

# Chiral method development kit and highly sulfated cyclodextrins.

## Chiral Analysis for Capillary Electrophoresis

**Capillary  
Electrophoresis**  
 Genomics  
 Cell Analysis  
 Centrifugation  
 Lab Tools  
 Lab Automation  
 Particle  
 Characterization

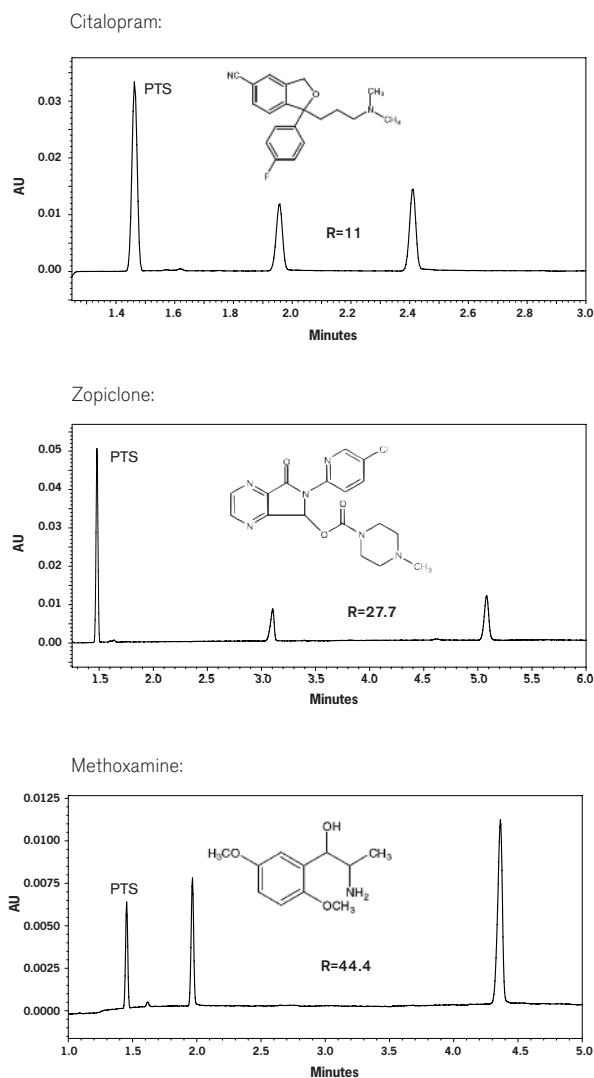
When capillary electrophoresis was first introduced it was seen as revolutionary. Now it is a well established technique in analytical laboratories world-wide and should be a researcher's first choice for analyzing chiral, polar or charged analytes.

Capillary electrophoresis (CE) offers highly efficient separations, short analysis times and minimal solvent and reagent consumption compared to other separation strategies. Now, with the Beckman Coulter Chiral Methods Development Kit, the separation of chiral molecules can be included among the many applications which demonstrate the strength of CE.

The separation of chiral molecules poses a unique challenge. In a symmetric environment, the chemical and physical properties of enantiomers (excepting the rotation of plane-polarized light) are identical. The key to resolving these molecules is therefore to construct the right chiral environment.

This is easily achieved in CE by filling the capillary with a separation buffer containing a chiral additive. Although many chiral selectors have been used successfully, the most comprehensive separation strategies have been achieved with highly sulfated cyclodextrins (HSCDs), a family of three chiral reagents. Among the chiral selectors that are available for CE today, HSCDs seem to provide not only the most general selectivity but also the fastest separations and the shortest method development time. It therefore makes good sense to try HSCDs first when developing chiral separation methods.

**Figure 1:** High-Resolution Chiral Separations in Short Analysis Times

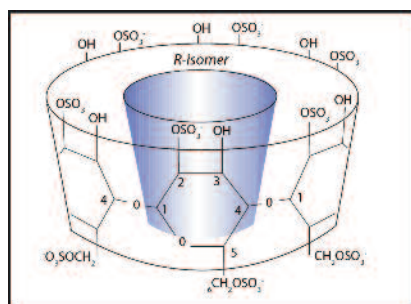


# Chiral Analysis for Capillary Electrophoresis

The Chiral Methods Development Kit from Beckman Coulter contains all three HSCDs, as well as a method development protocol for determining the optimal separation conditions for your chiral analytes. The protocol can be used for a variety of enantiomeric structures and has yielded successful separations for over 90% of the compounds that Beckman Coulter has analyzed (see [www.beckmancoulter.com/HSCDs](http://www.beckmancoulter.com/HSCDs)). In fact, CE with HSCDs has proven to be the ideal analytical tool for rapid chiral separations and for analytical-scale purity assessment of single-enantiomer preparations.

