User’s Guide

Separation of chiral compounds on
Chiral-AGP • Chiral-CBH • Chiral-HSA

Second Edition
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The CHIRAL-AGP column

$\alpha_1$-acid glycoprotein (AGP) is a very stable protein, which tolerates pure organic solvents, high temperatures and high and low pH. AGP is the chiral selector in the CHIRAL-AGP column. The selector has been immobilized on spherical 5 µm particles. The column is used in the reversed-phase mode. The CHIRAL-AGP column can be used for the resolution of an extremely broad range of chiral compounds, such as amines (primary, secondary, tertiary and quaternary ammonium), acids, esters, sulphoxides, amides, alcohols etc. The very broad applicability is demonstrated in the application section below and in the list of publications in the last part of the guide. In the applications you can find chromatograms together with the chromatographic conditions.

The enantioselectivity and the retention can easily be regulated by the pH of the mobile phase, the buffer concentration and the nature and the concentration of the organic modifier.

Stability of the CHIRAL-AGP column

The stability of the AGP column has been tested using bumadizon, an acidic drug, as test compound. In total 30.5 liters of mobile phase (10% isopropanol in phosph. buffer pH 6.0) was pumped through the column. During the test 2030 samples of bumadizon were injected. One of the chromatograms below is the starting chromatogram and the other one is the last chromatogram obtained in the test. No significant changes were observed.

The CHIRAL-CBH column

Cellulbiohydrolase(CBH) is the chiral selector in the CHIRAL-CBH column. CBH is a very stable enzyme, which has been immobilized onto spherical 5 µm silica particles. The column is used in the reversed-phase mode. The column is preferably used for the separation of enantiomers of basic drugs from many compound classes. The retention and the enantioselectivity can be regulated by changes in pH, buffer concentration and the nature and the concentration of organic modifier.

The CHIRAL-HSA column

The chiral selector used for this stationary phase is the human serum albumin (HSA). The protein has been immobilized onto spherical 5 µm silica particles. The column is used in the reversed-phase mode. Enantiomers of preferentially acidic compounds can be resolved on the column. As for the other two columns retention and enantioselectivity can be regulated by changing the mobile phase composition, see above.

Quality control of the columns

The silica used for the manufacturing of the chiral columns is tested according to an extensive test protocol. When approved the silica surface is modified. All the chemicals used for the surface modification are either purchased against certificate or tested and approved by ChromTech. After surface modification a batch test is performed. If the test parameters are within the specifications, the batch is approved and released for production of columns. The next step is the control of the final product. Each column is tested to control separation efficiency, retention and resolution.

Column selection guide

<table>
<thead>
<tr>
<th>Column</th>
<th>Applicability (type of samples)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHIRAL-AGP</td>
<td>Extremely broad applicability. Most likely the column with the broadest applicability of all chiral columns available. Separates all types of compounds: - amines (primary, secondary, tertiary and quaternary nitrogen) - acids (strong and weak) - non-protoplytes (amides, esters, alcohols, sulphoxides, etc.)</td>
</tr>
<tr>
<td>CHIRAL-CBH</td>
<td>More narrow applicability than CHIRAL-AGP. Separates preferably compounds containing one or more nitrogens together with one or more hydrogen accepting or hydrogen donating groups (alcohol, phenol, carbonyl, amide, ether, ester etc.).</td>
</tr>
<tr>
<td>CHIRAL-HSA</td>
<td>More narrow applicability than CHIRAL-AGP. Separates preferably weak and strong acids, zwitterionic and non-protoplytic compounds.</td>
</tr>
</tbody>
</table>

As can be seen the columns overlap for some types of compounds; basic compounds can be separated on both CHIRAL-AGP and CHIRAL-CBH, acidic and non-protoplytes 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 the first choice, if the analyte has not been resolved on any of the columns. There are, however, some types of compounds where one of the other columns might be the first choice:

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

See p. 35 for a list of available column dimensions.
Method development

The columns described here are reversed-phase columns giving many possibilities to affect both the retention and the enantioselectivity. The solutes are retained by three types of forces: ionic binding (charged solutes), hydrophobic interaction and hydrogen bonding. The relative contribution of the different forces to the retention of the solutes, depend of the nature of the analyte. Analytes containing charged groups, hydrogen bonding groups and hydrophobic parts can be retained by interaction with corresponding groups on the chiral selector. From this follows that a separation can be affected by:
- pH
- buffer concentration
- type of buffer
- organic modifier concentration
- type of organic modifier

Method development schemes

All columns are delivered with a method development scheme that makes the method development very simple. In this scheme you will find the starting mobile phase to use for a certain type of compound. When you have the first result with the starting mobile phase you can simply follow the scheme which in most cases gives a baseline separation.

