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Sparteine as mobile phase modifier in the chiral separation of hydrophobic basic drugs on an α_1-acid glycoprotein column

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Abstract

The effect of the chiral diamine sparteine as a cationic mobile phase modifier on the retention and enantioseparation of five hydrophobic basic drugs on an α_1-acid glycoprotein column was studied. Sparteine produced good results over the whole range of its concentration (1–15 m*M*) in the phosphate buffer mobile phase at pH 6.0 for the selected drugs (alprenolol, propranolol, promethazine, chlorpheniramine and disopyramide). When used as a sole modifier, sparteine greatly reduced retention without significant interference in the enantioseparation of the compounds. Sparteine was also applied in combination with 2.5% of the neutral modifier 2-propanol with good results as well. This study shows sparteine's potential both as a substitute for neutral mobile phase modifier and as an auxiliary in reducing amounts of neutral modifier.

Keywords: Enantiomer separation; Mobile phase composition; α_1-Acid glycoprotein; Sparteine; Alprenolol; Propranolol; Promethazine; Chlorpheniramine; Diisopyramide

1. Introduction

α_1-Acid glycoprotein (α-AGP) has been successfully applied as an immobilized chiral selector in high-performance liquid chromatography (HPLC) enantiomeric separations of many compounds with different structural characteristics [1–12].

The wide applicability of this chiral selector can be ascribed to the fact that it has binding sites, which contain many binding groups of different character. This gives it the possibility to interact stereoselectively with a broad range of solutes such as amines, acids and non-protolytes [2]. The selector, α-acid glycoprotein, is made up of a single 181-unit peptide chain linked to five carbohydrate units via the asparagine residues [13]. The carbohydrate moiety of α-AGP contains 14 sialic acid residues, which make it a very acidic protein with an isoelectric point of 2.7 [13].

The main binding site of this protein is thought to be a hydrophobic pocket which is formed by an enrichment of hydrophobic amino acid residues such tryptophan, phenylalanine and leucine [13]. Ionic interactions are also involved, because over the usual pH range 5–7 the protein has a net negative charge which is responsible for the high retention of cationic solutes [1].

Probably the most interesting property of immobilised α-AGP is that the character of this selector can be dramatically changed by a simple modification in

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the mobile phase composition [1,5–7]. These dynamic modifications on the immobilised α-AGP are also accompanied by dramatic changes in the retention and enantioresolution of the analytes [1,2,4]. In particular, retention and enantioseparation can be regulated by changing ionic strength, pH and concentration of organic charged or uncharged modifiers [3,5–7,12].

Charged modifiers such as dimethyloctylamine or octanoic acid have been used as mobile phase additives [2,3]. For some analytes they improved the enantioresolutions and for the others they totally destroyed it. Uncharged modifiers such as propanol, ethanol and acetonitrile have also been extensively used as mobile phase modifiers to shorten retention times, but in most cases an increase of their concentration results in progressive decrease in stereoselectivity. Moreover, high concentration of these modifiers (>20%) can compromise the protein column life.

Micellar mobile phases have also been used with fairly good results [3,4] and short chain aliphatic carboxylic acids proved to be useful for the enantioseparation of the β-blocker sotalol [12].

The aim of this study was to investigate the potential of sparteine as a mobile phase cationic modifier, in order to reduce retention on α-AGP column, while maintaining enantioselectivity for hydrophobic basic drugs. Sparteine, a chiral diamine, presents a basic bis-quinolizidine system made of four rings (Fig. 1). Rings A and B form a double chair system of *trans*-quinolizidine which is relatively resistant to conformational–configurational changes, while the second system C–D is more vulnerable to inversion on the N-16 atom and it may occur in *cis*-all chair conformation when N-16 is protonated [14] (Fig. 1). The structural properties of sparteine have been used by Okamoto et al. as chiral initiator to generate optically active polymers which after coating on a silica matrix, will give chiral stationary phases [15]. The chiral and hydrophobic (log P=2.15) properties of sparteine, used also as chiral auxiliary for asymmetric syntheses [16,17], aroused our interest on its utility as a cationic mobile phase modifier in chiral separations. The compound, moreover, is an inexpensive, commercially available product not absorbing over the usual UV detection range. Therefore, sparteine was investigated both as the sole mobile phase additive and also in combination with the neutral additive 2-propanol for the

