The Chiral Separation of Triazole Pesticides Enantiomers by Amylose-tris(3,5-dimethylphenylcarbamate) Chiral Stationary Phase

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Abstract

The amylose-*tris*(3,5-dimethylphenylcarbamate) chiral stationary phase was synthesized and used to separate the enantiomers of triazole pesticides by high-performance liquid chromatography. The mobile phase was *n*-hexane–isopropanol applying a flow rate of 1.0 mL/min. Six triazole pesticides were enantioselectively separated. Myclobutanil, paclobutrazol, tebuconazole, and uniconazole obtained complete separation with the resolution factors of 5.73, 2.99, 1.72, and 2.07, respectively, and imazalil and diniconazole obtained partial separation with the resolution factors of 0.79 and 0.77 under the optimized conditions. The effect of the content of isopropanol as well as column temperature on the separation was investigated. A circular dichroism detector was used to identify the enantiomers and determine the elution orders. The results showed the low temperature was good for the chiral separation except for diniconazole. The thermodynamic parameters calculated based on linear Van't Hoff plots showed the chiral separations were controlled by enthalpy.

Introduction

Chiral separations are playing an important role for the analysis of single enantioemers, especially in the field of drugs and pesticides (1). All kinds of chiral separation methods appeared in the past three decades, in which high-performance liquid chromatography (HPLC), gas chromatography (GC), capillary electrophoresis (CE), and supercritical fluid chromatography (SFC) were used frequently. HPLC using chiral stationary phases capable of separation of a wide range of enantiomers have proven to be the most useful among the methods currently used. There were lots of chiral stationary phases (CSPs) developed, in which polysaccharide-based CSPs such as cellulose-*tris*(3,5-dimethylphenylcarbamate) (CDMPC) and amylose-*tris*(3,5-dimethylphenylcarbamate) (ADMPC) showed powerful separation ability towards a great variety of chiral compounds (2–4).

Although chiral pesticides are very widely used (5), only a small part has achieved enantiomeric separation, because the same physical and chemical properties of the two enantiomers make discriminating and separating them very difficult. The two enantiomers usually show different behaviors in biological and environmental systems. Taking the fungicide metalaxyl (the fungicidal activity is concentrated almost exclusively in the *R*-form) for example, the environmental behaviors of *R*- and *S*-enantiomer in soil, plants, and animals were found different (6–11). But few data about the bioactivity, toxicology, and environment behaviors of the single enantiomer is available because of the lack of separation methods.

Triazole pesticides are one of the most important categories of fungicides, which show excellent protective, curative, and eradicant power towards a wide spectrum of crop diseases. The common moiety of this family of pesticides is a triazole ring, and most of them have chiral centers and thus have two or more isomers. At present, most of them are produced, sold, and used in the form of racemates (the mixture of two enantiomers). It is elucidated that the enantiomers of this kind of pesticide such as triadimenol, uniconazole, and diniconazole represent difference in term of bioactivity. However, the detailed information about the enantiomers of most of these fungicides was not yet illustrated well and this made the effective chiral separation an urgent work.

The open scientific literatures revealed several methods for chiral separation of triazole pesticides. The chiral separation of diniconazole was performed by liquid chromatography via β-cyclodextrin-bonded CSP (12), capillary electrophoresis via γ-cyclodextrin and dimethyl-β-cyclodextrin chiral selector (13), and supercritical fluid chromatography (14). Wu and co-workers had tried to resolved several triazole fungicides on sulfated β-cyclodextrin-mediated capillary electrophoresis (15). Our previous work showed diniconazole, tebuconazole, hexaconazole, triadimefon, and flutriafol could be separated on the CDMPC CSP by HPLC (16).

