

# INSTRUCTION MANUAL

## CHIRAL-AGP

### INSTALLATION

The column is filled with 15% 2-propanol in dist. water. Wash the column with dist. water. Start at low flow-rate and increase to 0.5 ml/min and maintain this flow during 2 min. Increase the flow-rate to 0.9 ml/min and continue the washing for 10 min. Equilibrate the column with the mobile phase to be used.

Recommended flow rate is 0.9 ml/min with a 4.0 mm I.D. column.

We recommend the use of a CHIRAL-AGP guard column in order to protect the analytical column from impurities with high affinity and particulate impurities. Exchange the guard column regularly, otherwise the column performance will be affected. This is of special importance in bioanalysis.

### STORAGE

The column can be stored at room temperature. When in use, the column can be left over night in the chromatographic system. However, be careful when buffers without organic modifiers, or buffers containing low concentrations of acetonitrile, are used, since in such mobile phases bacteria grows fast. Such mobile phases must be freshly prepared. 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 of time it is recommended to fill it with 15% 2-propanol in distilled water and place it in the refrigerator. Before use repeat the installation procedure.

### TEMPERATURE

The column should be used at room temperature or below.

### MOBILE PHASE COMPOSITION

**BUFFER** Different kinds of buffers can be used to obtain the best separation. For example: Sodium- or potassium phosphate buffer, ammonium- or sodium acetate buffer and citrate buffer. We recommend the concentration range 0.01 - 0.1 M. Normally 0.01-0.02 M.

**pH** The column can be used in the pH-range 4 - 7. Use of the column at pH > 7.0 or < 4 for longer periods, may decrease the column lifetime, due to silica decomposition.

**ORGANIC MODIFIER** We recommend the use of the following organic modifiers: 1-propanol, 2-propanol, methanol, ethanol and acetonitrile. 1- and 2-propanol and acetonitrile are the most frequently used modifiers.

The separation factor can be strongly affected by the type and the concentration of the uncharged modifier. For example, 1-propanol, 2-propanol and acetonitrile can give large differences in enantioselectivity. See the method development part

Cationic and anionic modifiers have also been used to regulate retention and enantioselectivity. However, some of these modifiers may be difficult to remove totally, due to very high affinity to the matrix. Thus, the properties of the column may be affected. We recommend the use of separate columns for uncharged, anionic and cationic modifiers.  
**Clean the column as described on the next page.**

**Guidelines for choosing separation conditions are described on the next pages.**

## SAMPLES

The recommended sample concentration is less than 0.10 mg/ml with an injection volume of 10-20 µl. If possible, dissolve the sample in the mobile phase. If the sample is insoluble in the mobile phase, add a higher concentration of the organic modifier. However, be aware of that a too high organic modifier concentration might precipitate the buffer salts. Avoid to dissolve the sample in pure solvents. 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.

## CLEANING OF THE COLUMN

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

## METHOD DEVELOPMENT

Attached you will find a method development scheme which means that it is very simple to develop a new method. When starting method development you only have to characterize your sample according to the groups in the scheme. If, for example, the sample is a hydrophobic amine, use the scheme for hydrophobic amines and start the method development work with the mobile phase recommended there. Depending on the results you obtain, follow the instructions in the scheme. You can also visit our chiral website, [www.chromtech.se](http://www.chromtech.se), where you can find hundreds of applications including all conditions, chromatograms and structures of the separated compounds. You can also find almost two hundred literature references. The information on the website can be used to get ideas on how to resolve compounds with widely different structures.

### CHIRAL-AGP products:

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



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Visit our chiral website to get ideas on how to resolve an extremely wide variety of chiral compounds

[www.chromtech.se](http://www.chromtech.se)

# Method development CHIRAL-AGP

Follow this scheme and method development will be much easier.

