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Direct TLC Optical Resolution of Two Sulphur-Containing DL-α-Amino Acids Using Vancomycin as Chiral Selector

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Direct TLC enantioseparation for naturally occurring two sulphur-containing α-amino acids, cysteine and methionine, were achieved on silica gel plates using vancomycin as chiral impregnating reagent with only one developing solvent *n*-butanol-methanol-water- acetic acid (5:1.6:1.2:0.4, v/v/v/v). The effects of developing solvents, the ratio of mobile phase, concentration of chiral selector and temperature on the enantioseparation had been studied. This is the first report on TLC direct resolution of cysteine and methionine using vancomycin as chiral selector with same developing solvent.

Keywords: Cysteine, Methionine, Vancomycin, Chiral selector, Optical resolution, TLC.

INTRODUCTION

In nature, chiral phenomenon is ubiquitous. Many pharmaceutical and bioactive compounds are racemic mixtures with chiral isomers having nearly identical physical and chemical properties, which are much difference amount the biological activity, toxicity and metabolize mechanism of racemates. Enantioseparations play an important role in many domains. The racemates have been resolved into their enantiomers either directly or indirectly using different chromatographic techniques. TLC has many advantages over other chromatographic techniques due to simple and inexpensive. Some reviews for TLC enantioseparations have already been published[1-4].

Naturally occurring amino acids, which are differ only in the nature of side chain residue R, are important biological compounds that are associated with peptides and proteins and occupy an important position in the food and pharmaceutical industries. Using macrocyclic antibiotic erythromycin[5], vancomycin[6] and bovine serum albumin[7] *etc*, the TLC plates has also been used to resolve dansyl-DL-amino acids. Impregnated TLC plates with (-)-brucine[8], (1R,3R,5R)-2-azabicyclo [3,3,0] octan-3-carboxylic acid[9], L-proline-Cu(II)-complex[10], (2S,4R,2'RS)-4-hydroxy-l-(2'-hydroxydodecyl)-proline-Cu-(II)-complex[11], vancomycin[12] and teicoplanin[13] have been used for resolution of DL-amino acids. Further, enantiomeric RP-TLC separations of amino acids and their derivatives are achieved with cyclodextrins[14], bovine serum[15] and vancomycin[16] in chiral mobile phase.

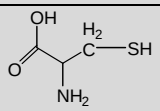
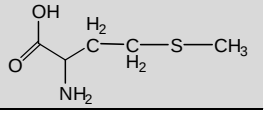
The powerful enantioseparation capability of glycopeptide antibiotics was first introduced by Armstrong and coworkers as chiral selectors and later on widely applied to chromatography for separation of enantiomers. In which, vancomycin and teicoplanin are the most frequently used and have success fully applied to HPLC and also to TLC for chiral separation of compounds of pharmaceutical interest[17-21].

Naturally occurring sulphur-containing α-amino acids only include cysteine and methionine (Table-1). With their simple structures and the ready availability of both enantiomers, they not only serve as a chiral pool for synthesis but also provide an inexpensive pool for resolution studies, especially for enantiomers of cysteine widely used in science and technology of gold nanomaterial. The objective of the present study is to present results on the resolution of two sulphur-containing DL-α-amino acids into their enantiomers on thin layer silica gel plates impregnated wancomycin as chiral selector with the same developing solvent. To the best of our knowledge, this is the first report on thin layer chromatographic direct resolution of two naturally occurring sulphur-containing DL-α-amino acids using vancomycin as chiral selector with same developing solvent[12,13].

EXPERIMENTAL

L-, D- and DL-cysteine and DL-methionine were from Fluka. Vancomycin was obtained from Suzhou Famu Import and Export Corporation (China). Silica gel G, with 14 % calcium sulphate as binder, having chloride, iron impurities

TABLE-1
CYSTEINE AND METHIONINE

Name	Abbreviation	Chemical structure
Cysteine	Cys	
Methionine	Met	

up to 0.02 % and with a pH 7 in a 10 % aqueous suspension, was supplied by Qingdao Ocean Chemical Factory (China). All other organic solvents and chemical reagents were of at least analytical-reagent grade (Beijing Chemical Factory, China).

