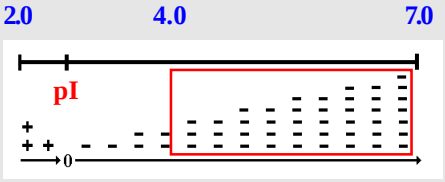
The Magic Tool for Method Development

CHIRAL-AGP

The **CHIRAL-AGP** column has the broadest applicability of all chiral columns. This broad applicability is a unique property. It is possible to separate enantiomers from many different compound classes; amines (primary, seconday, tertiary as well as quarternary ammonium compounds), acids (strong and weak) and nonprotolytes (esters, sulphoxides, amides, alcohols etc.). The unique properties has made the column one of the most widely used chiral columns worldwide.
One reason for this broad applicability is the possibility to increase or induce enantioselectivity by changing the composition of the mobile phase. Especially important are changes in the **pH**.

The stationary phase on the **CHIRAL-AGP** column is an extremely stable protein, α -acid glycoprotein (AGP). The protein contains many different [1]protolytic groups that are affected by changes in the pH of the mobile phase. AGP is a very acidic protein with a pI-value of 2.7, i.e. at this pH the net charge of AGP is zero. At a pH above 2.7 the net charge of AGP is negative. The charge of the protein at different pH can be seen in the picture below. The column is used between pH 4 - 7, indicated by the red frame.

Increasing net negative charge of AGP at higher pH



= *pH range used in chromatography*

Effect of pH on acidic analytes

When an acidic analyte is chromatographed at pH 7, both the acid and AGP has a net negative charge. As a result there is a repulsion between the acid and the stationary phase, leading to low or no retention. As pH is decreased, the negative charge is decreased on both the acid and AGP, giving higher retention and possibility for chiral recognition. The acids are bound by hydrogen bonding and hydrophobic interaction.

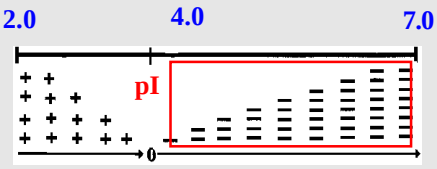
Effect of pH on hydrophobic basic analytes

When a hydrophobic amine is chromatographed at pH 7, the retention is high. The reason is that AGP has a strong negative charge and the amine is positively charged, leading to ionic bonding. If pH is decreased to 4 or 5, retention is much shorter, due to the decrease in negative charge of AGP. Also the enantioselectivity may be increased when decreasing pH from 7 to 4 or 5. The main reason is that at lower pH the organic modifier (2-propanol, acetonitrile etc.) content of the mobile phase can be reduced. This reduction is possible as the retention is lower due to less competition for hydrogen bonding with the analyte. The lower content of organic modifier results in a higher α-value, which leads to higher resolution. The basic analytes are bound to the stationary phase by ionic bonding, hydrogen bonding and hydrophobic interaction.

CHIRAL-CBH

The stationary phase on the CHIRAL-CBH column is another very stable protein, cellobiohydrolase (CBH). Also this protein contains many different protolytic groups affected by pH changes. The pI-value of CBH is 3.9. When the mobile phase has a pH above 3.9, the net charge of CBH is negative. The charge of the CBH protein at different pH can be seen in the picture below.

Increasing net negative charge of CBH at higher pH



= *pH range used in chromatography*

Effect of pH on basic analytes

The CHIRAL-CBH column is used between pH 4 and 7, indicated by the red frame in the picture above. At pH 7 a basic analyte has a higher retention than at lower pH, due to the strong negative charge on CBH. If retention is too high, pH should be decreased towards 4.

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