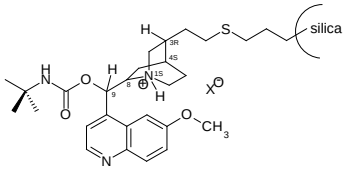
INSTRUCTION MANUAL FOR CHIRALPAK® QN-AX and CHIRALPAK QD-AX

Please read this instruction sheet completely before using these columns

Column Description

Packing composition: O-9-(*tert*-butylcarbamoyl) quinine for CHIRALPAK® QN-AX
or
O-9-(*tert*-butylcarbamoyl) quinidine for CHIRALPAK® QD-AX
immobilized on **5µm silica-gel**.



CHIRALPAK® QN-AX: (8S,9R)
CHIRALPAK® QD-AX: (8R,9S)

Shipping solvent: **100% Methanol**

All columns have been pre-tested before packaging. Test parameters and results, as well as the Column Lot Number, are included on a separate (enclosed) page.

Operating Conditions

	150 x 2.1 mm i.d. Analytical columns	**150 x 4.6 mm i.d.** Analytical columns	**150 x 21 mm i.d.** Semi-prep. columns
Flow rate direction	As indicated on the column label		
Typical Flow rate ①	~ 0.1 - 0.2 ml/min Do not exceed 0.3 ml/min	~ 1 ml/min Do not exceed 2 ml/min	~ 18 ml/min Do not exceed 25 ml/min
Pressure limitation ②	Should be maintained < 150 Bar (~2100 psi)③ for maximum column life Adapt flow rates to the size of the column. Do not exceed 180 Bar (~2500 psi)		
Temperature	0 to 40°C		

① The maximum flow rate depends on the mobile phase viscosity (mobile phase composition), and should be adjusted in accordance with the pressure upper's limit (i.e. 180 Bar).

Examples	**Column 150 x 4.6 mm i.d.**	**Column 150 x 21 mm i.d.**
100% Methanol	0.5 to 2 ml/min	9 to 25 ml/min
Methanol/aqueous buffer~ 80:20	0.5 to 1.5 ml/min	9 to 18 ml/min
Alkane/organic modifier~ 90:10	0.5 to 2 ml/min	9 to 25 ml/min

② The back pressure value that should be taken into account is the one generated by the column itself. This value is measured by calculating the difference between the pressure of [LC system + column] and the pressure of the LC system free of the column.

③ Ideal value for maximum column life, but stable up to 180 Bar.

- ❑ The use of a guard cartridge is highly recommended for maximum column life.
- ❑ Samples should be filtered through a membrane filter of approximately 0.5 µm porosity.
- ❑ Mobile phases (in particular RP-mode) should be filtered through an appropriate filtration membrane.

Operating Procedure

CHIRALPAK® QN-AX and CHIRALPAK® QD-AX have been developed as enantioselective weak anion-exchange (AX) HPLC columns most useful for the separation of acidic chiral compounds, based on two complementary stereoisomeric quinine (QN) and quinidine (QD) derivatives. Owing to their pseudo-enantiomeric character they usually reveal reversed elution order for opposite enantiomers.

They can be used in polar organic mode (non-aqueous, polar organic solvents containing organic acids and bases as buffer constituents) or in reversed-phase (RP) mode. CHIRALPAK® QN-AX and CHIRALPAK® QD-AX are designed specifically for enantioselective HPLC of chiral acids and possess exceptional enantiomer separation capabilities for acidic chiral compounds containing carboxylic, phosphonic, phosphinic, phosphoric or sulfonic acid groups. In some cases, weakly acidic compounds such as phenols (e.g. coumarols) can also be separated.

They are compatible with all common HPLC solvents (e.g. methanol, acetonitrile, tetrahydrofuran, dioxane or chloroform) as well as in a wide pH range spanning from pH 2 to 8. Typical buffers used in hydro-organic mode are acetate, formate, citrate and phosphate. They are capable for use in LC-MS detection of chiral acids with compatible mobile phases and buffers (e.g. ammonium acetate, ammonium formate).

In addition, the separation of chiral basic and neutral compounds may also be possible, but usually under normal phase (NP) conditions or if preferred in RP-mode with higher aqueous content. In NP-mode, CHIRALPAK® QN-AX and CHIRALPAK® QD-AX behave like a standard Pirkle type chiral stationary phase.

Column Care / Maintenance

Before initial use, the column should be flushed with at least 20 column volumes (ca. 30 ml for a 150 x 4.6 mm i.d. column) of 1% (v/v) acetic acid in methanol. The column should then be equilibrated in 20 column volumes of the initial mobile phase.

