HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC SEPARATION OF THE ENANTIOMERS OF AMINO COMPOUNDS ON CHIRAL STATIONARY PHASES

Summary of PhD Thesis

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Institute of Pharmaceutical Chemistry, University of Szeged
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INTRODUCTION

The separation of enantiomers of chiral compounds by chromatographic methods and related techniques is one of the most important tasks in modern analytical chemistry, especially in the analysis of compounds of biological and pharmaceutical interest.

On a molecular level of living organisms, chirality is an important property of the building blocks of life, such as amino acids and sugars, and therefore of peptides, proteins and polysaccharides. In biological systems, these biomolecules exist in only one of the possible enantiomeric forms and display different responses to a pair of enantiomers of drugs, agrochemicals, food additives, fragrances, *etc*. Stereoselectivity is often a characteristic feature of enzymatic reactions, messenger-receptor interactions and metabolic processes.

Accordingly, chirality is also a major concern in the pharmaceutical industry. When the enantiomers of a drug are administered into a chirally selective living system, these enantiomers frequently exhibit differences in bioavailability, distribution, metabolic and excretion behavior and action. One of the enantiomers is often the more active stereoisomer for a given action (eutomer), while the other, less active one (distomer) may either contribute side-effects, display toxicity or act as an antagonist. The differences in biological properties of enantiomers arise from the differences in protein transport and binding, the kinetics of their metabolism and their stability in the environment.

Single enantiomers can be obtained via (*a*) the selective synthesis of one enantiomer or (*b*) the separation of racemic mixtures. Stereoselective syntheses are both expensive and time-consuming and they are rarely selected for large-scale separations. The enantiomers of a racemic mixture can be separated *indirectly* when diastereomer pairs are formed covalently; their separation can be achieved by taking advantage of their different chemical or physical properties on crystallization, nonstereoselective chromatography or distillation. Alternatively, *direct* processes based on the formation of noncovalent diastereomeric pairs of molecules can be achieved by interaction of a racemic mixture with a chiral selector, either as part of a chiral stationary phase (CSP) or as a chiral mobile phase additive. Enantioseparation can also be achieved by kinetic resolution procedures, with enantioselective membranes, simulated moving bed techniques or biotransformations involving the application of enzymes.

To control the enantiomeric purity of starting materials and products, reliable and accurate analytical methods are necessary. At an analytical level, sensitivity and selectivity are important requirements in many fields of academic, industrial and pharmaceutical research. Of all the existing separation methods adapted for analytical purposes, high-

performance liquid chromatography (HPLC) is the most widespread chiral separation technique in analytical and preparative resolutions and drug discovery.

AIMS

The primary aim of this work was to develop simple, convenient, readily available direct HPLC methods that are suitable not only for the separation of the enantiomers of racemic aminonaphthols and *β*-amino acids, but also for monitoring their synthesis and for identifying their absolute configuration. Enantiomers of the 1-(aminobenzyl)-2-naphthol, 2-(aminobenzyl)-1-naphthol and 1-(aminoalkyl)-2-naphthol derivatives are of significance since they can serve as chiral catalysts, chiral auxiliaries or synthetic building blocks. To the best of our knowledge, to date there has been no publication dealing with the HPLC separation of enantiomers of these aminonaphthol analogs. *β*-Amino acids have gained in importance in the past few decades with regard to their unique biological activity and their meaningful application in synthetic chemistry and drug research. Besides their own pharmacological activity, they can be used as building blocks for the preparation of modified (unnatural) analogs of biologically active peptides or starting substances of different heterocycles with the aim of preparing potential pharmacons.

