ELSEVIER

Journal of Chromatography A, 839 (1999) 23–39

JOURNAL OF CHROMATOGRAPHY A

Direct high-performance liquid chromatographic separations of metoprolol analogues on a Chiralcel OD column using chemometrics

S. Svensson, J. Vessman, A. Karlsson*

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

Received 25 November 1998; received in revised form 26 January 1999; accepted 27 January 1999

Abstract

The chromatographic behaviour of sixteen racemic amino alcohols was studied under normal-phase liquid chromatography conditions using Chiralcel OD as the chiral stationary phase. The use of addition of water to the mobile phase sped up the equilibration time of the chromatographic system. The influence of content of mobile phase 2-propanol, water and acetic acid on enantioselective retention was investigated using a statistical factorial experimental design and the experimental results were evaluated using multivariate analyses. The impact of small changes in chemical structure of the tested amino alcohols on enantioseparation was also investigated in order to give some insight into the chiral recognition mechanism. The structure of the amino alcohols differ in distance between the chiral centre and the nitrogen atom, type and position of substituents in the aromatic ring and type of substituent attached to the nitrogen atom. Appropriate concentrations of water and acetic acid in the mobile phase made it possible to control the elution order of the enantiomers of an analogue to metoprolol. The high enantioselectivity obtained for several of the tested amines makes it possible to determine enantiomeric purity even if the main peak elutes before the minor enantiomeric impurity peak.

Keywords: Enantiomer separation; Chemometrics; Metoprolol; Amino alcohols

1. Introduction

The chiral amino alcohols which have β-adrenergic blocking activity are generally administered as racemates. As enantiomers may have different biological effects in vivo the possibility of enantioseparation is of importance [1]. Generally, the (*S*)-enantiomer has a higher biological activity than the (*R*)-enantiomer [2].

There are numerous chiral stationary phases developed for chromatography. The cellulose and amylose derivatives are widely used, due to great applicability for several techniques; liquid chromatography [3–15], supercritical fluid chromatography (SFC) [6,16–18] and capillary electrophoresis [19].

A parallel study using cellulose and amylose derivatives as stationary phases for packed column SFC showed that the cellulose based stationary phase provided much higher enantioresolution for a number of selected amino alcohols (metoprolol analogues) than the amylose stationary phase [20]. Therefore, the cellulose stationary phase, Chiralcel OD, was used throughout this study.

Most of the published studies regarding chiral

*Corresponding author.

0021-9673/99/$ – see front matter
PII: S0021-9673(99)00159-4

separations in liquid chromatography report the influence of different experimental factors such as modifier and additive concentration in a univariate mode. More systematic approaches for the evaluations of experimental parameters were reported by Andersson et al. [21].

A factorial design with centrepoints, [22,23] followed by multivariate analyses using partial least squares (PLS) [24], as the statistical method was utilised in order to study the chromatography of the racemic amino alcohols.

The purpose of this work was to study the influence of 2-propanol, water and acetic acid on enantioresolution in normal-phase liquid chromatography for a number of amino alcohols using chemometrics.

The influence of water on the equilibration time of a chromatographic system based on isohexane and 2-propanol will be shown.

Finally, the influence of solute structure on enantioselectivity has also been discussed in this work.

2. Experimental

2.1. Chromatographic equipment

A Hewlett–Packard SFC G 1205A, (Little Falls, Wilmington, DE, USA), was slightly rebuilt to function as a liquid chromatography instrument. The modifier pump was used to pump the mobile phase and the nozzle used in SFC for pressure regulation was decoupled. The system was equipped with a HP 1050 multiple wavelength UV-detector, a gas chromatography oven and an autosampler. The samples were loaded on a pneumatically actuated Rheodyne valve, equipped with a 5 μl loop. Flow-rate and column temperature were controlled by the HP ChemStation software, which also generated and evaluated the chromatograms.

2.2. Column and chemicals

The column used was Chiralcel OD [tris(3,5-dimethylphenylcarbamate) cellulose] with the dimension 10 μm, 250×4.6 mm ID, manufactured by Daicel (Tokyo, Japan).

All racemates and the pure enantiomers of metoprolol, Fig. 1, were all synthesised by Medicinal Chemistry, Astra Hässle, Mölndal, Sweden.

Ethanolamine, triethylamine, *tert*.-octylamine, propanoic acid, butanoic acid, pentanoic acid, *n*-hexane and isohexane were obtained from Fisher (Loughborough, Leicestershire, UK). Formic acid, acetic acid, octanoic acid and 2-propanol (p.a.) were purchased from Merck (Darmstadt, Germany). N,N-Dimethyloctylamine, (DMOA, >95% pure) was obtained from Aldrich (Gillingham, Dorset, UK) and glass distilled. Propylamine, *n*-hexylamine and heptanoic acid were manufactured by Fluka (Buchs, Switzerland). Nonanic acid was delivered by Labkemi (Stockholm, Sweden). Diethylamine (DEA) was obtained from BLD Laboratories (UK).

2.3. Chromatographic method

The racemates were dissolved in methanol and then diluted with 15% 2-propanol and 10 m*M* DEA in isohexane to a sample concentration of 1 mg ml^{-1}. The racemates were chromatographed and detected at 273 nm and the mobile phase flow-rate was 1 ml min^{-1}. All chiral separations were carried out at 30°C unless otherwise stated.

The t_0 value used ($t_0 = 2.9$ min) was calculated from the column volume and in accordance with the time of the first disturbance of the baseline obtained after injection. The retention times of the enantiomeric solutes, t_r, were measured from the time of injection.

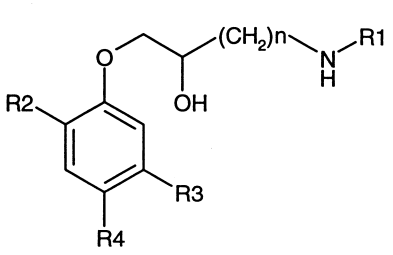
The chromatographic responses; capacity factors $[k = (t_r - t_0)/t_0]$ and selectivity factors $[\alpha = k_2/k_1]$ used in this work were the mean values from duplicate injections. Column efficiency was calculated using the equation $[N = 16t_r^2/wt^2]$.

A preliminary screening of the variables (mobile phase 2-propanol, water and acetic acid) resulted in the ranges of the mobile phase compositions given in Table 1.

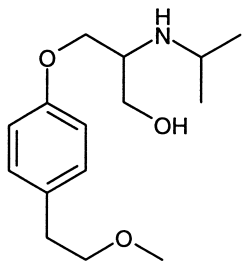
The complete dataset is available in Excel format from the authors.

2.4. Statistical methods

Evaluation of the experimental variables studied was performed by using statistical multivariate regression.



Solute 1-10, 12-16



Solute 11

Solute	R_1	R_2	R_3	R_4
1	$CH(CH_3)_2$ n=1	H	H	$CH_2CH_2OCH_3$
2	$CH(CH_3)_2$ n=2	H	H	$CH_2CH_2OCH_3$
3	$CH(CH_3)_2$ n=3	H	H	$CH_2CH_2OCH_3$
4	$CH(CH_3)_2$	H	$CH_2CH_2OCH_3$	H
5	$CH(CH_3)_2$	$CH_2CH_2OCH_3$	H	H
6	$CH(CH_3)_2$	H	H	CH_2CH_2 NONC$(CH_3)_3$
7	$CH_2CH_2CH_3$	H	H	$CH_2CH_2OCH_3$
8	$CH(CH_3)_3$	H	H	$CH_2CH_2OCH_3$
9	$CH(CH_3)_2$	CH_2CHCH_2	H	H
10	$CH(CH_3)_2$	$CH_2CH_2CH_3$	H	H
12	$CH(CH_3)_2$	CHO	H	H
13	$CH(CH_3)_2$	H	CHO	H
14	$CH(CH_3)_2$	H	H	H
15	$CH(CH_3)_2$	H	H	CH_2OCH_3
16	$CH(CH_3)_2$	H	H	CH_2 $CONH_2$

Fig. 1. Solute structures.