☞ ***Column cleaning and regeneration procedures***

- ❑ After use of counter-ions with high affinity towards the selector such as *citrate and phosphate* or when *multiply charged solute species* from the sample are trapped on the column and do not come off due to the use of a weak counter-ion in the eluent, it is recommended to rinse the column for 30 min with a solution of **methanol/triethylamine (TEA) (100:2, v/v)** before switching back to an eluent with a counter-ion having low affinity to the selector such as acetate. After the methanol/TEA rinsing, the column should be washed with methanol/water (50:50, v/v) or acetonitrile/water (50:50, v/v) before it is equilibrated with the new mobile phase or stored.

- ❑ If *highly hydrophobic compounds* are trapped on the column by virtue of strong hydrophobic interactions, they can be eluted with **acetonitrile/acetic acid (100:1, v/v)** or with **THF**, **dioxane** or **dimethylsulfoxide**. The column should then be washed with methanol/water (50:50, v/v) or acetonitrile/water (50:50, v/v) before it is equilibrated with the new mobile phase or stored.

Column Storage

- ❑ If the column is not used it should be washed for 5-10 min with a mixture of methanol/water (50:50, v/v) or pure methanol (or acetonitrile) for overnight storage (this is in particular recommended if phosphate or citrate is used as buffer).

☞ If high content of salts has been used, rinse the column first with an organic/aqueous mixture, before switching to pure organic solvents in order to avoid salt precipitation.

- ❑ For long-term storage, the column should be flushed with 20 column volumes of pure methanol or acetonitrile, but it can also be stored in a methanol or acetonitrile mixture in water (50:50, v/v). It can be stored at room temperature.

Practical Method Development Scheme

The solute to be separated into individual enantiomers is:

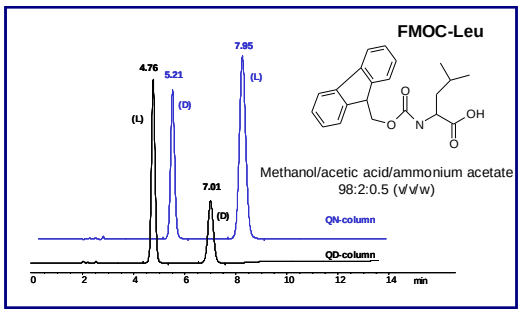
- a chiral **acidic** compound: Use polar organic mode or RP-mode (Charts 1 and 2).
- a chiral **sulfonic**, **phosphonic**, **phosphoric acid** and **di- or multicarboxylic acid**: Use RP-mode (retention will be too strong in the polar organic mode) (Chart 2).
- an **amphoteric** chiral compound: Use RP-mode, but study extended pH-range up to pH 8 and possibly lower flow rates e.g. 0.5 ml/min for analytical columns (Chart 2).
- a **neutral** or **basic** chiral compound: Use NP-mode or, if preferred, try RP-mode with higher water content.

Acidic Compounds in Polar Organic (PO) Mode *(Chart 1)*

1) Test a mobile phase of methanol/acetic acid/ammonium acetate (98:2:0.5, v/v/w). Start the screening at 1 ml/min (at 0.1 ml/min for microbore columns) and 25 °C.

2) A mixture of acetonitrile/methanol 50:50 (v/v) containing the same amount of organic acid and ammonium acetate can be tested. If with this mixture higher separation factors are obtained continue with it and optimize the percentage of methanol in acetonitrile. For *N-derivatized amino acids* methanol proved in general to be the better choice, while for *arylcarboxylic acids* acetonitrile turned out to generate higher enantioselectivity values. For *hydroxyl carboxylic acids* a mixture of acetonitrile/methanol may be the preferred solvent.

⇨ As soon as a separation is detected, the **elution order** can be checked. If necessary, switch from CHIRALPAK® QN-AX to QD-AX and vice versa to pursuit the method development, as they show inversion of elution order in most cases.



