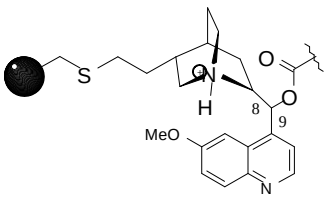
INSTRUCTION MANUAL FOR CHIRALPAK® ZWIX(+) and CHIRALPAK® ZWIX(-)

Please read this instruction sheet completely before using these columns

Column Description

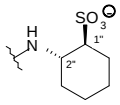
Packing composition:

⇨ 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 3µm silica-gel.*



CHIRALPAK® ZWIX(+)

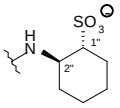
Quinine -derived (8S, 9R)



(1"S, 2"S)

CHIRALPAK® ZWIX(-)

Quinidine -derived (8R, 9S)



(1"R, 2"R)

Covalently bonded on 3µm 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 3 mm i.d. 250 x 3 mm i.d.	150 x 4 mm i.d. 250 x 4 mm i.d.
Flow rate direction	As indicated on the column label	
Flow rate range	~ 0.2 - 0.5 ml/min	~ 0.3 – 1.0 ml/min
Temperature range	5 to 45°C	

- 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

In zwitterionic mode, the mobile phase should provide efficient solvation to all the ionised 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 counter-ion 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)

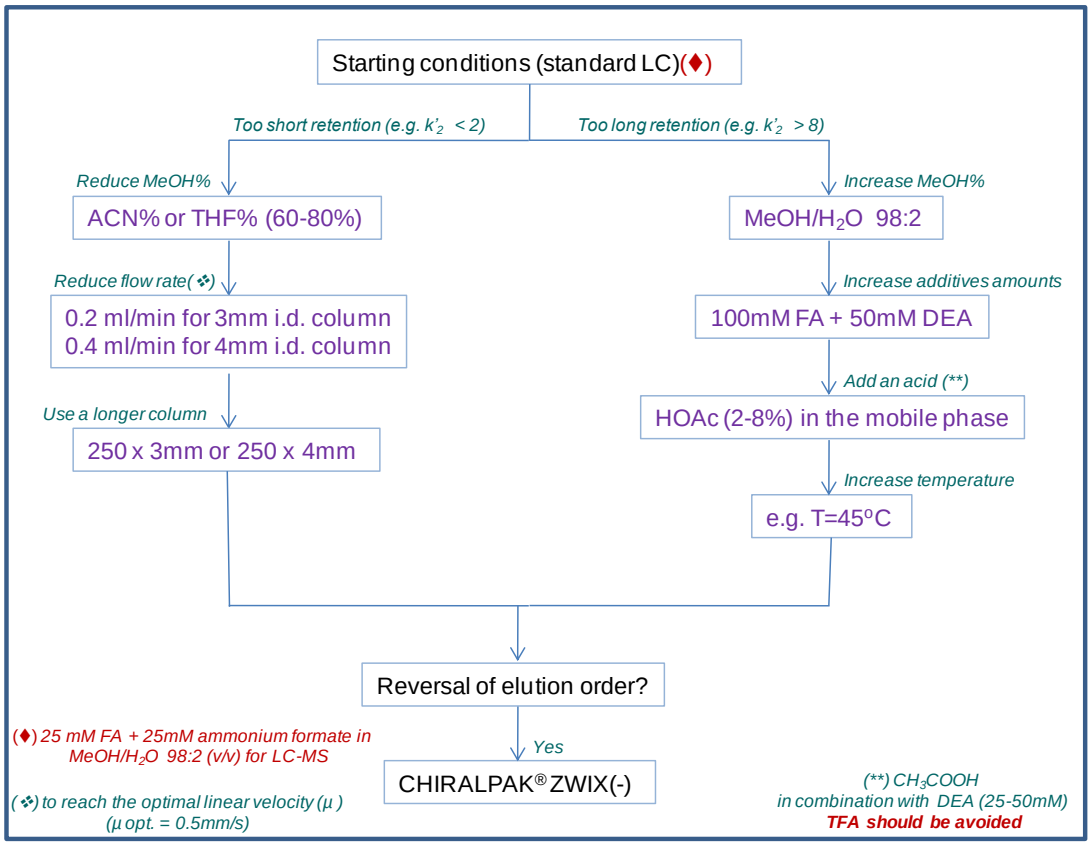
- Mobile Phases: (1). MeOH / ACN / H_2O 49:49:2 (v/v/v)
  50mM FA + 25mM DEA(*)

  (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



Column Care / Maintenance

☞ *Starting:*

Before initial use, the column should be equilibrated with 20 column volumes of the mobile phase (ca. 30 -40ml).

☞ *Cleaning AND Storage:*

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. We recommend flushing the column with 100% MeOH before storage (20 column volumes). 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.

