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Short Communication

Enantiomeric Separation of Underivatized Amino Acids: Predictability of Chiral Recognition on Ristocetin A Chiral Stationary Phase

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ABSTRACT The present work aimed to investigate the predictability of the chromatographic behavior for the separation of underivatized amino acids on ristocetin A, known as Chirobiotic R, using a DryLab high-performance liquid chromatography (HPLC) method development software, which is typically used to predict the effect of changing various chromatographic parameters on resolution in the reversed phase mode. After implementing the basic runs, and judging the predictability via the computed resolution map, it can be deduced that the chiral recognition mechanisms tend towards a hydrophilic interaction chromatography rather than the reversed phase mode, which limits the ability of DryLab software to predict separations on Chirobiotic R. *Chirality 26:132–135, 2014*.

KEY WORDS: DryLab software; ristocetin A; amino acids; chiral recognition mechanisms

INTRODUCTION

The macrocyclic antibiotics chiral stationary phase (CSP), unlike other classes of chiral selectors, comprise a large variety of structural types, including ansamycins (rifamycins), the polypeptide antibiotic thiostrepton, and glycopeptides. Among these, the most common and promising are the macrocyclic glycopeptides, which have been introduced as chiral selectors by the pioneer work of Armstrong et al.[1] In a relatively short time after their introduction to the market, they were used successfully in most chromatographic and electrophoretic methods of analysis. Besides the derivatized linear or branched carbohydrates (e.g., cellulose and amylose), macrocyclic glycopeptides appear to be the most successful chiral selectors used to date.[2,3]

They represent a group of structurally diverse naturally occurring compounds. Their molecular masses are in the range of 1000–2100 g/mol. They all share a basket-shaped aglycon framework, which consists of either three or four fused macrocyclic rings but differ in size, shape, and the geometrical arrangement of their numerous stereogenic centers and functional groups responsible for their enantioselective properties. The carbohydrate moieties attached to the aglycon basket contain ionizable groups (carboxylic and amino groups), allowing ionic interactions involved in chiral recognitions, to take place, which are in fact considered important players in the whole process of enantioselectivity.[4,5]

Vancomycin,[1] teicoplanin,[6] ristocetin A,[7] and the aglycone of teicoplanin[8] are commercially available and marketed under the trade names of Chirobiotic V, T, R, and TAG, respectively. They are prepared by covalently binding the corresponding glycopeptides selectors to spherical silica gel via linkage chains employing a variety of chemistries that aim to ensure their stability without losing their chiral recognition properties.[9,10]

The constitutional complex structure of the macrocyclic glycopeptides chiral selectors has been an obstacle in understanding their underlining chiral recognition.[11] All possible molecular interactions are responsible for retention and enantioselectivity, as proposed by Berthod et al.,[12] who found that the interactions that may occur between the CSPs and the analyte are: a charge–charge interaction, hydrogen bonding, steric hindrances, π–π interactions, ion dipole, dipole–dipole, dipole-induced dipole, and Van der Waals forces.[12,13] Nevertheless, the detailed recognition mechanism on a molecular basis is not yet fully elaborated.[5]

The macrocyclic glycopeptides are multimodal, as they can be coupled with different mobile phase systems: reversed, normal, polar organic, polar ionic, or supercritical fluid chromatography mode. The enantioselectivity of the macrocyclic CSPs are different in each of the operating modes, because of the different separation mechanisms that govern in each of these modes. This work aimed to investigate whether the dominating mode of Chirobiotic R is reversed using DryLab software under reversed phase mode.

The earliest studies performed to predict the enantioselectivity of a number of chiral compounds on derivatized β-cyclodextrin stationary phase was based on free energy calculations of substituents present on the stereogenic center. This study was able to predict the possibility of enantiomeric separation on a specific stationary phase rather than the elution order on

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this phase.[14] On the other hand, ACD Lab software was able to predict the optimum %B required for the separation of enantiomers of eszopiclone on chiral AGP (α_1-acid glycoprotein stationary phase).[15] As well, Chromsword was used to separate chiral drugs on the polysaccharide-based stationary phase in normal phase mode (http://www.chromsword.com/en/publications/).

A few reported trials aimed to predict the enantioselectivity of chiral drugs using DryLab. The software was able to predict the separation of N-derivatized amino acids with 2,4-dinitrofluorobenzeneon quinine carbamate-based chiral anion-exchanger[16] and tert-butyl carbamoylated quinine[17] stationary phases, where N-derivatized amino acids using 3,5-dinitrobenzylchloroformate were successfully resolved. In both cases, the amino acids were derivatized.