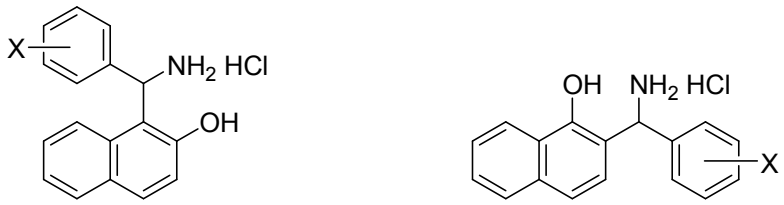
The first objective was to develop systematic methods for the optimized resolution of different *α*-aminobenzyl (Fig. 1) and *α*-aminoalkyl (Fig. 2) 1- and 2-naphthol analogs on 3,5-dimethylphenyl carbamoylated cellulose- and *β*-cyclodextrin (CD)-based CSPs. In order to investigate the thermodynamic functions of enantioselective adsorption, the effects of temperature on the chromatographic parameters were investigated on a cellulose *tris*-3,5-dimethylphenyl carbamoylated CSP.

The second objective was to optimize the enantioseparation of different *β*-substituted-*β*-amino acids (Fig. 3) on macrocyclic antibiotic CSPs and a recently designed chiral crown ether containing CSP.

The influence of different parameters, such as the nature and composition of the mobile phases and mobile phase modifiers, the buffer concentration, pH, *etc*. on chromatographic separations were extensively investigated for both substituted aminonaphthol analogs and *β*-amino acids. The effects of the structural features of the investigated analytes on the discrimination between the enantiomers were examined through the chromatographic parameters (retention, selectivity and resolution).

METHODS

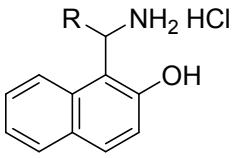
The enantiomers of the investigated analytes (Figs 1 and 2) were directly separated on different CSPs: 3,5-dimethylphenyl carbamoylated cellulose- and β-CD-based CSPs as well as macrocyclic antibiotics and chiral crown ether containing CSPs. The analytes were chromatographed without pre- or postcolumn derivatization.



1a-i **2a-f,h,i**

X = **a**: H, **b**: *p*-Me, **c**: *p*-OMe, **d**: *p*-F, **e**: *p*-Cl, **f**: *p*-Br, **g**: *p*-NO_2, **h**: *m*-Br, **i**: *m*-NO_2

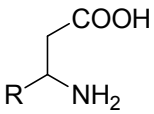
Figure 1.
Structures of 1-(aminobenzyl)-2-naphthol and 1-(aminobenzyl)-1-naphthol derivatives



3a-e

R = **a**: Me, **b**: Et, **c**: Pr, **d**: *i*Pr, **e**: *t*Bu

Figure 2.
Structures of 1-(aminoalkyl)-2-naphthol analogs



4a-r

X = **a**:Ph; **b**: *p*-Me-C_6H_4; **c**: *p*-CF_3-C_6H_4; **d**: *p*-OMe-C_6H_4; **e**: *m*-OMe-C_6H_4;
f: *o*-OMe-C_6H_4; **g**: *m,p*-diOMe-C_6H_3; **h**: *p*-F-C_6H_4; **i**: *p*-Cl-C_6H_4; **j**: *m*-Cl-C_6H_4;
k: *o*-Cl-C_6H_4; **l**: *m,p*-diCl-C_6H_3; **m**: *p*-Br-C_6H_4; **n**: *m*-Br-C_6H_4; **o**: 2-furyl;
p: 2-thienyl; **q**: 3-pyridyl; **r**: 1-naphthyl

Figure 3.
Structures of β-substituted-β-amino acids

Our measurements were carried out with three HPLC systems:

System I: An M-600 low-pressure gradient pump, equipped with an M-996 photodiode-array detector and a Millenium[32] 2.1 Chromatography Manager data system (all Waters Chromatography, Milford, MA, USA).

System II: A 1525 binary pump, a 487 dual-channel absorbance detector, a 717 plus autosampler and Breeze data manager software (all Waters Chromatography, Milford, MA, USA).

System III: An MD-2089 PLUS quaternary low-pressure gradient pump, an MD-2010 PLUS photodiode-array detector and a ChromPass 1.8 Chromatography Data System (all JASCO International Co., Tokyo, Japan).

