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Direct thin layer chromatography enantioresolution of some basic DL-amino acids using a pharmaceutical industry waste as chiral impregnating reagent

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Abstract

Direct enantiomeric resolution of DL-arginine, DL-histidine, DL-lysine, DL-valine and DL-leucine into their enantiomers was achieved by thin layer chromatography (TLC) on silica gel plates impregnated with optically pure (1*R*, 3*R*, 5*R*)-2-azabicyclo[3,3,0]octan-3-carbo-xylic acid (0.011 M) as a chiral selector which is a waste of a pharmaceutical industry. Different combinations of acetonitrile-methanol-water were found to be successful in resolving the DL-amino acids. The spots were detected by ninhydrin (0.2% in acetone) and the detection limit was 0.66 μg.

Keywords: Enantioresolution; DL-Amino acids; (1*R*, 3*R*, 5*R*)-2-azabicyclo [3,3,0]octan-3-carboxylic acid; Impregnated thin layer chromatography (TLC)

1. Introduction

The compound (1*R*, 3*R*, 5*R*)-2-azabicyclo[3,3,0]octan-3-carboxylic acid (Fig. 1(a)) is a non-proteinogenic amino acid recovered from waste streams of the resolution step in the production of the highly potent angiotensin converting enzyme (ACE) inhibitor ramipril [1]. This unnatu-

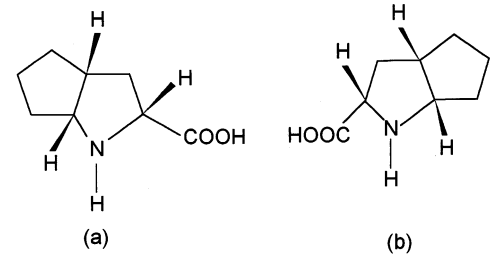

Fig. 1. (a) Chemical structure of (1*R*, 3*R*, 5*R*)-2-azabicyclo[3,3,0]-octan-3-carboxylic acid. (b) Chemical structure of (1*S*, 3*S*, 5*S*)-2-azabicyclo[3,3,0]-octan-3-carboxylic acid.

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Table 1
hR_F ($R_F \times 100$) values of enantiomers of DL-amino acids resolved on plates impregnated with (1*R*, 3*R*, 5*R*)-2-azabicyclo[3,3,0]octan-3-carboxylic acid (Fig. 1(a))[a]

DL-Amino acid	Solvent system, acetonitrile:methanol:water	hR_F values		
		Pure	From DL mixture	
		L	L	D
Arginine	7:6:3	13	13	6
Histidine	7:6:2	25	25	18
Lysine	10:5:2	31	31	15
Leucine	10:4:3	21	21	11
Valine[b]	10:5:2	50	50	38

[a] Time: 30–35 min; solvent front, 8.5 cm; detection; ninhydrin (0.2% in acetone); temperature, 26 ± 2°C.
[b] At 20 ± 2°C.

ral amino acid is advantageously available in the enantiomeric forms, i.e. (1*R*, 3*R*, 5*R*) (Fig. 1(a)) and (1*S*, 3*S*, 5*S*) (Fig. 1(b)) during the industrial production of ramipril. The compound has found applications in chiral synthesis [2–6], in ligand exchange thin layer chromatography (TLC) separation of amino acids [7], and as an impregnating reagent for TLC resolution of enantiomeric amino acids and their dansyl derivatives, with Cu(II) complex [8].

TLC resolution of enantiomers of amino acids and their different derivatives has been reviewed by Bhushan and Martens [9–11] and Günther and Möller [12]. The technique provides a direct method for resolution and analytical control of enantiomeric purity and has many advantages over other chromatographic techniques.

Search of the literature reveals that impregnation of TLC plates with (−)-brucine [13], L-proline-Cu(II)-complex [14] and L-arginine-Cu-(II)-complex [15] have been used for resolution of DL-amino acids, while (+)-tartaric acid or L-ascorbic acid [16] has been used for resolution of enantiomeric PTH amino acids. Impregnation of TLC plates has also been used to resolve dansyl-DL-amino acids using a macrocyclic antibiotic erythromycin [17] and bovine serum albumin [18]. Further, enantiomeric RP-TLC separations of amino acids and their derivatives were achieved with α- and β-cyclodextrins [19], bovine serum [20] and vancomycin [21] in chiral mobile phase.

The present studies have been aimed at finding analytical applications of this waste material, i.e. (1*R*, 3*R*, 5*R*)-2-azabicyclo[3,3,0]octan-3-carboxylic acid from pharmaceutical industry, simplifying the resolution process, extending its application for resolution of more DL amino acids and testing this compound as an ion-pair forming reagent because of its structural features. Some new solvent combinations have been worked out using very low concentration of the chiral selector. The results are described in the present communication.

