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Investigation of parameters in the separation of amino acid enantiomers by supercritical fluid chromatography

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ABSTRACT

The effects of column temperature (T) and pressure on enantioselectivity (α) and capacity factor (k') were investigated by supercritical fluid chromatography (SFC) with carbon dioxide as the mobile phase using a laboratory-made cross-linked chiral capillary column. The column temperature has considerable effects on both α and k' whereas the column pressure (*i.e.*, the density of mobile phase) shows no effect on α. Good linear relationships between $\ln\alpha$ and $1/T$ are obtained. The separation of some amino acid enantiomers is illustrated.

INTRODUCTION

Chromatographic separations of enantiomers have attracted wide attention since the important difference in biological activity of enantiomers became recognized[1]. Generally, gas chromatography (GC) is the method of choice for the separation of thermally stable and volatile molecules, but a high temperature is needed for the separation of amino acid enantiomers and the enantioselectivity decreases with increase in column temperature[2]. We therefore investigated supercritical fluid chromatography (SFC) for the separation of enantiomers under mild conditions and studied the parameters for the separatior of amino acid enantiomers. In this paper, the effects of column temperature (T) and pressure (P) (*i.e.*, the density of the mobile phase) on separation factors (α) and capacity factors (k') in SFC are considered.

EXPERIMENTAL

The chiral stationary phase OV-225–L-valine–*tert.*-butylamide was cross-linked within a glass capillary column (20 m × 100 μm I.D.)[3]. GC experiments were carried out with a GC R1A gas chromatograph and SFC with a laboratory made chromatograph with carbon dioxide as the mobile phase. Each chromatographs was equipped with a flame ionization detector. The enantiomers of amino acids were derivertized to N-trifluoroacetyl amino acid isopropyl esters before injection[4].

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RESULTS AND DISCUSSION

The α-values of some amino acid enantiomers were obtained at different pressures by SFC (see Table I) and compared with those given by GC at the same column temperature (see Table II).

The effects of column temperature on α and k' values in SFC and GC are shown in Tables III and Table IV, respectively. The relative deviations of the data for both α and k' are within 2%. Fig. 1 shows the plots of ln α *vs.* $1/T$ and Fig. 2 of ln k' *vs.* $1/T$ for different amino acid enantiomers.

TABLE I

EFFECT OF PRESSURE ON α VALUES IN SFC (60°C)

Amino acid	*Pressure (MPa)*				
	8.0	*9.0*	*10.0*	*10.5*	*11.0*
Leu	1.132	1.129	1.129	1.129	
Met			1.098	1.100	1.098

TABLE II

COMPARISON OF α VALUES IN GC AND SFC

Method	*Ala (70°C)*	*Val (70°C)*	*NVal (80°C)*	*Leu (80°C)*	*NLeu (80°C)*
GC[a]	1.078	1.061	1.075	1.093	1.080
SFC (8.0 Mpa)	1.080	1.060	1.075	1.091	1.080

[a] Carrier gas nitrogen.

TABLE III

EFFECTS OF COLUMN TEMPERATURE ON α AND k' VALUES IN SFC

T(°C)	*Parameter*	*Ala (8.0 MPa)*	*Val (8.0 MPa)*	*NVal (8.0 MPa)*	*Leu (9.0 MPa)*	*NLe (8.0 MPa)*	*Met (10.0 MPa)*
50	α	1.098	1.080	1.107	1.143	1.118	1.115
	k'(L)	0.58	0.67	0.92	0.50	1.13	0.48
60	α	1.090	1.070	1.094	1.129	1.106	1.098
	k'(L)	0.79	0.71	1.13	0.76	1.53	1.27
70	α	1.080	1.060	1.083	1.107	1.091	1.088
	k'(L)	0.69	0.60	1.06	0.80	1.48	2.09
80	α			1.075	1.091	1.080	1.076
	k'(L)			0.91	0.75	1.24	2.22
90	α				1.083		1.066
	k'(L)				0.65		2.15
100	α				1.068		1.058
	k'(L)				0.53		1.95

TABLE IV

EFFECTS OF COLUMN TEMPERATURE ON α AND k' VALUES IN GC

T(°C)	*Parameter*	*Ala*	*Val*	*NVa*	*Leu*	*NLe*	*Met*
70	α	1.078	1.061				
	k'(L)	21.4	28.9				
80	α	1.068	1.054	1.075	1.093	1.080	
	k'(L)	11.7	15.4	27.9	39.2	46.8	
90	α	1.059	1.045	1.067	1.083	1.068	
	k'(L)	6.81	8.70	15.2	21.2	24.9	
100	α	1.051	1.039	1.056	1.068	1.058	
	k'(L)	4.02	5.01	8.49	11.6	13.5	
110	α	1.045	1.034	1.048	1.059	1.048	1.048
	k'(L)	2.61	3.21	5.19	6.70	7.89	59.2
120	α	1.038	1.027	1.040	1.049	1.040	1.040
	k'(L)	1.70	2.01	3.19	4.22	4.89	34.5
130	α						1.034
	k'(L)						19.0
140	α						1.026
	k'(L)						11.5
150	α						1.021
	k'(L)						7.10

As is well known, the enantioselectivity (α) is defined by

$$\alpha = k'_L/k'_D \quad (1)$$

Other than the polarization property, there is no difference between L- and D-antipodes in most of their physical and chemical properties[5]. It is also true that direct separation of enantiomers could only be achieved by chiral–chiral recognition so far. It is reasonable to assume that the effects of an achiral mobile phase (such as carbon

