INSTRUCTION MANUAL FOR CHIRALPAK® ZWIX(+) and CHIRALPAK® ZWIX(-)

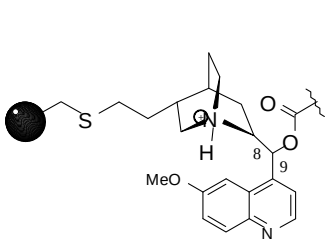
Semi-Preparative Columns

Please read this instruction sheet completely before using these columns

Column Description

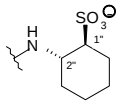
Packing composition:

- ⇨ Quinine combined with (S,S)-ACHSA(*) for CHIRALPAK® ZWIX(+),
- ⇨ Quinidine combined with (R,R)-ACHSA(*) for CHIRALPAK® ZWIX(-),
- ⇨ *Both of the chiral selectors are immobilized on **5µm** silica-gel.*



CHIRALPAK® ZWIX(+)

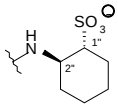
Quinine -derived (8S, 9R)



(1"S, 2"S)

CHIRALPAK® ZWIX(-)

Quinidine -derived (8R, 9S)



(1"R, 2"R)

Covalently bonded on **5µm** spherical silica gel

(*) *trans-2-aminocyclohexanesulfonic acid (ACHSA)*

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 Recommendations

	150 x 10 mm i.d. 250 x 10 mm i.d.	150 x 20 mm i.d. 250 x 20 mm i.d.
Flow rate direction	As indicated on the column label	
Flow rate range	2 to 6 ml/min	8 to 24 ml/min
Temperature range	5 to 45°C	

- Pressure drop over the column should be maintained <150 Bar (2100psi) for maximum column life time.
- Samples should be filtered through a membrane filter of approximately 0.5 µm porosity.
- Mobile phases should be filtered through an appropriate filtration membrane.

Operating Procedure

CHIRALPAK® ZWIX(+) and CHIRALPAK® ZWIX(-) are zwitterionic chiral stationary phases developed mainly for chiral separations of free amino acids. They exhibit remarkable stereoselectivity for zwitterionic molecules, especially amino acids and peptides, without derivatization.

CHIRALPAK® ZWIX(+) and CHIRALPAK® ZWIX(-) columns are compatible for use in LC-MS detection. The suitability of the mobile phase systems to MS detection/identification makes the chromatographic method from the zwitterionic columns extremely valuable in analyzing numerous amino acids which are deficient of chromophors for UV detection.

Owing to the feature of pseudo-enantiomers of the two chiral selectors, the elution order of enantiomers can be systematically reversed on CHIRALPAK® ZWIX(+) and CHIRALPAK® ZWIX(-), although their column performance may not be exactly equal towards each analyte.
They are compatible with all common HPLC solvents (e.g. methanol, acetonitrile, tetrahydrofuran, water).

Practical Method Development Scheme / Analytical Column

In zwitterionic mode, the mobile phase should provide efficient solvation to all the ionized species involved in the double ion-exchange equilibria. This requires the consequent proton activities of the mobile phase media.

☞ *Bulk mobile phase:*

- Owing to its pronounced protic properties, MeOH is an essential mobile phase component for chiral separations on CHIRALPAK® ZWIX(+) and CHIRALPAK®ZWIX(-).
- To adjust the eluting strength and separation degree, MeOH can be mixed with acetonitrile (ACN) or THF at various proportions (preferably with MeOH ≥ 20%, v/v) as the bulk stationary phase. Higher MeOH contents lead to decrease in retention time of zwitterionic compounds.
- Addition of a low percentage of water (e.g. 2%) to the mobile phase has no detrimental effect on enantio-selectivity. On the contrary, this gives the benefits of improving MS detection, increasing sample solubility (avoiding on-line precipitation) and reducing peak tailing when working with relatively low amount of MeOH in the mobile phase.

☞ *Additives:*

- Due to the intra-molecular counterion effect of the chiral selectors, the combined presence of acidic and basic additives in eluent is necessary. The additive pair of formic acid (FA)-diethyl amine (DEA) at 50mM-25mM is proved to be versatile for operating the zwitterionic CSPs. They contribute to the proton activity of mobile phase as well.
- For fully LC-MS compatible conditions, FA/DEA can be replaced by FA/ammonium formate or a mixture of FA/ammonia. For MS applications, we would recommend the following starting conditions:

  25mM FA + 25mM ammonium formate in MeOH/H_2O 98:2 (v/v).