3) If a **baseline separation** is achieved but **retention times are too long**, select the mobile phase combination that gave suitable selectivity for further optimization and adjust retention factors by:

   a. *increasing the concentration of the buffer in the eluent*: a change of the concentration of the competing acid (counter-ion) in the mobile phase at identical acid-basic ratio usually has a negligible or only minor effect on the enantioselectivity. It will, however, affect retention considerably by modulation of the actual ion-exchange capacity. Increasing counter-ion concentration will decrease retention. For example, a mixture of methanol/acetic acid/ammonium acetate (98:4:1, v/v/w) or simply methanol/acetic acid/triethylamine (TEA) 100:3:1 (v/v/v) (or methanol/acetic acid/concentrated aqueous ammonia (NH_3) 100:3:0.7 (v/v/v)) can be tested.

   b. *changing the type of counter-ion (competing acid)*: the buffer type may have a strong influence on retention and also affect enantioselectivity significantly. If retention is too long, the acetate buffer may often be replaced conveniently by formate buffer, which has a higher elution strength (Try for example, methanol/formic acid/TEA 100:1:3 (v/v/v) or methanol/formic acid/NH_3 100:1:1.5 (v/v/v)).

   c. *modifying acid-basic ratio (apparent pH or pHa):* by variation of the acid-base ratio (e.g. the acetic acid to TEA or NH_3 ratio), the apparent pH can be altered. This may have a significant effect on retention and enantioselectivity. With increasing excess of acid the retention will increase. The optimal concentration in terms of selectivity will depend on the solute structure. For example, compare first the best mobile phase at pHa 5, 6 and 7 by adjusting with TEA or NH_3 (see Table for advised acid-basic ratios).

Acid-base ratios (examples)

	pHa < 6	pHa ≈ 6	pHa ≈ 6.5	pHa > 7
Acetic acid/TEA	2 : 0.2	3 : 1 2 : 0.6 1 : 0.3	2 : 1.6 1 : 0.8	2 : 3 1 : 1.5
Acetic acid/NH3	2 : 0.2	3 : 0.7 2 : 0.5 1 : 0.2	2 : 1.2 1 : 0.5	2 : 2 1 : 1
Formic acid/TEA	0.5 : 0.5	1 : 3 0.5 : 1.5	1 : 3.5	1 : 4 0.5 : 1.8
Formic acid/NH3	0.5 : 0.3	1 : 1.5 0.5 : 0.8	0.5 : 1	0.5 : 1.5

⇩ **Starting conditions**

d. *evaluating flow rate:* with a baseline resolution flow rate can be increased at 1.5 ml/min (at 0.3 ml/min for microbore columns), but if this is not enough, try to re-adjust retention times by further increasing the concentration of the counter-ion or changing the buffer type in the mobile phase.

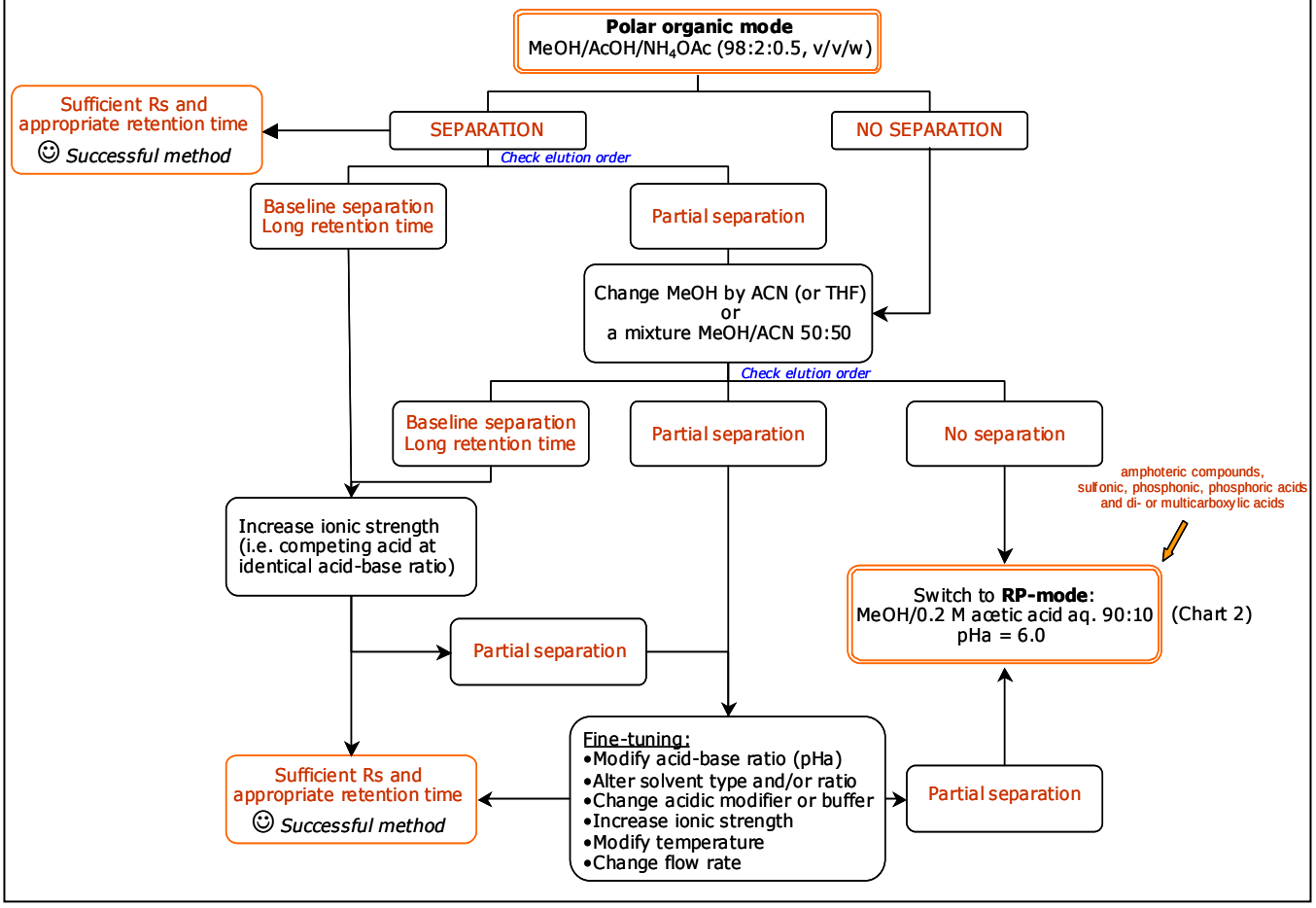
e. *increasing temperature:* except for a few solutes, higher temperature decreases retention times, but also diminishes enantioselectivity. If retention is too high at 25°C, try at 30°C (maximum 40°C).

