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Impact of amines as co-modifiers on the enantioseparation of various amino acid derivatives on a *tert*-butyl carbamoylated quinine-based chiral stationary phase

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Abstract

A *tert*-butyl carbamoylated quinine-based chiral stationary phase (CSP) for direct enantiomer separation of various natural and unnatural amino acid derivatives was studied. The influence of functional groups in the amino acid side chains upon the enantioseparation is discussed with the aim of realizing contributions to their overall chiral recognition. The effects of various amines as co-modifiers upon retention and overall enantioselectivity of amino acid derivatives in polar organic solvents was systematically investigated. In general, retention times decreased with increasing amine concentrations without a distinct alteration of enantioselectivity. All analytes were rapidly resolved on the CSP with the methanol-based mobile phase containing 87 mM acetic acid and 7 mM triethylamine.

Keywords: *Tert*-butyl carbamoylated quinine selector; Chiral stationary phase; Weak chiral anion-exchanger; Amino acids; Amines

1. Introduction

Enantioseparations using chiral stationary phases (CSPs) in high-performance liquid chromatography (HPLC) are the subject of continuing interest. Cinchona alkaloid carbamate-type chiral selectors (SOs) and thereof derived CSPs have been previously developed for HPLC enantiomer separation of chiral acidic analytes (selectands, SAs) in reversed or polar organic phases. These new chiral selectors are classified as weak anion-exchangers, due to the presence of a tertiary amino group within the quinuclidine ring ($pK_a \approx 9$) [1], which is protonated at the usual working pH of the mobile phase. This primary ionic interaction between the anionic solutes (SAs) and the cationic SO is significantly accompanied by additional intermolecular interactions as hydrogen bonding, dipole-dipole, charge transfer (π–π), hydrophobic and steric interactions [1–5].

Since, most of the efforts have been focused on the development of various quinine (QN) or quinidine (QD)-derived CSPs [3,4,6–8] and enantioseparation of chiral analytes [2,9–13] in recent years, less effort has been devoted to investigate the effect of mobile phase variables, such as the mobile phase composition, which also play a crucial role in chiral selectivity. Lubda and Lindner [5] compared reversed phase and polar organic phase using *tert*-butyl carbamoylated quinine (*t*-BuCQN)-based CSP. Higher efficiencies were found in the polar organic phase than in the reversed phase, however, the enantioselectivity (α) values were relatively similar although slightly better for the polar organic phase [5]. For acidic compounds, acidic additives, such as acetic acid and formic acid, are required in any polar organic phase according to the anion-exchange retention mechanism [5,14]. Gyimesi-forrás et al. made a systematic study of the effect of various acidic additives in the chiral separation of 2-methoxy-2-(1-naphthyl)propionic acid using quinine

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carbamate-type CSPs [14]. The benefit of achiral basic additives is generally considered to improve efficiency and peak shape for polar analytes in chiral HPLC [15]. Amines are generally used as their basic additives, numerous studies have been reported in macrocyclic antibiotic- [16,17], crown ether- [18], Pirkle type- [19] and human serum albumin-based [20] CSPs using amines to control chiral selectivity. Recently, Lämmerhofer et al. demonstrated that amines had a dramatic effect on the peak performance for amino acids in capillary electrochromatography (CEC) using *t*-BuCQN as chiral SO [21]. However, a systematic study of the effect of various amines as co-modifiers on the chiral separation of acidic analytes using quinine carbamate-type CSPs has not been done. Well understanding the specific functions of mobile phase additives could help to achieve the goal of elucidating the chiral recognition mechanism for the quinine carbamate-type CSPs. Such fundamental guidance may be useful to other practitioners in the analytical field.

The target compounds of this work, amino acids, are of high biological interest, both as single components and as constituents of peptides and proteins. Although L-amino acids are predominant, D-amino acids that have also been discovered in nature have recently been shown to play as well an important role in human physiology. Several amino acids show pharmacological effects, and in many cases, the biological or pharmacological activity is restricted to one enantiomer, while the other is inactive, may show side effects or may be even toxic [22,23]. The development of analytical methods for chiral separation of amino acids and their derivatives increasingly attracted attention during the past two decades. HPLC is, besides capillary electrophoresis (CE), the most common separation technique employed to enantioseparate chiral amino acids. In this context, several enantioselective HPLC applications utilizing columns packed with chiral sorbents have been reported, comprising cyclodextrins-bonded CSPs [24], norvancomycin-bonded [25], teicoplanin-based [26], macrocyclic antibiotic ristocetin A [23] and chiral crown ethers containing a dinaphthyl moiety-based CSP [18], and QN or QD-derived CSPs [1–3]. Of the many CSPs described, *t*-BuCQN as a selector (SO) (Fig. 1) immobilized onto a silica support has been successfully applied as CSP to enantioseparate chiral amino acids [3,5,6].