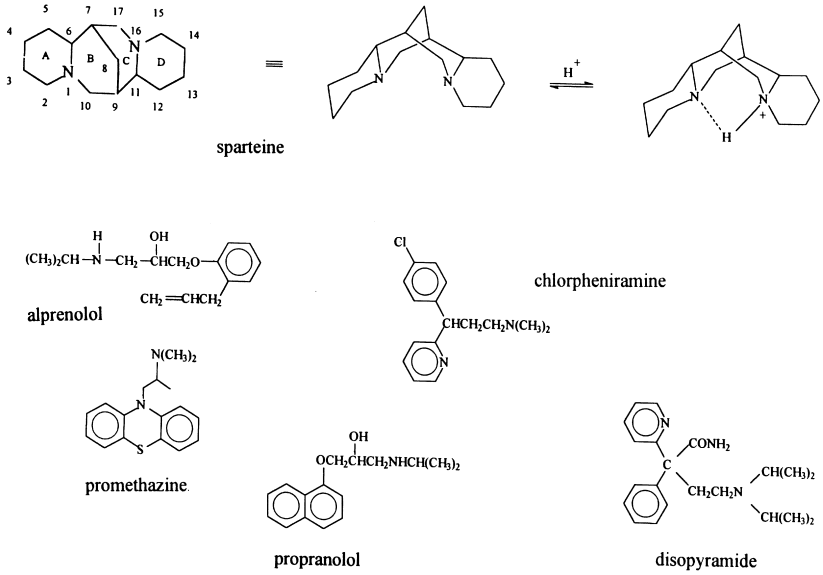

Fig. 1. Structural formulas of sparteine and the examined racemic basic drugs.

enantioresolution of five selected hydrophobic basic drugs which usually are too strongly retained on an α-AGP column [1].

2. Experimental

2.1. Materials

Chlorpheniramine maleate, disopyramide phosphate, promethazine hydrochloride and propanolol hydrochloride were bought from Sigma (St. Louis, MO, USA). Alprenolol hydrochloride was obtained from Euderma (Cerasolo di Coriano, Italy).

Sparteine sulphate was purchased from Fluka (Buchs, Switzerland).

Potassium monohydrogen phosphate came from Merck (Darmstadt, Germany). 2-Propanol and phosphoric acid were bought from Carlo Erba Reagents (Milan, Italy) and 1-propanol was bought from Romils (Cambridge, UK).

The structures of the compounds are shown in Fig. 1.

2.2. Apparatus

The chromatographic system consisted of a Waters Automated gradient controller connected to a Waters 501 pump and equipped with a Rheodyne Model 7125 injector with a 20-μl loop and an ERC-3312 degasser. The detector used was a Jasco 875 UV and was connected to an HP 3396 series II integrator. For all the analyses, the detection wavelength was 275 nm.

2.3. Chromatographic conditions

Analyses were conducted at room temperature. All buffers used were 20 m*M* in phosphate. The column used was a chiral α-AGP prepared as previously described [8]. The flow-rate was 0.8 ml/min.

2.4. Sample preparation

The solutions were prepared by dissolving about 2 mg of each analyte in 10 ml of 1-propanol. These solutions were then diluted four times with pH 6.0 phosphate buffer solution. A 20 μl volume of the diluted solution was injected into the column.

2.5. Mobile phase preparation

All mobile phases with and without sparteine consisted of 20 m*M* phosphate buffer solution of appropriate pH.

The buffer stock solution was prepared by dissolving the appropriate amount of potassium monohydrogen phosphate in bidistilled water.

To prepare a mobile phase with sparteine as the only modifier, appropriate amounts of sparteine sulphate were dissolved in about 200 ml of the basic buffer stock solution in a beaker. The pH went down and was adjusted to the required value by adding the same phosphate stock solution. This sparteine solution was then transferred into a volumetric flask (1000 ml) and put to volume with a 20 m*M* phosphate buffer solution already adjusted to the same pH as the sparteine solution. The pH value of 20 m*M* phosphate buffers was adjusted by adding phosphoric acid to the basic buffer stock solution.

To prepare the hydro–organic mobile phases, 2-propanol was first put into the volumetric flask. The appropriate buffer (prepared as described above) was then added to the 2-propanol up to the volume mark. Before use each mobile phase was sonicated for about 2 min.

3. Results and discussion

It is well known that at pH 7.0 basic drugs are strongly retained by α-AGP [1] owing to ionic binding; a reduction in mobile phase pH reduces the degree of negative charge of the protein and this results in decreased retention of protonated basic drugs [1,11].

On the other hand, previous works have shown that organic charged modifiers in the mobile phase can affect retention and enantioselectively when using immobilised protein stationary phases. This has been shown in the analysis of the basic compounds alimemazine, trimipramime, promethazine and propriomazine, using dimethyloctylamine (DMOA) as additive in the mobile phase and α-AGP column [2]. Increasing concentrations of DMOA

caused an increase in the enantioseparation of propriomazine and promethazine whereas it decreased the enantioseparation of trimipramine and alimemazine [2]. This shows how the effect of a charged modifier can be difficult to predict, given that alimemazine and promethazine are structural analogues but are affected differently by DMOA.