f. *changing the co-ion*: some minor differences may be observed between the adjustment of pH with TEA or NH_3.

4) If only a **partial separation** is achieved with the initial methanol mobile phase, check also the methanol/acetonitrile mixture (or even other solvents as THF), but type of buffer, co-ion, flow rate or temperature may help for nearly baseline separations. Otherwise, switch to RP-mode.

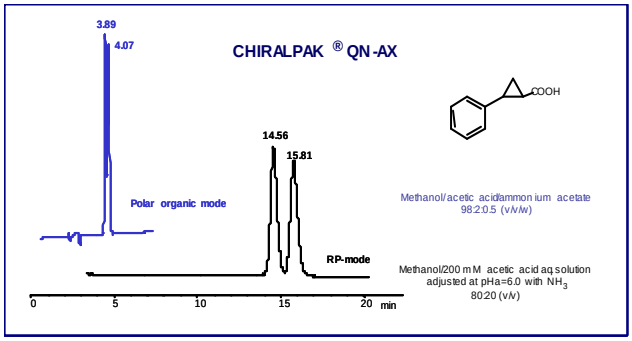
5) If **no separation** is achieved with the initial methanol mobile phase, try acetonitrile or methanol/acetonitrile mixture containing the above specified buffer or switch directly to RP-mode.

Chart 1. Acidic compounds in polar mode.



Factors affecting elution times (examples)

To *increase* elution times	To *decrease* elution times
⇓ Counter-ion concentration	⇑ Counter-ion concentration
Acid-base ratio: ⇓ base (PO)	Acid-base ratio: ⇑ base (PO)
⇓ pHa from 6 to 5 (RP) (even to 2, depending on pKa of compound)	⇑ pHa from 6 to 8 (RP)
⇓ Organic content (RP)	⇑ Organic content (RP)
⇓ Flow rate	⇑ Flow rate
Change acid type retention: acetic acid > succinic acid ≥ formic acid > glycolic acid > malonic acid (PO)	
Change buffer type retention: acetate > formate > phosphate > citrate (RP)	



General conditions: CHIRALPAK® QN-AX and QD-AX, 150 x 4.6 mm, Flow rate: 1 ml/min, 25°C

Acidic Compounds in Reversed-Phase (RP) Mode *(Chart 2)*

1) Test a mobile phase of methanol/0.2 M acetic acid aq. solution (90:10, v/v) (pHa of the mixture adjusted to 6 with concentrated aq. ammonia). Start the screening at 1 ml/min (at 0.1 ml/min for microbore columns) and 25 °C. If the screening was previously carried out in the polar mode and an acetonitrile mixture proved to be superior, use this organic modifier for further experiments and optimization.

