

# INSTRUCTION MANUAL

## CHIRAL-CBH

CHIRAL-CBH is a patented HPLC-column, used for the direct separation of enantiomers. Cellobiohydrolase (CBH 1) is a stable enzyme, which has been immobilized onto 5 µm spherical silica particles. The surface chemistry and the immobilization technique provide a stable column, which is used in the reversed phase mode.

The column is a very good tool for the determination of enantiomeric purity of drugs and for bioanalysis of drug enantiomers.

CHIRAL-CBH is an excellent column for the separation of, preferentially, basic drugs from many different compound classes.

**Temperature:** The column should be used at room temperature or below.

### **Mobile phase composition:**

The mobile phases are buffer solutions with a relatively low content of uncharged organic modifier. Note! Do not use charged organic modifiers in the mobile phase.

### pH and buffer solutions.

Phosphate or acetate buffers can be used in the concentration range 10-50 mM, preferentially 10 mM. The buffer concentration can affect the chromatographic properties of the solute, especially the retention. Changes of the mobile phase pH is the most powerful way to regulate the retention and the enantioselectivity of charged solutes. The column can be used in the pH range 3-7. Use of pH < 3 or > 7 may decrease the column lifetime, due to silica decomposition. Note! Always use buffer salts of analytical grade.

### Organic modifiers:

Examples of uncharged organic modifiers that can be used are 2-propanol (15%), acetonitrile (5%) and methanol (10%). Note! Do not use charged organic modifiers in the mobile phase. The concentration of the organic modifier affects the enantioselectivity, retention and the chromatographic performance. The nature of the modifier can affect the enantioselectivity and retention strongly. Note that, when going from high to low concentrations of organic modifier in the mobile phase, the equilibration time can be extended.

### Disodium EDTA ((ethylenedinitrilo))tetraacetic acid disodium salt.

It is recommended to use 50 µM disodium EDTA in the mobile phases in order to complex metal ions, which can deteriorate the column properties. Disodium EDTA in the mobile phase can cause system peaks when injecting samples dissolved in buffers with, for example, another pH.

### **Samples**

The recommended sample concentration is less than 0.10 mg/ml with an injection volume of 10-20 µl. Note that the medium in which the sample is dissolved can affect the chromatographic properties. Do not inject unclear sample solutions or samples containing undissolved compounds. In bioanalytical work use an isolation procedure that produces clear sample solutions, free from emulsions of fatty compounds. Exchange the guard column regularly.

**Flow rate:** Normally 0.9 ml/min with a 4.0 mm I.D. column.

### **Installation**

The column is filled with 15% 2-propanol in distilled water when delivered. Start with a flow rate of 0.5 ml/min in two minutes before increasing to 0.9 ml/min. In order to speed up the equilibration time of the column it is recommended to equilibrate the column for about 15 minutes with a mobile phase containing 50 mM buffer salts and 50 µM disodium EDTA (with the same pH as the analytical mobile phase), before changing to the appropriate mobile phase (normally containing 10 mM of buffer salts).

### **Maintenance**

In order to maintain the column performance it is necessary to use a CHIRAL-CBH guard column (10 x 3.0 mm), in front of the analytical column. The guard column must be replaced regularly. With daily continuous use, the guard column should be replaced after about 3 weeks (approximately 110-130 hours or 6-7 litres of mobile phase) or earlier if the separation efficiency decreases. It is important to note that the guard column should be replaced before the chromatographic performance is affected to a large extent.

### **Storage**

The column can be stored at room temperature. When the column is used daily, the column can be left over night in the chromatographic system. However, care must be taken when using mobile phases with no or low content of organic modifier, because bacteria grows fast in such mobile phases. Note! Prepare fresh mobile phases often. During weekends and other shorter periods it is recommended to fill the column with 10% 2-propanol in distilled water. When the column is stored for longer periods it is recommended to fill it with 15% 2-propanol in distilled water and place it in the refrigerator.

### **Cleaning of the column**

If the column has been contaminated, with deteriorated chromatographic properties as a result, backflush the column with the mobile phase containing 2.5 mM disodium EDTA (be sure that the salts will not precipitate). In case the column still shows bad chromatographic performance, backflush with 10 mM phosphate buffer pH 3.5 containing 15% 2-propanol at a flow rate of 0.5 ml/min (with a 4 mm I.D. column) for about 1 hour.



