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Introduction

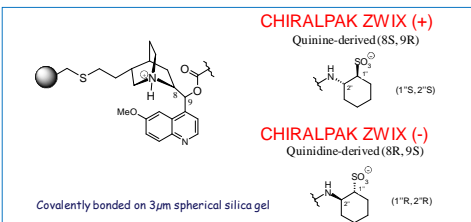
Amino acids are extremely important chemical species in life science. Most of them are chiral molecules containing a stereogenic carbon, to which the amino group is attached. The enantiomeric form of many amino acids can play very different roles in biological and pharmacological functions. The analysis of the composition and enantiomeric purity of amino acid samples by chromatography, without derivatisation, is of great importance in a large number of applications, including proteomics, API manufacturing and food industry.

CHIRALPAK® ZWIX (+) and CHIRALPAK® ZWIX (-) are zwitterionic chiral stationary phases (CSPs) recently developed mainly for chiral separations of free amino acids. They exhibit remarkable stereoselectivity for zwitterionic molecules, especially amino acids and peptides [1-3].

CHIRALPAK ZWIX (+) and CHIRALPAK ZWIX (-) can be operated in HPLC and in SFC. In the current study, we focus on the HPLC performance of these chiral selectors for direct separations of natural and synthetic free amino acids and of selected peptides using LC-MS compatible mobile phases. The viability of reversing the elution order and the approaches for method development on these columns are also addressed.

Chiral stationary phases (CSPs)

The chiral selectors are designed in a combinatorial approach by combining anion- and cation-exchangers in a single CSP. They are synthesized by the fusion of the cinchona alkaloids with trans-2-aminocyclohexanesulfonic acid (ACHSA) at the C-9 position via carbamate linkage. In CHIRALPAK ZWIX (+), Quinine combines with (S,S)-ACHSA. The chiral selector in CHIRALPAK ZWIX (-) is resulted from the fusion of Quinidine with (R,R)-ACHSA. Both chiral selectors are chemically bonded onto 3µm silica gel. They enable enantioseparation of zwitterionic compounds through a synergistic double ion-pairing process with high column efficiency.



Mobile phase conditions

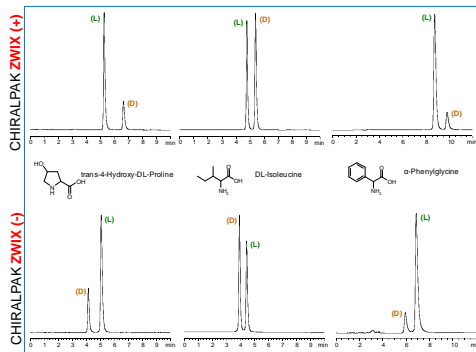
In zwitterionic mode, the mobile phase should provide efficient solvation to all the ionised species involved in the double ion-exchange equilibria. This requires the consequent proton activities of the mobile phase media. Owing to its pronounced protic properties, MeOH is an essential mobile phase component for chiral separations on CHIRALPAK ZWIX (+) and CHIRALPAK ZWIX (-). Typically, MeOH is mixed with ACN or THF at various proportions (preferably with MeOH ≥ 20% by volume) as the bulk mobile phase to adjust the eluting strength and separation degree. Higher MeOH% leads to decrease in retention time of zwitterionic compounds.

Addition of a low percentage of water (e.g. 2%) in bulk mobile phase has no detrimental effect on enantioselectivity. 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 MeOH% in the mobile phase.

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)-diethylamine (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.