☞ *Approaches for method development:*

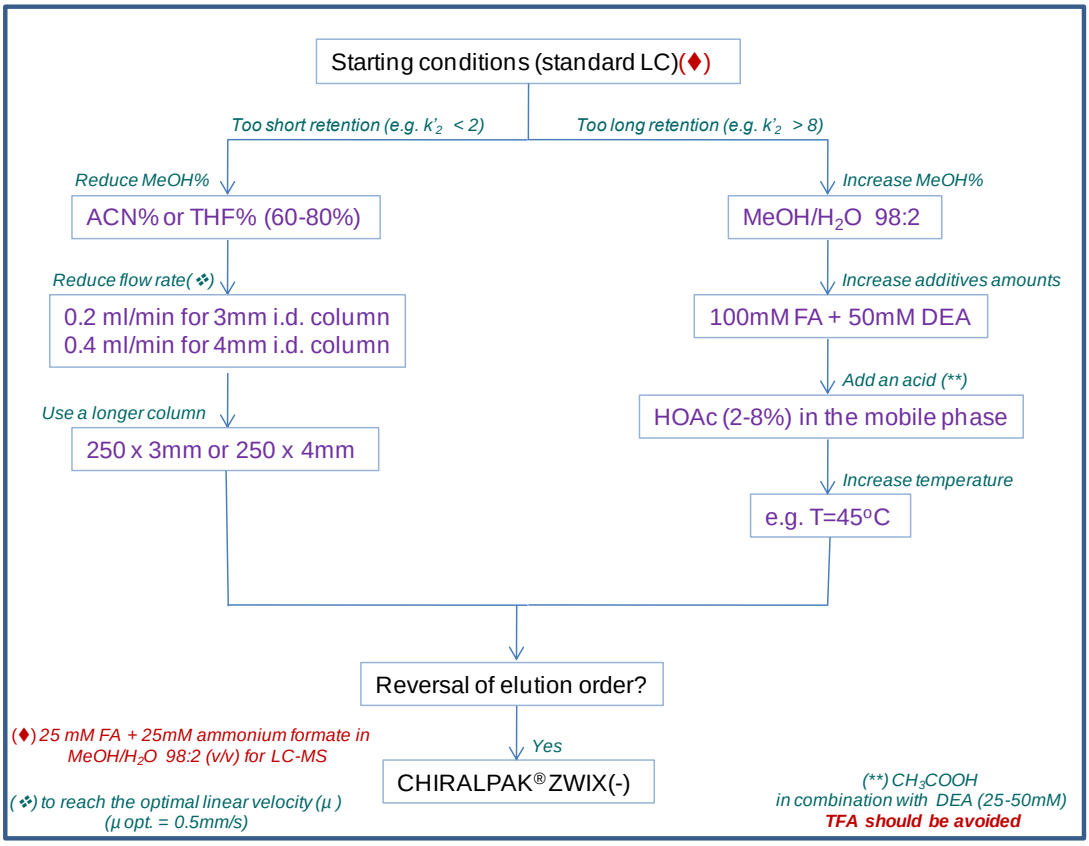
STARTING CONDITIONS (standard LC) / Analytical Column

- Mobile Phases:
  - (1). MeOH / ACN / H_2O 49:49:2 (v/v/v) + 50mM FA + 25mM DEA(*)
  - (2). MeOH / THF / H_2O 49:49:2 (v/v/v) + 50mM FA + 25mM DEA(*)

(*) *Add 1.9ml of formic acid and 2.6ml of diethyl amine to 1L of bulk mobile phase.*

- Column and flow rate: CHIRALPAK® ZWIX(+) 150 x 3mm i.d. / 0.4-0.5 ml/min or 150 x 4mm i.d. / 0.8-1.0 ml/min
- Temperature: 25°C

OPTIMIZATION STEPS / Analytical Column



Column Care / Maintenance

☞ *Starting:*

Before initial use, a 10 x 250 column should be equilibrated with 300mL of the mobile phase; a 20 x 250 column should be equilibrated with 1.2 Liters of the mobile phase.

☞ *Cleaning:*

100% MeOH and 100% ACN can be used to wash the column.
Mixtures of these solvents with H_2O (50:50, v/v) may also be efficient.

☞ *Storage*

We recommend flushing the column with 100% MeOH before storage: (10 x 250 300mL; 20 x 250 1.2 Liters)
The column can be stored at room temperature.