Spreading a slurry of silica gel G (25 g) in H_2O (50 mL) containing recrystallized vancomycin (2.45 g), impregnated thin-layer plates (10 × 20 cm × 0.5 mm) were prepared. The plates were dried overnight at 60 °C. Solutions of both DL-amino acids and their L- or D- forms (10^{-2} M) were prepared in water (methionine) or 40 % acetic acid (cysteine) and were applied side-by-side to the plates with a syringe.

Chromatograms were developed in solvent systems comprising of *n*-butanol-methanol-water-acetic acid 5:1.6:1.2:0.4 (v/v/v/v) in a paper-lined rectangular glass chamber at 4 °C. These were pre-equilibrated with developers for 15 min. The chromatograms were developed 8.5 cm. The plates were then sprayed with freshly prepared ninhydrin solution (0.2 % in ethanol) and heated about 110 °C for 10 min to reveal the characteristic spots of the amino acids.

RESULTS AND DISCUSSION

In order to investigate the effect of concentration of the impregnating reagent on resolution, 1.08, 1.57, 1.86 to 2.45 g of vancomycin were added to 25 g of silica gel to prepare thin layer, respectively. The experiments observe that the best resolution was at 2.45 g of chiral selector vancomycin for cysteine and methionine. However, more contents of vancomycin weren't studied due to enough optical resolution obtained for two animo acids. Fig. 1 shows the relationship between the weight of vancomycin and ΔR_f of two amino acids.

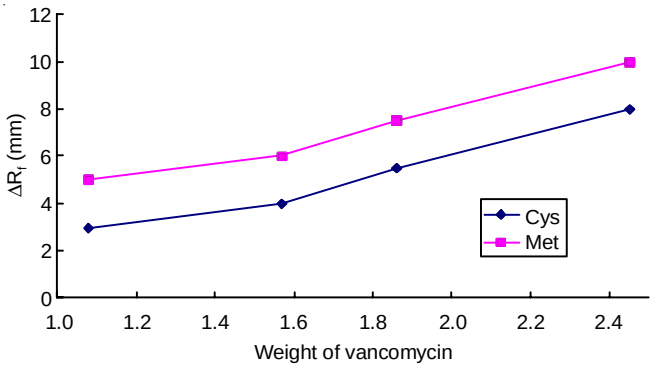


Fig. 1. Relationship between the weight of vancomycin and ΔR_f of two amino acids. Solvent front, 8.5 cm; temperature, 4 °C; solvent system, *n*-butanol-methanol-water-acetic acid 5:1.6:1.2:0.4 (v/v/v/v); weight of vancomycin: 1.08, 1.57, 1.86 and 2.45 g

For the resolution of DL-cysteine and DL-methionine into their enantiomers on plates impregnated with vancomycin, different solvent compositions of mixtures of water, methanol, butanol, ethyl acetate, acetonitrile, chloroform, benzene, hexane, acetic acid and propionic acid were systematically tried. The best solvent system was *n*-butanol-methanol-water-acetic acid 5:1.6:1.2:0.4 (v/v/v/v) for two DL-amino acid, whereas, the other compositions of mixtures resulted in poor or even no resolutions.