All of the HPLC systems were equipped with a Rheodyne Model 7125 injector with a 20-µl loop (Rheodyne, Cotati, CA, USA).

A Radelkis OP/20811 pH-meter (Budapest, Hungary) was employed for pH measurements.

The columns were thermostated in a water bath, a cooling-heating thermostat (MK70, Mechanik Prüfgeräte, Medlingen, Germany) being applied. The accuracy of temperature adjustment was ±0.1 °C.

RESULTS

I. AMINONAPHTHOLS

1. The enantiomers of analogs **1a**-**i** and **2a**-**f**,**h**,**i** were separated isothermally on a cellulose *tris*-3,5-dimethylphenyl carbamate-based CSP (Chiralcel OD-H) in normal-phase mode. The 2-propanol content of the mobile phase had a great influence on the retention factors (*k'*), selectivities (α) and resolutions (R_S) of both the 1- and 2-naphthol derivatives.

2. It was established that the position of the α-aminobenzyl subtituent in the 1- and 2-naphthol analogs influenced the retention and selectivity. At constant mobile phase composition, the difference between the retention factors of the 1- and 2-naphthol analogs revealed that the steric arrangements of the former are more favorable for chiral recognition. Methyl and halogen substitution on the α-aminobenzyl group merely slightly influenced *k'* and α, whereas the methoxy- and nitro-substituted analogs exhibited more pronounced effects.

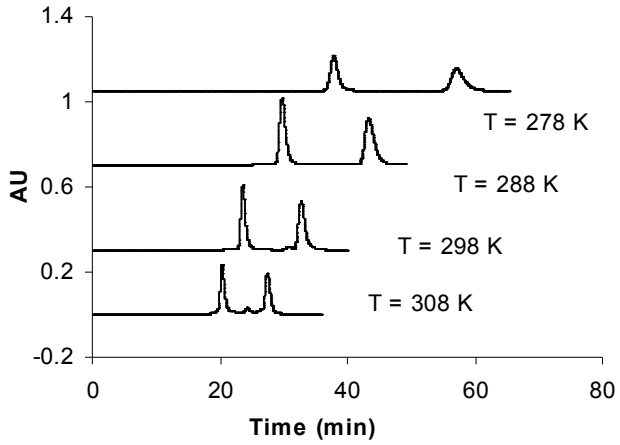


Figure 4.
Temperature dependence of the chiral separation of **2c** at the mobile phase composition *n*-hexane/IPA/DEA=55/45/0.1

3. The chromatographic data on the analogs **1a-i** and **2a-f**,**h**,**i** were measured and calculated at four temperatures in the range 278-308 K. The *k'*, α and R_S values for the enantiomers of all the investigated compounds decreased with increasing temperature (Fig. 4). The van't Hoff plots of $R \ln \alpha$ *vs* $1/T$ afforded the differences of the changes in enthalpy ($\Delta(\Delta H^o)$), entropy ($\Delta(\Delta S^o)$) and Gibbs free energy ($\Delta(\Delta G^o)$). The values of the thermodynamic parameters depended on the structures of the compounds: the 1-(aminobenzyl)-2-naphthol derivatives exhibited the more negative values. The thermodynamic data revealed that all the investigated analogs separated via the same enthalpy-driven chiral recognition mechanism.

4. The enantiomers of analogs **1a-i** and **2a-f**,**h**,**i** were separated on a 3,5-dimethylphenyl carbamoylated β-CD-based CSP (Cyclobond DMP) in normal-phase mode. Optimization of the separations was achieved by variation of the mobile phase additives and their composition. The position of the α-aminobenzyl substituent in the 1- and 2-naphthol analogs influenced the chromatographic parameters. Methyl, methoxy and halogen substituents on the α-aminobenzyl group only slightly influenced the retention and selectivity, while nitro substitution promoted chiral recognition on the Cyclobond DMP CSP.