The 2^3 factorial design and analysis of chromatographic data were fulfilled using Modde 3.0 as software (Umetri, Umeå, Sweden). The factorial design with three experimental variables resulted in eight runs and in addition centrepoints in triplicate.

Table 1
Mobile phase composition—experimental design and variable settings

Parameter	Type	Unit	Settings
Acetic acid (A)	Factor	m*M*	5–15
Water (W)	Factor	m*M*	28–83
2-Propanol (M)	Factor	%	15–25
Capacity factor, 1, (k_1)	Response		
Capacity factor, 2, (k_2)	Response		
Selectivity factor, (α)	Response		

The centrepoint experiments were executed in order to estimate the precision of the chromatographic system. The PLS method was used for calculation of regression, (R^2). Cross-validation, (Q^2), was used to estimate the quality of the statistical model [24]. R^2 is the fraction of variation of the responses explained by the statistical model and Q^2 is the fraction of variation of the response that can be predicted by the model. $R^2 = SS_{REG}/SS$ and $Q^2 = 1 - PRESS/SS$. SS_{REG} is the sum of squares of the response corrected for the mean and *PRESS* is the prediction residuals sum of squares. R^2 and Q^2 are used as indicative criteria of model fit, where values close to 1.0 indicate good fit and usability of the model.

The experimental domain was defined as the 2-propanol concentration from 15 to 25% (v/v), the

acetic acid concentration from 5 to 15 m*M* and the water concentration from 28 to 83 m*M*.

3. Results and discussion

3.1. Screening of effects of column temperature, 2-propanol, acetic acid, water and amine

A preliminary screening was performed with univariate conditions in order to study the influence of column temperature, content of modifier, addition of water and amines on enantioseparation of metoprolol, solute 1.

When varying the column temperature from −8 to 40°C, the temperature had practically no influence on the enantioselectivity as shown in Table 2. Therefore, the column temperature was kept constant at 30°C throughout the experiments in the statistical experimental design. The effect of the column temperature on enantioselective retention of amino alcohols has previously been studied using polysaccharides as chiral selectors [3–4]. Although the separation factors sometimes are independent of column temperature [3], the most common effect by using polysaccharide phases is that an increased column temperature results in decreased separation factors [4].

The influence of 2-propanol concentration on enantioselectivity was also studied univariately. The enantioselectivity of metoprolol, solute 1 was scarcely influenced by changing the mobile phase 2-propanol content of from 20% to 30%. The influence of different mobile phase modifiers on enantioselectivity has previously been studied using solutes 1 to 3 and the Chiralcel OD stationary phase as chiral selector [15]. 2-Propanol provided a higher enantioselectivity for these solutes compared to ethanol or 1-propanol, and was therefore chosen as modifier [15].

Table 2
Influence of column temperature on chromatographic parameters of metoprolol, 1[a]

Column temperature (°C)	k_R	k_S	α
−8	2.19	3.62	1.65
0	1.80	2.92	1.62
26	1.01	1.66	1.64
30	0.96	1.55	1.62
35	0.88	1.40	1.59
40	0.80	1.30	1.63

[a] Chromatographic conditions: mobile phase, 10 m*M* DEA and 56 m*M* water in *n*-hexane:2-propanol (85:15).

To control the system stability and reproducibility a small amount of water added to the mobile phase was found to be beneficial [15]. The influence of water on the equilibration time was studied using a chromatographic system based on isohexane and 2-propanol, Fig. 2. Metoprolol, solute 1 was repeatedly injected to the chromatographic system using mobile phases that contained an added amount of 44 m*M* water or no added water. The presence of water in the mobile phase had a high impact on the enantioselectivity. An addition of water (44 m*M*) decreased the enantioselectivity from $\alpha = 2.2$ to $\alpha = 1.6$. The equilibration time of the chosen system was fast and constant separation factors could be obtained almost immediately after a change of mobile phase, as shown in Fig. 2.

Addition of small and branched amines to the mobile phase is common in chromatography to improve column efficiency of basic solutes such as amino alcohols [17–18]. The contribution to column efficiency of six amines using a concentration level of 10 m*M* was studied. The chosen amines (DEA, DMOA, *n*-hexylamine, ethanolamine, triethylamine and *tert*.-octylamine) differed in hydrophobicity and degree of carbon chain branching. The enantioselectivity and retention were only slightly affected by the added amine, as shown in Table 3. However, column efficiency and general peak shape were improved using DEA compared to the other amines used. Therefore, DEA was chosen for the statistical experimental design experiments in a concentration level of 10 m*M*.

3.2. Statistical experimental design and evaluation of the statistical model

A preliminary screening of the studied variables (concentration of 2-propanol, acetic acid and water) resulted in the experimental ranges given in Table 1. The responses chosen were the capacity (k) and selectivity (α) factors.

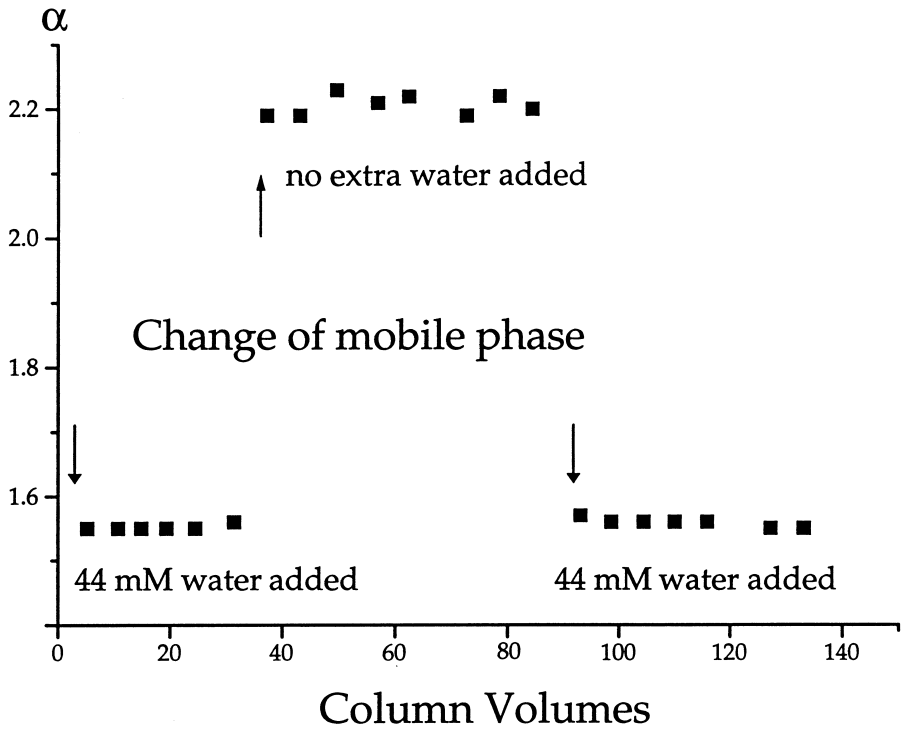


Fig. 2. Influence of water content on the equilibration of the chromatographic system. Chromatographic conditions: column temperature, 30°C; flow-rate, 1 ml min^{-1}; mobile phase, 10 m*M* DEA and 44 m*M* water in *n*-hexane–2-propanol (9:1).

