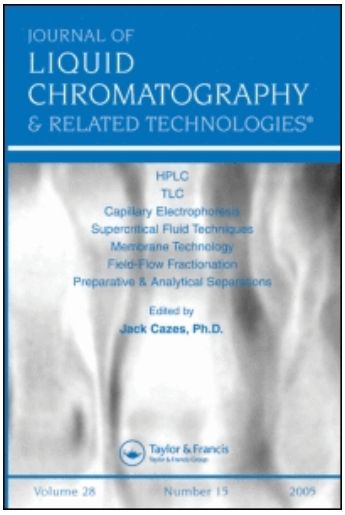

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Effect of Column Temperature on Retention of Dipeptide Isomers in Reversed-Phase High Performance Liquid Chromatography

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EFFECT OF COLUMN TEMPERATURE ON RETENTION OF DIPEPTIDE ISOMERS IN REVERSED-PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

The effect of column temperature on the capacity factor and selectivity of dipeptide isomers was investigated. It has been observed that the variation in the logarithm of the capacity factor of dipeptide isomers is linearly dependent on the reciprocal of the absolute column temperature. The standard enthalpic change for dipeptide isomers is almost a constant, and the selectivity value is almost independent of the column temperature. It can be concluded that (1) the enthalpy change of dipeptide isomers in RP-HPLC is mainly determined by the molecular interaction in the mobile phase and (2) the difference of their standard entropy change play more important role in the selectivity value than that of standard enthalpy change does.

INTRODUCTION

Reversed-phase high performance liquid chromatography (RP-HPLC) retention and thus, solute selectivity, is influenced by several chemical (e.g., type and concentration of organic modifier) and physical factors (e.g., column temperature and/or stationary phase chain length, density, surface area, pore volume, monomeric/polymeric coatings). The retention process in reversed-phase HPLC is thought to be primarily controlled by solute interactions within the

mobile phase[1-3]. Most research has focused on understanding the influence of mobile phase composition on retention. Studies has shown that variations in solvent composition (i.e., water with one or more organic solvents) can produce dramatic retention differences[4-7]. Stationary phase studies are limited because details of the bonded-stationary phase synthesis and silica substrates are not readily available. In addition to mobile-stationary phase effects on retention, changes in column temperature may alter retention characteristics as well[8-12].

In this investigation, the RP-HPLC of 10 dipeptide isomers at different column temperatures is described. It will be shown that there is linear relationship between the logarithm of the capacity factors and the reciprocal of absolute column temperature at constant composition of the mobile phase. The standard enthalpy change calculated from the slope of this relationship is almost constant, and column temperature has minor influence on the selectivity value for pair of dipeptide isomers, which is mainly determined by their difference of the standard entropy change.

EXPERIMENTAL

1. Materials

All of the dipeptide isomers used were purchased from Serva Feinbiochemica GmbH. (Heidelberg, Germany) and are listed in Table 1. These dipeptides form two series, series I contains dipeptides of Met-Gly, Val-Gly, Leu-Gly and Tyr-Gly, and their bonding was through the glycine amino group; and series II contains dipeptides of Gly-Met, Gly-Val, Gly-Leu, Gly-Phe and Gly-Tyr and their bonding was through the glycine carboxyl group. Methanol, KH_2PO_4 and all the other used chemical reagents are of analytical grade. Deionized and distilled water was used throughout the experiments.

2. Apparatus and Conditions

A Spherisorb silica gel ODS with particle diameter 10 μm (Phase Separation Ltd, Deeside, UK) was packed into a stainless steel column with 200X4.0 mm I.D.

The mobile phases was dilivered by a Waters Model 510 pump (Waters Assoc., Milford, MA, USA) equipped with the temperature-controlled eluent and column compartments. The eluates were detected by a homemade FS-100 UV detector at 210 nm. The samples were introduced by a U6K syringe loading-sample injector. The flow rate was set at 1.0 ml/min. The eluent pH was measured by the SA-720 pH meter (Orion Res. Inc., Chicago, IL, USA). Each sample was dissolved in the eluent. The void volume of columns was determined by the methanol peak at wavelength 210 nm. Capacity factors of the dipeptide isomers were measured under the eluent of methanol/water (0.1/0.9, v/v) containing KH_2PO_4 10 mmol/l (pH 2.20) with various column temperature of 12, 22, 32 and 42 °C respectively. All the experimental data were processed by a NEC-APCIV personal computer.