In this work, a *t*-BuCQN-based CSP was used to separate the enantiomers of various amino acid derivatives. The influence of the side chain of the amino acids and of seven additives as ammonia, diethylamine, ethylenediamine, triethanolamine, triethylamine, *N*-ethyldiisopropylamine (DIPEA) and tripropylamine as co-modifiers upon retention and overall enantioselectivity in polar organic phases were evaluated to gain more insight into the chromatographic mechanism for a given mobile phase. The nature of the organic solvents was likewise further investigated.

2. Experimental

2.1. Chemicals

The organic solvents were of analytical grade: acetonitrile, acetic acid, diethylamine, ethylenediamine, methanol, triethanolamine and triethylamine, as well as ammonia from Beijing Chemical Plant (Beijing, China), while *N*-ethyldiisopropylamine (DIPEA) was obtained from Alfa Aesar (Lancs, U.K.) and tripropylamine from Aldrich (Steinheim, Germany). The racemic and L-amino acids, alanine (Ala), glutamic acid (Glu), leucine (Leu), methionine (Met), phenylalanine (Phe), were purchased from Shanghai Chemical Reagents Factory (Shanghai, China), except DL-4-fluoro phenylalanine (4F-Phe), obtained from Acros Organics (New Jersey, USA); *N*-(9-fluorenylmethoxy carbonyloxy) succinimide (Fmoc-Osu) was from Fluka (Buchs, Switzerland) while dansyl chloride (DNS–Cl) was from Acros Organics. All other reagents used were of analytical grade.

2.2. Derivatization procedure

To synthesize the Fmoc-derivatized compounds, aqueous solutions of amino acids were derivatized with Fmoc-Osu [27]. Amino acids (2.4 mM) were prepared by dissolving the corresponding components in 0.2 M boric acid (pH 8.5). Amino acid stock solution (400 μL), 700 μL of 5 mM Fmoc-Osu acetonitrile solution and 800 μL 0.2 M boric acid (pH 8.5) were mixed together. The sample was shaken for approximately 0.5 min to ensure good mixing then heated for 30 min in a water-bath at 60 °C. To synthesize the DNS-derived compounds, 5 mM amino acids were dissolved in carbonate buffer (0.1 mol/L sodium hydrogencarbonate/0.1 mol/L sodium carbonate, 2/1, v/v), 700 μL amino acid and 300 μL of 24 mM DNS–Cl acetonitrile solution were mixed together. The reaction was allowed to proceed at room temperature for 40 min. These sample solutions were filtered with 0.45 μm syringe filters (Microfiltration, Systems, CA, USA). The structures of the studied compounds are shown in Fig. 1.

2.3. HPLC experimental conditions

HPLC experiments were performed with an Agilent 1100 series instrument (Agilent Technologies), equipped with a quaternary pump, a vacuum degasser, a thermostatted column compartment, a multiple wavelength UV detector, a sample injector of 20.0 μL, an HP Chemstation. A 150 mm × 20 mm i.d., 5 μm Chiris^{TM}-QN Chiral AX column was from Iris Technologies (Lawrence, KS, USA). Fig. 2 represents the structure of the CSP. Chromatographic studies were performed at 25 °C with a 0.8 mL/min flow-rate. The mobile phase compositions are specified in the tables and figures. The mobile phases were filtered through a 0.2 μm nylon membrane filter and the chromatograms were recorded by UV detection at 254 nm. All derivatization mixtures were diluted two times with mobile phase prior to injection. An aliquot of 20 μL sample was injected. Dead time was estimated from the first solvent disturbance peak.

3. Results and discussion

The studied cinchonan-derived CSPs have often been used in a non-aqueous polar organic mode exhibiting excellent enantiomer separation capabilities. In this system, the mobile phase