

# ChromTech Chiral HPLC Columns

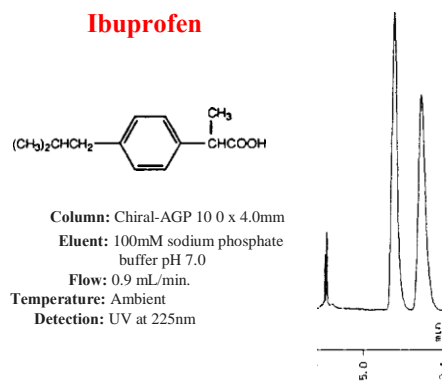
## Chiral Column Selection Guide

### Column Applicability (type of samples)

<b>Chiral-AGP</b>	Extremely broad applicability. Most likely the column with the broadest applicability of all chiral columns. Separates all kinds of compounds: <ul style="list-style-type: none"> <li>• amines (primary, secondary, tertiary and quaternary)</li> <li>• acids (strong and weak)</li> <li>• nonprotolytes (amides, esters, alcohols)</li> </ul>
<b>Chiral-CBH</b>	More narrow applicability than CHIRAL-AGP. Separates compounds containing one or more basic nitrogens together with one or more hydrogen accepting or hydrogen donating groups (alcohol, phenol, carbonyl, amide, ether, sulphoxide, ester, etc.).
<b>Chiral-HSA</b>	More narrow applicability than CHIRAL-AGP. Separates preferentially weak and strong acids and non-protolytic compounds.

The columns overlap for some types of compounds; basic compounds can be separated on both CHIRAL-AGP and CHIRALCBH, acidic and neutral compounds can be separated on both CHIRAL-AGP and CHIRAL-HSA. However, as **CHIRAL-AGP** is a column with an extremely broad applicability, this column should be chosen first, if the analyte has not been separated on any of the columns. There are, however, some types of compounds where one of the other columns may be the first choice:

CHIRAL-HSA: very hydrophilic acids  
 CHIRAL-CBH: very hydrophilic amines



## Columns for Drug Protein Binding Study

HPLC is a convenient method to use for the determination of the degree of the drug/protein binding. We supply a range of HPLC columns that can be used for these kind of studies:

- CHIRAL-HAS** : human serum albumin
- CHIRAL-AGP** :  $\alpha_1$ -acid glycoprotein
- CHIRAL-RSA** : rat serum albumin
- CHIRAL-DSA** : dog serum albumin
- CHIRAL-MSA**: mouse serum albumin

Albumins from other species also available, please contact ChromTech for pricing.

## Chiral Column Price List

### Chiral-AGP

Cat. No.	Description	Price
CT-20054	Chiral-AGP, 4.0 x 50mm, 5 $\mu$ m	
CT-20104	Chiral-AGP, 4.0 x 100mm, 5 $\mu$ m	
CT-20154	Chiral-AGP, 4.0 x 150mm, 5 $\mu$ m	
CT-200142	Chiral-AGP, 4.0 x 10mm, guard cart, 2/pk	
CT-20053	Chiral-AGP, 3.0 x 50mm, 5 $\mu$ m	
CT-20103	Chiral-AGP, 3.0 x 100mm, 5 $\mu$ m	
CT-20153	Chiral-AGP, 3.0 x 150mm, 5 $\mu$ m	
CT-200132	Chiral-AGP, 3.0 x 10mm, guard cart, 2/pk	
CT-20052	Chiral-AGP, 2.0 x 50mm, 5 $\mu$ m	
CT-20102	Chiral-AGP, 2.0 x 100mm, 5 $\mu$ m	
CT-20152	Chiral-AGP, 2.0 x 150mm, 5 $\mu$ m	
CT-200122	Chiral-AGP, 2.0 x 10mm, guard cart, 2/pk	
CT-201010	Chiral-AGP, 10.0 x 100mm, 5 $\mu$ m	
CT-201510	Chiral-AGP, 10.0 x 150mm, 5 $\mu$ m	

