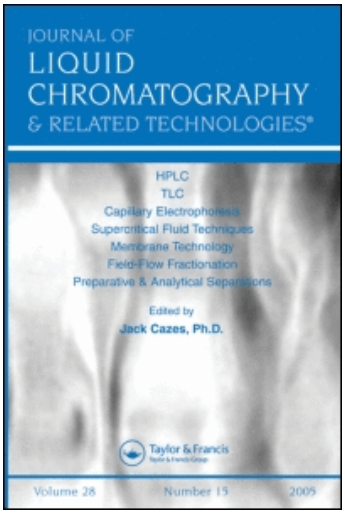

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Direct Chromatographic Separation of 2-(2-Amino-1, 3-thiazol-4-yl)methylglycine and Its Methyl Ester Enantiomers Using a Chiral Crown Ether Column

Sechoing Lin[a]; Nancy J. Maddox[a]
[a] Chemical Development Department, Parke-Davis Pharmaceutical Research Division Warner-Lambert Company, Holland, Michigan

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JOURNAL OF LIQUID CHROMATOGRAPHY, 18(10), 1947-1955 (1995)

DIRECT CHROMATOGRAPHIC SEPARATION OF 2-(2-AMINO-1,3-THIAZOL-4-YL)METHYLGLYCINE AND ITS METHYL ESTER ENANTIOMERS USING A CHIRAL CROWN ETHER COLUMN

SECHOING LIN* AND NANCY J. MADDOX
Chemical Development Department
Parke-Davis Pharmaceutical Research Division
Warner-Lambert Company
188 Howard Avenue
Holland, Michigan 49424

ABSTRACT

Direct liquid chromatographic separation of 2-(2-amino-1,3-thiazol-4-yl) methylglycine and its methyl ester enantiomers was achieved using a chiral crown ether column. The separation was strongly influenced by column temperature and the pH of mobile phase. The method can detect the undesired (+)-enantiomer down to a level of 0.5% and is routinely employed to determine the purity of the desired (-)-enantiomer.

INTRODUCTION

(-)-2-(2-Amino-1,3-thiazol-4-yl)methylglycineand its methyl ester are two intermediates in a process to synthesize CI-992, a renin inhibitor with potential antihypertensive activity (1). Chemical resolution produced the desired (-)-enantiomer. Optimization of the resolution and determination of enantiomeric

*Author to whom inquiries should be addressed

purity of the (-)-enantiomer required a chiral method that would allow the separation of the undesired (+)-enantiomer from a large excess of the (-)-enantiomer.

A crown ether-based stationary phase has been utilized for separation of primary amine-containing compounds (2-11). The same stationary phase was used to evaluate the enantiomeric separation of 2-(2-amino-1,3-thiazol-4-yl)methylglycine and its methyl ester. The effects of flow rate, column temperature, organic modifier, and mobile phase pH were also determined.

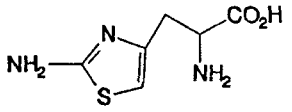
EXPERIMENTAL

Apparatus

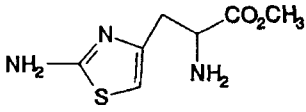
Chromatographic analysis was performed using a Waters 590 pump, a Micromeritics 728 autosampler and a Rheodyne 7010 injector with a 20 μl sampling loop, a Hitachi L-4000 variable wavelength UV detector, and a Hitachi D-2000 Chromato-Integrator. The chiral column is a Crownpak CR(+), (150 x 4.0 mm, 5 micron particle size) from Chiral Technologies, Inc., Exton, PA. A Brinkmann Lauda Model RMS-6 refrigerating circulator was used to control the column temperature. The mobile phase pH was determined using an Orion Model EA 940 expandable ionAnalyzer with a ROSS SURE-FLOW combination pH electrode.

Chemicals

Methanol (HPLC grade) was purchased from EM Science, Gibbstown, NJ. Perchloric acid (70%) was obtained from MCB Manufacturing Chemists, Inc., Cincinnati, OH. Water (HPLC grade) was from a Milli-Q water purification system. 2-(2-Amino-1,3-thiazol-4-yl)methylglycine, (+)-2-(2-amino-1,3-thiazol-4-yl)methylglycine, (-)-2-(2-amino-1,3-thiazol-4-yl)methylglycine, and the corresponding methyl esters were prepared at Parke-Davis Pharmaceutical Research Division, Holland, MI (12).



(A)



(B)

FIGURE 1. Chemical structures of (A) 2-(2-amino-1,3-thiazol-4-yl)methylglycine (abbreviated as acid) and (B) 2-(2-amino-1,3-thiazol-4-yl) methylglycine, methyl ester (abbreviated as ester).