The design was made for resolving linear and cross-terms (and also indicate the presence of quadratic terms) of the chromatographic variables.

A combination of capacity and selectivity factors resulted in a model that gave low predictor abilities, (Q^2), regardless if the cross-terms were included or not. Higher explanations of the statistical models were observed when the capacity factors and the selectivity factors were treated in separate models.

The predictor abilities, (Q^2), also increased when using only linear terms for building the statistical models for the selectivity and capacity factors. The linear models were based on two PLS components. However, even when using a model based on linear terms, the Q^2 values for some of the studied solutes were found to be close to zero. This could be explained by a correlation between the retention of the first eluted enantiomer and the second eluted enantiomer. Almost any chromatographic condition in the experimental domain could generate the same results for these solutes, thus the difficulty to model some of the amino alcohols, with low Q^2 values as result.

The centrepoint experiments (the three runs added to the eight corner experiments) estimated the pure experimental uncertainty of the study and proved to have low variability. The relative standard deviation

Table 3
Influence of mobile phase amine[a]

Amine	k_1	k_2	α	N_1	N_2
DEA	1.52	2.25	1.48	3450	5120
DMOA	1.82	2.59	1.42	730	890
n-Hexylamine	1.46	2.11	1.45	2690	3240
Ethanolamine	1.27	1.95	1.53	4560	1790
Triethylamine	1.76	2.48	1.41	1500	1650
tert.-Octylamine	1.64	2.31	1.41	1600	2200

[a] Chromatographic conditions: mobile phase, 10 m*M* amine in *n*-hexane:2-propanol (9:1); column temperature, 30°C.

($SD_{cp}/x_{cp} \times 100$) ranged from 0 to 0.73% for k and α.

3.3. Influence of content of 2-propanol, acetic acid and water on separation factors (α) and capacity factors (k)

3.3.1. Modelled separation factors (α)

The correlation between the chromatographic variables (2-propanol, acetic acid and water) and the selectivity factors is illustrated in the loading plot depicted in Fig. 3a. If a given variable has a negative position in the loading plot in comparison to a response, a decrease of the variable gives an increase of the response, e.g., solute 12 and acid concentration (A). Contrary if a descriptor variable is located close to a response, an increase of the variable increases the response, e.g., solute 15 and modifier concentration (M). The loading plot in Fig. 3a shows four subgroups, where each subgroup is differently influenced by the chromatographic variables. However, there are no simple relations for the members of the different subgroups in structural resemblance.

The coefficient plots in Fig. 4 show the influence of the mobile phase additives on four selected solutes from each of the four subgroups in Fig. 3a. The influence of the variables on enantioselectivity for the chosen solutes from the four subgroups differs as shown in Fig. 4. The coefficient plots in Fig. 4 give the influence of the experimental variables on the selectivity factors for the selected solutes.

The content of acetic acid (A) has a positive correlation to the enantioselectivity for solutes 1, 3, 4, 8, 11, 13 and 16. An increase of the acetic acid concentration in the mobile phase also increases the enantioselectivity as depicted in Table 4. On the other hand, there is a negative correlation of acetic acid to the solutes 5, 9 and 12. An increase of the acetic acid concentration decreases the enantioselectivity for these solutes.

The amount of added water (W) to the mobile phase is negatively correlated to the enantioselectivity of all of the studied solutes as shown in Fig. 3a. An increased amount of water added to the mobile phase decreased the enantioselectivity.

The concentration of 2-propanol (M) has a positive correlation to enantioselectivity of all of the studied solutes, that is increased content of mobile phase 2-propanol also increases enantioselectivity for all of the solutes, Fig. 3a.

As a supplement, the correlation between the chromatographic variables and the selectivity factors of all the solutes is further explained in Table 4. The first principal component (PC1, along the x-axis) of the chemometrical model is described by mobile phase water content and acetic acid concentration. The second principal component (PC2, along the y-axis) contains most of the information and is described by all three variables.

3.3.2. Modelled capacity factors (k)

The correlation between the mobile phase components (concentration of 2-propanol, acetic acid and water) and the capacity factors is illustrated in the loading plot depicted in Fig. 3b. The loading plot of the capacity factors indicates no subgroups, hence the capacity factors are similarly affected by the chromatographic variables.

The first principal component (PC1, along the x-axis) of the chemometrical model is described by mobile phase modifier content and acetic acid concentration. The second principal component (PC2, along the y-axis), which contains as much information as the first principal component is described by the same variables.

The content of acetic acid (A) in the mobile phase has a positive correlation to the retention for all of the solutes studied. This means that an increase in acetic acid concentration also increased the retention.

The water concentration (W) in the range used throughout this study, 28–83 mM, showed only a minor effect on the retention. This can also be shown in the loading plot in Fig. 3b where water is situated close to origin.

The effect of the mobile phase modifier concentration on enantioselective retention is given in Fig. 3b. An increase of the mobile phase 2-propanol concentration decreased the retention, which is the common effect in liquid chromatography.

3.4. Influence of solute structure on enantioselectivity

A comparison of the influence of solute structure on enantioselectivity was performed using the cen-