The aim of this work is to separate the enantiomers of triazole pesticides on the self-prepared ADMPC CSP; optimize the chromatographic conditions in terms of temperature and mobile phase composition; identify the enantiomers; and, thus, report effective chiral separation methods for this class of pesticides. Under the optimized condition, myclobutanil, imazalil, paclobutrazol, diniconazole, tebuconazole, and uniconazole obtained enantioselectivity. According to the literature (16), diniconazole,

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tebuconazole, and uniconazoled could also be separated on the CDMPC, but myclobutanil, imazalil, and paclobutrazol could not be separated on it. ADMPC could not separate the enantiomers of triadimefon and flutriafol, which obtained separation on CDMPC.

Experimental

Reagents

Macro-spherical silica was prepared in this laboratory with the following properties: particle size, 5–7 µm; average pore diameter, 6.7 nm; specific surface area, 110 m^2/g. 3-Aminopropyltriethoxysilane (99%) was from Acros Organics (Belgium). Amylose and 3,5-dimethylphenylisocyanate (99%) were purchased from Sigma-Aldrich Inc.

Pesticide samples (> 95%) were provided by the Lab of Pesticide Residual Analysis and Environmental Toxicology of China Agricultural University (Beijing, China), Institute for Control of Agrichemicals Ministry of Agriculture (Beijing, China), and several pesticide manufacturers (China). The sample solutions were prepared in isopropanol. All reagents were of analytical grade (Beijing Chemical Reagents Company, Beijing, China). The mobile phase eluents were distilled and filtered by 0.45-µm film before use.

Apparatus

Two HPLC systems were used. System 1: Agilent 1100 Series HPLC (Agilent Technologies Palo Alto, CA) equipped with G1322A degasser, G1311A pump, G1328A injector, a 20-µL sample loop, G1316A COLCOM (the part for column temperature control), G1315B DAD, and HP1100 workstation for signal acquiring and process. This system was used to separate the enantiomers and optimize the chromatographic conditions. System 2: JASCO 2000 HPLC (JASCO Co., Tokyo, Japan), equipped with a 20-µL sample loop, pu-2089 plus pump, CD-2095 plus circular dichroism detector, and Chrompass workstation. This system was used to identify the eluting enantiomers and determine the elution orders.

Chromatographic conditions

Mobile phase was *n*-hexane–isopropanol with a flow rate of 1.0 mL/min, injection volume of 20 µL and detection wavelength of 230 nm. The chiral column was 250 mm × 4.6 mm (i.d.). The following parameters were calculated:

k' (retention factor): $\frac{t - t_0}{t_0}$

Where t is the retention time and t_0 is the void time determined by tri-t-butylbenzene. α (separation factor: k_1'/k_2', k_1', and k_2' are the retention factors of the first and second eluted enantiomer respectively.

Rs (resolution factor): $\frac{2(t_2 - t_1)}{w_1 + w_2}$, where w is peak area.

Preparation of the CSP (17–19)

Amylose was dried and reacted with 3, 5-dimethylphenylisocyanate in pyridine at 105–110°C for 48 h. After cooling to room temperature the product ADMPC was precipitated, filtered, washed with methanol, and dried at 60°C for 12 h. Spherical silica (activated by hydrochloric acid) reacted with 3-aminopropyltriethoxysilane in toluene at 110°C for 24 h under nitrogen atmosphere. The product aminopropylsilica (APS) was filtered, washed with toluene and ethanol then dried at 80°C.

ADMPC (0.45 g) was dissolved in 60 mL tetrahydrofuran and the solution was added to APS (2.55 g) drop by drop. The mixture was stirred continuously, after evaporating solvent, dried at 60°C for 8 h. The percentage of the coated ADMPC was 15%.

n-Hexane-isopropanol (90:10 v/v) was used as slurry solvent. The CSP was packed into stainless steel column (250 mm × 4.6 mm i.d.) under 4.0 × 10^7 Pa to prepare HPLC chiral column.

Results and Discussion

The chemical structures of the chiral triazole pesticides, which had the common moiety triazole ring were listed in Figure 1. The chirality of the molecules was derived from the asymmetry carbon atom. Although paclobutrazol had two chiral carbon atoms, the technical material was a mixture of only one pair of enantiomers that were *R*,*R*- and *S*,*S*-form (15).

