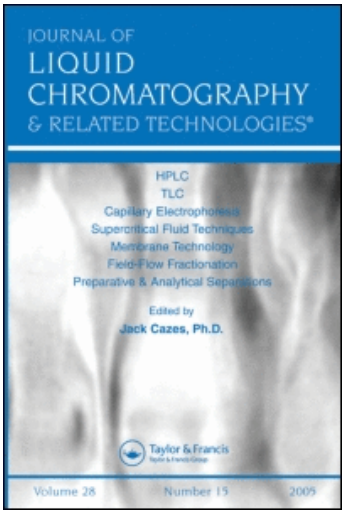

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Normal Phase TLC Separation of Enantiomers Using Chiral Ion Interaction Agents

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Original Article

NORMAL PHASE TLC SEPARATION OF ENANTIOMERS USING CHIRAL ION INTERACTION AGENTS

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ABSTRACT

A method for the thin layer chromatographic (TLC) separation of enantiomers and diastereomers involving the use of chiral ion interaction agents is described. Several aromatic amino alcohols were resolved by TLC on diol and/or high performance silica gel plates using a mobile phase containing (1R)-(-)- ammonium-10-camphorsulfonate or N-benzoxycarbonyl-glycyl-L-proline (ZGP). Many of these chiral aromatic amino alcohols are of pharmacological importance as α– and β–adrenergic blockers, adrenergic compounds, and anti-glaucoma agents. A comparison was made between various N-CBZ-amino acid derivatives as chiral counter ions/chiral mobile phase additives (CMAs). These separations could not be achieved on other normal phase TLC stationary phases including microcrystalline cellulose, alumina and ordinary silica gel plates.

INTRODUCTION

A substantial amount of research has been conducted in the area of chiral separations due to the demand for efficient, precise and dependable

methods for the determination of optical purity and/or preparative scale separation of optical isomers. One reason for this demand is the fact that two enantiomeric forms of a molecule can have different effects in biological systems (1) and as the need for information on the often widely variant activities of enantiomers of pharmologically important compounds increases, the demand for chiral separation methods will continue. Another reason is that synthetic organic chemists need efficient, accurate methods to check their reaction progress and purity (% composition of each enantiomer, quality control, etc.). Research has concentrated mainly on liquid (LC) and gas (GC) chromatography while progress in planar chromatography (PC) or thin layer chromatography (TLC) has been less pronounced. TLC, however, can be used to screen large numbers of compounds and solvent systems for HPLC and often produces unusual enantioselectivities.

Two methods are available for the separation of enantiomers by thin layer chromatography (TLC). One method involves the use of chiral stationary phases (CSPs) while the other entails the use of chiral mobile phase additives (CMAs). The only commercially available TLC plate is the ligand exchange type for amino acids (2). There have been reports on cyclodextrin and 3,5 - dinitrobenzoylphenylglycine bonded phase TLC plates, but as yet they have not been commercialized (3,4). Recent work in reversed-phase TLC entailed the use of β-cyclodextrin (β-CD) derivatives as CMAs. Armstrong, He, and Han separated a number of racemates using β-CD as a CMA in TLC (5). Armstrong, Faulkner, and Han separated a variety of racemic compounds using hydroxypropyl- and hydroxyethyl- derivatized β-CD as CMAs (6). Duncan and Armstrong reported the separation of various racemic compounds using maltosyl-β-CD as a CMA (7). Some of the problems in developing chiral TLC plates were discussed by Wilson (8). Consequently CMAs remain the mainstay for TLC chiral separations.

In this work, CMAs are employed in the separation of various racemic compounds of pharmacological importance. The formation of diastereomeric pairs of ions using chiral ion interaction agents is the basis for resolution of these enantiomers. It is possible to separate diastereomeric ion-pairs using non-chiral stationary phases because diastereomers display dissimiliar distribution properties in isotropic environments. Differences in the distribution properties of the diastereomeric complex may be due to differences in the binding strength to the adsorbent and/or to dissimiliarities in the complex solvation or solubility in the mobile phase. There has been much recent work done in normal phase HPLC involving the use of chiral counter ions. Pettersson and Schill reported the separation of enantiomers by "ion-pair" chromatography using quinine, camphorsulfonic acid, albumin, and n-dibutyl tartrate as chiral counter ions (9). Pettersson and Josefsson reported the separation of racemic aromatic amino alcohols in ion interaction chromatographic systems using N-benzoxycarbonyl-glycyl-L-proline (ZGP) (10). Knox and Jurad reported the separation of optical isomers by zwitterion-pair chromatography; the chiral agent in this case was L-leucyl-L-leucyl-L-leucine (11).