**Start with characterization of your sample. Decide which group (1a, 1b, 2a, 2b or 3) your compound belongs to:**

## 1. Amine

- a. hydrophobic
- b. hydrophilic

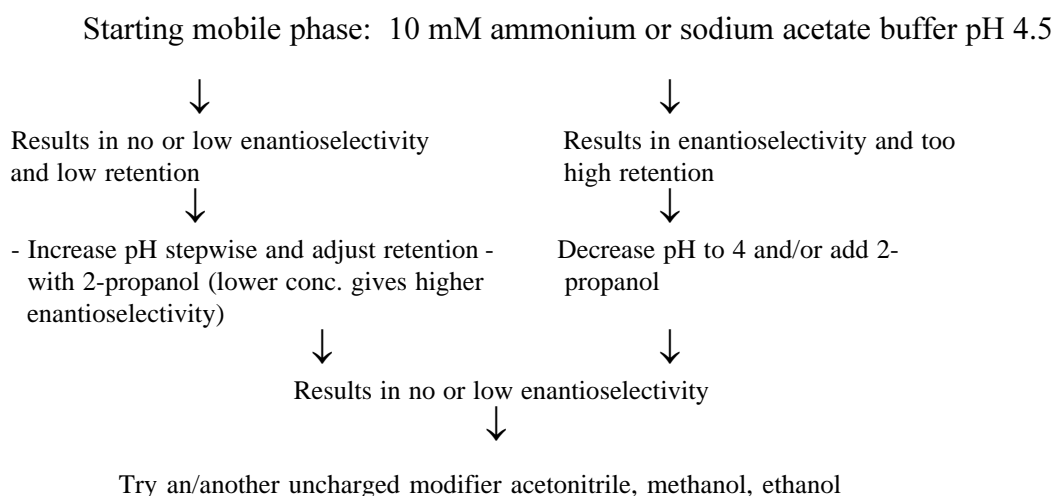
## 2. Acid

- a. weak
- b. strong

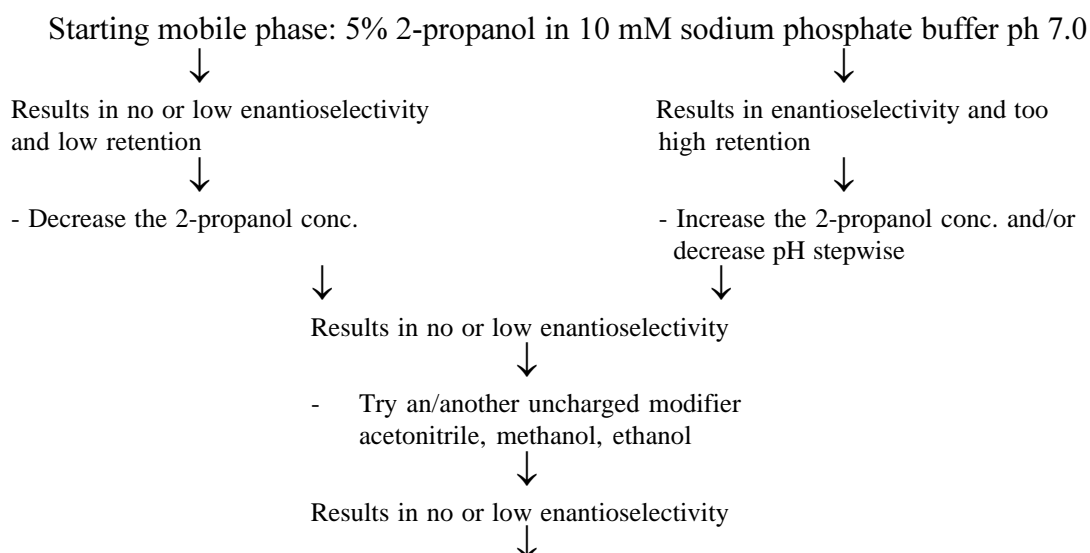
## 3. Non-protolyte

**When you have decided to which group your sample belongs, go to the appropriate scheme below:**

### 1a. Hydrophobic amine



### 1b, 2a and 3. Hydrophilic amine, weak acid and nonprotolyte



- For amines: try low conc. of a charged modifier  
octanoic acid 1-20 mM  
hexanoic or heptanoic acid 1-20 mM  
tetraethyl- and tetrapropylammonium bromide 1-5 mM

**Please note!**

If a column has been used with a mobile phase containing a charged modifier, it should not be used afterwards with mobile phases consisting of a pure buffer or a buffer containing an uncharged modifier. Different types of charged modifiers should not be used on the same column.

## 2b. Strong acid (for example carboxylic acids)

**Starting mobile phase: 10 mM sodium phosphate buffer pH 7.0**

↓  
Results in no or low enantioselectivity and low retention

↓  
- Decrease pH and/or increase buffer conc. up to approx. 0.1 M

↓  
Results in enantioselectivity and too high retention

↓  
- Add 2-propanol

↓  
Results in no or low enantioselectivity

↓  
Try an/another uncharged modifier  
2-propanol, acetonitrile, methanol, ethanol

↓  
Results in no or low enantioselectivity

↓  
Try low conc. of DMOA 1-5 mM  
(N,N-dimethyloctylamine)

**Please note!**

If a column has been used with a mobile phase containing a charged modifier, it should not be used afterwards with mobile phases consisting of a pure buffer or a buffer containing an uncharged modifier. Different types of charged modifiers should not be used on the same column.

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