### Chiral-CBH

CT-25054	Chiral-CBH, 4.0 x 50mm, 5 $\mu$ m
CT-25104	Chiral-CBH, 4.0 x 100mm, 5 $\mu$ m
CT-25154	Chiral-CBH, 4.0 x 150mm, 5 $\mu$ m
CT-250142	Chiral-CBH, 4.0 x 10mm, guard cart, 2/pk
CT-25053	Chiral-CBH, 3.0 x 50mm, 5 $\mu$ m
CT-25103	Chiral-CBH, 3.0 x 100mm, 5 $\mu$ m
CT-25153	Chiral-CBH, 3.0 x 150mm, 5 $\mu$ m
CT-250132	Chiral-CBH, 3.0 x 10mm, guard cart, 2/pk
CT-25052	Chiral-CBH, 2.0 x 50mm, 5 $\mu$ m
CT-25102	Chiral-CBH, 2.0 x 100mm, 5 $\mu$ m
CT-25152	Chiral-CBH, 2.0 x 150mm, 5 $\mu$ m
CT-250122	Chiral-CBH, 2.0 x 10mm, guard cart, 2/pk
CT-251010	Chiral-CBH, 10.0 x 100mm, 5 $\mu$ m
CT-251510	Chiral-CBH, 10.0 x 150mm, 5 $\mu$ m

### Chiral-HSA

CT-29054	Chiral-HSA, 4.0 x 50mm, 5 $\mu$ m
CT-29104	Chiral-HSA, 4.0 x 100mm, 5 $\mu$ m
CT-29154	Chiral-HSA, 4.0 x 150mm, 5 $\mu$ m
CT-290142	Chiral-HSA, 4.0 x 10mm, guard cart, 2/pk
CT-29053	Chiral-HSA, 3.0 x 50mm, 5 $\mu$ m
CT-29103	Chiral-HSA, 3.0 x 100mm, 5 $\mu$ m
CT-29153	Chiral-HSA, 3.0 x 150mm, 5 $\mu$ m
CT-290132	Chiral-HSA, 3.0 x 10mm, guard cart, 2/pk
CT-29052	Chiral-HSA, 2.0 x 50mm, 5 $\mu$ m
CT-29102	Chiral-HSA, 2.0 x 100mm, 5 $\mu$ m
CT-29152	Chiral-HSA, 2.0 x 150mm, 5 $\mu$ m
CT-290122	Chiral-HSA, 2.0 x 10mm, guard cart, 2/pk
CT-291010	Chiral-HSA, 10.0 x 100mm, 5 $\mu$ m
CT-291510	Chiral-HSA, 10.0 x 150mm, 5 $\mu$ m

### Chiral-RSA

RSA504	Rat serum albumin, 4.0 x 50mm, 5 $\mu$ m
RSA503	Rat serum albumin, 3.0 x 50mm, 5 $\mu$ m

### Chiral-MSA

MSA504	Mouse serum albumin, 4.0 x 50mm, 5 $\mu$ m
MSA503	Mouse serum albumin, 3.0 x 50mm, 5 $\mu$ m

### Chiral-DSA

DSA504	Dog serum albumin, 4.0 x 50mm, 5 $\mu$ m
DSA503	Dog serum albumin, 3.0 x 50mm, 5 $\mu$ m

### Accessories

731441	Guard cartridge holder
U-287	Column coupler (connects guard to column)

## Chiral-AGP

- Broadest applicability
- Acids, bases, and neutrals
- No derivatization

CHIRAL-AGP is the second generation chiral separation column based on the use of  $\alpha$ -1-acid glycoprotein (AGP) as the chiral stationary phase. Through a patented process  $\alpha$ -1-AGP has been immobilized on porous, spherical silica particles (5 $\mu$ m). The surface chemistry of the silica proves a stable chiral separation material with extremely broad applicability.