3.1. Sparteine as a charged modifier

In this study sparteine was used as cationic modifier varying its concentration from 0 to 15 m*M* in a mobile phase of phosphate buffer (0.02 *M*) at pH 6.0. This pH value was chosen according to previous studies [6,10–12] showing the pH range 5.0–7.0 to be optimal for the enantioselectivity of most cationic analytes.

Using mobile phase of only buffer solution (pH 6.0) the hydrophobic basic drugs alprenolol, propranolol, promethazine and disopyramide were not eluted after 115 min. For chlorpheniramine, the enantiomers were eluted at around 50 min with a very poor separation (α 1.13) and the peaks were broad (Fig. 2a).

With 1 m*M* sparteine in the mobile phase there was a dramatic decrease in the retention for chlorpheniramine and disopyramide. For chlorpeniramine, moreover, the enantiomers were completely separated within 26 min (α 1.45) with a simultaneous improvement in peak shape and symmetry (Fig. 2b).

Disopyramide enantiomers eluted with reasonable retention times (t_R 18.5 and 47.4) with wide peaks (Fig. 3a).

Successive increases in the concentration of sparteine produced narrower and more symmetric peaks with a slight loss in the enantioselectivity. For chlorpheniramine, acceptable separations were obtained up to 5 m*M* (Fig. 2c). For disopyramide (Fig. 3), promethazine (Fig. 4) and alprenolol (Fig. 5), increasing concentration of sparteine significantly reduced the retention times and maintained the separation factor virtually constant. This effect was also accompanied by an improvement in the peak shapes.

Of particular interest is the α value for alprenolol which remained at around 2, even with 15 m*M* sparteine, the highest concentration used (Fig. 5c). At this concentration, the alprenolol peaks were

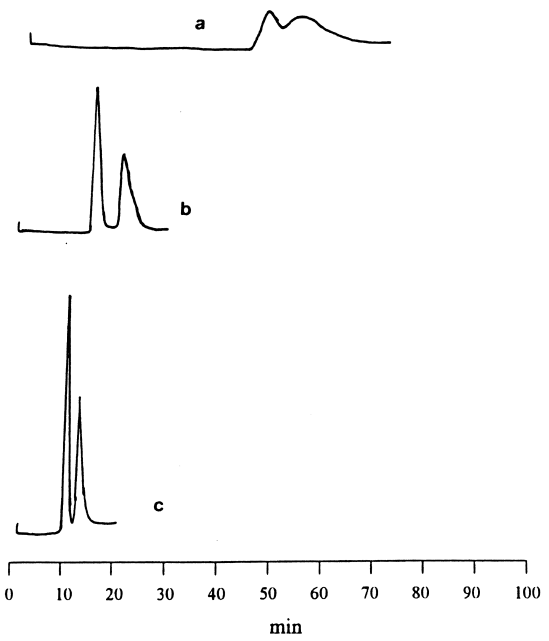

Fig. 2. Effect of sparteine concentration on the retention and enantioresolution of chlorpheniramine. Column: chiral AGP. Mobile phase: phosphate buffer (0.02 *M*; pH 6.0) without sparteine (a), with 1 m*M* sparteine (b) and 5 m*M* sparteine (c). Flow-rate: 0.8 ml/min. Detection: UV at 275 nm.

eluted in less than 30 min. At the same concentration the disopyramide peaks were eluted in 10 min and completely separated (Fig. 3c). For promethazine the elution was achieved within 30 min (Fig. 4).

For all concentrations of sparteine used, propranolol showed relatively poor chromatographic behaviour: the retention was high with wide peaks and α was 1.25 at 7 m*M* sparteine. Higher concentration of sparteine did not change both the retention and the enantioselectivity. Experiments at lower pH values (pH 5.0) confirmed the favourable results by Hermansson and Grahn [1] who obtained good enantioselectivity (α=1.52) working at pH 4.1. In fact, at pH 5.0 a significant reduction in the retention of propranolol was observed with α=1.20 (using 2.5% 2-propanol in phosphate buffer) and α=1.3 (using 8 m*M* sparteine in phosphate buffer).

