separation of some other β-blocking drugs. Chromatographic conditions: 10 m*M* phosphate buffer, pH 7.0, containing an aliphatic carboxylic acid (10 m*M*). (■) Atenolol; (□) metoprolol; (+) acebutolol; (♦) sotalol; (×) betaxolol; (◇) celiprolol.

Table 4
Within-day and between-day reproducibilities for sotalol enantiomers

Reproducibility	Concentration (ng/ml)	First enantiomer eluted (R.S.D., %; $n=5$)	Second enantiomer eluted (R.S.D., %; $n=5$)
Within-day	50	1.1	1.4
	250	0.38	0.47
	500	0.37	0.67
Between-day	50	7.8	4.6
	125	2.7	3.5
	250	2.4	2.1
	375	1.4	1.3
	500	2.0	2.4

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References

[1] S.R. Narayanan, J. Pharm. Biomed. Anal., 10 (1992) 251.
[2] J. Hermansson, Trends Anal. Chem., 8 (1989) 251.
[3] M. Enquist and J. Hermansson, Chirality, 1 (1989) 209.
[4] C. Rudolphi and G. Blaschke, J. Chromatogr. B, 663 (1995) 315.
[5] D.J. Jones and A.R. Bjorksten, J. Chromatogr. B, 661 (1994) 165.
[6] F. Li, S.F. Cooper, M. Cote and C. Ayotte, J. Chromatogr. B, 660 (1994) 327.
[7] G. Stagni and W.R. Gillespie, J. Chromatogr. B, 667 (1995) 349.
[8] A. Doroudian, K. Yeleswaram, D.W. Rurak, F.S. Abbott and J.E. Axelson, J. Chromatogr., 619 (1993) 79.
[9] V. Chapeau, A. Moulin, M. Caude and A. Dufour, J. Chromatogr. B, 660 (1994) 341.

[10] E. Delée, L. Le Garrec, I. Jullien, S. Béranger, J.C. Pascal and H. Pinhas, Chromatographia, 24 (1987) 357.
[11] K. Balmer, B. Persson and G. Schill, J. Chromatogr., 519 (1989) 107.
[12] M. Enquist and J. Hermansson, J. Chromatogr., 519 (1990) 285.
[13] C. Vandenbosch, D.L. Massart and W. Lindner, J. Pharm. Biomed. Anal., 10 (1992) 895.
[14] C. Vandenbosch, T. Hamoir, D.L. Massart and W. Lindner, Chromatographia, 33 (1992) 454.
[15] J. Ekelund, A. van Arkens, K. Bronnom-Hansen, K. Fich, L. Olsen and P. Vibholm Petersen, J. Chromatogr. A, 708 (1995) 253.
[16] I. Marle, S. Jönsson, R. Isaksson, C. Pettersson and G. Pettersson, J. Chromatogr., 648 (1993) 333.
[17] A.M. Dyas, J. Pharm. Biomed. Anal., 10 (1992) 383.
[18] E. Kuesters and D. Giron, J. High Resolut. Chromatogr. Chromatogr. Commun., 9 (1986) 531.
[19] J. Hermansson and A. Grahn, J. Chromatogr. A, 687 (1994) 45.
[20] R. Isaksson, C. Pettersson, G. Pettersson, S. Jönsson, J. Stalberg, J. Hermansson and I. Marle, Trends Anal. Chem., 13 (1994) 431.
[21] J. Haginaka, C. Seyama, H. Yasuda and K. Takahashi, J. Chromatogr., 598 (1992) 67.
[22] N. Mano, Y. Oda, N. Asakawa, Y. Yoshida, T. Sato and T. Miwa, J. Chromatogr. A, 687 (1994) 223.
[23] D.W. Armstrong, T.J. Ward, R.D. Armstrong and T.E. Beesley, Science, 232 (1986) 1132.
[24] D.W. Armstrong, S. Chen, C. Chang and S. Chang, J. Liq. Chromatogr., 15 (1992) 545.
[25] C. Hartmann, D. Krauss, H. Spahn and E. Mutschler, J. Chromatogr., 496 (1989) 387.
[26] Y. Okamoto, M. Kawashima, R. Aburatani, K. Hatada, T. Nishiyama and M. Masuda, Chem. Lett., (1986) 1237.
[27] C. Falcklam and A. Modler, J. Chromatogr. A, 664 (1994) 203.
[28] W.H. Pirkle and J.A. Burke, J. Chromatogr., 557 (1991) 173.
[29] A.F. Fell, M.L. Robinson and A.M. Dyas, Analyst, 116 (1991) 1343.
[30] I. Bechet, Ph. Paques, M. Fillet, Ph. Hubert and J. Crommen, Electrophoresis, 15 (1994) 818.
[31] Y. Okamoto, R. Aburatani, K. Hatano and K. Hatada, J. Liq. Chromatogr., 11 (1988) 2147.
[32] L. Le Garrec, E. Delée, J.-C. Pascal and I. Jullien, J. Liq. Chromatogr., 10 (1987) 3015.
[33] R.A. Carr, R.T. Foster and N.H. Bhanji, Pharm. Res., 8 (1991) 1195.
[34] R. Mehvar, J. Chromatogr., 492 (1989) 402.
[35] C. Fiset, F. Philippon, M. Gilbert and J. Turgeon, J. Chromatogr., 612 (1993) 231.
[36] M. Enquist and J. Hermansson, J. Chromatogr., 519 (1990) 271.
[37] G. Schill, I.W. Wainer and S.A. Barkan, J. Liq. Chromatogr., 9 (1986) 641.
[38] G. Schill, I.W. Wainer and S.A. Barkan, J. Chromatogr., 365 (1986) 73.
[39] J. Hermansson and M. Eriksson, J. Liq. Chromatogr., 9 (1986) 621.
[40] J. Hermansson and I. Hermansson, J. Chromatogr. A, 666 (1994) 181.
[41] J. Hermansson and A. Grahn, J. Chromatogr. A, 694 (1995) 57.
[42] J.C. Miller and J.N. Miller, in R.A. Chalmers and M. Masson (Editors), Statistics for Analytical Chemistry, Ellis Horwood, Chichester, 1984, p. 96.