




# Chiral Methods Development Kit

User's Guide

PN A54283 AB  
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Beckman Coulter, Inc.  
250 S. Kraemer Blvd.  
Brea, CA 92821



**BECKMAN  
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## **Chiral Method Development Kit User's Guide**

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# Method Development in Enantiomer Analysis



## Introduction

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Enantiomers are unique molecules that contain one or more asymmetric chiral centers. As a consequence, these molecules are mirror images of each other; however, the images are not superimposable. Enantiomers show identical physicochemical properties and differ only in the way they bend plane-polarized light. Their biological properties, however, can be significantly different.

The use of Highly Sulfated Cyclodextrins (HSCDs)<sup>1</sup> in Capillary Electrophoresis (CE) is an efficient way of providing an environment within the running buffer which allows for discrimination of chiral enantiomers. CE with HSCDs is a generic method development strategy for enantiomer analysis. Though it provides chiral separation in most cases, a number of other chiral selectors are available, if needed.

Highly Sulfated Cyclodextrins are cyclic oligosaccharides composed of six, seven, or eight repeating alpha ( $\alpha$ ) 1, 4-glucopyranose units (alpha ( $\alpha$ ), beta ( $\beta$ ), and gamma ( $\gamma$ ) respectively). Sulfonated ends are at positions 2 and 6, with position number 3 intact with the OH group (Figure 1). This plays a very important role in the resolution of the enantiomers. The average sulfate content of HS- $\alpha$ -CD, HS- $\beta$ -CD, and HS- $\gamma$ -CD is eleven, twelve, and thirteen sulfates per mole respectively.

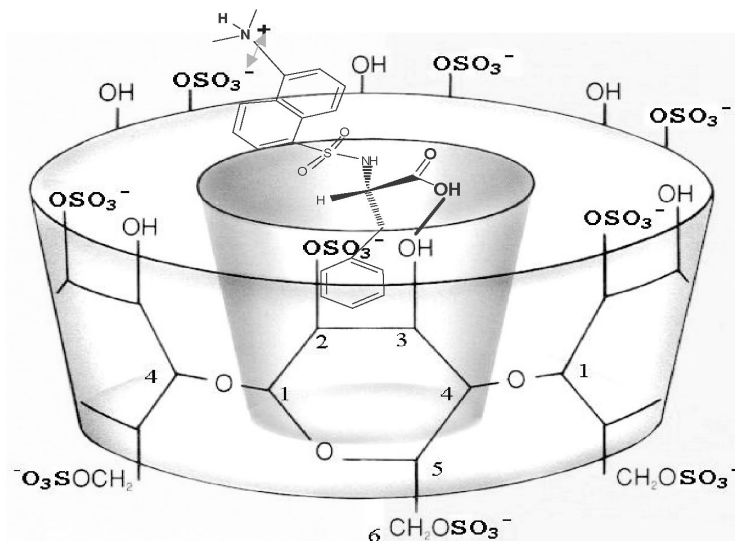
**Intended Use** This product is **for research use only**. It is not for use in diagnostic procedures. No clinical decision or patient notification may be made based on results using this research assay. This kit is intended to be used on a Beckman Coulter CE instrument equipped with a PDA detector and both cartridge and sample temperature controls. Use of this kit without liquid cooling of the capillary is not recommended.

**Safety** Refer to the Material Safety Data Sheets (MSDS) information, available at [www.beckman.com/customer-support/msds/msds.asp](http://www.beckman.com/customer-support/msds/msds.asp), regarding the proper handling of materials and reagents. Always follow standard laboratory safety guidelines.

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<sup>1</sup> Highly sulfated cyclodextrins (HSCDs) are covered under United States Patent 6316613

Figure 1. Pictorial representation of HS- $\alpha$ -CD



HS  $\alpha$ ,  $\beta$ , and  $\gamma$  CD complement each other with different chiral selectivity, allowing rapid enantiomeric resolution of neutral, basic, and acidic compounds.

Two types of methods are recommended for enantiomer analysis Method Development (MD):

1. Enantiomer separation methods, which enable you to achieve enantiomer resolution typically within ten minutes. These methods utilize an effective capillary length of 20 cm.
2. Enantiomer separation high-speed methods, which enable you to speed up and increase the throughput of your enantiomer separation. These methods utilize an effective capillary length of 10 cm.

This operating protocol discusses both methods, as well as the strategy for enantiomer separation method development.

## Enantiomer Method Strategy

Used as starting conditions, the parameters below have proven to be very effective and have successfully discriminated the vast majority of the chiral mixtures tested (See Chapman reference in Appendix E):

|                       | Parameters   |
|-----------------------|--|
| Capillary             | Bare fused-silica, 50 $\mu\text{m}$ i.d., 30 cm (20 cm effective length) |
| Field strength        | 500 V / cm   |
| Polarity              | Reversed   |
| Buffer                | 25 mM Triethylammonium Phosphate, pH 2.5                                 |
| Chiral selectors      | 5% (HS- $\alpha$ -CD, HS- $\beta$ -CD, and HS- $\gamma$ -CD)             |
| Detection             | PDA, Scan 190–350 nm with single-channel detection at 200 nm             |
| Sample injection      | 4 s at 0.3 psi   |
| Capillary temperature | 22°C   |
| Sample concentration  | 1–5 mM   |

The recommended method assumes that enantiomeric resolution can be obtained in less than ten minutes; however, some separations may take longer. If no peaks are observed in the first run, increase the running time to give ample time for the separation to occur.

In Appendix C, you will find information on how to optimize the chiral conditions in cases where additional optimization from these screening conditions is still needed.

## Kit Contents

### Contents of Chiral Method Development Kit (Reorder # A54279)

| Description  | Part Number |
|--|-------------|
| Highly Sulfated- $\alpha$ -Cyclodextrin 20% (w/v), 5 mL                        | A54280      |
| Highly Sulfated- $\beta$ -Cyclodextrin 20% (w/v), 5 mL                         | A54281      |
| Highly Sulfated- $\gamma$ -Cyclodextrin 20% (w/v), 5 mL                        | A54282      |
| 1, 3, 6, 8-Pyrene tetrasulfonate (PTS) reference marker – 10 mM in water, 1 mL | 713328      |
| 50 $\mu$ m (i.d.) bare fused-silica capillary – qty. 3                         | 338451      |
| Capillary conditioning solution, 10 mL   | 713333      |
| Phosphate Buffer pH 2.5 (50 mM triethylammonium phosphate) 100 mL              | 477422      |
| 1N Sodium Hydroxide Buffer (NaOH), 10 mL                                       | A54284      |

### Additional Reorder Components

| Description  | Part Number |
|--|-------------|
| Highly Sulfated- $\alpha$ -Cyclodextrin 20% (w/v), 30 mL | A50922      |
| Highly Sulfated- $\beta$ -Cyclodextrin 20% (w/v), 30 mL  | A50923      |
| Highly Sulfated- $\gamma$ -Cyclodextrin 20% (w/v), 30 mL | A50924      |