### Enantioselectivity

Racemic amines, acids and nonprotolytic compounds can be resolved directly, without derivatization. The column enables resolution of a very large number of chiral compounds from different compound classes. This is due to the unique nature of the chiral stationary phase, and the fact that enantioselectivity can be induced by choosing a proper mobile phase composition.

### Chromatographic Conditions

Phosphate buffers with addition of organic modifiers are used as mobile phase. (1- and 2-propanol and acetonitrile are the most frequently used modifiers.) Enantioselectivity and retention can be regulated by changing the mobile phase composition; ie: the pH, the concentration or the nature of the organic modifier. The column temperature also affects these parameters.

### Storage Conditions:

The column should be used at room temperature or below. During short periods of storage (ie: weekends) it is recommended to fill the column with 10% 2-propanol in distilled water. When the column is stored for longer periods of time it is recommended to fill with 15% 2-propanol in distilled water and place it in the refrigerator.

### Cleaning of the Column:

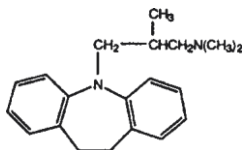
If the column has been contaminated with very hydrophobic material, wash the column over night with 25% 2-propanol in distilled water at a flow rate of 0.2mL/min.

See page 80 for ordering information.

Please note: The USP L-41 column is the CHIRAL-AGP.

For additional applications, please ask for our Chiral Users Guide.

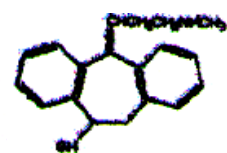
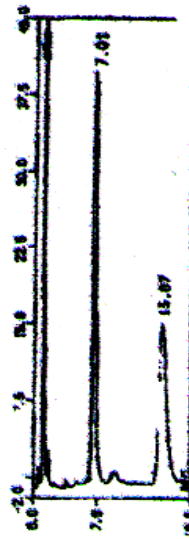
### Trimipramine



Column: Chiral-AGP 10 0 x 4.0mm  
 Eluent: 1% 2-propanol in  
 10mM sodium acetate pH4.0  
 total acetate conc.=60mM  
 Flow: 0.9mL/min.  
 Temperature: Ambient  
 Detection: UV at 225nm

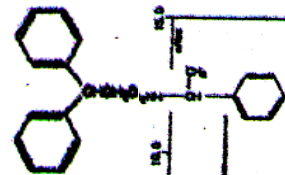
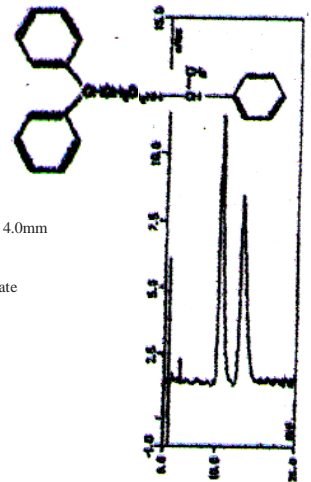


### E-10-Hydroxy nortriptyline



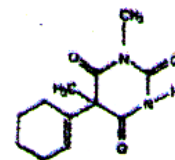
Column: Chiral-AGP 10 0 x 4.0mm and  
 Chiral-AGP guard column 10 x 3.0mm  
 Mobile phase: 7.5% 2-propanol  
 in 0.01M sod.ph.b.  
 pH 6.0  
 Detection: UV 225nm  
 Sample conc.: 0.03 mg/mL

### Fendiline



Column: Chiral-AGP 10 0 x 4.0mm  
 Mobile phase: 3% acetonitrile in  
 10mM sod.ac.b.  
 pH 4.1 (total acetate  
 conc. = 60mM)  
 Detection: UV 225nm  
 Sample conc.: 0.025 mg/mL

### Hexobarbital



Column: Chiral-AGP 10 0 x 4.0mm  
 Mobile phase: 2% 2-propanol  
 in 10mM sod.ph.b.  
 pH 7.0  
 Detection: UV 225nm  
 Sample conc.: 0.02 mg/mL

# ChromTech Chiral HPLC Columns

## Chiral-HSA

- **Acids and neutrals**
- **Excellent for hydrophilic acids**

The chiral selector in this stationary phase is human serum albumin (HSA). The protein has been immobilized onto spherical 5µm particles. Enantiomers of preferentially acidic compounds can be resolved directly, without derivatization. The column is operated in the reversed phase mode.

