INSTRUCTION MANUAL FOR CHIRALPAK® AGP

Please read this instruction sheet completely before using this column

Column Description

CHIRALPAK® AGP : α_1-acid glycoprotein immobilized on 5 µm silica-gel.

Shipping solvent: **Water / 2-Propanol (2-PrOH) solvent mixture (85/15 v/v)**

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

Application Scope

CHIRALPAK® AGP has very broad applicability and is suitable for enantiomer resolution of all types of compounds, including:

- amines (primary, secondary, tertiary and quaternary ammonium compounds)
- strong and weak acids
- non-ionisable compounds (amides, esters, alcohols, sulfoxides, etc)

Operating Conditions

	50 x 2.1 mm i.d.*1 100 x 2.1 mm i.d.*1 150 x 2.1 mm i.d.*1 Analytical column	**50 x 3 mm i.d.*1 100 x 3 mm i.d. 150 x 3 mm i.d.** Analytical column	**50 x 4 mm i.d.*1 100 x 4 mm i.d. 150 x 4 mm i.d.** Analytical column	**100 x 10 mm i.d. 150 x 10 mm i.d.** Semi-prep. column
Flow direction	As indicated on the column label			
Typical Flow rate	0.2 mL/min	0.5 mL/min	0.9 mL/min	4.0 mL/min
pH range	4.0 - 7.0			
Recommended temperature*2	20 - 30°C			
Buffer concentration	up to 100 mM, typically 10-20 mM			
Organic modifier ratio	0-15% by volume			
Charged additive concentration	up to 10 mM			

*1 It is very important that the HPLC system is optimized in terms of void volume for work with columns of small dimensions.

*2 The column lifetime might be reduced if used at higher temperature.

A - Mobile Phase Starting Conditions

	ACIDIC Compounds	**NEUTRAL** Compounds	**BASIC** Compounds
Typical starting conditions	**10 mM Ammonium acetate buffer (pH 5.8)❶ / 2-PrOH = 95 / 5 (v/v)**		

❶ Refer to section B for preparation of the buffer.

B – Buffer Preparation - Example

➢ *Preparation of 10 mM Ammonium acetate buffer (1Liter):*

1. Weigh 770.8 mg of ammonium acetate (CH_3COONH_4, purity > 99%) into a beaker.
2. Dissolve the salt with about 800 mL water (HPLC grade), equilibrated at room temperature (20-25°C).
3. Adjust pH to the target value by using either diluted acetic acid or a diluted ammonium hydroxide solution.
4. Filter the solution through a membrane of 0.22 µm into a measuring flask.
5. Add water until the limit line of the measuring flask. Place the stopper in the neck and homogenize the solution by agitation.

When buffer should be mixed with an organic modifier, the measurements are normally by volumes, using preferably volumetric flasks or measuring pipettes.
After mixing, de-gas the mobile phase in an ultrasonic bath.

Note that in the case where a charged additive is needed in the mobile phase, the charged additive should be added into the aqueous solution before the pH adjustment.

C – Mobile Phases

Bacteria grow fast in eluents containing no or low alcoholic organic modifier. Such mobiles phases must be freshly prepared.

❖ ***Buffer***
The salt concentration of ammonium acetate buffer is typically 10-20 mM but can be varied up to 100 mM. The other kinds of buffers, such as sodium or potassium phosphate buffers, sodium acetate buffers, formate or citrate buffers, can also be used. However, the LC-MS compatibility of the method may be sometimes compromised.

❖ ***Organic modifiers***
2-PrOH is the most frequently used. However, methanol, ethanol and acetonitrile can also be investigated. The relative eluting strength can be ranked as follows: 2-PrOH > EtOH ≥ ACN > MeOH

❖ ***Charged additives***
Cationic and anionic additives, such as *N,N*-dimethyloctyl amine (DMOA), trifluoroacetic acid (TFA), octanoic acid (OA), heptafluorobutyric acid (HFBA), can be usedin low concentration (≤ 10 mM) to regulate retention and enantioselectivity. However, some of these additives may be difficult to be removed totally from the column, due to very high affinity to the matrix. Thus, the properties of the column may be affected.

CAUTION: The miscibility of OA and DMOA to water is very limited. Only 2 mM OA or 5 mM DMOA can be homogeneously incorporated into the aqueous solution at ambient temperature. A phase separation may occur beyond these concentrations.

Once a charged additive is used in the mobile phase, the column should be dedicated for the purpose.