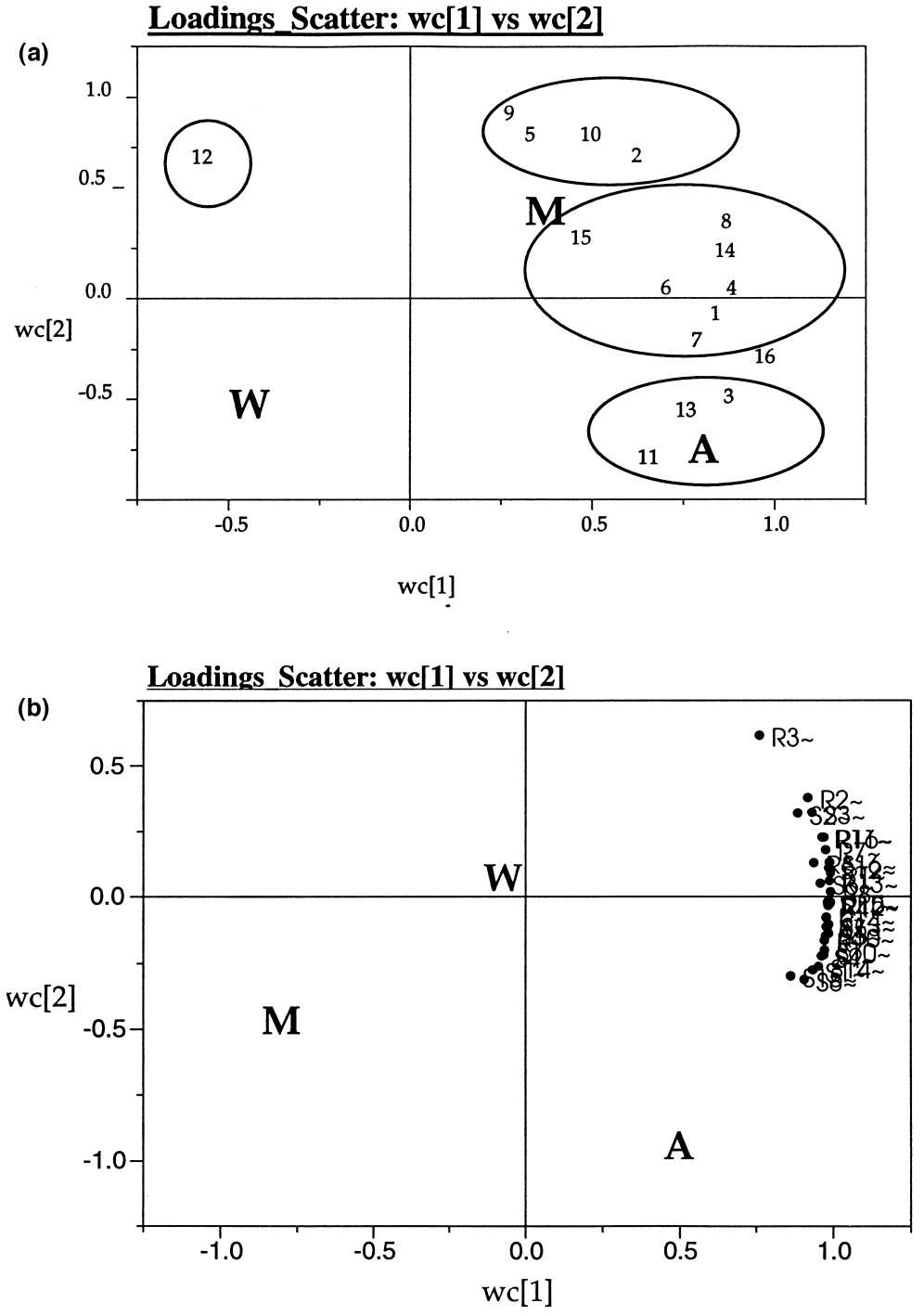


Fig. 3. Descriptors from mobile phases depicted in Table 1 (w) and response (c) variable intercorrelations and sizes (i.e. w1 versus w2 and c1 versus c2 superimposed) describing the influence of variables on responses; (a) selectivity factors, α, and (b) logarithmed capacity factors of the enantiomers, log k.

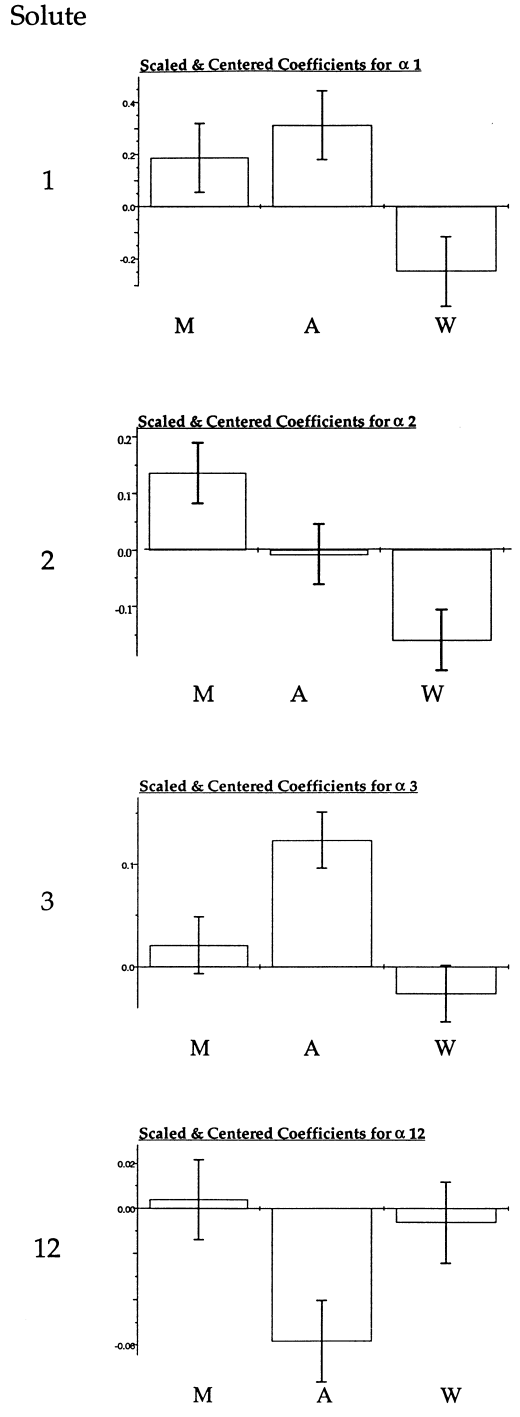


Fig. 4. Influence of mobile phase parameters on enantioselectivity for four selected solutes. Coefficients of model terms for four of the solutes selected from the subgroups in the loading plot in Fig. 3.

Table 4
Influence of mobile phase additives (2-propanol, M, water, W and acetic acid, A) giving significant influence on the selectivity—supplement to the loading plot in Fig. 3

Solute	Positive influence-increasing of α	Negative influence-decreasing of α
1	M, A	W
2	M	W
3	A	
4	M, A	W
5	M	A, W
8	M, A	W
9	M	A, W
10	M	W
11	A	
12		A
13	A	
16	A	

trepoint conditions. The mobile phase consisted of 10 m*M* DEA, 10 m*M* acetic acid, 56 m*M* water in 20% 2-propanol in isohexane.

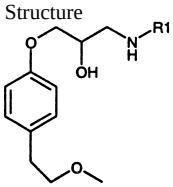
The enantioselectivity varied over a wide range in this study from $\alpha = 1.09$ to $\alpha = 9.10$. The largest α-value was obtained for solute 4 that had a methylethoxy substituent in *meta*-position and the smallest α-value was obtained for solute 12, an aldehyde substituted in *ortho*-position. However, in general, selectivity factors from 2 to 5 were observed.

Solutes of different size and flexibility regarding the substituent connected to the nitrogen atom were compared. Increased degree of branching of the substituent, from *n*-propyl (7) via isopropyl (1, metoprolol) to tertiary butyl (8) nearly doubled the α-value from $\alpha_{n\text{-propyl}} = 1.98$ to $\alpha_{\text{tertiary butyl}} = 3.56$ as shown in Table 5A. The enantioselectivity was found to be higher for solutes prepared with a less flexible and more bulky substituent. For example, when a tertiary butyl group is connected to the nitrogen atom compared to a more flexible substituent such as *n*-propyl, the separation factor is higher.

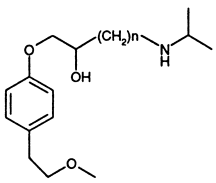
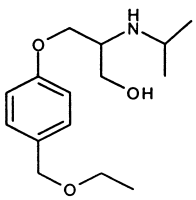
The influence of increased distance between the stereogenic centre and the nitrogen atom in the β-side chain was evaluated. The enantioselective retention for metoprolol, solute 1 and solutes 2, 3 and 11 were studied. An increased distance from 1 to 3 methylene groups between the stereogenic centre and the nitrogen atom decreased the enantioselec-

Table 5
Influence of solute structure on enantioselectivity[a]

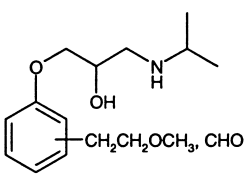
A. Different alkyl groups attached to the amine function

Solute	Structure	Substituent R_1	α
			
7		*n*-Propyl	1.98
Metoprolol (1)		Isopropyl	2.82
8		Tertiary butyl	3.56

B. Increased distance between the stereogenic centre and the amine function

Solute	Structure	Number of carbons in the β-side chain	α
			
Metoprolol (1)		$n=1$	2.82
2		$n=2$	2.03
3		$n=3$	1.18
			
11		β-Hydroxy	1.11

C. Positional isomers.

Solute	Structure	*Ortho*-, *meta*- or *para*-position	α
			
Metoprolol (1)		*Para*	2.82
4		*Meta*	9.10
5		*Ortho*	1.83
12		Aldehyde in *ortho*	1.08
13		Aldehyde in *meta*	2.65

