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THE APPLICATION OF POLYSACCHARIDE AND CYCLOFRUCTANE CHIRAL STATIONARY PHASES FOR THE SEPARATION OF ENANTIOMERS

Ph.D. THESIS

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1. INTRODUCTION

It is clearly necessary for analytical procedures to keep pace with the rapid developments in the natural sciences and the organic chemical industry. It is not easy to follow the changes in analytical techniques. A method which was progressive 10 years ago may still be useful today, but new techniques may no be available with which to determine analytical parameters in a much easier, cheaper and faster way. There well-separated groups of materials are usually examined by the qualitative control laboratory in the pharmaceutical industry: the incoming initial materials or basic substances, the intermediate samples which are produced during the manufacturing process, and the final products. The pharmacopoeias and their directives strictly control the characterization of the active agents and the final products, whereas only guidelines are given regarding the initial materials. As a result, it is clear that there are areas in the examination of basic materials where new and economical methods can be introduced to replace old, still-working, but not the cheapest procedures.

One of the most important tasks in modern analytical chemistry is the analysis of chiral compounds, and especially the separation of enantiomers of biological and pharmaceutical significance. Most of the organic compounds in the living world are chiral analytes, which exist in only one enantiomeric form; the different enantiomers may display different interactions with chiral drugs, food additives, agricultural chemicals, aromatics, etc. If a racemic drug comes into contact with a chirally selective living system, the effects of the enantiomers may be different, resulting in different biological consequences, division, metabolism, excretion, etc. For example, the *S* enantiomer of penicillinamine is an excellent drug against inflammation, while the *R* enantiomer is toxic (Fig.1). Similarly, Levodopa, applied in the therapy of Parkinson's disease, contains only the *S* enantiomer, because the *R* form causes a decrease in the level of the granulocytes in the blood.

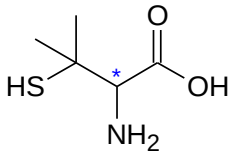

Figure 1. Penicillinamine

There are rare cases where the effects of the two enantiomers are the same, e.g. both enantiomers of ibuprophen are active against inflammation. (Fig. 2).

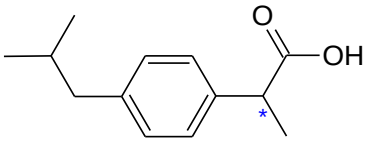

Figure 2. Ibuprophen

From a drug safety aspect, the role of chirality is crucial. For quite a long time a mixture of stereoisomers can be released as a drug only with good reason [Gross 1989]. In the case of racemates all the clinical and toxicological examinations must today be performed for both the enantiomers and the racemates. Releasing a racemic drug obviously involves extra costs, and the current developments therefore generally involve pure enantiomers.

2. AIMS

Our aims were to develop liquid chromatographic methods for the separation of different biologically and pharmacologically important stereoisomers, and to investigate the possibilities of their separation on newly developed chiral stationary phases.

The compounds investigated were the following:

- aminonaphthol enantiomers with two chiral centres on polysaccharide based chiral stationary phases
- amino 1- or 2-naphthol enantiomers with one chiral center on polysaccharide based chiral stationary phases
- amino 1- or 2-naphthol enantiomers with one chiral center on a newly developed cyclofructane based column
- amino acid amide, diamines and amino alcohols on cyclofructane based chiral stationary phases

The effects of the eluent composition, and the nature and quantity of the polar modifier on the separations were studied through the determination of the chromatographic parameters. The effects of the structure on the chiral discrimination were characterized through the use of compounds with different substituents. Conclusions relating to the separation mechanism were drawn by studying the effects of temperature on the chromatographic parameters.

3. EXPERIMENTALS

3.1. Apparatus

For the direct chromatographic methods polysaccharide and cyclofructane based chiral stationary phases were used. The HPLC apparatus applied were two Waters systems:

The first HPLC system consisted of a Waters Breeze system: a 1525 binary pump, a 487 dual-channel absorbance detector, a 717 plus autosampler, and Breeze data manager software (Waters Chromatography, Milford, MA).