CHIRAL-AGP

The most important tool in method development is the pH. The reason is that by changing the pH the net charge of the chiral selector as well as the charge of the solute can be changed, which affects the way the analyte interacts with the chiral selector. AGP has a low isoelectric point of 2.7. This means that using the column at pH 2.7 gives a net charge of zero of the chiral stationary phase. Increasing the pH from 2.7 up to 7 means that the degree of net negative charge of the chiral selector increases. This gives the prerequisites for ionic binding of positively charged solutes, resulting in a high affinity and high retention of the solute. Reducing the pH towards the isoelectric point reduces the negative charge of the stationary phase, resulting in lower retention of the solute. A change of the net charge of the chiral selector strongly affects the interaction between the solute and the chiral stationary phase. It has been demonstrated that ionic binding of amines to the AGP column is a very important type of interaction for retention of this category of compounds. The solutes are also retained by hydrophobic interaction and hydrogen bonding. The relative influence of the different types of binding forces depends on the nature of the solute, i.e. what kind of structure elements are present in the analyte.

Below you will find examples of the effect of changing the composition of the mobile phase, i.e. the pH, the modifier concentration and the modifier nature etc.

Changing the pH

When chromatographing hydrophobic amines a pH of 4-5 is preferred compared to a pH of 7. The explanation to this finding is that chromatography of the amine at a pH of 7, where the protein has a strong degree of net negative charge and the analyte is positively charged, gives a strong ionic binding of the analyte. However, reducing the pH to the range 4-5 reduces the degree of net negative charge of the protein (the analyte is still fully ionized) which gives a reduction of the ionic bonding of the analyte and the retention is strongly reduced. For some compounds even a decrease to pH 6 might give large improvements compared to pH 7.

The pH effects are demonstrated below for propranolol, chromatographed at pH 4 and 7. Note the very strong reduction of the retention and the improvement of the chromatographic performance at pH 4. See also the numerous application examples of compounds chromatographed at pH 4-5.

The pH can also be an effective tool for affecting the resolution of acids which is demonstrated below for 2-phenoxypropionic acid. The compound has been chromatographed at three different pH, 5, 6 and 7. The analyte is totally ionized (negatively charged) at pH 7, but the charge is reduced at lower pH since the pKa-value is about 4. Furthermore, a decrease in pH reduces the degree of net negative charge of the protein, resulting in higher retention due to reduction of the repulsion between the analyte and the chiral stationary phase. The solute is retained by hydrophobic interaction and hydrogen bonding.
Changing the buffer concentration

By changing the buffer concentration, it is possible to affect both the retention and the enantioselectivity. Such effects have been observed for acids and for certain amines. The chromatograms below an example for the acidic drug naproxen.

![Chromatograms showing the effect of buffer concentration on retention and enantioselectivity.]

Changing the modifier concentration

2-propanol, acetonitrile, methanol, ethanol and 1-propanol is the most frequently used organic modifiers. Higher modifier concentration reduces the retention and the enantioselectivity for both amines and acids. However, for certain types of acids the enantioselectivity can be strongly improved by increasing the modifier concentration, as is demonstrated for warfarin below.

![Diagram showing the effect of modifier concentration on enantioselectivity.]

**Mobile phase:** 2-propanol in 0.01 M phosphate buffer, pH 7.0

<table>
<thead>
<tr>
<th>Conc. 2-propanol (%)</th>
<th>$k'$</th>
<th>$\alpha$</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>4.73</td>
<td>1.33</td>
</tr>
<tr>
<td>10</td>
<td>2.45</td>
<td>1.42</td>
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<tr>
<td>12</td>
<td>1.19</td>
<td>1.53</td>
</tr>
<tr>
<td>14</td>
<td>0.76</td>
<td>1.57</td>
</tr>
</tbody>
</table>

Changing the nature of the modifier

By changing from one organic modifier to another with different hydrogen bonding properties, i.e. from acetonitrile (hydrogen ac-
cepting properties) to 2-propanol (hydrogen accepting and donating properties), it is possible to strongly affect the enantioselectivity as demonstrated below for pindolol. Using 1-propanol results in no chiral selectivity, while acetonitrile gives a complete base-line resolution.

