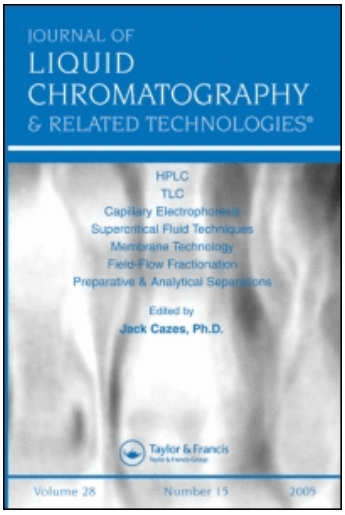

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ENANTIOMERIC SEPARATION OF AROMATIC AMINO ALCOHOL DRUGS BY CHIRAL ION-PAIR CHROMATOGRAPHY ON A SILICA GEL PLATE

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ABSTRACT

Four chiral aromatic amino alcohol drugs were separated by TLC on a general-purpose silica gel plate with ammonium-D-10-camphorsulfonate (CSA) as chiral ion-interaction agent. The chiral aromatic amino alcohols are all of pharmacological importance as ß-adrenergic blockers. The influence of eluent composition, temperature and concentration of CSA on the chiral separation is discussed. It is found that the temperature is also one of the important parameters to be varied for optimum separation in ion-pair chiral resolution. Among four drugs, three enantiomeric drugs were resolved at lower temperature (5°C). In this study, analytical reagent grade methanol and dichlormethane can be directly used as mobile phase without using molecular sieve before use.

INTRODUCTION

Chiral stationary phases (CSP's) or chiral mobile phase additives (CMA's) have been used for chiral separations by thin layer chromatography (TLC). In general, the CSP's have been used less, probably because the commercially available TLC plates were too small and relatively expensive. ß-Cyclodextrin (ß-CD) and its derivatives as CMA's have been most widely used in the chiral TLC separation.[1-5]

Ion pair chromatography is also an important chromatographic technique commonly used for separation of ionized compounds in liquid chromatography. This separation technique is based on the fact that a chiral counter-ion can bind an enantiomeric substrate as diastereomeric ion pairs which will have different distribution properties if the counter-ion has a suitable structure.

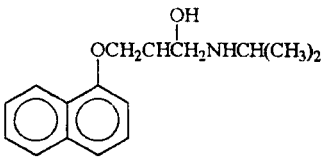
Pettersson et al.[6-7] separated a variety of enantiomers of organic amine derivatives, organic acids, and aromatic amino alcohols by ion-pair chromatography. They used camphorsulfonic acid (CSA), quinine, albumin, n-dibutyl tartrate, N-benzoxycarbonyl-glycyl-L-proline (ZGP) as chiral counter-ions in HPLC.

Recently, Duncan et al.[8] reported the separation of several racemic aromatic amino alcohols by TLC on diol or high performance silica gel plates using a mobile phase containing (IR)-(-)-ammonium-10-camphorsulfonate (CSA) or ZGP. In this work, based on reference 8, we reported on the enantiomeric separation of four aromatic amino alcohol drugs on silica gel plates, using a solution containing ammonium-D-10-camphorsulfonate as a mobile phase. The dependence of eluent composition, temperature and concentration of the counter ion on the separation was described. Some separations occurred only at lower temperature (5°C).

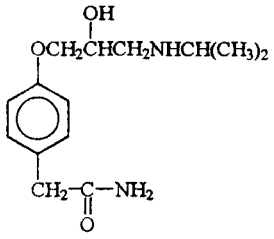
EXPERIMENTAL

Materials

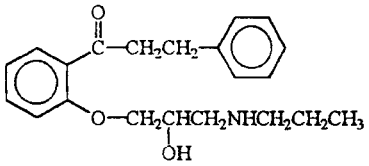
Silica gel GF_{254} (TLC, d_p = 10-40 μm, Qingdao Haiyong Chemical Factory, China), propranolol, pindolol and propafenone (Shanghai Huanghe Pharmacological Factory, China) and atenolol (Tianzhong Junyang Pharmacological Factory, China), D-10-camphorsulfonic acid (J. T. Baker Chemical Co.), methanol and dichloromethane (CH_2Cl_2) were analytical reagent grade and were used as received, without further purification.



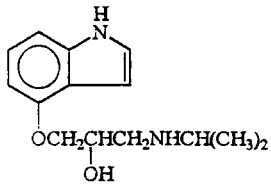
Propranolol



Atenolol



Propafenoni



Pindolol

Figure 1. The structure of test compounds.