The second system consisted of a Waters M-600 gradient HPLC pump, Waters M-2996 photodioda array detector, Waters „Millenium 32 Chromatography Manager" data manager system (Waters) and Spark Mistral column thermostate (Spark Holland, Emmen, Netherlands). Both chromatographic systems were equipped with a Rheodyne Model 7125 injector (Cotati, CA) with a 20-µl loop.

3.2. Investigated analytes

- aminonaphthol enantiomers with two chiral centers
- amino-1- or 2-naphthol enantiomers with one chiral center
- amino acid amide
- diamines
- amino alcohols

4. RESULTS

The enantioseparation of several biologically and pharmaceutically important compounds containing one or two chiral centres was investigated, e.g. aminonaphthols, aminoalcohols and the stereoisomers of diamines. Direct liquid chromatographic methods were introduced for newly-developed cyclofructane- and polysaccharide-based columns.

Enantiomer separation on polysaccharide-based chiral stationary phases

1. The separation of aminonaphthols with one or two chiral centres was achieved on polysaccharide-based columns. For the liquid chromatographic measurements, normal-phase methods were chosen, where the eluent contained 98-70% heptane, as apolar component and

2-30% of a polar modifier. Three types of polysaccharide columns were applied for the investigation of the first group of 2-naphthol analogues (**1-5**), while for the 1- and 2-naphthol (**6-15**) analogues all five available columns were used. On our results indicated that the Cellulose-1 column with the 3,5-dimethylphenylcarbamate selector was the most effective, since all the investigated analytes could be separated through the choice of appropriate conditions.

2. The effects of the eluent composition and the nature of the polar modifier (alcohol) on the separations were also studied. With increasing alcohol concentration in the mobile phase, the retention decreased in every case, which reflects typical normal-phase behaviour. The nature of the polar modifier had a strong influence on the retention factor, the selectivity and the resolution, which might be attributed to the differences in polarity and solvation capability of the alcohols.

3. The effect of temperature was investigated in the relatively wide range between 5 °C and 50 °C. In most cases, the chromatographic parameters decreased with increasing temperature. From van't Hoff plots, entropy and enthalpy changes were calculated. In most cases, enthalpy-controlled separations were achieved, but for certain analogues entropy-controlled separations were also observed. (In entropy-controlled separations, higher resolution can be achieved in a shorter analysis time.) For analogues **2c-d,** an extended temperature range (-5 °C to 40 °C) was applied and temperature-induced elution sequence changes could be observed. The elution sequence of the enantiomers is an important feature in chiral analysis because the biological and catalytic activities of the enantiomers depend significantly on the configurations of the analogues.

4. Structure – retention relationships were studied to be able to draw conclusions as to how the analyte structure influences the chromatographic behaviour. The results revealed that the nature and the position of the substituent exerted a significant influence on the sample polarity, the geometrical structure and hence the chromatographic behaviour. However, we should not ignore the functional groups of the chiral selector and the structure of the polysaccharide, which affect the interaction between the sample and selector, and thus the separation. It can be stated that the presence of a naphthyl group resulted in a stronger π-π interaction, and hence the retention increased. On the amylose-based column higher retention was generally observed mainly due to the rigid, helical structures. When a heteroatom (nitrogen) was present at different positions of the aromatic ring (ortho, meta or para), different chromatographic results were observed. In order to study the effects of alkyl substituents, the Meyer parameters were examined as a function of the retention factor. It

could be concluded that the chromatographic parameters depend strongly on the size and the geometry of the alkyl group.