Previous work involving the use of CMAs in HPLC revealed several important parameters: the nature of the solid phase, the concentration and nature of the CMA and the amount of polar modifier in the mobile phase (9). In this work, the use of a variety of chiral ion interaction agents for TLC enantiomeric separations is examined. Various mobile phase compositions and numerous commercially available TLC stationary phases were evaluated. (1R)-(-)-ammonium-10-camphorsulfonate (CSA) and N-benzoxycarbonylglycyl-L-proline (ZGP) seemed to be the most widely useful CMAs. When conditions are optimized, these chiral agents can be used for the separation and resolution of enantiomers and diastereomers of aromatic amino-alcohols including a variety of β-adrenergic blocking drugs.

EXPERIMENTAL

Silica gel (K5F, 250 μm layer thickness, 5 x 20 cm), high performance silica gel (HP-KF, 200 μm layer thickness, 5 x 5 cm), cellulose (K2F,250 μm layer thickness, 5 x 20 cm) and ethyl (KC2F, 200μm layer thickness, 5 x 20 cm) thin layer chromatography (TLC) plates were obtained from Whatman Chemical Separation Division, Inc. (Clifton, NJ). Aluminum oxide (250 μm layer thickness, 5 x 20 cm) and diol (HPTLC Diol $F_{254}s$, 200 μm layer thickness, 10 x 10 cm) TLC plates were obtained through Alltech Associates, Inc. (Deerfield, IL). Phenylpropanolamine, timolol, isoproterenol, metoprolol, propranolol, labetalol, octopamine, pindolol, N-CBZ-ALA-PRO, N-CBZ-ILE-PRO, N-CBZ-L-proline and N-CBZ-GLY-PRO were obtained from Sigma (St. Louis, MO). Norphenylephrine, 1-pentanol, molecular sieves (5 A,4-8 mesh) and (1R)-(-)-ammonium-10-camphorsulfonate were obtained from Aldrich (Milwaukee, WI). Methylene chloride(CH_2Cl_2), ethylene glycol, acetonitrile, ethanol, 2-propanol, methanol(MeOH), and 1-butanol were obtained from Fisher Scientific (Fair Lawn, NJ). The molecular sieve (Type 5A) was dried for 24 hours at 350°C and stored in a drying oven at 125°C. Fifty grams of molecular sieve were used to dry 250 ml of solvent. The solvents were dryed for 24 hours before use. The TLC plates were placed in a drying oven at 125°C for 3-4 hours and then stored in a dessicator.

It took approximately 30-45 minutes to completely develop a 5 x 5 cm TLC plate. All development was done at room temperature (20°C) in 6 (i.d.) x 23 cm cylindrical glass chambers. Spot visualization was performed by use of a fixed wavelength (254 nm) UV lamp. A Schimadzu dual wavelength TLC scanner (CS-910) was used to measure resolution. Various wavelengths were scanned to provide supporting evidence as to the validity of the enantiomeric separation.

RESULTS AND DISCUSSION

No enantiomeric separations could be achieved with any of the ion interaction agents unless the solvents and the TLC plates were thoroughly dried. Presumably, this was because an intimate, multi-point interaction must occur between the chiral ion interaction agent and the racemic substrate. Hydrogen bonding is particularly important when these associations occur (π-π interactions, dipole-dipole interactions and steric interactions also are pertinent). If too much water is present, it may preferentially associate via hydrogen bonding with the chiral selector and/or racemate thereby negating interactions between these species that are important for enantioselectivity. Dichloromethane was the major component of the mobile phase. However, it was difficult to dissolve ammonium camphorsulfonate (CSA) in dry dichloromethane. Several organic co-solvents were tested to evaluate their ability to solubilize CSA. These solvents included: 1-pentanol, 1-butanol, 2-propanol, ethanol, methanol, acetonitrile, and ethylene glycol. CSA was most soluble if ethylene glycol was used but the solution appeared to form two layers and thus was impractical. The CSA also was soluble in solutions which contained the lower molecular weight alcohols. As was expected, the higher the percent of alcohol in the mobile phase, the greater the solubility of the chiral ion interaction agent. It was possible to attain resolution of metoprolol at 95/5 (v/v) dichloromethane/methanol although CSA displayed limited solubility in mobile phases which contained less than 10% alcohol by volume. There was no solubility problem encountered with these mixed mobile phases when using ZGP as a chiral mobile phase additive. Solute movement was restricted to mobile phases which contained an alcohol as a co-solvent whether the chiral mobile phase additive (CMA) was ZGP or CSA. The best results were obtained when dry methanol or 2-propanol were present in the mobile phase.