![Diagram showing the effect of modifier type on enantioselectivity.]

CHIRAL-CBH

The majority of the compounds chromatographed on the CHIRAL-CBH column are amines. See the applications. The CBH column is used in the reversed-phase mode.

The same type of mobile phases can be used on both the AGP and the CBH columns. The retention and the enantioselectivity is affected by the pH, the buffer concentration, the nature and the concentration of the organic modifier. The same types of forces are involved in the retention process of the solute as was described for the AGP column above.

Changing the pH

A decrease in pH will result in decreasing retention and in most cases lower enantioselectivity, as is demonstrated for epanolol below.

![Diagram showing the effect of pH on retention and enantioselectivity.]

**Column:** CHIRAL-CBH
100 x 4.0 mm

**Mobile phase:**
5 % 2-propanol in 10 mM sodium acetate buffer + 50 μM disodium EDTA
Changing the modifier concentration

The most widely used organic modifiers on the CBH column are 2-propanol and acetonitrile. Normally, increasing modifier concentration results in reduction of the retention and increasing enantioselectivity. These effects are illustrated below for atenolol and talinolol.

![Retention vs. Modifier Concentration](image1)

![Separation Factor vs. Modifier Concentration](image2)

Addition of an organic modifier has in almost all cases a positive influence on the chromatographic performance compared to chromatography in pure buffers. See below for laudanosine.

![Laudanosine](image3)

Mobile phases:
1. 10 mM sod. phosph. b., pH 6.0 + 50 μM disodium EDTA
2. 10 % 2-propanol in 10 mM sod. phosph. b., pH 6.0 + 50 μM disodium EDTA

Changing the pH

Depending on the nature of the analyte, a change in pH will have different effects. For an acid, a decreasing pH will result in higher retention and increasing resolution. If the analyte is an ampholyte as tryptophan, the result can be seen in the table:

<table>
<thead>
<tr>
<th>pH</th>
<th>k'1</th>
<th>k'2</th>
<th>α</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.0</td>
<td>1.44</td>
<td>1.82</td>
<td>1.26</td>
</tr>
<tr>
<td>6.0</td>
<td>1.30</td>
<td>1.87</td>
<td>1.44</td>
</tr>
<tr>
<td>7.0</td>
<td>0.75</td>
<td>3.72</td>
<td>4.97</td>
</tr>
</tbody>
</table>

Changing the modifier concentration

2-propanol, 1-propanol and acetonitrile are frequently used modifiers on the CHIRAL-HSA column. A higher organic modifier concentration reduces the retention. Normally, also the enantioselectivity will decrease. These effects are exemplified below for kynurenine.

![Capacity Factor vs. 2-Propanol Concentration](image4)

![Separation Factor vs. 2-Propanol Concentration](image5)

However, for certain acidic compounds it has been observed that the enantioselectivity is increasing when an organic modifier is added to the mobile phase as is demonstrated below for absic acid.

Abscisic acid, effect of 2-propanol

<table>
<thead>
<tr>
<th>% 2-propanol</th>
<th>k'1</th>
<th>k'2</th>
<th>α</th>
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<tr>
<td>0</td>
<td>3.62</td>
<td>4.56</td>
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<tr>
<td>1</td>
<td>1.96</td>
<td>3.37</td>
<td>1.92</td>
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</tbody>
</table>

CHIRAL-HSA

The majority of the compounds that have been resolved on the CHIRAL-HSA column are acids, ampholytes and non-protolytes. See the applications. The HSA column is used in the reversed-phase mode.