D. Saturation or unsaturation.

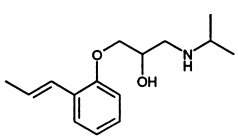
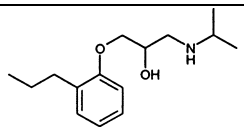
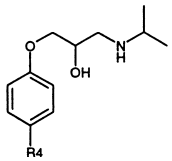
Solute	Structure	Substituent in *ortho*-position	α
Alprenolol (9)		Unsaturated in *ortho*	2.39

Table 5 (*continued*)

10		Saturated in *ortho*	1.93

E. Different *para*-substituents.

Solute	Structure	Substituent in *para*-position	α
			
Metoprolol (1)		Ethylmethoxy	2.82
6		Urea	1.67
14		Unsubstituted	7.99
15		Phenyl	5.02
Atenolol (16)		Amide	1.53

[a] Chromatographic conditions: mobile phase, 10 m*M* DEA, 10 m*M* acetic acid and 56 m*M* water in isohexane–2-propanol (4:1), (centrepoint conditions); column temperature, 30°C.

tivity from $\alpha_{n=1}=2.82$ to $\alpha_{n=2}=1.18$ as shown in Table 5B. The solute without methylene groups, 11, had an α-value of 1.11. A possible explanation for the large difference in enantioselectivity can be that the flexibility of a shorter β-side chain in for example metoprolol, solute 1 ($n=1$) is lower compared for the longer β-side chain in for example solute 3 ($n=3$). This may give a less pronounced difference in binding to the chiral selector for the (*R*) enantiomers compared to the (*S*) enantiomers depending on the flexibility of the solutes. Another explanation might be that the distance between the stereogenic centre and the nitrogen atom for metoprolol ($n=1$) solute 1, favours a better fit into the chiral adsorption site.

Positional stereoisomers differing in *ortho*-, *meta*- and *para*-position substituted with either ethylmethoxy substituents (solutes 1, 4 and 5) or aldehyde substituents (solutes 12 and 13) were compared regarding enantioselectivity. There is a large variation in enantioselectivity for the metoprolol positional isomers as shown in Table 5C. The enantioselectivity increases in order *ortho*<*para*<*meta* from $\alpha=1.83$ (*ortho*) compared to $\alpha=9.10$ (*meta*). The enantioselectivity is also larger for the *meta* analogue substituted with an aldehyde functionality compared to the *ortho* analogue, $\alpha=1.08$ to $\alpha=2.65$. The difference in enantioselectivity for the *ortho*, *para* and *meta* analogues may be due to differences in surface properties. The *ortho* analogue has a smaller surface area than the *meta* analogue due to hydrogen bonding between the oxygen atom in the *ortho* substituent and the hydroxyl group in the β-chain. Therefore, the enantiomers of an *ortho* substituted solute may be more similar than the enantiomers of a molecule with a larger surface area, for example a *meta* substituted solute.

The small difference in enantioselectivity for two solutes differing in level of saturation of their *ortho* positioned side chain was examined as shown in Table 5D. The highest enantioselectivity was obtained for the unsaturated solute (alprenolol, solute 9) substituted in *ortho*-position with an $\alpha=2.39$ compared to $\alpha=1.93$ for the saturated solute (10). This minor difference in enantioselectivity was expected as previous results [20] indicated that the *ortho*-positioned side chain also had little influence on the chiral recognition.

The enantioselectivity of stereoisomers differing in *para*-position was compared for five amino alcohols (metoprolol, atenolol and solutes 6, 14, 15) as shown in Table 5E. Enantioselectivity decreases with increasing steric hindrance and electron withdrawing properties of the *para* substituent, from $\alpha=7.99$ for

the unsubstituted solute 14 to $\alpha = 1.53$ for the amide substituted solute 16. Solute 15, substituted with a biphenyl had rather high separation factor; $\alpha = 5.02$, probably due to good fit of the substituent into the chiral selector.

The experimental results obtained in this study using liquid chromatography was in accordance with previous results using packed column SFC and Chiralcel OD as the chiral stationary phase [20].

The chiral recognition mechanism was previously studied for various chiral compounds using the Chiralcel OD stationary phase in combination with SFC [6] and liquid chromatography [6,11–13]. A number of propanol analogues have been examined [11] revealing that the (*R*)-enantiomers elutes before the (*S*)-enantiomers. This chromatographic behaviour was also found for the amino alcohols in this study. The impact of substituents of a series of 2-amidotetralins using the Chiralcel OD stationary phases in SFC have recently been studied. Small changes in the chemical structure may have a large effect on the enantioselectivity [12].

3.5. Reversal of retention order

The enantioseparation of the amino alcohols were differently affected by the chromatographic variables depending on subgroup participation in the loading plot as shown in Fig. 3a. Four solutes were selected, one from each subgroup in order to study reversal of retention order. The influence of chromatographic variables (acetic acid, water, 2-propanol and column temperature) on the enantioselectivity of the selected solutes (1, 2, 3, 12) was then studied univariately.

Addition of acetic acid in a concentration range of 0 to 25 m*M* resulted in reversal of retention order of the enantiomers of solute 3 as shown in the chromatograms in Fig. 5 and also in Fig. 6a. The enantioselective retention of solute 2, which contains one methylene group less than solute 3, was not influenced by an increased content of acetic acid in the mobile phase, as shown in Fig. 6b.

The concentration level of 5 m*M* acetic acid was thereafter kept constant while the content of 2-propanol, water and column temperature was changed in order to investigate if these parameters also could influence the retention order of the enantiomers of solute 3.

Of the variables examined, the amount of water proved to be very important for the enantioselectivity of solute 3 as shown in Fig. 7. Reversal of retention order could be obtained when the content of water was changed from 28–112 m*M*. The influence of water content in the mobile phase on the enantioselectivity of amino alcohols has earlier been discussed by Balmér et al. [8].

The effect of column temperature on the enantioselectivity for solutes 1 and 3 in a range of temperature between −8 to 40°C was studied. Separation of the enantiomers of solute 3 was observed using temperatures below 25°C but no reversal of retention order could be obtained. However, previously reversal of retention order has been observed for solute 3 using a mobile phase without acetic acid [8].

The rather small effects of column temperature on retention and selectivity of metoprolol, solute 1 in this study, are shown in Table 2. There is almost no change in selectivity using a temperature in the range −8°C to 40°C. This was rather surprising as column temperature generally is known to be an important variable in normal-phase chromatography. The enantioselectivity typically decreases as the column temperature increases [8].

The influence of content of 2-propanol on the enantioselectivity of solute 3 was also studied. A change in content of 2-propanol also changes the content of water. A change in concentration of 2-propanol from 20% to 30% had no influence of the enantioseparation of solute 3.

3.6. Influence of carboxylic acids on the enantioselectivity

The influence of addition of carboxylic acids of different chain length to the mobile phase on the enantioselectivity of solute 3 was investigated. The carboxylic acids, from formic acid, ($n = 1$) to nonanic acid, ($n = 9$) were added to the mobile phase in two concentration levels, 5 and 25 m*M* as shown in Fig. 8. The enantioselective retention of solute 3 decreased with increasing number of methylene groups in the carboxylic acids.