### Materials Required but not Included in this Kit

| Description   | Part Number |
|---|-------------|
| <b>Drug Test Mixture (not provided, user prepared). See the Note on page 8.</b>         | <b>N/A</b>  |
| 2 mL glass vials (pack of 100)  | 144980      |
| Vial caps for 2 mL glass vials, red caps (pack of 100)                                  | 144648      |
| 200 $\mu$ L PCR vials (pack of 50)  | 144709      |
| Caps for PCR vials – gray (pack of 50)  | 144656      |
| PCR vial springs (pack of 10)   | 358821      |
| Adjustable-volume pipettors, 200 $\mu$ L, 1000 $\mu$ L, 5 mL and 10 mL                  | N/A         |
| Pipette tips for adjustable-volume pipettors, 200 $\mu$ L, 1000 $\mu$ L, 5 mL and 10 mL | N/A         |
| 10 mL polypropylene conical bottom centrifuge tubes or 16 x 100 mm glass culture tubes  | N/A         |
| Tube Rack, Vortexer, Microfuge  | N/A         |
| Distilled and deionized (DDI) or 18 M $\Omega$ water                                    | N/A         |
| Reagent-grade methanol  | N/A         |



#### NOTE

To obtain a Certificate of Analysis (COA), go to [http://www.beckman.com/customersupport/CofA/cert\\_analysis.asp](http://www.beckman.com/customersupport/CofA/cert_analysis.asp)



## Storing the Kit Components

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All kit components are shipped at room temperature and, upon receipt, should be stored in a refrigerator at 2–8°C. At this temperature, all reagents are stable until at least the expiration date on the kit.

## Preparing the P/ACE MDQ

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Before proceeding, you must understand the following procedures as described in the *P/ACE MDQ Installation and Maintenance Manual* (A36419) and the *32 Karat User Manual* (A33113)

- Capillary Cartridge rebuild
- Photo Diode Array (PDA) Detector installation
- PDA Detector calibration
- Sample and Buffer Tray installation and removal
- P/ACE MDQ maintenance
- 32 Karat sequence and method preparation



### NOTE

Either a PDA detector or UV detector can be used. The PDA detector is advantageous for differentiating the undesired diastomer from other impurities since it provides spectral information. The spectrum of a diastomer is identical to that of the desired enantiomer. All data in this guide were generated on a P/ACE MDQ instrument equipped with a PDA detector.

## Preparing the Capillary

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To prepare a 50  $\mu\text{m}$  i.d., 30 cm (20 cm to detector window) capillary, use a permanent marker to mark one of the supplied capillaries at 20 cm from the center of the window (inlet side). On the other side of the column (outlet side), mark 10 cm from the center of the same window.

Using the proper capillary dimensions (20 cm inlet to detector and a total capillary length of 30 cm) is essential to achieve reproducible mobility data. To enter the capillary dimension data, select **Method** from the drop-down menu, then **Advanced**>**Capillary/Performance**.

Install a capillary in the cartridge, making sure the window is centered in the aperture area. Use a  $100 \times 800 \mu\text{m}$  aperture. Cut the capillary to length at the marks and examine the ends under magnification for a right-angled cut. The cut can be improved by lightly rubbing the end of the capillary against a clean ceramic cutter held at a right angle. This will “square” the cut and remove any jagged edges that result from the cutting procedure. If this procedure is used, ensure that the capillary is rinsed with water in both directions for five minutes at 50 psi to remove any material that may be in the capillary as a result of the cutting process.

### **IMPORTANT**

A new capillary should be cleaned and conditioned by performing the following sequence of rinses, each at 50 psi for five minutes:

1. Reagent-grade methanol
2. Distilled and deionized (DDI) water
3. 1N NaOH (A25774)
4. Distilled and deionized (DDI) water again
5. 25 mM Phosphate Buffer (prepared by diluting the phosphate buffer supplied in the kit in a 1:1 ratio with DDI water as described on page 6)

Note that only the 1N NaOH is supplied in the kit. This procedure should not have to be repeated unless the capillary becomes plugged or contaminated.

After filling the capillary with 25 mM phosphate buffer, check the current produced in the capillary by applying a voltage of 15 kV between the two buffer vials. The observed current should be about 20  $\mu\text{amps}$ . This check will ensure that the column is functional after the cleaning process.

## Inserting the Cartridge and Calibrating the PDA Detector

Insert the cartridge into the system as described in the *P/ACE MDQ Installation and Maintenance Manual*. Close the front panel and calibrate the PDA detector.

## Preparing Buffers, Test Samples, and Unknowns

A suggested procedure to follow includes these steps:

1. Prepare a 5% solution of  $\alpha$ ,  $\beta$ , and  $\gamma$  HSCD, as described in the table below.
2. Prepare test samples and unknowns for System Suitability (SS) and Method Development (MD).
3. Perform the SS runs and compare to the typical results obtained, as shown in Appendix A.
4. Unknown samples can be included at the end of SS runs to save time and allow concentration adjustments to be made prior to method optimization at other concentrations (if necessary).
5. Mix the volumes of each HSCD, water, and phosphate buffer as shown in the table below to prepare a series of three dilutions of each HSCD solution to be used in the Method Development (the 5% solution may already be available from the SS runs).
6. These solutions can be used for up to 30 runs per set of vials.
7. Prepare a 25 mM solution of phosphate buffer by diluting the phosphate buffer supplied in the kit 1:1 with DDI water. Prepare 20 mL of the diluted buffer per set of runs.

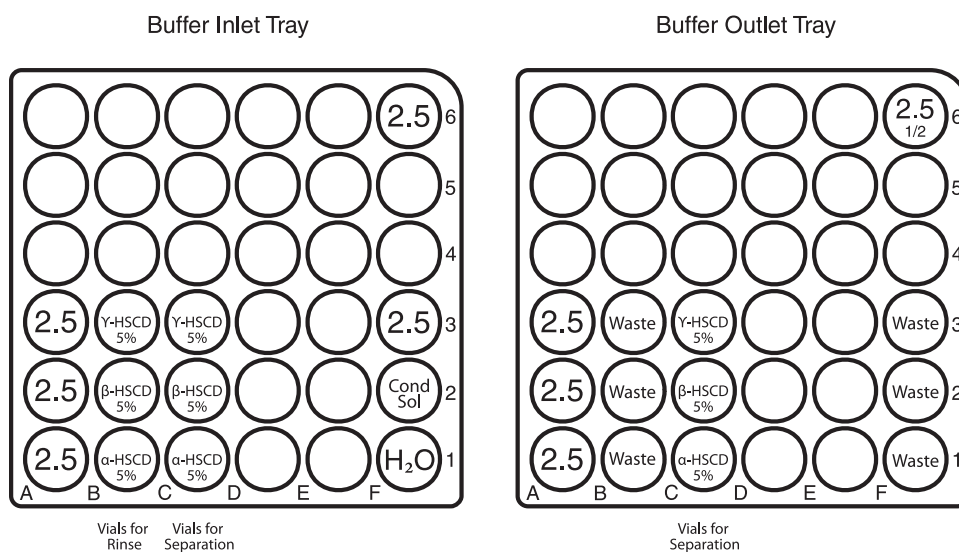
| Concentration of each HSCD                               | Volume and concentration of HSCD | Volume of 50 mM phosphate buffer | Volume of Water* | Final volumes |
|--|----------------------------------|----------------------------------|------------------|---------------|
| 7.5%   | 2.25 mL of 20%                   | 3 mL                             | 0.75 mL          | 6 mL          |
| 5%   | 1.5 mL of 20%                    | 3 mL                             | 1.5 mL           | 6 mL          |
| 2.5%   | 0.75 mL of 20%                   | 3 mL                             | 2.25 mL          | 6 mL          |
| *Use only distilled and deionized or 18 M $\Omega$ water |                                  |                                  |                  |               |

## Assessing Instrument and Kit Performance with System Suitability (SS)

See Appendix A for example electropherograms and suggested system suitability resolution criteria. Use the 5% concentration of each HSCD ( $\alpha$ ,  $\beta$ , and  $\gamma$ ) as the running buffer to measure the performance of the system.