Enantiomer separation on cyclofructane - based chiral stationary phases

5. The separations of 1- and 2-naphthol analogues, acid amides, diamines and amino alcohols were investigated on three different cyclofructane - based chiral columns. The normal - phase mode was applied, where the apolar component of the eluent was 40-80% heptane, and 60-20% of polar modifier was used with 0.1% TFA as additive. The separations of 1- and 2-naphthol enantiomers were achieved on the CF6-IP column, while the diamines, acid amides and amino alcohols were studied on the CF6-IP, CF6-RN and CF6-DMP chiral columns. For the naphthol analogues (except for **25, 26, 30, 34**), separation proved successful with higher than 1.0 resolution by choosing the appropriate conditions. The other group of examined analogues could be partially resolved on CF6-IP, but better separations were achieved with the use of the CF6-RN and CF7-DMP columns. The effects of polar components in the mobile phase were studied. With decreasing alcohol concentration, the retention factors increased, but in most cases there were no significant changes in the selectivity. As regards the resolution, the results were different. In the cases of the naphthol and amino acid amide analogues, the resolution usually increased with decreasing alcohol concentration, while for the amino alcohol analogues lower resolutions were observed. No relevant changes were observed in the resolution of the diamines. The results obtained demonstrate that the application of branched - chain alcohols as polar modifiers resulted in higher selectivity and resolution through better solvation capabilities.

6. The effects of the concentration of the acidic modifier applied in the mobile phase were also investigated. On increase of the TFA concentration, the retention factor decreased slightly in every case. This might be attributed to the increased ionic interactions between the sample and the mobile phase. For all of the examined analogues, the application of 0.1% TFA proved to be the most effective. Six different acidic modifiers were applied for the separation of amino acid amide, diamine and amino alcohol analogues. On the use of sulphuric acid and perchloric acid, we could achieve separations with acceptable retention times. On the application of acetic acid in the eluent, higher retention factors were observed, but the selectivity and resolution did not follow this trend. This might be explained in terms of the increasing non-enantioselective interactions.

7. Furthermore, the effects of temperature were studied in the range 5–40 °C. With

increasing temperature, the chromatographic parameters decreased in every case. When van't Hoff plots were used for the determination of the thermodynamic parameters, negative values were obtained in all cases, which points to enthalpy - controlled enantioseparations.

8. Our observations permit several conclusions on the structure – chromatographic behaviour relationships. The investigated naphthol analogues contained a phenyl ring with different chloro, fluoro and bromo substituents. For these compounds (except for **23** and **38)** the chromatographic parameters were higher relative to the non-substituted analogues. The various steric positions of the substituents led to further differences. The presence of a nitro group resulted in a higher retention time, but without significant differences in selectivity and resolution, probably because of the increased ratio of the non-enantioselective interactions.

For 2-naphthol analogues with different alkyl substituents, the retentions were higher than for the analogues with a phenyl substituent. Alkyl substituents have different effects on the selectivity and resolution. For larger analytes, lower chromatographic parameters were obtained. The geometrical effects of the alkyl substituents as a function of the Meyer parameters were also studied. It was found that the larger substituents prevented the interaction between the sample and the selector.

9. From studies of the various groups of analogues it was evident that, besides the π-π and polar interactions, H-bonding has an important role in chiral discrimination. Better results were observed for the analytes possessing carbonyl substituents. Moreover, the separation of amino alcohols resulted in better resolution on both columns, which might be explained by the H-bonding ability of the OH group.

5. LIST OF PUBLICATIONS

5.1. The thesis is based on the following publications

1. A. Aranyi, I. Ilisz, Z. Pataj, I. Szatmári, F. Fülöp, A. Péter

High-performance liquid chromatographic enantioseparation of 1-(phenylethylamino)- or 1-(naphthylethylamino)methyl-2-naphthol analogs and a temperature-induced inversion of the elution sequence on polysaccharide-based chiral stationary phases

Journal of Chromatography A, 1218, 4869-4876 2011

Impact factor: **4,582**

2. A. Aranyi, I. Ilisz, I., Z. Pataj, I: Szatmári, F. Fülöp, D. W. Armstrong, A. Péter

High-performance liquid chromatographic enantioseparation of Betti base analogs on a newly

developed isopropyl carbamate-cyclofructan6-based (IP-CF6) chiral stationary phase
Chirality, 23, 549-556 2011
Impact factor: **2,350**

