Journal of Chromatography A, 666 (1994) 375–379
Elsevier Science B.V., Amsterdam

CHROM. 25 448

Short Communication

Chiral separation by capillary zone electrophoresis of an optically active drug and amino acids by host–guest complexation with cyclodextrins☆

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ABSTRACT

Capillary zone electrophoresis with γ-cyclodextrin as buffer constituent was successfully applied to the enantiomeric separation of the neuroactive chiral drug SDZ EAA 494 s, which is a high-affinity competitive N-methyl-D-aspartate antagonist. A borate buffer containing 20 mmol/l γ-cyclodextrin (pH 11.0) was used (voltage 15 kV). The detection limit of SDZ EAA 494 s was improved by derivatization with dansyl chloride (0.1% of the antipode can be determined). The dansylation of the drug is also responsible for the enantioselectivity obtained. The electrophoretic behaviour of racemates of dansylated amino acids (Asp, Glu, Lys, Arg, Gln, His, Asn, Pro, Val, Phe) was investigated with α,β,γ-cyclodextrins at different pH values.

INTRODUCTION

The understanding of the action of drugs in relation to their stereochemistry is important for the assessment of the pharmacological and toxicological properties of new drugs. Chromatographic techniques are the method of choice for this purpose. In addition to GC and HPLC, CE offers an alternative for the chiral separation of polar and non-polar compounds. The chiral separation is obtained by the formation of diastereomeric molecular complexes of the enantiomers with the chiral selector.

Cyclodextrins (CDs) are suitable for the chiral separation of a large number of drugs and amino acids. Cyclodextrins are widely used in GC and LC and as chiral additives for open-tube CE. The formation of host–guest complexes permits the separation of enantiomers by their complexation constants. Especially in the case of charged compounds capillary zone electrophoresis (CZE) is a highly efficient separation technique for enantiomers.

SDZ EAA 494, [*R*-(*E*)]-4-(3-phosphono-2-propenyl)-2-piperazinecarboxylic acid, is a highly specific and potent competitive antagonist at the N-methyl-D-aspartate (NMDA) type of excitatory amino acid receptor. The compound antagonizes NMDA-mediated effects in the spinal cord, striatum and cerebral cortex [1]. The separation of the enantiomers of this drug was impossible with conventional Cyclobond I or II columns.

Free solution CE with cyclodextrins as chiral recognition agents has been reported for a number of racemic pharmaceuticals [2–4]. Recently, data on the chiral separation of dansylated amino acids by micellar electrokinetic

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0021-9673/94/$07.00
SSDI 0021-9673(93)E0843-J

capillary chromatography with taurodeoxycholic acid and β-cyclodextrin were published [5].

This paper considers quantitative aspects of the chiral separation of racemic SDZ EAA 494 s. The chiral separation of different amino acids as a function of pH was investigated in order to examine the possibilities of the separation of racemic pharmaceuticals with structures similar to those of amino acids.

EXPERIMENTAL

All separations were performed at a Waters Quanta 4000 instrument in fused-silica capillary tubes (60 cm × 75 μm I.D.). The applied voltages were 10 and 15 kV. Sample injection was performed hydrostatically for 5 s. Detection was performed at 214 nm.

All reagents were of analytical-reagent grade. Borate buffer (0.1 mol/l) was obtained from Merck and the pH was adjusted to 9.5 with NaOH. Phosphate buffers were used at pH 2.6. The concentrations of α-, β- and γ-CD were 20, 10 and 20 mmol/l, respectively (Fluka). Different γ-CD concentrations (10, 15 and 20 mmol/l) were tested at pH 11, and it was found that the best resolution of the racemate of SDZ EAA 494 was obtained at the highest concentration.

Derivatization with dansyl chloride

D,L-Amino acids and SDZ EAA 494 s were derivatized with dansyl chloride according to Tapui *et al.* [6]. The samples were dissolved in borate buffer (pH 9.5) and reacted with dansyl chloride for 30 min at 30°C. The reaction was stopped by addition of methylamine hydrochloride. The yield for SDZ EAA 494 s was 91.5 ± 2.1%.

Normalization of peak areas to their migration times was performed prior to calculation of area percentages.

RESULTS AND DISCUSSION

The separation of dansylated D,L-amino acids was investigated under acidic (pH 2.6) and alkaline (pH 9.5) conditions. Further, the influence of the different cyclodextrins on the electrophoretic separation was studied. Figs. 1 and 2 demonstrate the separation of D,L-amino acids with γ-CD in the mobile phase at pH 2.6 and 9.5, respectively. An overview of the separation of the racemates of dansylated amino acids in using the different cyclodextrins is given in Fig. 3. With γ- and β-CD baseline separations of D,L-amino acids were possible. Aspartate and glutamate were well resolved at pH 9.5 with