The same type of mobile phases can be used on both the AGP, the CBH and the HSA columns. The retention and the enantioselectivity is affected by the pH, the buffer concentration, the nature and the concentration of the organic modifier. The same types of forces are involved in the retention process of the solute as was described for the AGP column above.
<table>
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<tr>
<th>Substance</th>
<th>Column</th>
<th>Page</th>
<th>References</th>
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<td>Abscisic acid</td>
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<td>Acetobulol</td>
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<td>11</td>
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<td>β-alanine-N-[2-(3,4-dihydro-2H-1-benzo-pyran-3-yl)-ethyl] methyl ester hydrochloride</td>
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<td>76, 129, 149</td>
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<td>Alfuzosin</td>
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<td>8-Aza[4,5]decane-7,9-dione-8-(2-[[2,3-dihydro-1,4-benzodioxin-2-yl]-methyl]aminoethyl) monomethanesulfonate</td>
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<td>α,α’-bis[3-(N-benzyl-N-methylcarbamoyl)-piperidino]-p-xylene dihydrobromide</td>
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Abscisic acid

Column: CHIRAL-AGP 100 x 4.0 mm
Mobile phase: 75 mM sod.ph.b.
Detection: UV 225 nm
Sample conc.: 0.02 mg/ml

Acetbutolol

Column: CHIRAL-CB 100 x 4.0 mm
Mobile phase: 8% 2-propanol in 10 mM sod.ac.b.
Detection: UV 210 nm
Sample conc.: 0.02 mg/ml

β-alamin-N-[2-(3,4-dihydro-2H-1-benzo[4,5]cyclohepta[1,2-$d$]-1,3-oxazin-5-yl-methylamino)ethyl]methylester hydrochloride (Ref. 129)

Column: CHIRAL-AGP 150 x 4.0 mm
Mobile phase: phosph. buffer, pH 7.0
Detection: UV 225 nm
Sample conc.: 0.03 mg/ml

Alfuzosin (Ref. 30)

Column: CHIRAL-AGP 100 x 4.0 mm
Mobile phase: 6% acetonitrile in 0.025 M potassium phos. b. pH 7.4 containing 0.025 M TBA/Br
Flow: 0.9 ml/min
Detection: Fluorescence
Ex = 290 nm
Em = 400 nm

Alimemazine

Column: CHIRAL-AGP 100 x 4.0 mm
Mobile phase: 1% acetonitrile in 10 mM sod.ac.b.
Detection: UV 225 nm
Sample conc.: 0.025 mg/ml

Alprenolol

Column: CHIRAL-AGP 100 x 4.0 mm
Mobile phase: 3% acetonitrile in 10 mM sod.ac.b.
Detection: UV 225 nm
Sample conc.: 0.025 mg/ml

Aminoglutethimide

Column: CHIRAL-AGP 100 x 4.0 mm
Mobile phase: 9 mM amm.ac.b.
Detection: UV 225 nm
Sample conc.: 0.02 mg/ml

Amodipine (Ref. 155)

Column: CHIRAL-AGP 150 x 4.0 mm
Mobile phase: 1% 1-propanol in 10 mM acetate buffer, pH 4.5
Temp.: 30°C
Column switching system

Atenolol

Column: CHIRAL-CB 100 x 4.0 mm
Mobile phase: 8% 2-propanol in 10 mM sod.ph.b.
Detection: UV 265 nm
Sample conc.: 0.03 mg/ml

Atropine

Column: CHIRAL-AGP 100 x 4.0 mm
Mobile phase: 2% 2-propanol and 5 mM ocatonic acid in 0.01 M sod.ph.b.
Detection: UV 255 nm
Sample conc.: 0.08 mg/ml

8-Azaspiro[4,5]decan-7,9-dione-8-(2-[[2,3-dihydro-1,4-benzodioxin-2-yl]-methyl]amino)ethyl monomethanesulfonate (Ref. 127)

Column: CHIRAL-AGP 100 x 4.0 mm
Mobile phase: 27.5% methanol in 50 mM phos. buffer, pH 5.0
Flow: 1.0 ml/min
Detection: UV 210 nm

Bendroflumethiazide

Column: CHIRAL-AGP 100 x 4.0 mm
Mobile phase: 3% 1-propanol in 10 mM sod.ph.b.
Detection: UV 225 nm
Sample conc.: 0.02 mg/ml
Benflourex

