

ULTRAHIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC  
ENANTIOSEPARATION OF SOME  $\beta^2$ -AMINO ACIDS

Dániel Tanács<sup>1</sup>, Ferenc Fülöp<sup>2</sup>, Antal Péter<sup>1</sup>, István Ilisz<sup>1</sup>

<sup>1</sup>*Institute of Pharmaceutical Analysis, Interdisciplinary Excellence Centre, University of Szeged, H-6720 Szeged, Somogyi utca 4, Hungary*

<sup>2</sup>*Institute of Pharmaceutical Chemistry, Interdisciplinary Excellence Centre, University of Szeged, H-6720 Szeged, Eötvös u. 6, Hungary*

$\beta$ -Amino acids have an extra carbon atom between the amino and carboxylic groups. This difference in the structure can cause various effects in  $\beta$ -amino acid-containing peptides, both in the structure [1] and in the stability in an organism. For example, these  $\beta$ -amino acids have different susceptibility to hydrolysis or enzymatic degradation than their  $\alpha$  analogs [2]. Monosubstituted  $\beta$ -amino acids can be subdivided into  $\beta^2$ - and  $\beta^3$ -amino acids, depending upon the position of the side-chain on the 3-aminoalkanoic acid skeleton.

Literature data relate mainly to the separation and identification of  $\beta^3$ -amino acid enantiomers and are summarized in several review papers [3-5]. However, relatively few data are available for the separation of  $\beta^2$ -amino acid enantiomers. Recently core-shell particles (superficially porous particles, SPPs) and sub-2  $\mu\text{m}$  fully porous particles have been proven to provide high-throughput and effective separations of a variety of chiral molecules in ultrahigh-performance liquid chromatography (UHPLC). Macrocyclic glycopeptide based stationary phases are known for their highly selective amino acid and peptide separations and there are readily available SPPs with covalently bonded teicoplanin, teicoplanin aglycone and vancomycin.

In this study we used 2.7  $\mu\text{m}$  SPPs bonded with macrocyclic glycopeptides to investigate the enantiomeric separation of 19  $\beta^2$ -amino acids. We found that the teicoplanin and teicoplanin aglycone-based stationary phases were effective in the enantiomeric separation for all of the examined  $\beta^2$ -amino acids. We also studied the effect of different solvent mixtures and the effect of the nature of buffers and effect of their concentrations.

## References

- [1] D.F. Hook, F. Gessier, C. Noti, P. Kast, D. Seebach, Probing the proteolytic stability of  $\beta$ -peptides containing  $\alpha$ -fluoro- and  $\alpha$ -hydroxy- $\beta$ -amino acids, *Chem. BioChem.* 5 (2004) 691.
- [2] M.-I. Aguilar, A.W. Purcell, R. Devi, R. Lew, J. Rossjohn, A.I. Smith, P. Perlmutter,  $\beta$ -Amino acid-containing hybrid peptides—new opportunities in peptidomimetics, *Org. Biomol. Chem.* 5 (2007) 2884.
- [3] I. Ilisz, A. Péter, W. Lindner, State-of-the-art enantioseparations of natural and unnatural amino acids by high-performance liquid chromatography. *TRAC Trends. Anal. Chem.* 81 (2016) 11-22.
- [4] I. Ilisz, A. Bajtai, W. Lindner, A. Péter, Liquid chromatographic enantiomer separations applying chiral ion-exchangers based on Cinchona alkaloids. *J. Pharm. Biomed. Anal.* 159 (2018) 127-152.
- [5] I. Ilisz, T. Orosz, A. Péter, High-performance liquid chromatography enantioseparations using macrocyclic glycopeptide-based chiral stationary phases – An Overview. in Ed. G.K. Scriba, *Chiral Separations: Methods and Protocols, Methods in Molecular Biology* 1985, 2019, pp. 201-237.