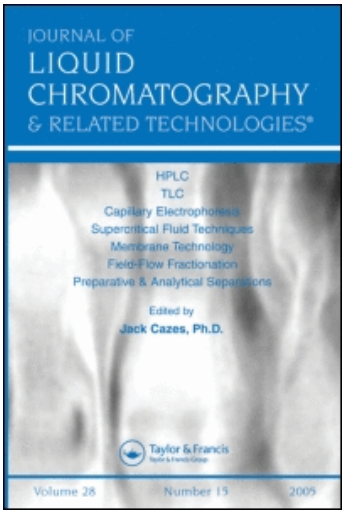

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Resolution of D,L-Dansyl Amino Acids by HELC with a Cu(II)-L-proline Eluant

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RESOLUTION OF D,L-DANSYL AMINO ACIDS BY HPLC WITH A Cu(II)-L-PROLINE ELUANT

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ABSTRACT

Highly fluorescent dansyl derivatives of D and L amino acids were resolved on a reversed phase column by using an aqueous eluant containing copper(II)-L-proline.

INTRODUCTION

The separation of optical isomers, especially D and L amino acids, has been a challenge and a topic of great research interest in liquid chromatography. To facilitate the enantiomeric differentiation, a chiral center has to be present in the chromatographic system; whether it is bonded to a solid support or added to the eluant. Optically active proline has been a classical reagent for the resolution of amino acid racemates. Davankov and Semechkin (1) have reviewed some aspects of ligand exchange chromatography of amino acid racemates on stationary supports to which one of the proline enantiomers were bonded. More recently, Gil-Av and Hare separated free D and L amino acids on cation exchange (2) and reversed phase columns (3) using an aqueous eluant that contained chiral copper(II)-proline complexes.

Dansyl amino acids are strongly fluorescent, and can be detected in the deep blue colored copper eluant with no difficulty.

This paper reports our initial successes in using a Cu(II)-L-proline eluant to separate D and L amino acids as their dansyl derivatives on a reversed phase column.

EXPERIMENTAL

Instrumentation. The chromatograph was a Perkin Elmer Model 601 LC equipped with a Rheodyne 7105 injection valve, a Model LC 650-10 fluorescence spectrophotometer, and a Model 56 chart recorder. The analytical column was 25.0 x 0.3 cm packed with C_{18} Partisil-5 support prepared as described below. All separations were performed at room temperatures.

Reagents. Acetonitrile, distilled in glass was obtained from Burdick and Jackson Laboratories, Inc., Muskegan, MI. D,L-dansyl amino acids were bought from Sigma Chemical Co., St. Louis, MO and Pierce Chemical Co., Rockford, IL. Silylation reagents for preparing the stationary support were purchased from Petrarch System Inc., Levittown, PA.

Preparation of stationary phase. Partisil-5, 5g was dried in a muffle oven at 400° C for 2 hours, and then transferred into a 250 ml round bottom flask to which 100 ml of 10% dimethyloctadecylchlorosilane in dry toluene was added. After shaken for 8 hours, the packing material was washed and dried. Unreacted surface silanol groups were end-capped with 100 ml of 10% trimethylchlorosilane in dry toluene to ensure a total non-polar surface (4). The column was packed by the downward slurry technique. Before analysis, the column was equilibrated with an aqueous solution that was 0.1M in proline and 0.05M in $CuSO_4 \cdot 5H_2O$.

Chromatographic procedure. The mobile phase consisted of 20% acetonitrile in an aqueous solution that was 5×10^{-3}M proline and ammonium acetate, and 2.5×10^{-3}M $CuSO_4 \cdot 5H_2O$. The pH of the aqueous portion of the mobile phase was 7 by the addition of ammonium hydroxide. The effluent flow rate was 1 ml/min. The fluorescence at 480 nm was monitored with excitation at 340 nm.

RESULTS

Enantiomers of aliphatic, hetercyclic and aromatic amino acids could be resolved by the use of Cu(II)-L-proline mobile phase. However, the separation of D and L phenylalanine, and D and L tryptophan dansyl derivatives were particularly striking (Figs 1 and 2). The elution of L before D isomer in our study, agrees with the results reported by other workers (3).

DISCUSSION

HPLC with metal solute complexes are highly selective for isomeric separations. Some works in using Ag(I) (5) and other metal ion complexes (6) to achieve isomeric resolutions have recently been reviewed. In this report, using an aqueous eluant of Cu(II)-L-proline complexes, a 'dynamic' rather than bonded optically active stationary phase is obtained. Dynamic stationary phases on hydrocarbonaceous solid supports, as reported by other workers (7,8), usually give better chromatographic efficiency than covalently bonded stationary phases. The dynamic Cu(II)-L-proline phase provides the site for chiral recognition. Based on the formation of more stable complexes of the Cu(II)-L-proline with L dansyl amino acids, optical resolution of the L amino acids from their D mirror images is accomplished.

Besides the present application to dansyl amino acids, the chiral additive being a secondary amine, allowed for fluorescence detection of the separated D and L amino acids by post column derivatization with O-phthaldehyde (3). The reagents are readily available, and the chromatographic conditions are mild. All these advantages together with the efficiency and selectivity of the analysis have demonstrated that the Cu(II)-L-proline system is a very promising approach to the resolution of amino acid racemates.

This communication have presented our preliminary successes. Detailed investigation of the system and the use of other potential chiral additives is being studied, and the results will appear in a latter publication.

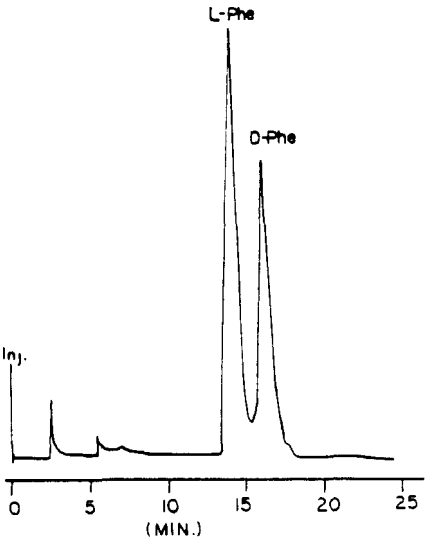

Figure 1

Separation of D,L dansyl phenylalanine with Cu(II)-L-proline eluant. Column : Partisil-5 C_{18}. Mobile phase : 20% acetonitrile in an aqueous solution that was 5 x 10^{-3}M proline and ammonium acetate, and 2.5 x 10^{-3}M $CuSO_4 \cdot 5H_2O$, pH 7.0. Flow rate 1.0 ml/min.

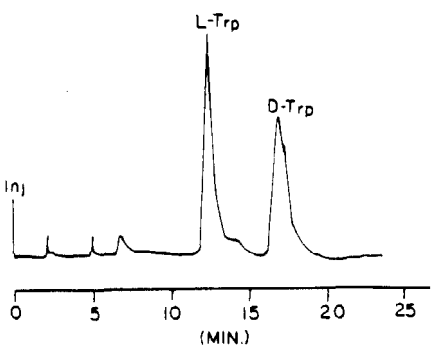

Figure 2

Separation of D,L dansyl tryptophan with Cu(II)-L-proline eluant. Conditions as in Figure 1.

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