With the Chiral-HSA column, both racemic acids and amino acids can be resolved directly, without derivatization.

### Chromatographic Conditions

The column is operated in reversed phase mode: phosphate buffers (normally 0.01 to 0.1 M, pH 5-7) with the addition of less than 10% of an organic modifier such as 2-propanol, acetonitrile, methanol, or ethanol. Charged organic modifiers such as octanoic acid (1-5µM) may also be used. Enantio-selectivity and retention can be regulated by changing the mobile phase composition; ie: pH, buffer concentration and/or nature of the organic modifier.

### Storage Conditions

The column should be used at room temperature or below. When the column is stored for long periods of time it is recommended to fill with 10% 2-propanol in distilled water and place it in the refrigerator.

### Cleaning of the Column

If the column has been contaminated, wash the column over night with 10% 2-propanol in distilled water at a flow rate of 0.2mL/min.

## Chiral-CBH

- **Basic compounds**
- **Excellent for hydrophilic amines**

Cellobiohydrolase (CBH) is a stable enzyme which has been immobilized onto 5µm spherical silica particles creating the chiral stationary phase in the Chiral-CBH column. This is also a reversed phase column, used for the direct separation of enantiomers. The column is preferentially used for the separation of the enantiomers of basic drugs from many compound classes.

### Chromatographic Conditions

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.** The mobile phases are mixtures of phosphate or acetate buffers and organic solvents as 2-propanol or acetonitrile. The retention and the enantioselectivity can be regulated by changes in pH, buffer concentration and organic modifier (nature and concentration).

### Storage Conditions

The column should be used at room temperature or below. When the column is stored for long periods of time it is recommended to fill with 10% 2-propanol in distilled water and place it in the refrigerator.

### Cleaning of the Column

If the column has been contaminated, wash the column over night with 10% 2-propanol in distilled water at a flow rate of 0.2mL/min.

See page 80 for ordering information.

# ChromTech Chiral Columns for Drug-Protein Binding Study

## Columns for Drug-Protein Binding Study

HPLC is a convenient method to use for the determination of the degree of the drug/protein binding. We supply a range of HPLC columns that can be used for these kind of studies:

**CHIRAL-HSA:** human serum albumin

**CHIRAL-AGP:**  $\alpha_1$ -acid glycoprotein

**CHIRAL-RSA:** rat serum albumin

**CHIRAL-DSA:** dog serum albumin

**CHIRAL-MSA:** mouse serum albumin

Albumins from other species also available, please contact Chrom Tech for pricing.

## Calculation of % Protein Binding

Retention data ( $k'$ ) is used to calculate the percentage of protein binding. A  $t_m$ -marker is injected (an unretained compound). The retention factor ( $k'$ ) for a drug is calculated by:

$$k' = \frac{t_r - t_m}{t_m} \text{ where } t_r = \text{retention time for the drug}$$

$$\text{where } t_m = \text{retention time for the } t_m\text{-marker}$$

The % protein binding (P) is calculated by:

$$P = 100(k'/(k' + 1))$$

## Mobile Phases

Different types of mobile phases can be used. A mobile phase consisting of 5% 2-propanol in 20mM potassium phosphate buffer pH 7.0 gives data in good agreement with literature data. **Table 1** lists recommendations on pH and solvent content in the mobile phases for chiral columns. The mobile phase conditions should be chosen to suit the drugs to be tested, ie: for high protein binding drugs a mobile phase with higher eluting strength might be needed in order to reduce the retention times.