2. Experimental

Amino acids were from SISCO Research Laboratory (Bombay, India). Silica gel G (from Merck (India), Bombay), with calcium sulphate (13%), iron and chloride (0.03%, each) and giving pH 7.0 in a 10% aqueous suspension was used. The other reagents were obtained from Merck, India.

Impregnated thin-layer plates (10 × 20 cm × 0.5 mm) were prepared using a Stahl type applicator for spreading a slurry of silica gel G (30 g) in methanol-H_2O (8.3 + 91.7, v/v 60 ml) containing optically pure (1*R*, 3*R*, 5*R*)-2-azabicyclo [3,3,0]-octan-3-carboxylic acid (Fig. 1(a)) (0.011 M). A

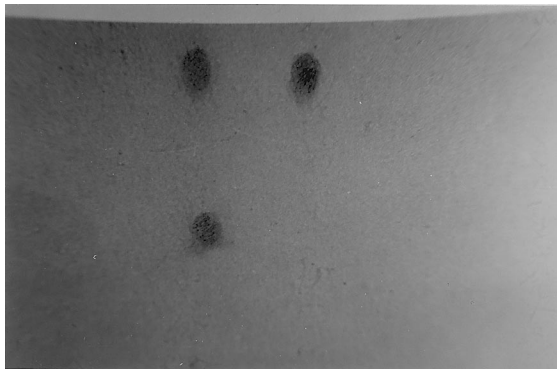


Fig. 2. Photograph of an actual chromatogram showing resolution of DL-histidine as typical results. Left to right: (1) resolved, DL-histidine; (2) pure L-histidine. Temperature, 26 ± 2°C; solvent front, 8.5 cm; detection, ninhydrin; solvent system, acetonitrile-methanol-water (7:6:2 v/v).

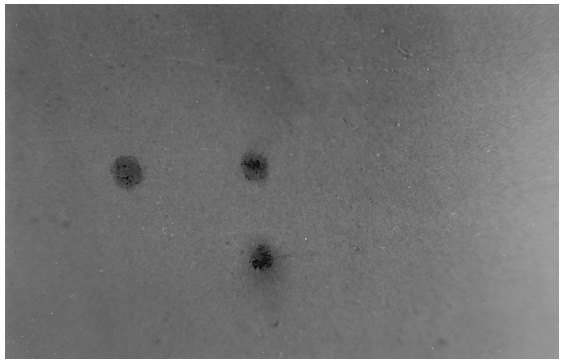


Fig. 3. Photograph of an actual chromatogram showing resolution of DL-lysine, as typical results. Left to right: (1) pure L-lysine; (2) resolved DL-lysine. Temperature, 26 ± 2°C; solvent front, 8.5 cm; detection, ninhydrin; solvent system, acetonitrile-methanol-water (10:5:2 v/v).

few drops of ammonia were added to make a slurry of pH 8. The plates were dried overnight at 60°C. The solutions of both DL-amino acids and their L enantiomers (10^{-3} M) were prepared in 70% ethanol and were applied side-by-side to the plates with 25-μl Hamilton syringe.

Chromatograms were developed in solvent systems comprising of acetonitrile-methanol-water in various proportions (v/v) in a paper-lined rectangular glass chamber pre-equilibrated with the solvent system for 10–15 min. The developed plates were dried at 40°C, cooled to room temperature, sprayed with dilute HCl, activated and then cooled to room temperature. The plates were then sprayed with freshly prepared ninhydrin solution (0.2% in acetone) and heated between 100 and 110°C for 10 min to reveal the characteristic spots of the amino acids.

3. Results and discussion

Various solvent compositions of mixtures of butanol, chloroform, acetic acid, acetonitrile, methanol and water were systematically tried. Extensive experimental work was carried out by varying the ratio of acetonitrile/H_2O/MeOH in the ternary mixture to work out the successful combinations, and only these are reported. The successful solvent systems were acetonitrile-methanol-water (7:6:3 v/v) for arginine, (7:6:2 v/v) for histidine, (10:5:2 v/v) for lysine, (10:4:3 v/v) for leucine, and (10:5:2 v/v) for valine. The amino acids were resolved on plates maintained at pH 8. The hR_F values of the resolved amino acids with their respective solvent compositions are given in Table 1. The results are averages of at least five identical runs. It was required to mark the spots as soon as possible otherwise the whole plate turned dark pink within 30 min. A photograph of the actual chromatogram showing resolution of DL-histidine is given in Fig. 2, Fig. 3 shows the actual chromatogram of resolution of DL-lysine, while Fig. 4 shows the actual chromatogram of resolution of DL-leucine, as typical results.