D – Samples

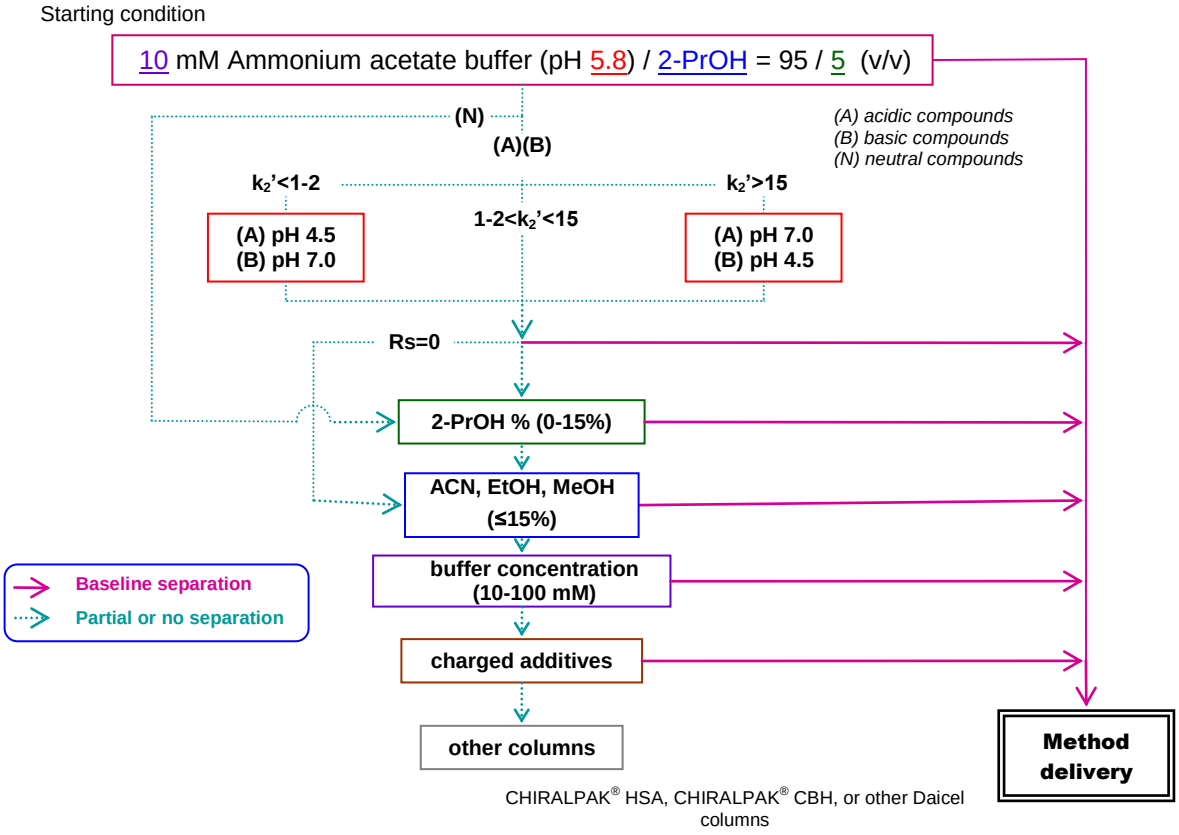
The sample amount injected onto the column should be kept low. The recommended sample concentration is 0.20 mg/mL or lower with an injection volume of 5-10 µL, preferably.

Dissolve the sample in the mobile phase when it is possible. If the sample is insoluble in the mobile phase, add a higher concentration of the organic modifier. The sample solution should be filtered through a membrane filter of approximately 0.5 µm porosity to ensure that there is no precipitate before using.

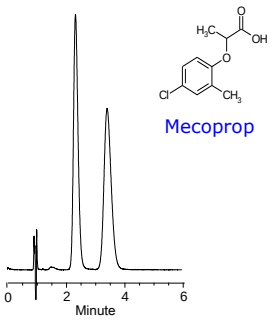
CAUTION: Dissolution of the sample in pure or high percentage of organic solvents may cause on-line sample precipitation.
Do not inject unclear sample solutions or solutions containing undissolved compounds.

Method Development

The following scheme offers a guide for method development and method optimization.

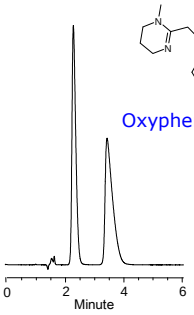


CHIRALPAK® AGP
100 x 4 mm i.d.



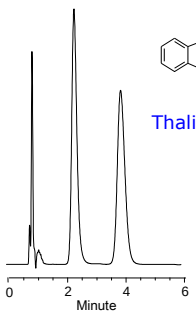
Mecoprop

Buffer (pH 5.8) / 2-PrOH = 95 / 5
Buffer : 10 mM Ammonium acetate



Oxyphencyclimine

Buffer (pH 4.5) / 2-PrOH = 90 / 10
Buffer : 10 mM Ammonium acetate



Thalidomide

Buffer (pH 7.0) / 2-PrOH = 98 / 2
Buffer : 10 mM Ammonium acetate
+ 5mM DMOA

Column Care / Maintenance

- The use of a guard cartridge is highly recommended for maximum column life.
- If the column has been contaminated with very hydrophobic material, wash the column backwards (no detector connected) over night with Water/2-PrOH 75/25(v/v) at a reduced flow-rate (e.g. 0.3 mL/min for 4 mm ID columns).
- Before disconnecting the column from the HPLC system, flush the column with a mobile phase that does not contain any salts / buffers, e.g. Water/2-PrOH 90/10(v/v).
- For the storage of the column, it is recommended to fill it with Water/2-PrOH 85/15(v/v).
  For short storage period, the column can be placed at ambient temperature (<30°C).
  For longer storage periods, however, it is recommended to place it in a refrigerator.

Important Notice

We recommend the use of a CHIRALPAK® AGP guard column in order to protect the analytical column from any particulates and impurities with high affinity to the stationary phase. Change the guard column regularly, especially in bioanalysis.

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

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