

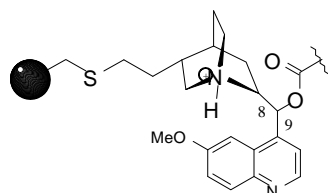
## INSTRUCTION MANUAL FOR CHIRALPAK® ZWIX(+) and CHIRALPAK® ZWIX(-)

**Please read this instruction sheet completely before using these columns**

### Column Description

**Packing composition:**

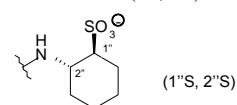
- ⇒ Quinine combined with (S,S)-ACHSA<sup>(\*)</sup> for CHIRALPAK® ZWIX(+),
- ⇒ Quinidine combined with (R,R)-ACHSA<sup>(\*)</sup> for CHIRALPAK® ZWIX(-),
- ⇒ Both of the chiral selectors are immobilized on 3µm silica-gel.



Covalently bonded on 3µm spherical silica gel

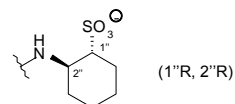
#### CHIRALPAK® ZWIX(+)

Quinine -derived (8S, 9R)



#### CHIRALPAK® ZWIX(-)

Quinidine -derived (8R, 9S)



<sup>(\*)</sup> *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<sub>2</sub>O 98:2 (v/v).*

## Approaches for method development:

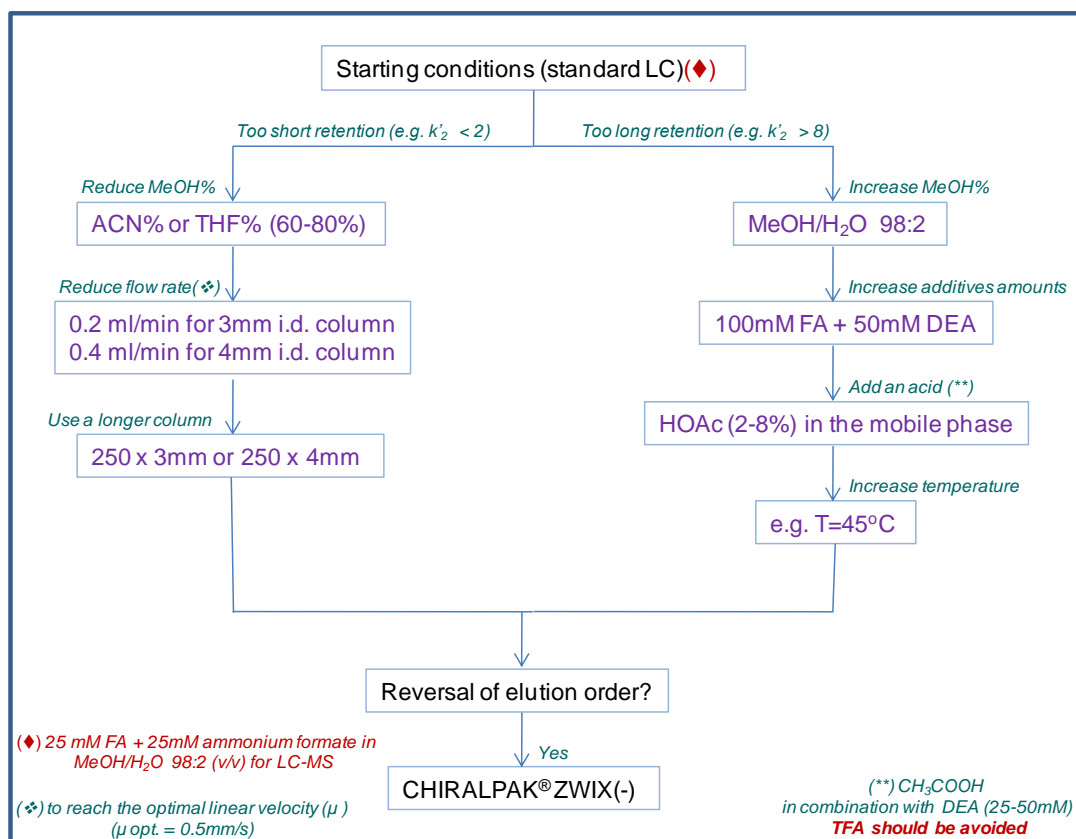
### STARTING CONDITIONS (standard LC)

- ❖ Mobile Phases:
  - (1). MeOH / ACN / H<sub>2</sub>O 49:49:2 (v/v/v)  
50mM FA + 25mM DEA<sup>(\*)</sup>
  - (2). MeOH / THF / H<sub>2</sub>O 49:49:2 (v/v/v)  
50mM FA + 25mM DEA<sup>(\*)</sup>

<sup>(\*)</sup> 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<sub>2</sub>O (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@cti.daicel.com](mailto:questions@cti.daicel.com) or call 800-6-CHIRAL