The effect of the concentration of sparteine on retention and enantioselectivity for the selected basic drugs is graphically illustrated in Fig. 6. As can be seen, for alprenolol the good (α value of 2 is maintained with the simultaneous reduction in re-

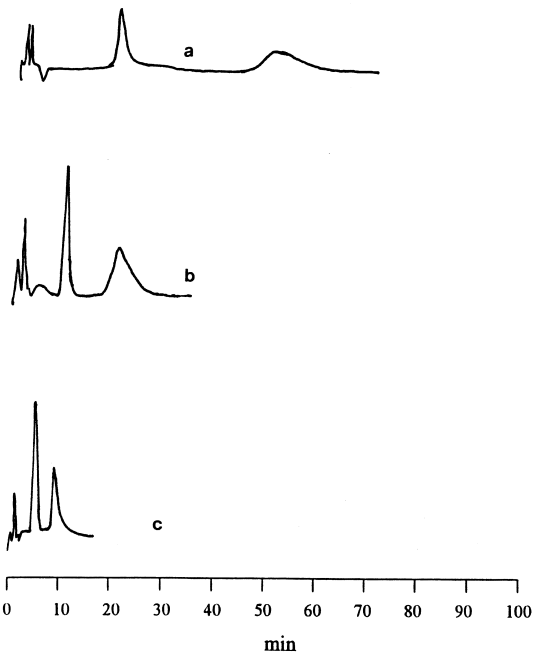

Fig. 3. Effect of sparteine concentration on the retention and enantioresolution of disopyramide. Column, detection and flow-rate as in Fig. 2. Mobile phase: phosphate buffer (0.02 *M*; pH 6.0) with 1 m*M* sparteine (a), 3 m*M* sparteine (b) and 15 m*M* sparteine (c).

tention time of both enantiomers. For promethazine and chlorpheniramine there is also a significant reduction in retention but with the selectivity well maintained at around 1.25. The same applies for disopyramide.

Sparteine acts similarly for all the components of this limited series of basic hydrophobic drugs. That is, there is a progressive decrease in retention for both enantiomers with increase in the sparteine concentration (Figs. 2, 3, 4a–c, 5a–c). Sparteine ($pK_{a1}=2.24$, $pK_{a2}=9.46$) in the monoprotonated form (Fig. 1) may be competing for hydrophobic non-stereoselective sites because even relatively high concentrations of the additive do not destroy the enantioselectivity. This hydrophobic competition by sparteine was confirmed in a conventional reversed-phase system. Using C_{18} Ultracarb stationary phase (30% of carbon loading), the introduction of sparteine in the mobile phase at 5 m*M* level (pH 6.0) resulted in a marked reduction of the retention of alprenolol, propranolol and pindolol.

Not to be excluded also is the possible competition by sparteine at cationic exchange sites on the α-AGP column, which has a predominantly negative charge at pH 6.0. It has to be pointed out that sparteine exhibits a characteristic convex shape. This leads to a partial shielding of the positive charge by the extended hydrophobic moiety of the molecule (Fig. 7). Calculations of the electronic distribution [19] of sparteine showed that the partial positive electrostatic charges are localized on the hydrogens of the methylene groups nearest to the protonated N-16 atom. Therefore, monoprotonated sparteine could predominantly compete with the analytes for the non-stereoselective hydrophobic sites of α-AGP and, to a less extent, for the anionic sites. It can be assumed that the ionic interactions, like the hydrophobic interactions, are of no or little enantioselectivity. However, further investigations on a larger variety of analytes and experimental conditions are necessary to better understand the role played by sparteine as a charged mobile phase modifier.

3.2. 2-Propanol as neutral modifier

The adsorption of uncharged modifiers on the protein affects the retention and the enantioselectivity. For certain solutes it is possible to improve the enantioselectivity by increasing the modifier concentration [7], but generally, increasing the concentration of neutral modifiers decreases retention and enantioselectivity.

In this work 2-propanol was chosen as a representative organic modifier to compare its effect with those of sparteine at pH 6.0. There was no enantioresolution for chlorpheniramine over the whole range of 2-propanol concentrations in the mobile phase (Fig. 8). This result contrasts with the enantioseparations obtained in the presence of sparteine (Figs. 6 and 2).