RESULTS AND DISCUSSION

1. Dipeptide Isomer Retention as a Function of Colum Temperature

The extent to which individual dipeptide isomer retention decreased was a function of column temperature. Eqn.(1) describes the relationship between solute retention and column temperature:

$$\ln k' = -\Delta H^\circ/RT + \Delta S^\circ/R + \ln\Phi \qquad (1)$$

where R is the gas constant, T is the absolute temperature, Φ is the mobile-stationary phase ratio, ΔH° is the standard enthalpy change of solute transfer from the mobile phase to stationary phase, and ΔS° is the associated change in the standard entropy.

Table 1 listed the capacity factors of dipeptide isomers experimentally measured at different column temperatures. Fig.1 showed a typical chromatogram for separation of 10 dipeptide isomers. Summarised in Table 2 are the results of lnk' vs. 1/T plots at constant mobile phase composition and the standard enthalpy calculated from the slope of lnk' vs. 1/T relationship. The relative standard deviation of intercept and slope in Table 2 are smaller than 5% at the most cases, which means that the intercept and slope values can be kept in three significant digits. The standard enthalpy change, ΔH°, was constant and

TABLE 1
Experimentally Measured Capacity Factors of Dipeptide Isomers at Different Column Temperature in Reversed-Phase HPLC with Spherisorb-ODS as the Stationary Phase and Methanol/Phosphate Buffer (0.1/0.9, v/v) as the Eluent.

Solute	Column Temperature (°C)			
	12	22	32	42
Val-Gly	0.89	0.76	0.62	0.53
Gly-Val	1.74	1.54	1.24	1.12
Met-Gly	1.27	1.06	0.84	0.69
Gly-Met	1.95	1.58	1.27	1.08
Leu-Gly	2.74	2.25	1.91	1.65
Gly-Leu	6.19	5.33	4.34	3.74
Phe-Gly	7.04	5.64	1.31	3.47
Gly-Phe	11.5	9.28	7.05	5.68
Tyr-Gly	2.94	2.18	1.46	1.11
Gly-Tyr	4.88	3.60	2.47	1.88

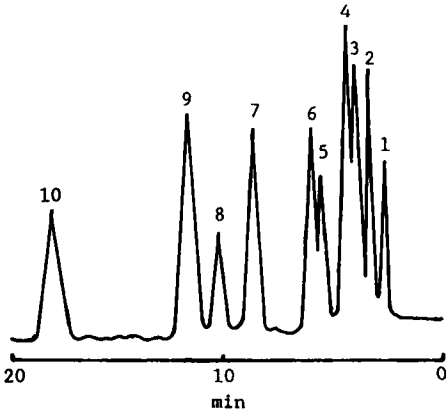

FIGURE 1 Chromatogram of ten dipeptide isomers at column temperature 12 °C. Other experimental conditions see text. Chromatogarphic peak: 1=Val-Gly; 2=Met-Gly; 3=Gly-Val; 4=Gly-Met; 5=Leu-Gly; 6=Tyr-Gly; 7=Gly-Tyr; 8=Gly-Leu; 9=Phe-Gly; 10=Gly-Phe .

TABLE 2
Intercept and Slope Value of lnk' vs. 1/T and Calculated Standard Enthalpy Change ΔH°, r Is Linear Regression Coefficient.

Solute	Intercept	Slope (10^3)	r	ΔH° (Kcal/mol)
Val-Gly	-5.68(0.02)	1.59(0.08)	0.995	-3.16
Gly-Val	-4.28(0.03)	1.38(0.13)	0.991	-2.75
Met-Gly	-6.21(0.02)	1.84(0.09)	0.995	-3.67
Gly-Met	-5.61(0.01)	1.79(0.04)	0.999	-3.56
Leu-Gly	-4.31(0.01)	1.52(0.03)	0.999	-3.02
Gly-Leu	-3.57(0.02)	1.54(0.08)	0.996	-3.06
Phe-Gly	-5.57(0.02)	2.15(0.09)	0.998	-4.28
Gly-Phe	-5.07(0.03)	2.14(0.11)	0.996	-4.28
Tyr-Gly	-9.37(0.04)	2.98(0.15)	0.997	-5.93
Gly-Tyr	-8.60(0.03)	2.91(0.12)	0.998	-5.79

independent of column temperature in the range studied. The negative value indicated that the dipeptide isomers transfer from the mobile phase to ODS stationary phase was enthalpically favored. However, the evaluation of corresponding entropy change, ΔS°, from the intercept is difficult because the phase ratio is not known.