The *n*-hexane-isopropanol mobile phase was used applying a flow rate of 1.0 mL/min. The retention factor (k'), separation factor (α), and resolution factor (*Rs*) were used to evaluate the resolution. The dead time t_0 determined by tri-t-butylbenzene was 2.06 min. Rs value of 1.5 was used as criteria for complete separation.

Chiral separation

The difference between the interactions of the two enantiomers and the CSP causes the chiral resolution. It is widely believed a combination of interactions such as hydrogen bonding, hydrophobic interactions, dipole–dipole interactions, and charge transfer complex (π-π) might be the key point for the chiral recognition (20). The main chiral adsorbing sites on ADMPC are the polar carbamate groups (21).

The chiral separation and the effect of isopropanol percentage in the mobile phase are listed in Table I, which shows the retention and the separation differed greatly despite of the similar structures they share.

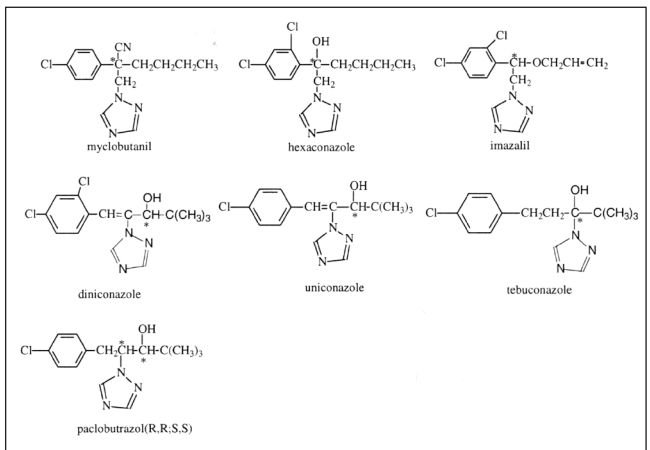

Figure 1. The chemical structures of the triazole pesticides.

The enantionselectivity of the samples on the ADMPC in the ascend trend was myclobutanil > paclobutrazol > uniconazole > tebuconazole > imazalil > diniconazole, and the retention in the ascend trend was myclobutanil > tebuconazole > uniconazole > paclobutrazol > diniconazole > imazalil. For most of the samples, decreasing the content of isopropanol resulted in a higher retention factor, separation factor, and resolution factor. Figure 2 shows the chromatograms of the chiral separations.

Myclobutanil obtained the best separation and the strongest retention on the CSP among the samples. The content of isopropanol from 15–5% was investigated to optimize the chromatographic condition, and the highest Rs value was 5.73 using 5% isopropanol. The separation factors decreased with isopropanol content decreasing; however, the retention factor and the resolutions were increased. The chiral separation of hexaconazole whose structure was very similar to myclobutanil was also conducted, but its two enantiomers were not separated. The fact that myclobutanil had a CN group on the asymmetry carbon but hexaconazole had an OH group might be the main reason for this great difference. The CN group could form a dipole–dipole interaction and hydrogen bonding as well, and OH could form a hydrogen bonding interaction. Furthermore, the additional substitutional chloric atoms on the phenyl ring of hexaconazole might decrease the chiral recognition because it decreased the π-π interaction. Imazalil was poorly separated, so the effect of the content of isopropanol of 15–2% on the separation was studied. Three parameters increased when the content of isopropanol decreased. The enantiomers got a higher Rs value of 0.79 using 2% isopropanol. The two enantiomers of paclobutrazol could be easily completely separated with the Rs value of 1.81 even using 15% isopropanol and of 2.99 when the content decreased to 5%. The parameters such as retention factor, separation factor and resolution factor increased with the isopropanol content increasing. Diniconazole and uniconazole had very similar structures, but the separations also differed greatly. Uniconazole had a good separation, while diniconazole obtained the worst separation in the samples. As Table I shows, uniconazole reached baseline separation when the isopropanol content decreased to 10%, and the separation parameters all increased with isopropanol content decreasing. The two enantiomers of diniconazole only had an Rs value of 0.77, even using 2% isopropanol in the mobile phase, and this parameter increased with the content of isopropanol decreasing. The difference of the described two triazole compounds in term of structure was that: uniconazole had a 4-chlorophenyl and diniconazole had a 2,4-dichlorophenyl group. The electron attractive chloric atoms on phenyl weakened the π-π interaction and might decrease the special fit of the enantiomers with the CSP. This may be the main reason for the separation. The two enantiomers of tebuconazole also obtained complete separation. The k', α, and Rs values all increased with the isopropanol content decreasing, and the Rs value reached 1.71 when the content decreased to 5%.