3. A. Aranyi, Á. Bagi, I. Ilisz, Z. Pataj, F. Fülöp, D. W. Armstrong, A. Péter
High-performance liquid chromatographic enantioseparation of amino compounds on a newly developed isopropyl carbamate-cyclofructan6-based (IP-CF6) chiral stationary phase
Journal of Separation Science, 35, 617-624 2012
Impact factor: **2,733**

4. A. Aranyi, I. Ilisz, N. Grecsó, R. Csütörtöki, I. Szatmári, F. Fülöp, A. Péter
Development of the high-performance liquid chromatographic method for the enantioseparation of unusual glycine ester analogs on polysaccharide-based chiral stationary phases.
Journal of Pharmaceutical and Biomedical Analysis, 76, 183-191 2013
Impact factor: 2,967
Cumulative impact factors: 12,632

5.2. Other publications

5. Z. Pataj, I. Ilisz, **A. Aranyi**, E. Forró, F. Fülöp, D. W. Armstrong, A. Péter
LC Separation of γ-Amino Acid Enantiomers
Chromatographia, 71, 13-19 2010
Impact factor: **1,075**

6. I. Ilisz, Z. Pataj, **A. Aranyi**, A. Péter
Chiral HPLC separation of amino acid enantiomers and epimers of small, biologically important peptides
Mini Reviews in Medicinal Chemistry, 10, 287-298 2010
Impact factor: **2,622**

7. I. Ilisz, **A. Aranyi**, Z. Pataj, A. Péter
Enantioseparations by High-Performance Liquid Chromatography Using Macrocyclic Glycopeptide-Based Chiral Stationary Phases
Chiral Separations-Methods and Protocols

Szerkesztő: G. Scriba, Humana Press, Totowa, NJ, USA, Könyvfejezet

8. I. Ilisz, Z. Pataj, **A. Aranyi**, A. Péter
Macrocyclic antibiotic selectors in direct HPLC enantioseparations
Separation and Purification Reviews, 41, 207-249 2012
Impact factors: **2,429**

9. I. Ilisz, **A. Aranyi**, Z. Pataj, A. Péter
Recent advances in the enantioseparation of amino acids and related compounds: A review
Journal of Pharmaceutical and Biomedical Analysis, 69, 28-41 2012
Impact factor: **2,967**

10. I. Ilisz, **A. Aranyi**, Z. Pataj, A. Péter
Enantiomeric separation of nonproteinogenic amino acids by high-performance liquid chromatography
Journal of Chromatography A, 1269, 94-121 2012
Impact factor: **4,612**

11. L. Sipos, I. Ilisz, **A. Aranyi**, Zs. Gecse, M. Nonn, F. Fülöp, M.H. Hyun, A. Péter
High-performance liquid chromatographic enantioseparation of unusual isoxazoline-fused 2-aminocyclopentanecarboxylic acids on (+)-(18-Crown-6)-2,3,11,12-tetracarboxylic acid-based chiral stationary phases
Chirality, 24, 817-824 2012
Impact factor: **2,350**

12. I. Ilisz, **A. Aranyi**, A. Péter
Chiral derivatizations applied for the separation of unusual amino acid enantiomers by liquid chromatography and related techniques
Journal of Chromatography A, 1296, 119-139 2013
Impact factor: **4,582**

13. I. Ilisz, N. Grecsó, **A. Aranyi**, P. Suchotin, D. Tymecka, B. Wilenska, A. Misicka, F. Fülöp, W. Lindner, A. Péter
Enantioseparation of β^2-amino acids on cinchona alkaloid-based zwitterionic chiral stationary

phases. Structural and temperature effects.
Journal of Chromatography A, 133, 44–54 2013
Impact factor: **4,582**