Keeping the ratio of *n*-butanol in the developing solvent, the effect of methanol content in the mobile phase has been studied by using *n*-butanol-methanol-water-acetic acid 5:1.3:1.2:0.4 (v/v/v/v), *n*-butanol-methanol-water-acetic acid 5:1.6:1.2:0.4 (v/v/v/v), *n*-butanol-methanol-water-acetic acid 5:2.0:1.2:0.4 (v/v/v/v) and *n*-butanol-methanol-water-acetic acid 5:2.4:1.2:0.4 (v/v/v/v) as mobile phase. The effect of concentration of water and acetic acid has also been studied with solvent systems of *n*-butanol-methanol-water-acetic acid 5:1.6:1.0:0.4 (v/v/v/v), *n*-butanol-methanol-water-acetic acid 5:1.6:1.4:0.4 (v/v/v/v), *n*-butanol-methanol-water-acetic acid 5:1.6:1.2:0 (v/v/v/v) and *n*-butanol-methanol-water-acetic acid 5:1.6:1.2:0.8 (v/v/v/v). The composition of developers and the distance of the two spots center are presented in Fig. 2-4, respectively. All experiments except Fig. 4 show that the best composition of mobile phase is *n*-butanol-methanol-water-acetic acid 5:1.6:1.2:0.4 (v/v/v/v) for two amino acids. When the concentration of acetic acid was zero in Fig. 4, the tailing of spot of cysteine was obvious. Therefore, the ratio of acetic acid was chosen to be 0.4 in all experiments. The different composition of solvent system also influence the separation time.

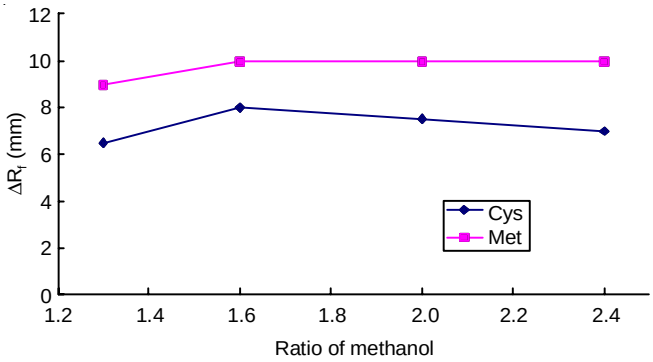


Fig. 2. Relationship between the ratio of methanol and ΔR_f of two amino acids racemates. Solvent front, 8.5 cm; temperature, 4 °C; solvent system, *n*-butanol-methanol-water-acetic acid 5:1.3:1.2:0.4 (v/v/v/v), *n*-butanol-methanol-water-acetic acid 5:1.6:1.2:0.4 (v/v/v/v), *n*-butanol-methanol-water-acetic acid 5:2.0:1.2:0.4 (v/v/v/v) and *n*-butanol-methanol-water-acetic acid 5:2.4:1.2:0.4 (v/v/v/v)

The temperature affect on the chiral interaction between the chiral selector and the analyte. Additional experiments with successful mobile phases were performed at 20 and 35 °C. Each temperature was maintained inside an incubator and the chamber was pre-equilibrated for 15 min at each temperature. Studies on the effect of temperature on enantioseparation of racemic amino acid in TLC with vancomycin as chiral selector revealed that the best resolution was obtained at 4 °C. The higher temperature got poor resolution. The relationship between temperature and ΔR_f of the two amino acids is presented in Fig. 5.

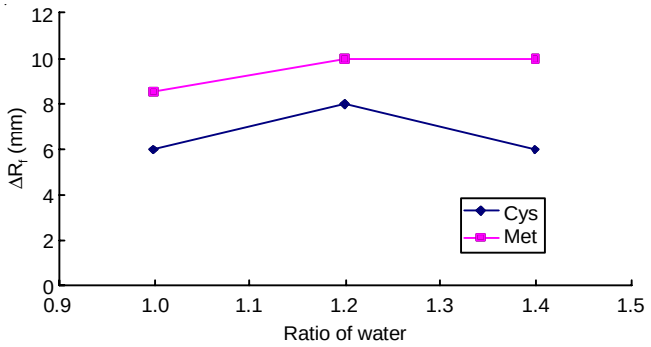


Fig. 3. Relationship between the ratio of water and ΔR_f of two amino acids racemates. Solvent front, 8.5 cm; temperature, 4 °C; solvent system, *n*-butanol-methanol-water-acetic acid 5:1.6:1.0:0.4 (v/v/v/v), *n*-butanol-methanol-water-acetic acid 5:1.6:1.2:0.4 (v/v/v/v) and *n*-butanol-methanol-water-acetic acid 5:1.6:1.4:0.4 (v/v/v/v)

