ChromTech Chiral HPLC Columns

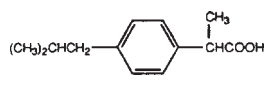
Chiral Column Selection Guide

Column	Applicability (type of samples)
Chiral-AGP	Extremely broad applicability. Most likely the column with the broadest applicability of all chiral columns. Separates all kinds of compounds: • amines (primary, secondary, tertiary and quartenary) • acids (strong and weak) • nonprotolytes (amides, esters, alcohols)
Chiral-CBH	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.).
Chiral-HSA	More narrow applicability than CHIRAL-AGP. Separates preferentially weak and strong acids and non-protolytic compounds.

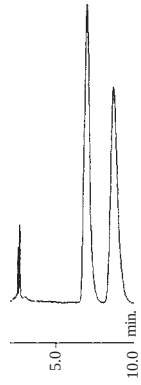
The columns overlap for some types of compounds; basic compounds can be separated on both CHIRAL-AGP and CHIRAL-CBH, acidic and neutral compounds can be separated on both CHIRAL-AGP and CHIRAL-HSA. However, as **CHIRAL-AGP** is a column with an extremely broad applicability, this column should be chosen 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

Ibuprofen



Column: Chiral-AGP 100 x 4.0mm
Eluent: 100mM sodium phosphate buffer pH 7.0
Flow: 0.9 mL/min.
Temperature: Ambient
Detection: UV at 225nm



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: α_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.

Chiral Column Price List

Chiral-AGP

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

Chiral-CBH

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

Chiral-HSA

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

Chiral-RSA

RSA504	Rat serum albumin, 4.0 x 50mm, 5µm
RSA503	Rat serum albumin, 3.0 x 50mm, 5µm

Chiral-MSA

MSA504	Mouse serum albumin, 4.0 x 50mm, 5µm
MSA503	Mouse serum albumin, 3.0 x 50mm, 5µm

Chiral-DSA

DSA504	Dog serum albumin, 4.0 x 50mm, 5µm
DSA503	Dog serum albumin, 3.0 x 50mm, 5µm

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 α_1-acid glycoprotein (AGP) as the chiral stationary phase. Through a patented process α_1-AGP has been immobilized on porous, spherical silica particles (5µm). 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 recommneded 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:

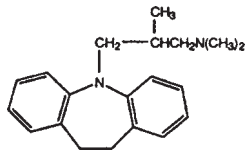
If the column has been contaminated with very hydrophobic material, wash the column over night with 25% 2-propanol in distilled water at a flow rate of 0.2mL/min.

See page 80 for ordering information.

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

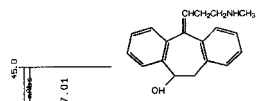
For additional applications, please ask for our Chiral Users Guide.

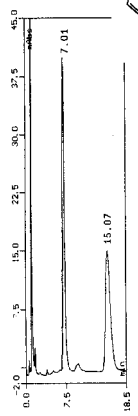
Trimipramine



Column: Chiral-AGP 100 x 4.0mm
Eluent: 1% 2-propanol in 10mM sodium acetate pH4.0 total acetatte conc.=60mM
Flow: 0.9mL/min.
Temperature: Ambient
Detection: UV at 225nm

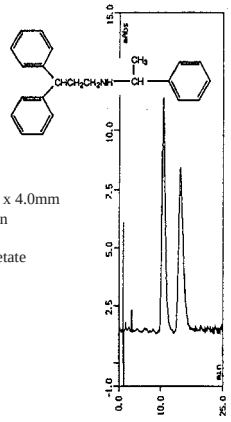
E-10-Hydroxy nortriptyline





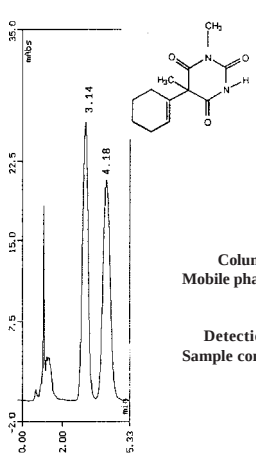
Column: Chiral-AGP 100 x 4.0mm and Chiral-AGP guard column 10 x 3.0mm
Mobile phase: 7.5% 2-propanol in 0.01M sod.ph.b. pH 6.0
Detection: UV 225nm
Sample conc.: 0.03 mg/mL

Fendiline



Column: Chiral-AGP 100 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 100 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.

See page 80 for ordering information.