## Highly Sulfated Cyclodextrins

The highly sulfated cyclodextrins (HSCDs) in the Chiral Methods Development Kit consists of three cyclodextrins



( $\alpha$ -,  $\beta$ - and  $\gamma$ -) with attached sulfate groups. The average number of sulfates per molecule is 11, 12 or 13 for  $\alpha$ -,  $\beta$ -, and  $\gamma$ -cyclodextrin respectively. This

distribution was “designed in” to provide increased resolution using the kit-defined separation conditions. HSCDs are negatively charged and have strong electrophoretic mobility toward the positive electrode (anode) in the CE environment. The low pH buffer (pH 2.5) provided in the chiral methods development kit suppresses the electroosmotic flow (EOF) within the capillary. Therefore, if the enantiomeric analytes interact with the HSCDs they will be swept toward the anode regardless of their charge state. This is the fundamental principle of HSCD-mediated chiral separations. Under the conditions defined by the kit, neutral compounds will interact with the hydrophobic cavity of the HSCDs, while basic compounds (which are strongly cationic at low pH) will interact with the hydrophobic cavity as well as ionically with the negatively charged sulfates. At pH 2.5, Zwitterionic analytes will be positively charged and behave similarly to basic compounds, while acidic compounds will be primarily protonated and behave as neutral species. Our experience shows that the use of HSCDs in our low pH buffer can achieve the majority of chiral separations.

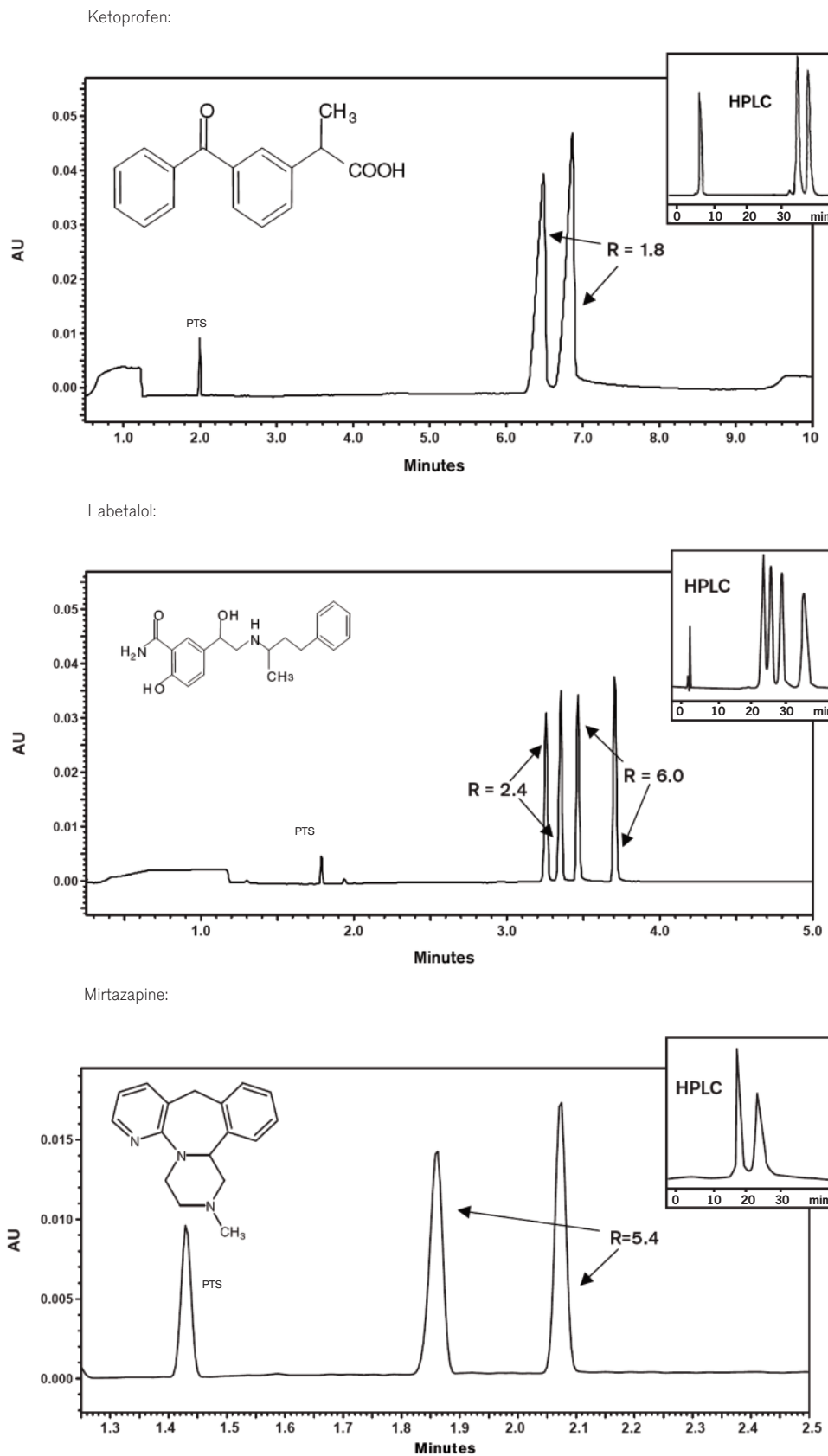
In summary, highly sulfated cyclodextrins in low pH buffer provide not only broad selectivity but also the fastest separations and the shortest method development time among chiral selectors available for CE today. The cyclodextrins perform well for the separation of many neutral, basic and weakly acidic compounds of pharmaceutical and biological interest. Additionally, our HSCDs can also be employed in the chiral separation of flavors and pesticides. It is our impression that enantioselective CE is an important tool for pharmaceutical analysis and perhaps the most important tool for analytical-scale chiral separations in particular.

