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High-performance liquid chromatographic enantioseparation of α-substituted glycine analogs on a quinine-based anion-exchanger chiral stationary phase under variable temperature conditions

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Abstract

The retention of enantiomers of chiral analytes, i.e. α-substituted glycine analogs, on a quinine-based anion-exchanger chiral stationary phase was studied in the temperature range of 5–70 °C and at different mobile phase compositions, using isocratic elution in the reversed-phase mode. By variation of both mobile phase composition and temperature, baseline separations could be achieved for these enantiomers. Separation could be optimized more quickly by adjusting the column temperature rather than the mobile phase composition. The dependence of the natural logarithms of retention and selectivity factors ($\ln k'$ and $\ln \alpha$) on the inverse of temperature, $1/T$ (van't Hoff plots) was used to determine thermodynamic data on the enantiomers. Calculated thermodynamic constants ($\Delta(\Delta H^\circ)$, $\Delta(\Delta S^\circ)$ and $\Delta(\Delta G^\circ)$) were applied to promote an understanding of the thermodynamic driving forces for retention in this chromatographic system. The elution sequence of the enantiomers in most cases was determined.

Keywords: Direct enantiomeric separation; Gly analogs; Cinchona alkaloid-based stationary phase; Temperature effects; van't Hoff plots

1. Introduction

Bioactive peptides adopt a specific conformation in order to bind to an acceptor molecule. The evaluation of specific binding conformations is therefore one of the most important processes involved in efforts to obtain potent and selective therapeutic agents. For this purpose, constrained peptidomimetics based on cyclic structures, constrained amino acids or amides and mimics of peptide secondary structures have been employed [1–4]. A number of α-substituted Gly analogs, including alkyl and aryl substituents, have been applied as potential building blocks (Fig. 1). The synthesis of these Gly analogs led either to racemic mixtures or via asymmetric synthesis to one enantiomer [5–8]. Since the biological and physicochemical properties of peptides are strongly related to the stereochemistry of the incorporated amino acids, it is very important to have at hand effective analytical methods that are suitable for the separation and identification of the amino acid enantiomers.

Among the possible methods, high-performance liquid chromatography (HPLC) is routinely used for the discrimination of enantiomers. A number of review articles and books deal with the methods and results of the direct enantioseparation of various compounds on chiral stationary phases (CSPs) and many attempts have been made to interpret how these CSPs operate with respect to molecular recognition [9–11]. Of the many CSPs described, those involving cinchona alkaloid derivatives, and in particular quinine carbamates, immobilized on porous silica have been successfully applied as CSPs with an anion-exchange character. The racemic acids that have been resolved range from chiral aryl-, aryloxy- and arylthiocarboxylic acids to *N*-derivatized α- and β-amino acids and many other chiral acids, including sulfonic, phosphonic and phosphoric acids [12–18].

In all chromatographic modes the selectivity and retention factors are mainly controlled by the concentration and nature of the mobile phase components, together with other variables, such as the flow rate and the pH of the mobile phase. Enantioselective retention mechanisms are influenced by temperature to a major extent than are ordinary separations. This has been noted for some time in chiral gas chromatography [19–22]. In addition, it is known that besides enantioselective interactions also non-enantioselective retention contributions affect enantioselectivity of chiral stationary phases that can be varied with a wide variety of experimental parameters [23–29]. Several papers have been

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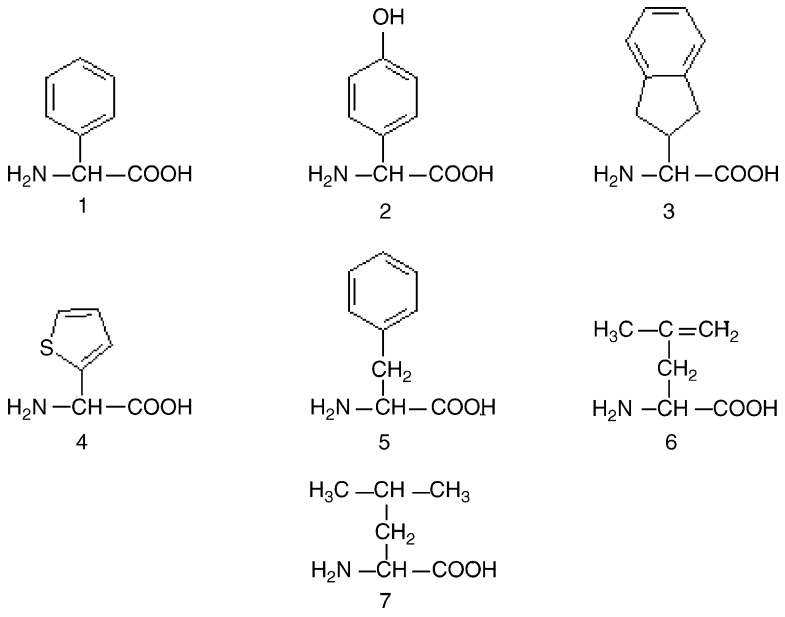