For promethazine there was an increase in the enantioselectivity from 1% to 2.5% 2-propanol with a decrease in retention. Further increases in 2-propanol reduced both retention and enantioselectivity. There was high retention for propranolol and alprenolol at 1% 2-propanol with a good enantioselectivity for alprenolol (Fig. 8). High concentration of 2-propanol produced sharp decreases in retention, while the enantioselectivity steadily decreased with a

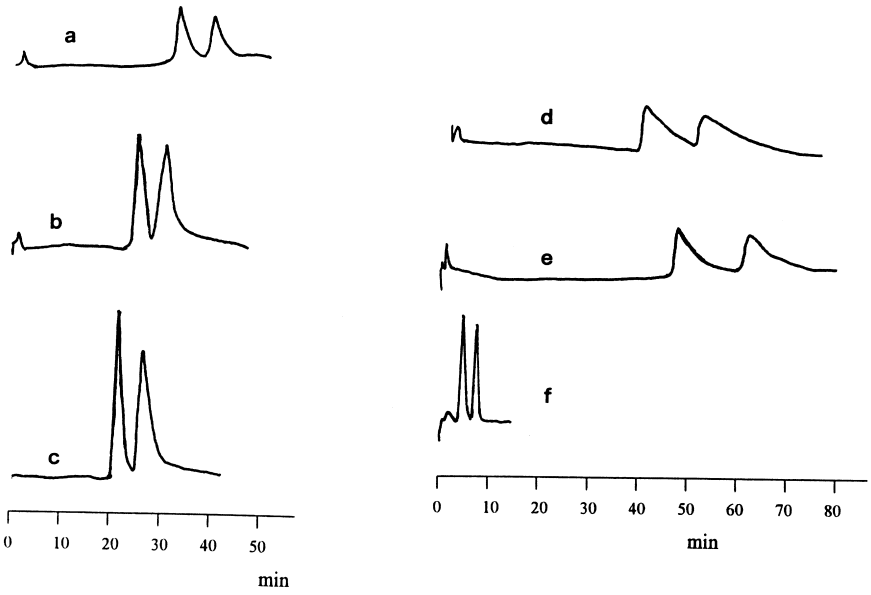

Fig. 4. Effect of sparteine concentration on retention and enantioresolution of promethazine. Column, detection and flow-rate as in Fig. 2. Mobile phase: phosphate buffer (0.02 *M*; pH 6.0) with 7 m*M* sparteine (a), 11 m*M* sparteine (b), 15 m*M* sparteine (c), with 2.5% 2-propanol and 5 m*M* 1,8-diaminooctane (d), 2.5% 2-propanol (e), and 2-propanol–5 m*M* sparteine in buffer solution (2.5:97.5, v/v) (f).

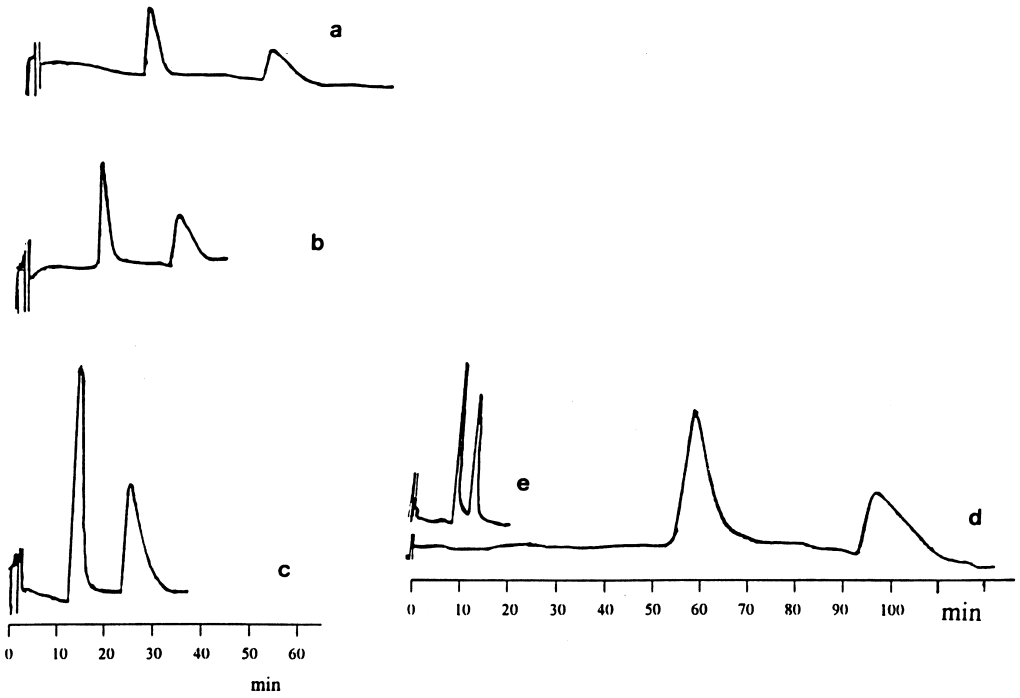

Fig. 5. Effect of sparteine concentration on the retention and enantioresolution of racemic alprenolol. Column, detection and flow-rate as in Fig. 2. Mobile phase A: phosphate buffer (0.02 *M*; pH 6.0) with 7 m*M* sparteine (a), 11 m*M* sparteine (b) and 15 m*M* sparteine (c). Mobile phase B: phosphate buffer (0.02 *M*; pH 7.0) with 2.5% 2-propanol (d) and with 2.5% 2-propanol and 5 m*M* sparteine (e).

