Separation of Sympathomimetic Drug Enantiomers by Capillary Electrophoresis

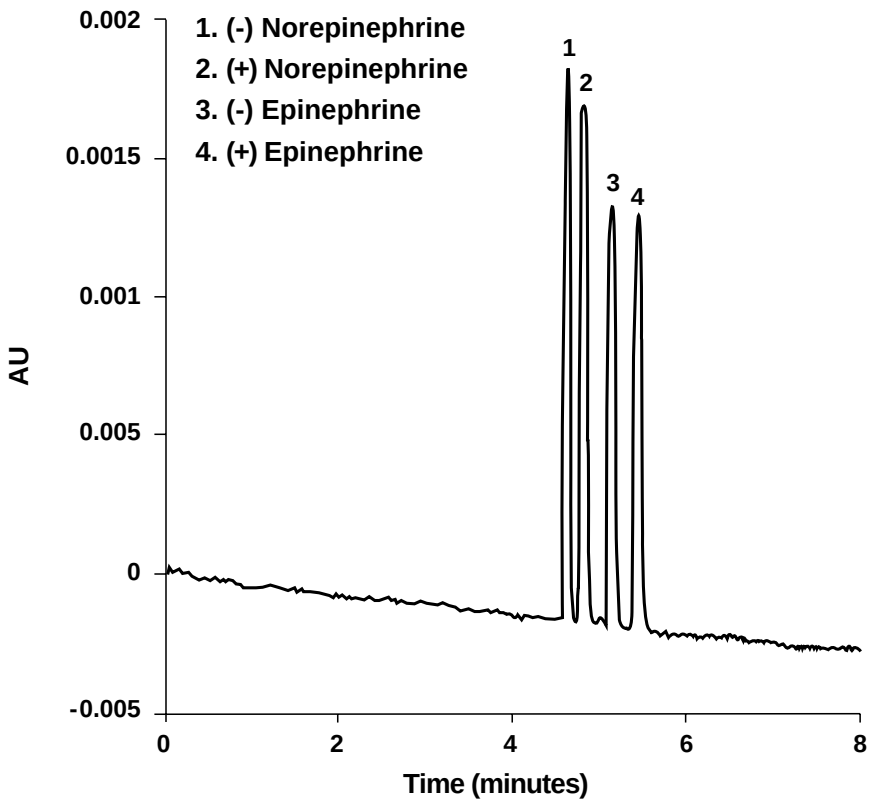

Fig. 1. Separation of racemic epinephrine and norepinephrine.

Analysis Conditions

Instrument	BioFocus® 3000 system
Capillary	24 cm x 25 µm, LPA-coated
Analysis buffer	10 mM Tris (adjusted to pH 2.4 with H_3PO_4) + 18 mM Heptakis (2,6-di-O-methyl-β-cyclodextrin)
Purge protocol	120 sec with analysis buffer
Injection	electrophoretic, 6 kV for 6 sec
Polarity	positive to negative
Voltage	9.6 kV
Current	5 µA
Capillary temperature	20 °C
Carousel temperature	20 °C
Detection	UV, 206 nm

Introduction

Enantiomers are isomeric forms of the same compound which have identical physical properties but differ in their spacial orientation. A pair of enantiomers (D- and L- isomers) are non-superimposable mirror images of each other.

The need to perform chiral analysis exists in many fields, including pharmaceuticals, environmental science, and physiology. Often, both enantiomers do not possess the same physiological effects, and therefore, analytical methods are required to differentiate between the two isomeric forms. Methods typically employed for chiral separations include high performance liquid chromatography (HPLC), thin layer chromatography (TLC), gas chromatography (GLC), and more recently, capillary electrophoresis (CE).[1]

Chiral analysis by capillary zone electrophoresis (CZE) usually involves the addition of a chiral selector to a low pH run buffer. Chiral selectors include native and derivatized cyclodextrins, crown ethers, bile salts, proteins, and carbohydrates. Selectivity can be altered by adjusting the type and concentration of the chiral additive and also by the addition of achiral modifiers such as alcohols, surfactants, urea, and metal ions.

Cyclodextrins (CDs) are the most widely used chiral selectors. They are nonionic cyclic oligosaccharides consisting of six, seven, eight, or nine glucose units, designated as α-, β-, γ-, and δ-CDs, respectively. Modified CDs, such as di- and tri-O-methyl-β-CD are also available. Their shape is similar to a truncated cone, with a relatively apolar cavity, and two relatively hydrophilic

rims containing the hydroxyl groups. Chiral selectivity results from inclusion of a hydrophobic portion of the analyte in the CD cavity and also from hydrogen bonding to the chiral hydroxyl moities. At low pH, electro-osmotic flow is minimal and the uncharged CD does not migrate. However, basic compounds become protonated and migrate towards the detector. When the analyte becomes included into the CD cavity, its mobility is greatly reduced. The extent of the inclusion depends on the stability of the complex formed. If the two enantiomers have different stability constants, one enantiomer migrates more slowly than the other and chiral resolution is achieved.

This report describes a simple and rapid CZE method for the resolution of epinephrine and norepinephrine enantiomers at low pH, using coated capillaries and heptakis (2,6-di-O-methyl-β-cyclodextrin) as the chiral selector.

Standards

Heptakis (2,6-di-O-methyl-β-cyclodextrin), (±) epinephrine • HCl, (±) norepinephrine • HCl, (-) epinephrine, and (-) norepinephrine • HCl were all purchased from Sigma (St. Louis, MO).

Standard stock solutions (~ 3 mM for each enantiomer) were prepared by dissolving the HCl salts of the drugs in 2 mM Tris-H_3PO_4, pH 2.4 buffer, and the free base form of the drugs in 0.1 N HCl. A standard mixture at 10 µM concentration was prepared by diluting the stock solution 300-fold with 2 mM Tris-H_3PO_4, pH 2.4 buffer.

Results

Figure 1 illustrates the resolution of racemic mixtures of two sympathomimetic drugs, epinephrine and norepinephrine. The separation was performed on a linear polyacrylamide-coated capillary (25 µm x 24 cm l) using a pH 2.4 buffer containing 18 mM heptakis (2,6-di-O-methyl-β-cyclodextrin) as the chiral selector. The use of coated capillaries enhances chiral selectivity to allow the resolution of compounds with similar effective mobilities by eliminating the electroosmotic flow (EOF). Complete resolution of the two racemates was achieved in less than 6 minutes. With both drugs, the (+) isomer showed a longer migration time, indicating that the interaction with the CD was higher than that obtained with the (-) isomer. Column efficiencies were in excess of 40,000 plates.

This study illustrates that chiral analysis by CZE is a promising analytical technique for the simple and rapid resolution of optical isomers in small sample volumes. CZE eliminates the drawbacks of other techniques such as HPLC where expensive stationary phases and/or high quantities of chiral reagents are utilized. In addition, HPLC methods development is often time consuming and separations suffer from low separation efficiencies.

References

1. Fanali, S., *J. Chromatogr.*, 474, 441 (1989).

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