Table I. The Chiral Resolutions and the Effect of the Isopropanol Content on the Separation

Sample	Content of IPA %	k_1	k_2	α	Rs
Myclobutanil	15	5.47	9.37	1.71	4.14
	10	9.18	15.57	1.70	4.90
	5	21.32	35.05	1.64	5.73
Imazalil	15	2.37	2.48	1.05	0.40
	10	3.68	3.90	1.06	0.59
	5	7.35	7.86	1.07	0.73
	2	15.67	16.77	1.07	0.79
Paclobutrazol	15	2.71	3.54	1.31	1.81
	10	4.42	5.99	1.35	2.24
	5	9.96	13.93	1.40	2.99
Diniconazole	15	2.43	2.52	1.04	0.27
	10	3.94	4.20	1.07	0.58
	5	9.06	9.69	1.07	0.66
	2	24.05	25.36	1.05	0.77
Tebuconazole	15	3.28	3.82	1.16	1.14
	10	5.25	6.19	1.18	1.31
	5	11.46	13.78	1.20	1.72
Uniconazole	15	2.74	3.34	1.22	1.32
	10	4.63	5.77	1.25	1.59
	5	11.15	14.42	1.29	2.07

Effect of temperature and thermodynamic parameters

Temperature played the most important role on the chiral resolution and was always an important parameter for chromatographic condition optimization. The influence of temperature in the range of 0–40°C or 0–30°C on the chiral separation of the triazole pesticides was studied, and the results

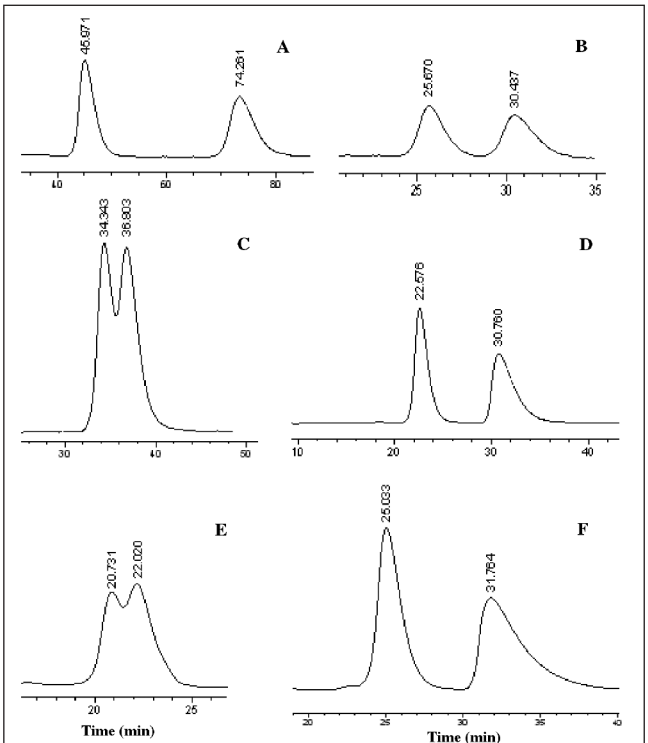

Figure 2. The chromatograms of the chiral separations *n*-hexane–isopropanol mobile phase, flow rate 1.0 mL/min, wavelength 230 nm, 20 degree A: myclobutanil, 5% isopropanol; B: tebuconazole l, 5% isopropanol; C: imazali, 2% isopropanol; D: paclobutrazol, 5% isopropanol; E: diniconazole, 5% isopropanol; F: uniconazole, 5% isopropanol.