Methods

Silica gel plates (2.5 x 10 cm) were prepared with 300-400 μm layer thickness and were placed in a drying oven at 120-130°C for one hour, then stored in a desiccator. All developments were carried out at 5°C (in a refrigerator) or at room temperature (25-30°C) in small glass jars of 250 mL volume. The distance of development was 8 cm. It took approximately 30-40 min. to completely develop the plate. The spot visualization was performed by use of a fixed wavelength (254 nm) UV lamp.

All samples were dissolved in the methanol. Counter-ion was added to the mobile phase in the ammonium form.

RESULTS AND DISCUSSION

The structures of the four test drugs are shown in Figure 1. Note that all of the compounds have three functionalities in common: an aromatic ring, an α-hydroxyl group and a ß-amino group. Figure 2 shows the TLC

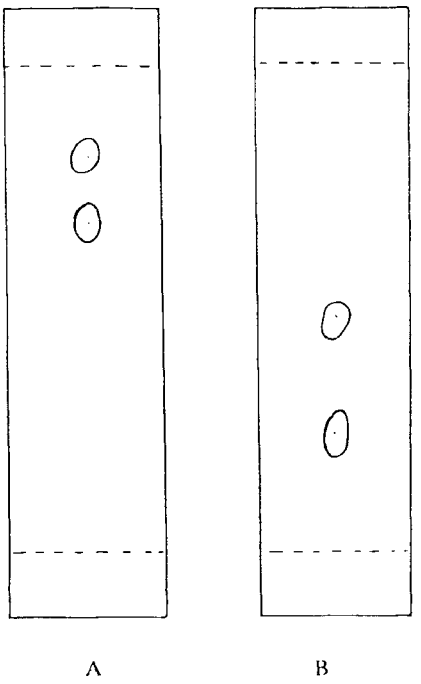


Figure 2. TLC chromatogram showing the resolution of the racemates. A: propafenone, B: atenolol. Mobile phase: A: methanol-dichloromethane = 50:50; B:methanol-dichloromethane = 70:30. Other conditions, see Fig. 3.

Table 1

Enantiomers Separated using CSA as Chiral Mobile Phase Additive with a Silica Gel Plate

Compound	R_{f1}	R_{f2}	α	Mobile Phase * (v/v) CH_2Cl_2 : MeOH
Propranolol	0.62	0.81	1.31	50 : 50
Propafenone	0.69	0.84	1.22	50 : 50
Pindolol	0.61	0.79	1.30	60 : 40
Atenolol	0.24	0.48	2.00	70 : 30

* 6.8 mM CSA in the mobile phase. Development was done at 5°C.

chromatogram of the chiral separation of propafenone and atenolol. The data for the enantiomeric separations are listed in Table 1. As can be seen in Table 1, it was possible to achieve very efficient separations using CSA as chiral mobile phase additive on the silica gel plate.

Figure 3 shows the influence of varying the ratio of dichloromethane and methanol in the mobile phase on the chiral separation. The separation occurs over a range of 30 % to 60 % dichloromethane content in the mobile phase (v/v) for propranolol (Fig. 3A). The ΔR_f value is the largest when the content of dichlormethane in the mobile phase is 30 % (v/v), but a poorer separation is obtained because of the tailing spots. The optimum resolution is achieved when the content of dichloromethane in the mobile phase is 50 %. The curves and the optimum eluent composition for chiral separation of propafenone are very similar to propranolol, probably due to similar molecular structure, and the optimum eluent composition for chiral separation of propafenone is 50 % dichloromethane in the mobile phase.

The chiral separation of pindolol and atenolol is also similar. An analogous curve to that shown in Fig. 3B is generated for the effect of the content of dichloromethane in the mobile phase on the chiral separation of atenolol. The optimum enantiomeric resolution occurs at approximately 60 % dichloromethane in the mobile phase for pindolol and at 70 % dichloromethane in the mobile phase for atenolol. The aforementioned results indicate that the eluent composition plays an important role in the enantiomeric separation.

In gas chromatography, temperature is the main parameter to be varied for optimizing separation. Recently, the effect of temperature has also been recognized as an important parameter in improving and optimizing chiral resolution by HPLC[9-12]. Duncan et al.[8] reported the enantiomeric separation of pindolol on a diol TLC plate using ZGP as chiral ion interaction agent and the enantiomeric separation of propranolol on a high performance silica gel plate using ZGP or CSA as chiral ion interaction agent. No chiral separation occurred on the silica gel plate. In this work, the pindolol and propranolol were also not separated on the silica gel plate using CSA as chiral ion interaction agent at room temperature (25-30°C), but the chiral separations of these two drugs and propafenone were achieved at 5°C in the refrigerator. Among the four drugs, only atenolol can be separated at 30°C, $R_{f1} = 0.33$, $R_{f2} = 0.53$, $\alpha = 1.61$. The R_f value at 30°C was larger than that at 5°C, and the separation at 30°C was poorer than that at 5°C due to slightly tailing spots and smaller R_f value. The reason for this (separation at room temperature for atenolol) probably is due to including a higher polarity amide group in the