Separations done on silica gel (K5F) and high performance silica gel (HP-KF) gave approximately the same R_f values but the K5F did not offer the same selectivity as HP-KF. Separations on the ethyl (KC2F) plates gave higher R_f values than the HP-KF plates but no enantiomers were resolved on the KC2F plates. The ethyl and diol plates gave similiar R_f values except there was much greater streaking on the ethyl plates. There was very little spot movement on either the aluminum oxide plates or the cellulosic plates. The only TLC plates on which effective, reproducible enantiomeric separations could be obtained were the diol and the high performance silica gel plates(HP-KF).

The structure of the resolved compounds as well as pertinent separation data are given in Table 1. Note that all of the compounds have three functionalities in common: an aromatic ring, an α-hydroxyl group and a β-amine. Table 1 shows that it was possible to achieve very efficient separations with the use of CSA and ZGP as chiral mobile phase additives (CMAs). Several compounds were separated with both CMAs. In four out of five of these compounds (Table I) a higher selectivity was obtained by adding 5 mM triethylamine (TEA) when ZGP was the CMA. A majority of the racemates were resolved on the HP-KF plates. Retention on the stationary phases is due to the OH-groups present on both the HP silica gel and the diol plates. These types of plates display a certain similiarity in their chromatographic behavior, however the silica gel plates are more strongly adsorbing than the diol plates (12). Note that the mobile phase which was used in conjunction with the diol plates had a lower percentage of alcohol and used a less polar alcohol in comparison with the mobile phases used with the HP silica gel plates. There were two similiarities among the compounds resolved with the diol plates: none of these compounds contained a primary amine and each racemate had a terminal branched hydrocarbon group. The

Table I. Compounds Separated Using ZGP or CSA as Chiral Mobile Phase Additives

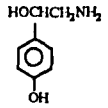
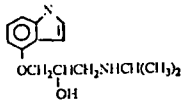
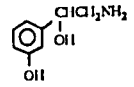
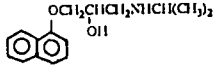
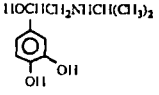
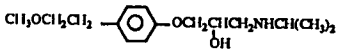
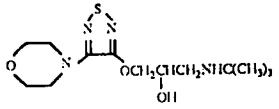
Compounds	R_{f1}	R_{f2}	α	Conditions
Phenylpropanol-amine 	0.06	0.26	4.3	6.9 mM ZGP + 5 mM TEA[a]
Octopamine 	0.15	0.33	2.2	7.9 mM CAS[a]
Pindolol 	0.07	0.12	1.7	6.7 mM ZGP + 5 mM TEA[b]
Norphenylephrine 	0.05	0.26	5.2	5.9 mM ZGP + 5 mM TEA[a]
	0.03	0.14	4.7	6.8 mM CAS[c]
Propranolol 	0.08	0.20	2.5	6.7 mM ZGP + 5 mM TEA[c]
	0.05	0.20	4.0	9.3 mM CAS[c]
Isoproterenol 	0.14	0.38	2.7	5.9 mM ZGP + 5 mM TEA[a]
	0.12	0.30	2.5	6.8 mM CAS[a]

Table 1 (continued)

Compounds	Rf1	Rf2	α	Conditions
Metoprolol 	0.11	0.17	1.5	10.7 mM CAS[b]
Timolol 	0.26	0.51	2.0	5.8 mM ZGP[a]
	0.39	0.55	1.4	13.9 mM CAS[b]

[a]HP Silica gel plates with 75/25 (v/v) methylene chloride/methanol
[b]DIOL plates with 95/5 (v/v) methylene chloride/2-propanol
[c]HP Silica gel plates with 90/10 (v/v) methylene chloride/methanol

NOTE: All separations achieved with ZGP contained 5 mM TEA.

separations achieved on the diol plates gave lower α values than those attained with the HP-KF plates. None of the adrenergic compounds were resolved on the diol plates. Separations were optimized for most of the adrenergic compounds by using a higher alcohol content in the mobile phase whereas most of the separations for the β-adrenergic blockers were optimized with a lower percentage alcohol content and/or a less polar alcohol in the mobile phase.