Fig. 1. Structures of investigated analytes. 2-Phenylglycine (**1**, Phg), 4-hydroxyphenylglycine (**2**, 4-OH-Phg), indanylglycine (**3**, Igl) 2-thienylglycine (**4**, Thg), phenylalanine (**5**, Phe), methallylglycine (**6**, Mag), leucine (**7**, Leu).

published that discuss the effect of temperature on enantiomer HPLC separations [30–44].

The dependence of the retention of an analyte on the temperature can be expressed by the van't Hoff equation, which may be interpreted in terms of mechanistic aspects of chiral recognition:

$$\ln k' = -\frac{\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R} + \ln \phi \quad (1)$$

This equation reveals that a plot of $\ln k'$ versus $1/T$ is linear, with a slope of $-\Delta H^\circ/R$ and an intercept of $\Delta S^\circ/R + \ln \phi$, if ΔH° is invariant with temperature. The calculation of ΔS° from the intercept requires the knowledge of the phase ratio (ϕ). Since the value of ϕ is often not known, the $\Delta S^{\circ\#}$ values [$\Delta S^{\circ\#} = \Delta S^\circ + R \ln \phi$] calculated from the intercepts of the plots via Eq. (1) are generally used. Any uncertainty in the phase ratio affects the $\Delta S^{\circ\#}$ values virtually equally, and the trends in $\Delta S^{\circ\#}$ as a function of molecular structure are therefore largely unaffected.

The corresponding $\Delta(\Delta H^\circ)$ and $\Delta(\Delta S^\circ)$ values for the separated enantiomers, can be determined from a modification of Eq. (1):

$$\ln \alpha = -\frac{\Delta(\Delta H^\circ)}{RT} + \frac{\Delta(\Delta S^\circ)}{R} \quad (2)$$

where α is the selectivity factor ($\alpha = k'_2/k'_1$).

The aim of the present work was to separate the enantiomers of α-substituted Gly analogs and to investigate the effects of temperature on enantioselective separations on a weak anion-exchanger column, Prontosil 120-5 tBuCQN (Fig. 2), in a reversed-phase chromatographic mode. The direct HPLC enantioresolution of some of these analogs on crown ether and macrocyclic glycopeptide-based CSPs was earlier reported by Péter et al. [45]. The amino acids analyzed were used in *N*-benzyloxycarbonyl (Z), *N*-3,5-dinitrobenzyloxycarbonyl (DNZ), *N*-benzoyl (Bz) and *N*-3,5-dinitrobenzoyl (DNB) derivatized forms. The separations were carried out at constant mobile phase compositions at different temperatures. Thermodynamic constants were obtained from the van't Hoff plots and were used to discuss the mechanistic aspects of chiral recognition.

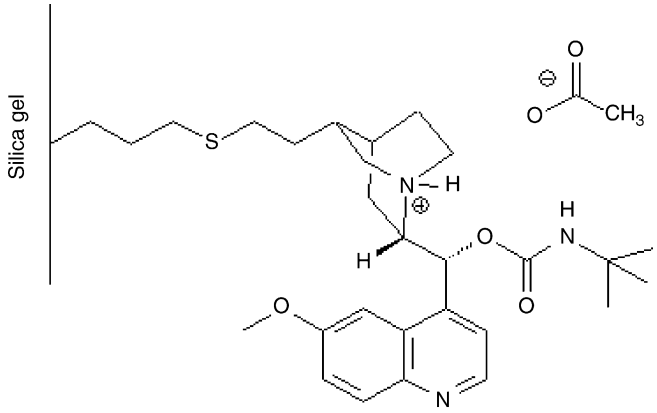

Fig. 2. Chemical structure of weak-anion exchanger stationary phase.

2. Experimental

2.1. Apparatus

The HPLC measurements were carried out on Waters systems. One consisted of an M-600 low-pressure gradient pump, an M-996 photodiode-array detector and a Millenium [32] Chromatography Manager data system; the Waters Breeze system consisted of a 1525 binary pump, a 487 dual-channel absorbance detector, a 717 plus autosampler, a column thermostat Model 5CH and Breeze data manager software (both systems from Waters Chromatography, Milford, MA, USA). Both chromatographic systems were equipped with Rheodyne Model 7125 injectors (Cotati, CA, USA) with 20 μl loops, too.