Column: CHIRAL-AGP 100 x 4.0 mm
Mobile phase: 4% 2-propanol in 10 mM sodium acetate, pH 5.0 (total acetate conc. = 15 mM)
Detection: UV 225 nm
Sample conc.: 0.02 mg/ml

Benzoin

Column: CHIRAL-AGP 100 x 4.0 mm and CHIRAL-AGP guard column 10 x 3.0 mm
Mobile phase: 5% methanol in 10 mM sodium phosphate, pH 6.0
Detection: UV 225 nm
Sample conc.: 0.02 mg/ml

N-benzoyl-DL-alanine

Column: CHIRAL-AGP 100 x 4.0 mm
Mobile phase: 10 mM sodium phosphate, pH 5.5
Detection: UV 210 nm
Sample conc.: 0.1 mg/ml

N-benzoyl-DL-leucine

Column: CHIRAL-HSA 100 x 4.0 mm
Mobile phase: 10% 2-propanol in 100 mM sodium phosphate, pH 7.0
Detection: UV 225 nm
Sample conc.: 0.02 mg/ml

N-benzoyl-DL-valine

Column: CHIRAL-AGP 100 x 4.0 mm
Mobile phase: 3% 2-propanol in 10 mM sodium phosphate, pH 5.0
Detection: UV 200 nm
Sample conc.: 0.1 mg/ml

α,α'-bis[3-(N-benzyl-N-methylcarbamoyl)-piperidino]-p-xylene dihydrobromide (Ref. 82)

Column: CHIRAL-AGP 100 x 4.0 mm
Mobile phase: 0.5 M sodium acetate in 20 mM Na2HPO4, pH 7.0
Flow: 0.5 mL/min
Detection: Fluorescence, Ex = 282 nm, Em = 304 nm

Berabrost sodium (Ref. 91)

Column: CHIRAL-CBH 100 x 4.0 mm
Mobile phase: 5% 2-propanol in 10 mM sodium phosphate, pH 5.5 + 50 mM diethylamine EDTA
Sample conc.: 0.05 mg/ml

β-Taxonol

N-t-BOC-DL-valine

Column: CHIRAL-AGP 100 x 4.0 mm
Mobile phase: 3% acetonitrile in 10 mM sodium phosphate, pH 5.0
Detection: UV 200 nm
Sample conc.: 0.2 mg/ml

Bumadizon

Column: CHIRAL-AGP 100 x 4.0 mm and CHIRAL-AGP guard column 10 x 3.0 mm
Mobile phase: 10% acetonitrile in 10 mM sodium phosphate, pH 7.0
Detection: UV 225 nm
Sample conc.: 0.02 mg/ml

Bunolol (Ref. 119)

Column: CHIRAL-AGP 100 x 4.0 mm
Mobile phase: 2% 2-propanol in 10 mM phosphate buffer, pH 7.0, 1 mM DMOA
Flow: 0.9 mL/min
Detection: UV 223 nm