Chromatographic Conditions

The mobile phase was aqueous perchloric acid except for the organic modifier study in which methanol was mixed with aqueous perchloric acid. The UV detection wavelength was set at 210 nm. Sample amount injected for the determination of enantiomeric purity was about 0.12 μmole.

RESULTS AND DISCUSSION

The mechanism for enantioselective retention of various amino acids on the crown ether stationary phase has been detailed (10). 2-(2-Amino-1,3-thiazol-4-yl) methylglycine, a synthetic amino acid, and its methyl ester as shown in Figure 1 each has a free amine adjacent to the chiral center. This structural characteristic makes them candidates for enantiomeric separation using a crown ether HPLC column. It would be desirable to resolve all four enantiomers.

Effect of the pH of Mobile Phase

As shown in Figure 2, the pH of mobile phase has a dramatic effect on the enantiomeric separation. The enantiomeric resolution increases with decrease in mobile phase pH. This is consistent with the earlier observations (3,9). At pH 1.60 or above, the acid was partially resolved and the ester was well resolved. At pH 1.00, all four enantiomers were well resolved.

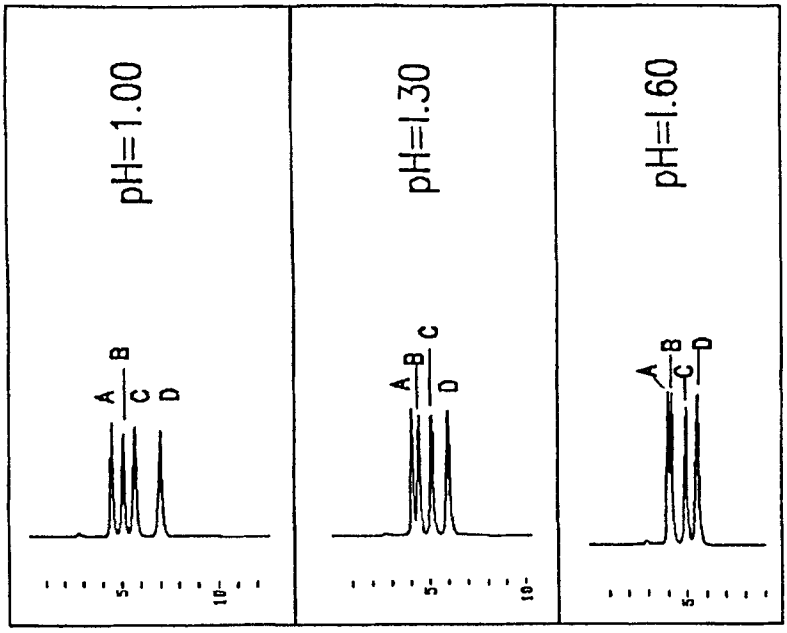


FIGURE 2. Effect of the pH of mobile phase on separation of A=(+)-acid; B=(-)-acid; C=(+)-ester; (D)=(-)-ester. The column temperature was 25°C and flow rate was 0.5 mL/min.

Effect of Column Temperature

The enantiomeric resolution increased as the column temperature decreased for acid and ester as shown in Figure 3. This is mainly attributed to the combined effects of increased chiral recognition and increased retention at lower temperature (10,11). All four enantiomers were resolved at 25°C. Partial separation was obtained for (-)-acid and (+)-ester at 15°C. All four enantiomers were resolved again at 5°C with (+)-ester eluted earlier than (-)-acid.

Effect of Flow Rate

Changes in flow rate from 0.3 mL/min. to 0.7 mL/min. showed little effect on separation and resolution as seen in Figure 4.

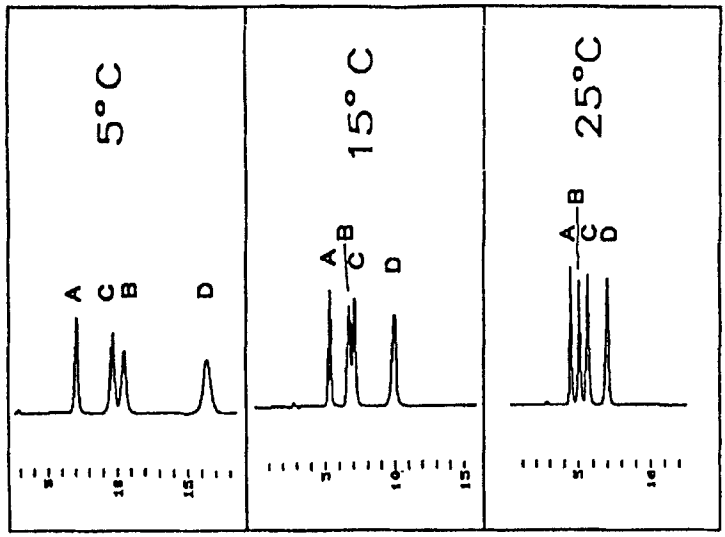


FIGURE 3. Effect of column temperature on separation of A=(+)-acid; B=(-)-acid; C=(+)-ester; D=(-)-ester. The pH of mobile phase was 1.00 and flow rate was 0.5 mL/min.