**Table 1:**

### Recommended Chromatography Mobile Phase Conditions

	Albumin columns	AGP columns
pH range:	5-7	4-7
2-propanol (rec. conc.)	0-10% (v/v)	0-30% (v/v)*
Acetonitrile (rec. conc.)	0-10% (v/v)	normally 0-10% 0-30% (v/v)*
		normally 0-10%

\*Higher concentrations give high back pressure.

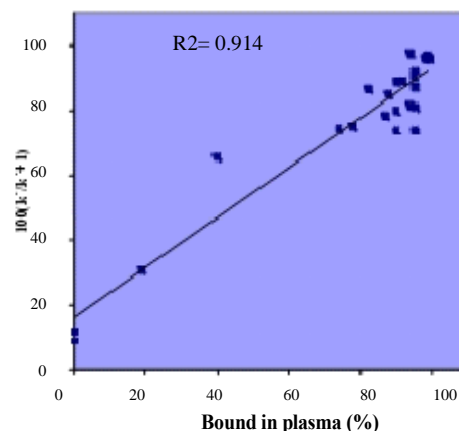
## Drug Protein Binding Columns Ordering Information

Cat.No.	Description	Price
CT-20054	Chiral-AGP, 4.0 x 50mm, 5 $\mu$ m	
CT-20053	Chiral-AGP, 3.0 x 50mm, 5 $\mu$ m	
CT-29054	Human serum albumin, 4.0 x 50mm, 5 $\mu$ m	
CT-29053	Human serum albumin, 3.0 x 50mm, 5 $\mu$ m	
RSA504	Rat serum albumin, 4.0 x 50mm, 5 $\mu$ m	
RSA503	Rat serum albumin, 3.0 x 50mm, 5 $\mu$ m	
MSA504	Mouse serum albumin, 4.0 x 50mm, 5 $\mu$ m	
MSA503	Mouse serum albumin, 3.0 x 50mm, 5 $\mu$ m	
DSA504	Dog serum albumin, 4.0 x 50mm, 5 $\mu$ m	
DSA503	Dog serum albumin, 3.0 x 50mm, 5 $\mu$ m	

## Correlation of Chromatographic Data

It is recommended to include a set of standard drugs to correlate the chromatographic data against published protein binding data. Literature values for plasma protein binding to use for correlation of binding to HSA can be obtained from Goodman A. and Gilman A.G., The Pharmacological Basis of Therapeutics, 9th Edition, McGrawHill, New York, p. 1712-1792 (1996).

Below is a plot with results obtained from chromatography of standard drugs on a Chiral-HSA column. In **Table 2** the results are correlated to data from Goodman & Gilman. All data is shown in the table.



**Table 2: Chromatographic Results vs. Literature Values for Plasma Protein Binding**

Drug Compound	% Bound in Plasma**	% Protein Binding
Isaniazid	0	11.8
Ethosuximid	0	9.2
Primidone	19	31
Folinic acid	40	65.9
Carbamazepine	74	74.8
Diltiazem	78	75.3
Desipramine	82	86.8
Propranolol	87	78.8
Budesonide	88	85.4
Indometacin	90	80.2
Verapamil	90	74.4
Imipramine	90.1	88.9
Nortriptyline	92	89.2
Sulindac	94	97.9
Fluoxetine	94	81.7
Amitriptyline	94.8	90.8
Propafenone	95	81.4
Carvedilol	95	92.6
Paroxetine	95	87.3
Omeprazole	95	74.2
Nitrendipine	98	96.4
Nicardipine	98.8	97.5
Ketoconazol	99	96.3

\*\* Values from Goodman A. and Gilman A.G., The Pharmacological Basis of Therapeutics, 9th Edition, McGraw-Hill, New York, p. 1712-1792 (1996). The percent protein binding values were calculated from the chromatographic data obtained on the Chiral-HSA, as described.

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