

## Very simple method development

**CHIRAL-AGP** is a reversed phase column which means that method development is simple and straightforward, with many possibilities to affect both the retention and the enantioselectivity. The solutes are retained by three types of forces:

- ionic bonding (charged solutes)
- hydrophobic interaction
- 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
- organic modifier concentration and nature
- buffer concentration and type

These are exactly the tools that are used in ordinary reversed phase chromatography, the most used chromatographic technique.

In order to use the **CHIRAL-AGP** column and develop methods on it, there is no need to learn and adopt to special techniques. Use the knowledge from reversed phase chromatography and it will be very easy to develop methods. The scheme at the top of the next column can be used as a starting point. Look at the structure of the compound of interest, characterize it and choose the corresponding starting mobile phase. Start the work and then use the Method Development Scheme, that is shipped with each column, to optimize the method.

Compound type	Starting mobile phase
Hydrophobic amine	10 mM ammonium or sodium acetate buffer pH 4.5
Hydrophilic amine	5% 2-propanol in 10 mM sodium phosphate buffer pH 7.0
Weak acid (phenol etc) or nonprotolyte (amide, ester, alcohol etc)	5% 2-propanol in 10 mM sodium phosphate buffer pH 7.0
Strong acid (carboxylic acid)	10 mM sodium phosphate buffer pH 7.0

Below is an **illustration**: The compound is a strong acid, **2-phenoxypropionic acid**. The suggested starting mobile phase is 10 mM sodium phosphate buffer pH 7.0, which gives the chromatogram to the left. The advise in the Method Development Scheme is to decrease the pH, which is shown on the middle and the right chromatogram. At pH 5.0 a successful separation is obtained.

