

## **CHIRAL-AGP - a robust chiral column**

### ***The chiral selector - AGP***

The chiral selector  $\alpha_1$ -acid glycoprotein (AGP) is immobilized onto silica particles.

AGP is an extremely stable protein, even in the native state; it tolerates:

- high temperatures
- high and low pH
- high concentrations of organic solvents used in HPLC.

On the **CHIRAL-AGP** column, in the immobilized state, AGP is even more stable.

### ***The silica particles***

The silica particles used for the **CHIRAL-AGP** columns have the same properties as silica particles used for other types of reversed phase columns (C18, C8 etc.):

- mechanically strong
- limited stability at pH >7 and at pH <4

### ***CHIRAL-AGP column stability in buffer-organic solvent mixtures***

Normally the following organic solvents are used:

#### ***Alcohols:***

- propanols (pure 2-propanol has been pumped through the column)
- methanol
- ethanol

#### ***Nitriles:***

- acetonitrile (up to 50% has been used)

#### ***Other organic solvents tested (limited experience):***

- butanols
- tetrahydrofuran
- DMSO (dimethylsulfoxide)

As a conclusion, a lot of different organic solvents, at high concentrations, can be used without deterioration of the column. However, most compounds give too low retention at high concentrations. Normally, concentrations below 15% are used.

### ***Pressure stability***

The **CHIRAL-AGP** column is packed at high pressure. At normal operating flow rate (0.8 - 0.9 ml/min) and 2-propanol concentrations at 5% or below, the back pressure on a column 100x4.0 mm is below 80 bar. The standard analytical **CHIRAL-AGP** column has an I.D. of 4 mm. The flow rate of 0.8-0.9 ml/min is used in order to obtain the same linear flow rate as on a column with I.D. 4.6 mm at flow rate of 1.0 ml/min.

### ***Temperature***

The normal temperature operating range is room temperature (20-25°C). The protein, AGP, is stable at higher temperatures. However, the silica is the most sensitive part in this type of columns, as in many other types of reversed phase columns. Increasing temperature speeds up the dissolution of the silica backbone, especially in combination with a pH around 7. The **CHIRAL-AGP** column has been used at temperatures up to 80°C without noticeable deterioration. Although the temperature can be used as a tool to regulate retention and enantioselectivity, such high temperatures are of no practical use, as the retention will be too low.

### ***Stability studies***

In order to show the good stability of the **CHIRAL-AGP** column, several stability studies have been performed, all with excellent results. In one of those studies, over 30 liters of mobile phase was pumped through the column. During the same time more than 2000 samples were injected. When comparing the first and the last chromatograms, no difference could be seen in retention time, efficiency or selectivity.

More question? Try [support@chromtech.co.uk](mailto:support@chromtech.co.uk) for help.

[www.chromtech.co.uk](http://www.chromtech.co.uk)