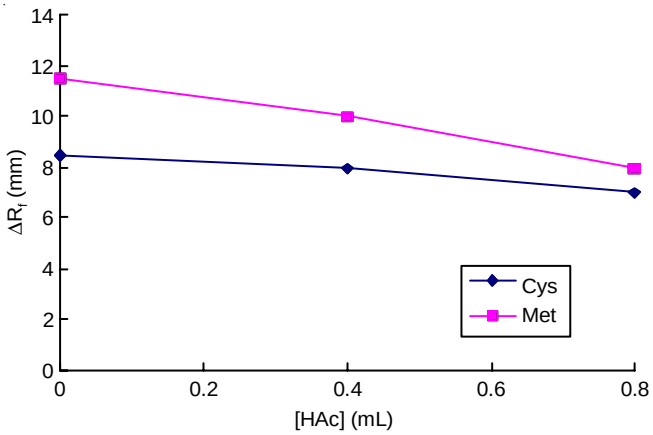


Fig. 4. Relationship between the ratio of acetic acid and ΔR_f of two amino acid racemates. Solvent front, 8.5 cm; temperature, 4 °C; solvent system, *n*-butanol-methanol-water 5:1.6:1.2 (v/v/v), *n*-butanol-methanol-water-acetic acid 5:1.6:1.2:0.4 (v/v/v/v) and *n*-butanol-methanol-water-acetic acid 5:1.6:1.2:0.8 (v/v/v/v)

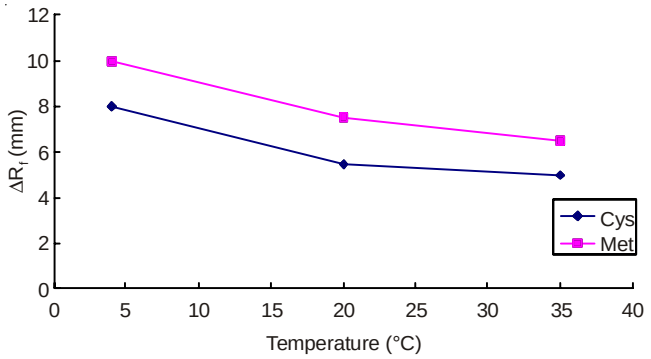


Fig. 5. Relationship between the temperature and ΔR_f of two amino acid racemates. Solvent front, 8.5 cm; temperature, 4, 20 and 35 °C; solvent system, *n*-butanol-methanol-water-acetic acid 5:1.6:1.2:0.4 (v/v/v/v)

Chiral recognition requires a minimum of three-points of interaction[21]. Earlier studies showed[17] that (1) vancomycin contains ionizable functional groups which can be either acidic or basic; (2) vancomycin has multiple stereogenic centers; (3) vancomycin processes numerous functional groups contributive to stereos-electivity; (4) vancomycin contains both hydrophobic and hydrophilic group. The chiral separation mechanism is based on electrostatic interactions as well as secondary interactions such as hydrophobic, hydrogen bonds, dipole-dipole, π-π interactions and steric repulsion because the racemate can form transient noncovalent diastereomeric complexes with glycopeptides antibiotic.

Conclusion

According to the present study, vancomycin is sufficiently stable, had good enantioselective property. The impregnated vancomycin TLC can separate simultaneously the racemates of cysteine and methionine with only one developing solvent *n*-butanol-methanol-water-acetic acid (5:1.6:1.2:0.4, v/v/v/v). This method can be considered as an improvement compared with earlier reports. The technique is versatile, flexible, simple, direct and economical and may become the method of choice compared with other chromatographic techniques for fast routine analysis.

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