2. Selectivity for Pair of Dipeptide Isomers as a Function of Temperature

It is assumed that the phase ratio is independent of the column temperature , and from eqn.(1), it can be derived an equation to describe the effect of column themperature on the selectivity (α) for pair of dipeptide isomers as follow:

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

where $\Delta\Delta H^{\circ}$ and $\Delta\Delta S^{\circ}$ are the difference of the standard enthalpy and entropy

TABLE 3
Values of $\Delta\Delta H^\circ$ and $\Delta\Delta S^\circ$ Calculated by Eqns.(3) and (4) as Well as Value of $|T\Delta\Delta S^\circ|$ at 300 °K.

Pair of Dipeptide Isomers	$\Delta\Delta H^\circ$ (Kcal/mol)	$\Delta\Delta S^\circ$ (Cal/mol·°K)	$\lvert T\Delta\Delta S^\circ \rvert$ (Kcal/mol)
Gly-Val/Val-Gly	0.39	2.8	0.84
Gly-Met/Met-Gly	0.09	1.2	0.36
Gly-Leu/Leu-Gly	-0.04	1.5	0.45
Gly-Phe/Phe-Gly	0.00	1.0	0.30
Gly-Tyr/Tyr-Gly	0.16	1.5	0.45

change and can be calculated from the enthalpy change and intercept of lnk' vs. 1/T shown in Table 2 by following equations:

$$\Delta\Delta H^\circ = \Delta H^\circ_{II} - \Delta H^\circ_{I} \quad (3)$$

$$\Delta\Delta S^\circ = R*(\text{Intercept}_{II} - \text{Intercept}_{I}) \quad (4)$$

where ΔH°_I, ΔH°_{II} and Intercept_I, Intercept_{II} are the enthalpy change and intercept of lnk' vs. 1/T for dipeptide isomers of series I and II respectively. Table 3 listed the values of $\Delta\Delta H^\circ$ and $\Delta\Delta S^\circ$ as well as value of $|T\Delta\Delta S^\circ|$ at 300 °K. It can be seen that there is little change in the value of standard enthalpy for the dipeptide isomers, there should be no serious change of the selectivity value for pair of the dipeptide isomers with a change of the column temperature in the chromatographic system studied. Table 4 illustrated the selectivity values for pair of dipeptide isomers experimentally measured and those calculated from eqn.(2) at different column temperature, these data basically agree with this assumption. The enthalpy change in reversed-phase HPLC is mainly determined by the molecular interaction between the solute and the mobile and stationary phases. It has been reported that the separation of dipeptide isomers is based on the fact that closeness of the charged protonated

TABLE 4
Selectivity Values for Pair of Dipeptide Isomers Experimentally Measured and Calculated from Eqn.(2) at Different Column Temperatures.

Pair of Dipeptid Isomers	12 °C		22 °C		32 °C		42 °C	
	(exp)	(cal)	(exp)	(cal)	(exp)	(cal)	(exp)	(cal)
Gly-Val/Val-Gly	1.95	2.05	2.02	2.10	1.99	2.15	2.12	2.19
Gly-Met/Met-Gly	1.54	1.56	1.49	1.57	1.52	1.58	1.56	1.58
Gly-Leu/Leu-Gly	2.26	2.28	2.37	2.27	2.24	2.27	2.27	2.26
Gly-Phe/Phe-Gly	1.63	1.65	1.65	1.65	1.64	1.65	1.64	1.65
Gly-Tyr/Tyr-Gly	1.66	1.60	1.65	1.62	1.69	1.63	1.69	1.65

amine group would diminish the interaction between the non-polar stationary phase and non-polar amino acid subunit in acidic eluent[13,14], but this factor does not make an important contribution to the enthalpy change of dipeptides, because there is little change of standard enthalpy for dipeptide isomers. So it may be deduced that the enthalpy change of dipeptide isomers is mainly determined by the molecular interaction between solute and mobile phase.

On the other hand, it can be seen that the entropy change of dipeptide isomers of series I is smaller than that of dipeptide isomers of series II in acidic eluent, which means that the closeness of the charged protonated amine group decreases the entropy change. It can also be expected that the closeness of the diprotonated carboxyl group will decrease the entropy change in the basic eluent. In the range of experimented column temperature from 12 °C to 42 °C, it can be known that:

$$|\Delta\Delta H^{\circ}| < |T\Delta\Delta S^{\circ}| \qquad (5)$$

This equation means that the difference of entropy change more strongly affects the selectivity for pair of dipeptide isomers than the difference of enthalpy

change does. Therefore, it can concluded that the separation of the dipeptide isomers in RP-HPLC is mainly based on the difference of their entropy change.

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