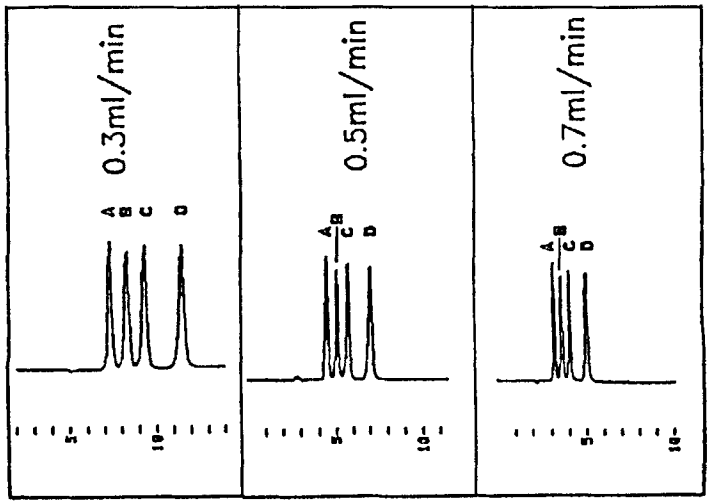


FIGURE 4. Effect of flow rate on separation of A=(+)-acid; B=(-)-acid; C=(+)-ester; D=(-)-ester. The pH of mobile phase was 1.00 and column temperature was 25°C.

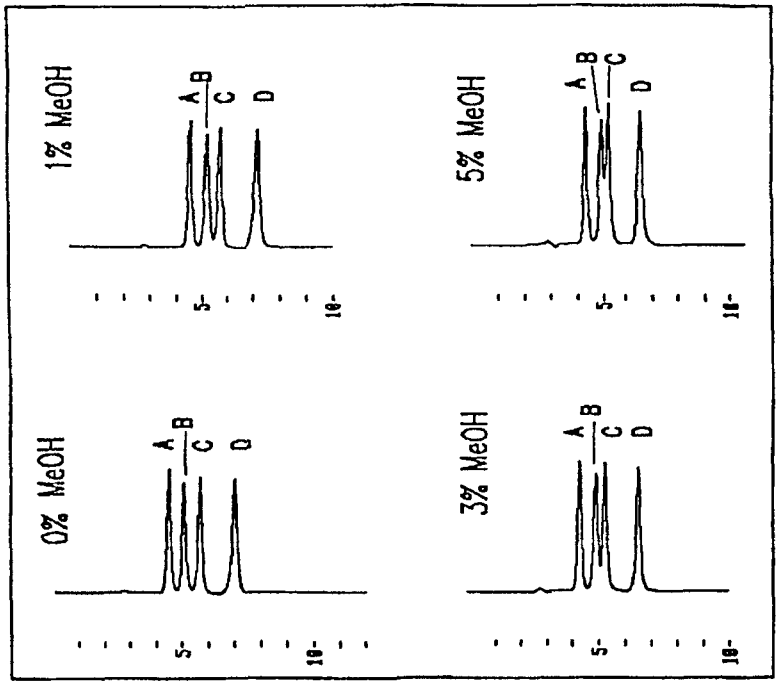


FIGURE 5. Effect of methanol content on separation of A=(+)-acid; B=(-)-acid; C=(+)-ester; D=(-)-ester. The pH of mobile phase was 1.00 and flow rate was 0.5 mL/min.

Effect of Methanol

Compounds with greater hydrophobicity in general have longer retention times on a crown ether stationary phase (10). Methanol has been used as a modifier in mobile phase to reduce retention (6,10). The increase of methanol content in mobile phase from 0 to 5% did not significantly change the enantiomeric resolution for acid and ester as shown in Figure 5. However, the appreciable decrease in retention for ester led to a partial separation between (-)-acid and (+)-ester when methanol content was increased to above 3%.