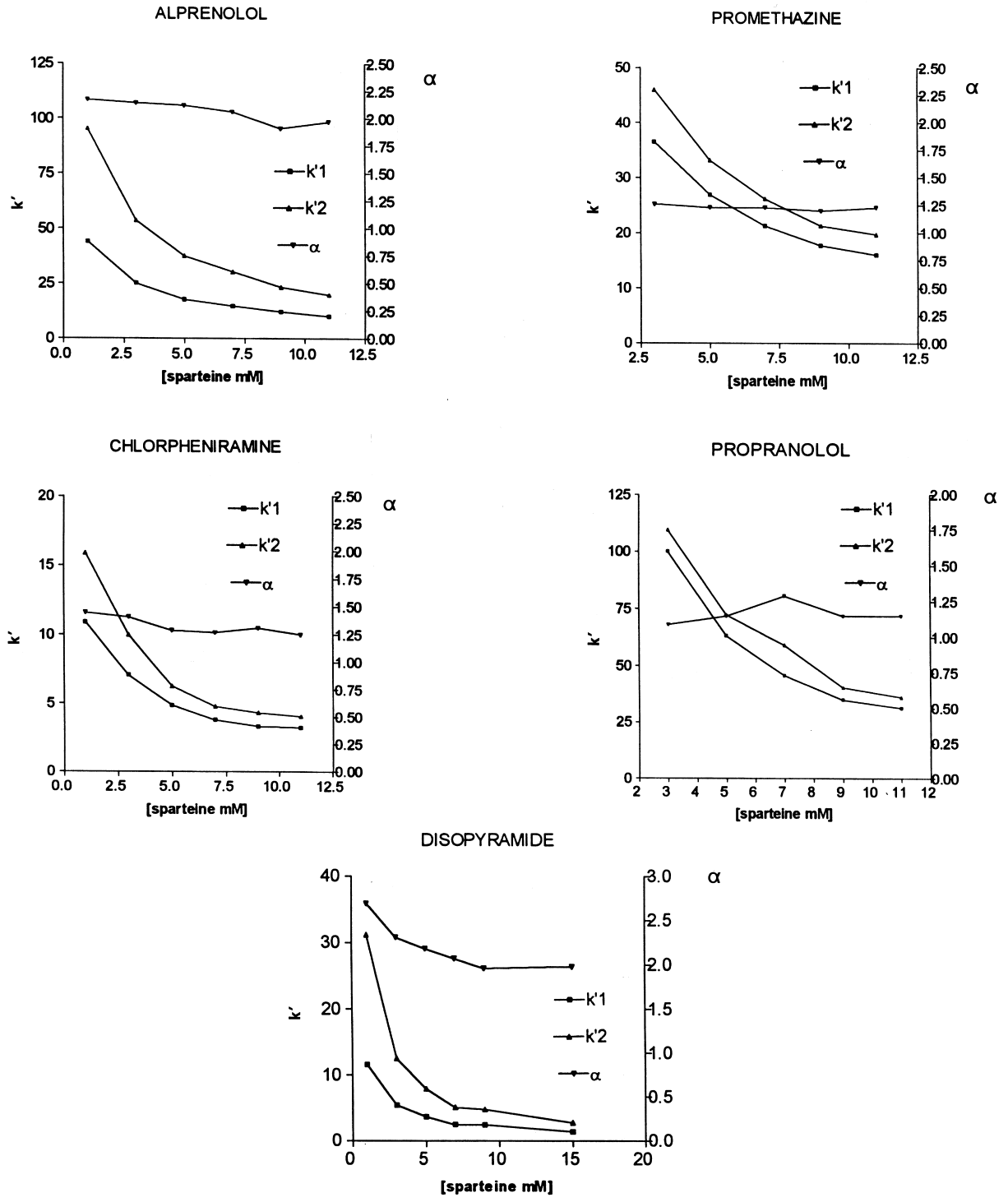

Fig. 6. Graphical representation of the influence of sparteine concentration in the mobile phase (pH 6.0; 0.02 *M* phosphate buffer) on the retention (k') and enantioselectivity (α) for selected basic racemic drugs.