5. The enantiomers of various 1-(aminoalkyl)-2-naphthol analogs (**3a-e**, Fig. 2) were separated on cellulose *tris*-3,5-dimethylphenyl carbamate-based CSPs (Chiralcel OD-H and Chiralcel OD-RH), in normal- and reversed-phase modes. Baseline separations were achieved in all cases in both chromatographic modes.

6. In the normal-phase mode, the chromatographic parameters (*k'*, α and R_S) depended on the 2-propanol content of the mobile phase. The dependence of the experimental chromatographic *k'* data on the volume in the anchor sphere of the substituents (V^a, Meyer parameter) was investigated by linear regression analysis, which revealed that the bulkier alkyl substituents inhibited the interaction with the selector of the Chiralcel OD-H CSP, but enhanced the selectivity and resolution. In reversed-phase mode, the pH of the mobile phase, the buffer concentration and the organic modifier content influenced the retention factors,

while the selectivities and resolutions exhibited only slight changes. The *k'*, α and R_S values increased with increasing length and bulkiness of the alkyl chain substituents.

II. β-AMINO ACIDS

7. The direct separation of the β-substituted-β-amino acid enantiomers (**4a**-**r**, Fig. 3) was performed on six CSPs: teicoplanin-based (Chirobiotic T and T2) columns, a teicoplanin aglycone-based (Chirobiotic TAG) column, vancomycin-based (Chirobiotic V and V2) columns and a ristocetin A-based (Chirobiotic R) column with polar-ionic and reversed-phase mobile phases. Among the applied eluent systems, the polar-ionic mode was the most successful for the resolution of the analytes.

8. Each of the Chirobiotic CSPs showed some selectivity for the investigated analytes, but the newly developed Chirobiotic T2 proved to be the most suitable of the six applied CSPs. The results observed point to the fact that the macrocyclic glycopeptides are to some extent complementary to one another, due to the subtle differences in diastereomeric binding sites between the different Chirobiotic phases. It was found that the substitution on the aromatic rings slightly influenced the chromatographic parameters.

9. Application of the (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid-based CSP was noteworthy: it provided a very valuable means of enantioseparation of the differently substituted β-amino acids. Among the acidic mobile phase modifiers applied for optimization of the separations, AcOH proved to be the most suitable. The organic content of the mobile phase influenced the chromatographic parameters of the analytes.

10. The chromatographic retention behavior was found to be dependent on the natures and positions of the substituents on the phenyl ring of the β-substituted-β-amino acid enantiomers. The *meta*-substituted analogs were generally more strongly retained than the *para*-substituted ones and the *ortho* position of the substituents was unfavorable for enantioseparation.

11. The elution sequences in such cases were determined, when configurally known samples were available.

PUBLICATIONS

I. **Anita Sztojkov-Ivanov**, István Szatmári, Antal Péter, Ferenc Fülöp
Structural and temperature effects in the high-performance liquid chromatographic enantioseparation of 1-(α-aminobenzyl)-2-naphthol and 2-(α-aminobenzyl)-1-naphthol analogs
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IV. Róbert Berkecz, István Ilisz, **Anita Sztojkov-Ivanov**, István Szatmári, Ferenc Fülöp, Daniel W. Armstrong, Antal Péter
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CONFERENCE LECTURES

VII. **Anita Sztojkov-Ivanov**, István Szatmári, Antal Péter, Ferenc Fülöp
Structural and temperature effects in the high-performance liquid chromatographic enantioseparation of 1-(*α*-aminobenzyl)-2-naphthol and 2-(*α*-aminobenzyl)-1-naphthol analogs
6th Balaton Symposium, Siófok, 7-9 September **2005**, Abstr.: P-62.

VIII. **Anita Sztojkov-Ivanov**, István Szatmári, Antal Péter, Ferenc Fülöp
Structural and temperature effects in the high-performance liquid chromatographic enantioseparation of 1-(*α*-aminobenzyl)-2-naphthol and 2-(*α*-aminobenzyl)-1-naphthol analogs
Pharmacy: Smart Molecules for Therapy, Budapest, 12-14 October **2005**, Abstr.: P-90.