Place the 5% vials for each HSCD in inlet and outlet trays, as shown in Figure 2.

**Figure 2.** Buffer Trays for system suitability



Vials marked 2.5 contain a 1:1 dilution of 50 mM phosphate buffer (477422). The diluted buffer at 25 mM is used to fill the capillary from the BI:F6 position. The vial at position BO:F6 should only be filled one-half full, indicated by the notation 1/2 in the figure. Ensure that there is approximately 20  $\mu$ m of current in the column when 15 kV is applied between vials in the BI:F6 and BO:F6 positions. The remainder of the 2.5 vials are used to clean the ends of the capillary and prevent carryover of the HSCD to other vials.

## Preparing Test Samples for System Suitability

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### NOTE

It is recommended that pseudoephedrine and glutethimide be used as test samples to assess kit performance. However, pseudoephedrine and glutethimide are controlled substances in some countries and cannot be included in the kit. Instructions are provided below for preparation of pseudoephedrine and glutethimide solutions and can be adapted for any other test materials you wish to substitute.

Use locally prepared pseudoephedrine and glutethimide test mixtures to represent basic and neutral compounds. They are used to assess system suitability prior to starting the enantiomer protocol for screening unknowns during method development.

Other compounds may be used as test mixtures and should be independently verified as performance compounds from the figures included at:

<http://www.beckman.com/resourcecenter/labresources/ce/pdf/chiral38.alpha.pdf>

The sample preparation instructions outlined below can be used for another selected test compound with a starting concentration of 1 mg/mL.

### Preparing the PTS Marker Solution

1. In a clean screw-cap vial, dilute 0.5 mL of the 10 mM PTS marker solution (713328) supplied in the kit with 0.5 mL distilled and deionized (DDI) water. Cap, refrigerate, and retain for future use.

### Preparing the Pseudoephedrine (PSE) Test Mixture

1. Prepare a pseudoephedrine test mixture in a racemic mixture of 2:1 (d:-l-) between the two enantiomers. Pseudoephedrine is used as the basic compound test mixture for  $\alpha$ ,  $\beta$ , and  $\gamma$ HSCD systems.
2. Add 50  $\mu$ L of a 1 mg/mL in MeOH solution of d-pseudoephedrine (also called S,S (+)-pseudoephedrine) and 25  $\mu$ L of l-pseudoephedrine (R,R(-)-pseudoephedrine) to a clean vial and evaporate. Add 1 mL of DDI water and vortex. The resulting solution contains 50 ng/ $\mu$ L of d-PSE and 25 ng/ $\mu$ L of l-PSE.
3. Add 4  $\mu$ L of the diluted PTS solution to 100  $\mu$ L of the pseudoephedrine test mixture in a sample vial, as shown in Figure 4.
4. Position it in the sample tray at SI:A1.

### **Preparing the Glutethimide (GLU) Test Mixture**

1. Prepare a glutethimide test mixture from a racemic mixture of 1:1 (d:-l-) between the two enantiomers. Glutethimide is used as the neutral test compound for the  $\beta$ -HSCD system.
2. Add 50  $\mu\text{L}$  of a 1 mg/mL in MeOH solution of d,l-glutethimide to a clean vial and evaporate. Add 1 mL of DDI water and vortex. The resulting solution contains 50 ng/ $\mu\text{L}$  of glutethimide.
3. Add 4  $\mu\text{L}$  of the diluted PTS solution to 100  $\mu\text{L}$  of the glutethimide test mixture in a sample vial, as shown in Figure 4.
4. Position it in the sample tray at SI:A2.

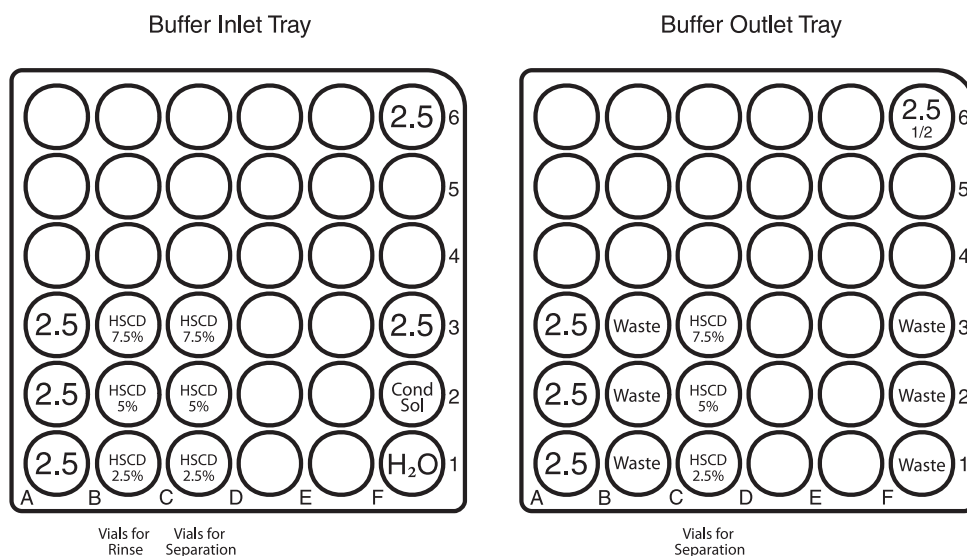
### **Preparing Samples For Chiral Analysis**

1. Add 4  $\mu\text{L}$  of diluted PTS reference marker to 100  $\mu\text{L}$  solutions of the chiral compound of interest prepared at approximately 50 ng/ $\mu\text{L}$ .
2. Run these samples in each of the  $\alpha$ ,  $\beta$ , and  $\gamma$  HSCD buffers at the 5% concentration. If further optimization is required, run the samples at the other concentrations, or refer to Appendix C for other suggestions.
3. Position the sample vials in unused positions starting at SI:F1.

## Buffer and Sample Tray Configuration

1. Set aside three groups of  $3 \times 2$  mL glass vials.
2. For each HSCD, dispense 1.8 mL of each of the three concentrations prepared into three 2 mL glass vials and properly cap the vials with red silicone caps.
3. Place the vials in buffer trays according to Figure 3.
4. Dilute 10 mL of the 50 mM phosphate buffer 1:1 with distilled and deionized water that has been filtered through a 0.2  $\mu$ m filter.
5. Dispense 1.8 mL of this 25 mM buffer into eight 2 mL glass vials and seal the vials with red silicone caps.
6. Place these buffer vials, along with a water vial, into the areas of the buffer trays marked with a 2.5, as shown in Figure 3. Fill one 2 mL glass vial half full with 25 mM phosphate buffer and place it in position BO:F6.
7. Dispense 1.8 mL of capillary conditioning solution into one 2 mL glass vial and properly cap the vial with a red silicone cap. This vial should be placed in position F2 of the buffer inlet tray.
8. Capped empty vials should be placed on positions BO:B1 to BO:B3 and BO:F1 to BO:F3 of the buffer outlet tray.