Control of elution order

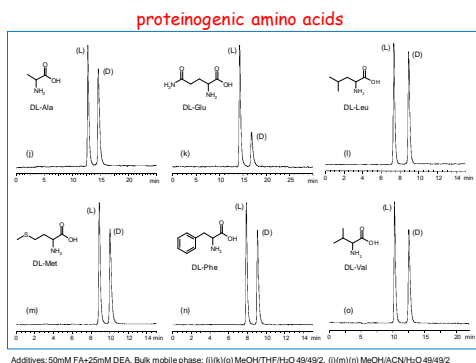
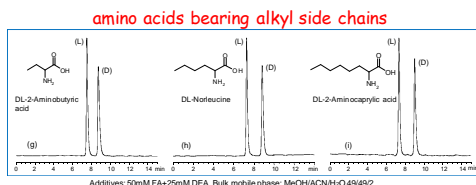
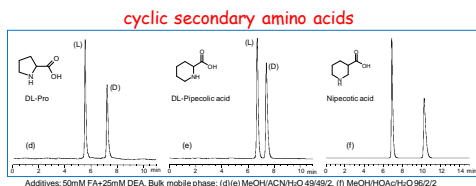
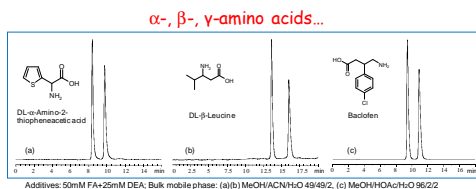
Owing to the feature of pseudo-enantiomers of the two chiral selectors, the elution order of enantiomers can be systematically reversed on CHIRALPAK ZWIX (+) and CHIRALPAK ZWIX (-), although their column performance may not be exactly equal towards each analyte.



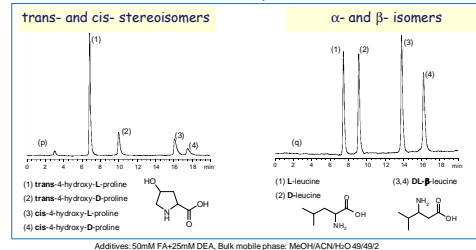
The feasibility to get the enantiomers eluted in a desired order upon the column choice is a valuable and instrumental tool for impurity profiling or accurate quantification of a given enantiomer in trace amount.

Examples of chiral analyses (on CHIRALPAK ZWIX (+))

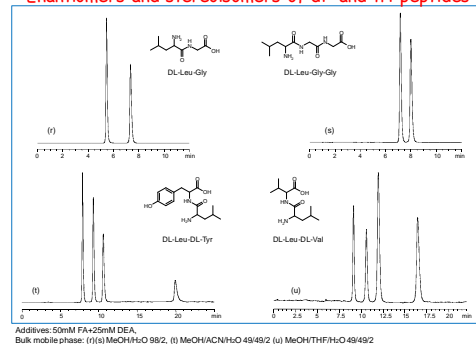
Common condition: ELSD;
 Column size: 250 x 3 mm (i.d.); T=25°C; Flow rate: 0.45 or 0.50 ml/min



Mixtures of structurally related amino acids



Enantiomers and stereoisomers of di- and tri-peptides



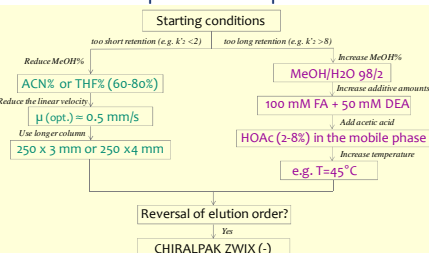
and many other types of zwitterionic compounds...

Approaches for method development

Starting chromatographic conditions

Column: CHIRALPAK ZWIX (+)
 Column dimension and flow rate: 150 x 3 mm (i.d.); 0.4-0.5ml/min
 150 x 4 mm (i.d.); 0.8-1.0ml/min
 Mobile phases: 50mM FA + 25mM DEA
 Bulk eluent: (1) MeOH/ACN/H₂O 49/49/2 v/v/v
 (2) MeOH/THF/H₂O 49/49/2 v/v/v
 Temperature: 25°C

Optimization steps



Conclusion

CHIRALPAK ZWIX (+) and CHIRALPAK ZWIX (-) are versatile in chiral separation of a broad range of zwitterionic compounds, including proteinogetic, non-proteinogetic, primary, secondary, cyclic, acyclic, α-, β-, γ-amino acids, di- and tri-peptides as well as many other types of zwitterionic compounds. The method development and optimization on these columns are easy and straightforward.

Elution order of enantiomers can be controlled by the column switch between CHIRALPAK ZWIX (+) and CHIRALPAK ZWIX (-).

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.

References

- [1] C.V. Hoffmann, R. Pell, M. Lämmerhofer, W. Lindner, Anal. Chem. 80 (2008) 8780-8789.
- [2] C.V. Hoffmann, R. Reischl, N.M. Maier, M. Lämmerhofer, W. Lindner, J. Chromatogr. A, 1216 (2009) 1167-1166.
- [3] S. Wernisch, R. Pell, W. Lindner, J. Sep. Sci., in press.