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### **IMPORTANT!**

Please read the manual before using the column

### **NOTE!**

In order to maintain the column performance it is necessary to use a CHIRAL-CBH guard column (10x3 mm) in front of the analytical column.



#### **ChromTech Ltd**

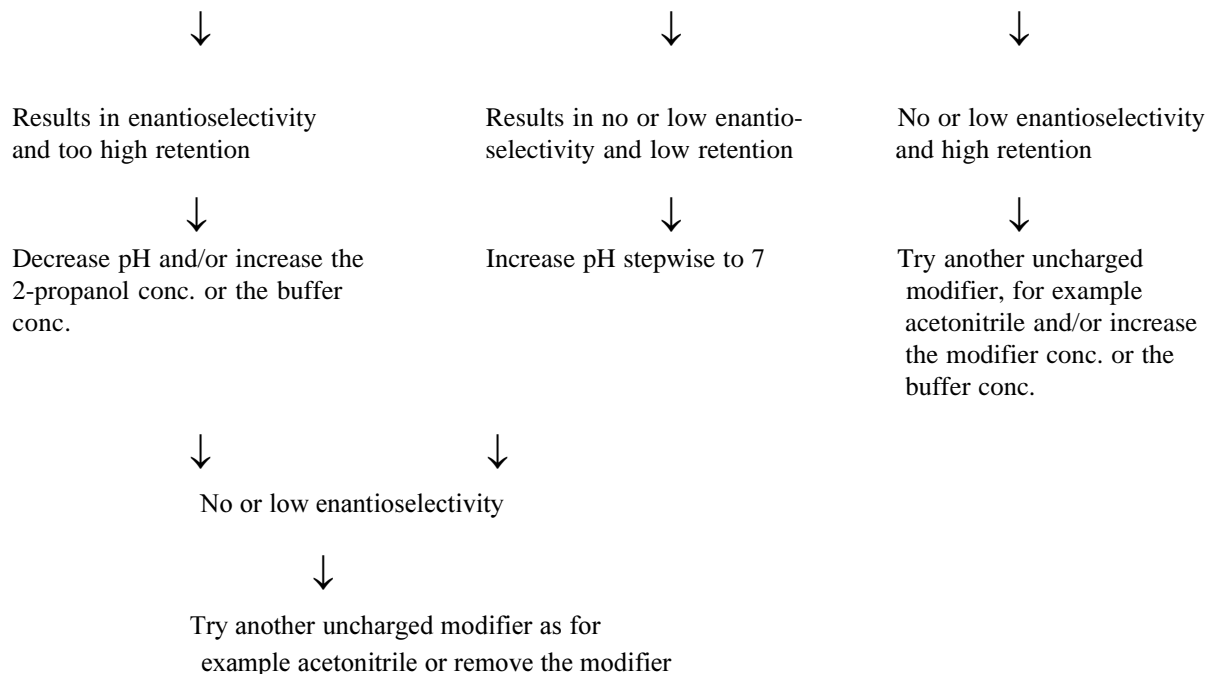
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# Method development CHIRAL-CBH

## Hydrophilic and hydrophobic amines

**Starting mobile phase: 5% 2-propanol in 10 mM sodium phosphate buffer pH 6.0 with 50  $\mu$ M disodium EDTA**



### CHIRAL-CBH products:

CBH 100.4	CHIRAL-CBH 100x4.0 mm	CBH 100.10	CHIRAL-CBH 100x10.0 mm
CBH 150.4	CHIRAL-CBH 150x4.0 mm	CBH 150.10	CHIRAL-CBH 150x10.0 mm
CBH 50.4	CHIRAL-CBH 50x4.0 mm	CBH 10.3	CHIRAL-CBH 10x3.0 mm guard
CBH 100.3	CHIRAL-CBH 100x3.0 mm	CBH 10.2	CHIRAL-CBH 10x2.0 mm guard
CBH 150.3	CHIRAL-CBH 150x3.0 mm	CH 10.3	Guard column holder
CBH 100.2	CHIRAL-CBH 100x2.0 mm	CON 2	Guard column coupler
CBH 150.2	CHIRAL-CBH 150x2.0 mm	CON 4	Micro guard column coupler
CBH 50.2	CHIRAL-CBH 50x2.0 mm		



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