are listed in Table II. Because the samples had different enantioselectivity and retention on the different CSP mobile phase composition was adopted. For those that obtained a good separation, the mobile phase contained a high percentage of isopropanol, which also saved time. The retention and the separation increased when the temperature decreased for the pesticides except diniconazole, and low temperature should be used for optimization of the chromatographic conditions for most of the samples. Taking myclobutanil for example, the influence of temperature from 0–40°C on the separation was investigated using the *n*-hexane–isopropanol (85:15) mobile phase, and the retention factor, separation factor, and resolution factor showed a decrease with an increase in temperature. Figure 3 shows the chromatograms as a function of temperature on the chiral separation of myclobutanil. The retention factor decreased from 2.08 to 1.47 when the temperature turned from 0°C to 40°C, and the resolution factor decreased from 5.43 to 3.67. However, the chiral resolution of diniconazole did not changed much with temperature, the retention represented a slight decreased when the temperature increased, but the separation factor and resolution factor seemed un-changed. The similar result was also found on the CDMPC CSP (16), suggesting that the intrinsic properties of the analytes may play the main role for the influence of temperature on the separation. In this instance, the chromatographic condition optimization should not involve temperature control.

It was considered that two different effects of temperature could affect the chiral separation of enantiomers on CSP (22). One was a kinetic effect that influenced the viscosity and the diffusion coefficient of the solute. Another was the thermodynamic effect (enthalpic and entropic contributions) that changed the Gibbs free energy change (ΔG) of transfer of the enantiomer between the stationary phase and the mobile phase. The fact that the separation factor usually increased with decreasing temperature might because that the Gibbs free energy change (ΔG) of transfer of the analyte between the two phases increased at low temperature.

The enthalpic and entropic contributions could be calculated by van't Hoff equations (23–26):

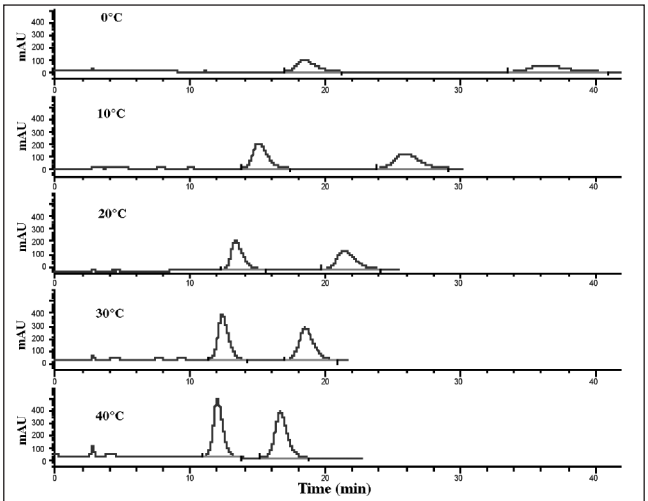

Figure 3. The influence of temperature on the chiral separation of myclobutanil.

$$\ln k = \frac{-\Delta H}{RT} + \frac{\Delta S}{R} + \ln\phi$$

and

$$\ln\alpha = -\frac{\Delta_{R,S}\Delta H^\circ}{RT} + \frac{\Delta_{R,S}\Delta S^\circ}{R}$$

Where ΔH and ΔS were the standard enthalpy and entropy of transfer of the solute from the mobile phase to the stationary phase. $\Delta_{R,S}\Delta H^\circ$ and $\Delta_{R,S}\Delta S^\circ$ were the differences $\Delta H_2 - \Delta H_1$ and $\Delta S_2 - \Delta S_1$ respectively. If plots of lnk versus 1/T were linear, the slope and intercept were $-\Delta H/R$ and $\Delta S/R + \ln\varphi(\Delta S^*)$ respectively. For a linear plot of lnα versus 1/T, the slope and intercept were respectively, $-\Delta_{R,S}\Delta H/R$ and $\Delta_{R,S}\Delta S/R$. However Van't Hoff plots were not always linear (16,27).