Bupivacaine

Column: CHIRAL-AGP 100 x 4.0 mm
Mobile phase: 8% tetrahydrofuran in 10 mM sodium phosphate, pH 7.0
Detection: UV 225 nm
Sample conc.: 0.02 mg/ml
<table>
<thead>
<tr>
<th>Dihydropyridines</th>
<th>Dihydropyridines</th>
<th>Diltiazem</th>
<th>Dimethindene</th>
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<tbody>
<tr>
<td>H 324/38, H 324/78, H 125/66 and H 152/80 (Ref. 148)</td>
<td>H 152/81, H 172/99 and H 324/38 (Ref. 148)</td>
<td>Column: CHIRAL-AGP 100 x 4.0 mm Mobile phase: 10% 2-propanol in 0.01 M sod.ph.b. pH 7.0 Detection: UV 225 nm Sample conc.: 0.02 mg/ml</td>
<td>Column: CHIRAL-AGP 100 x 4.0 mm Mobile phase: 10% 2-propanol in 0.01 M sod.ph.b. pH 7.0 Detection: UV 225 nm Sample conc.: 0.02 mg/ml</td>
</tr>
<tr>
<td>Column: CHIRAL-AGP 100 x 4.0 mm Mobile phase: 25 % methanol in 10 mM phosph. b., pH 4.51 Detection: UV 242 nm Flow: 1 ml/min</td>
<td>Column: CHIRAL-AGP 100 x 4.0 mm Mobile phase: 4% acetonitrile, 18% methanol in 10 mM ph.b., pH 5.5 Detection: UV 242 nm</td>
<td>Column: CHIRAL-AGP 100 x 4.0 mm Mobile phase: 1% acetonitrile in 10 mM sod.acb. pH 4.0 (total acetic conc. = 60mM) Detection: UV 225 nm Sample conc.: 0.03 mg/ml</td>
<td>Column: CHIRAL-AGP 100 x 4.0 mm Mobile phase: 8% 2-propanol in 0.01 M sod.ph.b. pH 6.0 Detection: UV 225 nm Sample conc.: 0.1 mg/ml</td>
</tr>
<tr>
<td>Diperodon</td>
<td>Disopyramide</td>
<td>Dixyrazine</td>
<td>N-2,4-DNP-DL-a-amino-n-butyric acid</td>
</tr>
<tr>
<td>Column: CHIRAL-AGP 100 x 4.0 mm Mobile phase: 0.5% 2-propanol in 30 mM amm.ac.b. pH 4.1 (total acetate conc. = 110 mM) Detection: UV 225 nm Sample conc.: 0.02 mg/ml</td>
<td>Column: CHIRAL-AGP 100 x 4.0 mm and CHIRAL-AGP guard column 10 x 3.0 mm Mobile phase: 10% 2-propanol in 0.01 M sod.ph.b. pH 7.0 Detection: UV 225 nm Sample conc.: 0.02 mg/ml</td>
<td>Column: CHIRAL-AGP 100 x 4.0 mm Mobile phase: 1% acetonitrile in 10 mM sod.acb. pH 4.0 (total acetic conc. = 60mM) Detection: UV 225 nm Sample conc.: 0.03 mg/ml</td>
<td>Column: CHIRAL-AGP 100 x 4.0 mm Mobile phase: 100 mM sod.ph.b. pH 6.0 Detection: UV 210 nm Sample conc.: 0.1 mg/ml</td>
</tr>
<tr>
<td>N-2,4-DNP-DL-a-amino-n-butyric acid</td>
<td>N-2,4-DNP-DL-citrulline</td>
<td>N-2,4-DNP-DL-ethionine</td>
<td>N-2,4-DNP-DL-glutamic acid</td>
</tr>
<tr>
<td>Column: CHIRAL-HSA 100 x 4.0 mm Mobile phase: 15% 2-propanol in 10 mM sod.ph.b. pH 7.0 Detection: UV 210 nm Sample conc.: 0.1 mg/ml</td>
<td>Column: CHIRAL-HSA 100 x 4.0 mm Mobile phase: 15% 2-propanol in 10 mM sod.ph.b. pH 7.0 Detection: UV 210 nm Sample conc.: 0.1 mg/ml</td>
<td>Column: CHIRAL-AGP 100 x 4.0 mm Mobile phase: 1% 2-propanol in 10 mM sod.ph.b. pH 7.0 Detection: UV 210 nm Sample conc.: 0.1 mg/ml</td>
<td>Column: CHIRAL-HSA 100 x 4.0 mm Mobile phase: 15% 2-propanol in 10 mM sod.ph.b. pH 7.0 Detection: UV 210 nm Sample conc.: 0.1 mg/ml</td>
</tr>
<tr>
<td>Compound</td>
<td>Ref.</td>
<td>Column</td>
<td>Mobile phase</td>
</tr>
<tr>
<td>----------------</td>
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<td>---------------------------------------------</td>
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<tr>
<td>Meprolol</td>
<td></td>
<td>CHIRAL-CBH 100 x 4.0 mm</td>
<td>5% 2-propanol in 10 mM sod.ph.b ph 6.0 + 50 μM di-sodium EDTA</td>
</tr>
<tr>
<td>Mosapride (Ref. 134)</td>
<td></td>
<td>CHIRAL-AGP 100 x 4.0 mm</td>
<td>2.5% 2-propanol in 10 mM sod. phos.ph. buffer, pH 7.0</td>
</tr>
<tr>
<td>1-(1-Naphthyl)-ethylamine</td>
<td></td>
<td>CHIRAL-AGP 100 x 4.0 mm</td>
<td>2.5% 2-propanol in 10 mM sod. phos.ph. buffer, pH 7.0</td>
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<tr>
<td>Naproxen</td>
<td></td>
<td>CHIRAL-AGP 100 x 4.0 mm</td>
<td>25 mM sod.ph.b ph 7.0</td>
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<tr>
<td>Nefopam</td>
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<td>CHIRAL-AGP 100 x 4.0 mm</td>
<td>1% 2-propanol in 10 mM sod.ac.b. ph 4.5 (total acetate conc. = 25 mM)</td>
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<tr>
<td>Nicotine (Ref. 93)</td>
<td></td>
<td>CHIRAL-AGP 100 x 4.0 mm</td>
<td>For conditions see reference no. 93</td>
</tr>
<tr>
<td>Nitrendipine</td>
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<td>CHIRAL-AGP 100 x 4.0 mm</td>
<td>10% 2-propanol in 10 mM sod.ph.b ph 7.0</td>
</tr>
<tr>
<td>Norepinephrine</td>
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<td>CHIRAL-CBH 100 x 4.0 mm</td>
<td>5% 2-propanol in 10 mM sod.ph.b ph 6.0 + 50 μM di-sodium EDTA</td>
</tr>
<tr>
<td>Normethanephine</td>
<td></td>
<td>CHIRAL-CBH 100 x 4.0 mm</td>
<td>5% 2-propanol in 10 mM sod.ph.b ph 6.0 + 50 μM di-sodium EDTA</td>
</tr>
<tr>
<td>Octopamine</td>
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<td>CHIRAL-CBH 100 x 4.0 mm</td>
<td>5% 2-propanol in 10 mM sod.ph.b ph 6.0 + 50 μM di-sodium EDTA</td>
</tr>
<tr>
<td>Omeprazole (Ref. 144)</td>
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<td>CHIRAL-AGP 100 x 4.0 mm</td>
<td>Mobile phase: 10% acetonitrile in 10 mM sod.ph.b ph 6.8</td>
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<tr>
<td>Omeprazole</td>
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<td>CHIRAL-AGP 100 x 4.0 mm</td>
<td>Mobile phase: 10% acetonitrile in 10 mM sod.ph.b ph 6.8</td>
</tr>
<tr>
<td>Chemical Name</td>
<td>Description</td>
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<td>Oxamnique (Ref. 34)</td>
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<td>Oxfendazole (Ref. 47)</td>
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<td>Oxodipine (Ref. 118)</td>
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<td>Oxyphenonium (Ref. 48)</td>
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<td>Pamatolol</td>
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<td>Pargyline N-oxide</td>
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<tr>
<td>Penthiobarbital</td>
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<tr>
<td>Pentobarbitone (Ref. 128)</td>
<td><img src="image12" alt="Pentobarbitone" /></td>
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</tbody>
</table>
Warfarin

