Enantioseparation Condition of tryptophan enantiomers using Ligand Exchange Chromatography

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A simple and precise method for the chiral separation of tryptophan enantiomers was developed by high performance liquid chromatography using ligand exchange mobile phase. The effect of different kinds and concentration of ligands, bivalent copper ion, organic modifier, pH of mobile phase, and temperature on enantioseparation were investigated and evaluated. Under the optimum condition, baseline separation of the two enantiomers with a resolution of 3.42 in less than 30 min was obtained on the C18 column using methanol-water solution (80:20 v/v) as mobile phase, which containing 3.0 mmol·L-1 L-phenylalanine and 0.5 mmol·L-1 copper sulphate. Thermodynamic data ($\triangle \triangle H$ and $\triangle \triangle S$) obtained by Van't Hoff plots revealed the enantioseparation is an enthalpy-controlled process. The mechanism of chiral discrimination is based on the stabilities of the copper (II) binary complexes and their ternary diasteremeric complexes with amino acids formed in solution and stationary phase. The proposed method was successfully used for the quality evaluation of tryptophan enantiomers.