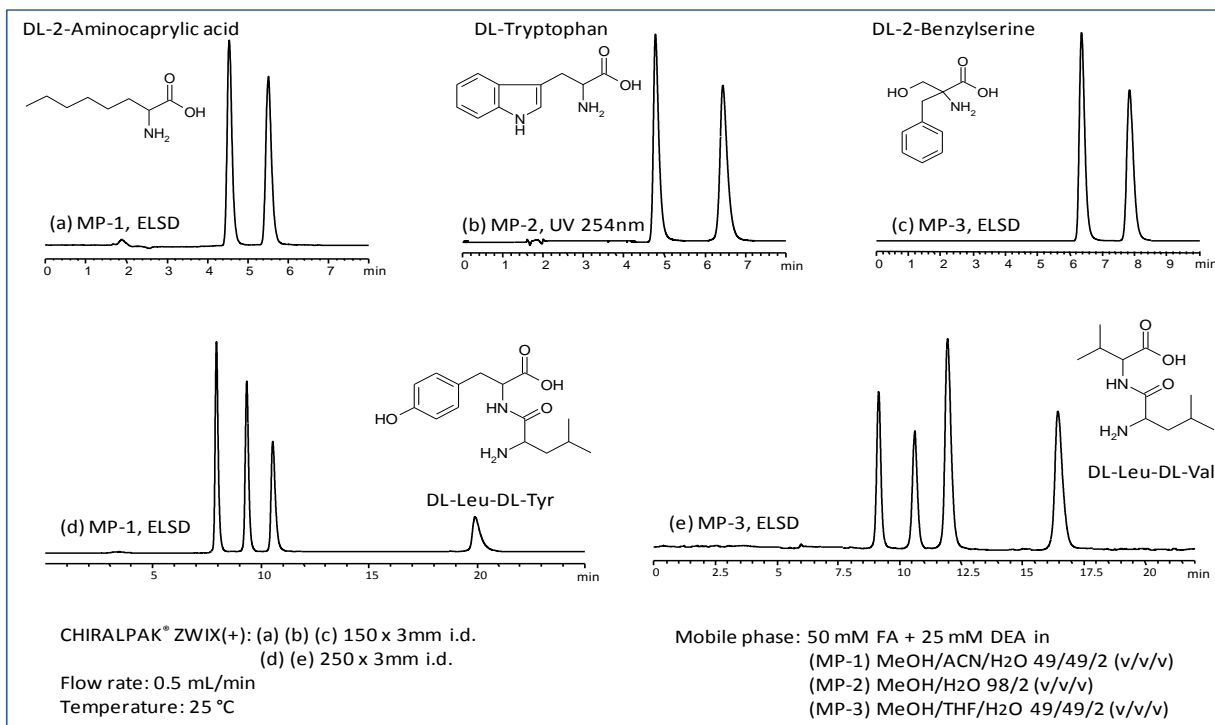
In the EU: [cte@cte.daicel.com](mailto:cte@cte.daicel.com) or call +33 (0)3 88 79 52 00

In India: [chiral@chiral.daicel.com](mailto:chiral@chiral.daicel.com) or call +91 84 1866 0700 & 703

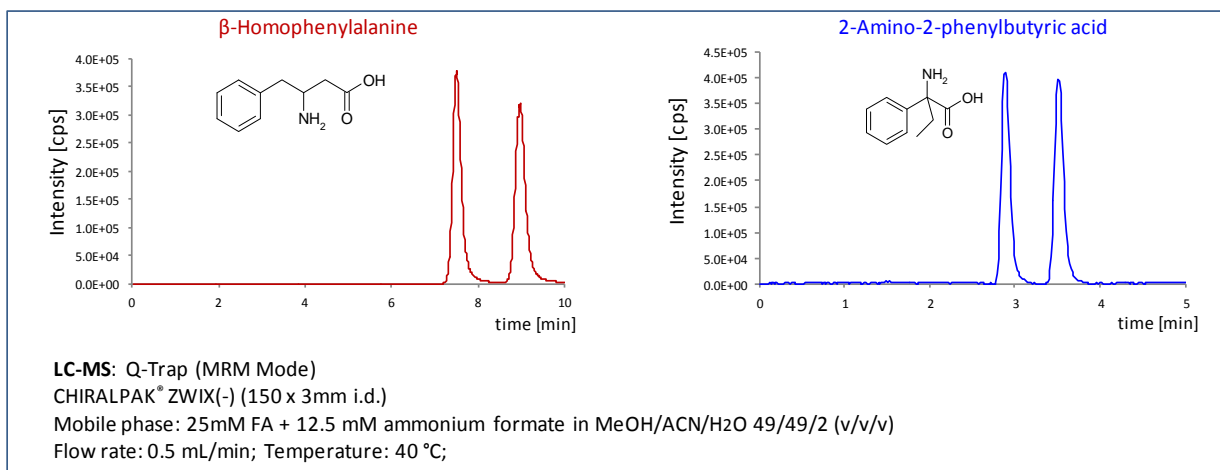
## Examples of Chiral Separations for Standard Amino Acids

Column: CHIRALPAK® ZWIX(+)/ 250 x 3mm i.d.						
Mobile phase: MeOH/ACN/H <sub>2</sub> O 49:49:2 (50mM FA + 25mM DEA); 0.5ml/min; 25°C						
Amino acid	t <sub>1</sub> (min)	t <sub>2</sub> (min)	$\alpha$	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



### Locations:

#### North/Latin America

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