Column: CHIRAL-AGP 100 x 4.0 mm
Mobile phase: 10% 2-propanol in 10 mM sod ph.b. pH 7.0
Detection: UV 225 nm
Sample conc.: 0.02 mg/ml

Reference 19

Column: CHIRAL-AGP 100 x 4.0 mm
Mobile phase: 10% 2-propanol in 8 mM sod ph.b. pH 7.0
Flow: 0.9 ml/min
Detection: UV 220 nm

Reference 83

Column: CHIRAL-AGP 100 x 4.0 mm
Mobile phase: 1% 2-propanol in sod ph.b. pH 7.0
Flow: 0.9 ml/min
Detection: UV 220 nm

Reference 97

Column: CHIRAL-AGP 100 x 4.0 mm
Mobile phase: 10 mM sod ph.b. pH 4.0 with acetonitrile (100/15)
Flow: 1.0 ml/min
Detection: UV 220 nm

H 310/83 and H 309/40 (Ref. 147)

Column: CHIRAL-AGP 100 x 4.0 mm
Mobile phase: 10% acetonitrile in phosphate buffer, ionic strength I=0.01, pH 7.5
Temp.: 40 °C
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chromatography  

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enantiomers in human plasma in the nanogram per milliliter  
range  

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# Chiral Column Ordering Guide

## Chiral-AGP

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## Chiral-CBH

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## Accessories

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