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SCIENCE DIRECT
Talanta 68 (2006) 1032–1036
Talanta
www.elsevier.com/locate/talanta

Reversal of the enantiomeric elution order of some aromatic amino acids using reversed-phase chromatographic supports coated with the teicoplanin chiral selector

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Received 8 March 2005; received in revised form 25 May 2005; accepted 13 July 2005
Available online 10 August 2005

Abstract

In this paper, two chiral stationary phases were prepared by coating the surface of both C8 and C18 high-performance liquid chromatography (HPLC) supports with the teicoplanin chiral selector. The hydrophobic C11 acyl side chain, attached to the D-glucosamine group of teicoplanin, served as anchor moiety for the immobilization of the chiral selector on the apolar support material. The retention and enantioselectivity of these coated stationary phases were studied using some aromatic amino acids as probe solutes and an aqueous solution as mobile phase. It was found that the enantiomer elution order on the modified C8 and C18 stationary phases was reversed (L > D) relatively to that classically observed with a teicoplanin covalently immobilized on a silica support (D > L). Such a dynamic coating on the reversed-phase supports was found to be of interest since the apparent enantioselectivity was not significantly changed by the use during an extended period of time or following a long-term storage of the columns.

Keywords: Chiral separation; Teicoplanin; Coating; Aromatic amino acids

1. Introduction

Coating methodology of apolar chromatographic surfaces with appropriate ligands has been widely exploited in HPLC notably for the separation of ionic species by ion chromatography [1] or the study of the interactions between bioactive compounds and "membrane-like" systems [2]. Such an approach has been also reported for HPLC chiral separation. Previous papers have shown that reversed-phase chromatographic supports such as C18 or porous graphitic carbon stationary phases, coated with chiral selectors covalently bonded to a suitable non-polar anchor molecule, can be used successfully as chiral stationary phases (CSPs). Various chiral selectors such as amino acids derivatives, tartaramide, lasalocid or acylcarnitine have been immobilized via such a methodology and successfully used for the resolution of various racemates [3–7].

During the past decade, the macrocyclic antibiotics have been widely used as chiral selectors in both capillary electrophoresis (CE) and high-performance liquid chromatography (HPLC), especially for the resolution of native amino acid racemates. Although these glycopeptides have been used in some cases as chiral mobile phase additives (CMPA) [8–11], the design of chiral stationary phases is the most popular methodology reported for the HPLC applications. To date, the commercially available glycopeptidic CSPs are silica based, with the chiral selector covalently bound [12,13]. The cyclic antibiotics have been attached to silica gel via carboxylic acid or epoxy-terminated organosilanes [12]. Teicoplanin is unique among the glycopeptides in that it has a C11 hydrophobic acyl side chain attached to the glucopyranosyl group. This characteristic is notably responsible for the formation of micelles and specific pharmacological

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doi:10.1016/j.talanta.2005.07.009

properties [14]. It is expected that such hydrophobic tail could be used to immobilize the chiral selector on hydrophobic chromatographic supports and then create a new type of chiral coating.

The aim of this paper was to investigate the feasibility of developing an apolar solid support permanently coated with teicoplanin. Both C18 and C8 reversed-phase chromatographic supports were tested to immobilize the chiral selector. The retention behaviour and enantioselective properties as well as the stability of these modified stationary phases were analyzed under aqueous mobile phase conditions using some aromatic amino acids as probe solutes.

2. Experimental and methods

2.1. Apparatus

The HPLC system consisted of a LC Shimadzu pump 10AT (Sarreguemines, France), a Rheodyne injection valve model 7125 (Interchim, Montluçon, France) fitted with a 20 μL sample loop, a Shimadzu SPD-10A UV–vis detector ($\lambda = 260$ nm for the compound detection and $\lambda = 310$ nm for the detection of the teicoplanin breakthrough curve during the coating procedure). The C18 (250 mm × 4.0 mm) and C8 (250 mm × 4.6 mm) reversed-phase columns (dp: 5 μm, pore size: 100 Å) were purchased from Merck (Darmstadt, Germany) and Macherey-Nagel (Düren, Germany), respectively. These columns were used with controlled temperature (25 °C) in an oven Igloocil (Interchim).