Chiral-CBH

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

Cellobiohydrolase (CBH) is a stable enzyme which has been immobolized 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

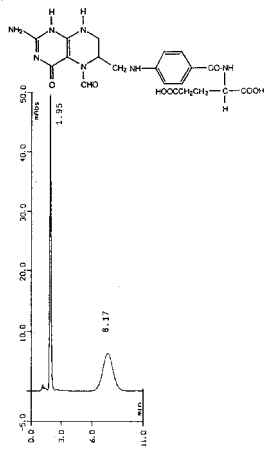
The column should be used at room temperature or below. When the column is stored for long periods of time it is recommended to fill with 10% 2-propanol in distilled water and place it in the refrigerator.

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.

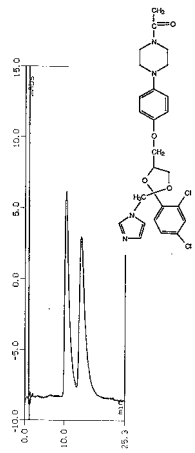
See page 80 for ordering information.

Folinic acid (Leucovorin)



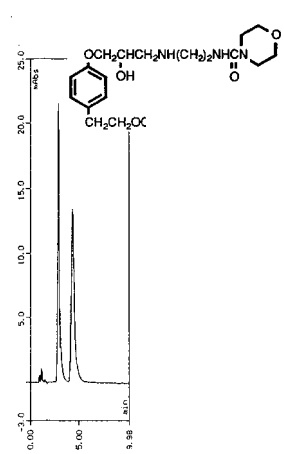
Column: Chiral-HSA, 100 x 4.0mm
Mobile phase: 6% 2-propanol in 100mM sod.ph.b. pH 7.0
Detection: UV 225nm
Sample concentration: 0.02 mg/mL

Ketoconazole



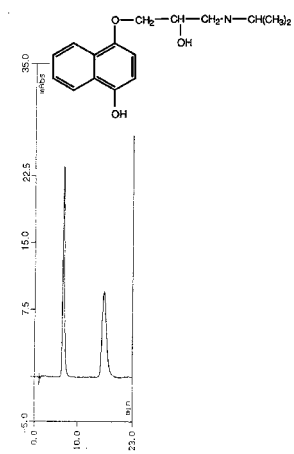
Column: Chiral-HSA, 100 x 4.0mm
Mobile phase: 10% 2-propanol in 50mM sod.ph.b. pH 7.0

H 174/48



Column: Chiral-CBH, 100 x 4.0mm
Mobile phase: 5% 2-propanol in 10mM sod.ph.b. pH 6.0 + 50µM disodium EDTA
Sample conc.: 0.03 mg/mL

4-Hydroxypropranolol



Column: Chiral-CBH, 100 x 4.0mm
Mobile phase: 5% 2-propanol in 10mM sod.ac. buffer, pH 5.0
Flow: 0.9mL/min.
Detection: UV 210nm

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: α_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:

$$k' = t_r - t_m/t_m$$ where t_r= retention time for the drug

where t_m= retention time for the t_m-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)* normally 0-10%
Acetonitrile *(rec. conc.)*	0-10% (v/v)	0-30% (v/v)* normally 0-10%

**Higher concentrations give high back pressure.*

Drug Protein Binding Columns Ordering Information

Cat.No.	Description
CT-20054	Chiral-AGP, 4.0 x 50mm, 5µm
CT-20053	Chiral-AGP, 3.0 x 50mm, 5µm
CT-20052	Chiral-AGP, 2.0 x 50mm, 5µm
CT-29054	Human serum albumin, 4.0 x 50mm, 5µm
CT-29053	Human serum albumin, 3.0 x 50mm, 5µm
CT-29052	Human serum albumin, 2.0 x 50mm, 5µm
RSA504	Rat serum albumin, 4.0 x 50mm, 5µm
RSA503	Rat serum albumin, 3.0 x 50mm, 5µm
MSA504	Mouse serum albumin, 4.0 x 50mm, 5µm
MSA503	Mouse serum albumin, 3.0 x 50mm, 5µm
DSA504	Dog serum albumin, 4.0 x 50mm, 5µm
DSA503	Dog serum albumin, 3.0 x 50mm, 5µm

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, McGraw-Hill, 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.

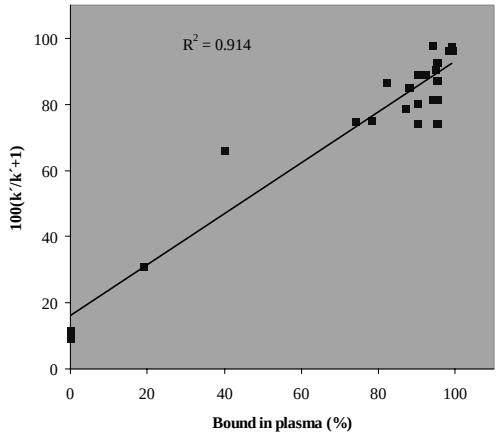


Table 2: Chromatographic Results vs. Literature Values for Plasma Protein Binding

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.