**Figure 3.** Buffer inlet (left) and outlet (right) trays

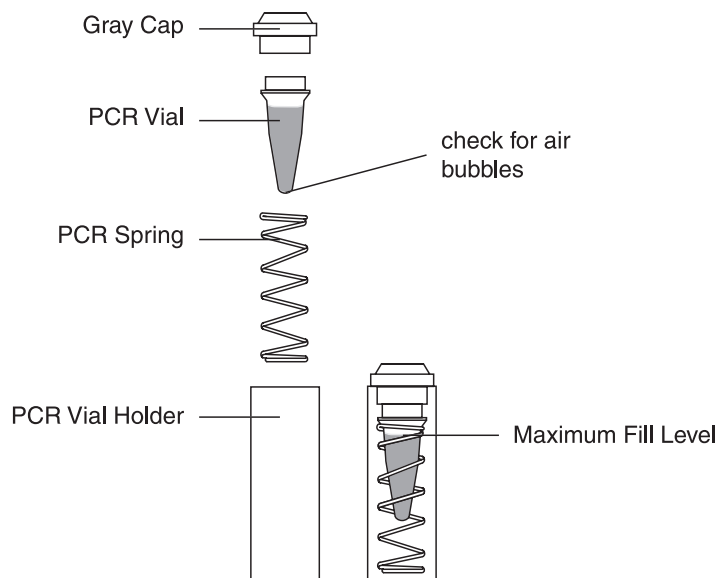


## Setting Up the Sample Vials

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1. Dispense 100  $\mu\text{L}$  of the test mixtures for system suitability and the entire contents of the prepared samples of interest into 200  $\mu\text{L}$  PCR sample vials.
2. Ensure that there are no bubbles at the bottom of the PCR vials, as shown in Figure 4.
3. Place the PCR vial onto the spring in the vial holder.
4. Cap the sample vial holder with a gray cap and ensure that a good seal is formed.

**Figure 4.** Sample vial assembly



5. Place the test sample vials in the inlet sample tray starting at position SI:A1.
6. Place the sample vial holders for the unknown samples in the same inlet sample tray starting in SI:F1. The sequence may be customized for alternate vial positioning or vial incrementing.



## Running the Protocol on the P/ACE MDQ



### NOTE

There are individual methods for the three HSCDs for both system suitability and method development, as well as common methods for capillary conditioning and instrument shutdown. These methods, along with example sequences, are available for download at: [www.celeader.com/downloads](http://www.celeader.com/downloads)

To use this assay, sequences should be prepared and will include these steps and corresponding methods:

- Capillary conditioning
- System suitability check
- Analysis of the compounds of interest
- Instrument shutdown procedure

### Instrument Conditions

General instrument and detector conditions are the same for all methods and are shown in Figures 5 and 6:

Figure 5. Initial conditions

The screenshot shows the 'Initial Conditions' dialog box with three tabs: 'Initial Conditions', 'PDA Detector Initial Conditions', and 'Time Program'. The 'Initial Conditions' tab is active. It contains several sections of controls:

- Auxiliary data channels:** Includes checkboxes for 'Voltage' (unchecked), 'Current' (checked), 'Power' (unchecked), and 'Pressure' (unchecked). 'Current' has a maximum value of 300.0  $\mu\text{A}$ .
- Mobility channels:** Includes checkboxes for 'Mobility' (unchecked), 'Apparent Mobility' (unchecked), and 'Plot trace after voltage ramp' (checked).
- Analog output scaling:** A 'Factor' dropdown menu is set to 1.
- Temperature:** 'Cartridge' and 'Sample storage' are both set to 22.0  $^{\circ}\text{C}$ .
- Peak detect parameters:** 'Threshold' is set to 2, and 'Peak width' is set to 9.
- Trigger settings:** Includes checkboxes for 'Wait for external trigger' (unchecked), 'Wait until cartridge coolant temperature is reached' (checked), and 'Wait until sample storage temperature is reached' (checked).
- Inlet trays:** 'Buffer' and 'Sample' are both set to 48 vials.
- Outlet trays:** 'Buffer' and 'Sample' are both set to 36 vials.

Figure 6. PDA detector initial conditions

The screenshot shows the 'PDA Detector Initial Conditions' window. It features three tabs: 'Initial Conditions', 'PDA Detector Initial Conditions' (selected), and 'Time Program'. The 'Electropherogram scan data' section includes a checked 'Acquisition enabled' box, a 'Data rate' of 2 Hz, and a 'Scan range' from 190 to 350 nm. The 'Electropherogram channel data' section shows a 'Data Rate' of 4 Hz and a table with columns for 'Acquisition enabled', 'Reference channel', 'Wavelength (nm)', and 'Bandwidth (nm)'. Channel 1 is active with a wavelength of 200 nm and a bandwidth of 10 nm. Channels 2 and 3 are inactive. A 'Peak detect' section is also present with a wavelength of 250 nm and a bandwidth of 120 nm. The 'Filter' section has radio buttons for 'High sensitivity', 'Normal', and 'High resolution' (selected), along with a 'Peak width (points)' dropdown set to '16-25'. 'Relay 1' and 'Relay 2' are both set to 'Off'. The 'Reference channel' section has a 'Wavelength' of 375 nm and a 'Bandwidth' of 75 nm. The 'Absorbance signal' section has radio buttons for 'Direct' (selected) and 'Indirect'.

**Capillary Conditioning in System Suitability (SS) and Method Development (MD)  
 (Example method names: SS Cond.met or MD Cond.met)**

This method should be run at least once a day to coat the capillary surface, which allows a much faster equilibration (see Figure 7).

Figure 7. Capillary conditioning program tab

|   | Time (min) | Event               | Value    | Duration | Inlet vial | Outlet vial | Summary | Comments                           |
|---|------------|---------------------|----------|----------|------------|-------------|---------|------------------------------------|
| 1 |            | Rinse - Pressure    | 50.0 psi | 5.00 min | BI:F1      | BO:F1       | forward | Water Rinse                        |
| 2 |            | Rinse - Pressure    | 50.0 psi | 5.00 min | BI:F2      | BO:F2       | forward | Column conditioning solution rinse |
| 3 | 0.00       | Separate - Pressure | 50.0 psi | 5.00 min | BI:F3      | BO:F3       | forward | Buffer rinse                       |
| 4 | 5.00       | Stop data           |          |          |            |             |         |                                    |
| 5 | 5.10       | End                 |          |          |            |             |         |                                    |
| 6 |            |                     |          |          |            |             |         |                                    |

### System Suitability (SS) and Method Development (MD) (Example method names: SS Alpha.met or MD Alpha.met)

Similar methods are used for each HSCD (see Figure 8).

