Analysis of Steroid Isomers by Capillary Electrochromatography

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Abstract

Capillary electrochromatography (CEC) is a fusion of capillary electrophoresis and liquid chromatography which preserves and exploits the best aspects of both techniques. The separation is achieved for neutral molecules essentially through chromatographic selectivity mechanisms, while the mobile phase is propelled through the packed capillary by electromotive forces. Efficiencies can be increased by as much as ten-fold, compared with conventional LC systems. Such increases in efficiency can allow faster method development times and also separations of very closely related compounds. This application brief contrasts the first-pass separation of α and β isomers of 17-hydroxyestradiol using HPLC and CEC. These isomers differ only by the position of the 17-OH group (figure 1).

Figure 1
Structure of 17-OH estradiol

Figure 2
Separation of alpha and beta 17-OH estradiol by HPLC

Conditions

Column 3 µm ODS 125 mm x 2 mm i.d.
Mobile Phase
NH₄Ac pH 7.0/acetonitrile (50/50)
Flow 0.26 ml/min
Temp 38 °C
**Experimental**

The CEC separation was performed using the Agilent Capillary Electrophoresis system equipped with diode array detector and computer controlled via Agilent ChemStation software. CEC capillary columns were Agilent CEC C18. The Agilent CE system is uniquely designed for operating CEC in that it can apply up to 12 bar pressure simultaneously to both vials in order to suppress bubble formation and maximize reliability.

HPLC of the isomers was carried out using a HP 1090 Series II liquid chromatograph equipped with diode-array detection and computer controlled via Agilent ChemStation software.

Figure 2 shows the separation obtained from HPLC analysis of these compounds. However in obtaining a rapid analysis the compounds are only partially resolved. When using CEC (figure 3) for separation, it should be noted that the retention time is markedly increased. This is due to the difference in stationary phase since the CEC phase has been specifically designed to meet the requirements of CEC. The two isomers are however well resolved and fully capable of quantitation.