Direct stereo-selective separations of amino acids and small peptides on chincopa derived zwitterionic chiral columns by HPLC

Tong Zhang, Emilie Holder, Pilar Franco, Elena Eksteen

Introduction

Synthetic peptides occupy an important position in therapy and drug discovery. They represent the effective and innovative solutions for new and/or unmet medical requirements.

In concert with the peptide synthesis and their applications, appropriate analytical methods are needed for assays of the chiral amino acid and small peptide building blocks, for monitoring and controlling the stereochemistry of the synthetic process, for assessing the structural integrity (amino acid composition, sequence and chirality) of the final peptides, as well as for determining the constituents of the samples back from biological media. HPLC, often combined to MS detection, has been the most frequently used technique for these purposes. However, developing efficient and reliable methods involving the molecular chirality information for control and characterization of synthetic peptides remains challenging.

In response to such challenges, the newly developed zwitterionic chiral stationary phases, namely CHIRALPAK® ZWIX(+) and CHIRALPAK® ZWIX(-), have been explored in HPLC for direct stereo-selective resolution of amino acids and oligopeptides (di- and tripeptides mainly) using LC-MS compatible mobile phase conditions.

The chiral stationary phases (CSPs)

CHIRALPAK® ZWIX(+) and CHIRALPAK® ZWIX(-) are chincopa aldehyd-derived zwitterionic CSPs. The chiral selectors are designed in a combinatorial approach, incorporating a weak anionic and a strong cationic interaction sites into a single chemical moiety [1-2]. They are synthesized by the fusion of quinidine with (R)- or (S)-(AChSA) at C-9 position via a carbamoyl linkage. Quinine combined with (S,S)-(AChSA) to form the ZWIX(+) chiral selector unit then immobilized onto mercaptopropyl silica (particle size: 3µm for analytical columns: 5µm for semi-preparative columns). The ZWIX(-) CSP is made in a similar way but via the combination of quinine with (R)-AChSA. Both columns are commercially available from Daicel Corporation.

MS-compatible mobile phase system

Due to its appropriate proton activities, methanol is the basic mobile phase component for ZWIX(+) and ZWIX(-) columns. In combination with a low percentage of water, it provides generally good solubility to amino acids and peptides, is a suitable medium for regulation of the chiral selector and the amphoteric affinities and affords efficient solution to all the ions groups involved in the dual ion-pairing chiral amino acid and peptide equilibria. The eluting strength of the mobile phase can be adjusted for weaker by incorporating polar non-protic solvents (e.g. ACN or THF), for stronger by additives (e.g. TFA).

In addition, the combined presence of acidic and basic additives (being the co- and counter-ions) in a suitable ratio can regulate the interactions via dipole-dipole effects. The combination of 50 mM formic acid (FA) and 25 mM diethylamine (DEA) has been effectively used as the generic mobile phase additives. The replacement of DEA with ammonia is straightforward in the sake of the improved ionization performance for MS.

Direct enantiomer resolution of amino acids

A large series of chiral amino acids of diverse structures have been successfully resolved into enantiomers using ZWIX(+) and ZWIX(-) columns by following a generic experimental scheme [5]. The examples are given in Figure 2.

Stereo-selective separation of small peptides

Due to the very limited access to the peptide samples, only the commercially available di- and tripeptide standards containing one or two stereogenic centers are tested (Figure 3) on the same columns. Based on the combination of chiral recognition for N-derivatives of amino acids as well [1-2].

Control of elution order

Due to their opposite configuration of four stereogenic centers (R,S,R,S; S,S,R,S) for ZWIX(+) and ZWIX(-) in a quasi-systematic way, although their separation performance may not be exactly equal towards each analyte. This can be a valuable instrumental tool for reliable determination of enantiomeric excess, for adjusting the impurity profile of the synthetic peptides and for adapting the analytical method to the real application requirements.

References