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

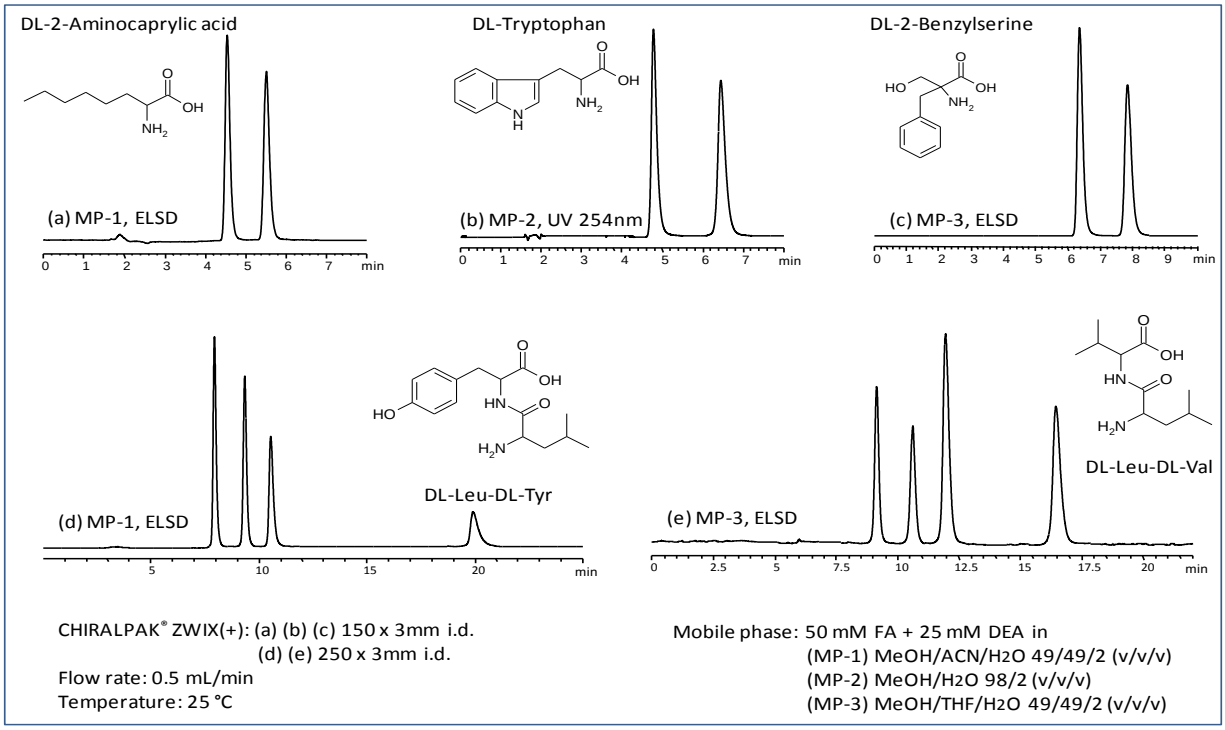
In the USA: questions@chiraltech.com or call 800-6-CHIRAL
In the EU: cte@chiral.fr or call +33 (0)3 88 79 52 00
In India: chiral@chiral.daicel.com or call +91-40-2338-3700

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

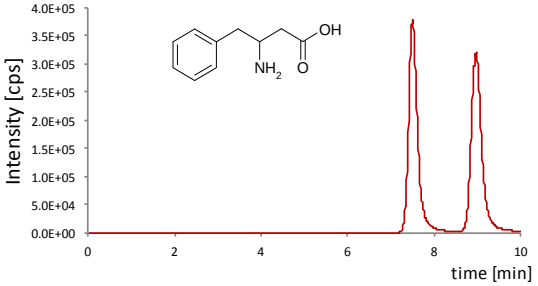
Amino acid	t_1 (min)	t_2 (min)	α	Rs	Elution order	Detection
Leucine	7.3	8.9	1.36	5.1	L/D	ELSD
Methionine	8.9	10.0	1.19	3.6	L/D	ELSD
Phenylalanine	7.9	9.1	1.24	4.1	L/D	ELSD
Proline	6.6	9.8	1.86	12.0	L/D	ELSD
Tyrosine	9.3	11.2	1.29	4.1	L/D	UV 230
Threonine	9.1	10.9	1.29	3.5	L/D	ELSD
Valine	7.3	8.8	1.34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

Examples of Chiral Analyses

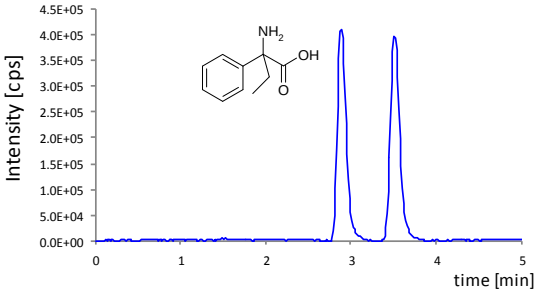


LC-MS applications

β-Homophenylalanine



2-Amino-2-phenylbutyric acid



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;

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