Letters to the Editor

Determination of Malic Acid Enantiomers by Ligand-Exchange Photometric Ion Chromatography

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Studies on the differences in physiological effects of enantiomers require both separative and quantitative techniques for them. High performance liquid chromatographic (HPLC) methods for the enantiomeric resolution using a chiral metal complex as an additive to the eluent have been reported.[1,2] In these systems, however, the alterations in absorbance on the elutions of enantiomers were too small to be detected directly.

Photometric ion chromatography (PIC) has an advantage that both chromophoric and nonchromophoric analytes are determined with a conventional HPLC system equipped with a UV detector.[3-5] In the study of metal complexes as effective eluents for PIC[6,7], the authors found that several enantiomers could be separated and directly detected by using chiral copper complex eluent. The method was based on a ligand-exchange mechanism in PIC.

In this paper, the authors demonstrate the determination of malate enantiomers by using a hydrophilic material-based anion exchange column and chiral coper(II)-tartrate complex as an eluent.

Experimental

The PIC system included a Shimadzu (Kyoto, Japan) LC-9A pump, a Rheodyne (Cotati, CA, USA) Model 7125 injector (loop of 100 µl), a Shimadzu SPD-6AV UV-visible detector and a Shimadzu C-R4A Chromatopac calculator. The anion-exchange column used was a Tosoh (Tokyo, Japan) TSK gel IC-Anion-PW (5 cm×4.6 mm i.d.). A Tokyo Rika Kikai (Tokyo, Japan) Model S-14 carboxylic acid analyzer was used for the determination of total malic acid.

All reagents were of guaranteed grade. D-Malic acid was purchased from Aldrich Chem. (Milwaukee, WI, USA), sodium L-malate from Wako (Osaka, Japan), L-tartaric acid from Tokyo Kasei (Tokyo, Japan) and copper(II) hydroxide from Kanto Chemical (Tokyo, Japan). Test-Combinations for the enzymatic determination of L-malic acid were purchased from Boehringer Mannheim Yamanouchi (Tokyo, Japan).

Results and Discussion

The selection of the ligand in the eluent is the most important factor in this system. Tartaric acid was used for the determination of malic acid enantiomers, since the copper(II) complex formation constant of tartaric acid[8] is similar to that of malic acid.[9] Figure 1(A) shows the separation of malic acid enantiomers by using 1.5 mM copper(II) hydroxide+3 mM L-tartaric

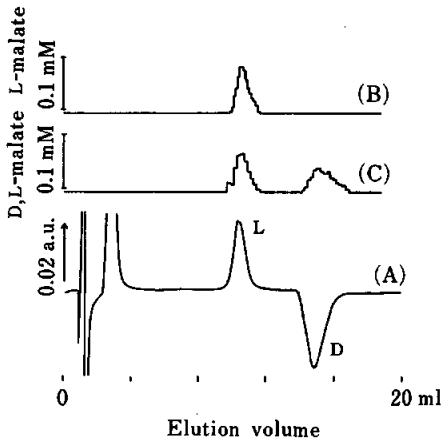

Fig. 1 Typical chromatogram of malic acid enantiomers (A) and verification of their elutions. Conditions: column, IC-Anion-PW (5 cm×4.6 mm i.d.); eluent, 1.5 mM $Cu(OH)_2$+3 mM L-tartrate (pH 4.7); flow rate, 0.8 ml/min; detection, 281 nm; column temp., 40°C; sample, 0.1 µM each of D,L-malate. Column effluent malic acid elution profiles were analyzed by enzymatic method (B) and carboxylic acid analyzer (C).

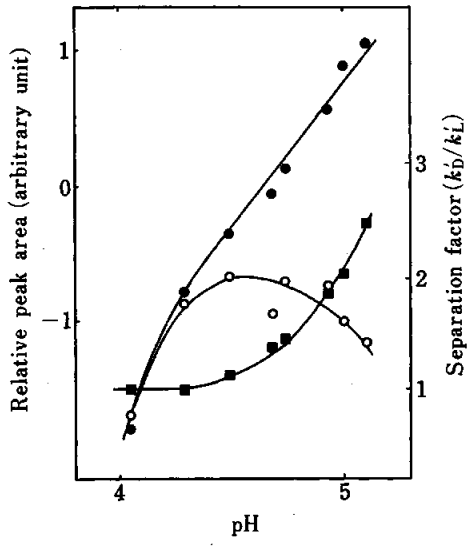

Fig. 2 Relative peak areas and separation factors of malic acid enantiomers as a function of eluent pH. Symbols: ●, relative peak area of L-malate; ○, relative peak area of D-malate; ■, separation factor. Sodium hydroxide solution was used for eluent pH adjustment.

acid (pH 4.7) as the eluent. The detection wavelength was 281 nm, at which the absorbance of eluent was approximately 1. Both Figs. 1(B) and 1(C), which were obtained by the enzymatic method and carboxylic acid analyzer, respectively, showed that the elutions of malic acid enantiomers were restricted to their corresponding peaks on the chromatogram. Figure 2 shows the relative peak areas and separation factor of malic acid enantiomers as a function of eluent pH of 1 mM copper +2 mM L-tartrate solution. The separation factor of enantiomers was improved as the pH increased from 4 to 5. D-Malate showed a negative peak at all pH values, while the inversion of peak direction was observed for L-malate.

At pH 5.0, where the peak height and depth of malic acid enantiomers were approximately equal, 1 nmol of each enantiomer could be determined. The straight calibration curves were obtained for peak areas against two orders of concentration. Other organic acids and inorganic anions caused no interference, since these complex formation constants of copper differ greatly from that of malate. This PIC system with chiral copper(II)-tartrate eluent is highly specific for malic acid.

The above results suggested that a ligand-exchange occurred in this PIC. At present, the authors are developing a theory based on stoichiometry to elucidate the mechanism of the enantiomeric separation. The details will be reported elsewhere.

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