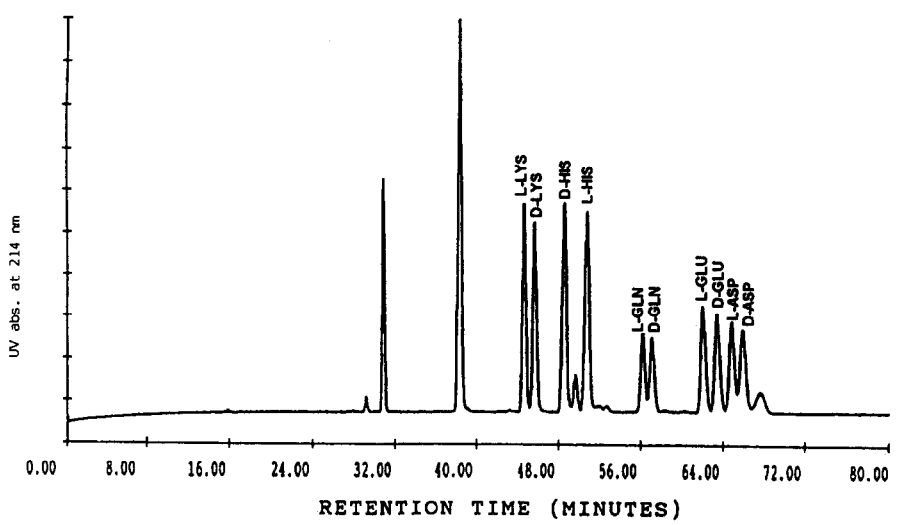

Fig. 1. Separation of dansylated D,L-amino acids with 0.1 mol/l phosphate buffer of pH 2.6 and 20 mmol/l γ-CD at 10 kV with a sampling time of 5 s. Capillary, 60 cm × 75 μm I.D.

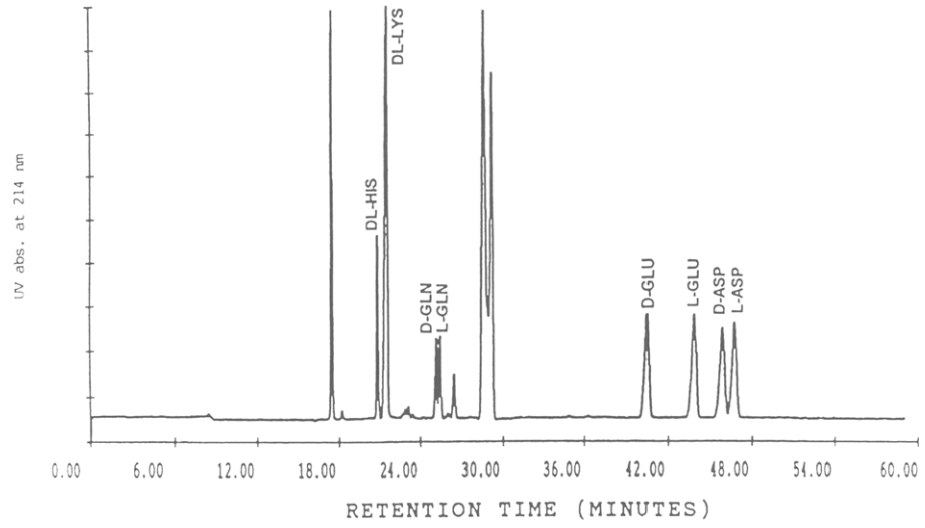

Fig. 2. Separation of dansylated D,L-amino acids with 0.1 mol/l borate buffer of pH 9.5 and 20 mmol/l γ-CD at 10 kV with a sampling time of 5 s. Capillary, 60 cm × 75 μm I.D.

β-CD and histidine and valine were well resolved at pH 2.6 with γ-CD. The naphthyl group of the dansylated amino acids fits well into the cavities of β- and γ-CD. This spatial enclosure by the host molecule allows the separation of the racemates. The cyclodextrins are not charged and thus move with the velocity of the electroosmotic flow. The migration time of the enantiomers is controlled by their interaction with the host and their own electrophoretic mobility. At pH 2.6 the L-amino acids eluted before the corresponding D-forms, with the exception of the racemate of histidine.