DryLab software was successfully used to predict the chiral chromatographic behavior of several compounds on Chirobiotic V, which is expected to dramatically ease method development of enantiomer separations of some racemic drugs on this CSP.[18] This also led to a better understanding of the chiral recognition of the CSPs regarding the studied analyte and parameters.[18]

Investigating the predictability of enantioseparation of underivatized amino acids on ristocetin A chiral stationary phase using the DryLab software will provide more information regarding the chiral recognition mechanisms involved in the enantioseparation of the amino acids used in this study.

MATERIALS AND METHODS

Chemicals and Reagents

Regents used were methanol (high-performance liquid chromatography [HPLC] grade; Sigma-Aldrich, Germany) and ultrapure water (Elga lab.). *Rac*–Phenylalanine and *rac*–valine were purchased from Merck Chemicals (Germany).

Equipment and Software

HPLC (Thermo Finnigan Spectrasystem, UK) consists of: pump model P2000, detector model UV3000, autosampler model AS3000 (UK). The column used was Chirobiotic R bonded ristocetin A-based phase (250 x 4.6 mm, 5 μm particle size) purchased from Supelco (Bellefonte, PA). Softwares used were Chromquest 4.2 data system (UK) data acquisition for, DryLab2000Plus and PeakMatch v. 3.60 (Molnár Institute for Applied Chromatography, Germany).

Determination of the Predictability of the CSPs

Experimental limitations. The parameters that can be optimized by DryLab software are: percent organic phase, temperature, pH, and flow rate. Macrocyclic glycopeptides (Chirobiotic R) have limitations regarding operating pressure (max 240 bar) and temperature (max 45 °C), and hence considerable variations of temperature and flow rate intervals were not possible to consider. Accordingly, the percent organic phase was the only investigated parameter.

Experimental preliminary runs. Preliminary runs involved injecting both racemic amino acids (phenylalanine and valine) into two isocratic runs at 40% and 60%B at 30 °C.

The systematic approach involved: two isocratic runs with 20% change in organic phase.[19] The isocratic runs were 40% B and 60% B. Repetitive injections of the analytes sample solution (1 mg/mL) were performed under each condition, until at least two consecutive reproducible chromatograms were obtained regarding peak areas (±10%) and retention times (±0.02 min). Mobile phase conditions were A = ultrapure water and B = methanol, at a flow rate 1.0 ml/min and wavelength of detection at 225 nm. The column oven was adjusted to 30 °C.[7]

RESULTS AND DISCUSSION

Racemic phenylalanine and valine were used to investigate the predictability of retention and resolution on Chirobiotic R using DryLab and deducing the possible reversed phase behavior as the main mode of enantiorecognition on this phase. Temperature was not a studied variable on Chirobiotic R (due to temperature limitations).

For phenylalanine, the preliminary run conditions were performed with a mobile phase composed of A: water and B: methanol at 1 mL/min and 30 °C. The two isocratic runs involved 40% and 60% B.

At 40% B, phenylalanine enantiomers were separated at t_R 4.452 min and 5.257 min, while at 60% B, t_R were 4.524 min

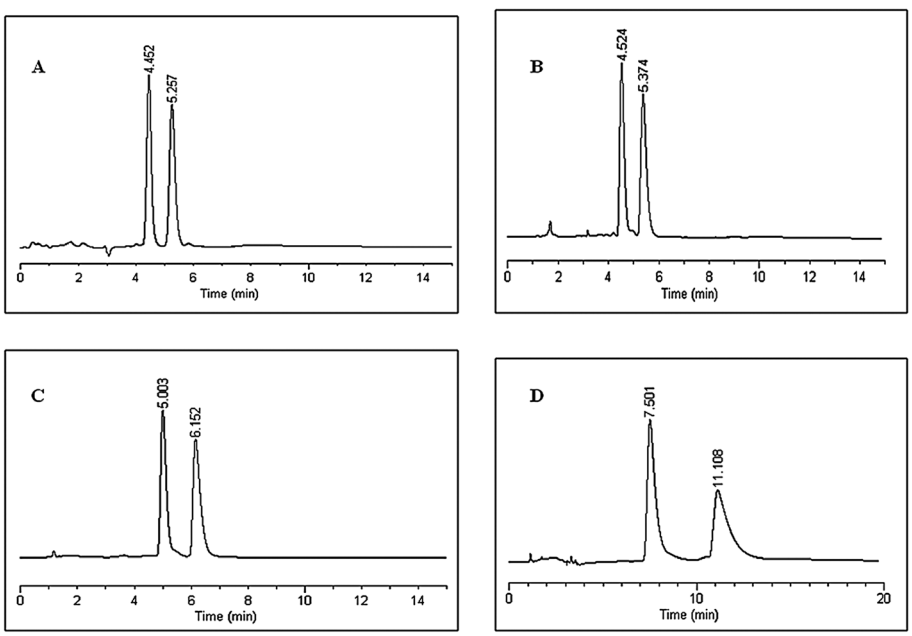

Fig. 1. Chromatograms of phenylalanine enantiomers on Chirobiotic R at different %B: (**A**) 40% B, (**B**) 60% B, (**C**) 80% B, and (**D**) 100% B.