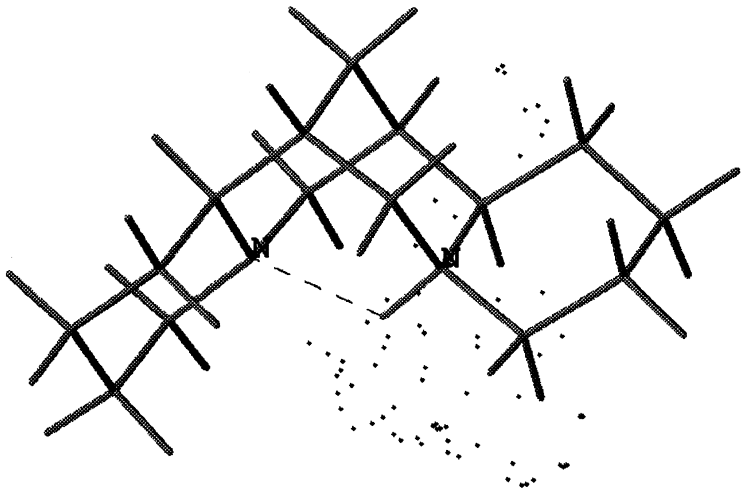

Fig. 7. Structure of monoprotonated sparteine. The dashed line represents the H-bond between the nitrogen atoms. The dots represent the distribution of positive potential (50 kcal/mol) over the atoms close to the protonated N (1 cal=4.184 J). The geometry optimization and the calculation of the electrostatic properties were performed by means of AM1 [19].

total loss at about 7% 2-propanol. Clearly, when using 2-propanol as modifier, the decrease in retention for all the examined compounds is accompanied by a loss of enantioselectivity resulting in a worse enantioresolution. A similar effect by ethanol, 1-propanol, 2-propanol and 1-butanol on the enantioselectivity has been observed in the other works [2].

3.3. Sparteine in the presence of 2-propanol

In this study 2-propanol was used as neutral modifier and was kept at a constant concentration of 2.5%. With a mobile phase of 2.5% 2-propanol and phosphate buffer pH 6, alprenolol eluted at k' values of 13.63 and 20.33 with an α value of 1.49. Inclusion of 5 m*M* sparteine reduced the k' values by more than three-fold with an α value of 1.43. With propranolol under the same conditions there was a small decrease in retention without improvement in the enantioseparation. The best results were obtained for promethazine; using 2.5% 2-propanol and pH 6.0 buffer the enantiomers eluted in about 1 h. On addition of 5 m*M* of sparteine to the mobile phase the enantiomers were eluted in less than 10 min and the α value increased from 1.31, without sparteine, to 1.76 with sparteine (Fig. 4f). The peak shape and symmetry were greatly improved.

Hydrophobic amines with tricyclic ring structure, such as promethazine, are difficult to enantioseparate with low retention [1]. Thus, the result illustrated in Fig. 4f shows that the conditions we used look promising for the complete enantioseparation of such compounds in a short time.

A few trials were also done to compare the effect of some commonly used amines to that of sparteine. 1,8-Diaminooctane (5 m*M*) was used in a mobile phase with 2.5% 2-propanol at pH 6.0. The effect of this diamine, useful for separation of basic compounds on C_{18} reversed-phase [18], proved to be insignificant for all the examined basic drugs on the α-AGP column (Fig. 4d,e, for promethazine). There was practically no change in retention times and peak shape. Therefore, it can be seen that sparteine is a better, interesting and useful modifier for the improvement of chromatographic performance when using α-AGP columns.

Finally, the effect of sparteine was also studied at pH 7.0. Increase in pH increases the negative charge on the α-AGP and the cationic drugs are more strongly retained by ionic binding to the anionic groups in the binding sites of the protein [1]. Therefore in order to obtain acceptable retention times 2-propanol was used at the constant 2.5% level in the mobile phase (pH 7.0, 0.02 *M*) phosphate

