ENANTIOSEPARATION OF CYCLIC β-AMINO ACIDS ON ION-EXCHANGER-BASED CHIRAL STATIONARY PHASES

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Abstract
In the present work a direct HPLC method is described for the separation of the stereoisomers of the ampholytic non-methylated, *N*-methyl-, *N*-dimethyl- and *N*-amidino protected cyclic *β*-amino acids through the application of novel *Cinchona* alkaloid and sulfonic acid-based zwitterionic chiral stationary phases Chiralpak ZWIX(+)™ and ZWIX(-)™. The enantioseparations were carried out in polar-ionic mobile phase mode in the temperature range 5–40 °C. The effects of the composition of the bulk solvent, the acid and base additive, the temperature, the structures of the ampholytic analytes on the separations were investigated.

Introduction
Nowadays, one of the most interesting challenges of the modern analytical chemistry is the separation of chiral compounds. Chirality is momentous for the modern pharmaceutical industry since many drug compounds are chiral whose stereoisomers usually dispose of variant pharmacological and toxicological properties. One of the enantiomers (eutomer) have the desired pharmacological activity, while the other isomer (distomer) is inactive or in worst cases some undesirable effects or even toxic effect can also be produced. Consequently, it is important that the enantiomerically pure form become available, preeminently in the pharmaceutical field. For the separations of the enantiomers chiral high performance liquid chromatography (HPLC) is one of the most frequently applied techniques.
In recent years, cyclic *β*-amino acids have been intensively investigated due to their potential biological activity and their benefits in synthetic chemistry. The simplest carbocyclic *β*-amino acid - (1*R*,2*S*)-2-aminocyclopentanecarboxylic acid (cispentacin) - is an antifungal antibiotic [1], while its methylene derivative (icofungipen, PLD-118) is active *in vitro* against *Candida species* [2]. Cyclic *β*-amino acids can also serve as building blocks for the synthesis of modified peptides.

Experimental
An 1100 Series HPLC system equipped with a solvent degasser, a pump, an autosampler, a column thermostat, a multiwavelength UV–Vis detector and ChemStation chromatographic data software (Agilent Technologies, Waldbronn, Germany), and a corona-charged aerosol detector from ESA Biosciences Inc. (Chelmsford, MA, USA) was applied for chromatographic measurements. Analyses were also performed on a Waters chromatographic system consisted of a M600 pump, a 2996 PDA detector (Waters Chromatography, Milford,

MA, USA) and connected with a Jasco 2031 Plus refractive index (RI) detector (Jasco Tokyo, Japan) and Empower 2 data manager software (Waters Chromatography). The HPLC columns used were Chiralpak ZWIX(+)™ and ZWIX(-)™ (150 x 3.0 mm I.D., 3-µm particle size for both columns). Chromatography was performed in isocratic mode at a flow rate of 0.6 mL min^{-1} and a column temperature of 25 °C. The dead-time (t_0) of the column was determined via the injection of acetonic solution of methanol.

Results and discussion

Influence of mobile phase composition on chromatographic parameters

Cyclic *β*-amino acids investigated in this study were chromatographed on Chiralpak ZWIX(+)™ and ZWIX(-)™ columns. In polar-ionic mobile phase mode, a mixture of MeOH as a protic solvent and MeCN as aprotic solvent in combination with acid and base additives provides the best enantioseparation of zwitterionic chiral CSPs. The increased retention observed for the investigated analytes at higher MeCN content were accompanied with increased selectivity and resolution in most cases. The observed chromatographic behavior can likely be explained on the basis of decreased solvation of the ionizable compounds. The values of selectivity increased monotonously in most cases, when the MeCN content in mobile phase changed from 20 to 60 *v%* due to enhanced electrostatic and H-bonding interactions. The best separation performances were achieved applying mobile phase composition of MeOH/MeCN (60/40 v/v) containing 25 mM TEA and 50 mM FA, thus most of the measurements were performed by using the above mentioned mobile phase system.
The applied *Chincona*-based selectors are diastereoisomers, but they often behave as pseudo-enantiomers. This extraordinarily useful property makes these chiral columns greatly effective in enantiomeric excess determination. Hence, the sequence of elution of the enantiomers can be reversal on change from the quinine-, to the quinidine-based CSP. The elution sequence was determined in all cases (when enantioseparation was achived) and was found to be reversal in every case on switching from ZWIX(+)™ to ZWIX(-)™. It should be mentioned that for Fmoc-substituted analytes no separation could be achieved on these stationary phases.

