High sensitivity chiral excess analysis

Application Note

Pharmaceutical

Author

Gordon Ross
Agilent Technologies,
Waldbronn, Germany

Abstract

Analysis of chiral drug compounds is easily performed by capillary electrophoresis (CE) since the chiral selector (e.g. cyclodextrin) is dissolved in the buffer rather than bound to a stationary phase as in HPLC. This enables a more rapid method development, improved efficiency and resolution, and reduced cost of both the analysis and the method development. CE is frequently the method of choice for chiral analyses. Regulatory requirements demand the demonstration of chiral purity of any chiral drug substance and the acceptable criteria for the presence of excess enantiomer is around 0.1 %. This, therefore demands that the analysis not only has an adequate sensitivity but also that the linear range is such that the minor component may be quantitatively reported as a corrected (area/area) percent of the main component. To date, CE has been stretched to produce such quantification.

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Experimental

All experiments were performed on the Agilent Capillary Electrophoresis system which is computer controlled via Agilent ChemStation software. High sensitivity detection was achieved by using the Agilent CE High Sensitivity Detection Cell kit (part number G1600-68713) and capillaries.

Figure 1 compares the chiral separation of epinephrine on a 75 µm id standard capillary and using the high sensitivity cell. The signal to noise obtained for a 75 mbars injection of a 100 µM solution of (±) epinephrine was 62.5.

When using exactly the same conditions with the high sensitivity cell coupled to capillaries of identical dimensions the signal response was increased over 20 times. As a truer reflection of the increased sensitivity of the high sensitivity cell the signal to noise ratio was determined at 650 therefore giving a true increase in sensitivity of over 10-fold. The combination of extended linear dynamic range and increased sensitivity makes the Agilent CE High Sensitivity Cell suited to the quantitative analysis of chiral excess. Figure 2 shows the analysis of the R–form of a basic drug in the presence of trace amounts of the S–enantiomer. The S–enantiomer is present at a level of less than 0.5 % corrected (area/area) with a signal to noise of around 10.

Conclusions

The high sensitivity cell is of considerable use in the determination of chiral excess at levels of 0.1 % and lower which is of great benefit to pharmaceutical manufacturers who must demonstrate such purity for regulatory compliance.
The basic drug was the kind gift of Dr. Mel Euerby, Astra-Charnwood U.K.

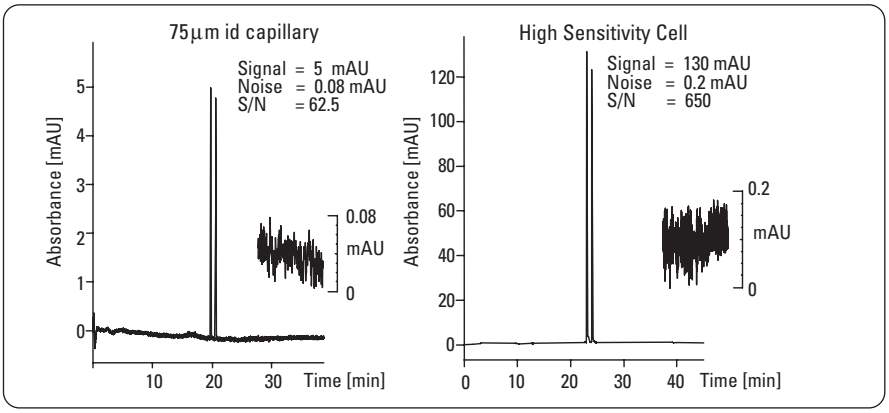

Figure 1
Comparison of the chiral separation of epinephrine with a 75 µm id standard capillary and high sensitivity cell.

Chromatographic conditions

Buffer:	50 mM tris-phosphate pH 2.4, 20 mM dimethyl-ß-cyclodextrin
Capillary:	56 cm eff x 75 µm id
Injection:	75 mbars
Run:	30 kV, 20 °C
Detection:	200 nm/10 nm (with/out high sensitivity cell)

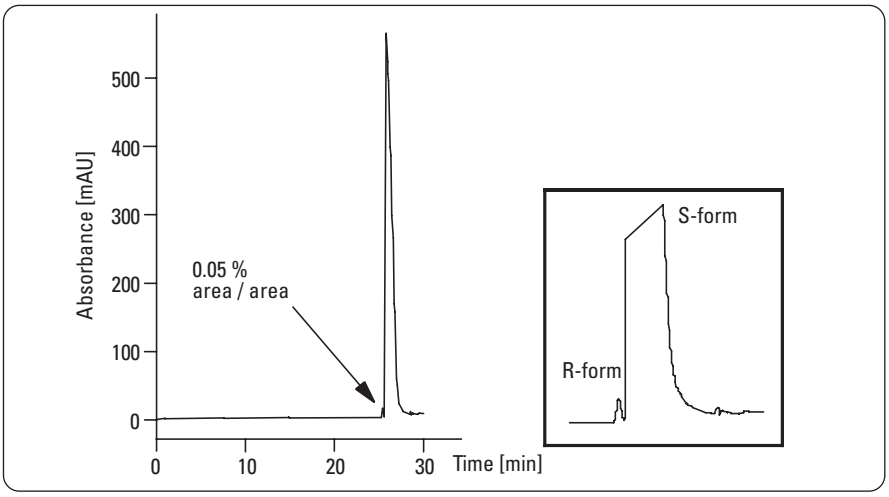

Figure 2
Analysis of the R-form of a basic drug in the presence of trace amounts of the S-enantiomer.

Chromatographic conditions

Buffer:	50 mM tris-phosphate pH 2.5, 5 mM gamma-cyclodextrin
Capillary:	56 cm eff x 75 µm id
Injection:	150 mbars
Run:	30 kV, 10 °C
Detection:	200 nm/10 nm (with/out high sensitivity cell)

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