   ⇨ As soon as a separation is detected, the **elution order** can be checked. If necessary, switch from CHIRALPAK® QN-AX to QD-AX and vice versa to pursuit the method development, as they show inversion of elution order in most cases.

2) If a **baseline separation** is achieved but **retention times are too long**, select the mobile phase combination that gave suitable selectivity for further optimization and adjust retention factors by:
   a. *increasing the concentration of the buffer in the eluent:* it will decrease retention. For example, a mixture of methanol/0.5 M acetic acid aq. solution (90:10, v/v) (pHa of the mixture adjusted to 6 with concentrated aq. ammonia) can be tested. For multiply charged analytes the buffer concentration should be at least 0.5 M or 1 M ammonium acetate buffer, in order to be able to elute the analytes within reasonable time. For phosphonic and phosphoric acids 20-50 mM phosphate buffer (pHa 6) may perform better in terms of peak shapes and elution times. Higher volume percentages of aqueous component are recommended to avoid solubility problems of phosphate buffer in the hydroorganic mixture.

   b. *changing the type of counter-ion (competing acid):* if the pH optimum is lower than pH 4, acetate could be replaced by formate. A change to phosphate or citrate, which have considerably higher elution strength, has in the majority of cases a negative effect on enantioselectivity. These buffers should solely be used if reasonable elution times cannot be achieved even with highly concentrated acetate or formate buffers. This may be the case e.g. for phosphonic acids and di- or tricarboxylic acids. If citrate or any other buffer with high affinity towards the chiral selector was used, it is recommended to regenerate the column after use by rinsing for 20 min with a solution of methanol/TEA (100:2, v/v) and then washing with methanol/water (50:50, v/v) for 30 min before switching to another mobile phase.

   c. *increasing the organic content*: study the effect of organic modifier content (90-95%). Consider also in such a case the polar organic mode for evaluation.

   d. *modifying pHa:* The optimal pH for carboxylic acids is mostly found between pH 5 and 6, for stronger acids it is often shifted towards lower pH, and for amphoteric compounds on the contrary towards higher pH (allowed range between 2-8). An increase of pHa between 6 and 7 will reduce retention times with small impact on selectivity, in the range of 7-8 alpha values may decrease dramatically (for example, compare first the best mobile phase at pHa 5, 6 and 7 by adjusting with TEA or NH_3). For strongly acidic compounds such as phosphonic acids or sulfonic acids and amphoteric compounds as well, the pH optimum may be shifted and the useful pH range may be extended between 2 and 8.

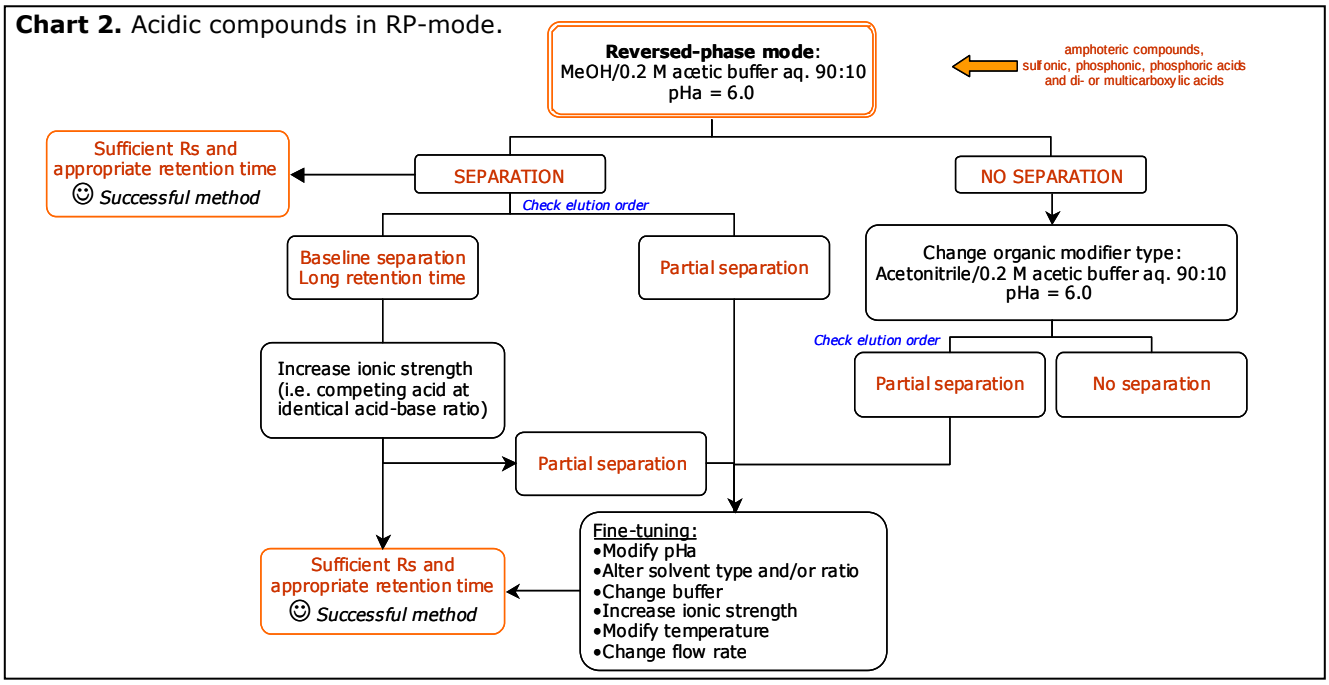
   e. *evaluating flow rate:* with a baseline resolution flow rate can be increased to accelerate the separation depending on the viscosity of the mobile phase until 180 bar, but if this is not enough, try to re-adjust retention times by further increasing the concentration of the counter-ion or changing the buffer type in the mobile phase. A reduction of the flow rate will increase the plate number.