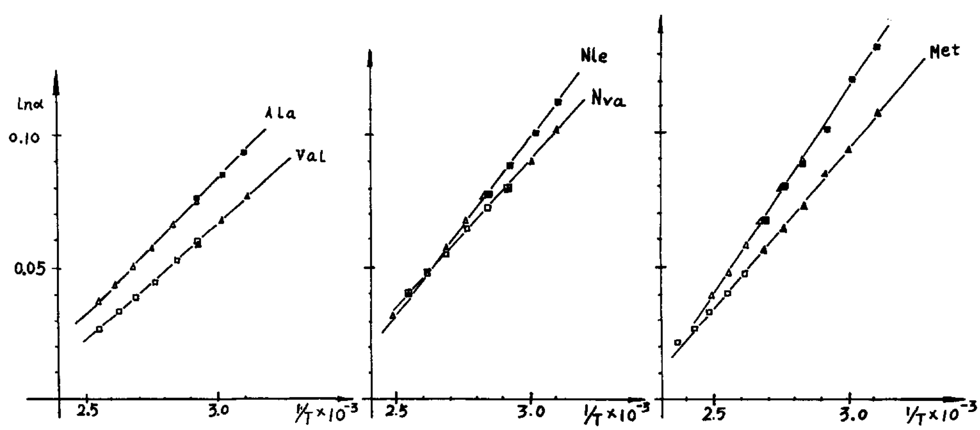

Fig. 1. Plots of ln α *vs.* $1/T$ obtained by (△, □) GC and (▲, ■) SFC.

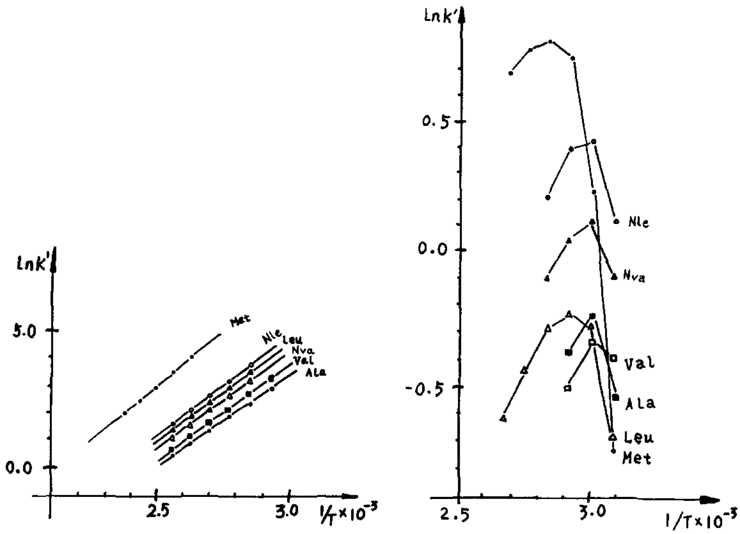

Fig. 2. Plots of ln k' *vs.* $1/T$: (left) GC; (right) SFC.

dioxide) on L- and D-antipodes will be identical, so according to the equations derived by Martire and Boehm[6] the following equations can be easily derived:

$$(\ln \alpha)_{SFC} = (\ln \alpha)_{GC} \tag{2}$$

$$(\ln \alpha)_{SFC} = -(\Delta H_L - \Delta H_D)_{SFC}/RT + (\Delta S_L - \Delta S_D)_{SFC}/R$$
$$= -(\Delta H_L - \Delta H_D)_{GC}/RT + (\Delta S_L - \Delta S_D)_{GC}/R \tag{3}$$

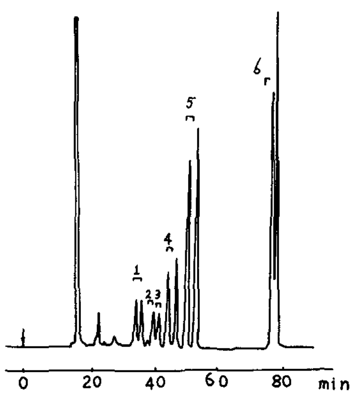

Fig. 3. Chromatogram of amino acid enantiomers obtained by SFC. Column, 20 m × 0.10 mm I.D. cross-linked OV-225-L-Val-tert.-butylamide; mobile phase, CO_2; detector, flame ionization; column temperature, 60°C; pressure, increased from 8 to 16 MPa at 0.1 MPa/min. Peaks: 1 = Val; 2 = allo-Isoleucine; 3 = Ile; 4 = Nva; 5 = Leu; 6 = Met.

(where R is the molar gas constant). Eqns. 2 and 3 indicate that the plots of ln α *versus* $1/T$ are linear in SFC and they are the same as those obtained by GC if the same column is used. The data in Tables II–IV and Fig. 1 confirm and support these points.

The effects of column temperature on capacity factors in SFC are more complicated than those in GC (see Fig. 2), probably because column temperature can affect the solute solubility in the mobile phase and the retention in the stationary phase simultaneously, but the two effects on k' values are in opposite directions. The results shown in Fig. 2 are the consequence of the competition of these effects.

Using SFC, enantiomers could be separated at fairly low column temperatures and have higher α values than in GC. The separation of some amino acid enantiomers with pressure programming by SFC is shown in Fig. 3.

CONCLUSION

The density of the mobile phase has no influence on enantioselectivity under the operating conditions applied. The results further confirm that the linear relationships between ln α and $1/T$ in SFC for the amino acids tested coincide with those obtained in GC. Higher α values are obtained by SFC than GC owing to the lower column temperature used.

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