**Figure 8.** SS or MD method time program tab

| Initial Conditions |            | PDA Detector Initial Conditions |          | Time Program |            |             |                                  |                                   |  |
|--------------------|------------|---------------------------------|----------|--------------|------------|-------------|----------------------------------|-----------------------------------|--|
|                    | Time (min) | Event                           | Value    | Duration     | Inlet vial | Outlet vial | Summary                          | Comments                          |  |
| 1                  |            | Rinse - Pressure                | 25.0 psi | 3.00 min     | BI:B1      | BO:B1       | forward, In / Out vial inc 15    | Buffer rinse                      |  |
| 2                  |            | Inject - Pressure               | 0.3 psi  | 4.0 sec      | SI:A1      | BO:C1       | Override, forward                | Sample injection                  |  |
| 3                  |            | Wait                            |          | 0.00 min     | BI:A1      | BO:A1       | In / Out vial inc 15             | Buffer dip to avoid carry over    |  |
| 4                  | 0.00       | Separate - Voltage              | 15.0 kV  | 10.00 min    | BI:C1      | BO:C1       | 0.17 Min ramp, reverse polarity. | Enantiomer separation step        |  |
| 5                  | 1.25       | Autozero                        |          |              |            |             |                                  |                                   |  |
| 6                  | 10.10      | Wait                            |          | 0.00 min     | BI:A1      | BO:A1       | In / Out vial inc 15             | Buffer dip at end of run to avoid |  |
| 7                  | 10.20      | Stop data                       |          |              |            |             |                                  |                                   |  |
| 8                  | 10.30      | End                             |          |              |            |             |                                  |                                   |  |
| 9                  |            |                                 |          |              |            |             |                                  |                                   |  |

### System Suitability (SS) and Method Development (MD) (Example method name: Shutdown.met)

This method should be run at the end of every sequence (see Figure 9).

**Figure 9.** Shutdown program tab

| Initial Conditions |            | PDA Detector Initial Conditions |          | Time Program |            |             |         |                                |  |
|--------------------|------------|---------------------------------|----------|--------------|------------|-------------|---------|--------------------------------|--|
|                    | Time (min) | Event                           | Value    | Duration     | Inlet vial | Outlet vial | Summary | Comments                       |  |
| 1                  | 0.00       | Separate - Pressure             | 20.0 psi | 10.00 min    | BI:F6      | BO:F6       | forward | Buffer rinse to half-full vial |  |
| 2                  | 10.00      | Lamp - Off                      |          |              |            |             |         |                                |  |
| 3                  | 10.00      | Stop data                       |          |              |            |             |         |                                |  |
| 4                  | 10.10      | End                             |          |              |            |             |         |                                |  |
| 5                  |            |                                 |          |              |            |             |         |                                |  |

## Sequence Preparation for System Suitability and Unknowns

Prepare a sequence for system suitability and compounds of interest, as shown in Figure 10. The number of repetitions, usually two to six, can be adjusted as necessary.

**Figure 10.** SS sequence

| Run # | Reps | Sample Inject Inlet | Sample Inject Outlet | Sample Inject Duration | Sample ID    | Method                | Filename         |
|-------|------|---------------------|----------------------|------------------------|--------------|-----------------------|------------------|
| 1     | 1    |                     |                      |                        |              | SS Cond.met           |                  |
| 2     | 6    | SI:A1               | BO:C1                | 4.0                    | SS PSE Alpha | SS PSE alpha HSCD.met | 001 SS PSE Alpha |
| 3     | 6    | SI:A1               | BO:C2                | 4.0                    | SS PSE Beta  | SS PSE beta HSCD.met  | 002 SS PSE Beta  |
| 4     | 6    | SI:A1               | BO:C3                | 4.0                    | SS PSE Gamma | SS PSE gamma HSCD.met | 003 SS PSE Gamma |
| 5     | 6    | SI:A2               | BO:C2                | 4.0                    | SS Glu Beta  | SS Glu beta HSCD.met  | 004 SS Glu Beta  |
| 6     | 1    |                     |                      |                        |              | Shutdown.met          |                  |
| 7     |      |                     |                      |                        |              |                       |                  |

Samples of unknowns can be inserted as part of the System Suitability Sequence, as shown in Figure 11.

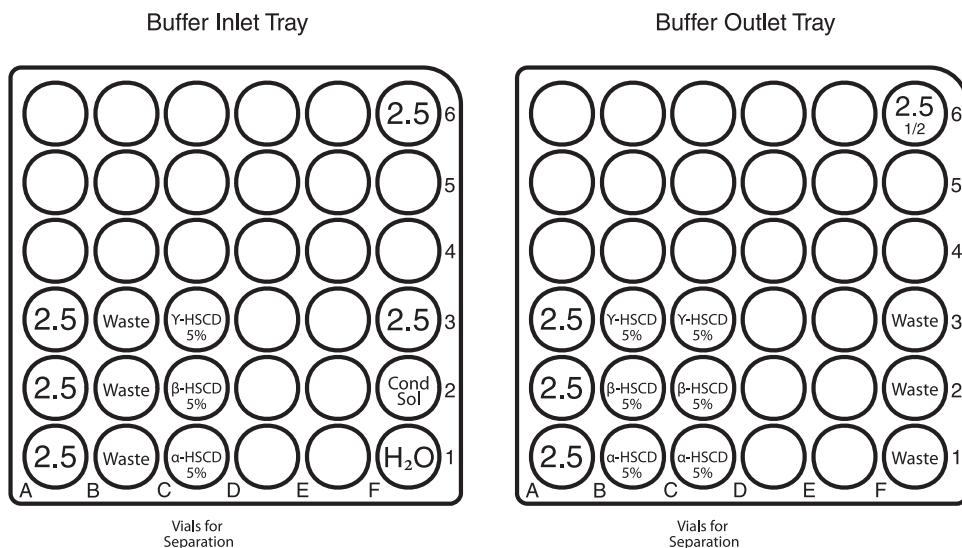
**Figure 11.** SS sequence with unknowns inserted

| Run # | Reps | Sample Inject Inlet | Sample Inject Outlet | Sample Inject Duration | Sample ID      | Method                | Filename           |
|-------|------|---------------------|----------------------|------------------------|----------------|-----------------------|--------------------|
| 1     | 1    |                     |                      |                        |                | SS Cond.met           |                    |
| 2     | 6    | SI:A1               | BO:C1                | 4.0                    | SS PSE Alpha   | SS PSE alpha HSCD.met | 001 SS PSE Alpha   |
| 3     | 2    | SI:F1               | BO:C1                | 4.0                    | Cpd X in Alpha | SS PSE alpha HSCD.met | 002 Cpd X in Alpha |
| 4     | 6    | SI:A1               | BO:C2                | 4.0                    | SS PSE Beta    | SS PSE beta HSCD.met  | 003 SS PSE Beta    |
| 5     | 2    | SI:F1               | BO:C2                | 4.0                    | Cpd X in Beta  | SS PSE beta HSCD.met  | 004 Cpd X in Beta  |
| 6     | 6    | SI:A1               | BO:C3                | 4.0                    | SS PSE Gamma   | SS PSE gamma HSCD.met | 005 SS PSE Gamma   |
| 7     | 2    | SI:F1               | BO:C3                | 4.0                    | Cpd X in Gamma | SS PSE gamma HSCD.met | 006 Cpd X in Gamma |
| 8     | 6    | SI:A2               | BO:C2                | 4.0                    | SS Glut Beta   | SS Glu beta HSCD.met  | 007 SS Glut Beta   |
| 9     | 1    |                     |                      |                        |                | Shutdown.met          |                    |
| 10    |      |                     |                      |                        |                |                       |                    |

## Fast Method Development for Enantiomer Separations

To improve throughput and significantly decrease separation time, there is an option to set up the runs so that the separation takes place in the shorter end of the capillary (the 10 cm between the outlet end and the window in the capillary). Figure 12 shows the vial locations in the trays used in fast enantiomer analysis.