Figure 1 shows two TLC chromatograms. These chromatograms illustrate the high degree of stereoselectivity obtainable with this method. Both chromatograms were developed on HP-KF plates using 75:25 (v/v) dichloromethane/methanol but each uses a different CMA. The plate on the right illustrates the separation of isoproterenol and propranolol using CSA as a chiral ion interaction agent. The plate on the left shows the resolution of methoxamine, norphenylephrine, phenylpropranolamine and octopamine

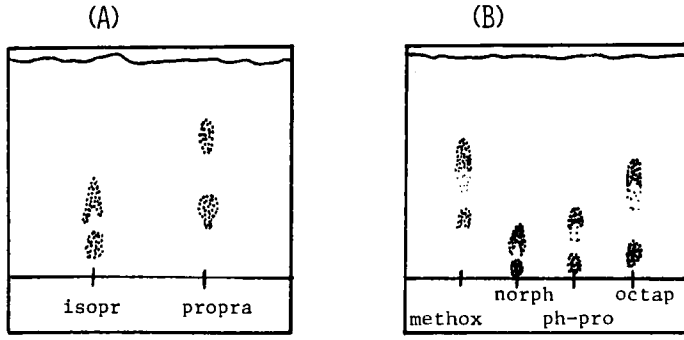


Figure 1: Thin layer chromatograms on 5 x 5 cm HPTLC silica gel. (A) Mobile phase 75:25 CH_2Cl_2: MeOH containing 6.8 mM ammonium camphorsulfonate (CSA). Isopr (isoproterenol); propra (propranolol). (B) Mobile phase 75:25 CH_2Cl_2: MeOH containing 6.9 mM N-benzoxycarbonyl-glycyl-L-proline (ZGP) and 5 mM triethylamine. Methox (methoxamine); norph (norphenylephrine; ph-pro (phenylpropanolamine); octop (octopamine).

employing ZGP and triethylamine (TEA). Methoxamine and phenylpropranolamine are diastereomers. The TLC densitometric scans for this plate showed three peaks for methoxamine and two peaks for phenylpropranolamine. There was an enantiomeric and a diastereomeric separation for methoxamine. It was not possible to determine which separation was enantiomeric and which was diastereomeric using the TLC scanner so this separation is not reported in Table 1.

Figures 2(A) and 2(B) show three TLC densitometric profiles of TLC plates scanned at 254 nm, 275 nm and 300 nm, respectively. Notice that the peak areas maintain the same relative proportions at different wavelengths thereby making it probable that these are enantiomeric separations. The stationary phase was HP silica gel and the mobile phase was 75:25 (v/v)

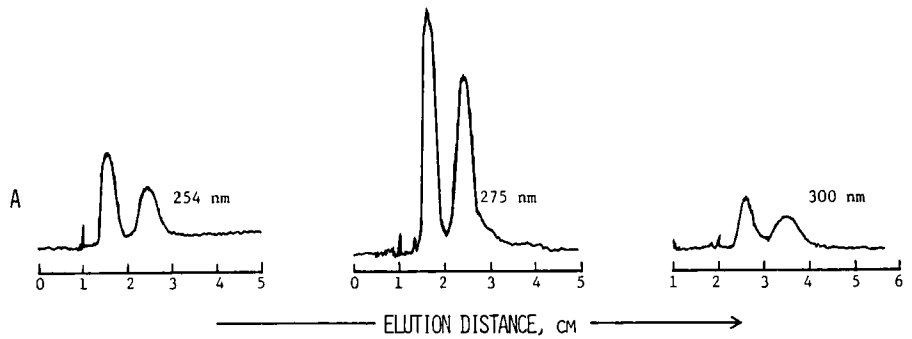


Figure 2(A): TLC densitometric scans (using a Shimadzu CS-910 at three different wavelengths) showing the resolution of racemic norphenylephrine. The stationary phase was a HPTLC silica gel plate (5x5 cm). The mobile phase consisted of 6.6 mM N-CBZ-gly-L-proline + 5 mM triethylamine dissolved in 75:25 (v/v) CH_2Cl_2: MeOH.