14. A. Aranyi, I. Ilisz, A. Péter; F. Fülöp, G. K. E. Scriba
Cyclodextrin-mediated enantioseparation of phenylalanine amide
derivatives and amino alcohols by capillary electrophoresis—Role of complexation constants and complex mobilities
Electrophoreses – accepted manuscript – DOI 10.1002/elps.201400142
Impact factor: 3,261
***Cumulative impact factors*: 41,112**

5.3. Congress abstracts and posters

Aranyi A., Ilisz I., Pataj Z., Szatmári I., Fülöp F., Péter A.
Aminonaftol sztereoizomerek nagyhatékonyságú folyadékkromatográfiás elválasztása poliszacharid alapú királis kolonnákon
Elválasztástudományi Vándorgyűlés
10-12 November 2010, Tapolca, Hungary.

A. Péter, **A. Aranyi,** I. Ilisz, Z. Pataj, I. Szatmári, F. Fülöp
High-Performance Liquid Chromatographic Enantioseparation of Aminonaphthol Analogs on Polysaccharide-Based Chiral Stationary Phases
ISC 28th International Symposium on Chromatography,
12-16 September 2010. Valencia, Spain.

A. Aranyi, I. Ilisz, Z. Pataj, I. Szatmári, F. Fülöp, A. Péter
HPLC Enantioseparation and a Temperature-Induced Inversion of the Elution Sequence of 1-(Phenylethylamino)- or 1-(Naphthylethylamino)methyl-2-Naphthol Analogs
36th International Symposium on High-Performance Liquid Phase Separations and Related Techniques, 19-23 Jun 2011. Budapest, Hungary.

A. Aranyi, I. Ilisz, Z. Pataj, I. Szatmári, F.Fülöp, A. Péter
HPLC Enantioseparation and a Temperature-Induced Inversion of the Elution Sequence of Aminonaphthol Analogs

17th International Symposium on Separation Sciences,
5-9 September 2011. ClujNapoca, Romania.

A. Aranyi, I. Ilisz, Z. Pataj, I. Szatmári, F. Fülöp, A. Péter
HPLC Enantioseparation of Aminonaphthol Analogs on Polysaccharide-Based Chiral Stationary Phases
ISC 29th International Symposium on Chromatography,
9-13 September 2012. Torun, Poland.

A. Aranyi, I. Ilisz, N. Grecsó, M. Alexandra, D. Tymecka, S. Wernisch, W. Lindner, A. Péter
ß-aminosavak folyadékkromatográfiás elválasztása, új zwitterion típusú állófázison
Vegyészkonferencia,
23-25 Jun 2013. Hajdúszoboszló, Hungary.

A. Aranyi, I. Ilisz, A. Péter; F. Fülöp, G. K. E. Scriba
Determination of binding constants and the influence of the cyclodextrin cavity size for the EMO on the separation of amino compounds by capillary electrophoresis
MicroBioseparation Conference,
27 Apryl- 1 May 2014. Pécs, Hungary.

5.4. Presentations

Separation of aminonaphthol analogs by chiral liquid chromatography
Ifjú Szerves Kémikusok Támogatásáért Alapítvány Előadóülése, Szeged, 2010.

Separation of Betti-bases analogs by chiral liquid chromatography
XXXII. Kémiai Előadói Napok, Szeged, 2010.

Separation of aminonaphthol analogs by chiral liquid chromatography
Műszaki TDK, Temesvár, 2011.

Separation of aminonaphthol analogs by chiral liquid chromatography
XXX. OTDK, Kémiai és Vegyipari Szekció, Analitikai Kémia szekció, Pécs, 2011.

Separation of Betti-bases analogs by chiral liquid chromatography on polysaccharide stationary phases

Ifjú Szerves Kémikusok Támogatásáért Alapítvány Előadóülése, Szeged, 2011.

Separation of ß^2-amino acids on zwitterionic based chiral stationary phases

MTA Peptidkémiai Munkabizottság Tudományos Ülése, Balatonszemes, 2013.