3.7. Enantiomeric purity

The enantiomeric purity could be determined also

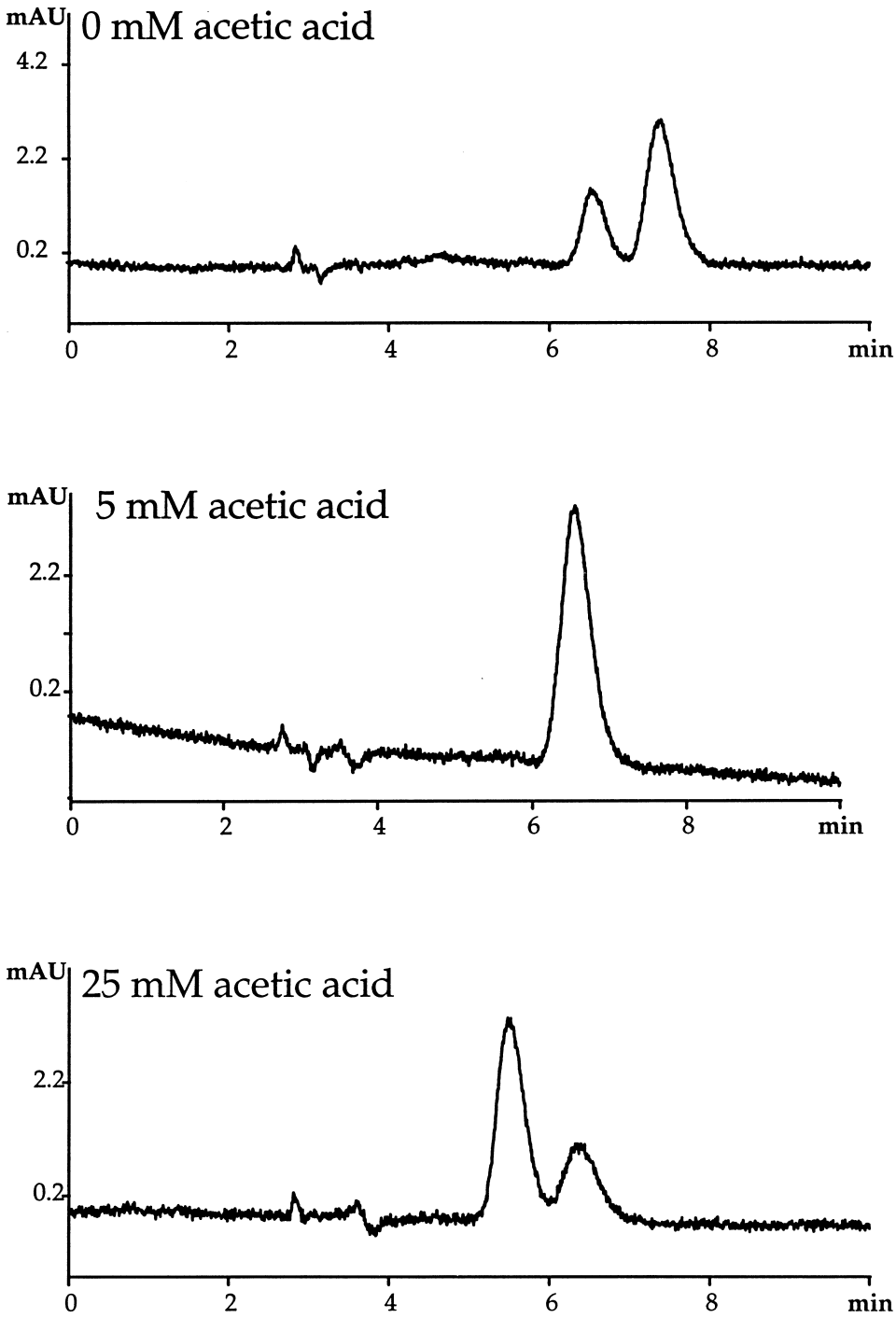


Fig. 5. Influence of acetic acid on enantioselectivity of solute 3. Chromatographic conditions: column temperature, 30°C; flow-rate, 1 ml min^{-1}; mobile phase, 10 m*M* DEA and 83 m*M* water in *n*-hexane–2-propanol (3:1).

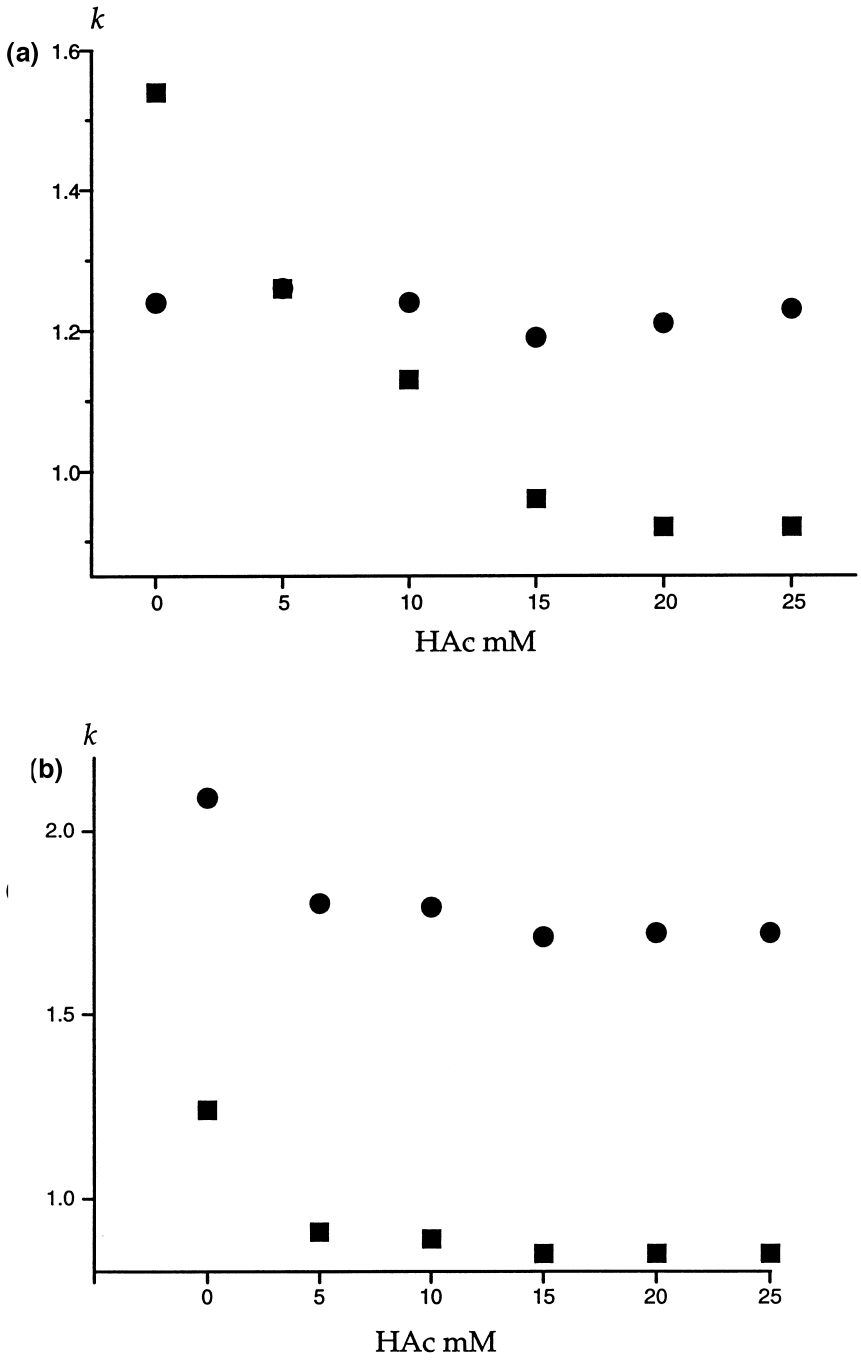


Fig. 6. Influence of acetic acid on retention of (a) solute 2, (b) solute 3. Chromatographic conditions: column temperature, 30°C; flow-rate, 1 ml min^{-1}; mobile phase, 10 m*M* DEA and 83 m*M* water in *n*-hexane–2-propanol (3:1).