The plot of lnα versus 1/T for diniconazole at 0–40°C was not linear and thus the $\Delta_{R,S}\Delta H^\circ$ and $\Delta_{R,S}\Delta S^\circ$ values could not be calculated by experimental data. For the other 5 samples, the Van't Hoff plots were linear (R > 0.94) and the thermodynamic parameters were thus calculated. Figure 4 shows the van't Hoff plot of myclobutanil. The thermodynamic parameters of the pesticides are listed in Table III. The results indicated the resolutions were controlled by enthalpy except diniconazole, and the absolute values of $\Delta_{R,S}\Delta H^\circ$of the pesticides on ADMPC were consistent

Table II. The Effect of Temperature on the Resolutions

Sample	Temperature (°C)	k_1'	k_2'	α	Rs
Myclobutanil	0	7.93	16.53	2.08	5.43
(85:15)*	10	6.30	11.53	1.83	4.98
	20	5.47	9.37	1.71	4.14
	30	4.99	7.98	1.60	4.15
	40	4.82	7.08	1.47	3.67
Imazalil	0	9.05	9.87	1.09	0.81
(95:5)	10	8.17	8.83	1.08	0.79
	20	7.35	7.86	1.07	0.73
	30	6.78	7.18	1.06	0.67
	40	6.66	6.89	1.03	0.42
Paclobutrazol	0	3.04	4.27	1.40	2.00
(85:15)	10	2.84	3.86	1.36	1.76
	20	2.71	3.54	1.31	1.87
	30	2.63	3.32	1.26	1.66
Diniconazole	0	9.59	10.24	1.07	0.66
(95:5)	10	9.12	9.80	1.08	0.71
	20	9.06	9.69	1.07	0.71
	30	8.86	9.36	1.06	0.62
	40	8.80	9.44	1.07	0.73
Tebuconazole	0	6.28	7.76	1.24	1.64
(90:10)	10	5.62	6.73	1.20	1.44
	20	5.25	6.19	1.18	1.31
	30	4.97	5.85	1.18	1.55
	40	5.05	5.85	1.16	1.39
Uniconazole	0	4.94	6.49	1.31	1.58
(90:10)	10	4.71	5.99	1.27	1.62
	20	4.63	5.77	1.25	1.59
	30	4.54	5.58	1.23	1.58

* *n*-hexane–IPA.

with the resolutions. For example, myclobutanil obtained better separation than paclobutrazol, and the absolute $\Delta_{R,S}\Delta H°$ value of myclobutanil was higher than that of paclobutrazol. The values were comparative because they were obtained using the same mobile phase composition. The chiral separation of uniconazole and tebuconazole and the thermodynamic parameters also showed the trend.

Elution orders

HPLC using CD detector is a convenient technique for identifying low amounts of enantiomer (28,29). The elution orders of the enantiomers were studied and the results are listed in Table IV. The CD signal [(+) and (–)] was the differential absorbance for right circularly polarized light versus left circularly polarized light, and this differential absorbance was tightly related to wavelength. The elution orders of myclobutanil, imazalil, and paclobutrazol at the monitoring wavelength listed in Table IV were ±, and those of diniconazole, tebuconazole, and uniconazole were ±.