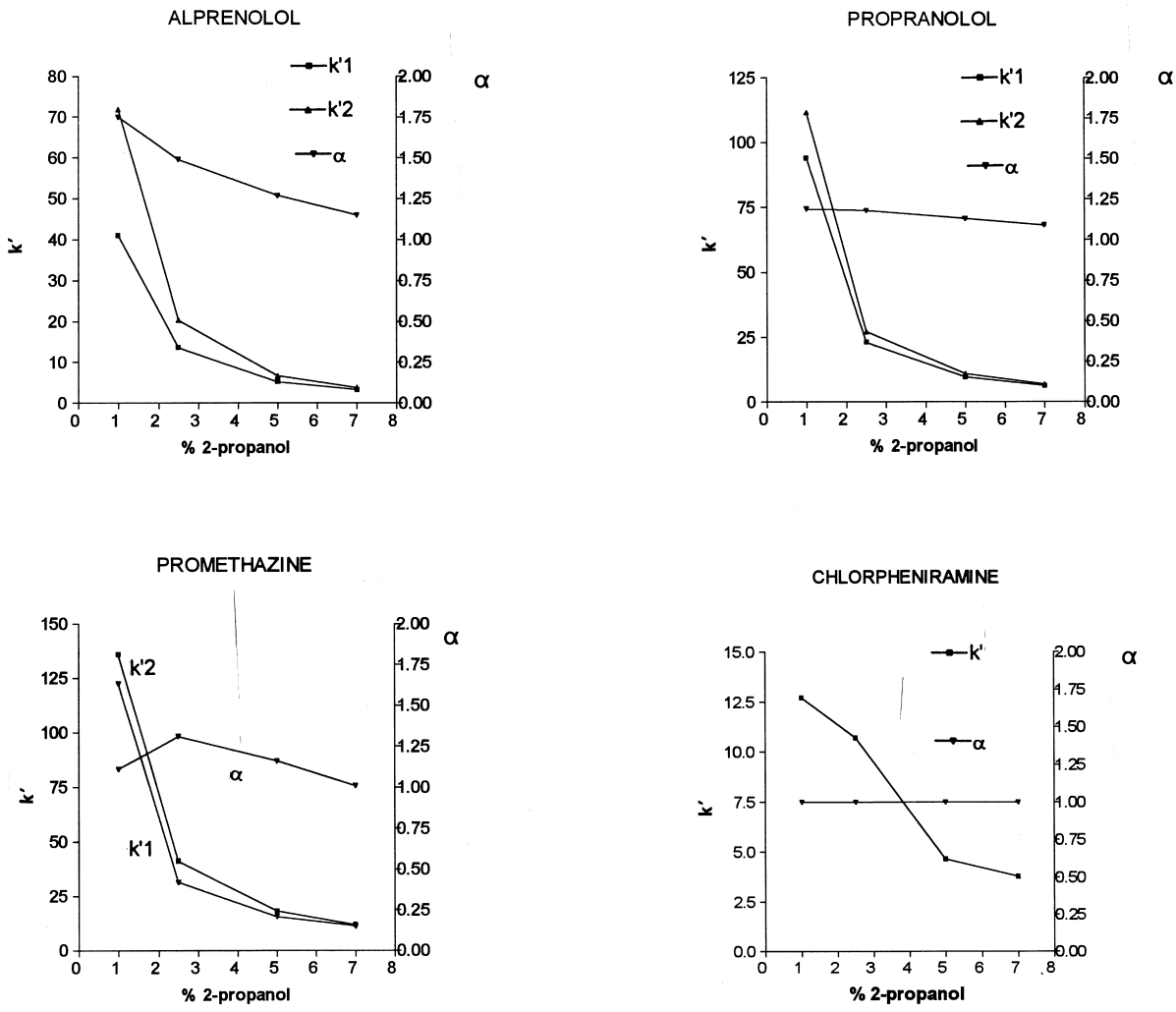

Fig. 8. Graphical representation of the influence of 2-propanol percentage in the mobile phase on the retention and enantioselectivity of the selected drugs.

buffer. Under these conditions, most of the analytes were eluted in about 1 h. Only chlorpheniramine (not enantioresolved) was eluted in a reasonable time (29 min). Moreover, the peaks were wide and tailed. When the mobile phase was modified by adding sparteine at the 5 m*M* level, there was a big decrease in retention times for all the analytes with a general improvement in peak shape and symmetry, while enantioselectivity was only slightly reduced. Alprenolol in particular had excellent results as illustrated in Fig. 5d,e, where the complete separation of the enantiomers in very short times is shown. This effect suggests that sparteine may compete for the anionic non-stereoselective sites of the α-AGP besides the hydrophobic ones.

4. Conclusions

On the whole the study produced interesting results for the enantioresolution of some basic hydrophobic drugs (alprenolol, promethazine, disopyramide and chlorpheniramine). Less favourable are the results for propranolol.

Even if the study was done on a limited series of compounds, it is possible to note the utility of sparteine as a modifier for chiral separations on an α-AGP column when compared to other known modifiers. It is worth noting that sparteine seems to act on non stereoselective hydrophobic and anionic sites without involving its chirality. Sparteine can be used alone in the mobile phase (pH 6.0) as a substitute for organic solvents and relatively low concentrations are required. It can be also used (pH 6.0 and 7.0) in combination with a neutral modifier such as 2-propanol, allowing good enantioselectivity with low retention times to be achieved using minimum concentrations of the organic solvent. Of course appropriate combinations of sparteine and organic modifier have to be optimized to obtain the required enantioresolution.

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