## Chiral Method Development Kit

Since HSCDs seem to provide not only the most general selectivity, but also the fastest separations and the shortest method development time, it makes sense to develop chiral methods with these charged cyclodextrins first. This generic method development strategy first involves screening the compound for separation using all three ( $\alpha$ ,  $\beta$  and  $\gamma$ ) HSCDs and then optimizing on the chiral selector which yielded the best resolution.

Our experience with HSCDs shows that the majority of separations may be achieved at low pH. Under these conditions of suppressed electroosmotic flow (EOF), the negatively charged cyclodextrins have strong electrophoretic mobility toward the positive electrode (anode). If the enantiomers interact with these HSCDs, they will be swept toward the anode regardless of charge state. Neutral compounds will interact with the hydrophobic cavity of the HSCDs, while basic compounds will be strong cations at low pH—interacting with the hydrophobic cavity and ionically with the negatively charged sulfates. At pH 2.5, acidic compounds will be primarily protonated, behaving as neutral species. Zwitterionic analytes will be positively charged and behave in a fashion similar to basic compounds.

**Figure 2:** Chiral Separations with HSCDs and a P/ACE™ MDQ Capillary Electrophoresis System versus classical HPLC



## Kit Contents

Our method development kit (part #A54279) includes 5 mL of each of the three HSCDs, 3 capillaries, a reference marker and other required reagents. Our manufacturing processes, in combination with a rigorous quality-control program, ensure consistent performance of the reagents and all lots are performance-matched to a Gold Standard on our CE platform.

<b>Part #</b>	<b>Item Description</b>
713348	HS- $\alpha$ -Cyclodextrin, 20% (w/v), 5 mL
713331	HS- $\beta$ -Cyclodextrin, 20% (w/v), 5 mL
713350	HS- $\gamma$ -Cyclodextrin, 20% (w/v), 5 mL
713328	PTS Reference Marker, 1 mL
713333	Capillary Conditioning Solution, 10 mL
477422	Phosphate Buffer pH 2.5, 100 mL
A54284	1 N NaOH, 10 mL
338451	50 $\mu$ m (id) bare fused-silica capillaries, Qty 3



Each of the kit items can be ordered separately, whether for further method development or for routine analysis.

For the routine user, it is more practical to acquire the 30 mL HSCDs (listed below).

<b>Part #</b>	<b>Item Description</b>
A50922	HS- $\alpha$ -Cyclodextrin, 20% (w/v), 30 mL
A50923	HS- $\beta$ -Cyclodextrin, 20% (w/v), 30 mL
A50924	HS- $\gamma$ -Cyclodextrin, 20% (w/v), 30 mL



## Instrumentation

A P/ACE™ MDQ CE system with liquid capillary cooling and photodiode array (PDA) detection is recommended for chiral methods development using HSCDs. The HSCDs generate high current and demand effective capillary thermoregulation; therefore, rapid and efficient chiral separations require the advanced liquid cooling of the P/ACE MDQ CE system. Enantiomers have identical physiochemical properties in a symmetric environment and will produce similar absorption spectra. Spectral analysis is therefore useful in the identification of enantiomers and in their discrimination from contaminants. A P/ACE MDQ with photodiode array (PDA) detection will provide this valuable spectral data. The P/ACE MDQ CE system comes with a variety of sampling formats, including a 96-well plate, which allow this system to be compatible with many forms of laboratory automation.



P/ACE MDQ Capillary Electrophoresis System

Australia, Gladesville (61) 2 9844-6000 Canada, Mississauga (1) 905 819 1234 China, Beijing (86) 10 6515 6028  
Czech Republic, Prague (420) 272 01 73 32 Eastern Europe, Middle East, North Africa, South West Asia: Switzerland, Nyon (41) 22 365 3707  
France, Villepinte (33) 1 49 90 90 00 Germany, Krefeld (49) 2151 33 35 Hong Kong (852) 2814 7431  
India, Mumbai (91) 22 3080 5101 Italy, Cassina de' Pecchi, Milan (39) 02 953921 Japan, Tokyo (81) 3 5530 8500 Korea, Seoul (82) 2 404 2146  
Latin America (1) (305) 380 4709 Mexico, Mexico City (001) 52 55 9183 2800 Netherlands, Mijdrecht (31) 297 230630 Puerto Rico (787) 747 3335  
Singapore (65) 6339 3633 South Africa/Sub-Saharan Africa, Johannesburg (27) 11 564 3203 Spain, Madrid (34) 91 3836080  
Sweden, Bromma (46) 8 564 85 900 Switzerland, Nyon (41) 0800 850 810 Taiwan, Taipei (886) 2 2378 3456 Turkey, Istanbul (90) 216 309 1900  
UK, High Wycombe (44) 01494 441181 USA, Fullerton, CA (1) 800 742 2345