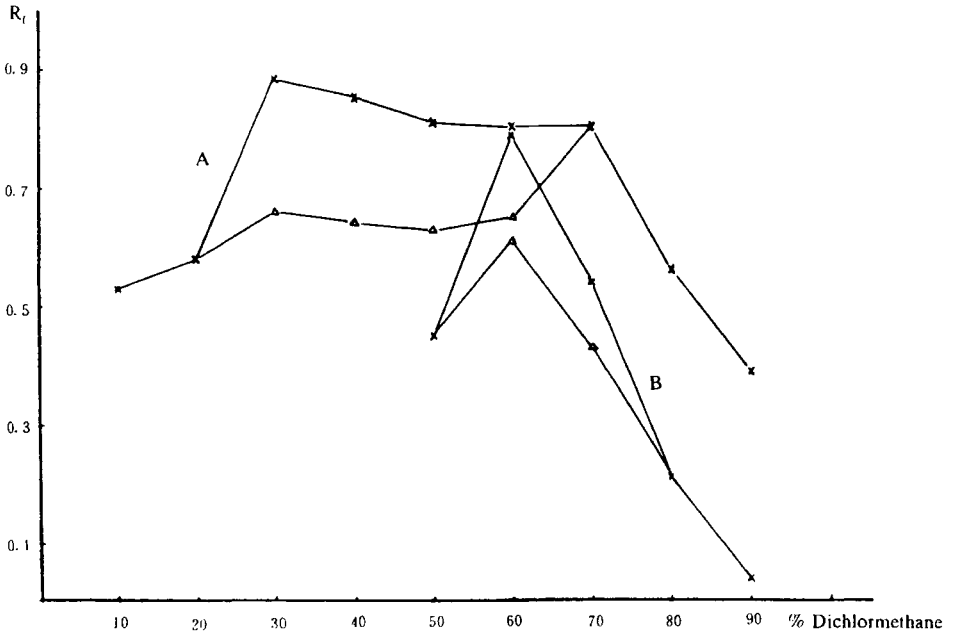


Figure 3. Plot showing the effect of the content of the dichloromethane in the mobile phase on chiral TLC separation. –×–: first eluted enantiomer, –Δ–: second eluted enantiomer. The concentration of CSA is 0.68 mM. Stationary phase: silica gel plate, temperature: 5°C. A: propranolol, B: pindolol.

molecular structure of the atenolol and more strength in the binding to the absorbent. The R_f value of atenolol in Table 1 was minimum (the largest retention) and the content of dichloromethane in the mobile phase was the largest.

From the results above, it is obvious that the temperature is also one of the main parameters to be varied for optimizing separation in the ion-pair TLC chiral resolution, besides the stationary phase, eluent composition and counter ion.

According to the report by Duncan et al., originally, molecular sieve (Type 5A) was dried for 24 hours at 350°C and then was used to dry solvent. The plates were placed in a drying oven at 125°C for 3-4 hours. Later it was found that one hour heating for the plates is necessary and adequate. If the plates were heated a longer time, the plates were easily broken during use. This is probably due to fact that the particle diameter of silica gel is ununiform

(10-40°) in this work. It was also found that the separation at 5°C can be achieved when the mobile phase solvents were not dried with the molecular sieve before use. The content of water in the methanol and dichloromethane was determined by GC and was about 1000-2000 ppm. Therefore, the methanol and dichloromethane were directly used. In addition, the R_f value was hardly changed with a decrease of the concentration (8.8-4.8 mM) of CSA. The concentration of CSA in the mobile phase was kept at 6.8 mM in this study because the resultant spot was smaller (or more concentrated) and there was no tailing.

The shapes of the spots on TLC plate were visualized with a UV lamp, then the spots were scraped out and dissolved in methanol. The methanolic solution, containing sample, was scanned from 190 nm to 600 nm with a Model UV 240 (Shimadzu, Kyoto, Japan). The UV spectrum of the two spots are identical, thereby indicating they are enantiomers.

CONCLUSION

The chiral separation can be achieved with a general purpose silica gel plate using CSA as a mobile phase additive. Besides eluent composition, stationary phase and counter-ion, it is found that the temperature is also an important parameter to be adjusted for optimizing a separation in this work. The chiral separations of propranolol, pindolol and propafenone only are achieved at 5°C. It appears that the presence of 1000-2000 ppm water in the analytical system does not influence chiral separation. Once the separation was optimized, it was possible to attain enantiomeric separation with good α values. These separation conditions were easily obtained. This method is relatively inexpensive and attractive.

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