Method Development Schemes CHIRAL-AGP™ (conventional detection UV etc)

Characterize your sample and find the starting mobile phase

Compound type	Starting mobile phase
Hydrophobic amine	10 mM ammonium or sodium acetate buffer pH 4.5
Hydrophilic amine Weak acid (phenol etc) 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
If the compound contains many functional groups, the protolytic function decides which of the following schemes to use	

Hydrophobic amine

Depending on the result obtained with the starting mobile phase, continue as indicated below:

Retention and enantioselectivity	No or low enantioselectivity and low retention	Enantioselectivity and too high retention.	Too high retention. No enantioselectivity.
Optimize with pH and/or uncharged modifiers	Increase pH stepwise and adjust retention with 2-propanol (lower conc. gives higher enantioselectivity ↓ Test another uncharged modifier: acetonitrile, methanol, 1-propanol, ethanol ↓ Test low conc. of a charged modifier*: - octanoic acid 1-20 mM - hexanoic or heptanoic acid 1-20 mM - tetraethyl- and tetra propyl-ammonium bromide 1-5 mM	Decrease pH to 4 and/or add 2-propanol ↓ Test another uncharged modifier, acetonitrile, methanol, 1-propanol, ethanol	Test different uncharged modifiers: 2-propanol, acetonitrile, methanol, 1-propanol, ethanol ↓ Test low conc. of a charged modifier*: - octanoic acid 1-20 mM - hexanoic or heptanoic acid 1-20 mM - tetraethyl- and tetra-propylammonium bromide 1-5 mM

Hydrophilic amines, weak acids and non-protolytes

Depending on the result obtained with the starting mobile phase, continue as indicated below:

Retention and enantioselectivity	No or low enantioselectivity and low retention	Enantioselectivity and too high retention.	Too high retention. No enantioselectivity.
Optimize with pH and/or uncharged modifiers and/or buffer conc.	Decrease the 2-propanol conc ↓ Test another uncharged modifier: acetonitrile, methanol, 1-propanol, ethanol ↓ Amines: Test low conc. of a chargedmodifier*: - octanoic acid 1-20 mM - hexanoic or heptanoic acid 1-20 mM - tetraethyl- and tetra-propylammonium bro mide 1-5 mM	Decrease pH stepwise and/or increase the 2-propanol conc. ↓ Test another uncharged modifier: acetonitrile, methanol, 1-propanol, ethanol	Go to the scheme for hydrophobic amines

Strong acid (for example carboxylic acids)

Depending on the result obtained with the starting mobile phase, continue as indicated below:

Retention and enantioselectivity	No or low enantioselectivity and low retention	Enantioselectivity and too high retention.	Too high retention. No enantioselectivity.
Optimize with pH and/or uncharged modifiers and/or buffer conc	Decrease pH and/or increase buffer conc. up to approx. 50-75 mM (max. 100 mM) ↓ Try low conc. (1-5 mM) of the charged modifier* DMOA (N,N-dimethyl-octylamine)	Add 2-propanol ↓ Test different uncharged modifiers: acetonitrile, methanol, 1-propanol, ethanol	Test different uncharged modifiers: 2-propanol, acetonitrile, methanol, 1-propanol, ethanol ↓ Try low conc. (1-5 mM) of the charged modifier* DMOA (N,N-dimethyl-octylamine)

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***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.