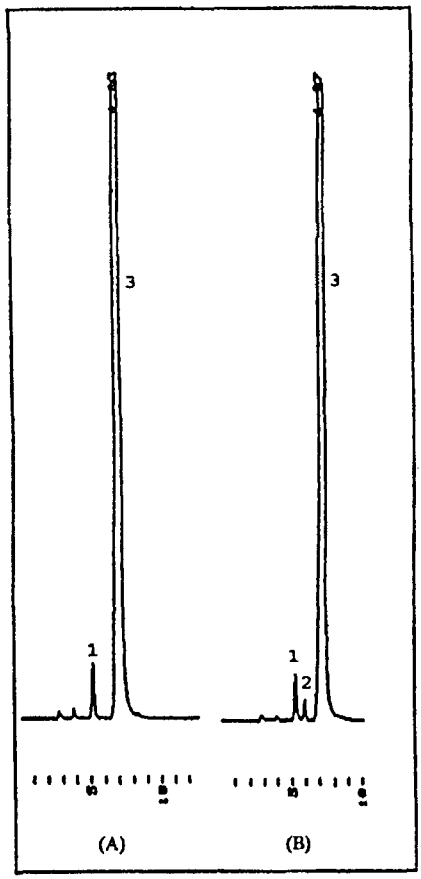


FIGURE 6. (A) Chromatogram of an enantiomerically pure (-)-ester sample (B) chromatogram of the same sample spiked with 0.5% (+)-ester. 1=(-)-acid; 2=(+)-ester; 3=(-)-ester. Mobile phase was an aqueous perchloric acid (pH 1.00), column temperature was 25°C and flow rate was 0.5 mL/min.

Applications

As a result of the study above, the crown ether stationary phase was found to be useful for direct chiral separation of 2-(2-amino-1,3-thiazol-4-yl) methylglycine and 2-(2-amino-1,3,thiazol-4-yl)methylglycine, methyl ester. The chromatogram obtained from an (-)-ester sample using the conditions given in Figure 6(A) indicated that a small amount of (-)-acid as an impurity was well resolved from a large amount of desired (-)-ester. The same sample when spiked with 0.5% (+)-ester as shown in the chromatogram in Figure 6(B) further demonstrated that the method can detect the undesired enantiomer to the level of 0.5%.

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REFERENCES

1. Repine, J.T.; Himmelsbach, R.J.; Hodges, J.C.; Kaltenbronn, J.S.; Sircar, I.; Skeean, R.W.; Brennan, S.T.; Hurley, T.R.; Lunney, E.; Humblet, C.C.; Weishaar, R.E.; Rapundalo, S.; Ryan, M.J.; Taylor, Jr., D.G.; Olson, S.C.; Michniewicz, B.M.; Kornberg, B.E.; Belmont, D.T.; Taylor, M.D., Renin Inhibitors Containing Esters at the P_2-position. Oral Activity in a Derivative of Methyl Aminomalonate, J. Med. Chem., 34, 1935 (1991).

2. Daicel's Application Guide for Chiral Column Selection (1989).

3. Udvarhelyi, P. M.; Watkins, J. C.; Direct Resolution of some Phenylglycines by Liquid Chromatography on a Chiral Crown Ether Phase, Chirality, 2, 200 (1990).

4. Motellier, S.; Wainer, I.W., Direct Stereochemical Resolution of Aspartame Stereoisomers and their Degradation Products by High-performance Liquid Chromatography on a Chiral Crown Ether based Stationary Phase, J. Chromatogr., 516, 365 (1990).

5. Aboul-enein, H.Y.; Bakr, S.A.; Islam, M.R.; Rothchild, R., Direct Chiral Liquid Chromatographic Separation of Tocainide Enantiomers on a Crownpak (CR) Column and its Application to Pharmaceutical Formulations and Biological Fluids, J. Liquid Chromatogr., 14, 3475 (1991).

6. Hilton, M.; Armstrong, D.W., Evaluation of a Chiral Crown Ether LC Column for the Separation of Racemic Amines, J. Liquid Chromatogr., 14, 9 (1991).

7. Hilton, M.; Armstrong, D.W., Evaluation of the Enantiomeric Separation of Dipeptides Using a Chiral Crown Ether LC Column, J. Liquid Chromatogr., 14, 3673 (1991).

8. Okamoto, M.; Takahashi, K.I.; Doi, T., Direct Stereochemical Resolution of 3,4-Dihydroxyphenylserine using a Chiral Crown Ether Stationary Phase, J. Chromatogr. A, 675, 244 (1994).

9. Kersten, B.S., HPLC Chiral Optimization of a Unique β-Amino Acid and its Ester, J. Liquid Chromatogr., 17, 33 (1994).

10. Shinbo, T.; Yamaguchi, T.; Yanagishita, H.; Kitamoto, D.; Sakaki, K.; Sugiura, M., Improved Crown Ether-based Chiral Stationary Phase, J. Chromatogr., 625, 101 (1992).

11. Shinbo, T.; Yamaguchi, T.; Nishimura, K.; Sugiura, M.; Chromatographic Separation of Racemic Amino Acids by use of Chiral Crown Ether-coated Reversed-phase Packings, J. Chromatogr., 405, 145 (1987).

12. 2-(2-Amino-1,3-thiazol-4-yl)methylglycine, (+)-2-(2-amino-1,3-thiazol-4-yl)methylglycine, (-)-2-(2-amino-1,3-thiazol-4-yl)methylglycine and the methyl esters were prepared by Mr. Mark Hoekman at Parke-Davis.

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