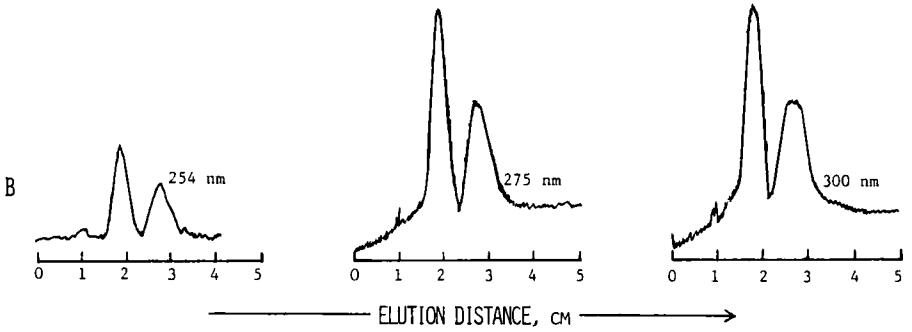


Figure 2(B): TLC densitometric scans (using a Shimadzu CS-910 scanner) showing the resolution of racemic isoproterenol (a bronchodilator). The stationary phase was a HPTLC silica gel plate (5x5 cm). The mobile phase consisted of 6.8 mM ammonium camphorsulfonate dissolved in 75:25 (v/v) CH_2Cl_2:MeOH.

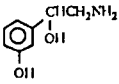
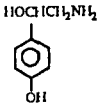
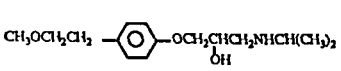
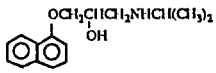
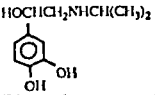
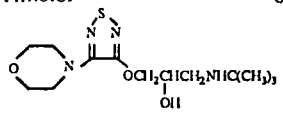
dichloromethane/methanol for both separations. In Figure 2(A), norphenylephrine was resolved using ZGP and TEA. Figure 2(B) illustrates the resolution of racemic isoproterenol; the chiral ion interaction agent was CSA.

A comparison was made between various N-CBZ-amino acid derivatives as CMAs: N-CBZ-alanyl-L-proline (ZAP), N-CBZ-isoleucyl-L-proline (ZIP), N-CBZ-L-proline (ZP) and N-CBZ-glycyl-L-proline (ZGP). Table 2 shows that it was possible to attain some separations with all of the N-CBZ-amino acid derivatives. However, ZGP separated the largest number of compounds and gave higher α values than the other N-CBZ-amino acid derivatives. Note that the only compound resolved using diol plates and 2-propanol with an N-CBZ derivative was metoprolol. Also, metoprolol was the only compound resolved using ZAP or ZP as a CMA. It was possible to separate two adrenergic compounds and one β-adrenergic blocker using ZIP as a CMA (Table 2). From these results it is clear that ZGP was the best chiral ion interaction agent of the N-CBZ- amino acid derivatives in resolving the racemates of interest in this study.

CONCLUSIONS

The high performance silica gel and diol TLC plates were the only stationary phase plates on which it was possible to attain enantiomeric separations. The most effective chiral ion interaction agents were N-benzoxycarbonyl-glycyl-L-proline (ZGP) and (1R)-(-)-ammonium-10-camphorsulfonate (CSA). This approach was highly successful in separating chiral aromatic amino alcohols. Once the separation was optimized, it was possible to attain enantiomeric separations with good α values in approximately 30 minutes. This appears to be a good method for screening specific types of racemic compounds and different solvent systems for HPLC in a relatively short time. Many of these chiral ion interaction agents are relatively inexpensive and provide an easily accessible pathway for

Table 2. Compounds Separated Using Other N-CBZ Amino Acid Derivatives as CMAs.

Compounds	R_{f1}	R_{f2}	α	Conditions
Norphenylephrine 	0.20	0.48	2.4	7.5 mM ZIP[a]
Octopamine 	0.20	0.52	2.6	7.5 mM ZIP[a]
Metoprolol 	0.17	0.28	1.6	6.9 mM ZIP[b]
	0.17	0.28	1.6	7.0 mM ZAP[b]
	0.17	0.28	1.6	7.8 mM ZP[b]
Propranolol 	0.10	0.30	3.0	6.3 mM ZGP[a]
Isoproterenol 	0.04	0.10	2.5	6.3 mM ZGP[a]
Phenylpropranolamine 	0.08	0.32	4.0	6.3 mM ZGP[a]
Timolol 	0.20	0.40	2.0	5.8 mM ZGP[a]

[a]HP Silica Gel Plates with 75/25 CH_2Cl_2/MeOH

[b]Diol plates with 95/5 CH_2Cl_2/2-propanol

NOTE: The TEA was not used as an additive for these separations.

ZP: N-CBZ-L-proline
ZIP: N-CBZ-isoleucyl-L-proline
ZAP: N-CBZ-alanyl-L-proline
ZGP: N-CBZ-glycyl-L-proline

enantiomeric separations while the supply of chiral stationary phases for TLC remains limited.

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