**Figure 12.** Buffer tray configuration for fast enantiomer analysis



### NOTE

The positions of the vials used for capillary conditioning have not been changed. Methods used for capillary conditioning and instrument shutdown are shown in Figures 7 and 9.

The system suitability samples are placed in the outlet sample tray positions SO:A1, A2, A3, and so on. The unknown samples can be placed in similar outlet tray positions such as SO:F1, F2, and F3.

Methods for fast method development must be created by the user such that the pressure is applied in the reverse direction and the sample is injected into the short (10 cm) end of the capillary.

See Appendix B for an example using the fast method development.



# System Suitability

## System Suitability

**IMPORTANT**

System suitability should be assessed before any method development is attempted. Pseudoephedrine and glutethimide in test mixtures containing PTS are recommended for system suitability assessment. Only PTS is included in the kit. If pseudoephedrine and glutethimide, which are controlled substances, are available locally, they should be run in the appropriate 5% HSCD buffer. Resolution values should be equal to or better than the values in the figure titles for Figures A.1 through A.4.

**Figure A.1 Pseudoephedrine in HS- $\alpha$ -CD, expected resolution  $\geq 2.0$**

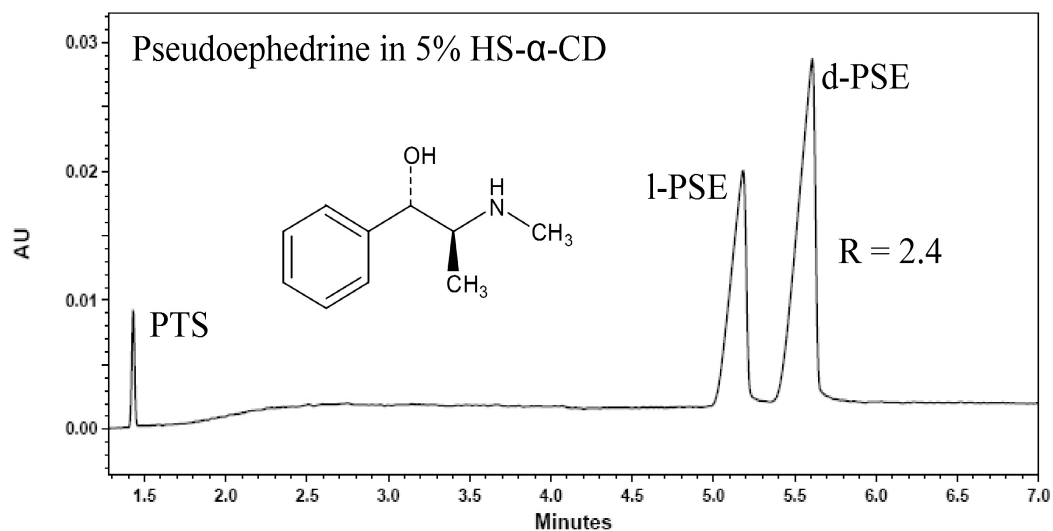


Figure A.2 Pseudoephedrine in HS- $\beta$ -CD, expected resolution  $\geq 4.0$

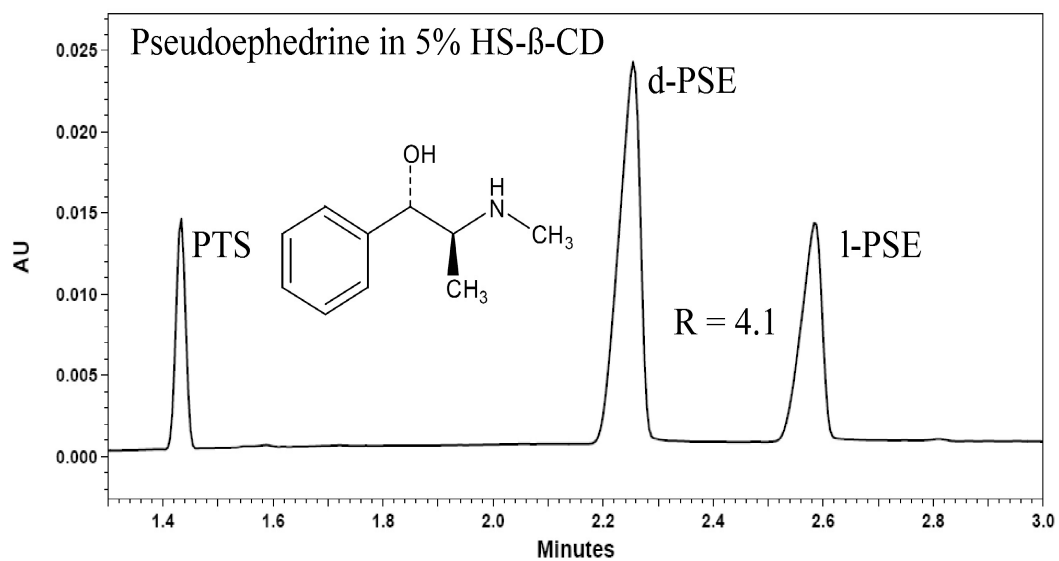


Figure A.3 Pseudoephedrine in HS- $\gamma$ -CD, expected resolution  $\geq 4.0$

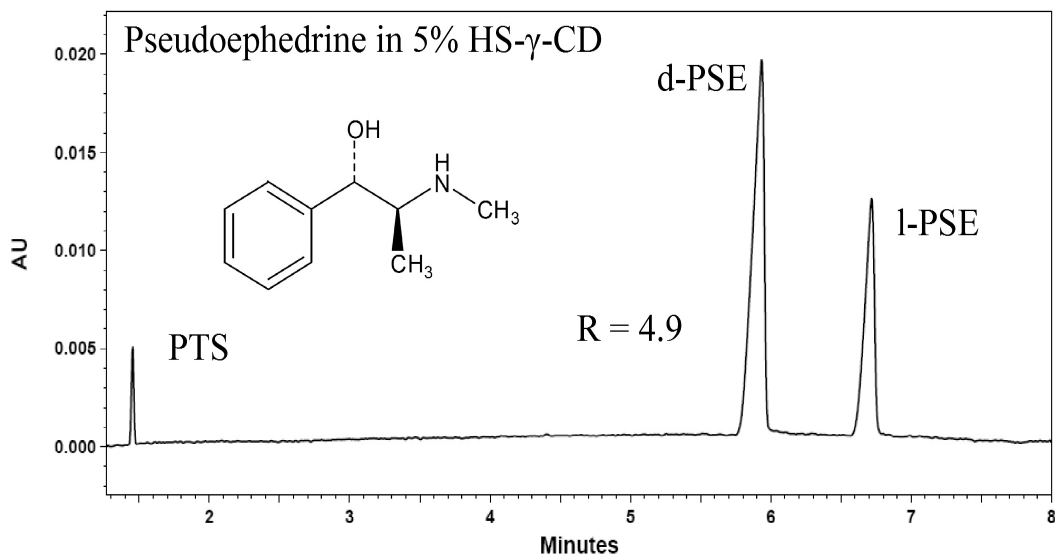
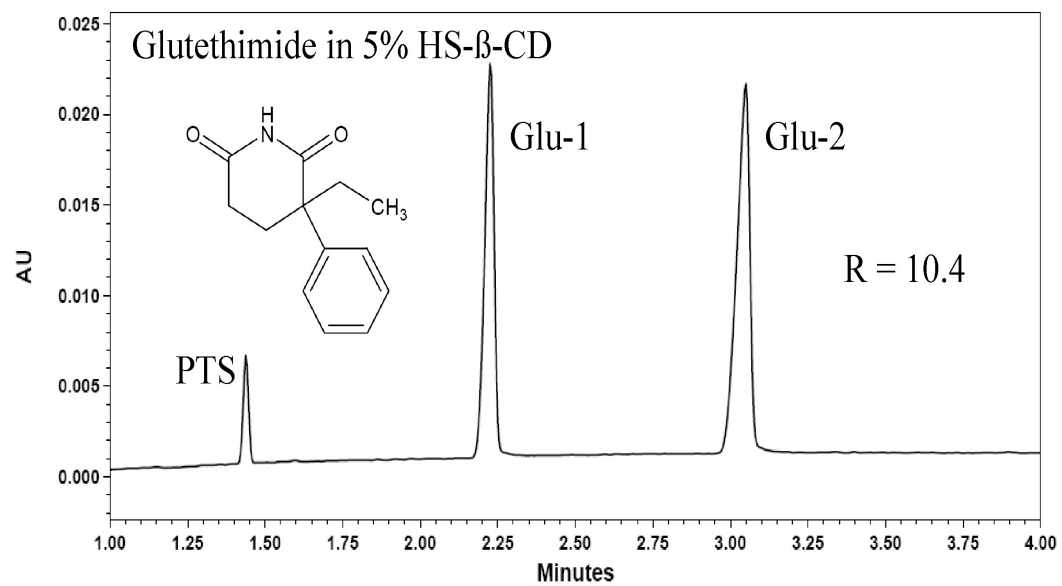




Figure A.4 Glutethimide in HS- $\beta$ -CD, expected resolution  $\geq 7.0$





# Analyzing Unknown Samples Using Fast Method Development

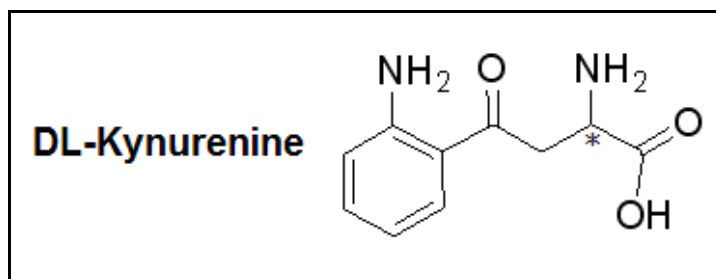
## Analyzing Unknown Samples Using Fast Method Development

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As an example, a racemic mixture of D and L isomers of Kynurenine at a concentration of 2 mg/mL will be used as an unknown molecule, and HS-CD  $\alpha$ ,  $\beta$ , and  $\gamma$  will be used as the enantiomer discriminator at concentrations of 2.5, 5, and 7.5% (v/v) in 25 mM phosphate buffer pH2.5.

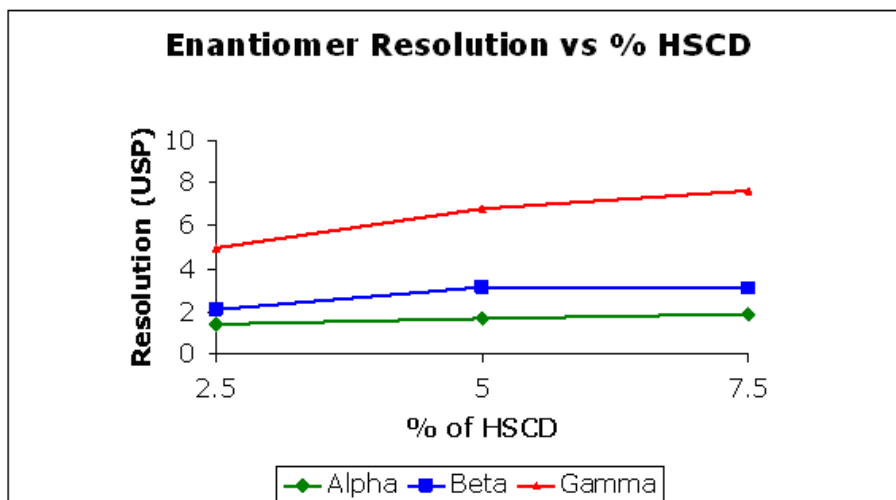
DL-Kynurenine contains one chiral center, highlighted by \*, as shown in Figure B.1.

**Figure B.1** Chemical structure of DL-Kynurenine



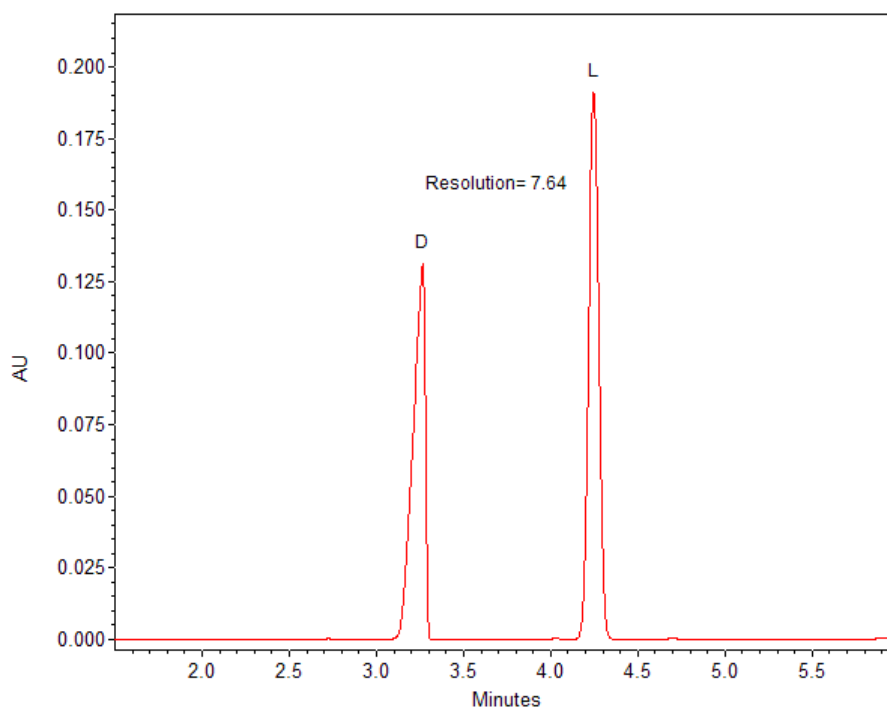
The resolution achieved by the three types of HSCDs at three different concentrations is shown in Figure B.2. HS- $\gamma$ -CD achieved the highest resolution.