Effects of structures of N-substitution

The structure (substituent position and size) have a strong influence on the chiral recognition. In order to study the structure - retention (selectivity) relationships the same mobile phase system (MeOH/MeCN (60/40 *v*/*v*) containing 25 mM TEA and 50 mM FA) was applied. It was observed that with increase of the degree of substitution of the amino group and the size of the substituent the retention decreased appreciably. The values of k_1 were smaller for *N*-methylated, *N*-dimethylated and *N*-amidino substituted cyclic *β*-amino acids compared to the non-substituted ones for both CSPs. The lowest k_1 values were observed in the case of Fmoc-protected analytes. The Fmoc protection of the amino group results in decreased basicity with the consequence that the double ion pairing interaction mechanism is not possible anymore. Investigated the free amino acids it can be presumed that steric effects have a great influence on the selector-analyte interactions. The obtained results show that selectivity changed in different ways. The highest α values were observed for *N*-amidino substituted analytes. In addition, the values of selectivity was higher in the case of *N*-methylated compounds compared to *N*-dimethylated and non-methylated ones. Comparing the analytes containing cyclopentane- or cyclohexane skeleton the same trend was observed considering k_1 and α values. The *cis-trans* configuration and the degree of substitution possess marked influence on

the retention and selectivity. In the case of compounds with *trans* configuration k_1 values were higher for the studied analytes on both CSPs. This observation can be explained by more tight interactions between the cationic- and anionic-sight of the selector and the *trans*-isomers. Comparing the two CSPs, in most cases ZWIX(-)™ CSP was more suitable for the enantioseparation of the studied analytes. However, ZWIX(+)™ exhibited more effective separation for the *N*-amidino protected analytes.

Effects of temperature and thermodynamic parameters

In order to investigate the effects of temperature on the chromatographic parameters, a variable-temperature study was carried out on ZWIX(+)™ and ZWIX(-)™ over the temperature range 5–40 °C. The retention factors and selectivities generally decreased with increasing temperature in most cases. The changes observed in the selectivity with increasing temperature were not consistent. Resolution usually decreased with the increase of temperature on both ZWIX(+)™ and ZWIX(-)™ CSPs. On the other hand, an increasing tendency was also registered on ZWIX(-)™. All of the van't Hoff plots (ln α vs. 1/T curves) were linear. Under the conditions when Δ(ΔH°) was negative, Δ(ΔS°) was also negative, and positive Δ(ΔH°) was accompanied by positive Δ(ΔS°). The obtained results revealed that enantioselective separations were in most cases enthalpically-driven, but entropically-driven separation was also observed.

Conclusion

The chromatographic retention behavior and resolution proved to depend the composition of the bulk solvent, the acid and base additive, the temperature and the positions of the substituents. The retention behavior and resolution proved to dependent on the mobile phase compositions and the temperature. The studied CSPs, ZWIX(+)™ and ZWIX(-)™ exhibited complementary behavior in the direct enantioseparation of the non-methylated, *N*-methyl, *N*-dimethyl and *N*-amidino substituted analytes, while separation for the enantiomers of *N*-Fmoc protected cyclic *β*-amino acids could not be achieved on these CSPs. The values of thermodynamic parameters such as the changes in Δ(ΔH°), Δ(ΔS°) and Δ(ΔG°) proved to depend on the structures of the analytes and on the chiral selector used.

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