2.2. Reagents

All racemates and enantiomers were obtained from Sigma–Aldrich (Saint-Quentin, France) or Bachem (Weil am Rhein, Germany). Na_2HPO_4 and NaH_2PO_4 were supplied by Sigma–Aldrich. Teicoplanin was provided by Astec (Whippany, USA). Acetonitrile HPLC grade (ACN) was purchased from Fisher Scientific (Leicestershine, UK). Water was obtained from an Elgastat option water purification system (Odil, Talant, France) fitted with a reverse osmosis cartridge.

2.3. Coating procedure

The immobilization of the teicoplanin chiral selector was performed in situ, by frontal chromatography on the reversed-phase columns. A 1 mM aqueous solution of teicoplanin was pumped onto the column at a flow rate of 0.1 mL/min and a column temperature of 25 °C until a breakthrough was detected with a stable detector response at $\lambda = 310$ nm. Before the chromatographic experiments, the columns were washed with the aqueous mobile phase (phosphate buffer 5 mM, pH 7.0) until stable baseline was observed. The amount of teicoplanin immobilized on the chromatographic supports was estimated by subtracting the UV absorbance of the unbound teicoplanin solution from that of the initial solution, at 280 nm. It was estimated to be about 0.150 mmol for the two columns each. When not in use for an extended period of time (long-term storage experiment), the columns were stored in the aqueous buffer containing sodium azide (0.05%) in order to prevent microbial contamination.

2.4. Chromatographic operating conditions

The mobile phase consisted of phosphate buffer (5 mM, pH 7.0). The flow rate varied from 0.25 to 1.70 mL/min. Samples were prepared in the mobile phase at a concentration of 2 mM. Twenty microliters was injected in triplicate and the retention times were measured. The apparent retention factor k was determined using the following relation: $k = (t_R - t_0)/t_0$, where t_R is the retention time of the respective enantiomer and t_0 is the retention time of an unretained species. Although this is not the most accurate approach for estimating the retention factor, t_R was determined through the solute peak position. This simplification is justified because no thermodynamic or kinetic data were extracted from this chromatographic parameter. t_0 was determined using methanol as void time marker. The retention times and column void time were corrected for the extra-column void time. They were assessed by injections of solute onto the chromatographic system when no column was present. The apparent enantioselectivity α was calculated as follows: $\alpha = k_2/k_1$, where k_2 is the retention factor for the more retained enantiomer and k_1 is the retention factor for the less retained enantiomer. The efficiency of the column was characterized by calculating the number of theoretical plates $N = 5.54(t_R/\delta)$, where δ is the peak width at half-height. The resolution R_s was calculated using the following relation: $R_s = [1.18(t_{R2} - t_{R1})]/(\delta_2 + \delta_1)$. The asymmetry factor A_s was determined by calculating the width ratio of the second (or right) part of the peak over the first (or early) part of the peak at 10% of the peak height.

3. Results and discussion

3.1. Chromatographic properties of the reversed-phase supports coated with the teicoplanin chiral selector

Table 1 presents the various aromatic amino acids which were used as probe solutes. The retention and enantioselective properties of the dynamically modified supports were investigated using an aqueous mobile phase which consisted of phosphate buffer adjusted to pH 7.0. Preliminary results showed that such operating conditions were optimal for the enantiomeric separation. A decrease in the mobile phase pH or the addition of an organic modifier (acetonitrile) in the eluent decreased the solute retention and altered significantly the enantioselective properties of the two CSPs. It can be noted that acetonitrile was responsible for the teicoplanin desorption from the column since different solute retention factors were obtained before and after the addition of the