Figure B.2 Enantiomer resolution vs % of HS- $\alpha$ -CD, HS- $\beta$ -CD, and HS- $\gamma$ -CD.



A typical electropherogram obtained of racemic mixture of DL-Kynurenine at 7.5% concentration of HS- $\gamma$ -CD using the high-speed method is shown in Figure B.3.

Figure B.3 Separation of DL-Kynurenine using 7.5% HS- $\gamma$ -CD



# Important Remarks

## Important Remarks

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If baseline separation is not achieved under the conditions described in this guide, the HSCD concentration can be increased or decreased to produce better resolution.

The capillary length can be increased to provide better resolution. It is recommended that the voltage be adjusted to maintain the same field strength. For example, for a 30 cm capillary operated at 15 kV, the field strength is 500 volts per cm. To maintain the same field strength with a 40 cm capillary, voltage would have to be increased to 20 kV.

Chiral neutral drug samples can be prepared and analyzed under conditions similar to that used for D, L-Glutethimide. Typically, chiral amine separations are achieved within ten minutes, and neutral enantiomers are separated within twenty minutes.

For all HSCD systems, pseudoephedrine is recommended as a test analyte. Under the described electrophoretic conditions, the chiral separation time for the pseudoephedrine enantiomers may be achieved in less than nine minutes. These same conditions are also recommended for chiral amine salts.

**Important Remarks**  
*Important Remarks*

# Troubleshooting

## Enantiomer Analysis Troubleshooting

| Symptom   | Possible Causes   | Corrective Action  |
|---|---|--|
| Migration times are drifting longer                             | Capillary surface changing over time                          | Condition the capillary more frequently  |
|   | Buffer depletion  | Replenish buffer   |
| Software gives the following error message:<br>Current Leakage  | Vial caps are not present, not correctly in place, or are wet | Check all vials in the trays to make sure they are properly capped and are clean and dry.  |
|   | Interface block is dirty                                      | Use a mirror to help identify the presence of any dried buffer in the interface block. Clean the interface block, if necessary.  |
|   | Capillary is broken   | Carefully inspect the capillary ends, as well as the capillary window. When using high ionic strength buffers, broken windows can cause buffer leakage. When voltage is applied, voltaic arcing will occur and the software will flag the current leakage. Replace the capillary, if broken. |
| Software gives the following error message:<br>Pressure Failure | Vial caps are not present or are not correctly in place       | Check all vials in the trays to make sure they are properly capped.  |
|   | Capillary is bent or broken                                   | Inspect the capillary very carefully to make sure that it did not bend or break. Replace the capillary if necessary.   |
|   | Electrode is bent or broken                                   | Inspect the electrode very carefully to make sure that it did not bend or break. Replace the electrode if necessary.   |
| Current trace is erratic or drifting                            | Heat is not being dissipated efficiently                      | Check the level of coolant in your instrument. Refill with appropriate cooling liquid if level is below the mark. For instructions on how to refill the coolant reservoir, please refer to <i>P/ACE MDQ Installation and Maintenance Manual</i> .  |
|   |   | Decrease the applied voltage, the HSCD concentration, or both if necessary.  |

## Enantiomer Analysis Troubleshooting (con't.)

|   |  |  |
|---|--|--|
| Current trace is faulty, eventually dropping to zero      | Buffer depletion   | Each running buffer can be used for up to 30 runs. If running more than 30 samples, make sure that you have more running buffer vials in the instrument and that you are also properly using the sequence table with the increment feature. For more details on how to use the increment feature, please refer to 32 Karat manual. |
|   | Plugged capillary or broken window                                 | This requires corrective action, such as replacing the capillary.  |
| Software gives the following error message: PDA light low | UV lamp hours exceeded 1,000 hours                                 | Check the lamp hours on the PDA diagnostics screen. If 1,000 hours or more, replace the lamp. Refer to the <i>P/ACE MDQ Installation and Maintenance Manual</i> .  |
|   | Capillary is broken at the window                                  | Check the integrity of the capillary. If broken at the window, replace the capillary.  |
|   | Fiber optic is dirty or broken                                     | Refer to the <i>P/ACE MDQ Installation and Maintenance Manual</i> for instructions on cleaning and verifying the integrity of the fiber optics.  |
|   | Window is not centered in the aperture                             | Adjust window position.  |
| Current is much higher than expected                      | Incorrect dilution of 20% HSCD aqueous solution                    | Make sure you are mixing the volumes of the 20% HSCD solution, the distilled and deionized water, and the phosphate buffer in the correct proportions.   |
|   | The temperature is set at a higher value than the recommended 22°C | Verify on the control that the actual temperature is the recommended 22°C. If not, right click on the cartridge hot spot and set the temperature for the cartridge to 22°C.  |
|   | Capillary has a larger inner diameter than specified               | Check the inner diameter of the capillary. Table D.1 shows the average current values as a function of capillary inner diameter when running HSCD.   |

**Table D.1** Average Current Values for 5% HSCD Solutions

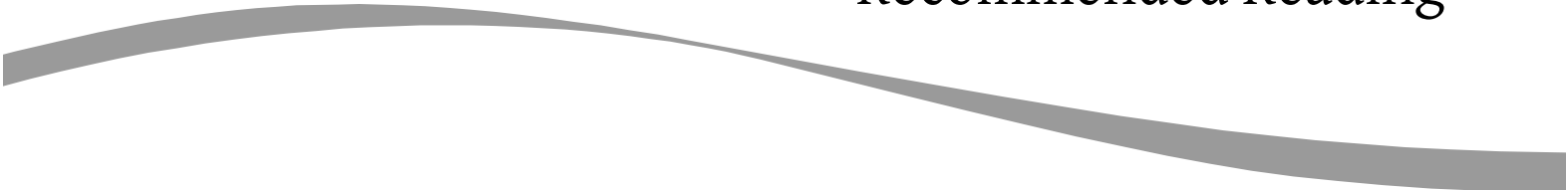
| Capillary Inner Diameter (µm) | Current (µA) |
|-------------------------------|--------------|
| 25                            | 30–50        |
| 50                            | 150–180      |
| 75*                           | Above 300    |



\*The current generated using HSCD buffers in a capillary with an inner diameter of 75 µm exceeds the current capacity of the instrument. Do not use 75 µm i.d. columns in this application. Instrument and capillary damage may result.



# References and Recommended Reading



## References and Recommended Reading

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- Beckman Coulter Inc. web page on chiral separations,  
<http://www.beckmancoulter.com/products/splashpage/chiral38/default.asp>
- Chapman, J., H. Watley, and F-T A. Chen, "Methods Development Strategy for Enantiomer Analysis Using the P/ACE MDQ Chiral System", *Beckman Coulter Application Information*, A-1889A.
- Chen, F-T A., G. Shen, and R. A. Evangelista, "Characterization of Highly Sulfated Cyclodextrins", *J. Chromatogr. A*, 924 (2001): 523-32.
- Chen, F-T A., and R. A. Evangelista, "Applications of Highly Sulfated Cyclodextrins for Enantiomeric Separation by Capillary Electrophoresis", *Journal of the Chinese Chemistry Society*, 46 (1999): 847-60.
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## Chiral Methods Development User's Guide

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