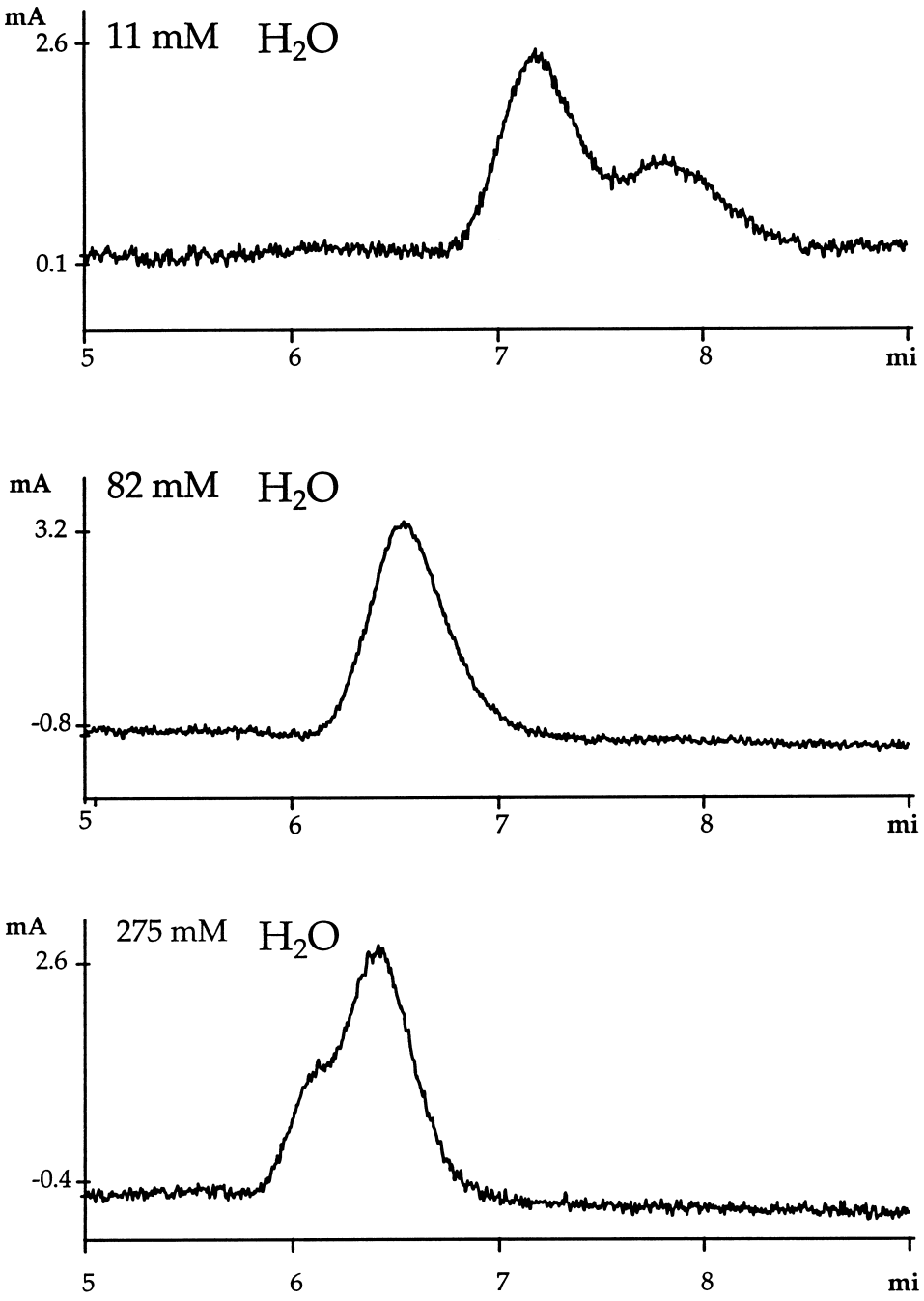


Fig. 7. Influence of water content on the enantioselectivity of solute 3. Chromatographic conditions: column temperature, 30°C; flow-rate, 1 ml min^{-1}; mobile phase, 10 m*M* DEA and 5 m*M* acetic in *n*-hexane–2-propanol (3:1).

