



# High-Throughput Chiral Separations Using the cePRO 9600™ Multiplexed Capillary Electrophoresis System

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**For the analytical scale separation of enantiomers, multiplexed capillary electrophoresis (CE) performed with the cePRO 9600™ system offers a significant increase in sample throughput relative to single column methods.**

It is well established that enantiomers of a chiral drug compound can possess different pharmacological effects, with one enantiomer producing the desired effect and the other enantiomer being inactive or even toxic. The rising importance of chirality in pharmaceutical development is noted by the observation that a third of all drug sales worldwide in 2000 were in single isomer form. Chiral chromatography methods, although extremely popular, employ expensive stationary phases and lack general applicability, in that often a number of columns are required to cover a wide application range.

Capillary electrophoresis (CE) has emerged as an extremely popular method for obtaining the analytical scale separation of enantiomers. When performing chiral CE, a universal capillary can be used and chiral selectors are added directly to the background electrolyte (BGE) run buffer. Advantages of CE for chiral analysis include high separation efficiency, reduced method development time, small chiral reagent consumption, and a low cost of analysis. Multiplexed 96-capillary electrophoresis using the cePRO 9600™ system provides the capability to perform up to 96 independent CE separations in parallel, significantly improving sample throughput and increasing laboratory efficiency.

## Experimental

All test compounds and buffer components were purchased from Sigma-Aldrich (St. Louis, Missouri). Highly sulfated  $\alpha$ -,  $\beta$ -, and  $\gamma$ -CD solutions were purchased from Beckman (Fullerton, California) and diluted to 2.5% or 5% (w/v) concentrations prior to use. Sulfated  $\beta$ -CD (S- $\beta$ -CD; D.S. 7–11) was obtained from Sigma and prepared at concentrations of 2.5% or 5% (w/v).

A cePRO 9600™ multiplexed CE system employing UV absorbance detection at 214 nm was employed for all separations. A 96-capillary array of 33 cm effective length and 55 cm total length (50  $\mu$ m i.d., 200  $\mu$ m o.d.) was used. Air chilled to 15 °C by a water chiller was circulated across the capillary array during the CE experiments to minimize the effects of Joule heating.

## Results

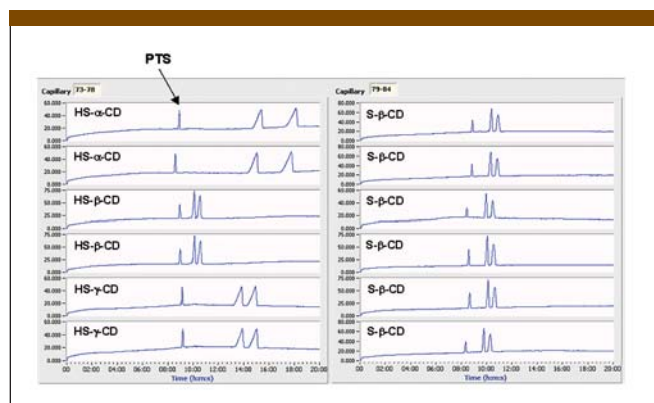
Development of a suitable method for the chiral separation of a new chemical entity (NCE) often involves a trial-and-error approach, whereby several different chiral selectors, selector concentrations,

and/or buffer conditions are varied in an attempt to achieve adequate enantiomeric resolution. The majority of chiral CE methods employ neutral or charged cyclodextrin (CD) selector buffer additives. For example, the recent introduction of highly sulfated CDs possessing various cavity sizes ( $\alpha$ ,  $\beta$ , and  $\gamma$ ) has shown much promise for achieving enantioseparations of NCEs.

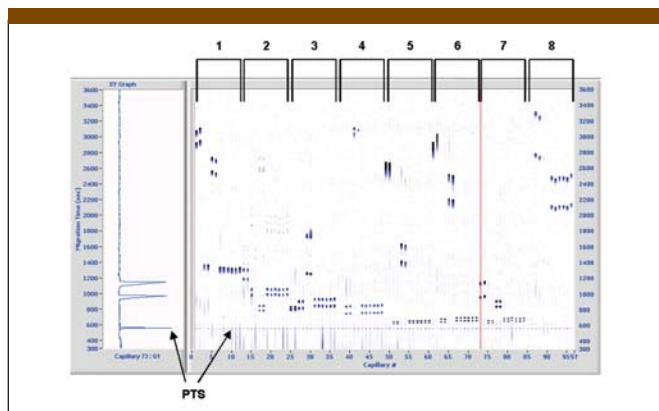
Single capillary CE separations during method development can range from a few minutes up to 30 min. The cePRO 9600™ allows for the simultaneous screening of up to 96 different NCE/selector combinations in a single experiment, significantly reducing method development times. Figure 1 shows 12 capillaries of a 96-capillary array where fixed concentrations of 2.5% (w/v) sulfated CD selectors were screened against a racemic mixture of nefopam. In this case, HS- $\alpha$ -CD provides the highest resolution relative to the other selectors.