The dansylated racemate of SDZ EAA 494 s

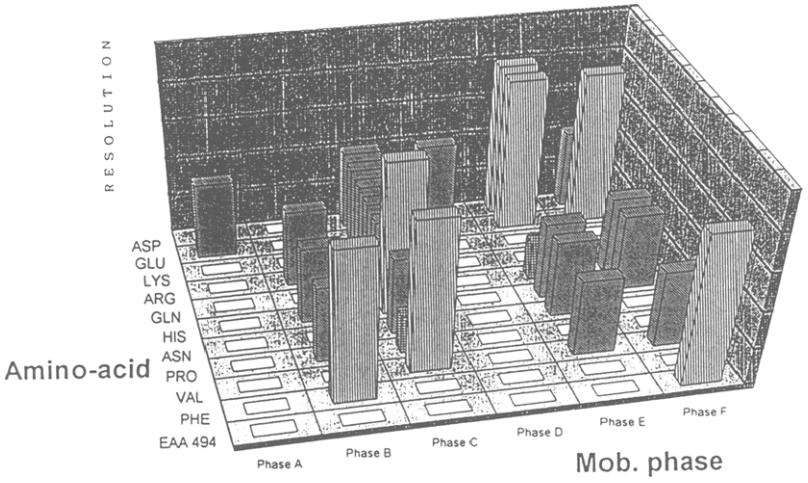

Fig. 3. Diagram of the separation of racemic mixtures of D,L-amino acids using different cyclodextrins. Large columns, baseline separation ($R > 1.25$); medium columns, partly separated ($R = 1$); small columns, shoulder ($R < 0.8$). Mobile phases: A = phosphate buffer (pH 2.6) with 20 mmol/l α-CD; B = phosphate buffer (pH 2.6) with 10 mmol/l β-CD; C = phosphate buffer (pH 2.6) with 20 mmol/l γ-CD; D = borate buffer (pH 9.5) with 20 mmol/l α-CD; E = borate buffer (pH 9.5) with 10 mmol/l β-CD; F = borate buffer (pH 9.5) with 20 mmol/l γ-CD.

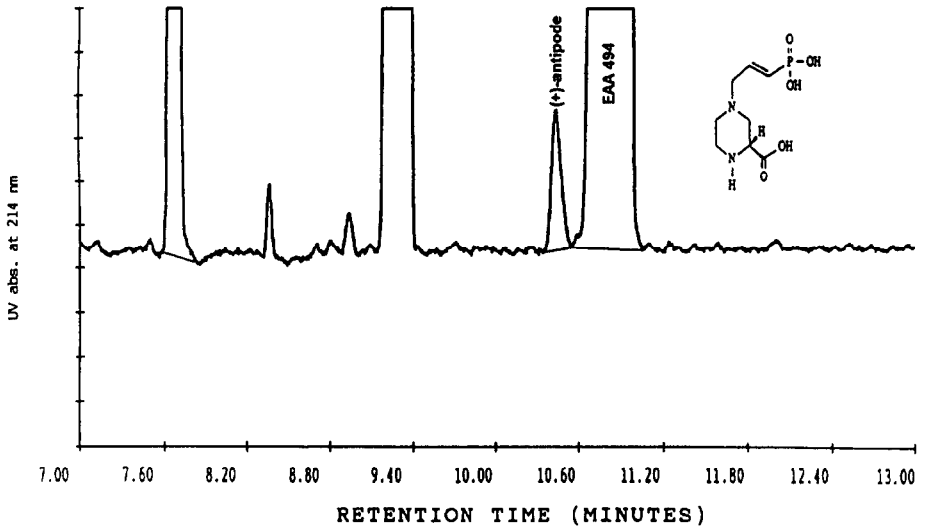

Fig. 4. Separation of SDZ EAA 494 s and the (+)-antipode with 0.1 mol/l borate buffer of pH 11 and 20 mmol/l γ-CD at 15 kV with a sampling time of 5 s. Capillary, 60 cm × 75 μm I.D. The structural formula of SDZ EAA 494 s is shown. The breakthrough of the electroosmotic flow was at 7.85 min.

could only be separated with γ-CD at pH 9.5–11 in borate buffer. It is known that acidic enantiomers are best resolved in buffers of high pH whereas basic enantiomers are best resolved in buffers of low pH [7].

Specifity, linearity and limit of detection

A sample of SDZ EAA 494 s was spiked with the (+)-enantiomer to confirm the migration order. Fig. 4 shows a separation of this mixture, with the (+)-enantiomer migrating first. Peak heights and normalized peak area measurements quantitatively confirmed the spiking levels with additions between 0.1 and 1.5%. Over this range, detector linearity for the CE method was demonstrated (Fig. 5). A limit of detection of 0.1% for the undesired enantiomer was obtained.

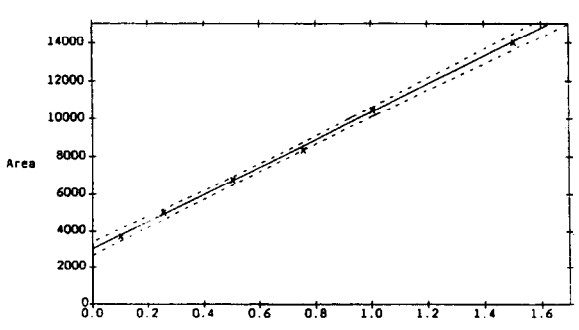

Fig. 5. Linearity of the CE method for the determination of the (+)-antipode of SDZ EAA 494 s. The correlation coefficient was 0.9995. $y = ax + b$; $a = 7327.8 \pm 332.52$; $b = 3052.1 \pm 276.04$; $r = 0.99947$; $n = 6$. Confidence level 95%.

CONCLUSION

The potential of host–guest complexation by cyclodextrins for the chiral separation of pharmaceutical drugs has been demonstrated. The interaction of the complex formation and the electrophoretic mobility of the sample influences the separation of the racemates of amino acids and SDZ EAA 494 s. The separation could not be achieved if the differences in the mobilities of the free and complexed solute were too small. For the separation of dansylated amino acids, β- and γ-CD are universally applicable.

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