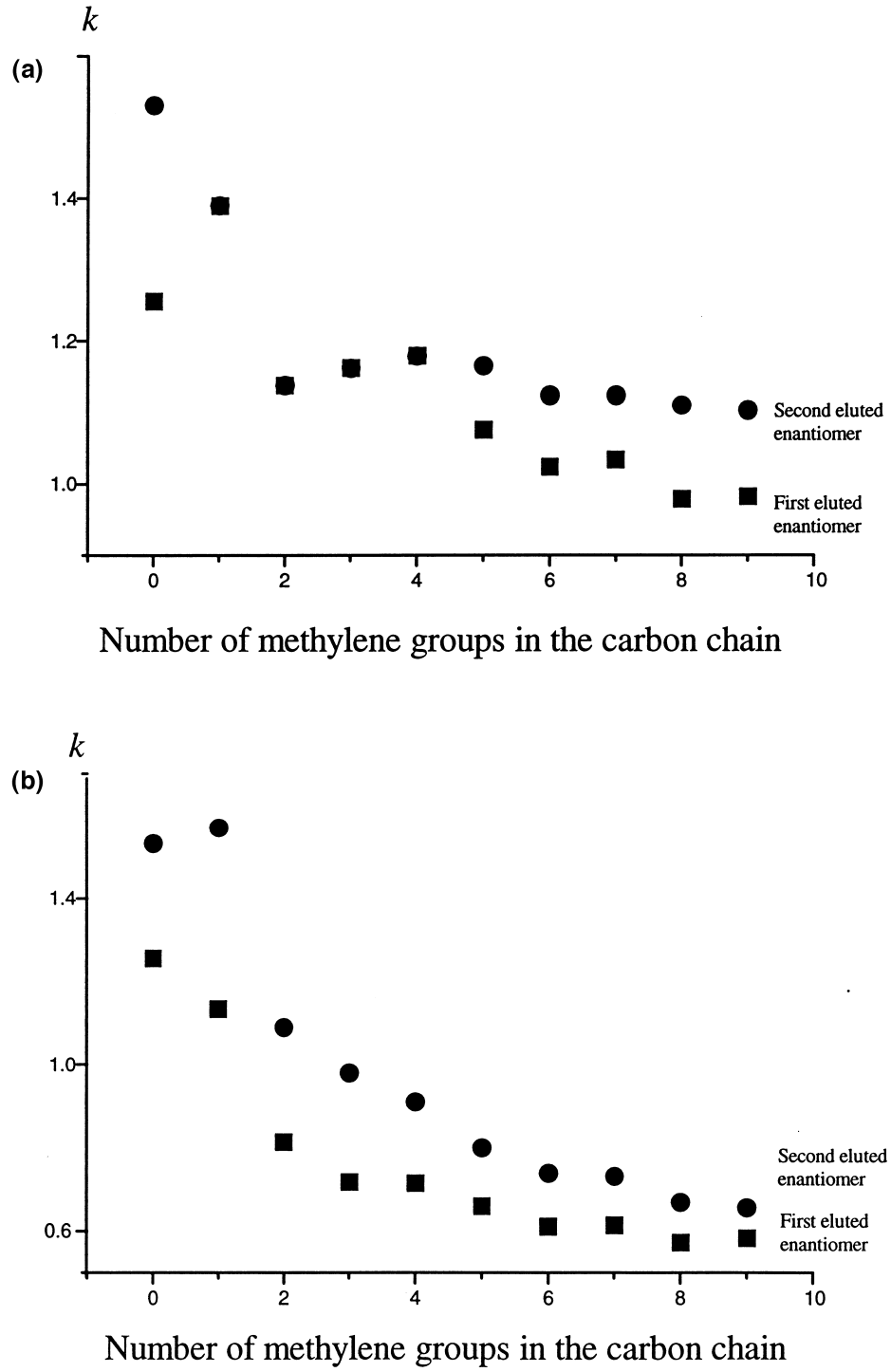


Fig. 8. Influence of carboxylic acids differing in chain length on enantioselectivity of solute 3. Chromatographic conditions: column temperature, 30°C; flow-rate, 1 ml min^{-1}; mobile phase, 10 m*M* DEA and 83 m*M* water in isohexane–2-propanol (3:1). (a) 5 m*M* Carboxylic acid. (b) 25 m*M* Carboxylic acid.

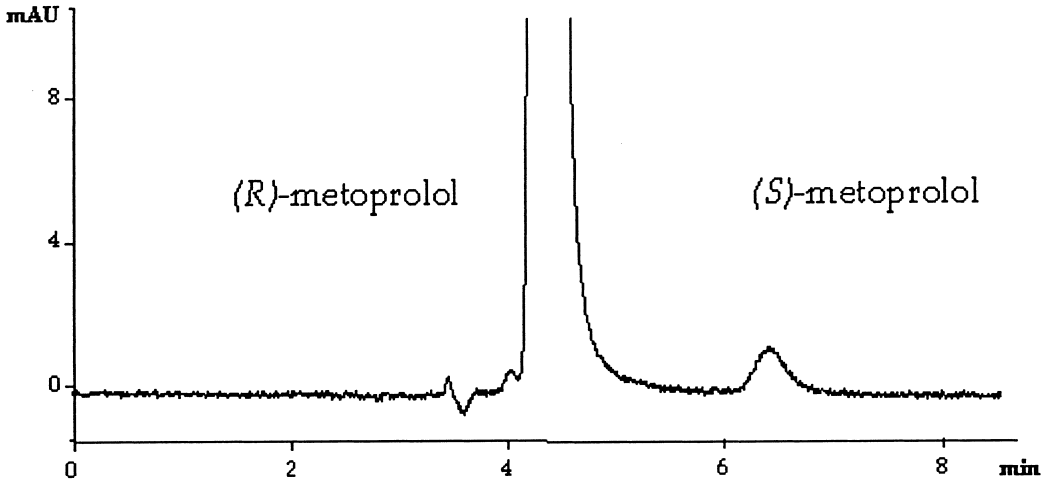


Fig. 9. Enantiomeric purity of (*R*)-metoprolol, solute 1. Chromatographic conditions: column temperature, 30°C; flow-rate, 1 ml min^{-1}; mobile phase, 10 m*M* DEA, 28 m*M* water and 5 m*M* acetic acid in *n*-hexane:2-propanol (85:15); 0.9% (*S*)-metoprolol in (*R*)-metoprolol.

when the chiral impurity eluted after the main peak due to high enantioselectivity in the studied chromatographic systems. The enantiomeric purity of (*R*)-metoprolol, solute 1 is shown in Fig. 9. The studied sample contained 0.9% (*S*)-metoprolol.

4. Conclusions

The chromatography of sixteen amino alcohols has been studied using Chiralcel OD as chiral stationary phase in normal-phase liquid chromatography.

A statistical experimental design with centrepoints was used in order to evaluate the influence of content of 2-propanol, water and acetic acid on selectivity and capacity factors. The experimental data were evaluated with multivariate analyses. The statistical models obtained were described using linear terms, without interaction- and quadratic-terms. The chromatographic variables were all important for the enantioselectivity and the studied amino alcohols could be divided into four subgroups. High separation factors, generally between 2 and 9 were obtained.

The influence of water on the equilibration time was studied and fast equilibration times were observed. Constant separation factors were obtained almost immediately.

The retention order of the (*R*)-3 and (*S*)-3 could be controlled by either the content of water or acetic acid. The influence of addition of carboxylic acids to the mobile phase on the enantioselectivity has been investigated.

The influence of solute structure on enantioselectivity has been discussed in this work.

The chromatographic systems could also be used to evaluate the enantiomeric purity of the amino alcohols also when the chiral impurity eluted after the main peak.

References

[1] E.J. Ariens, Eur. J. Clin. Pharmacol. 26 (1984) 663.
[2] A.M. Barett, V.A. Cullman, Br. J. Pharmacol. 34 (1968) 43.
[3] H.Y. Aboul-Enein, M.R. Islam, Anal. Lett. 23 (1990) 937.
[4] H.Y. Aboul-Enein, M.R. Islam, J. Chromatogr. A 511 (1990) 109.
[5] Y. Okamoto, M. Kawashima, R. Aburatani, K. Hatada, T. Nishiyama, M. Masuda, Chem. Lett. 7 (1986) 1237.
[6] N. Bargmann-Leyder, A. Tambuté, M. Caude, Chirality 7 (1995) 311.
[7] Y. Okamoto, R. Aburatani, Y. Kaida, K. Hatada, N. Inotsume, M. Nakano, Chirality 1 (1989) 239.
[8] K. Balmér, P.-O. Lagerström, S. Larsson, B.-A. Persson, G. Schill, J. Chromatogr. A 592 (1992) 331.
[9] E. Küsters, C. Spöndlin, J. Chromatogr. A 737 (1996) 333.
[10] Y. Okamoto, Y. Kaida, J. Chromatogr. A 666 (1994) 403.
[11] H.Y. Aboul-Enein, L.I. Abou-Basha, S.A. Bakr, Chirality 8 (1996) 153.

[12] U. Selditz, S. Copinga, J.P. Franke, H. Wikström, R.A. de Zeeuw, Chirality 8 (1996) 574.
[13] D.T. Witte, J.P. Franke, F.J. Bruggeman, D. Dijkstra, R.A. de Zeeuw, Chirality 4 (1992) 389.
[14] K.M. Kirkland, J. Chromatogr. A 718 (1995) 9.
[15] K. Balmér, A. Persson, P.O. Lagerström, B.A. Persson, G. Schill, J. Chromatogr. A 553 (1991) 391.
[16] A. Kot, P. Sandra, A. Venema, J. Chromatogr. Sci. 32 (1994) 439.
[17] P. Biermanns, C. Miller, V. Lyon, W. Wilson, LC·GC 11 (1993) 744.
[18] Y. Kaida, Y. Okamoto, Bull. Chem. Soc. Jpn. 65 (1992) 2286.
[19] H. Nishi, S. Terabe, J. Chromatogr. A 694 (1995) 245.
[20] S. Svensson, A. Karlsson, O. Gyllenhaal, in manuscript.
[21] A.M. Andersson, A. Karlsson, M. Josefson, J. Gottfries, Chromatographia 38 (1994) 715.
[22] E. Morgan, Chemometrics: Experimental Design: Analytical Chemistry by Open Learning, Wiley, Chichester, 1991.
[23] G.E.P. Box, W.G. Hunter, J.S. Hunter, Statistics For Experimenters, An Introduction to Design, Data Analysis and Model Building, Wiley, Chichester, 1978.
[24] A. Höskuldsson, J. Chemometr. 2 (1988) 211.