## Conclusions

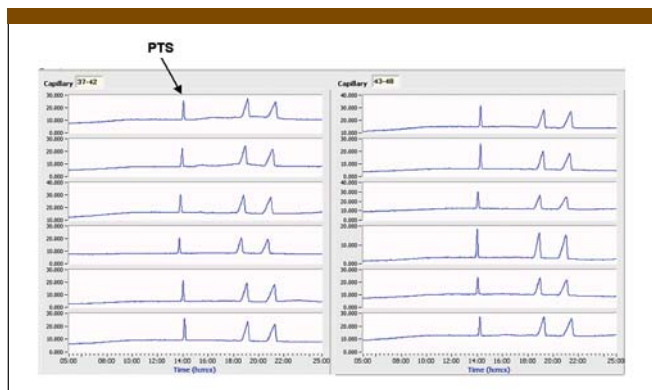
The cePRO 9600™ can provide a means for high-throughput chiral method development and parallel enantioseparation. The ability to perform up to 96 independent chiral separations in parallel, even in the presence of up to 5% (w/v) HSCDs, has widespread application in chiral analysis.



**Figure 1:** Parallel screening of different chiral selectors against a racemic mixture of nefopam using the cePRO 9600™ system. BGE: 2.5% (w/v) selector in 25 mM H<sub>3</sub>PO<sub>4</sub>/triethylamine pH 2.5 CE: 150 V/cm.



**Figure 2:** Two-dimensional “gel-like” display of 96-capillary CE data from the experiment in Figure 1, where eight compounds were simultaneously screened against four types of sulfated CD selectors. The arrangement of selectors is the same as Figure 1 for each compound. The migration times have been normalized to PTS internal standard. BGE: 2.5% (w/v) selector in 25 mM H<sub>3</sub>PO<sub>4</sub>/triethylamine pH 2.5. CE: 150 V/cm. The electropherogram for capillary #73 (nefopam, HS- $\alpha$ -CD) is shown to the left.



**Figure 3:** Parallel separation of a racemic mixture of isoproterenol using the cePRO 9600™ system. Peaks: 1 = PTS internal standard, 2 = (+) isoproterenol, 3 = (-) isoproterenol. BGE: 5% (w/v) S- $\beta$ -CD in 25 mM H<sub>3</sub>PO<sub>4</sub>/triethylamine pH 2.5. CE: 100 V/cm.

**Table I:** Results from the screening of eight different compounds against four types of sulfated CD selectors (see Figure 2)

| # | Compound                    | Selector         | Separation Time (min) | Avg R <sub>s</sub> |
|---|-----------------------------|------------------|-----------------------|--------------------|
| 1 | Atenol                      | HS- $\alpha$ -CD | 52                    | 1.25               |
|   |                             | HS- $\beta$ -CD  |                       |                    |
|   |                             | HS- $\gamma$ -CD | 47                    | 1.60               |
|   |                             | S- $\beta$ -CD   |                       |                    |
| 2 | Alprenolol                  | HS- $\alpha$ -CD | 22                    | 1.70               |
|   |                             | HS- $\beta$ -CD  | 18                    | 1.33               |
|   |                             | HS- $\gamma$ -CD | 14                    | 1.38               |
|   |                             | S- $\beta$ -CD   | 17                    | 1.25               |
| 3 | <i>p</i> -Chloroamphetamine | HS- $\alpha$ -CD | 15                    | 0.75               |
|   |                             | HS- $\beta$ -CD  | 13                    | 1.58               |
|   |                             | HS- $\gamma$ -CD | 30                    | 4.26               |
|   |                             | S- $\beta$ -CD   | 15                    | 1.48               |
| 4 | Isoproterenol               | HS- $\alpha$ -CD |                       |                    |
|   |                             | HS- $\beta$ -CD  | 13                    | 2.05               |
|   |                             | HS- $\gamma$ -CD | 49                    | 0.63               |
|   |                             | S- $\beta$ -CD   | 14                    | 1.98               |
| 5 | Metaproterenol              | HS- $\alpha$ -CD |                       |                    |
|   |                             | HS- $\beta$ -CD  | 10                    | 1.03               |
|   |                             | HS- $\gamma$ -CD | 25                    | 2.05               |
|   |                             | S- $\beta$ -CD   | 11                    | 1.03               |
| 6 | Terbutaline                 | HS- $\alpha$ -CD |                       |                    |
|   |                             | HS- $\beta$ -CD  | 11                    | 1.34               |
|   |                             | HS- $\gamma$ -CD | 40                    | 2.13               |
|   |                             | S- $\beta$ -CD   | 11                    | 1.37               |
| 7 | Nefopam                     | HS- $\alpha$ -CD | 18                    | 2.86               |
|   |                             | HS- $\beta$ -CD  | 11                    | 1.29               |
|   |                             | HS- $\gamma$ -CD | 15                    | 1.38               |
|   |                             | S- $\beta$ -CD   | 11                    | 1.50               |
| 8 | Warfarin                    | HS- $\alpha$ -CD |                       |                    |
|   |                             | HS- $\beta$ -CD  | 51                    | 4.73               |
|   |                             | HS- $\gamma$ -CD |                       |                    |
|   |                             | S- $\beta$ -CD   | 40                    | 3.86               |

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