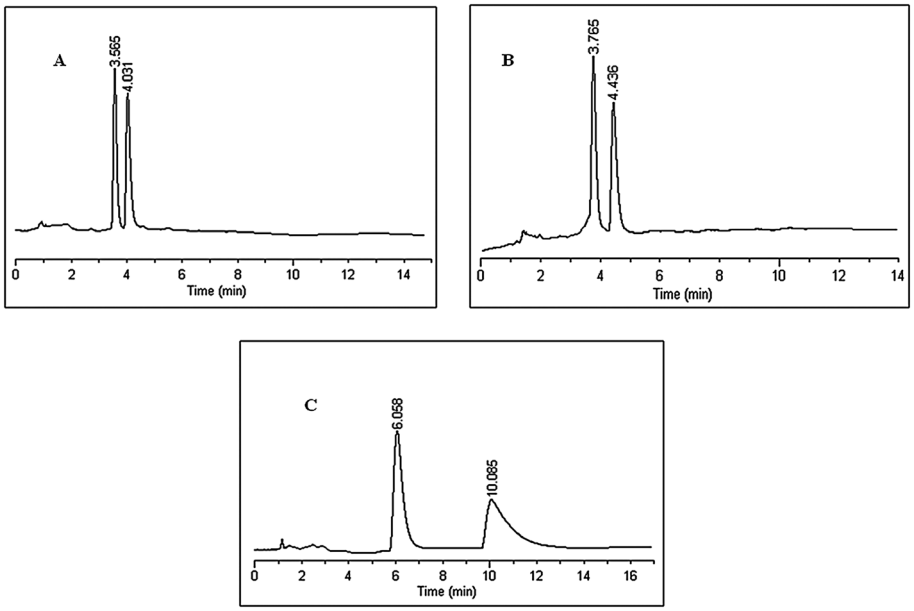

Fig. 2. Chromatograms of valine enantiomers on Chirobiotic R at different %B, (**A**) 40% B, (**B**) 60% B, and (**C**) 100% B.

and 5.374 min, for the first and the second enantiomers, respectively (Fig. 1). The effect of an increase 20% B on t_R of phenylalanine was minimal. It was also observed that the behavior was atypical to reversed phase mode, because the enantiomers were slightly retarded when %B increased. It was important to increase %B further to verify this observation. It was confirmed that the enantiomers were significantly retarded at higher %Bwhen it was increased to 80% B, where t_R were 5.003 min and 6.152 min. Furthermore, at 100% B, t_R were 7.501 min and 11.108 min for the first and the second enantiomers, respectively. It was also observed that R_s of phenylalanine enantiomers was enhanced at a higher percentage of the organic modifier.

All the chromatographic conditions performed for phenylalanine were repeated for the enantiomeric resolution of racemic valine. At 40% B, valine enantiomers were separated at t_R 3.565 min and 4.031 min for the first and the second enantiomers, respectively. While at 60% B, t_R were 3.765 min and 4.436 min (Fig. 2). Increasing %B by 20% was found to have small effect on retention, which was unlike the reversed phase mode. A higher percentage of B (100%) t_R were 6.058 min and 10.085 min, for the first and the second enantiomers, respectively, and R_s improved at increasing amounts of the organic modifier (Fig. 2).

It can be concluded that the retention mode on Chirobiotic R, in the conditions studied in this work, is a hydrophilic interaction chromatography rather than reversed phase mode, where amino acids being highly polar and charged analytes are retained predominantly by charge–charge interactions between the amino acids carboxylate group of the analyte and the ammonium group on the chiral selector. The secondary interactions involved would be π–π and H-bonding,[3] with no classic reversed phase retention mechanism involved. Hence, DryLab, which mainly predicts reversed phase behavior, and not hydrophilic interaction chromatography, would fail to predict retentions on Chirobiotic R.

CONCLUSION

It can be concluded that the chiral recognition mechanism of Chirobiotic R, in the cases under study in this work, belongs to hydrophilic interaction chromatography rather than reversed phase mode. This accounts for the inability of software such as DryLab to predict chiral behavior on Chirobiotic R.

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