   f. *Increasing temperature:* except for a few solutes, higher temperature decreases retention times, but also diminishes enantioselectivity. If retention is too high at 25°C, try at 30°C (maximum 40°C).

   g. *changing the co-ion*: some minor differences may be observed between the adjustment of pH with TEA or NH_3.

3) If **no separation** or only a **partial separation** is achieved with the initial methanol or acetonitrile/aqueous (90:10) mobile phase, several situations are possible:
   a. *Very short retention times*: if k < 1 reduce the concentration of counter-ion in the mobile phase by a factor of 2 to 5 and proceed with optimization of the other parameters (see above). Alternatively, reduce the organic content (e.g. to 80 and 70%).
   b. *Reasonable retention times (1≤k<10)*: study the effect of decreasing the organic content (e.g. to 80 and 70%). If a baseline separation is attained, use the guidelines to adjust retention time described in section 2. In all cases, if resolution is nearly baseline decrease the flow rate from 1 ml/min to 0.5 ml/min and decrease the temperature. If this leads to baseline resolution, but run times are too long, try to re-adjust retention times by further increasing the concentration of the counter-ion in the mobile phase.

Chart 2. Acidic compounds in RP-mode.



Neutral and Basic Compounds in Normal-Phase (NP) Mode

Before the column can be used in this mode it has to be washed with methanol/water exhaustively, then methanol/TEA (100:2, v/v) (30 min) and finally with 2-propanol, which is miscible with NP eluents.

1) Start the optimization with a mobile phase consisting of hexane/2-propanol (90:10, v/v). If elution is too fast, adjust the polar modifier to only 1% (e.g. for arylcarbinols). If it was too slow, increase to 30% 2-propanol.
2) If no resolution of enantiomers is achieved, replace the polar modifier e.g. instead of 2-propanol use ethanol, dichloromethane, dioxane and so forth. Also the sole use of the pure polar solvent component may afford resolution.
3) If tailing is observed e.g. for basic compounds, add 0.1% of TEA or another basic modifier.
4)

Neutral and Basic Compounds in Reversed-Phase (RP) Mode

If for practical reasons hydro-organic conditions are preferred over the NP-mode, it may be attempted to achieve resolution of enantiomers of neutral or basic solutes also in the reversed-phase mode. Successful examples have been reported previously.

1) Start with a linear gradient of acetonitrile from 5% to 100% in water with 30 min gradient time, to determine the suitable organic modifier content for a following isocratic separation.
2) Run an isocratic separation with a mixture of acetonitrile and water having a composition that the capacity factor is between 5 and 10.
3) If no resolution or beginning of a resolution is observed, repeat the procedure with methanol.
4) If retention factors have been too low or if a beginning separation is identified, it is advisable to reduce the linear flow rate of the eluent.

5) For ionizable compounds such as chiral bases a buffer must be added and the pH of the eluent needs to be optimized. At higher pH retention will be stronger.
6) If no separation or beginning of a separation is obtained, it is suggested to continue with the NP-mode.

Operating these columns in accordance with the guidelines outlined here will result in a long column life.

⇨ If you have any questions about the use of these columns, or encounter a problem, contact:

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