Conclusion

The chiral separation of several triazole pesticides were performed on the self-prepared ADMPC CSP using the HPLC system. Normal mode mobile phase was used. Four of six samples achieved complete resolution, and two obtained partial resolution. For most of the samples, to achieve better separation, a low content of isopropanol and low temperature should be used, but the retention and peak tailing increased as well and it is time consuming. For the chiral separation of diniconazole, the parameters did not change much with the temperature, suggesting it was not a valid parameter for optimization. The

Table IV. The Elution Orders of the Enantiomers on the CSP

Compound	W.L. (nm)	Elution order Pk1/Pk2	CD signal
Myclobutanil	235	±	
Imazalil	230	±	
Paclobutrazol	220	±	
Diniconazole	230	±	
Tebuconazole	225	±	
Uniconazole	240	±	

Mobile phase: *n*-hexane/isopropanol

Table III. The Thermodynamic Parameters for the Chiral Separation

Sample (*n*-hexane–IPA)	Enantiomer	$\ln k = -\Delta H/RT + \Delta S^*$	ΔH kJ/mol	ΔS*	$\ln\alpha = \Delta_{R,S}\Delta H/RT + \Delta_{R,S}\Delta S/R$	ΔΔH kJ/mol	ΔΔS J/mol/K
Myclobutanil (85:15)	E1*	$\ln k_1 = 1062/T - 1.88$, $R = 0.9677$	–8.83	–1.88	$\ln\alpha = 709.96/T - 1.88$, $R = 0.9972$	–5.90	–15.63
	E2*	$\ln k_2 = 1777.4/T - 3.78$, $R = 0.9837$	–14.78	–3.78			
Imazalil (95:5)	E1	$\ln k_1 = 688.34/T - 0.33$, $R = 0.9836$	–5.72	–0.33	$\ln\alpha = 111.51/T - 0.32$, R = 0.9741	–0.93	–2.66
	E2	$\ln k_2 = 795.05/T - 0.63$, $R = 0.9922$	–6.61	– 0.63			
Diniconazole (95:5)	E1	not linear			not linear	–	–
	E2	not linear					
Paclobutrazol (85:15)	E1	$\ln k_1 = 400.17/T - 0.36$, $R = 0.9896$	–3.33	–0.36	$\ln\alpha = 291.66/T - 0.73$, $R = 0.9954$	–2.42	–6.07
	E2	$\ln k_2 = 697.67/T - 1.11$, $R = 0.9978$	–5.80	–1.11			
Tebuconazole (90:10)	E1	$\ln k_1 = 484.731/T + 0.03$, $R = 0.9381$	–4.03	0.03	$\ln\alpha = 127.36/T - 0.26$, $R = 0.9677$	–1.06	–2.16
	E2	$\ln k_2 = 612.091/T - 0.23$, $R = 0.9502$	–5.09	–0.23			
Uniconazole (90:10)	E1	$\ln k_1 = 225.181/T + 0.77$, $R = 0.9739$	–1.87	0.77	$\ln\alpha = 183.081/T - 0.40$, $R = 0.9875$	–1.52	–3.33
	E2	$\ln k_2 = 408.261/T + 0.36$, $R = 0.9812$	–3.39	0.36			

* E1 and E2 represent the first and second eluted enantiomers, respectively.

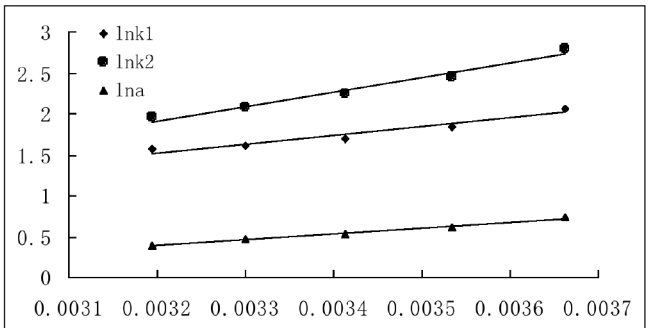

Figure 4. The Van't Hoff plot of myclobutanil.

enantiomers were identified by the CD detector and the elution orders were determined. The excellent chiral separation of myclobutanil, paclobutrazol, uniconazole, and tebuconazole, which were still produced and used in racemates provided a convenient method for enantiomeric semipreparation, optical purity determination of the technical and the chiral residual analysis of real samples. Diniconazole and imazalil obtained part separation and the chromatographic conditions needed further optimization.

Acknowledgments

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