Chiral interactions between the chiral selector and the analyte are known to be affected by temperature [22]. So the effect of temperature on enantioresolution was studied. It was revealed that the best resolution was at 26 ± 2°C. Only DL-valine was resolved at 20°C, while no resolution of DL-amino acids was observed at 34 and 15°C.

Changes in pH are also known to affect chiral recognition [11,22]. Consequently, effect of pH in the present study was investigated. Thin layer plates were prepared at pH 4, pH 6, and pH 8. No resolutions of DL-amino acids were observed at pH 4 and pH 6. At pH 8, DL-histidine, -arginine, -lysine, -valine and -leucine were re-

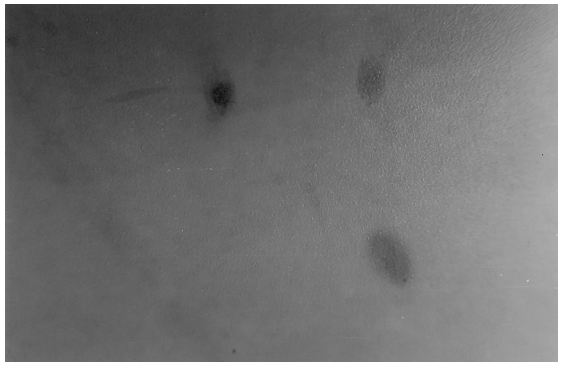
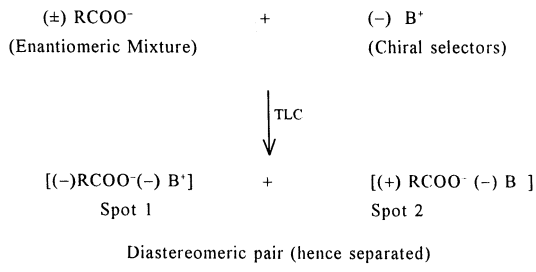


Fig. 4. Photograph of an actual chromatogram showing resolution of DL-leucine, as typical results. Left to right: (1) pure L-leucine; (2) resolved DL-leucine. Temperature, 26 ± 2°C; solvent front, 8.5 cm; detection, ninhydrin; solvent system, acetonitrile-methanol-water (10:4:3 v/v).



Scheme 1. Illustration of ion-pair separation mechanism.

solved, while DL-asparagine, -isoleucine, -methionine, -norleucine, -phenylanine, -alanine and -tryptophan showed a tendency to resolve as demonstrated by an eight shaped spot in the solvent system acetonitrile-methanol-water (10:4:2 v/v).

In the present study no enantioresolution was observed at pH 4 and pH 6. The p*I* values for the resolved amino acids are: histidine 7.8; arginine 10.8; lysine 9.8; valine 6.0 and leucine 6.0. At pH 4 all these amino acids exist as cations and at pH 6 both leucine and valine exist as a zwitterions, while the others exist as cations. Enantiomeric resolution was observed at pH 8. At this pH, leucine, valine and histidine exist as anions, while arginine and lysine would exist as cations. The $-$COOH group of the chiral selector would be expected to be COO^- and its $>$NH may be $>NH_2^+$ at pH 8. Consequently, a plausible explanation for enantiomeric resolution for lysine and arginine would be through ionic interactions between $-COO^-$ of the chiral selector and $-NH_3^+$ of the amino acids, hydrogen bonding and van der Walls forces. Enantioresolution may have been observed for histidine, leucine and valine due to ionic interactions between $-COO^-$ groups of the amino acid and $>NH_2^+$ groups of the chiral selector, hydrogen bonding and van der Walls bonds.

The chiral discrimination is due to in situ formation of diastereomeric ion-pairs. Since the formation of the diastereomers is not being carried out prior to spotting on TLC plate the method is regarded as a direct separation. The mechanism is illustrated in Scheme 1. (1*R*, 3*R*, 5*R*)-2-azabicyclo[3,3,0]octan-3-carboxylic acid (Fig. 1(a)) contains ionizable and hydrogen bonding forming groups (COOH and NH) which are in the vicinity of the chiral center. These properties make this chiral selector a good chiral ion-pairing agent.

The method described here provides a direct method for resolving DL-amino acids. The method is simple, less expensive and fast, uses a far less amount of chiral selector (0.011 M) compared to the reported amount (0.032 M) of the same chiral selector [8]. The method can be adapted for routine analysis of DL-amino acids, for control of enantiomeric purity, and in particular basic amino acids. The detection limit was 0.66 μg.

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