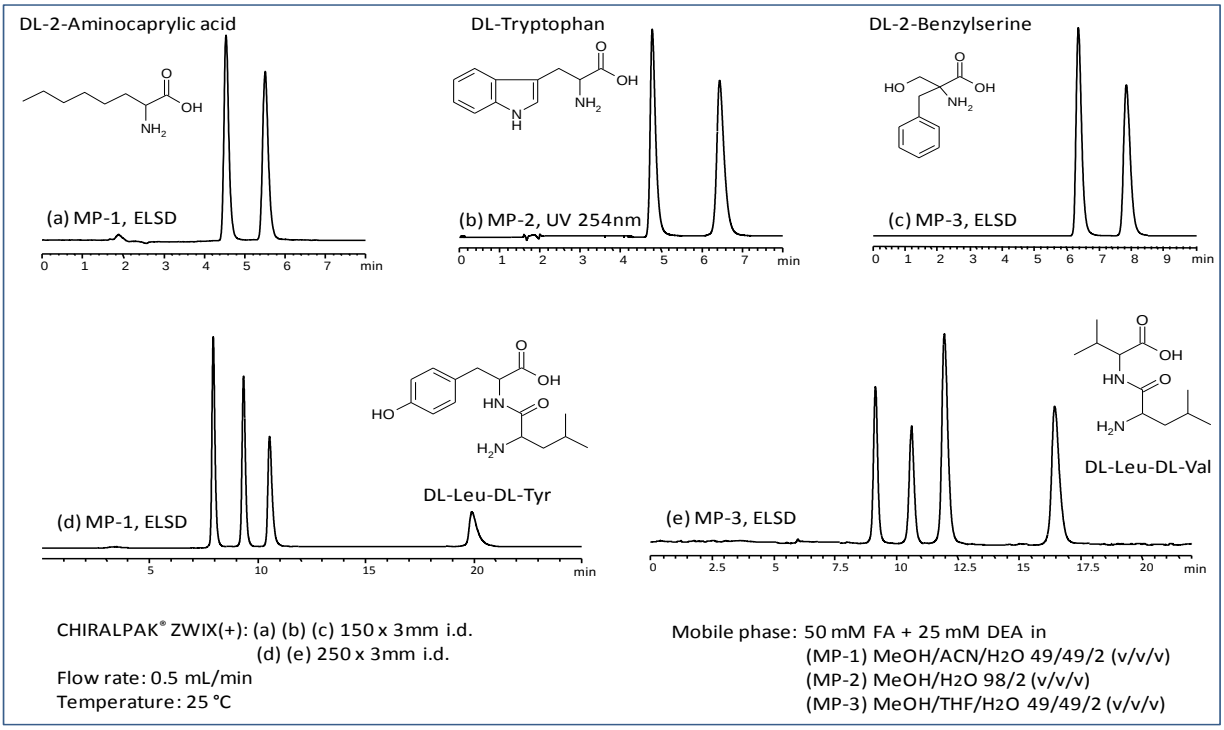
Operating this column in accordance with the guidelines outlined here will result in a long column life.

Examples of Chiral Separations for Standard Amino Acids

Column: CHIRALPAK® ZWIX(+) / 250 x 3mm i.d.
Mobile phase: MeOH/ACN/H_2O 49:49:2 (50mM FA + 25mM DEA); 0.5ml/min; 25°C

Amino acid	t_1 (min)	t_2 (min)	α	Rs	Elution order	Detection
Leucine	7.3	8.9	1.36	5.1	L/D	ELSD
Methionine	8.9	10.0	1.19	3.6	L/D	ELSD
Phenylalanine	7.9	9.1	1.24	4.1	L/D	ELSD
Proline	6.6	9.8	1.86	12.0	L/D	ELSD
Tyrosine	9.3	11.2	1.29	4.1	L/D	UV 230
Threonine	9.1	10.9	1.29	3.5	L/D	ELSD
Valine	7.3	8.8	1.34	4.8	L/D	ELSD

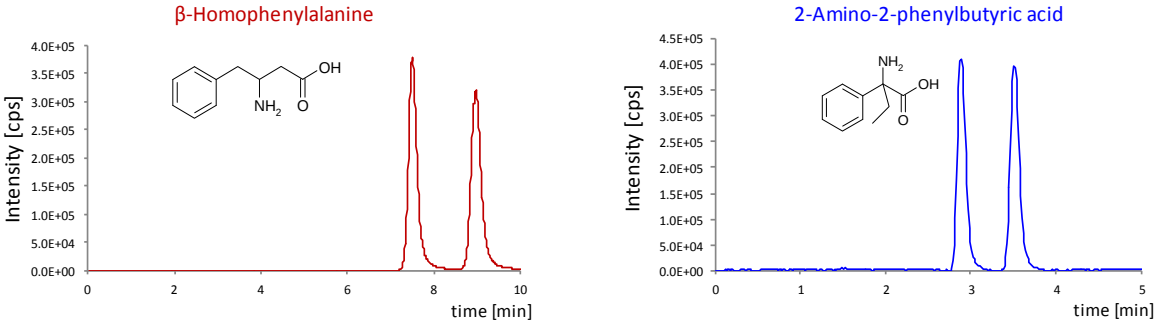
Examples of Chiral Analyses



CHIRALPAK® ZWIX(+): (a) (b) (c) 150 x 3mm i.d.
(d) (e) 250 x 3mm i.d.
Flow rate: 0.5 mL/min
Temperature: 25 °C

Mobile phase: 50 mM FA + 25 mM DEA in
(MP-1) MeOH/ACN/H_2O 49/49/2 (v/v/v)
(MP-2) MeOH/H_2O 98/2 (v/v/v)
(MP-3) MeOH/THF/H_2O 49/49/2 (v/v/v)

LC-MS applications



LC-MS: Q-Trap (MRM Mode)
CHIRALPAK® ZWIX(-) (150 x 3mm i.d.)
Mobile phase: 25mM FA + 12.5 mM ammonium formate in MeOH/ACN/H_2O 49/49/2 (v/v/v)
Flow rate: 0.5 mL/min; Temperature: 40 °C;

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

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